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First report of contamination of public water system by *Giardia duodenalis* and *Cryptosporidium* spp. in Bahia, northeastern Brazil

Primeiro relato de contaminação de sistema público de água por *Giardia duodenalis* e *Cryptosporidium* spp. na Bahia, nordeste do Brasil

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Highlights

First detection of *Giardia* and *Cryptosporidium* in water samples in Bahia.
Giardia and *Cryptosporidium* contaminate water from the public supply network.
Water contamination increases the risk of disease transmission in the region.

Abstract

Waterborne diseases such as cryptosporidiosis and giardiasis are among the leading public health problems worldwide because these protozoa are more resistant to water disinfection methods. This study aimed to evaluate the presence of oocysts of *Cryptosporidium* spp. and cysts of *Giardia* spp. in samples of raw and treated water. During one year, 24 raw and treated water samples were collected from the public water supply system in Canavieiras, Bahia, Brazil. Cysts and oocysts were concentrated using membrane filtration and visualized through Direct immunofluorescence assay (DFA). DNA isolated with a commercial kit was subjected to nested PCR (nPCR). The DFA obtained a better result than the nPCR in detecting both protozoa. By DFA, *Cryptosporidium* spp. was present in 25% (6/24) of the raw water and 4.2% (1/24) of the treated water samples. *Giardia* spp. was detected in 41.6% (10/24) of the raw water and 16.6% (4/24) of the treated water samples. In nPCR, *Giardia* spp. was detected in treated and raw water at 16.6% (4/24) and 16.6% (4/24), respectively. *Cryptosporidium* spp. was detected in

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raw water at 8.3% (2/24). The DFA and nPCR results differed despite the molecular analysis having greater sensitivity. Many inhibitors in the samples may have influenced the result. The physicochemical parameters pH and turbidity were not under the standards established by the legislation. In raw water, microbiological parameters were above the required legal standard. Precipitation and pH correlated with *Cryptosporidium* nPCR results in raw water. The presence of these protozoa in the water samples constitutes a potential risk for transmitting diarrheal diseases to this municipality's human and animal populations.
Key words: Contaminated water. Water potability. Protozoa. Public Health. Watersheds.

Resumo

As doenças de veiculação hídrica como a Criptosporidiose e a Giardiase estão entre os principais problemas de saúde pública em todo mundo em razão de que estes protozoários são mais resistentes aos métodos de desinfecção da água. O objetivo deste estudo foi avaliar a presença de oocistos de *Cryptosporidium* spp. e cistos de *Giardia* spp. em amostras de água bruta e tratada. Durante um ano, foram coletadas 24 amostras de água bruta e água tratada da rede pública de abastecimento do município de Canavieiras, Bahia, Brasil. Os cistos e oocistos foram concentrados pela técnica de filtração por membrana. Ensaio de imunofluorescência direta (DFA) foi realizado para visualizar cistos e oocistos. A extração do DNA foi realizada com kit comercial, e o DNA extraído foi submetido à nested-PCR (nPCR). O DFA obteve resultado superior que o nPCR na detecção de ambos os protozoários. Por DFA, *Cryptosporidium* spp. estava presente em 25% (6/24) das amostras de água bruta e 4,2% (1/24) das amostras de água tratada. *Giardia* spp. foi detectado em 41,6% (10/24) das amostras de água bruta e 16,6% (4/24) das amostras de água tratada. Na nPCR *Giardia* spp. foi detectado em água tratada e bruta 16,6% (4/24) e 16,6% (4/24) respectivamente, e, *Cryptosporidium* spp. somente foi detectado em água bruta com 8,3% (2/24). Os resultados do DFA e da nPCR não foram similares, apesar da análise molecular apresentar maior sensibilidade, muitos inibidores presentes nas amostras possam ter influenciado o resultado. Os parâmetros físico-químicos pH e turbidez não estavam de acordo com os padrões estabelecidos pela legislação. Na água bruta, os parâmetros microbiológicos ficaram acima do padrão legal exigido. A presença de *Cryptosporidium* na água bruta foi relacionada com a precipitação e o pH. A presença desses protozoários nas amostras de água constitui um risco potencial de transmissão de doenças diarreicas à população humana e animal deste município.
Palavras-chave: Água contaminada. Potabilidade. Protozoários. Saúde pública. Bacias hidrográficas.

Introduction

Contaminated water is the main route of transmission of diarrheal diseases such as Giardiasis and Cryptosporidiosis to men, pets, and farm animals from urban and rural centers around the world (Buret et al., 2020; Widmer et al., 2020). Especially in developing countries, where legal and economic aspects hamper health development and where these parasites are neglected, these diseases remain among the leading public health problems, mainly affecting populations of children and the immunosuppressed (Thompson & Ash, 2016; Coelho et al., 2017).

In human and animal populations, the spectral clinical manifestation of these protozooses varies from asymptomatic to severe clinical conditions, which lead to impairments in physical and cognitive development. The clinical severity in patients with a higher parasitic burden results from dysbiosis, which evolves into malnutrition and can even culminate in death (Niehaus et al., 2002; Prado et al., 2005; Thompson et al., 2008; Feng & Xiao, 2011; Ryan & Cacciò, 2013; Beatty et al., 2017). As for lethality, according to Fundo das Nações Unidas para a Infância [UNICEF] (2017), 8% of deaths of children up to 5 years of age are due to diarrheal diseases, with *Giardia duodenalis* and *Cryptosporidium* ssp. among the main zoonotic agents that cause these conditions (Thompson & Smith, 2011).

Multiple factors contribute to the dissemination of *Giardia* cysts and *Cryptosporidium* oocysts, as well as their resistance to environmental conditions and their infectious duration. The situation is exacerbated by the resistance of these protozoa to conventional water treatment processes such as filtration and chlorination (Fayer et al., 2000; Karanis et al., 2007; Dorny et al., 2009). These observations are relevant to Public Health due to the many at-risk people (Franco et al., 2012a).

In recent decades, outbreaks of waterborne cryptosporidiosis and Giardiasis have been reported in several countries (Buret et al., 2020; Widmer et al., 2020), related to factors such as untreated surface water consumption, inadequate sanitation, inefficient water management, and even drinking water that does not meet standards established by specific legislation (Baldursson & Karanis, 2011).

In Brazil, controlling and monitoring drinking water quality and the water potability standard is established by Ordinance 2914/11 (Ministério da Saúde [MS], 2011). The absence of *Escherichia coli*/100 mL, an indicator of fecal contamination, defines the microbiological standard of water for human consumption. Based on the treatment efficiency indicator, the absence of fecal coliforms/100mL characterizes the treated water. Regarding pathogenic protozoa, the law establishes that when an annual geometric mean greater than or equal to 1000 *E. coli*/100mL, the presence of cysts of *Giardia* spp. and oocysts of *Cryptosporidium* spp. should be investigated, aiming to reduce its occurrence (MS, 2011). In fact, in the last 20 years, *Giardia duodenalis* has been the most prevalent enteric parasite in Brazilian children (Pacheco et al., 2014; Ignacio et al., 2017; Harvey et al., 2020). However, water treatment systems in Bahia do not monitor these pathogens at their catchment points or in the final water distributed to the population.

This study aimed to evaluate the presence of *Cryptosporidium* spp. oocysts and *Giardia* spp. cysts in raw and treated water samples intended for public supply in the Canavieiras municipality.

Materials and Methods

Study area

This study was conducted in the municipality of Canavieiras (Figure 1), located in the Immediate Geographic Region of Camacan (ImGR Camacan), in the Intermediate Geographic Region of Ilhéus-Itabuna (InGR Ilhéus-Itabuna), in the south of

the state of Bahia, Brazil (15°39'S - 38°57'W) (Instituto Brasileiro de Geografia e Estatística [IBGE], 2017). Canavieiras has 33,130 inhabitants distributed in 9,714 households in an area of approximately 1,381 km², allocated in the Atlantic Forest Biome (IBGE, 2010).

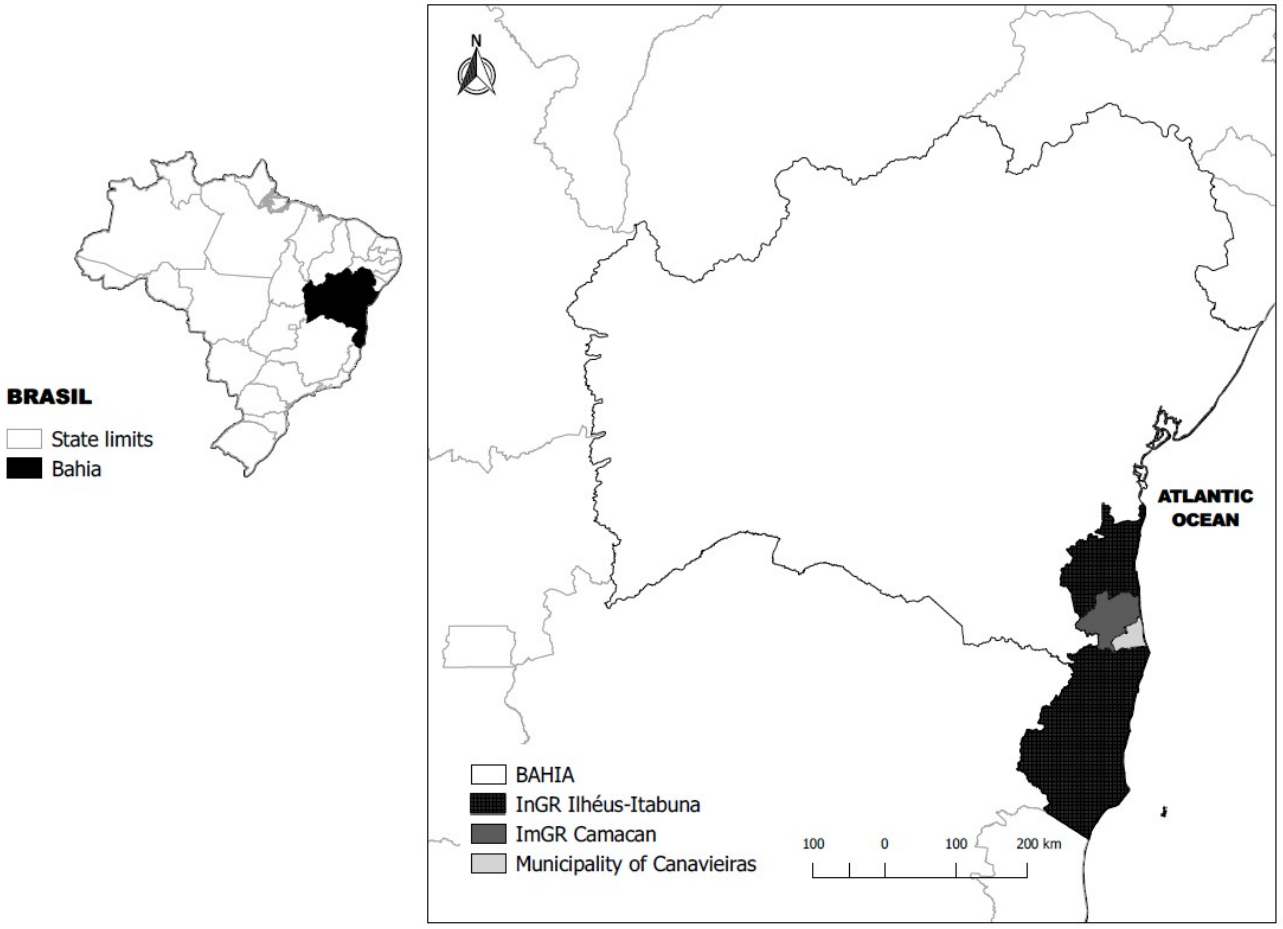

Figure 1. Location Map of the Municipality of Canavieiras, Bahia, Brazil. This map was prepared using the QGIS software (version 2.18).

The municipality has an average HDI (Human Development Index) of 0.59, G (Gini Index) of 0.46, and a poverty match rate of 55.8%. As for the sanitary scope, 55.3% of households have adequate sewage, 5.6% do not provide an adequate destination for garbage, and 9% need a bathroom or toilet. Regarding the water supply of the households, 76.9% use it connected to the water treatment plant (WTP), 16.6% use water from wells or springs, and 6.5% use water through other means, including

river water consumption (3.2%). The infant mortality rate is 10.5/1,000 live births, and the hospitalization rate for diarrheal disease is 1.2/1,000hab. Its primary economic activity is agriculture (IBGE, 2010).

The Cipó River is the source of public water supply in Canavieiras. The catch capacity is 61 liters per second (l/s), and the nominal treatment volume of the system is 80 liters per second (l/s). The operating regime is 13 hours per day, and the station can produce an average of 3.083 m^3/day. WTP uses a rapid water filtration system, which requires a turbidity standard of 0.5 uT in 95% of the samples (MS, 2011). Chemicals are common and employed worldwide (Empresa Baiana de Águas e Saneamento [EMBASA], 2015).

Water samples

Two-liter samples of raw water (RW) and 20 liters of treated water (TW) (Brandão et al., 2012; Cantusio & Bueno Franco, 2004) were collected twice a month from March 2016 to February 2017. The distance from the water catchment point (WCP) to WTP is approximately 5.3 km. The treated water collection point (hydrometer) was 600m from the WTP (Figure 2). The 48 samples, 24 from RW and 24 from TW were stored in plastic containers and transported to the State University of Santa Cruz, Ilhéus, BA. The samples were collected in two sterile 500 mL polypropylene vessels for physicochemical and bacteriological analyses. The samples were stored in identified isothermal boxes and transported from the collection site to the laboratory under refrigeration. The time between sample collection in the field and laboratory processing did not exceed 12 hours.

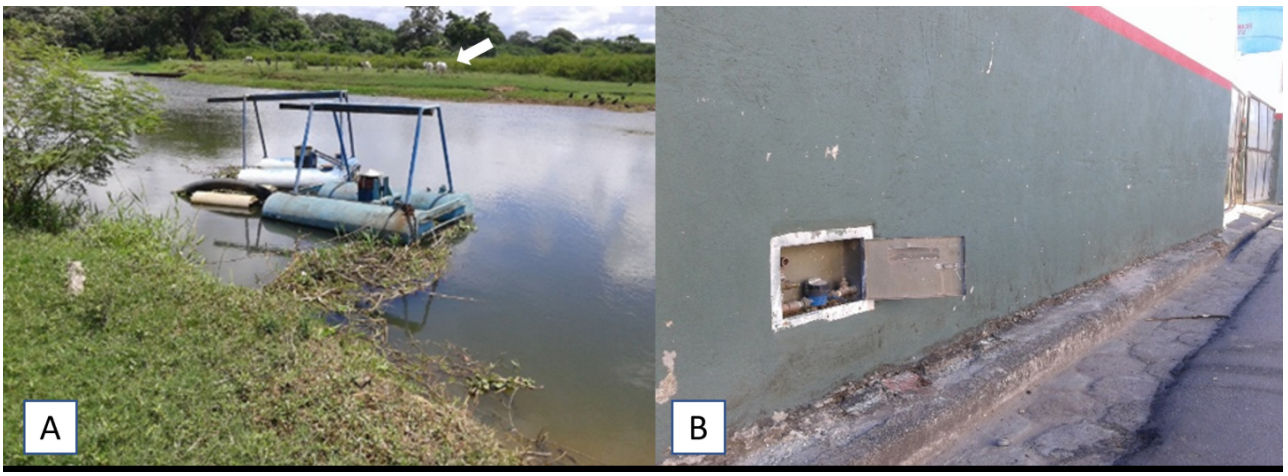

Figure 2. A) Water catchment point. Note the presence of cattle raised on the Cipó River. B) Point of collection (water meter) of treated water. Collection performed in the Municipality of Canavieiras, Bahia, Brazil.

To investigate *Cryptosporidium* spp. oocysts and *G. duodenalis* cysts, samples were initially filtered on cellulose ester membranes with a diameter of 47 mm and nominal porosity of 3.0 µm (Millipore, Brazil). Filtration occurred using a pressure pump, changing the membranes approximately every 250 mL (United States Environmental Protection Agency [USEPA], 2005). Elution by mechanical extraction occurred with scraping and washing of the membrane surface. All liquids resulting from this step were collected in a centrifuge tube (12-15 ml volume and centrifuged at 1500xg for 15 minutes). Subsequently, the final sediment was stored in previously identified microtubes, maintaining the final volume of 1mL (USEPA, 2005).

Physical, chemical, microbiological, and climatic parameters

Water pH was determined using a pHMB 10 meter of Automatic Calibration (Mars Scales Ltda). Water turbidity was measured using a turbidimeter (Solar, model SL 2K).

Aliquots of 100 mL of each raw and treated water sample were diluted in buffered peptide water in the same volume for total coliform counts. After inoculation, the samples were stored at 37 ° C for 3 hours. Subsequently, serial dilutions 10^{-1}, 10^{-2}, and 10^{-3} in 9mL of the tryptose lauryl sulfate (LST) medium were prepared and stored at 35°C for 48 hours to observe turbidity and gas production. After detecting growth in this medium, the samples were inoculated in bright green medium (BGM), using a 2-3 mm diameter platinum handle and 24 - 26 gauge; subsequently, they were inoculated in *E. coli* medium (CEM) in a water bath, for 48 hours, at temperatures of 37°C and 45°C, respectively (Brandão et al., 2012). Precipitation data were collected from the Meteorology National Institute.

Direct fluorescent antibody (DFA) assay

Visualization and quantification of the protozoa were performed using 10 µL aliquots of each sample for the direct immunofluorescence (DFA) test. The Merifluor commercial kit (Meridian Bioscience, Cincinnati, Ohio) with simultaneous detection of *Cryptosporidium / Giardia* was used, following the manufacturer's instructions, with positive and negative controls. Slides were examined using an Olympus BX51 epifluorescence microscope equipped with a simultaneous Differential Interferential Contrast (DIC) system, with a magnification of 100-200X. The characteristics used in the identification by direct immunofluorescence assay were size, color, and ring. The characteristics used in differential interferential contrast were related to empty or amorphous structures and the number of nuclei and axonemes.

Positive samples of *Giardia* spp. were between 8 and 18 µm in size and oval shape, and oocysts of *Cryptosporidium* spp. were between 4 and 6 µm in size and spherical shape. The fluorescence intensity in bright apple-green color was dominant in the cysts' and oocyst's walls (Redlinger et al., 2002). The use of differential interferential contrast allowed us to visualize image detail, considering the positivity criteria of the samples (size and shape of the cysts

and oocysts present in the control sample), the presence of suture in the oocysts, the presence of sporozoites in oocysts and the presence of axonemes, medium bodies, and nuclei in cysts (USEPA, 2005). After enumerating the cysts and oocysts present in the samples, the number of cysts and oocysts per liter of water was estimated according to the following equation (Cantusio & Bueno Franco, 2004):

$$X = \frac{\text{Number of cysts x } 10^6}{\text{Vol. Sediment (L) in the well}} \text{ x } \frac{\text{Vol. sediment (mL) obtained}}{\text{Vol. sample (mL)}}$$

Nested-PCR for Cryptosporidium spp. and Giardia duodenalis

All samples were submitted for DNA extraction using the DNA Stool Mini Kit (QIAGEN, USA) with adaptations (Grecca et al., 2013; Ribeiro et al., 2015). Nested PCR was used in the investigation of both protozoa.

Cryptosporidium spp. - In the first and second reactions, products of 1325 bp and 819-825 bp (SSU-rRNA) were amplified, respectively (Xiao et al., 1999). Reactions were made in 50 µL solutions consisting of 5.0 µL of buffer, 1.5 µL MgCl2, 1.0 µL of dNTP, 0.5 U of Taq Platinum DNA polymerase, and 0,5µL of each *primer* and 3.0µL of target DNA. Samples were subjected to initial denaturation at 94ºC for three minutes, followed by 35 cycles of denaturation at 94ºC for 45 seconds, annealing at 55ºC for 45 seconds, and extension at 72ºC for 1 minute, followed by final extension at 72ºC for seven minutes.

G. duodenalis - In the first reaction, the 16S rRNA gene amplified a 497 bp fragment (Appelbee et al., 2003). In the second reaction, a second pair of oligonucleotide primers amplified a fragment ranging in size from 292 to 297 bp (Hopkins et al., 1997). Reactions (50 µL) consisted of 6.0 µL of buffer, 3.0 µL MgCl2, 2.4 µL of dNTP, 0.25U of Taq Platinum DNA polymerase, 1.2 µL of each oligonucleotide, and 3.0µL of target DNA, for both primary and secondary reactions. The samples were subjected to initial denaturation at 96ºC for four minutes, followed by 35 cycles of denaturation at 96ºC for 45 seconds, annealing at 55ºC for 30 seconds, and extension at 72ºC for 45 seconds, followed by a final extension at 72ºC for four minutes (Appelbee et al., 2003).

A positive control extracted from positive fecal samples from the UESC Parasitology Laboratory was used for each PCR reaction. Ultrapure water was the negative control. The amplicons were visualized using an ultraviolet transilluminator (L.PIX®) on a 1.5% agarose gel in 1x TAE buffer and photographed. Agarose gels were stained with SYBER® Safe DNA Gel Stain (Invitrogen, USA) and visualized on a transilluminator.

Data analysis

The correlation between contamination by *G. duodenalis* spp. and *Cryptosporidium* spp. and physicochemical and microbiological variables in treated and untreated water were analyzed using

the Spearman coefficient (r) using BioEstat 5 statistical package (Ayres et al., 2007). Statistics associated with the sensitivity and specificity of the tests were calculated using the Dag_Stat worksheet (Mackinnon, 2000).

Results and Discussion

The analysis results using the DFA and nPCR methods are shown in Table 1. The mean of 10 Giardia cysts and 08 *Cryptosporidium* oocysts was found per slide.

Physicochemical parameters - In raw water, the pH varied from 2.51 to 8.62 (mean = 5.2 ± 1.63), and the turbidity varied from 3.82 to 37 (mean = 10.0 ± 8.14). In treated water, the pH varied from 3.39 to 7.58 (mean = 5.6 ± 1.14), and the turbidity varied from 2.91 to 14.9 (mean = 5.7 ± 2.62).

Microbiological parameters - *Escherichia coli* was found only in the raw water samples (19/24; 79.2%), with a mean of 560.3 MPN/100mL and median of 271.5 MPN/100mL, ranging from 21 to 1,600 MPN/100mL. Coliforms were found in raw water samples (20.2; 83%), ranging from 140 to 1,600 MPN/100mL, with a mean of 858.7 MPN/100mL and median of 1,050 NMP/100mL, and in only one sample of treated water (4.2%); in this sample, it was not possible to count units. There was no correlation between the microbiological parameters and the presence of protozoa (Brandão et al., 2012).

Table 1
Contamination by *Giardia duodenalis* and *Cryptosporidium* sp. in samples of treated (n=24) and untreated water (n=24) in the municipality of Canavieiras, Bahia, Brazil. March / 2016 to February / 2017

Sample	*Giardia duodenalis*		*Cryptoporidium* spp.	
	DFA	nPCR	DFA	nPCR
Treated water (ps*)	4/24	4/24	1/24	-
Treated water (%)	16.6	16.6	4.2	-
Cysts or oocysts/L (mean)	0.05		0.04	
Raw water (ps*)	10/24	4/24	6/24	2/24
Raw water (%)	41.6	16.6	25	8.3
Cysts or oocysts/L (mean)	1.1		0.6	

*= positive samples.

Precipitation - The average rainfall was 142.5 mm per month, with the highest rain in November (374.2 mm) and the lowest in January (41.2 mm). Precipitation and pH significantly correlated with the results of nPCR for *Cryptosporidium* in raw water (Table 2).

Table 2
Spearman correlation coefficients (r) and correlation significance (p-Value) between the detection of *Cryptosporidium* and *Giardia* by direct immunofluorescence antibody (DFA) and nested-PCR (nPCR) methods and the physicochemical and rainfall measurements in crude and treated water samples

Parasite	Method	Water	Precipitation		Turbidity		pH	
			R	p-Value	r	p-Value	r	p-Value
Cryptosporidium spp	DFA	Raw	-0.0992	0.6448	0.2502	0.2382	-0.0348	0.8719
		Treated	0.3530	0.0906	-0.2560	0.2271	-0.1054	0.6239
	nPCR	Raw	**0.5362**	**0.0069**	-0.2594	0.2209	**-0.4522**	**0.0265**
		Treated	0.3995	0.0530	-0.0653	0.7616	-0.2396	0.2595
Giardia spp	DFA	Raw	-0.1058	0.6228	0.0997	0.6498	-0.0348	0.8719
		Treated	-0.1152	0.5919	0.2907	0.1681	-0.1054	0.6239
	nPCR	Raw	-0.0988	0.6462	0.1292	0.5473	0.0565	0.7930
		Treated	-0.0082	0.9696	0.3553	0.0883	0.2584	0.2227

p-Value < 0.05 indicates significative correlations.

The sensitivity and specificity values of nPCR were not satisfactory compared to those of DFA, as nPCR sensitivity values were consistently low regardless of parasite and water type. Similarly, the kappa index was low in all cases, indicating that the nPCR method did not faithfully reproduce the DFA gold standard results (Table 3). Cysts and oocysts must be viable to cause human infection and be considered a public health risk. Olson et al. (1999) concluded that Giardia cysts are viable for two weeks and Cryptosporidium oocysts for ten weeks in water at 25°C.

Table 3
Sensitivity (S), specificity (E), Efficiency (Ef), positive predictive value (PPV), negative predictive value (NPV), kappa (k) association coefficient, and associated p-value to the McNemar test between *Cryptosporidium* and *Giardia* detection in raw or treated water samples by nested-PCR (nPCR) in comparison to direct immunofluorescence (DFA)

Parasite	Water	S	E	Ef	PPV	NPV	k	p-Value
Cryptosporidium	Raw	16.17	77.78	62.50	20.00	73.68	-0.06	1.00
	Treated	0.00	91.30	87.50	0.00	95.45	-0.06	1.00
Giardia	Raw	30.00	92.86	66.67	75.00	65.00	0.25	0.08
	Treated	25.00	85.00	75.00	25.00	85.00	0.10	0.68

The occurrence of *Cryptosporidium* spp. and *Giardia duodenalis* in samples of treated and raw water has been reported in different regions of the world (Sato et al., 2013; Onichandran et al., 2014; Vermeulen et al., 2019; Borja-Serrano et al., 2020). Faced with this reality, the quality of water used for human consumption, whether for drinking or preparing food, must be established within stricter standards of quality control, effectively and periodically incorporating the investigation of zoonotic pathological agents as the evolutionary forms of cysts and oocysts of the protozoa mentioned in this work, have a significant resistance susceptibility related to the action of chlorine and other disinfectants for disinfecting drinking water (Boni, 2016). Thus, the magnitude of the damage caused by diarrheal diseases, such as Giardiasis and cryptosporidiosis, makes these conditions one of the significant challenges for Public and Animal Health, as their scope goes beyond the scope of health (Buret et al., 2020; Widmer et al., 2020; Branco et al., 2012; World Health Organization [WHO], 2013; Ligda et al., 2020), extending significantly to the productive chains of the agricultural sector (Thompson et al., 2008; Santin, 2020).

The presence of these protozoa in Brazilian underground and surface water sources has been reported for more than 20 years (Cantusio & Bueno Franco, 2004; Sato et al., 2013; Franco et al., 2001; Hachich et al., 2004; Costa & Mendoza- Sassi, 2007; Dias et al., 2008; Xavier et al., 2011; Almeida et al., 2015; Freitas et al., 2015; Grott et al., 2016). In this period, Giardia was predominant among the pathogens detected in treated and raw water, as we also observed in the Rio Cipó. However, in addition to the low number of studies, most research was concentrated, particularly in the southeastern region of Brazil. In addition, studies that meet the recommendation of Ordinance 2,914 / 11 and investigate the presence of these pathogens in treated water are even rarer. In the northeastern region, *Giardia* spp. and *Cryptosporidium* spp. were detected in rainwater stored in tanks and clay pots (Xavier et al., 2011) and in the water of one of the rivers used by the public water system (Freitas et al., 2015), in studies carried out in the state from Pernambuco. In this context, our study, in addition to being a pioneer in the investigation of these protozoa in a source of treated and raw water in the state of Bahia, provides relevant data for the reevaluation and reconstruction of public policies that envision the control of the spread of these parasites in human populations and animals in the municipality of Canavieiras.

The occurrence of *Giardia* spp. in surface water, including Brazilian rivers, lakes, and streams, varies from 5.5% (Santos et al., 2010) to 100% (Franco et al., 2001). However, in most studies, the occurrence is greater than 20%, as opposed to the percentage found in our study. These high percentages are likely related to the higher population density in the respective study areas, which can result in a flow of larger volumes of waste in the rivers, as was the case in the studies by Sato et al. (2013) and Freitas et al. (2015), for example, contrary to the reality observed along the Cipó River.

In comparison to the Brazilian study, which adopted a similar methodology (Almeida et al., 2015) and followed the sampling guidelines recommended by ordinance 2,914/11, we found some differences between the results of the diagnostic techniques, the physical-chemical parameters and the number of *G. duodenalis*

cysts by liter of raw water. DFA showed better results than nPCR in our study, especially in detecting *G. duodenalis.* In contrast, in the study cited above, which evaluated samples of raw and treated water from one of the public water treatment systems in Londrina-PR, the sensitivity of DFA and nPCR were equivalent in detecting *G. duodenalis* in raw water. However, nPCR was more sensitive in detecting *Cryptosporidium* (Almeida et al., 2015). DFA is the gold standard method in laboratories and one of the most used methods in detecting *Giardia* cysts and *Cryptosporidium* oocysts (Centers for Disease Control and Prevention [CDC], 2023). We emphasize that membrane filtration followed by direct immunofluorescence assay remains the most used method in the country to detect these parasites. Therefore, we assume that the techniques used in this study efficiently detect these protozoa in water samples. Although the sensitivity and specificity of the nPCR were low, it is necessary to consider that several other factors may have influenced our results, including DNA extraction, the presence of PCR inhibitors, and the number of samples evaluated.

The mean of cysts and oocysts found in raw water in this study was lower than the averages observed in studies in the south (Grott et al., 2016) and southeast (Sato et al., 2013; Franco et al., 2001; Dias et al., 2008; Cantusio et al., 2010) regions of the country, suggesting a lower level of degradation, probably due to the reduced anthropic pressure and to the lower amount of sewage drained along the Cipó River (WHO, 2009). The mean of *Cryptosporidium oocysts* per slide was the same as observed in samples from a river in Pernambuco (Freitas et al., 2015).

Ordinance 2914/11 establishes that the pH varies between 6.0 and 9.5 and that the turbidity, in a rapid filtration system, is equivalent to 0.5 uT in 95% of the samples. In this study, these parameters were outside the standard recommended in Brazil. The low pH and the high turbidity of the raw water suggest the presence of significant levels of pollution (Freitas et al., 2015). However, higher levels of turbidity may be due to specific characteristics of fluvial neosols (Grott et al., 2016), as well as the presence of algae, sand, clay, organic debris, household waste, and lack of rain (MS, 2014). In this context, this parameter should be reevaluated in Rio Cipó. We also emphasize that high levels of turbidity in rivers and streams have been reported in the southeastern (Franco et al., 2001; Cantusio & Bueno Franco, 2004; Cantusio et al., 2010) southern (Grott et al., 2016) and northeastern (Freitas et al., 2015) watersheds of Brazil, suggestively influenced by the degradation due to the pollution of its beds. The high turbidity and pH of the treated water detected in our study suggest flaws in the treatment process at the municipal supply station. We emphasize that the determination of pH is an essential element in controlling decomposition and treating sewage and industrial waste (MS, 2014). Turbidity is a parameter of acceptance or rejection of the product according to the presence of material suspended in the water. Turbid particles lead to concentrated organic matter that can impart an unpleasant taste and odor to the waters (MS, 2011). Another critical aspect of turbidity is the potential influence on the sensitivity of membrane filtration techniques, which may have underestimated the presence of protozoa in our samples since the more significant the presence of dirt, the faster the membrane

pores are clogged, increasing the need for replacement during the filtration process (Franco et al., 2012b).

The correlation between *Giardia* and *Cryptosporidium* and physical-chemical and microbiological parameters is still controversial and needs further investigation. Contrary to what was observed in our study and the study by Kumar et al. (2016), the correlation between the presence of *Cryptosporidium* and pH has not been identified in some national (Franco et al., 2001; Almeida et al., 2015; Grott et al., 2016) and international studies (Rose et al., 1988; Atherholt et al., 1998; Ithoi, 2009). Considering the correlation of the presence of *Cryptosporidium* with the precipitation in raw water observed in our study, several studies point to the postulation of this relationship (Atherholt et al., 1998; Curriero et al., 2001; Davies et al., 2004; Carmena et al., 2007; Dias et al., 2008). It is important to note that the increase in water turbidity, associated with the ample water supply from runoff during periods of rain, increases the number of oocysts, which confirms the decrease in the parasitological quality of raw water during this period (Davies et al., 2004). In contrast, Ithoi (2009) did not find such a correlation. In treated water, our study showed a tendency to correlate with this variable, which should be further investigated.

High fecal contamination of Brazilian surface water sources has been reported in the south (Grott et al., 2016; Toledo et al., 2017), southeast (Franco et al., 2001; Cantusio et al., 2010), and northeast (Freitas et al., 2015) of Brazil. In Bahia, the impact of anthropic pressure on the degradation of watersheds was demonstrated through the dissonance of the presence of thermotolerant coliforms with the legislation (Pessoa et al., 2018). In this study, although few samples concentrate amounts of *E. coli* beyond legal limits in raw water, the presence of this bioagent, plus the presence of total coliforms in more than 80% of the samples and the presence of *Giardia* and *Cryptosporidium,* demonstrates a high level of degradation and fecal contamination of the municipal water supply, representing a risk to human and animal health. It is essential to consider that more than 20% of Canavieiras households do not receive treated water and use alternative forms of supply, such as the consumption of water from the river. In addition, we emphasize that the presence of these contaminants suggests the presence of other fecal parasites. It is important to note that the concentration of these bioagents in raw water was lower than the concentrations observed in rivers in São Paulo (Franco et al., 2001; Cantusio et al., 2010) and in a river in Pernambuco (Cantusio et al., 2010). In the case of the Rio Cipó, this finding is probably due to a reduced anthropic impact along the river, which differs from what occurs in the rivers of the states mentioned above. The presence of total coliforms in the treated water, added to the standardization of physicochemical parameters, shows flaws in the water filtration and treatment process (MS, 2011).

In this study, contamination of raw water and treated water can result from several factors, including the lack or deficiency of basic sanitation infrastructure in the communities bordering the Cipó River, the flow of garbage and waste in the river (Pessoa et al., 2018), the access of cattle and wild animals to the water (Fayer et al., 2004; Thompson et al., 2008; Dias et al., 2008; Toledo et al., 2017), as well as failures

in the process of filtering and treating water from the distribution network. *G. duodenalis* and *Cryptosporidum* spp. infect human populations (Mariano et al., 2015; Harvey et al., 2020) and animals, including dogs (Harvey et al., 2020) and cattle (Muniz Neta et al., 2010) from municipalities in the same geographic region to which they belong the municipality of Canavieiras. In this context, it must be considered that humans and animals may be contributing to river contamination (Thompson et al., 2008). In addition, it is essential to note that both protozoa are resistant to conventional treatment methods used in water treatment plants, which may also explain the presence of these agents in treated water (Karanis et al., 2007). It is also noteworthy that the southern region of the state, where the municipality of Canavieiras is located, concentrates one of the highest percentages of cases of acute diarrheal diseases (Secretaria da Saúde [SESAB], 2019).

This scenario highlights the need for the integration of all sectors involved in the water supply process, aiming to meet the water potability standards and to reach the permitted levels of risk to the health of human and animal populations. Currently, the importance of implementing actions based on the principles of One Health for monitoring, responding, and understanding the ecological dynamics of diseases is a fact (Muniz Neta et al., 2010; SESAB, 2019). Thus, there is a consensus among the authors of this study on the actions that should be prioritized to enable an increase in the quality of treated and raw water, as well as on actions that promote the control of diarrheal diseases in locations with similar water profiles. Among the actions, we include the adaptation to the water treatment process in WTP, recommended by Ordinance 2914/2011 (MS, 2011); the periodic monitoring of the presence of pathogenic protozoa at the points of water collection and distribution; the detection of local hotspots for cases of diarrheal diseases in humans and animals; the collection and proper treatment of effluents in order to avoid their outflow in the sources used for public supply; investment in health infrastructure in riverside communities; continuous health education actions for the general population, animal producers and pet owners, through the Primary Health Care departments. We also emphasize that the population must be oriented to avoid access to superficial watersheds, especially after the rainy period.

Conclusion

Cryptosporidium and *Giardia* are present in the public water supply network and the water distribution network in the municipality of Canavieiras, state of Bahia, with physicochemical and microbiological parameters in disagreement with current legislation. The DFA method had a better result than the nPCR in detecting these protozoa, mainly for *Giardia.* The presence of PCR inhibitors in the samples may be a plausible explanation for the result since molecular analysis is more sensitive in detecting microorganisms.

Contamination of the Cipó River and the public water supply network may increase the risk of transmission of diarrheal diseases in the region. To reduce the risk of infection by *Cryptosporidium,* health agencies should guide the population not to access the river after periods of rain.

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