

Asymptomatic carriage of cysts of *Entamoeba Histolytica* among patients attending two hospitals in Maiduguri, Northeastern, Nigeria: the need for adequate tool for specific diagnosis.

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ABSTRACT

Amoebiasis is the third common parasitic infection in about 10% of the world's total population causing death after Schistosomiasis and malaria. A study was under taken to determine the prevalence of asymptomatic carriage of *Entamoeba histolytica* (*E.histolytica*). Two hundred (200) samples of stool were analyzed between March to October, 2010. Formol-ether concentration technique and iodine wet preparation was used for stool analysis. An overall prevalence of *E. histolytica* among asymptomatic patients was 56% out of the 200 samples examined. Males recorded a prevalence rate of (66%) and lower infection rate was observed among females (45.4%). Highest prevalence 73.9% was observed among age group 0-10 years while lowest (31.6%) was observed among age group 51-60 years. Subjects with secondary education had highest prevalence (82.1%) while those with tertiary education had lowest (30.2%). School-aged children had the highest cyst carriage (80.6%) while students had lowest (28.2%). Individuals whose source of drinking water is pipe borne water had highest cyst carriage (97.8%) while lowest cyst carriage (66.7%) was observed among those who drank well water. Highest cyst carriage (78.7%) was observed among males while lowest (30.8%) was observed among females who ate home-made food more often. Peri-urban settlers had the highest prevalence (65.9%) while lowest (36.5%) was observed among those who reside in highbrow areas. The 56% prevalence of asymptomatic cyst carriage calls for concern because of the possible health implications of amoebiasis and the risk of transmission to healthy individuals. There is need for a more sophisticated tool for specific diagnosis of the parasite.

1. INTRODUCTION

Amoebiasis is the third common parasitic infection in about 10% of the world's total population causing death after Schistosomiasis and malaria [1]. Amoebiasis has been a big problem of human civilization at the beginning of 21st century, so every study from this field is valuable [2]. In tropical areas with poor sanitation, infection rates are estimated to reach 50% of the total population [1]. Yet, about 90% of the infections are found to be asymptomatic, while the remaining 10% produce a spectrum of symptoms varying from dysentery to amoebic liver abscess.

[3] reported that *E.histolytica* was by far the most frequently encountered pathogen found in association with bloody diarrhoea. The method used to come to that conclusion consisted of microscopic examination of a simple wet smear, which is nonspecific in the diagnosis of amoebiasis.

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Over the years, lots of work has been done on *Entamoeba histolytica* in other parts of the world and parts of Nigeria, but very little has been done from this part of Nigeria and more so the asymptomatic cases of this area are not known which can put the larger community at risk of its outbreak since the carrier serve as the reservoir (i.e. source of infection to others) through food and water contaminations. Hence this work aimed to determine the prevalence of *E. histolytica* among asymptomatic carriers in patients attending hospitals in Maiduguri. The information obtained would be used in creating awareness of the disease and emphasize the need for good personal hygiene.

Keywords: Asymptomatic, diagnostic tool, *Entamoeba histolytica*, Maiduguri, Nigeria.

1. MATERIALS AND METHOD

A prospective study was carried out between March – October 2010.

Study area

Two sites were selected for the purpose of this research within Maiduguri Metropolis. Site A is University of Maiduguri Teaching Hospital and site

B is the State Specialist Hospital, Maiduguri. These hospitals were chosen because they are the largest hospitals in Maiduguri with the former serving as a referral hospital for the North Eastern Nigeria while the latter serves as a General Hospital mostly for the Low Socio-economic class. Therefore data from these hospitals can be a representative of subjects of various socio-economic groups.

Maiduguri, the state capital of Borno state is the largest of the six states in the north eastern zone of Nigeria. It lies on latitude 11° North and longitude 15° East. It occupies an area of 50,778 square kilometer. Borno state shares borders with republic of Niger to the North, Chad to the North East and Cameroun to the East. Borno states have an estimated population of 4,098,391 people according to 2006 national population census.

Ethical consideration

Approval was obtained from the research and ethical committees of the two hospitals.

Recruitment of patients

Patients seen at the General Outpatient Clinics of the hospitals referred to the Medical microbiology laboratories for test different from parasitological investigations were enrolled after seeking an informed consent from them. One hundred patients were enrolled from each hospital.

Questionnaire design

A questionnaire was filled for each patient that carries the following questions - Age, Sex,

Educational background, Residential address, marital status, Occupation, Source of drinking water, usual diet and History of recent diarrhoea.

Sample collection

Stool sample was collected from patients using the universal container for sample collection. Two hundred (200) samples of stool, 100 from each hospital, were collected in a clean screw cap bottle. Accordingly, the sample bottles were labeled based on the site of collection, A and B. The samples were always taken to the laboratory and immediately analyzed microscopically in accordance with [4].

Analysis of Samples

Each specimen was prepared using the formol-ether concentration technique. A piece of stick was used to emulsify about 1g of the stool sample in 7ml of formol-saline in a universal bottle. The mixture was sieved with a wire mesh sieve and the filtrate was transferred to a centrifuge tube to which 3mls of ether was added and mixed vigorously for 15 seconds. The preparation was then centrifuged at 3000 revolutions for 1 minute. The supernatant was discarded and a small amount of the deposit was dropped on a clean grease-free slide, a drop of Lugol's iodine was added and covered with a cover slip. The entire preparation was examined systematically for cysts of *E. histolytica* using X10 objective of the light microscope. Several microscopic fields were always examined with x40 objective before reporting "no cyst found". The

iodine preparation was used to assist in the identification of the cysts.

Statistical Analysis

Statistical analysis was carried out using GraphPad InStat.

3. RESULT

An overall prevalence of *Entamoeba histolytica* among asymptomatic patients was 56% out of the 200 samples examined. Males recorded a prevalence rate of (66%) and lower infection rate was observed among females (45.4%) (Table 1). Highest prevalence (73.9%) was observed among age group 0-10 years while lowest (31.6%) was observed among individuals with age 51-60 (Table 2). Subjects with secondary education had highest prevalence (83.6%) while those with primary education had lowest (34.9%) (Table 3). School-aged children had the highest cyst carriage (80.6%) while students had lowest (28.2%) (Table 4). Individuals whose source of drinking water is pipe borne water had the highest cyst carriage (75.3%) while lowest cyst carriage (45.5%) was observed among those who drank well water (Table 5). Highest cyst carriage (78.7%) was observed among males who ate commercially sold food more often while lowest (30.8%) was observed among females who ate home food often (Table 6). Highest prevalence (65.9%) was observed among those who reside in Peri-urban setting while lowest (36.5%) was observed among those who reside in highbrow areas (Table 7).

Table 1: Prevalence based on collection sites

	NUMBER TESTED		NUMBER POSITIVE (%)	
	M	F	M	F
SITE A	54	46	35 (64.8)	27 (58.7)
SITE B	49	51	33 (67.3)	17 (27.5)
TOTAL	103	97	68 (66)	44 (45.4)

($X^2=0.6955$, $P>0.05$)

Table 2: Age specific prevalence

Age (years)	Number tested	Number of <i>E.histolytica</i> cysts seen (%)
0-10	23	17 (73.9)
11-20	29	16 (55.2)
21-30	52	31 (59.6)
31-40	44	29 (65.9)
41-50	28	11 (39.3)
51-60	19	6 (31.6)
>60	5	2 (40)
TOTAL	200	112 (56)

($X^2 = 4.69$, $P > 0.05$)

Table 3: Prevalence based on educational background

Educational Background	Number examined	No. Positive (%)
Primary	47	26 (55.3)
Secondary	67	56 (83.6)
Tertiary	86	30 (34.9)
Total	200	112(56%)

($X^2 = 10.04$, $P > 0.05$)

Table 4: Occupational prevalence

Occupation	Number examined	Number positive (%)
Civil servant	55	20 (36.4)
Business	41	22 (53.7)
Farmers	13	4 (30.8)
Students	39	11 (28.2)
School-aged children	31	13 (80.6)
Artisan	21	25 (61.9)
Total	200	112

($X^2 = 6.76$, $P > 0.05$)

Table 5: Prevalence based on source of drinking

water.

Source of drinking water	Number examined	Number positive (%)
Pipe borne water	89	67 (75.3)
Well water	33	15 (45.5)
Bore hole	78	40 (51.3)
TOTAL	200	112

($X^2 = 3.39$, $P > 0.05$)

Table 6: Prevalence in relation to usual diet
among male and female patients.

Sex	Usual diet	Number tested	No. of Parasite seen (%)
Male	Home food	33	19 (57.6)
	Commercial Food	75	59 (78.7)
Female	Home food	53	12 (30.8)
	Commercial Food	39	22 (41.5)
Total		200	112

($X^2=0.2662, P>0.05$)

Table 7: Prevalence in relation to residence.

Residence	Number tested	Number of Parasite seen (%)
High brow	52	19 (36.5)
Peri-urban	94	62 (65.9)
Low class	54	31 (57.4)
Total	200	112

$(X^2 = 3.59, P > 0.05)$

4. DISCUSSION

This study provides an insight of the asymptomatic carriage of *E.histolytica* among patients who do not present with diarrhoea in Maiduguri. The 56% prevalence is relatively high and calls for concern since most of these patients do not present any symptoms of amoebiasis. This prevalence is higher than the estimated 50% prevalence rate of a total population in Tropical areas [1] because the study was carried out on patients that attended hospitals and not a population study. [5], [6] and [7] further added that *E.histolytica* is quite rare not only in asymptomatic cases, but even in patients with bloody diarrhoea, the great majority of trophozoites and cysts found appeared to belong to either *E.dispar* or entirely different intestinal amoeba.

Prevalence based on sex revealed 66% in males and 45.4% in females respectively. This is in agreement with previous workers [8]. However, any observed differences that have been reported in the incidence and prevalence of amoebiasis between sexes are probably related to exposure than a true sex susceptibility to infections [9].

Highest prevalence 73.9% was observed among the age group 0-10 years. This is in agreement with previous work by [10]. They observed that generally, children are more susceptible to food-borne and water-borne infections because their playing and hygiene practices predispose them to infections than older age groups, more so their level

of health education is not sufficient to distinguish the dangers of contamination.

With regards to level of education, subjects with secondary education had the highest number of infection (83.6%). This could be due to the relatively low level of hygiene among this group of individuals coupled with the poor environmental sanitation that affects almost everyone in Nigeria. The low rate of infection (34.9%) observed among patients that attended a tertiary institution might be due to the fact they are more often enlightened and economically stable [11].

Occupational prevalence reveal highest among the school-aged children (80.6%). Observed reasons may be due to children's habits of playing outdoors that make their hands to be contaminated with soil and consequently with unwashed hands [11]. Prevalence based on source of drinking water reveals highest (75.3%) among people who drink pipe borne water. Reasons for this observation may be because tap water gets contaminated by sewage due to defects of plumbing and sanitation. [9] reported that leakages of pipes of public water supplies result in low water pressure and consequent contamination of the chlorinated piped water. Moreover, protozoan cysts are resistant to the concentrations of chlorine normally used for the treatment of domestic water supply [4].

Prevalence in relation to usual diet reveals highest among males (78.8%) and (41.5%) among females that eat commercially sold food. Several reasons for this abound in literature. [13] reported that cyst of *E.histolytica* remains viable for up to 5 minutes on

the surface of the hand and for about 45 minutes under the fingernails. Also, in liquid foods (Milk, yoghurt etc), the cyst may survive for as long as 15 days [14] The selling of ready - cooked foods on the streets of towns and cities as well as in rural areas is now a common practice; and the habit of purchasing and consuming meals and snacks prepared outside the home is typical of the present lifestyle of some homes in this country [9]. The majority of food vendors use pit latrines and other related structures and/or dung hills for defecation. These structures may however, facilitate contact with stools from which they can either acquire infection or carry eggs and cysts of parasites with which contamination of their wares may be inevitable thereby leading to widespread contamination of food and drink [2].

Prevalence in relation to residence reveals highest (65.9%) among those living in peri-urban areas. Reasons may be as [15] observed that several factors are responsible for high prevalence of amoebiasis in urban and semi-urban areas of Nigeria. These include; poor hygiene, poor housing and overcrowding, poor environmental sanitation and inadequate methods of disposal of human faeces and public refuse.[16]reported that the prevalence and presentation of both symptomatic and asymptomatic amoebiasis vary geographically and with the population of individuals affected; differing between countries and between areas with different socio-economic conditions.

5. CONCLUSION

AND

RECOMMENDATIONS

The 56% prevalence of asymptomatic carriage of cyst of *E.histolytica* carriage calls for concern because of the possible health implications of amoebiasis and the risk of transmission to healthy individuals. Promotion of better environmental conditions with serious emphasis on health education and personal hygiene especially eating and defecating habits will enhance the prospects for the control of enteric faeco-oral diseases in Nigeria. There is need for a more sophisticated tool for specific diagnosis of the parasite.

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