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Groundwater contamination by *Cryptosporidium* oocysts from animal faeces in the city of Les Cayes, Haiti

Ketty Balthazard-Accou*[1), 2), 3)], Evens Emmanuel*[1), 2), 4)],
Max François Millien[4), 5)], Bénédique Paul[3), 6), 7)]

1) Université Quisqueya, Laboratoire de Qualité de l'Eau et de l'Environnement (LAQUE),
218 Jean Paul II Ave, Haut de Turgeau, HT6113, Port-au-Prince, Haiti

2) Université Quisqueya, Association Haïtienne Femmes, Science et Technologie (AHFST),
218 Jean Paul II Ave, Haut de Turgeau, HT6113, Port-au-Prince, Haiti

3) Université des Antilles, UR 6-1 AIHP-GEODE Caraïbe, HT6113, Port-au-Prince, Pôle, Haiti

4) Université Quisqueya, Espace Universitaire One Health, 218 Jean Paul II Ave, Haut de Turgeau, HT6113, Port-au-Prince, Haiti

5) Université Quisqueya, Laboratoire de Recherche sur les Zoonoses et Intoxications
Alimentaires (LAREZIA), 218 Jean Paul II Ave, Haut de Turgeau, HT6113, Port-au-Prince, Haiti

6) Université Quisqueya, CREGED, 218 Jean Paul II Ave, Haut de Turgeau, HT6113, Port-au-Prince, Haiti

7) Université Quisqueya, AgroUniQ Lab, 218 Jean Paul II Ave, Haut de Turgeau, HT6113, Port-au-Prince, Haiti

* Corresponding author

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Abstract: The vast majority of large Haitian urban areas are coastal cities and draw their water from groundwater. Located downstream from their watersheds, these aquifers are receptacles for multiple downward materials, including oocysts, which threaten the One Health preservation. Cryptosporidiosis infection from livestock is one of the most important health issues. This study investigates the environmental contamination risk posed by *Cryptosporidium* oocysts from animal faeces in Les Cayes, Haiti, with a particular focus on water resources quality. A total of 129 stool samples from six animal species were collected, with *Cryptosporidium* coproantigens detected in 27 samples, supporting previous findings from the same locations. Groundwater samples from wells and boreholes also showed significant concentrations of *Cryptosporidium* oocysts. To better understand oocyst transmission from soil to groundwater, soil samples were analysed for granulometric properties, physico-chemical characteristics, and percolation behaviour. No oocysts were detected in leachates from percolation tests. This may be explained by the soil's high content of fine particles, which likely interact with and retain oocysts. Environmental transmission was assessed through a tripartite analysis of water, soil, and seasonal climatic factors. These findings contribute to understanding *Cryptosporidium* transmission pathways and offer a foundation for environmental risk assessment and mitigation strategies in the perspective of a One Health approach.

Keywords: animal faeces, cattle, *Cryptosporidium* oocysts, Haiti, soil properties, transmission, water contamination

INTRODUCTION

Cryptosporidium is a globally distributed enteric protozoan infecting humans, domestic animals, and wildlife (Mensah *et al.*, 2023). It causes cryptosporidiosis, typically asymptomatic or mild (Ryan, Fayer and Xiao, 2014; Liu *et al.*, 2019), but potentially severe in immunocompromised individuals (Webb Jr, 2019), and is a major cause of diarrhoea-related death in children under five (Khalil *et al.*, 2018). Treatment options are limited, no vaccine exists (Prabakaran *et al.*, 2023), and preventive measures are scarce (Morris *et al.*, 2019). Transmission occurs via the faecal-oral route through direct contact or ingestion of contaminated food or water with an infectious dose as low as 10 oocysts (Gharpure, 2019; Hatam-Nahavandi *et al.*, 2019). The disease is zoonotic, with *Cryptosporidium parvum* being the primary species of concern (Johnson *et al.*, 1999; Walter *et al.*, 2021), causing outbreaks linked to livestock and child-care settings (Gharpure, 2019). Animals, especially calves, serve as significant reservoirs (Graaf de *et al.*, 1999; Damiani *et al.*, 2013), and children involved in herding increase exposure risk (Qi *et al.*, 2015). The species of *C. parvum* has also been detected in yaks in China (Nydam *et al.*, 2001), while the zoonotic role of sheep and goats remains debated (Ryan *et al.*, 2005).

Oocysts contaminate water bodies via runoff and wastewater discharge (Liu *et al.*, 2019), and are found in recreational waters, groundwater, and drinking supplies (Liu *et al.*, 2019; Chique *et al.*, 2020), posing a global health threat (Bourli *et al.*, 2023), with over 1,000 cases reported in around 100 countries. In Haiti, oocysts have been detected in surface and drinking water in Port-au-Prince (Brasseur *et al.*, 2011), and in groundwater in Cap-Haïtien ($(741–6088)\cdot(100\ \mathrm{dm}^3)^{-1}$) (Balthazard-Accou *et al.*, 2009; Balthazard-Accou *et al.*, 2010; Balthazard-Accou, 2011) and Les Cayes ($(5–100)\cdot(100\ \mathrm{dm}^3)^{-1}$) (Balthazard-Accou *et al.*, 2020), indicating faecal contamination. A single oocyst can cause infection (Messner and Berger, 2016). Their persistence and chlorine resistance hinder control (Ramo *et al.*, 2017). Oocysts also affect soils (Nag *et al.*, 2020) and aquaculture (Koinari *et al.*, 2013), and their exact environmental transport pathways remain unclear (Innes *et al.*, 2020). Runoff during rainfall is a likely vector (Brankston *et al.*, 2018), though hydrodynamic processes are also implicated. Environmental factors such as temperature, rainfall, humidity, and solar radiation impact oocyst survival and disease spread (Wang *et al.*, 2021), with incidence peaking in warmer months in temperate climates. This study aims to improve understanding of the transport and fate of *Cryptosporidium* oocysts in soil and groundwater systems, providing a foundation for risk assessment and mitigation strategies.

MATERIALS AND METHODS

STUDY AREA

The study was carried out in Les Cayes, a major urban centre in southern Haiti (18°34'00"N, 72°21'00"W) with an estimated population of about 150,000 inhabitants (IHSI, 2009). The city lies on a coastal plain with high annual rainfall (>2000 mm), average temperatures between 24°C and 28°C, and a climate marked by two distinct seasons – rainy and dry. Groundwater resources in the area include unconfined alluvial and karst aquifers, which serve as the main sources of drinking water and also sustain local rivers and lakes (UNDP, 1991; USACE *et al.*, 1999). Water is extracted from wells, boreholes, and spring catchments, and distributed through private connections, networks, and public standpipes. The municipal water system, for instance, is supplied by two wells with a combined flow of $66\ \mathrm{dm}^3\cdot\mathrm{s}^{-1}$, producing about $10{,}134\ \mathrm{m}^3\cdot\mathrm{d}^{-1}$. However, the city's coastal topography makes groundwater particularly vulnerable to contamination. Latrines are often in close contact with aquifers, especially during the rainy season, facilitating the infiltration of microorganisms. Moreover, the area is highly prone to flooding, which further increases the risk of groundwater contamination.

Collection of animal stool samples and sampling method

Fresh faecal samples were randomly collected from domestic animals living in close proximity to communities that rely on local water sources for drinking. Sampling was carried out in backyards, farms, and grazing areas during two field missions: the first in February 2010, during the rainy season, and the second in April, during the dry season. Only freshly excreted faeces were collected using sterile spatulas and transferred into labelled containers, then preserved at 4°C in 2.5% potassium dichromate until analysis. A total of 129 samples were collected from six species, with cattle (*Bos taurus*, 52 samples, 40.3%) and goats (*Capra hircus*, 33 samples, 25.6%) predominating, followed by pigs (*Sus scrofa domestica*, 19 samples, 14.7%), horses (*Equus caballus*, 13 samples, 10.1%), and dogs (*Canis familiaris*) and sheep (*Ovis aries*), each with 6 samples (4.7%). The six species were selected because they represent the main domestic animals in the Les Cayes region, are frequently raised under free-ranging conditions in close contact with human settlements and water sources, and have been consistently identified in the literature as important reservoirs of *Cryptosporidium*. Detection of *Cryptosporidium* oocysts was carried out at the Laboratory of Parasitology and Medical Mycology, Amiens South University Hospital. This distribution reflects the predominance of ruminants in the study areas, which are considered major potential sources of *Cryptosporidium* contamination.

Detection of *Cryptosporidium* oocysts in stool samples

Methods for coproantigen detection. Coproantigen detection was carried out using the RIDA®QUICK Cryptosporidium lateral flow immunochromatographic test (R-Biopharm). According to the manufacturer and published evaluations, the immunochromatographic test has a sensitivity of 91–95% and specificity of 97–99%. Approximately 50 mg of fresh faeces were transferred into a sterile $1.5\ \mathrm{cm}^3$ microtube containing $1\ \mathrm{cm}^3$ of extraction buffer supplied with the kit. Samples were thoroughly homogenised using a vortex mixer for 30 s and then allowed to sediment for 3 min at room temperature. Subsequently, four drops ($\sim 100\ \mathrm{mm}^3$) of the clarified supernatant were dispensed into the sample well of the test cassette. The assay is based on capillary migration across a nitrocellulose membrane coated with monoclonal antibodies directed against Cryptosporidium antigen complexes. The appearance of both a red test line (T) and a control line (C) within 5–10 min was interpreted as positive, while the presence of only the control line indicated a negative result. Tests without a control line were considered invalid. To ensure reliability, the presence of coproantigens was further confirmed by thin-layer immunochromatography as recommended by the manufacturer.

Detection of oocyst by the Ziehl–Neelsen staining technique. Microscopic detection of *Cryptosporidium* oocysts in faecal smears was performed using the modified Ziehl–Neelsen (mZN) staining technique as described by Henriksen and Pohlenz (1981), which is considered a reference method. A small amount of stool (~20 mg) was emulsified in a drop of distilled water on a clean glass slide and spread to form a thin smear. After air-drying, smears were fixed in absolute methanol for 5 min and allowed to dry. Slides were then stained with Ziehl's carbol fuchsin (RAL Reagents) for 1 h in a humid chamber at room temperature. After staining, slides were rinsed with distilled water and briefly decolourised with

2% sulfuric acid for 20 s, followed by counterstaining with 5% malachite green (RAL Reagents) for 5 min. Finally, slides were rinsed, air-dried, and mounted with Eukitt® mounting medium. Microscopic examination was performed under an oil immersion objective (×1000 magnification). *Cryptosporidium* oocysts were identified as spherical to ovoid bodies, 4–6 μm in diameter, staining bright red against a green background. For quality assurance, each staining series included positive control slides containing known *Cryptosporidium* oocysts and negative controls.

SOIL CHARACTERISTICS

Soil samples were collected from the alluvial aquifer of Les Cayes (southwestern Haiti) at five random points, under potential emission sources of *Cryptosporidium* oocysts. Samples were taken manually at depths of 40–80 cm, excluding the top 5 cm to avoid debris. All samples were air-dried at 35°C, sieved through a 2 mm mesh, homogenised, and stored at room temperature. Coarse fractions (>2 mm) were excluded since fine sediments (<2 mm) are more reactive and favour microbial adsorption**.** The five subsamples were pooled into a composite soil characterised by 35.6% coarse sand, 7.1% fine sand, 25.4% fine silt, 6% coarse silt, and 25.9% clay. Clay and organic matter fractions were emphasised as key drivers of adsorption in aquifer environments. Physicochemical parameters measured included pH, organic matter, clay content, $CaCO_3$, and cation exchange capacity (*CEC*), using standard analytical methods. No chemical treatments were applied to preserve natural clay minerals and organic matter. Analyses were carried out at National Laboratory for Building and Public Works (Fr.: LNBTP – Laboratoire National du Bâtiment et des Travaux Publics) (Haiti), National Institute for Agricultural Research (Fr.: INRA – Institut National de la Recherche Agronomique) Arras (France), and Nuclear Safety and Radiation Protection Authority (Fr.: IRSN – Institut de Radioprotection et de Sûreté Nucléaire) (France). The prepared soil was used for granulometric, physicochemical and percolation column experiments to assess oocyst–soil interactions.

COLUMN PERCOLATION TESTS

Filling protocol

Percolation tests were conducted at the Water and Environmental Quality Laboratory (Fr.: LAQUE – Laboratoire de Qualité de l'Eau et de l'Environnement) in Haiti using polycarbonate columns measuring 4.5 cm in diameter and 20 cm in height, with an estimated surface area of 15.9 cm^2. Each column featured a fixed cylindrical polycarbonate disk at the base, connected to a glass tube (inner diameter: 0.42 mm; outer diameter: 8 cm). To enable oocyst passage (3–6 μm), 2 mm glass beads and polyvinyl chloride (PVC) grids were placed at both the inlet and outlet. Tests were performed in duplicate. The soil was rewetted with distilled water and added in successive 3 cm layers, compacted uniformly. The final soil height in each column was approximately 16 cm, and rubber stoppers were used to seal the columns.

Column saturation by distilled water injection

Percolation tests were performed using soil columns saturated by capillary action with distilled water from a Mariotte bottle over 24 h. The total water volume was determined by weighing the columns before and after percolation. Distilled water was then injected using a peristaltic pump (ISMATEC, IPC-8) to maintain a constant flow rate at the column inlets. The leachates were collected at the outlet in plastic containers and immediately filtered through Envirochek® capsules until clogging occurred. The capsules were subsequently processed following standardised procedures for oocyst purification/elution, detection and enumeration. Positive controls consisting of sterile soil inoculated with a reference suspension of *Cryptosporidium* oocysts were included in each experimental run to validate recovery efficiency.

MICROBIOLOGICAL CHARACTERISATION OF WATER SAMPLES

Collection of water samples

During the rainy season and the onset of the long dry season, 25 water samples were collected from 5 drinking water points (CA03, CA05, CA07, CA09, CA13). To prevent cross-contamination, new sampling equipment (bucket, tumbler, and funnel) was used at each site. For each groundwater sample, 100 dm^3 of water were collected and immediately filtered through a 1 μm pore size polyethersulfone capsule (Environchek, Pall Gelman, Saint Germain en Laye, France), which was then stored at 4°C until processing.

Purification of *Cryptosporidium* oocysts. *Cryptosporidium* oocyst purification followed the NF T90-455 standard method (AFNOR, 2015). Capsules stored at 4°C were eluted with 240 cm^3 of detergent buffer (PBS, pH 7.4, with 0.1% Tween 80), and eluates were centrifuged at 3500 g for 30 min at 4°C. The resulting sediment was resuspended in double-distilled water to a final volume of ~5 cm^3. Oocysts were then purified using immunomagnetic separation (IMS) with Dynabeads® coated with anti-Cryptosporidium monoclonal antibodies (Dynal) according to the manufacturer's instructions.

Detection and enumeration of oocysts. For enumeration, a 20 mm^3 aliquot of the IMS-treated suspension was dried on glass slides, fixed, and stained with a FITC-conjugated monoclonal antibody (Monofluokit Cryptosporidium, Bio-Rad). Slides were examined under epifluorescence microscopy, and oocyst counts were expressed as the number of oocysts per 100 dm^3 of filtered water. Positive control slides were systematically included to validate immunofluorescence assay results.

Identifying the modes of contamination in water sampling campaigns

In developing countries, the interconnections between ruminants, open defecation, and water resources are complex and significantly impact human and animal health, as well as water availability. The widespread lack of adequate sanitation exposes water sources to contamination, increasing the risk of waterborne diseases. Following the 2010 earthquake in Haiti, a cholera epidemic claimed 8,494 lives, with its spread linked to poor hygiene and sanitation practices (Aibana *et al.*, 2013; UN, 2013). Similarly, open defecation by ruminants, particularly cattle, contributes to environmental contamination and the risk of zoonotic disease transmission (Brankston *et al.*, 2018). Water contamination sustains a cycle of disease transmission between humans, animals, and the environment. Breaking this cycle requires improved sanitation infrastructure and hygiene promotion for both people and livestock. The impacts of open defecation underscore the urgency for integrated interventions to ensure clean water access and protect public health. Furthermore, socio-economic vulnerability exacerbates disease risk, emphasising the need to consider the interdependence of humans, animals, and water in sustainable development planning (Yonkeu *et al.*, 2003; Fayer, Santín and Trout, 2008). In this context, the One Health approach is essential, promoting collaboration across human, animal, and environmental health sectors to implement holistic strategies that improve the well-being of both ecosystems and communities.

DATA ANALYSIS

Statistical analyses were conducted using Excel software. Descriptive statistics, including frequencies, cumulative frequencies, means, medians, quartiles, minimum and maximum values, and standard deviations, were calculated for oocyst concentrations in animal faeces, soil, and water samples, as well as for soil physico-chemical parameters. Data distributions were assessed visually using histograms, Q–Q plots, and boxplots to identify skewness, asymmetry, and potential outliers. Normality was formally tested using the Kolmogorov–Smirnov and Shapiro–Wilk tests. While visual inspection suggested right-skewed distributions, numerical tests confirmed approximate normality ($p > 0.05$). Oocyst concentrations were presented in proportional tables, displaying minimum, maximum, and average values across the study period. These analyses provided the basis for comparisons between animal species and environmental matrices, and for interpreting factors influencing *Cryptosporidium* contamination.

RESULTS

PRESENCE OF *Cryptosporidium* OOCYSTS IN ANIMAL FAECES

Of the 129 animal faeces samples from 6 different species: *Bos taurus*, *Capra hircus*, *Sus scrofa domestica*, *Equus caballus*, *Canis familiaris* and *Ovis aries*, 27 tested positive for coproantigens by thin-layer immunochromatography (Tab. 1).

Microscopic examination of modified Ziehl–Neelsen-stained slides confirmed the presence of *Cryptosporidium* oocysts in 17 samples, representing 13.2% of the total analysed. This discrepancy highlights the occurrence of false positives in the coproantigen tests. Prevalence varied among host species, with the highest rates observed in *Sus scrofa domestica* (42%), followed by *Capra hircus* (18%), *Ovis aries* (17%), *Equus caballus* (8%), and *Bos taurus* (2%), while no oocysts were detected in *Canis familiaris*. Oocyst concentrations in faecal samples ranged from 0 to 52 oocysts per 100 g (Tab. 2).

OOCYST CONCENTRATION IN WATER

Oocyst concentrations in water samples varied between seasons, with higher counts observed during the dry season. Values are expressed as oocysts per 100 dm^3 of water, with minimum, maximum, and mean values reported for each sampling site.

QUALITY CONTROL

Each batch included known positive and negative samples to validate staining and immunochromatography procedures. All counts and observations were performed independently by two trained technicians to ensure reproducibility.

Table 1. Positive stool samples in coproantigen tests

Species	*Sus scrofa domestica*		*Capra hircus*		*Canis familiaris*		*Bos taurus*		*Equus caballus*		*Ovis aries*	
Sample	19		33		6		52		13		6	
Test strip	pos.	neg.	pos.	neg.	pos.	neg.	pos.	neg.	pos.	neg.	pos.	neg.
Result	10	9	8	25	2	4	3	49	2	11	2	4

Explanations: pos. = positive, neg. = negative.
Source: own study.

Table 2. Overall presentation of results

Species	*Sus scrofa domestica*		*Capra hircus*		*Canis familiaris*		*Bos taurus*		*Equus caballus*		*Ovis aries*	
Samples	19		33		6		52		13		6	
Results	pos.	neg.	pos.	neg.	pos.	neg.	pos.	neg.	pos.	neg.	pos.	neg.
Number of oocysts	8	11	6	27	0	6	1	51	1	12	1	5
Species (%)	42	58	18	82	0	100	2	98	8	92	17	83

Explanations: pos., neg. as in Tab. 1.
Source: own study.

PHYSICO-CHEMICAL CHARACTERISTICS OF SOIL IN LES CAYES

The results of physico-chemical analysis of the soil are presented in Table 3.

The results of particle size analysis of the soil samples revealed an alluvial soil type and clay loams texture. Particle size analysis was conducted to determine the particle size distribution of the reconstituted soil. Therefore, we found 42.7% of sand, 31.4% of silt and 25.9% of clay. The percentage of organic carbon was 10.3 and the pH was 8.52. The unit of charge per weight of soil or cation exchange capacity (CEC) measured was 15.2 cmol·kg^{-1} and the specific surface was 16.32.

Table 3. Physico-chemical analysis of soil

Parameters		Laboratory	Protocol
Sedimentometry		National Laboratory of Building and Public Works – LNBTP	AFNOR (2003)
Size		–	AFNOR (2003)
Water content		–	AFNOR (1994)
Soil pH	pH in H_2O	National Institute of Agronomic Research – INRA	AFNOR (2022)
	pH in KCl		
Carbonate content	total limestone	–	AFNOR (2002), AFNOR (2014)
	active limestone		
Organic carbon content		–	AFNOR (1995)
Clay content		–	AFNOR (2002)
CEC		–	AFNOR (2022)
Specific soil surface		Institute for Radiological Protection and Nuclear Safety – IRSN	AFNOR (2022)

Explanations: *CEC* = cation exchange capacity (units of charge per weight of soil).
Source: own study.

Table 4. Leaching test results

Saturated soil samples	Volume of water filtered (dm^3)	Dry soil mass (g)		Flow rate (cm^3·min^{-1})	Number of oocysts per 100 dm^3
		before saturation	after saturation		
1	41.58	350	470	48	0
2	31.62	330	380	48	0
3	29.92	400	510	36	0
4	28.46	370	430	36	0
5	27.16	350	460	36	0

Source: own study.

RESULTS OF COLUMN PERCOLATION TESTS

The main results of the leaching tests carried out on four types of soil and a mixture of these soils from the study site are presented in Table 4.

The results obtained during these preliminary tests revealed no presence of *Cryptosporidium* oocysts in the leachates.

OOCYST CONCENTRATION IN WATER BETWEEN SEASONS

Oocyst concentrations in groundwater samples varied between the rainy and dry seasons, with generally higher counts observed during the dry season. Values are expressed as oocysts per 100 dm^3 of water, with minimum, maximum, and mean values reported for each sampling site (Tab. 5).

STATISTICAL ANALYSIS OF SAMPLES

A descriptive statistical analysis was performed on the distribution of faecal samples among the six animal species investigated (Tab. 6, Fig. 1). The mean number of samples per species was 21.5 (median = 16.0; minimum = 6; maximum = 52; *SD* = 18.0), with cattle representing the largest proportion (40.3%).

Graphical analyses of the sample distribution indicated deviations from the normality test. The histogram did not display

Table 5. Number of oocysts per 100 dm^3 in wet and dry seasons

Site	Rainy season (mean [min.–max.])	Dry season (mean [min.–max.])
CA03	3.33 [0–10]	2 [0–4]
CA05	6.33 [5–9]	121.5 [3–240]
CA07	1.33 [2–2]	506.5 [24–989]
CA09	34.33 [1–100]	0 [0–0]
CA13	9.26 [3–23]	18 [0–36]

Source: own study.

Table 6. Sample descriptive statistics

Species	Sample No.	Cumulative headcount		Frequency	Cumulative frequencies	
		increasing	decreasing		increasing	decreasing
Bos taurus	52	52	129	0.4031	0.4031	0.9998
Canis familiaris	6	58	77	0.0465	0.4496	0.5967
Capra hircus	33	91	71	0.2558	0.7054	0.5502
Equus caballus	13	104	38	0.1007	0.8061	0.2944
Ovis aries	6	110	25	0.0465	0.8526	0.1937
Sus scrofa domestica	19	129	19	0.1472	0.9998	0.1472

Source: own study.

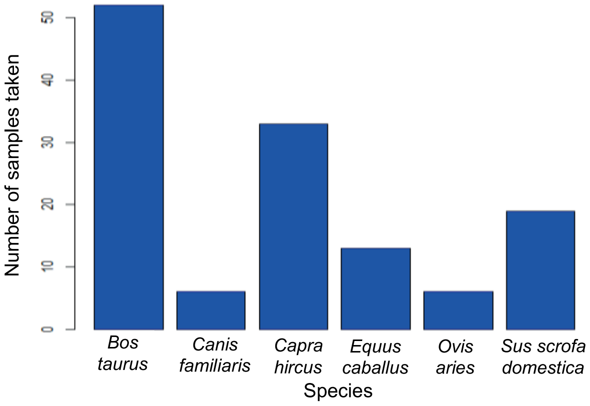

Fig. 1. Composition of the sample; source: own study

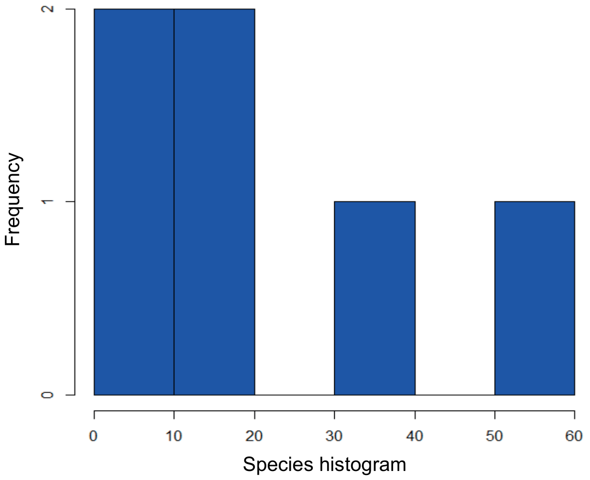

Fig. 2. Histogram distribution of the sample; source: own study

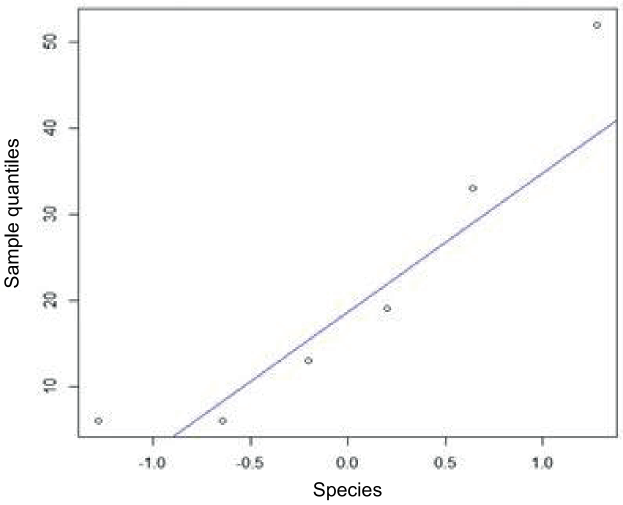

Fig. 3. Q-Q plot distribution of the sample in quantiles; source: own study

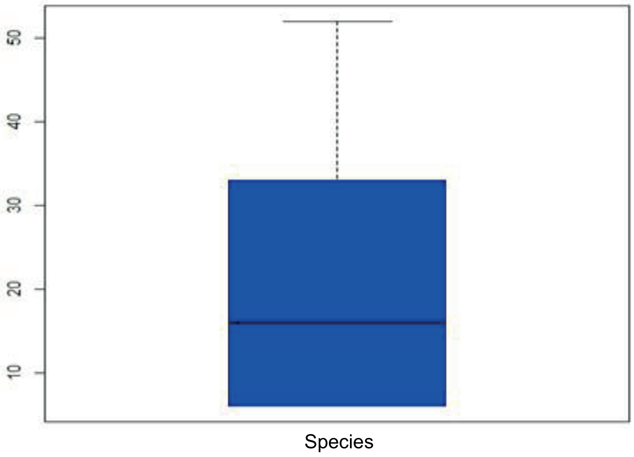

Fig. 4. Box plot of the sample; source: own study

the expected bell-shaped curve (Fig. 2), the Q–Q plot revealed several points deviating from the reference diagonal (Fig. 3), and the box plot emphasised asymmetry and the presence of outliers (Fig. 4). Collectively, these empirical observations suggested a right-skewed distribution.

Nevertheless, statistical normality tests provided contrasting results. Both the Kolmogorov–Smirnov test ($D = 0.2219$, $p = 0.9292$) and the Shapiro–Wilk test ($W = 0.8759$, $p = 0.2505$) failed to reject the null hypothesis of normality ($p > 0.05$). This apparent contradiction between graphical and numerical methods can be explained by the limited sample size ($n = 6$) and the presence of repeated values in the dataset. Overall, although graphical exploration suggested skewness, the results of statistical testing supported the assumption of approximate normality. Accordingly, parametric methods were applied for subsequent analyses, while acknowledging the limitations associated with sample size.

DISCUSSION

This study provides new insights into the potential impact of domestic animals on groundwater quality in Les Cayes, Haiti. The Rida®Quick Cryptosporidium lateral flow immunochromatographic assay has been shown to have high diagnostic performance, with a sensitivity of 88.9%, specificity of 95.5%, positive predictive value of 94.1%, negative predictive value of 91.3%, and overall accuracy of 92.5% (Mohamed, El-Hady and Ahmed, 2020). Analysis of 129 faecal samples from six domestic animal species revealed that *Bos taurus*, *Capra hircus*, the most abundant livestock, accounted for the largest proportion of samples, reflecting their numerical dominance in the local populations (MARNDR, 2012; FAO, 2020). Overall, 13.2% of the faecal samples tested positive for *Cryptosporidium* oocysts, confirming that domestic animals serve as important reservoirs of this protozoan pathogen. These results align with global prevalence data, as a recent meta-analysis reported an overall infection rate of 18.9% across common livestock species, including *Bos taurus*, *Capra hircus*, *Sus scrofa domestica*, *Equus caballus*, *Ovis aries* and *Bubalus bubalis* (Hatam-Nahavandi *et al.*, 2019), with infection rates in cattle specifically ranging from 6.3 to 39.7% in different regions worldwide (Buchanan *et al.*, 2025). Taken together, these findings highlight domestic animals as key contributors to potential groundwater contamination in Les Cayes and provide a foundation for investigating environmental and hydrological factors that influence oocyst transport.

To understand the distribution of contamination, statistical analysis of the faecal sample data was conducted. The dataset showed a right-skewed pattern, primarily driven by the predominance of cattle samples. Graphical analyses, including histograms, Q–Q plots, and box plots, suggested deviations from a normal distribution; however, numerical tests, namely the Kolmogorov–Smirnov and Shapiro–Wilk tests, indicated approximate normality, supporting the use of parametric methods for subsequent comparisons (Zar, 2010; Field, 2013). This combination of visual and statistical evaluation underscores the importance of rigorous data analysis, particularly in studies with relatively small sample sizes, and sets the stage for interpreting the interaction between soil, water, and pathogen distribution.

Building on this, soil analyses showed that fine particles (clay, fine silt, and coarse silt) represented 57.3% of the total composition, corresponding to a silty-clay texture with high plasticity and low permeability (Sayyad *et al.*, 2010). The soils also displayed a basic pH (8.52), high carbonate content, elevated cation exchange capacity, substantial organic matter, and a large specific surface area, all properties known to enhance the adsorption and retention of *Cryptosporidium* oocysts (Jenkins *et al.*, 2002). Although soils are often considered natural filters, oocyst transport is strongly influenced by texture, pH, and organic matter content (Petersen *et al.*, 2012). *Cryptosporidium* oocysts survive longer in soil than in water, with particularly high viability in loamy soils (up to 1096 days at 30°C, compared to 211 days in water). Thus, faecal matter accumulated in soil constitutes a long-lasting reservoir of infective oocysts, likely to contaminate surface waters through rainfall, runoff, or manure spreading (King and Monis, 2007). While adsorption capacity and survival times were not directly measured in this study, recent evidence indicates that soils rich in clay and organic matter significantly improve oocyst retention and prolong their viability (Balthazard-Accou *et al.*, 2014). Thus, the silty-clay soils of Les Cayes may provide only partial protection against pathogen migration into aquifers, with soil properties interacting closely with faecal contamination patterns to shape groundwater vulnerability.

Water analyses further support this connection. According to Jagai *et al.* (2009), seasonal variations in oocyst concentrations were observed, with higher levels during the dry season. Limited water availability during this period may concentrate oocysts in aquifers, whereas heavy rainfall during the wet season can mobilise pathogens and increase the risk of groundwater contamination. These seasonal dynamics highlight the importance of considering hydrological and climatic factors when assessing the risk of waterborne *Cryptosporidium* transmission in tropical regions like Les Cayes. However, no specific climatic parameters were evaluated in this study.

The presence of *Cryptosporidium* in both natural aquatic environments and drinking water represents a substantial biological hazard. Its persistence and infectivity, coupled with complex interactions among biological, environmental, climatic, and socio-behavioural factors, amplify public health risks (Wang, Wang and Cao, 2023). Environmental factors such as water quality, soil characteristics, and climatic conditions play a key role in the transmission of *Cryptosporidium.* Indeed, the survival and dispersion of oocysts are strongly influenced by these parameters, which affect both water source contamination and the incidence of diarrheal diseases (Wang, Wang and Cao, 2023). The presence and concentration of oocysts may fluctuate considerably, since they are modulated by multiple factors, including flooding events, agricultural practices, free-roaming livestock (Paul, 2023), on-site sanitation systems such as pit latrines in karstic aquifers, and the sporadic deposition of animal faeces containing oocysts (Balthazard-Accou *et al.*, 2009). In Les Cayes, anthropogenic land-use practices exacerbate these risks, as surface waters impacted by runoff from livestock operations or untreated sewage frequently exhibit markedly elevated concentrations of *Cryptosporidium* oocysts, often several orders of magnitude higher than those in unimpacted sites (Hamilton *et al.*, 2018). These pressures are compounded by the city's specific socio-environmental context: a high prevalence of open defecation in the southern region (35.2%) (Paul *et al.*, 2022), widespread use of latrines and septic systems often in contact with the water table, recurrent discharges of untreated urban effluents, and inadequate waste management, with solid waste evacuated either by truck collection or discharged into waterways, drains, and wastelands (Balthazard-Accou *et al.*, 2020). Moreover, the coastal topography of Les Cayes, with several low-lying districts below sea level, makes the city particularly vulnerable to flooding and backflow of domestic and rainwater into urban drainage channels (Balthazard-Accou, 2011). Altogether, the percolation of leachates from uncontrolled landfills, effluents from latrines and septic tanks, contamination of surface waters, and interactions between surface and groundwater systems contribute to amplifying the risk of waterborne pathogen transmission and, consequently, pose a serious threat to public health.

From an environmental health and policy perspective, these findings highlight critical gaps in urban planning, sanitation infrastructure, and livestock management. The coexistence of free-ranging animals, unregulated waste disposal, and insufficient wastewater treatment infrastructure significantly increases the risk of waterborne disease outbreaks (Graaf de *et al.*, 1999). Addressing these challenges requires integrated interventions, including regulated livestock management, enhanced wastewater treatment, protection of aquifer recharge zones, and community education on safe water practices. By simultaneously targeting anthropogenic pressures and inherent environmental vulnerabilities, such strategies can substantially reduce the risk of *Cryptosporidium* transmission and support sustainable water resource management in Les Cayes. Finally, all domestic species examined in this study are confirmed as potential hosts of *Cryptosporidium* oocysts, emphasising the pivotal role of animals in groundwater contamination. Incorporating knowledge of soil characteristics, seasonal climatic variability, and livestock management practices into water protection strategies is essential to mitigate environmental and public health risks. This study further demonstrates the utility of combining quantitative, graphical, and microbiological analyses to inform evidence-based interventions, even when working with relatively small sample sizes, by applying robust statistical approaches adapted to limited datasets (Morgan, 2017).

CONCLUSIONS

Cryptosporidium transmission in Les Cayes is a multifactorial process involving multiple animal hosts, environmental pathways, and seasonal influences. Among the 129 faecal samples collected from six animal species, *Bos taurus*, *Capra hircus*, *Sus scrofa domestica*, *Equus caballus*, *Canis familiaris* and *Ovis aries*, 27 tested positive for coproantigens by thin-layer immunochromatography, of which 17 were confirmed microscopically. This corresponds to an overall prevalence of 13.2%, with species-specific prevalence as follows: *Sus scrofa domestica* 42%, *Capra hircus* 18%, *Bos taurus* 2%, *Equus caballus* 8%, *Canis familiaris* 0%, and *Ovis aries* 17%. Oocyst concentrations ranged from 0 to 52 per 100 g of faeces, highlighting substantial heterogeneity among host species. Seasonal monitoring of groundwater revealed fluctuations in oocyst concentrations, with higher counts during the dry season, suggesting that rainfall patterns, runoff, and soil-water interactions strongly influence oocyst mobilisation and accumulation. Soil properties, including high clay and silt content, basic pH, elevated organic matter, and large specific surface area, further modulate oocyst retention and transport, providing partial but incomplete protection against contamination. These findings demonstrate that free-range animal husbandry, inadequate waste management, and environmental degradation are key contributors to *Cryptosporidium* contamination in groundwater. In line with a One Health approach, targeted interventions are recommended: molecular characterisation of *Cryptosporidium* species, identification of contamination sources, assessment of well vulnerability, continuous water quality monitoring, improved livestock and waste management, community education, and implementation of mitigation measures such as controlled grazing and protection of water sources. Collectively, these measures will reduce transmission risks and enhance the safety and quality of drinking water in Les Cayes, ultimately improving environmental health outcomes for the local population.

CONFLICT OF INTERESTS

All authors declare that they have no conflict of interests.

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