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ORIGINAL PAPER

Copro prevalence and estimated risk of *Entamoeba histolytica* in Diarrheic patients at Beni-Suef, Egypt

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Abstract Amoebiasis diagnosis is usually based on microscopy that cannot differentiate pathogenic *E. histolytica* from morphologically identical non-pathogenic species. 194 fecal samples were collected from diarrheic &/or dysenteric patients and examined for *Entamoeba* complex microscopically, *E. histolytica/E. dispar* coproantigen using ICT and *E. histolytica* coproantigen using Tech lab *E. histolytica II* ELISA test. *Entamoeba* complex trophozoites/cysts, *E. histolytica/E. dispar* coproantigen and *E. histolytica* coproantigen were detected in 22.2, 14.4 and 3.6 % of samples, respectively. Microscopy and ICT method had limited sensitivity with poor PPV (9.3 and 7.1 %, respectively) and both slightly agree with ELISA test. The prevalence of *E. histolytica* was low (3.6 %) in studied individuals and was 14 times lower than non-pathogenic amoebae. *E. histolytica* detection studied individuals was positively associated with mucoid and bloody stool, which makes them disease predictors. *E. histolytica* fecal ELISA assay for *E. histolytica* detection surpassed microscopy and *E. histolytica/E. dispar* ICT assay. This has highlighted the need for practical non-microscopic detection methods that can differentiate between amoeba infections to avoid unnecessary and possibly harmful therapies and to determine the true prevalence and epidemiology of *E. histolytica*.

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Introduction

Intestinal amebiasis is a frequent disease and endemic among tropics and subtropics countries with poor and socio-economically deprived communities (Walsh 1986). The most common method of diagnosis is microscopic detection of trophozoites and/or cysts in stool specimens that cannot differentiate pathogenic *E. histolytica* from morphologically identical but non-pathogenic species (Ackers 2002; Johnston et al. 2003). In addition, many studies have shown that excretion of the parasite, in the feces may be intermittent leading to missed infections due to the low numbers of trophozoites and/or cysts in the stool sample (Danciger and Lopez 1975). Differentiation between the pathogenic *E. histolytica* and non-pathogenic amoebae is essential for accurate diagnosis and to avoid unnecessary treatment (Ackers 2002; Kebede et al. 2004; Gutiérrez-Cisneros et al. 2010).

Recently, variety of fecal immunoassays depend on detection of coproantigen using monoclonal antibodies, such as enzyme immunoassays (EIAs) and immunochromatography (ICT), has been developed to establish more sensitive methods for diagnosis of *Entamoeba* and differentiation of *E. histolytica* and *E. histolytica/dispar* (Sharp et al. 2001; Garcia et al. 2003; Johnston et al. 2003). A number of commercial kits are available with a good range of sensitivity and specificity. Although ELISAs are the commonly used diagnostic immunoassays for detection of *Entamoeba* species, only the Techlab *E. histolytica II* test reported that it could identify pathogenic *E. histolytica*. It

is reported that, the *E. histolytica* II test is more sensitive than the combination of microscopy and culture as PCR identified parasite DNA in 27 of 34 (79 %) of the antigen-positive, culture-negative stool specimens and only one stool specimen was positive by microcopy and culture (Haque et al. 2000).

The risk of exposure or prevalence of amoebiasis may be influenced by socio-economic, environmental, sanitary and population behavioral hygienic factors that contribute to the transmission and distribution of amoebiasis (Norhayati et al. 2003; Gatei et al. 2006; Al-Harthi and Jamjoom 2007; Van Eijk et al. 2010). The present study aimed to determine the copro-prevalence of *E. histolytica* and the non-pathogenic *Entamoeba*e, among diarrheic/dysenteric stool samples from clinically suspected individuals from Beni-Suef, Egypt using microscopic and two immunoassays. Also, to evaluate the diagnostic effectiveness of used diagnostic tests. The second objective was to analyze the associated study individual variables as the estimated risk for *E. histolytica* infections.

Materials and methods

Study population

Hundred and ninety-four fecal samples were collected from patients suffering from diarrhea or dysentery of both sexes and any age, attending Internal and Tropical Medicine Departments at Beni-Suef University Hospital Outpatient's Clinics, Faculty of Medicine, Beni-Suef University in the period from November 2012 to May 2013.

Study design and ethical consideration

A cross sectional study was performed to achieve first objective and a case control model was used to estimate risks that may influence detection of *E. histolytica*. The study was ethically approved by Faculty of Medicine, Cairo University. All patients included were informed verbally about the purpose of the study and the collection of stool samples was performed after obtaining their consent.

Sample collection and processing

Single stool sample was collected from each patient included in the study in dry clean containers, urine and water contaminations were avoided. Related data, including demographic data, age, gender, diarrhea characters, associated clinical manifestations, and stool consistency and contents, were recorded using a questionnaire. Samples were transferred immediately to the lab. All samples were examined macroscopically and microscopically. Then part of sample was kept frozen at −20° C for further Copro-immunoassays and rest of sample preserved in sodium acetate acetic acid formalin (SAF) for further staining by Iron hematoxilin stain.

Copro-parasitological examination

All samples were examined by direct wet mount prior and after concentration technique to detect trophozoites and/or cysts of *Entamoeba* complex using 40× and then 100× objectives. Detected other parasites were reported.

Copro-antigen detection of *Entamoeba*

Frozen stool samples were subjected to *E. histolytica/dispar* coproantigen detection using RIDA®QUICK *Entamoeba* Test (R-Biopharm AG, Germany) and *E. histolytica* coproantigen using Wampole™ *E. histolytica* II A 2nd generation monoclonal ELISA kit [Catalog No. T5017 (96 Tests), (TechLab, Blacksburg, Virginia, USA) according to the manufacturer's instructions].

Statistical analysis

The results were analyzed using the SPSS computer software, version 17.0 (Chicago, IL, USA). Since Techlab *E. histolytica II* ELISA test was reported that it could identify pathogenic *E. histolytica*, it was nominated as a reference standard test. Sensitivity, specificity, PVP, NPV and accuracy were calculated with the following formula to analyze data: sensitivity: $100 \times [a/(a + c)]$; Specificity: $100 \times [d/(b + d)]$; PVP: $100 \times [a/(a + b)]$; NPV: $100 \times [d/(c + d)]$, and accuracy: $100 \times [a + d/(a + b+c + d)]$, where a = true positive samples, b = false positive samples, c = false negative samples and d = true negative samples. Kappa test was done to measure the agreement between the methods. P value ≤ 0.05 was statistically significant. An univariable conditional logistic regression analysis was carried out to associate potentially estimated risk of independent variables with detection of *E. histolytica* (dependent variable = outcome) for which matched OR and its 95 % confidence interval (CI) and P value were calculated. Variables in the univariable analysis with $P < 0.2$ were included in the multivariable conditional logistic regression analysis (Hosmer and Lemeshow 1989) (Table 1).

Results

Entamoeba complex trophozoites/cysts was detected in 20.6 % (n = 40) by wet mount and in 22.2 % (n = 43)

Table 1 Microscopic examination of the studied group

	All study individuals (n = 194)	
	n.	%
Parasitic stage other than *Entamoeba complex*		
Helminthes		
E. vermicularis eggs	5	2.6
Taenia eggs	1	0.5
H. nana eggs	4	2.1
C. phillippenensis eggs	2	1
Protozoa		
G. lamblia cysts/trophozoites	14	4.7
E. coli cysts[a]	12	6.2
Blastocystis	6	3.1
I. butschii cysts	1	0.5

[a] There were no cases with multiple parasites except only one case of the diagnosed 12 cases of *E.coli* cysts was a co-pathogen with *E. histolytica* case

after concentration, morphology was confirmed by Iron Hematoxilin stain with sensitivity of 57.1 % and specify of 79.1 %. ICT revealed *E. histolytica/E. dispar* in 14.4 % (n = 28) (sensitivity, 28.6 % and specificity 86.1 %, respectively) and ELISA detected *E. histolytica* in only 3.6 % (n = 7) (Tables 2, 3). Microscopy and ICT reported recovery of *E. histolytica* with poor PPV (9.3 and 7.1 %, respectively). There was slight agreement between ELISA test and both the microscopy and ICT (Table 3).

On microscopic examination, none of *E. histolytica* positive cases had pus in their stool, while RBCs was detected in one case. Only one case of *E. histolytica* cases showed co-pathogen with *E. coli* cysts (Table 1).

Among studied variables (age groups, gender, diarrhea characters, associated clinical manifestations, and stool consistency and contents) (Table 4) of *E. histolytica* positive cases, only presence of mucus and blood in stool showed statistical significant positive association with the probability of having *E. histolytica*, (*P* value = 0.01 and 0.02, respectively) in their stool (Tables 4, 5).

Table 3 Diagnostic yield, accuracy and Kappa agreement of the used diagnostic tests among study individuals using ELISA as a reference standard

	Microscopy of wet mount of fecal smear:		ICT
	Direct (before conc.)	After conc.	
Sensitivity	42.9 %	57.1 %	28.6 %
Specificity	80.2 %	79.1 %	86.1 %
PPV	7.5 %	9.3 %	7.1 %
NPV	97.4 %	98.0 %	97.0 %
Accuracy	79.0 %	78.4 %	84.0 %
Kappa (κ)*	0.07	0.10	0.06

(κ)* Interpretation; < 0: Poor agreement; 0.01–0.20: Slight agreement; 0.21–0.40: Fair agreement; 0.41–0.60: Moderate agreement; 0.61–0.80: Substantial agreement; 0.81–1.00: Almost perfect agreement

Discussion

Our obtained results clearly showed that *E. histolytica* prevalence using ELISA was low (3.6 %, n = 7) among studied individuals and obviously demonstrated that most *Entamoeba* infections diagnosed by ICT and microscopic examination, 26 and 39 samples, respectively were non-pathogenic and negative for *E. histolytica* using *E. histolytica II* ELISA.

E. histolytica is the causative agent of amebiasis disease, thus differentiating it from non-pathogenic species is important (Diamond and Clark 1993). The World Health Organization suggests that diagnosis of amebiasis based only on microscopy is inadequate (WHO 1997). The TechLab *E. histolytica II* ELISA for *E. histolytica* detection in feces, demonstrated satisfactory sensitivity and specificity when was compared to culture (sensitivity of 14.3 % and a specificity of 98.4 %) (Gatti et al. 2002) and real-time PCR (79–71 % sensitive and 96–100 % specific) (Roy et al. 2005; Visser et al. 2006) as a reference test.

E. histolytica prevalence differs among countries, a fact that was attributed to environmental, socioeconomic, demographic, host hygiene-related behavioral factors (Norhayati et al. 2003; Al-Harthi and Jamjoom 2007).

Table 2 Results of used diagnostic methods for detection of *Entamoeba* among study individuals

	Microscopy of wet mount of fecal smear				ICT		Total
	Direct (before conc.)		After conc.				
	Positive	Negative	Positive	Negative	Positive	Negative	
ELISA *E. histolytica* (n = 194)							
Positive	3	4	4	3	2	5	7
Negative	37	150	39	148	26	161	187
Total	40	154	43	151	28	166	194

Data presented as n. (conc. = concentration)

Table 4 Distribution and association of related data variables with cases of *E. histolytica*

	Frequency (n = 7)	*P* value
Age group		
Infants (up to 2 year)	0	0.86
Children (>2–12 year)	2	
Teen age (>12–20 year)	3	
Young (>20–40 year)	1	
Middle age (>40–60 year)	1	
Old age (>60 year)	0	
Gender		
Male	3	0.52
Female	4	
Onset of diarrhoea/dysentery		
Sudden	0	0.42
Acute	4	
Gradual	3	
Duration of diarrhoea/dysentery		
2 days	2	0.89
3 days	3	
5 days	1	
7 days	1	
Diarrhoea/dysentery motion (frequency)/day		
2	0	0.81
3	2	
4	2	
5	2	
6	1	
7	0	
Clinical symptoms		
Fever	4	0.47
Nausea	5	
Flatulence	4	
Vomiting	3	
Colic	7	
Stool consistency		
Liquid	4	0.19
Soft	3	
Stool contents		
Mucus	3	0.01
Blood	2	0.02
Pus cells	0	0.66
RBCs	1	0.49

Table 5 Associations of multivariate analysis of cases of *E. histolytica*

	Frequency		OR	95 % CI	*P* value
	No.	%			
Stool consistency					
Liquid	5	5.5	2.9	0.6–15.5	0.21
Soft	2	1.9			
Stool contents					
Mucus					
Yes	3	16.7	8.6	1.8–42.1	0.01[#]
No	4	2.3			
Blood					
Yes	2	15.4	6.4	1.1–36.8	0.02[#]
No	5	2.8			

[#] = significant *P* value

OR odds ratio, *CI* confidence interval

Interestingly we obtained low prevalence among study individuals; however most of studies in Egypt and developed countries suggesting a higher rate; it was 57 and 25 % in patients with acute and prolonged diarrhea, respectively using ELISA (Abd-Alla and Ravdin 2002); El-Shazly et al. (2006) reported that the *Entamoeba histolytica/E. dispar* prevalence in Mansoura, Egypt was 19 %, Makhlouf et al. (1994) reported it in 9 % of children living orphanages. In Venezula E. histolytica/E. dispar prevalences were 20.09 % using microscopy; while prevalences of E. histolytica and E. dispar were 6.31 and 4.44 %, respectively using PCR (Mora et al. 2008). However recent studies especially using PCR came in agreement with our results, in Egypt, El-Naggar et al. (2006) detected E. histolytica in 5.0 % (cyst) and 0.3 % (vegetative) of examined patients by the direct wet smear. Heckendorn et al. (2002); reported that the prevalence of E. histolytica was low (0.83 %), while E. dispar was 15 % in schoolchildren in central Côte d'Ivoire and on testing only microscopically positive samples by PCR (n = 129), most of Entamoeba infections were E. histolytica with a very low E. histolytica:E. dispar ratio (1:46). Malatyal et al. (2011) detected E. histolytica/dispar cysts in 22 (1.5 %) stool samples of primary school children living in the rural areas around Sivas but E. histolytica specific antigen based ELISA gave negative reactions for all the samples.

In the light of earlier reports, interpretation is very difficult because older data did not differentiate between morphologically identical species. The true prevalence of *E. histolytica* in the study population with the specific antigen detection ELISA test was 13–11 times lower than the ICT test and microscopy. Being the prevalence and true epidemiology of amoebiasis are still unclear and had a high degree of divergence. Because of the low specificity of direct microscopy immunoassays (ICT and most of ELISAs) tests to confirm diagnosis of amoebiasis and to differentiate between pathogenic/non-pathogenic amoeba species. This highlighted the need to use non-microscopic methods; specific fecal ELISA kits or molecular methods

to prevent patients from being given an unnecessary treatment. Since this study was conducted on a cross-sectional sampling, a controlled prospective study should be done to determine the true prevalence of *E. histolytica* infection.

Despite microscopy outperformed ICT (57.1 and 28.6 % sensitivity, respectively) compared with ELISA in this study, as it cannot differentiate between pathogenic *E. histolytica* and other nonpathogenic as *E. moshkowski, E. poleki, E. coli*, *and E. hartmanii* in addition to *E. dispar* (Pariya and Rao 1995; Kebede et al. 2004). But both in accordance with other studies reported limited sensitivity, low recovery of *E. histolytica* and with poor specificity (Gatti et al. 2002; Kebede et al. 2004; Lebbad and Svard 2005). Besides that, ICT assay require fresh or fresh-frozen stool. However the ease of use and adaptability to batches are important criteria in determining the method of choice for immunoassays as diagnostic alternatives (Nuran et al. 2004). *E. histolytica* specific antigens detection in feces by ELISA could be useful for clinical and epidemiological studies where molecular assays cannot be used (Haque et al. 1993).

Apart from mucoid and bloody stool, there was no statistical significant association between other variables including; age group, gender, associated clinical manifestations, diarrhea characters and stool consistency and the possibility of *E. histolytica* detection in stool.

Detection of *E. histolytica* was found among large proportion of mucoid (95 % CI 1.8–42.1, OR 8.6) and bloody stool (95 % CI 1.1–36.8, OR 6.4), with statistical significance positive association in-between (P value = 0.01 and 0.02, respectively). There was estimated 8.6 and 6.4 times increase in the risk of *E. histolytica* among cases who had mucus and blood in their stool, respectively than those didn't had. Such epidemiological study is limited by estimates of population, migration of population from study areas, and the ecological fallacy. Mora et al. (2008) stated that the infections by E. histolytica and E. dispar were statistically associated with age but not with sex and mucus, blood and abdominal pain were only associated with E. histolytica infection.

Environmental, clinical and host socio-economic and behavioral factors known to influence the transmission and distribution of intestinal parasitic infections (Norhayati et al. 2003) and were reported as important risk factors for amoebiasis in many studies (Benetton et al. 2005; Nyarango et al. 2008; Pham Duc et al. 2011) however they used techniques did not differentiate between pathogenic and non-pathogenic *Entamoeba*, this may explain our obtained results. These factors may influence the risk of exposure, prevalence or affect susceptibility of amoebiasis but not the pathogenicity or course of the disease (Gatei et al. 2006; Van Eijk et al. 2010).

Parasites other than *E.* complex were detected in 85 (43.8 %) individuals, 17 (8.8 %) of them had multiple parasites, interestingly, among them only one of *E.coli* cysts cases was a co-pathogen with *E. histolytica*. Similarly Zali et al. (2004), Haghighi et al. (2009) and Solaymani-Mohammadi et al. (2006) observed that *E.coli* frequently associated with *E. histolytica*.

Beside the studied variables in the present study that may induce susceptibility of *E. histolytica* detection in stool samples and influence its prevalence there are added variables in Egypt including varies in environmental and socioeconomic status within the same geographical areas. These may have an important role in differences in reported disease prevalence and estimated risks.

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Conflict of interest None.

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