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The Frequency of *Cryptosporidium* spp. in Immunocompromised Patients by Modified Acid-Fast Staining, Cassette Kit and ELISA Methods: Comparison of the Diagnostic Techniques

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Abstract

Background: Cryptosporidiosis has been reported in both immunocompetent and immunocompromised patients from over 40 countries in six continents.
Objectives: This study was carried out to determine the prevalence of *Cryptosporidium* spp. in immunocompromised patients by methods of modified acid fast staining, ELISA and Cassette Kit, and to also compare the three methods.
Methods: The patients in different age groups admitted to Bitlis state hospital between June 23, 2011 and January 13, 2015 were enrolled. The study group was composed of 300 immunocompromised volunteer patients and the control group of 100 volunteers with normal immune system. In the study group, patients with hemodialysis, chronic renal failure, diabetes mellitus, diabetes insipidus, and cancer were enrolled. Formalin-ethyl acetate, modified acid fast staining and native-lugol were used for all stool samples; they were also investigated for *Cryptosporidium* antigens by the ELISA and Cassette Kit.
Results: The highest prevalence rate of *Cryptosporidium* spp. is found to be 11.3%, which is obtained by ELISA and the lowest (0.7%) by Cassette Kit in the study group. All patients with cryptosporidiosis had diarrhea. The highest prevalence rate (20%) was observed in patients with diabetes insipidus and the second highest in patients with chronic renal failure (11.5%) by ELISA. The sensitivities and specificities of Cassette Kit and modified acid fast staining were 5.9%, 100%; and 50%, 100%, respectively.
Conclusions: It is concluded that *Cryptosporidium* spp. should be considered in immunocompromised diarrheal patients and ELISA must be chosen for detection of this parasite. Modified acid fast staining and Cassette Kit techniques could be performed if ELISA is not available. The single use of the Cassette Kit will be inadequate for the diagnosis of cryptosporidiosis.

Keywords: Cryptosporidiosis, Immunocompromised Host, Clinical Laboratory Techniques

1. Background

The first case of cryptosporidiosis in humans was reported in 1976. A few cases, subsequently, mostly of immunocompromised patients, were reported between 1976 and 1981. During 1981 - 1982, however, *Cryptosporidium* infections causing severe enteritis in AIDS patients were encountered. The parasite was reported in animal keepers, tourists, and also in some immunocompetent persons in the following years (1-3).

Duration of the symptoms and outcome typically vary according to the immunologic health of the host. In the AIDS/AIDS-related complex group, long duration infections followed by death are most frequent, although spontaneous clinical recovery has been reported and treatment may modify symptoms. Immunologically healthy people normally have a shorter duration of symptoms (< 20 days) and a spontaneous complete recovery. Whereas *Cryptosporodium* causes asymptomatic infection or transient diarrhea in healthy people, may lead to severe chronic diarrhea, abdominal pain, nausea, vomiting, subfebrile fever, malaise, weight loss, pancreatitis, cholecystitis, gastritis, esophagitis, and respiratory infections in immunocompromised people, which may lead to even death (3-8).

Cryptosporidiosis has now been reported from over 40 countries in six continents in both immunocompetent and immunocompromised patients around the world (9). In a review of over 130,000 presumably immunocompetent patients with diarrhea, 43 studies were done in developing areas (Asia, Africa and Latin America) and 35 studies in industrialized countries (in Europe, North America and Australia) (8, 10).

A large number of staining techniques have been used to detect *Cryptosporidium* oocysts. The most commonly used have been the modified acid-fast procedures. Mala-

chite green, Giemsa, and hematoxylin and eosin staining techniques have been used to detect the organism with varying success and are inferior to modified acid fast staining (4). It may be impossible to detect the parasite in stool samples, which usually harbor few or distorted oocysts, leading a false negativity of the microscopy. The false negativity of stool antigen tests is also due to scarcity of parasite. ELISA kits, however, employing *Cryptosporidium* specific monoclonal antibodies, have a sensitivity and specificity of 93% - 100% (2, 11).

2. Objectives

This study aims to determine the prevalence of *Cryptosporidium* spp. in immunocompromised patients by the modified acid-fast staining (MAFS), ELISA, and Cassette Kit (CK) diagnostic techniques, and to also compare the three methods.

3. Methods

The patients in different age groups admitted to the Bitlis state hospital between June 23, 2011 and January 13, 2015 were enrolled. The study group included 300 voluntary immunocompromised patients (141 female, 159 male; mean age: 51.24 $\pm$ 15.25 years) and the control group included 100 healthy people (45 female, 55 male; mean age: 50.75 $\pm$ 16.55 years). In the study group 49 patients were $\leq$ 35 and 251 were above 36. The patients were either positive for chronic renal failure (CRF), diabetes mellitus (DM), diabetes insipidus (DI) or cancer (CA).

All stool samples were examined by native-lugol, formalin-ethyl acetate, and MAFS (11). The samples were also tested by ELISA (R-Biopharm, Germany) and CK (R-Biopharm, Germany) for *Cryptosporidium* antigens at Yuzuncu Yil University, medicine faculty parasitology laboratory. The study was approved by Yuzuncu Yil University, medicine faculty research ethics committee (Meeting Date: 07 October 2010, Decision No: 07). Written informed consent was obtained from all patients.

3.1. Statistical Analysis

Descriptive statistics for the categorical variables (characteristics) were presented as count and percent. Chi-Square test was calculated for determination of relationships between the categorical variables. The Z test was also used for comparison of proportions. ELISA test was accepted as gold standard test for detection of *Cryptosporidium* oocysts in fecal specimens. Furthermore, the diagnostics test statistics (sensitivity and specificity etc.) were also computed for determination of performances for the new tests (MAFS and CK methods). The statistical significance level was considered as 5% and the MINITAB statistical program was used for all statistical computations.

4. Results

ELISA detected *Cryptosporidium* in stool samples in 11.3% of the immunosuppressed patients, while the CK method found it in only 0.7%. MAFS, however, revealed oocysts in 5.7% of the patients. The ones with MAFS and CK positivity were also found to be positive by ELISA. All positive patients for the parasite had diarrhea. ELISA detected *Cryptosporidium* spp. in only 3% of the control group (Tables 1 and 2). The parasite was detected by ELISA in mostly DI patients (20%) and secondly in patients with CRF (11.5%). Another pathogenic intestinal parasite was not detected in *Cryptosporidium* positive patients.

4.1. Statistical Findings

No statistically significant relation was obtained between *Cryptosporidium* positivity and patients' age or gender. The differences between *Cryptosporidium* incidence and DM ($P < 0.05$), CRF ($P < 0.05$), and immunodeficiency ($P < 0.01$; our patient group, 300 patients, is taken into consideration) were statistically significant. ELISA was accepted as the gold standard technique. CK had a sensitivity and specificity of 5.9%, and 100% respectively. MAFS, however, had a sensitivity of 50% and a specificity of 100% (Table 2). The negative results of MAFS, ELISA, and CK for *Cryptosporidium* spp. detection are consistent with each other. However, the positive results of the CK method was found to be quite low compared to MAFS and ELISA. MAFS was found to be an alternative to ELISA for detection of the parasite, while CK was not.

5. Discussion

Human infections caused by *Cryptosporidium* spp. are seen in rural or urban areas of underdeveloped or developing countries in 6 continents. Large series involving adult or children patients presented with diarrhea or gastrointestinal symptoms demonstrated that the parasite can be seen as high as 1% - 2% in Europe, 0.6% - 4.3% in North America (except outbreaks), and 3% - 4% to 10% - 20% in Asia, Australia, Africa, Latin and South America (4).

Cryptosporidium spp. is shown to be an important cause of chronic diarrhea in immunocompromised patients by numerous international and local studies. Nahrevanian

Table 1. *Cryptosporidium* spp. Positivity by Patient Subgroups

Patient Groups	ELISA		CK Method		MAFS	
	N	%	N	%	N	%
DM (n: 141)	15	10.6	-	-	7	5
DI (n: 10)	2	20	-	-	-	-
CRF (n: 96)	11	11.5	2	2.1	7	7.3
CA (n: 53)	6	11.3	-	-	3	5.7
Total (n:300)	34	11.3	2	0.7	17	5.7

Abbrviations: CA, Cancer; CRF, Chronic renal failure; DM, Diabetes mellitus; DI, Diabetes insipidus; N, Number.

Table 2. *Cryptosporidium* spp. Positivity in Both Groups by Three Methods as well as the Sensitivities and Specificities of the Methods

Methods	Patient Group							
	35 ≤ (n: 49)		36 ≥ (n: 251)		Female (n: 141)		Male (n: 159)	
	N	%	N	%	N	%	N	%
ELISA	7	14.3	27	10.8	13	9.2	21	13.2
MAFS	1	2	16	6.4	9	6.4	8	5
CK	-	-	2	0.8	2	1.4	-	-
Control Group								
	35 ≤ (n: 23)		36 ≥ (n: 77)		Female (n: 45)		Male (n: 55)	
	N	%	N	%	N	%	N	%
ELISA	2	8.7	1	1.3	1	2.2	2	3.6
The Results of the Diagnostic Test Criteria (%)								
	Sensitivity		Specificity		False Positives		False Negatives	
MAFS[a]	50		100		0		50	
CK[a]	5.9		100		0		94.1	

Abbreviations: CA, Cancer; CRF, Chronic renal failure; DI, Diabetes insipidus; DM, Diabetes mellitus; N, Number.
[a] ELISA was accepted as the gold standard technique.

and Assmar (7) showed the parasite in 1.4% of 214 immunocompromised patients by acid fast stain, auramin phenol fluorescence, and direct fluorescence; Abaza et al. (12) showed it in 6.3% of 427 immunocompromised patients with Kinyoun acid-fast stain; Baqai et al. (13) did in 80% of 10 patients with CA, 25% of 20 DM patients, 35% of 20 CRF patients by Kinyoun acid fast method; Hassanein et al. (3) in 24% of 25 children with cancer by modified Ziehl-Neelsen; Seyrafian et al. (14) in 11.5% of 104 CRF patients by MAFS; Gil et al. (15) in 26.4% of 110 CRF patients by ELISA; Raja et al. (16) in 53% of 644 patients with kidney transplantation by modified Ziehl-Neelsen stain; Sulzyc-Bielicka et al. (17) in 12.6% of 87 cancer patients by immunoenzymatic tests; Al-Qobati et al. (18) in 30.1% of 206 cancer patients by staining methods; Kulkarni et al. (19) in 12% of 137 AIDS patients with diarrheal by staining, and finally Dehkordy et al. (20) in 5.1% of 176 immunocompromised patients with ELISA.

Local studies from Turkey using serological test and/or stain methods to detect *Cryptosporidium* spp. in various immunocompromised patients also exist. Tamer et al. found cryptosporidiosis in 12.35% of their patients by the ELISA method and 7.86% by Kinyoun acid fast staining in a group of 89 children with a diagnosis of leukemia/lymphoma and diarrhea. No cryptosporidiosis was reported to be detected in the 60 patients of the control group (21). There are several other studies, one of which was done b Tanyüksel et al. and reported that the parasite was detected using Ziehl-Neelsen and Giemsa staining methods in 17% of the 106 patients with neoplasia and diarrhea (22), another study was done by Ok et al. (23) and reported 39.1% positivity out of 69 renal transplant recipients, an additional study done by

Sari et al. (24) found 6.4% positivity of 47 patients with CRF by Kinyoun acid fast stain, and Sönmez Tamer et al. (25) showed the parasite in 12.35% of 89 patients with leukemia and lymphoma by the ELISA method.

We found *Cryptosporidium* spp. positivity in 11.3% of 300 immunocompromised patients ($P < 0.01$). The parasite was encountered in the 3 control volunteers (3%) by only the ELISA method. While DI patients (20% of them) mostly had the parasite, the CRF group came in second (11.5%). The best methods to detect the parasite were found to be ELISA, MAFS, and CK, in a descending order.

Cryptosporidiosis, which is rarely seen in individuals with normal immunity, is found at a much higher rate in immunocompromised patients, as could be observed in the abovementioned studies. In some studies, either serological or staining methods have been used to determine the positivity of the parasite. However, in others, serological and staining methods were used together, which is similar to our study. Different results were obtained with these different methods. When few oocysts were found in the stool, the staining methods might not be sufficient for diagnosis. Thus, serological tests with high sensitivity and specificity such as the ELISA method should be used together with the conventional staining tests.

Rosenblayt and Sloan (25) determined *Cryptosporidium* spp. positivity in 100 of the 296 stool specimens with ELISA. These researchers found that the ELISA sensitivity was 93%, specificity was 99%, and the positive predictive value was 99%. Sonmez Tamer and Gulenc (26) reported that out of 80 stool samples, 3.75% were found to be positive for oocysts of *Cryptosporidium* spp. with the acid-fast stain and 6.25% were found to be positive with ELISA. In the study, the sensitivity, specificity, negative predictive, and positive predictive with *Cryptosporidium* ELISA kit were 60%, 100%, 97.4%, and 100%, respectively. On the other hand, we have not found any study regarding the CK method to determine the prevalence of *Cryptosporidium* spp. in the literature.

In this study, the highest prevalence rate of *Cryptosporidium* spp. is found, which is obtained by the ELISA method. Assuming ELISA as a gold standard with 100% sensitivity, MAFS is resulted to have 50% sensitivity, whereas CK had 5.9%. The specificity of both the MAFS and CK methods were found to be 100%. Thus, it is not possible for the CK method to be used as an alternative to ELISA and MAFS. To our knowledge, there are no previous studies considering all three methods together. A number of diagnostic modalities with varying sensitivities and specificities are now available. Acid-fast stains out of conventional staining methods for detection of *Cryptosporidium* spp. are more reliable, specific, and accurate. The ELISA method with a standardized antigen-detection capacity in stool specimens is highly preferred since it is rapid and easy to perform, having a higher sensitivity and specificity compared to other conventional microscopic methods (2, 9, 11).

5.1. Conclusion

In brief, it is concluded that *Cryptosporidium* spp. should be considered in immunocompromised patients who have diarrhea, and ELISA is the method that must be chosen for detection of the parasite. MAFS and CK should be performed together if ELISA is not available. The single use of the CK method will be inadequate for diagnosis of cryptosporidiosis.

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Footnotes

Authors' Contribution: Study concept and design, Zeynep Tas Cengiz and Hasan Yilmaz; acquisition of patient materials, Ibrahim Halil Sahin and Mahir Kapmaz; analysis and interpretation of the results, Zeynep Tas Cengiz, Hasan Yilmaz, Ibrahim Halil Sahin, Mahir Kapmaz and Pinar Ekici; drafting of the manuscript, Zeynep Tas Cengiz; critical revision of the manuscript, Zeynep Tas Cengiz and Hasan Yilmaz; study supervision, Zeynep Tas Cengiz and Hasan Yilmaz.

Conflict of Interest: Authors mention that there is no conflict of interest in this study.

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References

1. Xiao L, Fayer R, Ryan U, Upton SJ. Cryptosporidium taxonomy: recent advances and implications for public health. *Clin Microbiol Rev.* 2004;**17**(1):72–97. [PubMed: 14726456].
2. Mehlhorn H. Progress in parasitology. Berlin: Springer; 2011.
3. Hassanein SM, Abd-El-Latif MM, Hassanin OM, Abd-El-Latif LM, Ramadan NI. Cryptosporidium gastroenteritis in Egyptian children with acute lymphoblastic leukemia: magnitude of the problem. *Infection.* 2012;**40**(3):279–84. doi: 10.1007/s15010-011-0230-5. [PubMed: 22189516].
4. Fayer R, Ungar BL. Cryptosporidium spp. and cryptosporidiosis. *Microbiol Rev.* 1986;**50**(4):458–83. [PubMed: 3540573].
5. Bouzid M, Hunter PR, Chalmers RM, Tyler KM. Cryptosporidium pathogenicity and virulence. *Clin Microbiol Rev.* 2013;**26**(1):115–34. doi: 10.1128/CMR.00076-12. [PubMed: 23297262].
6. Caccio SM, Widmer GC. Parasite and disease. mycopy softcover edition ed. New York: Springer Wien Heidelberg; 2014.

7. Nahrevanian H, Assmar M. Cryptosporidiosis in immunocompromised patients in the Islamic Republic of Iran. *J Microbiol Immunol Infect.* 2008;**41**(1):74–7. [PubMed: 18327430].
8. Dillingham RA, Lima AA, Guerrant RL. Cryptosporidiosis: epidemiology and impact. *Microbes Infect.* 2002;**4**(10):1059–66. [PubMed: 12191656].
9. Satoskar AR, Simon GL, Hotez PJ, Tsuji M. Medical Parasitology. Texas: Landes Bioscience; 2009.
10. Adal KASC, Guerrant RL. Cryptosporidium and related species. In: Blaser PDS MJ, Ravdin JI, Greenberg HB, Guerrant RL, editors. Infections of the gastrointestinal tract. New York: Raven Press; 1995. .
11. Korkmaz M, Ok UZ. Parazitolojide laboratuvar. Izmir: Meta Basim; 2011.
12. Abaza SM, Makhlouf LM, el-Shewy KA, el-Moamly AA. Intestinal opportunistic parasites among different groups of immunocompromised hosts. *J Egypt Soc Parasitol.* 1995;**25**(3):713–27. [PubMed: 8586866].
13. Baqai R, Anwar S, Kazmi SU. Detection of cryptosporidium in immunosuppressed patients. *J Ayub Med Coll Abbottabad.* 2005;**17**(3):38–40. [PubMed: 16320794].
14. Seyrafian S, Pestehchian N, Kerdegari M, Yousefi HA, Bastani B. Prevalence rate of Cryptosporidium infection in hemodialysis patients in Iran. *Hemodial Int.* 2006;**10**(4):375–9. doi: 10.1111/j.1542-4758.2006.00133.x. [PubMed: 17014515].
15. Gil FF, Barros MJ, Macedo NA, Junior CG, Redoan R, Busatti H, et al. Prevalence of intestinal parasitism and associated symptomatology among hemodialysis patients. *Rev Inst Med Trop Sao Paulo.* 2013;**55**(2):69–74. [PubMed: 23563757].
16. Raja K, Abbas Z, Hassan SM, Luck NH, Aziz T, Mubarak M. Prevalence of cryptosporidiosis in renal transplant recipients presenting with acute diarrhea at a single center in Pakistan. *J Nephropathol.* 2014;**3**(4):127–31. doi: 10.12860/jnp.2014.25. [PubMed: 25374881].
17. Sulzyc-Bielicka V, Kolodziejczyk L, Jaczewska S, Bielicki D, Kladny J, Safranow K. Prevalence of Cryptosporidium sp. in patients with colorectal cancer. *Pol Przegl Chir.* 2012;**84**(7):348–51. doi: 10.2478/v10035-012-0058-4. [PubMed: 22935456].
18. Al-Qobati SA, Al-Maktari MT, Al-Zoa AM, Derhim M. Intestinal parasitosis among Yemeni patients with cancer, Sana'a, Yemen. *J Egypt Soc Parasitol.* 2012;**42**(3):727–34. [PubMed: 23469646].
19. Kulkarni SV, Kairon R, Sane SS, Padmawar PS, Kale VA, Thakar MR, et al. Opportunistic parasitic infections in HIV/AIDS patients presenting with diarrhoea by the level of immunesuppression. *Indian J Med Res.* 2009;**130**(1):63–6. [PubMed: 19700803].
20. Dehkordy AB, Rafiei A, Alavi S, Latifi S. Prevalence of cryptosporidium infection in immunocompromised patients, in South-west of iran, 2009-10. *Iran J Parasitol.* 2010;**5**(4):42–7. [PubMed: 22347265].
21. Tamer GS, Balikci E, Erbay A. [The prevalence of cryptosporidiosis in children who were diagnosed with leukemia and lymphoma]. *Turkiye Parazitol Derg.* 2008;**32**(3):192–7. [PubMed: 18985569].
22. Tanyuksel M, Gun H, Doganci L. Prevalence of Cryptosporidium sp. in patients with neoplasia and diarrhea. *Scand J Infect Dis.* 1995;**27**(1):69–70. [PubMed: 7784817].
23. Ok UZ, Cirit M, Uner A, Ok E, Akcicek F, Basci A, et al. Cryptosporidiosis and blastocystosis in renal transplant recipients. *Nephron.* 1997;**75**(2):171–4. [PubMed: 9041537].
24. Sari C, Sari K, Ertug S. Investigation of the frequency of Cryptosporidium spp. and Blastocystis hominis in patients with chronic kidney failure.. *Turkish J Parasitol.* 2003;**27**(3):187–90.
25. Rosenblatt JE, Sloan LM. Evaluation of an enzyme-linked immunosorbent assay for detection of Cryptosporidium spp. in stool specimens. *J Clin Microbiol.* 1993;**31**(6):1468–71. [PubMed: 8314988].
26. Tamer GS, Gulenc S. [The investigation of the presence of antibodies for Cryptosporidium spp. in fecal samples using ELISA]. *Turkiye Parazitol Derg.* 2008;**32**(3):198–201. [PubMed: 18985570].