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Epidemiology and Comparison of Methods for Diagnosis of *Entamoeba Histolytica* in Stool and Serum Specimens among School Children in Lagos State, Southwest, Nigeria

Muoneke Peter Declan[1] PhD, *Okanlawom Babatunde Meshach[2] M.Sc, Adekunle Oluwatoyin Christianah[1] PhD, Rachel Adekemi Akanni1 M.Sc, Sanusi Tawakalit Omotayo[3] M.Sc, Adefioye Olusegun Adelowo[1] M.Sc, Ojurongbe Olusola[1] PhD

[1] Department of Medical Microbiology and Parasitology, College of Health Sciences, Ladoke Akintola University of Technology, P.M.B. 4400, Osogbo Nigeria.
[2] Department of Medical Laboratory Science, College of Health Sciences, Ladoke Akintola University of Technology, P.M.B. 4400, Osogbo, Nigeria.
[3] Department of Community Medicine, College of Health Sciences, Ladoke Akintola University of Technology, P.M.B. 4400, Osogbo, Nigeria.
***Corresponding Author**
Mr. Okanlawon Babatunde Meshach
Department of Medical Laboratory Science
Ladoke Akintola University of Technology
Osogbo, Osun State
Nigeria
Email: bmokanlawon@lautech.edu.ng

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Abstract

Entamoeba histolytica, the causative agent of amoebiasis infection is second to malaria as a major protozoan cause of morbidity and mortality worldwide, especially in developing countries. This study was designed to determine the prevalence of *Entamoeba histolytica* infection using microscopy and Enzyme Linked Immunosorbent Assay (ELISA) techniques and its contribution to the development of anaemia among primary school children. Five hundred and forty faecal and blood samples were collected and examined according to World Health Organisation standard using direct saline-iodine and floatation techniques and ELISA kit was used to determine *E. histolytica* antibody quantitatively. Overall prevalence by microscopy and ELISA were 10.9%, 49.6% respectively. Male (13.3%) were more infected than female (8.6%) (P> 0.05) and age group 2-5years had the highest prevalence rate of 12.2%, while age group 6-9years (10.2%) had the least (P> 0.05). The mean serum ferritin of uninfected and infected school children are 73.61 ±11.58, 76.00 ±11.51 respectively (P<0 .05), while the mean packed cell volume of uninfected and infected school children are 38.19 ± 2.74, 38.06 ± 3.92 respectively (P> 0.05). Personal and environmental hygiene, public awareness and regular mass treatment programme should be sustained as this would reduce the burden of intestinal protozoal infection among school children.

Keywords: Epidemiology, Comparison, Diagnosis, *Entamoeba histolytica*

1. Introduction

Entamoeba histolytica is the causative agent of amoebiasis which is pseudopod-forming nonflagellated protozoan parasite [15]. The *E. histolytica* life cycle consists of an infective cyst and an invasive trophozoite form. Infection is acquired by ingestion of food or water containing the cyst form of *Entamoeba histolytica* parasite, which is responsible for amoebic colitis and liver abscess [16]. In developing world, *Entamoeba histolytica* infections are second to malaria as a protozoan cause of death worldwide [19]. About 50 million people are believed to be infected at any one time, and up to 100,000 deaths occur per year [12]. Although cosmopolitan in distribution, it mainly occurs in the tropics and sub tropics and other places especially in areas where there is low level of sanitation and very poor personal hygiene practices [13]. The prevalence of *Entamoeba histolytica* infection is as high as 50% in areas of Central and South America, Africa and Asia [16]. In Nigeria, amoebiasis is prevalent and widespread which has been attributed to quite a number of multiple environmental sources of transmission such as poor education, poverty, overcrowding, contaminated water supply, and unsanitary conditions [9,14]. The several surveys have indicated a high prevalence of *Entamoeba histolytica* infections among Nigerian children in different localities [10]. In Anambra State, Southeastern part of Nigeria, the prevalence of *E. histolytica* infection was found to be 12.6% [10].

Infection with *E. histolytica* may be asymptomatic or cause intestinal or extraintestinal disease [10]. Asymptomatic infection with *E. histolytica* is associated with a positive serum amoebic anti-amoebic antibody and a positive stool antigen test. Clinical syndromes associated with intestinal *E. histolytica* disease are bloody diarrhoea, weight loss, fatigue, abdominal pain, acute rectocolitis, fulminant colitis (acute necrotizing colitis) with perforation, toxic megacolon, chronic non dysenteric colitis, amoeboma, and perianal ulceration [10]. Liver abscesses due to amoebiasis are ten times more frequent in adults than in children. Very young seem to be pre-disposed to fulminant colitis. Amoebic colitis affects both sexes equally [15].

Various virulence factors of *E. histolytica* such as adhesins, toxins, amoebapores and proteases are powerful weapons that lead to the lysis, death and eventually destruction of the host tissues [5]. *Entamoeba dispar* which is non-pathogenic, non-invasive protozoa and cannot be distinguished morphologically from *E. histolytica* is ten times more common than *Entamoeba histolytica* and can only be detected by molecular-based techniques [6]. However, only 10% of *E. histolytica* infections cause invasive disease. Hence, only 1% of persons with stool microscopy findings that reveal *Entamoeba* develop symptomatic amoebiasis. *E. histolytica* can be diagnosed routinely using faecal samples because results are available quickly but certain other species are impossible to distinguish by microscopy alone [1]. There are presently several commercially available antigen kits for detecting *Entamoeba* antigen in stools, these tests however require fresh stool for analysis, and only a few kits have been reported to be specific for *E.histolytica* not *E. dispar*.

The aim of the study was therefore, designed to determine the prevalence of *E. histolytica* among school children in Lagos State, Nigeria using two different methods of diagnosis and also to determine the association of *E. histolytica* infection in relation to packed cell volume (PCV) and serum ferritin.

2. Materials and Methods

2.1 Study Location

The study was carried out in five local Government Areas within Lagos State which comprise: Mushin, Surulere, Ajeromi-Ifelodun, Badagry and Ojo Local Government Areas with estimated populations of 633,009; 503, 975; 684,105; 426,735 and 598,071 respectively according to the 2006 population census. These areas are located in the rain forest area with distinct rainy and dry seasons. These local government areas are predominantly with clustered homes, poor sanitary conditions and are densely populated with indiscriminate disposal of human wastes in

drainages, streams and rivers. School children within the age bracket 2-13 years were recruited for the study. The children were randomly selected from the schools in all the five local government areas.

2.2 Study design

A total number of five hundred and forty (540) school children were examined for *Entamoeba histolytica* infection. The parasitological survey was preceded by a pre-survey contact during which verbal permission was obtained from the Ethical review Committee and parents of the participating school children.

2.3 Sample Collection

School children were educated on how to collect fresh morning stool sample into sterile containers which were labeled and distributed to each of the participating school children a day preceding the program. Stool samples were collected as soon as they arrived at the school premises, and were packaged and transported to the laboratory for examination [8]. Three millilitres of venous blood sample was collected from each of the participants and transferred into EDTA containers and mixed gently [7].

2.4 Sample Analysis and Identification

Stool samples were visually examined to note the consistency, presence of abnormal features; whether watery, bloody, with mucus, formed or unformed [8]. Direct smear saline-iodine preparation and floatation techniques of stool samples were examined for cyst or trophozoite of *Entamoeba histolytica* parasites under the microscope using x10 and x40 objectives lenses as recommended by World Health Organisation [8].

2.5 Determination of Packed Cell Volume and Serum Ferritin

The packed cell volume (PCV %) was determined by centrifuging microhematocrit tubes filled with whole blood in a microhaematocrit rotor for 5 min at 10,000rpm and was read with the haematocrit reader [7] while serum ferritin was estimated using commercially available rapid test kit produced by TECO DIAGNOSTICS, Lakeview Ave. Anaheim CA U. S.A. The procedure was according to the manufacturer's instruction.

2.6 Enzyme Immunoassay for the detection of *E. histolytica*

Enzyme immunoassay was used for the quantitative determination of *E. histolytica* antibody in serum using commercially available test kits by Diagnostic Automation/ Cortez Diagnostics, Inc. Calabsas, CA USA. The assay was carried out according to the manufacturer's instruction.

2.7 Statistical analysis

Data analysis was carried out using SPSS version 16. Frequency tables and cross tabulations were produced for each of the study variables. Relationship between independent and dependent variables was assessed using chi-square test. Statistical significance was achieved if $P < 0.05$.

3. Results

A total number of five hundred and forty (540) stool and blood samples were examined from school children in the study areas. The mean age and mean packed cell volume were 7.5 ± 2.78 and 37.81 ± 2.4 respectively while the mean serum ferritin $\pm$ SD was 74.6 ± 9.62. Age group 6-9 years had the highest number of participants (43.5%) while age group 10-13 years had the least (24.6%). The male to female ratio was 27.1: 26.9 (Table 1).

Prevalence of *Entamoeba histolytica* infection among school children by age and sex is shown in Table 2. The prevalence of *Entamoeba histolytica* was 10.9%. Age group 2-5years had the highest prevalence rate of 12.2% while age group 6-9years had the least (10.2%). There was no significant difference between age group and *Entamoeba histolytica* infection ($p=0.3707$). Male (13.3%) were more infected than their female counterpart (8.6%) and there was no significant difference between *Entamoeba histolytica* infection and age ($p = 0.723$).

The association of *Entamoeba histolytica* infection in relation to packed cell volume and serum ferritin is shown in Table 3. Non infected school children had more mean packed cell volume (38.19 ± 2.74) than the infected ones (38.06 ± 3.92) and there is no significant difference between

packed cell volume and *Entamoeba histolytica* infection (P= 0.5897). The case was not the same with serum ferritin in which infected school children had more mean serum ferritin (76.00 ± 11.51) than the uninfected ones (73.61 ± 11.58), thus there was significant association between serum ferritin and *Entamoeba histolytica* infection (P= 0.0026)

Table 4 shows the detection of *E.histolytica* infection among school children using ELISA and microscopy methods. With microscopy, the prevalence of *E.histolytica* was 10.9% while it was 49.6% using ELISA. Detection rate for microscopy method was 22.1% while the ELISA method was 100%.

Table 1: Characteristics of study subjects of *Entamoeba histolytica* based on different diagnostic methods

Characteristics	Number (%) n= 540
Age Group	
2-5	172 (31.9)
6-9	235 (43.5)
10-13	133 (24.6)
Sex	
Male	271 (51.0)
Female	269 (49.0)
Mean age ±SD	7.5 ±2.78
Mean PCV ±SD	37.81 ± 2.4
Mean Serum ferritin ±SD	74. 63 ± 9.62
***E. histolytica* positive by Microscopy**	59 (10.9)
***E. histolytica* positive by ELISA**	268 (49.6)

Table 2: Prevalence of *Entamoeba histolytica* infection among school children by age and sex

Parameter	No. Examined	No. Infected	% Infected	P-value
Age Group				
2-5	172	21	12.2	
6-9	235	24	10.2	0.3707
10-13	133	14	10.5	
Total	540	59	10.9	
Sex				
Male	271	36	13.3	
Female	269	23	8.6	0.723
Total	540	59	10.9	

Table 3: Association of *Entamoeba histolytica* infection in relation to Packed Cell Volume and serum ferritin

Parameter	Volume	P- value
Mean Pack Cell Volume (%)		
Infected ±SD	38.06 ±3.92	0.5897
Non infected ±SD	38.19 ±2.74	
Mean Serum Ferritin /Ug		
Infected ±SD	76.00 ±11.58	0.0026
Non infected ±SD	73.61 ±11.51	

Table 4: Detection of *Entamoeba histolytica* infection among School children Using ELISA and Microscopy methods

Methods	Number		
	Positive	**Negative**	**Detection rate among positive participants (n= 268)**
Microscopy	59	481	59 (22.1%)
ELISA	268	272	268 (100%)

4. Discussion and Conclusion

Entamoeba histolytica is second to malaria as a protozoan cause of death worldwide [19]. About 50 million people are believed to be infected at any one time, and up to 100,000 deaths occur per year [12]. Although cosmopolitan in distribution, it mainly occurs in the tropics and sub tropics and other places especially in areas where there is low level of sanitation and very poor personal hygiene practices [13]. In this study, the prevalence of *Entamoeba histolytica* by direct saline-iodine examination of stool samples microscopically was 10.9% while by ELISA using serum samples was 49.6%.The difference in the prevalence of *E. histolytica* showed that direct stool microscopy method alone was not effective any longer for the diagnosis of amoebiasis and this is in tandem with the report of Haque et. al. [11]. The prevalence rate of 10.9% by direct saline-iodine method was similar to the work conducted in Anambra State, Southeastern part of Nigeria by Aribodor et.al., [10] with prevalence rate of 12.6% and almost similar with Akingbade et. al., [18] in Abeokuta, Southwestern part of Nigeria with prevalence rate of 19.4%. The prevalence of *E. histolytica* recorded in this study is quite lower than those obtained in Kano by Ibrahim, [13] (45.0%) and in Kaduna by Obadiah [17] (37.6%). In Nigeria, amoebiasis is prevalent and widespread which has been attributed to quite a number of multiple environmental sources of transmission [9,14]. Several surveys have indicated a high prevalence of *Entamoeba histolytica* infections among Nigerian children in different localities [10].

In this study, the highest prevalence of *Entamoeba histolytica* infection was recorded in age group 2-5years (12.2%), followed by age group 10-13years (10.5%) and the least age group was 6-9years (10.2%). This result is in agreement with the work of Simon-Oke and Ogunleye, [22] which also recorded highest prevalence among the same age group but not in tandem with the work of Oti et. al., [23] who recorded highest prevalence among the age group 6-10. Besides, there was no significant association between age and *Entamoeba histolytica* infection (P = 0.3707). The reduction in prevalence as the age increases could be associated with a higher level of awareness of personal hygiene as they grow older. It was observed that male (13.3%) school children have higher prevalence than their female counterparts (8.6%) and the difference was not statistically significant (P = 0.723). This agreed with the work of Aribodor et al. [10] that showed that males (16.1%) were more infected than females who had a prevalence rate of 9.3%. Although, gender is not a significant risk factor for the prevalence of *Entamoeba histolytica* infection, it is however important to note that more males were enlisted in the study than females.

Mean serum ferritin levels was higher in infected school children (76.00 ±11.58 ug/ml) than their non-infected counterparts (73.61 ±11.51 ug/ml) and there was no significant difference between packed cell volume and *Entamoeba histolytica* infection (P= 0.5897). This result agreed with Silva et al., [20] but not with Le et. al., [21] who showed that mean serum ferritin was concentrated more among non-infected school children than infected with *Entamoeba histolytica*. Statistical analysis showed that there was significant association between serum ferritin and *E.histolytica* infecti.on (P = 0.0026). The packed cell volume (PCV) recorded in this study varied between infected school children when compared with non-infected school children. The results showed that mean packed cell volume was higher in non- infected school children (38.19 ±2.74) than in infected ones (38.06 ±3.92) and there was no significant association between packed cell volume and *E.histolytica* infection (P= 0.5897).

In this study, ELISA was able to detect *E. histolytica* when compared with microscopy. ELISA showed a better sensitivity than microscopy because microscopy cannot distinguish between *E. histolytica* and other forms of amoebiasis such as *E. dispar*. Generally in terms of sensitivity and specificity, most studies have shown microscopy to be more sensitivity and specific than ELISA. A study conducted in Turkey reported microscopy as being more sensitive and specific than ELISA in the detection of *E. histolytica* [4]. Another sets of studies in Kilimanjaro and New Delhi also reported microscopy as being more sensitive than ELISA in the detection of *E. histolytica* [2,3]. The higher detection rate in this study is attributed to the fact that ELISA IgG was used which is an indication of high exposure rate rather than high positivity rate in the study area. The high IgG rate means that most of the participants have been exposed to the parasite which is an indication that the parasite may be endemic in this environment. This study is limited in several aspects as we did not cover most senatorial area and this is due to financial constraint. A future study needs to be done in order to include other senatorial districts in the study area, so that a larger, more representative sample size for the state would be collected for analysis.

Amoebiasis can be prevented by increased sanitation, effective and safe disposal of human waste. Travelers should avoid unwholesome fruits and fresh vegetables of doubtful sources. They should drink only boiled or bottled water. Avoiding sexual practices that involve faecal-oral contact can reduce infection in homosexuals. In mental institutions recurrent outbreaks of amoebiasis can be prevented by routine screening of stool and adequately treating infected patients.

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