Original Research Article

THE SERO-EPIDEMIOLOGY AND RISK FACTORS OF *E.HISTOLYTICA* INFECTION IN CALABAR, NIGERIA

Abstract

Aim of the study: Aim of the present study was to investigate the sero-epidemiology and risk-factors of *Entamoeba histolytica* infection among dysentery patients presenting at general hospital calabar, Cross river.

Methods: The Sero-epidemiology of *E. histolytica* was determined in three hundred and eighty-one subjects in calabar,Cross river state using an enzyme-linked immunoassay. In addition, sero-prevalence association with the socio-demographic and risk factors of the subjects studied was also investigated.

Results: Forty five (45) out of the three hundred and eight one (381) samples were positive for *E.histolytica*, 45(11.8%). Subjects in the age group 1-10 years had the highest prevalence (32.8%). The study also investigated that *E. histolytica* infections was associated with age, educational status, occupational status, source of water, toilet facility, hand washing and contact with domestic animal/faecal matter($p<0.05$). Females were more infected (13.5%) than male (9.9%) but the difference was not statistically significant ($p> 0.05$). The infection rate was higher in the wet season (18.1%) than the dry season (4.9%). However, *E. histolytica* infection was statistically associated with season ($p<0.05$).

Conclusions: The sero prevalence of *E. histolytica* infection found in this study is moderate compared with those reported in other Nigerian population. The data of *E. histolytica* sero-positivity found in the present study may be useful for the planning of optimal preventive measures against *E. histolytica* infection.

Keywords: *E. histolytica* sero-positivity infection prevalence

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INTRODUCTION

Entamoeba histolytica is a protozoan parasite that causes amoebiasis, high morbidity and mortality of amoebisis have been reported worldwide. Infections with *E. histolytica* are prevalent in the tropics and is a major health problem in developing countries[3,4], Majority of the *E.histolytica* infection morbidity and mortality occurs in Africa, Central and South America and the Indian sub-

continent. The prevalence of amoebiasis due to *E. histolytica* has been difficult to establish because there is a probability of overestimating it in endemic areas, where cases of dysentery or bloody diarrhea are often misdiagnosed as amebiasis due to the non-pathogenic *E.dispar*[6].

In non-endemic areas with a low incidence of the disease, there is a tendency to underestimate, the prevalence of *E. histolytica* infection due to other bacterial and viral pathogen causing dysentery and diarrhea [7]. Studies in parts of Africa reported prevalence rates of 22% and 21% in South Africa and Egypt, respectively [8]. In Nigeria, prevalence rates of 21.6% in Enugu [9]and 13.7% in Ilesha have been reported[10].The rate of infection by *E. histolytica* differs among countries, socio-economic and sanitary conditions and population's [8].It is highly endemic throughout the tropics and subtropics [11]. Environmental, socio-economic, demographic and hygiene-related behavior is known to influence the transmission and distribution of intestinal parasitic infections. Humans are the host of *E. histolytica* and there are no other known animal reservoirs of this parasite [5] Besides humans, E. histolytica also infects Nonhuman primates (NHPs), cats, and dogs [reference added) but no cross transmission vice versa

Most persons infected with *E. histolytica* are carriers [13]. Infection with *E. histolytica* is responsible for most cases of prolonged diarrhea in the travellers[14]. In addition to this infection with *E. histolytica* may lead to the development of life-threatening abscess in liver, brain [15] or lungs[5]. It is very important to investigate the sero-epidemiology of *E.histolytica* parasite in the population. Transmission of *E. histolytica* occurs in areas with poor sanitation by contamination of drinking water or food with human feces[16]. Water-associated outbreaks of *E. histolytica* disease have been reported[17].

There is paucity of data on the sero-epidemiology of *E. histolytica* infection in calabar, Cross river state. Most of data are based on microscopic diagnosis of *E.histolytica* in stool which could be non-Pathogenic *E.dispar*[18]. Furthermore, socio-demographic and risk factors of the subjects associated with *E. histolytica* and sero-prevalence was investigated for public health intervention.

Comment [d3]: Add the reference corrected as you are writing that prevalence is more in people living in the vicinity of animals so find which animals are affected by ameobiasis

MATERIAL AND METHOD

STUDY AREA

Cross river state derives its name from the river which passes through the state .It is a coastal state located in the Niger Delta,and occupies 20,156 square kilometers. It shares boundaries with Benue state to the North, Ebonyi and Abia state to the west, its shares boundaries with Cameroun to the east and to the south west by Akwa Ibom State, Calabar is located in The South South geopolitical zone of Nigeria with a population of 3,737,517 inhabitants, Calabar is often described as the tourism capital of Nigeria(19).

Sample size

The minimum sample size was determined by using the formula described by Naing *et al*,[20],Therefore, to obtain a more reliable result, A total of 381 patients sample was collected.

Ethical Consideration

Informed consent was obtained from each of the patient or their parents. The work was also approved by the Ethical Committee of the Ministry of Health of Cross River State. The general hospital Calabar used in this study.

Enrolment of patients

All patients presenting to the general hospital in Calabar with acute and persistent diarrhoea or dysentery within the 12 months (January to December, 2013) period of study were enlisted having consent to participate and fulfill the inclusion criteria which included acute or persistent diarrhoea and dysenteric syndrome. Patients with diarrhoea or dysentery on antimicrobial agents were excluded. Patients visiting the hospital for reasons other than diarrhoea and had no diarrhoeal illness within the last 2 weeks were used as control.

Data collection

A well-Structured questionnaire was used to obtain information from each patients on the demographics and risk factors. Demographics include; Age, sex, Occupational status and education. Risk factors includes; Toilet facilities, Source of water and Contact with domestic animals/Faecal matter.

Specimen collection and processing

Three hundred and eighty one samples (n=381) blood specimens were aseptically collected, One hundred and eighty three(n=183) samples were collected during the dry season (November-March)and One hundred and ninety eight samples(n=198) collected during the wet season(April-October) ,respectively. The sera obtained from clotted blood were centrifuged to obtain the serum from each patient presenting with dysentery or diarrhoea at general hospital calabar,Cross river State.

Analysis of samples

Detection of *E. Histolytica* antibody was carried out using the Enzyme linked Immunosorbent assay.

ELISA Antibody Detection Technique

The sera from the blood samples were analyzed using the Enyzme Linked Immunosorbent Assay (ELISA) made by TechLab (USA). The first well of the microplate was left blank (control) while 100ul of negative and positive controls were added to the second and third wells respectively. Two drops of the diluted test samples were added to the remaining wells and incubated at room temperature (15-20^{o}C) for 10 minutes. The contents were then shaken and washed 3 times with

diluted buffer. After washing, 2 drops of enzyme conjugate were added to each well and again incubated at room temperature for 5 minutes. This was followed by another shaking and washing again with buffer after which 2 drops of chromagen was added to each well and again incubated at room temperature. Finally, 2 drops of the stop solution were added to each well and mixed by tapping the strip holder. The results were read with a microplate reader machine set for biochromatic readings at 450/650-620nm. Positive and negative control sample was included for each batch of sample assayed.

Data analysis

The prevalence of *E. histolytica* was determined by the percentage of patients who tested positive, while the Chi-square was used to determine the association between *E histolytica* and the selected variables. All statistical analyses were carried out using the SPSS statistical software.

Table 1: Sero -Prevalence based on socio demographic factors among study population

Parameters/	**Number**	**Number**	**x^2dfp.value**

factors	Examined	Positive (%)	
Age group			
1 -10	61	20(32.8)	33.5 5 0.0012
11 -20	83	10(12.1)	
21 -30	104	5(4.8)	
31 -40	63	6(9.5)	
41 -50	40	2(5.0)	
>50	30	2(6.7)	
Sex			
Male	180	18(9.9)	1.2 1 0.283
Female	200	27(13.5)	
Occupational status			
Students	112	5(4.5)	
Unemployed	54	7(13.0)	
Farmers	43	14(32.6)	
Civil servants	63	3(4.8)	
Artisans	25	6(24.0)	
Business	84	10(11.9)	
Educational status			
Not educated	68	15(22.1)	11.6 2 0.003
Primary	70	5(7.1)	
Secondary	158	12(7.6)	
Tertiary	85	8(9.4)	

Table 2: Sero- Prevalence of *E. histolytica* among the study population based on risk factors

Risk Factors	Number Examined	Number Examined	x^2dfp.value
Source of water			

Bore hole	201	28(13.9)	49.3	2	0.003
Tap water	164	7(4.3)			
Well	16	10(62.5)			
Toilet facility					
Pit	26	10(38.5)	19.0	1	0.0012
Water cistern	355	35(9.9)			
Hand washing /personal hygiene					
Washes hands with soap	281	25(8.9)	8.7	1	0.002
Washes Hand without soap	100	20(20)			
Contact with domestic animal/fecal matter					
Yes	256	40(15.6)	10.9	1	0.001
No	125	5(4.0)			
Season					
Dry	198	36(18.1)	16.1	1	0.014
Wet	183	9(4.9)			

RESULTS

Out of the (n=381) subjects studied,45(11.8%) were sero-positive for *E.histolytica* infection, based on age. The age group 1-10 years of the study population had the highest prevalence 32.8% ,while those in age group 40-50 years had the lowest prevalence rate of 4.8%. Statistical analysis showed that the difference was significant (P=0.0012)(Table 1).

E.histolytica infection among the subjects studied showed no significant association (P=0.283) with respect to gender in the study population. The female subjects had a prevalence rate of 13.5% and the male subjects have a prevalence rate of 9.9%(Table 1).The subjects whose occupation were farmers, had the highest prevalence rate of *E.histolytica* infection of 32.6% while the subjects whose occupation were students had the lowest prevalence rate of 4.5%.This difference was statistically significant(P=0.002)(Table 1).Subjects not educated had the highest prevalence rate of *E,histolytica* infection of 22.1%, while subjects with primary education level had the lowest prevalence rate of 7.1%.*E.histolytica* infection was statistically associated with educational status (P=0.0014),(Table 1).

Subjects whose source of drinking water was wells had the highest prevalence rate of 62.5% of *E.histolytica* infection , while subjects whose source of drinking water was tap water and bore hole had the prevalence rates of 4.3% and 13.9%, respectively. This difference was significant (P=0.003),(Table 2). The highest prevalence rate of *E.histolytica* infection was recorded in subjects that use pit latrine, there was a significant association between the type of toilet facility and *E.histolytica* infection (P=0.0012)(Table 2).Subject that washes their hands without

soap had the highest prevalence rate of 20.0%, while those that washes their hands with soap had a prevalence rate of 8.9%. This difference was significant(P=0.002) (Table2). *E.histolytica* infection was associated with contact of domestic animal/faecalMatter (P=0.001)(Table 2). The prevalence of *E.histolytica* infection among participants in the study population was highest in the rainy season 18.1% than in the dry season 4.9%. The association of *E.histolytica* infection with seasons was significant (P=0.0014).(Table 2).

Discussion

E. histolytica infection predominates in developing countries and represents a major public health problem in tropical countries [21]. This study established a low sero-prevalence rate of *E.histolytica* infection (11.8%) in the study population when compared with 22% and 21% reported in south Africa and Egypt respectively. In Nigeria, 21.6% in Enugu 14.3% ,in Kaduna and 13.7% in Ilesa have been reported by the researchers[8,9,10,22]. Although, slightly higher than 10 % reported by World health organization in developing countries[23].The reasons for the disparity in the variation of prevalence rate of *E.histolytica* infection could be attributed to geographical, study design, seasonal, diagnostic methods, patients selection and behavioral factors in the different study population.
The age group 1-10 years were among the most infected with *Entamoeba histolytica* infection with a prevalence rate of 32.8% in agreement with Zahida *et al.* (2010)[24], their study, reported that age is an important risk factor for many infectious diseases especially those that are transmitted orally such as *E. histolytica*(24 ,25). The current study findings are also in consistence with previous studies done in Pakistan and Bangladesh which reported that infection with *E. histolytica* is most common among young children who are predisposed to contact with infected material as they crawl on the ground or play games outdoors [5,25] In addition, Children are less acquainted with hygiene habits which also makes them more vulnerable to infection [21]. The result from this study,found people from all age groups were infected with *E.histolytica*, Although, there is variation of prevalence rate among the different age groups. This is consistent with the results by Zahida *et al.* (2010)[24]. The association of *E.histolytica* infection with age is in agreement with Al-Harthi and Jamjoom, (2007) and Zahida *et al*,(2010) [5,7]. who reported similar results.
The lack of significant association of *E.histolytica* infection with gender observed in this study is consistent with Dawah *et al*,2010 [22], attributed to equal exposure of both sex in the study area to the risk factors of *E.histolytica* infection. Although, prevalence of 13.5 % of *E.histolytica* infection in females was higher than male ,it is inconsistence with other previous studies on amoebiasis. Jamaiah

and Shekhar(1999) and Stauffer *et al*(2006)[8,27] who reported higher prevalence in males.

The high prevalence rate reported in females could be attributed to women being culturally expected to be involved in more domestic chores than males, These may bring them into constant contact with contaminated fruits, vegetables and water which potentially promote oral transmission of the disease through contaminated hands. This might account for the slightly higher level of infection among the female population similar to report by Haque*et al*. (2006)[5].

Interestingly, occupation was associated with an increased risk of *E.histolytica* infection. Farmers had the highest prevalence rate of 32.6% of *E.histolytica* infection, which is attributed to the nature of their occupation where they expose themselves with human and animal excreta especially when using it as manure. This findings from the current study, is in contrast with Pham duc *et al*,(2011)[28]. In their study of the risk factors of *E.histolytica* in agricultural communities in Vietnam.

The non-educated subject had highest number of *E. histolytic*a prevalence rate 22.1% when compared with primary, secondary and tertiary educated subjects, Education was significantly associated with *E.histolytica* infection in this study. Education is regarded as one of the parameter of determining personal well-being especially in improving hygiene. Ross *et al*. (2003) studied that people with higher level of education tend to be healthier than those of similar income who are less educated because they seek medical attention early[29,30,31] found ignorance, toilet habit and degree of literacy as serious risk factors for amoebiasis. This is in consistence with our findings in the present study.

The results from this study showed significant association of *E.histolytica* infection with source of drinking water. The association of *E. histolytica* with the source of drinking water of the patients agreed with the findings of Cox (2001), Olsen *et al*,(2001), Ogunlesi *et al*,(2005) and Rinne *et al*,(2005)[10,32,33,34],all of them reported that the source of water is a risk factor for amoebiasis. Most of the subjects in this study obtained water from bore hole which are less likely to be contaminated with *E.histolytica* parasites when compared with well water. The subject who use well water as a source of drinking water had the highest prevalence rate of *E.histolytica* infection. Most of the wells in the study area were manually dug, uncemented with no casing or covering. Sometimes, the well is contaminated with surface run-off which may be faecally contaminated.

There was significant association between personal hygiene and *E.histolytica* infection, this is attributed to the route of transmission of *E.histolytica* infection which is faecal –oral transmission. This is in agreement with Espinosa-Cantellano 2000; Ryan and Ray, 2004, which reported similar findings[30,35].Close contact with domestic animals/faecal matter was associated with an important risk factor

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for *E.histolytica* infection in this study . The subject with the highest prevalence of 15.6% are respondent with contact domestic animals/faecal matter. It is possible that cysts of *Entamoeba histolytica* deposited on the surface (fur) of the animals during close contact with humans and then later transmitted to a next person via faecal-oral route of transmission.

The seasonal variation was observed in this study, there was association of *E. histolytica infection* with the season. Higher prevalence rate of 18.1% *E. histolytica* infection was reported during the wet season in this study but slightly lower than 26.9% reported by Dawah *et al*,2010 in Kano[22], but consistent with some of the earlier studies of Park (2002) and Mawashi (2003),who reported similar prevalence rates in their studies[36,37]. Higher rate of fecal-oral contamination may be implicated during wet season and coincides with intensive farming activities. Low prevalence rate of parasite during the dry season is attributed by high temperature and low humidity which is not favourable for parasite growth.

Conclusions To curb the relatively high levels of amoebiasis in Calabar there is need for surveillance systems and health education targeting parents and guardians of children under five years age which are aimed at early and proper treatment of the disease. The Ministry of Health should intensify health campaign especially in children less than five years of age and their parents / guardians, particularly females, on ways to improve hygiene practices at home to avoid infection. There is need for residents in Calabar, Cross River State to emphasize use of safe water for all domestic chores if the benefit of personal hygiene is to be realized.

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