Article presented at 2nd International Nineveh Conference on Medical Sciences (2nd INCMS 2023)

Original Article

Detection of *Cryptosporidium* spp. and *Giardia* spp. from Water Samples in Mosul City, Iraq

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Abstract

Background: Protozoan parasites are significant in waterborne parasitic diseases because they are resistant to common disinfection methods and have stable cystic phases in the environment. **Objectives:** The present study aimed to investigate protozoan parasites in water samples collected from different regions that collect rainwater. **Materials and Methods:** A total of 150 water samples were collected from different regions in Mosul City, Iraq, from November 2019 to the end of March 2020. After sample collection, the water samples were examined to investigate oocysts or cysts of protozoan parasites using direct wet smear, Sheather's sugar solution, and the Modified Ziehle–Nelseen stain method. **Results:** The results showed that 43.3% of the samples examined were infected with protozoan parasites, and the detected parasites were found in different ratios across the various regions. Were high percentage was 65.5% Al-Kossiat region and the low percentage was 20% in the Ajaamea region. **Conclusion:** This study recorded the high number of (oo)cysts of these protozoan parasites in water samples at a percentage (58.4%).

Keywords: *Cryptosporidium* spp., *Giardia* spp., protozoa parasite, rainwater

Introduction

Intestinal protozoan parasites of the genera *Cryptosporidium* (phylum Apicomplexa) and *Giardia* (phylum Sarcomastigophora) have an important role as waterborne pathogens responsible for diarrhea worldwide.[1-4] These protozoa are spread by the oral–fecal pathway when contaminated drinking water is consumed.[5,6] In contrast to *Giardia*, which accounts for 20%–30% of diarrheal cases in children in developing nations, *Cryptosporidium* causes roughly 20% of those cases.[7]

The transmission stages (oocysts and cysts) of *Cryptosporidium* and *Giardia*, respectively, may persist for several months under adverse environmental conditions.[8] Infectious diarrhea is a major cause of death in humans, especially in children under 5 years old in Africa.[9] Unsafe water sources as well as inadequate standards of hygiene and sanitation contribute to an increase in the spread of infectious diarrhea.[10] Parasitic intestinal protozoa, such as *Giardia* and *Cryptosporidium* were not the most significant long-term causes of diarrhea.[3,4,11]

Multiple studies have examined the occurrence or concentrations of these pathogens in the environment. Cattle, especially calves, are a substantial source of environmental contamination and human illness; the calves are reservoirs of cysts of the potentially zoonotic parasite *Cryptosporidium parvum.*[11-13] Therefore, the fecal–oral route of transmission can lead to human infection through various water sources, particularly those contaminated by animal and human waste.[14,15] By releasing a large number of (oo)cysts containing feces into the environment, calves are thought to have a significant role in environmental contamination. These (oo)cysts can contaminate water sources used for human and animal

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Website: https://journals.lww.com/mjby

DOI: 10.4103/MJBL.MJBL_1311_23

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Submission: 30-Aug-2023 **Accepted:** 08-Oct-2024 **Published:** 21-Nov-2024

How to cite this article: Al-Jarjary SAA, Hasan MH, Hussain BI. Detection of *Cryptosporidium* spp. and *Giardia* spp. from water samples in Mosul City, Iraq. Med J Babylon 2024;21:S282-7.

consumption.[14,16] The present investigation identifies the species of *Cryptosporidium* and *Giardia* in water samples from different regions that collect rainwater, as the public health implications of these protozoa's pollution of water sources are significant.

Materials and Methods

Study location

Water samples were collected from different regions that collect rainwater [Figure 1] at two points in each water region: One from the surface water and the other from the bottom of the collected water.

Microscopic examination was conducted on the rainwater samples

A total of 150 rainwater samples (100–500 mL) were collected during the study period from November 2019 to March 2020. The samples were transported to the laboratory for further investigation. About 10 mL of each rainwater sample was placed in a centrifuge tube and centrifuged (for 10 min) to concentrate the sediment into a single pellet. The samples were then examined using direct and flotation methods with Sheather's sugar solution[17,18] as follows:

About 5 ml of each 25 mL sample was then mixed with 10 mL Sheather's solution (500 g sucrose, 320 mL distilled water, and 9.7 mL phenol, 1.29 g/mL). Five tubes for each sample were left for 15 min, then, the scummed uppermost layer was collected, and water was on a clean slide for examination under the microscope.

The scummed uppermost layer was collected from each tube and transferred to other tubes, and added 5 mL of distilled water was added and centrifuged for 10 min. To concentrate the sediment the drop from the sediment water was smeared on two clean slides, and one slide by a modified Ziehl–Neelson technique. After drying, the slides were examined for detecting *Cryptosporidium* oocysts, and the number of oocysts in the upper layer of the supernatant. The slides were stained using the following steps[19]:

1. Fix in 96% methanol for 2–5 min.
2. Dry at room temperature.
3. Stain with concentrated carbol fuchsin for 5 min.
4. Thorough rinsing in water.
5. Decolorization by dipping the slide in sulfuric acid H_2SO_4 at a concentration of 10% for 1 min.
6. Counterstain with methylene blue for 2–6 min.
7. Rinse with water to remove the excess dye and place in the slide holder to dry, then, examined by microscope at 100×.

In smears stained by this technique, cryptosporidia appear as 3–6 µm large, densely stained red bodies, clearly distinguishable against a blue background and other slides stained by Geimsa stain after drying. Slides examined for detecting *Giardia* cysts. Counting the number of cysts in the uppermost layer of the supernatant water.[20]

Examined the staining slides using the light microscope under ×100 and the number of (oo)cysts of parasites was calculated according to Bakir[21] considering the number of (oo)cysts more than 5 (high number in sample) a score

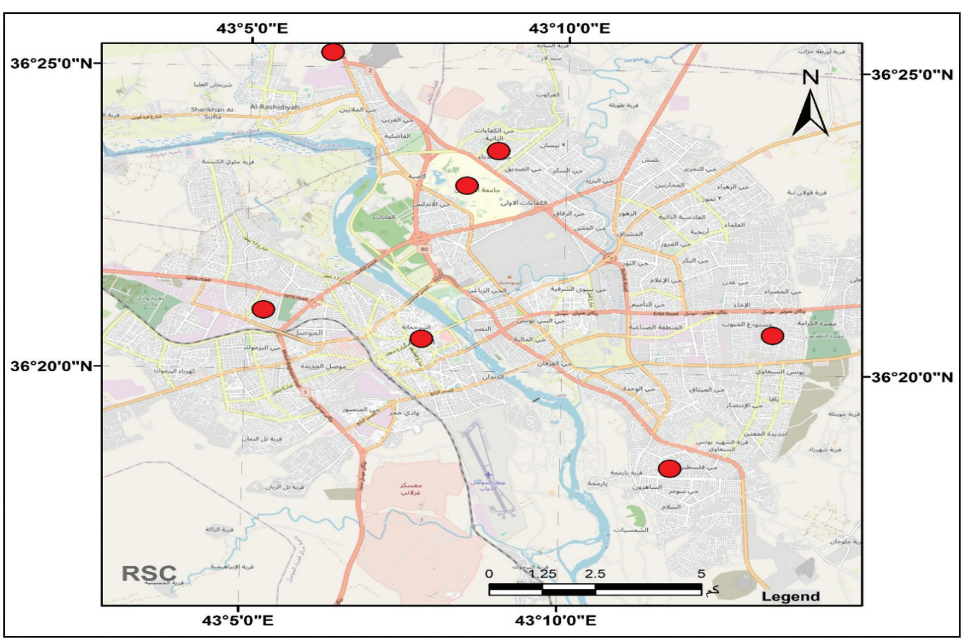

Figure 1: Location of collected rainwater samples from different regions in Mosul city

1, the number of (oo)cysts <5 (mild number in sample) a score 2 at 10 microscopic fields.

Results

In this study, *Giardia* cysts and *Cryptosporidium* oocysts were investigated in a total of 150 water samples, and both were frequently found in 65 samples (43.3%). Different percentages from different regions were high percentages of 65.5% Al-Kossiat region and low percentages of 20% in the Ajaamea region [Table 1 and Figures 2–4].

In addition, differences in percentages of both protozoa were found in the water samples during the study months [Table 2].

During the examination of water samples, there were *Cryptosporidium* oocysts, *Giardia* cysts, and both *Cryptosporidium* and *Giardia* cysts contamination in the studied water samples. In percentages 49.2%, 12.3%, and 35.4%, respectively [Table 3].

Table 1: *Cryptosporidium* and *Giardia* (oo)cysts in samples of water in different regions

Region	Number of samples	Number of positive samples	%
Alhadbaa	26	9	34.6
Domeeze	34	14	41.2
Alkudis	20	8	40
Al-Eslah Alzeraee	18	10	55.6
Aljaamea	20	4	20
Al-Kossiat	32	20	62.5
	150	65	43.3%

The result of this study shows the high number of (oo) cysts of protozoan parasites in water samples at a percentage (58.4%), and *Cryptosporidium* oocysts at a high no. in percentage (90.6%). While the presence of oo(cysts) of *Cryptosporidium* and *Giardia* at a percentage of 26%. The mild number of oo(cysts) of *Cryptosporidium* at a percentage of 9.3%, whereas the presence of oo(cysts) of *Cryptosporidium* and *Giardia* at a percentage of 73.9% [Table 4].

Discussion

In this research, samples from rainwater collections from various places in Mosul city contained 43.3% *Cryptosporidium* and *Giardia* (oo)cysts.

The presence of *Giardia* (oo)cysts and *Cryptosporidium* in samples of rainwater collection suggests that the oocysts or cysts of these parasites are being passed into rainwater collections by infected feces of different animals, birds, and humans. These results were in agreement with other investigations estimating global parasite contamination of water sources in Japan,[22] Malaysia,[23] Iran,[24] and Iraq.[18,19,25] The presence of oo(cysts) of these parasites may occur through sewage effluent discharges, septic tank leakage, agricultural runoff, or soils exposed to infected feces.[26,27]

These (oo)cysts could be contaminating surface water used as a source of drinking water for humans, animals, and birds. Moreover, *Cryptosporidium* oocysts retain viability when exposed to many commonly used disinfectants, including hypochlorite solutions with a concentration of 3%.[28] In addition, the examined rainwater samples show differences in the rates of presence of (oo)cysts of both protozoa in different regions in Mosul city, with high rates recorded in the Al-Kossiat region (62.5%), their results reveal that

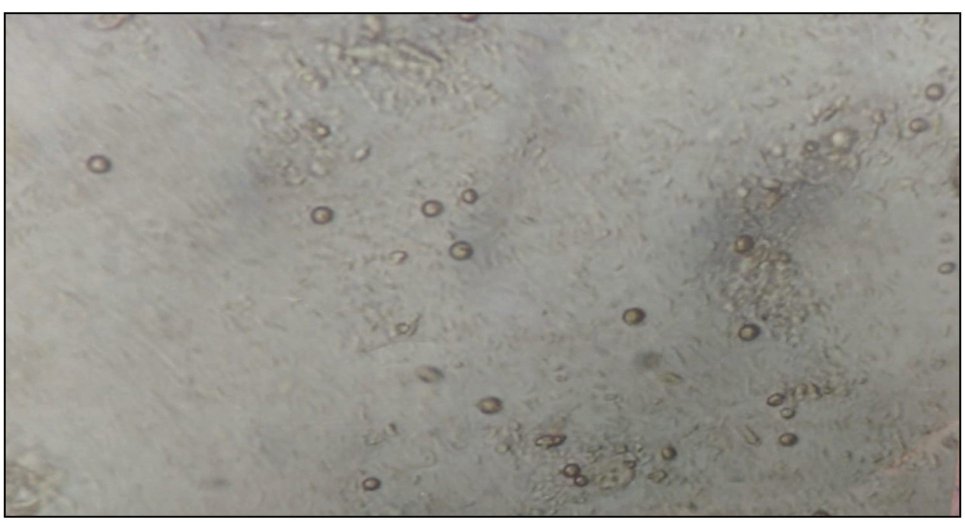

Figure 2: *Cryptosporidium* oocysts by flotation method (40×)

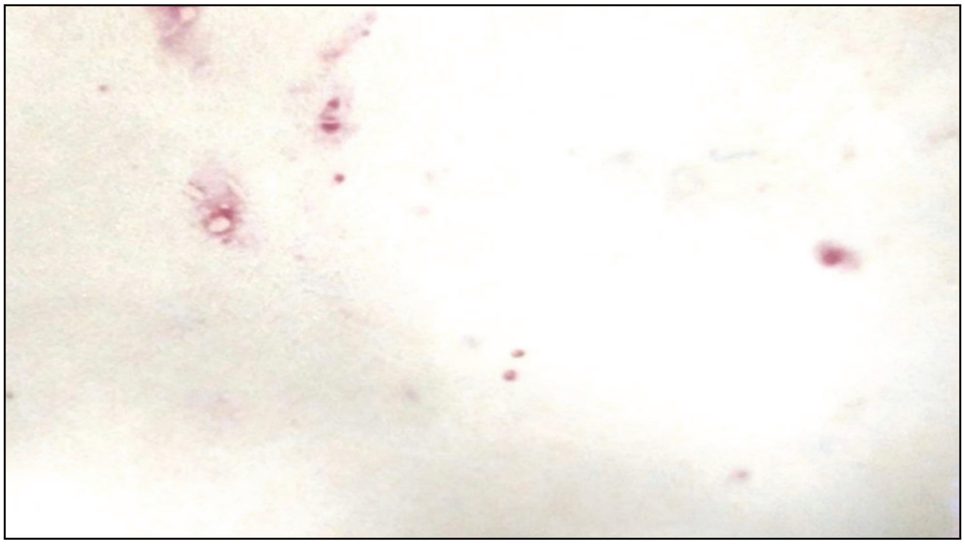

Figure 3: *Cryptosporidium* oocysts (bright red color) isolated from water samples (acid-fast stain; 100×)

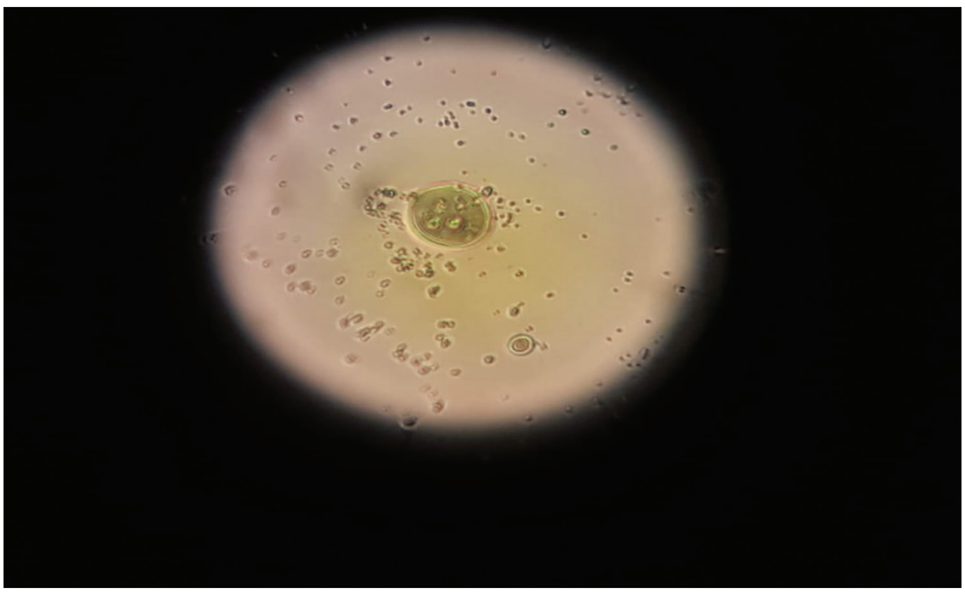

Figure 4: *Giardia* cysts isolated from water samples (40×)

Table 2: *Cryptosporidium* and *Giardia* (oo)cysts in water samples

Time of sampling	Number of sample examination	Number of positive samples	%
November 2019	30	12	40
December 2019	31	12	38.7
January 2020	26	8	30.8
February2020	33	17	51.5
March 2020	30	16	53.3
	150	65	43.3

environmental contamination with both protozoa, this condition perform a potential risk for waterborne diseases for different animals and birds. The results show the incidence of both protozoa in the studied water samples during the study months (November, December, January, February, and March) at high rates (30.8%–53.3%). The results were in line with previous studies.[29-31]

Identically, a Turkish study from Ankara that examined municipal water supplies, wells, river water, and untreated dams found that 5.8% of samples from the wells, dams, and rivers were *Giardia*-positive overall.[32] *Cryptosporidium* oocysts were found in 63.5% of river samples and 15.4% and 22.6% of raw water samples from large and minor water treatment facilities, respectively. *Giardia* cysts were found in 55.5% of reservoir samples and 92.3% of river samples.[30]

The present study shows that the high number of (oo)cysts of protozoan parasites in water samples, at a percentage

Table 3: Detection of *Cryptosporidium* and *Giardia* (oo)cyst in water samples

Regions	Number samples examined	Number of positive	Number of positive *Cryptosporidium*	Number of positive *Giardia*	Number of *Cryptosporidium* + *Giardia*
Alhadbaa	26	9	1	3	7
Domeeze	34	14	2	2	8
Alkudis	20	8	8	0	0
Al-Eslah AIzeraaee	18	10	6	3	1
Alchameaa	20	4	4	0	0
Al-Kossiat	32	20	11	2	7
	150	65	32 (49.2%)	10 (15.3%)	23 (35.3%)

Table 4: Number of *Cryptosporidium* oocysts and *Giardia* cysts in rainwater samples

Parasite	Number of positive samples	Score 1 (high no.)		Score 2 (mild no.)	
		%	*N*	%	*N*
Cryptosporidium	32	90.6	29	9.3	3
Giardia	10	30	3	70	7
Cryptosporidium + *Giardia*	23	26	6	73.9	17
Total	65	58.4	38	41.5	27

(58.4%), were *Cryptosporidium oocysts* at a high number. In percentage (90.6%) this agreement with Carmena *et al.*[30] shown that *Cryptosporidium* and *Giardia* (oo)cysts are present in surface water throughout the year, with the highest frequency of occurrence during the autumn. A second peak for *Cryptosporidium* was recorded during the summer.

The results in this study indicate that *Cryptosporidium* and *Giardia* (oo)cysts can be frequently isolated from a variety of water sources, suggesting that these protozoan parasites could play an important role in waterborne disease outbreaks.[33]

Conclusion

The results obtained in this study might elucidate the presence of (oo)cysts *Cryptosporidium* and *Giardia* in samples of rainwater collections with high numbers. These (oo)cysts could contaminate surface water, which is used as a source of drinking water for humans, animals, and birds. However, rainwater could play an important role in the transmission of these protozoan parasites.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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