Turkiye Parazitol Derg 2023;47(2):71-7
DOI: 10.4274/tpd.galenos.2023.74936

Investigation of *Giardia* spp., *Cryptosporidium* spp. and *Cyclospora cayetanensis* in Samples Collected from Different Spring Waters Iğdır, Türkiye

Farklı Kaynak Sularından Alınan Örneklerde Giardia spp., Cryptosporidium spp. ve Cyclospora cayetanensis'in Araştırılması Iğdır, Türkiye

Önder Akkaş[1], Esra Gürbüz[2], Selahattin Aydemir[3], Maksut Şahin[4], Abdurrahman Ekici[3]

[1]Erzincan University Faculty of Medicine, Department of Medical Microbiology, Erzincan, Türkiye
[2]University of Health Sciences Türkiye, Van Training and Research Hospital, Clinic of Infectious Diseases and Clinical Microbiology, Van, Türkiye
[3]Van Yüzüncü Yıl University Faculty of Medicine, Department of Parasitology, Van, Türkiye
[4]Van Yüzüncü Yıl University Faculty of Medicine, Dursun Odabaş Medical Center, Van, Türkiye

Cite this article as: Akkaş Ö, Gürbüz E, Aydemir S, Şahin M, Ekici A. Investigation of *Giardia* spp., *Cryptosporidium* spp. and *Cyclospora cayetanensis* in Samples Collected from Different Spring Waters Iğdır, Türkiye. Turkiye Parazitol Derg 2023;47(2):71-7.

ABSTRACT

Objective: In this study, it was aimed to investigate the physical and chemical properties of different spring waters and parasitic factors with different methods.

Methods: This study was carried out on 69 water samples collected from different spring waters in and around Iğdır Province in April and June 2021. The samples were analyzed by native-Lugol, modified acid-fast staining, and nested polymerase chain reaction (nPCR). In addition, altitude (meter) and pressure (mmHg) measurements were made at the point where the water samples were taken.

Results: One or more parasites were detected in 27.5% of the 69 water samples examined. Only *C. cayetanensis* was found in 13% of the samples, only *Cryptosporidium* spp. in 10.1%, only *Giardia* spp. in 1.4%, only *C. cayetanensis* and *Giardia* spp. in 1.4%, only *C. cayetanensis*, *Cryptosporidium* spp., and *Giardia* spp. in 1.4%. Only *Giardia* spp. cyst (4.3%) was detected by the direct examination method. While *C. cayetanensis* and *Cryptosporidium* spp. oocysts were detected in 8.7% and 7.2% of the samples by the modified acid-fast staining method, *C. cayetanensis* was detected in 15.9% and *Cryptosporidium* spp. was detected in 11.6% of the samples by nPCR. When the *C. cayetanensis* and *Cryptosporidium* spp. positivity rates were compared according to the characteristics of the water, there was no statistical difference between the altitude, salinity, pH, mmHg, and temperature (kelvin) values, but a significant correlation was found between the amount of dissolved oxygen and *Cryptosporidium* spp. positivity (p=0.047).

Conclusion: *Cryptosporidium* spp., *C. cayetanensis*, and *G. intestinalis* are important waterborne pathogens that can cause epidemics. It is our belief that in order to reduce the risk of contamination of these parasitic factors with spring waters, public awareness should be raised, infrastructures should be improved, and new water treatment techniques, such as ultraviolet, ozonation and monitoring systems, should be used.

Keywords: Iğdır, water, parasite, native-Lugol, nested PCR

ÖZ

Amaç: Bu çalışmada, farklı kaynak sularının fiziksel ve kimyasal özellikleri ile paraziter etkenlerin farklı yöntemlerle araştırılması amaçlandı.

Yöntemler: Bu çalışma, 2021 yılı Nisan ve Haziran aylarında Iğdır ili ve çevresinden farklı kaynak sularından toplanan 69 su örneği üzerinde yürütüldü. Su numunelerinin ısı, pH, çözünmüş oksijen ve tuzluluk gibi fiziksel ve kimyasal özellikleri ölçüldü.

Received/Geliş Tarihi: 04.08.2022 **Accepted/Kabul Tarihi:** 14.02.2023

Address for Correspondence/Yazar Adresi: Abdurrahman Ekici, Van Yüzüncü Yıl University Faculty of Medicine, Department of Parasitology, Van, Türkiye
Phone/Tel: +90 507 704 24 00 **E-mail/E-Posta:** abdurrahman2400@gmail.com **ORCID ID:** orcid.org/0000-0001-6034-513X

Türkiye PARAZİTOLOJİ Dergisi

Numuneler nativ-Lugol, modifiye asit fast boyama ve nested polimeraz zincir reaksiyon (nPCR) yöntemleri ile incelendi. Ayrıca su örneği alınan noktada rakım (metre) ve basınç (mmHg) ölçümleri yapıldı.

Bulgular: İncelenen 69 su örneğinin 19'unda (%27,5) bir veya birden fazla parazit saptandı. Örneklerin 9'unda (%13) sadece *C. cayetanensis*, 7'sinde (10,1) sadece *Cryptosporidium* spp., birinde (%1,4) sadece *Giardia* spp., birinde (%1,4) *C. cayetanensis* ve *Giardia* spp., birinde (%1,4) ise *C. cayetanensis*, *Cryptosporidium* spp. ve *Giardia* spp. saptandı. Direkt bakı yöntemi ile sadece *Giardia* spp. kisti (%4,3) saptandı. Modifiye asit fast boyama yöntemi ile 6 (%8,7) örnekte *C. cayetanensis*, 5 (%7,2) örnekte *Cryptosporidium* spp. ookisti saptanırken, nPCR yöntemi ile 11 (%15,9) örnekte *C. cayetanensis*, 8 (%11,6) örnekte *Cryptosporidium* spp. DNA'sı saptandı. Suyun özelliklerine göre *C. cayetenansis* ve *Cryptosporidium* spp. pozitiflik oranları karşılaştırıldığında istatistiksel olarak rakım, tuzluluk oranı, pH, mmHg ve sıcaklık (kelvin) değerleri arasında bir fark saptanmadı, ancak çözünmüş oksijen miktarı ile *Cryptosporidium* spp. pozitifliği arasında anlamlı bir ilişki saptandı (p=0,047).

Sonuç: *Cryptosporidium* spp., *C. cayetanensis* ve *G. intestinalis*, salgınlara neden olabilen önemli su kaynaklı patojenlerdir. Bu paraziter etkenlerin kaynak suları ile bulaşma riskini azaltmak için toplumun bilinçlendirilmesi, alt yapıların iyileştirilmesi ile birlikte ultraviyole, ozonlama ve monitoring sistem gibi yeni su arıtma tekniklerinin kullanılması gerektiği kanaatindeyiz.

Anahtar Kelimeler: Iğdır, su, parazit, nativ-Lugol, nested PCR

INTRODUCTION

The safety of drinking water plays an important role in improving the quality of life in modern societies because water has a significant impact on public health. Water can be contaminated with viruses, pathogenic bacteria, parasites, and many other biological factors that are harmful to human health (1,2). In addition to the direct consumption of contaminated water, transmission to humans can occur, especially through the cleaning and preparation of raw vegetables and foods with contaminated water (3-5).

According to the World Health Organization, it is estimated that around 1.1 billion people worldwide use unsafe water. The vast majority (88%) of diarrhea cases worldwide are due to unsafe water, sanitation, and hygiene (6). About 3.1% (1.7 million) of annual deaths and 3.7% of annual health burden (disability, etc.) are due to unsafe water, hygiene, and sanitation (7).

Water has a major role in the transmission of parasitic agents *Giardia intestinalis*, *Cryptosporidium* spp., and *Cyclospora cayetanensis* to humans (1,2). The International Organization for Standardization (ISO) has specified a method (ISO 15553:2006) that is valid for the detection and enumeration of *Cryptosporidium* spp. oocysts and *G. intestinalis* cysts in surface and ground waters, treated waters, mineral waters, and swimming pool and recreational waters (8,9).

G. intestinalis are parasitic protozoans that are one of the leading agents of waterborne intestinal infection epidemics worldwide. *G. intestinalis* cysts spread to the environment through the feces of the hosts, causing the contamination of water and food with the cysts (5).

Cryptosporidiosis, which is generally zoonotic in character, is a worldwide infection. Contamination occurs as a result of the fecal-oral ingestion of oocysts excreted in the feces of infected animals and humans. Many vertebrate species, including humans, can be infected this way. The feces of domestic animals, especially calves, have a great role in transmission to humans. Contamination occurs as a result of the contamination of the environment and water by oocysts of human and animal origin. The oocysts of *Cryptosporidium* spp. can remain infective for months in both fresh and salt water. Routine chlorination or ozonation of drinking water has little or no lethal effect on the oocysts of *Cryptosporidium* spp. (10-12).

Cyclospora infections are a diarrheal disease that are especially seen in countries such as Nepal, Pakistan, and India (13). Transmission to humans occurs by fecal-oral ingestion of food and water contaminated with infective oocysts, which are highly resistant to the external environment (14). How food and water become contaminated with *C. cayetanensis* is still unknown. The oocysts of this parasite become infective by sporulating in suitable environmental conditions a few days or weeks after they are excreted. This feature is of great importance in the epidemiology of infection (15-17). It has been reported that transmission and spread in *C. cayetanensis* outbreaks may be water-borne as well as food-borne and mostly fresh produce (3,18,19).

The aim of this study was to investigate *Giardia* spp. by microscopic method and *Cryptosporidium* spp., and *C. cayetanensis* by modified acid fast and molecular methods in different spring waters.

METHODS

This study was carried out on 69 water samples collected from different spring waters from Iğdır Province and its surroundings in April and June 2021. The water samples taken were placed in clean 5-L plastic containers and brought to the laboratory. The samples were first filtered through a vacuum pump filtration device (Rocker-167400-22 Rocker 400 Model Vacuum Pump) equipped with a 0.22-µm cellulose acetate membrane filter (Sortorius AG, Germany). After the filtering process was completed, the part of the filter that the water passed through was scraped thoroughly using a sterile scalpel, and the same 15-mL water sample was placed into falcon tubes. In addition, the filter was washed with 5% tween 80 and the washed solution was transferred to falcon tubes. The falcon tubes were centrifuged at 3500 rpm for 15 min and the supernatant was discarded. The underlying precipitate was first examined by native-Lugol, and then by modified acid-fast staining and nested polymerase chain reaction (nPCR). In addition, altitude (meter) and pressure (mmHg) measurements were made at the point where the water samples were taken (Model:Testo 511).

Water analysis: The physical and chemical characteristics of the monitored water samples included the temperature, pH, dissolved oxygen (DO), and salinity. The dissolved oxygen meter of the model, which was a Hanna HI9142, was used in measuring the dissolved oxygen and temperature. The pH was measured using a pH meter (model: Hanna Instrument model no. H1 8915 ATC) while the salinity was measured using a salinometer, model: S-200 IP67.

The probe end of the meter was dipped into the water samples while the value at the pointer of the scale was read and recorded. The DO was measured in milligrams per liter (mg/L), temperature was measured in Kelvin, and the salinity was measured in mg/L.

nPCR for *C. cayetanensis*

DNA extraction was performed as described in the GeneMATRIX Stool DNA Purification Kit (Gdańsk, Poland) manual from whole water samples. The Lyticase enzyme from Arthrobacter Luteus (L2524; Sigma-Aldrich, St. Louis, MO, USA) was used to weaken or break down the oocyst wall before extraction. Enzymes were added to the samples and incubated at 25 °C for 15 min. The samples were then incubated at 95 °C for 30 min in a dry block heater and vortexed at 5-min intervals during the incubation period. All of the other procedures were carried out according to the manufacturer's instructions in the kit.

nPCR was performed using the methods and primers specified by Orlandi et al. (20). In the first stage of the nPCR, F1E 5′-TACCCAATGAAAACAGTTT-3′ and R2B 5′-CAGGAGAAGCCAAGGTAGG-3′ were the primers used to amplify the ~636 bp region of the *18S rRNA* gene region of *Cyclospora* and Eimeria species. The reaction was adjusted to a total volume of 50 µL, containing 25 µL of Tag 2x Master Mix (12.5 mM $MgCl_2$), 0.5 mM $MgCl_2$ and 0.2 µM of each primer, and 1 µL of sample DNA. Next, 1 µL of the amplicon obtained for the second stage of the nPCR was used. In the second stage of the nPCR, the primers were used to amplify the region of ~298 bp from the 18S rRNA gene region of *Cycylospora* species. The second nPCR reaction was carried out under the conditions specified in the previous step.

Reactions were performed on an Applied Biosystems SimpliAmp Thermal Cycler (Thermo Fisher Scientific Inc., Waltham, MA, USA). The first PCR was programmed for a total of 35 cycles, each at 94 °C for 30 s, then 53 °C for 30 s, and 72 °C for 90 s. The nPCR was adjusted for a total of 25 cycles, at 94 °C for 15 s and 66 °C for 15 s. Since the primary binding temperature in the nPCR phase was close to the activity temperature of the Tag polymerase, no extension temperature (72 °C) was required. In both PCR procedures, an additional administration was done at 95 °C for 15 min before the first cycle for the denaturation step. Following the last cycle, an extension step was performed at 72 °C (66 °C for nPCR) for 10 min.

To display the results of the nPCR procedure, 15 µL of PCR reaction products was run on agarose gel (1%) electrophoresis and visualized in a UVP Gel documentation system (Ultra-Violet Products Ltd., Upland, CA, USA).

nPCR for *Cryptosporidium* spp.

The GeneMATRIX stool DNA purification kit (EURx Ltd., Gdansk, Poland) was used for DNA extraction, which was performed in accordance with the instructions provided with the kit. However, before starting the purification procedures, the samples were incubated at 95 °C for 30 min in a dry block heater (Bio-TDB-100, BioSan) at 5-min intervals in order to effectively weaken the oocyst wall. nPCR was used for the molecular investigation. First, primers CryptoF 5′-TTCTAGAGCTAATACATGCG-3′ and CryptoR 5′- CCCATTTCCTTCGAAACAGGA-3′ were used, which amplified the DNA fragment, encoding the SSU rRNA as 1.325 bp in length (21). For the nPCR reaction, 25 µL of Solis Biodyne 5X Firepol (12.5 mM MgCl2) master mix was used, which comprised 100 nM of each primer and 2 µL of the sample DNA, and 1 µL of these reaction products was later subjected to nPCR. During the nPCR, a base set of CryptoNF 5′- GGAAGGGTTGTATTTATTAGATAAAG-3′ and CryptoNR 5′- AAGGAGTAAGGAACAACCTCCA-3′ was used, which amplified a region about 826–864 bp in length (21). The reactions were performed in a thermo thermal cycler with a heater lid (SimpliAmp Thermal Cycler, Thermo Fisher). The first step of the PCR consisted of a total of 18 cycles (33 s at 95 °C, 45 s at 55 °C, and 45 s at 72 °C), and a 10-min extension period at 72 °C. The second step of the nPCR was performed the same as the first step, but with an annealing process that comprised 35 cycles. The nPCR products were run for 45 min at 90 V in 1% agarose gel with ethidium bromide, and the images were recorded. From the pool with positive results, 10 samples were randomly selected and subjected to sequence analysis, which confirmed *Cryptosporidium* spp.

Statistical Analysis

In the statistical analysis, the incidence of parasites according to the relevant categorical variables was expressed as numbers and percentages, and whether there was a relationship between categorical variables was determined by the chi-square (χ^2) test. The Z-test was used to compare the incidence of parasites and the calculations were made in the MINITAB (version: 14) statistical package program.

RESULTS

One or more parasites were detected in 19 (27.5%) of the 69 water samples examined. Of the samples, 9 (13%) contained only *C. cayetanensis*, 7 (10.1%) contained only *Cryptosporidium* spp., 1 (1.4%) contained only *Giardia* spp., 1 (1.4%) contained *C. cayetanensis* and *Giardia* spp., 1 (1.4%) contained *C. cayetanensis*, *Cryptosporidium* spp. and *Giardia* spp.

Only *Giardia* spp. cyst (4.3%) was detected by direct examination method. While *C. cayetanensis* was detected in 6 (8.7%) samples and *Cryptosporidium* spp. oocyst was detected in 5 (7.2%) samples by modified acid-fast staining, *C. cayetanensis* (Figure 1) was detected in 11 (15.9%) samples and *Cryptosporidium* spp. (Figure 2) was detected in 8 (11.6%) samples by nPCR (Table 1).

When the *C. cayetanensis* and *Cryptosporidium* spp. positivity rates were compared according to the water sources, no statistically significant difference was found (Table 2). When the *C. cayetanensis* and *Cryptosporidium* spp. positivity rates were compared according to the characteristics of the water, there was no statistical difference between the altitude, salinity, pH, pressure (mmHg), and temperature (kelvin) values, but a significant correlation was found between the amount of DO and *Cryptosporidium* spp. positivity (p=0.047) (Table 3).

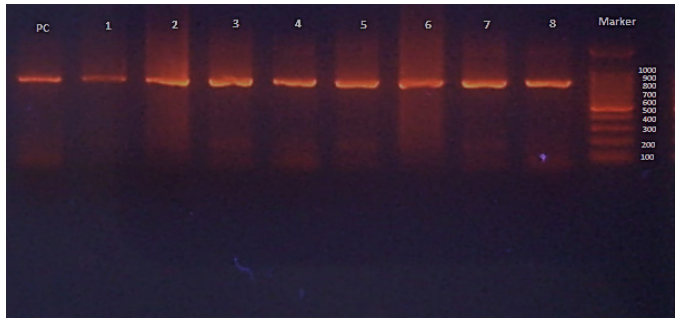

Figure 1. Band images of the *Cryptosporidium* spp. positive samples, which were amplified by PCR and visualized in gel electrophoresis (in the presence of 0.15% ethidium bromide and 1X tris boric acid-EDTA buffer) [Marker: 100 bp DNA marker (Grisp Mark), PC: Positive control]

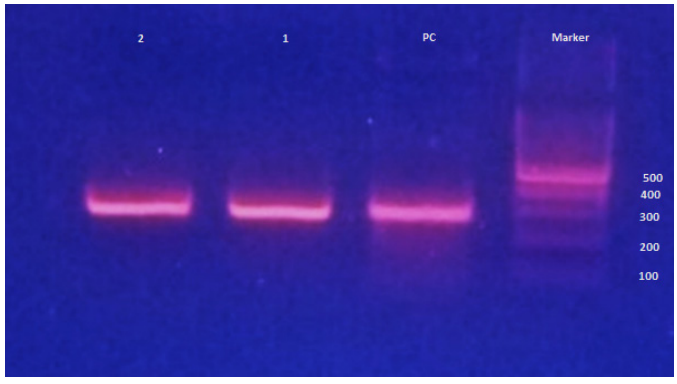

Figure 2. Band images of the *C. cayetanensis* positive samples, which were amplified by PCR and visualized in gel electrophoresis (in the presence of 0.15% ethidium bromide and 1X tris boric acid-EDTA buffer) [Marker: 100 bp DNA marker (Grisp Mark), PC: Positive control]

DISCUSSION

In the prevalence of infections caused by protozoa, Türkiye's location in a temperate geographical region, its inadequate infrastructure, and low education level of society, as well as the lack of adequate information about the transmission routes of parasitic diseases are also effective. In Türkiye, studies on parasitic infections that cause waterborne outbreaks are limited, since research on parasitic infections is mostly conducted with stool and blood samples from humans and animals.

It was reported that *Cryptosporidium* spp. oocysts were detected in 58 (39%) of 147 gastroenteritis patients in a gastroenteritis epidemic thought to affect 13,000 people in a town with a population of 64,900 in Georgia (22).

From 1986 to 1988, 50 disease outbreaks were reported in 24 the USA and Puerto Rico, affecting 25,846 people from drinking water sources. *G. intestinalis*, a protozoal parasite, is the most common cause of epidemics; most of these outbreaks have been associated with the consumption of chlorinated but unfiltered

Table 1. Distribution of parasites seen according to the methods used

Parasites seen	Native-Lugol n (%)	MAF staining n (%)	nPCR n (%)	Total n (%)
Giardia spp.	3 (4.3)	-	-	3 (4.3)
C. cayetanensis	-	6 (8.7%)	11 (15.9%)	11 (15.9%)
Cryptosporidium spp.	-	5 (7.2%)	8 (11.6%)	8 (11.6%)
Total	3 (4.3)	11 (15.9%)	18 (26.1%)	19 (27.5%)

Table 2. Prevalence of *C. cayetanensis* and *Cryptosporidium* spp. by type of water source

Source of water sample	*C. cayetanensis*		*Cryptosporidium* spp.		p-value*
	Positive n (%)	Negative n (%)	Positive n (%)	Negative n (%)	
Drilling-network (7)	1 (14.3)	6 (85.7)	-	7 (100)	a =0.975 b =0.296
Source-network (41)	7 (17.1)	34 (82.9)	7 (17.1)	34 (82.9)	
Stream (12)	2 (16.7)	10 (83.3)	-	12 (100)	
Well water (9)	1 (11.1)	8 (88.9)	1 (11.1)	8 (88.9)	

n: Number of samples, a: Significance value for *C. cayetanensis*, b: Significance value for *Cryptosporidium* spp., Significance levels according to the chi-square test

Table 3. Prevalence of *C. cayetanensis* and *Cryptosporidium* spp. according to water properties

	C. cayetanensis		*Cryptosporidium* spp.		p-value*
	Positive (mean ± SD)	Negative (mean ± SD)	Positive (mean ± SD)	Negative (mean ± SD)	
Altitude (meters)	1256.82±496.53	1216.14±462.27	1220.38±532.51	1222.92±459.64	a =0.792 b =0.989
Salinity (mg/L)	0.28±0.19	0.29±0.23	0.23±0.17	0.30±0.23	a =0.849 b =0.452
pH	7.51±0.59	7.34±0.58	7.48±0.54	7.35±0.59	a =0.380 b =0.554
DO (mg/L)	64.68±35.67	56.84±26.67	39.56±14.63	60.52±28.65	a =0.401 **b =0.047**
Pressure (mmHg)	652.00±38.84	653.21±37.07	655.50±41.27	652.69±36.85	a =0.922 b =0.842
Temperature (kelvin)	286.00±4.52	284.98±3.85	285.38±4.07	285.11±3.69	a =0.437 b =0.862

SD: Standard deviation, a: Significance value for *C. cayetanensis*, b: Significance value for *Cryptosporidium* spp., *Significance levels according to independent t-test

surface water. Moreover, during this period, one of the biggest epidemics affecting 13,000 people occurred as a result of the contamination of a chlorinated and filtered public water source with *Cryptosporidium* spp. (23).

In the 2-year period between 1989 and 1990, 26 outbreaks of drinking water were reported in 16 states, and an estimated 4288 people were affected in these outbreaks. *G. intestinalis* was cited as the etiologic agent in 7 of 12 outbreaks in which a single agent was identified. All outbreaks of giardiasis were associated with the consumption of filtered surface water or surface-affected groundwater (24).

G. intestinalis is the leading parasitic etiology of human enteric infections in the USA. About 1.2 million cases occur annually. Between 1971 and 2011, 242 outbreaks of giardiasis were reported to the CDC. These outbreaks, which affected approximately 41,000 people, were caused by waterborne (74.8%), foodborne (15.7%), person-to-person (2.5%), and animal contact (1.2%). Most waterborne outbreaks (74.6%) were associated with drinking water, followed by recreational water (18.2%) (25).

Diarrhea is one of the major factors that cause mortality and morbidity in children. While it causes the death of approximately 2.5 million people every year, it causes growth and developmental retardation in children in the long term. One of the important causes of diarrhea is intestinal parasitic infections (26).

Globally, cryptosporidiosis is estimated to be responsible for the majority of deaths among children under the age of five. In the Global E Haque Enteric Multicentre Study, a 3-year matched case-control study of more than 22,000 infants and children 0-59 months of age with moderate-to-severe diarrhea in seven regions in Africa and Asia, *Cryptosporidium* spp. was the second leading cause of severe diarrhea after rotavirus (27).

Diarrhea is a commonly reported disease in young animals and remains a major cause of productivity and economic loss for cattle producers worldwide. Diarrhea is a clinical condition that causes serious economic losses because it can cause high mortality, weight loss or even delayed growth in different animals and even humans (28).

Many pathogens are transmitted by water. Many studies have been conducted on pathogenic agents in waters. Many studies have been carried out around the world on the detection of parasitic agents in water samples.

In a study conducted with different methods on 166 water samples in Russia and Bulgaria, *G. intestinalis* was detected in 9.6% of the samples (29). Water samples taken from a total of six rivers, four in Washington and two in California, were examined and *Cryptosporidium* spp. oocysts were detected in all of the samples (30). In North America from 1985 to 1994, it was reported that 12 epidemics have occurred due to the contamination of drinking water sources by *Cryptosporidium* spp. In two of these 12 outbreaks (Milwaukee and Las Vegas), the mortality rate in immunocompromised individuals was between 52% and 68%, and the largest *Cryptosporidium* spp. epidemic was found in the USA, with 403,000 symptomatic cases (31). It was stated that 13,000 people were infected with *Cryptosporidium* spp. in 1987 in Carrolton, USA, due to the contamination of drinking water sources It was also reported in 1992 that *Cryptosporidium* spp. Infection affected the health of 15,000 people in Jankson County and Oregon, USA (32). Another study conducted in Las Vegas, USA, in 1998 reported that 20 AIDS patients died in 1994 due to drinking water contaminated with *Cryptosporidium* spp. (33).

The first parasitological study in waters in Türkiye was carried out between 1997 and 1999 at Kağıthane, Büyükçekmece, Ömerli and Elmalı dams in İstanbul. 40 raw water samples obtained from these dams were examined for *Cryptosporidium* spp. and *G. intestinalis* and it was reported that *Giardia* cyst and *Cryptosporidium* oocyst were not detected in any of them. In a study conducted in Mersin, the prevalence of *C. parvum* in water resources was determined as 5.2% (7/135) (16). In a study conducted in Samsun, 11% of *Cryptosporidium* spp. oocysts were detected in 100 water samples (34). In another study conducted in the environmental waters of Samsun Province and its districts, 142 of 228 water samples contained *Giardia* spp., 132 contained *Cryptosporidium* spp., 56 contained *Cyclospora* spp., 38 contained *Microsporidia*, 47 contained *Blastocystis* spp., 38 contained *Entamoeba coli* cyst, 18 contained *Dientemoeba*, 9 contained *Chilomastix*, 9 contained *Strongyloides* spp., and 6 contained hookworm (35). In a study carried out with 40 water samples in Isparta, *C. parvum* cyst was found in 13 (32.5%) samples and *G. intestinalis* cysts were found in 8 samples (20%) (36). In a study conducted by Çiçek et al. (37) in the Van region, it was reported that *Cryptosporidium* spp. oocysts were detected in 1.13% of 440 water samples. In another study conducted in Erzurum, it was reported that *Cryptosporidium* spp. oocysts were detected in 15% of 120 water samples (38).

Different results were obtained in studies conducted in Türkiye. *C. cayetanensis* was detected in 13%, *Giardia* spp. in 1.4%, and *Cryptosporidium* spp. in 10.1% of 69 water samples examined in this study. The rate of *Cryptosporidium* spp. in the study was compatible with the studies conducted in Erzurum and Samsun, and it was higher than the results of the studies conducted in Sivas, Mersin, and Van. The rate of *Giardia* spp. and C. cay*etanensis* in the study was less than the rates in other studies. It is known that epidemics originating from drinking water are important problems in terms of public health, and the contamination rate (27.5%) in this study supported this.

CONCLUSION

As a result, although the direct examination method is one of the most used methods in the investigation of parasitic agents, it was concluded that it is not sufficient alone in the detection of parasites and that different diagnostic methods should be used together. In the literature reviews conducted herein, it was concluded that since the research and methods used in this subject in Türkiye are very limited, they do not reflect the truth and there is a need for comprehensive research. It is our belief that the prevalence of parasitic agents in drinking water will be high if more comprehensive studies are conducted in Türkiye.

Cryptosporidium spp., *C. cayetanensis* and *G. intestinalis* are important waterborne pathogens that can cause epidemics. It is our belief that in order to reduce the risk of contamination of these parasitic factors with spring waters, public awareness should be raised, infrastructures should be improved, and new water treatment techniques, such as ultraviolet, ozonation, and monitoring systems, should be used.

ACKNOWLEDGEMENTS

The authors would like to thank Iğdır University Scientific Research Projects Coordination Unit for providing financial support to this study (project number: 2020-SBE-AP01).

* Ethics

Ethics Committee Approval: Ethics committee approval is not required for this study.

Informed Consent: No patients were included in the study.

Peer-review: Evaluated by the editorial board.

* Authorship Contributions

Concept: Ö.A., A.E., E.G., Design: Ö.A., A.E., S.A., Data Collection or Processing: S.A., Ö.A., M.Ş., A.E., Analysis or Interpretation: A.E., M.Ş., S.A., Ö.A., Literature Search: Ö.A, M.Ş., S.A., Posted by: Ö.A., M.Ş., S.A.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: This research has been refunded by Scientific Research Projects Unit of Iğdır University (project number: 2020-SBE-AP01).

REFERENCES

1. Franco RMB, Rocha-Eberhardt R, Cantusio NR. Occurrence of *Cryptosporidium* oocysts and *Giardia* cysts in raw water from the Atibaia river, Campinas, Brazil. Rev Inst Med Trop Sao Paulo 2001; 43: 109-11.
2. Moreira NA, Bondelind M. Safe drinking water and waterborne outbreaks. J Water Health 2017; 15: 83-96.
3. Terzi G. Gıda kaynaklı protozoon enfeksiyonların insan sağlığı açısından önemi. Yüzüncü Yıl Üniversitesi Veteriner Fakültesi Dergisi 2005; 16: 47-55.
4. Alemu G, Mama M, Misker D, Haftu D. Parasitic contamination of vegetables marketed in Arba Minch town, southern Ethiopia. BMC Infect Dis 2019; 19: 410.
5. Ahlinder J, Svedberg AL, Nystedt A, Dryselius R, Jacobsson K, Hagglund M, et al. Use of metagenomic microbial source tracking to investigate the source of a foodborne outbreak of cryptosporidiosis. Food Waterborne Parasitol 2022; 26: e00142.
6. WHO 2003a. Quantifying selected major risks to health. The World Health Report 2002. World Health Organization, Geneva (Chapter 4).
7. Ashbolt NJ. Microbial contamination of drinking water and disease outcomes in developing regions.Toxicology 2004; 198: 229-38.
8. Schreiber C, Heinkel SB, Zacharias N, Mertens FM, Christoffels E, Gayer U, et al. Infectious rain? Evaluation of human pathogen concentrations in stormwater in separate sewer systems. Water Science & Technology 2019; 80: 1022-30.
9. Cirkovica V, Kluna I, Utaakerb KS, Uzelaca A, Tysnesb KR, Robertsonb LJ, et al. Surface waters as a potential source of *Giardia* and *Cryptosporidium* in Serbia. Experimental Parasitology 2020; 209: 1-6.
10. Casemore DP. Epidemiological aspects of human cryptosporidiosis. Epidemiol Infect 1990; 104: 1-28.
11. Ramirez NE, Ward LA, Sreevatsan S. A review of the biology and epidemiology of cryptosporidiosis in humans and animals. Microbes and Infection 2004; 6: 773-85.
12. Nasser AM. Removal of Cryptosporidium by wastewater treatment processes: a review. J Water Health 2016; 14: 1-13.
13. Hassan EM, Örmeci B, DeRosa MC, Dixon BR, Sattar SA, Iqbal A. A review of *Cryptosporidium* spp. and their detection in water. Water Research 2021; 83: 1-25.
14. Drenaggi D, Cirioni O, Giacometti A, Fiorentini A, Scalise G. Cyclosporiasis in a traveler returning from South America. J Travel Med 1998; 5: 153-5.
15. Yu RJ, Sohn WM. A case of human cyclosporiasis causing traveler's diarrhea after visiting Indonesia. J Korean Med Sci 2003; 18: 738-41.
16. Aslan G, Bayram G, Otağ F, Direkel Ş, Özkan At, Çeber K, et al. Mersin ilinde farklı su kaynaklarında *Cryptosporidium* spp. varlığının araştırılması. Mikrobiyoloji Bülteni 2012; 46: 93-100.
17. Kłudkowska M, Pielok L, Frąckowiak K, Paul M. Intestinal coccidian parasites as an underestimated cause of travellers' diarrhoea in Polish immunocompetent patients. Acta Parasitol 2017; 62: 630-8.
18. Ortega YR, Sanchez R. Update on *Cyclospora cayetanensis*, a Food-Borne and Waterborne Parasite. Clin Microbiol Rev 2010; 23: 218-34.
19. Kahler AM, Mattioli MC, Silva AJ, Hill V. Detection of *Cyclospora cayetanensis* in produce irrigation and wash water using large-volume sampling techniques. Food and Waterborne Parasitology 2021; 22: 1-8.
20. Orlandi PA, Frazar C, Carter L, Chu D. BAM: detection of *Cyclospora* and *Cryptosporidium*. U.S. Food and Drug Administration 2004.
21. Sakarya Y, Kar S, Tanyüksel M, Karaer Z, Babur C, Vatansever Z. Detection of *Cryptosporidium* sp. in humans and calves through nested PCR and carbol fuchsin staining methods in Ankara, Turkey. Kafkas Univ Vet Fak Derg 2010; 16: 977-80.
22. Hayes EB, Mat TD, O'Brien TR, McKinley TW, Logsdon GS, Gül JB, et al. Large community outbreak of cryptosporidiosis due to contamination of a filtered public water supply. N Engl J Med 1989; 320: 1372-6.
23. Levine WC, Stephenson WT, Craun GF. Waterborne disease outbreaks, 1986-1988. Journal of Food Protection 1991; 54: 71-8.
24. Herwaldt BL, Craun GF, Stokes SL, Juranek DD. Waterborne-Disease Outbreaks, 1989-1990. MMWR CDC Surveill Summ 1991; 40: 1-21.
25. Adam EA, Yoder JS, Gould LH, Hlavsa MC, Gargano JW. Giardiasis outbreaks in the United States, 1971-2011. Epidemiol Infect 2016; 144: 2790-801.
26. Haque R, Mondal D, Duggal P, Kabir M, Roy S, Farr BM, et al. Entamoeba histolytica infection in children and protection from subsequent amebiasis. Infect Immun 2006; 74: 904-9.
27. Ryan U, Zahedi A, Paparini A. *Cryptosporidium* in humans and animals-a one health approach to prophylaxis. Parasite Immunol 2016; 38: 535-47.
28. Haggag YN, Samaha HA. Nossair MA, Awad WK. Epidemiological Studies on Diarrhea in Some Dairy Cattle Farms AJVS 2016; 51: 282-9.
29. Karanis P, Sotiriadou B, Kartashev V, Kurenti C, Tsvetkova N, Stojanova K. Occurrence of *Giardia* and *Cryptosporidium* in water supplies of Russia and Bulgaria. Environmental Research 2006; 102: 260-71.
30. Ilardi I, Leone F. Technics for identifying *Cryptosporidium* spp. in feces: synthetic review and personal experience on anti-HIV seropositive, AIDS-related complex and AIDS subjects. Quaderni Sclavo di diagnostica clinica e di laboratorio 1988; 24: 133.
31. Mac Kenzie WR, Hoxie NJ, Proctor ME, Gradus SM, Blair KA, Peterson DE, et al. A massive outbreak in Milwaukee of *Cryptosporidium* infection transmitted through the public water supply. N Engl J Med 1994; 331: 161-7.
32. Stein PL. The great Sydney water crisis of 1998. Water, air, and soil pollution 2000; 123: 419-36.
33. Steiner TS, Thielman NM, Guerrant RL. Protozoal agents: what are the dangers for the public water supply? Annu Rev Med 1997; 48: 329-40.
34. Çeber K, Aslan G, Otağ F, Delialioğlu N, Öztürk C, Babür C, ve ark. Mersin İlinde İçme Suyu, Kullanma Suyu, Atık Su ve Deniz Sularında *Cryptosporidium* spp. Ookistlerinin Araştırılması. Türkiye Parazitoloji Dergisi 2005; 29: 224-8.
35. Karaman Ü, Kolören Z, Seferoğlu O, Ayaz E, Demirel E. Samsun İl ve İlçelerinden Alınan Çevresel Sularda Parazitlerin Varlığı. Turkiye Parazitol Derg 2017; 41: 19-21.

36. Rad AY, Aysal S, Taner M. Investigation of *Cryptosporidium parvum* and *Giardia intestinalis* in Various Water Sources in Isparta Area, Turkey. Hacettepe J Biol & Chem 2007; 35: 209-17.

37. Çiçek M, Körkoca H, Akkaş Ö. Investigation for *Cryptosporidium* spp. oocysts of drinking water in Van. Turkish Bulletin of Hygiene and Experimental Biology 2011; 68: 122-6.

38. Yılmaz A, Akkaş Ö, Uslu H. Investigation of *Cryptosporidium* sp. Oocysts in Erzurum's Potable Water On Different Months. Middle Black Sea Journal of Health Science 2016; 2: 1-6.