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RESEARCH ARTICLE

Investigation of Cryptosporidium sp. Oocysts in Erzurum's Potable Water on Different Months

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Abstract

Objective: Cryptosporidium sp. is a protozoan which is highly resistant to external environmental conditions and chlorination and can lead to severe diarrhea in immunosuppressed persons. Oocysts of this parasite, is excreted by human and animal feces, lead to contamination of potable (drinking) water supplies in environments with poor sanitation. Our aim is to investigate the presence of oocysts in some potable water samples taken from different points of Erzurum city center and around on different months.

Methods: Totally 120 water samples were collected from 40 random different points of Erzurum city center and around on April, May and July. Of the 120 samples, 45 were from city water system and 75 from fountain and wells water. Water samples collected within 5 liter tanks were filtered by using membrane filter. From each sample preparation were done by using modified acid fast staining method and then examined under microscope.

Results: *Cryptosporidium* sp. oocysts were detected on 18 (15.0%) of total 120 water samples. 6 (13.3%) of these positive samples were from city water system and the other 12 (16.0%) were from fountains and wells. According to the seasonal distribution of positive samples 9 (22.5%) have been taken on April, 7 (17.5%) on May and 2 (5.0%) on July.

Conclusion: Results of this study have shown that source of water supplies in our region are notably contaminated with *Cryptosporidium* sp. oocysts, and the rate of contamination is higher on April and May when compared with July.

Key words: Cryptosporidium sp, water, modified acid fast

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Introduction

Cryptosporidium sp. is an intracellular protozoa which can lead to diarrhea in both human and animals by ingestion of contaminated food and water (Fayer, 2004). It has a typical coxcidian (merogony, gametogony, chizogony) life cycle. The parasite is very common in the world, and currently 26 species are reported. But among them only the six have a special importance in human cryptosporidiosis cases (Griffiths et al., 1998; Clark, 1999; Chalmers et al., 2013). They are *C. hominis, C. parvum, C. meleagridis, C. cuniculus, C. felis* and *C. canis* (Chalmers et al., 2013). Oocysts of this parasite excreted by human and animal feces leads to contamination of drinking water sources in bad sanitized environments. On the other hand being resistant to disinfectants and heat changes of the oocysts make the wastewater treatment processes difficult. Although, boiling of water for about one minute or treating it with iodine for about 20 minutes or filtering are effective methods for inactivity of oocysts, none of them are practical to apply in city system of water (Redlinger et al., 2002). Additionally chlorinating and ozone-treating of the water can't provide sufficient prevention. As a result of this fact, waterborne epidemics may arise due to the drinking of water contaminated with oocyst (Ekinci, 2012). The epidemic of Milwaukee, in which about 400.000 people are affected in 1993 in USA, is one of the best examples that the agent may lead to the outbreaks (Mac Kenzie et al., 1994; Eisenberg et al., 2005).

The infection dose of the parasite is low, and it is reported that taking 10 to 30 oocysts can lead to infection (DuPont et al., 1995; Okhuysen et al., 1999). Since the disease can be seen in animals, infected animals may act as a reservoir. Therefore some occupation employers, such as veterinarians and livestock rangers are at risk for this disease. The places such as school, dormitory, water parks, day light care centers are risky places for epidemics. Clinical table varies depending on the age and immune status of the host. It is asymptomatic in immunocompetent individuals (Egyed et al., 2003). In a number of vertebral organisms including human being, in particular immunosuppressive individuals, it leads cholera-like enteritis continuing with extreme dehydration in which water loss may reach up to 20 ml by the destruction of microvillus lining of gastrointestinal epithelium. This life threating clinical picture is commonly seen in developing countries especially in children at the age of under-five (Inceboz et al., 2002; Dirim et al., 2003). Disease can be diagnosed by detecting the cryptosporidium in feces and tissue biopsy using IFAT, DFA, ELISA, PCR methods (Eren, 2011).

This study was planned to investigate the drinking water supplied from different sources in Erzurum city center and its surroundings for the presence of Cryptosporidium sp. oocysts, and if detected, to determine the seasonal distribution of the parasite.

Methods

Selecting the sampling points: People, living in Erzurum city center and in its surrounding, obtain their drinking water mainly from two different sources. One of these sources is Çat dam belonging to metropolitan municipality. The water produced from this natural source is distributed to the city water system The second source is ground water which comes from unknown origin. People, living in villages and in city use this water by means of wells and fountains.

In this study, 15 points at which the water is supplied from dam by city water system, and 25 points at which water is supplied from wells and fountains were selected by simple randomized method. Water samples were taken from each selected points. Sampling from the same city water system and fountain and wells was repeated three times on April, May and July.

Collection of water samples and laboratory analyses: The process of all samplings was carried out by using 5 lt sterilized plastic containers. Water samples taken from the sources are filtered using vacuum-pumped filtration device with a 0.45 µm cellulose acetate membrane filter (Sortorius AG, Germany). Then, the particles remaining on membrane were washed with 20 ml of the same sample by centrifuging for 15 minutes at 3500 rpm. Supernatant was discharged and the sediments were taken into 1.5 ml Eppendorf tube. Two preparations from each sample were done by putting 100 µl samples onto clean slide, then let them to dry. Dried preparations were kept in pure methanol for three minutes, and fixed. Modified acid fast staining method was applied. Stained slides were then examined under microscope at x40, x100 magnification. Due to easy application, being able to show the fine structure of the oocysts in detail, and the fact that red oocysts can be seen easily on blue ground, this method was used in the diagnosis of cryptosporidium (Ok et al., 1997; Cicek et al., 2011).

Statistical analysis: SPSS 17 packet program was used for statistical analysis. Categorical variables in the study were expressed as percentage (%) and numeric values (n)

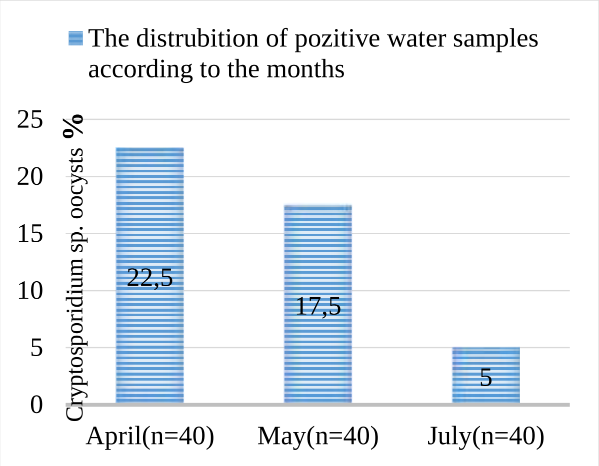
Results

Cryptosporidium sp. oocysts were detected in 18 of 120 water samples examined (15.0%). When the distribution is considered according to the sources in which the oocysts are available, oocysts is detected in 6 of 45 city water systems (13.3%) and 12 of 75 fountains (16.0%) (table 1).

Table 1. The distributions of *Cryptosporidium* sp. oocysts observed in water samples according to months and sources

Water sources	City water system			Fountain and wells		
Months	Sample (n)	Positive (n)	%	Sample (n)	Positive (n)	%
April	15	3	20	25	6	24
May	15	2	13.3	25	5	20
July	15	1	6.7	25	1	4
Total	45	6	13.3	75	12	16

While *Cryptosporidium* sp. oocysts was observed in total 9 drinking water samples in April (22.5%), this was 7 in May (17.5%) and 2 in July (5.0%) (Graphic 1)



Graphic 1. The distribution of *Cryptosporidium* sp. oocysts in drinking waters according to the months

When we evaluated the positive results according to the months and the sources of the samples, it is seen that oocysts were detected in 6 of 25 samples from fountain and wells (24.0%) and 3 of 15 from city water system (20.0%) in April. We also found out that there was oocysts in 2 of 15 (13.3%) samples taken from city water system and in 5 of 25 (20.0%) samples taken from fountains and wells in May. Also in 1 of 15 (6.7%) samples taken from city water systems and in 1 of 25 (4.0%) samples taken from fountain and wells in July oocysts were detected (Table 1). *Cryptosporidium* sp. oocysts were detected in exactly different sources in April, May and July. During microscopic examinations, we also observed *Cyclospora* sp. oocysts in some of the samples as well as *Cryptosporidium* sp. (2 in April, 2 in May and 2 in July) (Figure1- 3).

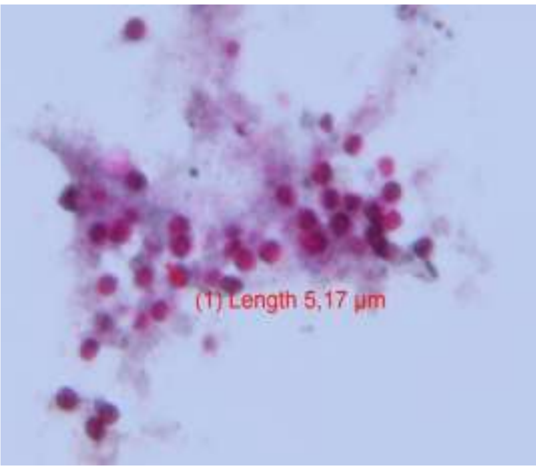


Figure 1. *Cryptosporidium sp*. oocysts (100X)

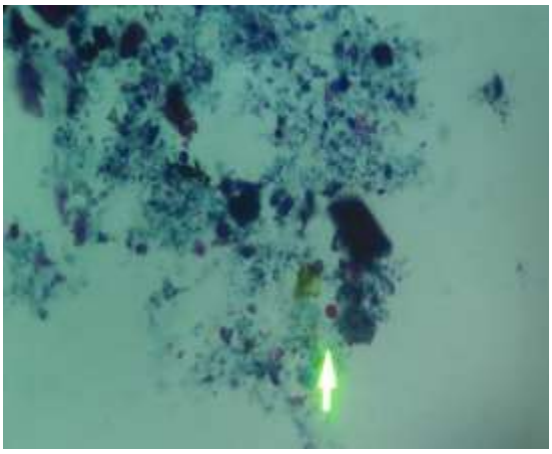


Figure 2. *Cryptosporidium* sp. oocysts (40X)

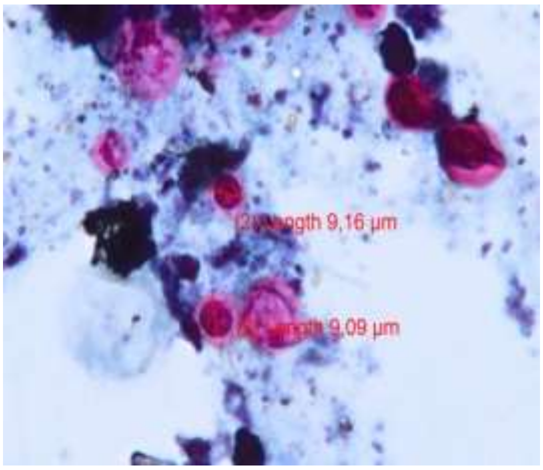


Figure 3. *Cyclospora sp*. oocysts (100X)

Discussion

Cryptosporidium sp. is considered to be one of the three pathogens which is the most common and causing gastroenteritis, especially in developing countries. Among the many species *C. parvum* and *C. hominis* are more prevalent than the others (Current et al., 1991; Wilson, 2004). This protozoon may transmit to the humans by different ways but waterborne transmission is the most important one. As the time passes, the numbers of cases in both immunocompromised and immunocompetent persons increased and this make the disease more important (Juranek, 2000; Fayer et al., 2000; Ozcel et al., 2007).

In some countries such as USA and Australia, Cryptosporidium takes place at the upper ranks among the waterborne epidemics, however, in Europe, frequency and importance of infection is highly variable. It is reported that even in only one epidemic in USA, while over thousands of people are affected, this number may be the most 27-575 in Europe (Mac Kenzie et al., 1994; Smith, 1998; Eisenberg et al., 2005). In Turkey one waterborne epidemic of Cryptosporidium sp. and *Cyclospora* sp. which was experienced in a village of Izmir was reported by Aksoy et al. (2007).

In a study carried out in Germany, Cryptosporidium sp. has been detected in 90% of sand-filtered portable waters, and in 78 % surface waters (Karanis et al., 1996). Also, in a multicenter study carried out in Europa, *Cryptosporidium* sp. has been detected in one third of drinking waters (Ward et al., 2002). In the studies carried out in Mediterranean Region particularly in Greece, Spain and İtaly, high rate of Cryptosporidium sp. oocysts was detected in lakes, rivers and wastewater treatment pools (Conio et al., 1999; Karanis et al., 2002). LeChevallier et al. (1991) reported that they detected Giardia cysts and *Cryptosporidium* sp. oocysts at the rate of 17% and 27% in filtered waters, 81% and 87% in non-filtered waters in wastewater treatment centers at 14 states of USA. Almeida et al. (2010) reported that they met Giardia cysts in 8.4% of 167 drinking waters coming from 44 sources and *Cryptosporidium* sp. oocysts in 10.2% of drinking water in Portugal. In another study carried out by Galván et al. (2014) in Spain, they reported that *Cryptosporidium* sp. oocysts were most often encountered in winter and spring season.

The study by Koksal et al. (2002) in which *Giardia* sp. and *Cryptosporidium* sp. oocysts were searched in 40 untreated water samples obtained from different dams in İstanbul was known to be the first study in Turkey. But the authors reported that they couldn't find any parasite. In Mersin, Ceber et al. (2005) investigated *Cryptosporidium* sp. oocysts in total 100 water samples including drinking water, wastewater, sea water and potable water. In the result of study, they reported to have found Cryptosporidium sp. oocysts in 11.4% of 44 drinking water, in 21.0% of 19 wastewater, in 50.0% of 2 well water and in 2.9% of 35 sea water samples. In Van of Turkey, Cicek et al. (2011) observed that there was Cryptosporidium sp. oocysts in 1.1% of total 440 water samples.

In our study, we observed *Cryptosporidium* sp. oocysts in 16.0% of 75 samples from fountains and wells, and in 13.3% of 45 samples from city water system. When we evaluated the results according to the months, we found out that in 22.5% of 40 water samples taken in April, 17.5% of 40 samples taken in May, and 5.0% of 40 samples taken in July were contaminated with oocysts. These results that *Cryptoporidium* sp. oocysts are more prevalent in spring than in summer are accordance with the study results by Galvan et al. (2014) in Spain.

In conclusion, we can say that city water system and fountain and wells water used as drinking water in our region is contaminated with *Cryptosporidium* sp. oocysts. One another conclusion of our study is that more *Cryptosporidium* sp. oocysts is seen in April and May than in July. The reason of this situation may be due to the more fecal contamination occurred in these months resulting from melting of snow mass and more rainfall in these months. In order to obtain healthy drinking water, contamination of water sources with human and animal fecal wastes should be prevented. Because the *Cryptosporidium* sp. oocysts are resistant to chlorine and other disinfectant, using of filtered water or water which is boiled or heated at least one at 72 °C will be protective for people especially small children and immunosuppressive patients.

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