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Transmission Dynamics of Water-borne Protozoa: An Insight into Current Challenges and Control Measures in Developing Countries

AUTHORS DETAIL

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INTRODUCTION

Water-borne parasitic infections are one of the main health related problems of developing countries. This is because of the reason that there is no proper sewage and drinking water supply system for their community. These parasites are the main cause of diarrhea, dysentery, fever, malabsorption, lymphadenopathy, hepatitis, lactose intolerance, enteritis, and peritonitis in both humans as well as in animals. It has been reported that till 2007, 325 outbreaks of water-borne parasitic diseases occurred worldwide. Among these, 93% outbreaks were documented from North America and Europe. According to a study, during the period of 1948-2012, round about 537 outbreaks of water related protozoa have been reported (Khan et al. 2019).

Water-borne GIT Protozoa

Most of the etiological agents for gastrointestinal infections are protozoa and belong to the phylum Apicomplexa which includes mainly *Giardia* spp., *Isospora* spp., *Sarcocystis* spp., *Cyclospora* spp., *Entamoeba histolytica*, *Cryptosporidium* spp., *Balantidium coli*, *Toxoplasma gondii* and *Acanthamoeba*, in exception of a spore forming unicellular parasite i.e. *Enterocytozoon bieneusi* (Microsporidia) (Schets et al. 2008). In this study, *Cryptosporidium* spp. and *Giardia* spp. were dominant pathogenic protozoa (Kumar et al. 2014).

Life cycle of these protozoa is very simple and usually need only single host for their multiplication. Transmission mostly occurs by feco-oral route and they multiply within the host asexually and thousands of protozoa in the form of cyst or oocyst excrete out with feces. These oocysts are their infective stage and can survive in harsh conditions like temperature, chemicals, enzymes and chlorine treatment. This simple lifecycle of protozoa makes water very favorable for their transmission. Interestingly, they are very small in size and can easily passthrough physical barriers during filtration, making difficult to purify water from these pathogens. The outbreaks by these parasites occur when water bodies like lakes, dug wells and canals got polluted with the rainfall and overflow of the sewage system. Divers and other people particularly in summer season jump fall and get pushed in the canals and ultimately got exposed to canal water. Sometime accidental ingestion of canal water also occurs (Schets et al. 2008). In urban areas of Pakistan, the drainage of sewage water in canals is a common practice. In peri-urban and rural areas, this situation is worst because of non-availability of proper municipal supply for drinking purpose and people in these areas are dependent on the use of dug wells and canal water for drinking. Moreover, this contaminated canal water is used for irrigation purpose by which our vegetables and fodders got contaminated. Humans and animals got infected when they eat them in raw form (Mumtaz et al. 2010; Alam et al. 2014).

Among these protozoa, most pathogenic are *Cryptosporidium* spp., *Giardia* spp., and *Entamoeba* spp. In acute infections, most common conditions are enteritis, diarrhea and dysentery but in chronic cases, peritonitis, enteritis, hepatitis and lymphadenopathy mostly seen. Approximately 500 million people are suffering from amoebic dysentery per year. Out of theses, 0.1 million people die every year (Ananthakrishnan and Xavier 2020). Chances of *Cryptosporidium* and *Giardia* infections are mostly seen in children and immune-compromised patients, such as AIDS patients. These protozoa in these patients cause abdominal distension, malnutrition, fever, vomiting and diarrhea. *Toxoplasma gondii* and *Sarcosystis* also have a public health concern. Except of intestinal disturbances, these parasites also cause muscle fatigue, eosinophilia and neurological disorders in humans and animals. Sporulated oocysts are their infective stage which is ingested by the humans and animals by drinking improperly purified water. *T. gondii* is an opportunistic parasite of humans and cause neurological

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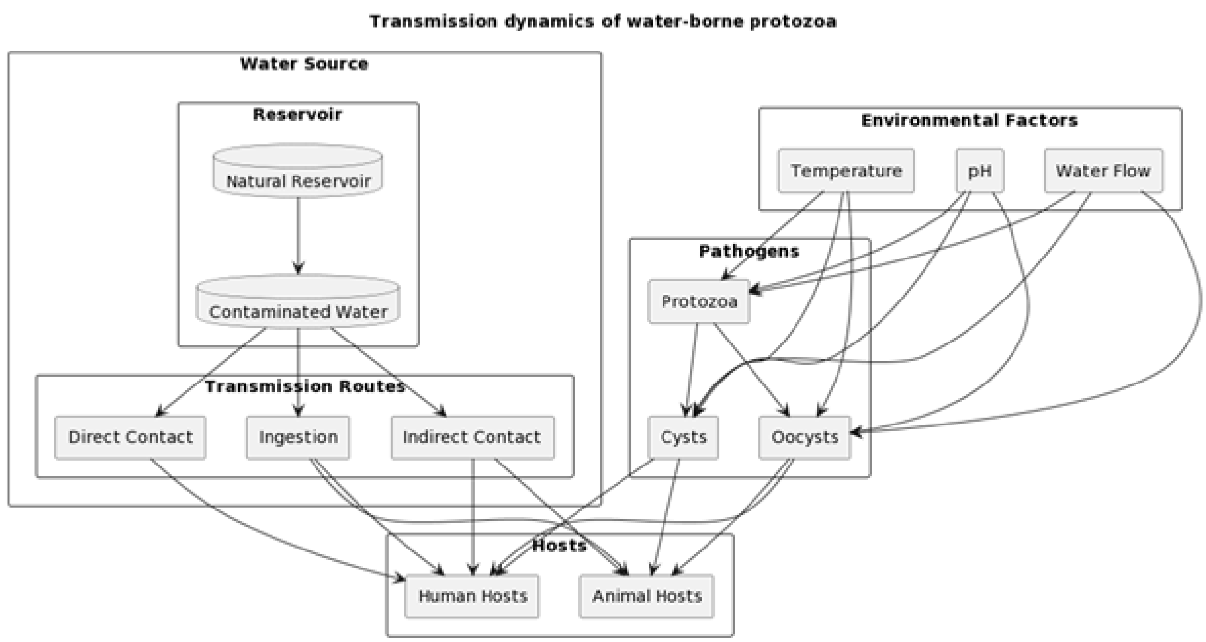

Fig. 1: Water-borne Protozoa Transmission to Hosts

diseases in newborn and abortion in adults (Squire and Ryan 2017). The Fig. 1 provides an overview of the transmission dynamics of water-borne protozoa to human and animal hosts, and the environmental factors that affect their spread.

Several methods are used for the detection and diagnosis of these parasites. Most commonly, conventional method is used in which protozoal cysts and sporulated oocysts are detected microscopically from water samples. Some serological tests like ELISA, CFT are also common in practice for parasitic detection with more sensitivity. However, this method can only detect these parasites at genus level. Molecular methods like PCR have the advantage of diagnosing these parasites at species level. Moreover, these advanced techniques have comparatively much more sensitivity and specificity (Slater et al. 2022).

Global Distribution of Water-borne Protozoa

Water borne protozoan infection is a global issue and is reported from a number of countries including Australia (Ma et al. 2022), Africa (Abuseir 2023), Bangladesh (Alam et al. 2014), Brazil (Taverne 2002), Bulgeria (Sotiriadou and Karanis 2008), Canada (Herwaldt 2000; Wallis et al. 2001; Ho et al. 2002; Murrow et al. 2002; Hopkins et al. 2013), China (Zhang et al. 2011; Lv et al. 2013; Liu et al. 2014), Ethiopia (Ayalew et al. 2011), France (Dalle et al. 2003; Villena et al. 2004; Aubert and Villena 2009), Germany (Gornik et al. 2000; Gallas-Lindemann et al. 2013), Iraq (Raza and Sami 2009), Iran (Mahmoudi et al. 2015), Ireland (Glaberman et al. 2002; Jennings and Rhatigan 2002), India (Kiran et al. 2014; Jain and Nahri 2015), Japan (Uga et al. 2005; Kourenti and Karanis 2006), Korea (Cheun et al. 2013; Moon et al. 2013), Malaysia (Mahsol et al. 2008; Kumar et al. 2014), Nepal (Sah et al. 2013), Netherland (Schets et al. 2008), New Zealand (Webber 2002), Pakistan (Ahsan-ul-Wadood et al. 2005; Mumtaz et al. 2010; Chaudhary and Chandra 2012; Khan et al. 2013; Masood et al. 2013; Alam et al. 2014), Poland (Sroka et al. 2006), Portugal (Lobo et al. 2012), Philippines (Baldo et al. 2004; Al-Hindi and El-Kichaoi 2008; Onichandran et al. 2014), Russia (Sotiriadou and Karanis 2008), Scotland (Wells et al. 2015), Spain (Perez et al. 2000), Sweden (Widerstr€om et al. 2014; Rehn et al. 2015), Taiwan (Chen et al. 2001), Thailand (Kumar et al. 2013), Turkey (Koloren and Demirel 2013; Demirel et al. 2014), Uganda (Tumwine et al. 2002),USA (Barwick et al. 2000; Ho et al. 2002; Lee et al. 2002; Murrow et al. 2002; Cope et al. 2015; Bedard et al. 2016; DeSilva et al. 2016), United Kingdom (Puleston et al. 2014; McCann et al. 2014).

Prevalence

Many research have been carried out to study the water borne protozoa due to their public health significance. According to a literature, 524 outbreaks have been documented till 2010 and most of their prevalence was found in America, Europe and Australia. In Asia, their prevalence is also significant (Karanis et al. 2007). Moreover, their prevalence is very high in peri-urban and rural areas of developing countries where people tend to use contaminated municipal water, dug well water and unfiltered canal water (Mumtaz et al. 2010; Baldursson and Karanis 2011; Masood et al. 2013; Alam et al. 2014; Kumar et al. 2016). Prevalence of various water-borne zoonotic protozoa in different countries from year 2000-2018 has been listed in Table 1.

Table 1: Worldwide prevalence of different water borne protozoa

Year	Country	Est. Cases/% Prevalence	References
		Giardia lamblia	
2009	USA	36	Bedard et al. 2016
2010	Korea	25	Cheun et al. 2013
	Bangladesh	>37%	Alam et al. 2014
	Ethiopia	41.9%	Ayalew et al. 2011
	India	55%	Jain and Nahri 2015
		Cryptosporidium Spp.	
2008	UK	422	Puleston et al. 2014
2010	Wales, UK	48	McCann et al. 2014
2010	Sweden	27,000	Widerström et al. 2014
2010	Canada	12	Hopkins et al. 2013
2011	Sweden	20,000	Rehn et al. 2015
2012	Korea	126	Moon et al. 2013
2013	USA	2780	DeSilva et al. 2016
		Entamoeba histolytica	
2009	Tajikstan	25.9%	Matthys et al. 2011
2011-2012	Nepal	6.1%	Sah et al. 2013
2013	India	25.4%	Kiran et al. 2014
2013-2014	Pakistan	5.9%	Chaudhary and Chandra 2012
		Toxoplasma Gondii	
2009-2010	Iran	5.9%	Mahmoudi et al. 2015
2012	Pakistan	7%	Khan et al. 2013
	Turkey	51.6%	Koloren and Demirel, 2013
2013	Turkey	13.2%	Demirel et al. 2014
2013	Scotland	8.8%	Wells et al. 2015
2015	Colombia	76.9%	Triviño-Valencia et al. 2016
		Enterocytozoon bieneusi	
-	China	9	Zhang et al. 2011
-	Portugal	54	Lobo et al. 2012

Transmission of GIT Protozoa through Water

Water is a necessity for almost all living beings. But it also provides a suitable and favorable route for the transmission of gastrointestinal protozoa. Once an animal or human got infected by any of the protozoa, it starts shedding a massive amount of infected cyst/oocyst in the environment. Due to close interaction of animals and humans with the natural sources of water, there are greater chances of infecting these sources (Bozorg-Haddad et al. 2021). Additionally, these water-borne protozoa may reach to ground water by infiltration of contaminated surface waters. Most reported concentrations of infected cyst/oocyst in water are up to 150/liter of water. However, greater concentrations have also been reported from different lakes, ponds, rivers, canals, furrows, sewage systems, municipal water and even in mineral water. *Giardia* and *Cryptosporidium* have been reported as the most frequently associated water-borne pathogens. Most deadly episode of *Cryptosporidium* outbreak was occurred in 1993 in USA when 0.4 million people got hospitalized causing an estimated economic loss of $96.2 million (Lee 2019). Several outbreaks of other water-borne protozoa have also been documented in different regions of the world (Mchardy et al. 2014).

The most common cause of diarrhea is protozoan infections in humans as well as in animals. *Cryptosporidium* spp., *Giardia* spp., *Enterocytozoon* and *Cyclospora* spp. are the main GIT protozoa causing diarrhea. This is the conclusion of a research done in China during 2012-2013. Fecal samples of 252 diarrheal patients had been collected and examined with nested PCR. Out of these 252, 76 samples were positive for any one of these four parasites (Liu et al. 2014). A study was conducted in Philippines for the awareness of water contamination with protozoa most likely *Cryptosporidium* spp., *Giardia* spp., *Acanthamoeba* and *Naegleria*. 33 samples from rivers, lakes, ponds, swimming pools and drinking water of peri-urban and rural areas were collected, and tests were positive for *Cryptosporidium* spp. and *Giardia* spp. by counting oocysts/liter. And PCR test for *Acanthamoeba* were also positive as well (Onichandran et al. 2014).

In France, a case was presented by in a hospital with severe peritonitis and severe abdominal pain. The patient was a butcher and was addicted to alcohol. When the case was studied, they found that he was suffering from *Balantidium coli*. This parasite is very common in wild animals and pork. This parasite can easily be transmitted by ingestion of food and drinking contaminated water. For this patient, specific antibiotic with metronidazole was given for peritonitis and to stop bloody diarrhea (Ananthakrishnan and Xavier 2020). A comprehensive study was performed on outbreaks of water-borne protozoan infections during the period of 2004-2010. A total of 199 outbreaks were reported during this time. These outbreaks occurred in Australia, South America, and Europe. Prevalence of *Cryptosporidium* spp., *Giardia lambia*, *Toxoplasma gondii*, *Cyclospora cayetanensis* and *Acanthamoeba* was reported as 60.3%, 35.2%, 2%, 1.5% and 1%, respectively (Baldursson and Karanis 2011).

Pregnant women were found the most susceptible host for the opportunistic parasites and these parasites were found to be very dangerous for not only the mother but also for the new borne babies. *Toxoplasma gondii* is found to be very prevalent in many European countries such as Belgium with 48.75% prevalence (Gebremedhin 2019) in pregnant women or those which were just given birth to babies. Similarly, 25.4% (Glynou et al. 2005), 21.2% (Kansouzidou et al. 2008) in Greece, 24.6% in Ireland (Ferguson et al. 2008) and 19.8% (Masini et al. 2008) prevalence was recorded in Italy. This parasite was found to cause neurological disorders in new borne babies and children of young ones.

Another study was conducted to check the prevalence of *Cryptosporidium parvum* and *Giardia lambia* in water samples from different countries of Southeast Asia. Total 221 samples of size 10 litter each from Malaysia, Thailand, Philippines and Vietnam were collected. Theses water samples were examined with respect to the methods of United states Environmental Protection Agency microscopically observed and subsequently screened using RT-PCR assays. From treated water samples *Cryptosporidium* oocysts were detected at the rate of 0.06 ± 0.19 oocyst/Liter concentrations while from non-treated water samples at the range of 0.13 ±

0.18 to 0.57 ± 1.41 oocyst/Liter concentrations. Similarly, *Giardial* cysts which were detected in treated water of Philippines at concentration of 0.02 ± 0.06 cyst/L while from untreated water samples at concentration of 0.12 ± 0.3 to 8.90 ± 19.65 cyst/L. This study revealed the potential risk to human population of these countries (Kumar et al. 2016).

Toxoplasmosis is a worldwide problem now a days and it is most common in females. Situation is worst in pregnant females around the globe. Toxoplasmosis was found in different states of America. Prevalence of *Toxoplasma gondii*, in Brazil was recorded 51.2% (Avelino et al. 2004), 60% (Olbrich-Neto and Meira, 2004), 70.6% (Leao et al. 2004), 77.5% (Porto et al. 2008), 61.2% (Carellos et al. 2008) and 48.7% (Rosso et al. 2008) was documented in Columbia. In Pakistan, a few years ago, drinking and surface waters have been examined and the occurrence of *Cryptosporidium* spp. and *Giardia lamblia* in these samples has been associated with diarrhea in animal and human population. In recent, samples of tap water, pond water, dug well, bore well water, hand pump water from KPK were examined and the prevalence of *Cryptosporidium parvum* and *Giardia lamblia* has been documented 36% (Alam et al. 2014).

Similarly, samples were taken from patients who were suffering from diarrhea with acute abdominal pain, and they were found positive for *Entamoeba histolytica*, *Giardia lamblia*, *and Cryptosporidium parvum.* It has been observed that these people had poor socio-economic status and lack of facilities for purified or drinking water and they were tending to use contaminated water. Another study was conducted in Pakistan and the water samples were examined for the prevalence of water borne parasites. It has been observed that the prevalence of *Cryptosporidium parvum* and *Giardia lamblia* was highest in humans as well as in animals causing a huge economic loss (Masood et al. 2013). *Toxoplasma gondii* is an important zoonotic and opportunistic parasite. Basically, it is transmitted by several routes and water is also a source for its transmission. To study the sero-prevalence of this parasite in human population, studies conducted in different countries in last decade were compiled just to overview the worldwide occurrence of this parasite (Pappas et al. 2009).

Toxoplasma gondii is an opportunistic parasite of human. It infects men, women and even children. Having an opportunistic property, this parasite was found to be very prevalent in Immuno-compromised people such as HIV/AIDS patients. Because of the Immune deficiency of such people, this parasite attacked the central nervous system and causes nervous disorders and histopathology of the samples collected from their brain tissues, showed numerous lesions in the brain cells of *Toxoplasma* infected patients (Lago et al. 2009).

In 2006, two lakes and three rivers were suspected to have contamination with water borne protozoa. So, total 57 samples were collected from these natural sources and examined with molecular methods such as Immunofluorescence (IMS-IF) for *Cryptosporidium* and *Giardia* followed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and it has been observed that out of 57 samples, *Giardia* and *Cryptosporidium* cyst were detected at the rate of 165cyst/10L. Meanwhile, from these samples *Enterocytozoon bieneusi* was also found in 2 river samples. No respective co-relation was found in prevalence of bacteria and protozoa (Coupe et al. 2006).

Suitability of Protozoan Parasites to Waterborne Transmission

Many of the protozoan parasites have common physical and biochemical features which make them resistant to ecological stresses and help in successful dispersal in the aquatic environment. Following are some characteristic features which make these parasites to survive in the aquatic environment:

Shedding of Cysts/oocysts in Huge Amount

One of the characteristic features of these parasites is the asexual reproduction in which one cyst/oocyst can produce thousands of protozoa within the infective host. It enhances the probability of survival and transmission of these parasites in the environment. For example, infected cattle with *Cryptosporidium* shed 10^{6-8} oocysts/g of feces for 3-12 days which clearly indicates the huge impact of cattle in transmitting infective *Cryptosporidium* to the environment. Similarly, humans also play a significant role in spreading these parasites to the environment and contamination of different water bodies and recreational water sources. A clean example of contribution to contamination is that infected humans can shed 10^{9} cysts of *Giardia* every day (Savioli et al. 2006).

Persistence in the Aquatic Environment

Protozoan parasites especially, *Giardia lamblia*, *Cryptosporidium parvum*, *Toxoplasma gondii*, *Entamoeba histolytica* and *Balantidium coli* are highly resistant to the harsh environmental conditions. They can survive for months due to their outer protective shell. However, in the aquatic environment, their survival is significantly affected by increase in temperature. Most of these parasites usually survive for 45 days at 30^{0}C. But their survival goes on decreasing with increase in temperature and at 22^{0}C, they can only survive for 45 days. Similarly, very low temperature also affects the viability of oocysts of these parasites. For example, cysts/oocysts of these parasites can live only for 24 hours at -20ºC. The viability and infectivity of cysts/oocysts of these parasites is also affected by solar radiation, freeze-thaw cycles, and desiccation (Smith et al. 2006).

Smaller Size of their Cysts/oocysts

Most of the protozoa have a very smaller size ranging from 1µm to 50µm. However, Balantidium coli is about 150µm long. Due to their smaller size, they have a very low specific gravity due to which they continue floating in the water. Some researchers stated that sedimentation rate is higher regarding the occurrence of these parasites in water due to attachment of their cysts/oocysts with suspended particles. However, other researchers stated controversially and stated that they live freely which makes them more consistent and facilitates their transport to other water bodies. Due to this characteristic feature, they can pass any physical barrier like filtration process. Even, these parasites can also pass-through well-designed treatment systems which allow these parasites to expose the public communities (Savioli et al. 2006).

Resistance to Chemical Disinfectants

Protozoan parasites are highly resistant to chlorine-based disinfectants at optimum concentrations and exposure times which are commonly used practices in water filtration industries. Even, if the chlorine concentration is increased which might help in killing these parasites, it may lead to increased concentration of toxic byproducts within the water such as halomethanes. It illustrated the failure of the disinfection method used for cleaning the water. The best method to disinfect the drinking water is by using absolute-sized filtration paper (smaller pour size than parasitic cyst/oocyst) and appropriate disinfectant under optimum conditions (Betancourt and Rose 2004).

High Infectivity Rate

Generally, the infection after exposure to these parasites depends upon immune status of the host, number of cysts/oocysts ingested and associated risk factors. In any case and condition, a very few cysts/oocysts (5-40) are enough to cause infection in the host. For example, 10-30 oocysts of *Cryptosporidium parvum* are enough of cause infection in any kind of host including animals and humans. Similarly, 25-100 cysts are enough to cause medium infection in humans. Nevertheless, it is even unclear how many cysts and oocysts of parasites are present in the drinking water, but they do cause infection after ingestion. The reason behind this infection by a single cyst/oocyst is the asexual reproduction by which they can multiply in hundreds and thousands (Smith et al. 2006).

Surveillance and Control Measures

Due to high public health concern, water-borne parasites have become a major challenge for the sewage disposal and water industry which is responsible for providing safe drinking water to the world population. In this regard, different developed countries like USA, New Zealand, Australia and Canada have established some standards and regulation to their water industries including turbidity monitoring, removal of cyst/oocyst through proper filtration process and inactivation of detected water-borne pathogens. Unfortunately, none of these authorities made a standard for cyst/oocyst monitoring of water-borne protozoa. Moreover, these authorities were also unable to provide information regarding the protozoon species as well as their infectivity to the human population. In contrast, monitoring of cyst/oocyst in the drinking water is compulsory on regular basis in England, Ireland and wales. These countries have made a standard of existing less than one cyst/oocyst $10L^{-1}$ in drinking water provided by the water industry regardless of their viability and infectivity to humans. Regardless of their public health concern, presence of more than one cyst/oocyst $10L^{-1}$ in the water has been considered a critical question on the quality and standards of water-providing company in these countries (Carmena 2010).

Based on epidemiological studies of water-borne parasites and their worldwide outbreaks, scientists have made an action threshold level for the presence of cysts/oocysts in the water. It means that if the concentration of cyst/oocyst exceeds 3-30 cysts/oocysts $100L^{-1}$ of provided water, immediate action should be taken for the detection of these cysts/oocysts through most appropriate method to get the information regarding the infectivity as well as exact concentration of cyst/oocyst in the provide water. Mathematical and statistical methods have been a useful tool for checking the probability of outbreaks associated with water-borne protozoa (Casman et al. 2000; Pouillot et al. 2004).

Conclusion

Water is a main source for the transmission of gastrointestinal parasites. Most important gastrointestinal parasites are *Giardia* spp., *Isospora* spp., *Sarcocystis* spp., *Cyclospora* spp., *Entamoeba histolytica*, *Cryptosporidium* spp., *Balantidium coli*, *Toxoplasma gondii* and *Acanthamoeba* and *Enterocytozoon bieneusi*. These parasites have a cosmopolitan distribution and cause huge morbidity and mortality. These parasites mostly cause diarrhea and dysentery. The situation of illness is worse in young children and immunocompromised patients. Due to some characteristic features like smaller size, resistance to chemicals, high reproductivity and infectivity rate, they are suitable for water-borne transmission. There is no appropriate method for the removal and inactivation of cyst/oocyst in the water. However, surveillance and control measures are the only options to control the parasitic transmission through water. Exposure of animals to the natural sources of water should be stopped or minimized. Sewage water should be properly disposed of and irrigation of agricultural land with the sewage water should be stopped. There should be two-way treatment of water before use. Firstly, proper filtration and secondly should be treated with UV light, ozonization and again membrane filtration. By using such preventive and treatment measures, water-borne transmission of gastrointestinal parasites could be stopped or minimized.

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