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Scientific Paper

Occurrence of *Cryptosporidium* oocysts and *Giardia* cysts in public water supplies in Vitória, ES, Brazil

Ocorrência de oocistos de Cryptosporidium *e cistos de* Giardia *em águas de abastecimento público em Vitória, ES, Brasil*

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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 concentrated by flocculation, identified by direct immunofluorescence microscopy and confirmed by DAPI staining and phase-contrast microscopy. In raw water, the occurrence of cysts was from 7.5 (WTP A) to 100% (WTP B) of the samples, and of oocysts from 66.6 (WTP A) to 83.3% (WTP B). Both protozoa were detected in water treated by direct filtration (cysts: < 0.27 to 20.0 cysts L^{-1}; oocysts: < 0.48 to 22.5 oocysts L^{-1}) and flotation-filtration (cysts: < 0.27 to 5.0 cysts L^{-1}; oocysts: < 0.48 to 17.5 oocysts L^{-1}). The absence of cysts and oocysts in chlorinated water does not exclude risks, as the limitations of concentration and identification techniques must be considered, given the low recovery rates, especially in water with low turbidity (15.5 – 72.7% of *Giardia*; 3.6 – 38.5% of *Cryptosporidium*). In the raw water samples from WTP A, a moderate correlation was observed between the protozoa, and these with the conventional parameters of water quality. In the raw water samples from WTP B, the correlation was insignificant. These results reinforce the importance of monitoring protozoa in water destined for public supply.

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

RESUMO

Este estudo teve como objetivo investigar a ocorrência de oocistos de *Cryptosporidium* e cistos de Giardia em águas brutas, filtradas e cloradas, coletadas de duas estações de tratamento de água potável (ETA A e ETA B). A ETA A utiliza filtração direta ou flotação-filtração dependendo da turbidez da água bruta. A ETA B possui duas linhas de tratamento independentes, uma de filtração direta e outra de tratamento convencional. Os oocistos de *Cryptosporidium* e cistos de Giardia foram concentrados por floculação, identificados por microscopia de imunofluorescência direta e confirmados por coloração com DAPI e microscopia de contraste de fase. Na água bruta, a ocorrência de cistos variou de 7,5% (ETA A) a 100% (ETA B) das amostras, e de oocistos de 66,6% (ETA A) a 83,3% (ETA B). Ambos os protozoários foram detectados na água tratada por filtração direta (cistos: ETA A e B < 0,27 a 20,0 cistos L^{-1}; oocistos: < 0,48 a 22,5 oocistos L^{-1}) e flotação-filtração (cistos: < 0,27 a 5,0 cistos L^{-1}; oocistos: < 0,48 a 17,5 oocistos L^{-1}) na ETA A. A ausência de cistos e oocistos na água clorada não exclui riscos, pois as limitações das técnicas de concentração e identificação devem ser consideradas, dados os baixos índices de recuperação, especialmente em água com baixa turbidez (15,5 – 72,7% de Giardia; 3,6 – 38,5% de *Cryptosporidium*). Nas amostras de água bruta da ETA A, foi observada uma correlação moderada entre os protozoários e destes com os parâmetros convencionais de qualidade da água. Nas amostras de água bruta da ETA B, a correlação foi insignificante. Esses resultados reforçam a importância de monitorar protozoários em água destinada ao abastecimento público e a otimização dos processos de tratamento de água para produzir água de baixa turbidez.

Palavras-chave: protozoários; filtração direta; flotação-filtração; tratamento convencional de água.

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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; KOURENTI; SMITH, 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, 2019), 89.0% of worldwide deaths from diarrhea are caused by ingestion of contaminated water or inadequate sanitation services. In 2019, 525,000 children aged zero to five years died from diarrhea; in Brazil, the number of deaths totaled 1,318 (WHO, 2016, 2019). Brazil also had more than 130 thousand hospitalizations in 2021 due to water-borne diseases (DATASUS, 2021). Only 51.2% of sewage is treated in Brazil (SNIS, 2021). The state of Espírito Santo, southeastern Brazil, collects 56.9% of domestic wastewater and treats only 45.16% (SNIS, 2021).

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; KOURENTI; SMITH, 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, small cyst or oocyst size have been shown to facilitate parasite transmission, and weakness of Brazilian regulations regarding the criteria for monitoring protozoa in water for public supply, such as researching protozoa only in raw water and controlling filtration efficiency by analyzing turbidity in filtered water (CAREY; LEE; TREVORS, 2004; RAMIREZ; WARD; SREEVATSAN, 2004; SMITH *et al.*, 2006; CARMENA, 2010; RAZZOLINI; SANTOS, BASTOS, 2010; BALDURSSON; KARANIS, 2011; REEVEA *et al.*, 2018; ZINI *et al.*, 2021; BRASIL, 2021). 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).

In Brazil, the presence of *Cryptosporidium* and *Giardia* in clinical samples, food, and animals has been widely reported (FRANCO; ROCHA-EBERHARDT; CANTUSIO, 2001; RAZZOLINI; SANTOS, BASTOS, 2010; SATO *et al.*, 2013; ALMEIDA *et al.*, 2015; SANTOS *et al.*, 2016). Important studies highlight the occurrence of *Giardia* and *Cryptosporidium* in public water sources, such as in the cities of Belo Horizonte (LOPES *et al.*, 2017), Campinas (FRANCO *et al.*, 2016), Londrina (ALMEIDA *et al.*, 2015), 11 cities in the state of São Paulo (BRETERNITZ *et al.*, 2020), 15 cities in the state of Goiás (SILVA; SCALIZE, 2020) and 48 cities in the state of Rio Grande do Sul (ZINI *et al.*, 2021).

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 region.

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 (Figure 1). These plants supply water to 1.5 million inhabitants in the metropolitan region of Vitória, Espírito Santo, Brazil. The use and occupation of the soil in the two hydrographic basins are defined by urban, industrial, agricultural and livestock activities. Both rivers receive daily sanitary and industrial effluents, have a high level of siltation, and low vegetation cover.

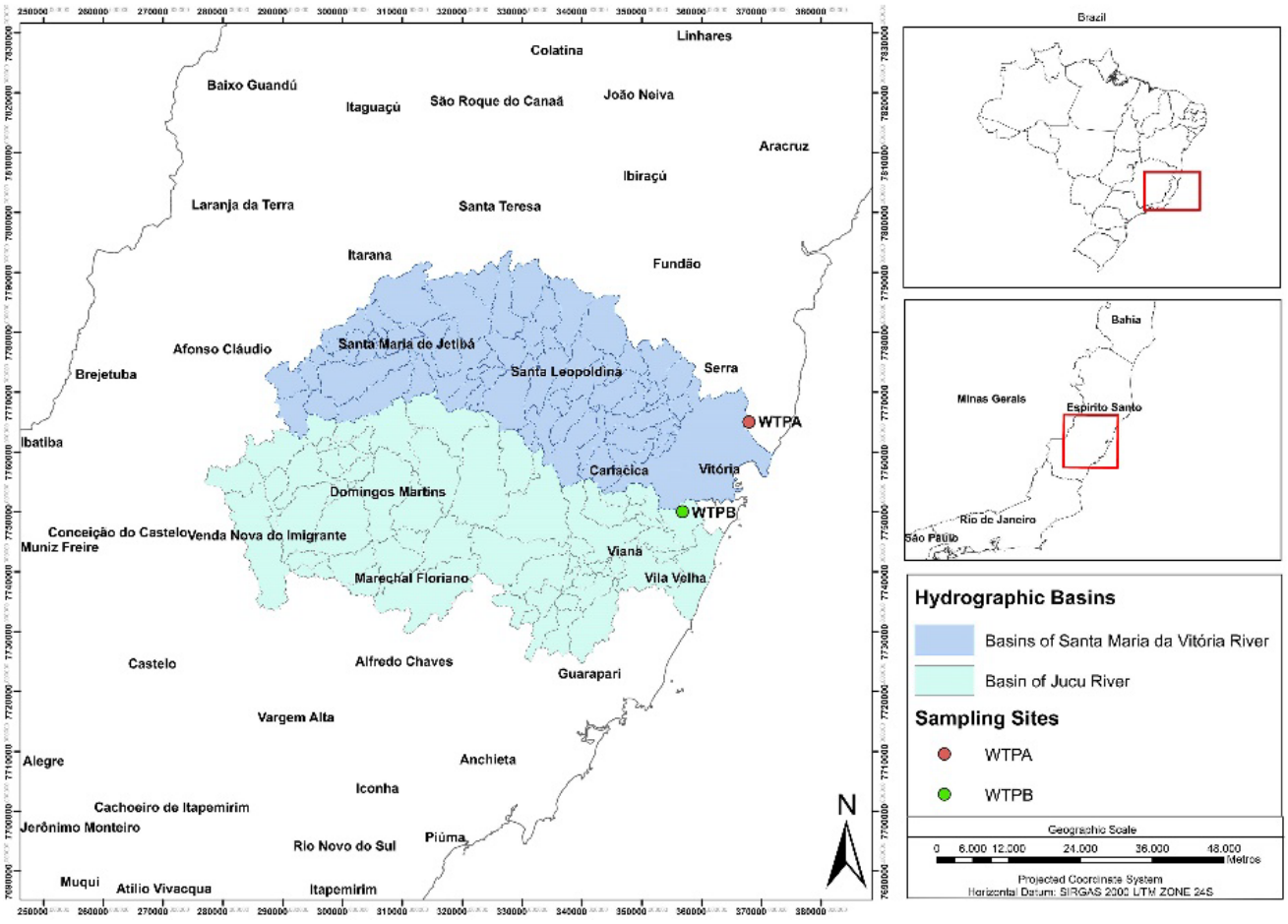
Description of water treatment plants

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). The water treatment processes and sampling points in WTP A and B are presented in Figure 2. Sampling times were adjusted so that samples could be collected at the beginning of each process.

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

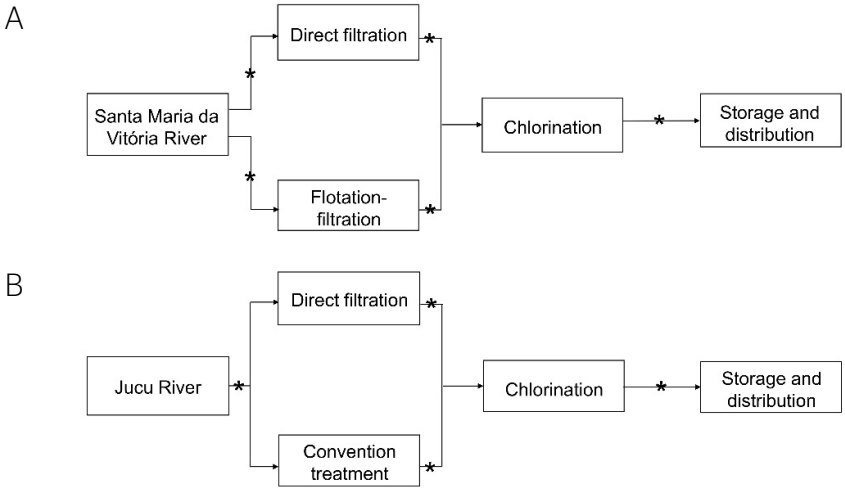
Samples (10 L) of raw water (n = 24), filtered water (n = 36), and chlorinated water ($n = 20$) were collected monthly from each sampling point for 12 months (April 2008 to March 2009) and analyzed for the presence of *Cryptosporidium* oocysts and *Giardia* cysts. Sample collection, storage, and transportation 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 12L 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). Of the final sample, 10 µL were added to each well slide for identification and quantification of cysts and oocysts. Protozoa were identified by direct immunofluorescence microscopy



Source: the authors (2023).

Figure 1 – Map showing the location of drinking water treatment plants A (WTPA) and B (WTPB) in the Santa Maria da Vitória basin (blue area) and Jucu basin (green area), Espírito Santo, Brazil.



Source: the authors (2023).

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

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. Four slides were examined for each sample 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. Each slide was viewed in duplicate. Positive and negative controls were also prepared and analyzed.

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

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

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

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. *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 (Equation 3):

$$\text{RE} = \frac{\text{Number of (oo)cysts recovered}}{\text{Number of (oo)cysts inoculated}} \times 100 \quad (3)$$

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. Parameter analyses were performed in triplicate, on 24 samples of raw water, 36 samples of filtered water and 20 samples of chlorinated water.

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 water samples

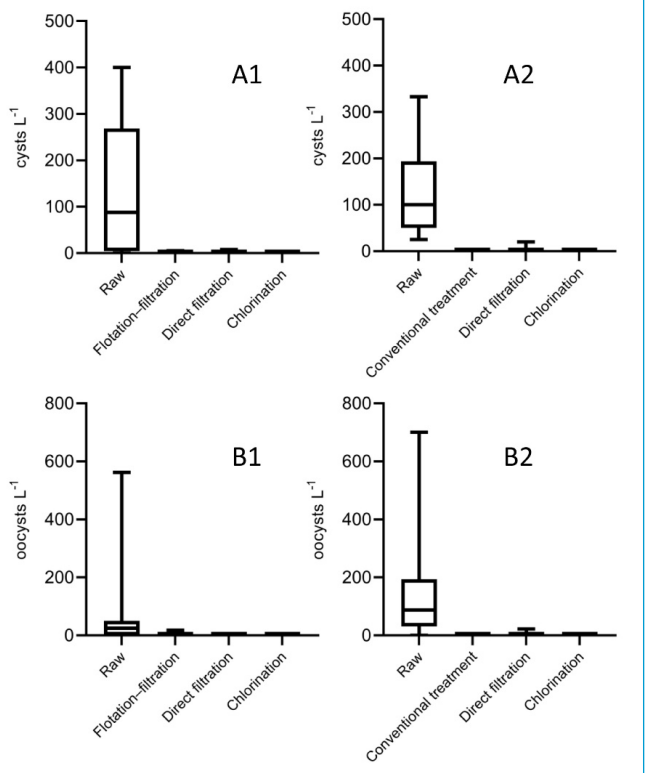
In raw water supplying WTP A, cysts were detected in 75.0% of samples and oocysts in 66.7%, whereas in water supplying WTP B, cysts and oocysts were found in 100.0 and 83.3% of water samples, respectively. Raw water samples did not differ in *Cryptosporidium* ($p = 0.1190$) and *Giardia* ($p = 0.5067$) concentrations. Figure 3 shows the boxplot of concentrations of cysts and oocysts in raw, filtered, and chlorinated waters from WTP A and B.

Physicochemical and bacteriological characteristics

Table 1 shows the median physicochemical parameters (turbidity, pH, alkalinity, temperature, and residual chlorine) of raw and treated water from both treatment plants, and Figure 4 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, and the Environmental Protection Agency (EPA) limit of *E. coli* in raw water for protozoa research. In WTP A, the median concentrations of oocysts, cysts, and *E. coli* were 25.0 oocysts L^{-1}, 87.5 cysts L^{-1}, and 4.1×10^2 MPN 100 mL^{-1}, respectively. In WTP B, oocysts were detected at 87.5 oocysts L^{-1}, cysts at 100.0 cysts L^{-1}, and *E. coli* at 3.05×10^2 MPN 100 mL^{-1}, respectively.

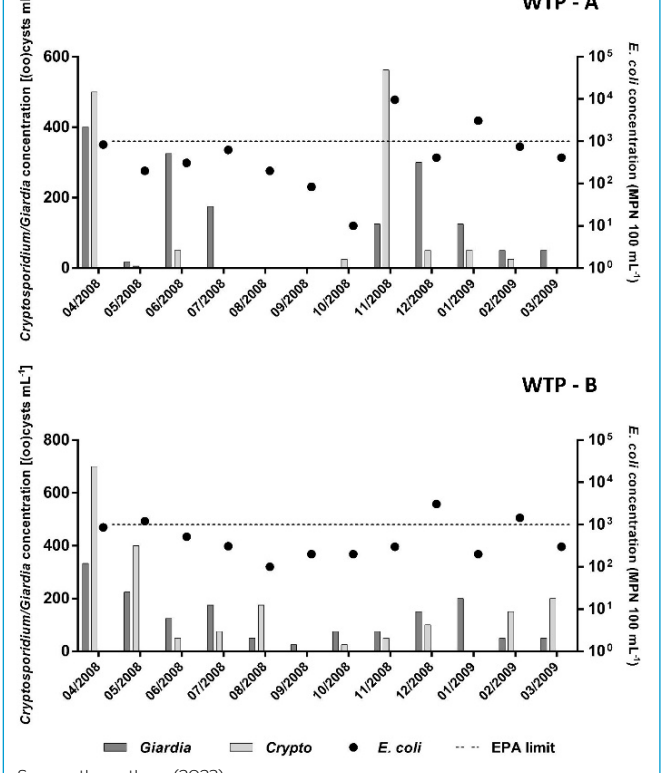
In raw water samples from WTP A, a moderate correlation was observed between occurrence of *Cryptosporidium* and *Giardia* ($r_s = 0.628$). *Giardia* cyst levels were positively correlated with *E. coli* levels ($r_s = 0.637$) and true color

(r_s = 0.602), where as *Cryptosporidium* levels showed a positive moderate correlation with total coliforms (r_s = 0.585), *E. coli* levels (r_s = 0.620), turbidity (r_s = 0.668), true color (r_s = 0.769), and apparent color (r_s = 0.736) (Figure 5A). In samples of raw water supplying WTP B, no correlations were observed between *Giardia* cyst and *Cryptosporidium* oocyst levels (r_s = 0.271). *Giardia* did not correlate with any physicochemical or bacteriological parameter, and *Cryptosporidium* showed a positive moderate correlation only with total coliforms (r_s = 0.593) (Figure 5B).



Source: the authors (2023).

Figure 3 – Boxplot of concentrations of Giardia cysts and *Cryptosporidium* oocysts and in raw, filtered, and chlorinated waters from water supplying treatment plants A and B (A1 and A2, Giardia cysts in WTPA and WTPB, respectively; B1 and B2, *Cryptosporidium* oocysts in WTPA and WTPB, respectively).



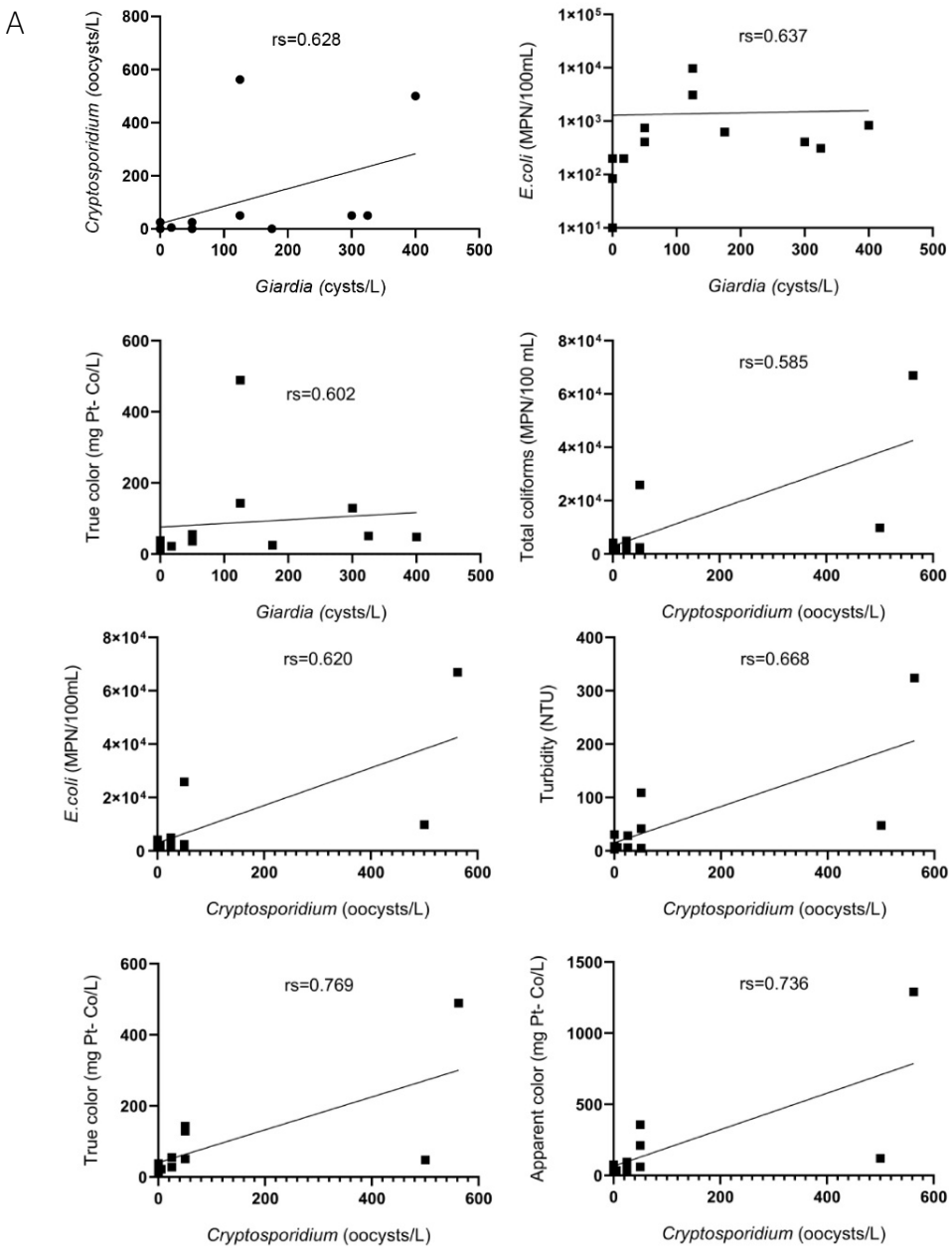
Source: the authors (2023).
MPN: most probable number; EPA: United States Environmental Protection Agency.

Figure 4 – Concentration of *Cryptosporidium* oocysts, Giardia cysts, and *Escherichia coli* in raw water supplying treatment plants A (WTP-A) and B (WTP-B) from April 2008 to March 2009.

Table 1 – Physicochemical and microbiological parameters of raw and treated water from water treatment plants A and B. Values are presented as median 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)	44.8 ± 115.1	92.0 ± 172.0	166.0 ± 468.5	nd	7.3×10^3	7.9×10^2
	Raw (low turbidity)	5.8 ± 1.8	26.5 ± 13.6	43.0 ± 14.1	nd	1.8×10^3	2.0×10^2
	Direct filtration	0.2 ± 0.3	10.5 ± 9.5	22.0 ± 11.8	0.02 ± 0.01	nd	nd
	Flotation-filtration	1.4 ± 2.0	2.0 ± 2.1	6.0 ± 10.1	0.02 ± 0.12	nd	nd
	Chlorination	0.7 ± 2.5	6.0 ± 2.7	10.5 ± 7.6	1.42 ± 0.38	nd	nd
B	Raw	42.8 ± 23.4	62.5 ± 34.7	215.0 ± 96.1	nd	5.16×10^3	3.05×10^2
	Direct filtration	1.2 ± 1.3	2.5 ± 2.1	9.0 ± 7.7	0.03 ± 0.08	nd	nd
	Conventional treatment	0.3 ± 0.2	1.5 ± 4.6	5.5 ± 6.2	0.04 ± 0.05	nd	nd
	Chlorination	0.6 ± 0.4	3.0 ± 1.8	4.0 ± 2.4	1.39 ± 0.47	nd	nd

WTP: water treatment plant; *nd: not detected.



Source: the authors (2023).

Figure 5 – Correlations between concentrations of *Cryptosporidium* oocysts and Giardia cysts with physicochemical and bacteriological parameters in raw water samples from WTP A (A) and WTPB (B).

DISCUSSION

Recovery of *Giardia cysts* and *Cryptosporidium oocysts*

The methodology for detecting *Giardia* cysts and *Cryptosporidium* oocysts showed a higher recovery rate in high turbidity water (65 NTU), 72.7% for cysts and 43.0% for oocysts, than in low turbidity water (0.3 NTU), 36.1% for cysts and 20.9% for oocysts. According to LeChevallier e Norton (1995), the presence of suspended particles in raw water helps the precipitation of organisms in the sediment, increasing recovery, especially when flocculation with $CaCO_3$ is used. On the other hand, excess particles can cover cysts and oocysts, preventing antigen-antibody binding and, consequently, leading to false-negative results (VESEY *et al.*, 1993; FRANCO *et al.*, 2012; USEPA, 2012).

The tests carried out by Vesey *et al.* (1993) showed a recovery of *Cryptosporidium* oocysts of 76% for deionized water, 73.7% for tap water and 75.6% for spring water. Shepherd and Wyn-Jones (1996) recovered 71.3% *Cryptosporidium* oocysts and 72.5% *Giardia* cysts in river water, and 73.6% oocysts and 77.1% cysts in treated water. In the tests carried out by Cantusio Neto *et al.* (2010), the recovery rates of *Cryptosporidium* oocysts were 26.8% and *Giardia* cysts were 14.3% in the environmental matrix of the study area.

The results found in the recovery tests for *Giardia* cysts and *Cryptosporidium* oocysts demonstrate that the efficiency of the concentration and detection techniques depends on the quality of the water used in the tests, the storage time of the water, the method of conservation, the skills of the technical staff, and the different counting techniques adopted.

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 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. Currently, 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 10^3 100 mL^{-1} and the efficiency of the WTP in removing spores of aerobic bacteria is less than 2.5 log. (BRAZIL 2021). This study preceded the last publication of the Brazilian standard, but it is important to highlight that the concentrations of *E. coli* in raw water, for the most part, did not exceed the limits established by the EPA and Brazilian regulations for research on protozoa. Protozoan cysts and oocysts were frequently detected in water catchment areas, mainly in the waters of the Jucu River that supplies ETA B.

Cryptosporidium accounts for most waterborne outbreaks of protozoan parasitic diseases even when bacteriological results were in accordance with regulatory standards (KARANIS; KOURENTI; SMITH, 2007; BALDURSSON; KARANIS, 2011; CHECKLEY *et al.*, 2015; EFSTRATIOU; ONGERTH; KARANIS, 2017). 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 to 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 that, 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 that evaluated the presence of *Giardia* and *Cryptosporidium* during the seasons of the year reported only a relatively weak correlation, 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 and 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; KUNIKANE; HIRATA, 2002; RAMO *et al.*, 2017; NASCIMENTO; GINORIS; BRANDÃO, 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 was 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 an association between the presence of *Cryptosporidium* 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 an 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 United States Environmental Protection Agency (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 the absence of protozoa (ALLEN; CLANCY; RICE, 2000; VERNILE *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 determination of protozoa viability is important, because low numbers of viable cysts and oocysts in water can present risks (EHSAN, 2015). However, commonly used methodologies do not assess infectivity.

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 reduction of 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 and determination of the viability of cysts and oocysts in drinking source water and the need for preventive and corrective measures to minimize watershed contamination.

AUTHORS' CONTRIBUTIONS

Covre, M.A.: Conceptualization, Data Curation, Investigation, Methodology, Writing – Original Draft, Writing – Review & Editing. Santos, R.P.: Data Curation, Software, Formal Analysis, Writing – Review & Editing. Keller, R.P.: Data Curation, Investigation, Formal Analysis, Writing – Original Draft, Writing – Review & Editing. Coelho, E.R.: Conceptualization, Data Curation, Funding Acquisition, Investigation, Project Administration, Writing – Original Draft, Writing – Review & Editing.

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