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Review Paper

Research progress on the contamination status and control policy of *Giardia* and *Cryptosporidium* in drinking water

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ABSTRACT

Cryptosporidium and *Giardia* are globally recognized protozoa that directly cause human diarrhea. Their transmission route mainly involves drinking contaminated water, thus needing proper water treatment to avoid human infection. At present, there is a lack of review on the infection status and control measures of the two protozoa. Hence, this article summarizes and compares the infection status and the role of drinking water in transmitting the *Cryptosporidium* and *Giardia* in some key countries in Asia, Africa, Europe, and the Americas. With collected data, this review offers recommendations for sanitary control and provides theoretical support for the application of drinking water treatment projects.

Key words: *Cryptosporidium*, disinfection, drinking water, *Giardia*, water treatment

HIGHLIGHTS

- Need to strengthen preventive measures of *Giardia* and *Cryptosporidium* in animals.
- Improve sanitation facilities, pay attention to non-core areas, and avoid concentrated outbreaks.
- Reasonable selection of testing methods according to the needs and conditions of each country.
- The water treatment process parameters need to be reasonably selected according to the natural water quality conditions.

INTRODUCTION

Giardia lamblia and *Cryptosporidium* are two parasitic protozoa that directly cause human diarrhea (Smith *et al.* 2006). Giardiasis and cryptosporidiosis are common non-viral infectious diseases, ranking highest among diarrhea-causing parasites (Cai 2005). *Giardia* and *Cryptosporidium* are commonly found in natural water bodies, especially in areas polluted with agriculture and animal husbandry wastes (Bukhari *et al.* 1997). Although the global distribution of water resources is uneven, the common feature is that natural water bodies are used as the main source of domestic water. Therefore, the water treatment industry should emphasize biosafety risks caused by these two protozoa (Cui *et al.* 2006; Xiao *et al.* 2013; Zhang *et al.* 2010; Ma *et al.* 2014; Sun *et al.* 2014).

Giardia is a genus of the anaerobic flagellated protozoa and a common parasite that cause human intestinal infections. The tetranuclear cyst is the infective stage following a fecal-oral transmission. Once humans and animals ingest the infective cyst, it decapsulates through the action of the digestive juice and then develops into a trophozoite. Trophozoites parasitize the duodenum or the front end of the small intestine and multiply by longitudinal binary fission. Encystation occurs as the trophozoites move to the colon and then form the cysts excreted through the feces (Kofoid & Christiansen 1915). The *Giardia* cysts measure (8–12) μm × (7–10) μm and become infective after two weeks at 25 °C or 11 weeks at 4 °C (Graczyk *et al.* 2008; Silva & Sabogal-Paz 2020; Singer *et al.* 2020).

Cryptosporidium is one of the apicomplexan parasitic alveolates, causing a zoonotic disease called cryptosporidiosis manifested clinically by watery diarrhea. Cryptosporidiosis is one of the six common diseases that cause diarrhea in the world

(Cheng 2015). The life history of *Cryptosporidium* involves five developmental stages: the trophozoite, schizont, gametocyte, zygote, and oocyst. Oocyst is the infective stage of *Cryptosporidium*. Oocysts are round or elliptical with a diameter of 4–6 μm. The wall is smooth and colorless. A mature oocyst contains four crescent-shaped sporozoites and one residual body. After humans and animals swallow the oocysts, the sporozoites escape through the digestive juice and invade the intestinal epithelial cells. Sporozoites develop into trophozoites and type I schizonts. After three nuclear divisions, type I schizonts will have eight merozoites. After being released, the merozoites invade the intestinal epithelial cells and develop into second-generation trophozoites, which develop into type II schizonts after two nuclear divisions. Mature type II merozoites contain 4 merozoites. After the merozoites are released, they invade epithelial cells and develop into female and male gametocytes, further developing into female and male gametes. After fertilization of gametes, it forms the zygote that develops into oocysts. Mature oocysts contain four naked sporozoites. There are two types of oocysts: thin-walled and thick-walled oocysts. Sporozoites of the thin-walled oocysts (~20%) invade the intestinal epithelial cells directly after escaping and proliferate to form an autologous infection. Meanwhile, thick-walled oocysts (~80%) are formed in the intestinal epithelial cells or intestinal cavity and are excreted with the host's feces. It takes 5–11 days to complete the entire life cycle of *Cryptosporidium* (DuPont *et al.* 1995; Xue 2006; Wang & Luo 2016).

HARM AND CONTAMINATION STATUS OF *GIARDIA* AND *CRYPTOSPORIDIUM*

Hazards and transmission of *Giardia* and *Cryptosporidium*

Giardia and *Cryptosporidium* have a high biosecurity risk due to their wide-spreading routes and difficulty inactivating their infective stages. These intestinal pathogenic microorganisms grow and reproduce in the host body and are finally excreted in the feces in the form of oocysts and cysts, respectively. They can infect other hosts through contaminated water or food (Figure 1).

The transmission routes of *Giardia* and *Cryptosporidium* are complex and diverse, including contact transmission, water transmission, food transmission, and respiratory transmission (Thompson 2008). Among them, water transmission is the most important route. Interestingly, a few studies have shown that unclean sex is also a transmission route (Escobedo *et al.* 2014). Even developed and developing countries with comprehensive water treatment technologies can also be vulnerable to *Giardia* and *Cryptosporidium* environmental contamination. Some of these reasons are described below.

First, *Giardia* and *Cryptosporidium* have a wide transmission route. Although countries with comprehensive water treatment technology can guarantee drinking water quality, other ways such as vegetable pollution and contact with livestock and pets still pose greater risks (Fayer *et al.* 2000; Chen *et al.* 2002; Nahhas & Aboualchamat 2020). Some studies have shown that raw vegetables play a role in spreading parasitic food-borne diseases (Blackburn & McClure 2002; Eraky *et al.*

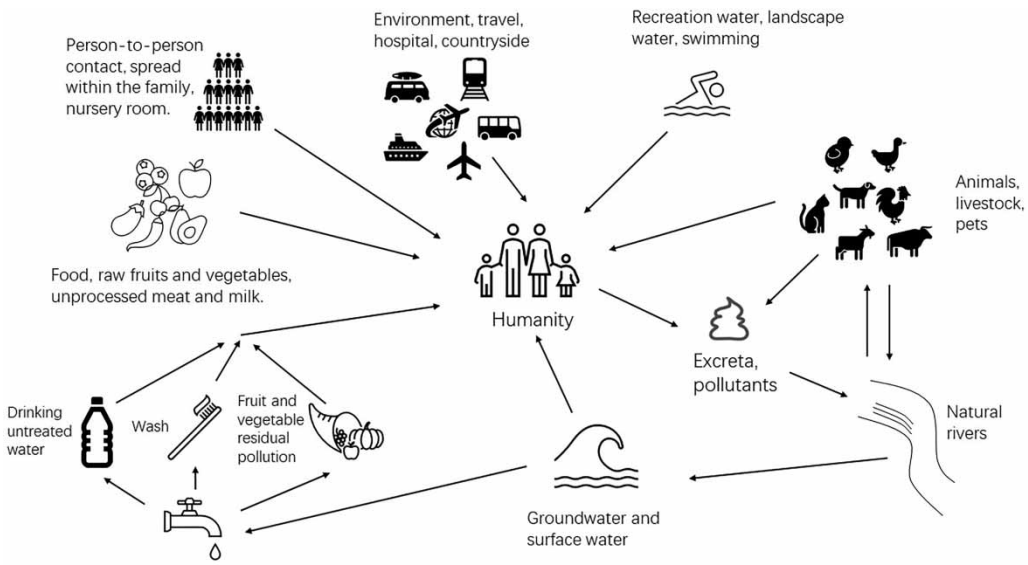

Figure 1 | The transmission route of *Giardia* and *Cryptosporidium*.

2014). Several studies have also shown that people are more susceptible to intestinal worms and protozoa by eating fruits or vegetables (Al-Shawa & Mwafy 2007; Adanir & Tasci 2013; Ismail 2016). Researches have revealed that the reason for this phenomenon may be that vegetables are polluted by wastewater during irrigation or directly contaminated by animals and humans during harvesting, packaging, transportation, processing, distribution, and sales (Amoah *et al.* 2007; Gabre & Shakir 2016). The increase in the number of pets raised has also increased the infection rate of humans with *Giardia* and *Cryptosporidium*. In particular, common pets in most areas of China are likely to be infected with *Giardia* and *Cryptosporidium*, which increases the risk of infection by human contact. A summary of reports on pet infections in China and some foreign countries is shown in Table 1.

Second, *Giardia* and *Cryptosporidium* are highly contagious. A study by Hunter *et al.* (2009) has shown that when there is a problem with water safety, individuals will substitute emergency water (untreated or improperly treated water), which are of great risks to *Giardia* cysts and *Cryptosporidium* oocysts contaminations. Many studies have pointed out that the pathogenic doses of *Giardia* cysts and *Cryptosporidium* oocysts to humans are 10–100 live cysts and 1–10 live oocysts, respectively (Xu & Hu 2007). Under *Giardia* infection, patients can excrete as many as 1×10^{10} cysts per day, thus increasing the spread of the parasite in the environment and the risk of transmission.

Third, humans are highly susceptible to *Giardia* and *Cryptosporidium* infections. Except for children, the high-risk group includes the elderly, immunodeficient individuals, hospitalized individuals, travelers, gay men, people in post-disaster areas, and homeless people in the outbreak area (Escobedo *et al.* 2010).

Fourth, giardiasis and cryptosporidiosis are hard to cure. At present, there is no specific medicine for giardiasis and cryptosporidiosis (Chen *et al.* 2018). In immunodeficient patients, the death rate after protozoan infection is extremely high (Kotloff *et al.* 2013; Utami *et al.* 2020). A few studies have shown that children infected with cryptosporidiosis will have a short-term developmental delay with varying degrees of cognitive development impairment (Bushen *et al.* 2007).

Table 1 | Statistics of *Giardia* and *Cryptosporidium* in pets in some areas at home and abroad

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)

The contamination status of *Giardia* and *Cryptosporidium*

Contamination status of *Giardia* and *Cryptosporidium* in China

China first detected a case of *Cryptosporidium* in Nanjing in 1987. After that, *Giardia* and *Cryptosporidium* infections investigations have been carried out in many areas, specifically in Xuzhou, Anhui, Inner Mongolia, Fujian, Shandong, Hunan, and other provinces and cities (Cai 1995; Fu *et al.* 1998; Gao 2008). In 2005, an epidemiological survey of *Cryptosporidium* was conducted for the first time in Chinese rural populations in Jiangshan, Zhejiang Province. The results showed that the infection rate was as high as 56.72% (Shan 2007). In 2010, the survey of the contamination of *Giardia* and *Cryptosporidium* in drinking water and environmental water in Shanghai showed that oocysts were not detected in 156 water samples collected from 16 districts in Shanghai, including factory water, pipe network water, and community drinking water. Among the 70 water samples collected from the water supply plant, water source, Huangpu river, animal feed farm surrounding a river, sewage treatment plant effluent, and domestic sewage collected in 5 districts, the total detection rate of *Cryptosporidium* oocysts was 17.1%. The total detection rate of *Giardia* cysts was 20.0% (Zhang *et al.* 2010). In 2014, survey results showed that *Cryptosporidium* oocysts and *Giardia* cysts in the water source of the centralized water supply source in rural areas in southern China had a prevalence of 23.33% and 33.33%, respectively (Sun *et al.* 2014). In the same year, the survey results of *Giardia* and *Cryptosporidium* contamination in source and drinking water in typical areas of Jiangsu Province showed that 7 out of 222 samples were positive, of which the detection rates of *Cryptosporidium* and *Giardia* were 0.5% and 2.7%, respectively (Zheng *et al.* 2014). In 2019, Wang Lu *et al.* summarized *Cryptosporidium* studies and analyzed the map of *Cryptosporidiu*m infection in China using GIS technology. Results showed that infected people were concentrated in Jiangsu, Anhui, Shandong, Henan, and other places (Wang *et al.* 2019a, 2019b). In addition, a large survey indicated that the water environment of some provinces in China (Liaoning, Northeast, Qinghai, Shaanxi, and Inner Mongolia) is seriously contaminated by *Giardia* cysts and *Cryptosporidium* oocysts. Even the water environment of first-tier cities and key projects (Shanghai, Three Gorges Reservoir) had exceeded the standard contamination rate of the two protozoa (Hou *et al.* 2011; Liu *et al.* 2011; Wang *et al.* 2012, 2019a, 2019b; Wang 2013; Xiao *et al.* 2013).

Contamination status of *Giardia* and *Cryptosporidium* in other countries

Most of the research on *Giardia* and *Cryptosporidium* infections in countries other than China is concentrated in Asia and Africa. The backward health system of most countries in Asia and Africa is the main reason for the serious situation of *Giardia* and *Cryptosporidium* infections. Several studies have shown that children under five years of age in developing countries with poor basic health conditions account for cryptosporidiosis to 30–50% of overall child mortality (Checkley *et al.* 2015; Platts-Mills *et al.* 2015). Because children and immunodeficient patients are high-risk and susceptible groups, most of the researches centered on these two groups. A summary of related studies on children and immunodeficient patients in Asian and African countries infected with *Giardia* and *Cryptosporidium* is shown in Table 2.

Infections with *Giardia* and *Cryptosporidium* are common in children in Southeast Asia. For instance, Thailand's large water systems provide a good environment for transmission. Recent research reports showed that the infection rate of *Cryptosporidium* in children in Thailand was as high as 15% (Chokephaibulkit *et al.* 2001), while the infection rate of *Giardia* was as high as 10.2% (Sagnuankiat *et al.* 2014). At the same time, the study also pointed out that the infection rate of *Cryptosporidium* in children with AIDS was 33%, twice that of normal children and 11.5% higher than that of normal AIDS patients. Children in Cambodia, Malaysia, Myanmar, the Philippines, Laos, and Indonesia are also at risk of being infected by the two parasitic protozoa. In South Asia, India and Bangladesh have the most serious infections in children, especially in terms of *Cryptosporidium* infection in which the prevalence was recorded at 27.4 and 64%, respectively (Mahmoudi *et al.* 2017; Steiner *et al.* 2018). In Bangladesh, the reported *Giardia* infection rate was as high as 40% (Berendes *et al.* 2020). West Asia is the hardest-hit area in Asia, with most infections occurring in children. For example, in a study in Kuwait, the infection rate among the surveyed groups was as high as 94%.

In Africa, the child infection rates in Ethiopia, Kenya, and Egypt were higher than 20%, far higher than that of other countries, with both ordinary AIDS and child AIDS patients showing the highest infection rates. However, there was no obvious difference in infection rate between the two AIDS patient types, indicating that these patients could be both infected by *Giardia* and *Cryptosporidium*. It is worth noting that the infection rate of *Giardia* and *Cryptosporidium* is closely related to the sanitary conditions and water environment of these countries.

Compared with Asian and African regions, other countries have fewer studies on the infection status of *Giardia* and *Cryptosporidium*. Results of the epidemiological data of some European and American countries are shown in Table 3. There are

Table 2 | Epidemiological statistics of children and immunodeficiency patients infected with the *Giardia* and *Cryptosporidium* in some Asian and African countries

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)	Kurniawan *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),
		AIDS patient	11–35.70%	*Cryptosporidium*(uncategorized)	Paudyal *et al.* (2013)
	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)	

(*Continued.*)

Table 2 | Continued

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)

Note: Child(CWD): Children with diarrhea; Child(HIV): Children with AIDS.

Table 3 | Epidemiological statistics of population infections with *Giardia* and *Cryptosporidium* in some European and American countries

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), Teixeira *et al.* (2007)

Note: Child(CWD): Children with diarrhea; Child(HIV): Children with AIDS;-: The survey population is not clearly indicated.

little data on human infections, and most of the relevant research directions are concentrated in agriculture, with research focusing mainly on animal or livestock scenario. In the case of human infection, the infection rate of *Giardia* and *Cryptosporidium* in European and American countries is extremely low. In Slovakia, the relatively higher infection rate (6.3%) can be explained by the survey participants in poor areas in Northeast Slovakia. This place lacks water supply and sewage treatment systems and has low sanitation, education, and health awareness.

In South America, Brazil has the highest infection rate (13.7–18%). The main reason is that the survey was performed in 29 slum areas in the city of Zfífola in southeastern Brazil. Secondly, Brazil has rich water resources, with freshwater resources accounting for about 13% of the world's total. In addition, the number of rivers entering the sea accounts for about 20% of the total rivers in the world. Thus, the large water system provides unique environmental conditions for the spread of *Giardia* and *Cryptosporidium*.

Tables 2 and 3 show that research data from Asia and Africa are relatively rich. Data suggest that *Giardia* and *Cryptosporidium* contamination improvements have been slow across time, and there has been a sharp increase in recent years. In some regions, such as India, Bangladesh, Iraq, and Pakistan, the infection rate in children is relatively high, directly related to the country's health system conditions. Although few relevant studies in Europe and America, the infection rate is far lower than

that of Asia and Africa. However, the data of Europe and America have not shown a steady decline. In New Zealand, human *Cryptosporidium* infection rates in 2001 and 2002 revealed a total of 33.2 cases per 100,000 and 26.1 cases per 100,000, respectively. In addition, infection rates in 2013 and 2014 were 30.3 cases per 100,000 and 12.9 cases per 100,000, respectively (Garcia-R *et al.* 2020). In Stockholm, Sweden, there were more than 300 cases of cryptosporidiosis between October and November 2019. In France, the water supply system was polluted by heavy rain, causing 92 cases of cryptosporidiosis in October 2019. Therefore, European and American countries maintain a low infection rate while steadily reducing the infection rate.

DETECTION METHOD OF *GIARDIA* AND *CRYPTOSPORIDIUM* IN WATER

The earliest monitoring method for *Giardia* and *Cryptosporidium* was the Information Collection Rule (ICR) protozoan method proposed by U.S. Environmental Protection Agency (USEPA). However, this method is prone to sample loss in the elution, concentration, and purification stages. Also, it needs experienced staff to operate the fluorescence microscope, which could lead to relatively large subjective detection results. Moreover, this method cannot evaluate the activity of *Giardia* and *Cryptosporidium* and cannot further distinguish the protozoan species (Zong *et al.* 2005).

Since 1996, USEPA began to use immunomagnetic separation technology to optimize the detection methods of *Giardia* and *Cryptosporidium*. The first release of the EPA1622 method for the detection of *Cryptosporidium* was in 1999. Then, the EPA1623 method that can detect two types of protozoa was released. This method benefits from filter cartridge filtration, immunomagnetic bead separation, and immunofluorescence microscopy detection, improving this method's recovery rate and accuracy. In addition, with the help of DAPI staining and differential interference microscope, the internal structure can now be observed to confirm the presence of oocysts and cysts (USEPA, Method 1623 2001; Sun 2007). However, this method has several drawbacks. First, the binding site of the immunofluorescence antibody is located on the outer surface of oocysts and cysts, and the cyst wall after decapsulation will still be detected, which may increase the detection rate. Second, chlorine disinfection, increasing age of parasites, and environmental changes are likely to cause inactivation of binding sites and reduce the detection rate. Third, the method still cannot clearly distinguish between activity and protozoan species. However, some researches showing the optimization of the EPA1623 process could improve the system. For example, Ye *et al.* optimized the immunomagnetic separation stage, which replaced the *Filta-Max Xpress* filter element with a filter membrane for rapid filtration. It also replaced the air compressor with an ultrasonic elution filter membrane to quickly elute the filter element. After optimization, the recovery rate of *Giardia* and *Cryptosporidium* standard addition is much higher than the recovery rate obtained by operating following the national standard (Ye *et al.* 2017).

Enzyme-linked immunosorbent assay (ELISA) is an immunological detection technique. Because of its good specificity and sensitivity, ELISA has been used often in epidemiological studies for *Giardia* and *Cryptosporidium* detection (Li 2015). Although it can be qualitatively or quantitatively measured, the operation is complicated in quantitative measurement, and many factors affect the reaction. Moreover, each group of experiments needs to measure multiple concentrations to draw a positive standard curve.

Due to the limitations of immunological technology, molecular technology has been rapidly developed and showed great potential in detecting pathogenic protozoa. Polymerase chain reaction (PCR) is a molecular biology detection method developed in the mid-1980s (Lu 2016). However, this method has strict requirements on the test environment and is often sensitive to DNA contamination. Therefore, it can be combined with immunomagnetic separation technology (IMS-PCR) to improve PCR specificity (Giovanni *et al.* 1999; Rimhanen-Finne *et al.* 2002). Other molecular biology techniques include the Nested PCR, reverse transcription-polymerase chain reaction (RT-PCR), Quantitative Real-time PCR (qRT-PCR), reverse transcription PCR (RT-PCR), multiplex fluorescent PCR, loop-mediated isothermal amplification (LAMP), and PCR-restriction fragment polymorphism analysis (PCR-PFLP).

The Nested PCR uses two pairs of PCR primers based on traditional PCR, making it highly specific and sensitive. This technique is widely used in the detection of pathogenic microorganisms (Wang *et al.* 2014). For example, Meng *et al.* (2011) used nested PCR to detect water-borne *Cryptosporidium* in Xinjiang, China. Li *et al.* (2010) also established a nested PCR method to detect cryptosporidiosis.

Quantitative Real-time PCR (qRT-PCR) is the real-time monitoring of the entire PCR process through fluorescent signals in the traditional PCR amplification process. Kumar *et al.* (2016) used Real-time PCR to detect *Cryptosporidium* in the water environment in Malaysia, the Philippines, Thailand, and Vietnam in Southeast Asia. Results showed that Real-time PCR is

sensitive and specific in the quantitative detection of *Cryptosporidium*. But this method still cannot distinguish and detect the activity of live oocysts. Reverse transcription PCR (RT-PCR) is based on mRNA for detection. The above PCR-derived methods are based on DNA for detection. DNA can still be stored intact for a long time after the oocysts or cysts die, so the detection rate is relatively high. Only living cells can produce mRNA. This method overcomes the shortcomings of other DNA-based molecular biology detection methods.

Traditional PCR technology can only detect one kind of pathogenic microorganism in each PCR reaction tube, which has low efficiency and high cost. To address such shortcomings, Chamberlain *et al.* (1988) first proposed the concept of multiplex PCR (multiplex-PCR) in their research in 1988. This method can detect multiple pathogenic microorganisms at the same time, saving workforce and material resources. For example, Moniot *et al.* (2020) established a simultaneous detection method for intestinal microsporidia and *Cryptosporidium* using multiplex PCR technology. PCR-restriction fragment polymorphism analysis (PCR-RFLP) is a technique that combines PCR amplification and restriction enzyme digestion to detect polymorphisms. It uses a specific restriction enzyme to cut the amplified product and run on gel electrophoresis. The technique is simple and can analyze samples in a short time. Therefore, it is suitable for biotyping and identifying multiple protozoan species, such as *Cryptosporidium* and *Giardia*. Rafiei *et al.* (2014) used PCR-RFLP technology to identify the types of *Cryptosporidium* infecting Iranians. The study identified three genotypes *C. parvum*, *C. hominis*, and *C. meleagridis*, of which *C. meleagridis* was the first case in Iran.

Loop-mediated isothermal amplification technology (LAMP) is a relatively new technology. In 2000, a Japanese scholar Notomi proposed a constant temperature accounting amplification technology suitable for genetic diagnosis. It uses strand displacement DNA polymerase to amplify a large amount in a short time under a constant temperature environment, and the product produces a large amount of magnesium pyrophosphate white precipitate. As a result, the presence of the target gene can be observed by naked eyes. Karanis *et al.* (2007) applied this technology to detect *Cryptosporidium* for the first time due to its high specificity, simple operation, and low requirements for equipment (PCR requires expensive equipment, LAMP only needs a water bath or incubator). Thus, this method is of great significance at the practical application level.

The above technology is the basic detection technology for *Giardia* and *Cryptosporidium* (as shown in Table 4). According to the characteristics of each detection method, combined with the actual conditions and requirements of the test, researchers from various countries often use a combination of multiple detection methods, thus proposing a variety of effective combinations. For example, Hallier-Soulier & Guillot (2000) combined immunomagnetic separation and PCR technology to detect the number of *Cryptosporidium* oocysts in rivers. Fontaine & Guillot (2003) used immunomagnetic separation technology combined with real-time PCR technology to detect *Cryptosporidium* oocysts in tap water and Seine water in France. Liao *et al.* (2014) combined real-time fluorescent PCR and multiplex PCR to establish a dual real-time fluorescent PCR detection method for *Cryptosporidium* and *Giardia*, creating a much faster and more accurate system.

CONTROL OF *GIARDIA* AND *CRYPTOSPORIDIUM* BY DRINKING WATER TREATMENT PROCESS

Compared with *Giardia* cysts, *Cryptosporidium* oocysts are smaller, have a lower pathogenic dose, and are more resistant to disinfectants (Betancourt & Rose 2004). Researchers believe that when *Cryptosporidium* oocysts are removed from the water, it could also lead to the removal of *Giardia* cysts. Hence, *Cryptosporidium* oocysts are generally used as control targets, which is why there are more studies on *Cryptosporidium* than *Giardia*. The removal rate of *Giardia* and *Cryptosporidium* is often expressed in logarithm. For example, the logarithmic removal rate of 2.0 log corresponds to an inactivation rate of 99%. These two types of protozoa are ubiquitous in the natural water environment. Thus, certain control indicators need to be reached in each stage of water treatment to ensure that subsequent processes can run well and ensure that the final effluent reaches the standard.

Coagulation-sedimentation-filtration

The outer surfaces of *Cryptosporidium* oocysts and *Giardia* cysts are negatively charged, similar to other low-density negatively charged colloids. As a result, a coagulation-sedimentation process can remove them to a certain extent. However, at the same time, because the impurity particles have a protective effect on pathogenic microorganisms, it affects the mechanism of disinfectants and the removal of pathogens (Yan & Chen 2004). Therefore, what kind of coagulant to choose, what type of filtration method, and how much effluent turbidity is controlled have become the main research directions.

The USEPA promulgated the 'Interim Enhanced Surface Water Treatment Rule' (IESWTR) on December 6, 1998. The regulation's core is that the maximum pollutant level indicator (MCLG) of *Cryptosporidium* must be set to zero. Furthermore,

Table 4 | List of *Cryptosporidium* and *Giardia* detection methods

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

(*Continued.*)

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

it is required to use only the filtration process without disinfection process, and the removal rate of *Cryptosporidium* oocysts needs to reach 2.0 log. When the content of *Cryptosporidium* oocysts in the raw water ($C_{(Cry)}$) is greater than 0.075 oocysts per litter (oocysts/L), the oocyst removal rate must reach 3.0 log. Meanwhile, when $C_{(Cry)}$ is greater than one oocysts/L, the removal rate must reach 4.0 log. When $C_{(Cry)}$ is greater than three oocysts/L, the removal rate needs to reach 4.5 log (USEPA IESWTR).

States *et al.* (2002) pointed out that the three coagulants of ferric chloride, alum, and polyaluminum chloride can effectively remove *Cryptosporidium* oocysts based on the effect of pH on the removal of *Cryptosporidium* oocysts and Total Organic Carbon (TOC). Here, the removal rate is up to 4.3 log, and pH does not affect the removal of *Cryptosporidium*. Alum is the most commonly used water treatment coagulant in Australia, with a few studies showing its good removal effect on oocysts. The removal rate of *Cryptosporidium* oocysts is greater than 1.0 log when alum dosage is at 40–100 mg/L (Keegan *et al.* 2008). Cornwell *et al.* (2003) and Logsdon & Johnson (2010) showed that the use of the lime softening method in water treatment could greatly reduce the content of *Giardia* and *Cryptosporidium* in water (2.5–3.5 log). Ongerth & Pecoraro (1995) used a designed filter (anthracite + silica sand + garnet) to explore the ability of the probability pool to treat the two protozoa. They found that when the influent turbidity is 0.38 NTU and the effluent turbidity is 0.03 NTU at room temperature, the removal rates of *Cryptosporidium* and *Giardia* can reach 3.1 log and 3.6 log, respectively.

Based on many studies, the three conventional water treatment processes of coagulation-sedimentation-filtration can effectively remove *Giardia* and *Cryptosporidium*. In contrast to the disinfection method, these two types of protozoa are removed as suspended particles. However, filtered water effluent needs that the removal rate reaches at least 2.0 log. Therefore, as the concentration of oocysts and cysts in the raw water increases, the process needs to be changed to achieve a higher removal rate.

Disinfection

Disinfection is the most critical link in the water treatment process. Choosing the appropriate disinfectant and working conditions is key to ensuring the safety of the effluent. *Giardia* and *Cryptosporidium*, as a special type of protozoa in the water environment, are important indices to evaluate the disinfection effect and the quality of the effluent. Researchers have carried out many experimental studies on removing *Giardia* and *Cryptosporidium to provide theoretical support for engineering practice*, particularly on the impact of different disinfection methods.

Chlorine disinfection

In 1981, Rice *et al.* (1982) first applied chlorine disinfection to the inactivation of *Giardia* cysts. The research object was the cysts excreted by *Giardia* cyst carriers, and the chlorine (Cl_2) concentration used was 2.5 mg/L. Although this is medical research, it is a milestone for applying disinfection technology to inactivate *Giardia* and *Cryptosporidium*. The Cl_2 has a

slight killing effect on *Giardia* and *Cryptosporidium*. The concentration of Cl_2 added to the water plant cannot completely kill the protozoa cysts and oocysts. If the inactivation rate reaches 99%, the CT value needs to be 7,200 mg.min/L. Moreover, because Cl_2 is used for drinking water disinfection, there are many hidden health and safety hazards, so there is less research on inactivating *Giardia* and *Cryptosporidium* using Cl_2 disinfectant. Compared to Cl_2, chlorine dioxide (ClO_2) has a stronger killing effect. Ran *et al.* (2011) explored the influencing factors of ClO_2 in the inactivation of *Cryptosporidium* and found that the best disinfection effect (inactivation rate is greater than 99%) is pH = 7, T = 25 °C, NTU = 1, $C_{(ClO2)}$ = 3 mg/L, and t (contact time) = 120 min. Moreover, turbidity was revealed as the main influencing factor, of which the higher the turbidity, the worse the inactivation effect. Clark *et al.* (2003) optimized the CT value equation for ClO_2 inactivation of *Cryptosporidium* and proposed a CT value equation to ensure drinking water safety effectively. The data given by the current specifications are also instructive for engineering practice. The USEPA LT2ESWTR: Toolbox Guidance Manual clearly shows the relationship between the inactivation rate of *Cryptosporidium*, temperature, and the CT value of ClO2, as shown in Table 5.

Ozone disinfection

Yu *et al.* (2008) pointed out that when the ozone dosage is 1 mg/L and the contact time is 5–10 min, the inactivation rate of *Cryptosporidium* oocysts can reach 1.0 log. Cho & Yoon (2007) showed that under the conditions of pH 5.6, 7.1, and 8.2, the inactivation rate of *Cryptosporidium* oocysts was 3.25, 3.10, and 2.78 mg/(L.min). Sivaganesan & Mariñas (2005) established a model based on the first-order reaction kinetics that can predict and calculate the minimum CT value with a confidence interval under the conditions of a given temperature and target survival rate. This model can provide technical and theoretical support for engineering practice. The USEPA LT2ESWTR: Toolbox Guidance Manual has developed a comparison table of CT values for inactivating *Cryptosporidium* oocysts with ozone, as shown in Table 6. The CT value calculation formulas suitable for inactivating *Cryptosporidium* (1) and *Giardia* (2) in water treatment are also given.

$$\textit{Cryptosporidium}\ \text{Log Credit} = 0.0397 \times (1.09757)^{\text{Temp}} \times \text{CT} \quad (1)$$

$$\textit{Giardia}\ \text{Log Credit} = 1.0380 \times (1.0741)^{\text{Temp}} \times \text{CT} \quad (2)$$

UV disinfection

The chemical reagents used in the disinfection stage of water treatment often produce disinfection by-products and bring potential harm to human health. Ultraviolet disinfection is gradually applied to the water treatment industry because of its broad-spectrum antibacterial properties and no by-product formation. Many scholars began to study the effect of ultraviolet rays on the removal of *Giardia* and *Cryptosporidium*. King *et al.* (2008) studied the impact of tap water and environmental water on *C. parvum* after being irradiated by the sun. Results showed that solar radiation could reduce the infectivity of *C. parvum* oocysts. Soliman *et al.* (2018) studied the inactivation of *Cryptosporidium* oocysts by solar ultraviolet and artificial ultraviolet radiation. The experimental results indicate that *Cryptosporidium* does not have the ability to infect mice again after 4 hours of artificial ultraviolet radiation of 10 mJ/cm^2 or natural sunlight of 8 hours ultraviolet radiation. This conclusion provides a simple, convenient, and economical method to inactivate *Giardia* cysts and *Cryptosporidium* oocysts. Entrala *et al.*

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

(2007) studied the effect of ultraviolet disinfection on the removal of *Cryptosporidium* oocysts by designing a medium-pressure ultraviolet reactor and a low-pressure reactor. Results showed that the flow rates in the two types of reactors are 15 m^3/h and 42 m^3/h, respectively. The inactivation rate of *Cryptosporidium* exposed to an effective UV dose of 400 J/m^2 can reach 4.92 log. The U.S. and German guidelines for UV disinfection are widely recognized internationally (Ru & Pan 2011). The U.S. Environmental Protection Agency's UV Disinfection Manual (USEPA-UVDGM 2006) provides the corresponding relationship between the inactivation rate of *Giardia*, *Cryptosporidium*, and viruses with the irradiation measurement, as shown in Table 7. Ru & Pan (2011) combined USEPA-UVDGM and German standard DVGM to apply UV disinfection to a water plant in Shanghai. The designed UV reactor has a maximum water volume of 6,627 m^3/h and a minimum water volume of 3,313 m^3/h. The ultraviolet dose in the early stage of the operation is 270 J/m^2. The long-term ultraviolet measurement is 400 J/m^2, making the removal rates of *Giardia* and *Cryptosporidium* reach 2.5–3.0 log. However, the data provided by USEPA-UVDGM suggests that, although ultraviolet rays can effectively kill the *Giardia* cysts and *Cryptosporidium* oocysts, the amount of exposure required for viruses in the water is extremely large.

CONCLUSIONS

1. As intestinal pathogenic microorganisms, *Giardia* and *Cryptosporidium* have a wide range of transmission routes and high biosafety risks. Studies have found that pets are commonly infected with *Giardia* and *Cryptosporidium*. Therefore, the health department should initiate investigations and prevention of pets infected with zoonotic protozoa. At the same time, water source transmission is their main way of transmission, and attention should be paid to their removal and monitoring in drinking water treatment. The pollution of *Giardia* and *Cryptosporidium* is more serious in the water environment of many places in China, and there are more reports of infections in humans. The number of reports on *Giardia* and *Cryptosporidium* in other countries is uneven. Countries in Asia and Africa have more serious pollution problems. Thailand, India, Bangladesh, Jordan, and Egypt have high infection rates among children and immunodeficient patients. The high infection rate is closely related to the country's sanitary conditions and water environment. Therefore, it is necessary to improve the construction of sanitation facilities and close attention to high-risk susceptible groups.
2. The detection technology for *Giardia* and *Cryptosporidium* has matured through decades of diagnostic developments. The detection methods are abundant, and many research contents are combining multiple detection methods. Researchers should choose suitable detection methods according to their experimental conditions. International organizations should promote the LAMP method in Asia and Africa as the core detection method for such high-infection poverty

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

areas and apply it to water environment detection and medical emergency detection in poor areas. All stages of drinking water treatment have a certain removal effect on *Giardia* and *Cryptosporidium*. The coagulation-sedimentation-filtration method is to remove them as suspended particles. The removal rate is related to the selection of coagulant, filtration method, turbidity control, among other factors. Commonly used coagulants, such as ferric chloride, alum, and polyaluminum chloride, can effectively remove *Giardia* and *Cryptosporidium* and are not affected by environmental pH. It is recommended to use the combination of anthracite + silica sand + garnet to remove the cysts and oocysts of the two protozoa. The minimum limit of the removal rate of *Giardia* and *Cryptosporidium* in the filtered water is 2.0 log, taking into account that the removal rate needs to be increased according to the content of the raw water. The different disinfection processes have different killing intensities for *Giardia* and *Cryptosporidium*. Compared with Cl_2, ClO_2 has a stronger killing effect, while ozone and ultraviolet disinfection have better killing effects among them. However, it is not recommended to use a single disinfectant. Moreover, the operating parameters should be implemented according to the USEPA standard.

3. Improved drinking water treatment process is a key link to prevent *Giardia* and *Cryptosporidium* from spreading through water sources and causing harm to human health. However, this method requires strict treatment management and close monitoring. In the absence of a water treatment facility, protozoa infections still occur frequently in some regions of various countries. As an emerging water treatment technology, membrane separation technology can achieve excellent control for *Giardia* and *Cryptosporidium*. The most widely used microfiltration membrane can reach a removal rate of up to 6.0 log. Therefore, it is recommended that the conventional water treatment methods for *Giardia* and *Cryptosporidium* involve an appropriate amount of ozone or ultraviolet based on controlling the effluent of the filtered water. It should also use a microfiltration membrane to intercept the protozoa cysts and oocysts to ensure safety. In the emergency water source treatment, the treatment method of ultraviolet + microfiltration can be directly adapted to provide a higher water treatment per unit time under the premise of ensuring water quality safety.

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CONFLICT OF INTEREST

We declare that we have no financial and personal conflicts of interest to this work.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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