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Waterborne transmission of protozoan parasites: a review of water resources in Iran – an update 2020

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ABSTRACT

Waterborne protozoan parasitic diseases are considered a global disease. With no comprehensive review for the country, we evaluated only the available studies (n = 27) related to the waterborne transmission of protozoan parasites in Iran. The most commonly reported protozoan parasites were *Acanthamoeba* spp., *Naegleria* spp., *Vahlkampfiid* spp., *Cryptosporidium* spp., *Giardia* spp., *Hartmannella* spp., *Saccamoeba* spp., *Entamoeba* spp., *Blastocystis* spp., and other free-living amoeba. Immunofluorescence assay (IFA), polymerase chain reaction (PCR), loop-mediated isothermal amplification (LAMP), differential interference contrast (DIC), microscopic detection, and enumeration methods are the most reported methods to identify protozoan parasites in water samples. The literature showed untreated water, recreational water, and contaminated water supply sources by animal feces as the main routes of waterborne parasites transmission. No study reported standard protocols to identify protozoan parasites. In addition, the investigation of protozoan parasites in water resource has not been performed all around the country. Iran, as a developing country, use surface water to supply drinking water. Many recreational rivers and springs can be potentially contaminated with parasitic protozoa and thus reliable surveillance systems need to be established. Furthermore, quick and applicable methods to survey the parasitic protozoa in water are necessary.

Keywords: Water contamination; Waterborne disease; *Giardia*; *Cryptosporidium*; Entamoeba; Balantidium; *Acanthamoeba*; *Naegleria*

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1. Introduction

Drinking water is vital for our survival, but if the physical, chemical, and microbial quality of water does not meet the regulatory standards, it can cause serious complications and threaten human welfare and health [1]. Many of the health problems associated with drinking water in developing countries are due to a lack of access to safe drinking water [2]. Drinking water can be considered as one of the most important environments for the transmission of pathogens [3]. It is estimated that about one-third of the world's population suffers from parasitic infections [4,5]. Consumption of contaminated water is one of the ways of water-borne parasitic agent transmission [6]. Although the prevalence of waterborne diseases is not common in developed countries, waterborne diseases still occur and can lead to serious consequences for acute, chronic, and sometimes fatal health, especially in sensitive populations.

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From 1971 to 2006, there were 833 drinking water-associated disease outbreaks in the United States, leading to 577,991 individuals having parasitosis and 106 deaths [7]. In developing countries, the most common cause of food-borne associated death is diarrhea [8]. In 2004, for example, 1.87 million children under 5 y of age died as a result of diarrhea worldwide, of which 1.46 million were estimated to be from developing countries [9]. It accounted that 1.7 billion cases were caused by water-related parasitic diseases responsible for 842,000 deaths/y [10]. According to the World Health Organization (WHO) mortality database, protozoan parasites ranked in the second common etiology cause of death among children under 5 y of age [10,11]. The waterborne protozoan parasites cause disease through outbreaks in both developed and developing countries [10,12]. There is a global increasing trend for diseases caused by waterborne or water-washed parasites [12]. The water resources quality, water treatment system, distribution network, and system maintenance are the possible cause of waterborne protozoan parasites transmission [12,13]. The presence of protozoan parasites in water is categorized as microbiological contamination of water. Although there are microbial tests that should be performed daily, there is no standard procedure to test for the presence of protozoan parasites in water treatment facilities. On the other hand, the most diagnostic method is limited to clinical samples that may be not be applicable to identify of protozoan parasites in water samples [14]. The available methods need threshold concentrations of cysts or oocysts and water samples have a lower concentration than clinical samples and as such are difficult to detect [14]. Additionally, the water samples have unknown interfering factors that affect the diagnostic method, which are generally expensive. Among the 15,000 identified protozoa species, four categories of parasitic protozoa are known to cause health impacts including Sarcodina, Flagellata, Sporozoa, and Infusoria [10]. Important protozoa that are prone to water transmission include *Giardia duodenalis* (syn. *Giardia intestinalis*, *Giardia lamblia*) (cause of giardiasis), *Cryptosporidium parvum* (human cryptosporidiosis), *Entamoeba histolytica* (cause of dysentery), and *Balantidium coli* (cause of Balantidiasis, also known as balantidiosis) [10,11]. These pathogens enter drinking water in various ways and cause disease in humans. Table 1 presents some of the most important protozoan parasites transmitted through water and their clinical symptoms.

The following sections will describe cryptosporidiosis and giardiasis as the most important protozoan illnesses that can be transmitted through water. In addition, we have focused on acanthamba and *Naegleria* as they have been identified as the most prevalent protozoan parasites in water resources in Iran.

1.1. Cryptosporidiosis and giardiasis

Cryptosporidiosis and giardiasis are the most prevalent diseases that can be transmitted by water [10,11]. These zoonotic parasites are the most commonly reported during outbreaks related to contaminated water resources [11]. Among the causes of water contamination, human wastewater or animal feces remain the main source of waterborne parasites [16–18]. In case of incomplete or insufficient raw water treatment, the resulting drinking water may contain a number of parasitic agents. Old or broken water pipes also allow pathogens to enter the water sources. *Cryptosporidium* oocysts and *Giardia* cysts are found in groundwater and surface water resources, respectively, globally [10,14]. Conventional water treatment are unable to destroy or filter the cysts and oocysts of protozoa the size of *Cryptosporidium* oocyst and *Giardia* cysts ranged 4–6 and 10–15 μm, respectively [10,19,20]. Even a small quantity of oocysts or cysts can produce severe changes in the gut and stomach causing diarrhea, headaches, and nausea [21]. Immunocompromised individuals and malnourished children are particularly vulnerable to the *Cryptosporidium* parasite [22,23]. Symptoms appear 2–25 d after infection and can last one to 2 weeks [21]. The prevalence of giardiasis in Iran is estimated at 14.7% [24].

1.2. Acanthamoeba and Naegleria

Acanthamoeba and *Naegleria* species are one of the most opportunistic and pathogenic amoebae transmitted from water sources [25]. *Acanthamoeba* shows two forms: trophozoites and cysts; while *Naegleria* has three forms: cysts, trophozoites, and flagellated forms [10]. The prevalence of *Acanthamoeba* spp. in environmental samples (water and soil) has been reported to be up to 42.7% [26]. *Naegleria fowleri* causes primary amebic meningoencephalitis, which is a fatal brain infection [27].

1.3. Standards/guidelines for waterborne protozoan parasites

In 1985, the WHO recommended the evaluation of nematode parasite eggs and fecal coliforms in urban effluents (e.g., irrigation of green spaces and agriculture) as health indicators [28]. The standard for parasite eggs in treated effluent for urban landscape irrigation is less than one egg per liter [29]. The presence of parasites in water resources is associated with high mortality potential and rapid spread rate in the community. Therefore, it is ideal that drinking water does not contain any pathogenic microorganisms. In order to prevent the transmission of waterborne parasitic diseases, it is necessary to control and monitor every step from treatment to consumption, which are currently not investigated routinely. In some rural areas of the Iran, it is difficult to provide ample good drinking water. Sewage collection and treatment systems are also not properly developed, which in turn increases the possibility of parasites in water resources. A reviewing of available sources suggests that the presence of waterborne pathogens can lead to flooding and epidemics of water-borne diseases, which can lead to significant costs to the health care system. There is a surveillance system in Iran (Department of Water and Foodborne Diseases and Hospital Infections in the Center for Communicable Disease Control) such as many other countries [USA Waterborne Disease and Outbreak Surveillance System (WBDOSS), Sweden and Japan's National Epidemiological Surveillance of Infectious Diseases (NESID), National Notifiable Diseases Surveillance System (NNDSS) in Australia, and the European Centre for Disease Control and Prevention (ECDC)] that collects water and foodborne outbreak

Table 1
Some of the most important protozoan parasites transmitted through water and their clinical symptoms [10,11,15]

Parasite	Disease/clinical symptoms	Transmission route(s)	Stage of transmission and pathogenesis
Giardia duodenalis	Giardiasis. Symptoms including: diarrhea, bloating, abdominal cramps, weight loss, malabsorption of fat-soluble vitamins such as vitamin A and B12, changes in wear and tear of intestinal villi, and excretion of fatty stools. It may have no symptoms. Clinical symptoms of giardiasis usually appear 1–2 weeks after infection.	Drinking water and contaminated food, pool water, contaminated surfaces in public places.	*Giardia* spp. is transmitted to humans through fecal-oral route, so swallowing a *Giardia* spp. trophozoites can cause the parasite to settle in the small bowel and develop giardiasis.
Cryptosporidium parvum	Cryptosporidiosis. Symptoms include acute, chronic and prolonged diarrhea followed by dehydration.	Water amusement parks, swimming pools, eating contaminated food, contact with infected animals, people, or solid surface.	The oocysts are transmitted to humans through contaminated drinking water.
Entamoeba histolytica	Clinical symptoms usually begin 2–6 weeks after ingesting a cyst and manifests as diarrhea (which may occur 6–12 times during a day), abdominal pain, and palpitations. The most severe form of gastrointestinal disease is amoebic dysentery, which is characterized by the presence of blood in the stool, fever, and abdominal pain.	Contaminated water or food, surface stagnant water (river).	The disease is caused by the mature cysts, through water, or contaminated food. The parasite's cysts are very resistant and can survive for weeks in moist soil. After the cyst enters the gastrointestinal tract, it becomes active and causes disease.
Balantidium coli	Clinical symptoms of infection are non-specific and include diarrhea, abdominal cramps, and nausea. In more severe cases, the symptoms include severe watery diarrhea, fever and fatigue, and nonspecific symptoms.		The disease is caused by the cyst through water or contaminated food. After digestion, the cyst becomes vacuolar or granular or remains the same.

(Continued)

Table 1 Continued

Parasite	Disease/clinical symptoms	Transmission route(s)	Stage of transmission and pathogenesis
Acanthamoeba	Acanthambiasis. Brain, skin, lung, and eye complications are symptoms of acanthambiasis. Brain infection causes granulomatous amebic meningoencephalitis (GAE), which is a chronic and fatal complication. Another type of infection with this amoeba is *Acanthamoeba keratitis*. Symptoms include severe eye pain, fear of light, and annular inflammation of the cornea.	The opportunistic parasite is found in soil, dust, fresh water, seawater, stagnant surface water, swimming pools, dental units, air conditioners and hospital spaces.	The first step in acanthambiasis is to stick to the cell surface. In these cases, the amoeba may enter the bloodstream through the olfactory epithelium, lungs, skin lesions, or mucosa and spread to the brain.
Naegleria fowleri	Primary amoebic meningoencephalitis (PAM); the incubation period of this parasite in the human body is 2–5 d. Some patients initially experience changes in their sense of smell and taste, but the most important symptoms in most patients are sudden severe headache, high fever, vomiting, neck stiffness, lightheadedness, paralysis cerebral nerves, and lethargy (confusion and drowsiness). It quickly leads to seizures, decreased levels of consciousness and coma, and most of them die within a week of the onset of symptoms.	This amoeba is abundant in lakes, rivers, and hot springs, ponds, swimming pools and even urban water sources that are not well-disinfected. Although it is "thermophile", but also identifies in the natural waters of tropical climate and is more likely to be affected during the warm seasons.	It never leads to disease by "drinking" contaminated water. When *Naegleria fowleri* enters the nasal cavity, it passes through the nasal mucosa and the cribriform plate in the ethmoid bone through the skull and enters the brain. It first affects the frontal lobe and the olfactory bulb, causing extensive damage in those areas. It then spreads to the base of the brain, the brainstem and cerebellum, possibly damaging these areas by secreting enzymes. Finding trophozoites in biopsy confirm diagnosis.
Entamoeba coli	Non-pathogenic	Surface stagnant water	This protozoan lives in the intestine as a companion and does not cause any symptoms
Hartmannella vermiformis	*H. vermiformis* has direct and indirect public health significance. *H. vermiformis* can cause keratitis. The indirect public health significance of the organism is related to its role as a host for *Legionella pneumophila*, the causative agent of Legionnaires' disease.	Free-living amoeba, is widespread in the environment and has been isolated from soil, freshwater, air, and a variety of engineered water systems.	Two distinct life cycle forms are known for *H. vermiformis*, viz. the trophozoite, an active feeding cell that also multiplies, and cysts, which are inactive dormant cells.

reports for the health chancellor of all medical universities. However, these documents are available for outbreaks only and there is no continued program to monitor waterborne protozoan parasites. Efstratiou et al. [11] reviewed all global outbreaks caused by protozoan parasites for the following countries: New Zealand (48%), the USA (14%), the UK (5.5%), Ireland (2.4%), Australia (0.79%), Sweden (0.52%), the Republic of Korea (0.52%), Norway (0.26%), Belgium (0.26%), Germany (0.26%), and China (0.26%). Many of the published papers were research work and not collected by governmental surveillance systems. Generally, information on parasitic diseases transmitted by water is not available in Iran as a comprehensive study that can be compared with other countries. In the present study, we aim to determine the frequency of the presence of parasites based on the type of water resources.

2. Methods

2.1. Search strategy

We collected the data from published articles in reputable national and international databases in both Persian and English. MEDLINE/PubMed, Science Direct, Scopus, and Google Scholar databases were used to search for English articles, and SID, Magiran, Iranmedex, Irandoc, and ISC national databases were used to search for Persian and English articles. The information on the website of the Department of Water and Foodborne Diseases and Hospital Infections in the Center for Communicable Disease Control was also used to access reports that may not have been published as an article. To search for articles, we used the following keywords: *Cryptosporidium*; cryptosporidiosis; *Giardia*; giardiasis; *Blastocystis*; *Entamoeba*; *Acanthamoeba*; Amoebiasis; toxoplasma; microsporidia; Sarcocystis; *Naegleria*; and *B. coli*. The criteria for entering articles for the study was the prevalence of the aforementioned parasites in water resources, while the criteria for excluding the articles were: unrelated to the subject, the repetition of the article in both Persian and English, the prevalence of parasites in sources other than water, and insufficient data.

2.2. Systematic review of collected and screened articles

At this stage, all the articles collected were read and categorized as described by Manoochehry et al. [30]. The number of articles found was sorted by each database. During the screening phase, all articles that did not have a thematic relevance to the study entry criteria in terms of title, abstract, or text were not available, or duplicate search titles were deleted. Then, the rest of the articles were reviewed in terms of content and goals. After the final screening stage, the required information including study goals, year of publication, place of study, type of water source under study, type of parasite or parasites identified, and other relevant information was extracted and analyzed.

3. Results and discussion

3.1. Included papers in the study

According to Fig. 1, a total of 132 articles were retrieved based on keywords. After reviewing the titles and abstracts and deleting duplicates, 69 articles were obtained, and only

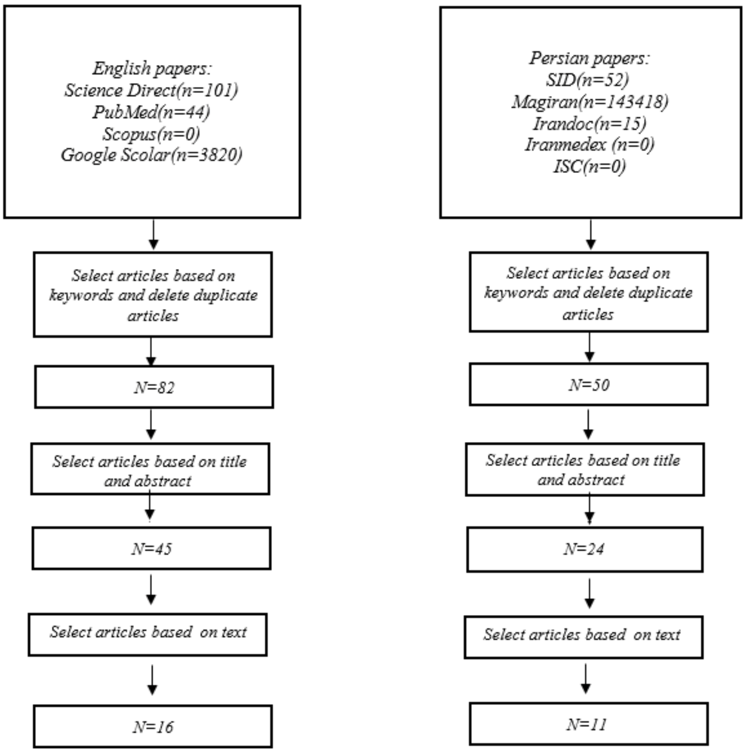

Fig. 1. Database search based on the topic and their classification.

27 were relevant to the present study. Another 42 articles examined the prevalence of protozoan parasites in other sources, such as feces, which have cited water as one way to transmit the parasite to humans.

3.2. Geographic distribution of identified protozoan parasites in Iran water resources

Infection by intestinal parasites can be found global. These parasites are highly prevalent in developing countries because they are economically and culturally inferior to developed countries. For this reason, parasitic diseases are considered an important health problem in these countries, which limits economic and social growth, employs a large amount of capital, labor, and energy, and are time-consuming. In Iran, the prevalence of these parasites depends on geographical location, weather conditions, people's culture, waste management, and human and animal wastewater. The studies reviewed here (Fig. 2, Table 2) only covered some provinces/cities and not the entire country. Most of the studies were performed in the north, western, and eastern north and western south of Iran provinces.

3.3. Most identified protozoan parasites in Iran water resource

The majority of the reported protozoan parasites (47.64% as the average percent of positive samples) included *Acanthamoeba* spp. The other protozoan parasites identified included *Naegleria* spp., *Vahlkampfiid* spp., *Cryptosporidium* spp., *Giardia* spp., *Hartmannella* spp., *Saccamoeba* spp., *Entamoeba* spp., *Blastocystis* spp., and other free-living amoebae (FLA). Positive cases included 12.21% for 4 studies, 37.64% for 2 studies, 18.62% for 12 studies, 29.97% for 13 studies, 12.5% for one study, 8.69% for one study, 16.07% for 4 studies, 7.5% for 2 studies, and 88.4% for 1 study, respectively. There were found no other protozoan parasites in the water samples. Efstratiou et al. [11] reviewed all reported waterborne protozoan parasites between 2011 and 2016 worldwide. They found *Cryptosporidium* spp. and *Giardia* spp. as the most identified etiological agents (63% of 239 reported outbreaks and 37% of 142 reported outbreaks, respectively), which is not in accordance with our results. *Acanthamoeba* spp. changes in the form of cysts in the cold season and is present in river sediments [54,55], so sampling in winter would show better results. Generally, *Acanthamoeba* spp. is thermophile [56,57]. According to studies, there is a significant relationship between sampling season, water temperature, and water contaminated to free living amoebae [58,59], which the contamination with *Acanthamoeba* spp. is highest in summer and in waters with temperatures of 26°C–30°C and pH = 7. In Tonekabon (in the Mazandaran province, central-northern Iran), sampling of the river surface took place in the summer. In the warm season, this amoeba is actively transformed into transphozoite [60] and located on the surface. *Acanthamoeba* spp. has been studied for the cities of Mashhad, Bojnourd, Birjand, Sari, Tonekabon, Rasht, Sarein, Tabriz, Urmia, Qazvin, Karaj, Tehran, Kashan, Isfahan, Hamadan, Shahrekord, Ahvaz, Shiraz, Kazerun, and Bushehr [16–18,32–38,47,48,51–53]. The lowest prevalence was reported in Sarein (in Ardabil Province, northwest of Iran) [53], Kazerun (located in Fars Province, southwestern Iran) [18], Rasht (capital city of Guilan province, northern Iran) [17], and Tonekabon (in Mazandaran province, central-northern Iran) [37], respectively. In Kashan (a city in the northern part of Isfahan province, center of Iran), there is a 900 m long aqueduct that passes alongside houses and some parts of it are open. People tend to use the aqueduct water, which is not monitored hygienically and has parasitic contamination. The city's tap water is supplied by groundwater sources and it meets the regulatory standards, but it has a high hardness and a low tendency to use it as drinking water. In Kashan, ablution pools in mosques as well as pools in urban parks have been surveyed for *Acanthamoeba* spp. [38]. According to the available studies, the ablution basins of the mosques contained free-living amoeba and contaminated amoebae compared to the ponds in urban parks. It can be caused by unhealthy habits, such as ablution water entering the nose and causing these amoebae to infect the pool water [61,62]. In a study from Guilan province (2014), the prevalence rate of *Acanthamoeba* spp. was 78.26%, higher than the average reported for the city of Rasht [17]. *Acanthamoeba* spp. found in Bojnurd river (the capital city of north Khorasan province, northeastern Iran) [34] can be dangerous for people who settle along the river and swim in it. A study between 2008 and 2009 investigated the presence of *Acanthamoeba* spp. in hospital taps [52]. The water of 14 hospitals in Iran was sampled and the culture method was used to identify *Acanthamoeba* spp., showing positive samples for Mashhad hospitals [52]. Generally, free residual chlorine can destroy most free-living amebae and is the most used disinfectant to treat drinking water worldwide especially in developing country. A study done on tap water in Shiraz, showed 42 positive samples for free living amebae, of which 39 did not have free residual chlorine. *Acanthamoeba* spp. prevalence was reported in 50% of sampled water in Ahvaz (the southwest of Iran and the capital of Khuzestan province), 50% in Tehran, 20% in Sari (the capital of Mazandaran Province), 75% in Hamadan (the capital city of Hamadan Province, west of Iran), and 20% in Rasht. The results are different from other studies conducted on other sources of water. Also, a study conducted for Tehran (2007–2006) reported 33% prevalence of *Acanthamoeba* spp. [33]. The cities of Tehran and Qazvin (capital of the province of Qazvin, north-west of Iran) are comparable in terms of the frequency of free-living amoebae because they are adjacent to each other and have relatively similar climatic conditions. According to a report from the two cities, the frequency of free amoebae in Qazvin [32] was 25% higher than Tehran. One of the reasons could be the sampling time, which was done in two different seasons. Since it was done for Qazvin in the fall, but not for Tehran. The difference in sampling season affects the frequency of *Acanthamoeba* spp. Also, the number of samples can be effective. In Qazvin, the number of samples was two times that of Tehran. It is important to note that the identification method can also be very effective in the results obtained, as the frequency of *Acanthamoeba* spp. with the polymerase chain reaction (PCR) identification method was almost the same in both cities (Tehran and Qazvin). Other studies conducted in Rasht (capital of Guilan province) during 2009–2010 [41], showed 40% positive for *G. duodenalis*. There were

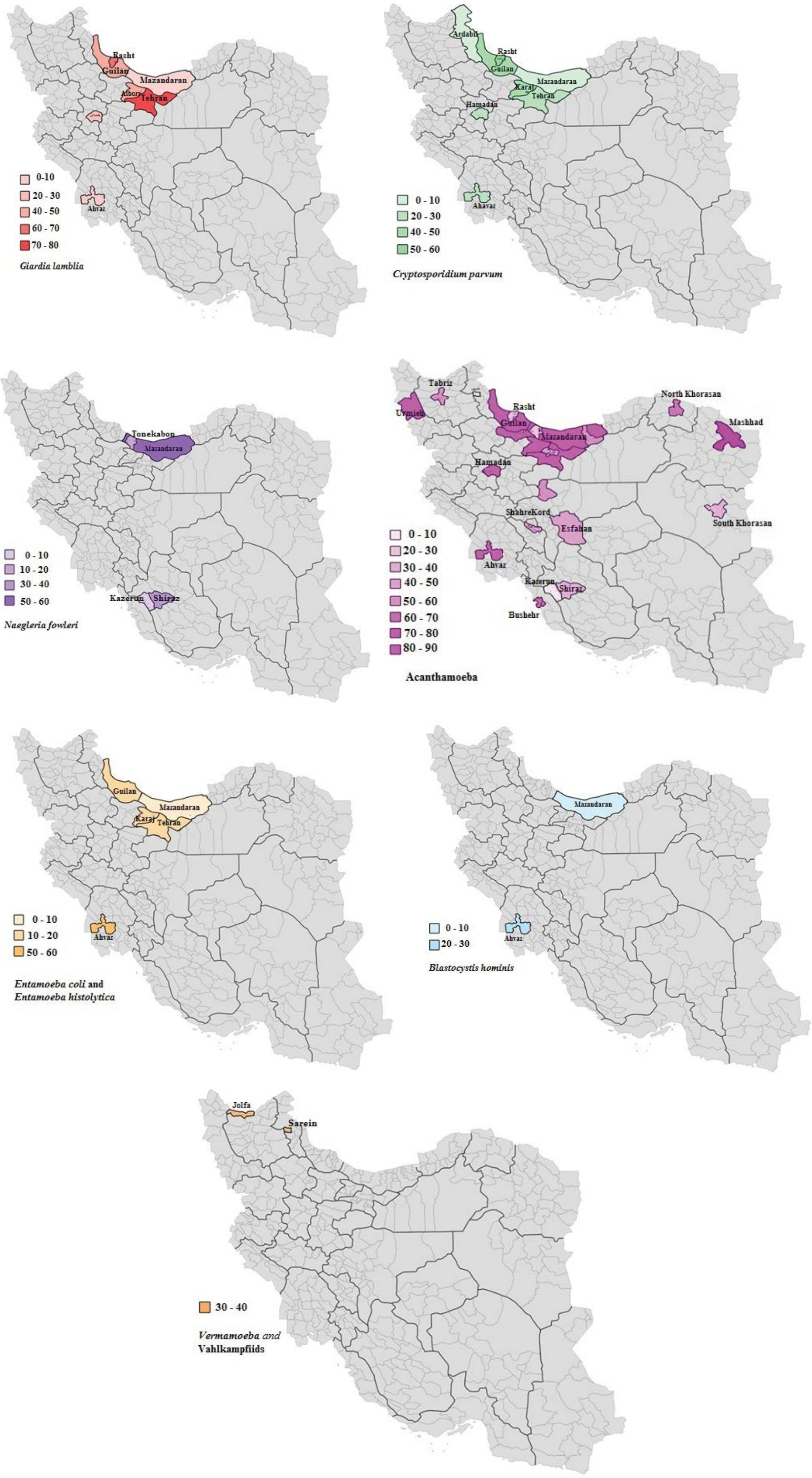

Fig. 2. Distribution of water-borne protozoan parasites in water resources in Iran.

Table 2
List of waterborne protozoan parasites identified in different studies

Province/city	Studied water resource	Etiological agent	Diagnostic life stage of parasite	Geographical region in the country	Used diagnostic method	Prevalence (%)	Reference
Mazandaran (12 cities)	Water wells (989 samples)	*Cryptosporidium parvum*	Oocysts	Central-northern	Membrane filter (0.8 microns); direct method; lugol staining; in case of *Cryptosporidium* spp. detection Sheather's sugar flotation method and Ziehl-Neelsen staining were used	0.6	[31]
		Giardia duodenalis	Cysts			5.1	
		Blastocystis hominis	Mature parasite			4.1	
		Entamoeba histolytica	Mature parasite			6.1	
		Naegleria fowleri	Mature parasite			0	
		Entamoeba coli	Mature parasite			0.4	
Qazvin	Surface stagnant water (water in ponds of urban parks and squares of the city); 40 samples	*Acanthamoeba* spp.	Cysts – trophozoite	North-western	PCR	8.43	[32]
Tehran	Surface stagnant water (water in the ponds of urban park and squares of the city); 22 samples	*Acanthamoeba* spp.	Cysts	Northern	0.45 micron cellulose nitrate filter; monoxenic media for isolation; non-nutritious agar medium containing saline page; incubation at 28°C–30°C for 1–2 weeks; PCR	27.3	[33]
					PCR	46	
Bojnourd	Water transfer canal to farms-swimming pool-river-tap water (50 samples)	*Acanthamoeba* spp.	Cysts	East	0.45 micron cellulose nitrate filter; monoxenic media for isolation; non-nutritious agar medium containing saline page; incubation at 28°C–30°C for 1–2 weeks; PCR	68	[34]
Shiraz	Wells, reservoirs, water distribution network (120 samples)	*Acanthamoeba* spp., *Naegleria fowleri*	Cysts – trophozoite	South-central	0.45 micron nitrocellulose filter; non-nutritive agar culture medium containing *Escherichia coli* K12; incubation at 25°C–30°C for 4 d; identification of amoebae based on morphological features	35	[35]
Ardabil	Surface waters, Balkhlu-Chai River, Samian River, Shourabil Lake, tape water (200 samples)	*Cryptosporidium parvum*	Oocysts	West	Filtration by 1.2 micron paper filter; PCR method; species determination with RFLP	4	[36]

Tonekabon, Mazandaran	River (100 samples)	*Acanthamoeba* spp.	Cysts – trophozoite	North	0.45 micron cellulose nitrate filter; monoxenic media for isolation; non-nutritious agar medium containing saline page; incubation at 28°C–30°C for 1–2 weeks; PCR	23	[37]
Kashan	Ablution pools in mosques as well as pools in urban park (138 samples)	Free-living amebae	Trophozoite	Center	0.45 micron cellulose nitrate filter; monoxenic media for isolation; non-nutritious agar medium containing saline page; incubation at 28°C–30°C for 1–2 weeks; PCR	4.88	[38]
		Acanthamoeba spp.				4.59	
Tonekabon, Mazandaran	River (100 samples)	*Naegleria fowleri*	Cysts – trophozoite	Central-northern	0.45 micron cellulose nitrate filter; monoxenic media for isolation; non-nutritious agar medium containing saline page; incubation at 28°C–30°C for 1–2 weeks; PCR	13	[39]
Jolfa, East Azerbaijan	Drinking water, pool water, water in urban park ponds, hot springs (50 samples)	Free-living amebae (*Vermamoeba* and Vahlkampfiids)	Cysts – trophozoite	West	1.6 microns cellulose nitrate filter; culture medium containing non-nutritive agar and 1.5% *Escherichia coli*; incubation at 25°C–30°C for 1 month; cloning	36	[40]
Rasht	Lagoon (1 sample); dam (6 samples); river (13 samples)	*Cryptosporidium parvum*	Oocysts; cysts	Northern	PCR	10	[41]
		Giardia duodenalis				50	
		Cryptosporidium parvum			LAMP	55	
		Giardia duodenalis				40	
		Cryptosporidium parvum			IFA	30	
		Giardia duodenalis				65	
Ahvaz	River, tap water, purified water at the water treatment plant, purified water sales station (44 samples)	*Entamoeba (histolyticane* and *coli*)	Oocysts; cysts	Southwestern	Keep the samples for 24 h; Ziehl–Neelson staining method	50	[42]
		Cryptosporidium parvum				27.27	
		Giardia duodenalis				9.09	
		Blastocystis hominis				13.63	
Tehran	Surface water, dam (not said)	*Cryptosporidium parvum*	Oocysts; Cysts	Northern	Filtering; separation; immunofluorescence method	21.05	[43]
		Giardia duodenalis				73.68	

(Continued)

Table 2 Continued

Province/city	Studied water resource	Etiological agent	Diagnostic life stage of parasite	Geographical region in the country	Used diagnostic method	Prevalence (%)	Reference
Rasht	Surface waters (rivers, wetlands) 45 samples	*Giardia duodenalis*	Cysts	Northern	PCR	40	[44]
					Nitrocellulose filtering; microscopic observation	33.33	
Guilan; Tehran; Alborz	Surface waters (49 samples)	*Cryptosporidium parvum*	Oocysts; Cysts	Northern	1.2 micron cellulose filtration; separation; PCR and IMS	48.97	[45]
Guilan	Surface waters (rivers, wetlands, springs, and lakes) and other freshwater resources (27 samples)	*Acanthamoeba* spp.	Cysts – trophozoite	Northern	PCR	73.7	[17]
					0.45 micron cellulose filtration; non-nutritive agar culture containing *Escherichia coli*; incubation for 2–7 d at 30°C–42°C; microscopic examination	70.3	
Guilan; Tehran; Alborz	55 samples of river water (Jajrud, Karaj, and Guilan rivers)	*Giardia duodenalis*	Mature parasite	Northern	Separation by methods (IMS) and flotation (FS); PCR	49	[46]
		Entamoeba (histolyticane and *coli*)			Flotation separation (FS); PCR	14.28	
Guilan; Mazandaran; Alborz; Tehran	Surface water resources (river) 49 water samples	*Acanthamoeba* spp.	Mature parasite	Northern; central-northern; northern; northern	0.45 micron cellulose nitrate filtration; non-nutrient agar culture containing *Escherichia coli*; PCR	78.26	[47]
		Hartmannella vermiformis				12.5	
		Saccamoeba limax				8.69	
Birjand	Surface waters; ponds in urban parks and squares and water stations (50 samples)	*Acanthamoeba* spp.	Cysts – trophozoite	Eastern	Filtration by nitrocellulose filters; non-nutrient agar culture containing *Escherichia coli*; incubation for 1 week to 2 months; morphological identification by microscope; PCR	38	[48]

Hamadan	Raw water (3 samples)	*Giardia duodenalis*	Cysts	Western	IFA	2.5	[49]
					Staining by hematoxylin eosin method	0.5	
		Cryptosporidium parvum	Oocysts		IFA	1.5	
					Staining by hematoxylin eosin method	0.1	
	Treated water (3 samples)	*Giardia duodenalis*	Cysts		IFA	25	
					Staining by hematoxylin eosin method	0	
		Cryptosporidium parvum	Oocysts		IFA	25	
					Staining by hematoxylin eosin method	0	
Mazandaran	Therapeutic hot springs (22 hot spring)	*Naegleria fowleri*	Cysts	Central-northern	Based on morphological features, PCR method	54	[50]
Sari (in Mazandaran province)	77 water samples of water resources	*Acanthamoeba* spp.	Cysts – trophozoite	Central-northern	Filtration by nitrocellulose filters; non-nutrient agar culture containing *Escherichia coli*; Incubation; PCR	55.8	[51]
14 city around Iran	Hot and cold tap water in hospitals (94 samples)	*Acanthamoeba* spp.	Cysts – trophozoite	–	Filtration by nitrocellulose filters; non-nutrient agar culture containing *Escherichia coli*; incubation; morphological identification by microscope	48	[52]
Ahvaz	Water canals to farms, water ponds in urban parks, rivers, and pools (60 samples)	*Acanthamoeba* spp.	Cysts – trophozoite	Southwestern	Filtration by nitrocellulose filters; non-nutrient agar culture containing *Escherichia coli*; incubation; PCR	71.6	[16]
	Soil (50 samples)				Non-nutrient agar culture containing *Escherichia coli*; Incubation; PCR	26	
Kazerun (Fars province)	Soil and water (354 samples)	*Acanthamoeba* spp.	Cysts - trophozoite	Southwestern	The samples were cultured on non-nutrient agar and the amoeba were collected and stained by Giemsa stain for morphological studies	2.8	[18]
		Naegleria fowleri				0.84	
Sarein (Ardabil province)	Hot spring water (28 samples)	*Acanthamoeba* spp.	Cysts - trophozoite	Northwest	Filtration; culture was done in non-nutrient agar medium enriched with *Escherichia coli*; identified by FLA based on morphological criteria of cysts and trophozoites; genotype identification done using sequencing-based method	3.57	[53]
		Vahlkampfiid amoebae	Cysts			39.28	

two studies for Tehran [43,46], too. In one study from 2011, the prevalence of *G. duodenalis* was reported as 49%. The C*ryptosporidium* spp. protozoan parasite in Iran has been studied in water resources of Ardabil, Mazandaran, Guilan, Tehran, Alborz, and Khuzestan provinces (Table 2). As can be shown from the map (Fig. 2), the lowest prevalence of this parasite has been reported in Mazandaran and Ardabil provinces and the highest prevalence rate has been reported in Guilan province (Rasht). The probability of the higher incidence of protozoan parasites in water reservoirs in the northern provinces, such as Mazandaran, might be due to the following reasons: the level of groundwater in this province is high; parasitic contamination in the province is significant; the distance between waste disposal, toilets, and agricultural lands with water tanks is low.

According to the Center for Disease Control and Prevention (CDC), cryptosporidiosis is the second most common waterborne protozoan parasite in the world causing 2%–7% diarrhea in the population, especially among children [11,63]. A governmental surveillance program is thus necessary to monitor all water resources. There are two studies for waterborne protozoan parasites in Tehran. In the study conducted by Mahmoudi et al. [45], the prevalence of *Cryptosporidium* spp. is reported as 48.97%. In addition, a study conducted in Tehran, parasitic contamination was higher in children who used tap water rather than underground (well) water, but there was no significant relationship between water resources type and giardiasis [43]. In developing countries, *Giardia* spp. and *Cryptosporidium* spp. are the two major pathogens transmitted by water [64], because these two parasites are present in most native animals and the parasites enter the water sources through the feces of these animals [11,65]. These cysts and oocyst are resistant to disinfectants and disinfectants [10]. Ardabil province (northwestern Iran) has many husbandry and livestock centers. There are different species of *Cryptosporidium* spp. in animals and livestock (as animal reservoirs) [66,67]. Water sources may be contaminated with parasite-infected animal waste. As the established process in conventional water treatment (coagulation, flocculation, clarification, and filtration) is typically followed by disinfection at full-scale to remove more than 90% of *Cryptosporidium* oocysts and *Giardia* cysts, it can be concluded that treated water can effectively be free of protozoan parasites. Based on available reports, the slow sand filters, especially diatom type, have high efficiency for removal of *Cryptosporidium* oocysts and *Giardia* cysts, and are recommended for use in filtration setups in water treatment plants [68,69]. Membrane filtration (microfiltration 0.1–5 μm, nanofiltration >1 nm, ultrafiltration 0.1 μm–20 nm, and reverse osmosis 0.1–1 nm) have pore size smaller than waterborne protozoan parasites with a diameter of 4–15 μm that can result in high protozoan cyst removal efficiency. In Ardabil, tap water supplied from groundwater sources were free of *Cryptosporidium* spp. contamination, but the rivers (surface water sources) that passed through the villages were contaminated with animal waste [36]. Among protozoan parasites, the *Cryptosporidium* spp. cyst is most resistant to chlorine, so it can be used as protozoan disinfection indicator [36]. The results of this study show the importance of contamination of surface water resources through livestock species. Among the various routes of transmission of *Cryptosporidium* spp., transmission through drinking water is the main route. Therefore, identifying and seeking for different species of *Cryptosporidium* spp. in water resources of Iran is needed. Provinces with livestock husbandry such as Ardabil, Azerbaijan (western and eastern), Shahrekord, Kohgiluyeh, Boyer Ahmad, Shiraz, Hamadan, Lorestan, Khuzestan, and Khorasan can become endemic areas for cryptosporidiosis. Efstratiou et al. [11] found that waterborne protozoan parasites contaminate water resources by different routes. They found that recreational water, and other contaminated sources (14% and 1.8%, respectively) were the main way of transmission of *Cryptosporidium* spp. that led to outbreaks. The use of raw water (without any water treatment process), contamination of water resources by protozoan parasites, ineffective water treatment process, and contamination during distribution network have been described as the main waterborne parasite transmission routes. *N. fowleri*, *Blastocystis hominis*, *Vermamoeba*, and Vahlkampfiids are the least researched parasites, only reported for one or two places. In Hamadan, the water leaving the treatment plant has been checked for the presence of water-borne parasites [49]. The water sample of Shahid Beheshti treatment plant contained only one *Giardia* spp. cyst, which was not alive. Ozone is used as a disinfectant in this treatment plant, so this may have been the cause of death for the cysts. Ozone have high toxicity against most waterborne microorganisms and most waterborne protozoan parasites because of the high oxidant potential. In the Ekbatan Dam treatment plant, only sand filters are used, and the number of parasites identified in the water sample was higher than Shahid Beheshti effluent water. Although parasites in raw water are usually removed by conventional treatment, purified water may contain parasites too. It is thus important to check the water for parasites after conventional water treatment process.

3.4. Methods for the identification of waterborne protozoan parasites

The reported data show all the available methods used to identify waterborne parasite cysts and oocysts including: concentration, purification, and detection. Some methods are not suitable for use in water samples when the concentration of cysts and oocysts is low. In order to resolve this limitation for some waterborne protozoan parasites (*Giardia* and *Cryptosporidium* for example), the United States Environmental Protection Agency (USEPA) recommended two methods (1,622 and 1,623 for *Cryptosporidium* spp. oocysts and *Giardia* spp. cysts, respectively) that are specific to detect any concentration of these parasites in water samples. There is no specific method to detect other protozoan parasites in water samples other than *Cryptosporidium* spp. oocysts and *Giardia* spp. As shown in Table 2, immunofluorescence assay (IFA), PCR and loop-mediated isothermal amplification (LAMP), differential interference contrast (DIC), microscopic detection, and enumeration methods are the most commonly reported methods to identify protozoan parasites in water samples. PCR is able to identify the parasites species that no other non-molecular method can. It also can determine the origin and pathogenicity of the detected parasite. Although molecular based methods can identify waterborne parasites, water, and other aqueous samples can include impurities such as organic and inorganic matter that interfere with the tests result or lead to false positives, so protocols should be standardized to be used in water.

4. Conclusion

We reviewed waterborne transmission of protozoan parasites in Iran water resources. One of the limitations is that some regions of the country have not studied protozoan parasites. Most available reports show that protozoan parasites are considered a health risk. The reports showed all available literature was academic research and not data from governmental surveillance systems, which shows that the country has no established monitoring program for waterborne protozoan parasites with the potential for an outbreak. The literature showed untreated water, recreational water, and contaminated water by animal feces as the main routes of waterborne parasite transmission. Different detection methods are available but they lead to different results for the same location. The need to establish and standardize surveillance programs as well as detection methods in the country in accordance to global protocols could be a first step to prevent waterborne protozoan parasite outbreaks. Additionally, national collaboration among the Department of Water and Foodborne Diseases, the Hospital Infection Center for Communicable Disease Control, and other offices, such as the Ministry of Energy, National Water, and Wastewater Co., against waterborne protozoan parasites should be established.

Data Availability Statement

All datasets generated for this study are included in the article/supplementary material.

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Author Contributions

EH did conception and design, acquisition of data, or analysis and interpretation of data, and writing. AR, CW, and MR did conception and design, interpretation of data.

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