Detection of *Giardia duodenalis* and *Toxoplasma gondii* in soil and water samples in the Quindío River basin, Colombia

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ABSTRACT

Two zoonotic protozoan pathogens, *Giardia duodenalis* and *Toxoplasma gondii*, are important causes of waterborne infections in the Quindío region in Colombia. No previous data exist on how contamination occurs at the source for drinking water consumed by the human population in this region. Our aim was to describe the frequency of *G. duodenalis* and *T. gondii* DNA in 11 sampling points during a five-month period in water and adjacent soil at the Quindío River basin (Andean region in the central western part of Colombia). The study employed nested PCR for *T. gondii*, using the B1 gene as the amplification target, and single-round PCR for *G. duodenalis* assemblage A and assemblage B, amplifying the *gdh* gene, followed by DNA sequencing. In 50 soil samples, 28% (14/50) were positive for *T. gondii*. For *G. duodenalis*, distribution was in equal parts for assemblage A (8%; 4/50) and assemblage B (8%, 4/50). Genotyping of *T. gondii* sequences showed two soil samples with type I strain, another two samples of soil with type III strain, but most samples were of unidentified strains. In water samples, *T. gondii* was detected in 9.1% (5/55), *G. duodenalis* assemblage A in 34.5% (19/55), and *G. duodenalis* assemblage B in 12.7% (7/55). *T. gondii* DNA positivity was associated with lower soil temperature ($p = 0.0239$). Presence of *G. duodenalis* and *T. gondii* was evidenced in soil and water samples in the Quindío River basin, indicating soil as the potential source of contamination for the river that it is destined for human consumption. Monitoring these protozoa in drinking water is necessary to prevent public health risks in human populations.

1. Introduction

The most common causal agents of waterborne parasite infections in developing countries are *Giardia duodenalis*, a cosmopolitan organism and causative agent of giardiasis (Cai et al., 2021), and *Toxoplasma gondii*, an obligate intracellular apicomplexan parasite that can infect all warm-blooded vertebrates (Shapiro et al., 2019a). Both are zoonotic protozoan pathogens whose principal causes of transmission are attributed to factors linked to environmental sanitation, inadequate disposal of wastes, and poor health education

(Cai et al., 2021; Djurković-Djaković et al., 2019).

For *G. duodenalis* and *T. gondii*, it is presumed that the predominant transmission routes for humans are consumption of contaminated water or foods (Hald et al., 2016; Ma et al., 2022; Robertson, 2016). Felids are the only hosts that shed *T. gondii* oocysts, and humans and animals can excrete *Giardia* cysts in their stool (Gómez-Marín, 2010). Events like direct exposure to soil contaminated with feces from asymptomatic carriers of the infective stage of protozoa can be the origin of contamination of water resources (Dumètre et al., 2012; dos Santos et al., 2010; Taghipour et al., 2022). Also, direct contact of vegetable crops, which are usually consumed raw, with soils and water carrying cysts and/or oocysts of parasitic protozoa (Ismail, 2016; Srikanth and Naik, 2004; Utaaker et al., 2017) through accidental exposure or by using manure as fertilizer can contaminate water and produce (Avazpour et al., 2013). Besides water and food, exposure to contaminated soil also has been identified as an important risk factor for indirect parasitic contamination of humans (Deng et al., 2021; Ferreira et al., 2018).

Soil aridity reduces the viability of protozoa in soil. The contrary occurs with intense rainfall, which increases the time of viability of cysts or oocysts. Environmental conditions thus play an important role in parasite distribution (Deng et al., 2021; Dumètre et al., 2012; Krusor et al., 2015). This viability and infectivity of *Giardia* cysts and *Toxoplasma* oocysts is mediated by the morphology of the cyst/oocyst, which involves a highly resistant wall that allows it to survive adverse weather conditions, as well as disinfection processes (Dumètre et al., 2012; Shapiro et al., 2019a). As such, environmental factors play a decisive role in the appearance of infectious diseases, especially with regards to indirect transmission of pathogenic microorganisms (Blackburn et al., 2019).

Between 2017 and 2020, at least 251 outbreaks were attributed to transmission of parasitic protozoa through water, linking their presence in bodies of water to contamination derived from waste waters, including urban and industrial, as well as livestock and agricultural waste waters (Ma et al., 2022; Wang et al., 2017). Considering the aforementioned, purification of water resources is increasingly complex because of frequent and probable contamination by protozoa transmitted through water, which represents a great challenge for its treatment (Betancourt and Rose, 2004; Triviño-Valencia et al., 2016).

In Colombia, the characteristics of water for human consumption are regulated by Resolution 2115 of 2007 from the Ministry of the Environment, Housing, and Territorial Development, which determines that the acceptable value for *Giardia* spp. in water for human consumption is zero cysts (*Ministerio de Protección Social; Ministerio de Vivienda Ambiente y Desarrollo Territorial*, 2007). Nationally, this protozoon has an estimated prevalence of 29.6% in school-age children (Escuela Nacional de Salud Publica, 20155) and, of 13% in children between 1 and 7 years of age specifically for the department of Quindío (Giraldo-Gómez et al., 2005; Londoño et al., 2009b). Regarding *T. gondii,* there is a high prevalence of 47% in the overall human population at the national level and 60% in the department of Quindío (Cañón-Franco et al., 2014). Although *T. gondii* oocysts have been reported in treated water, with estimates that drinking water-related factors could explain up to 50% of toxoplasmosis infections in Colombia (Gómez-Marín, 2007; López-Castillo et al., 2005; Triviño-Valencia et al., 2016), still no legislation exists to monitor this protozoon in aquatic matrices. No prior data exist on how contamination occurs at the source for drinking water in Colombian cities where human infection occurs. Therefore, our aim was to describe the frequency of *G. duodenalis* and *T. gondii* DNA in soil and water associated with the Quindío River and the frequency of contaminated soil and water from this basin.

2. Materials and methods

2.1. Study area and characterization of human population residing in the area

The study samples were collected in the municipality of Salento, department of Quindío, located on the western flank of the central mountain range, where the Quindío River starts, which is the supply surface tributary for the city of Armenia. The sampling sites are located at an altitude between 1800 and 2400 m above sea level (masl) within Los Nevados National Park. The sampling points were defined considering proximity to to the sampling zone and possible carriers of the protozoa of interest, such as grazing cattle for *Giardia* (Taghipour et al., 2022) and human settlements where domestic cats are present for *Toxoplasma* (Afonso et al., 2008) (Vásquez Palacios et al., 2019). The study area corresponds to 11 points (Table 1) that covers the course of the river basin to the point of

Table 1
Coordinates and altitude in meters above sea level of the sampling points for water and soil selected in this study.

Sampling point	Altitude masl*	Coordinates	
		N	W
001	1713	4° 37′ 41.2″	75° 35′ 42.2″
002	1754	4° 38′ 8.6″	75° 35′ 18.1″
003	1986	4° 38′ 23.9″	75° 32′ 34.4″
004	2290	4° 37′ 57.5″	75° 29′ 51.3‴
005	2292	4° 37′ 56.9″	75° 29′ 50.5″
006	2589	4° 38′ 37.8″	75° 28′ 46.2″
007	2473	4° 38′ 39″	75° 28′ 40″
008	3190	4° 37′ 22.8″	75° 25′ 50.3″
009	2385	4° 37′ 55.1″	75° 28′ 35.7′
010	2385	4° 37′ 57.1″	75° 28′ 34.5″
011	2396	4° 38′ 12.8″	75° 29′ 1.9″

* masl = Meters Above Sea Level.

collection for drinking water for the city. Samples of water and soil were collected between August and November 2019 and February 2020, during a single day of the month; thus, there were five samples for each of the 10 points for water and for each of the 10 points for soil. The soil samples were collected at the nearest land site to the river (1 to 10 m). The distance was dependent on the terrain topography (rocks, inclination, accessibility). One additional point of water collection was a household wastewater discharging directly into the river (point number 009). In total, 55 water samples and 50 soil samples were collected. As part of the characterization of the sampling sites, we performed a survey of the human population residing in the Quindio River basin by using a questionnaire on the sociodemographic characteristics (age, socieconomic strata, level of education) of the people residing in this area.

2.2. Collection of water samples and physicochemical properties

From each point described in Table 1, a 10-L sample was taken, using a sterile plastic bag, for a total of 55 samples. The samples were taken from the center of the brooks, by submerging the container upstream to a depth not >1 m. Upon taking the water samples, measurements were made of physical-chemical variables using a multiparameter equipment model HQ40d (Hach Colombia S.A.S. Bogotá) to measure temperature (°C), pH, conductivity (µS/cm), dissolved oxygen (mg/l), and percentage of oxygen saturation (%). The samples were transported and processed at the Biomedical Research Center, Universidad del Quindío.

2.3. Collection and processing of soil samples and physicochemical properties

The 50 soil samples were collected at a distance of 1 to 10 m from the river. This distance varied depending on the terrain's topography. Prior to collecting each soil sample, pH and soil temperature were measured, and a thermo hygrometer reference 445,815 (Extech Instruments Division, Flir Company, Boston MA, USA) recorded environmental temperature and relative humidity. In each of these locations, 200 g of soil were collected from horizon A. In the points selected, 5 m × 5 m quadrants were made, along with a systematic sampling that consisted in taking five subsamples within each quadrant, corresponding to corners and the center. The samples were transported in a styrofoam cooler at room temperature to the parasitology lab at Universidad del Quindío, where they were dried for 24 h and individually sieved. For the samples collected at the private property, appropriate informed consent was obtained.

2.4. Processing of water samples

Water samples were processed by using our previously published method (Lora-Suarez et al., 2016). This process is based on concentrating 10 L of water to 5 ml by centrifugation, followed by formalin/ether sedimentation for a final volume of 1 ml.

2.5. Validation of a modified method to detect protozoa DNA in soil

To process a greater amount of soil sample to detect protozoa DNA, we modified a method already described (Ferreira et al., 2018). A sample of 200 g of surface soil was sieved, autoclaved for 15 min at 15 PSI, and exposed to UV irradiation at 40 µW/cm^2 for 30 min. The sterilized soil samples were inoculated with 10 oocysts of *T. gondii* and 10 *G. duodenalis* cysts, as enumerated by the USEPA 1623 method U.S. Environmental Protection Agency (U.S. EPA) Office of Water, 2012. Soil samples were spiked separately for each protozoon, and these samples were incubated at room temperature for 24 h. *G. duodenalis* cysts from human stools were donated by Dr. Fidel Angel Nuñez from Instituto Pedro Kouri in Havana, Cuba, and the *T. gondii* oocysts of the ME49 strain (clonal type II) collected from cats in June 2014 were donated by Dr. Dubey (USDA, Beltsville). The samples were dried at 37 °C for 24 h, mixed by manual shaking and 25 g samples were collected in BagFilter 400 P bag for analysis. Then, 75 mL of 1 M glycine were added to each sample and submitted to mechanical stirring for 30 min at 100 x g. Following a 5 min undisturbed period the supernatant was poured into 50-mL conical tubes and centrifuged 1500 x*g* for 15 min, and the sediment processed by the formalin-ether technique as described (Lora-Suarez et al., 2016). The assay was conducted in triplicate and with a negative control using the inoculation.

2.6. DNA extraction of Giardia cysts and Toxoplasma oocysts

For DNA extraction, 300 µl of the concentrated samples was combined with 600 µl of DNAzol, 10 µl isoamyl alcohol, and 0.3 g of 1-mm zirconium beads (BioSpec product Inc., USA) and homogenized in a mini-bead beater (Stratech UK) for 1 min then placed in ice for 1 min as previously described (Triviño-Valencia et al., 2016). Additionally, concentrations of bovine serum albumin (BSA) at 200 µg/µl, 400 µg/µl, 600 µg/µl, and 800 µg/µl were evaluated in triplicate to determine optimal PCR amplification conditions of the sample matrix.

2.7. PCR of the B1 gene for Toxoplasma gondii and of the gdh gene for Giardia duodenalis

To detect DNA from *T. gondii*, a nested PCR was performed as described (Luna et al., 2019; Triviño-Valencia et al., 2016). The first amplification used oligonucleotides (Toxo N1) 5′-GGAACTGCATCCGTTCATGAG-3′ and (Toxo C1) 5′-TCTTTAAAGCGTTCGTGGTC-3′. The nested PCR used the oligonucleotides (Toxo N2) 5′-TGCATAGGTTGCCAGTCACTG-3′ and (Toxo C2) 5′-GGCGACC AATCTGC-GAATACACC-3′.

Conventional PCR was performed to detect the glutamate dehydrogenase (gdh) gene from assemblages A and B of *G. duodenalis*,

with the GoTaq Green Master Mix kit, following manufacturer's recommendations. Oligonucleotide sequences were used, as described (Read et al., 2004): for Assemblage A, forward 5′-CCGCGAGATCGGGTACCTGTA-3′ and reverse 5′-GCCGGAGAC AGAGACACCG-3′ (637 bp) and for Assemblage B, forward 5′-ATCCTT AAGTTCCTCGGC-3′ and reverse 5′-ATCGGTTATCTGTTTGGAC-3′ (232 bp). The products were visualized in agarose gel at 1.5% by running 30 min at 100 V.

Amplifications were made in Veriti 96-well Thermocycler (Applied Biosystems, Waltham, MASS, USA). During each PCR assay, a negative control of the reaction was available that consisted in adding all the reagents, except for the DNA and a positive control that contained DNA from the parasite.

2.8. Genetic sequencing and clustering

The PCR products were sequenced in ABI3730XL DNA sequencer with the same PCR primers by using the standard Sanger sequencing service by Psomagen (Rockville, MD 20850). We removed reads containing >10% 'N's and with >50% bases having low quality value (Phred score ≤ 5). The *T. gondii* and *G. duodenalis* sequences were analyzed in BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and aligned with the MUSCLE algorithm, in the "Molecular Evolutionary Genetics Analysis" (MEGA X) software (available at: http://www.megasoftware.net/). The evolutionary history of *T. gondii* was inferred by using the UPGMA method; the calculations were made using the Kimura 2-parameter model, employing 2000 replicas. For *G. duodenalis*, the nearest-neighbor method and the Kimura 2-parameter model were used with 2000 replicas. To construct the phylogenetic tree, sequences reported in the GenBank for the three types of *T. gondii* strains were used as reference strains: RH strain for type I (KC607827.1), ME49 strain for type II (XM_002370240.2), and VEG strain for type III (LN714499.1). As an outgroup, the sequence homologous to B1 from *Neospora caninum* (AY941177.1) was used.

2.9. Statistical analysis

A data matrix was constructed where the response variable was the presence/absence of the parasite. The explanatory variables were the environmental variables registered in each of the sampling points. Associations among the variables were made through logistic regression in the Statgraphics Centurion XVII program. Values below $p < 0.05$ were considered statistically significant.

3. Results

3.1. Characterization of human population residing in the analysis zone

From a population of 82 people in this zone, 54 responded to the questionnaire: 28 were women (51.8%) and 26 were men, with a mean age of 40.4 ± 0.8 and 42.1 ± 0.8 years, respectively. Of the respondents, 46% had completed secondary education and only 2% had not finished primary school; 58% were wage-earning agricultural workers, 35% were farmers with their own farms, and 7% were retired. Moreover, 14% had lived for over 20 years in the site and 34% for less than one year; 48% of the people were home owners; and all had basic public services (water, sewage, power). This zone is used for livestock grazing of bovine cattle and tourism (Granada, 2019).

3.2. Validation of the modification of the method to detect protozoa DNA in soil

For *T. gondii*, a band was obtained at the expected weight (97 bp), employing a concentration of 600 ng/μl of BSA. For *G. duodenalis*, inoculation of 10 cysts in 25 g of soil was performed, and a band of 232 bp was obtained, corresponding to Assemblage B, only when using 800 ng/μl of BSA. These conditions were then used for the molecular assays with the field samples.

3.3. PCR amplification of the B1 gene for toxoplasma gondii and the gdh gene for Giardia duodenalis

The study collected 105 samples from August 2019 to February 2020, comprised of 50 soil samples and 55 water samples: 18.1% (19/105) were positive for detection of DNA from *T. gondii* and 32.3% (34/105) for DNA from *G. duodenalis*. *T. gondii* was detected more often in soil samples than in water samples, and for *Giardia* most of the positive samples were found in water. Of the 50 soil samples analyzed, 28% (14/50) were positive for *T. gondii* and 16% (8/50) for *G. duodenalis* (Table 2).

In *Giardia* Assemblage specific PCR, 21.9% (23/105) were *G. duodenalis* Assemblage A and 10.5% (11/105) *G. duodenalis* Assemblage B. The study detected both protozoa, *T. gondii* and *G. duodenalis,* Assemblage B at the same time in 2% (1/50) of the soil

Table 2

PCR-positive samples for *Toxoplasma gondii* and *Giardia duodenalis* in soil and water samples collected during the five sampling months.

Type of sample	Toxoplasma gondii		Giardia assemblage A		Giardia assemblage B	
	N°	%	N°	%	N°	%
Soil	14/50	28	4/50	8	4/50	8
Water	5/55	9,1	19/55	34,5	7/55	12,7

samples, corresponding to point 007 in September. In water samples, *T. gondii* was detected in 9.1% (5/55), *Giardia* Assemblage A in 34.5% (19/55), and *Giardia* Assemblage B in 12.7% (7/55) (Table 2). In some months, both protozoa and both Assemblages were found in the same sample. In 5.4% (3/55) of the samples, *T. gondii* and *Giardia* Assemblage A were found simultaneously, and in 3.6% (2/55) of samples, DNA from *Giardia* Assemblages A and B were detected, corresponding to September for point 009 and for February for point 006.

3.4. Phylogenetic analysis of DNA sequences from soil and water samples of Giardia duodenalis and Toxoplasma gondii

We sequenced 16 samples of the 19 PCR *T. gondii* positive samples. The phylogenetic analysis indicated that two soil samples and the positive controls were the RH type I strain; likewise, two additional samples of soil were the VEG type III strain. However, most of the samples did not correspond with the sequences from reference strains used in this study (Fig. 1).

For *G. duodenalis*, sequencing was obtained in only six of 23 samples from Assemblage A, five from water, and one from soil (Fig. 2). Sequencing was not possible for any of 11 samples from Assemblage B. *G. duodenalis* sequences found in three water samples belonged to Assemblage AI (010A10, 010A09, and 009A09), corresponding to points 009 and 010 for September and point 010 for October. Likewise, three samples were grouped into Assemblage AII, two from water and one from soil. The first samples correspond to point 008 for August and to point 006 for February; regarding soil, it belongs to point 004 for November.

3.5. Seasonal variation of T. gondii and G. duodenalis in soil and water

In soil, the month in which the highest number of *T. gondii* positive samples were detected was November (Fig. 3) with 50% (5/10), except for points 002 and 011, with no detection of *T. gondii* DNA, although in the other points it was found at least once. Most of the positive samples for this protozoon were in point 007, followed by points 003, 006, 008, and 010 (Fig. 4). In water samples taken from the Quindío River, DNA from *T. gondii* was detected in five sampling points (001, 002, 004, 008, and 011) of the 11 points, with a positive sample for each. The month with the greatest number of positive PCR was August, with 27.2% (3/11), and there was no DNA
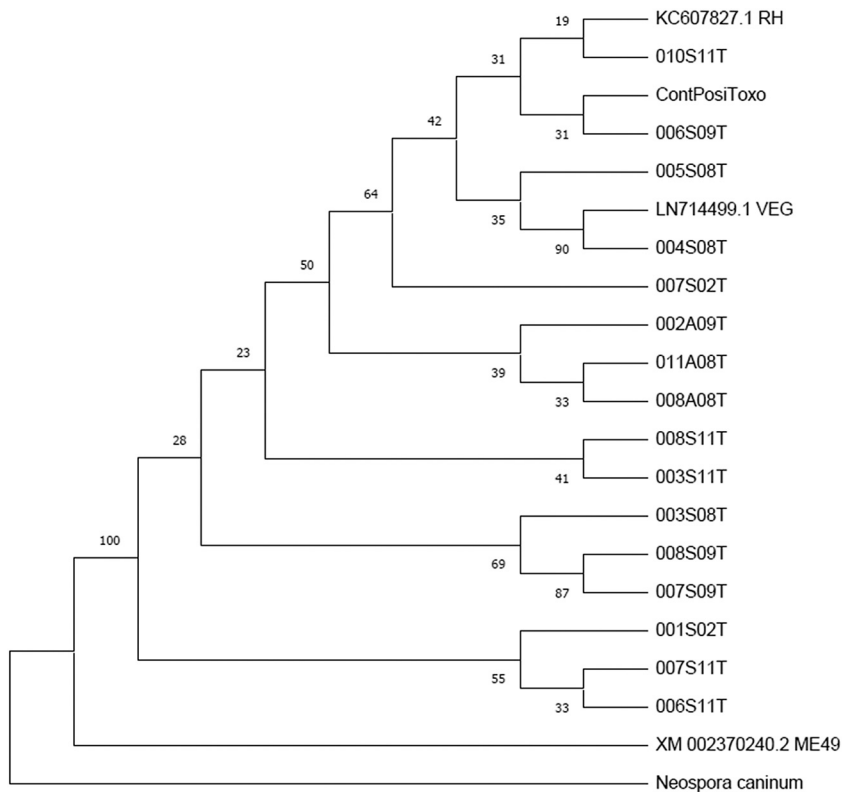

Fig. 1. Bootstrap consensus tree inferred by the UPGMA method with 2000 replicates. Analysis of the sequences of the B1 gene of *Toxoplasma gondii* sequences obtained from water and soil samples. Reference sequences obtained from GenBank were: KC607827.1 (clonal group type I, RH strain); XM 002370240 (clonal type II, ME49 strain), and LN/114499.1 (Clonal group type III, VEG strain). The sequence from the reference strain cultured at the laboratory was from the RH strain (ContPosiToxo). The outgroup sequence was from B1 homologous of *Neospora caninum* (GenBank: AY941177.1). Water sequences include the letter A within its code (example: 002A09T) and soil sequence the S within name (example: 0120S11T). There were 70 positions in the final dataset. Evolutionary analyses were conducted in MEGA X software (available at: http://www.megasoftware. net/). Values at each interior branch of the original UPGMA tree are the BCL (bootstrap confidence level).
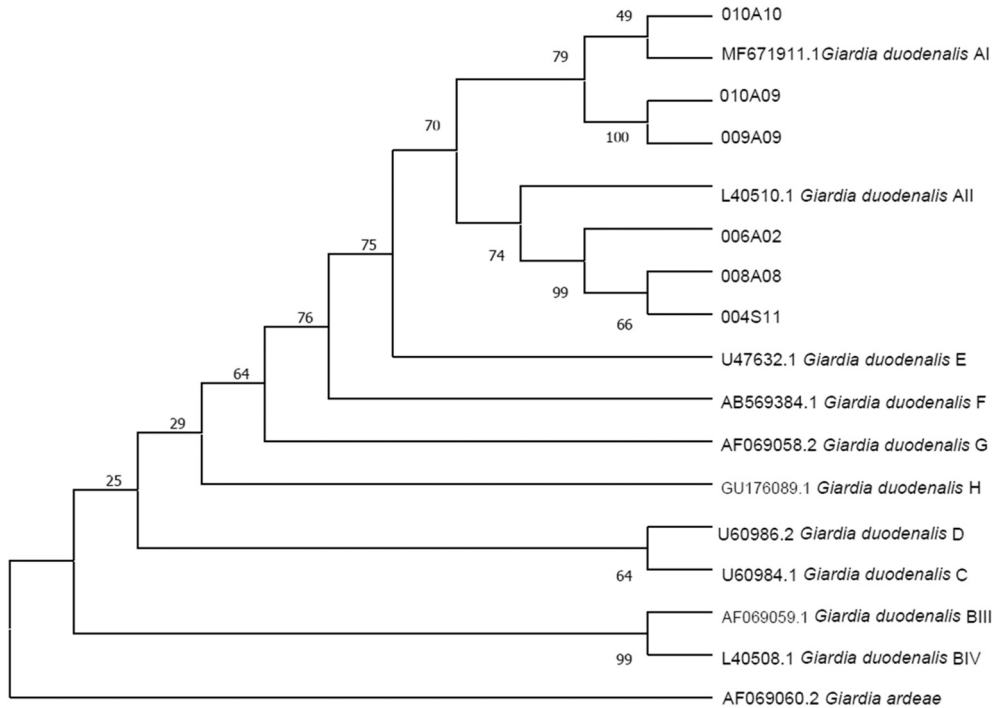
Fig. 2. Bootstrap consensus tree inferred by Neighbor Joining method with 2000 replicates. Analysis of the sequences of the *gdh* gene of *Giardia duodenalis* sequences obtained from water and soil by using the nearest-neighbor method. Reference sequences were obtained from GenBank from *G. duodenalis* assemblages AI, AII, BIII, BIV, C, D, E, F, G and H. The outgroup sequence was from *gdh* gene homologous of *Giardia ardeae*. Water sequences include the letter A within its code (example: 010A10) and soil sequence the S within name (example: 004S11). There were 609 positions in the final dataset. Evolutionary analyses were conducted in MEGA X software (available at: http://www.megasoftware.net/). Values at each interior branch of the original UPGMA tree are the BCL (bootstrap confidence level).
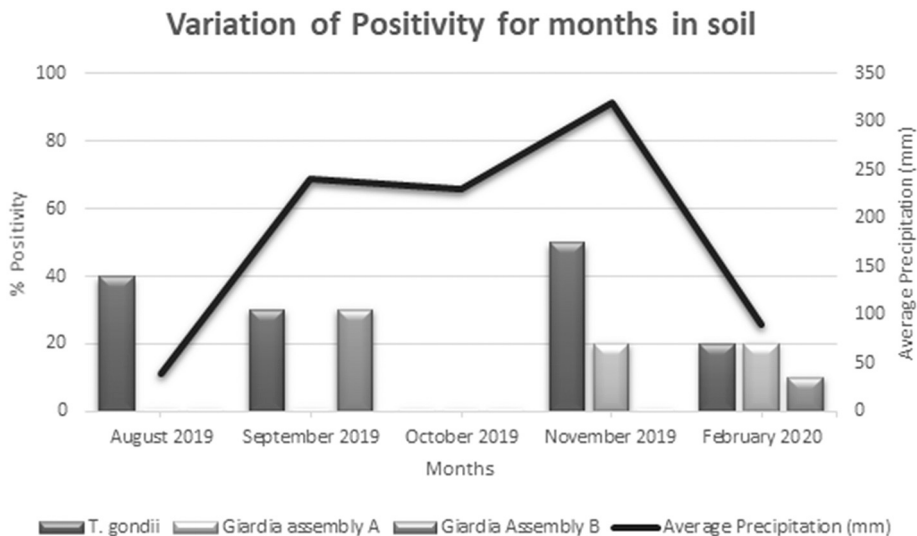

Fig. 3. Percentage of positivity for *Toxoplasma* and *Giardia* Assemblages A and B during the sampling months in relation to the average precipitation of each month in soil.

detected in October 2019 and February 2020 (Fig. 5).

Assemblages A or B of *G. duodenalis* were not detected in soil samples during August and October. For *G. duodenalis*, Assemblage A, November and February had the most positive samples obtained in soil, with 20% each. During these months, the points where this Assemblage was detected were 001, 003, 004, and 005.
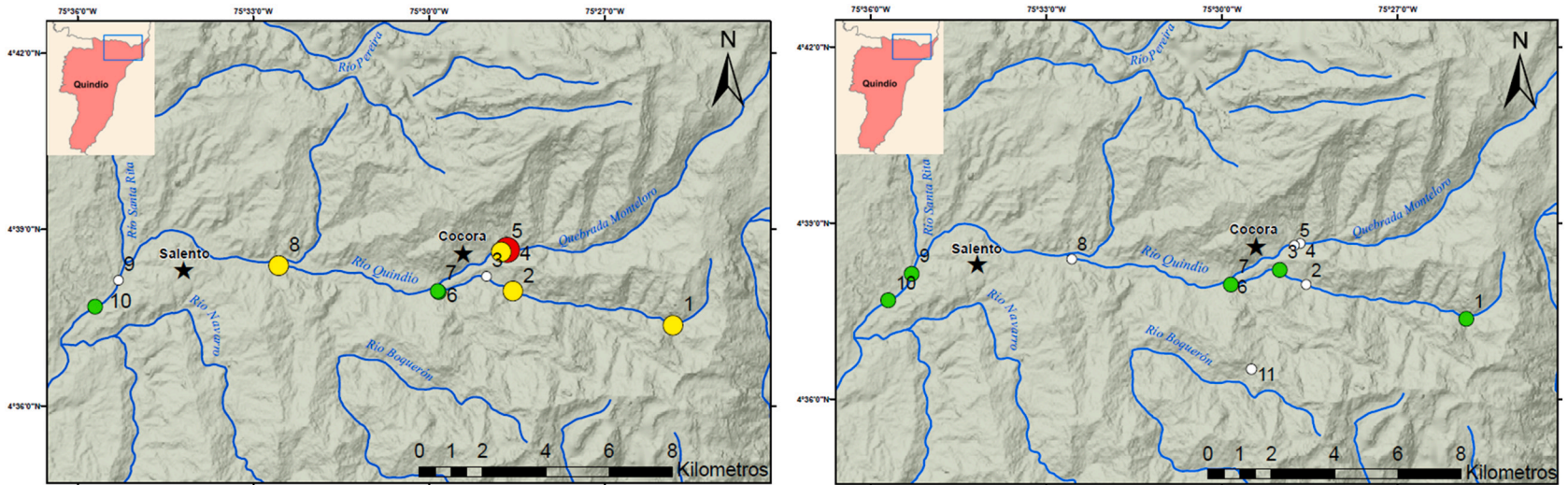
Fig. 4. Map of the Quindio River basin, indicating the percentage of presence through PCR of *Toxoplasma gondii* in **(A)** soil and **(B)** water samples collected between August 2019 and February 2020. The numbers correspond to sampling points, the red color represents >60% positivity, yellow from 40% to 59%, green from 20% to 39%, and white from 0% to 19%. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
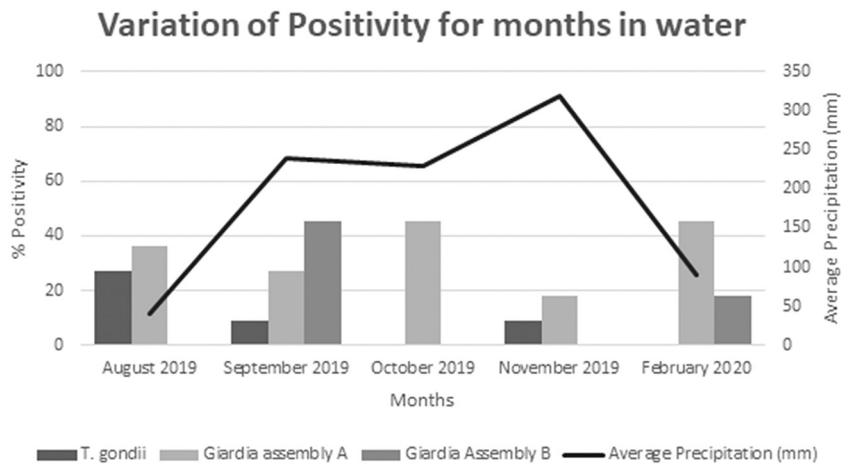
Variation of Positivity for months in water

Fig. 5. Percentage of positivity for *Toxoplasma* and *Giardia* assemblages A and B in relation to the average precipitation of each month in water.

For the *G. duodenalis* Assemblage A water samples, DNA was found at least once in the 11 sampling points, with point 010 reporting a higher number of positive samples (Fig. 6). During October and February, 45.4% (5/55) of the samples were found positive for the DNA in this assemblage.

Giardia Assemblage B was only detected in four (002, 006, 007, and 011) of the 10 points studied in soil (Fig. 7). The month with the highest number of positive samples was September with 30%. Also, DNA was detected from *G. duodenalis* Assemblage B in water in seven sampling points (001, 002, 003, 004, 006, 007, and 009). In each of these points, this assemblage was found once, with September being the most representative month with 45.4% (5/55).

3.6. Concurrence of T. gondii and G. duodenalis in soil and water

An analysis was performed to test for any association between the detection of each protozoa in soil and water, considering that the point taken from the Quindío River basin was next to the site where the soil sample was collected. The presence in specific water points was not associated with the simultaneous presence of *T. gondii* in nearby soil (X^2 test 0.176 $p = 0.6745$) or *G. duodenalis* Assemblage A (X^2 test 0.157; $p = 0.6920$), contrary to that occurring with Assemblage B (X^2 test 5.945; $p = 0.0148$), where the association was statistically significant. Thus, it may be inferred that for this Assemblage B, that which is observed in water for a particular case is related to that found in soil.

3.7. Association between the presence of T. gondii and G. duodenalis in soil and water with physical or chemical variables

A logistic regression analysis was performed to test if association existed between the presence of *T. gondii* or *G. duodenalis* with the four physical or chemical variables of soil (Table 3). Only one significant association was found for the presence of *T. gondii* DNA with soil temperature ($p = 0.037$), with a mean temperature of 19.8 °C for the soil with positive samples (range 12–25) and 22.3 °C for the negative samples (range 14.5–27). Similarly, in water, there were no physical or chemical variables that served as significant predictors of presence of *T. gondii* or *G. duodenalis* Assemblages A and B (Table 4).

4. Discussion

This work is the first to study the simultaneous occurence of *Giardia* and *Toxoplasma*, two protozoan pathogens in the source of the drinking water for a city where human infection of both protozoa is related to water consumption (Arias et al., 2010; Giraldo-Gómez et al., 2005; Gómez-Marín et al., 2021; Londoño et al., 2009a; Lora-Suarez et al., 2002). While *Giardia* and *Toxoplasma* DNA presence are described in the present study, *Cryptosporidium sp* will be a matter for our next report, which will include correlation with stools from cattle. *Toxoplasma, Giardia,* and *Cryptosporidium* are the most frequent protozoa in water samples (Triviño-Valencia et al., 2016), all of which significantly impact the human population in the Quindío region (Luna et al., 2019; Muñoz-Sánchez et al., 2019).

It is important to highlight that soil is increasingly recognized as an important source of transmission of *T. gondii* (Afonso et al., 2008; Escotte-Binet et al., 2019) and *G. duodenalis* (Balderrama-Carmona et al., 2014; Lee et al., 2021; Omeragić et al., 2021; Paller et al., 2022). However, detecting the presence and estimating the load of *T. gondii* in soil has been difficult given limitations in the availability of detection methods (Shapiro et al., 2019a, 2019b). Hence, a rapid, low-cost, and sensitive method to detect cysts and oocysts in soil samples is needed, even if they are present at low densities and with inhibitors that impede DNA detection (Escotte-Binet et al., 2019; Yan et al., 2016). Environmental samples generally have low amounts of target DNA and contain high amounts of PCR inhibitors, compared with other types of samples, like crop isolates (Hamilton et al., 2018; Thurston-Enriquez et al., 2002). Nevertheless, the effect of PCR inhibitors could be attenuated significantly by adding BSA (Plutzer et al., 2008), which increases the stability
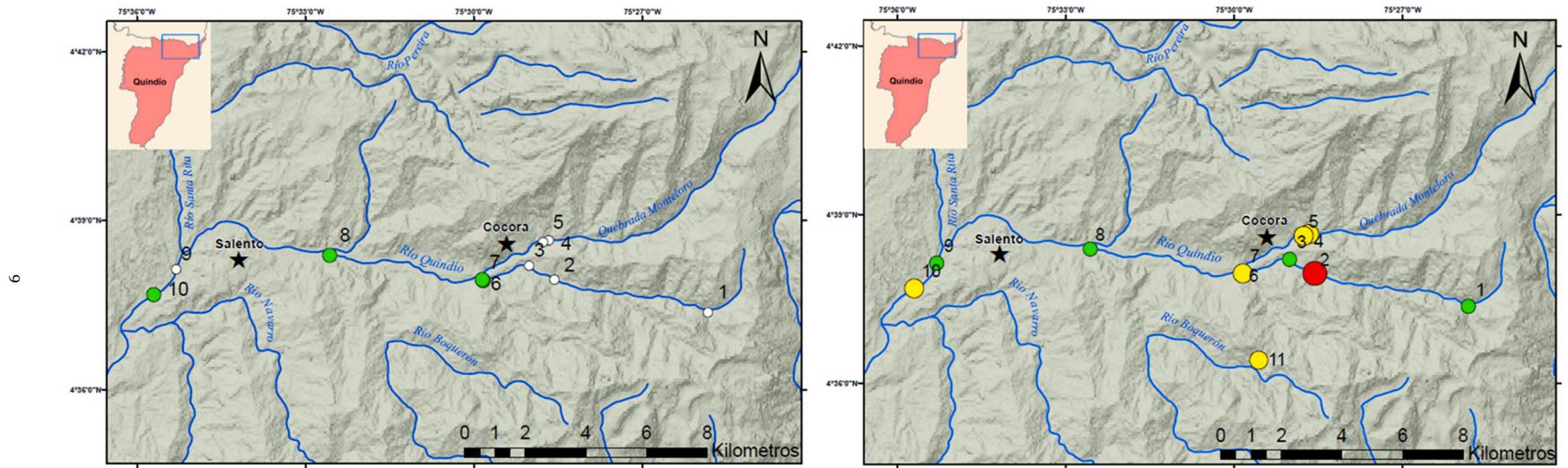
Fig. 6. Map of the Quindio River basin, indicating the percentage of presence through PCR of *Giardia duodenalis* assemblage A in **(A)** soil and **(B)** water samples collected between August 2019 and February 2020. The numbers correspond to sampling points, the red color represents >60% positivity, yellow from 40% to 59%, green from 20% to 39%, and white from 0% to 19%. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Fig. 7. Map of the Quindio River basin, indicating the percentage of presence through PCR of *Giardia duodenalis* assemblage B in **(A)** soil and **(B)** water collected between August 2019 and February 2020. The numbers correspond to sampling points, the green color represents from 20% to 39% positivity and white from 0% to 19%. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
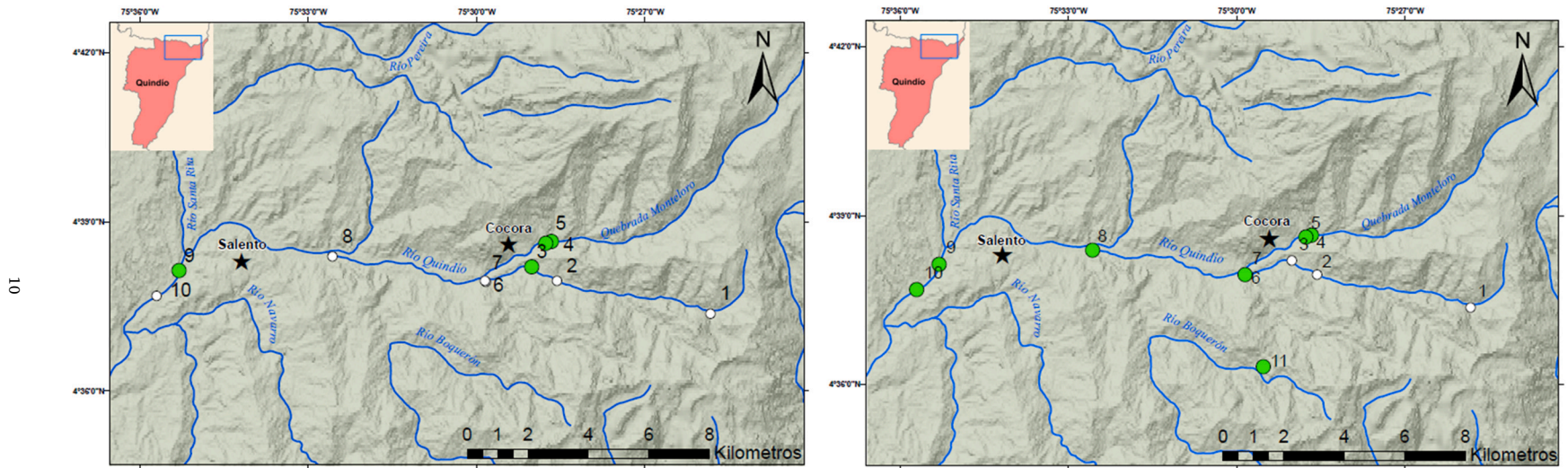
Table 3

Logistic regression analysis to assess physical-chemical variables of the soil associated with PCR positivity for *T. gondii* and *G. duodenalis* assemblages A and B.

Physical-chemical variables	Mean	Range	*T. gondii*	*G. duodenalis* assemblage A	*G. duodenalis* assemblage B
			p value	p value	p value
Soil temperature °C	21.6	(12–27.5)	0.037*	0.1365	0.2497
Ambient temperature °C	18.4	(10–28.1)	0.281	0.2835	0.2893
relative humidity %	73.7	(38–98)	0.062	0.4034	0.6472
pH	7.8	(7–8)	0.931	0.3837	0.3458

* Variable associated in statistically significant manner.

Table 4

Logistic regression analysis to assess physical-chemical variables of the water associated with PCR positivity for *T. gondii* and *G. duodenalis* assemblages A and B.

Physical-chemical variables	Median	Range	*T. gondii*	*G. duodenalis* assemblage A	*G. duodenalis* assemblage B
			p value	p value	p value
pH	7.4	(6.24–8.67)	0.068	0.5572	0.4985
Conductivity µS/cm	89.25	(9.92–165)	0.259	0.9331	0.4777
Mg/l oxygen	7.2	(5.34–11.2)	0.939	0.8455	0.5265
% Saturation	90.7	(59.8–106)	0.719	0.6564	0.6191
Temperature °C	13.2	(9.1–17.5)	0.100	0.6996	0.9375

of the polymerase enzyme activity (Strien et al., 2013) and its half-life in the reactions (Farell and Alexandre, 2012).

Documenting the degree of soil contamination with *T. gondii* oocysts and *G. dudodenalis* cysts is fundamental to propose efficacious measures to prevent toxoplasmosis and giardiasis (Slifko et al., 2000; Escotte-Binet et al., 2019; Dumètre et al., 2012; Lee et al., 2021; Paller et al., 2022). Molecular assays are currently the most sensitive and effective methods to detect *T. gondii* and *G. dudoenalis* in soil (Orlofsky et al., 2013; Shapiro et al., 2019a; Slifko et al., 2000).

The present study found that 28% of the soil samples had *T. gondii* DNA, similar to that reported in Brazil with 20% (Ferreira et al., 2018), France with 29.2% (Lélu et al., 2012), and China in urban areas with 30.4% (Gao et al., 2016). Nevertheless, variations exist according to land use, and another study in China found frequencies ranging between 4% and 16% (Cong et al., 2020). Presence of this protozoon in soil had never been reported in Colombia; however, a study in the region of the Quindío River basin in free-range chicken (a good biological monitor for soil contamination) found 44.4% positivity for anti-*Toxoplasma* antibodies ((Dubey et al., 2005).

Various studies have reported the presence of *G. duodenalis* in soil. Mexico reported 57% in a study based only on microscopy (Balderrama-Carmona et al., 2014). Similarly, a microscopy study conducted in Spain detected *G. duodenalis* cysts in 4.5% of soil samples (Dado et al., 2012). In Brazil, a 20% rate was detected by PCR but did not report the assemblages (Ferreira et al., 2018). In Colombia, another study reported *Giardia* in 20.8% of soil samples, employing conventional microscopy (Villafañe Ferrer and Pinilla Pérez, 2016).

Although PCR does not reveal infectivity, it is a good indicator to estimate the exposure of water and soil to pathogenic protozoa (Rousseau et al., 2018; Shapiro et al., 2019a). Various studies have documented the presence of *Giardia* in surface waters, such as reports from China in a recreational lake that indicated *Giardia* Assemblage A and Assemblage B at 5.7% and 1.9%, respectively (Xiao et al., 2017); very low prevalence similar to that reported in Serbia with 3.2% for Assemblage A and 6.4% for Assemblage B (Ćirković et al., 2020). A similar study in Quindío (Triviño-Valencia et al., 2016) reported *Giardia* Assemblage A at 7.7% compared with the 34.5% found in this research. The earlier study reported 38.4% for Assemblage B, a higher prevalence compared with that detected in this study at 12.7%. This is similar for *T. gondii,* which was reported at a high frequency of 76.9% in surface water samples upstream from a treatment plant (Triviño-Valencia et al., 2016), compared with the 9.1% reported in the present study. The difference of results from the same region (by the same laboratory and by using the same methods) suggests that occurrence of the protozoa in water is dynamic and the changing situation merits continuous monitoring and analysis to identify factors contributing to these changes in frequency.

Identification of specific genotypes is crucial for identifying the zoonotic or anthropic sources of infection. For example, sub-Assemblage AI is mainly found in animals and sub-Assemblage AII in humans. By applying genotyping methods, active transmission pathways may be revealed in different areas (Capewell et al., 2021; Hopkins et al., 1997; Lebbad et al., 2011; Taghipour et al., 2022).

Our study results of *T. gondii* in water samples agree with those reported in Pakistan, which revealed 7% positive samples (Khan et al., 2013), 12.5% in Bulgaria (Kourenti and Karanis, 2006), and 13% in Norway with real-time PCR (Harito et al., 2017). In contrast, studies conducted in Greece (Kourenti and Karanis, 2006) and Nariño, Colombia (Sanchez et al., 2014) yielded no positive results for *T. gondii*.

This study is the first to demonstrate a significant association of the presence of Assemblage B of *Giardia* in soil with its presence in a

nearby body of water. Additional studies are needed to relate the presence of different protozoa in these two environments because sampling river water to detect protozoa is complicated by the attachment of the oocyst and cyst to fecal or soil particles before entering rivers (Dumètre et al., 2012). Binding of protozoa cysts or oocysts to organic particles causes a rapid precipitation of parasites in the sediments of water, decreasing the freely suspended parasites available in water samples (Ferguson et al., 2003). This can explain why no association was found in the frequency of *T. gondii* in soil with the nearby water sample (Shapiro et al., 2019a; VanWormer et al., 2016).

By using the sequencing analysis of B1 amplicons, it was found that two *T. gondii* samples showed similarity with type I and two with type III strains, but most of the sequences did not correspond to any of the three types, suggesting that the remaining isolates had recombinant or atypical genotypes. This is similar to previous findings using multilocus or single-locus analysis in this region in human (de-la-Torre et al., 2013; Gallego et al., 2006), animal (Dubey et al., 2005), or environmental samples (Triviño-Valencia et al., 2016). Although B1 is not the best locus for high-resolution molecular typing, it is useful when there is not enough DNA to perform a multilocus analysis (Costa et al., 2013). In the present study *G. duodenalis* assemblages displayed similarity with both sub-assemblages AI and AII, which have zoonotic potential (Cai et al., 2021; Taghipour et al., 2022). This assemblage has been found in humans and in non-human primates, domestic and wild ruminants, alpacas, pigs, horses, domestic and wild canines, cats, ferrets, rodents, marsupials, and other mammals (Cai et al., 2021; Sarria-Guzmán et al., 2022; Taghipour et al., 2022; Zajaczkowski et al., 2021). Sub-assemblage AI has been reported predominantly in livestock and pets, and sub-assemblage AII in humans (Colli et al., 2015; Lebbad et al., 2011; Taghipour et al., 2022). The great diversity of hosts means that this assemblage can be found more commonly in the study zone due to the presence of livestock and farms near to the basin (CRQ, 2015; Granada, 2019).

With regards to the physical and chemical variables of the sampled soil, a significant relation was found with soil temperature. Similar to our findings, other studies have reported that the viability and persistence of the *T. gondii* oocysts in soil can be influenced by environmental factors, such as humidity and temperature (Afonso et al., 2013; Shapiro et al., 2019a). Humidity and high precipitation rates have also been classified as critical parameters for infectivity of *T. gondii* oocysts (Afonso et al., 2009; Frenkel et al., 1975; Gómez-Marín et al., 2011; Gotteland et al., 2014), which can remain infectious for months or years, especially in humid environments, shade, and regulated temperature environments (Frenkel et al., 1975).

Although no significant variables in water were found to be associated with *T. gondii* detection, a previous PCR study for *Giardia* spp. and *Cryptosporidium parvum* in the region found that pH was strongly correlated with positivity, and temperature was associated with the detection of *Cryptosporidium* spp. (Triviño-Valencia et al., 2016). Further studies should be conducted to determine the conditions that can have a strong association with presence of pathogenic protozoa in this region. Also, it is important to note that this region is the habitat for wild felids, such as *Leopardus tigrinus*, *Puma concolor*, and *Herpailurus yagouaroundi* (Vásquez Palacios et al., 2019).

Little information is available on the levels of protozoa present in water sources according to temporal and climate variations. The Latin American region is subject to alternating rain and dry seasons; therefore, research is needed to delve into this topic (Bonilla-Aldana et al., 2019; Yan et al., 2016). Surveillance programs should be established to determine the presence of cysts and oocysts of *Toxoplasma*, *Giardia*, *Cryptosporidium*, and *Cyclospora* in water and soil (Shapiro et al., 2019a). This monitoring is critical at the present time, given the impact of climate change on the increase and re-emergence of these and other parasitic infections in this region and others around the world where fresh produce can be contaminated (Gomez-Marin et al., 2022; Gómez-Marín et al., 2020; Rodriguez-Morales et al., 2021). Recently, we described presence of *Giardia* Assemblage B and Assemblage E in fresh produce (Muñoz-Sánchez et al., 2019). It is estimated that approximately 15% (95% UI 7–27%) of *Giardia* infections are transmitted via contaminated food (Foodborne Disease Burden Epidemiology Reference Group 2007–2015, 2015). Therefore, the monitoring of presence of *Giardia* sub-assemblages in the sites of culture for fresh produce should be incorporated.

In conclusion, this work established that the modified protocol to recover oocysts and cysts in soil, as well as the amplification protocol used, have been effective for detecting the presence of *T. gondii* DNA in 28% of soil samples and in 9% of water samples, and *G. dudoenalis* in 43% of water samples and in 16% of soil samples. An association was identified between low soil temperature and presence of *T. gondii* DNA in soil samples, as well as between presence of *Giardia* in soil and positivity in water in nearby points.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

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