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CASE REPORT

THE FIRST REPORT OF A NON-AUTOCHTHONOUS HUMAN CASE OF DIPHYLLOBOTHRIOSIS IN THE STATE OF PARANÁ, BRAZIL

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ABSTRACT

Diphyllobothriasis is a zoonotic disease caused by consuming raw or undercooked fish contaminated with fish tapeworms, and it is emerging as a global public health issue. Although cases have been reported in several Brazilian States, this study describes the first non-autochthonous human case of Diphyllobothriasis in Paraná, Brazil, specifically in Curitiba. The following case is presented: a woman developed symptoms after eating raw fish during a trip to Pucón, Chile. Molecular analysis of the expelled proglottids confirmed the presence of *Diphyllobothrium* species, with epidemiological evidence supporting the identification of *D. latum*. This study is the first to report a non-autochthonous human case of Diphyllobothriasis in Paraná, Brazil. This case highlights the importance of considering parasitic infections in the differential diagnosis of patients with gastrointestinal symptoms and a history of international travel, especially to regions where the disease is endemic.

KEY WORDS: Diphyllobothriasis; *Diphyllobothrium latum*; fish-borne infection; raw meal; one health; zoonosis.

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INTRODUCTION

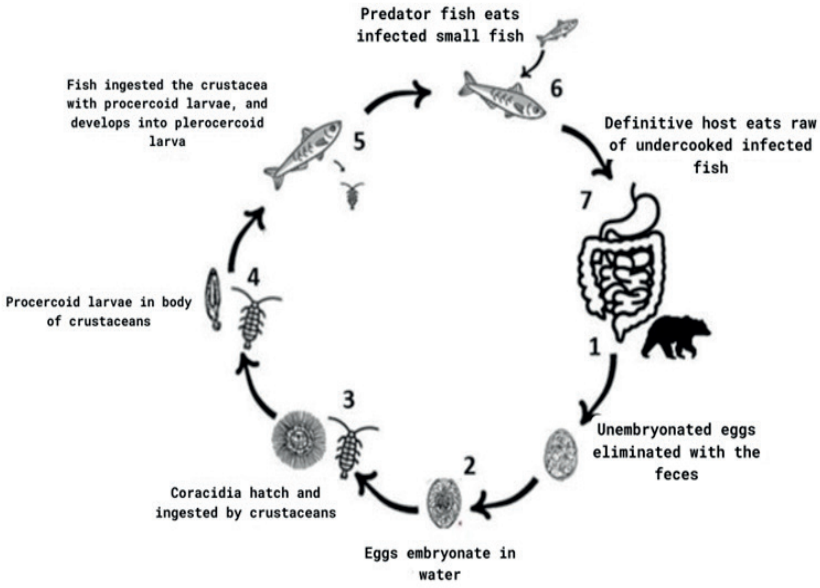
Diphyllobothriasis is a zoonosis caused by the ingestion of raw or undercooked fish contaminated with cestode Diphyllobothriidae parasites of different genera, such as *Adenocephalus*, *Dibothriocephalus*, and *Diphyllobothrium* (Cobbold, 1858; Nybelin et al., 1931; Hernández-Orts et al., 2015; Waeschenbach et al., 2017).

Research has demonstrated that there are over 16 species capable of causing human infections; however, only four of these are more prevalent in such cases. *Adenocephalus pacificus* (Nybelin et al., 1931), endemic to the South Pacific, *Diphyllobothrium latum* (Cobbold, 1858), which is distributed in Chile, *D. nihonkaiense* (Cobbold, 1858), commonly found in the North Pacific region, and *D. dendriticum* (Hernández-Orts et al., 2015). The distribution of the species under consideration is known to extend as far as the Arctic (see Osorio et al., 1974; Llaguno et al., 2008; Kuchta et al., 2013; Scholz et al., 2016; Caira & Jensen, 2017).

The biological cycle of this parasite involves the presence of intermediate and definitive hosts (Figure 1). The intermediate hosts comprise microcrustaceans of the genera *Cyclops* spp. and *Diaptomus* spp., as well as medium and large fish. The definitive hosts include mammals, including humans (Flores et al., 2002; Oyarzún-Ruiz & González-Acuña, 2021).

It is generally accepted that the confirmation of this parasitosis is achieved through the implementation of coproparasitological tests. However, to ensure the precise identification of the species, it is imperative to conduct molecular analysis (Osorio et al., 1974; Leštinová et al., 2016). However, some structures and characteristics of the morphology of eggs, shell thickness, color, operculum diameter, presence of apical bud or size of embryonic hooks and proglottids, presence of protuberances, transverse grooves, depressions and/ or folds anterior to the male gonopore in pregnant proglottids, have been discussed for a primary identification, but only molecular analysis can reach the species level (Kuchta et al., 2013; Hernández-Orts et al., 2015; Leštinová et al., 2016).

Figure 1. Biological cycle of the parasite (Cestoda: Diphyllobothriidea): 1) eggs are released into environment by the definitive host; 2) eggs in contact with water are embryonated and release a coracidia larva; 3) coracidia hatch from eggs and are ingested by the first intermediate host, crustaceans; 4) procercoid larvae develop within the body cavity of crustaceans; 5) infected crustacean ingested by the second intermediate host, usually a small fish. Procercoid larva released from crustacean develops into plerocercoid larva; 6) predator fish eats small fish, and plerocercoid larva invades the tissue; 7) humans and other fish-eating mammals.



Source: Authors

According to Oliveira et al. (2017), the State of São Paulo experienced 68 cases of Diphyllobothriasis between 2004 and 2008, with a progressive increase of 16 cases in 2004, 39 cases in 2005, nine cases in 2006, one case in 2007, and three cases in 2008. Moreover, during this same period, the official institution, *Secretaria de Saúde de São Paulo*, reported cases in various Brazilian cities, showing the total number of occurrences: one case in Salvador (Bahia), one in Ribeirão Preto (São Paulo), 13 in Rio de Janeiro, five in Porto Alegre (Rio Grande do Sul), one in João Pessoa (Paraíba), two in Vitória (Espírito Santo), and one in Brasília (Distrito Federal). It is noteworthy that no new cases of Diphyllobothriasis were reported in the country between 2009 and 2012 (Emmel et al., 2006; Hack et al., 2021; SESP, 2025).

The present case report has two objectives. Firstly, it aims to provide a detailed description of the first reported instance of Diphyllobothriasis in a non-autochthonous human case in the State of Paraná, Brazil, specifically in the

city of Curitiba. Secondly, it seeks to facilitate a discussion of the implications of this case for public health in the country within the context of a One Health approach, taking into consideration other cases that have been documented both nationally and globally.

CASE REPORT

A 31-year-old woman patient living in Curitiba, Paraná, traveled to Pucón, Chile, in December 2021. She and her family ate a typical regional dish called ceviche, served with raw fish caught in Pucón, Chile. The patient began to show symptoms a week after returning from Pucón, these being epigastric pain and abdominal distension. Due to these symptoms, the patient sought help from a gastrointestinal specialist who required blood counts instead of parasitological tests. Laboratory tests revealed only eosinophilia, which is suggestive of parasite infection.

In July, while defecating, the patient spontaneously released a strobile with a part of the parasite's proglottids. A stool sample and proglottids were sent to the laboratory for analysis.

The biological material was fixed in acetic formaldehyde. The tests consisted of spontaneous sedimentation and centrifugal-float techniques. The parasitological examination of the feces by microscopy identified the presence of oval eggs with opercula, with an average size of 64 × 45µm, and a yellowish-brown color (Figure 2). Measurements of eggs were made using an ocular millimeter (Leštinová et al., 2016).

From the strobile expelled by the patient, three proglottids were separated to make a slide. The proglottids were stained in crimson borax and mounted in Canada balsam, and an image was obtained using a Nikon Confocal A1R MP+ microscope (Hernández-Orts et al., 2015).

Molecular analysis

Diphyllobothriasis is a parasitic disease usually confirmed by coproparasitological methods in conventional laboratories. However, molecular analysis helps to obtain more accurate results regarding genus and species (Osorio et al., 1974; Leštinová et al., 2016).

For molecular identification, part of the proglottids was used for amplification and partial sequencing of the 18S rDNA gene. Approximately 100 mg of the sample was used for DNA extraction with the Pathogen Gold kit (Loccus-Extracta® Kit - DNA and RNA Pathogens), according to the manufacturer's recommendations. The DNA was quantified and assessed for purity using a Nanodrop2000® (Thermo Fisher).

Amplification of the 18S rDNA region was performed using a PCR

reaction containing 20 ng of genomic DNA and the primers 83F 5'GAT ACC GTC CTA GTT CTG ACC A3' and 81R 5'TTC ACC TAC GGA AAC CTT GTT ACG3' (Mariaux, 1998). For the PCR mix, 1.25 µL of PCR buffer, 0.25µL of each primer, 1 µL of dNTP mix (10 mM), 0.35 µL of MgCl (25 mM), 2.5U of taq DNA polymerase, 8 µL of sterile MilliQ water, and 1 µL of template DNA (20 ng) were used, totaling a volume of 12.5 µL. The reaction conditions included an initial denaturation step at 95 °C for 5 min, followed by 30 cycles of denaturation at 95 °C for 30 s, annealing for 30 s at 51 °C, extension at 72°C for 1 min, and a final extension at 72 °C for 10 min.

The products were analyzed by agarose electrophoresis. The reactions were enzymatically purified with ExoI/SAP® (Thermo Fisher), according to the manufacturer's recommendations.

The PCR products were labeled BigDye v3.1® (Thermo Fisher) according to the manufacturer's instructions. Samples were sequenced on the ABI3500R automated sequencer® (Applied Biosystems, Foster City, CA, USA). Consensus sequences were analyzed and aligned using Mega 6.0 (Tamura et al., 2013) and BioEdit and compared to sequences available in the GenBank database (http://www.ncbi.nlm.nih. gov/BLAST/).

Ethics approval

This work was approved by CHC/UFPR Ethics Committee for Research in Human Beings under number CAAE91542618.0.0000.0102 on 14/06/2019.

DISCUSSION

Diphyllobothriasis is a rare condition that is not well documented in this country. However, there is evidence to suggest that it has appeared in some regions of Brazil and caused severe outbreaks (Osorio et al., 1974; Caira & Jensen, 2017). The infection may be transmitted in two ways: through the ingestion of contaminated fish originating from endemic regions, and through consumption in the endemic region itself (Osorio et al., 1974; Llaguno et al., 2008). This case report details of the infection of a woman by fish tapeworms (Cestoda: Diphyllobothriidae) after a trip to Chile, a case akin to those previously documented by Osorio et al. (1974) and Waeschenbach et al. (2017).

Using BLAST analysis of the GenBank database, the isolate was classified as belonging to the genus *Diphyllobothrium*, presenting 100% similarity of the 18S rDNA sequence obtained with the species *Diphyllobothrium latum, D. nihonkaiense*, *D. tetrapterum*, *D. schistochilo*,

and *Dibothriocephalus ditremus*. Therefore, due to the site of infection and the likely intermediate host, we suggest that the causative species may be *D. latum*, since previous studies showed that *D. latum* is the most common species associated with the consumption of raw fish or infections in Chile (Torres et al., 2004; Emmel et al., 2006; Mercado et al., 2010; Rosas & Weitzel, 2014; Rubilar et al., 2022).

The eggs were measured (n= 20); the average egg size was 47.27 × 65.54 µm. The proglottids (n= 3) had an average size of 2316.51 × 4283.77 µm (Figure 2). They presented the typical characteristics for some species of the genus Diphyllobothriidae, such as gravid proglottids wider than long, a rosette-shaped uterus, and median uterine and genital pores in the anterior third of the proglottid. As already pointed out by several authors, the measurements and morphological characteristics observed in the eggs and proglottids did not allow specific identification (Scholz et al., 2016; Caira & Jensen, 2017; Waeschenbach et al., 2017).

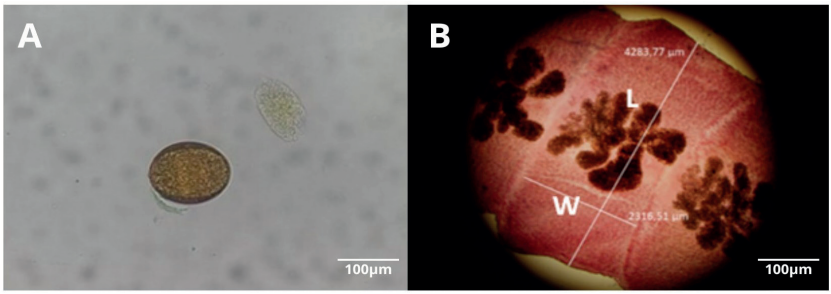


Figure 2. Egg (A) and part of the parasite strobila (proglottid) by Diphyllobothriidae. B= Strobila with three proglottids. W= width and L= length.

The first documented instance of Diphyllobothriasis in Brazil was reported by Pirajá da Silva in the northeastern State of Bahia (Caira & Jensen, 2017). Subsequent studies have identified a small number of additional cases, all of which pertain to individuals who have travelled to other countries (Osorio et al., 1974; Flores et al., 2002; Llaguno et al., 2008; Caira & Jensen, 2017). In Brazil, from 2004 to 2005, there were approximately 92 cases of Diphyllobothriasis reported in the States of São Paulo, Rio de Janeiro, Minas Gerais, Bahia, and Rio Grande do Sul. However, no cases were reported from 2009 to 2012, with the most recent case reported in the literature occurring in 2018 in Rio Grande do Sul (Osório et al., 1974; Caira & Jensen, 2017; Hack et al., 2021).

Although Oliveira et al., (2017), Emmel et al., (2006), Hack et al., (2021), and SESP, 2025, described a total of 92 cases of Diphyllobothriasis reported in Brazil between 2004 and 2008, distributed in São Paulo (with 68 cases, including 39 in 2005), Rio de Janeiro (13 cases), Rio Grande do Sul (five

cases in Porto Alegre) and others in Salvador, Ribeirão Preto, João Pessoa, Vitória and Brasília, it is crucial to consider that these numbers may represent significant underreporting.

The lack of records between 2009 and 2012, for example, does not necessarily indicate the absence of parasitic disease, but may reflect a lack of awareness of the disease or difficulties in diagnosis and reporting by healthcare professionals.

The emergence of a case in a new location, such as the first report in Paraná, reinforces the hypothesis that Diphyllobothriasis may be entering our country and not being correctly diagnosed or reported. This scenario highlights the urgency of improving epidemiological surveillance and training in disease recognition to obtain a more accurate understanding of its true prevalence and impact on Brazilian public health.

This is the first report of a non-autochthonous case of Diphyllobothriasis in the State of Paraná. The increase in Diphyllobothriasis cases in non-endemic countries has attracted the attention of public health authorities, because imported infections can allow the parasite to establish itself in new regions (Flores et al., 2002; Llaguno et al., 2008). In Brazil, moreover, both the farming and consumption of imported fish are involved in the life cycle of *Adenocephalus* spp., *Dibothriocephalus* spp., and *Diphyllobothrium* spp. pose an additional risk.

A further salient point emphasized by this report is the necessity for more precise laboratorial diagnoses. As previously documented, the patient sought consultation with a gastroenterologist, who had the chance to order parasitological examinations. These investigative procedures would likely have revealed parasitemia, thereby enabling timely treatment (Wendt et al., 2025). This scenario highlights the significance of requesting appropriate parasitological tests, particularly for patients exhibiting gastrointestinal symptoms and/ or having recently travelled to regions endemic for parasites such as *Adenocephalus* spp., *Dibothriocephalus* spp., and *Diphyllobothrium* spp.

In conclusion, this case report, in conjunction with preceding studies, calls upon public health authorities to devise and initiate control measures against fish tapeworms to prevent their establishment as endemic parasites in Brazil. It is strongly recommended that patients presenting with intestinal manifestations undergo routine parasitological testing.

CONFLICTS OF INTEREST

All authors declare that there are no conflicts of interest.

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