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OCCURRENCE OF *CRYPTOSPORIDIUM* OOCYSTS AND *GIARDIA* CYSTS IN PUBLIC WATER SUPPLIES IN VITÓRIA, ES, BRAZIL

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Abstract: This study aimed to investigate the occurrence of *Cryptosporidium* oocysts and *Giardia* cysts in raw, filtered, and chlorinated waters collected from two drinking water treatment plants (WTP A and WTP B). WTP A uses either direct filtration or flotation–filtration depending on the turbidity of raw water. WTP B has two independent treatment lines, a direct filtration and a conventional treatment line. *Cryptosporidium* oocysts and *Giardia* cysts were identified by direct immunofluorescence microscopy and confirmed by DAPI staining and phase-contrast microscopy. Both protozoa were detected in water treated by direct filtration (WTP A and B) and flotation–filtration (WTP A). The absence of cysts and oocysts in chlorinated water does not exclude risks, as the limitations of concentration and identification techniques must be considered. These results reinforce the importance of monitoring protozoa in water destined for public supply, and the optimization of water treatment processes to produce low turbidity water.

Keywords: Protozoa; direct filtration; flotation-filtration; conventional water treatment.

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INTRODUCTION

Safe drinking water is a basic human need that contributes to ensuring proper health conditions and quality of life. Inadequate water and wastewater treatment, associated with low-quality public health services and disorderly growth of metropolitan regions, facilitate the transmission of infectious diseases that can have profound social and economic repercussions (Karanis *et al*., 2007, Sato *et al*., 2013). Water contaminated with pathogenic microorganisms, including bacteria, viruses, and protozoa, can cause diarrhea and vomiting within a few days of ingestion (SES/SP, 2013). In immunocompromised individuals, children, and the elderly, such exposure can result in long-term or even fatal infections (Chinen & Shearer, 2010).

According to the World Health Organization (WHO, 2015), 88.0% of worldwide deaths from diarrhea are caused by ingestion of contaminated water or inadequate sanitation services. In 2016, 525,977 children aged 0 to 4 years died from diarrhea; in Brazil, the number of deaths totaled 1,318 (WHO, 2016). Only 54,1% of sewage is collected in Brazil, of which 49,1% is treated (SNIS, 2019). The state of Espírito Santo, southeastern Brazil, collects 55,9% of domestic wastewater and treats only 42,5% (SNIS, 2018).

Waterborne enteric protozoa, such as *Cryptosporidium* and *Giardia*, are among the major etiological agents of diarrhea (Fletcher *et al*., 2012). These parasites are widely distributed in both developed and developing countries (Baldursson & Karanis, 2011, Fletcher *et al*., 2012). Although the life cycle, sources of contamination, and transmission routes of these pathogens are well known, waterborne disease outbreaks occur every year in several countries (Karanis *et al*., 2007). *Cryptosporidium* spp. were responsible for 60.3% of global diarrhea outbreaks caused by waterborne protozoa in 2004–2010, *Giardia* spp. were involved in 35.1% of outbreaks, and other protozoa were implicated in 4.5% of cases (Baldursson & Karanis, 2011). In the United States of America (USA), from 1971 to 2006, parasites were responsible for 18.0% of outbreaks associated with drinking water (n = 780), with *Giardia* intestinalis identified in 86.0% of cases (Craun *et al*., 2010).

Several factors may contribute to the spread of pathogenic protozoa. For instance, high contamination levels in the environment, emergence of highly infective strains, resistance to widely used disinfection processes, and small cyst or oocyst size have been shown to facilitate parasite transmission (Carey *et al*., 2004, Ramirez *et al*., 2004, Smith *et al*., 2006, Carmena, 2010, Razzolini *et al*., 2010, Baldursson & Karanis, 2011, Reevea *et al*., 2018). Therefore, periodic monitoring and quantification of pathogenic protozoa in water supply systems are extremely important for the adoption of management measures to reduce health risks and ensure the quality of water distributed to the population (Ongerth, 2013, Santos *et al*., 2016, Lo *et al*., 2018).

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In Brazil, the presence of *Cryptosporidium* and *Giardia* in clinical samples, food, and animals has been widely reported (Franco *et al*., 2001, Razzolini *et al*., 2010, Sato *et al*., 2013, Almeida *et al*., 2015, Santos *et al*., 2016); however, little is known about their presence in public water supplies. This study aimed to investigate the occurrence of *Cryptosporidium* oocysts and *Giardia* cysts in two public drinking water treatment plants in the metropolitan region of Vitória, Espírito Santo, Brazil.

This is the first study on the detection of cysts and oocysts in catchment water and water treatment systems in the State of Espírito Santo. The results provide information for decision making in the management of water resources used for public supply in the State of Espirito Santo.

MATERIALS AND METHODS

Water collection sites

Water samples were collected from the water treatment plants of Carapina (WTP A) and Vale Esperança (WTP B), located in the Santa Maria da Vitória River and Jucu River basins, respectively (**Fig. 1**). These plants supply water to 1.5 million inhabitants in the metropolitan region of Vitória, Espírito Santo, Brazil.

Description of water treatment plants

WTP A uses either direct filtration (coagulation, filtration, and disinfection) or flotation–filtration (coagulation, flotation–filtration, and disinfection) depending on the turbidity of raw water. Direct filtration is the treatment of choice when turbidity is below 50 NTU. WTP B has two treatment lines that operate independently, a direct filtration line and a conventional treatment line (coagulation, flocculation, decantation, filtration, and disinfection). A flowchart of the water treatment processes and sampling points in WTP A and B is presented in **Fig. 2**. Sampling times were adjusted so that samples could be collected at the beginning of each process.

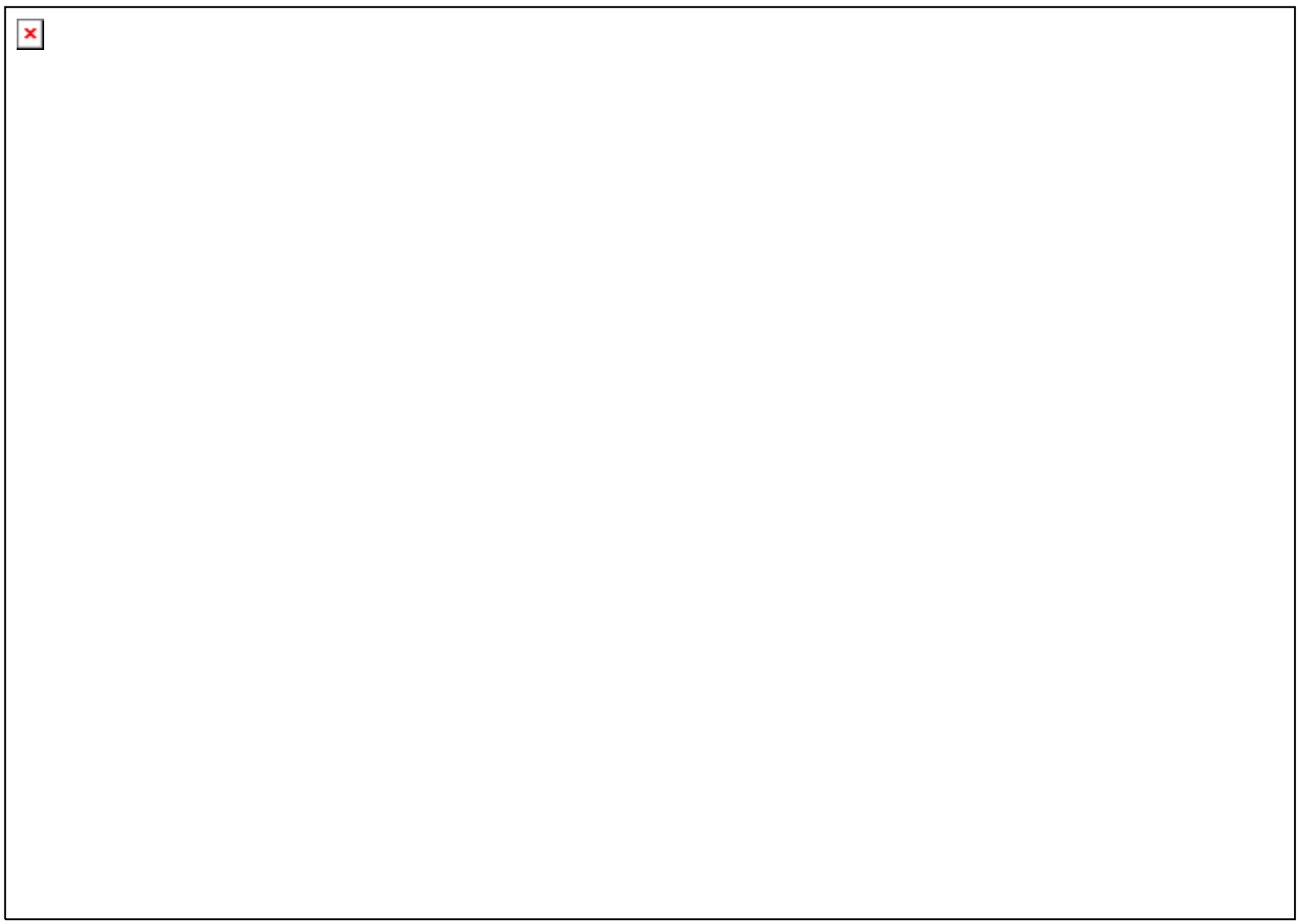

Fig. 1 Map showing the location of drinking water treatment plants (WTP) A and B (black triangle) in the Santa Maria da Vitória basin (green area) and Jucu basin (blue area), Espírito Santo, Brazil. Source: AGERH, 2020.

Detection and enumeration of *Cryptosporidium* oocysts and *Giardia* cysts in environmental samples

Samples (10 L) of raw water (n = 24), filtered water (n = 36), and chlorinated water (n = 20) were collected monthly from each sampling point for 12 months (April 2008 to March 2009) and analyzed for the presence of *Cryptosporidium* oocysts and *Giardia* cysts. Sample collection, storage, and transportation were performed in accordance with the recommendations of the Guidelines for Collection and Preservation of Water Samples (CETESB 1987) and the Standard Methods for the Examination of Water and Wastewater (APHA 2005). All analyses were carried out at the Laboratory of Sanitation of the Federal University of Espírito Santo, Vitória, Brazil.

Samples were concentrated in 12 L flat-bottomed flasks by the calcium carbonate flocculation method (Vesey *et al*. 1993), followed by centrifugation at 3,000 × g for 10 min. This concentration method limits the sample volume to up to 10 L. Pellets were resuspended to 8 mL with elution fluid (1% Tween 80, 1% sodium dodecyl sulfate, 10 × PBS, and 0.1% antifoam A. 10 µL of the final sample were added to each well slides for identification and quantification of cysts and oocysts. Protozoa were identified by direct immunofluorescence microscopy using the Merifluor C/G kit (Meridian Bioscience, Cincinnati, OH, USA) and confirmed by phase-contrast microscopy with 4,6-diamidino-2-phenylindole (DAPI) (Sigma–Aldrich, St. Louis, MO, USA) staining. Slides were examined under an epifluorescence microscope (ZEISS Axioplan HBO 50, excitation wavelength of 450–490 nm, 510 nm suppression filter; Oberkochen, Germany) at 200, 400, and 630 × magnification. Positive and negative controls were also prepared and analyzed.

The detection limit (Eq. 1) and concentration (Eq. 2) of *Cryptosporidium* oocysts and *Giardia* cysts were calculated from the results of the recovery tests according to the formula of Ongerth (2013):

$$\text{Detection limit} = \text{One (oo)cyst} / \text{Sample volume} \times \text{Recovery efficiency} \quad (1)$$

$$\text{Protozoan concentration} = \text{Number of (oo)cysts detected} / \text{Sample volume} \times \text{Recovery efficiency} \quad (2)$$

Recovery of *Giardia* cysts and *Cryptosporidium* oocysts

Recovery tests were conducted in high-turbidity raw water (65 NTU) and low-turbidity filtered water (0.3 NTU) using the calcium carbonate flocculation method (Vesey *et al*., 1993), as described in the previous topic.

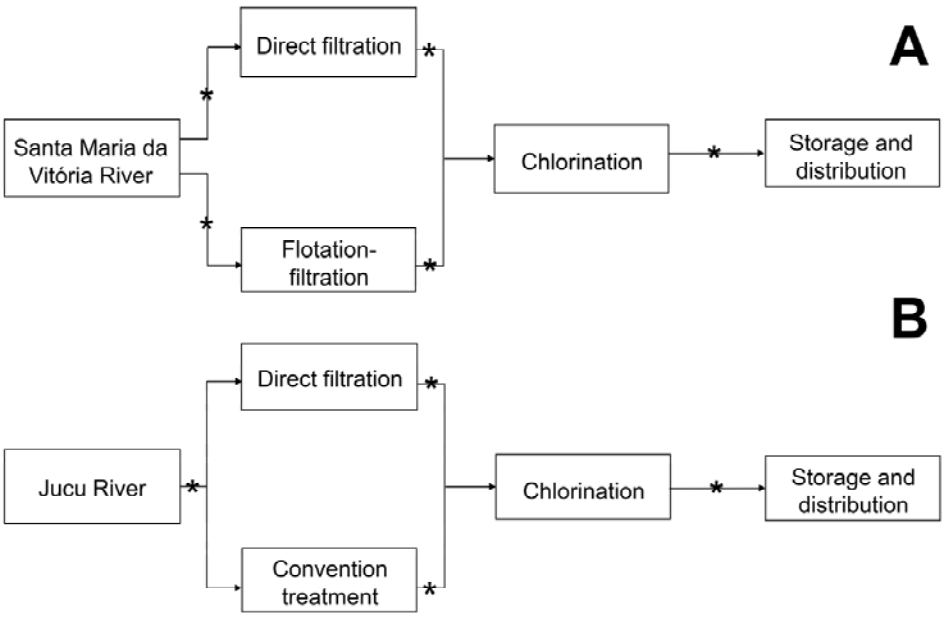

Fig. 2 Flowchart of drinking water treatment processes at plants A and B (Asterisks indicate sampling points).

Cryptosporidium oocysts were purified from feces of newborn calves by sucrose gradient centrifugation, washed with phosphate-buffered saline (PBS), and suspended in PBS containing 10 g L^{-1} penicillin-streptomycin and 0.01% Tween 20. Isolated oocysts were kindly donated by the Department of Biological Sciences of the Federal University of Triângulo Mineiro, Uberaba, Minas Gerais, Brazil. *Giardia* cysts were separated from human feces using sucrose gradient solution and suspended in PBS containing 25 µg mL^{-1} miconazole and 125 µg mL^{-1} enrofloxacin, according to Roberts-Thompson *et al*. (1976). Isolated cysts were kindly provided by the Department of Basic Pathology of the Federal University of Paraná, Curitiba, Brazil. After purification, *Cryptosporidium* oocysts and *Giardia* cysts were enumerated by flow cytometry and inoculated into water samples, in triplicate, at two concentrations, 10^2 and 10^3 (oo)cysts L^{-1} Cysts and oocysts supplied with the kit MeriFluor® (Meridian Diagnostics, Cincinnati, Ohio, EUA) were used as positive controls, and sterile distilled water was used as negative control. For biosafety reasons, all materials were disinfected with 5% sodium hypochlorite and autoclaved at the end of the experiment. Recovery efficiency (RE) was estimated by the following equation (Eq. 3):

RE = Number of (oo)cysts recovered / Number of (oo)cysts inoculated × 100 (3)

Because water samples used to assess recovery efficiency might be naturally contaminated, the samples were also subjected to protozoan quantification prior to inoculation. The number of naturally occurring protozoa was subtracted from the number of (oo)cysts recovered.

Physicochemical and microbial analyses

Water pH, turbidity, temperature, alkalinity, true and apparent color, and free residual chlorine were measured in the field using portable equipment, according to APHA (2005). Total coliforms and Escherichia coli were quantified by a chromo-fluorogenic method (Colilert, IDEXX), according to APHA (2005). Raw and filtered water were dechlorinated with 1.8% sodium thiosulfate before microbiological analysis.

Statistical analysis

Data were analyzed using descriptive statistics. The Shapiro–Wilk test was applied to assess the normality of the distribution of positional errors. Differences in protozoan concentrations between water sampling points were determined by the nonparametric Mann–Whitney U test (also known as the Wilcoxon rank-sum test). Associations between protozoan concentrations and physicochemical and bacteriological indicators of water quality were assessed by the nonparametric Spearman's correlation test. The level of significance was set at $p < 0.05$. All statistical analyses were performed using GraphPad Prism version 6.1 (GraphPad Software, La Jolla, CA, USA).

RESULTS

Recovery of *Giardia* cysts and *Cryptosporidium* oocysts from turbid water

Recovery efficiencies were determined in high- and low-turbidity water samples. Significant differences ($p = 0.0065$, high-turbidity; $p = 0.0166$, low-turbidity) in protozoan recoveries were observed. The highest recoveries were obtained from high-turbidity water (65

NTU): 72.7% (62.5–83.3%) for *Giardia* cysts and 43.0% (20.8–65.7%) for *Cryptosporidium* oocysts. From the low-turbidity sample (0.3 NTU), 36.1% (15.5–72.7%) of *Giardia* and 20.9% (3.6–38.5%) of *Cryptosporidium* were recovered.

Detection of *Giardia* cysts and *Cryptosporidium* oocysts in raw, filtered, and chlorinated water

In raw water supplying WTP A, cysts were detected in 75.0% of samples and oocysts in 66.7%, whereas in water supplying WTP B, cysts and oocysts were found in 100.0 and 83.3% of water samples, respectively. Raw water samples did not differ in *Cryptosporidium* ($p = 0.1190$) and *Giardia* ($p = 0.5067$) concentrations. **Table 1** shows the concentrations of cysts and oocysts in raw, filtered, and chlorinated waters from WTP A and B.

Physicochemical and bacteriological characteristics of raw and treated water from WTP A and B

Table 2 shows the mean physicochemical parameters (turbidity, pH, alkalinity, temperature, and residual chlorine) of raw and treated water from both treatment plants, and **Fig. 3** shows the concentrations of *Cryptosporidium* oocysts, *Giardia* cysts, and *E. coli* in raw water supplying WTP A and B during the 12-month monitoring period.

In WTP A, the mean concentrations of oocysts, cysts, and *E. coli* were 105.6 oocysts L^{-1}, 130.6 cysts L^{-1}, and 1.4 $\times 10^3$ MPN 100 mL^{-1}, respectively. In WTP B, oocysts were detected at 160.4 oocysts L−1, cysts at 127.8 cysts L^{-1}, and *E. coli* at 7.3 $\times 10^2$ MPN 100 mL^{-1}, respectively.

In raw water samples from WTP A, a moderate correlation was observed between occurrence of *Cryptosporidium* and *Giardia* ($rs = 0.628$). *Giardia* cyst levels were positively correlated with *E. coli* levels ($rs = 0.637$) and true color ($rs = 0.602$), where as *Cryptosporidium* levels showed a positive moderate correlation with total coliforms ($rs = 0.585$), *E. coli* levels ($rs = 0.620$), turbidity ($rs = 0.668$), true color ($rs = 0.769$), and apparent color ($rs = 0.736$). In samples of raw water supplying WTP B, no correlations were observed between *Giardia* cyst and *Cryptosporidium* oocyst levels ($rs = 0.271$). *Giardia* did not correlate with any physicochemical or bacteriological parameter, and *Cryptosporidium* showed a positive moderate correlation only with total coliforms ($rs = 0.593$).

DISCUSSION

Detection of oocysts and cysts in raw water

Cryptosporidium oocysts and *Giardia* cysts were detected with high frequency in water sources that supply the region of Vitória, Espírito Santo, Brazil, throughout the 12-month monitoring period. In raw water supplying WTP A, the occurrence of cysts and oocysts was 75 and 66.6%, respectively. Regarding raw water supplying WTP B, all samples (100.0%) were positive for *Giardia* cysts and 83.3% of samples were positive for *Cryptosporidium* oocysts. The high frequencies of detection indicate that current watershed protection measures are ineffective. It is important to highlight that the rivers that supply the Vitória metropolitan region (Santa Maria da Vitória River and Jucu River) cross many agricultural and livestock areas. Therefore, it is probable that water bodies were contaminated with *Giardia* cysts and *Cryptosporidium* oocysts excreted by cattle and other animals, which are hosts to these protozoa (Hansen & Ongerth 1991, Geurden *et al*. 2004, 2006, Castro-Hermida *et al*. 2009, Ligda *et al*. 2020).

It is essential to define limits for these protozoa in source water so as to (i) ensure that treatments used by plants are compatible with the microbiological quality of water and (ii) assess the risk of contamination if waters are to be used for recreation. The Brazilian legislation establishes that *Giardia* cysts and *Cryptosporidium* oocysts should be monitored monthly in water catchment areas (for a period of 12 months) when the concentration of *E. coli* is greater than or equal to $10^3 \times 100$ mL^{-1} and the efficiency of the WTP in removing spores of aerobic bacteria is less than 2.5 log (Brazil, 2021). Throughout the 12 months of monitoring of this study, the concentrations of E. coli in

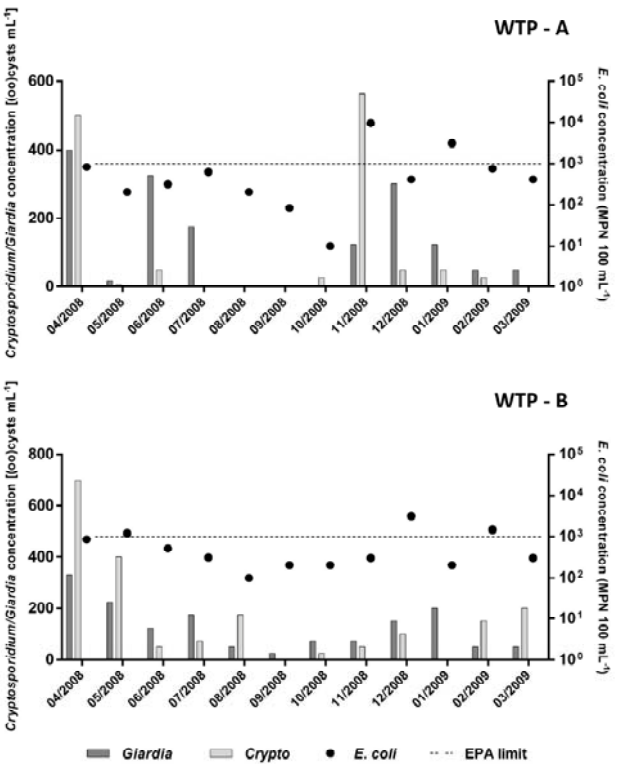

Fig. 3 Concentration of *Cryptosporidium* oocysts, *Giardia* cysts, and *Escherichia coli* in raw water supplying treatment plants (WTP) A and B from April 2008 to March 2009.
Abbreviations: MPN, most probable number; EPA, United States Environmental Protection Agency.

Table 1. Descriptive statistics of *Cryptosporidium* oocyst and *Giardia* cyst concentrations in raw and treated water from plants A and B.

Sample	*n*	*Cryptosporidium* (oocysts L^{-1})				*Giardia* (cysts L^{-1})			
		Min	Max	Mean	SD	Min	Max	Mean	SD
Plant A									
Raw water	12	*	562.5	105.6	200.3	*	400.0	130.6	140.8
Direct filtration	6	*	*	*	*	*	7.5	1.2	3.1
Flotation–filtration	6	*	17.5	5.0	6.5	*	5.0	1.6	2.0
Chlorination	10	*	*	*	*	*	*	*	*
Plant B									
Raw water	12	*	700.0	160.4	203.5	25.0	333.3	127.7	92.4
Direct filtration	12	*	22.5	3.3	6.4	*	20.0	2.7	6.5
Conventional treatment	12	*	*	*	*	*	*	*	*
Chlorination	10	*	*	*	*	*	*	*	*

SD, standard deviation; *, lower than the limit of detection; limit of detection in high-turbidity water: *cryptosporidium*, 0.23 oocysts L^{-1} and *giardia*, 0.13 cysts L^{-1}; limit of detection in low-turbidity water: *cryptosporidium*, 0.48 oocysts L^{-1} and *giardia*, 0.27 cysts L^{-1}).

Table 2. Physicochemical and microbiological parameters of raw and treated water from water treatment plants (WTP) A and B. Values are presented as mean and standard deviation.

WTP	Water sample	Turbidity (NTU)	Real color (mg Pt-Co L^{-1})	Apparent color (mg Pt-Co L^{-1})	Chlorine residual (mg L^{-1})	Total coliforms (MPN 100 mL^{-1})	*Escherichia coli* (MPN 100 mL^{-1})
A	Raw (high turbidity)	97.1 ± 115.1	150.0 ± 172.0	358.3 ± 468.5	0	9.7×10^3	1.2×10^3
	Raw (low turbidity)	5.7 ± 1.8	29.3 ± 13.6	43.8 ± 14.1	0	1.8×10^3	1.4×10^2
	Direct filtration	0.4 ± 0.3	10.2 ± 9.5	18.3 ± 11.8	0.018 ± 0.011	0	0
	Flotation–filtration	2.5 ± 2.1	2.7 ± 2.1	9.3 ± 10.1	0.064 ± 0.121	2.2×10^0	0
	Chlorination	1.7 ± 2.5	6.1 ± 2.7	11.8 ± 7.6	1.466 ± 0.377	0	0
B	Raw	38.7 ± 26.6	67.7 ± 34.8	215.5 ± 96.1	0	4.4×10^2	4.4×10^2
	Direct filtration	1.4 ± 1.4	2.3 ± 2.1	11.8 ± 7.7	0.074 ± 0.085	9.4×10^0	1.1×10^0
	Conventional treatment	0.4 ± 0.2	2.8 ± 4.6	7.2 ± 6.2	0.069 ± 0.055	0	0
	Chlorination	0.7 ± 0.3	2.5 ± 1.8	3.6 ± 2.4	1.321 ± 0.456	0	0

raw water varied, but, for the most part, did not surpass the limits established by the Environmental Protection Agency (EPA) and Brazilian regulation. Nevertheless, protozoan cysts and oocysts were frequently detected in water catchment areas, particularly in the waters of the Jucu River supplying WTP B.

Cryptosporidium accounts for a majority of waterborne outbreaks of protozoan parasitic diseases even when bacteriological results were in accordance with regulatory standards (Baldursson & Karanis, 2011; Checkley *et al*., 2015; Efstratiou *et al*., 2017; Karanis *et al*., 2007). Protozoa and bacteria differ in cell structure, biology, and environmental resistance; thus, the commonly analyzed bacterial groups are not good indicators of the presence of protozoa in water.

According Benedict *et al*. (2017), *Cryptosporidium* was the second most common cause of both outbreaks and illnesses in USA, demonstrating the continued threat from this chlorine-tolerant pathogen when drinking water supplies are contaminated. De Silva *et al*. (2016) claim to prevent waterborne outbreaks, it is essential to monitor the quality of both raw water and drinking water and to evaluate the efficiency of current barriers in water treatment plants.

Several factors may affect the quality of source water. Rainfall, for instance, influenced the turbidity of raw water supplying WTP. In the study region, water basins received an average annual rainfall of 1,500 mm, with episodes of heavy and constant rainfall in the summer (IEMA, 2020). Rainfall was not correlated with the occurrence of protozoa (data not shown), but peaks of cysts, oocysts, turbidity, and coliform bacteria were observed in the rainy season (October to March).

Kifleyohannes and Robertson (2020) comment that it is possible that the concentration of cysts and oocysts is higher in the water source after precipitation. However, other studies evaluated the presence of *Giardia* and *Cryptosporidium* during the seasons of the year reported only a relatively weak correlations, or correlations with only one of the parasites (Carmena *et al*., 2007; Mons *et al*., 2009; Utaaker *et al*., 2019). Davies *et al*. (2004), in a pilot-scale experiment, observed that, after heavy rainfall, floodwater passing through soils without vegetation cover had higher levels of oocysts than floodwater passing through covered soils. In the present study, animal feces containing *Giardia* cysts and *Cryptosporidium* oocysts were likely a source of water contamination.

Controversial results have been reported regarding the correlation between occurrence of protozoa in water turbidity. Some authors reported a significant correlation (Hsu *et al*., 2000, Hu, 2002, Carmena *et al*., 2007, Burnet *et al*., 2014, Ligda *et al*., 2020), whereas others reported a lack of correlation (Menge *et al*., 2001, Bastos *et al*., 2002, Hashimoto *et al*., 2002, Ramo *et al*., 2017, Nascimento *et al*., 2020). Monitoring of protozoan levels in raw and drinking water should not be replaced by turbidity control.

Detection of cysts and oocysts in treated water

Giardia cysts and *Cryptosporidium* oocysts were detected in filtered water by direct filtration (WTP A and B) and flotation–filtration (WTP A). In WTP A, direct filtration is used to treat low-turbidity water, and flotation–filtration for high-turbidity water. The short time of direct filtration and the lack of clarification prior to filtration may have reduced protozoan removal efficiency. Moreover, an increase in filter washing during periods of high water turbidity reduces treatment efficiency, especially in the first hours after washing (Libânio, 2005). The combination of flotation and filtration was not sufficient to improve oocyst removal.

Brazilian drinking water legislation (PRC no. 888/2021, Ministry of Health) states that the turbidity of filtered water should not exceed 0.3 NTU in 95% of samples when the concentration of Cryptosporidium is greater than 1 oocyst L^{-1} (Brazil, 2021). The turbidity of water treated in WTP A and WTP B was higher than the limit defined by Brazilian drinking water legislation. In filtered samples containing *Cryptosporidium* oocysts, protozoan concentration were detected at concentrations above the alert levels of 0.1 oocysts L^{-1} (The Water Supply Regulations, 2007) in all samples in which they were identified.

The presence of protozoa in treated water is not uncommon in developed countries. In the United Kingdom, Mason *et al*. (2010) found a significant association between the presence of *Cryptosporidium* hominis in treated drinking water and the 2005 waterborne outbreak. The authors stated that, although low, the oocyst count in treated water (<0.08 oocysts 10 L^{-1}) was sufficient for infection. Widerström *et al*. (2014) detected 0.20 - 0.32 oocysts 10 L^{-1} of *Cryptosporidium* in treated drinking water during a cryptosporidiosis outbreak in Östersund, Sweden.

The high costs and methodological limitations of detecting *Giardia* and *Cryptosporidium* in water stimulate the search for indirect indicators of these protozoa. However, the scientific community has not yet identified a reliable indicator of protozoan occurrence in water. The USEPA established *Escherichia coli* limits for water sources that if exceeded require sampling for *Cryptosporidium*, but many studies have found no correlation between fecal indicators such as *E. coli* and *Cryptosporidium* in water (Bonadonna *et al*., 2002; Harwood *et al*., 2005; Mons *et al*., 2009; Nieminski *et al*., 2010). The discrepancy in reports on the correlation between physicochemical and biological parameters can be attributed to differences in water quality, analytical methods, and equipment used for parasite detection (Vernile *et al*., 2008).

The USEPA suggests that aerobic bacterial spores be utilized as a surrogate for *Cryptosporidium*, because they are not pathogenic, can be produced and analyzed cheaply and easily in the laboratory, are persistent in the environment, and remain unchanged during transport, sampling, and laboratory analysis (USEPA, 2010).

Some aspects of the current study must be considered. The non-detection of cysts and oocysts in chlorinated water samples from WTP A and B does not imply absence of protozoa (Allen *et al*., 2000, Vernille *et al*., 2008). The methods used for Giardia and *Cryptosporidium* quantification, added to the small sample volume, resulted in low recovery efficiencies from low-turbidity waters. Factors related to water quality and chemical compounds used in water treatment processes, such as iron and aluminum coagulants, polymers, and chlorine, may interfere with parasite separation and detection with antibodies (USEPA, 2001).

The results showed that direct filtration and flotation–filtration alone are not effective in removing protozoa from waters supplying WTP A and B; and post-treatment with chlorine does not guarantee a reduce infection risks. The already proven resistance of *Cryptosporidium* oocysts and *Giardia* cysts to chlorination combined with the methodological limitations in detecting protozoa in chlorinated water reinforces the importance of continuous monitoring of *Giardia* and *Cryptosporidium* in drinking source water and the need for preventive and corrective measures to minimize watershed contamination.

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Declaration of Competing Interest

The authors declare that they have no competing financial interests.

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