PREVALENCE OF *ENTAMOEBA HISTOLYTICA* AMONG PATIENTS WITH GASTROENTERITIS ATTENDING SOME SELECTED HOSPITALS IN ZARIA METROPOLIS, KADUNA STATE

[1]Sulaiman, M. A., [1]Musa, B., [1]Ibrahim, A.Y., [2]Ibrahim, A., [1]Tijjani, M.B. and [1]Aliyu, M.S.
[1]Department of Microbiology, Faculty of Life Sciences, Ahmadu Bello University, Zaria
[2]Department of Medical Laboratory Science, Bayero University, Kano
Correspondence author: mamunuabdulkadir@yahoo.com; +2348038937309

Abstract
Background: Amoebiasis is a cosmopolitan infection caused *by Entamoeba histolytica* and ranks third among parasitic infections that lead to death especially in children. Clinical features of amoebiasis range from asymptomatic colonization of amoebic colitis (dysentery or diarrhoea) and invasive extra intestinal amoebiasis, which is manifested most commonly in the form of liver abscesses.
Aim: The prevalence of *E. histolytica* infection in patients having gastroenteritis attending three selected hospitals in Zaria, Kaduna state was studied using direct wet mount and formol-ether concentration method. The hospitals were Hajiya Gambo Sawaba General Hospital Kofan Gayan (Gambo Sawaba), Major Ibrahim B. Abdullahi Memorial Hospital Sabon Gari, Zaria (Limi hospital) and University Health Services ABU Zaria (Sick Bay).
Methods: One hundred and forty stool samples from gastroenteritis patients of all ages attending the selected hospitals were examined for *E. histolytica* trophozoites/cysts via direct wet mount and formol-ether concentration method.
Results: Eight samples were positive giving an overall prevalence of 5.71%. Highest prevalence (10.4%) was obtained from patients attending Gambo Sawaba Hospital. The prevalence of 2.4% and 4.0% were recorded among gastroenteritis patients attending Limi Hospital and University (A.B.U, Zaria) health service respectively. With respect to age, the prevalence (11.4%) was highest in the age group 11-15 (years) followed by 6-10 years with prevalence of 6.38%. However, none of the socio-demographic factors examined showed significant statistical association (P>0.05).
Keywords: *Entamoeba histolytica*, Trophozoite, Formol-ether, Prevalence, Zaria, Amoebiasis

Introduction

Amoebiasis is an infection with protozoan parasite *Entamoeba histolytica* of the class Sarcodina with or without the presence of clinical symptoms. It ranks third among parasitic diseases that results to death worldwide; being second to malaria as a protozoan cause of death (Schmidt and Roberts, 2000). About 500 million people are believed to be infected at one time, and up to 100,000 deaths occur per year (Haque and Petri, 2006). 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 (Ogbe and Isichei, 2002). Many species of the genus *Entamoeba* infect humans. These include: *Entamoeba histolytica, Entamoeba dispar, Entamoeba coli, Entamoeba hartmanni, Entamoeba polecki, and Entamoeba gingivalis* (Ash and Orihel, 1980). Among these, only *E. histolytica* is considered pathogenic and the disease it causes is called amoebiasis or amoebic dysentery (Sateriale *et al.,* 2011). It parasitizes man causing amoebic dysentery, amoebic hepatitis, and pulmonary amoebiasis.

Citation: Sulaiman, M. A., B. Musa, A.Y. Ibrahim, Ibrahim Aminu M.B. Tijjani, M.S. Aliyu (2016): Prevalence of *Entamoeba histolytica* among Patients with Gastroenteritis Attending Some Selected Hospitals in Zaria Metropolis, Kaduna State. *BJMLS. 1(1): xxx*

E. histolytica inhabits the large intestine of man, but can also establish itself in the liver, lungs, brain and other organs where secondary lesions are produced. Amoebiasis remains an important health problem in Nigeria due to inadequacies in sanitation infrastructure and health care facilities (Udonsi, 1999). Clinical features of amoebiasis range from asymptomatic colonization of amoebic colitis (dysentery or diarrhoea) and invasive extra intestinal amoebiasis, which is manifested most commonly in the form of liver abscesses (Fotedar *et al.,* 2007). The prevalence of *E. histolytica* infection indicates that 90% of individuals remain asymptomatic while the other 10 % develop clinically overt disease (Haque *et al.,* 1995). Once the parasites invade the intestinal wall, they reach the sub mucosa and the underlying blood vessels. From there, trophozoites travel in the blood to sites such as the liver, lungs or skin. Encystation occurs in the intestinal lumen, and cyst formation is complete when four nuclei are present. These infective cysts are passed into the environment in human faeces and are resistant to a variety of physical conditions. On some occasions, trophozoites may exist in the stool, but cannot survive outside the human host (Smith, 1999). *E. histolytica* is basically transmitted through the ingestion of food and water that are faecally contaminated with the cysts of parasite (Ibrahim, 2008). In Nigeria, amoebiasis is prevalent and widespread which has been attributed to quite a number of multiple environmental sources of transmission (Ajero *et al.,* 2008). Several reports have recognized amoebiasis as an important health problem especially among young growing children of school age. The several surveys have indicated a high prevalence of intestinal parasitic infections among Nigerian children in different localities (Ogbe and Isichei, 2002; Ukpai and Ugwu, 2003). The aim of the study was to identify the cysts and trophozoites of *E.histolytica* in patients having gastroenteritis using direct wet mount preparation and formol-ether concentration method and to determine the risk factors and socio-demographic factors associated with amoebiasis among the study population using structured questionnaires.

.

Materials and Methods

Study area

This study was conducted in Zaria (11° 3' N; 7° 42' E) Nigeria, which is located about 83km north of Kaduna, along the Kaduna-Kano highway (Mortimore, 1970). Zaria comprised of two Local Government Areas namely; Zaria and Sabon Gari. By the existing pattern of settlement, Zaria urban is composed mainly of four districts namely; Zaria City, Tudun Wada, Sabon Gari and Samaru. Zaria is made up of a natural and stable ecosystem in the northern Guinea Savannah zone, with a discontinuous layer of sparsely distributed short trees followed by relatively continuous layers of tall, medium and short grasses; rainfall is below 125mm (Mortimore, 1970). Zaria is an old commercial, administrative and academic town in Northern Nigeria, but most parts have poor environmental hygiene as evident by heaps of refuse dumps coupled with indiscriminate disposal of human and animal wastes, even by the side of streets (Aina, 2002).

Ethical Clearance

Three hospitals in Zaria were selected for the study; Hajiya Gambo SawabaGeneral Hospital Kofan Gayan, Major Ibrahim B. Abdullahi Memorial Hospital, Sabon Gari Zaria (Limi Hospital) and University Health Services ABU Zaria (Sick Bay). Ethical approval was obtained to undertake the study from Kaduna state Ministry of Health.

Sample Size

Sample size was calculated using a the formula described by Mshana *et al.* (2009). The prevalence used in the calculation was 8.51% (Enabo *et al.,* 2000).

$n = Z^2P(1-P)/d^2$

.

Where n = Sample size

P = Prevalence of the disease in the previous study = 8.51% = 0.0851

Z = Standard normal distribution at 95% confidence limit = 1.96

d = allowable error of 5% = 0.05

$$n = \frac{(1.96)^2 \times 0.0851 \times 0.9149}{0.05^2}$$

$$= 119.6$$

A total of 140 samples were collected.

Sample Collection

Faecal sample of patients with gastroenteritis were collected from the selected hospitals, using a sterilized clean well labelled specimen container and then transported to the laboratory for processing.

Administration of questionnaires

Questionnaires were distributed to the patients during sample collection by the health worker in charge and were requested to fill it. Respondents who could not read or write in the English language were interviewed in Hausa by the researcher. The questionnaire took into account socio-demographic and risk factors such as water supply/sources, hygienic situation at home and presence and use of latrines at home, gender etc.

Laboratory Analysis Technique

After transporting the sample from hospitals to the laboratory, the collected stool specimens were first observed physically for consistency, presence of blood stains and any macroscopic parasites (Wakid, 2010).

Direct smear method was used to process the stool as described by Cheesbrough (2006) and then observed microscopically using X10 or X40 objective for cysts or trophozoites. Formol-ether concentration technique was also employed for more quantitative examination.

Direct Faecal Smear (Wet Mount) Technique

A drop of normal saline was placed on a clean slide. Small sample of stool was emulsified on the normal saline using an applicator stick and a cover slip was placed on it. The slide was placed on the microscope slide and examine for trophozoite systematically using X10 followed by X40 objectives (Cheesbrough, 2006).

Formol-ether Concentration Technique

One to two grams (1-2g) of each specimen was emulsified in about 7mls with 10% formal saline by stirring with a clean glass rod in a test tube. The suspension was filtered over two layers of surgical gauze into a 10mls-capacity centrifuge tubes. Then, 3mls of diethyl-ether was then added into each tube and shaken vigorously. This was centrifuged using a centrifuge machine at increasing speed to a maximum of 1500 rpm for about 5min. The supernatant was discarded and a drop of Lugol's iodine was added to the residue. After tapping each tube, smears were made on clean slides, after which they were examined using a microscope at magnifications of X10 and X40 for the presence of *E. histolytica* cyst complex cysts (National Committee for clinical laboratory, NCCLS, 1997).

Results

Prevalence of *E. histolytica* among the patients attending the three hospitals selected; Gambo Sawaba, Sick Bay, was reported in Table 1. The highest prevalence was recorded in Gambo Sawaba, 5 (10.4%), while prevalences of 4% and 2.4% in Sickbay and Limi Hospital respectively. The prevalences for females and males were (6.4%) and (4.8%) respectively as presented in Table 2. The prevalence of *E. histolytica* in relation to age of the patients was reported with the highest prevalence among age group 11-15 (11.4%) followed by patients of age group 6-10 (6.38%) as shown in Table 3. The risk factors examined were found to have various levels association with prevalence of *E. histolytica,* however, none is statistically significant.

Table 1: Prevalence of *E. histolytica* by hospitals in Zaria metropolis.

Hospitals	No. Examined	No. Positive (%)	P – value
Sick bay	50	2 (4)	
Gambo Sawaba	48	5 (10.4)	0.193
Limi hospital	42	1 (2.4)	
Total	140	8 (5.7)	

$X^2 = 4.729$, df = 2.

Table 2: Prevalence of *E. histolytica* in relation to gender

Gender	No. Examined	No. Positive (%)	P – value
Female	78	5 (6.4)	
Male	62	3 (4.8)	0.0691
Total	140	8 (5.71)	

$X^2 = 0.158$, df = 1.

Table 3: Prevalence of *E. histolytica* in relation to age

Age Range (Years)	No. Examined	No. Positive (%)	P - value
1 – 5	8	0 (0.00)	
6 – 10	47	3 (6.38)	
11 – 15	35	4 (11.4)	
16 – 20	30	1 (3.3)	0.756
21 – 25	11	0 (0.00)	
26 – above	9	0 (0.00)	
Total	140	8 (5.71)	

$X^2 = 2.636$, df = 5.

Table 4: Occurrence of *E. histolytica* infection in relation to Risk factors

Risk factors	**No. Examined**	**No. Positive (%)**	**X^2 (Chi-Square)**	**P value**
Main source of water?				
Bore hole	95	6 (6.30)		
Tap	30	1 (3.3)		
River	3	0 (0.00)	3.635	0.603
Well	4	0 (0.00)		
Packaged water	2	0 (0.00)		
Others	4	1 (25)		
Do you treat drinking water?				
Yes	63	2 (3.17)		
No	77	6 (7.9)	0.371	0.242
Type of toilet?				
Latrine	49	3 (6.1)	0.692	0.708
Water system	71	4 (5.6)		
Bush/open space	11	0 (0.00)		
Washing of hands after toilet use?				
Always	105	6 (5.7)		
Sometimes	20	1 (5.0)	0.254	
Never	4	0 (0.00)		0.881

Table 4 continue

Risk factors	No. Examined	No. Positive (%)	X^2 (Chi-Square)	P value
Refuse disposal?				
Garbage Pit	13	0 (0.00)		
Outside compound	19	0 (0.00)	4.208	0.379
Dust Bin	92	6 (6.52)		
Others	6	1 (16.7)		
Economic status?				
Civil servant	13	1 (7.7)		
Trader	9	0 (0.00)		
Farmer	3	0 (0.00)	1.036	16.6
Unemployed	17	1 (5.9)		
Students/Pupil	86	5 (5.8)		
Others	3	0 (0.00)		
Washing of fruits/veg. before use?				
Always	100	6 (6.0)		
Sometimes	27	1 (3.7)	0.254	0.797
Never	4	0 (0.00)		

Discussion

The overall prevalence of *E. histolytica* infection in patients having gastroenteritis attending the selected hospitals was found to be 8(5.71%). Gambo Sawaba had the highest prevalence of 5 (10.4%), Limi hospital had lowest prevalence of 1(2.4%) and Sick Bay recorded 2(4%). The total prevalence in this study agrees with findings by Inabo *et al.* (2000), who reported an overall prevalence of 8.51% in Zaria, Kaduna State, Nigeria. This may be an indication of risk factors and management measures status quo in the study location. The difference in prevalence of amoebiasis in male patients (4.8%) was not statistically significant from that of females (6.4%), suggesting that both males and females patients in the study area have the same predisposition to amoebiasis. This agrees with findings by Brelet, (2000), who reported higher prevalence of (42.5%) in females than in males (41.6%). This was attributed to socio-cultural life style in this part of the world which got females to be more involved in domestic activities that may expose them to contaminated water, food or washing of babies after defecation. There was a higher prevalence (11.4%) among patients of age group 11-15 (years), however, the association is not statistically significant (P = 0.756). The high prevalence may be attributed to the fact that persons of that age bracket are often inquisitive, carefree and are more likely to get involved in activities or chores involving contaminants; for example playing in areas where the sand may be infected with faecal sample or playing with contaminated water as the case may be. Also, this age group may also have poorer hygiene measures since they are allowed to wash themselves but younger ones were washed by older ones at home (Mamadou *et al.,* 2010). In addition, findings by (Nematian *et al.,* 2012; Oninla *et al.,* 2007) revealed that *E. histolytica* infection is highest in children of school age in developing countries.

The risk factors examined like water source was not statistically associated with the prevalence of amoebiasis in our study (P value >0.05), this is in contradiction with the report of Kinuthia *et al.* (2012), who identified drinking well water as a risk factor of amoebiasis. Other sources, where water is usually enclosed such as bore holes and tap water; there was no significant association between the use of the water and prevalence.

No significant association was also found among patients who treat drinking water or not, types of toilet used, washing of hands after toilet use, refuse disposal habit, economic status and washing of vegetables and fruits before consumption. This can be attributed to awareness, improved water supply in the areas and use of drug for treatment of amoebiasis.

Conclusion

The finding of this study revealed an overall prevalence of 5.7% of *E. histolytica* infection among patients in the study area. None of the socio-demographic and risk factors examined was statistically associated with amoebiasis.

Recommendations

Health education programs should be emphasized especially in schools and communities to target children as well as care givers, teaching them the importance of basic hygiene practices.

More preventive measures for the uninfected and the treatment of the infected ones should me put in place.

Further studies involving a larger sample size should be carried out.

References

Aina, O. E. (2002). Solid waste management in Zaria. Unpublished B.Sc. (project) of Department of Geography, A.B.U. Zaria, .2-7.

Ajero, C.M., Nwoko, B.E.B., Nwoke, E.A. and Ukaga, C.N. (2008). Human Amoebiasis: Distribution and Burden; and the Nigerian Environment. *International Science Research Journal*, 1(2):130-134.

Ash, L. and Orihel, C. (1980). Atlas of human parasitology. *American society of clinical pathologists,* Chicago 2nd edition, 22-32.

Brelet, C. (2000). Women, Education and water in Africa. *Technical document on hydrology*/UNESCO Paris, 55-61.

Cheesbrough M. (2006). District Laboratory Practice in Tropical Countries. Second edition (part 2). Cambridge University Press, Cambridge, New York, United State of America, 56-109.

Fotedar, R., Stark, D., Beebe, N., Marriott, D., Ellis, J. and Harkness, J. (2007). Laboratory diagnostic techniques for Entamoeba species. *Clinical Microbiology Review, **20***(3):511-32.

Haque, R. and Petri Jr, W.A. (2006). Diagnosis of amoebiasis in Bangladesh. *Archives of Medical Research*, **37**:273-276.

Haque, R., Neville, L.M., Hahn, P. and Petri, W. A. Jr. (1995).Rapid diagnosis of *Entamoeba* infection by using *Entamoeba histolytica* stool antigen detection kits. *Journal of Clinical Microbiology*, **33**: 2558-25.

Kinuthia, G., Afolayan, F., Ngure, V. and Anjili, C. (2012). Selected practices among rural residents versus the prevalence of Amoebisis and Giardiasis in Njoro District, Kenya. *African Journal of Health Sciences*, **20**:11-20.

Mamadou, O., Nicaise, O., Guessan, N., Ahoua, Y. and Eliezer, K. (2010). Prevalence and spatial distribution of *Entamoeba histolytica/dispar* and *Giardia lamblia* among School children in Agboville area (Côte d'ivoire), *PLOS Neglected Tropical Diseases,* **4**(1):1-30.

Mshana, S.E., Imirzalioglu,C., Hossain, H., Torstein, H., Domann, E. and Chakraborty, T. (2009). Conjugative IncFI plasmids carrying CTX-M-15 among *Escherichia coli*ESBL producing isolates at a university hospital in Germany. *Infectious Diseases*,**9**(97):1471-2334.

Mortimore, M. J. (1970). Zaria and its regions. *Annals of the Association of America Geographers*, **60**(1):73-80.

National Committee for clinical laboratory (NCCLS).Procedures for the recovery and identification of parasites from the intestinal tract. Approved guideline. Pennsylvania, 1997.

Nematian, J., Nematian, E., Gholamrezanezhad, A. and Asgari, A.A. (2012). Prevalence of intestinal parasitic infections and their relation with socio-economic factors and hygiene habits in Tehran Primary School students. *Acta Tropica*, **92**(3):179-186.

Ogbe, G.E. and Isichei, M. (2002).Intestinal helminth infection in Primary school children in areas of operation of shell petroleum Development Company of Nigeria (SPDC), Western Division in Delta State. *Nigerian Journal of Parasitology*, **23**:3-10.

Sateriale, A. and Huston, C. (2011).A sequential model of host cell killing and phagocytosis by *Entamoeba histolytica*. *Journal of parasitology Research*, Hindawi publishers corporation, University of Vermont, USA **1**: 1 – 10.

Schmidt, G.D. and Roberts, L.S. (2009). Foundations of Parasitology, 6th ed. New York: McGraw-Hill Companies.767

Smith, J.D. (1999). *Animal Parasitology*. 3rd Ed. Cambridge University Press, UK. 549.

Udonsi, J.K.U. (1999). Parasites and Parasitic Diseases. A Textbook of General and Medical Parasitology in Tropical Africa.Josany Press, UK. 275

Ukpai, O.M. and Ugwu, C.D. (2003). The Prevalence of gastro-intestinal tract parasites in Primary school children in Ikwuano Local Government Area of Abia State, Nigeria. *The Nigerian Journal of Parasitology*, **24**:129136

Wakid, M. (2010). Faecal occult blood test and gastro intestinal parasitic infection. *Journal of Parasitology*, King Abdullaziz University, Jeddah, Saudi Arabia 434801:1-4.