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Investigation of *Cryptosporidium parvum* and *Giardia intestinalis* in Various Water Sources in Isparta Area, Turkey

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Abstract

Cryptosporidium parvum (*C. parvum*) and *Giardia intestinalis* (*G. intestinalis*) were investigated in samples collected from lake, stream and drinking fountain within borders of Isparta city in Turkey. This study was conducted between June and October 2004 and 40 water samples were collected. Seven water samples were collected from Eğirdir Lake, six from Gölcük Lake, ten from Darı Stream of Isparta flowing into Karacaören Dam after uniting Aksu Stream. Seventeen samples from fountains were collected from different regions of the city. These samples were filtered using a membrane filtration system. After centrifuging, the filtered samples were investigated with direct microscopy for *Giardia* and with Modified Zielh-Nellsen (MZN) for *Cryptosporidium*. Existence of *C.parvum* was verified by immunoflourescence assay (IFA) method. After the direct microscopy and MZN, *C. parvum* existence susficied in 13 (32,5%) of the 40 samples, and six of them (15%) was confirmed by IFA method. *Giardia cysts* were found in 8 (20%) sample after direct microscopy.

Key Words: *Giardia intestinalis*, *Cryptosporidium parvum*, drinking water, immunoflourescence assay.

INTRODUCTION

Infectious diseases caused by pathogenic bacteria, viruses, and protozoa are the most common and wide spread health risks associated with drinking water. Drinking of water contaminated by human or animal faeces that contains pathogenic microorganisms, causes water borne diseases. Water borne diseases are the general term used to denote diseases associated with poor water supply or quality. Waterborne infection occurs by consuming contaminated surface water of lakes, ponds or rivers directly or accidentally during water sport activities. Swimming pool-associated outbreaks of *cryptosporidiosis* and *giardiasis* have also been widely reported [1-2]. Ground (well) water is usually free of these organisms but occasional contamination has been reported. Treated drinking water has been shown to be a cause of occasional disease outbreaks due to water treatment deficiencies. In developing countries, four-fifths of all the illnesses are caused by water-borne diseases, with diarrhea being the leading cause of childhood death [3].

In recent years, the occurrence of the water-borne parasites *Cryptosporidium parvum* (*C. parvum*) and *Giardia intestinalis* (*G. intestinalis*) have become a major focus for the drinking water industry [4].

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C. parvum is a coccidian gastrointestinal parasite that is ubiquitous in surface waters. It has been responsible for a number of disease outbreaks related to drinking water. *G. intestinalis* is another familiar water-borne parasite and lately, there has been a concern over the water-borne transmission potential of other eukaryotic enteric pathogens [5].

Exposure to these organisms can cause diarrhea, lasting days to weeks in susceptible persons. *Cryptosporidium* can be life-threatening for individuals with weakened immune systems including persons with AIDS or the AIDS virus, organ transplant or cancer chemotherapy patients, persons on high dose steroid therapy and persons with inherited immunodefficiencies. The two main routes of infection are: 1) "hand-to-mouth" following contact with a contaminated person, animal or object; and 2) consuming contaminated water. The infection due to contaminated food has also been observed on occasion [6].

Giardia and *Cryptosporidium* enter surface waters such as lakes, ponds and reservoirs as environmentally resistant cysts and oocysts (egg-like structures), respectively, in the faeces of infected people or animals. Contamination sources include sewage treatment plant effluents, septic tank discharges, and infected pets, farm and wild animals. Faecal material may contaminate water directly or following storm water runoff. *Giardia* and *Cryptosporidium* can survive in the environment for weeks or months.

According to the United Nations World Health Organization's (WHO) (2002) report, an estimated 1.7 million deaths per year can be attributed to unsafe water supplies. Most of these deaths are from diarrhoeal diseases; ninety percent of those who die from diarrhoeal diseases are children in developing countries [7]. The first documented waterborne outbreak of cryptosporidiosis occurred in July 1984 in Texas [8]. Recent significant Water-borne Disease Outbreaks or WBDO's include the 1993 Milwaukee cryptosporidiosis outbreak, which resulted in estimates of more than 400,000 sick and approximately 60 deaths. Ultimately the cause was thought to be high levels of pathogens in source water following flooding, combined with poor back flushing practices at one of the water treatment plants. Considering other WHO statistics, there are approximately 4 billion cases of diarrhoea each year, caused by a number of different pathogens, including *Cryptosporidium*, *Giardia*, *Shigella*, *Campylobacter jejuni*, *Escherichia coli*, *Salmonella*, and cholera, so these pathogens would have to be considered the most dangerous [9].

In Turkey, there are a few published data on the prevalence of *Giardia* and *Cryptosporidium* in wastewaters. The present study was to evaluate the prevalence of these parasites in the lake, stream and drinking fountain within provincial borders of Isparta.

MATERIALS AND METHODS

Province of Isparta is 400 km away from the capital of Turkey and is located in the Lake District in northern Mediterranean (Longitude/Latitude: E 320 20' and 310 33'- N 370 18' and 380 30'). Altitude is about 1050 metres (Figure 1) [10].

Water Sampling Sites and Procedures

Samples were collected at twenty-three points in Lake Eğirdir and Lake Gölcük and Isparta Stream from June 2004 to October 2004. Also, seventeen drinking water samples were collected from fountains open to public. Raw water samples of ten litres were concentrated through 1.2 µm pore size polypropylene filter.

Natural park of Lake Gölcük is in north west

Mediterranean and lays in south and south west of Isparta (Longtitude/Latitude: E 37 º 38' 33''- 38 º 03'38''- N 30º 22' 24''-30º 45' 34''). It is 13 km from the city centre. Water samples were collected from six points (Ga-Gf) in north, north-west and north east of the area (Figure 2).

Figure 1. Location of Isparta in Turkey.

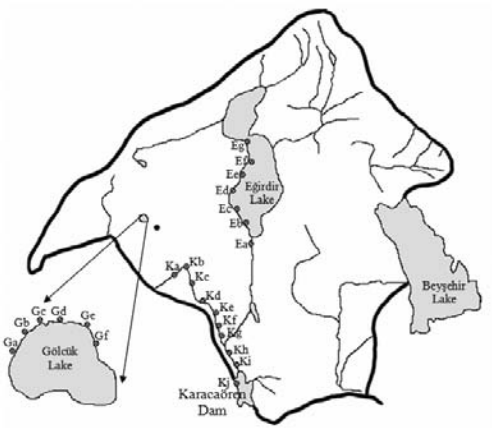

Figure 2. Locations of investigated water sources.

Lake Eğirdir is in the Lake District and the second fresh water in Turkey (Longitude/Latitude: E 30º 57' 43''- 30º 44' 39''- N 37º 50' 41''-38º 16' 55''). It is 25 km from the city centre. Water samples were collected from seven points (Ea-Eg) in west and south west of the area (Figure 2).

Isparta Creek: Around the river flowing from the city centre to Karacaören Dam there are a lot of villages and the creek is used for agricultural purposes. From ten points (Ka-Kj) of the creek, water samples were collected (Figure 2).

Determination of C. parvum and G. intestinalis

Collected samples were processed within 24 h. Ten litre water was filtered using polypropylene filter (1.2 µm in diameter) Millipore system.

After filtration, filter fibbers were placed in phosphate-buffered saline containing 0.1% (wt/vol) sodium dodecyl sulphate and 0.1% (v/v) Tween 80 [11].

The samples were placed vertically in the shaker and were shaken for five minutes at (maximum) 800 rpm. After shaking, the filters were taken away and water samples were centrifuged for fifteen minutes (maximum 1.0503 g). The supernatant was taken away and from the precipitated pellet two preparations were obtained to determine *Giardia* cysts and *Cryptosporidium* oocysts. One of them was used to determine *Giardia oocysts*; one drop was taken from the solution precipitated on a clean microslide for a direct microscopic examination and the thin glass cover was put to examine it under a light microscope. The samples with *Giardia cysts* were accepted as *Giardia* positive. The other preparation was for *Cryptosporidium*. One drop was taken from the precipitate and put onto the microslide. Then it was stained with Ziehl-Neelsen (MZN) and examined under an immersion objective. A new preparation was made in order to discriminate *C.parvum* oocysts from *Cyclospora cayetanesis oocysts* in MZN positive samples. It was fixed with methanol. The immunoflouresans (IFA; Cellabs-Australia) was stained in accordance with examination and assessed under a UV microscope (Microscope Zisse Germany) [12].

RESULTS AND DISCUSSION

Forty water samples were collected from various sources such as lakes, creeks and fountains in Isparta between June 2004 and October 2004 and the samples were grouped according to the place of collection (Figure 1). Ten of the total 40 water samples (25%) were from Isparta Creek, seven (17.5%) from Lake Eğirdir, six (15%) from Lake Gölcük and 17 (42.5%) from fountains open to public (Table 1). In four of the samples from ten different points of Isparta Creek (40%) *C.parvum* was determined and *G.intestinalis* was seen in five samples (50%) (Table 2) . In one of the six samples from Lake Gölcük (20%), *G.intestinalis* was determined, whereas there was no *C. parvum* observed (Table 3). In two of the seven samples from Lake Eğirdir (28.6%) *G.intestinalis* was seen and in the other two (28.6) *C. parvum* was observed(Table 4). In 17 samples from fountains open to public found in different streets in Isparta city centre, neither *Cryptosporidium* nor *Giardia* was found.

Table 1. Water samples from stations.

Sampling stations	Number of samples	(%)
Isparta Creek	10	(25)
Lake Eğirdir	7	(17.5)
Lake Gölcük	6	(15)
Fountains open to public	17	(42.5)
TOTAL	40	(100)

The water sampling sites are listed in Table 1. Forty samples in total were collected. Samples of the Isparta stream were collected from Ka to Kj (Figure 2). *Cryptosporidium oocysts* were come across between Ka and Ke, and *Giardia oocysts* between Ka and Kg. Around the Isparta River, are there few settlement areas where people are interested in agriculture and raising animals, refinery plants and garbage areas. In a study conducted, *Cryptosporidium oocysts Giardia oocysts* at high levels were come across in a river into which waste from stables, sheds and barns flows and it was stated that it might be due to animal faeces [12].

In a study conducted in Sivas, Turkey faeces from 457 cows and calves on 30 different farms was examined in terms of and *Giardia oocysts*. According to the results of the study, on all of the farms there were animals infected with *Cryptosporidium* (64.3%), whereas *Giardia oocysts* were determined in 17 animals (3.7%) on 11 farms. These findings clearly demonstrate that asymptomatic infections in calves and cows infected with *Giardia spp*. and *Cryptosporidium* spp. play a potential role as reservoirs for humans [13].

Another sampling area is Lake Eğirdir, the third biggest lake in Turkey, and around this lake there are beaches, restaurants and picnic sites. Water samples were collected from the areas of these activities (Ea-Eg) (Figure 2). *Cryptosporidium* and *Giardia oocysts* were determined in “Ea” and “Ee” areas. These areas, especially “Ee”, are the main entrance to a public beach which is very crowded during summer time. Parasite oocysts were not found in further areas (Table 4). Water samples were also collected from Lake Gölcük, included in the natural park and around which there are restaurants and picnic sites. In Table 3 the areas from which the water samples were collected are listed. Water samples were collected from the North of the lake, because picnic sites and toilets are in that area. *Giardia oocysts* were only found in the samples from “Ge” point of the lake. *Cryptosporidium* oocysts were not found. It is likely for the area to have a low percentage of parasite oocysts, because there are not beaches, settlements, stables, sheds or barns. Water samples were collected from ten fountains open to public in Isparta city (ten litres from each) in addition to these natural sources. After examination, no *C. parvum* or *Giardia* was found. Natural conditions, as well as human activities, in catchment areas of surface reservoirs significantly affect the quality and safety of running and stored water. In

Table 2. Results of the samples from Isparta Creek.

Stations	C. *parvum* *ARB*	*DFA*	G. *intes.*	Area
Davraz district bypass starting from Isparta Creek	+	+	+	Ka
Refinery plant exit	+	+	+	Kb
Marble factory exit before garbage	+	+	+	Kc
Water near villages of Kadılı and Direkli	+	-	+	Kd
Intersection point of water villages of Kadılı and Direkli and the 4th station	+	+	-	Ke
40th km of Isparta-Antalya highway creek under fountain	+	-	-	Kf
Water sample from Ağlasun fork in the road under the bridge	+	-	+	Kg
45th km of Isparta Antalya highway goes to the trout farm	-	-	-	Kh
Creek in the west of Karacaören dam 60 km away from Antalya	-	-	-	Kj
30th km of Isparta-Antalya highway fountain water	-	-	-	Ki
Total number of stations	7	4 (40%)	5 (50%)	

Table 3. Results of the samples from Lake Gölcük.

Stations	C. *parvum* *ARB*	*DFA*	G. *intes.*	Area
Lake Gölcük entrance	0	0	0	Ga
North of Gölcük 250 km from the state water affairs pomp	0	0	0	Gb
North of Gölcük 200 km from the state water affairs pomp	0	0	0	Gc
North of Gölcük 150 km from the state water affairs pomp	+	-	+	Gd
North of Gölcük 100 km from the state water affairs pomp	0	0	0	Ge
Gölcük near the State water affairs pomp	0	0	0	Gf
Total station number	1	0	1 (20%)	

Table 4. Results of the samples from Lake Eğirdir.

Stations	C. *parvum* *ARB*	C. *parvum* *DFA*	G. *intes.*	Area
Lake Eğirdir island (1)	+	-	0	Ea
Lake Eğirdir island (2)	0	0	0	Eb
Eğirdir beach of the teacher's guesthouse	+	+	0	Ec
Eğirdir beach of the Göltür block	0	0	+	Ed
Eğirdir Altınkum beach (entrance)	+	+	+	Ee
Eğirdir Altınkum beach (50 m after entrance)	+	0	0	Ef
Eğirdir Altınkum beach (100 m after entrance)	+	0	0	Eg
Total station number:7	5	2 (28.6%)	2	

drinking water, the values for microbial parameters, especially the parasite load, increased to the maximum levels during heavy rainfall and runoff events. Relationships between meteorological parameters and microbial loads of fecal origin have been described. The concentrations of *E.coli*, coliform bacteria, and enterococci, as well as turbidity, increased after rainfall. The concentrations of *Cryptosporidium oocysts* were found to be lower during dry periods, such as summer [14].

The present study was conducted during summer, between June and October. Isparta has a geography where it rarely or never rains in summer.

In a periodical study covering a whole year may give different results. That's why such a periodical study on drinking water is needed. *Cryptosporidium spp*. has been known as a protozoon causing Cryptosporidiosis through contamination from different water sources to people since 1980s.

In *Cryptosporidial* infections water contamination is through drinking water, irrigation water in agriculture and water used for entertainment. Out of 40 included in the study, six water samples (15%) had *C. parvum* and eight (20%) had *G. intestinalis*. In this study, it is shown that there are *Cryptosporidium oocysts* in 65-97% of surface water sources [15]. *Cryptosporidium* infections are observed very often during summer, especially between August and September. The reason might be high contamination in these months that rain, humidity and hot weather bring in drinking water and water from rivers, swimming pools, lakes and seas, thus leading to an increase in concentration with *Cryptosporidium oocysts* [16]. Since oocysts are resistant to disinfectants and cannot be got rid of through chlorinating drinking water or water consumed, epidemics are reported caused by water contamination [17]. The most important factor of contamination is human or animal faeces in surface water, swimming pools, lakes, seas and especially from rivers from which water is used for drinking or consumption. As for the reason of human infection, animals were suspected for the first time and in a study it was reported that twelve people with sound immune systems were infected because they were consuming water from contaminated rivers with infected animal faeces [18]. The authors reported that infections were much common especially among those who consume water from rivers in terms of contamination of the infection. Ilordi et.al. showed the existence of *Cryptosporidium oocysts* in all of the water samples collected from four rivers in Washington and two in California [19]. There were 12 epidemics caused by contamination in drinking water sources with *C.parvum* between 1985 and 1994 in North America. In two of the epidemics death rate was between 52-68% in population with immune systems under constraints [20].

In 1987, *Cryptosporidium* infection was observed in 13.000 people in Carrolton, America due to contamination of drinking water sources with *C. parvum*. In 1992, it was a health threat for 15.000 people in Jankson County and Oregon [21]. The biggest epidemics up to now happened in America, and there were 403.000 symptomatic cases seen [20]. In 1994, infection with HIV caused 20 people to die in Las Vegas and those deaths were possibly related to contaminated water consumption [1]. In a study conducted in Isparta in 2004, G. Intestinalis was reported as 26.3% in faeces samples from eight hundred people [22]. This finding shows that there is a contamination source in the city examined in our study. However, in Turkey studies on *C. parvum* are generally conducted with an orientation to clinical *Cryptosporidium* infection. Cases of *Cryptosporidium* are reported but the ways of contamination in those cases remain unknown. Therefore, in the present study, we examined different water sources in Isparta, where there have been parasite infections, as the causes of *Cryptosporidium* and *Giardia* infections. Since 1990, there have been various modern methods developed for diagnosis of *Cryptosporidium* [23,24]. The latest one is Method 1623, EPA (2001). This method is a combined method to determine *Cryptosporidium oocysts* and *Giardia cysts* and it was used in our study after modification. *Cryptosporidium oocysts* were determined but the number of oocysts is not known [25].

Various staining methods have been developed for diagnosis of *Cryptosporidium* both in water and faeces. The most common of these methods is modified Ziehl-Neelson staning method [26]. However, in this method it is likely for *C. parvum* oocysts to be combined with other structures. These might be fungal spors, fat structures and bacteria spor sor *Cylospora cayetanensis*. An IFA technique was applied during colouring stage of our analysis in order to reach a certain result, taking 13 possible formations (32.5%) out of the 40 samples into account. Specification of IFA test, which is done using monoclonal anticores, one of the immunological methods, is high and it is more sensitive than other staining techniques. IFA technique might be even used for water samples including few oocysts. It is important for early determination of oocysts in water [27]. As a result, after application of IFA technique, *C. parvum* oocysts were determined in six of the forty water samples (15%). There have been different methods developed to determine *Giardia* in water samples, including immunological and molecular methods [23-27]. In the present study, we used direct microscopic method which is still regularly used in many laboratories today. After filtration and centrifuge, pellet on the filter was evaluated in the water samples collected and then it was examined under a 10x40 microscope. As a result, in developing countries, inadequate sewage disposal and drinking water treatment facilities are primary causes of waterborne infectious disease. In industrialized nations, while treated drinking water is generally available to the entire population, organisms such as *Cryptosporidium* are challenging existing treatment Technologies. To protect water quality, we must pay attention to microbial fauna and flora; outbreaks of diseases such as *Cryptosporidiosis* remind us that we cannot overlook traditional public health concerns. Clearly, there is a need to reduce runoff of microbe containing wastes into water supplies, while also strengthening water treatment programs and public health surveillance for infectious diseases [28].

CONCLUSION

In conclusion, numerous outbreaks of waterborne *cryptosporidiosis* and *giardiasis* have been reported in many countries. We present the results of various water sources for *Giardia sp*. and *Cryptosporidium sp*. in an Isparta region, Turkey. The size and extent of the problem of cryptosporidiosis and giardiosis in Turkey is not well characterized. These data provide important information on the occurrence and determinants of the two most important intestinal parasitizes in a Isparta region, Turkey.

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