Comparison of methods investigating *Giardia intestinalis*, *Entamoeba histolytica*, and *Cryptosporidium* spp. in stool samples of patients with diarrhea

Original Article

Ayşe Hümeyra Taşkin Kafa[1], Fatih Çubuk[2], Rukiye Aslan[1,3], Mürşit Hasbek[1]

Sivas Cumhuriyet University, Faculty of Medicine, Department of Medical Microbiology, Sivas[1], Ministry of Health General Directorate of Public Health, Department of Microbiology Reference Laboratory and Biological Products, Ankara[2], Sivas Cumhuriyet University, Vocational School of Health Services, Department of Medical Services and Techniques, Sivas[3], Türkiye

ABSTRACT

Background: Intestinal protozoan infections (IPIs), common all over the world, are an important public health problem, especially in developing countries. Different diagnostic methods are used for the diagnosis of causative agents in diarrhea cases.
Objective: This study aims to analyze results of direct microscopy, coproantigen detection test, and PCR technique in diagnosis of *G. intestinalis*, *E. histolytica/dispar*, and *Cryptosporidium* spp. in stool samples of patients with diarrhea.
Subjects and Methods: Fresh stool samples were collected from 683 patients complaining of diarrhea, and simultaneously examined by direct microscopy, commercial rapid diagnostic tests (RDTs) for detection of coproantigens, and molecularly using PCR technique.
Results: The overall detection rate of parasites was 3.7% by direct microscopy, 6.6% by RDTs and 2% by PCR technique. Moderate, and weak fits were recorded between direct microscopy and RDTs results (Kappa=0.46, $P<0.001$), and between direct microscopy and PCR technique results (Kappa=0.236, $P<0.001$), respectively. No fit (Kappa=0.108, $P=0.001$) was recorded between coproantigen detection test and PCR technique results.
Conclusion: It was concluded that direct microscopy and RDTs will be the correct approach in the first instance in the suspicion of IPIs. Despite high cost of PCR technique, it should be considered in differentiation between pathogenic and non-pathogenic amoeba, and genotyping of *Cryptosporidium* spp.

Keywords: coproantigens; diagnostic methods; intestinal protozoa; microscopy; PCR; rapid diagnostic tests.

Received: 22 August, 2023; **Accepted:** 10 October, 2023.

Corresponding Author: Ayşe H. Taşkin Kafa; **Tel.:** +90 3464870000; **Email:** ahtaskin@cumhuriyet.edu.tr

Print ISSN: 1687-7942, **Online ISSN:** 2090-2646, **Vol. 16, No. 3, December, 2023.**

INTRODUCTION

Amoebiasis, giardiasis, and cryptosporidiosis are common all over the world, and constitute an important public health problem especially in developing countries. Several factors contribute in their widespread epidemiological distribution, such as contamination of drinking water and food, substructure deficiencies, environmental and climatic characteristics, socioeconomic level, educational status, nutrition, and clean habits[1-5]. The most important route of transmission of the disease is the ingestion of infective parasitic stages by consuming contaminated water and food. The disease can progress asymptomatically or symptomatically with complaints of varying severity. Significant medical and social problems affecting the quality of life, such as diarrhea, malabsorption, bloody stools, and loss of workforce, are seen in symptomatic people. Although the mortality rate is low in such infections, complications that require hospitalization may develop in some cases[1-4].

Considering the medical complications resulting from intestinal protozoan infections, treatment costs, and negative social adaptation, it is critically important to quickly and accurately test for the presence of possible parasitic agents in individuals with diarrhea, taking protective measures, and applying an effective treatment[6-8].

Different diagnostic methods such as microscopy, coproantigen detection tests, and PCR technique are used for the diagnosis of these agents in diarrhea cases. In addition to sensitivity, specificity, and diagnostic accuracy, other parameters such as ease of application, time to obtain the result, cost, and

 DOI: 10.21608/puj.2023.231013.1220

the necessity for trained technical personnel, are among the factors affecting the method selection of laboratories[8-10].

In this study, it was aimed to analyze and compare between the results of direct microscopic examination, coproantigen detection, and PCR technique for the presence of *G. intestinalis*, *E. histolytica/dispar*, and *Cryptosporidium* spp. in stool samples collected from patients complaining with diarrhea.

SUBJECTS AND METHODS

This retrospective analytical study was carried out at Sivas Cumhuriyet University Medical Faculty Hospital, during the period from January 2017-December 2021.

Study design: Included in the study were the test results of patients who complained of diarrhea during this date range. Stool specimens were simultaneously analyzed by direct microscopic examination, coproantigen detection, and PCR technique, for the presence of protozoan stages in a fresh stool sample in the Medical Microbiology Laboratory. Patients not tested simultaneously for all three diagnostic tests were not included in the study. In addition, repeated tests for the same patients were not included in the study.

Study sample size: A total of 683 stool samples were examined using all three methods. The patients' age ranged from one year to 74 years. Among them 331 (48.5%) were females, and 352 (51.5%) were males. The study sample included 454 (66.5%) children, and 229 (33.5%) adults.

Microscopy: Fresh stool samples were examined under a light microscope in two stages using the Native (saline)-Lugol method. This microscopic examination includes low power examination (X10) using saline to detect helminthic ova, and high power (X40) using Lugol iodine to detect protozoan cysts. The modified Ehrlich-Ziehl-Neelsen (EZN) staining method was used to examine all specimens for the presence of *Cryptosporidium* spp.[11].

Coproantigen detection tests: Fresh stool samples were examined using the qualitative-chromatographic-immunoassay triple antigen test kits *Crypto+Giardia+Entamoeba*[12] (CerTest Biotec SL, Spain). According to the manufacturer's instructions, fecal samples were transferred to the kit's sample collection tubes containing the extraction buffer, and mixed to homogenize. Five drops were then dropped into a sample well of the test cassette. After 10 min, the formation of a colored line in the control (C) region on the test strips indicates that the test was correctly performed, i.e., valid. The formation of a colored line only in the C region is interpreted as negative, and the formation of a colored line in the C region and the strip region signifies a positive result.

Molecular diagnosis: Stool samples were examined using the MAX Enteric Parasite Panel; BD MAX (Becton Dickinson, USA) kits[13]. The genetic materials of *G. intestinalis* (ssu rRNA gene), *E. histolytica* (ssu rRNA gene), and *Cryptosporidium* spp. (*Cryptosporidium*-specific DNA fragment) in fresh or formalin-fixed stool samples were qualitatively determined using the real-time PCR (RT-PCR) technique. Accordingly, ten ul vortexed stool sample were transferred to a sample buffer tube using a disposable sterile loop. The tubes contained 1.5 ml of suitable sample diluent formulated to minimize PCR technique inhibition associated with stool samples. After the sample buffer tubes were inserted in the instrument, further steps were performed automatically by the BD MAX. At the end of the study, based on internal control and target gene region amplification by the BD MAX system software program, the test results of the samples for all three microorganisms were automatically interpreted as positive, negative, or invalid result.

Statistical method: IBM SPSS version 22.0 program was used for data statistical analysis. Numerical variables are shown as frequency (percentage). The chi-square test was used in the evaluation of the data, and $P<0.05$ was considered significant. Concordance between the test results of the methods used was analyzed by calculating Cohen's Kappa value. Kappa value was categorized as <0.20: no fit; 0.21-0.40: weak; 0.41-0.60: moderate; 0.61-0.80: good; and 0.81-1.00: best fit.

Ethical consideration: This study was conducted with the approval of the Sivas Cumhuriyet University Non-Invasive Clinical Research Ethics Committee (Date: 22.02.2023 and Decision No: 2023-02/16).

RESULTS

Microscopic forms compatible with *G. intestinalis* cysts, *E. histolytica/dispar* cysts, and *Cryptosporidium* spp. oocysts were detected in stool samples of 18 (2.7%), 6 (0.9%), and 1 (0.1%), respectively. In addition, helminth eggs were detected in 4 (0.6%) samples (two of each of *E. vermicularis* and *Taenia* spp.) As a result of direct microscopic examination, the overall detection rate of *G. intestinalis*, *E. histolytica/dispar*, and *Cryptosporidium* spp. parasites were amounted to be 3.7% (25/683) (Table 1).

Result of coproantigen detection test performed for the same samples are presented in table (2). Accordingly, antigens of *G. intestinalis*, *E. histolytica/dispar*, and *Cryptosporidium* spp. were detected in 35 (5.1%), 4 (0.6%) and 6 (0.9%), stool samples, respectively. Result of coproantigen detection test

revealed a 6.6% (45/683) overall detection rate of the three parasites (Table 2).

Number of PCR technique positive results for the three parasites are presented in table (3). The genetic materials of *G. intestinalis*, and *Cryptosporidium* spp. were detected in 9 (1.3%) and 5 (0.7%) samples, respectively. *E. histolytica/dispar* genetic material was not detected in any of the samples. The overall detection rate of *G. intestinalis*, *E. histolytica/dispar*, and *Cryptosporidium* spp. parasites was found to be 2% (14/683) by PCR technique (Table 3).

Gender distribution of patients with positive results of direct microscopy, coproantigen detection, and PCR technique tests are presented in table (4). Of the 331 female patients included in the study direct microscopy was positive in 11 (3.3%), coproantigen detection test in 23 (6.9%), and 7 (2.1%) by PCR technique. In the 352 male patients parasite microscopy was positive in 14 (4%), coproantigen detection tests in 22 (6.3%) and PCR technique in 7 (2%). There was no statistical difference between both genders in terms of the positivity of the tests (Table 4).

All three test results were positive for *G. intestinalis* in only one patient's stool sample. No patients with positive results in all three tests for *E. histolytica/dispar* and *Cryptosporidium* spp. were identified. The number of patients with compatible results in stool samples was determined as 45 (6.6%) for *G. intestinalis*, 7 (1%) for *E. histolytica/dispar* and 10 (1.5%) for *Cryptosporidium* spp. The overall detection rate for these three agents was 9.1% (62/683) (Table 5).

Table 1. Stool samples in which the parasite forms (cyst, trophozoite, oocyst, egg) were detected by microscopic examination.

Microorganism	Child (454)	Adult (229)	Total (683)
	No. (%)	No. (%)	No. (%)
G. intestinalis	11 (2.4)	7 (3.1)	18 (2.7)
E. histolytica/dispar	2 (0.4)	4 (1.7)	6 (0.9)
***Cryptosporidium* spp.**	1 (0.2)	0 (0.0)	1 (0.1)
E. vermicularis	2 (0.4)	0 (0.0)	2 (0.3)
***Taenia* spp.**	0 (0.0)	2 (0.9)	2 (0.3)
Total	16 (3.5)	13 (5.7)	29 (4.3)

Table 2. Number of positive stool samples by coproantigen detection test in children and adults.

Microorganism	Child (454)	Adult (229)	Total (683)
	No. (%)	No. (%)	No. (%)
G. intestinalis	20 (4.4)	15 (6.6)	35 (5.1)
E. histolytica/dispar	2 (0.4)	2 (0.9)	4 (0.6)
***Cryptosporidium* spp.**	4 (0.9)	2 (0.9)	6 (0.9)
Total	26 (5.7)	19 (8.4)	45 (6.6)

Table 3. Number of positive stool samples identified by PCR technique.

Microorganism	Child (454)	Adult (229)	Total (683)
	No. (%)	No. (%)	No. (%)
G. intestinalis	7 (1.5)	2 (0.9)	9 (1.3)
E. histolytica/dispar	0 (0.0)	0 (0.0)	0 (0.0)
***Cryptosporidium* spp.**	4 (0.9)	1 (0.4)	5 (0.7)
Total	11 (2.4)	3 (1.3)	14 (2.0)

Table 4. The correlation of positive results of microscopy, coproantigen detection, and PCR technique tests with patient gender.

Tachnique	Female (331)	Male (352)	Total (683)	Statistical analysis
	No. (%)	No. (%)	No. (%)	*P* value
Microscopy	11 (3.3)	14 (4.0)	25 (3.7)	0.649
Coproantigen detection	23 (6.9)	22 (6.3)	45 (6.6)	0.713
PCR technique	7 (2.1)	7 (2.0)	14 (2.0)	0.907

Evaluation of the methods used for detection of parasites in stool samples, showed "moderate fit" (Kappa=0.46, *P*<0.001) between direct microscopy and coproantigen detection test, "weak fit" (Kappa=0.236), *P*<0.001) between direct microscopy and PCR technique results, and no fit (Kappa=0.108, *P*=0.001) was found between the coproantigen detection test and PCR technique test results.

Table 5. Number of positive stool samples identified by PCR technique.

Tachnique	*G. intestinalis* (45)	*E. histolytica/dispar* (7)	*Cryptosporidium* spp. (10)
	No. (%)	No. (%)	No. (%)
Microscopy	18 (40.0)	6 (85.7)	1 (10.0)
Coproantigen detection	35 (77.8)	4 (57.1)	6 (60.0)
PCR technique	9 (20.0)	0 (0.0)	5 (50.0)
Total	45 (6.6)	7 (1.0)	10 (1.5)

DISCUSSION

The first traditional approach to the microbiological diagnosis of intestinal protozoa such as *G. intestinalis*, *E. histolytica/dispar*, and *Cryptosporidium* spp. is the microscopic examination of the stool sample. Specific morphological differences in oocyst, cyst and trophozoite forms are used for the microscopic diagnosis of intestinal protozoa. However, it is not always possible to distinguish these particular morphologies by direct microscopy. Microscopic diagnosis requires technical staining methods and trained technical personnel, which cannot be easily applied in routine microbiology laboratories. The success of the evaluation depends on the microorganism density in the sample and the technical personnel who evaluated it, and request for three consecutive stool samples directly affects the sensitivity of microscopy[14-17]. For these reasons, methods such as coproantigen and molecular diagnosis in stool examination have been developed as an alternative to microscopy especially for the diagnosis of intestinal protozoa[14,15].

Coproantigen detection tests are practical diagnostic methods that give fast and easily interpreted results that do not require special equipment or trained personnel. The high specificity of these tests ensures that a positive test result can be accepted with confidence. The disadvantages of these tests are that they can be used for the diagnosis of a limited number of protozoa, and their effectiveness may be affected by the local detection rate of the microorganism from which they were developed[14].

Molecular PCR techniques are based on the amplification of a specific genome region of the parasite. These tests have the advantage of providing the opportunity for molecular parasite load detection and subtyping as the differentiation between *E. histolytica* and *E. dispar*. However, the PCR kit used in our study could not distinguish between the *Entamoeba* subtypes. In addition, molecular methods can contribute to the diagnosis of other intestinal protozoa such as *D. fragilis, B. coli*, or *Blastocystis* spp. Two significant advantages of PCR technique are that a single stool sample is sufficient for diagnosis, and it is a less labor-intensive technique compared to staining methods. Lack of standardization, presence of organic/inorganic inhibitors, and high cost are reported as the main disadvantages of molecular tests[14].

In recent studies using different diagnostic methods in our country, the detection rate of intestinal protozoa was reported as *G. intestinalis* 1.8-6.9%, *E. histolytica/dispar* 0.2-2.6%, and *Cryptosporidium* spp. 0.02-0.3%[18-21]. In our study, the detection rate was determined as *G. intestinalis*, 6.6%; *E. histolytica/dispar*, 1%; and *Cryptosporidium* spp., 1.5%. The results of our study seem to be compatible with the studies conducted in our country, except for the detection rate of *Cryptosporidium* spp.

Goudal *et al*.[22], in their study in France, compared a different coproantigen detection test kit with microscopy and reported that the two methods performed closely. They also stated that coproantigen detection test can be used to detect the presence of *G. intestinalis* and *Cryptosporidium* spp. in stool samples and can provide a time-saving alternative to microscopy methods. In our study, in 45 stool samples *G. intestinalis* was detected in 20% (18/45) by microscopic examination and in 77.8% (35/45) by coproantigen detection test. In addition, while 6 (60%) of 10 stool samples proved positive for *Cryptosporidium* spp. by coproantigen detection test only 1 (10%) sample was recorded by microscopic examination.

The data obtained in our study indicated that coproantigen detection test results were more successful than microscopy tests. In a study by Dandapani *et al*.[23] in India, they examined stool samples of patients with diarrhea for the presence of *G. intestinalis* and *E. histolytica/dispar*. They reported that the coproantigen detection test proved to be statistically more successful in detecting these two parasites. They also showed that positive results can

be obtained with coproantigen detection tests for non-pathogenic protozoon such as *E. dispar*. For this reason, confirmation with clinical correlation or PCR technique in cases where coproantigen detection test results are positive for E. histolytica will be advantageous.

In a study conducted in Turkey, the same BD MAX Enteric Parasite Panel kit, which was also employed in our study, was used for comparison with microscopic examination. The researchers included a total of 362 stool samples in their study, and obtained positive results in 40 (11%) samples by microscopic evaluation and in 23 (6.3%) samples by molecular testing. They reported that similar performance was obtained for *G. intestinalis* and *Cryptosporidium* spp. using the two methods. On the other hand, success was different for *E. histolytica*. The researchers stated that microscopic evaluation could not differentiate between *E. histolytica* and other non-pathogenic *Entamoeba* spp. and that the molecular approach is more advantageous than microscopy in the diagnosis of *E. histolytica*[24]. In our study, the number of samples positive for *E. histolytica/dispar* was seven (1%). Six (85.7%) of these samples were positive by microscopy, and four (57.1%) of them were positive by coproantigen detection test.

However, all samples positive with coproantigen detection tests, were negative by PCR technique. This suggests that cost-effective and easy-to-perform coproantigen detection tests may be a more advantageous practice for the detection of *E. histolytica/dispar* in stool samples. In a recent study, the presence of *G. intestinalis* and *E. histolytica/dispar* in stool samples was investigated by microscopy and PCR technique methods. In that study, the PCR technique proved to be more successful reporting moderate fit" for both test results (Kappa=0.51 for *G. intestinalis*, and Kappa=0.47 for *E. histolytica*)[25].

In our study, "weak fit" (Kappa=0.236, $P<0.001$) was found between the microscopy and PCR technique test results. In the routine laboratory, problems such as carelessness in the staining method and microscopic evaluation requires professional experience since *E. histolytica/dispar* distinction cannot be made by microscopy. Notably, lack of standardization in PCR technique tests, and the presence of organic/inorganic inhibitors; may have led to the detection of "weak fit" between direct microscopy and PCR technique test results in our study.

Another study by Shimelis *et al*.[26] investigated the presence of *Cryptosporidium* spp. in stool samples by microscopic examination and coproantigen detection testing. The researchers examined microscopic preparations stained with the modified EZN staining method for ≤10 min and >10 min for samples with negative results in the first stage. The researchers stated that there was a diagnostic fit between results of microscopy and the coproantigen detection test (Kappa value: 0.75 for microscopic examination for ≤10 min; 0.60 for microscopic examination of >10 min).

In our study, "moderate fit" (Kappa=0.46, $P<0.001$) was found between the results of direct microscopy and coproantigen detection tests. It is thought that the incompatible results in microscopy may be due to the lack of professional experience. In addition, a no fit (Kappa=0.108, $P=0.001$) was found between the coproantigen detection test and PCR technique test results in our study. PCR technique tests are methods that should be studied carefully by experienced laboratory personnel, and it is not always possible to provide this situation in routine laboratories.

In conclusion, the routine experience of a microbiology laboratory is reviewed. Accordingly, we suggested using direct microscopy and RDTs in diagnosis of suspected IPIs in diarrheic patients Besides, molecular diagnosis should be recommended in differentiation between pathogenic and non-pathogenic amoeba.

Author contribution: Taşkın Kafa AH, and Çubuk F designed the research topic. Çubuk F, Hasbek M, and Taşkın Kafa AH were responsible for acquisition, analysis, and interpretation of the research data. Taşkın Kafa AH, Çubuk F, and Aslan R wrote the draft of the manuscript, while Taşkın Kafa AH revised it for final publication. All authors approved the final revision.

Conflict of interest: None to declare.

Funding statement: None.

REFERENCES

1. Ahmed SA, Guerrero Flórez M, Karanis P. The impact of water crises and climate changes on the transmission of protozoan parasites in Africa. Pathog Glob Health 2018; 112(6):281-293.
2. Horton B, Bridle H, Alexander CL, Katzer F. *Giardia duodenalis* in the UK: Current knowledge of risk factors and public health implications. Parasitology 2019; 146(4):413-424.
3. Tharmaratnam T, Kumanan T, Iskandar MA, D'Urzo K, Gopee-Ramanan P, Loganathan M, *et al*. *Entamoeba histolytica* and amoebic liver abscess in northern Sri Lanka: a public health problem. Trop Med Health 2020; 48:2.
4. Mahmoudi MR, Ongerth JE, Karanis P. *Cryptosporidium* and cryptosporidiosis: The Asian perspective. Int J Hyg Environ Health 2017; 220(7):1098-1109.
5. World Health Organisation (WHO). Prevention and control of intestinal parasitic infections: WHO

Technical Report Series N°749, WHO, https://www.who.int/publications/i/item/WHO-TRS-749
6. Turkeltaub JA, McCarty TR 3rd, Hotez PJ. The intestinal protozoa: Emerging impact on global health and development. Curr Opin Gastroenterol 2015; 31(1):38-44.
7. Allain T, Buret AG. Pathogenesis and post-infectious complications in giardiasis. Adv Parasitol 2020; 107:173-199.
8. Khurana S, Gur R, Gupta N. Chronic diarrhea, and parasitic infections: Diagnostic challenges. Indian J Med Microbiol 2021; 39(4):413-416.
9. Fitri LE, Candradikusuma D, Setia YD, Wibawa PA, Iskandar A, Winaris N, *et al*. Diagnostic methods of common intestinal protozoa: Current and future immunological and molecular methods. Trop Med Infect Dis 2022; 7(10):253.
10. van Lieshout L, Roestenberg M. Clinical consequences of new diagnostic tools for intestinal parasites. Clin Microbiol Infect 2015; 21(6):520-528.
11. Fayer R, Morgan U, Upton SJ. Epidemiology of *Cryptosporidium*: transmission, detection, and identification. Int J Parasitol 2000; 30(12-13):1305-1322.
12. Basmaciyan L, François A, Vincent A, Valot S, Bonnin A, Costa D, *et al*. Commercial simplex and multiplex PCR assays for the detection of intestinal parasites *Giardia intestinalis*, *Entamoeba* spp., and *Cryptosporidium* spp.: Comparative evaluation of seven commercial PCR kits with routine in-house simplex PCR Assays. Microorganisms 2021; 9(11):2325.
13. Madison-Antenucci S, Relich RF, Doyle L, Espina N, Fuller D, Karchmer T, *et al*. Multicenter evaluation of BD Max enteric parasite real-time PCR assay for detection of *Giardia duodenalis*, *Cryptosporidium hominis*, *Cryptosporidium parvum*, and *Entamoeba histolytica*. J Clin Microbiol 2016; 54(11):2681-2688.
14. Rijsman LH, Monkelbaan JF, Kusters JG. Clinical consequences of polymerase chain reaction-based diagnosis of intestinal parasitic infections. J Gastroenterol Hepatol 2016; 31(11):1808-1815.
15. Hooshyar H, Rostamkhani P, Arbabi M, Delavari M. *Giardia lamblia* infection: Review of current diagnostic strategies. Gastroenterol Hepatol Bed Bench 2019; 12(1):3-12.
16. Saidin S, Othman N, Noordin R. Update on laboratory diagnosis of amoebiasis. Eur J Clin Microbiol Infect Dis 2019; 38(1):15-38.
17. O'Leary JK, Sleator RD, Lucey B. *Cryptosporidium* spp. diagnosis and research in the 21st century. Food Waterborne Parasitol 2021; 24:e00131.
18. Akpolat N, Çakır F, Çiçek M, Bilden A. retrospective analysis of the distribution of intestinal parasites in patients admitted to Dicle University Faculty of Medicine between the years 2011-2020. Turkiye Parazitol Derg 2022; 46(2):119-123.
19. İnal N, Ünalan Altıntop T, Ergüven S, Akyön Yılmaz Y. Retrospective results of Hacettepe University, Faculty of Medicine Parasitology Laboratory between 2014-2019. Turkiye Parazitol Derg 2022; 46(2):114-118.
20. Usluca S, Babür C, Kılıç S. Current status in intestinal parasitic infections: A reference laboratory results. Klimik Derg 2020; 33(3): 307-13.
21. Ataş AD. The distribution of pathogenic intestinal parasites in Sivas Cumhuriyet University, Faculty of Medicine, Research and application hospital between 2006-2018. Turkiye Parazitol Derg 2020; 44(1):25-30.
22. Goudal A, Laude A, Valot S, Desoubeaux G, Argy N, Nourrisson C, *et al*. Rapid diagnostic tests relying on antigen detection from stool as an efficient point of care testing strategy for giardiasis and cryptosporidiosis? Evaluation of a new immunochromatographic duplex assay. Diagn Microbiol Infect Dis 2019; 93(1):33-36.
23. Dandapani S, Rajkumari N, Gopichand P. Comparison of detection of *Giardia lamblia* and *Entamoeba histolytica* using microscopy and antigen detection rapid cards in patients with diarrhea. J Gastrointest Infect 2020; 10(1):4.
24. Akgun S, Celik T. Evaluation of *Giardia intestinalis*, *Entamoeba histolytica* and *Cryptosporidium hominis/ Cryptosporidium parvum* in human stool samples by the BD MAXTM Enteric Parasite Panel. Folia Parasitol (Praha) 2020; 67:2020.
25. Emisiko J, Shaviya N, Shiluli C, Kiboi N, Wamalwa R, Jumba B, *et al*. Comparison of microscopy and PCR for detection of *Giardia lamblia* and *Entamoeba histolytica* in human stool specimens in a resource limited setting in western Kenya. Ethiop J Health Sci 2020; 30(6):891-896.
26. Shimelis T, Tadesse E. Performance evaluation of point-of-care test for detection of *Cryptosporidium* stool antigen in children and HIV infected adults. Parasit Vectors 2014; 7:227.