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Original

Prevalence of cryptosporidiosis in humans and calves, and molecular detection of *Cryptosporidium parvum*

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ABSTRACT

Objective. To investigate of the prevalence of *Cryptosporidium* species in humans and calves in the province of Van, Turkey. **Materials and methods.** Included in this research were 150 patients, comprising 50 hemodialysis patients, 40 immunosuppressed patients with diarrhea, 30 patients with diarrhea only, and 30 immunocompetent patients. Collected were stool rectal samples from 50 calves that were housed in stables and farms in 10 central villages of Van, Turkey. **Results.** *Cryptosporidium parvum* was detected in 17.3% of the 150 human stool samples. *C. parvum* was observed in 20% of the 50 samples from the hemodialysis patients, 32.5% of the 40 samples from the immunosuppressed patients with diarrhea, and 10% of the 30 samples from patients with diarrhea only, whereas no *Cryptosporidium* spp. was detected in the samples from the immunocompetent patients. *C. parvum* was observed in only 6% of the samples from the diarrheic 30 calves. **Conclusions.** It was clearly understood that cryptosporidiosis was detected at a high rate in the samples from the immunosuppressed patients and those who were immunosuppressed with diarrhea, and that the active and effective species that causes cryptosporidiosis in the Van region is *C. parvum*. Hence, these patient groups should be evaluated in terms of cryptosporidiosis.

Keywords: *Cryptosporidium* parvum; PCR; prevalence (*Source CAB*).

RESUMEN

Objetivo. El objetivo de este estudio fue investigar la prevalencia de especies de *Cryptosporidium* en humanos y terneros en la provincia de Van, Turquía. **Materiales y métodos.** Se incluyeron en el estudio un total de 150 pacientes, incluidos 50 pacientes en hemodiálisis, 40 pacientes inmunosuprimidos con diarrea, 30 pacientes con diarrea solamente y 30 pacientes inmunocompetentes. Se recolectaron muestras de heces rectales de un total de 50 terneros alojados en establos y granjas en 10 aldeas centrales de Van, Turquía. **Resultados.** Se detectó *Cryptosporidium* parvum en el 17.3% de las 150 muestras de heces tomadas de seres humanos. *C. parvum* se observó en el 20% de los 50 pacientes

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en hemodiálisis, el 32.5% de los 40 pacientes inmunosuprimidos con diarrea y el 10% de los 30 pacientes con diarrea solamente, mientras que no hubo *Cryptosporidium* spp. detectado en los pacientes inmunocompetentes. *C. parvum* se observó en sólo el 6% de los 30 terneros diarreicos. **Conclusiones.** Claramente se entendio que la Criptosporidiosis fue detectada en una alta tasa en las muestras de los pacientes inmunosuprimidos sin y con sintomas de diarrea, y que además la especie activa que causó la enfermedad fue el agente etiologico Criptosporidium parvum. Por lo tanto, estos dos grupos de pacientes deben ser evaluados en lo que a términos de Criptosporidiosis se refiere.

Palabras clave: *Cryptosporidium parvum*; PCR; prevalencia (*Fuente: CAB*).

INTRODUCTION

Parasitic diseases are one of the most significant worldwide health problems and are most commonly seen in countries that are still developing. Parasitic infections are more dangerous for adolescents and immunocompromised patients (1).

Some intestinal parasites, which are not pathogenic, do not show any symptoms, or cause mild diarrhea in individuals with a healthy immune system, can progress much more severely in patients with a suppressed immune system, and cause fatal diarrhea. Among the most common intestinal parasites that can cause fatal diarrhea in these patient groups are species of *Cryptosporidium*. *Cryptosporidium* species live in the microvilli of the stomach and intestinal mucosa epithelial cells. They show an intracellular extrastoplasmic location and provide energy and nutrition to the cell. *Cryptosporidium parvum* does not have intermediate hosts in its life cycle. *Cryptosporidium* is transmitted orally and fecally. Methods of transmission include coming into direct contact with an infected person (person to person) or from animal to person, as well as by the oral intake of contaminated food and beverages. This parasitosis affects the entire digestive system, but the part that is most heavily affected is the jejunum. Apart from the digestive tract, the respiratory and bile ducts are also affected. In cryptosporidiosis, atrophy of the villi, elongation of the length of the crypts, and infiltration of the mononuclear cells into the lamina propria occur. Due to the inflammatory damage in the small intestines, absorption is impaired and with increased secretion, diarrhea and growth retardation occur (1-4).

The examination material in the diagnosis of cryptosporidiosis is feces. Cryptosporidiosis is diagnosed by painted microscopic examination of the stool, fluorescent methods, tests based on antigen detection, and molecular methods (5). Diagnosis is made via the appearance of oocysts in the painted stool using the modified acid-fast staining method. In the commonly used painted microscopic examination evaluation, the requirement of experienced personnel and its low sensitivity are important disadvantages. Immunofluorescence microscopy is used as the gold standard in the reference laboratory of the USA and Europe. Molecular methods are used in laboratories with infrastructure due to their high sensitivity and specificity and they allow for the separation of the species (1,5).

The diagnosis of infections due to *Cryptosporidium* species has generally been based on the presence of the agent in the stool and bile samples by various methods. Methods such as endoscopy, mucosa biopsy, small intestine aspirate, and bronchoalveolar lavage are also used for diagnosis. However, using these techniques alone is sometimes insufficient to precisely find the agent (1,5).

As faecal samples from clinical cases generally have within large numbers of cysts and parasite antigenic material, even methods that have a low sensitivity can give a positive diagnosis. In contrast, when testing samples having in it few cysts, as may be needed for an epidemiological investigation, the use of an initial screening careful way, went after by a confirmatory careful way such as immunofluorescence or molecular views can augment secrets in the diagnosis. For this purpose the immunofluorescent staining of cysts with fluorescein isothiocyanateconjugated anti *Cryptosporidium* monoclonal antibody has been stated to be particularly specific (96–100%) and sensitive (98.5–100%). On the other hand, *Cryptosporidium* fecal antigen can be detected in stool samples even before the oocysts start to be excreted. There are many studies on ELISA and immunochromatographic

tests with different fecal antigen specificities, and the reported specificity and sensitivity are between 97% and 100%. Another advantage of these fecal antigen detection assays is that they can be used to test large numbers of samples in a fast and cost-effective manner. However, for more detailed epidemiological studies, these tests are not suitable because they do not provide any (6).

Different studies have been conducted on *Cryptosporidium* spp. in humans and calves in Van Province. In a study conducted on children, *Cryptosporidium* spp. was found at a rate of 4.9% (7). In another study conducted on children with diarrhea, *Cryptosporidium* spp. was found at a rate of 28% (8). In a study conducted on asymptomatic food workers, the incidence of *Cryptosporidium* spp. was 1.3% (9). *Cryptosporidium* spp. was found in 1.1% of workers, 13.2% of slaughtered sheep, 10.7% of goats, and 8.1% of cattle working in the municipality of Van (10). In another study conducted on calves, *Cryptosporidium* spp. was found in 37.2% of calves without diarrhea and 43.9% of calves with diarrhea (11).

The aim of the current research was to investigate the prevalence of cryptosporidiosis in stool samples that were collected from both humans and calves in the Van region of Turkey using the modified acid-fast staining method, immunochromatographic test, and nested polymerase chain reaction (PCR) method. In addition, it was aimed to compare the methods and characterize the *Cryptosporidium*-positive samples using the PCR-restriction fragment length polymorphism (PCR-RFLP) method.

MATERIALS AND METHODS

Sample collection. This study was performed between June 2016 and February 2017 at the Department of Parasitology Research Laboratory, Faculty of Medicine, Van Yüzüncü Yıl University. Stool samples were obtained from both humans and calves. A total of 200 stool samples, comprising 150 from humans (consisting of 50 from patients with chronic kidney failure, 40 from immunosuppressed patients with diarrhea, 30 from patients with only diarrhea, and 30 from immunocompetent patients) and 50 from calves (30 from calves with diarrhea and 20 from calves without diarrhea) were included in the study. Collection of the calf feces samples was performed directly from the animal rectums. The epidemiological history and clinical findings of the patients were recorded from the hospital automation system.

In this study, the modified acid-fast staining method, immunochromatographic test, nested PCR method, and RFLP were used (12,13).

Genomic DNA isolation. The DNA needed for the molecular methods to be used in the study was obtained using an extraction kit (GeneMATRIX stool DNA purification kit, Poland). At this stage, the oocysts were vortexed at intervals of 5 min and incubated at 95 °C for 30 min in a dry block heater. DNA extraction was then performed in accordance with the manufacturer's instructions

Nested PCR. BCOWPF: 5′-ACCGCTTCTCAA CAACCATCTTGTCCTC-3′ and BCOWPR: 5′-CGCACCTGTTCCCACTCAATGTAAACCC-3′ were used in the first step of the nested PCR method. For the second stage of the PCR procedure, the desired region was amplified using primers Cry-15: 5′-GTAGATAATGGAAGAGATTGTG-3′ and Cry-9: 5′-GGACTGAAATACAGGCATTATCTTG-3′, which amplified the region of the 553 bp length (13). The PCR protocol was created using Solis Biodyne 5X Firepol (12.5 mM of $MgCl_2$) ready-made master mix. The reaction mixture was prepared at a final concentration of 20 µL for the first primer set and 30 µL for the second primer set. The primers were diluted to 10 nM with sterile distilled water. The reaction mixture was prepared in the first PCR procedure with 12 µL of H_2O, 4 µL of 5X firepol master mix, 1 µL of forward primer and the reverse primer, and 2 µL of DNA. For the second stage of the PCR procedure, 20 µL of H_2O, 6 µL of 5X firepol master mix, and 1.5 µL of the forward primer and the reverse primer were prepared. The PCR heat cycle comprised 95°C for 4 min and 95°C for 33 s, and then 18 cycles at 58°C for 45 s, 72°C for 45 s, 72°C for 10 min, and 4°C ∞. For the second PCR procedure, only a primary binding temperature of 45°C for 33 cycles was used.

Bidirectional sequence analysis of the *Cryptosporidium* sp. oocyst wall protein (COWP) gene region was made by sending it to a commercial firm. The sequence analysis results obtained were checked using SnapGene 4.1 software (GSL Biotech LLC, San Diego, CA, USA). The sequence data obtained were entered in the relevant places in GenBank.

The PCR products that were found to be positive using the nested PCR method were subjected to enzyme cutting for the detection of *Cryptosporidium* species. Fast digest restriction *Rsa*I enzyme was used for this procedure (Thermo Scientific, Waltham, MA, USA). The procedure was modified by combining 16.8 µL of H_2O, 2.5 µL of Buffer, 0.7 µL of RsaI enzyme, and 10 µL of DNA, and the total volume was adjusted to 30 µL. Incubation of the prepared mix was performed for 30 min at 37°C. The samples that had been subjected to PCR-RFLP were then run on agarose gel (2%).

This study was approved by the Van Yuzuncu Yil University Non-Interventional Clinical Research Ethics Committee (21.09.2017/ Decision no: 2017/09) and Animal Experiments Local Ethics Committee (29.12.2016/Decision no: 2016/12). This research was supported by the Scientific Research Projects Directorate of Yüzüncü Yıl University as the project numbered "TDK-2017-5796".

Statistical analyses. In the statistical evaluation, the incidence of the disease was expressed as numbers and percentages according to the relevant categorical variables. Calculation of the relationship between the categorical variables was performed with the chi-square (χ^2) test. The Z-ratio test was utilized in the determination of the average of the sample mean. It was accepted that $p<0.05$ indicated statistical significance and the MINITAB (Ver:14, Minitab Inc., State College, PA, USA) statistics program was also utilized in the calculations.

RESULTS

In this study, *C. parvum* was detected in 10 (20%) of the samples from the 50 hemodialysis patients, 13 (32.5%) of the 40 samples from the immunosuppressed patients with diarrhea, and only 3 (10%) of the 30 samples from the patients with diarrhea. No *Cryptosporidium* species were found in the samples from the immunocompetent patients. Thus, *C. parvum* was detected in 26 (17.3%) of the 150 human samples in total. While parasites were observed in 3 (10%) of the 30 samples from the calves with diarrhea, no parasites were found in the 20 samples from the calves without diarrhea, and *C. parvum* was detected in 3 (6%) of the 50 samples from the calves in total.

When a comparison of the results obtained using the immunochromatographic test and the modified acid-fast staining methods was performed with the results obtained using the nested PCR method, it was determined that there was a statistically significant difference between them ($p<0.05$, Table 1). The samples found to be positive using the modified acid-fast staining method were also found to be positive by the immunochromatographic test and the nested PCR method. However, 8 samples that were found to be negative using the modified acid-fast staining method were found to be positive with the other 2 methods. In this study, it was found that the sensitivity was 72.4% and the specificity was 100% using the modified acid-fast staining method. It was observed that the immunochromatographic test, which gives much more practical and rapid results, and works with the principle of antigen screening in stool, exhibited more sensitivity when compared to the modified acid-fast staining method, the sensitivity and specificity were both 100%, and the same results were obtained with the nested-PCR method.

Table 1. Incidence rates of *Cryptosporidium* species according to the diagnosis methods.

Method	MAFS	ICGT (%)	Nested PCR	Total
MAFS	-	21	21	200
ICGT test	21	-	29	200
Nested PCR	21	29	-	200

MAFS: Modified acid-fast staining; ICGT: Immunochromatographic test

In this study, significant relationships were found between the *Cryptosporidium* species and these symptoms; abdominal pain ($p=0.0001$), nausea ($p=0.0001$), vomiting ($p=0.0001$), weakness ($p=0.0001$), and anorexia ($p=0.0001$), in individuals infected with cryptosporidiosis ($p=0.001$), and in the statistical evaluation, significant relationships were found between this disease and these symptoms separately (Table 2). Evaluation of the prevalence of *Cryptosporidium* species according to the place of residence of the individuals was also made (Table 3; Figure 1).

Table 2. Relationship of the prevalence of *Cryptosporidium* species with some clinical symptoms.

Clinical signs and symptoms	Cryptosporidium sp.		Total	P-value
	Positive (%)	Negative (%)		
Vomiting	13 (65)	7 (35)	20	0.0001
	13 (10)	117 (90)	130	
Nausea	15 (55.6)	12 (44.4)	27	0.0001
	11 (8.9)	112 (91.1)	123	
Weakness	17 (65.4)	9 (34.6)	26	0.0001
	9 (7.3)	115 (92.7)	124	
Anorexia	10 (41.7)	14 (58.3)	24	0.001
	16 (12.7)	110 (87.3)	126	
Abdominal pain	16 (66.7)	8 (33.3)	24	0.0001
	10 (7.9)	116 (92.1)	126	
Mucus	17 (73.9)	6 (26.1)	23	0.0001
	9 (7.1)	118 (92.9)	127	

Table 3. Evaluation of the prevalence of *Cryptosporidium* species according to the place of residence of the individuals.

Place of residence	Cryptosporidium spp.		Total	P-value
	Positive (%)	Negative (%)		
Countryside	9 (45)	11 (55)	20	0.0001
Town center	17 (13.1)	113 (86.9)	130	
Total	26 (17.3)	124 (82.7)	150	

No statistically significant results were found between cryptosporidiosis and gender, growth retardation, constipation, presence of other parasites, fever, leukocytes, or age.

One or more parasite species were found in 65 (43.3%) of the human stool samples. Herein, *Cryptosporidium* sp. was found in 26 human samples (17.3%), *B. hominis* was found in 21 human samples (14%), *G. intestinalis* was found in 12 human samples (8%), *C. cayetanensis* was found in 4 human samples (2.7%), and *C. mesnili* was found in 2 (1.3%) human samples. The microscopic examination of the preparations stained using the modified acid-fast staining method showed an oocyst in 10 different areas, evaluated as low-density oocysts (+), 2–10 as medium density oocysts (++), 11–25 as high-density oocysts (+++), and >25 oocysts as very high intensity (++++) (12). Of the 21 patients that were determined to be positive in terms of parasites using modified acid-fast staining method, 10 (47.6%) had high density oocysts, 6 (28.6%) had medium density oocysts, and 5 (23.8%) had low density oocysts. Moreover, 9 (42.9%) patients with high concentrations of oocysts were also found to have immunosuppression and diarrhea.

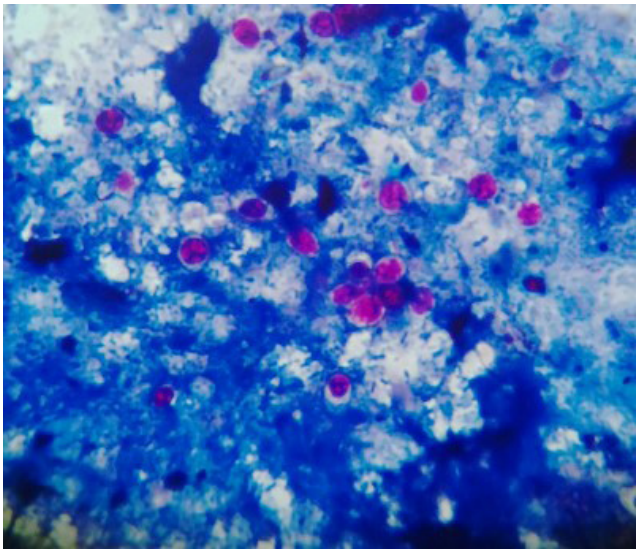

Figure 1. *Cryptosporidium* sp. detected in the stool of a patient (X1000).

***Cryptosporidium* sp. oocyst wall protein (COWP) gene nested-PCR and PCR-RFLP findings.** In the study, the COWP gene region of the genomic DNA obtained from the stool samples was amplified using the nested PCR method and the 553 bp region was amplified (Figure 2A). The second set of PCR products obtained were performed using gel electrophoresis at 90 V for 45 min, and amplification of the samples was observed.

Positive samples were determined using the nested PCR method. The samples were sent to a DNA sequence analysis laboratory and subjected to bidirectional sequence analysis. The sequence analysis results were found in the www.ncbi.nlm.nih.gov database, and all of the samples that were found to be positive

were infected with *C. parvum*. The sequence sequences of the samples, which comprised MG255781, MG255782, MG255783, MG255784, MG255785, MG255786, MG255787, MG255788, MG255789, MG255790, MG255791, MG255792, MG255793, MG255794, MG255795, MG255796, MG255797, MG2557, MG255786, MG255797, and MG255788, were added to the www.ncbi.nlm.nih.gov database. They were registered under accession numbers (GenBank accession number) MG255806, MG255807, MG255808, and MG255809. The names of the 3 species, VANH6, VANH9, and VANH10, in which various nucleotide differences were detected in the studied COWP gene region, were given.

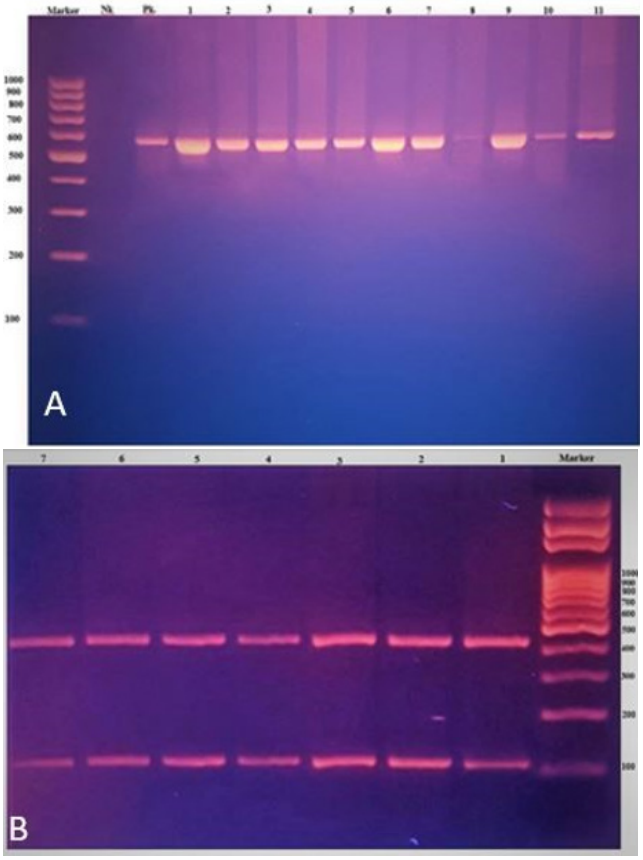

Figure 2. A. *Cryptosporidium* sp. COWP gene region amplification of the 553 bp region using the nested PCR method in agarose gel (marker (100 bp ladder) Nk: negative control, Pk: positive control, positive samples: 1–11). **B.** Bands formed as a result of detecting *Cryptosporidium* species in electrophoresis using the PCR-RFLP method using the *RsaI* enzyme marker (100 bp ladder); *C. parvum*: 1–7.

When the PCR-RFLP method was applied using the *RsaI* enzyme after using the nested PCR method, bands were formed in the 34, 106, and 410 bp regions in *C. parvum*. and in the 34, 106, 125, and 285 bp regions in *C. hominis* (13). These samples were found to be *C. parvum*-positive using agarose gel imaging.

DISCUSSION

In the many studies that have been conducted on cryptosporidiosis to date, it was reported that the most infectious species in human and vertebrate animals was *C. parvum* (1). Moreover, *Cryptosporidium* sp. oocysts were found at a rate of 1.8%–46.8% (14-17).

In our study, *Cryptosporidium* sp. was found in 26 (17.3%) of the 150 human stool samples examined, which was higher than the results obtained other studies conducted in Turkey (16, 18). The low socioeconomic status of the Van region, insufficient infrastructure, lack of attention to personal hygiene, and widespread animal husbandry are among the reasons why it is higher there than in other regions. The fact that the parasite species was *C. parvum* in all of the human samples in the study supported this opinion. In addition, was believed herein that similar high results would be found if the methods used in the current study were used in many regions of Turkey where cattle breeding is widespread.

Cryptosporidiosis can cause fatal diarrhea in immunocompromised patients, diarrhea in children, severe fluid loss, electrolyte imbalance, and even circulatory failure in advanced cases (19). *Cryptosporidium* species, which are opportunistic protozoans, are usually asymptomatic in immune-compromised individuals, and sometimes cause limited diarrhea. However, it can cause fatal diarrhea with a much more severe course, especially in immunosuppressed patient groups, patients with AIDS or cancer, dialysis patients or patients whose immune system is suppressed for any reason (20). In the current research, clinical symptoms such as nausea (p=0.0001), vomiting (p=0.0001), abdominal pain (p=0.0001), malaise (p=0.0001), mucus (p=0.0001), and anorexia (p=0.001) were evaluated with cryptosporidiosis. Statistically significant correlations were found between the symptoms.

It was concluded that *Cryptosporidium* sp., an opportunistic protozoan, should be evaluated in terms of cryptosporidiosis, especially in patients with immunosuppression and diarrhea.

Microscopy methods used to investigate *Cryptosporidium* species in stool are of low sensitivity and require experienced personnel and time. In addition, different staining methods are needed to detect *Cryptosporidium* and some other parasites that cannot be detected by direct examination. In the current study, 21 cases of *Cryptosporidium* sp. were detected in a total of 200 stool samples collected from humans and calves using the modified acid-fast staining method, and 8 cases that could not be detected using this method were detected using the immunochromatographic test and PCR methods, which showed that there may be cases that were overlooked using the modified acid-fast staining method.

Herein, it was found that 9 (42.9%) of the immunosuppressed patients with diarrhea were evaluated as having high density oocysts by examining 10 microscope fields. It was believed that *Cryptosporidium* sp., which is an opportunistic protozoan, should be taken into consideration, especially in immunosuppressed patients, and if diarrhea is seen in this patient group, these patients should also be evaluated in terms of cryptosporidiosis.

All of the samples herein that were found to be positive for *Cryptosporidium* sp. using the modified acid-fast staining method were also found to be positive using the immunochromatographic test method. However, 8 samples that were found positive using the immunochromatographic test method were not found to be positive using the modified acid-fast staining method. Although the cost of the immunochromatographic test method, which looks for specific antigens in stool, is higher than that of the modified acid-fast staining method, it was concluded that it would be the more preferred method because it can be applied more easily than other diagnostic methods. At the same time, it was seen that the results obtained with the Biotek Certest Crypto (Spain) brand cassette test, which is a rapid diagnostic kit, were exactly the same as those of the nested-PCR method. It was observed that the samples with low positive results using the immunochromatographic test also exhibited silent bands when they were run in agarose gel after the nested-PCR method. It was determined that the immunochromatographic test method used provided results in a short time, it did not require experienced personnel, and its reliability was high. Cryptosporidiosis can lead to fatal diarrhea in immunocompromised patients, and also cause diarrhea in children, and severe fluid loss, electrolyte imbalance, and even circulatory failure in advanced cases. Since cryptosporidiosis cannot be diagnosed using only clinical examinations of the patients, ineffective and long-term treatments are generally attempted. For this reason, unnecessary medication will be given to the patients and their conditions will become more serious, because the patients cannot be treated due to incorrect treatment. In addition, the economy of the country is harmed due to this unnecessary and ineffective drug usage. For this reason, the importance of using rapid diagnostic tests in every hospital is better understood when an assessment is made in terms of both protecting patient health and not harming the economy of the country.

DNA extraction is the first step of molecular studies. Since feces has a structure that contains many organisms, it directly affects the purity and quality of DNA in extraction. The health of the study is directly related to the quality of the DNA extraction and the purity of the DNA that is obtained. The storage conditions of the stool, kits to be used in the extraction process, characteristics of the agent sought, and lysis method used are factors that directly affect the success of DNA extraction. The intact oocyst wall of some parasites, such as *Cryptosporidium* species, negatively affects the success of DNA extraction (10). While there is no problem when DNA extraction is made with other parasites, when DNA extraction from *Cryptosporidium* sp. is attempted using the same methods, the process may fail. For these reasons, researchers working with *Cryptosporidium* species may need to conduct some procedures to weaken the oocyst wall or make various modifications to the kit procedure before beginning the DNA extraction (11). Herein, *Cryptosporidium* sp. DNA could not be obtained as a result of the incubation process performed by heating the stool samples that were kept at –20 °C for a certain period of time at 95 °C for 30 min. However, as soon as fresh stool samples were brought into the

laboratory, they were subjected to the same incubation process, and DNA was successfully obtained from all of the samples. DNA could not be obtained when the fresh stool samples, from which *Cryptosporidium* sp. DNA was obtained, were subjected to ta temperature of −20 °C, and then the extraction process was repeated in the same way. It is extremely important for researchers who are going to extract DNA from *Cryptosporidium* species to consider these issues in terms of cost, labor, and saving time.

Cryptosporidium has many species, and it is not possible to separate these species from each other without using molecular methods. Although many studies have been conducted to determine the *Cryptosporidium* species found in humans around the world, studies using molecular methods in Turkey remain limited and comprehensive studies are needed (12,13).

In Turkey, in a study conducted at the Department of Parasitology of Dokuz Eylül University Faculty of Medicine, it was reported that 4.8% of the 21 *Cryptosporidium* sp. detected were *C. meleagridis* and 95.2% were *C. Parvum* (12). Additionally, in a study conducted at the Department of Parasitology of Erciyes University Faculty of Medicine, *Cryptosporidium* sp. was detected in a patient with renal transplantation that was determined to be *C. parvum* using the nested PCR method (13). In the research conducted herein, 29 positive samples were evaluated using the PCR-RFLP method and it was determined that the causative species in all of the cases was *C. parvum*. In addition, these samples were sent to a DNA sequence analysis laboratory where sequence analysis was performed, and it was confirmed that they were *C. parvum*. The presence of these specified species as *C. parvum* was similar to that of many studies conducted in Turkey. However, since there have not been many studies conducted about the typing of *Cryptosporidium* species in Turkey, it is not possible to give information about other species that have infected humans. In order to provide more accurate and detailed information, many studies should be conducted on the typing of *Cryptosporidium* species in places that have different geographical features.

As a result, Cryptosporidiosis gains more importance in patients with low socioeconomic status, inadequate infrastructure, insufficient attention to personal hygiene and livestock farming, especially in patients with immune system deficiency and when accompanied by diarrhea, as in the Van region. It was concluded that studies on the typing of *Cryptosporidium* species in Turkey are limited and research in this area should be increased.

Conflict of interests

The authors declare no conflict of interest with publication of this manuscript

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