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Immunoassay Method as Diagnostic Tool for Enteric Amoebiasis and Cryptosporidiosis in Some Rural Communities, of Kwara State, Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Author SKB developed the idea and reframe work of the research, authors EAA and RMA carried out the bench work, while authors MAA and SKB did the write up and revisions of the manuscript.

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ABSTRACT

Background: Laboratory diagnosis of enteric amoebiasis and cryptosporidiosis in rural communities and urban cities in sub-Saharan Africa depends on use of microscopic method that has been adjured to be unspecific, more sensitive and specific methods are needed such as immunoassay method.
Objective: This study compared the use of antigen capture immunoassay and microscopy techniques in detecting antigen and cysts of *Entamoeba histolytica* and oocysts of Cryptosporidium respectively in fecal specimens of individuals in rural communities of Kwara State, Nigeria.
Methods: Wet preparation and formol ether concentration of fecal specimens were examined for cysts of *Entamoeba histolytica* and Modified Ziehl Neelson (MZN) stained fecal concentration were examined for oocysts of Cryptosporidium using microscope, then human Cryptosporidium and *Entamoeba histolytica* antigen capture immunoassay were also used for diagnosis of these parasites in the fecal specimens.

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Results: Microscopic method detected 25 (15.6%) cysts of *Entamoeba histolytica/dispar* complex, and 42 (26.2%) of oocysts Cryptosporidium, while immunoassay method detected significantly higher values of 31 (19.4%) and 47 (29.4%) respectively. Microscopy cannot be used to differentiate pathogenic *Entamoeba histolytica* from non-pathogenic *Enatamoeba dispar* which immunoassay method can do.
Conclusion: Antigen capture enzyme immunoassay method is a better tool in diagnosis of amoebiasis and cryptosporidiosis in rural communities, preventing unnecessary treatment of individuals harboring nonpathogenic amoeba species and higher detection of pathogenic species.

Keywords: Amoebiasis; cryptosporidiosis; microscopic; enzyme immunoassay; diagnosis.

1. INTRODUCTION

Protozoan parasitic diseases contribute significantly to the burden of infectious diseases worldwide. Most of these infections and death from protozoan parasitic diseases affect people in low income areas and rural communities of the developing countries. Conditions such as inadequate methods of fecal disposal, poor water supply, poor personal hygienic practice and low level of health education are contributory factors to disease occurrence [1,2]. These parasites can cause significant illness even in developed countries. The WHO [3] reported that diarrheal disease affects far more individual than any other illness, even in regions that include high-income countries.

Several species of enteric protozoa are associated with diarrheal illness in rural human communities; such include *Giardia lamblia, Entamoeba histolytica, Cryptosporidium, Isospora belli, Cyclospora and Microsporidium*. The commonest causes of diarrheal disease among these are *E. histolytica* and *Cryptosporidium.* They are well recognized as agents of diarrheal episodes in children and adults in developing countries [2,4]. The Walsh [5] reported further that about approximately 500 million people worldwide are infected annually with *E. histolytica*, resulting in symptomatic illness and death in about 50 million and 100,000 persons respectively. The vast majority (about 90%) of individual infected with *Entamoeba* species are colonized by nonpathogenic strain *E. dispar* [6]. The traditional method of diagnosis of enteric amoebiasis in rural communities of developing countries relies on microscopic examination of feces for typical morphology of trophozoites or cysts of the parasite. This makes diagnosis difficult because the pathogenic species *E. histolytica* is morphologically identical to the nonpathogenic species, *E. dispar* and *E. moshkovskii*, hence microscopy is generally considered insufficient to differentiate these species [7].

The diagnosis of cryptosporidiosis is generally undertaken by identification of oocysts in feces of the patient using modified Ziehl-Neelsen staining. The small size and subtle staining characteristic of *Cryptosporidium* species have contributed to the difficulties of identifying this parasite in routine stool preparations [8]. Other authors have opinioned that identification of morphological characters of Cryptosporidium is unreliable and relatively time-consuming with light microscopy [9,10].

The aim of this study was to evaluate the use of enzyme-immunoassay method in diagnosis of amoebiasis and cryptosporidiosis in rural communities of Ogboro and Malete, Moro Local Government Area of Kwara State, Nigeria. This method was compared with the microscopic method of wet mount preparation with Lugols iodine stained, formol ether concentrated method for cysts *Entamoeba* species and MZN stained formol ether concentrated sample for oocysts *Cryptosporidium* species respectively.

2. MATERIALS AND METHODS

2.1 Study Site

This study was carried out in two communities of Moro Local Government Area of Kwara State. The two communities were Malete and Ogboro, both consisted of predominantly farmer, with few school teachers, community health staff; while others are petty traders and artisans.

2.2 Sample Collection

The subjects were the pupils of the two primary schools and adults in the communities. Consent was obtained from the village heads, clan/family heads or their representative at community level;

from the local health authority and teaching service commission/school heads. Signed consent forms from parents were received with samples from pupils. Adult samples were from patients that required laboratory investigation for intestinal ailments through primary healthcare centers in the communities. The assistance of community health officers and laboratory technicians in charge of the centers was obtained. Institutional consent was obtained from Community Development Centre (CDC) of Kwara State University. Subjects were given labeled, universal screw capped plastic bottle to bring early morning fecal sample. Samples were collected both at the school assembly for the pupils and samples for adults were collected from the health centers. As many as consented to be included among the school children were included while adults that have complain intestinal illness were recruited by healthcare staff. These were examined as soon as they reached the Microbiology laboratory of Kwara State University, Malete. The samples were collected from August, 2014 through May, 2015.

2.3 Sample Examination

Macroscopic and microscopic examinations were carried out as previously described [8]. Wet preparation of fecal samples in normal saline stained with Lugols iodine; Ridley modified formol ether concentration technique also was used to improve recovery of the cysts and oocysts of protozoan parasites from the samples that were missed on routine wet mount preparation. The sediments were examined for cysts with aid of Lugols iodine and the second slide was stained with modified Ziehl-Neelson (MZN) method to detect oocysts of cryptosporidium species [9].

2.4 Immunoassay Methods

Fresh fecal sample in 5 ml of normal saline were preserved in the refrigerator at 4℃ until all the samples were harvested. Human cryptosporidium antigen detection (Cry-Ag) ELISA kit (MBS2600132) sourced from MY Bioscience Company (California) was removed from the refrigerator and left at room temperature to acclimatize.

Human *Entamoeba histolytica* antigen detection (EH-Ag) ELISA kit (BS108889) purchased from MY Bioscience Company (California) was used to detect antigen of *E. histolytica* according to manufacturer's protocol from the preserved stool samples. The tests were carried out according to manufacturer's manual using automated ELISA Bio-Rad microplate reader.

2.5 Statistical Analysis

The data were analyzed by using statistical package of SPSS 21.0 software and Wilks' Lambda, Chi-Square test was used for comparison of qualitative data, the risk was estimated by using odds ratio and 95% confidence interval. Significance was considered at *p* value less than 0.05. Sensitivity and specificity were calculated.

3. RESULTS

3.1 *Entamoeba histolytica*

A total of 160 fecal samples were collected and analyzed in this study. Entamoeba cysts identified using microscopic method were *Entamoeba histolytica/dispar, E. coli* and *Iodamoeba buetschlii*. A total of 25 (15.6%) of the samples had *Entamoeba histolytica/dispar* and 35 (21.9%) had non-pathogenic Entamoeba. Fecal distribution of the amoebae detected using microscopy and immunoassay method is presented in Table 1.

The microscopy method detected 25 (15.6%) of *E. histolytica/dispar* complex while the immunoassay method detected 31 (19.4%). Immunoassay method is more specific for *E. histolytica* and had 6 (3.75%) more than what microscopy detected, in addition microscopy method cannot distinguish *E. histolytica* and *E. dispar*. The detection of *E. histolytica* by microscopy (using concentrated wet mount) compared with immunoassay showed sensitivity and specificity of 83.8% and 76.6% respectively. Using Chi-Square ($_{\chi}2$) to test for efficacy of the two techniques in detecting *E. histolytica/dispar* complex and *E. histolytica* by microscopy and immunoassay respectively, there was a significant difference between microscopy and immunoassay at $_{\chi}2$ (1df, n=160) =95.328, p< 0.05. Immunoassay method detected 31 (19.4%) of *E. histolytica* while microscopy detected 25 (15.6%) *E. histolytica/dispar* complex, there was significant sensitivity variation (Wilks' Lambda = 0.656, F (1984) = 9.2 p=0.000).

Table 1. Distribution of amoebae in fecal samples and immunoassay detection of *Entamoeba histolytica*

E. histolytica/dispar		Microscopy		Immunoassay
		E. coli	***I. buetschlii***	**Elisa**
Positive	25 (15.6%)	24 (15.0%)	11 (6.9%)	31 (19.4%)
Negative	135	136	149 (93.1%)	129 (80.6%)
(84.4%)				
(85.0%)				
Total	**160**	**160**	**160**	**160**

3.2 *Cryptosporidium*

The formol ether concentration of fecal samples stained with modified Ziehl-Neelsen (MZN) method detected 42 (26.2%) while the immunoassay method detected 47 (29.4%) in the subjects that participated in this study. Fecal detection of *Cryptosporidium* in the methods is shown in Table 2.

There was higher detection of *Cryptosporidium* by immunoassay method 47 (29.4%) than MZN stained method 42 (26.2%). There was significant relationship between MZN stained and immunoassay using Chi-Square (χ 2) (1df, n = 160) = 0.3894, $p < 0.05$.

4. DISCUSSION

This study included pupils were asymptomatic carrier of Entamoebae and Cryptosporidium and patients presenting with symptoms of intestinal illness that sought for medical attention in rural communities of Moro Local Government (LGA) area. The prevalence of *E. histolytica* and *Cryptosporidium* species is relatively high 31% and 29.4% respectively in immunoassay method; this is comparable to similar result by Babatunde and colleagues [2]. In these communities and several others in developing countries, the major laboratory method of diagnosis of intestinal parasitic illness is by use of microscopy. Direct microscopic examination is undoubtedly the gold standard for the diagnosis of intestinal parasitic diseases with advantages of being inexpensive and easy to operate compared to immunoassay method. Accurate diagnosis of *E. histolytica* is difficult because it is based on morphology and size of iodine stained/trichome stained cysts and nuclei of *Entamoeba* species, thus depends on proficiency and experience. Although cysts of *E. coli, E. moshkovski* and *E. poleki* may readily be differentiate by experienced microscopist, *E. histolytica* and *E. dispar* cannot be differentiated on basis of microscopic morphology [5]. In addition *E. dispar* is recognized as a non-pathogenic species of amoeba; it has to be differentiated form pathogenic species *E. histolytica*. In this study microscopy detected 25 (15.6%) of *E. histolytica/dispar* complex while the immunoassay method detected 31 (19.4%) of *E. histolytica*; there was significant difference between the two methods. This indicated that a significant individual that harbor this pathogenic species may not be detected and so not treated even though they may have symptoms.

Previous studies indicated that *E. dispar* can be ten times as common as *E. histolytica* in endemic regions of West and South Africa [2,10]. This report and our current finding support the need for use of immunoassay method that is more specific for diagnosis of pathogenic species. A more sensitive and specific technique that even differentiate other nonpathogenic species of Entamoeba complex such as *E. coli, E. moshkovski, E. polecki, E. dispar* and *E. hartmanni* is Multiplex PCR [11]. However, this molecular method is more expensive and required highly trained personal than the immunoassay method [12].

Table 2. Fecal detection of *Cryptosporidium* in both MZN stained and immunoassay methods

Fecal MZN stained samples		Fecal immunoassay samples	
Positive	**Negative**	**Positive**	**Negative**
42 (26.2%)	118 (73.8%)	47 (29.4%)	113 (70.6%)
Total	160		160

There was huge difference in detection of *Cryptosporidium* using modified ZN stained with 42 out of 160 (26.2%) to immunoassay with 47 out of 160 (29.4%). This difference may lead to significant margin of error and individuals that were infected but not detected by microscopy. Reasons that may be adduced to low detection by modified ZN stained method are the small size and subtle staining characteristics of *Cryptosporidium* in stool preparations [13].

Previous reports also corroborated out finding, that antigen detection method by immunoassays are more sensitive than the conventional staining method and more effective in cases where oocysts numbers are low [14,15]. However, this antigen detection immunoassay is not as specific and sensitive as molecular methods, which can further differentiate *Cryptosporidium* into various species [16]. Both immunoassay and molecular methods are more expensive and required trained hands that are not readily available in rural communities.

5. CONCLUSION

In conclusion, *E. histolytica* and *Cryptosporidium* are important intestinal protozoan parasites associated with diarrheic conditions in sub-Saharan African. Diagnosis based on use of microscopy and MZN stained slides for *E. histolytica* and *Cryptosporidium* respectively are not specific and sensitive enough for detection of intestinal illnesses caused by these parasites. We have demonstrated that immunoassay method is more sensitive and specific method of diagnosis of these parasites even in rural settings.

ETHICAL APPROVAL

Ethical approval for the research was obtained from village heads, Schools board and health department of Moro LGA. Institutional approval was given through Community Development Centre and ethic committee of Kwara State University, therefore, all authors hereby declare that all experiment have been examined and approved by the appropriate ethics committee and have been performed in accordance with the ethical standard laid down in the 1964 Declaration of Helsinki).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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