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Technical Article

Risk to human health from protozoan (oo)cysts in water treatment plants

Risco à saúde humana por (oo)cistos de protozoários em estações de tratamento de água

Débora Pereira da Silva[1*], Ysabella de Paula dos Reis[1], Nolan Ribeiro Bezerra[1], Paulo Sérgio Scalize[1], Lilian Carla Carneiro[1]

ABSTRACT

The presence of waterborne pathogens, when associated with the water supply system, poses risks to public health. This study investigated the occurrence of (oo)cysts of *Cryptosporidium* spp. and *Giardia* spp. in two Water Treatment Plants, with full-cycle technology, and assessed the microbiological risk to consumers' health. The membrane filtration technique was employed to identify the protozoan load in 24 samples of raw and filtered water. Physicochemical and bacteriological parameters were also analyzed. The Quantitative Microbial Risk Assessment model was used to determine the risk of daily and annual infection associated with the ingestion of filtered water. A total of 66.67% of raw water samples were positive for *Cryptosporidium*; and 33.33%, for *Giardia*. The maximum concentrations of 200 (oo)cysts/L and 50 cysts/L were detected in the Cerrado stream (located in Sanclerlândia, state of Goiás, Brazil), due to the predominance of grazing areas and intense agricultural activity. Water Treatment Plants did not completely remove the protozoa and the retention efficiency was lower than that recommended by the United States Environmental Protection Agency, with average values of 1.27 log for cysts and 1.04 log for oocysts. The probability of annual infection by cysts (100%) was higher than that of oocysts (86.61 – 98.32%) as for consumption of filtered water, and in the dry season, there was a higher risk of infection, due to the low performance of the Water Treatment Plants and higher concentration of pathogens. According to the results, the continuous intake of filtered water above the warning level can cause infectious diseases in the supplied population.

Keywords: *Cryptosporidium*; *Giardia*; risk of infection; public health; Quantitative Microbial Risk Assessment.

RESUMO

A presença de patógenos de veiculação hídrica quando associados ao sistema de abastecimento de água causam risco à saúde pública. Esse estudo investigou a ocorrência de (oo)cistos de *Cryptosporidium spp.* e *Giardia spp.* em duas Estações de Tratamento de Água com tecnologia do tipo ciclo completo e avaliou o risco microbiológico à saúde dos consumidores. Utilizou-se a técnica de filtração em membranas para identificar a carga dos protozoários nas 24 amostras de água bruta e filtrada. Os parâmetros físico-químicos e bacteriológicos também foram analisados. O modelo de Avaliação Quantitativa de Risco Microbiológico foi empregado para determinar o risco de infecção diária e anual associada à ingestão de água filtrada. Um total de 66.67% das amostras de água bruta foram positivas para *Cryptosporidium*; e 33.33% para *Giardia*. As concentrações máximas de 200 oocistos/L e 50 cistos/L foram detectadas no ribeirão Cerrado (localizado em Sanclerlândia, Estado de Goiás, Brasil), devido ao predomínio de área de pastagens e à intensa atividade agropecuária. As Estações de Tratamento de Água não removeram completamente os protozoários e a eficiência de retenção foi inferior ao recomendado pela Agência de Proteção Ambiental dos Estados Unidos, com valores médios de 1,27 log para cistos e 1,04 log para oocistos. A probabilidade de infecção anual por cistos (100%) foi superior aos oocistos (86,61% a 98,32%) para o consumo da água filtrada, sendo que a estação seca, o risco de infecção foi maior, devido ao baixo desempenho das Estações de Tratamento de Água e à maior concentração de patógenos. Esses resultados indicam que a ingestão contínua de água filtrada acima do nível de alerta pode provocar doenças infecciosas na população abastecida.

Palavras-chave: *Cryptosporidium*; *Giardia*; risco de infecção; saúde pública; Avaliação Quantitativa de Risco Microbiológico.

[1]Universidade Federal de Goiás – Goiânia (GO), Brazil.
***Corresponding author:** debora_pdsilva@hotmail.com
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INTRODUCTION

Access to drinking water is crucial for the promotion and protection of human health, as safe water ensures the prevention of waterborne diseases and improves the population's quality of life. According to the World Health Organization (WHO), approximately two billion people use contaminated water sources, and this consumption can cause 485 thousand deaths from diarrhea each year (WHO, 2019).

Giardia spp. and *Cryptosporidium* spp. are parasitic protozoa that infect a wide variety of vertebrate hosts, including humans, and their transmission occurs via the fecal-oral route, through indirect contact, due to the ingestion of contaminated water and/or food, or direct contact from feces of infected people. These protozoa are considered relevant to public health, as they are responsible for waterborne diarrheal diseases called giardiasis and cryptosporidiosis, whose number of cases has increased in recent years (KARANIS, KOURENTI & SMITH, 2007; EFSTRATIOU, ONGERTH & KARANIS, 2017; ĆIRKOVIĆA *et al.*, 2020). Between the years 2011 to 2016, there were at least 381 outbreaks caused by (oo)cysts of *Giardia* spp. and *Cryptosporidium* spp. transmitted by treated water in New Zealand, North America, and Europe (EFSTRATIOU, ONGERTH & KARANIS, 2017).

In Brazil, cases of giardiasis and cryptosporidiosis are underreported, as they are not deemed compulsory notifiable diseases by Decree No. 264 of the Brazilian Ministry of Health (BRAZIL, 2020b). However, the number of infected people is reported as a public health event, as provided by Ordinance No. 104 (BRAZIL, 2011). Nevertheless, (oo)cysts of *Giardia* spp. and *Cryptosporidium* spp. have been detected in surface water in different Brazilian cities (SATO *et al.*, 2013; OLIVEIRA, 2017; SILVA; SCALIZE, 2020) and regions worldwide (NISHI *et al.*, 2009; NAKADA *et al.*, 2019). Outbreaks caused by the (oo)cysts of these pathogens occur due to their high infectivity in addition to resistance to environmental conditions and the treatment of conventional chlorination (THOMPSON, 2004; KARANIS, 2011).

Moreover, Iglói *et al.* (2018) recorded complications and long-term sequelae after an episode of acute gastroenteritis. Thus, it is evident the need to assess the adverse health effects in a population prone to ingest water contaminated with the infectious stages of these protozoa.

The Quantitative Microbial Risk Assessment (QMRA) model can be used to estimate the population infection probability, raising awareness about this situation. The estimates from this assessment are important to subsidize and encourage managers to preventively act in terms of health protection, as well as for implementing processes and treatments aimed at reducing the number of these pathogens in the water supply sources.

Thus, this study aimed to investigate the concentration of (oo)cysts of *Giardia* spp. and *Cryptosporidium* spp. in two water treatment plants (WTPs), with full-cycle technology, and to estimate the risks to health of the population subjected to the ingestion of water contaminated with these pathogens.

METHOD

Study area

The study area consisted of two WTPs, located in the state of Goiás (Brazil), which use full-cycle treatment technology (Figure 1), comprising the following steps: coagulation with aluminum sulphate, rapid mixing using Parshall gutter, flocculation using a baffled hydraulic flocculator, decantation by a conventional decanter of horizontal flow, rapid downward filtration by double-layer filters, disinfection and fluoridation in a contact chamber.

WTP I, located in the city of Sanclerlândia, at the coordinates 16°12'56.69"S and 50°18'38.59"W, catches water from the Cerrado stream, with minimum and maximum flows of 23 L·s and 33 L·s, respectively. WTP II, located in the city of São Luís dos Montes Belos, at the coordinates 16°32'15.34"S and 50°21'14.94"W, catches water from the Santana stream, with minimum and maximum flows of 25 L·s and 77 L·s, respectively.

Sample collection

Six samples of raw water and six samples of filtered water (without disinfection) were collected from each WTP in specific places, i.e., 12 samples from each WTP, totaling 24 samples. The collections were performed in the dry (September) and rainy (December) seasons in 2019, according to the Köppen (1948) classification.

For sample collection, the volume was determined according to the type of analysis as follows:

- Parasitological analysis: 20 L of water in a polyethylene container, previously cleaned with elution solution (0.1% Tween® 80);
- Physicochemical analysis: 1.0 L of water in a polypropylene flask;
- Bacteriological analysis: 100 ml of sample in a sterile flask.

Parasitological analysis

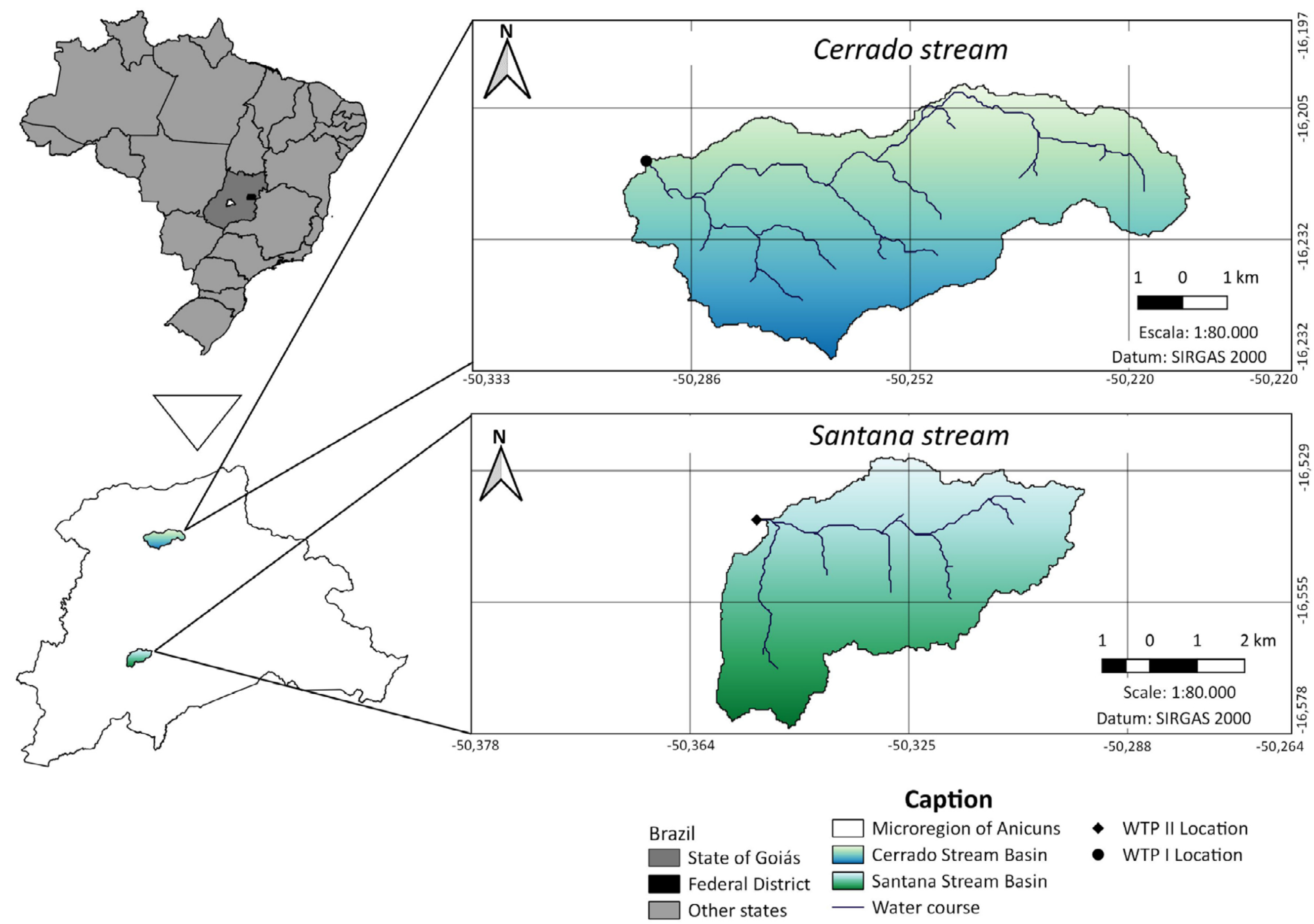
The samples were analyzed using the membrane filtration method, adapted from Franco, Rocha-Eberhardt and Cantusio (2001), for the detection and enumeration of *Cryptosporidium* spp. and *Giardia* spp. (oo)cysts. This technique was adopted due to the low cost of implementation, when compared with standard methods recommended by the United States Environmental Protection Agency (USEPA, 2012), and due to the possibility of being widely used in developing countries such as Brazil.

The ease of implementation, the possibility of preserving (oo)cysts for future infectivity tests (MACIEL & SABOGAL-PAZ, 2016), and the use in research involving water supply sources in different regions in the world (ALMEIDA *et al.*, 2015; BAUTISTA *et al.*, 2018; SILVA & SCALIZE, 2020) were other criteria for choosing the aforementioned technique.

In quality control tests to verify the reliability of the method, three samples of ultrapure water (2 L) were contaminated with 268 oocysts and 32 cysts obtained from the protozoa inoculum, using the Merifluor® kit. Thus, the average recovery rates and standard deviation values of 78.1 ± 0.0%, for *Giardia* spp. Cysts, and of 60.6 ± 32.6% for *Cryptosporidium* spp. oocysts, were obtained. These values are in accordance with those recommended by USEPA (2012), which are 27–100% ± 39% and 38–100% ± 37%, respectively.

The method was applied according to the following steps:

- Filtration of 2 L of sample in mixed cellulose esters membranes (Millipore®), with diameter of 47 mm and nominal porosity of 3 µm (raw water) and 1.2 µm (filtered water), for the concentration of (oo)cysts;
- In order to elute the (oo)cysts, scraping and washing of the material retained on the membrane, using elution solution (0.1% Tween® 80), were performed;
- Double centrifugation and concentration of the material, using a centrifuge (Excelsa® 4, model 280-R) with a rotation of 1500 x g, for 15 min;



WTP: Water Treatment Plants.
Source: prepared by the authors.

Figure 1 – Delimitation of hydrographic basins of the Cerrado and Santana streams, located in the state of Goiás (Brazil), and identification of the sampling sites.

- Preparation of the slide by the immunofluorescence assay (FA) technique, using the Merifluor® kit (Meridian Bioscience Diagnostics, Cincinnati, USA), with 10-µL aliquots;
- Reading of the slides using the Axio Imager.M2 microscope for epifluorescence (Zeiss®), with 400x magnification, 450–490-nm excitation filter, and 520-nm barrier filter.

The results of protozoa concentrations (PC) were expressed in (oo)cysts/L according to Equation (1) described by Cantusio Neto and Franco (2004).

$$PC = \frac{\text{No. of (oo)cysts observed}}{\text{sediment volume analyzed } (\mu L)} x \frac{\text{sediment volume obtained } (\mu L)}{\text{filtered sample volume (L)}} \quad (1)$$

For data analysis, the Detection Limit (DL) (Equation 2) was determined, which consists of the minimum number of recoverable organisms in an analyzed sample volume (ONGERTH, 2013).

$$DL = \frac{1}{\text{sediment volume analyzed } (\mu L)} x \frac{\text{sediment volume obtained } (\mu L)}{\text{filtered sample volume (L)}} \quad (2)$$

The protozoan removal efficiency in the WTPs was analyzed using Equation 3.

$$\text{Removal log} = -\frac{\text{PC in filtered water}}{\text{PC in raw water}} \quad (3)$$

Where: PC in raw water = protozoa concentration in raw water ([oo cysts/L); PC in filtered water = protozoa concentration in filtered water ([oo]cysts/L).

According to the USEPA (2021), when using the health risk method, concentrations of dangerous agents below DL cannot be considered absent, as they may hide health threats. In this study, a minimum concentration of 1 (oo)cysts/L, in raw or filtered water, was adopted and the detected value was below the DL to avoid overestimation of risk value.

Physicochemical and bacteriological analyses

Turbidity, apparent color, true color, pH, total coliforms, and *Escherichia Coli* analyses were performed according to the *Standard Methods for the Examination of Water and Wastewater* (APHA, AWWA & WEF, 2012).

Turbidity was continuously monitored in the filtered water, for seven days, with an interval of 5 min, using a TU5300sc® Low Range Laser Turbidimeter connected to an SC200® controller, both from the HACH brand.

Statistical analysis

Results of protozoan concentrations in the samples of raw and filtered water from the WTPs were analyzed using descriptive statistics, i.e., arithmetic mean and maximum and minimum values, adopting a minimum concentration of 1 (oo) cysts/L for values below the detection limit. The arithmetic mean and standard deviation were calculated for the physicochemical and bacteriological analyses.

In order to verify the correlation between the protozoa and the physical and bacteriological parameters, Spearman's correlation coefficient (r) was calculated using the XLSTAT® software. The seasonal variation of protozoa concentration was analyzed using the single-factor ANOVA test. The considered level of significance was $p < 0.05$ (95%).

Probability of infection

The risk of infection was estimated using the model recommended by Haas, Rose, and Gerba (2014), according to the following stages:

- Identification of the "reference pathogen";
- Evaluation of the exposure route;
- Evaluation of the exposure pathway;
- Evaluation of the dose-response relationship;
- Risk characterization.

The (oo)cysts of *Giardia* spp. and *Cryptosporidium* spp. were the reference pathogens selected. For the exposure route, the hydric environmental compartment was considered, and the probable sources of contamination of the water source were identified through data on land use and occupation.

The analyzed exposure route was the ingestion of filtered water, without disinfection. To determine the number of (oo)cysts ingested by consumers in a single event, Equation 4 was considered, adapted from Haas, Rose and Gerba (2014):

$$d = \frac{N}{Tr}.V \quad (4)$$

Where: d = "reference pathogen" mean dose ingested at each exposure event ([oo]cysts/d);
N = mean concentration of (oo)cysts of *Giardia* spp. and *Cryptosporidium* spp. in filtered water ([oo]cysts/L);
Tr = recovery rate of the membrane filtration method for (oo)cyst pathogens of *Giardia* spp. (60.6%) and *Cryptosporidium* spp. (78.1%);
V= per capita consumption of water ingested per day (L/day), adopting 2 L/day (WHO, 2006).

To assess adverse health effects (dose-response relationship), the exponential mathematical model (Equation 5) proposed by Haas, Rose and Gerba (2014) was considered.

$$P_{Inf\,(d)} = 1 - e^{(-d.\kappa)} \quad (5)$$

Where: PInf(d) = probability of daily infection from a single exposure;
d = mean dose of the "reference pathogen" ([oo]cysts/d) (Equation 4);
κ = characteristic parameters of the agent-host interaction, using the parameters defined by Haas, Rose and Gerba (2014) of 50.23 for *Giardia* spp cysts and 238.0 for *Cryptosporidium* spp. oocysts.

It is worth mentioning that values of the κ parameter were defined by Haas, Rose and Gerba (2014) considering the species *Cryptosporidium parvum* and *Giardia lamblia*.

Regarding risk characterization, the probability of annual infection, referring to the dry and rainy seasons, was analyzed using Equation 6 (HAAS, ROSE & GERBA, 2014).

$$P_{Inf\,(a)} = 1 - [1 - P_{Inf\,(d)}]^n \quad (6)$$

Where: PInf(a) = probability of annual infection, referring to the dry and rainy seasons, in relation to *n* exposure events at the same dose (d);
PInf(d) = probability of infection from a single exposure (Equation 5);
n = number of exposures in each analyzed period. To specify the exposure in each season, 153 days were considered for the dry period, and 212 for the rainy period (KÖPPEN, 1948).

It is noteworthy that there are two approaches to estimate microbiological risks, namely the models of static and dynamic risks. According to Soller (2006), static models assess the risk of infection based on direct exposure (person-environment), and generally immunological issues and secondary transmission are not considered. To determine the risk, this model considers that the concentration of pathogens in the analyzed media does not change over time; therefore, the individual is exposed to a constant concentration of pathogens.

Concerning dynamic models, the risks of infection are assessed in a population exposed to a variable concentration of microorganisms over time. In addition, this method considers the potential for direct (environment-person) and secondary (person-person) transmissions and immunological issues of exposed individuals (SOLLER, 2006).

Dynamic risk models can be deterministic or stochastic. The deterministic approach is appropriate for large population studies, as epidemiological data are used to determine the secondary transmission rate, using models expressed in a set of differential equations (EISENBERG *et al.*, 1998; SOLLER *et al.*, 2003). In contrast, the stochastic approach is more appropriate for small populations, as it determines the interaction between individuals by probability analysis using methods such as Markov Chain or Monte Carlo Simulation (SOLLER, 2006).

Soller and Eisenberg (2008) concluded that the static model provides satisfactory risk estimates when risks associated with direct contamination are low, considering that uncertainties related to the study are lower.

Taking this into consideration, the present study used the static model due to the direct transmission route and the scarcity of epidemiological data on "reference pathogens" in the study areas.

RESULTS

Detection of protozoa

Among the 24 analyzed samples, *Cryptosporidium* spp. oocysts were detected in 66.67% of the samples collected from the Cerrado stream, and in 100% of those collected from

Table 1 –Concentration of *Giardia* spp. and *Cryptosporidium* spp. in raw and filtered water, and Detection Limit in Water Treatment Plants I and II.

Collection area	Collection	Concentration ([oo]cysts/L)		
		Giardia spp.	*Cryptosporidium* spp.	DL
Raw water from Cerrado stream	Collection 1	50.00	66.67	16.67
	Collection 2	< DL	33.33	8.33
	Collection 3	< DL	< DL	16.67
	Collection 4	< DL	< DL	10.42
	Collection 5	8.33	8.33	8.33
	Collection 6	< DL	200.00	100.00
Raw water from Santana stream	Collection 1	8.33	8.33	8.33
	Collection 2	< DL	16.67	16.67
	Collection 3	< DL	16.67	16.67
	Collection 4	< DL	35.09	17.54
	Collection 5	< DL	8.33	8.33
	Collection 6	16.67	33.33	8.33
Filtered water from WTP I	Collection 1	< DL	4.35	4.35
	Collection 2	< DL	< DL	5.00
	Collection 3	< DL	2.08	2.08
	Collection 4	< DL	< DL	1.25
	Collection 5	< DL	< DL	1.25
	Collection 6	< DL	1.25	1.25
Filtered water from WTP II	Collection 1	4.17	< DL	4.17
	Collection 2	< DL	1.67	1.25
	Collection 3	< DL	< DL	1.25
	Collection 4	< DL	< DL	1.79
	Collection 5	< DL	< DL	1.25
	Collection 6	< DL	1.25	1.25

WTP: Water Treatment Plant; DL: Detection Limit.
When protozoan concentrations were < DL, a value equal to 1 was adopted.
Source: prepared by the authors.

the Santana stream; the concentrations of each collection shown in Table 1 were lower than the DL. After treatment by the respective WTPs (WTP I and WTP II), detection in the filtered water was reduced to 50% and 33.33% respectively. The *Giardia* spp. cysts were less frequently in raw water, with 33.33% of positive samples in both water sources and, after treatment, no cysts were detected in the WTP I filtered water, and only one sample from the WTP II had cysts, corresponding to 16.67%.

Due to seasonality, the raw water from the Cerrado stream had mean concentrations of 69.78 and 33.67 of *Cryptosporidium* spp. oocysts/L (Figure 2A) and 3.44 and 17.33 of *Giardia* spp. cysts/L (Figure 2B), in the rainy and dry seasons, respectively. In contrast, the raw water from the Santana stream had a higher concentration of pathogens in the rainy season, with mean concentrations of 25.58 oocysts/L2 (Figure 2C) and 6.22 cysts/L (Figure 2D). The single-factor ANOVA test showed that the occurrence of these protozoa had no significant differences between seasons ($p > 0.05$).

Regarding the filtered water from WTP I, the mean oocyst loads of *Cryptosporidium* spp. were 1.08 oocysts/L in the rainy season and 2.48 oocysts/L in the dry season (Figure 2A). Conversely, *Giardia* spp. cysts values were lower than the DL (Figure 2B), in such a way that the concentration of 1.0 cyst/L was adopted for analyzing the results. In the filtered water from WTP II, the mean oocyst loads of *Cryptosporidium* spp. were 1.08 and 1.22 oocysts/L (Figure 2C), referring to the rainy and dry seasons, respectively. *Giardia* spp. cysts were detected in only one sample, in the dry season, with a concentration of 4.17 cysts/L, resulting in an average of 2.06 cysts/L (Figure 2D). The concentration of other samples with values lower than DL (Table 1) was 1 cyst/L.

The concentrations of (oo)cysts in the water from the Cerrado stream were higher than those detected in the Santana stream water (Figures 2 and 3), with maximum values of 200 oocysts/L (Figure 2A) and 50 cysts/L (Figure 2B).

The results shown in Figure 3 demonstrate an average retention efficiency of 1.27 log for cysts, which was higher than the one found for oocysts (1.04 log). The WTPs had better efficiency in the rainy season, except for the removal of *Giardia* spp. cysts in WTP I (Figure 3).

Physicochemical and bacteriological analyses

The results of physicochemical and bacteriological analyses are shown in Table 2. The mean values of turbidity (16.90 and 527 NTU) and apparent color (74.50 and 409.50 uC) regarding raw water from the Cerrado stream were higher than those found in the Santana stream water, which feeds WTP II.

The following Spearman's correlation coefficients (r) were obtained: between *Cryptosporidium* spp. and the presence of turbidity ($r = 0.006$, $p = 0.773$), apparent color ($r = 0.001$, $p = 0.897$), total coliforms ($r = 0.023$, $p = 0.574$), and *E. Coli* ($r = 0.001$, $p = 0.897$), and between *Giardia* spp. and the values of turbidity and apparent color ($r = 0.002$, $p = 0.883$) and total coliforms and *E. Coli* ($r = 0.007$, $p = 0.764$).

Regarding the continuous monitoring of turbidity, Figures 2e and 2f show the turbidity variation in the filtered water, which had higher mean values in the rainy season, with 0.56 NTU in WTP I (Figure 2E) and 0.35 NTU in WTP II (Figure 2F).

Risk assessment

Table 3 shows the results regarding the probability of infection by *Giardia* spp. cysts and *Cryptosporidium* spp. oocysts due to the intake of filtered water (without disinfection) supplied by the WTPs located in Sanclerlândia (I) and São Luís dos Montes (II).

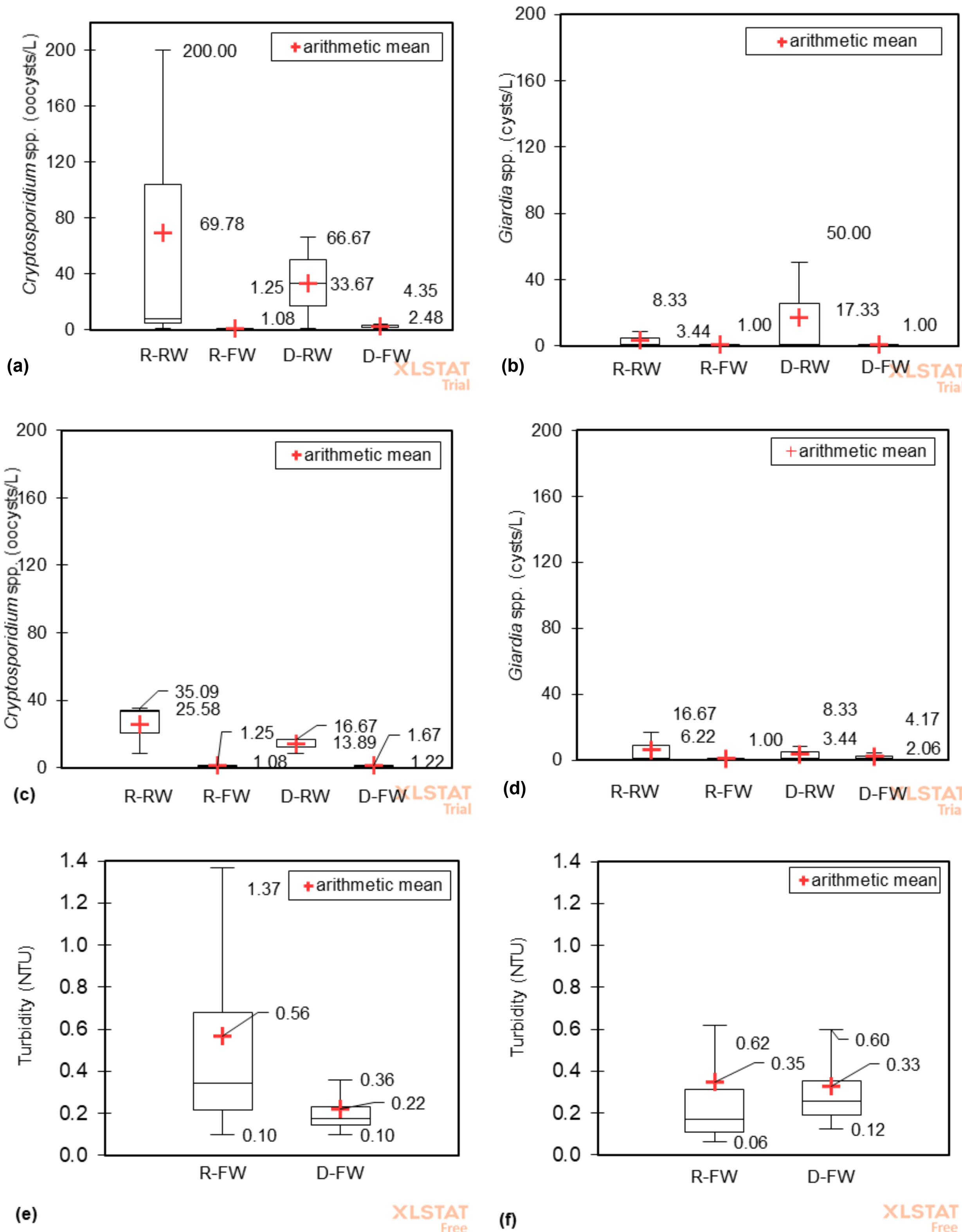
Considering a daily consumption of two liters of filtered water (without disinfection), consumers would ingest mean doses of 3.33 cysts/d and 4.56 oocysts/d when drinking water from WTP I. Regarding WTP II, consumers would ingest mean doses of 5.10 cysts/d and 2.95 oocysts/d.

The highest risk probabilities of daily infection were 12.78% cysts in WTP II and 2.64% oocysts in WTP I. The annual probability of infection by cysts was 100% for both WTPs. About the oocysts, the probabilities range from 86.61% to 98.32%, in WTP II and WTP I, respectively, both in the dry period.

DISCUSSION

Among the 24 analyzed samples, there was a higher frequency of oocyst detection, thus indicating that the studied water sources have a high potential for environmental contamination by this pathogen.

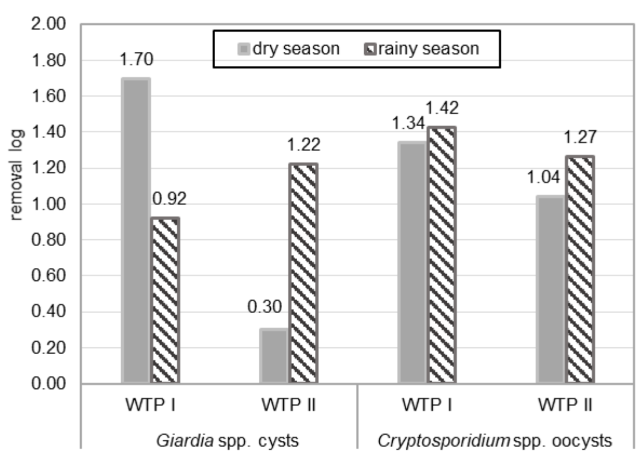
The higher concentration of pathogens in the Cerrado stream water may be associated with the predominance of grazing areas in 79.33% and the presence of 5,533 bovines in the hydrographic basin (SILVA & SCALIZE, 2020), as the intense agricultural activities upstream from the water catchment area influence water contamination by bovine feces (ADAMSKA, 2015), considering that an infected bovine can release from 50 to 3.9×10^5 (oo)cysts/g of feces (FARIZAWATI *et al.*, 2005). The presence of the main hosts of *Giardia*



R-RW: raw water in the rainy season; R-FW: filtered water in the rainy season; D-RW: raw water in the dry season; D-FW: filtered water in the dry season. In the case of protozoa concentrations < DL (Detection Limit), values equal to 1 were adopted for the arithmetic mean calculation.

Source: prepared by the authors.

Figure 2 – Concentration of (oo)cysts (arithmetic mean and maximum values) in the raw water from the Cerrado stream and in the filtered water from Water Treatment Plant I, in Sanclerlândia, state of Goiás, Brazil (A, B), in the raw water from the Santana stream and in the filtered water from Water Treatment Plant II, in São Luís dos Montes Belos, state of Goiás, Brazil (C, D), and Box plot graph without outliers of turbidity values in filtered water from Water Treatment Plant I (E) and Water Treatment Plant II (F).



WTP: Water Treatment Plant.
Source: prepared by the authors.

Figure 3 – Log of removal of protozoan (oo)cysts and cysts in Water Treatment Plants with full-cycle technology, considering raw water and filtered water (without disinfection), in the rainy and dry seasons.

Table 2 – Results of physicochemical and bacteriological analyses of raw water and filtered water, in Water Treatment Plants I and II, expressed as arithmetic mean [± standard deviation].

Season	Parameter	WTP I		WTP II	
		Raw water	Filtered water	Raw water	Filtered water
dry	T(1)		0.223 [0.150]		0.325 [0.277]
	T(2)	16.90 [2.97]	0.72 [0.37]	9.40 [0.33]	0.51 [0.29]
	AC(3)	74.50 [2.55]	7.80 [3.54]	54.45 [0.21]	9.50 [3.68]
	pH	7.64 [0.08]	7.33 [0.13]	7.60 [0.14]	7.33 [0.20]
	TC(4)	7576 [1538.66]	2976 [3985.25]	15732.50 [2257.79]	356.50 [184.55]
	EC(5)	842 [93.34]	36.50 [37.48]	2206.5 [2047.07]	15 [7.07]
rainy	T(1)		0.565 [0.723]		0.345 [0.765]
	T(2)	527 [453.96]	0.64 [0.42]	87.70 [85.28]	0.78 [0.70]
	AC(3)	409.50 [154.86]	6.35 [1.06]	119 [8.49]	5.75 [1.20]
	pH	6.91 [0.18]	6.34 [0.30]	7.51 [0.18]	6.59 [0.44]
	TC(4)	36540 [0]	115.50 [26.16]	-	136 [24.04]
	EC(5)	7,408 [3,388.46]	25 [7.07]	59,845.5 [73,743.46]	46 [22.63]

T(1): turbidity data continuously monitored in NTU; T(2): Turbidity in NTU; AC(3): apparent color in uC; TC(4): total coliforms in NMP/100 ml; EC(5): *Escherichia Coli* in NMP/100 ml; WTP: Water Treatment Plant.
Source: prepared by the authors.

Table 3 – Results of the mean dose and probability of infection, daily and yearly, regarding dry and rainy seasons.

Pathogen	Period	d ([oo]cysts/d)		$P_{Inf\,(d)}$ (%)		$P_{Inf\,(a)}$ (%)	
		WTP I	WTP II	WTP I	WTP II	WTP I	WTP II
Giardia spp.	Dry	3.33	6.87	6.42	12.78	100.00	100.00
	Rainy	3.33	3.33	6.42	6.42	100.00	100.00
Cryptosporidium spp.	Dry	6.36	3.13	2.64	1.31	98.32	86.61
	Rainy	2.77	2.77	1.16	1.16	91.51	91.51

D: dose ingested in a single event; PInf (d): daily infection probability; PInf (d): annual infection probability; WTP: Water Treatment Plant. The results were obtained from filtered water samples from Water Treatment Plant I, in Sanclerlândia, state of Goiás, Brazil, and Water Treatment Plant II, in São Luís dos Montes Belos, state of Goiás, Brazil, for arithmetic means of *Giardia* spp. cysts and *Cryptosporidium* spp. oocysts.
Source: prepared by the authors.

duodenalis in the catchment watershed can also cause outbreaks of giardiasis in the population.

The highest occurrences of these two protozoa in the filtered water from WTP I can be associated with the high concentrations of these pathogens in the Cerrado stream (Figures 2A e 2B), considering that, according to Almeida *et al.* (2015), there is a correlation between the quantities of these organisms in raw and treated water; therefore, monitoring the supply source is paramount, as established in Annex XX of Consolidation Ordinance No. 5 (BRAZIL, 2017).

The protozoan loads in the filtered water (Figures 2A–2D) were higher than the action levels suggested in the literature for treated water: 0.1–0.3 oocysts/L (HAAS & ROSE, 1995) and 0.03–0.05 cysts/L (WALLIS *et al.*, 1996), in which a single sample above these limits increases the exposure to outbreaks of parasitic diseases. Therefore, the passage of (oo)cysts to filtered water can compromise the health of the population and cause outbreaks.

During the rainy season, filtered water contamination by both protozoa was lower (Figures 2A–2D). According to Dugan *et al.* (2001), the greater amount of colloidal material in the raw water assists in the removal of oocysts during the treatment process as well as higher concentrations of turbidity increase the removal of solids in the sedimentation stage (ASSAVASILAVASUKUL *et al.*, 2008).

The lowest rates of efficiency of retention of oocysts (Figure 3) indicated that the WTPs had difficulty removing them due to their small size (4.0–6.0 µm), lower density (1036.2 kg·m³), and lower sedimentation speed (0.3–0.4 µm·s) when compared with the speed of cysts (0.7–1.4 µm·s) (MEDEMA *et al.*, 1998).

Overall, the studied WTPs proved to be unable to completely remove (oo) cysts, as the mean efficiencies were lower than the removal ranges of 1.4–4.0 log for *Cryptosporidium* spp., and 1.5–4.0 log for *Giardia* spp. found by Betancourt and Rose (2004) in WTPs with full-cycle technology, as well as below the minimum removal limits recommended by USEPA (2006) of 2.5 log for cysts and 2.0 log for oocysts. Considering that these pathogens are often found in systems with full-cycle technology, they are deemed extremely important for public health (KARANIS, KOURENTI & SMITH, 2007).

The better performance of WTPs in the rainy season can be associated with a higher load of protozoa in the catchment water sources in this period (Figures 2A–2D) as, according to Assavasilavasukul *et al.* (2008), such concentration is directly associated with removal efficiency.

WTP I had better performance than WTP II, regardless of operating with a flow overload that varies from 15% to 65% (design flow of 20 L·s), which can be associated with higher concentration of both protozoa in the Cerrado stream water.

Concerning water quality, deterioration can be related to the characteristics of land occupation, as verified by Dias *et al.* (2008), as grazing area is predominant in 79.33% of the Cerrado stream basin and in 78.46% of the Santana stream basin.

The deterioration of raw water in the studied sources (Table 2) was intensified in the rainy season due to the dragging of particles during superficial runoff. However, physical and bacteriological parameters showed a weak correlation ($0 < r < 0.4$) with parasitological variables. A moderate correlation between color and turbidity and the presence of (oo)cysts was reported by Almeida *et al.* (2015).

The pH was within the range established by the National Environment Council – CONAMA Resolution No. 357 (BRAZIL, 2005) for rivers with freshwater intended for human consumption (6–9), whereas the turbidity and apparent color values recorded in the rainy season were not in conformity.

The turbidity parameter of filtered water is considered a good indicator of the efficiency of the filtration unit for removing these pathogens from WTPs by treatments with full-cycle technology (DUGAN *et al.*, 2001). Hence, the results shown in Figures 2E and 2F may compromise the quality of life of the supplied population.

Therefore, special attention should be payed to the filtration units, mainly in the rainy season, because 37.21% (WTP I) and 16.06% (WTP II) of the samples exceeded the value of 0.5 NTU and did not meet the limit of 5% established in Annex XX of the Consolidation Ordinance No. 5 (BRAZIL, 2017).

Regarding the probability of infection, during the dry season, the risks of infection (Table 3) are high due to the lower efficiency of retention of protozoa by the treatment units (Figure 3) and the greater presence of (oo)cysts of protozoa in the filtered water supplied by the WTPs (Figures 2A–2D).

In addition, the intake of filtered water containing cysts represents a greater risk to health in comparison with water containing oocysts, with probabilities of daily and annual infection of 12.78%, in WTP II, and 100% in both WTPs, respectively. These results are related to the ingested doses and the greater virulence of *Giardia* spp. regarding the *Cryptosporidium* spp. oocysts, as evidenced by Haas, Rose, and Gerba (2014).

Sato *et al.* (2013) estimated risks of infection of 0.29%–2.47% and 0.15%–0.29% for cysts and oocysts, respectively, in a WTP located in São Paulo (Brazil). These results are lower than the ones recorded in the present research, which can be explained by the low removal efficiency of WTPs, whose removal rates were lower than 1.7 log for cysts and 1.4 log for oocysts, whereas Sato *et al.* (2013) found removal rates of 3.0 log and 2.0 log for *Giardia* ssp. and *Cryptosporidium* spp., respectively.

The values presented in this study (Table 3) exceed the acceptable risk of 10^{-4}, recommended by USEPA (2006), thus indicating a high risk of infection. Similar results were obtained in studies carried out in the Southeast region of Brazil (SATO *et al.*, 2013, RAZZOLINI *et al.*, 2016).

Nevertheless, neither outbreaks of giardiasis nor cryptosporidiosis were reported in the study municipalities, which may be related to the nonrecognition of the disease or its aggravation due to surveillance procedures (BRAZIL, 2020a) and overestimation of the risk, as it is unlikely that all (oo)cysts are viable and pathogenic to humans (SATO *et al.*, 2013).

It should be noted that the studied water is submitted to a disinfection process with gaseous chlorine prior to distribution; however, according to Ramo *et al.* (2017), the chlorine-based disinfectant is not efficient in inactivating some protozoa, such as *Cryptosporidium* spp. and *Giardia* spp., which requires longer contact time and high concentrations of residual chlorine (BWTANCOURT & ROSE, 2004).

In addition, the positive results found for infection probability do not necessarily indicate risk due to these protozoa, considering that the employed method can detect both infectious and noninfectious particles, and it is not possible to confirm the infectious potential in these cases. Thus, to confirm whether the estimated infection probability is accurate, the presence of the pathogen in the population must be analyzed by performing molecular analysis and genetic characterization to eliminate false-positive results found in microscopy.

CONCLUSIONS

Based on the results, the authors conclude that:

- Public water sources had higher contamination by *Cryptosporidium* spp. oocysts than by *Giardia* spp. cysts;
- The water quality from the Cerrado stream is deteriorated, which may be mainly related to the predominance of grazing areas and the intense agricultural activities upstream from the catchment area that may impact the public water supply;
- The treatment with full-cycle technology, focusing on the filtration stage, proved to be insufficient to remove all (oo)cysts of the studied protozoa;
- The treatment with full-cycle technology had a lower removal log for *Cryptosporidium* spp., probably due to the difficulty in retaining the oocysts;
- The filtered water turbidity values, in noncompliance with the legislation, indicate that companies responsible for sanitation services should pay special attention to the last physical barrier of the treatment, i.e., the filtration unit, as the chlorination process, using a chlorine-based disinfectant, may not be fully effective in inactivating the researched protozoa;
- The pathogen loads found in the filtered water were higher than the action level, being necessary to monitor the water distributed to the population to verify the efficiency of inactivation of these microorganisms by the time of contact with the disinfectant agent;
- The risk of annual infection to consumers is high in both WTPs, which can cause a serious public health problem in this geographic area. This risk proved to be higher for *Giardia* spp. cysts due to their greater virulence;
- The high-risk values are associated with the low log of removal of (oo)cysts by the WTPs;
- In addition, overestimation of risk may have occurred, considering that not all (oo)cysts are pathogenic to humans;
- The results of infection risk can support the development of public policies for protecting health and water resources, aimed at reducing pathogens in water supply sources;
- The results show the need to improve the recovery of analytical methods and to analyze their viability.

AUTHORS' CONTRIBUTIONS

Silva, D. P: Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization, Writing – original draft, Writing – review & editing. Reis, Y. P: Methodology, Formal analysis, Data curation, Conceptualization, Writing – original draft, Writing – review & editing. Bezerra, N. R.: Validation, Supervision, Data curation. Carneiro, L. C.: Validation, Supervision. Scalize, P. S.: Validation, Supervision, Project administration, Resources.

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