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Intestinal Amebiasis and associated risk factors among pregnant Women attending University of Maiduguri Teaching Hospital, Nigeria

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Abstract

Background
Entameba histolytica (*E. histolytica*), the etiological agent of intestinal amebiasis poses significant community health concern especially in pregnant woman due to their physiological status. Pregnancy-induced immunodeficiency can lead to reduced IgA activities which makes pregnant women susceptible to penetration of intestinal mucosal linings and placental barrier especially by *E. histolytica*.

Objective of study
This study aimed to determine the prevalence and associated risk factors of intestinal amebiasis among pregnant women with acute diarrhea (<3 days post onset) attending University of Maiduguri Teaching Hospital (UMTH), Maiduguri, Nigeria.

Materials and Methods
This hospital-based cross-sectional study was conducted among 200 randomly selected acute diarrheic pregnant women attending UMTH. Fresh fecal samples were collected and immediately analyzed microscopically for *E. histolytica oocytes* using iodine wet preparation method. Structured questionnaires were used to assess sociodemographic variables and risk factors for amebiasis. Data analysis was done using Statistical Package for Social Sciences (SPSS) version 22 (IBM, California Inc., USA).

Results
Out of the 200 fecal samples analyzed for *E. histolytica,* 10 (5%) were positive. Those infected by moderate *E. histolytica* cysts had the highest occurrence rate, 4 (40%) and least by those infected by many *E. histolytica* cysts, 1 (10%). *E. histolytica* infection was associated with rural residence (OR=6.86 [95% CI:1.42-33.2]), lower Socioeconomic status (OR=22.0 [95% CI: 4.69-103.1]) and primigravity (OR= 5.76 [95% CI:1.19-27.8]) ($p<0.05$). Drinking water from borehole (OR=0.24 [95% CI: 0.06-0.95], p=0.043) and large household (OR=0.1 [95% CI: 0.02-0.41], p=0.001) were significant risk factors of intestinal amebiasis.

Conclusion
The results indicate that *E. histolytica* infection is one of the etiological agents of acute diarrhea in study participants. Its recommended that pregnant women should avoid risk factors for intestinal amebiasis identified in this study.

Keywords: Immunosuppression, Amebiasis, Risk factors, Diarrhea.

Introduction

Entameba histolytica (E. histolytica) is an amoebic protozoan of genus *Entamoeba* and belong to the phylum *Sarcomastigophora,* subphylum, Sarcodina. [1] *E. histolytica* is the causative agent of amoebiasis. Amoebic dysentery occurs when *E. histolytica* trophozoites invade the wall of the large intestine and multiply in the submucosa, forming large flask-shaped ulcers referred to as bottle neck ulcers. [2] If the parasite reaches the blood stream, it can spread through the body most frequently ending up in the liver, where it causes extra-intestinal infection called liver abscess. [3] The incidence of liver abscess is less common in women and is rare in children under ten years. [3]

E. histolytica commonly colonizes the human gut asymptomatically. [4] Immunity plays a very critical role in an individual's protection against infection. [5] Intestinal amebiasis leads to significant morbidity and mortality in pediatric and adult populations worldwide. [6] Intestinal parasitism during pregnancy is of interest as it may affect the health of pregnant women and their offspring. Amoebiasis is known to be more severe in pregnant women. [6] This is associated with poorer maternal iron status and reduced fetal growth. [7] When infection of *E. histolytica* results in clinical symptoms, the disease is either intestinal or invasive amoebiasis. [3, 7]

Amoebiasis caused by *E. histolytica* kills between 40,000 and 100,000 people per year. [8] The infection occurs when man ingests food stuffs, vegetables or drinks water, milk, yoghurt, etc. fecally contaminated by cysts of the parasite. [9] The incubation period of *E. histolytica* varies greatly but is generally four to five days. [10] Housing conditions are an important determinant for developing intestinal amebiasis. [11] Risk factors for these infections include deprivation of access to clean water, inadequate hygiene habits, and inferior sanitary conditions. [12] As in the case of other neglected diseases, poverty in general is a condition correlating with intestinal amebiasis. [13] Although factors associated with parasitism in pregnant women are mainly the same as in other population groups, multiparity is an additional risk factor. [14] On a larger scale, people living below the poverty line in low-income countries,

especially young women and pregnant women, their infants, and children, are at a high risk of amebiasis. [15]

The role of food-handlers and vendors in the spread of faeco-oral parasitic diseases such as amoebiasis is epidemiologically significant. In Nigeria, as in many other developing countries, the selling of ready-cooked foods on the streets of towns and cities as well as in rural area is a common practice. The role of these food-handlers and vendors, with poor personal hygiene and dirty habits is very significant in transmission of amoebiasis. It was reported that 20-30% of the households' expenditure in developing countries (including Nigeria) is on street foods. [16] Pathogenic microorganisms that are indicator of fecal contamination, such, as *E. histolytica and Escherichia coli* are key in determining food and water safety. [17] Similarly, water contaminated with sewage waste has been reported as cause of outbreaks of amoebiasis. [16] The major cause of this contamination is usually due to defects of plumbing and sanitation.

It is a common occurrence in Maiduguri to observe leakages of pipes of public water supplies due to bursting of old pipes and destruction during road and house construction activities. These leakages result in low water pressure and consequent contamination of the chlorinated pipe water. In addition, *E. histolytica* survives for a long period in unchlorinated water, hence fecal contamination of springs and streams in our rural communities can be a source of major outbreaks of amoebiasis. Sanitation provision in Maiduguri is grossly deficient. Most people do not have access to hygienic toilet and large amount of fecal waste are discharge to the environment; thereby predisposing inhabitants to the spread of amoebiasis.

In Maiduguri, Balla *et al* reported an overall 56% prevalence of *E. histolytica* among asymptomatic persons attending two government hospitals. [18] This represents a significant health implication and risk of transmission to healthy and immunocompromised individuals, especially younger children and pregnant women. Amebiasis could be common among pregnant women due their physiologically reduced immunity. [18]

Pregnant women infected by *H histolytica* may come with bloody, dysenteric stools with moderate abdominal pain and tenderness. The diarrhea is marked, and secondary signs include fluid loss and electrolyte imbalance, which may adversely affect the outcome of pregnancy. [19] However, there has been no documentation of placental involvement or transmission of the parasite to the fetus. [18, 19] This study was designed to determine the prevalence of *E. histolytica* infection among pregnant women attending antenatal clinics of the University of Maiduguri Teaching Hospital, Maiduguri, Northeastern Nigeria.

Materials and Methods

Study Area and Design

The cross-sectional hospital-based study was conducted at the Parasitology laboratory of university of Maiduguri Teaching Hospital Maiduguri Borno, Nigeria. This hospital was chosen because it's the largest hospital and serves as the referral hospital in the North Eastern Nigeria.

Maiduguri, the state capital of Borno state is the largest city of the six states in the Northeastern Nigeria. It lies on latitude 11° north and longitude 15° east. It occupies an area of 50,778 square kilometers. Borno state shares borders with republic of Niger to the North, Chad to the North East and Cameroun to the East. Maiduguri have an estimated population of 1,112,449 people according to GeoNames geographical database. The occupation of most people in the area are farming, fishing, and rearing of live stocks. The inhabitants of Maiduguri often depend on well, borehole, rain water and stream for domestic use. According to the 2016 Nigerian National Population census, less than 5% of Maiduguri populace had access to portable tap water.

Study Population

This study was conducted on acute diarrheic pregnant women attending University of Maiduguri Teaching Hospital, for medical assistance.

Sample Size Calculation

Using the *E. histolytica* prevalence of 11.1% reported by Usip *et al* [6], a minimum sample size of 154 was calculated. By adding 20% attrition, two hundred (200) stool samples were collected from 200 randomly selected pregnant women attending antenatal clinic at UMTH between 15th January and 16th February 2013.

Specimen Collection

The participants were educated on how to collect fresh fecal sample. Prior to enrolment, the study was clearly explained to every participant and that gave informed consent before they were enrolled. Questionnaire was used to obtained demographic and obstetric information which include: age, gestational age and gravidity.

All study participants received stool sample containers and standard instructions on proper and safe collection and preservation of the samples. By agreement, researchers contacted participants by phone to insure that, once the sample was collected and stored in proper dark low-temperature home location, the assistants could pick it up from the participants' homes within 4 hours after evacuation. The researchers who were trained in biosafety and handling potential biohazard specimen standards, followed a protocol for stool sample collection, safe handling and delivery to the laboratory.

Study variables

The dependent variables were the prevalence of amebiasis while the sociodemographic and risk factors were the independent variables. Indices of socioeconomic status were defined by educational attainment, income, housing, and employment variables. Persons who had tertiary education, earn more than 200 US$ per month, resides in urban settlement and works in civil services were considered upper SES, while those that had had tertiary education, earn between 100 - 200 US$ per month, resides in rural settlement and employed through business were considered middle SES, and persons who had at most high school education, earn

less than 100 US$ per month, resides in rural settlement and works as peasant farmer or unemployed were considered lower SES.

Data Collection

Interviewer-administered questionnaire was used to assess sociodemographic conditions. These included were age, occupation, education level, civil status, monthly income, household, conditions, water availability, supply, washing fruit and vegetables.

Laboratory Analysis

Analysis of stool samples was carried by the researcher assisted by qualified laboratory

technicians. The consistency of the stool was checked and noted as formed, loose, watery, with mucus, with blood as previously explained by Cheesbrough [17]. Wet mount microscopy was used to analyze the samples. A drop of physiological saline was placed at the center of the left half of a microscope slide and another drop of Dobell's iodine in the center of the right half of the slide. About 2 grams of stool sample was picked using an applicator stick and mixed with the Dobell's iodine and an equal amount with normal saline until a smooth thin preparation was obtained. Each preparation was covered with a cover slip. The entire preparation was examined at a magnification of x40 under light microscope. The iodine preparations assist in the identification of the cysts. Normal saline preparation was used to examine for trophozoites on fresh diarrheic samples to avoid their encysting. The grading of cysts found per preparation was reported as follows: Scanty (1–3), few (4–10), moderate (11-20), many (21–40) and very many (>40) as described by Cheesbrough. [17]

Statistical analysis

Data were analyzed using Statistical Package for Social Sciences (SPSS) version 16 (California Inc., USA). Association between prevalence of amoebiasis and level of education, age, occupation, place of residence, water source, socioeconomic status and risk factors of amebiasis of the respondents were analyzed using Chi-square

and univariate logistics. P value ≤ 0.05 was considered statistically significant. Tables and charts were plotted where appropriate. Association between categorical variables (such as socio-demographic variable) and amebiasis prevalence were compared using Chi-square test. Data were subjected for univariate logistic regression to compute Odd Ratio (OR) at 95% confidence interval (95%CI) between *E histolyica* infected and uninfected subjects. P value <0.05 at 95% CI were considered statistically significant.

Ethical Consideration

Ethical approval for the study was obtained from the Human Ethical Research Committee (HREC) of University of Maiduguri Teaching Hospital, Maiduguri, Nigeria. Study was conducted in accordance with Helsinki declaration. All data were analyzed anonymously. A copy of the result was provided to the hospital management.

Results

This study was conducted on 200 pregnant women with acute diarrhea. Majority of them 62% were less than 26 years and reside in urban settlements, 61% (Table 1). One hundred and four (52%) were middle income earners, while 5% of them were multigravidae (Table 1). Out of the 200 fecal samples analyzed for *E. histolytica,* 10 (5%) were positive. Those infected by many *E. histolytica* cysts had the highest occurrence rate, 4 (40%) and least by those infected by 21 – 40 cysts (Figure 1). The prevalence of *E. histolytica* was highest in pregnant women >35 years, 2 (25%) and none in those 26 – 30 years (Table 2). It was also highest among those without formal education, 6 (15.8%) and least in those with primary education level. Intestinal amebiasis prevalence was relatively higher in rural resident than those who reside urban settlement (10.3% Vs 1.6%, respectively). Lower income earners had the highest prevalence of amebiasis, 6 (40.0%) than middle income and higher income earners. Those in 1st trimester and primigravidae had the highest prevalence of amebiasis, 1 (5.6%) and 8 (8.9%), respectively (**Table 2**). Lower Socioeconomic

status and primigravity were significant risk factors for intestinal amebiasis among pregnant women (p<0.05). (**Table 1)**

Pregnant women who had daily consumption vegetables food had higher cases of *E. histolytica*, 5.1% than those who do not, 4.6%. Those who had borehole as their only source of drinking water had relatively lower cases of *E histolytica*, 2.4 % than those who had tap water as their source of drinking water, 9.3%. Pregnant women with pit latrine as their toilet system had relatively higher cases of *E. histolytica*, 6.1% than those who used the water closet system, 4.5%. Those with dense household size had relatively lower cases of E histolytica, 1.9% than those with sparse house hold size, 16.3% (table 2). *E. histolytica* infection was associated with rural residence (OR=6.86 [95% CI:1.42-33.2]), lower Socioeconomic status (OR=22.0 [95% CI: 4.69-103.1]) and primigravity (OR= 5.76 [95% CI:1.19-27.8]) (p<0.05) (Table 2). Drinking water from borehole (OR=0.24 [95% CI: 0.06-0.95], p=0.043) and large household (OR=0.1 [95% CI: 0.02-0.41], p=0.001) were significant risk factors of intestinal amebiasis. (**Table 3)**

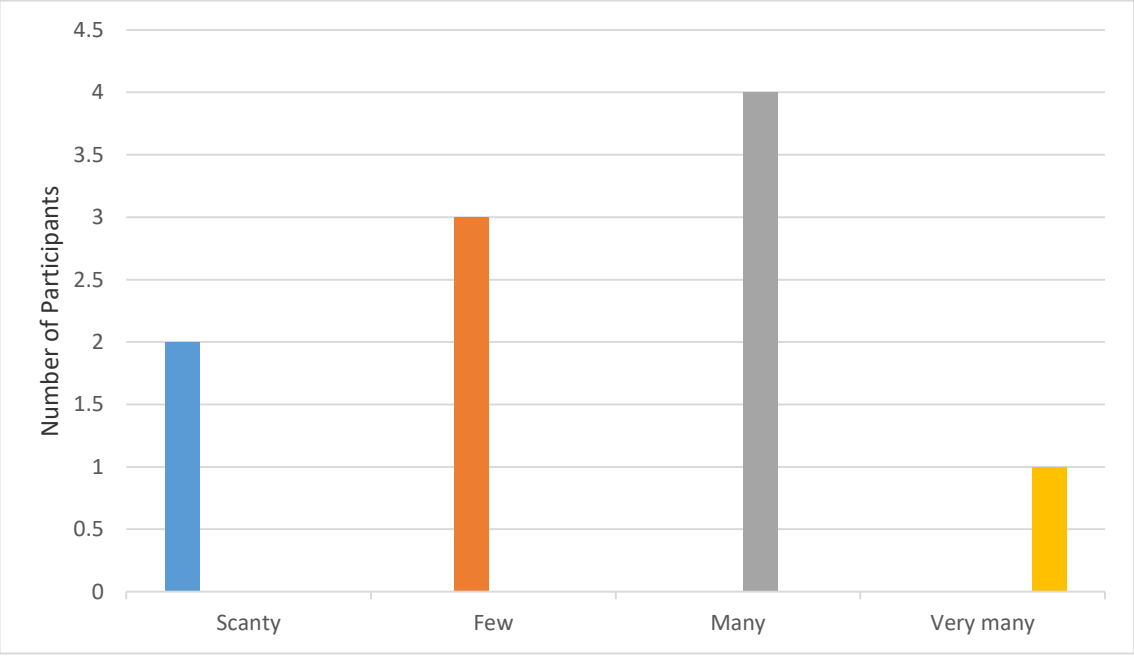

Figure 1: Distribution of *Entameba histolytica* load among pregnant women (N= 200)

Table 1: Sociodemographic Distribution of Pregnant women (N = 200)

Characteristics	Observation	No. tested
Age range (years)	a. <20	60
	b. 20 – 25	64
	c. 26 – 30	49
	d. 31 – 35	19
	e. >35	8
	Total	200
Educational level	a. No formal education.	38
	b. Primary	39
	c. High school	39
	d. Tertiary	25
	Total	200
Residential area	a. Rural	78
	b. Urban	122
	Total	200
Socioeconomic status	a. Lower	15
	b. Middle	102
	c. Upper	83
	Total	200
Occupation	a. Trader	56
	b. Civil servant	20
	c. Self-employed	59
	d. Student	16
	e. Unemployed	91
	Total	200
Gestational age	a. 1st Trimester	18
	b. 2nd Trimester	84
	c. 3rd Trimester	98
	Total	200
Gravidity	Primigravidae	90
	Multigravidae	110
	Total	200

Table 2: Distribution of Odd ratios and 95% confidence intervals (95% CI) for Intestinal Amebiasis among Pregnant Women (N = 200)

Characteristics	Observation	No. tested	No. (%) positive	OR (95%CI)1*
Age range (years)	a. <20	60	2 (3.3)	1
	b. 20 – 25	64	4 (6.3)	0.51 (0.09-2.93)
	c. 26 – 30	49	0 (0.0)	7.36 (0.39-140.1)
	d. 31 – 35	19	2 (10.5)	0007 (0.01-1.55)
	e. >35	8	2 (25.0)	0.35 (0.04-3.91)
Educational level	a. No formal education.	38	6 (15.8)	1
	b. Primary	39	0 (0.0)	15.8 (0.86-291.1)
	c.High school	39	2 (5.1)	0.19 (0.001-4.09)
	d. Tertiary	25	2 (8.0)	0.62 (0.08-4.72)
Residential area	a. Rural	78	8 (10.3)	1
	b. Urban	122	2 (1.6)	6.86 (1.42-33.2)*
Socioeconomic status	a. Lower	15	6 (40.0)	1
	b. Middle	102	3 (2.9)	22.0 (4.69-103.1)*
	c. Upper	83	1 (1.2)	2.5 (0.67 - 5.93)
Occupation	a. Trader	56	2 (3.6)	1
	b. Civil servant	20	3 (15.0)	0.21 (0.03-1.36)
	c.Self-employed	59	1 (1.7)	10.2 (0.99-104.9)
	d. Student	16	0 (0.0)	0.8 (0.03-21.8)
	e. Unemployed	91	4 (4.4)	0.9 (0.03-11.5)
Gestational age	a. 1st Trimester	18	1 (5.6)	1
	b. 2nd Trimester	84	4 (4.8)	1.18 (0.12-11.2)
	c. 3rd Trimester	98	5 (5.1)	0.93 (0.24-3.58)
				0.98 (0.24-0.13)
Gravidity	Primigravidae	90	8 (8.9)	1
	Multigravidae	110	2 (1.8)	5.76 (1.19-27.8)*

**Significant association determined by Multivariate Logistic Regression*

[1]Adjusting for the clustering effect was done in all analyses

Table 3: Bivariate Logistic Analysis of Intestinal Amebiasis in Pregnant Women (N =200)

Variable	No. tested	No.(%) positive	OR (95% CI)	*p* value
Daily consumption vegetables food				
a. Yes	156	8 (5.1)	1.14 (0.23-5.55)	0.876
b. No	44	2 (4.6)		
Total	200	10 (5.0)		
Source of drinking water				
a. Borehole	125	3 (2.4)		
b. Tap	75	7 (9.3)		
Total	200	10 (5.0)	0.24 (0.06-0.95)	0.043*
Toilet System				
a. Water Closet	134	6 (4.5)		
b. Pit latrine	66	4 (6.1)		
Total	200	10 (5.0)	0.73 (0.19-2.67)	0.6304
Household population size				
a. Dense(≥8 persons)	157	3 (1.9)		
b. Sparse(<8persons)	43	7 (16.3)		
Total	200	10 (5.0)	0.10 (0.02-0.41)	0.0013*

**Significant association determined by Bivariate analysis.*

Discussion

The current study recorded relatively low prevalence of amoebiasis at 5.0% for the period of the study. The low prevalence rate may be due to majority of pregnant women self-medicate themselves with antiprotozoal chemotherapy (e.g. metronidazole) whenever they feel signs of sickness. Probably, the commonly used antiprotozoal metronidazole might have affected the chances of cysts recovery from their fecal samples. However, this value is similar to the 2.7% and 6.26% prevalence of *E. histolytica* reported by Akinbo *et al* [9] and Usip *et al* [6] in pregnant women and children in South-southern Nigeria, respectively. When compared to other studies, this is lower than the prevalence of 21% reported in Nakuru County by Kinuthia *et al* [20] and 27% by Reuben *et al* [21]. Majority of intestinal amebiasis occur in Africa,

Central and South America and the Indian sub-continent where prevalence as high as 50% have been reported. [22] The reason for the disparity was unclear but it could be attributable to differences in study design participant selection and environmental conditions in the various study canters.

The transmission risk factors associated with amoebiasis were found to be source of water for drinking which is prone to contamination, poor personal hygiene, type of housing and monthly earning. These factors played a significant role in acquiring *E. histolytica*. Other significant factors reported by previous studies such as level of education, occupation, place of residence and diet were not statistically different in the present study.

Pregnant women and immunodeficient persons are believed to be susceptible to amebiasis, but individuals with physiological affected immunity such as those in pregnancy may suffer more severe forms of the disease. [19, 22, 23] This is thought to be due to raised progesterone levels and failure of immunoglobulin levels to neutralize *E. histolytica.* [5] In consonance to these older pregnant women had more prevalence of intestinal amebiasis compared to the younger aged counterparts. However, according to the findings of Zahida *et al* [24], people of all ages in developing countries were at risk of amoebiasis infection although prevalence among the ages varies greatly.

Women with no formal educations and those with tertiary level were all predisposed to amoebiasis infection. This could be attributed to the fact that most of them could be in formal employment and working away from their homes and therefore more likely to eat away from home and especially from canteens, kiosks and food joints near their work places. This concurs with a study that was conducted in Nigeria, which reported that those who eat away from home were more likely to be infected with the parasite than those who ate at their homes. [24] Conversely, those with no formal education also had higher prevalence because they have inadequate

awareness of the possible transmission routes and preventive measures of amebiasis.

Findings from this study revealed that civil servants and traders were more prone to amebiasis. This means more women in formal employment were infected with amoebiasis compared to unemployed. The reason why those with formal employments were more likely to acquire the parasite could be that, they often eat away from home and that they may not get time to prepare their own food. [25] The findings of this study indicated that rural residents and urban residents were both likely to be infected with amoebiasis (p=0.05). The prevalence rate was found to be 10.3% and 1.6% in rural and urban women, respectively. The possible explanation for this finding is that, urban residents have better social and financial conditions that may allow them to seek treatment in other health facilities including private facilities other than at UMTH. For rural residents, they engaged in farming/ trading, hence their hands being in contact with soil which may be contaminated promoting transmission of the parasite through oral route. This is similar to the findings of a study that showed that place of residence had a significant influence on amoebiasis. [26]

Amebiasis during pregnancy is believed to be more severe than in non-pregnant women. This is partly because pregnant women are susceptible to penetration of intestinal mucosa linings and placental barrier especially by *E. histolytica.* [11] During pregnancy, reduced production of IgA which may lead to sub-chronic inflammation and placenta dysfunction have been associated with preeclampsia, risk of preterm delivery and fetal growth restriction. [27] However, there is need for further investigation on etiopathogenesis of amebiasis in chronic inflammation.

Generally, the effect of amebiasis on pregnancy outcomes largely depends on trimester when the infections occur. In this study, women at their 1st and 3rd trimester had highest prevalence of intestinal amebiasis. These stages represent period of high risk of neonatal involvement than the 2nd trimester. [11] Due to absence of routine amebiasis screening for pregnant women, majority of women will be misdiagnosis for amebiasis, thus increases chances of neonatal morbidity.

Conclusion

The results indicate that *E. histolytica* infection is one of the etiological agents of acute diarrhea in pregnant women in Maiduguri, Nigeria. It is recommended that pregnant women should avoid risk factors for intestinal amebiasis identified in this study.

Conflict of Interest

None.

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