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ORIGINAL ARTICLE

Comparison of microscopic and immunoassay examination in the diagnosis of intestinal protozoa of humans in Mansoura, Egypt

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Abstract Protozoal diseases are prevalent globally and especially in developing countries that have relatively lower socioeconomic populations such as Egypt. Direct microscopic examination (DME) is used for the detection and identification of protozoa but lacks sufficient reliability, and thus may be detrimental in obtaining accurate diagnostic or epidemiological data. In this study, we determine the prevalence of infections by *Giardia intestinalis*, *Cryptosporidium* sp., and *Entamoeba histolytica* in humans in Egypt. Furthermore, we determine the reliability of DME in determining infections caused by these protozoa and compare the results to enzyme linked Immunosorbent assays (ELISA). Our results indicate that the prevalence of giardiasis, cryptosporidiosis, and entamoebiasis is 38, 22, and 16 %, respectively. The sensitivity and specificity of DME for detection of *G. intestinalis* is 45 and 99 %, for *Cryptosporidium* 66 and 99 %, and for *Entamoeba* 45 and 100 %, respectively. Our findings demonstrate that ELISA is more reliable for diagnostic and epidemiologic study purposes.

Keywords Prevalence · Diagnosis · Entamoeba · Cryptosporidium · Giardia · ELISA

Introduction

Intestinal protozoal diseases are known to occur frequently in Egypt (Smith-Palmer and Cowden 2009; Smith-Palmer and Locking 2011). Among the most frequent are giardiasis, cryptosporidiosis, and entamoebiasis (Hegab et al. 2003; Zaki 2009). While few studies have attempted to investigate the prevalence and distribution of these infections in Egypt, these studies are incomplete, apply to different geographical locations (El-Shazly et al. 2006; Ibrahium 2011; Sabry et al. 2009), or involve distinct populations that do not represent the general population in Egypt (Antonios et al. 2010; El-Mahallawy et al. 2013; El-Sherbini et al. 2008). In addition, studies are often performed using variable, inaccurate, or inconsistent identification methods and few studies have addressed the reliability of these methods (Feng and Xiao 2011; Gaafar 2011; Selim et al. 2009). Furthermore, many studies have investigated *Giardia*, *Cryptosporidium,* and *Entamoeba* infections in combination with other parasitic infections and have therefore not focused sufficiently on these intestinal protozoa.

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Symptoms of intestinal parasitic infections are usually general symptoms of gastrointestinal ailment including diarrhea, abdominal pain, nausea, vomiting, flatulence, anorexia, and fever (Katz et al. 2006). *Entamoeba histolytica, G. Intestinalisand Cryptosporidium* spp. are three of the most important and common diarrhea-causing parasitic protozoa, and often have a similar clinical presentations (Haque et al. 2007). However, the common symptoms and clinical presentations of these infections have not been sufficiently investigated. Examination of the literature on giardiasis revealed that only few contained information on symptoms or clinical presentations of the disease in Egypt (Muhsen and Levine 2012). This information is required for the study of these diseases and for the ability of healthcare providers to identify the protozoa and perform critical differential diagnoses onpatients.

Direct microscopic examination (DME) remains the most commonly used method for detecting and identifying intestinal parasites as DME is relatively inexpensive and appropriate for resource-limited developing countries (Utzinger et al. 2010). However, DME is prone to a number of limitations such as requiring trained personneland considerable effort for preparing, staining, and examining smears. In addition, there is a frequent need to examine multiple fecal samples to find a suspected organism (Johnston et al. 2003; Palmieri et al. 2011). Misdiagnosis by DME can significantly impact patient care (Newman et al. 1993; Palmieri et al. 2011). When DME was used, infection rates appeared 51.3 % lower when compared to rates detected by enzyme linked Immunosorbent assays (ELISA) and 17 % lower when compared to rates detected by polymerase chain reaction (PCR) (Feng and Xiao 2011). Immunoassays for the detection of stool copro-antigens have replaced DME as the routine diagnostic procedure in many laboratories worldwide (Garcia and Shimizu 1997), providing adequate sensitivity and specificity. The assays have also been used in the study of the epidemiology of asymptomatic disease (Gonzalez et al. 1995). However, the routine use of immunoassays for diagnosis of protozoal diseases is not common in Egypt. Furthermore, few studies have investigated the reliability of microscopic examination compared to serological or molecular based methods. There are no reported studies comparing *Giardia*, *Cryptosporidium,* and *Entamoeba* infections in Egypt.

In this study, we determine the prevalence of *G. intestinalis*, *Cryptosporidium*, and *E.histolytica* in humans. We investigate the prevalence of symptoms associated with each infection and their association with human disease. We also determine the reliability of DME in identifying these organisms from clinical samples compared to ELISA-based assays using commercially available diagnostic test kits.

Materials and methods

Study populations

Human stool samples from 185 patients at Mansoura University Hospital out-patient clinics were collected as part of their clinical evaluation. Patients were visiting the hospital complaining of a variety of gastrointestinal and non-gastrointestinal symptoms. Gastrointestinal symptoms at the time of sample submission were recorded for each patient. Sample collection and clinical information followed ethical medical research guidelines of Mansoura University, Egypt. Patients were between 2 and 58 years old and included 86 females and 99 males. Samples were tested for the presence of *G. intestinalis*, *Cryptosporidium*, and *E. histolytica*.

Microscopy

For determining the reliability of DME in identification of organisms, fecal samples were processed as per the standard procedures in the Mansoura University clinical diagnostic laboratory. Samples were preserved in 10 % buffered neutral formalin and concentrated by centrifugation at $500\times g$ for 10 min and the resulting supernatant located directly above the concentrated stool sediment was discarded. For identification of *G. intestinalis* or *Entamoeba* spp., microscopic examination consisted of evaluating two wet mount preparations for each fecal specimen; one non-stained and the other stained with iodine. For identification of *Cryptosporidium* spp. concentrated samples were stained using Modified Ziehl-Neelson acid fast stain (Garcia 2001) before microscopic examination. All samples were examined at $1000\times$ for the presence of trophozoites or cysts. Only findings of *Giardia*, *Cryptosporidium*, or *Entamoeba* Spp. Were included in the results and analysis of this study.

ELISA based testing

TechLab's *GIARDIA II*, *CRYPTOSPORIDIUM II*, and *E. HISTOLYTICA II* ELISA-based diagnostic kits (TechLab, Blacksburg, VA, USA) were used to identify *G. intestinalis, Cryptosporidium* spp., or *E. histolytica* antigens, respectively. The *GIARDIA II* test relies on monoclonal antibodies for detection of *Giardia* cyst antigen and the *CRYPTOSPORIDIUM II* test detects *Cryptosporidium*oocysts. The *E. HISTOLYTICA II* test relies on monoclonal antibodies for detection of *E. histolytica*adhesin and does not cross react with *Entamoeba dispar.* The specificity of each kit is determined by the manufacturer to be 100 % and no cross reactivity was found. All kits were used according

to the manufacturer's instructions. Only non-preserved samples were used for ELISA testing.

Statistical analysis

To determine the reliability of DME, the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated as described by Altman and Bland (1994a, 1994b). Sensitivity was determined by calculating the proportion of positive samples as determined by ELISA that are correctly identified by DME, and specificity is determined by calculating the portion of negative samples using ELISA that are also negative using DME. PPV = (*sensitivity* × *prevalence*)/(*sensitivity* × *prevalence* + (1 − *specificity*) × (1 − *prevalence*). NPV = (specificity × (1 − prevalence)/(1 − sensitivity) × prevalence + specificity × (1 − prevalence).

The findings of DME were considered the test results and the findings of the ELISA-based kits were considered the correct diagnostic results. To determine whether infection with *G. intestinalis*, *Cryptosporidium*, or *E. histolytica* is associated with absence of symptoms the Chi Square test (χ^2) test was used. Significance was defined as $P < 0.001$.

Results

Disease prevalence and clinical presentation

The prevalence of giardiasis, cryptosporidiosis, and entamoebiasis using ELISA assays was 38 % (71 cases), 22 % (41 cases) and 16 % (29 cases) respectively. The prevalence of giardiasis, cryptosporidiosis, and entamoebiasis using DME was 18 % (33 cases), 15 % (28 cases), and 7 % (13 cases), respectively. In the majority of cases diagnosed with each disease, patients were asymptomatic. Diarrhea, abdominal distention, and colic were the most frequently reported symptoms while nausea and constipation were less frequently reported (Fig. 1). To determine whether infection with each of the organisms tended to present with gastrointestinal symptoms and whether diarrhea was as an important symptom of each infection, the association between infection and the presence of symptoms, or specifically diarrhea, were determined for infection with each organism. In patients with giardiasis, there was an association between infection and asymptomatic presentation (Table 1), suggesting that infections tended to be asymptomatic. In patients who presented with gastrointestinal symptoms, there was an association with diarrhea (Table 2), suggesting that in patients who presented with gastrointestinal symptoms, diarrhea tended to occur.

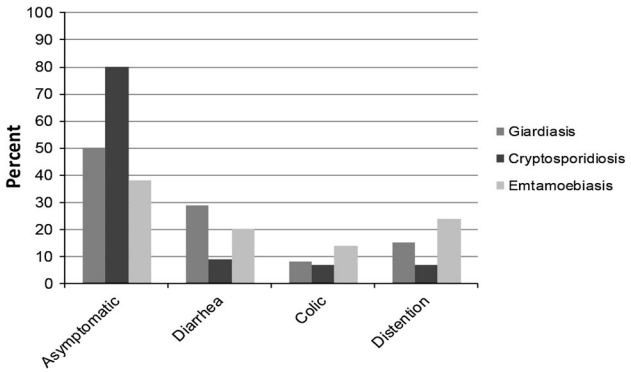

Fig. 1 Clinical symptoms associated with intestinal protozoal infection. Asymptomatic presentation was more frequent than each of the individual symptoms and diarrhea was the most common presenting symptom

Table 1 Association of asymptomatic gastrointestinal presentation with giardiasis, cryptosporidiosis, or entamoebiasis

	Number of patients	Percent infected	χ^2	*P*
Asymptomatic presentation (vs. giardiasis)			13.890	<0.001
Yes	124	29		
No	61	57		
Asymptomatic presentation (vs. cryptosporidiosis)			0.321	0.567
Yes	124	23		
No	61	20		
Asymptomatic presentation (vs. entamoebiasis)			13.174	<0.001
Yes	124	9		
No	61	29		

Table 2 Association of diarrhea with giardiasis, cryptosporidiosis, or entamoebiasis

	Number of patients	Percent infected	χ^2	*P*
Diarrhea (vs. giardiasis)			22.734	<0.001
Yes	26	81		
No	159	31		
Diarrhea (vs. cryptosporidiosis)			0.805	0.369
Yes	26	15		
No	159	23		
Diarrhea (vs. entamoebiasis)			1.220	0.269
Yes	26			
No	159	14		

Table 3 Reliability of microscopic examination for identification of intestinal protozoa in fecal samples

Organism	Sensitivity (%)	Specificity (%)	PPV (%)[a]	NPV (%)[b]
G. intestinalis	45	99	97	74
Cryptosporidium	66	99	96	91
E. histolytica	45	100	100	91

[a] Positive predictive value

[b] Negative predictive value

There was an association between infection with *E. histolytica* and absence of gastrointestinal symptoms (Table 1). However, there was no association between the infection and the presence of diarrhea when symptoms were present (Table 2), suggesting that infections tended to be asymptomatic and diarrhea was not especially present when symptoms occurred.

Reliability of microscopic examination for diagnosis

Enzyme linked Immunosorbent assays results were considered as the reference method when determining, the specificity, sensitivity, PPV, NPV of microscopical examination for diagnosis of giardiasis, cryptosporidiosis, or entamoebiasis. Results are shown in Table 3. No cross-reactivity with other parasites was detected.

Discussion

Intestinal protozoal infections are a public health problem in developing countries where they cause high morbidity and mortality (Dorny et al. 2009; Kenny and Kelly 2009).The prevalence of these infections and the extent of their public health effect in Egypt are not clearly understood. Lack of knowledge on their epidemiologic status is most likely due to incomplete understanding of the diseases' symptoms, the variation and overlap of those symptoms, and the ineffectiveness of DME as a commonly used diagnostic tool. Therefore, there is a need to study the prevalence of intestinal protozoal infections, determine theirmost common symptoms, and determine the efficiency of DME in their identification in clinical samples.

In our study, the prevalence of *G. intestinalis*, *Cryptosporidium,* and *E. histolytica*infections was 38, 22 and 16 %, respectively, when determinedby ELISA-based diagnostic kits. *Giardia intestinalis* infections had the highest prevalence among the examined protozoans, which is in-line with previous studies that demonstrate that giardiasis is the most common intestinal parasitic disease of humans in developing countries (Feng and Xiao 2011; Smith-Palmer and Locking 2011). The prevalence of giardiasis in our study (38 %) was higher than that reported in other studies from Egypt, which varied between 14.8 and 30.8 % (El-Kadi et al. 2006; Sabry et al. 2009). Cryptosporidiosis is mainly reported in animals in Egypt(Mahran and Taher 2010; Samaha et al. 2012). However, cryptosporidiosis is one of the least studied infectious disease in Egypt and, as with other parasitic diseases, its prevalence is thought to be underreported (Palmieri et al. 2011). The prevalence of *Cryptosporidium* infections obtained in our study by ELISA was high (22 %) in comparison with studies using the same method in other countries [1.7 % in Italy (Cirak and Bauer 2004) and 7.4 % in Canada (Rinaldi et al. 2008)] and approximates that of Germany 23 % (Shukla et al. 2006). Few studies have reported the prevalence of *Entamoeba* infections in Egypt. In these reports, DME was often used and the organisms were reported as either *E. histolytica* or *E. dispar.* However, DME alone is not capable of distinguishing the two species (Stauffer et al. 2006) and these reports are likely to include false negative and false positive results. In a study by El-Shazly et al. (2006)carried out in Dakahlia, Egypt among 3,180 patients, the authors reported a prevalence of 19.6, 19.0, and 14.3 % for *Giardia*, *Entamoeba* and *Cryptosporidium,* respectively, using microscopy with staining. These percentages are lower than our findings and this may be due to the use of different microscopic methods in other studies compared to ours, resulting in an increase in false negative cases.

In this study, a variety of symptoms were reported by patients with giardiasis. Symptoms of giardiasis may be typical or atypical. Typical symptoms include diarrhea, loose stools, malaise, abdominal cramps and weight loss. Atypical symptoms vary and patients may be asymptomaticorbemildlysymptomatic (Meyer and Radulescu 1979). However, common symptoms associated with giardiasis in Egypt have not been previously reported. Our findings demonstrate that 57.3 % of *Giardia* positive cases are asymptomatic while diarrhea is the main symptom in 29 % of symptomatic cases. Most cases of *Cryptosporidium* infection (80 %) were asymptomatic and diarrhea was the main complaintin symptomatic cases. This finding is consistent with that of previous studies (Huang and White 2006; Raccurt 2007). In our study the majority of entamoebiasis cases were symptomatic (29.5 %) while distension and diarrhea were the main symptoms. Only 8.9 % cases were asymptomatic. Our findings suggest that in our population, giardiasis tends to be asymptomatic but causes diarrhea if symptoms exist. Entamoebiasis also tends to be asymptomatic, but presenting with diarrhea was not associated with its symptoms. The variation of symptoms associated with each of the diseases we examined may be due to their intermittent nature or due to the presence of an undetermined underlying condition. The endemic nature of

these diseases in Egypt may also contribute to the presence of variable and atypical symptoms. The variety of symptoms and their atypical nature may also contribute to the diseases being commonly under-diagnosed or misdiagnosed by healthcare providers.

In this study routine microscopic examination of stool samples did not reveal as many positive specimens as the ELISA tests. We also demonstrated that the sensitivity of DME was 45, 66, and 45 % while the specificity was 99, 99, and 100 % for detection of *G. intestinalis*, *E. histolytica,* and *Cryptosporidium,* respectively. The specificity for detection and identification of each of the organisms was acceptable. However, the sensitivity was not acceptable. The sensitivity for identification of cryptosporidiosis was higher than that for giardiasis and entamoebiasis. This difference may be due to the use of staining for the routine identification of *Cryptosporidium* using DME, which enhances the ability to detect and correctly identify the organism. Our results demonstrate that ELISA is more sensitive than DME in detection of *G. intestinalis*, *E. histolytica,* and *Cryptosporidium*as ELISA is able to detect minimal amounts of antigen and canshow a positive result even when the parasite load is low. These findings are in accordance with reports that indicate that TechLab ELISA for detection of *E. histolytica* antigen in stool specimens have excellent correlation with PCR (Haque et al. 1998). Other studies have also found that ELISA is more sensitive (80–94 %) and more specific (94–100 %) than microscopy and culture (Haque et al. 2000) and DME was less effective in detection and identification of *Giardia* (Shatla et al. 2004). Other enzyme immune assays (EIA) have also been used in detection and identification of these protozoans. In one study in Egypt, microscopic examination detected *Giardia* in 19 %, *Cryptosporidium* in 4 %, and *E. histolytica/E. dispar* in 1 % of examined stool samples, while an EIA kit detected *Giardia* in 23 %, *Cryptosporidium* in 5 %, and *E. histolytica/E. dispar* in 2 % (Gaafar 2011). In another study, a *Giardia* EIA identified the organism in at least 30 % more specimens than microscopic examination (Rosoff et al. 1989). These studies also indicate that the immune assays are more reliable than microscopic examination.

Enzyme linked Immunosorbent assays has many significant advantages for the diagnosis of intestinal protozoa. The technique is able to differentiate *E. histolytica* from *E. dispar* and has excellent sensitivity and specificity. When used as commercial kits, the technique does not require a higher level of training and experience as often required for DME. Enzyme linked Immunosorbent assays can outperform microscopy in its potential as a large-scale diagnostic tool in epidemiological studies (Gaafar 2011). Most studies of *Giardia*, *Entamoeba,* and *Cryptosporidium* infections in Egypt use DME for diagnosis of the diseases and as the standard when determining the sensitivity and specificity of other tests (Antonios et al. 2010; El-Shazly et al. 2004). However, microscopy appears to be an unsuitable reference standard; techniques that have more sensitivity and specificity should be adopted instead. Enzyme linked Immunosorbent assays-based tools are more specific and sensitive, and are more affordable and thus appear to be the most suitable tests. Microscopy may continue to be used as a confirmatory test.

Overall, our results demonstrate that *G. intestinalis*, *E. histolytica*, and *Cryptosporidium* infections are prevalent in Egypt, indicating that a management strategy is needed to prevent and control infections. In our study, many infections were asymptomatic, necessitating more efficient methods of detection and identification of their causative organisms. Our findings suggest that DME is not adequate for identification of these organisms for the purpose of clinical diagnosis or epidemiological studies and should not be used as a reference technique to evaluate other diagnostic methods. Enzyme linked Immunosorbent assays-based or other serological detection and identification tests are more reliable. These tests, or other molecular testes, should be used for identification and diagnosis of these intestinal parasitic infections and as the reference tests when evaluating other commonly used techniques.

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