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Environmental surveillance of zoonotic protozoa and fecal indicators in surface waters of a subtropical transition zone in Southern Brazil

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Abstract

Waterborne protozoa pose a significant challenge to public health, particularly in regions with deficient sanitation. This study aimed to evaluate the occurrence of *Giardia lamblia*, *Cryptosporidium* spp., and *Cystoisospora* spp. in surface waters of the Pelotas microregion, Southern Brazil, and their correlation with fecal indicator bacteria (*Escherichia coli*) and rainfall events. From March to August 2025, eight sampling points (streams, urban canals, and recreational beaches) were monitored. Water samples were analyzed for physico-chemical parameters, *E. coli* quantification, and molecular detection (PCR) of protozoa. *Giardia lamblia* and *Cystoisospora* spp. were frequently detected in urban drainage canals and streams, showing a persistence that contrasted with the variable counts of *E. coli*. Notably, extreme rainfall events (> 180mm accumulated) and low water temperatures (< 14°C) in the final sampling campaign appeared to influence the detection rates, suggesting a dilution effect or reduced bacterial viability, while protozoan DNA persisted. *Cryptosporidium* spp. was not detected. These findings highlight the environmental resilience of protozoan cysts and the limitations of relying solely on bacterial indicators for water quality assessment in subtropical transition zones.

1. Introduction

Access to safe water is recognized as a fundamental human right; however, the fecal contamination of surface waters remains a pervasive public health challenge in many developing countries (Madriles et al., 2024; Forstinus et al., 2016). Anthropogenic activities, including the discharge of untreated urban sewage and agricultural runoff, introduce a range of pathogens into aquatic ecosystems, thereby exacerbating the risk of waterborne diseases. Enteric protozoa, notably *Giardia lamblia* and *Cryptosporidium* spp., are of particular concern because of their extremely low infectious doses and remarkable resistance to environmental stresses and chemical disinfection treatments (Nishi et al., 2009; Madriles et al., 2024). Moreover, the infrequent monitoring of *Cystoisospora* spp. in environmental studies suggests that its zoonotic potential, especially in areas characterized by frequent human–animal interactions, may be underestimated (Madriles et al., 2024; Forstinus et al., 2016).

In southern Brazil, where the Pampa biome is defined by a subtropical climate, distinct seasonal variations, and an extensive hydrological network, water quality challenges are accentuated. The region surrounding Pelotas, which serves as the drainage basin for the massive Patos Lagoon, is particularly vulnerable to shifts in water quality due to its unique hydrogeomorphological characteristics. The interplay between urban drainage infrastructures and episodic meteorological events, such as those associated with *El Niño* phenomena, further complicates the dynamics of pathogen transport and persistence (Nishi et al., 2009; Forstinus et al., 2016). Despite these critical factors, little is known about how rainfall patterns and temperature fluctuations modulate the presence of waterborne zoonotic protozoa in this transition zone.

Against this backdrop, the present study undertook an environmental surveillance assessment of zoonotic protozoa in raw water sources across southern Brazil. Central to our investigation was the hypothesis that urban drainage channels serve as persistent reservoirs for these pathogens, whose concentrations may be significantly modulated by meteorological events. In turn, these findings question the reliability of classical fecal indicators, such as *Escherichia coli*, and underscore the need for a more comprehensive approach to monitoring water quality and associated health risks (Nishi et al., 2009; Forstinus et al., 2016; Madriles et al., 2024).

2. Materials and Methods

2.1 Study Area and Sampling

The study was conducted in Pelotas, a municipality in Rio Grande do Sul, Brazil, where the interplay of urban, rural, and coastal influences creates distinct hydrological settings. Eight sampling sites were selected to capture this diversity of aquatic environments. These sites were categorized into four groups:

1. **Upstream/Rural Influence**: Arroio Moreira.
2. **Drainage Canals**: Canal do Pepino, Canal Santa Bárbara and Canal São Francisco.
3. **Receiving Water Bodies**: Arroio Pelotas, Canal São Gonçalo, and Barragem Santa Bárbara (supply reservoir).
4. **Recreational/Coastal**: Praia do Laranjal (located at Patos Lagoon).

This site selection strategy echoes previous work that emphasizes spatial heterogeneity in water quality monitoring (Khouja et al., 2010; Ramo et al., 2017).

2.2 Sampling Design

Sampling campaigns were performed over four occasions between March and August 2025, thereby capturing the transition from late summer to winter and the corresponding shifts in environmental conditions. From each site, 2-liter water samples were collected at depths ranging from 15 to 30 cm, ensuring representativeness of the water column as described in similar environmental surveillance studies (Jiang et al., 2024; Khouja et al., 2010). *In situ*, key physico-chemical parameters such as pH, water temperature, and turbidity were recorded to help correlate microbial dynamics with environmental variability (Table 1). Additionally, meteorological data, in particular, accumulated rainfall over the 30 days preceding sample collection and the duration between sampling events, were retrieved from the National Institute of Meteorology (INMET) to provide context for potential hydrological perturbations (Jiang et al., 2024; Ramo et al., 2017).

Table 1
Physical-chemical parameters, quantification of Escherichia coli, and molecular detection of protozoa in surface water samples from the Pelotas microregion, Southern Brazil (March–August 2025).

Sample Site	**Category**	**Water Temp. (°C)**	**Turbidity (NTU)**	***E. coli* (CFU/100mL)**	***Giardia spp.* (Pos/Total)**	***Cystoisospora spp.* (Pos/Total)**
Arroio Moreira	Upstream Reference	13.0–24.0	0.11–0.46	< 1–51	0/4	0/4
Barragem Santa Barbara	Water Supply Reservoir	14.0–20.0	0.21–0.38	< 1–16	0/4	0/4
Canal do Pepino	Urban Drainage	14.0–24.0	0.12–0.50	0.12–0.50	3/4	2/4
Canal Santa Barbara	Urban Drainage	12.0–24.0	0.33–0.79	1 – > 300	4/4	4/4
Canal São Francisco	Urban Drainage	14.0–21.0	0.44–1.86	< 1 – > 300	4/4	4/4
Arroio Pelotas	Receiving Body	14.0–19.0	0.28–1.42	< 1–83	4/4	4/4
Canal São Gonçalo	Receiving Body	13.0–19.0	0.29–0.63	< 1–58	0/4	0/4
Praia do Laranjal	Recreational	14.0–18.0	0.23–0.45	< 1–13	0/4	0/4

Legend: Temp: Temperature; **NTU**: Nephelometric Turbidity Units; **CFU**: Colony Forming Units. **Note**: Values for temperature, turbidity, and E. coli represent the range (minimum – maximum) observed across four sampling campaigns. Where E. coli counts were 0, they are expressed as < 1. *Cryptosporidium* spp. was not detected in any sample and is therefore excluded from the table.

2.3 Microbiological and Molecular Analysis

Fecal Indicator Analysis

As a baseline measure of water contamination, *Escherichia coli* was quantified by determining colony-forming units (CFUs) on selective media. This method, widely employed in water quality assessments, provides a robust measure for fecal contamination and complements the molecular detection of protozoa (Khouja et al., 2010; Chauret et al., 1995).

Sample Processing and Concentration

To recover protozoan cysts and oocysts from the collected water samples, water samples (2 L) were concentrated by membrane filtration. The samples were filtered through mixed cellulose ester membranes (47 mm diameter, 1.2 µm pore size) using a vacuum filtration system. The membranes were carefully scraped and rinsed with a 0.1% Tween 80 solution to recover the retained biomass. The eluate was then centrifuged at 1,500 × g for 15 minutes, and the supernatant was discarded to obtain the final pellet for DNA extraction. This dual-

step procedure maximizes the recovery of low-abundance microorganisms and has been validated in several studies investigating waterborne pathogens (Jiang et al., 2024; Khouja et al., 2010).

DNA Extraction

DNA was extracted from the concentrated biomass using the TRIzol® reagent, with modifications tailored to environmental samples. This protocol efficiently lyses cells and preserves nucleic acids for downstream molecular assays, as reported in analogous studies focusing on waterborne protozoa detection (Rochelle et al., 1997; Ramo et al., 2017).

PCR Detection

Molecular detection targeted three specific gene markers: (i) the small subunit ribosomal RNA (SSU-rRNA) gene for *Giardia lamblia* based on the protocol of Hopkins et al. (1997); (ii) the SSU-rRNA gene for *Cryptosporidium* spp. Based on the protocol of Xiao et al. (2000); (iii) the internal transcribed spacer 1 (ITS1) region for *Cystoisospora* spp. based on the protocol of Samarasinghe et al. (2008).

PCR assays were performed using previously established primer sets and protocols to ensure both sensitivity and specificity. This approach is consistent with optimization strategies described by Rochelle et al. (1997) and has been applied effectively in environmental monitoring contexts (Huang et al., 2017). The target genes were selected based on their proven utility in differentiating and genotyping protozoan pathogens in water samples (Rochelle et al., 1997; Ramo et al., 2017).

3. Results

3.1 Environmental Conditions

A distinct seasonal shift was observed during the study period. Water temperature dropped from a mean of 21.1°C in the first campaign (March) to 13.5°C in the fourth campaign (August) (Table 2). Rainfall patterns varied drastically: the first two campaigns occurred during a drier period (cumulative rainfall of 27.6 mm and 1.4 mm, respectively), while the third and fourth campaigns followed heavy rainfall events (Table 2).

Table 2
Seasonal variation of meteorological, physicochemical, and microbiological parameters across the four sampling campaigns (n = 8 sites per campaign).

Campaign (Date)	Season	Rainfall (mm)*	Mean Water Temp. (°C)	Mean Turbidity (NTU)	*Giardia* Detection Rate *(%)*	*Cystoisospora* Detection Rate *(%)*	*E. coli* Detection Trend**
1st (Mar 25)	Summer/Autumn	27.6	21.1	0.46	50.0% (4/8)	50.0% (4/8)	High (Up to > 300 CFU)
2nd (May 05)	Autumn	1.4	18.4	0.24	50.0% (4/8)	50.0% (4/8)	Variable (< 1 to > 300 CFU)
3rd (Jun 19)	Autumn/Winter	147.0	14.8	0.55	50.0% (4/8)	50.0% (4/8)	Low (Max 6 CFU)
4th (Aug 05)	Winter	180.4	13.5	0.63	37.5% (3/8)	37.5% (3/8)	Very Low (< 1 to 4 CFU)

Legend: * Rainfall data refers to the accumulated precipitation in the interval prior to sampling or the last 30 days (Source: INMET). ** Descriptive trend of E. coli counts observed across the sites. Note: Despite the heavy rainfall and temperature drop in the 3rd and 4th campaigns, protozoan DNA remained detectable in key urban sites, contrasting with the sharp decline in cultivable E. coli.

3.2 Bacteriological Quality (*E. coli*)

E. coli counts varied spatially and temporally. Urban canals (e.g., Canal Santa Bárbara, Canal São Francisco) presented the highest contamination levels (> 300 CFU/100mL) during the first two campaigns (Table 1). However, in the fourth campaign (August), despite high turbidity, *E. coli* counts dropped significantly, with several points testing negative or showing low counts (< 5 CFU).

3.3 Occurrence of Protozoa

Giardia lamblia was the most frequently detected protozoan. It was persistent in the Arroio Pelotas and urban canals (Pepino, Santa Bárbara, São Francisco) across almost all campaigns (Table 1). *Cystoisospora* spp. was detected in 50% (4/8) of the sites during the first two campaigns, showing a spatial distribution similar to *Giardia*. Conversely *Cryptosporidium* spp. was not detected in any of the analyzed samples (Table 1).

3.4 Impact of Rainfall and Temperature

Heavy rainfall preceding the third (147 mm) and fourth (180.4 mm) sampling events coincided with a sustained detection of protozoan pathogens, while a paradoxical reduction in *Escherichia coli* counts was observed during the final campaign. This counterintuitive trend may result from dilution effects and differential survival dynamics between protozoan cysts and bacterial cells under precipitation-induced hydrological changes (Maldonado et al., 2023; Kapoor et al., 2018). Notably, even when water temperatures ranged between 12°C and 14°C during the fourth campaign, both Arroio Pelotas and Canal Santa Bárbara continued to yield positive results for *Giardia* and *Cystoisospora*. The persistence of these enteric protozoa, despite low *E. coli* counts, underscores their resilience to environmental stress and highlights inherent limitations in relying solely on fecal indicator bacteria for water quality assessments (Maldonado et al., 2023; Kapoor et al., 2018).

4. Discussion

4.1 Persistence of Protozoa in Urban Drainage

The frequent detection of *Giardia lamblia* and *Cystoisospora* spp. in urban canals strongly indicates that these watercourses function as open sewers, receiving untreated wastewater discharge and subsequently transferring pathogens to larger receiving bodies such as the Patos Lagoon (Kistemann et al., 2002; Singh et al., 2024). Furthermore, the detection of *Cystoisospora* spp., an organism typically associated with animals yet inclusive of human pathogenic variants, such as *Cystoisospora belli*, suggests a mixed contamination profile involving both zoonotic and anthroponotic sources (Wilkes et al., 2013; Singh et al., 2024). This mixed profile is likely driven by surface runoff from areas with domestic animals and insufficient sanitation practices, thereby highlighting the complex interplay of environmental and anthropogenic factors affecting water quality.

4.2 The "Dilution Effect" vs. Persistence

During the fourth campaign (August), the accumulated rainfall of 180.4 mm apparently induced a wash-out effect, as evidenced by a dramatic reduction in *Escherichia coli* counts despite detectable turbidity. This phenomenon is likely attributable to a dilution effect and the reduced viability of vegetative bacterial cells at temperatures below 14°C. In contrast, protozoan DNA from *Giardia* and *Cystoisospora* persisted at key sampling points (e.g., Arroio Pelotas and urban canals), which suggests that the robust cyst forms of these protozoa exhibit superior environmental resilience compared to typical fecal bacteria (Fekete et al., 2021; Fernández et al., 2022). These findings support the notion that a decline in *E. coli* does not necessarily equate to reduced parasitological risk.

4.3 The Absence of *Cryptosporidium*

The consistent non-detection of *Cryptosporidium* spp. stands in stark contrast to the frequent recovery of *Giardia*. This discrepancy may be explained by several factors: (i) a relatively lower environmental shedding rate of *Cryptosporidium* compared to *Giardia*; (ii) the inherent resistance of *Cryptosporidium* oocysts, which may limit the efficiency of DNA extraction methods; and (iii) intermittent shedding patterns that complicate detection (Fernández et al., 2022; Fekete et al., 2021). This contrast underscores the importance of employing multiple diagnostic approaches to achieve an accurate assessment of waterborne protozoan pathogens.

4.4 One Health Implications

The presence of *Giardia* and *Cystoisospora* in waters utilized for irrigation and recreation raises significant One Health concerns. These waters serve as potential conduits for zoonotic transmission, thereby posing risks to both human and animal populations. In particular, the detection of *Cystoisospora* spp., which includes variants such as *C. belli* that are known to infect humans, indicates a contamination profile with both zoonotic and anthroponotic origins, likely driven by surface runoff from areas with domestic animals and inadequate sanitation (Andrade et al., 2019; Fernández et al., 2022). Furthermore, recent insights into host–parasite and gut microbiota interactions underscore the complex dynamics of pathogen persistence and transmission, reinforcing the imperative for integrated One Health surveillance measures (Grondin et al., 2024).

5. Conclusion

This study demonstrates that the surface waters in the Pelotas microregion serve as reservoirs for protozoan pathogens, specifically *Giardia lamblia* and *Cystoisospora* spp., with urban drainage channels acting as key conduits for contamination. The data reveal that heavy rainfall events may obscure conventional bacterial indicators, as evidenced by the substantial dilution or environmental inactivation of *Escherichia coli* under low temperature conditions (< 14°C), even as protozoan DNA persists in the water. This apparent dissociation between bacterial indicators and protozoan pathogen presence underscores the inherent resilience of the cystic forms relative to vegetative bacteria (Sha-Amu et al. 2024m et al., 2015). Considering these findings and the associated One Health risks, the results accentuate the urgency of incorporating protozoan surveillance into routine water quality monitoring programs. Such integrative approaches are essential for accurately assessing public health risks and guiding interventions in environments affected by both urban and agricultural influences (Richter et al., 2015m et al., 2015).

Declarations

Author Contributions

W.C.F.Z. designed the study, conducted the sampling and laboratory analyses, performed data analysis, and wrote the manuscript. G.C.F.K. assisted in sample collection and processing. E.R. and B.R.F. contributed to the molecular analyses. P.M.M.A., K.R.M., and N.M.R. assisted in the parasitological concentration techniques. R.C.C. supervised the study, provided resources, and reviewed the final manuscript. All authors read and approved the final version.

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Conflict of Interest:

The authors declare that they have no conflict of interest.

Ethical Approval:

Not applicable (Environmental study not involving human or animal subjects).

Data Availability:

The datasets generated during the current study are available from the corresponding author on reasonable request.

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