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Original Research Article

Investigation of the presence of *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis* in immunosuppressed patients with diarrhea by IFA and real time PCR methods

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ABSTRACT

Purpose: This study aimed to investigate the frequency of *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis* in patients with diarrhea in the immunosuppressed.
Methods: Patients between the ages of 18–85 who applied to different clinics of Muş Bulanık and Bitlis State Hospitals and were referred to the microbiology or parasitology laboratory were selected for this study. A total of 200 individuals, including 88 immunosuppressed with diarrhea patients, 38 immunocompetent with diarrhea patients, 38 immunosuppressed without diarrhea patients, and 36 immunocompetent without diarrhea individuals, were included. Collected stool samples were evaluated using IFA-MAbs and real-time PCR methods to determine the frequency of *E.intestinalis* and *E.bieneusi*.
Results: E. intestinalis was detected in 59 (29.5%) of 200 samples and *E. bieneusi* was detected in 46 (23.0%) of them. Mixed infection was detected in 16 (8%) of the positive samples. While there was no statistically significant difference between *E. intestinalis* positivity and gender, age, diarrhea status and immune system status, a statistically significant relationship was determined between *E. bieneusi* positivity and diarrhea. When the real-time PCR method was accepted as the gold standard, the sensitivity of the IFA-MAbs method in the diagnosis of *E. intestinalis* was 94.54%, the specificity was 97.24, the sensitivity in the diagnosis of *E. bieneusi* was 95.45%, and the specificity was 98.72%. The overall accuracy of the IFA-MAbs method was 96.5% for the diagnosis of *E. intestinalis* and 98% for the diagnosis of *E. bieneusi*.
Conclusions: The findings suggest that *E. intestinalis* and *E. bieneusi* should be considered in both immunosuppressed and healthy individuals with diarrhea. IFA-MAbs method can be used in addition to the real-time PCR method to diagnose *E. intestinalis* and *E. bieneusi*.

1. Introduction

Microsporidia are obligate intracellular eukaryotic parasites that may cause infection in vertebrate-invertebrate hosts and have more than 1000 species defined under 200 genera. These agents, opportunistic pathogens in immunocompromised patients, can also be seen in immunocompetent individuals. It can be transmitted to humans by oral-oral, fecal-oral, contaminated food and drink, water and respiratory tract. *Encephalitozoon intestinalis* and *Enterocytozoon bieneusi* are the most common of the 14 microsporidia species identified as human pathogens [1,2].

Microsporidia infection can be life-threatening in immunocompromised individuals. While these parasites may cause systemic and non-systemic diseases, the most common clinical finding in infected individuals is chronic diarrhea [1,2].

In the laboratory diagnosis of microsporidia, samples, such as biopsy materials, stool, urine, bile, broncho-alveolar lavage or nasal fluids, are used. The diagnosis can be made by staining the clinical samples with different methods and examining them under a light or fluorescent microscope. Although the trichrome staining method has a crucial place in the diagnosis of the parasite, it has the disadvantage that it requires

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experienced personnel and takes much time to examine with this method. In addition, if there is not enough parasite in the examined sample, it becomes challenging to detect the parasite. Moreover, it is not possible to distinguish species with different staining methods used for diagnosing microsporidia agents [2–4].

Polymerase Chain Reaction (PCR), among the molecular methods, comes to the fore in diagnosis today because it gives more sensitive results. The PCR method allows for detecting even a very small amount of parasite and identifying the type of parasite detected. However, due to the resistant wall structures of microsporidia spores, the DNA of these parasites is challenging to isolate. Today, there is a need to establish a standard protocol for the DNA isolation of these parasites [5].

Studies on the epidemiology of microsporidias generally include HIV-positive or immunosuppressive patients as the patient group, and few studies have been conducted in this area in our country [2,6–8]. However, only a few of these studies have been identified at the species level [2,7,8].

This study aimed to investigate the frequency of *E. bieneusi* and *E. intestinalis* in patients with diarrhea in the immunosuppressed using IFA and real-time PCR methods.

2. Material and method

A total of 200 patients, 88 immunosuppressed with diarrhea (ISD) patients, 38 immunocompetent with diarrhea (ICD) patients, 38 immunosuppressed without diarrhea (IS) patients, and 36 immunocompetent without diarrhea (IC) individuals, were included in the present study (Table 1). Those included in this study were selected from patients aged 18–85 years who applied to different clinics of Muş Bulanık and Bitlis State Hospitals and were referred to the microbiology or parasitology laboratory. Information about the patients and their immune system status were obtained from the automation system of the hospital to which the patient applied.

2.1. Preparation of samples

Approximately 250 mg of stool was placed in Eppendorf tubes and vortexed by adding 500 µl of PBS (1 M, pH: 7.4). The stools were then filtered using plastic tea strainers.

2.2. IFA-MAbs method

In this method, *E. intestinalis* and *E. bieneusi* spores were investigated in stool samples using monoclonal antibodies *anti-E. intestinalis* and *anti-E. bieneusi* kit (Bordier Affinity Products, Switzerland). Preparations were prepared according to the procedure recommended by the kit manufacturer. The preparations were examined under a fluorescent microscope (Olympus BX50) at 1000 magnification with a filter with a wavelength of 450–490 nm.

2.3. DNA isolation and real-time PCR method

The rest of the samples, which were dissolved and filtered in PBS, were centrifuged at 5000×*g* for 5 min and the supernatant was discarded. DNA isolation was performed from the pellet using the DNA extraction kit (EURx GeneMATRIX Stool DNA Purification Kit, Poland). The method was studied with some modifications to the procedure suggested by the kit manufacturer. After incubation at 70 °C for 5 min in the kit procedure, incubation was performed at 95 °C for 45 min in a dry block heater. During the incubation, vortexing was done at 5 min intervals. After incubation, the tubes were placed in a horizontal vortex and vortexed at maximum speed for 30 min. Afterwards, the kit procedure was applied.

SYBR Green real time PCR was performed with the obtained DNA using MsRTf1 5′-CAGGTTGATTCTGCCTGACG-3′ and MsRTr1 5′-CCATCTCTCAGGCTCCCTCT-3′ primers [9]. For PCR, a 20 µl mixture containing DNase and RNase-free distilled water, 10 µM right and left primers, 2X SYBR Green I Master mix, and genomic DNA was prepared. The PCR cycle was set at 95 °C for 10 s, at 55 °C for 20 s, and at 72 °C for 20 s. *E. intestinalis* and *E. bieneusi* species were determined by melting curve analysis [9]. For melting curve analysis, after the end of the PCR cycle, the final temperature was increased from 50 °C to 95 °C with a 0.2 °C temperature increase.

2.4. Statistical analysis

In this study, descriptive statistics for continuous variables were expressed as mean, standard deviation, minimum and maximum values, while categorical variables were expressed as numbers and percentages. The chi-square test was used to determine the relationship between categorical variables. Sensitivity and specificity values were found to determine the success criteria of the methods used. Statistical significance level was taken as 5% ($p < 0.05$) in the calculations and SPSS (IBM SPSS for Windows, Ver. 26) and Minitab statistical package programs were used for calculations.

3. Results

3.1. Findings of E. intestinalis

A total of 59 (29.5%) of the stool samples examined were *E. intestinalis* positive, 52 (26.0%) of them with both the IFA-Mabs and real-time PCR method, four (2.0%) with the IFA-MAbs method and three (1.5%) with the real-time PCR method. Of the 59 positive samples, 27 (45.8%) belonged to the ISD group, 12 (20.3%) to the ICD group, 10 (16.9%) to the IS group, and 10 (16.9%) to the IC group. No statistically significant difference was found between the groups in the statistical analysis. Likewise, no statistically significant correlation was found between *E. intestinalis* positivity and patients' gender, age, diarrhea or immune system status (Table 2).

When the real-time PCR method was accepted as the gold standard, the sensitivity of the IFA-MAbs method for diagnosing *E. intestinalis* was 94.5%, the specificity was 97.2%, and the overall accuracy was 96.5% (Table 3).

3.2. Findings of E. bieneusi

A total of 46 (23.0%) of the stool samples examined were *E. bieneusi* positive, 42 (21,0%) of them with both the IFA-Mabs and real-time PCR method, two (1.0%) with the IFA-MAbs method and two (1.0%) with the real-time PCR method. Of the 46 positive samples, 24 (52.2%) belonged

Table 1
The groups included in the study and their characteristics.

Groups	Group feature	Number of people	Gender Female-Male	Age Mean ± sd	Age Min.- Max.
ISD	Immunosuppressed with diarrhea	88	50–38	46.25 ± 16.68	18–74
ICD	Immunecompetent, with diarrhea	38	16–22	27.18 ± 9.14	18–47
IS	Immunosuppressed without diarrheal	38	18–20	47.90 ± 16.43	18–75
ID	Immunocompetant without diarrheal	36	16–20	27.91 ± 9.10	18–51
Total		200	100–100	39.46 ± 17.08	18–75

Table 2
Distribution of positive patients.

	Group	*E. intestinalis*			*E. bieneusi*		
		Number	Percent (%)	*p.*	Number	Percent (%)	*p.*
Research Group	ISD (N:88)	27	30.68	0.921	24	27.27	0.629
	ICD (N:38)	12	31.58		12	31.58	
	ISD (N:88)	27	30.68	0.615	24	27.27	0.911
	IS (N:38)	10	26.31		10	26.32	
	ISD (N:88)	27	30.68	0.745	24	27.27	**0.001**
	IC (N:36)	10	27.78		0	0.00	
Diarrhea	Yes (N:126)	39	30.95	0.552	36	28.57	**0.008**
	None (N:74)	20	27.03		10	13.51	
Immune System	Competent (N:74)	22	29.73	0.957	12	16.22	0.065
	Suppressed (N: 126)	37	29.36		34	26.98	
Gender	Female (N:100)	24	24.00	0.086	26	26,00	0.312
	Male (N:100)	35	35.00		20	20.00	
Age Group	18-44 (N:110)	35	31.82	0.424	24	21.82	0.662
	≥45 (N:90)	24	26.67		22	24.44	
Total (N:200)		**59**	**29.50**		**46**	**23.00**	

Table 3
Sensitivity and specificity values of the IFA-MAbs method when Real Time PCR is accepted as the gold standard in the diagnosis of *E. intestinalis* and *E. bieneusi*.

Parasite	Method		Real Time PCR		Sensitivity	Specificity	Overall Accuracy
			Positive	Negative			
E. intestinalis	**IFA-MAbs**	Positive	52	4	94.54%	97.24%	96.5%
		Negative	3	141			
E. bieneusi	**IFA-MAbs**	Positive	42	2	95.45%	98.72%	98%
		Negative	2	154			

to the ISD group, 12 (26.1%) to the ICD group, and 10 (21.7%) to the IS group. No positive sample was detected in the IC group (Table 2). There was no statistical difference between ISD, ICD and IS groups for *E. bieneusi* positivity. A statistically significant difference was found between the other groups and the IC group without positive samples ($p = 0.001$). There was a significant correlation between the positivity of this parasite and the complaint of diarrhea ($p = 0.008$). There was no significant difference between age, gender, immune system status and the frequency of the parasite (Table 2).

When the real-time PCR method was accepted as the gold standard, the sensitivity of the IFA-MAbs method for the diagnosis of *E. bieneusi* was 95.4%, the specificity was 98.7%, and the overall accuracy was 98.0% (Table 3).

3.3. Patients with mixed infections of E. intestinalis and E. bieneusi

Sixteen (8%) of the 200 stool samples analyzed were positive for both *E. intestinalis* and *E. bieneusi*. Of 16 patients who were positive for both parasites, 12 (75%) belonged to the ISD group, 2 (12.5%) to the ICD group, and 2 (12.5%) to the IS group. Mixed infections were not detected in any healthy individuals (IC group). A statistically significant correlation ($p = 0.034$) was found between mixed infection, immune system status and diarrhea complaint (Table 4).

Table 4
Distribution of mixed infections.

	Group	*E. intestinalis* + *E. bieneusi*		
		Number	Percent (%)	*p.*
Diarrhea	Yes (N:126)	14	30.95	**0.034**
	None (N:74)	2	27.03	
Immune System	Competent (N:74)	2	29.73	**0.034**
	Suppressed (N: 126)	14	29.36	
Total (N:200)		**16**	**29.50**	

4. Discussion and conclusion

There are 14 species of microsporidia identified as human pathogens. As the pathogenicity of these species differs, their treatments also differ [1]. Albendazole is generally used for treatment of infections due to *E. intestinalis* however it is not very effective against *E. bieneusi*. The current recommendation is to use Fumagillin in the treatment of infections due to *E. bieneusi* [10]. Due to the differences in treatment, it is important to detect the species of microsporidia that causes the infection.

The most common intestinal microsporidia species in humans are *E. intestinalis* and *E. bieneusi*. Many studies have been conducted on the frequency of *E. intestinalis* in immunosuppressed patients and the relationship between the positivity of the parasite and the patient's immune system has been evaluated. In the studies performed, *E. insteinalis* was detected in 33% of immunosuppressed patients [11], in 60.21% of patients diagnosed with cancer and undergoing chemotherapy [2], in 40% of patients receiving chemotherapy and those with acute gastrointestinal complaints, in 24% of patients with bone marrow transplantation and acute gastrointestinal complaints, and 20% of patients with urticaria [8]. In these studies, a statistically significant difference was found between the patient groups and the healthy control groups. In a study by Karimi et al. [12], *Encephalitozoon* spp. was detected in 3.54% of immunosuppressed patients and 4.91% of healthy individuals, and no statistically significant difference was found between the patient group and the control group. In this study, *E. insteinalis* was detected in 30.7% of immunosuppressed with diarrhea patients, 26.3% of immunosuppressed without diarrhea patients, 31.6% immunocompetent with diarrhea patients, and 27.8% of immunocompetent without diarrhea individuals. No statistically significant difference was found between the groups in this study. The fact that no statistically significant difference was determined showed that it would be appropriate to evaluate both healthy and immunosuppressed patients with at least one of the symptoms seen in the infection caused by this parasite.

E. bieneusi is an important intestinal microsporidia species that may cause infection in humans. Some researchers suggest that immunosuppressed patients are more at risk than immunocompetent individuals for this parasitosis [7]; others reported that there was no difference in positivity

between these two groups, as determined in this study [12]. Although asymptomatic infection is often seen in immunocompetent individuals, colonization of *E. bieneusi* may damage the intestinal barrier, and intestinal absorption may be impaired [13]. Diarrhea and malabsorption seem to be the most common clinical problems associated with *E. bieneusi* infections reported in the literature [14–16]. However, some studies did not find a statistically significant relationship between *E. bieneusi* and diarrhea [7]. In this study, the rate of *E. bieneusi* detected in patients with diarrhea (28.6%) was higher than in patients without diarrhea (13.5%) ($p = 0.009$). This parasite was not found in immunocompetent individuals without diarrhea. Given the statistical evaluation results obtained in this study, it was concluded that it would be appropriate to evaluate patients with diarrhea regarding *E. bieneusi*, regardless of the state of the immune system.

Mixed infections of *E. intestinalis* and *E. bieneusi* are common. In studies, mixed infections were at a rate of 9.5% in bone marrow transplant patients [7] and 14% in cancer patients receiving chemotherapy [2]. In another study, mixed infections were at a rate of 7.7% [17]. In this study, mixed infections were detected at a rate of 8%. However, mixed infections were not detected in immunocompetent without diarrhea individuals. Given that 87.5% of mixed infections are seen in immunosuppressed patients indicates that immunosuppressed patients are at risk for mixed infections. In addition, 87.5% of the patients with mixed infections had diarrhea. We believe this situation can be explained by the increase in pathogenicity in mixed infections.

The importance of gender and age in the epidemiology of microsporidian parasites has been discussed in previous studies, and different results have been obtained. Compatible with the results obtained in this study, in many studies, there was no statistically significant relationship between the incidence of *E. insteinalis* or *E. bieneusi* and gender [1,6,18,19] or age [6,20].

Parasites from intestinal microsporidia are diagnosed using light, fluorescent and TEM, or serological and molecular methods. The real-time PCR method, one of the molecular tests, has come to the fore in recent years due to its speed, sensitivity and specificity, and the ability to determine the target pathogen or gene region quantitatively. In studies using this method and other molecular methods, sensitivity and specificity were generally found at high rates [3].

In diagnosing microsporodia species, molecular methods, such as real-time PCR, were accepted as the "gold standard" method, and studies were conducted to evaluate the sensitivity and specificity of the IFA-MAbs method. Alfa Cisse et al. [3] found that the sensitivity and specificity of the IFA-MAbs method was 100% compared with the PCR method and stated that this method could be considered the "gold standard" because it is faster and cheaper than PCR. When Singh et al. [21] accepted nested PCR as the gold standard method, the sensitivity of the IFA-MAbs method was 87%, and the specificity 100%; when Ghoshal et al. [22] accepted the RFLP-PCR method as the gold standard, they found the sensitivity of the IFA-MAbs method as 95.2% and the specificity as 100%. In this study, when the real-time PCR method was accepted as the gold standard, the sensitivity of the IFA-MAbs method in the diagnosis of *E. intestinalis* was 94.54%, and the specificity was 97.24%; its sensitivity in the diagnosis of *E. bieneusi* was calculated as 95.45% and specificity as 98.72%. In addition, the overall accuracy of the IFA-MAbs method was 96.5% for the diagnosis of *E. intestinalis* and 98% for the diagnosis of *E. bieneusi*. Given these sensitivity and specificity values, it was concluded that the IFA-MAbs method could be used in the routine diagnosis of *E. intestinalis* and *E. bieneusi* species. However, when evaluating the results, structures with a bright green form that do not resemble *E. insteinalis* or *E. bieneusi* should not be confused with these parasites.

As a result, *E. bieneusi* and *E. intestinalis* should be considered in both immunosuppressed and immunocompetent individuals, especially *E. bieneusi* in patients with diarrhea. Since patients with immunosuppressed diarrhea are more sensitive, it has been concluded that they should be evaluated periodically, and necessary treatments should be administered. In addition, the IFA-MAbs method could be used because it is more practical in diagnosing *E. intestinalis* and *E. bieneusi*.

Author's contribution statement

SA, ZTC, AGH & AE performed the experiments and interpreted the results. SA, ZTC & AE analyzed the data and drafted the manuscript. ZTC, AGH & SA designed the study. SA & ZTC critically revised the manuscript. All the authors approved the final version of the manuscript to be published and agree to be accountable for all aspects of the work.

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Ethical clearance

The ethical approval for this study was obtained from the Scientific Research and Publication Ethics Committee of Muş Alparslan University (01/10/2019-E.13,580).

Declaration of competing interest

The authors declare that there are no conflicts of interest.

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References

[1] Türk S, Al FD, Karaman Ü, Kuştimur S. Investigation of microsporidia prevalence by different staining methods in cas es of diarrhea. Mikrobiyoloji Bulteni 2012;46(1):85–92.
[2] Hamamcı B, Çetinkaya Ü, Berk V, Kaynar L, Kuk S, Yazar S. Prevalence of *Encephalitozoon intestinalis* and *Enterocytozoon bieneusi* in cancer patients under chemotherapy. Mikrobiyoloji Bulteni 2015;49(1):105–13. https://doi.org/10.5578/mb.8787.
[3] Alfa Cisse O, Ouattara A, Thellier M, Accoceberry I, Biligui S, Minta D, et al. Evaluation of an immunofluorescent-antibody test using monoclonal antibodies directed against Enterocytozoon bieneusi and Encephalitozoon intestinalis for diagnosis of intestinal microsporidiosis in Bamako (Mali). J Clin Microbiol 2002;40(5):1715–8. https://doi.org/10.1128/JCM.40.5.1715-1718.2002.
[4] Al-Mekhlafi MA, Fatmah M, Anisah N, Azlin M, Al-Mekhlafi HM, Norhayati M. Species identification of intestinal microsporidia using immunofluorescence antibody assays. Southeast Asıan J Trop Med Publıc Health. 2011;42(1):19.
[5] Javanmard E, Mirjalali H, Niyyati M, Jalilzadeh E, Tabaei SJS, Aghdaei HA, et al. Molecular and phylogenetic evidences of dispersion of human-infecting microsporidia to vegetable farms via irrigation with treated wastewater: one-year follow up. Int J Hyg Environ Health 2018;221(4):642–51. https://doi.org/10.1016/j.ijheh.2018.03.007.
[6] Karaman Ü, Şener S, Çalık S, Şaşmaz S. Investigation of microsporidia in patients with acute and chronic urticaria. Mikrobiyoloji Bulteni 2011;45(1):168–73.
[7] Çetinkaya Ü, Hamamcı B, Kaynar L, Kuk S, Şahin İ, Yazar S. Investigation of the presence of *Encephalitozoon intestinalis* and *Enterocytozoon bieneusi* in bone marrow transplant patients by IFA-MAbs method. Mikrobiyoloji Bulteni 2015;49(3):432–8. https://doi.org/10.5578/mb.9809.
[8] Çetinkaya Ü, Kuk S, Sivcan E, Kaynar L, Arslan D, Şahin İ. The high prevalence of Encephalitozoon intestinalis in patients receiving chemotherapy and children with growth retardation and the validity of real-time PCR in its diagnosis. Turk J Med Sci 2016;46(4):1050–8. https://doi.org/10.3906/sag-1504-69.
[9] Polley SD, Boadi S, Watson J, Curry A, Chiodini PL. Detection and species identification of microsporidial infections using SYBR Green real-time PCR. J Med Microbiol 2011;60(4):459–66. https://doi.org/10.1099/jmm.0.026781-0.
[10] Saffo Z, Mirza N. Successful treatment of Enterocytozoon bieneusi gastrointestinal infection with nitazoxanide in a immunocompetent patient. IDCases 2019;18:e00586.
[11] Halánová M, Valenčáková A, Jarčuška P, Halán M, Danišová O, Babinská I, et al. Screening of opportunistic Encephalitozoon intestinalis and Enterocytozoon bieneusi in immunocompromised patients in Slovakia. Cent Eur J Publ Health 2019;27(4):330–4. https://doi.org/10.21101/cejph.a5407.
[12] Karimi K, Mirjalali H, Niyyati M, Haghighi A, Pourhoseingholi MA, Sharifdini M, et al. Molecular epidemiology of *Enterocytozoon bieneusi* and *Encephalitozoon* sp., among immunocompromised and immunocompetent subjects in Iran. Microb Pathog 2020;141:103988. https://doi.org/10.1016/j.micpath.2020.103988.

[13] Wang SS, Wang RJ, Fan XC, Liu TL, Zhang LX, Zhao G-H. Prevalence and genotypes of *Enterocytozoon bieneusi* in China. Acta Trop 2018;183:142–52. https://doi.org/10.1016/j.actatropica.2018.04.017.

[14] Matos O, Lobo ML, Xiao L. Epidemiology of *Enterocytozoon bieneusi* infection in humans. J Parasitol Res 2012;2012. https://doi.org/10.1155/2012/981424.

[15] Abreu-Acosta N, Lorenzo-Morales J, Leal-Guio Y, Coronado-Álvarez N, Foronda P, Alcoba-Florez J, et al. *Enterocytozoon bieneusi* (microsporidia) in clinical samples from immunocompetent individuals in Tenerife, Canary Islands, Spain. Trans R Soc Trop Med Hyg 2005;99(11):848–55. https://doi.org/10.1016/j.trstmh.2005.05.010.

[16] Endeshaw T, Kebede A, Verweij JJ, Zewide A, Tsige K, Abraham Y, et al. Intestinal microsporidiosis in diarrheal patients infected with human immunodeficiency virus-1 in Addis Ababa, Ethiopia. Jpn J Infect Dis 2006;59(5):306.

[17] Abd-Elbaki M, Arafa M, Hameed A-E, Habib K, Abdel Rahman A, Anwar M. Intestinal microsporidiosis: prevalence and genetic study of Egyptian isolates. Parasitologists United Journal 2020;13(1):35–45.

[18] Calik S, Karaman U, Colak C. Prevalence of microsporidium and other intestinal parasites in children from Malatya, Turkey. Indian J Microbiol 2011;51(3):345–9. https://doi.org/10.1007/s12088-011-0107-4.

[19] Mumcuoglu I, Cetin F, Dogruman Al F, Oguz I, Aksu N. Prevalence of microsporidia in healthy individuals and immunocompetent patients with acute and chronic diarrhea. Inf Disp 2016;48(2):133–7. https://doi.org/10.3109/23744235.2015.1094572.

[20] Karaman Ü, Daldal N, Atambay M, Çolak C. The epidemiology of microsporidias in humans (Malatya sample). Turk J Med Sci 2009;39(2):281–8. https://doi.org/10.3906/sag-0803-32.

[21] Singh I, Sheoran AS, Zhang Q, Carville A, Tzipori S. Sensitivity and specificity of a monoclonal antibody-based fluorescence assay for detecting *Enterocytozoon bieneusi* spores in feces of simian immunodeficiency virus-infected macaques. Clin Vaccine Immunol 2005;12(10):1141–4. https://doi.org/10.1128/CDLI.12.10.1141-1144.2005.

[22] Ghoshal U, Khanduja S, Pant P, Ghoshal UC. Evaluation of Immunoflourescence antibody assay for the detection of *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis*. Parasitol Res 2016;115(10):3709–13. https://doi.org/10.1007/s00436-016-5130-2.