İSTANBUL UNIVERSITY PRESS

RESEARCH ARTICLE / ARAŞTIRMA MAKALESİ
Journal of Advanced Research in Health Sciences /
Sağlık Bilimlerinde İleri Araştırmalar Dergisi 2024
DOI: 10.26650/JARHS2024-1496234

INVESTIGATION OF THE PREVALENCE OF PATHOGENIC INTESTINAL PROTOZOANS IN PATIENTS WITH GASTROINTESTINAL COMPLAINTS VIA MICROSCOPY AND MULTIPLEX REAL-TIME PCR

GASTROİNTESTİNAL ŞİKÂYETİ OLAN HASTALARDA BAĞIRSAK PATOJENİ PROTOZOONLARIN PREVALANSININ MİKROSKOPİ VE MULTİPLEKS REAL-TIME PCR İLE ARAŞTIRILMASI

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Citation/Atıf: Karacan B, Büyükbaba Boral Ö, İmadoğlu Yetkin G, Çifcibaşı Örmeci A, Özkaya O, Sütçü M, et al. Investigation of the prevalence of pathogenic intestinal protozoans in patients with gastrointestinal complaints via microscopy and multiplex real-time PCR. Journal of Advanced Research in Health Sciences 2024;7(3):180-184. https://doi.org/10.26650/JARHS2024-1496234

ABSTRACT

Objective: This study aimed to contribute to surveillance data by determining the prevalence of pathogenic intestinal protozoans in patients with gastrointestinal complaints and to comparatively evaluate the performance of microscopy and multiplex real-time PCR methods.

Materials and Methods: Forty adults (18 years or older) and 40 children (aged 5 to 12 years) who volunteered patients with at least one gastrointestinal complaint were included in the study. Stool samples collected three times every other day from each patient were examined under a light microscope using Native-Lugol, modified formol-ether concentration, Wheatley's modified trichrome staining, and Modified Ziehl-Neelsen staining techniques. In addition, the first stool samples were tested using multiplex real-time PCR with the Allplex™ GI Parasite Assay panel after DNA extraction. Differences between the sensitivities and specificities between the methods were analysed using Fisher's exact test. A p-value of <0.05 was considered statistically significant.

Results: The prevalence of pathogenic intestinal protozoans was 25% in adults and 35% in children. Differences in the sensitivities and specificities of the methods were not found to be statistically significant (p=0.999).

Conclusion: Multiplex real-time PCR method performed using the Allplex™ GI Parasite Assay panel was effective even when only the first sample tested, suggesting that this method would be used for the routine diagnosis of pathogenic intestinal protozoans.

Keywords: Pathogen, intestinal, protozoon, prevalence, microscopy, PCR

ÖZ

Amaç: Bu çalışmanın amacı, hem gastrointestinal şikâyeti olan hastalarda bağırsak patojeni protozoonların prevalansını tespit ederek sürveyans verilerine katkı sağlamak hem de mikroskopi ve multipleks real-time PCR yöntemlerinin performanslarını karşılaştırmalı olarak değerlendirmektir.

Gereç ve Yöntem: Çalışmaya, en az bir gastrointestinal şikâyeti olan 40 yetişkin (18 yaş ve üzerinde) ve 40 çocuk (5-12 yaş arasında) gönüllü hasta dâhil edildi. Her hastadan günaşırı olarak 3 kez alınan gaita örnekleri; Nativ-Lugol, modifiye formol-eter çöktürme, Wheatley'in trikrom boyama ve modifiye Ziehl-Neelsen boyama teknikleri uygulandıktan sonra ışık mikroskobunda incelendi. Ayrıca, yalnızca ilk olarak alınan gaita örnekleri; DNA ekstraksiyonundan sonra, Allplex™ GI Parasite Assay paneli kullanılarak multipleks real-time PCR yöntemiyle test edildi. Yöntemlerin duyarlılıkları ve özgüllükleri arasındaki farklılıklar, Fisher'in kesin testi ile analiz edildi. p değerinin <0,05 olması, istatistiksel olarak anlamlı kabul edildi.

Bulgular: Bağırsak patojeni protozoonların prevalansının yetişkin grubunda %25, çocuk grubunda ise %35 olduğu tespit edildi. Yöntemlerin duyarlılıkları ve özgüllükleri arasındaki farklılıklar, istatistiksel olarak anlamlı bulunmadı (p=0,999).

Sonuç: Allplex™ GI Parasite Assay paneli kullanılarak gerçekleştirilen multipleks real-time PCR yönteminin yalnızca ilk örnek test edildiğinde bile etkili olması; bu yöntemin, bağırsak patojeni protozoonların rutin tanısında kullanılabileceğini düşündürmektedir.

Anahtar Kelimeler: Patojen, bağırsak, protozoon, prevalans, mikroskopi, PCR

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Submitted/Başvuru: 05.06.2024 • **Revision Requested/Revizyon Talebi:** 09.07.2024 • **Published Online/Online Yayın:** 23.10.2024

INTRODUCTION

Gastrointestinal infections are a major cause of morbidity and mortality worldwide, posing a serious threat to public health (1, 2). In tropical and subtropical climate zones, including Türkiye, parasites are responsible for a significant proportion of gastrointestinal infections (2, 3). Pathogenic intestinal protozoans are the most common parasites causing gastrointestinal infections.

The diagnosis of pathogenic intestinal protozoans is generally made via microscopy. This method requires at least three stool samples every other day, along with experience, proper concentration, and permanent staining techniques for optimal results. Because the implementation of this method is both difficult and time-consuming, patients who can benefit from antiparasitic drugs or antibiotics may not receive appropriate treatment on time or at all (4-6). In addition, patients with chronic diseases/comorbidities may be unnecessarily hospitalized and may undergo extra examinations, such as gastroscopy and colonoscopy, until a diagnosis is complete (7-9). The Centers for Disease Control and Prevention (CDC) recommends that patients with suspected infectious diarrhea use contact precautions (10, 11). Thus, patients may be unnecessarily isolated and psychologically affected if diagnosis is delayed. Rapid and accurate diagnosis of gastrointestinal infections is crucial for infection control and prevention plans, public health interventions, and case management (1, 9, 12, 13). Therefore, a sensitive and specific method to rapidly and simultaneously detect protozoans that cause gastrointestinal infections is urgently needed. The Allplex™ Gastrointestinal (GI) Parasite Assay is the most comprehensive multiplex real-time panel for detecting pathogenic intestinal protozoans. The multiplex real-time Polymerase Chain Reaction (PCR) method performed using this panel may rapidly and accurately identify protozoans causing gastrointestinal complaints, ensuring timely initiation of appropriate treatment. Thus, the unnecessary and incorrect use of antiparasitic drugs and antibiotics, the development of resistance to antiparasitic drugs and antibiotics, needless isolation, redundant use of other diagnostic tests, length of hospital stays, and healthcare costs may be reduced.

In this study, we aimed to contribute to surveillance data by detecting the prevalence of pathogenic intestinal protozoans in patients with gastrointestinal complaints, and to comparatively evaluate the performances of microscopy and multiplex real-time PCR.

MATERIALS and METHODS

This study was conducted at İstanbul University İstanbul Faculty of Medicine Hospital, İstinye University Liv Hospital Bahçeşehir, and İstinye University Faculty of Medicine, after receiving approval from the İstanbul Medical Faculty Clinical Research Ethics Committee (Date: 24.09.2021, No: 17) and the İstinye University Clinical Research Ethics Committee (Date: 14.10.2021, No: 2/2021.K-75). The study was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants or their legal representatives.

Inclusion criteria

We focused on a specific age range for pediatric patients, in contrast to adult patients. In total, 80 volunteer patients who agreed to participate in the study, or whose legal representatives consented, and who met the criteria on items A-1, A-2, B-1, and B-2, were included in the study:

A-1: Twenty immunocompetent adults (≥18 years old) who applied to the Department of Internal Diseases at İstanbul University İstanbul Faculty of Medicine Hospital with at least one gastrointestinal complaint;

A-2: Twenty immunosuppressed adults (≥18 years old) who applied to the Department of Internal Diseases at İstanbul University İstanbul Faculty of Medicine Hospital with at least one gastrointestinal complaint;

B-1: Twenty immunocompetent children (aged 5 to 12 years) who applied to the Department of Pediatrics at İstinye University Liv Hospital Bahçeşehir with at least one gastrointestinal complaint;

B-2: Twenty immunosuppressed children (aged 5 to 12 years) who applied to the Department of Pediatrics at İstinye University Liv Hospital Bahçeşehir with at least one gastrointestinal complaint.

Sample collection

Stool samples were collected from adult patients between April 4 and May 8, 2022, and from pediatric patients between May 10 and June 16, 2022, three times every other day. Following the collection of the first stool sample from each patient, blood samples were also obtained.

Microscopic examination

Stool samples were examined under a binocular microscope (Nikon, Tokyo, Japan; Olympus, Tokyo, Japan) after applying Native-Lugol, modified formol-ether concentration (MF), Wheatley modified trichrome staining (WS), and modified Ziehl-Neelsen staining (ZN) techniques.

DNA extraction

DNA was extracted from the first samples using a QIAamp Fast DNA Stool Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. For amplification with the Allplex™ GI Parasite Assay (Seegene®, Seoul, South Korea), 10 µl of internal control (IC) DNA was added to the samples prior to extraction, as recommended by the manufacturer. After completing the DNA extraction procedure, samples were stored at -20°C until multiplex real-time PCR analysis.

Multiplex real-time PCR

Multiplex real-time PCR was performed using a CFX96 Touch real-time PCR detection system (Bio-Rad, Marnes-La-Coquette, France) and the Allplex™ GI Parasite Assay (Seegene®, Seoul, South Korea) panel, following the manufacturer's instructions. Amplifications were managed using the CFX Maestro Software (Seegene®, Seoul, South Korea), and results were analysed using the Seegene Viewer Software (v3 (Seegene®, Seoul, South

Korea). Samples with a Cycle threshold (Ct) value of ≤43 were interpreted as positive. A test that did not meet the positive and negative control criteria was repeated. In addition, the DNA extraction process and multiplex real-time PCR testing were repeated for four samples with IC Ct values interpreted as N/A.

Statistical analysis

All data were entered into an Excel (Microsoft Excel 2019) database, and statistical analysis was performed using IBM SPSS Statistics v26 (IBM SPSS Corp., Armonk, NY, USA). Differences in sensitivities and specificities between the methods were analysed using Fisher's exact test. A p-value of <0.05 was considered statistically significant.

RESULTS

Upon evaluating the results and all clinical parameters, 21 out of 80 samples were found to be positive. Among these positive samples, three exhibited co-infection: two samples were positive for both *Blastocystis* spp. and *D. fragilis*, and one sample was positive for both *Blastocystis* spp. and *G. lamblia*. The overall prevalence rates were 25% in adults and 35% in children. Detailed information on the prevalence of pathogenic intestinal protozoans is presented in Table 1.

Table 1: Information on the prevalence of pathogenic intestinal protozoans identified in this study

	Group			
	Adults (≥18 years)		Children (5-12 years)	
Pathogenic intestinal protozoans	**A-1**	**A-2**	**B-1**	**B-2**
Giardia lamblia	-	-	5%	5%
Dientamoeba fragilis	10%	10%	10%	15%
Entamoeba histolytica	-	5%	-	-
Cryptosporidium spp.	-	-	-	5%
Blastocystis spp.	10%	15%	15%	15%

It was found that the microscopy method yielded one false-negative result and three false-positive results for *Blastocystis* spp., as well as two false-negative results for *D. fragilis*. On the other hand, multiplex real-time PCR method yielded one false-negative result, besides two false-positive results with high Ct values (42.87 and 42.91) for *Blastocystis* spp., which were very close to the limit. The sensitivities and specificities of the methods are compared in Tables 2 and 3, respectively.

Compared with the examination of the first stool sample via microscopy, testing of the same sample via multiplex real-time PCR demonstrated remarkably higher sensitivity (45.83% vs. 95.83%), and the performance difference was statistically significant (p=0.0003). Compared with the examination of the first and second stool samples via microscopy, testing of the first stool sample via multiplex real-time PCR again demonstrated higher sensitivity (70.83% vs. 95.83%), and the performance difference remained statistically significant (p=0.049). Compared with the examination of the three stool samples via microscopy, testing of the first stool sample via multiplex real-time PCR also exhibited higher sensitivity (87.5% vs. 95.83%), but the performance difference was not statistically significant (p=0.6085). Compared with microscopy, multiplex real-time PCR showed higher specificity (94.92% vs 96.56%), but the performance difference was not statistically significant (p=0.999).

Table 2: Comparison of the sensitivities of the methods based on species level

	Sensitivity	
Pathogenic intestinal protozoan	**Microscopy (After examining the three samples)**	**Multiplex real-time PCR (After testing the first sample)**
Giardia lamblia	100% (2/2)	100% (2/2)
Dientamoeba fragilis	77.78% (7/9)	100% (9/9)
Entamoeba histolytica	100% (1/1)	100% (1/1)
Cryptosporidium spp.	100% (1/1)	100% (1/1)
Blastocystis spp.	90.91% (10/11)	90.91% (10/11)

Table 3: Comparison of the specificities of the methods based on species level

	Specificity	
Pathogenic intestinal protozoan	**Microscopy (After examining the three samples)**	**Multiplex real-time PCR (After testing the first sample)**
Giardia lamblia	100% (78/78)	100% (78/78)
Dientamoeba fragilis	100% (71/71)	100% (71/71)
Entamoeba histolytica	100% (79/79)	100% (79/79)
Cryptosporidium spp.	100% (79/79)	100% (79/79)
Blastocystis spp.	95.83% (69/72)	97.18% (69/71)

DISCUSSION

In our study, *Blastocystis* spp. was the pathogenic intestinal protozoan with the highest prevalence in both adults and children, followed by *Dientamoeba fragilis*. Our study supports the literature indicating that the most common protozoan causing gastrointestinal complaints in humans is *Blastocystis* spp., followed by *D. fragilis* (14).

The testing of the first stool sample via multiplex real-time PCR was statistically superior to the examination of two stool samples via microscopy. The testing of the first stool sample via multiplex real-time PCR was statistically equivalent to the examination of three stool samples via microscopy. For each case, examining three stool samples every other day using microscopy took approximately 5 days, while multiplex real-time PCR, including the DNA extraction procedure, took approximately 4.5 hours. Pathogenic intestinal protozoans can lead to epidemics, particularly in crowded areas. Co-infections may increase the likelihood of false-negative and false-positive results via microscopic diagnoses. Conversely, multiplex real-time PCR can rapidly detect multiple microorganisms simultaneously with high sensitivity and specificity, effectively overcoming the limitations of microscopy-based diagnosis.

In addition to Native-Lugol and MF, permanent staining techniques, such as WS (for *D. fragilis*, *E. histolytica and Blastocystis* spp.) and ZN (for *Cryptosporidium* spp.), were found to enhance the performance of the microscopy method. All *D. fragilis*, which were observed in 7 out of 9 cases via microscopy, could be detected using the WS technique. Thanks to the WS technique, we were able to observe erythrophagocytosis by *E. histolytica* trophozoites; the *E. histolytica*/*E. dispar* group could be separated as *E. histolytica* in one case and the *E. dispar* group in four cases. Separation of the *E. histolytica* and *E. dispar* group is crucial; treatment is unnecessary when the *E. dispar* group is diagnosed, while urgent treatment is required if *E. histolytica* is diagnosed (15). *Blastocystis* spp. were identified in 10 of 11 cases via microscopy, with 2 of these detected using the WS technique. *Cryptosporidium* spp. can lead to life-threatening complications, especially in immunosuppressed patients (16). *Cryptosporidium* spp. oocysts were detected in the first sample using the WS technique, and the immunosuppressed patient was immediately treated. However, these permanent staining techniques are not routinely utilized in many diagnostic laboratories worldwide. Their implementation is laborious and time-consuming, and successful results are not always achieved. In addition, no consensus has been reached on a complementary diagnostic test for the detection of pathogenic intestinal protozoans. Differences in the performance of various diagnostic methods and techniques can affect the reported prevalence rates (17). Therefore, multiplex real-time PCR should be employed as a first-line diagnostic method to ensure standardization in the diagnosis of pathogenic intestinal protozoans.

In our study, no infections caused by *Cyclospora cayetanensis*, *Cystoisospora* spp., *Sarcocystis* spp., or *Balantioides coli* were detected. *Cyclospora cayetanensis,* and *Cystoisospora* spp. are opportunistic protozoans that are especially prevalent in HIV/AIDS patients. A limitation of our study is that no HIV/AIDS patients or legal representatives participated in groups A-2 and B-2, which comprise the immunosuppressed groups. *Sarcocystis* spp. and *Balantioides coli* are commonly found in regions characterized by animal farming (especially pigs), poor sanitation, and the consumption of raw or undercooked meat (particularly pork). These pathogenic intestinal protozoans are rare in Türkiye. Although the panel used in our research is the most comprehensive multiplex real-time PCR panel for detecting pathogenic intestinal protozoans, and no cases caused by *Cystoisospora* spp., *Sarcocystis* spp., or *B. coli* were identified in our study, the panel must still be designed to detect these pathogenic intestinal protozoans for use in routine diagnosis. Since no cases of *C. cayetanensis* were identified, which is included in the Allplex™ GI Parasite Assay, we could not evaluate the sensitivities of either microscopy or multiplex real-time PCR methods for this pathogen. However, since no false-positive results were detected with either method, their specificity for detecting this pathogen was determined to be 100%.

By integrating automated DNA isolation, multiplex real-time PCR can be considered an almost entirely robotic process. This advancement facilitates the application of multiplex real-time PCR, saves extra time, and reduces staff costs. Recently, the manufacturer of the panel recommended the automated Seegene STARlet (Seegene®, Seoul, South Korea) device for DNA isolation. They stated that DNA isolation from 94 samples takes 155 minutes using this device.

Multiplex real-time PCR, which requires high-quality molecular laboratories, specialized equipment, and regular consumable supplies, is a more expensive method than microscopy. However, it may provide financial advantages by reducing unnecessary and incorrect use of antiparasitic drugs and antibiotics, decreasing the need for other diagnostic tests, and lowering overall healthcare costs. At this point, there is a need for studies evaluating the financial impacts of the multiplex real-time PCR in the diagnosis of pathogenic intestinal protozoans.

Apart from our study, only one prospective study (5) has comparatively evaluated the performance of microscopy and multiplex real-time PCR methods using Allplex™ GI Parasite Assay panel. However, unlike our study, the WS technique was not utilized in that research. The sensitivity value obtained in our study (95.83%) higher than that found in a prospective study (91.83%) conducted by Autier et al. (5). Two retrospective studies (5, 18) have evaluated the performance of multiplex real-time PCR using the Allplex™ GI Parasite Assay panel. The sensitivity value obtained in our study (95.83%) was higher than that found in a retrospective (90.35%) conducted by Autier et al. (5). Unlike our study, a comparison of specificity could not be performed in the study by Autier et al. (5) because specificity values were not stated. The sensitivity (95.83%) and specificity (96.56%) in our study were lower than the sensitivity (96.45%) and specificity (98.33%) reported by Argy et al. (18). These differences may arise from variations in the numbers of tested cases or the compatibility of the DNA extraction kit with the panel used. The sensitivity performance of the Allplex™ GI Parasite Assay against the most common *Cryptosporidium* species in human cases, as well as *C. cayetanensis*, was investigated by Autier et al. (5), and the panel's performance against these pathogens was found to be perfect. However, the performance against other *Cryptosporidium* species must also be evaluated to ensure that the method accurately detects all *Cryptosporidium* species.

CONCLUSION

The performance of multiplex real-time PCR performed using the Allplex™ GI Parasite Assay panel was effective even when only the first sample was tested. However, pathogenic intestinal protozoans not included in the panel, (*Cystoisospora* spp., *Sarcocystis* spp., and *B. coli*) must be added. In addition, it must be verified that the panel accurately detects all *Cryptosporidium* species. Additionally, an automated DNA isolation method should be integrated to maximize yield as soon as possible. We believe that if the panel demonstrates excellent performance in large-scale studies following these enhancements, the multiplex real-time PCR method would be used for the routine diagnosis of pathogenic intestinal protozoans.

Ethics Committee Approval: This study was approved by the İstanbul Medical Faculty Clinical Research Ethics Committee (Date: 24.09.2021, No: 17) and the İstinye University Clinical Research Ethics Committee (Date: 14.10.2021, No: 2/2021.K-75).

Informed Consent: Written informed consent was obtained from all study participants or their legal representatives.

Peer Review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- B.K., Ö.B.B., G.İ.Y.; Data Acquisition- B.K., Ö.B.B., G.İ.Y., A.Ç.Ö., O.Ö., M.S., M.A.; Data Analysis/Interpretation- B.K., Ö.B.B., G.İ.Y., A.Ç.Ö, O.Ö., M.S., M.A., B.E.; Drafting Manuscript- B.K., Ö.B.B., G.İ.Y.; Critical Revision of Manuscript- B.K., Ö.B.B., G.İ.Y., A.Ç.Ö., O.Ö., M.S., M.A., B.E.; Final Approval and Accountability- B.K., Ö.B.B., G.İ.Y, A.Ç.Ö., O.Ö., M.S., M.A., B.E.; Material or Technical Support- B.K., Ö.B.B., G.İ.Y., O.Ö., M.S., M.A, B.E; Supervision- B.K., Ö.B.B., G.İ.Y.

Conflict of Interest: The authors have no conflict of interest to declare.

Financial Disclosure: This work was supported by Scientific Research Projects Coordination Unit of İstanbul University (Project number: 38270).

REFERENCES

1. Axelrad JE, Freedberg DE, Whittier S, Greendyke W, Lebwohl B, Green DA. Impact of Gastrointestinal Panel Implementation on Health Care Utilization and Outcomes. J Clin Microbiol 2019;57(3):e01775-18.
2. Faria CP, Zanini GM, Dias GS, da Silva S, de Freitas MB, Almendra R, et al. Geospatial distribution of intestinal parasitic infections in Rio de Janeiro (Brazil) and its association with social determinants. PLoS Negl Trop Dis 2017;11(3):e0005445.
3. Short EE, Caminade C, Thomas BN. Climate Change Contribution to the Emergence or Re-Emergence of Parasitic Diseases. Infect Dis (Auckl) 2017;10:1178633617732296.
4. McHardy IH, Wu M, Shimizu-Cohen R, Couturier MR, Humphries RM. Detection of intestinal protozoa in the clinical laboratory. J Clin Microbiol 2014;52(3):712-20.
5. Autier B, Gangneux JP, Robert-Gangneux F. Evaluation of the AllplexTM Gastrointestinal Panel-Parasite Assay for protozoa detection in stool samples: A retrospective and prospective study. Microorganisms 2020;8(4):569.
6. Ghoshal U, Tejan N. Rationale of using multiplex polymerase chain reaction (PCR) panels for etiological diagnosis of infective diarrhea in the tropics. Indian J Gastroenterol 2018;37(5):381-4.
7. Goldenberg SD, Bacelar M, Brazier P, Bisnauthsing K, Edgeworth JD. A cost benefit analysis of the Luminex xTAG Gastrointestinal Pathogen Panel for detection of infectious gastroenteritis in hospitalised patients. J Infect 2015;70(5):504-11.
8. Beal SG, Tremblay EE, Toffel S, Velez L, Rand KH. A Gastrointestinal PCR Panel Improves Clinical Management and Lowers Health Care Costs. J Clin Microbiol 2017;56(1):e01457-17.
9. Machiels JD, Cremers AJH, van Bergen-Verkuyten MCGT, Paardekoper-Strijbosch SJM, Frijns KCJ, Wertheim HFL, et al. Impact of the BioFire FilmArray gastrointestinal panel on patient care and infection control. PLoS One 2020;15(2):e0228596.
10. https://www.cdc.gov/disasters/disease/diarrhea-evac.html (Access date: January 2, 2021).
11. https://www.cdc.gov/infectioncontrol/guidelines/isolation/precautions.html, (Access date: January 2, 2021).
12. Buss SN, Leber A, Chapin K, Fey PD, Bankowski MJ, Jones MK, et al. Multicenter evaluation of the BioFire FilmArray gastrointestinal panel for etiologic diagnosis of infectious gastroenteritis. J Clin Microbiol 2015;53(3):915-25.
13. Binnicker MJ. Multiplex molecular panels for diagnosis of gastrointestinal infection: performance, result interpretation, and cost-effectiveness. J Clin Microbiol 2015;53:3723-8.
14. Garcia LS. *Dientamoeba fragilis*, One of the Neglected Intestinal Protozoa. J Clin Microbiol 2016;54(9):2243-50.
15. uncay S, Inceboz T, Over L, Yalçin G, Usluca S, Şahin S, et al. Dışkıda *Entamoeba histolytica*'nın saptanmasında kullanılan yöntemlerin birlikte değerlendirilmesi. Turkiye Parazitol Derg 2007;31(3):188-93.
16. Acikgoz Y, Ozkaya O, Bek K, Genc G, Sensoy SG, Hokelek M. Cryptosporidiosis: a rare and severe infection in a pediatric renal transplant recipient. Pediatr Transplant 2012;16(4):E115-9.
17. Büyükbaba-Boral Ö, Çelik DG, İliaz R, Akgül A, İşsever H, Akyüz F. İnflamatuar Barsak Hastalığı Olan Hastalarda *Blastocystis* spp.'nin Farklı Tanı Yöntemleri ile Araştırılması. İst Tıp Fak Derg 2017;80(1):38-44.
18. Argy N, Nourrisson C, Aboubacar A, Poirier P, Valot S, Laude A, et al. Selecting a multiplex PCR panel for accurate molecular diagnosis of intestinal protists: a comparative study of Allplex® (Seegene®), G-DiaParaTrio (Diagenode®), and RIDA®GENE (R-Biopharm®) assays and microscopic examination. Parasite 2022;29:5.