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SHORT COMMUNICATION

First report of *Myxobolus* (Cnidaria: Myxozoa) spores in human feces in Brazil

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ABSTRACT

Most species of *Myxobolus* (Cnidaria: Myxozoa) infect freshwater and marine fish, and cause sporozoosis. Passage of myxozoan parasites into human feces has been described occasionally. We examined fecal samples from 97 dwellers of a riverine community in Amazonas State (Brazil), which revealed *Myxobolus* sp. in 13 (13%) samples**.** The discovery probably does not represent true parasitism as the myxospores – most likely ingested through the consumption of infected fish – were eliminated unchanged after passing through the gastrointestinal tract. This discovery represents the first report of this myxosporean in human fecal samples in Brazil.

KEYWORDS: myxosporean, human infection, Amazon, fish consumption

Primeiro relato de esporos de *Myxobolus* (Cnidaria: Myxozoa) em fezes humanas no Brasil

RESUMO

A maioria das espécies de *Myxobolus* (Cnidaria: Myxozoa) infecta principalmente peixes de água doce e marinhos, nos quais produz esporozoose. A passagem de parasitos mixozoários em fezes humanas tem sido ocasionalmente descrita. Nós examinamos amostras fecais de 97 moradores de uma comunidade ribeirinha no estado do Amazonas e identificamos *Myxobolus* sp. em 13 (13%) delas. O achado provavelmente não representa um parasitismo verdadeiro, uma vez que os mixosporos – mais provavelmente ingeridos ao consumir peixes infectados – foram eliminados inalterados após a passagem pelo trato gastrointestinal. O achado representa o primeiro relato deste mixosporídeo em amostras fecais humanas no Brasil.

PALAVRAS-CHAVE: mixosporídeo, infecção humana, Amazonas, consumo de peixes

Myxozoans of the genus *Myxobolus* (Bütschli 1882) belong to the phylum Cnidaria, sub-phylum Myxozoa, class Myxosporea. Myxosporea is divided in the orders Bivalvulida and Multivalvulida. Bivalvulida includes the family Myxobolidae, which includes the genera *Myxobolus* and *Henneguya (Okamura et al. 2015);* the order Multivalvulida includes the genus *Kudoa.* These three genera have already been found in human feces.

Myxozoa exhibits complex life cycles with sexual reproduction occurring within invertebrate definitive hosts such as aquatic annelids or freshwater bryozoans (Eszterbauer *et al*. 2015). In addition, it infects intermediary vertebrate hosts such as fish, amphibians, and reptiles (Feist 2008; Eiras *et al.* 2005). It has also been found to infect endotherms: mammals (Prunesco *et al*. 2007) and birds (Bartholomew *et al*. 2008).

Transmission of myxozoans between invertebrate and vertebrate hosts is mediated by spores (Feist 2008). The morphology and morphometry of the spore or myxospore is the basis for the identification of *Myxobolus* species (Feist 2008). However, molecular studies have improved diagnostic tools for this myxosporean and have allowed the description of new species (Eiras *et al*. 2014).

Myxozoan infections in fish can result in substantial economic losses to aquaculture and fisheries, because they can cause damage to wild and farmed fish (Okamura *et al*. 2015). The infection by *Myxobolus* sp. is characterized by the formation of cysts in tissues with spores containing only two polar capsules. The presence of these cysts has been associated with gross deformities, tissue lesions, and organ malfunction, while in the gills, they can cause bleeding and swelling, which leads to a decreased surface for oxygen absorption (Feist 2008).

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Myxospores of Myxozoa have been incidentally found in human fecal samples, which were collected to investigate soil-transmitted helminthiases or intestinal diseases caused by protozoa of medical importance (Bradbury *et al*. 2015; McClelland *et al*. 1997). The presence of myxospores in human feces has been associated with ingestion of contaminated food or undercooked fish (Kawai *et al*. 2012). Infections in humans are considered accidental and without association with clinical manifestations (Bradbury *et al*. 2015; McClelland *et al*. 1997; Boreham *et al*. 1998). Although association of the parasite with gastrointestinal disorders has been reported (Moncada *et al*. 2001; Kawai *et al*. 2012), a causal relation with myxospores could not be established due to the concomitant presence of other enteric pathogens (Hessen and Zamzame 2004).

The parasite has been recorded in several Brazilian states (Adriano and Oliveira 2018), including the northern region (Amazonas and Pará states), which belongs to the Amazon region. *Myxobolus* was identified in the gills of *Semaprochilodus insignis,* known in Brazil as jaraqui (Eiras *et al*. 2005), in the blood of tambaqui, *Colossoma macropomum* (Maciel *et al*. 2011), in the subcutaneous tissue of pacu, *Metynnis argenteus* (Casal *et al*. 2006), in the skin and gills of pirarara, *Phractocephalus hemioliopterus* (Naldoni *et al*. 2018), in the gills of dourada, *Brachyplatystoma rousseauxii* (Zatti *et al*. 2018), and in ornamental fish endemic to the Amazon basin (Mathews *et al*. 2016). However, *Myxobolus* sp. had never been reported in human feces in Brazil.

In October – November 2015, we carried out a field survey to evaluate the prevalence of intestinal parasites in inhabitants of a rural community in the state of Amazonas, in the Brazilian Amazon. The riverine community of Limão (03°11'0.99"S, 60°20'35.89"W) is located in the municipality of Iranduba, at 52 km from the capital city of Manaus (Figure 1), on the shores of Limão Lake, near the Ariaú River. The climate is characterized by a rainy season, when annual flooding occurs, and a dry season. The study was approved by the Ethics Committee (CEUA) of Universidade Federal do Amazonas (protocol 41067414.6.0000.5020).

Fecal samples from 97 asymptomatic dwellers were collected in transport boxes and sent to the laboratory for analysis on the same day of delivery. The Hoffman concentration method was used for the parasitological analysis (Hoffman *et al*. 1934). In 13 (13%) of the 97 samples, the parasitological examination of fecal samples resulted in the detection of Myxozoan spores, which were identified, based on their morphology and morphometry, as *Myxobolus* sp. (Figure 2). All samples presenting myxospores were from adults (22 to 71 years old) living in nine of the forty houses visited. Of these nine houses, three had 2 or 3 residents with positive samples (n = 7).

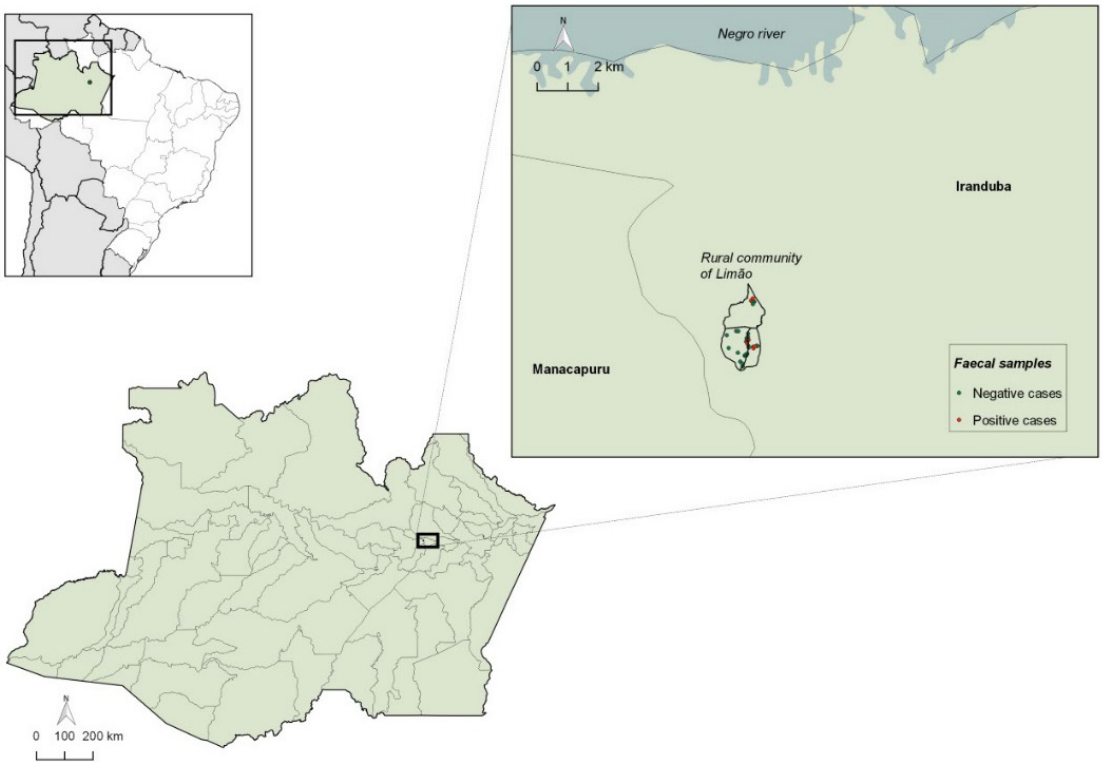

Figure 1. Location of the study site (Limão community) where *Myxobolus* sp. was recorded in human fecal samples (municipality of Iranduba, Amazonas state, Brazil). This figure is in color in the electronic version.

The myxospores were micro-photographed using a digital system (Synoptics™, Cambridge, UK) coupled to an optical microscope (Leica DMTM1000, Frankfurt, Germany). The program Auto Montage 4.0 was used to obtain the final images. The spores of *Myxobolus* sp. were pyriform in shape and exhibited thick walls (Figure 2), were bilaterally symmetric, had one suture, and two polar capsules on each finer side of the central axis of the structure. Fresh spores (n = 13) were 10 to 15 µm in length and 7.5 to 10 µm in width (average size, 12.7 µm by 8.1 µm); the polar capsules were 3.7 µm in length and 2.5 µm in width.

In the Amazonas state, some studies have reported the occurrence of *Myxobolus* species infecting freshwater fish from farmed and natural environments, including *Semaprochilodus insignis* (jaraqui) (Eiras *et al*. 2005) and *Corydoras melini* (coridora) (Mathews *et al*. 2016). Consumption of fish in this state is very high, and is the main source of protein, particularly for people living in rural areas and in riverine communities (Lopes *et al*. 2016). Therefore, we assume that the spores identified in human feces were probably acquired through the consumption of infected fish and passed intact through the human digestive tract. Thus, the parasite probably did not establish an infection in humans that was able to cause disease.

However, *Myxobolus* was identified as the probable cause of diarrhea in an immunocompromised patient (Moncada *et al*. 2001). Another Myxozoa, *Kudoa septempunctata*, was responsible for outbreaks of food poisoning in Japan, caused by the consumption of raw fish (Kawai *et al*. 2012), and its sporoplasms may invade human epithelial cells (Ohnishi *et al*. 2013), though evidence of its pathogenicity is still weak (Chung and Bae 2017). Consuming raw, undercooked, or smoked fish can also transmit other parasites such as *Diphyllobothrium latum*, a cestode that causes dipyllobothriasis in humans (Emmel *et al*. 2006). Parasitic nematodes of fishes also can infect humans (Eiras *et al*. 2016).

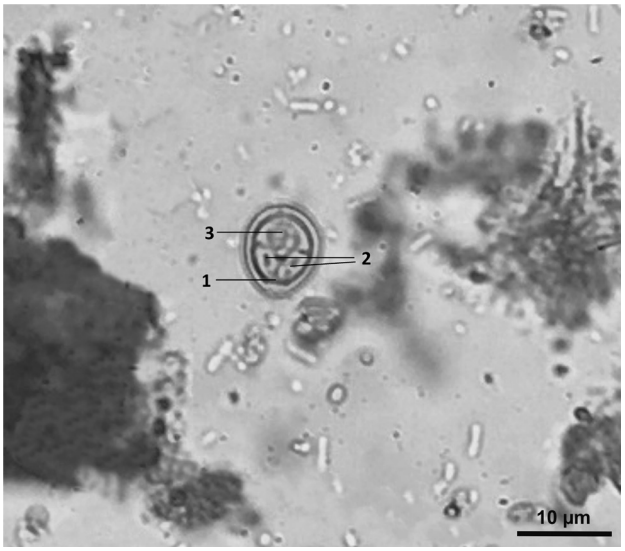

Figure 2. Microscopic image of a fresh spore of *Myxobolus* sp. in a human fecal sample from Limão community (Iranduba, Amazonas, Brazil): 1- suture; 2 - polar capsules; 3 - sporoplasm.

We must also consider that the presence of myxospores may lead to erroneous diagnoses in pathology laboratories that examine human stools due to possible confounding interpretations with other structures (McClelland *et al*. 1997), or even leading to concerns of a new, previously unidentified protozoan species infecting humans rather than an artefact caused by the passage of fish parasitic spores.

At present, there is no evidence that the myxosporean life cycle involves a human host, and no stages of the parasite development besides the spores have been detected in humans (Hallet *et al*. 2015). Nevertheless, it is important to consider the possibility of myxosporean spores being passed into human feces while analyzing and diagnosing intestinal parasites, as well as detecting any change in the biology or ecology of myxosporidia involving human populations.

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