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Rasha Khalid Abd Al-Baky
General Directorate of Wasit Education, Wasit, Iraq

Corresponding Author:
Rasha Khalid Abd Al-Baky
General Directorate of Wasit Education, Wasit, Iraq

The state of *G. lamblia* and *C. parvum* contaminants in water supply and their management strategy

Rasha Khalid Abd Al-Baky

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Abstract
Giardia spp and *Cryptosporidium* spp are two well-known diarrhea-causing protozoa in humans. Water treatment is necessary to prevent human illness as the primary means of transmission for these pathogens is through the use of tainted water. The state of infections and their management strategies for the two protozoa are not currently reviewed. As a result, This article examines and analyzes the *Giardia* and *Cryptosporidium* infection status and water consumption impact in a few significant Asian, African, European, and American nations.
This review uses the data gathered to provide suggestions for hygienic control and intellectual support for the execution of programs aimed at treating drinking water.

Keywords: *Giardia* spp, *Cryptosporidium* spp, water treatment, waterborne pathogens hygienic control

Introduction
Humans can contract diarrhea from two parasite protozoa: *Giardia* lamblia and *Cryptosporidium* (Smith *et al*. 2006) [80]. Among the parasites that cause diarrhea, the most frequent non-viral infectious illnesses are cryptosporidiosis and *Giardia*sis (Cai 2005) [19]. Natural water sources frequently include *Giardia* and *Cryptosporidium*, particularly in regions contaminated by agricultural as well as trash from the raising of animals (Bukhari *et al*., 1997) [17]. The primary supply of domestic water is natural bodies of water, notwithstanding the uneven distribution of water resources across the globe. The biosafety threats posed by these two protozoa should thus be emphasized by the water treatment industry (Xiao *et al*., 2013; Zhang *et al*., 2010; Ma *et al*., 2014; Sun *et al*., 2014; Cui *et al*. 2006) [75, 80, 53, 92, 27].
Giardia is a common parasite that causes intestinal illnesses in humans. It belongs to the genus of anaerobic flagellated protozoa. The infectious stage that comes after a fecal-oral transmission is called a tetranuclear cyst. Following ingestion by humans or animals, the infectious cyst decapsulates and transforms into a trophozoite thanks to the action of digestive juices. Trophozoites proliferate through longitudinal binary fission They parasitize the small intestine's first segment or the duodenum. The trophozoites finally transform into the cysts that are evacuated through the stool as they go to the colon. encystation takes place (Kofoid & Christiansen 1915) [40].
Giardia cysts, which range in size from 8 to 12 µm to 10 µm, spreads in two weeks at 25 °C or eleven weeks at 4 °C. (Graczyk *et al*., 2008; Silva & Sabogal-Paz., 2020; Singer *et al*., 2020) [34, 84, 85]. Watery diarrhea is the clinical manifestation of cryptosporidiosis, a zoonotic disease caused by the apicomplexan parasitic alveolate *Cryptosporidium*. One of the 6 prevalent infections that cause diarrhea worldwide is cryptosporidiosis (Cheng 2015) [23]. The five developmental stages of the *Cryptosporidium* life cycle are the oocyst, zygote, gametocyte, schizont, and trophozoite. Oocyst is the name of the infectious stage of *Cryptosporidium*.
Oocysts have a diameter of 4-6 µm and can be spherical or oval. The wall is colorless and smooth. One remnant body together with four moon-shaped sporozoites are present in a mature oocyst. Following oocyst ingestion by humans or animals, the sporozoites break free enter the digestive tract and penetrate the intestinal epithelial cells.

Damage and contamination status of *Cryptosporidium* and *Giardia*
Risks and how *Giardia* and *Cryptosporidium* spread Because of their ability to spread widely and the difficulty of deactivating their infectious phases, *Giardia* and *Cryptosporidium* pose a significant biosecurity danger. These intestinal pathogenic microorganisms develop and proliferate within the host before escaping the body and ending up in the feces as cysts or oocysts, respectively.

Through tainted food or water, they can spread to other hosts (Figure 1).

Giardia and *Cryptosporidium* have diverse routes of spread and intricate, encompassing touch, water, food, and respiratory routes (Thompson 2008) [94]. The most crucial path among them is the one for water transmission. It's interesting to note that some research has indicated that unclean sex can serve as a transmission channel (Escobedo *et al*. 2014) [30]. Even nations with advanced water treatment systems, both developed and developing, may still be susceptible to *Giardia* and environmental contamination by *Cryptosporidium*. Here are some explanations for some of these causes.

Firstly, there is a broad transmission pathway for *Giardia* and *Cryptosporidium*. Drinking water quality can be guaranteed in nations with extensive water treatment technologies, but there are also dangers associated with other methods, such as vegetable contamination and interaction with animals and pets (Zhao & Aboualchamat 2020; Fayer *et al*., 2000; Chen *et al*. 2002) [31, 21, 59]. According to certain research, raw veggies can contribute to the spread of parasitic foodborne illnesses (Blackburn & McClure. 2002; Eraky *et al*., 2014) [15].

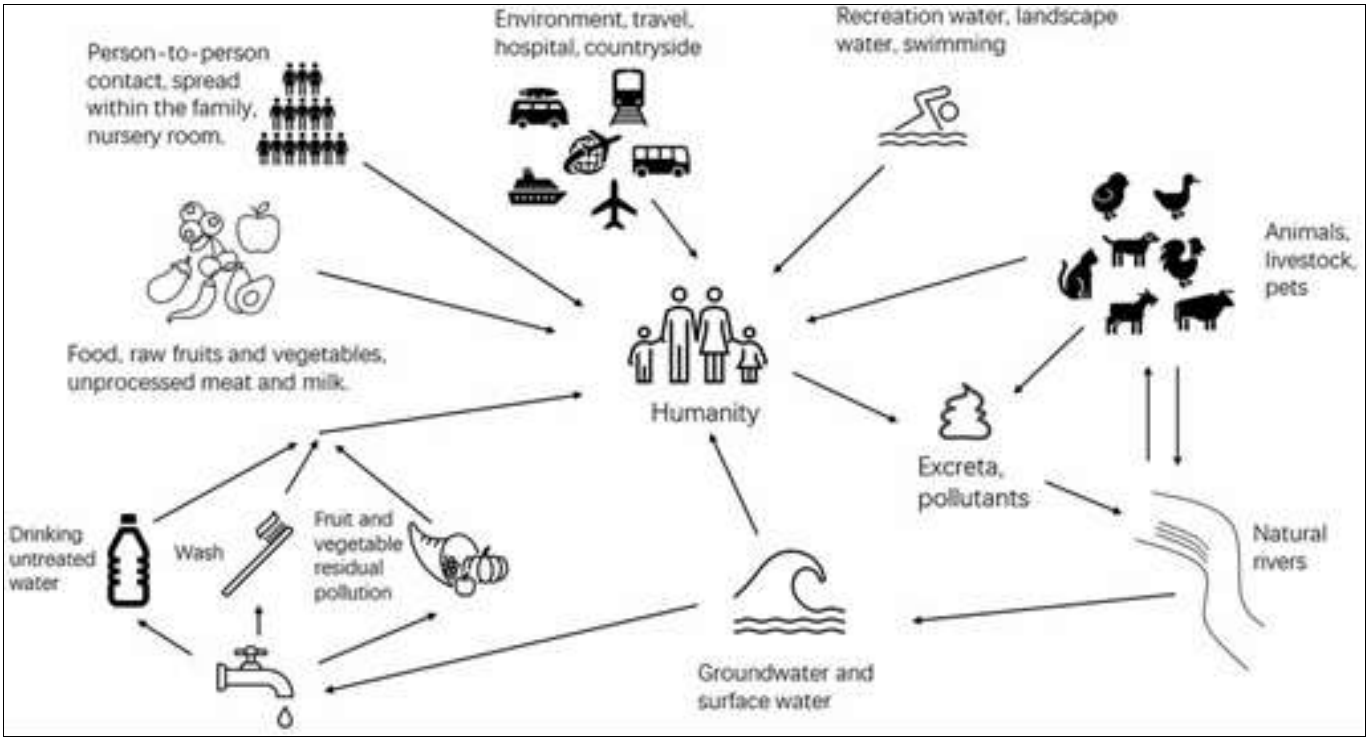

Fig 1: The transmission route of *Giardia* and *Cryptosporidium*

Secondly, G. lamblia and C. parvum are extremely contagious. According to 2009 studies by Hunter *et al*., People will use emergency water instead of properly treated or untreated water.-when there is an issue with water safety, which puts them at serious risk for contamination from cysts of *Giardia* and oocysts of *Cryptosporidium*. Xu and Hu (2007) [77] have conducted several investigations which have demonstrated that the hazardous concentrations of G. lamblia cysts and C. parvum oocysts for humans are, respectively, One to ten live oocysts and ten to one hundred live cysts. Up to one million cysts might be expelled everyday by those with *Giardia* infestations. which increases the risk of transmission and the parasite's ability to spread throughout the environment. Third, infection caused by *Giardia* and *Cryptosporidium* are very common in people. The elderly, immunocompromised persons, hospitalized people, travelers, gay men, residents of places affected by the crisis, and homeless people in the outbreak area are among the high-risk groups, with the exception of children (Escobedo *et al*. 2010) [29].

Fourth, it is difficult to treat cryptosporidiosis and *Giardia*sis. *Giardia*sis and cryptosporidiosis do not currently have a specific medication (Chen *et al*., 2018) [22]. The death rate following protozoa infection is very height in immunocompromised patients (Kotloff *et al*., 2013; Utami *et al*. 2020) [42, 95]. According to according to a few research, kids with cryptosporidiosis will experience varied degrees of cognitive development impairment along with a short-term developmental delay (Bushen *et al*., 2007) [18].

Table 1: Data on *Giardia* and *Cryptosporidium* in pets Various Domestic and International Areas Species of insects that are human can be

Parasite category	Country/region		Species	Infection rate	Human insect species can be infected in positive samples	References
Cryptosporidium	China	Henan	Totoro	10.00%	*C. parvum*	Qi *et al.* (2015)
			Birds	8.10%	*C. meleagridis*	Qi *et al.* (2011)
		Hubei	Birds	20.20%	*C. meleagridis*	Liao (2019)
		Guangzhou	Dogs	3.20–6.90%	*C. parvum*	Liao *et al.* (2020), Zheng *et al.* (2019)
			Cats	6.20%	*C. felis, C. parvum*	Zheng *et al.* (2019)
		Sichuan	Dogs	4.30%	*C. canis*	Hu (2011)
		Heilongjiang	Dogs	2.20%	*C. canis*	Yang (2015)
			Cats	3.80%	*C. felis, C. parvum*	
		Anhui	Dogs	1.50%	*C. canis*	Gu *et al.* (2015)
		Zhejiang	Dogs	1.50%	*C. canis*	
		Shanghai	Dogs	8.00%	*C. canis*	Xu (2016)
			Cats	3.80%	*C. felis*	
		Xinjiang	Dogs	6.80%	*C. canis, C. parvum*	Zhang *et al.* (2017)
	Ethiopia		Cattle	7.80%	–	Wegayehu *et al.* (2013)
Giardia	China	Guangdong	Dogs	3.10–9.40%	*G. lamblia(Aggregate A)*	Xiao *et al.* (2013), Zheng *et al.* (2019)
			Cats	3.60%	*G. lamblia(Aggregate A)*	Zheng *et al.* (2019)
		Henan	Totoro	37.50%	–	Lu (2009)
		Heilongjiang	Cats	1.90%	–	Yang (2015)
			Dogs	4.50%	*G. lamblia(Aggregate C)*	
		Anhui	Dogs	3.20%	*G. lamblia(Aggregate B & D)*	Gu *et al.* (2015)
		Zhejiang	Dogs	3.20%	*G. lamblia(Aggregate B & D)*	
		Shanghai	Dogs	6.00%	*G. lamblia(Aggregate A & B)*	Xu (2016)
			Cats	5.60%	*G. lamblia(Aggregate A & B)*	
	Russia		Dogs	4.60%	–	Kurnosova *et al.* (2019)
			Cats	9.80%	–	
			Totoro	47.40%	–	
	Colombia		Dogs	47.00%	–	Hernández *et al.* (2021)
	Ethiopia		Cattle	2.30%	–	Wegayehu *et al.* (2013)

Ethiopia, Kenya, and Egypt had child infection rates of 20%, which was significantly higher than the rates in other African nations. These countries had the highest rates of both adult AIDS and pediatric AIDS patients. The fact that there was not discernible variation in the infections rates of the 2 categories of AIDS patients suggests that *Giardia* and *Cryptosporidium* infections could coexist in these individuals. It is important to remember that the water quality and sanitation levels in these nations have a direct impact on the prevalence of *Giardia* and *Cryptosporidium* infections.

Table 2: Summarizes relevant research on immunocompromised patients and children in Asian & African nations afflicted with *Cryptosporidium* & *Giardia*

Country		Infected people	Infection rate	Infected species	References
Southeast Asia	Cambodia	Child	7.40%	*Cryptosporidium (C. hominis, C. parvum)*	Arthur *et al.* (1992)
		AIDS patient	45%	*Cryptosporidium (C. hominis, C. parvum)*	Chhin *et al.* (2006)
	Malaysia	Child	0.40–10.60%/2.60%	*Cryptosporidium/Giardia* (uncategorized)	Lai (1992), Mahmoudi *et al.* (2017), Lim *et al.* (2008)
		Child(CWD)	4.60%	*Cryptosporidium*(uncategorized)	Latif & Rossle (2015)
		AIDS patient	3–64%	*Cryptosporidium*(uncategorized)	Lim *et al.* (2005), Zaidah *et al.* (2008), Lim *et al.* (2011)
	Myanmar	Child	3.40%	*Cryptosporidium*(uncategorized)	Aye *et al.* (1994)
	Philippines	Child	2.50–2.90%	*Cryptosporidium*(uncategorized)	Mahmoudi *et al.* (2017)
		Cancer patient	28.30%	*Cryptosporidium(C. hominis, C. parvum)*	Rivera *et al.* (2005)
	Thailand	Child	15%/0.80–10.20%	*Cryptosporidium/Giardia* (uncategorized)	Chokephaibulkit *et al.* (2001), Sagnuankiat *et al.* (2014), Assavapongpaiboon *et al.* (2018), Sanprasert *et al.* (2016)
		Child(HIV)	33%	*Cryptosporidium*(uncategorized)	Chokephaibulkit *et al.* (2001)
		AIDS patient	11.50%	*Cryptosporidium(C. hominis, C. meleagridis, C. parvum, C. felis, C. canis)*	Pinlaor *et al.* (2005)
	Laos	AIDS patient	13.90%	*Cryptosporidium*(uncategorized)	Paboriboune *et al.* (2014)
	Indonesia	AIDS patient	4.90%	*Cryptosporidium*(uncategorized)	Kumiawan *et al.* (2009)
South Asia	India	Child	1.30–27.40%	*Cryptosporidium(C. hominis, C. parvum)*	Mahmoudi *et al.* (2017)
		AIDS patient	2–77%	*Cryptosporidium(C. parvum)*	
	Sri Lanka	Child(CWD)	5.70%	*Cryptosporidium*(uncategorized)	Sirisena *et al.* (2014)
	Bangladesh	Child	44–64%/40%	*Cryptosporidium(C. hominis, C. parvum, C. meleagridis)/Giardia* (uncategorized)	Steiner *et al.* (2018), Berendes *et al.* (2020)
		AIDS patient	47.10%	*Cryptosporidium*(uncategorized)	Mahmoudi *et al.* (2017)
	Nepal	Child	1–16%	*Cryptosporidium*(uncategorized)	Mahmoudi *et al.* (2017), Paudyal *et al.* (2013)
		AIDS patient	11–35.70%	*Cryptosporidium*(uncategorized)	
	Pakistan	Cancer, Diabetes, Dialysis	40%	*Cryptosporidium*(uncategorized)	Baqai *et al.* (2005)
		Child	3.30–10.30%	*Cryptosporidium*(uncategorized)	Mahmoudi *et al.* (2017)
West Asia	Iran	Child	2–7%	*Cryptosporidium(C. hominis, C. parvum)*	
		AIDS patient	1.50–7%	*Cryptosporidium(C. hominis, C. parvum)*	Meamar *et al.* (2006)
	Iraq	Child(CWD)	8.56%	*Cryptosporidium*(uncategorized)	Latif & Rossle (2015)
		Child	6–33.83%	*Cryptosporidium(C. parvum)*	Azeez & Alsakee (2017), Rahi *et al.* (2013)
	Israel	Child	1.30–31.90%	*Cryptosporidium*(uncategorized)	Mahmoudi *et al.* (2017)
	Jordan	Child	42.60%	*Giardia* (uncategorized)	Ammoura (2010)
		Child(CWD)	37.30%	*Cryptosporidium*(uncategorized)	Latif & Rossle (2015)
		AIDS patient	6%	*Cryptosporidium*(uncategorized)	
		Hemodialysis patients	11%	*Cryptosporidium*(uncategorized)	Zueter *et al.* (2019)
	Kuwait	Child	3.40–94%	*Cryptosporidium(C. hominis, C. parvum)*	Ahmed & Karanis (2020)
	Lebanon	Child	10.40%	*Cryptosporidium*(uncategorized)	Osman *et al.* (2018)
	Palestine	Child	11.60%	*Cryptosporidium*(uncategorized)	

Table 2: Continue

Country		Infected people	Infection rate	Infected species	References
		Child(CWD)	1%	*Giardia* (uncategorized)	Abu-Elamreen *et al.* (2008)
	Yemen	Child	43.70%	*Cryptosporidium(uncategorized)*	Mahmoudi *et al.* (2017)
		Cancer patient	30.1%/18%	*Cryptosporidium/Giardia* (uncategorized)	Al-Qobati *et al.* (2012)
	Saudi	Child	1.70–11%	*Cryptosporidium(C. hominis, C. parvum)*	El-Malky *et al.* (2018), Shalaby *et al.* (2014)
	Arabia	AIDS patient	8.10–69.70%	*Cryptosporidium (C. hominis, C. parvum, C. meleagridis, C. muris)*	Al-Megrin 2010, Al-Brikan *et al.* (2008)
Africa	Nigeria	Child	19.40%	*Cryptosporidium(C. hominis, C. parvum)*	Molloy *et al.* (2010)
	Egypt	Child	35%	*Cryptosporidium(C. hominis, C. parvum)*	Gharieb *et al.* (2018)
	Ethiopia	Child	4.60%/55%	*Cryptosporidium/Giardia* (uncategorized)	Wegayehu *et al.* (2016)
		Child(HIV)	9.60%	*Cryptosporidium*(uncategorized)	Gebre *et al.* (2019)
	Botswana	Child	10%/7%	*Cryptosporidium/Giardia* (uncategorized)	Alexander *et al.* (2012)
	Kenya	Child	45.20%	*Cryptosporidium*(uncategorized)	Mutai *et al.* (2020)
	Tanzania	Child	6%	*Cryptosporidium*(uncategorized)	Korpe *et al.* (2018)
	Gabon	Child	13.30%/ 15.60%	*Cryptosporidium/Giardia* (uncategorized)	Bouyou-Akotet *et al.* (2015)

Table 3: Data on the prevalence of G. lamblia and C. parvum infection in populations in certain European and American nations

Country	Infected people	Infection rate	Infected species	References
United States	Patients with diarrhea	$2.99/10^5$	*Cryptosporidium* (uncategorized)	Alleyne *et al.* (2020)
Croatia	Food industry personnel	0.07%	*Giardia* (uncategorized)	Plutzer *et al.* (2018)
	Symptoms of bowel disease	0.24%	*Giardia* (uncategorized)	
Czech Republic	Symptoms of bowel disease	0.52%	*Giardia* (uncategorized)	
Estonia	Patients with diarrhea	$(0.05/10^5)/(18.28/10^5)$	*Cryptosporidium/Giardia* (uncategorized)	
Hungary	–	0.03%/1.2%	*Cryptosporidium/Giardia* (uncategorized)	
Latvia	Patients with diarrhea	$(0.29/10^5)/(2.48/10^5)$	*Cryptosporidium/Giardia* (uncategorized)	
Poland	–	$(0.006/10^5)/(5.43/10^5)$	*Cryptosporidium/Giardia* (uncategorized)	
Romania	–	$(0.01/10^5)$	*Cryptosporidium* (uncategorized)	
Slovenia	Patients with diarrhea	1.53%	*Cryptosporidium* (uncategorized)	
Bosnia and Herzegovina	Symptoms of bowel disease	0.96%	*Giardia* (uncategorized)	
	Patients with diarrhea	9.09%	*Giardia* (uncategorized)	
Serbia	Food industry personnel	0.28%	*Giardia* (uncategorized)	
Slovakia	Child	6.30%	*Giardia (Aggregate A II, B, F)*	Pipiková *et al.* (2018)
Austria	Child	1.50%	*Cryptosporidium* (uncategorized)	Joachim (2004)
Italy	Child(CWD)	7.20%	*Cryptosporidium* (uncategorized)	
Switzerland	Symptoms of bowel disease	0.20%	*Cryptosporidium* (uncategorized)	
	Child(CWD)	5.50%	*Cryptosporidium* (uncategorized)	
New Zealand	–	$12.90/10^5$	*Cryptosporidium (C.hominis, C.parvum)*	Pipiková *et al.* (2018)
Brazil	Child	13.70–18%	*Giardia* (uncategorized)	Prado *et al.* (2003), Tei *et al.* (2007)

There is a dearth of information about infections in humans, and the majority of pertinent research is focused on animals or cattle scenarios in agriculture. In terms of human infection, very few people in European and American nations are infection with *Giardia* or *Cryptosporidium*. The fact that the majority of survey respondents were from impoverished areas in Northeast Slovakia helps to explain Slovakia's comparatively higher infected prevalence of 6.3%. This area has poor sanitation, low levels of education, and low health awareness. It also lacks sewage treatment infrastructure and water supplies

Research data are rather rich from Africa and Asia. As Tables 2 and 3 demonstrate. The data indicates that improvements in *Giardia* and *Cryptosporidium* contamination have been gradual over time, with a notable upsurge in recent years. The health system problems in certain areas including Pakistan, Bangladesh, Iraq, and India, are closely linked to the comparatively high infection prevalence among children.

Technique to Find *Cryptosporidium* and *Giardia* in Water

The U.S. Environmental Protection Agency (USEPA) first proposed the Information's Collections Rules (ICR) protozoan approach as means of monitoring *Giardia* and *Cryptosporidium*. Nevertheless, during the elution, concentration, and purification phases of this process, sample loss is common. Additionally, using the fluorescent microscope requires skilled personnel, which could result in comparatively high subjective detection results. Furthermore, *Giardia* and *Cryptosporidium* activity cannot be assessed by this method, and it is unable to further differentiate between protozoan species (Zong *et al*. 2005) [83].

The Nested PCR is extremely sensitive and specific because it employs two sets of PCR primers that are based on conventional PCR. According to Wang *et al*. (2014) [96], this method is frequently employed in the identification of harmful bacteria. For instance, Meng *et al*. (2011) [56] found water-borne *Cryptosporidium* in Xinjiang, China, using

nested PCR. In addition, Li *et al*. (2010) [100] developed a nested PCR technique for the detection of cryptosporidiosis.

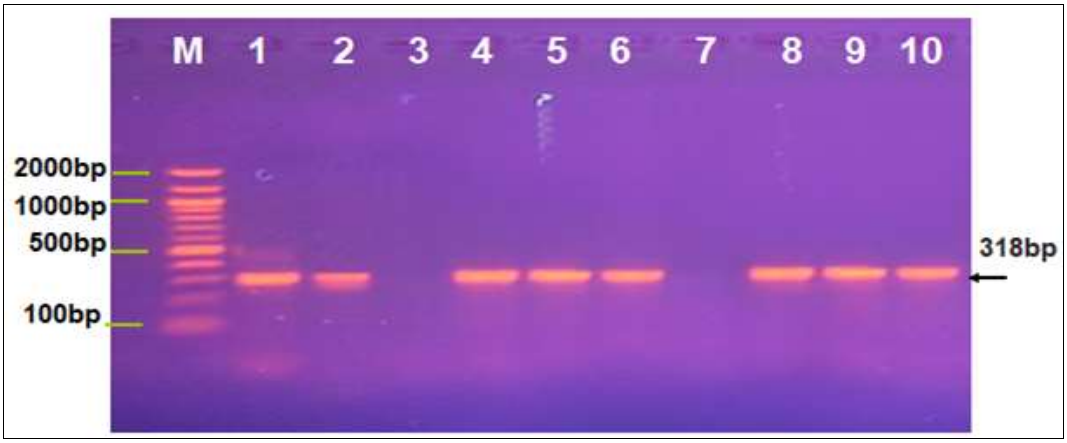

Fig 2: Agarose gel electrophoresis of a Nested PCR product subunit ribosomal RNA gene in *Cryptosporidium* spp. from 10 drinking water samples. M: Marker (2000-100bp), lanes 1-10 show *Cryptosporidium* spp. at 318bp Nested PCR product size

Figure 2 shows a Phylogenetic tree of C parvum, C hominis and C andersoni referenced against those of Gen-Bank which highlight differences by DNA STAR (Tamura et at, 2013). *Cryptosporidium* spp. 1, 3, 4, 6 and 9 were closely related to NCBI-Blast *Cryptosporidium* andersoni (10010086.1) 5, 8 and 10 to NCBI-Blast *Cryptosporidium hominis* (KT123173.1), and 2 and 7 to NCBI-Blast *Cryptosporidium parvum* isolates (MH341586.1) with a genetic difference of 0.01-0.04%. Datasets suggest strong genetic distinctiveness amongst species.

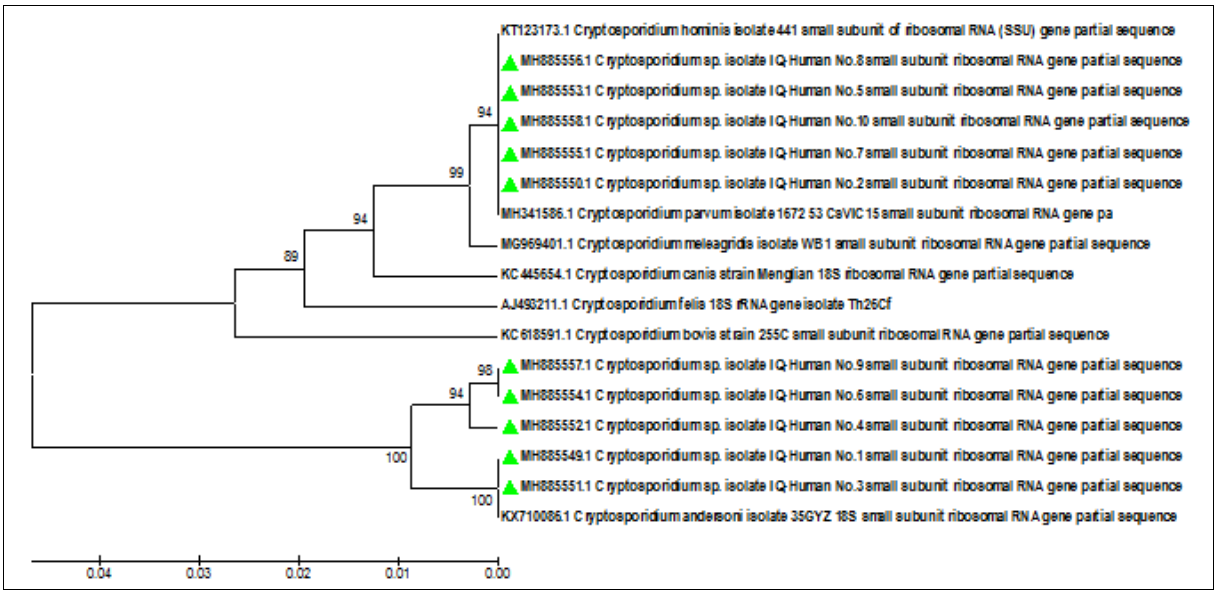

Fig 3: Phylogenetic tree analysis based on the partial sequence Small subunit rRNA gene in *Cryptosporidium* spp. isolated from stools of cattle handlers and *Cryptosporidium* species genetic Identification analysis.

In the conventional PCR amplification process, The PCR process is monitored in real time using fluorescent signals in a technique known as quantitative real-time PCR (qRT-PCR). In Southeast Asia, Malaysia, the Philippines, Thailand, and Vietnam are among the countries where *Cryptosporidium* can be found in aquatic habitats. Kumar *et al*., (2016) [43] employed Real-time PCR. The quantitative detection of *Cryptosporidium* using Real-time PCR has high specificity and sensitivity, according to the results. However, the activity of live oocysts is still neither distinguishable or detectable by this approach. The detection method known as reverse transcription PCR, or RT-PCR, uses mRNA. The PCR-derived techniques mentioned above rely on DNA for detection. The detection rate is comparatively rise because DNA can remain undamaged for a considerable amount of time after the oocysts or cysts pass away. mRNA can only be produced by live cells. This technique addresses the shortcoming of earlier Identification of molecular biology using DNA approaches.

With limited effectiveness and expensive cost, traditional PCR technique can only identify a single type of harmful bacteria included in every PCR reaction tube. Chamberlain *et al*., (1988) [20] initially introduced the idea of multiplex PCR, in their 1988 study in an effort to solve these problems. This technique can simultaneously identify several harmful bacteria,

Preserving labor and material resources is of utmost importance. As an expert in the field, Moniot *et al*. (2020) employed multiplex PCR technology to create a method that can detect intestinal microsporidia and *Cryptosporidium* simultaneously.

The investigation identified 3 strains: C. meleagridis, C. parvum, and C. hominis. Among these, C. meleagridis was the initial instance in Iran. Technology known as loop-

mediated isothermal amplification (LAMP) is relatively recent. A constant temperature accounting amplification technique suited for genetic diagnosis was presented in 2000 by the Japanese researcher Notomi. It amplifies a lot of DNA in a short amount of time at a steady temperature using strand displacement DNA polymerase, and the end product yields a lot of magnesium pyrophosphate white precipitate. Consequently, the target gene's existence is visible to the unaided eye. This approach was originally used to detect *Cryptosporidium* by Karanis *et al*. (2007) [98] because of its high specificity, ease of use, and minimal equipment requirements (PCR requires expensive equipment, whereas LAMP simply requires a water bath or incubator). As a result, this approach has enormous practical application value.

According to Table 4, the aforementioned technology is the fundamental method of detecting *Giardia* and *Cryptosporidium*. Researchers from different countries often mix many detection methods, offering a range of effective combinations, based on the features of each approach as well as the actual conditions and criteria of the test.

For instance, Hallier-Soulier & Guillot (2000) [99] used PCR technique in conjunction with immunomagnetic separation to determine the quantity of *Cryptosporidium* oocysts present in rivers. In 2003, Fontaine and Guillot employed a combination of real-time PCR technology and immunomagnetic separation technique to identify *Cryptosporidium* oocysts in Seine and tap water in France. In order to create a dual real-time fluorescent PCR detection approach for *Giardia* and *Cryptosporidium*, Liao *et al*. (2014) [49] integrated multiplex PCR with real-time fluorescence PCR, resulting in a considerably faster and more accurate system.

Drinking water treatment procedures effectively control the presence of Garidia and *Cryptosporidium*.

Oocysts of *Cryptosporidium* are smaller, less pathogenic, and more resistant to disinfectants than those of *Giardia* (Betancourt & Rose 2004) [14]. *Giardia* cysts may also be removed from the water when *Cryptosporidium* oocysts are eliminated, according to research. Because of this, investigations on *Cryptosporidium* rather than *Giardia* are more common because oocysts from the parasite are typically utilized as control targets. The logarithm is frequently used to indicate the clearance rate of *Giardia* and *Cryptosporidium*. For instance, a 99% inactivation rate is equivalent to a logarithmic elimination rate of 2.0 log. In the natural water environment, these two kinds of protozoa are widely distributed. Therefore, in order to guarantee the smooth operation of later processes and the achievement of the standard by the final effluent, Each step of the water treatment process requires that the control indications are satisfied process.

Filtration, sedimentation, and coagulation: *Giardia* cyst and *Cryptosporidium* oocyst have negatively charged exterior surfaces, Similar to other colloids with a negative charge and low density. A coagulation-sedimentation procedure can help to partially eliminate them. However, Understanding the workings of disinfectants and how they eliminate pathogens is influenced by impurity particles, as they offer a shield against harmful bacteria. (Yan & Chen 2004) [22]. Thus, the primary research directions now include selecting the right type of coagulant, selecting the right filtration technique, and controlling the amount of turbidity in the effluent.

Table 4: Compilation of methods for detecting *Cryptosporidium* and *Giardia*

Detection method	Introduction	Advantage	Disadvantage
ICR	The earliest method used to monitor Giardia and Cryptosporidium in the water environment	Qualitatively analysis	1. Large losses in the elution, concentration and purification stages can easily lead to low detection rates 2. High professional requirements and strong subjectivity 3. Unable to detect activity and distinguish types
EPA1623	The globally recognized detection method for Giardia and Cryptosporidium, which has confirmed the existence of oocysts and spore cysts, and has now been widely used	1. Higher detection rate 2. Simple operation 3. Observe the internal structure	1. More unstable factors 2. Unable to detect activity and distinguish protozoan species
ELISA	An immunological technique widely used in epidemiological testing	1. Both quantitatively and qualitatively analysis 2. High specificity and sensitivity	1. The operation is complicated in quantitative determination, and there are many influencing factors
PCR	The first molecular biology detection method applied to the detection of Giardia and Cryptosporidium	Detectable genotype	1. Unable to determine the activity of oocysts and cysts 2. Poor sensitivity and high DNA purity requirements
Nested PCR	PCR-derived detection method is one of the most effective molecular biology detections	1. High specificity and sensitivity 2. Detectable genotype	1. Difficult to quantify 2. Cross-infection may occur during secondary amplification 3. Unable to determine the activity of oocysts and cysts

qRT-PCR	A detection method for adding fluorescent chemicals in DNA amplification to monitor the total amount of products in each PCR process	1. Obvious data 2. Quantitative analysis	1. Expensive 2. Only suitable for detecting specific targets 3. Unable to determine the activity of oocysts and cysts
RT-PCR	MRNA-based detection method	1. The activity of oocysts and cysts can be determined 2. High sensitivity	1. A single reverse transcription can only amplify one gene 2. Strict operating environment requirements 3. RNA preservation is difficult
mutiplex PCR	One of the molecular biology detection methods in which multiple primers can be amplified in the same reaction system	1. High efficiency 2. Simple operation 3. Low experiment cost	1. The pairing and competitive amplification among multiple primers affect the multiplex PCR amplification 2. High quality requirements for DNA extraction 3. Unable to determine the activity of oocysts and cysts

Table 4: Continued

Detection method	Introduction	Advantage	Disadvantage
LAMP	The detection technology proposed by Japanese scholars has been applied to the detection of SARS, avian influenza, HIV and other diseases	1. Simple operation 2. High specificity and sensitivity 3. Low requirements for experimental environment and testing costs	1. Unable to quantitatively study 2. Unable to distinguish sample species
PCR-RFLP	A method that can accurately identify the genotypes and species of Giardia and Cryptosporidium	1. The operation is simple, fast and highly automated 2. Low amount of DNA required 3. Accurately perform typing research on samples	1. High requirements for digestion conditions 2. Inability to distinguish heterozygotes 3. Difficulty in distinguishing alleles

States *et al*. (2002) [90] It has been observed that the pH level has a significant impact about the elimination of Total Organic Carbon (TOC) and *Cryptosporidium* oocysts. Three coagulants-ferric chloride, alum, and polyaluminum chloride-can be used to successfully remove *Cryptosporidium* oocysts. In this case, the pH has no effect on the *Cryptosporidium* removal rates, which may exceed 4.3 log. Alum is the preferred coagulant for water treatment in Australia due to its proven ability to effectively eliminate oocysts.

According to a study by Keegan *et al*. in 2008, the clearance rates of *Cryptosporidium* oocyst is greatest from 1.0 log when the dose of alum falls between 40 and 100 mg/L. Applying the process of water purification using lime softening has the potential to Reduces a lot the presence of *Giardia* and *Cryptosporidium* in water by 2.5-3.5 log. These findings were shown in the studies conducted by Cornwell *et al*., (2003) and Logsdon & Johnson., (2010) [101, 102]. In 1995, Ongerth & Pecoraro conducted a study on the effectiveness of a specially designed filter (composed of anthracite, silica sand, and garnet) in treating two types of protozoa in a probability pool.

Many studies have shown that the three traditional waters treated procedures of coagulations, sedimentations, and filtrations are highly effective in removing *Giardia* and *Cryptosporidium*. These 2 kinds of protozoa are removal as particulates, unlike the disinfection procedure. However, it is crucial that the elimination rate of filtered water effluent is at least 2.0 log. Thus, to enhance the removal rate, adjustments must be made to the method like the amount of cysts and oocysts in the raw water rises.

Sanitization

The most important step in the water treatment process is disinfection. To guarantee the effluent's safety, the right disinfectant and operating conditions must be selected. As unique forms of protozoa in the aquatic environment, *Giardia* and *Cryptosporidium* are crucial markers for assessing the effluent's quality and disinfectant effectiveness. To give theoretical basis for engineering practices, researchers have conducted several experimental investigations on the removal of *Giardia* and *Cryptosporidium*, focusing on the effects of various disinfection techniques.

Disinfection with chlorine

In 1982, Rice *et al*. utilized chlorine disinfection to deactivate *Giardia* cysts, marking a significant milestone in the field. The study subject involved the cysts secreted by *Giardia* cyst carriers. And 2.5 mg/L of chlorine (Cl2) was employed. Despite being medical research, this is a significant advancement in the use of disinfection technologies to render *Giardia* and *Cryptosporidium* inactive. Cl2 has a negligible effect on *Cryptosporidium* and *Giardia*. The protozoa cysts and oocysts cannot be entirely destroyed by the concentration of Cl2 that was added to the water plant. When the inactivation rate hits 99%, 7,200 mg/min/L is the required CT value. Furthermore, little study has been done on using Cl2 disinfectant to inactivate *Giardia* and *Cryptosporidium* because of the numerous hidden health and safety risks associated with its use for disinfecting drinking water. Chlorine dioxide (ClO2) has a more potent lethal effect than

Cl2.

The information provided by the present requirements is useful for engineering practice as well. The link between the temperature, the CT value of ClO2, and the inactivation rate of *Cryptosporidium* is clearly indicated in Table 5 of the USEPA LT2ESWTR: Toolbox Guidance Manual.

Ozone decontamination

In a study conducted by Yu *et al*. (2008), When exposed to 1 mg/L of ozone and allowed to come into contact for 5-10 minutes, it was discovered that the inactivation rate of C. parvum oocyst might reaches 1.0 log. In a study conducted by Cho & Yoon (2007) [25], it was found that the rate of inactivation for *Cryptosporidium* oocysts varied at different pH levels. The inactivation rates were measured at 3.25, 3.10, and 2.78 mg/(L.min) for pH values of 5.6, 7.1, and 8.2, respectively. In 2005, Sivaganesan and Mariñas developed a model that utilized using first order reaction kinetics to estimate and determine the smallest CT value and provide an interval of confidence.

This was achieved by taking into account a goal percentage of survival and a particular temperature. This models provides valuable theoretical and technological assistance that can greatly benefit engineering practice. Table 6 presents an ozone-based table of comparison of CT readings for *Cryptosporidium* oocyst deactivation. The data is derived from the USEPA LT2ESWTR: Toolbox Guidance Manual. Furthermore, included Are the techniques for calculating CT values appropriate for rendering *Giardia* and *Cryptosporidium* inactive in water treatment?

Cryptosporidium Log Credit ¼ 0:0397 (1:09757)^{Temp} CT (1)

Giardia Log Credit ¼ 1:0380 (1:0741)^{Temp} CT (2)

UV protection

The chemical reagents employed in the water treatment process' disinfection step frequently result in byproducts of disinfection and pose a risk to public health. The water treatment industry is gradually implementing ultraviolet disinfection due to its broad-spectrum antibacterial characteristics and lack of byproduct generation. Numerous academics started investigating how UV radiation affects the elimination of G. lamblia and C. parvum. King *et al*., (2008) [39] investigated the effects of ambient and tap of water on C. parvum following solar radiation. The findings indicated that exposure to sunlight may lessen the infectiousness of C. parvum oocysts. The inactivation of *Cryptosporidium* oocyst by artificial and sun UV light was investigated by Soliman *et al*. (2018) [89]. According to the experimental findings, after four hours of artificial UV radiation (10 mJ/cm2) or eight hours of natural sunlight, *Cryptosporidium* is unable to re-infect mice. This conclusion offers an easy, practical, and affordable way to deactivate *Cryptosporidium* oocysts and *Giardia* cysts. Entrala and associates.

Table 5 | The CT value of inactivated *cryptosporidium* by ClO_2 (mg-min/L)

	Water temperature, (°C)										
Inactivation rate Log	<=0.5	1	2	3	5	7	10	15	20	25	30
0.25	159	153	140	128	107	90	69	45	29	19	12
0.5	319	305	279	256	214	180	138	89	58	38	24
1.0	637	610	558	511	429	360	277	179	116	75	49
1.5	956	915	838	767	643	539	415	268	174	113	73
2.0	1,275	1,220	1,117	1,023	858	719	553	357	232	150	98
2.5	1,594	1,525	1,396	1,278	1,072	899	691	447	289	188	122
3.0	1,912	1,830	1,675	1,534	1,286	1,079	830	536	347	226	147

Table 6 | The CT value of inactivated *cryptosporidium* by ozone (mg-min/L)

	Water temperature, (°C)										
Inactivation rate Log	<=0.5	1	2	3	5	7	10	15	20	25	>30
0.25	6.0	5.8	5.2	4.8	4.0	3.3	2.5	1.6	1.0	0.6	0.39
0.5	12	12	10	9.5	7.9	6.5	4.9	3.1	2.0	1.2	0.78
1.0	24	23	21	19	16	13	9.9	6.2	3.9	2.5	1.6
1.5	36	35	31	29	24	20	15	9.3	5.9	3.7	24
2.0	48	46	42	38	32	26	20	12	7.8	4.9	3.1
2.5	60	58	52	48	40	33	25	16	9.8	6.2	3.9
3.0	72	69	63	57	47	39	30	19	12	7.4	4.7

Employed a low-pressure reactor and a medium-pressure UV reactor to investigate how UV disinfection affected the clearance of *Cryptosporidium* oocysts. The two types of reactors had flow rates of 15 m3/h and 42 m3/h, respectively, according to the results. When ineffective UV radiation of 400 J/m2, *Cryptosporidium* can inactivate at a rate of 4.92

log. International recognition of the German and American UV disinfection norms is widespread (Ru & Pan 2011) [71]. Table 7 illustrates the related link between the irradiation measurement and the inactivation rate of *Giardia*, *Cryptosporidium*, and viruses, as provided by the U.S. Environmental Protection Agency's UV Disinfection Manual (USEPA-UVDGM 2006). Ru & Pan (2011) [71] applied UV disinfection to a Shanghai water facility by combining USEPA-UVDGM and German standard DVGM. The intended UV reactor can hold up to 6,627 m3/h of water at its maximum and 3,313 m3/h at its lowest. Early in the operation, the UV dose is 270 J/m2. With a long-term UV measurement of 400 J/m2, *Giardia* and *Cryptosporidium* clearance rates of 2.5-3.0 log are reached. Nevertheless, the information from USEPA-UVDGM indicates that while UV radiation can destroy *Giardia* cysts and *Cryptosporidium* oocysts, a very high exposure level is needed to destroy viruses in water.

Conclusion

1. *Giardia* and *Cryptosporidium* are intestine pathogenic bacteria with numerous modes of transmission and significant biosafety hazards. According to studies, pets frequently contract *Giardia* and *Cryptosporidium* infections. Consequently, the health department ought to start looking into cases and taking precautions against pets that have zoonotic protozoa infections. However, since drinking water treatment should remove and monitor them, since their primary mode of transmission is through water sources. There are increased instances of human diseases due to *Giardia* and *Cryptosporidium* pollution in numerous areas of China's water environments. There is variation in the amount of *Giardia* and *Cryptosporidium* reported from other nations. Pollution issues are more severe in Asian and African nations.
   Children and patients with impaired immune systems are more likely to contract infections in Thailand, India, Bangladesh, Jordan, and Egypt.
   The nation's water environment and hygienic standards are directly linked to the high occurrence of infection. As a result, it's imperative to pay special attention to high-risk sensitive groups and create better sanitary facilities.
2. Decades of advancements in diagnostics have led to the maturation of *Giardia* and *Cryptosporidium* detection technologies. There are many different detection techniques available, and many research publications combine different detection techniques. It is important for researchers to select appropriate detection techniques based on the specifics of their experiments. The LAMP approach should be promoted by international organizations in Asia and Africa as the primary means of detecting poverty with such high rates of illness.

Table 7 | Comparison table of inactivation rate and radiation dose of *Giardia* and *Cryptosporidium* by ultraviolet

Inactivation rate/log	0.5	1	1.5	2	2.5	3	3.5	4
Giardia/J/m^2	16	25	39	58	85	120	150	220
Cryptosporidium/J/m^2	15	21	30	52	77	110	150	220
Virus/J/m^2	390	580	790	1,000	1,210	1,430	1,630	1,860

3. A more effective method of treating drinking water is essential to halting the spread of *Giardia* and *Cryptosporidium* via aqueous environments and endangering public health. But this approach necessitates careful observation and stringent therapy control. In certain areas of various countries, protozoa infections still happen regularly without a facility for treating water. Membrane separation technology is a new water treatment technique that can effectively reduce *Giardia* and *Cryptosporidium*.

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