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# Occurrence of *Cryptosporidium* spp., *Giardia* spp. and other pathogenic intestinal parasites in the Beberibe River in the State of Pernambuco, Brazil

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## ABSTRACT

**Introduction:** Transmission of pathogenic protozoa and helminths by water is a serious public health problem. In this study, we analyzed the presence of these organisms in the Beberibe River in Pernambuco, Brazil. **Methods:** Parasite analysis was performed using the Hoffman, Pons, & Janer method followed by centrifugation and preparation of slides by staining with acetic acid and Lugol's solution. Protozoan oocysts were isolated by the modified Ziehl Neelsen method. **Results:** *Cryptosporidium* spp., *Giardia* spp. and other parasites were found in the Beberibe River. **Conclusions:** Sanitation companies must assess pathogenic intestinal parasites in water basins providing public water and subsequently develop improved treatment systems for removal of such parasites.

**Keywords**: Protozoa. Beberibe River. *Cryptosporidium* spp.

Transmission of pathogenic protozoa by water is a major public health concern[1] and therefore a challenge for producers and distributors of water systems[2]. For example, the pathogenic intestinal protozoa *Giardia* spp. and *Cryptosporidium* spp. exhibit resistance to chemical disinfectants and are long-lived in the environment; thus, these pathogenic parasites are highly problematic for sanitation companies and public health officials[3]. Although conventional water treatment stations are able to remove 99% of *Cryptosporidium* spp. oocysts from water sources, there is still a risk of transmission[4]. Indeed, because oocysts exhibit high compressibility during filtration, these oocysts can behave as small particles (2-4µm), allowing them to pass through commonly used filters and enter treated water provided to consumers[5]. Therefore, improved methods for detection and removal of such pathogenic parasites is necessary.

*Cryptosporidium* spp. and *Giardia* spp. have been found in surface water in several countries, such as the United States on Lake Texoma (on the border of Texas and Oklahoma)[6], in France on Seine River and its tributary Marne River[7], in Hungary on the Danube River[8], and in China in the reservoir of the Three Gorges Dam[9]. In Brazil, in accordance with decree number 2914 of the Health Ministry (year 2011), which describes water quality procedures for human consumption and potability patterns, sanitation companies are expected to survey water intended for human consumption for the presence of *Giardia* spp. and *Cryptosporidium* spp.[10]. However, methods for detection of *Cryptosporidium* spp. are complex, and proper survey of water supplies would require specialized human resources, which are scarce. Additionally, there is little information about these pathogens in national surface water bodies[1]. Therefore, while many researchers are studying *Cryptosporidium* spp. and *Giardia* spp., more studies are needed to further characterize these pathogens[6]. Indeed, in the northeast region of Brazil, few studies have characterized or identified pathogenic intestinal parasites, such as some species of helminths and protozoa. An initial report describing water supply sources showed contamination by *Cryptosporidium* spp. in the Capibaribe River and the Tapacura reservoir[11] in State of Pernambuco, Northeast Brazil.

In this study, we characterized the presence of intestinal parasites in the Beberibe River in Pernambuco. The human population at the Beberibe River basin is approximately 590,000 inhabitants, with an average population density of 7,300 inhabitants/km$^2$. Moreover, this area has many low-income settlements in the Recife metropolitan region (RMR); these settlements are scattered in risk areas, hills, and flooded areas and are characterized by poor urban infrastructure.

The Beberibe River is one of the most polluted rivers in Pernambuco due to the insufficient sanitary conditions of Olinda

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and Recife. Despite this, the Beberibe River is one of the water sources for the public water system, supplying about 10% of the volume of water distributed throughout the RMR. In particular, the Beberibe River contributes enough water for approximately 100,000 people in the RMR[12].

Between May 2012 and May 2013, water samples were collected in duplicate from the Beberibe River at two locations where sampling was easy (i.e., convenience sampling). Samples were collected from the Beberibe River in sterile 1-L plastic bottles and transported to the Laboratory of Parasitology, Department of Tropical Medicine of the Federal University of Pernambuco [*University Federal of Pernambuco* (UFPE)] for analysis of intestinal parasites. Protozoa and helminths were analyzed using the Hoffman, Pons, & Janer method or spontaneous sedimentation. The time to sedimentation was between 24 and 48h for 250-mL samples. Subsequently, aliquots of the sediment were centrifuged at 900rpm for 5-10 min. During the water sampling, turbidity (portable turbidity meter, Hanna Instruments model HI93703), water temperature, and depth of the river water were also analyzed. The pH of water samples was measured using the standard method (SM 4,500-H+) and electrometric method[13].

For each sample in duplicate, two slides were made with approximately 1mL of the centrifuged sample. Because collections were made at two points of the river, a total of eight slides were made for each collection day; samples were collected on 16 days, yielding a total of 128 slides. The slides were stained with Lugol iodine and analyzed under an optical microscope at 10× and 40× magnification for qualitative evaluation of protozoa and helminth parasite contamination.

A modified Ziehl Neelsen method was used for identification of *Cryptosporidium* spp. oocysts, with fuchsin-phenolated solution followed by spontaneous sedimentation. For the identification of C*ryptosporidium* spp. oocysts by optical microscopy, an oil-immersion 100× objective lens was used to visualize the whole smear, observing the shape, color, and size of oocysts. A micrometer was coupled to the microscope for confirmation of the morphometric identification of the parasite.

Bacteriological parameters were evaluated in water samples by determination of total coliforms and *Escherichia coli* between June 2012 and May 2013. Samples (250mL) were collected in sterilized polyethylene containers. Fermentation in multiple tubes was performed with chromogenic substrate[13], with results presented as the most probable number (MPN) in 100-mL samples (MPN/100mL).

**Table 1** shows parasite contamination in the Beberibe River and physicochemical parameters. *Cryptosporidium* spp. oocysts: 75% (12/16), *Giardia* ssp. cysts: 50% (8/16), complexes of *Entamoeba histolytica/E. dispar*: 50% (8/16), *Cystoisospora belli:* 6.3% (1/16), *Endolimax nana:* 81.3% (13/16), *Entamoeba coli*: 37.5% (6/16), and *Iodamoeba butschlii:* 6.3% (1/16) were detected in the water samples collected from the Beberibe River

**TABLE 1 - Occurrence of intestinal parasites in water samples from the Beberibe River.**

| | 2012 | | | | | | | | 2013 | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Microorganisms | April 14 | May 29 | June 11 | June 25 | August 20 | September 3 | October 15 | October 29 | February 5 | February 19 | March 5 | March 19 | April 4 | April 23 | May 15 | May 28 |
| *Cryptosporidium* spp. | P | P | P | P | P | P | A | A | A | P | P | A | P | P | P | P |
| *Giardia* spp. | P | P | A | P | P | P | A | A | P | A | A | P | A | A | P | A |
| *Complex Entamoeba histolytica/E. dispar* | P | A | P | P | A | A | A | P | A | A | P | A | P | P | P | A |
| *Isospora belli* | P | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A |
| *Endolimax nana* | P | P | P | P | P | A | P | P | A | A | P | P | P | P | P | P |
| *Entamoeba coli* | A | P | P | A | P | A | A | A | P | A | A | P | A | P | A | A |
| *Iodamoeba butschlii* | A | P | A | A | A | A | A | A | A | A | A | A | A | A | A | A |
| *Strongyloides* spp. *larvae* | P | P | P | P | P | A | P | A | P | P | P | P | A | P | P | P |
| *Ascaris* spp. eggs | P | P | P | A | P | A | A | P | A | A | P | P | P | P | P | P |
| *Ancilostomideo larvae* | A | P | P | A | A | A | A | A | A | A | A | A | A | A | A | A |
| *Ancilostomideo* eggs | P | P | A | A | P | P | A | A | A | A | A | A | A | P | A | A |
| *Hymenolepis nana* eggs | P | P | A | P | A | A | P | A | A | A | A | A | P | P | A | A |
| *Hymenolepis diminuta* eggs | A | A | A | A | A | A | A | P | A | A | A | A | A | A | A | A |

P: present; A: ausent; *E: Entamoeba*.

**TABLE 2 - Physicochemical parameters measured in the Beberibe River between May 2012 and May 2013.**

| Parameters | Unit | Mean ± standard deviation | Minimum – maximum |
|---|---|---|---|
| Temperature | °C | 27.6 ± 1.1 | 26.0 – 30.0 |
| pH | - | 6.9 ± 0.2 | 6.6 – 7.2 |
| Turbidity | uT | 35.32 ± 28.87 | 16.31 – 100.00 |

pH: Potential Hydrogenionic; uT: Turbidity units.

in this study. The sizes of *Cryptosporidium* spp. oocysts found in the Beberibe River ranged from 3.3 to 6.6µm, with 0-8 oocysts per slide. *Endolimax nana* with 81.3% (13/16) was frequently present, as shown by microscopic analysis of the water samples; these data confirmed that the Beberibe River was contaminated with human excreta.

Helminths were also present in water samples from the Beberibe River; we identified *Strongyloides* ssp.: 81.3% (13/16), *Ascaris* spp. eggs: 68.8% (11/16), hookworm eggs: 12.5% (2/16,) and larvae: 31.3% (5/16), *Hymenolepis nana*: 37.5% (6/16), and *Hymenolepis diminuta* 6.3% (1/16). The number of total coliforms in the Beberibe River ranged from ≥ 1,600 to ≥ 160,000 NMP/100mL, while that for *Escherichia coli* ranged from 50,000 to ≥ 160,000 NMP/100mL.

Similar results of intestinal parasites were observed in a study performed in the catchment area of the Danube River in the Galati region of Romania, with particularly high frequency of the protozoa *Cryptosporidium* spp., *Giardia* spp., *Iodamoeba butschli*, *Endolimax nana*, *Entamoeba coli*, and *Entamoeba histolytica* and helminths such as *Ascaris lumbricoides*, *Hymenolepis diminuta*, and *Strongyloides stercoralis*[14]. *Cryptosporidium* spp., *Giardia* spp., *Ascaris* spp., *Schistosoma* spp., and *Acantamoeba* spp. were also detected in water samples from two recreational lakes in the region of Selangor, Malaysia[15].

The Beberibe River is characterized by constant discharge of domestic wastewater; this may have influenced the pH values of the water samples, which were slightly alkaline values (**Table 2**).

Due to the detection of parasite contamination in the Beberibe river, we suggest that water production and distribution companies should assess the presence of intestinal parasites (particularly protozoa and helminths) in public water supplies and should consider improving their treatment systems to ensure removal of such pathogens, which are known to be highly resistant to chemical disinfectants and to be particularly long-lived in the environment.

We hope that the information presented in this study, i.e., the presence of pathogenic intestinal parasites in the Beberibe River in Pernambuco, Brazil, may contribute to improvements in public health. In addition, public authorities need to create documentation for informing the population of the occurrence of parasites in watersheds providing public water supplies.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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