Issues of the presence of parasitic protozoa in surface waters

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Abstract. Parasitic protozoa are very numerous organisms in the environment that play an important role in the spread of water-borne diseases. Water-borne epidemics caused by parasitic protozoa are noted throughout the world. Within these organisms, intestinal protozoa of the genera *Cryptosporidium* and *Giardia* are ones of the most serious health hazards for humans.

This paper focuses on the problem of the presence of parasitic protozoa in surface waters. Characteristics of the most frequently recognized pathogens responsible for water-borne outbreaks were described, as well as sources of contamination and surface waters contamination due to protozoa of the genus *Cryptosporidium* and *Giardia* were presented. The methods of destroying the cysts and oocysts of parasitic protozoa used nowadays in the world were also presented in a review.

1 Occurrence of parasitic protozoa in surface water

Parasitic protozoa are unicellular animal organisms that are very numerously isolated from natural waters, soils, food and media contaminated with animal manure and diseased humans. They are cosmopolitans, and are found in all countries of the world. They play an important role in the spread of water-borne diseases [1,2]. Water is the most common source of infection with protozoa, such as *Entamoeba histolytica*, *Giardia intestinalis*, *Cryptosporidium* sp., *Cyclospora cayetanensis*, *Toxoplasma gondii*. The first epidemics caused by *G. intestinalis* and *E. histolytica* were already described in 1946, and the etiology of human infection caused by *Cryptosporidium* sp. infection was presented in 1976. Since then, epidemics caused by parasitic protozoa have been recorded all over the world. Most of the infections and deaths caused by parasitic protozoan diseases affect people in developing countries, but they can also cause serious diseases in developed countries [3,4].

The most commonly diagnosed pathogens in water-borne epidemics are *Giardia* sp. and *Cryptosporidium* sp. Until 2010, 524 human lesions attributed to water-borne parasitic protozoa were described, of which 285 were caused by *Cryptosporidium* sp., and 202 cases were caused by protozoa *Giardia* sp. [3]. According to Efstratiou et al. [5], in the period 2011-2016, at least 381 cases of epidemics caused by parasitic protozoa transmitted by water were documented. Almost half of them (49%) occurred in New Zealand, 41% of

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outbreaks were recorded in North America and 9% in Europe. The most common etiologic factor was *Cryptosporidium* spp. reported in 63% (239) outbreaks, while *Giardia* spp. was mentioned in 37% (142) cases.

Increasingly common cryptosporidioses and giardioses result from infecting people with parasitic protozoa and occur as a result of direct or indirect contact through:

− consumption of water contaminated with protozoa (drinking water, surface water, swimming pool water, jacuzzi),
− consumption of undercooked contaminated food,
− contact with objects, surfaces contaminated with feces of humans or animals infected with parasitic protozoa.

It is assumed that the infective dose for healthy adults is low, and amounts to less than 10 oocysts, while in the case of immune-compromised people, a single oocyst may cause cryptosporidiosis [6,7].

Table 1. Parasitic diseases caused by protozoa [8].

Kind of the parasite	Triggered diseases
Entamoeba histolytica	Intestinal amebiasis, amoebic dysentery
Toxoplasma gondi	Toxoplasmosis
Giardia intestinalis	Giardiasis, lambliasis
Cryptosporidium parvum	Cryptosporidiosis
Naegleria fowleri	Naegleriasis

1.1 Protozoa of the genus *Cryptosporidium*

Cryptosporidium protozoa are organisms of *Apicomplexa* type, *Eucoccidiriorida* order, *Cryptospridae* family. They parasitize intracellularly, mainly in epithelial cells of the digestive tract of humans and animals. At least 19 species and several parasitic genotypes are known in many mammals [9]. These protozoa cause a disease called cryptosporidiosis in humans and animals. In immunologically healthy individuals, *Cryptosporidium* causes disease in the form of short-term diarrhea, and in people with immune deficiencies (mainly children and people with immune-compromised organisms), chronic, life-threatening diarrhea [10,11]. The most common parasite in Europe is *C. parvum*, the largest epidemiological significance is *C. parvum* type II [12], while *C. hominis* is most commonly found in the USA and Australia [2].

Invasive forms of *Cryptosporidium* (oocysts) are commonly found in surface waters, in which they survive for a long time [2]. There are two types of *Cryptosporidium* oocysts - thin-walled (there are about 20% of them), which are not excreted outside the host and thick-walled (80%), which escape into the environment, mainly to water and soil [13]. *Cryptosporidium* oocysts show very high resistance to adverse external conditions. They are able to survive freezing in the temperature range from -15 to -20°C, and in the isotonic solution at a temperature of 4°C, they survive even 18 months. In addition, oocysts are particularly resistant to chlorine [14]. Only after 4 hours at a concentration of 30 mg Cl_2/dm^3 of water, the number of oocysts decreased by 99% [6,15]. In addition, they show resistance to iodine and bromine in doses used in the water treatment process [16].

Estimates indicate that 65 million oocysts of *C. parvum* gets into the environment daily from the sewage treatment plant servicing about 50,000 inhabitants, and up to 14,000 oocysts can be detected in a liter of untreated wastewater [17]. Research conducted in the USA (Arizona) showed that in 24 samples of raw wastewater from 2 treatment plants, the average concentration of *Cryptosporidium* oocysts was 74-100 oocyst/dm^3 [18]. According to WHO [6] and Smith [19], *Cryptosporidium* oocysts were detected in the range from 3.3 to 20,000 oocyst/dm^3 in purified wastewater, 0.006-2.5 oocyst/dm^3 in surface waters contaminated with agricultural sewage, 0.66-500 oocyst/dm^3 in recreational waters, and 0.006-4.8 oocyst/dm^3 in drinking water.

1.2 Protozoa of the genus *Giardia*

The protozoa of the genus *Giardia* are flagellates belonging to the type *Sarcomastogophora*, order *Diplomonadida*, family *Hexamitidae* [9]. *G. intestinalis (G. lamblia)* is an invasive species for humans. It is believed that 2-5% of the population in developed countries is infected with this protozoan, while in Poland, a few to a dozen or so percent of adults are infected [20,21]. Other species of this genus, such as *G. muris, G. agilis, G. ardeae* and *G. psittaci*, are non-invasive to humans [22,23].

Protozoa of the genus *Giardia* are located in the digestive tract of humans and animals, and their vegetative forms (trophozoites) occur only in the host organism. In unfavorable conditions, they develop cysts that are excreted into the environment in feces. They are resistant to environmental factors - in chlorinated water at 4-18°C, they can survive up to 3 months, but only 4 days at 37°C [24]. In water from a river or lake, cysts survive up to several months. Among disinfection methods used, UV rays and ozonation are more effective than chlorine [6].

According to studies carried out by Kitajima et al. [18] and Hatam-Nahavandi et al. [25], 1600-4900 cysts from *Giardia* genus (Iranian area) may be present in 1 dm^3 of raw sewage or their number may even reach 4800-6400 cysts (USA, Arizona).

Giardiosis, like cryptosporidiosis, mainly occurs in children and people with reduced system immune. *Giardia* infection is considered a "dirty hands disease". One of the most common transmission routes is the human-environment system. *G. intestinalis* causes inflammation of the small intestine, morphological changes, disturbances in absorption of amino acids, fatty acids, simple sugars and some vitamins (e.g. A, B_{12}), which leads to significant malnutrition. The infection is manifested by the occurrence of increased nausea, vomiting and bloodless diarrhea [26,27].

2 Sources of water infections with protozoa of the genera *Cryptosporidium* and *Giardia*

The basic way to fight diseases caused by parasitic protozoa is to recognize the sources of their origin. Parasitic protozoa get into the water as a result of pollution, which can come from municipal and agricultural sewage, organic fertilizers, as well as manure of livestock and wild animals [16].

The reservoir of *Cryptosporidium* are people, farm animals (mainly cattle, sheep and goats) and free-living animals (beaver, muskrat, roe deer, deer, wild boar, fox and squirrel), as well as laboratory animals (guinea pig, mouse and rat). The occurrence of *Cryptosporidium* spp. in dogs and cats as well as in domestic and wild birds as well as breeding and wild boar rabbits has also been reported [12,20,28]. According to Siński [13], the main source of environmental contamination with *Cryptosporidium* spp. are infected calves and lambs, which can expel about 10 billion oocysts a day, which makes them widely present in the natural environment. Carriers of this protozoon can also be marine fish, crustaceans, molluscs and cephalopods. Research on the waters of the Cheasepeak Bay in the USA has shown that the mussels are contaminated with *C. parvum* oocysts due to anthropogenic and agricultural pollution entering the waters of the Bay. In addition, *Cryptosporidium* vectors can also be flies that carry most oocysts in their digestive tract and then contaminate the food [29,30].

Table 2. Protozoa from the *Cryptosporidium* genera pathogenic to human [13].

Species of the parasite	Main host	Casual host
Cryptosporidium andresoni	cattle	human, sheep, camel
Cryptosporidium baileyi	hen, turkey	duck
Cryptosporidium canis	dog	human
Cryptosporidium hominis	human	sheep, pigs, cattle
Cryptosporidium muris	rodents, other mammals	human
Cryptosporidium parvum	ruminants, human	rodents
Cryptosporidium suis	pigs	human

The main source of surface water pollution with *Giardia* protozoa cysts are animals infected with *G. intestinalis.* In Poland, infection by *Giardia* was found, among others, in cattle (2.2%), sheep (1.3%), dogs (6.1%) and cats (1.3%). The highest percentage of *Giardia* spp. infections was found in wild rodents, i.e. bank vole and common vole - 93.9% and 96.3%, respectively [31,32]. Earlier reports also mention the responsibility of beavers for water-borne giardioses - it was found that the intensity of water contamination in streams populated by beavers is from 1 to 65 cysts per 100 dm^3 of water [33].

3 Contamination of surface waters with parasitic forms of protozoa

Numerous analyses carried out in the USA, Great Britain and Canada clearly indicate that contamination with parasitic protozoa can occur in various types of waters, i.e. surface, ground, underground, treated, lakes, ponds and swimming pools [34]. The greatest biological pollution concerns flowing and standing surface waters used by people and animals (farmed and wild). Quality of water in a given water reservoir depends on many factors. Environmental studies have shown that water contamination by parasitic protozoa depends primarily on:

- degree of pollution and topography of the area, where the water reservoir is located,
- climate - rainfall can only slightly influence the number of oocysts in surface water, where their survival depends on temperature,
- social factors - infrastructure of water departments and their management.

In addition, the quality of water in the reservoirs is affected by the activity of recreational centers, changes in the direct reservoir catchment and transformation of the lakes' coastline [35,36,37].

Cryptosporidium spp. oocysts are quite common all over the world. Research carried out on approximately 6 thousand surface water samples from the USA showed that 20% of them were contaminated with oocytes, and their average density was 2 oocysts/100 dm^3 of water [38]. Surveys of water sources in Great Britain revealed the presence of *Giardia* cysts in as many as 46% of samples of raw drinking water, 57% of samples containing cysts were found in surface waters. At the same time, a higher percentage of positive tests were recorded always in summer and autumn [2].

Studies on the occurrence of *Cryptosporidium* spp. and *Giardia* spp. in the environment have been conducted in Poland for over 30 years. These are not routine tests, and they mostly cover analyzes of various types of waters. Although raw and purified sewage can be the most serious source of contamination with cysts and oocysts of parasitic protozoa, not much Polish research deals with determining the number of *Cryptosporidium* spp. and *Giardia* spp. in wastewater. Drinking water, as a source of transmission of pathogenic intestinal protozoa, has been studied by numerous authors. There are also frequent studies upon the presence of parasitic protozoa in recreational waters - lakes or swimming pools.

According to Bojar and Kłapeć [2], high contamination with *Cryptosporidium* oocysts is found in surface waters (83%), a lower percentage of positive samples is found in pre-treated (13%) and tap water (22%). With regard to protozoa of the genus *Giardia*, the surface water pollution with cysts is 57%, whereas in the case of pre-treated and tap water, it is identical to that of *Cryptosporidium*. Research on the waters of Lake Malta and the Warta river in Poznań showed that the largest amount of intestinal protozoa occurs between August and November, which indicates the seasonal nature of contamination [39]. Based on the analyzes carried out, Matuszewska et al. [40] found the presence of parasitic protozoa in the environmental samples of water originating from surface intakes. The *Giardia* cysts were detected in 100% of water samples in the number of 1.0-4.5 cysts per 10 dm^3 of water from the intake at Zalew Zegrzyński and from the intake on the Vistula river in the number of 1.0-38.9 cysts per 10 dm^3 of water. *Cryptosporidium* oocysts were present in 50% of surface water samples from the Zalew Zegrzyński intakes and 47.6% samples from the Vistula river, and their number was similar in both cases and ranged from 0.5 to 2.5 oocysts per 10 dm^3 of water. In other studies, Matuszewska et al. point to the presence of parasitic protozoa cysts in surface waters, from which water is subsequently captured by waterworks, the purpose is to supply the population with drinking water [41,42]. Research conducted on outdoor water reservoirs performing the bathing function in Cracow indicated the regular occurrence of live *Cryptosporidium* sp. Oocysts [43]. This allows to suppose that the problem of water contamination with parasitic protozoa of the genus *Cryptosporidum* and *Giardia* may apply to the entire territory of Poland.

4 Prevention of water-borne infection caused by protozoa of the genus *Cryptosporidium* and *Giardia*

Water disinfection, as an element of the treatment process, has various effects on the disposal of parasitic protozoa present in this medium. The action of chlorine is usually a sufficient barrier to protect water against pathogenic microorganisms. In the case of parasitic protozoa, it is necessary to use much higher doses of disinfectants than in the case of bacteria. With the chlorine dose of 0.08 mg/dm^3 at 1-2°C and pH 7, 99% reduction of most vegetative forms of bacteria can be achieved, while in the case of protozoa, similar effect can be achieved only when the dose of chlorine is increased to 3.3 mg/dm^3. At the same time, it is effective only against *Giardia*, but it does not kill *Cryptosporidium*. Also, when using monochloramine, a similar relationship is noted [1,44,45]. Among the disinfection methods used, UV and ozonation appear to be more effective than chlorine compounds in relation to parasitic protozoa. In order to inactivate 99% *Giardia*, the effective dose is 59 mJ/cm^2 and for *Cryptosporidium* 10 mJ/cm^2. In turn, effective doses of ozone are 1.9 mg min/dm^3 and 40 mg min/dm^3, respectively, at 1°C and pH 6-9. It should be emphasized that not only the use of chemicals, but also conventional methods of water treatment such as coagulation, flocculation, sedimentation, filtration cannot always ensure that water intended for consumption will be free from parasitic protozoa of the genus *Cryptosporidium* and *Giardia* [6].

Table 3. Disinfectants used to inactivate *Cryptosporidium parvum* and *Giardia intestinalis* [46]

Disinfectant C*t	*Cryptosporidium parvum*	*Giardia intestinalis*
UV ($mJcm^{-2}$)	19	80
chlorine	7.200	03-121
chloramines	7.200	1.470
chlorine dioxide	78	17
ozone	5-10	1.3

C*t – Factor that is the product of the concentration of disinfectant after time t from the introduction of the disinfectant to the water at its first recipient, expressed in mg/dm^3, providing 99% inactivation at 5°C and pH 7.0.

As mentioned before, the most serious source of parasitic protozoa reaching the environment (surface water) is wastewater, both raw and purified. This is due to the fact that the conducted wastewater treatment processes do not change the lifespan of the protozoan dispersal stages [16]. Studies on the effectiveness of the parasitic protozoa removal in the process of wastewater treatment conducted throughout the world illustrate the variation in the degree of reduction of cysts and oocysts reaching the aquatic environment. According to the literature, in the case of protozoa of *G. intestinalis* in the USA, cyst removal rate reaches 97% and 99% [18], while in Germany, the estimated average removal rate for all wastewater treatment plants is 92% [47], in Spain, the yield is 82% [48], and in Sweden, it is 67-87% [49]. These differences may result mainly from the dissimilarity of applied waste water treatment technologies and the amount of organisms contained in the raw sewage. One should be aware that detection, estimation of abundance and species identification of parasitic protozoa in sewage is an important issue from the point of view of engineering and environmental protection as well as health protection. No less important is the desire to develop more effective methods of destroying the cysts and oocysts in treated wastewater discharged into the waters of receivers.

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