

Evaluation of Conventional Drinking Water Treatment Plants in Removal of *Giardia* and *Cryptosporidium*.

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ABSTRACT

Enteric pathogenic protozoa like *Giardia* and *Cryptosporidium* pose significant health risks, especially in Egypt; they contaminate drinking water. *Giardia* is known for causing giardiasis, while *Cryptosporidium* leads to cryptosporidiosis, both of which can result in severe gastrointestinal symptoms. The presence of these protozoa in water sources is often linked to inadequate sanitation and water treatment practices, making it essential to address issues related to water quality. Control of waterborne diseases is critical for public health, and drinking water treatment plants (DWTPs) play a central role in this effort through employing various treatment processes such as coagulation, filtration, and disinfection. DWTPs effectively remove contaminants and pathogens from water sources. It is true that not much study has been done on the use of immunofluorescence assays (IFA) to monitor *Cryptosporidium* and *Giardia* species in developing countries including Egypt. These techniques can significantly enhance the detection and quantification of these parasites compared to traditional methods. Water samples (10 liters volume each) were collected monthly at the same time from each sampling site for a year from January 2023 to December 2023, from both the inlet (raw freshwater) and outlet (final treated drinking water) of two conventional DWTPs (Shebin and Tala). The samples were collected from the inlet and outlet of each DWTP and were processed by immunofluorescence assay. The examination of 48 collected water samples from inlet and outlet of Tala and Shebin DWTPs revealed the occurrence of *Giardia* cyst and *Cryptosporidium* oocyst in 58.3 % (14/24) and 54.2 % (13/24) of collected raw water respectively, on other hand the occurrence of *Giardia*, *Cryptosporidium* and in outlet samples were 16.7 % (4/24) and 12.5 % (3/24), respectively.

Keywords: *Giardia*, *Cryptosporidium*, drinking water treatment plants, immunofluorescence.

INTRODUCTION

Safe drinking water helps to maintain good health and is highly necessitate for living organisms, in addition to essential human requirements. Inadequate wastewater and water treatment, coupled with poor public health care and unruly urbanization, makes it easier for infectious diseases to spread, which can have serious social and financial consequences (Zin Eldin et al., 2023; Eissa, 2024 and Keller *et al.*, 2024).

Human health is in danger when biological agents contaminate water for public use, particularly when it comes to waterborne infectious illnesses (WHO, 2022). Companies that produce drinking water in impoverished nations face difficulties due to the existence of pathogenic parasites in these matrices, even with technological advancements for their removal from water catchment. In this sense, the most efficient and least costly methods of preserving water quality are monitoring the water-producing system and lowering contamination at the watershed, or safeguarding water sources.

(de Araújo *et al.*, 2018; Eissa and Harb, 2023; Zin Eldin et al., 2023 and Eissa, 2024).

Human fatality is primarily driven by poor sanitation and a lack of safe drinking water, above the combined impact of terrorism and chemical weapons (Byomi et al. 2018; 2019a; 2019b; Mousa *et al.*, 2023; Eissa and Harb, 2023; Zin Eldin et al., 2023 and Eissa, 2024).

Giardia and *Cryptosporidium* are currently the most common protozoa found in waterborne epidemics

throughout continents. Because of their resistance to traditional drinking water treatment methods and capacity to withstand environmental changes, they pose a serious threat to public health (Baldursson and Karanis, 2011; Byomi et al., 2018; Elgendy et al., 2024 and Ramzy et al., 2025).

Raw polluted water and the ability of certain pathogenic protozoa, such as *Giardia*, *Blastocystis*, and *Cryptosporidium*, to adapt to its surroundings necessitate a framework of drinking water treatment operations in order to totally eliminate these pathogens from the water supply system. Many digestive disorders have been caused by the protozoan parasites *Giardia duodenalis* and *Cryptosporidium parvum*.

(Moreno-Mesonero *et al.*, 2024 and Ramzy et al., 2025).

Approximately 12 billion is lost globally each year as a result of water-borne illnesses such as diarrhea, intestinal diseases, and systemic illness (Alhamlan *et al.*, 2015; Eissa and Harb, 2023; Zin Eldin et al., 2023; Eissa, 2024 and Ramzy et al., 2025). Protozoan parasites that involve *Giardia*, *Entamoeba*, and *Cryptosporidium* have been extensively attributed to diarrheal outbreaks in advanced countries (Karanis *et al.*, 2007; Moussa *et al.*, 2023 and Ramzy et al., 2025). Additionally, the two most frequent causes of water-borne parasite infections that result in diarrhea are *Giardia* and *Cryptosporidium* (Al-Rifai *et al.*, 2020 and Ramzy et al., 2025).

MATERIAL AND METHOD

1. Sampling sites and structure of DWTPs

Two DWTPs, Shebin Al-kom and Tala, are chosen for the present study; DWTPs were conventional DWTP that served a large city community.

Conventional DWTP is composed of an intake system, clarifiers, filters and product water tank.

The intake systems of Shebin and Tala DWTPs are Shebin and Al Bajouriya canal, respectively; each canal is branched from Nile River.

Shebin DWTP produces 65.000 M³/day and composed of 4 clarifiers, 10 filters and product water tank. In addition it serves 500,000 citizens in Kafr Al-

masalha, Shebin Al-kom city, Meyet khakaan and Kafr Al- Masalha localities in Monofeya governorate.

Tala DWTP is composed of 4 clarifiers and 12 filters ; it produce 60.000 M³/day; and it serves 450.000 people in Zanarah, Kafr snaded, Babil, Tabluha, Kafr Tabluha, Kammesh , Zawraqan and Samalij localities in Monofeya governorate.

Water samples (10 liters in volume each) were drawn monthly at the same time from each sampling site for a year, from January 2023 to December 2023, from the inlet (raw freshwater) and outlet (final treated drinking water) of two conventional DWTPs (Shebin and Tala).



Figure 1. Location of conventional drinking water treatment plants.

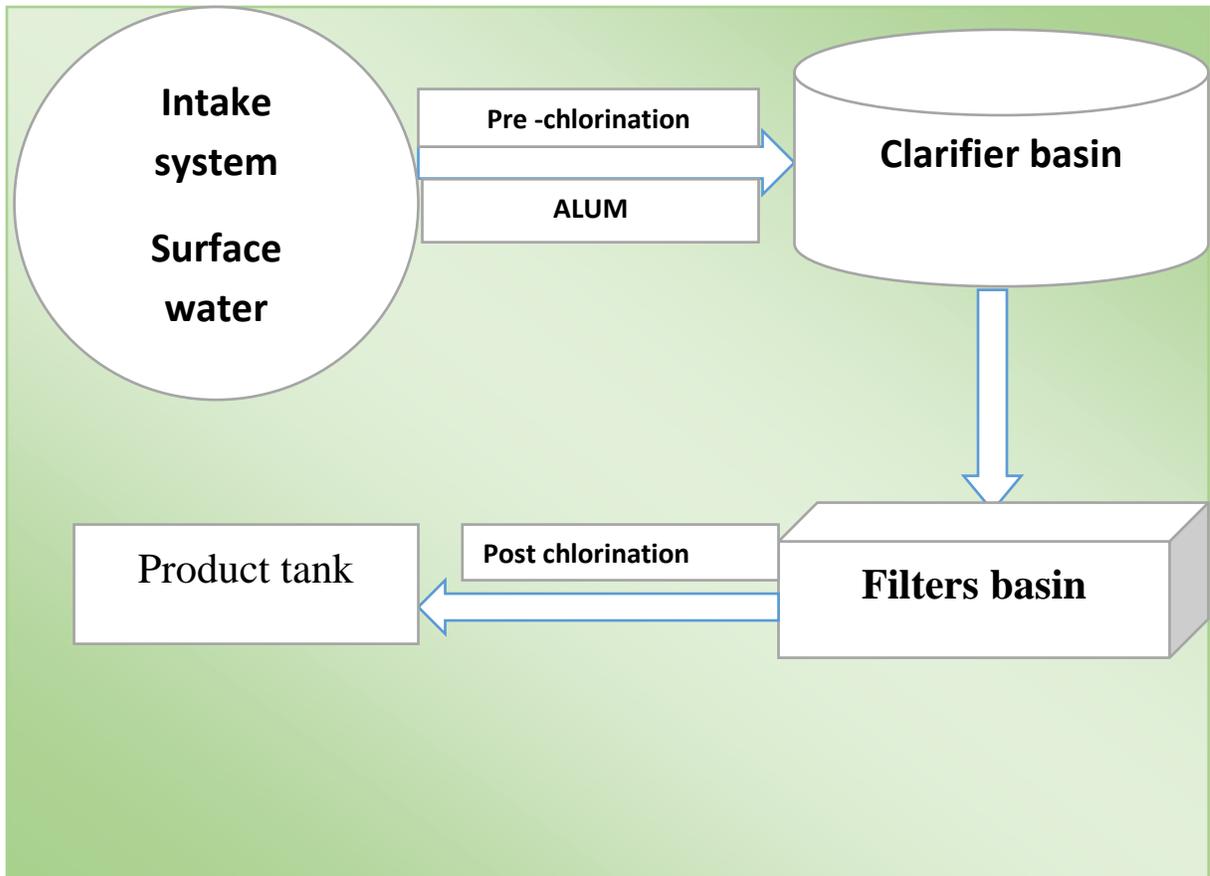


Figure 2. Diagrammatic Structure of conventional DWTP.

2. Method Immunofluorescence assay technique and microscopic examination.

The samples were collected in a volume 10 L from inlet and outlet of each DWTP in autoclavable polypropylene container and were processed by immunofluorescence assay (EPA method 1623, 2005; ISO/FDIS 15553:2006).

2.1. Filtration

Samples were filtered through sterile nitrocellulose membranes (142 mm diameter, 0.45 μm pore size) using a stainless-steel pressure filtration system (Millipore) to remove particulates and microorganisms. This step was essential to ensure the purity of the samples for

further analysis, which is included in the IFA technique.

2.2. Elution

The membrane filter was removed from its housing and placed in a 142 mm-diameter Petri dish.

2.2.1. Eluent

25 ml of a 0.1% Tween 80 solution was gently poured onto the membrane to help detach particulate material.

2.2.2. Repeat Washing:

The washing step was performed again to maximize recovery of the material.

2.3. Centrifugation:

The combined washing solution was subjected to centrifugation at $1500 \times g$ for 15 minutes to separate the particulate material.

2.4. Sample Purification (Immunomagnetic separation)

The resulting pellet had been shaken in ten milliliters of phosphate buffer saline (pH = 7.4), after the supernatant was disposed of, pellet was vortexed for 10 to 15 seconds before being placed in an L10 tube (Leighton tube). The L10 tube was filled with 1 mL of each of the 10X SL-buffers A and B. The solution in an L10 tube was then supplemented with 100 μ L of the Dynabeads *Giardia* and 100 μ L of the reconstituted Dynabeads *Cryptosporidium*. Microscopic analysis, immunostaining (DAPI and FITC), and immunomagnetic separation were carried out in accordance with EPA Method 1623 (2005).

RESULTS

Examination of 48 collected water samples from the inlet and outlet of Tala and Shebin DWTPs during a year period from January 2023 to December 2023 revealed the occurrence of *Giardia* cyst and *Cryptosporidium* oocyst evenly in the inlet samples (58.1%) (14/24) respectively; on the other hand, the occurrence of *Giardia*, *Cryptosporidium*, and in the outlet samples was 16.7% (4/24) and 12.5% (3/24).

(Table 1).

Giardia cyst appeared as oval brilliant apple green when stained with FTIC stain, and its size was 10–15 μ m in length and 5–8 μ m in width. In addition, the nucleus was appeared by staining with DAPI stain. *Cryptosporidium* oocyst appeared as rounded brilliant green when it was stained with FTIC stain; in addition, its size was 4–6 μ m (Figure 3,4,5).

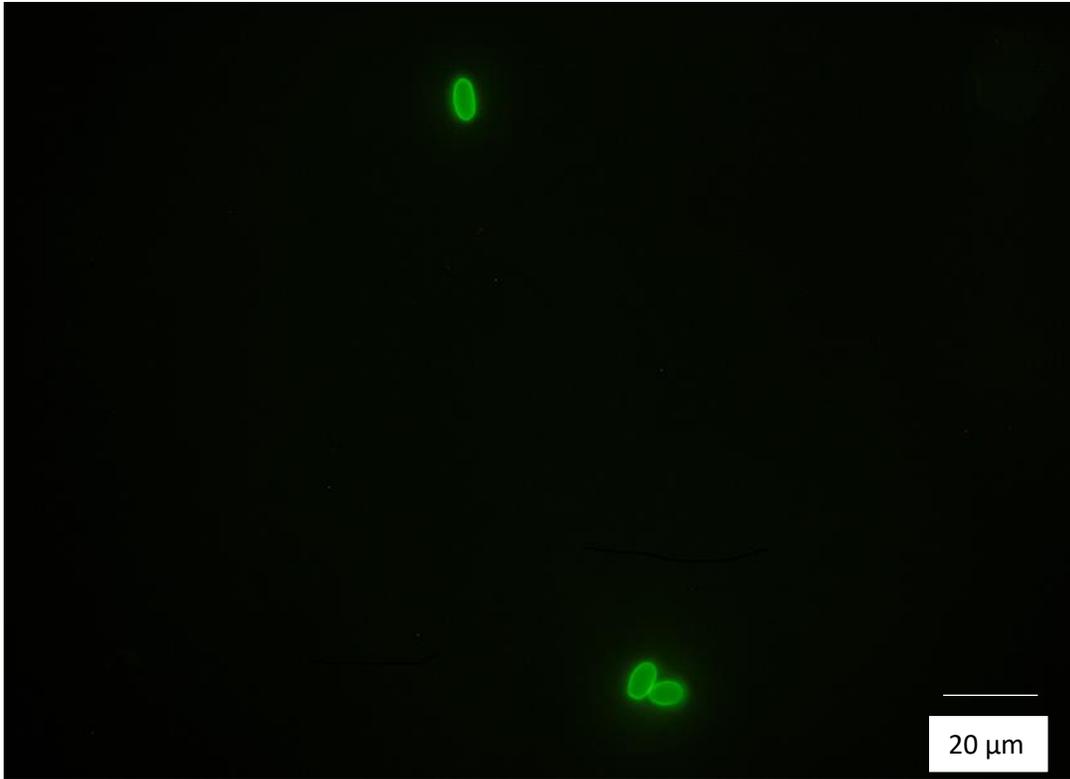


Figure 3. *Giardia* cyst stained with Fluorescence stain.



Figure 4. *Giardia* cyst's nucleus stained with DAPI stain.

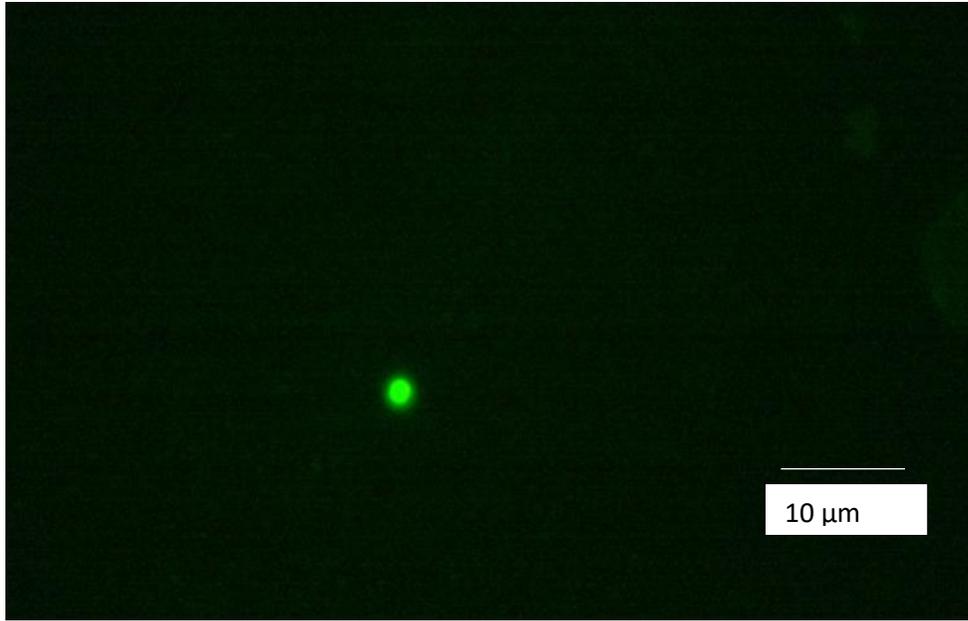


Figure 5. *Cryptosporidium* oocyst stained with Fluorescence stain.

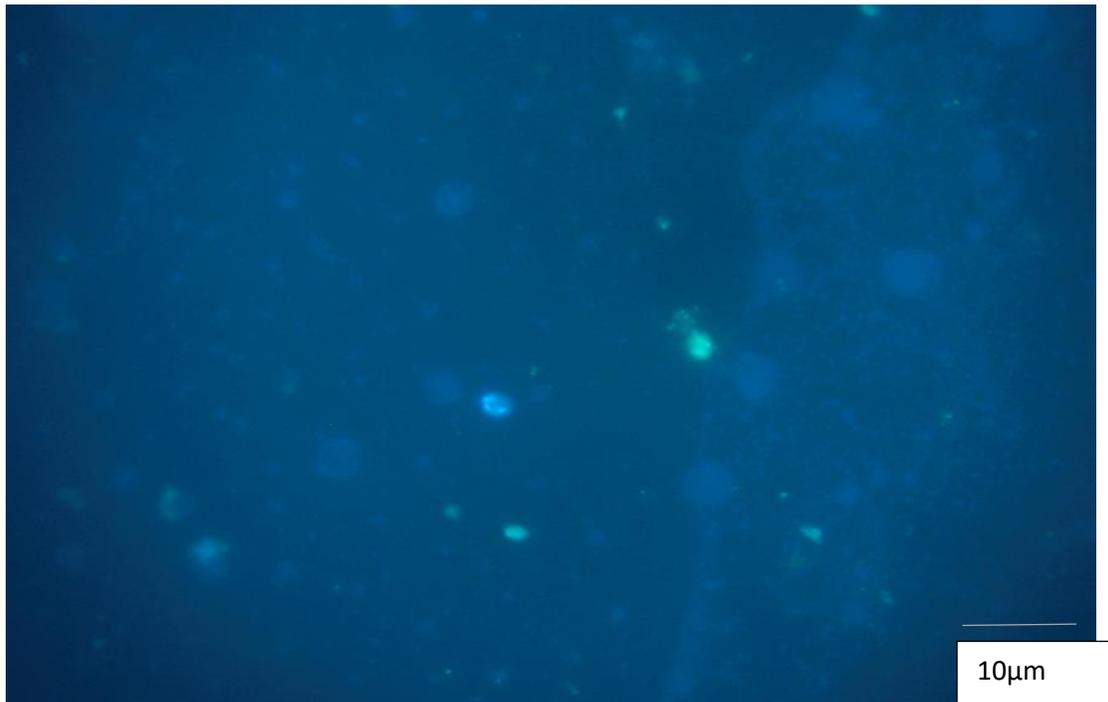


Figure 6. *Cryptosporidium* oocyst stained with Fluorescence stain.

The maximum count of *Giardia* cyst in raw water samples was detected in the inlet of Shebin DWTP (Shebin canal), which is branched from the Nile River;

the count was 7 cysts/L. The maximum count of *Cryptosporidium* oocyst in collected raw water samples was detected in the inlet of Tala DWTP (Al

Bajouriya canal), which is branched from the Nile River; the count was 22 oocysts/L, by immunofluorescent assay (Table 1).

The collected outlet samples showed the maximum count of *Giardia* cyst was detected evenly in the outlet of Shebin and Tala DWTPs. (2 cysts/L), where the maximum count of *Cryptosporidium*

oocysts was detected evenly in each DWTP (3 oocyst/L) (Table 1).

Concerning the seasonal variation in inlet samples collected from Shebin DWTP, the highest prevalence of *Giardia* was detected at 100% in the summer and autumn seasons, while the highest prevalence of *Cryptosporidium* was detected at 100% in the spring season (Figure 7).

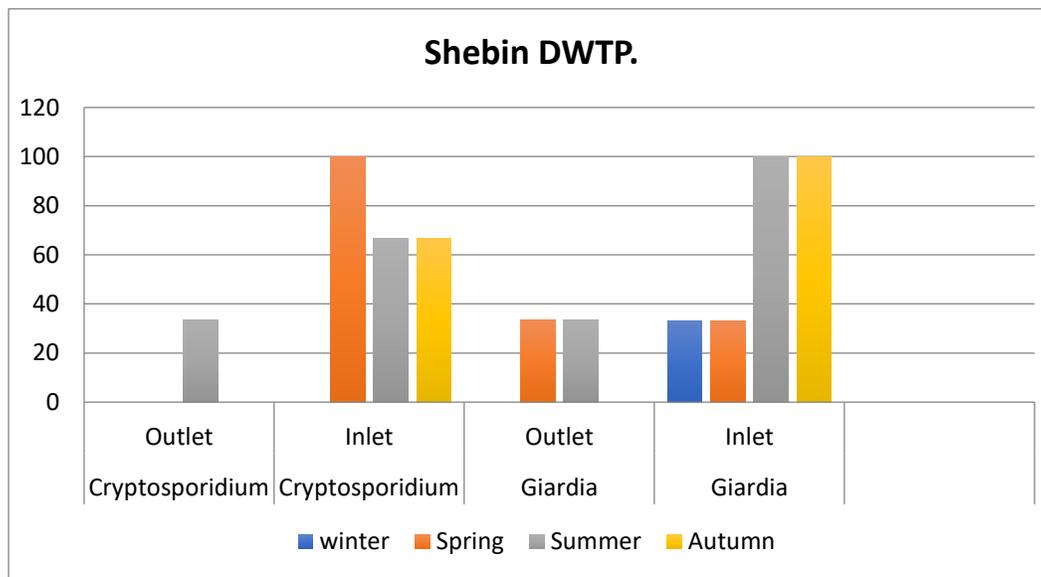


Figure 7. Seasonal variation of *Cryptosporidium* and *Giardia* in Shebin DWTP.

In the inlet samples collected from Tala DWTP, the highest occurrence of *Giardia* was detected in the spring season at 100%, while the highest occurrence of *Cryptosporidium* was detected in the summer season at 100%.

The outlet samples collected from Shebin and Tala DWTPs showed the maximum occurrence of *Giardia* was in spring and summer in 33.3% evenly; in addition, the maximum occurrence of *Cryptosporidium* in outlet samples was recorded in the summer season in Shebin and Tala DWTPs (Figure 8).

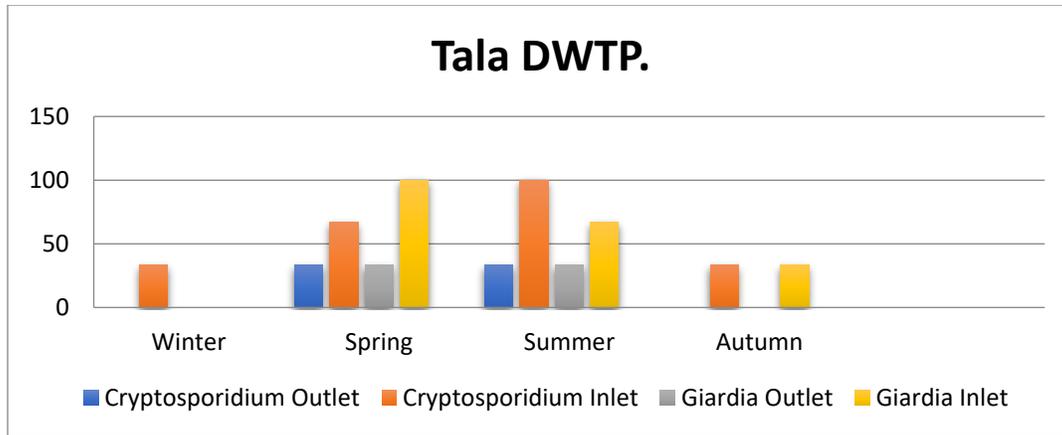


Figure 8. Seasonal variation of *Cryptosporidium* and *Giardia* in Tala DWTP.

The removal percentage of *Giardia* cyst and *Cryptosporidium* oocyst by Shebin conventional drinking water treatment plant reached 75 and 85.7 % respectively, in Tala conventional

drinking water treatment plant, the removal percentage of *Giardia* cyst and *Cryptosporidium* oocyst was 66.7 and 71.4% respectively, (Table 2).

Table 1. The count of *Cryptosporidium* oocyst and *Giardia* cyst in DWTPs.

Month	Shebin DWTP				Tala DWTP			
	<i>Cryptosporidium</i>		<i>Giardia</i>		<i>Cryptosporidium</i>		<i>Giardia</i>	
	Oocyst count /L		Cyst count/L		Oocyst count /L		Cyst count/L	
	Inlet	outlet	Inlet	outlet	Inlet	outlet	Inlet	outlet
January	0	0	0	0	0	0	0	0
February	0	0	0	0	0	0	0	0
March	0	0	1	0	11	0	0	0
April	6	0	0	0	22	1	5	0
May	3	0	0	0	0	0	6	2
June	8	0	3	2	7	0	2	0
July	9	0	2	0	6	0	3	0
August	18	3	4	1	20	3	4	2
September	0	0	5	0	10	0	0	0
October	0	0	5	0	0	0	0	0
November	7	0	7	0	10	0	0	0
December	10	0	7	0	0	0	2	0

Table 2. Removal percentage of *Giardia* and *Cryptosporidium* by Shebin and Tala DWTPs.

<i>Giardia</i>			<i>Cryptosporidium</i>		
Inlet positive samples	Outlet positive samples	Removal percentage	Inlet positive samples	Outlet positive samples	Removal percentage
8	2	75%	7	1	85.7%

<i>Giardia</i>			<i>Cryptosporidium</i>		
Inlet positive samples	Outlet positive samples	Removal percentage	Inlet positive samples	Outlet positive samples	Removal percentage
6	2	66.7%	7	2	71.4%

DISCUSSION

Water-related diseases are a major global health crisis, contributing to millions of deaths each year, especially in underdeveloped nations with inadequate access to sanitary facilities, hygienic practices, and clean water. These diseases include diarrhea, cholera, dysentery, typhoid, and other waterborne infections that thrive in environments where clean water is scarce and sanitation is poor. Protozoal contamination of water is a significant concern for public health, particularly because protozoa like *Cryptosporidium* and *Giardia* are highly resilient and can survive in water systems where traditional disinfection methods, such as chlorination, may not be effective. These protozoa can cause gastrointestinal illness, often resulting in outbreaks, especially in regions with inadequate water treatment infrastructure (Byomi et al., 2018; Zin Eldin et al., 2023; Eissa

and Harb, 2023; Eissa, 2024 and Ramzy et al., 2025)

In the present study the examination of 48 collected raw water samples revealed the occurrence of *Giardia* cyst and *Cryptosporidium* oocyst in 58.3 % (14/24) and 54.2 % (13/24) of collected raw water samples respectively, on other hand the occurrence of *Giardia*, *Cryptosporidium* and in outlet samples were 16.7 % (4/24) and 12.5 % (3/24), by immunofluorescence assay, the present study higher than the other studies such as in Colombia, the occurrence of *Giardia* (43.6%) in samples of raw water obtained from the Quindío River basin (Pinto-Duarte et al., 2022). In Malaysia and Philippines, the occurrence of *Cryptosporidium* in raw water was 4.5 and 4.3 % respectively, (Kumar et al., 2016).

In Shanghai, China, the prevalence of *Giardia* and *Cryptosporidium* in surface water (Huangpu River) samples

analyzed by IFA reached 18% (with cyst concentration 2-8 cysts/10L) and 32% (with an oocyst concentration 1.8 - 22 oocysts/10L), respectively (Feng *et al.*, 2011). *Cryptosporidium* spp were not found in the 48 water samples from Brazil (24 raw and 24 treated) according to the results of direct immunofluorescence assay. However, *Giardia* spp were detected in 8.33% (2/24) of the raw water samples (Almeida *et al.*, 2015).

Other studies higher than the present study such as in Taiwan, the occurrences of *Giardia* cysts and *Cryptosporidium* oocysts in raw water samples were 77.8% for *Giardia* and 72.2% for *Cryptosporidium* analyzed by IFA technique (Hsu *et al.*, 1999). These results were higher than raw water of the present study for *Cryptosporidium* and *Giardia*. Consequently, the occurrences of *Giardia* (76.9% with a mean concentration 1.8 cysts/10L) and *Cryptosporidium* (38.4% with a mean concentration 1.51 oocysts/10L) in Taiwan treated drinking water samples (Hsu *et al.*, 1999) were higher than that of the present study. The reason of these high results might be due to the presence of hog farming region near the domestic sewage and wastewater from agricultural practices significantly contaminated Taiwan's river water and the intakes of similar drinking water treatment facilities (Madore *et al.*, 1987).

In the current investigation, the removal percentage of *Giardia* cyst and *Cryptosporidium* oocyst by Shebin traditional drinking water treatment facility reached 75 and 85.7 % respectively, in Tala conventional drinking water treatment plant, the removal percentage of *Giardia* cyst and *Cryptosporidium* oocyst was 66.7 and

71.4% respectively, other study in Southern Brazil, DWTPs removed 100% of *Giardia* and *Cryptosporidium* (the full water treatment cycle includes catchment, coagulation, flocculation, decantation, flotation, chlorine disinfection, fluoridation, storage, and distribution) (Almeida *et al.*, 2015). This result was higher than the present.

In Taiwan the removal efficiency of *Giardia* and *Cryptosporidium* in nine potable DWTPs reached 92.5% and 95.6%, respectively (Hsu *et al.*, 1999). In Brazil, traditional DWTP had a poor removal percentage of *Giardia*, reaching 16.7%, but no removal of *Cryptosporidium* occurred. The reason for these poor statistics is that watershed catchments in Brazil's Southeast regions are made up of Water Resources Management Units (WRMUs). This watershed catchment system was in charge of delivering drinking water to millions of people following conventional treatment. It was surrounded by a metropolitan area experiencing significant population growth, with substandard urbanization and inadequate sanitation infrastructure that could have a direct impact on water quality. The sanitation conditions in the research region were deplorable, with a lack of wastewater collection, treatment, and disposal, as well as insufficient solid waste disposal (Razzolini *et al.*, 2010).

CONCLUSION

Giardia and *Cryptosporidium* are parasitic protozoa that have dangerous effects on human health. Drinking water treatment plants are considered as the first line of defense for preventing the disease occurrence accompanied by these parasites. Great effort was done by stalk holders in Egypt to eliminate any

outbreak of diseases. Drinking water treatment plants in this study eliminated around 85.7% for *Giardia* and *Cryptosporidium*. In the present study, the IMF assay was applied for getting the accurate results that represent the quality of water resources and the performance of drinking water treatment plants.

Drinking water resources protection is the critical issue. The outbreak of diseases is causing due to contaminating of water resources, which is the water supply for drinking water treatment plants. Parasitic protozoa have small size that enable them to pass through clarifiers and filters to the product water tank of the plants, so it is very important to spread awareness about keeping water resources clean to safe our health.

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