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Original Research Article

Detection and clinical characteristics of *Entamoeba histolytica* infection among children in Kirkuk-Iraq

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Abstract: Amoebiasis is one of important health problems in developing countries. It is commonly reported among human beings with different frequency of distribution in different parts of the world. The aim of this study was to detect clinical signs and symptoms and to compare sensitivity and specificity of conventional stool examination and qualitative enzyme immunoassay (EIA) panel kit to detect E. histolytica/ E. dispar among children in Kirkuk hospitals. The method in a study was carried on 800 stool samples from children attended Kirkuk hospitals for period from February 2007 to end of January 2009, as well as another 100 children samples of the near ages of the patients as a control group. The ages of children were ranging from below one month to 12 years old. The stool samples were examined by direct stool examination and qualitative enzyme immunoassay (EIA) panel kit. In results the morphology of E. histolytica cysts for which modified D'antoni's iodine and 1% lugol's iodine (weak iodine solution) was clearer than that strong iodine solution. It was found that the sensitivity, specificity, and efficiency of Enzyme immunoassay (EIA) test for E. histolytica/E. dispar were 91.07%, 98% and 93.2% respectively. The clinical signs and symptoms were increased when the bacterial infections associated with E. histolytica/E. dispar infections.
Keywords: Detection E. histolytica and E. dispar, clinical characteristics.

INTRODUCTION

It is now known that amoebiasis is caused by two very similar species E. histolytica and E. dispar. E. histolytica and E. dispar are genetically distinct but closely related protozoan species [1]. The former is the cause of all invasive diseases, with an estimated 100,000 fatalities each year [2]. Persistent diarrhea should not be confused with chronic diarrhea which is recurrent or long lasting-diarrhea due to noninfectious causes, such as sensitivity to gluten or inherited metabolic disorders [3].

Microscopic diagnosis of E. histolytica/E. dispar complex on stool samples requires technical expertise because of the existence of similar amoebas or artifacts that can be misdiagnosed as E. histolytica/E. dispar.

Entamoeba histolytica is a pathogen or invasive parasite, whereas E. dispar and E. moshkovskii are non-pathogenic and non-invasive parasites that are identical morphologically to E. histolytica [4]. There are at least eight amoebas (E. histolytica, E. dispar, E. moshkovskii, E. coli, E. hartmanni, E. polecki, Iodamoeba butschlii and Endolimax nana) which live in the human intestinal lumen, however, these are generally accepted as commensal organisms except E. histolytica [5].

Diagnosis via microscopic examination of single stool specimen has a low sensitivity and may be missed. Therefore, up to 50% of Entamoeba infections are because of the intermitted shedding of the parasites which take the microscopic examination of 3 consecutive stool-specimens to reach sensitivity over 90% [6]. It has been accepted that the positive predictive value (PPV) of microscopic diagnosis of E. histolytica is low and that alternative causes for the complaints with which the patient presented should always be taken seriously [7].

In order to find simple, inexpensive and reliable diagnostic techniques for detecting intestinal infections with E. histolytica, Triage Parasite panel Enzyme Immunoassay has been developed and tested in various studied [8,9]. For evaluating the performance of commercially available Triage parasite panel Enzyme Immunoassay kit for detecting E. histolytica/E. dispar. G. lamblia, C. parvum. It is rapid, easy to use and can

be used as a screen for immediate testing of stool specimens [10]. In Kenya [11] evaluated the Triage Micro Parasite Panel in detecting E. histolytica/E. dispar, G. lamblia and C. parvum compared to O&P examination in 266 stool samples, they found that the sensitivity and specificity results for Triage Micro Parasite Panel were for E. histolytica/E.disparand Giardia lamblia 100%, 100% for each and for C. parvum 73%, 100%. There was no evidence of cross reactivity using the kit with other parasites identified in the stool samples.

The aim of this study was to detect E. histolytica and E. dispar in children and clinical symptoms associated with this infection in Kirkuk City.

MATERIALS AND METHODS

The study was carried out on patients attended Kirkuk Pediatric hospital and pediatric wards in Al-Hawija and Kirkuk General Hospitals. The period of study was from beginning of February 2007 to the end of January 2009. A total of 800 children with invasive diarrhea who requested medical advice were included in this study, their age were ranging from below 2 years up to 12 years

In addition, one hundred children matched by age, sex, socio-economic strata were chosen as a control group amongst children brought to Al – Salam primary Health care center (PHCC) near kirkuk pediatric hospital for routine children medical care .

A full history was taken from the parents of each child regarding the clinical features like fever abdominal pain, tenesmus, vomiting and rectal prolaps. These patients that admitted to hospital not received antibiotics and patients who received antibiotics excluded from the study.

Fresh stool specimen were collected from the subjects into sterile containers and transported in to a cooled box (temperature approximately 10 $^{\circ}$C). Stool samples were divided into three portions within two hours of collection on arrival at Kirkuk pediatric hospital laboratory. One portion was for the direct examination of parasites; the second portion was cultured for bacteriological examination while the third portion was stored immediately at -20°C and tested later by a new qualitative enzyme immunoassay (EIA) panel kit.

Stools were examined macroscopically for parasites and microscopically for ova and cysts using wet mount technique, Stools were examined macroscopically for parasites and microscopically for ova and cysts of parasites by direct microscopy. Stool specimens were examined unstained or stained with Lugol's or D'Antonis Iodine iodine for Chlamydia. Iodine for Chlamydia. The pHs of stool specimens was determined with pH paper.

Stool specimens were cultured within the same day of collection on MacConkey agar (MA), Salmonella-Shigella agar (SSA), Sorbitol MacConkey agar (SMA), Thiosulphate citrate bile sucrose agar (TCBS). The specimens were also enriched in both tetrathionate and alkaline peptone water. The first is subcultered onto SSA and the second onto TCBS.

MA and SSA were used for isolation of Escherichia coli (EPEC, EIEC), Salmonella spp. and Shigella spp. TCBS is used for isolation of Vibrio species, SMA was used for isolation of Escherichia coli O157:H7 and alkali treatment method for Y. enterocolitica. Plates were incubated at 37^{0}C for 24-48 hours except those used to identify Y. enterocolitica by incubation at 28^{0}C [12].

Enzyme immunoassay (EIA) Test

Assay procedure Triage parasite panel is a qualitative enzyme immunoassay (EIA) which is a single immunochromatographic strip coated with monoclonal antibodies specific for E. histolytica/E. dispar antigen (29 KDA) and for antigens of G. lamblia and C. parvum (Biosite Diagnostics, San Diego, Calif).

A qualitative enzyme immunoassay (EIA) is used according to the following manufacturer's directions. The assay procedure involves the addition of 4.5 ml of specimen diluents to the specimen tube sample (0.5 ml) and the mixture is vortexed for at least 10 seconds. The mixed sample is centrifuged at 1.500 Xg for at least 5 minutes. The supernatant is poured into the sample filter device and is filtered into filtrate tube. The filtered sample (0.5 ml) is then added to the center of test device with a transfer pipette. Enzyme conjugate (140 µl) is added to the center of the membrane. Six drops of washing solution is added to the membrane. This step is repeated twice then four drops of the substrate is added to the membrane followed by 5 min incubation at 25 $^{\circ}$C. The device is then read and the results are interpreted. Positive results are visualized as purple black lines in the appropriate positions in the result window. The EIA test was carried on 343 samples (100 E. histolytica positive test, 100 E. histolytica, negative by Conventional microscopic examination, 124 E. histolytica/E. dispar associated with bacteria and 19 dual and triple infections).

The following term and equation were used for detecting the efficiency of laboratory methods in detecting Entamoeba histolytica/E. dispar; TP=True positive, TN=True negative, FP=False positive, FN=False negative. Sensitivity=TP/(TP+FN), Specificity=TN/(TN+FP).

To calculate sensitivity, specificity and efficiency of Triage parasite enzyme immunoassay kit, the following formulas [13].

	CME	
EIA▼	Positive	Negative
Positive	a (True positives)	b (False positives)
Negative	c (False negatives)	d (True negatives)

▼ Triage parasite enzyme immunoassay.

$$\text{Sensitivity} = \frac{a}{a+c} \times 100$$

$$\text{Specificity} = \frac{d}{b+d} \times 100 \qquad \text{Efficiency} = \frac{a+d}{a+b+c+d} \times 100$$

The specimens which are positive with both Triage parasite panel enzyme immunoassay and CME are considered true positive (a). A number of specimens which were negative for E. Histolytica by both methods were considered to be true negative (d). Specimens that were Positive by CME and negative by triage parasite enzyme immunoassay were considered false Negative (c). Specimen that is negative by CME and positive by Triage Parasite Panel Enzyme Immunoassay are considered false positive (b).

For the purpose, 324 selected specimens are tested for EIA test following CME. They were 124 specimens E. histolytica associated with bacteria, 100 E. histolytica alone and 100 E. histolytica negative. The triple and dual infections are not included due to possibility of cross reaction of G. lamblia and C. parvavum with E. histolytica/E. dispar. Statistical analysis is done using the chi-square (χ^2) of independency and homogeneity test with Yates correction. Probabilities of ($P< 0.05$) were considered statistically significant [14].

RESULTS

The prevalence of parasitic infection among patients was as the following: E. histolytica/E. dispar 42.5%, and E. vermicular is 0.62% while in control groups the rate of E. histolytica was 2%. From examination of 20 stool samples using 7 types of iodine solution, it was found that the best morphology of E. histolytica cyst was observed, using both modified D'antoni's iodine and routinely used 1% Lugols' iodine. While the morphology of parasite was not clearly visible using other types of iodine as Lugols iodine 5%, gram iodine, Alberts iodine, iodine for staining chlamydia. (Figures 1,2 &3), indicates that the structure of the parasite was best seen when using modified D'antoni's iodine and Lugols' iodine than using iodine for staining Chlamydia and their morphology of parasites was not significantly varied when using other types of iodine stains.

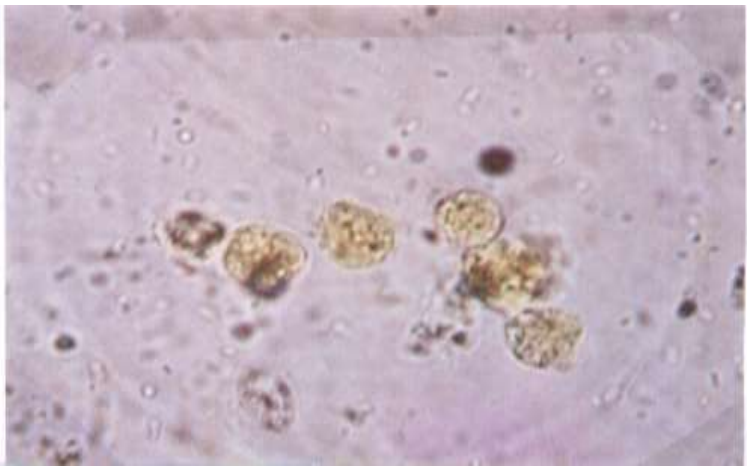

Fig-1: Photograph of E. histolytica Cyst Stained with Modified Da'ntonis' Iodine (X1000).

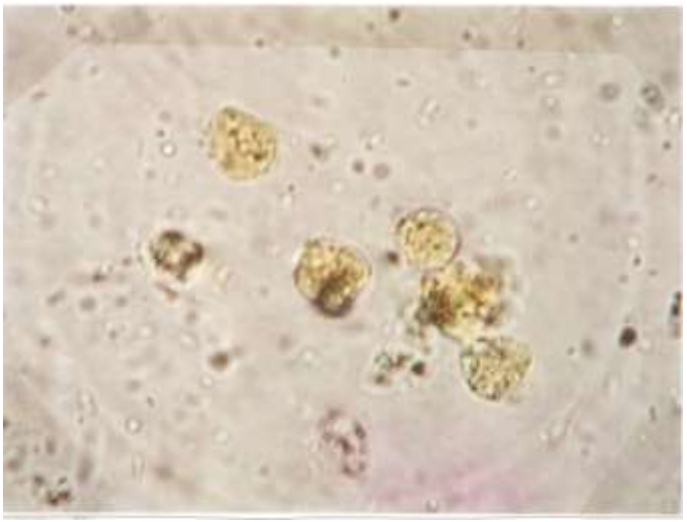

Fig-2: Photograph of E. histolytica Cyst Stained with Lugols' Photograph of E. histolytica Cyst Stained with Iodine for Chlamydia (X1000).

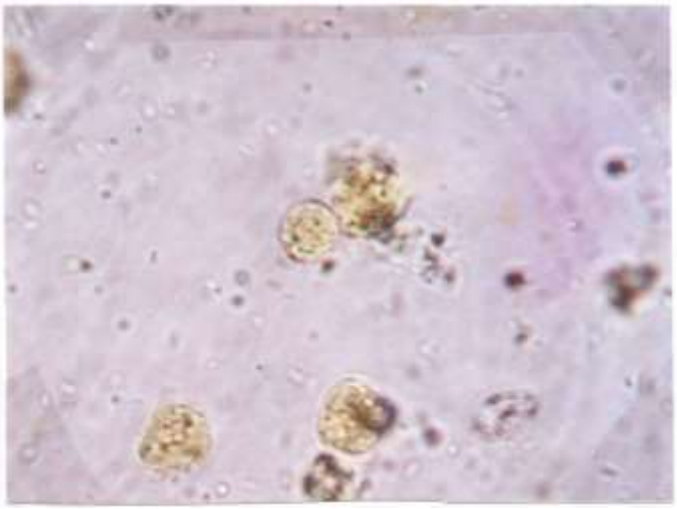

Fig-3: Photograph of E. histolytica Cyst Stained with with Iodine for Chlamydia (X1000).

Sensitivity and Specificity of EIA Test

The results of 124 samples which were positive for E. histolytica by CME and bacteria with culture methods, were compared with EIA test for E. histolytica/E. dispar indicates that out of 124 samples only 116 were positive for E. histolytica/E. dispar with EIA test and 8 were negative as shown in Table 1.

Table 1: Comparison between the Accuracy of CME▼ and EIA▪ Test for Enteropathogens

Type of pathogen	Bacteria associated with E. histolytica (CME)	Traige parasite panel (EIA) test for E. histolytica/E. dispar	
		Positive	Negative
P. aeruginosa	42	40	2
EIEC	25	24	1
EPECI	25	24	1
S. flexneri	12	12	0
S. typhi	12	10	2
Non O157:H7 E. coli	3	3	-
P. shigellodies	5	3	2
Total	124	116	8

▼CME: Conventional microscopic examination. ▪ EIA: Enzyme immunoassay.

To calculate sensitivity, specificity and efficiency of Triage parasite enzyme immunoassay kit, 324 specimens were tested for EIA test following CME. They were 124 specimens E. histolytica associated with bacteria, 100 E. histolytica alone and 100 E. histolytica negative. It was found the sensitivity of EIA was 91.07%, specificity 98% and efficiency 93.2% as follows:.

		CME	
+ *Triage*	+	-	*Total*
E. histolytica/	204 (a)	2 (b)	206
- *E. dispar*	20 (c)	98 (d)	118
(EIA)	224	100	324

The sensitivity of EIA test was: $\frac{204}{204+20}\times 100 = 91.07\%$

The specificity of the test was: $\frac{98}{2+98}\times 100 = 98\%$

The efficiency of the test was: $\frac{204+98}{204+2+20+98}\times 100 = 93.2\%$

For testing the sensitivity, specificity and efficiency of EIA for E. histolytica positive and E. histolytica negative specimens were tested. It was found that the sensitivity of the test was 88%, specificity 98% and efficiency was 93% as illustrated in the following formula.

		CME	
	+	-	*Total*
+	**88 (a)**	**2 (b)**	**90**
-	**12 (c)**	**98 (d)**	**110**
	100	**100**	**200**

Sensitivity = $\frac{88}{88+12} = 88\%$ Specificity = $\frac{98}{2+98} = 98\%$

Efficiency = $\frac{88+98}{88+2+12+98}\times 100 = 93\%$

Multiple infection are mostly encountered in those cases with E. histolytica/E. dispar that are 125 patients: 40 with P. aeruginosa, 24 with EPEC I, 24 with EIEC, 12 with S. flexner, 10 with S. typhi, 3 with Non O157:H7 E. coli and 3 with P. shigellodies (Table 4-11a), 6 with G. lamblia and (3) with C. parvum. The rate of bacterial infections associated with E. histolytica/ E. diapar was higher than parasitic infections (Table 2).

Table 2: Distribution of E. histolytica/ E. dispar with enteropathogena using EIA test among 125 patients

Enteropathogens	**Number**	**Percentage**
Pseudomonas Aeruginosa	40	32.0%
EPECI	24	19.2%
EIEC	24	19.2%
S. flexeneri	12	9.6%
S. typhi	10	8.0%
Non 0157:H7 E.coli	3	2.4%
P. shigellodies	3	2.4%
Total	116	92.8%
G. lamblia	6	4.8%
C. parvum	3	2.4%

Clinical Signs and Symptoms

The clinical signs and symptoms associated with E. histolytica are shown in Table 3. It was found that tenesmus (82.35% was predominantly seen in amoebiasis followed by mucus in stool (40.88%); fever (38.82%); vomiting (32.94%) and rectal prolaps (31.76%).

In general the clinical signs and symptoms were increased when the bacterial infections associated with E. histolytica/E. dispar infections. The detail of clinical signs and symptoms for each organisms associated with E. histolytica mixed infection are illustrated in Table 3. Statistically there was significant difference in clinical signs and symptoms between two main groups of infections E. histolytica, and E. histolytica/E. dispar associated with bacteria (P< 0.05).

Table 3: Clinical characteristics for E. histolytica and associated bacteria

Pathogens	**No**	**Mucus in stools No. (%)**	**Fever No. (%)**	**Vomiting No. (%)**	**Tenesmus No. (%)**	**Rectal Prolaps No. (%)**
E. histolytica / dispar	340	139 (40.88)	132 (38.82)	112 (32.94)	280 (82.35)	108 (31.76)
E. histolytica/dispar associated with bacteria						
P. aeruginosa	40	12 (30)	30 (75)	21 (52.5)	19 (47.5)	6 (15)
EPEC 1	24	12 (50)	21 (87.5)	9 37.5)	23 (95.8)	16 (66.6)
EIEC	24	23 (95.8)	24 (100)	15 (62.5)	24 (100)	12 (50)
Shigella flexneri	12	12 (100)	12 (100)	6 (50)	10 (83.3)	10 (83.3)
Salmonella typhi	10	8 (80)	10 (100)	4 (40)	10 (100)	7 (70)
Non O157:H7 E. coli	3	1 (33.3)	2 (66.6)	2 (66.6) 3 (100)		2 (66.6)
Plesiomonas shigelloides	3	3 (100)	3 (100)	1 (33.3)	3 (100)	1 (33.3)
Total	116	71 (61.20)	102 (87.93)	58 (50)	92 (79.31)	54 (46.55)
P<0.05						

DISCUSSION

In this study, different types of iodine solution are used in wet mount of stool samples examination. The structure of the parasite was most clearly seen using modified D'Antoni's iodine as shown in Figure 1 and 1% lugol's iodine as shown in Figure 2 which is routinely used in our diagnostic laboratory. The morphology of cysts in the use of this weak iodine solution is clearer than that of the use of Chlamydia and stock solution of lugol's iodine as shown in Figure 3. This reflects that the strong iodine is not as efficient as weak iodine solution. It has been reported that the strong iodine tend to coagulate the faecal particle and destroy the refractile nature of the organism [15]. It is also recommended by WHO [16] that the stock iodine solution 5% to be diluted to 1% and freshly prepared every two week for wet mounts technique. The other iodine solution were inferior than 1% iodine solution and modified D'Anton's iodine, therefore, these two iodine solutions were routinely used in general stool examination in this study. Shetting and Prabhu [17] found that D'Anton's iodine was much better than saline or buffered methylene blue for detection of E. histolytica cysts while saline and buffered methylene blue were equally good for detection of E. histolytica trophozoites.

In order to find simple, inexpensive and reliable diagnostic techniques for detecting intestinal infections with E. histolytica, Triage Parasite panel Enzyme Immunoassay has been developed recently and tested in various studied [8,9]. For evaluating the performance of commercially available Triage parasite panel Enzyme Immunoassay kit for detecting E. histolytica/E. dispar. G. lamblia, C. parvum, 324 specimens examined by conventional microscopy were compared with the results of Triage E. histolytica/E. dispar kit. Comparing the sensitivity and specificity of EIA test when applied on the whole 324 samples which include E. histolytica associated with bacteria, E. histolytica alone and negative samples, the sensitivity of EIA on the whole samples was 91.07% which is slightly higher than that of which tested on E. histolytica specimens alone that is 88% while the specificity of the test is 98% in both whole samples and E. histolytica alone and efficacy being 93%. The sensitivity and specificity of EIA test applied in this study is almost identical to that referred by the Biosite diagnostics San Diago Calf Company which referred to sensitivity 87% and specificity 99%. The finding of this study was in agreement with that reported by other studies who reported the sensitivity of the test ranging from 68.3% - 95% and specificity ranging from 97% - 99% [18, 19].

The high sensitivity and specificity of the EIA test and its simplicity to be used in our diagnostic laboratory in the future, because it is costly in the present time. As it is mentioned earlier, microscopic examinations of one single stool specimen has low sensitivity [20, 21]. This reflects that stool antigen assays are more sensitive and specific than microscopy for diagnosis of E. histolytica [22]. Diagnostic problems arise when only cysts are identified in stools of healthy or diarrhoeic individuals. A commercially available laboratory test based on the identification of specific E. histolytica antigens in stool is able to discriminate E. dispar cysts [18]. However, the high cost and lack of knowledge of this test have hindered its use in clinical laboratories, especially in countries where amoebiasis is endemic. Until these new diagnostic tests are widely available to clinical laboratories, these samples should be reported as containing E. histolytica/E. dispar [23].

Stool antigen assay has been shown to be as sensitive and specific as culture with isoenzyme analysis and to outperform microscopy for detection of E. histolytica in areas of endemicity [18].

Furthermore, detection of positive zones of E. histolytica/E. dispar, G. lambia and C. Yparvum by EIA test could be considered as an alternative method for performing simultaneous discrete detection of Giardia, Cryptosporidium and E. histolytica/E. dispar specific antigen in patient faecal specimens. G. lamblia or E. histolytica can be detected by EIA test even in the absence of intact organisms (cysts or trophozoites). This reflects to greater sensitivity of EIA tests compared with microscopy.

Another parasitic agent detected was G. lamblia with E. histolytica/ E. dispar in 6 patients (0.75%) and C. parvum with E. histolytica/E. dispar in 3 patients (0.37%). Cryptosporidium, and Giardia lamblia enteric pathogen, waterborne, which has been looked for in Iraq [6, 24, 25] of which reports are rare in the area which may be because of a specific diagnostic method is not being used routinely during stool examination in our country.

Clinical Signs and Symptoms

In this study several parameters are added to aid through them in trying to help the clinician to make a rapid judgment as to the probably causative agent in the presenting case of gastroenteritis. These parameters are: presence of blood, mucus in stool, consistency of the stool, presence or absence of fever, tenesmus and prolaps of the rectum.

The clinical signs and symptoms of amoebiasis are varied from one child to another. In general the main clinical signs and symptoms in case of amoebiasis were as the following: mucus in stool 40.88%, fever 38.8%, vomiting 32.9%, and tenesmus 82.35%. These findings are in accordance with that reported by Peter et al. [26]. Thus the researchers see that the wide spectrum in the rate of clinical signs and symptoms among the studied groups of children might be due to E. dispar infection misdiagnosed by E. histolytica [27]. Zaki *et al.;* [28] have been reported that the presence of both types of parasite (E. histolytica/E. dispar) and / or different strains of either parasite in the same patient could be one of the reasons for the differences in signs and symptoms in infected persons.

Detection of rectal prolaps in cases of children infected with amoebiasis may be due to complications such as intestinal stricture formation amoeboma, which lead to abdominal pain or difficulty with defecation. In addition to that the disease can progress to severe involvement of the colon with dilatation and paralysis resembling a toxic megacolon or ulcerative colitis [27]. The increase in clinical signs and symptoms in case of E. histolytica/E. dispar associated with bacterial infection reflects the synergistic effect of bacterial and Entamoeba infection. This finding is also observed by Wittner and Rosenbam [29] and Mirelmam [30] who found that the virulence of E. histolytica increased when the culture seeded with E. coli and S. typhi infections. Finding of mucus in all cases of diarrhoea in children suffering from shigellosis reflects the invasiveness of Shigella spp

It is concluded that the prevalence of E. histolytica/E. dispar was high in Kirkuk province. The morphology of E. histolytica/E. dispar cysts using modified D'antoni's iodine and 1% lugol's iodine is more evident than strong Iodine solution.

The sensitivity, specificity and efficiency of EIA test was high for detection of E. histolytica / E. dispar. E. histolytica/E. dispars were mostly associated with P. aeruginosa followed by EPEC, EIEC, S. flexneri, S. typhi, Non O157:H7 E. coli and P. shigellodies respectively.

REFERENCES

1. Nesbitt RA, Masha FW, Katki HA, Ashraf M, Assenga C, Lee CM; Amebiasis and comparison of microscopy to ELISA Technique in detection Entamoeba histolytica and Entamoeba dispar. J. Nat. Med. Assoc., 2004; 96 (5): 471-478.
2. Ali IKM, Clark CG, Petri JWA; Molecular epidemiology of Amebiasis. Infect Genet Evol. 2008; 8(5): 698-707.
3. Kliegman RM, Jenson HB, Marcdante KJ, Behrmen RE; Nelson Essentials of Pediatrics, 5th ed. Elsevier Saunders, Philadelphia, 2006; 587.

4. Diamond LS, Clark CG, Cunnick CC. YI-S; A casein-free medium for axenic cultivation of E. histolytica related Entamoeba, G. intestinal is and Trichomonas vaginalis. J Eukaryot Microbiol 1995; 42 (3): 277-278.
5. Tanyuksel M, Petri WWACaballero-Salcedo A, Viveros-Rogel M, Salvatierra B, Tapia-Conyer R, Sepulveda-Amor J, Gutterrez G, Ortiz-Ortiz L *et al.;* Sero epidemiology of amoebiasis in Mexico. Am J Trop Med Hyg 1994; 50: 412-419.
6. Kadir MA, Daoud ISh, Al-Bayati ZMS; Differentiation between Entamoeba histolytica and E. dispar using enzyme linked immunosorbent assay and wet mount method. Tikrit J. Pharmac Sci., 2013; 9(1): 122-129.
7. Kebede A, Verweij J, Petros B, Polderman AM; Short communication: Misleading microscopy in amoebiasis. Trop Med & int Hlth 2004; 9 (5): 651-652.
8. Gonzalez-Ruiz A, Haque R, Rehman T, Aguirre A, Hall A, Guhl F, Warhurst DC, Miles MA *et al.;* Diagnosis of amoebic dysentery by detection of Entamoeba histolytica fecal antigen by an invasive strain-specific, monoclonal antibody-based enzyme-linked immunosorbent assay. J Clin Microbiol 1994; 32: 964-970.
9. Jelinek T, Peyer G, Loscher T, Nothdurft HD; Evaluation of an antigen-capture enzyme immunoassay for detection of Entamoeba histolytica in stool samples. Eur J Clin Microbiol Infect Dis 1996; 15: 752-755.
10. Sharp SE, Suarez CA, Duran Y and Poppiti RJ; Evaluation of the Triage Micro Parasite Panel for detection of Giardia lamblia, Entamoeba histolytica/Entamoeba dispar, and Cryptosporidium parvum in patients stool specimens. J. Clin. Microb, 2001; 39: 332-334.
11. Swierczewski B, Odundo E, Ndonye J, Kirera R, Odhiambo C, Oaks E; Comparison of the Triage Micro Parasite Panel and Microscopy for the detection of Entamoeba histolytica/Entamoeba dispar, Giardia lamblia and Cryptosporidium parvum in stool samples collected in Kenya. J. Trop. Med., 2012; Article ID 564721, 1http://dx.doi.org/10.1155/2012/564721.
12. Turgeon DK, Fritsche TR; Laboratory approaches to infectious diarrhoea. Gastroenterol Clinics 2001; 30 (3): 47-63.
13. Forbes BA, Saham DF, Weissfeld AS; Bailey and Scott's diagnostic microbiology. 11thed. The C.V Mosby Company. 2002; 42-45, 155, 792.
14. Dunn OJ, Clark VA; Basic statistics a primer for the biomedical sciences. 4th ed. John Wily and Sons, New Jersey. 2009; 41-146.
15. Finegold, 1986 Finegold SM, Baron EJO; Bailey and Scotts Diagnostic Microbiology 7th ed. The C.V. Mosby Company. 1986; 73, 426, 785-787, 907-908.
16. National Guideline clearinglhouse. Diagnosing food borne Illnesses. Diagnosis and management of food borne illnesses: a primer for physicians MMWR Recomm. Rep 2001; 50 (RR-2): 1 – 69.
17. Shetty N, Prabbu; Evaluation of faecal preservation and staining methods in the diagnosis of acute amoebiasis and giardiasis. J Clin Pathol 1988; 41: 694-699.
18. Haque R, Ali IKM, Akther S, Petri WA Jr; Comparison of PCR, isoenzyme analysis and antigen detection for diagnosis of Entamoeba histolytica infection. J Clin Microbiol 1998; 36: 449-452.
19. Garcia LS, Shimizu RY, Bernard CN; Detection of Giardia lamblia, Entamoeba histolytica / Entamoeba dispar, and Cryptosporidium parvum antigens in human fecal specimens using the Triage parasite panel Enzyme Immunoassay. J Clin Microbiol 2000; 38 (9): 3337-3340.
20. Sanuki J, Asai T, Okuzawa E, Koba Yashi S, Takeuchi T; Identification of Entamoeba histolytica and E. dispar cysts in stool by polymerase chain reaction. Parasitol Res 1997; 83: 96-98.
21. Addis DG, Mathews HM, Stewart JM, Wahlquist SP, Williams RM; Evaluation of a commercially a available enzyme linked immunosorbent assay for Giardia lamblia antigen in stool. J Clin Microbiol 1991; 29: 1137-1142.
22. Pillai DR, Kain KC; Immuno chnomatographic strip-based detection of Entamoeba histolytica / E. dispar and Giardia lamblia coproantigen. J Clin Microbiol 1999; 37: 3017-3019.
23. Espinosa-Cantellano M, Martinez-Palomo A; Pathogenesis of intestinal amoebiasis: from molecules to disease. Clin Microbiol Rev 2000; 13 (2): 318-331.
24. Salman YJ and Mustafa MI; Evaluation of the employment of four laboratory diagnostic methods in detecting Giardia lamblia among children in Kirkuk city. J. Kirkuk Med. Coll., 2013; 1(2): 52-60.
25. Salman YJ, Sadek WS, Rashed ZK; Prevalence of Cryptosporidium among Iraqi displaced people in Kirkuk city using direct microscopy, flotation technique and ELISA-copro antigen test. Int. J. Microbiol. App. Sci., 2015, 4(11): 559-572.
26. Peter G, Halsey NA, Marcuse EK, Pickering LK; Red Book: Report of the committee on

infection diseases. Am Acad Pediatr 1994; 236: 517-518.
27. Gendrel D, Treluyer JM, Richard-Lenoble D; Parasitic diarrhoea in normal and malnourished children. Fund-Clin Pharm 2003; 17: 189-197.
28. Zaki M, Meelu P, Sun W, Clark CG; Simultaneous Differentiation and Typing of Entamoeba histolytica and Entamoeba dispar. J Clin Microbiol 2002; 40 (4): 1271-1276.
29. Wittner M, Rosenbaum RM; Role of bacteria in modifying virulence E. histolytica. Am J Trop Med Hyg 1970; 19 (5): 755-761.
30. Mirelman D; Amoeba-bacterium relationship in amoebiasis. Microbiol Rev 1987; 51 (2): 272-284.