Turkiye Parazitol Derg 2025;49(1):10-6
DOI: 10.4274/tpd.galenos.2025.88700

Investigation of the Frequency of *Cryptosporidium* spp. in Immunosuppressed Patients with Diarrhea

İmmünosüpresif İshalli Hastalarda Cryptosporidium spp. Sıklığının Araştırılması

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Cite this article as: Beder D, Esenkaya Taşbent F. Investigation of the frequency of *Cryptosporidium* spp. in immunosuppressed patients with diarrhea. Turkiye Parazitol Derg. 2025;49(1):10-6.

ABSTRACT

Objective: We aimed to investigate the frequency of *Cryptosporidium* spp. in immunosuppressed patients who were admitted with diarrhea by microscopic, serological, and molecular methods and to evaluate the results in comparison with the direct fluorescent antibody (DFA) method.

Methods: We analyzed 90 stool samples from immunosuppressed patients with diarrhea. All stool samples were examined using modified acid-fast staining, polymerase chain reaction (PCR) and enzyme-linked immunosorbent assay (ELISA) tests for the detection of *Cryptosporidium* spp. samples examined with the DFA method were selected randomly, including samples found positive in other diagnostic tests. Stool samples that were positive in any of these diagnostic tests were evaluated by immunochromatographic card test.

Results: Our study included 90 samples, of which 44 (48.8%) and 46 (51.2%) were from male and female patients, respectively. The mean age of the patients was 37.8±27.5. Thirty-one (34.4%) of the samples were from pediatric patients and their mean age was 5.3±4.34. The methods used did not show a statistically significant difference between the positivity status and patient age and gender ($p>0.05$). *Cryptosporidium* spp. positivity was detected in five samples (5.6%), two samples (2.2%), and one sample (1.1%) by using the modified acid-fast staining, PCR, and ELISA methods, respectively. Six of 51 samples to which the DFA method was applied were detected positive. In 4 of the 7 stool samples that were found positive by any of these methods, *Cryptosporidium* spp. was detected positive by the immunochromatographic card test.

Conclusion: When the DFA method is accepted as the gold standard method in the diagnosis of *Cryptosporidium* spp., the modified acid-fast method can be used in routine diagnosis with high sensitivity and specificity. Besides, it immunochromatographic diagnostic tests thought to be useful in laboratories with intensive workflow due to their practical use and rapid results.

Keywords: *Cryptosporidium*, diarrhea, direct fluorescent antibody, immunosuppressive

ÖZ

Amaç: Bu çalışmada ishal şikayeti ile başvuran immünosüpresif hastalarda *Cryptosporidium* spp. sıklığını mikroskobik, serolojik ve moleküler yöntemlerle araştırmayı ve sonuçları direkt floresan antikor (DFA) yöntemi ile karşılaştırarak değerlendirmeyi amaçladık.

Yöntemler: İmmünosüpresif ishalli hastalardan alınan 90 dışkı örneği çalışmaya alınmıştır. *Cryptosporidium* spp. tespiti amacıyla tüm dışkı örneklerine modifiye asit fast boyama, polimeraz zincir reaksiyonu (PZR) ve enzyme-linked immunosorbent assay (ELISA) testi uygulanmıştır. DFA yöntemi ile incelenen örnekler, diğer tanı testlerinde pozitif bulunan örneklere ek olarak diğer örneklerden rastgele seçilmiştir. Bu tanı yöntemlerinden herhangi biriyle pozitif saptanan dışkı örnekleri immünokromatografik kart test ile değerlendirilmiştir.

Bulgular: Çalışmaya alınan 90 örneğin 44'ü (%48,8) erkek, 46'sı (%51,2) kadın hastalara aittir. Hastaların yaş ortalaması 37,8±27,5 olarak bulunmuştur. Çalışmada çocuk hastalara ait 31 (%34,4) örnek bulunmaktadır ve bu hastaların yaş ortalamaları 5,3±4,34 olarak tespit edilmiştir. Kullanılan yöntemlerin hiçbirinde hastaların pozitiflik durumu ile yaşı ve cinsiyeti arasında istatistiksel olarak anlamlı bir farklılık bulunmamıştır ($p>0,05$). Çalışılan dışkı örneklerinde modifiye asit fast yöntemiyle 5 (%5,6), PZR ile 2 (%2,2), ELISA yöntemiyle 1 (%1,1) örnekte *Cryptosporidium* spp. pozitif tespit edilmiştir. DFA yöntemi uygulanan 51 örneğin 6'sı pozitif saptanmıştır. Bu yöntemlerden herhangi biri ile pozitif bulunan 7 dışkı örneğinin 4'ünde immünokromatografik kart test ile *Cryptosporidium* spp. pozitif tespit edilmiştir.

Received/Geliş Tarihi: 09.01.2025 **Accepted/Kabul Tarihi:** 14.04.2025 **Epub:** 17.04.2025 **Publication Date/Yayınlanma Tarihi:** 09.05.2025

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***The study was prested as an oral presentation at the 1st International MİKPEDKON Congress (15-18 May 2024, Bolu, Türkiye).**

Türkiye PARAZİTOLOJİ Dergisi

Sonuç: *Cryptosporidium* spp. tanısında DFA yöntemi altın standart yöntem kabul edildiğinde modifiye asit fast yöntemi yüksek duyarlılık ve özgüllükle rutin tanıda kullanılabilir. Ayrıca yoğun iş akışı olan laboratuvarlarda immünokromatografik tanı testlerinin pratik kullanımı ve hızlı sonuç sağlaması nedeniyle faydalı olacağı düşünülmektedir.
Anahtar Kelimeler: *Cryptosporidium*, ishal, direkt floresan antikor, immünosüpresif

INTRODUCTION

The severity and duration of infections caused by *Cryptosporidium* spp. vary depending on the type, immunity, and host age (1). Cryptosporidiosis involves the jejunum and ileum in patients, making diarrhea the most typical clinical finding of the infection (2). Other less frequently detected clinical findings are abdominal pain, nausea, and vomiting (3). Extraintestinal system involvement, such as cholecystitis, pancreatitis, and cholangitis can also be observed in immunosuppressive patient groups, such as acquired immunodeficiency syndrome (AIDS) (1).

Respiratory cryptosporidiosis commonly occurs in children, and although the presentation is generally asymptomatic, pulmonary infiltration and respiratory distress can be detected (4). Cough is the main symptom when the lungs or trachea is involved (1). In addition, cryptosporidiosis persisting for a long time in childhood may lead to growth and developmental retardation (5).

Additionally, asymptomatic infections have also been reported (3). Anorexia, depression, myalgia, headache, and fatigue have been reported in asymptomatic cases. Asymptomatic patients are more common than diagnosed patients in developed or developing countries (6-8).

Although clinical symptoms vary individually, the excreted amount of oocyst is closely related to the immune status and host age. The infection, which generally has an asymptomatic course in healthy people or heals spontaneously within 2-3 weeks causes more severe clinical symptoms, particularly in children, those with insufficient oral intake, and immunosuppressed and elderly people (9). Persistent diarrhea lasting for a long time can become chronic and lead to resulting in death (10).

Infections caused by intestinal parasites are commonly seen in immunosuppressed patients. Parasitic agents can cause diarrhea varying in severity and may result in mortality (11). In developing countries, the frequency of diarrhea due to parasitic agents was >90% in the immunosuppressed patient population. *Cryptosporidium* is one of the most frequently detected pathogens in these patients (12).

Cases of this agent may not be adequately detected due to symptoms of cryptosporidiosis being similar to other gastroenteritis symptoms, stool samples of patients complaining of diarrhea not being routinely evaluated for *Cryptosporidium*, and not using advanced diagnostic techniques in many cases (13).

Cryptosporidiosis can be diagnosed using histological and molecular methods, microscopic examination, and serological techniques, such as direct fluorescent antibody (DFA) and enzyme-linked immunosorbent assay (ELISA) (14,15). The development of microscopical techniques revolutionized the morphological sciences, progressively providing new levels of magnification and resolution for exploring biological and non-biological samples. Furthermore, the high resolution of microscopic techniques also allowed the detection and visualization of different types of microorganisms, by exploring in a deep way the causative agent of different pathologies (16).

Microscopic examination is the most commonly preferred method. However, evaluating *Cryptosporidium* oocysts using direct microscopic examination is challenging due to their small size which is very similar to yeasts. The chance of diagnosing *Cryptosporidium* spp. can be increased with concentration methods (17,18). Microscopic examination after acid-fast staining is preferable because it is cheap, allows the internal structure of oocysts to be evaluated, and preparations can be stored for a long time thanks to permanent staining. However, due to disadvantages such as the need for a long time and experience for evaluation and low reproducibility, different diagnostic methods are also needed (17).

Molecular techniques are diagnostic methods that provide rapid results with high specificity and sensitivity based on the amplification of the appropriate gene region. Nowadays, it has started to replace conventional diagnostic techniques based on the identification of phenotypic characteristics and even became the gold standard for the detection of some microorganisms. It can distinguish between closely species or subtypes. It is also of great importance in the diagnosis of microorganisms that have lost their viability during the transport phase (19).

The DFA method, which detects surface antigens, technique offers the highest combination of sensitivity and specificity and is considered the gold standard by many laboratories (20).

We aimed to investigate the frequency of *Cryptosporidium* spp. in immunosuppressed patients who were admitted with diarrhea by microscopic, serological, and molecular methods and to evaluate the results in comparison with the DFA method.

METHODS

This prepared study was evaluated and approved by Necmettin Erbakan University Faculty of Medicine Hospital Non-Drug and Non-Medical Device Research Ethics Committee on 15.04.2022 with decision number 2022/3755. Since our study was conducted on stool samples routinely sent to the microbiology laboratory, patient consent was not obtained. This study, with project number 221518022, was supported by Necmettin Erbakan University Scientific Research Project Coordination Office.

Gender, age, clinical and diagnostic information of the patients were obtained from our hospital's laboratory information system. The study included patients receiving immunosuppressive therapy after transplantation, patients receiving chemotherapy due to malignant solid tumors or hematological malignancies, patients immunosuppressed due to hematological malignancies, patients receiving corticosteroid therapy for more than two months, and patients diagnosed with primary immunodeficiency.

A simple sedimentation technique was applied to the samples for fecal concentration. Subsequently, direct microscopic examination was performed with physiological saline (0.9% NaCl) on the slides. For stained microscopic examination, a smear was prepared from the stool samples, and modified acid-resistant Ehrlich-Ziehl-Neelsen (EZN) was performed. We also recorded other parasitic agents detected during direct microscopic examination or stained

(modified acid-resistant staining) microscopic examination was recorded.

The remaining samples were divided into three for ELISA, DFA, and polymerase chain reaction (PCR) analyses without adding any preservative solution, divided into 1.5 mL eppendorf tubes, and kept at -80 °C to be stored until analyses.

In the ELISA method, the *Cryptosporidium* RIDASCREENR, C 1201 r-Biopharm commercial kit was used to detect *Cryptosporidium parvum* and *Cryptosporidium hominis* antigens.

Before the molecular study, the QIAamp DNA Stool Mini Kit (QIAGEN GmbH, Hilden, Germany) was used for DNA extraction of stool samples. Subsequently, probe-based real-time PCR analysis of the extracted samples was performed using the Roche real-time PCR device (Roche, Switzerland 2004). The probe and primer sequences defined in the PCR study in the literature were used for *Cryptosporidium* (22). These sequences are shown in Table 1.

PCR primers and probes (Table 1) were designed using the BioEdit software program, version 7.0.9.0 (http://www.mbio.ncsu.edu/BioEdit/bioedit.html) to create Clustal W (23) alignments of representative *Cryptosporidium* sequences from GenBank [National Center for Biotechnology Information (NCBI)]; http://www.ncbi.nlm.nih.gov/GenBank/). The Primer Express software program (Applied Biosystems, Warrington, United Kingdom) was used to calculate melting temperatures and check for undesirable inter and intramolecular binding. Primer and probe sequences were then checked for cross-reactions with non-target sequences on the GenBank database using the basic local alignment search tool (http://www.ncbi.nlm.nih.gov/blast/Blast.cgi).

Cryptosporidium and *Giardia* antigens were investigated using the DFA method with the Merifluor *Cryptosporidium/Giardia* Direct Immunofluorescence Assay (DFA; Meridian Diagnostics, Inc., Cincinnati, Ohio, USA) commercial kit. Samples examined with the DFA method were selected randomly by including samples found positive in other diagnostic tests. The samples detected as positive by any of the diagnostic methods were evaluated with an immunochromatographic card test as a using the commercial *Entamoeba/Giardia/Crypto* Combo Rapid Cassette test (Microcult, Biotech, China). ELISA, DFA, PCR and card tests were used following the manufacturer's recommendations.

Using the DFA method as the reference method, the positive and negative predictive values, specificity, sensitivity and accuracy rates of serological, molecular and microscopic methods were determined. The accuracy value was found by dividing the sum of the true positive and true negative values by the total data.

Statistical Analysis

Statistical analysis of the data was analyzed with SPSS (Statistical Package for Social Sciences) ver.23 statistical package program. Continuous variables are expressed as mean ± standard deviation, minimum and maximum values, and categorical variables are expressed as numbers and percentages. Differences between categorical variables were examined with Pearson chi-square and Fisher-Freeman-Halton Exact tests. The significance level was determined as $p<0.05$.

RESULTS

The frequency of *Cryptosporidium* spp. was investigated in 90 immunosuppressed patients with diarrhea, of whom 31 were children, between May 2022-December 2022.

The ages of the patients ranged between 0 and 81 years, and the mean age was 37.8±27.5 years. Moreover, 31 (34.4%) samples were from pediatric patients, of which 18 (58%) and 13 (42%) were from men and women, respectively, and their mean age was 5.3±4.34 years. Of the 59 adult patients, 26 (44%) and 33 (56%) were men and women, respectively. Of the 90 included samples, 44 (48.8%) and 46 (51.2%) were from men and women patients, respectively. The methods used did not show a statistically significant difference between the positivity status and patient age (p=0.688) or between the positivity status and sex (p=0.261).

The study patients frequently consist of patients in pediatrics, oncology and hematology clinics. The positivity rates of patients coming from these departments are 11.1%, 11.7% and 8.3% respectively. In this study, there were 4 patients from the immunology and allergic diseases clinics, and the positivity rate in this patient group was 25%. Table 2 shows the distribution and rates of patients included in the study according to clinics.

Among the study participants, 54.4%, 14.4%, 11.1%, 8.8%, and 7.7% were diagnosed with a malignant solid tumor, lymphoma, primary immunodeficiency, multiple myeloma, and leukemia, respectively.

In the microscopic examination using acid-fast modified EZN staining, *Cryptosporidium* spp. oocysts were detected in five samples (Figure 1). *Cyclospora* spp. oocyst was seen in one sample. In microscopic examination, *Giardia* spp. and *Blastocystis* spp. were detected in 2 and 26 samples, respectively.

Cryptosporidium positivity was detected in one and two samples using ELISA and PCR methods, respectively. In this study, in which *Cryptosporidium* and *Giardia* antigens were investigated by DFA in 51 stool samples, including those positive in any of the microscopic, ELISA or PCR evaluations, *Cryptosporidium* spp. was found positive in six samples and *Giardia* spp. was found positive in three samples (Figure 2).

Because the DFA is considered the gold standard method, the DFA results were considered as the main results in the comparative evaluation of the methods in statistical analysis. Based on the DFA method, the modified acid-fast staining method had a sensitivity, specificity, positive predictive value, negative predictive value, and accuracy rate of 83.33%, 100%, 100%, 97.82%, and 98.03%,

Table 1. Real-time PCR primers and probes used in this study

Target locus	Primer/probe	Position (nt)[a]	Sequence (5'-3')[b]
Cryptosporidium species SSU *rRNA* gene	CRU18SF[c]	440-468	GAGGTAGTGACAAGAAATAACAATACAGG
	CRU18SR[c]	710-738	CTGCTTTAAGCACTCTAATTTTCTCAAAG
	CRU18STM	587-609	FAM-TACGAGCTTTTTAACTGCAACAA MGB-NFQ

[a]: Positions on *Cryptosporidium* species SSU *rRNA* gene (GenBank accession numbers AF164102). nt: Nucleotide(s), [b]: MGB: Minor groove binder, NFQ: Non-fluorescent quencher, [c]: Modified from reference (21), PCR: Polymerase chain reaction

Table 2. Distribution and rates of patients included in the study according to clinics

Clinics	Number of samples (n)	Rate in the sample group (%)
Pediatrics	27	30
Medical oncology	17	18.9
Hematology	12	13.3
Gastroenterology	11	12.2
Pediatric hematology-oncology	7	7.7
Internal medicine	4	4.4
Immunology and allergic diseases	4	4.4
Radiation oncology	3	3.3
Bone marrow transplantation unit	2	2.2
Nephrology	1	1.1
Organ and tissue transplantation center	1	1.1
Chest diseases	1	1.1
Total	90	100

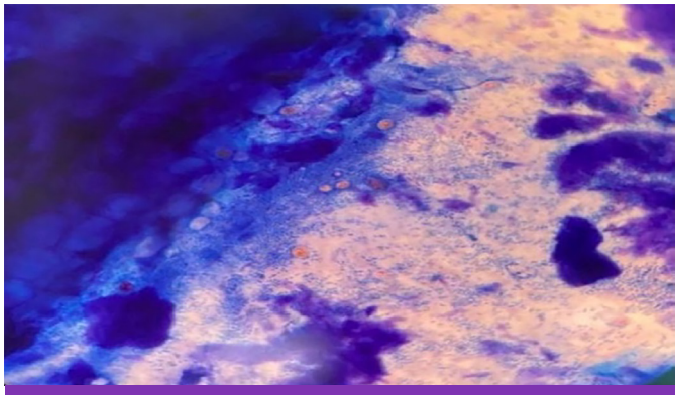

Figure 1. *Cryptosporidium* spp. oocysts in EZN staining (x100)
EZN: Ehrlich-Ziehl-Neelsen

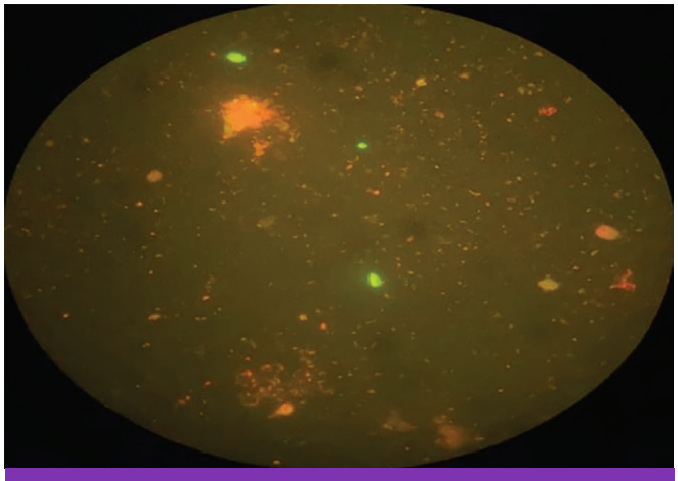

Figure 2. In the examination performed by the DFA method, *Cryptosporidium* spp. oocysts and *Giardia* spp. cysts (x100)
DFA: Direct fluorescent antibody

respectively. Based on the DFA method, PCR had a sensitivity, specificity, positive predictive value, negative predictive value, and accuracy rate of 33.3%, 100%, 100%, 91.83%, and 92.15%, respectively. Based on the DFA method, ELISA had a sensitivity, specificity, positive predictive value, negative predictive value, and accuracy rate of 0%, 97.77%, 0%, 88%, and 86.27%, respectively. The specificity and sensitivity, positive and negative predictive values and accuracy rates of serological, molecular and microscopic methods compared to the gold standard method the DFA method, are shown in Table 3. The stool samples of seven patients found to be positive with any of the other diagnostic tests were re-examined with the immunochromatographic card test, resulting in four samples being positive. The characteristics of patients with *Cryptosporidium* positivity are shown in Table 4.

DISCUSSION

Cryptosporidium spp., one of the factors causing infectious diarrhea, is an opportunistic agent that inhabits most commonly in the jejunum and less frequently in organs, such as the lungs, gallbladder, and liver. This parasite can cause mortality in older people and children, in the immunosuppressive patient group, and malnourished individuals (20). Initially, it causes watery diarrhea in immunosuppressive patients. Furthermore, it also causes gastroenteritis with a more benign course in immunocompetent individuals (24).

After evaluating the literature, sex was not associated with the incidence of *Cryptosporidium* (25). In our study, sex did not affect the positivity of *Cryptosporidium*. *Cryptosporidium* is an important cause of diarrhea in childhood, and the parasite is more symptomatic in children (4). In this study, the positivity rate was higher in children than in the adult age group, but the difference was not statistically significant.

This parasite is found worldwide and may be the underlying cause of diseases with diarrhea as the main symptom (26). *Cryptosporidium,* which is associated with premature deaths and growth and development retardation in childhood, caused a waterborne epidemic affecting 400,000 people in Milwaukee, USA, in the 1990s. Currently, it can be found almost everywhere, except in Antarctica. Although *Cryptosporidium* is detected in 1% of immunocompetent people in developed countries, it is 5-10% in countries with low socioeconomic levels. This rate varies between 10% and 25% in children who consult with complaints of diarrhea (4).

Because this pathogen is more commonly detected in immunocompromised patients, epidemiological studies were mostly conducted on immunosuppressed patients with diarrhea (27). Therefore, the data were highly inconsistent, which may be due to the stage of the infection, characteristics and number of the evaluated patient population, sensitivity of the diagnostic methods, and the climatic conditions (28).

Cryptosporidium spp. positivity was found in 6.3% of 427 immunocompromised patients with diarrhea in a study conducted in Egypt, 12.6% of 87 patients with colorectal malignancy and diarrhea in a study conducted in Poland (29,30), 12% of 137 HIV patients with diarrhea, and 12.4% of 84 immunocompromised patients with diarrhea (31,32).

In the studies evaluating *Cryptosporidium* spp. incidence in the immunocompromised patient population in Türkiye, *Cryptosporidium* spp. positivity was found in 6.4% of 47

Table 3. Specificity and sensitivity, positive and negative predictive values and accuracy rates of serological, molecular and microscopic methods compared to the gold standard method DFA method

	Microscopy (%)	PCR (%)	ELISA (%)
Sensitivity	83.33	33.3	
Specificity	100	100	97.77
Positive predictive value	100	100	0
Negative predictive value	97.82	91.83	88
Accuracy rate	98.03	92.15	86.27

PCR: Polymerase chain reaction, ELISA: Enzyme-linked immunosorbent assay, DFA: Direct fluorescent antibody

Table 4. Characteristics of patients with *Cryptosporidium* positivity

Age	Sex	Department	Diagnosis	Microscopy	PCR	DFA	ELISA	Card test
0	Men	Pediatrics	Lymphoma	Positive	Negative	Positive	Negative	Positive
0	Women	Pediatrics	ALL	Positive	Negative	Positive	Negative	Positive
7	Men	Pediatrics	Solid tumor	Positive	Negative	Positive	Negative	Negative
54	Men	Medical oncology	Solid tumor	Negative	Negative	Negative	Positive	Negative
61	Men	Hematology	Lymphoma	Positive	Positive	Positive	Negative	Negative
67	Women	Medical oncology	Solid tumor	Positive	Negative	Positive	Negative	Positive
77	Men	Immunology and allergic diseases	Primary immune deficiency	Negative	Positive	Positive	Negative	Positive

PCR: Polymerase chain reaction, DFA: Direct fluorescent antibody, ELISA: Enzyme-linked immunosorbent assay

patients diagnosed with chronic renal failure (33), 5.3% of 94 immunosuppressive patients with malignant solid tumors receiving chemotherapy and complaining of diarrhea for >5 days, and 8.3% in 72 stool samples of immunosuppressive patients with diarrhea (34,35).

In our study, *Cryptosporidium* spp. was detected in 7 (7.8%) of 90 patients with complaints of immunosuppressive diarrhea, by at least one of the methods used.

The DFA method is used as the gold standard in reference laboratories in the USA and Europe (4). Therefore, in our study, based on DFA, in the modified acid-fast EZN staining method, the sensitivity and specificity were 83.33% and 100%, respectively. The high cost of DFA tests is the most important problem that limits their use in routine laboratories. Modified EZN staining, which has high sensitivity and specificity, can be used to detect *Cryptosporidium* spp. as a more cost-effective method in experienced laboratories, particularly in sensitive groups, such as immunosuppressed and pediatric patients.

Depending on the commercial kit used, the efficacy of the ELISA method varies. In our study, ELISA was unsuccessful in detecting *Cryptosporidium* spp. In another study, PCR was considered 10^3-10^4 times more sensitive than the ELISA method (36). Moreover, false-positive results can be due to cross-reactions against common antigens in many pathogens, particularly apicomplexan parasites (27). The high cost of this method is another disadvantage, as well as sample collection before the examination (34).

A previous study evaluated 80 stool samples of patients presenting with gastrointestinal system complaints, and *Cryptosporidium* spp. positivity was found in three (3.75%) and five samples (6.25%) by using acid-fast staining and ELISA, respectively. Three positive samples detected using acid-fast staining were also positive with the ELISA technique (37). In another study, the ELISA method detected *Cryptosporidium* spp. in 2.8% of 723 stool samples in the patient group presenting with the same complaints (27). In our study, the ELISA method detected positivity in only one patient, which could not be confirmed by other diagnostic methods. The low sensitivity of the ELISA method may be related to the kit used or the *Cryptosporidium* spp. oocysts may not have been distributed homogeneously in the stool of the positive samples.

Recently, molecular methods have been preferred because they can perform genotyping based on nucleic acid analysis, and the opportunity to easily evaluate many samples, and guide the treatment process (38). In order to apply molecular biology techniques in studies with genetic material, DNA and RNA molecules must be obtained in pure form before PCR, taking into account that they will also contain inhibitors. Otherwise, results may be masked by taq polymerase enzyme inhibitors in the sample. The basic components used in PCR are target DNA or RNA (template), taq DNA polymerase enzyme, primers, deoxynucleotides, buffer liquid, pH, Mg+2 ions. Temperature parameters (especially annealing temperature) and Mg+2 concentration that are not in the appropriate range, and the use of poor quality polymerase enzyme may lead to erroneous PCR results. Additionally, the design of oligonucleotide primers is only possible with known strains of microorganisms and known sequences of these strains. False negatives may occur in inappropriate primer selections. Another factor that may cause problems in the functions of PCR is unexpected mutations that occur in microbial genomes (19). In conditions where parasite oocysts are not homogeneously distributed throughout the stool, molecular techniques detect fewer positive samples than microscopic examination (39). Furthermore, polysaccharides, bile salts, or bilirubin in the stool can inhibit the PCR study (34). If oocyst damage occurs before DNA isolation PCR can not detect *Cryptosporidium* spp., resulting in discrepant data

between microscopic evaluation data and PCR results (39,40). The inclusion of molecular techniques in routine diagnosis will only be possible when these problems are solved and ensure a certain standardization (40).

In our study, only two patients were positive using PCR, and based on the DFA test, the sensitivity and specificity were 33.3% and 100%, respectively. False negative results in PCR studies are thought to be caused by inhibitors in the stool sample, problems in the DNA extraction stage, or primer probe optimization. Some studies in the literature also showed that PCR had lower sensitivity than microscopic evaluation (38,41).

In a study conducted in our country on 36 immunosuppressed patients with diarrhea, the *Cryptosporidium* was investigated using microscopic examination with modified acid-fast staining, molecular techniques, and DFA testing. Based on the data, one sample and three samples were positive with acid-fast staining and DFA method, respectively; however, no positive sample could be detected with the molecular technique used (41). Another study evaluating 80 immunosuppressed patients with diarrhea, three, five, and four positive samples were found by microscopic examination, ELISA, and DFA, respectively, whereas PCR did not detect positivity (38).

In the stool samples of 90 immunosuppressive patients in our study, positivity was detected in five, one, and two samples by microscopic examination, ELISA, and PCR, respectively. All the positive samples were positive with DFA, except for the positive sample detected by ELISA, and DFA positivity was detected in 6 of the 51 samples studied. An immunochromatographic rapid diagnostic test was used in seven positive samples by any of the methods, and four samples were positive with the rapid diagnostic test. Rapid diagnostic tests will be useful in rapid diagnosis in laboratories with intensive sample studies, particularly in sensitive groups with chronic diarrhea. In the study, *Cyclospora* spp., another opportunistic pathogen, were detected in one patient, and *Giardia* spp., an important causative agent of diarrhea, were detected in three patients.

The inadequate effectiveness of the commercial kit used in the ELISA test and the problems experienced in the DNA extraction phase and/or primer probe optimization in PCR caused limitations in our study. In addition, DFA and immunochromatographic rapid diagnostic tests could not be applied to all samples. The small number of positive samples does not allow for a fully accurate assessment of the usefulness of these methods. Studies in which all diagnostic techniques are applied on more samples are needed.

CONCLUSION

Our study especially showed that the probability of detection of *Cryptosporidium* spp. in stool samples from immunosuppressed patients with diarrhea should not be underestimated. Considering that the number of immunosuppressive patients increases daily, rapid and reliable detection of this agent is extremely important in the routine work of laboratories, particularly for the immunosuppressive patient group. Therefore, diagnostic algorithms should be created for the diagnosis of *Cryptosporidium* spp. in secondary and tertiary care hospitals with more immunosuppressive patient follow-up.

Although diagnostic methods used to detect *Cryptosporidium* oocysts have advantages, each has its disadvantages. Based on the gold standard method DFA, modified acid-fast staining has high sensitivity and specificity, but it requires labor and technical experience. Performing modified acid-fast staining and evaluating all stool samples received in laboratories with intensive sample flow may not be possible. However, in high-risk patient groups, evaluation of samples using modified acid-fast staining as a minimum diagnostic method will be useful for the diagnosis of *Cryptosporidium*. Besides, it immunochromatographic diagnostic tests will be useful in laboratories with intensive workflow due to their practical use and rapid results.

*Ethics

Ethics Committee Approval: Non-Drug and Non-Medical Device Research Ethics Committee on 15.04.2022 with decision number 2022/3755.

Informed Consent: Since our study was conducted on stool samples routinely sent to the microbiology laboratory, patient consent was not obtained.

Footnotes

*Authorship Contributions

Surgical and Medical Practices: D.B., Concept: F.E.T., Design: F.E.T., Data Collection or Processing: D.B., Analysis or Interpretation: F.E.T., Literature Search: D.B., Writing: D.B.

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

Financial Disclosure: This study, with project number 221518022, was supported by Necmettin Erbakan University Scientific Research Project Coordination Office.

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