



Prevalence of *Entamoeba histolytica* Infection Using Microscopy and Adhesin Detection Methods among School Children in Central Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

This study aimed to evaluate the prevalence of *Entamoeba histolytica* infection using microscopy and adhesin detection methods among school children in Central Nigeria. After ethical clearance, stool samples of three hundred and sixty (360) children aged 5-16 years old as adopted by FMOH (2013) were randomly collected between September-December 2015 and were examined for *E. histolytica* cysts/trophozoites using microscopy and TechLab *E. histolytica* II ELISA test (Sigma Diagnostic Inc, USA) in six public primary schools in Keffi, Nasarawa State. The TechLab *E. histolytica* II ELISA result showed that out of the 88 (24.4%) stool samples positive by microscopy, 85 (96.6%) samples were positive for *E. histolytica* antigens while 3 (3.4%) were negative. The overall prevalence of the infection using microscopy method was 24.4%. Males recorded a higher prevalence 64 (30.5%) than females 24 (16.0%). Children aged 6-10 years recorded the highest prevalence of 37 (28.5%) while the lowest 22 (21.6%) was showed in those aged <6 years. There was significant difference in the infection rate among the sex of the children ($p \leq 0.05$). The association was also significant based on location of the children's homes and finger sucking habit.

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Children living outside Keffi town had a higher prevalence 34 (25.4%) than those living in Keffi town 54 (23.9%). Out of the children infected, 23 (32.9%) suck their finger while 65 (22.4%) did not. In this study age, pupil's class, occupations of caregivers, sources of drinking water and types of toilet facility had no statistical significance with *E. histolytica* infection ($p > 0.05$). Health education programmes that will promote personal and environmental hygiene and regular mass treatment are suggested strategies to mitigate transmission of the infection especially in children who are usually asymptomatic.

Keywords: Prevalence; *Entamoeba histolytica*; microscopy; ELISA; Keffi.

1. INTRODUCTION

Entamoeba histolytica infection also known as Amoebiasis, is an anaerobic protozoan parasite infection which is cosmopolitan parasite that causes human morbidity after Malaria and Schistosomiasis [1,2,3]. Also, amebiasis, caused by this intestinal parasite, has an estimated global prevalence rate of 500 million cases of symptomatic disease, and 40,000 to 110,000 deaths annually [4,5]. It is a serious health challenge, especially in developing countries. The rates of infections vary among countries on the basis of socioeconomic status, sanitary conditions and population [6]. It is endemic in socio-economically deprived communities in the tropics and subtropics. Lack of environmental sanitation, socio-economic deprived communities, demographic factors, and hygiene-related behavior facilitates the spread and distribution of this intestinal parasite among individuals [3]. Transmission is often associated with contaminated food, water and finger. Therefore, children are not mostly expected to develop amoebiasis. More severe disease is common among the young, aged, malnourished and immunosuppressed individuals. In developed countries, amoebiasis tends to be found mostly in aged people and among homosexual men and in institutions [3]. However, in tropical regions, the epidemiology of the disease is mostly found among the general population especially in patients attending health care facilities with cases of diarrhoea [6]. Clinical features of amoebiasis ranges from asymptomatic colonization, amoebic dysentery and invasive extraintestinal amoebiasis which is shown most commonly in the form of liver abscess [7,5]. Out of 10% of the world's population infected by *E. histolytica*, only 1% develops the invasive form of the disease [3]. Symptom ranges from mild diarrhea, dysentery with blood and mucus in stool. Severe amoebic infections occur in two major forms. Invasion of the intestinal lining causes amoebic dysentery or amoebic colitis. If the parasite reaches the bloodstream it can spread through

the body, most frequently ending up in the liver where it causes amoebic liver abscesses [6]. Liver abscesses can occur without previous development of amoebic dysentery [3]. In asymptomatic case, the infected individual is still a carrier, potentially capable to transmit the parasite to others through poor hygienic practices and symptoms at onset can be similar to bacillary dysentery [8]. There is a dearth of information concerning the prevalence of *E. histolytica* using microscopy and adhesin detection methods in Keffi, Nasarawa State, Nigeria. This study was therefore conducted to evaluate the prevalence of *E. histolytica* infection using microscopy and adhesin detection methods among school children in Central Nigeria.

2. MATERIALS AND METHODS

2.1 Study Area

The study was conducted in Keffi, Nasarawa State, Nigeria. Keffi is approximately 68 Km East of Abuja the Federal Capital Territory and 128 Km from Lafia the capital of Nasarawa State. The State is bounded on the North by Kaduna State, on the South by Benue State, East by Plateau and on the West by the Federal Capital Territory. Keffi lies between 7°45' and 9°25'N of the equator and latitude 7° and 9°37'E of the Greenwich Meridian and located on an altitude of 850 M above sea level. Keffi which is a stockade or fenced settlement shares common borders with Karu and Kokona in the South East and North respectively occupying an area of 3,019 Km with a population of 242,764 [9]. It is a fertile land with Agriculture as its main stay of the economy and subsistence of peasant farming is greatly enhanced among the people and also rearing of domestic animals. Trading is also an important economic activity in the community [10].

2.2 Study Population

The study population comprises of children of 5-16 years old as adopted by FMOH [11] in public

primary schools in Keffi who agreed to participate in the study from a period of September-December 2015. Structured questionnaire was administered to the parents/guardians of the pupils; information on age, sex, occupations of parents and risk factors such as, sources of drinking water and types of toilet facilities was obtained from each participant.

Six public primary schools were randomly selected in the study area for the study. They are: Yelwa Primary school (YPS), ECWA transfer Primary School (ETPS), Majema Primary school (MPS), Baptist Primary school (BPS), Kofar-Hausa Primary school (KHPS) and St. Williams Primary school (SWPS). The total population in the six primary schools is 1860 pupils. A total of 360 pupils comprising of 210 (58.3%) males and 150 (41.7%) females was randomly sampled for the study where 60 pupils were selected from each school based on willingness of the pupil to participate in the study.

2.3 Ethical Permission and Administrative Clearance

This work received ethical permission from the Nasarawa State Ministry of Health, Department of Primary Health Care, Keffi Local Government Council, Nasarawa State. Introduction letter for this study was obtained from the Education Secretary and Educational inspectorate zone of Nasarawa State. Further consent for the work was sorted and obtained from the Heads of the schools. Head Masters, Teachers, Parents/Guardians of pupils and Pupils were informed properly on the objectives of the study.

2.4 Administration of Research Questionnaire

Structured questionnaire were administered to the parents/guardians of the three hundred and sixty (360) children recruited into the study. Respondents who could not read or write in English Language were interviewed in Hausa by the researcher. The questionnaire was designed to provide information such as age, gender, occupation of parent's, educational background of parents and risk factors such as sources of drinking water, types of toilet facility and finger sucking habit.

2.5 Sample Collection

About 20 g of faecal samples was collected from each of the 360 pupils. A clean dry sample

container was given to each pupil for their faecal specimen. The samples collected were then transported in ice packs to the Microbiology/Parasitology Unit of the Laboratory Department, School of Health Technology, Keffi where they were examined for the parasites.

2.6 Sample Size Determination

Due to unavailability of data on the true prevalence of the infection among school children in Keffi, a prevalence of 8.51% based on the previous study of Inabo et al. [12] was used to determine the sample size required for the study using the formula;

$$N = Z^2 Pq / L^2$$

Where;

N=Sample size

Z=Standard Normal distribution at 95% confidence interval=1.96

P= Prevalence of 8.51% (0.0851) from Inabo et al. [12]

q= 1-P=1-0.0851=0.915

L= Allowable error =5% or 0.05

Substituting the values in the formula therefore:

$$N = 1.96^2 \times 0.0851 \times 0.915 \div 0.05^2$$

$$N = 3.84 \times 0.0779 \div 0.0025$$

$$N = 120$$

2.7 Methods

The faecal samples were checked macroscopically to note the colour, odour, presence of mucus and/or blood. Examination of the parasite under the microscope was subsequently done using direct smears methods with (normal saline and iodine solution) and formal-ether concentration techniques. The presence of one to four nuclei cysts and/or trophozoites of amoeba were detected as *E. histolytica* [13].

Positive *E. histolytica* stool was kept into 2ml tubes in a deep freezer prior to the ELISA analysis.

2.7.1 Direct smear methods

For the microscopic examination, both saline and iodine preparation of the stools were made and examined. The former was used for the identification of the trophozoites while the later for the identification of cysts.

Wet preparation using 3% iodine was the method of choice because the nucleus of *E. histolytica* retains the dye and thus allows easy identification of the cyst. A little portion of the formed stool sample was fetched and emulsified with the dye to form a smear. This was covered with a cover slip and viewed under the microscope using x10 and x40 objectives for examination and identification of the parasite respectively [13].

Another stool specimen was also prepared using a drop of physiological saline. A cover slip was applied before examining the preparation microscopically. The presence of ingested erythrocyte and characteristic directional movement are diagnostic of *E. histolytica*. The cyst of the parasite was identified based on the diagnostic characters described by Cheesbrough, [14].

2.7.2 Formalin/ether concentration techniques

Formalin/ether concentration technique was adopted to concentrate cysts in the stool samples based on the principle of the force of gravity. Four millilitre of formol water was placed in the screw capped test tube and the applicator stick was used to collect 1 gram of the sample from the container and was mixed with the water in the tube. The tube was capped and mixed vigorously after which it was sieved with the gauze into the beaker as suspension. The suspension was harvested into another tube, 3ml of ether was added and content was mixed properly for about 5 minutes. The tube was uncapped gradually and the preparation was then centrifuged at 3000 rpm for 2 minutes. An applicator stick was used to loosen the thick layer of faecal debris at the side of the tube and it was inverted to discard the any suspended particles and solutions with the sediment remaining at the bottom of the tube. The tube was turned upright with the droplet of water on the side draining back into the tube and tapped at the base to re-suspend the sedimented parasites. A Pasteur pipette was used to harvest and transfer the sediment onto a clean glass slide. The preparation was viewed with the x 10 and x 40 objectives for the parasites. A drop of iodine solution was dropped under the cover slip to enhance the identification of the parasites [14].

2.8 ELISA Method

The TechLab (*E. histolytica* II kit) (Sigma Diagnostic, Inc. USA) was used to detect

adhesin antigen from the positive faecal samples according to the manufacturer's instructions as described by Saeed and Manal, [15].

2.8.1 Spectrophotometry

The microplate ELISA reader was set to read at 450 nm and referenced/blanked against air at 630 nm. The absorbance values for the positive and negative controls were evaluated. A sample was considered positive when value read was higher than the negative control value (but lower than the positive control value), and negative when the value read was lower than the negative control value. A positive test result showed that the antigen was present in the faecal specimen, while a negative test indicated that *E. histolytica* was not in the faecal sample [16].

2.9 Statistical Analysis

Descriptive statistics and frequency tables were used to describe the results. The data gathered was analyzed using Chi square test to determine the association of the prevalence of *E. histolytica* infections among school children with the studied risk factors. Values obtained were considered significant at $P \leq 0.05$.

3. RESULTS

A total of 360 school children in six public primary schools in Keffi participated in this study. The TechLab *E. histolytica* II ELISA analysis showed that out of the 88 (24.4%) stool samples positive by microscopy, 85 (96.6%) samples were positive for the parasite antigens and 3 (3.4%) were negative. The overall prevalence of the infection using microscopy method was 88(24.4%). Males recorded a higher prevalence 64(30.5%) than females 24(16.0%). Children aged 6-10 years recorded the highest prevalence of 37(28.5%) while the lowest 22(21.6%) was showed in those aged <6 years. There was a significant difference in the infection among the sex of the children ($p \leq 0.05$). The association was also significant based on location of the children's homes and finger sucking habit. Children living outside Keffi town had a higher prevalence 34(25.4%) than those living in Keffi town 54(23.9%). Of the children infected, 23(32.9%) suck their finger while 65(22.4%) did not. In this study age, pupil's class, occupations of caregivers, sources of drinking water and types of toilet facility had no statistical significance with *E. histolytica* infection ($p > 0.05$).

Table 1. Prevalence of *E. histolytica* infection among schools sampled in Keffi

School no.	No. examined	No. positive (%)
YPS	60	21 (35.0)
ETPS	60	18 (30.0)
MPS	60	12 (20.0)
BPS	60	10 (16.7)
KHPS	60	13 (21.7)
SWPS	60	14 (23.3)
TOTAL	360	88 (24.4)

Legend: YPS; Yelwa Primary School, ETPS; ECWA Transfer Primary School,

MPS; Majema Primary School, BPS; Baptist Primary School, KHPS; Kofar-Hausa Primary School, 249, SWPS; St. Williams Primary School

Table 2. Prevalence of *E. histolytica* infection among the classes of school children in Keffi

Class	No. Examined	No. Positive (%)	p value
Class 1	60	12 (20.0)	>0.05
Class 2	60	15 (25.0)	
Class 3	60	13 (21.7)	
Class 4	60	18 (30.0)	
Class 5	60	20 (33.3)	
Class 6	60	10 (16.7)	
TOTAL	360	88 (24.4)	

4. DISCUSSION

E. histolytica infection is a common occurrence in developing countries such as Nigeria with school age children carrying the hardest hit of the associated morbidity [3]. An overall prevalence rate of 24.4% was recorded for all the schools surveyed in this study which is in agreement with the reports of Reuben et al. [1] in Lafia, Nasarawa State, Simon-Oke and Ogunleye, [5] in Ondo state, Amaechi et al. [3] in Abia, South East Nigeria, Inabo et al. [12] in Zaria, Aribodor et al. [13] in Anambra State and Houmsou et al. [17] in Makurdi, Benue State. Lower rates compared to findings in the present study have been reported in other countries like 2.6% from Makkah, Saudi Arabia [16], 21.0% from Malaysia [18], 0.19% from Turkey [19], 18.8% from Cote d' Ivoire [20]. Higher rates than reported in this study have been found in South Jeddah, Saudi Arabia where it was 50% [21] and 52.1% in Pakistan [22]. The relatively high prevalence of *E. histolytica* infection reported in this study might relates to poverty, lack of proper drainage system, indiscriminate disposal of sewage, lack of potable water, indiscriminate defecation, ignorance and low standard of personal hygiene

in the study area and among school children. These factors facilitate the spread of the parasite to children who moves around with other children since the portal of entry are by faecal-oral route.

The highest prevalence of the infection (35.0%) was recorded in children attending Yelwa Primary School (YPS). The reason might be as a result of the school location being in a rural setting and the awareness of personal and environmental hygiene is limited. Obviously, the infection remains asymptomatic among school children in the study area.

The high prevalence rates of 33.3% and 30.0% showed among children in Class 5 and Class 4 respectively shows a common pattern of adventurous behavior among children in that age group and thus plays a lot in sand and nearby streams/ponds with little or no care [3].

The present study showed a higher prevalence of *E. histolytica* infection among males (30.5%) than their female's counterparts (16.0%). This is in agreement with the work of Amaechi et al. [3] from Abia State which recorded 18.7% for males and 13.3% for females. Reuben et al. [1] in Lafia city reported higher prevalence in males (27.7%) than females (24.3%). The significantly high prevalence of infection observed among male children may be attributed to the fact that they are more adventurous than their female counterparts and have a greater chance of indulging in outdoor activities [12,1,8]. It could also be maintained that males engage more in activities that predisposes them to the infections such as playing football, farming, fishing, hunting and also playing in the streams or ponds. These activities necessitated more contact and exposure to the infections. On the other hand, female children are more pre-occupied with household activities which limit their level of exposure to the possible sources of infection [13]. This is however contrary to the report of Nyenke et al. [23] in Degema and environs where they reported that females have more infections than males.

The result revealed a high prevalence rate of 28.5% among children between age group 6-10 years, followed by those aged 11-14 years (22.7%) and 0-5 years recorded (21.6%) respectively. There was no significant difference in age and the protozoan infection among the study population ($p > 0.05$). This study correlates with the works of Gimba et al. [8], Reuben et al. [1] and Amaechi et al. [3]. This could be

Table 3. Prevalence of *E. histolytica* infection with respect to Risk Factors among the study population in Keffi, Nigeria

Risk factors	No. Examined	No. Positive (%)	p value
Gender			
Male	210	64 (30.5)	< 0.05
Female	150	24 (16.0)	
Age groups (Years)			
<6	102	22 (21.6)	>0.05
6-10	130	37 (28.5)	
11-14	128	29 (22.7)	
Occupation of Parents/Guardians			
Civil servants	69	18 (26.1)	>0.05
Farmers	63	20 (31.7)	
Traders	108	23 (21.3)	
Artisans	120	27 (22.5)	
Educational status of Parents/Guardians			
Primary	111	30 (27.0)	>0.05
Secondary	130	35 (26.9)	
Tertiary	119	23 (19.3)	
Sources of Drinking Water			
Tap	76	16 (21.1)	>0.05
Borehole	103	28 (27.2)	
Well	123	23 (18.7)	
Stream/River	58	21 (36.2)	
Types of Toilet Facility			
Water closet system	124	28 (22.6)	>0.05
Pit latrine	132	39 (29.5)	
Open field	104	21 (20.2)	
Finger Sucking habit			
Yes	70	23 (32.9)	< 0.05
No	290	65 (22.4)	

Table 4. *E. histolytica* positive samples detected by microscopy and ELISA methods

TechLab ELISA +ve	ELISA -ve	Microscopy +ve	Microscopy -ve
85	3	88	0

attributed to the fact that children within this age group are found to be playing on the sand with no care. They tend to be ignorant to the principle of cleanliness and personal hygiene.

The prevalence of infection among the different occupational groups of the parents/guardians of the children in this study also varied insignificantly. This means that, the prevalence rate of the infection cut across the socio economic background of their parents/guardians. This is in agreement with the epidemiological study which shows that low socioeconomic status is a risk factor for infection and that infection; particularly the parasitic ones, are seen in regions with low socioeconomic status [19].

In this study, significant association was observed among children in and outside Keffi, even though both areas apparently have similar sanitary and other environmental challenges. Keffi town and its environs are characterized by poor environmental hygiene as evidence by heaps of refuse dumps coupled with indiscriminate disposal of human wastes, even by the road side. Also, active children are often seen playing and picking objects from such heaps of refuse dumps and thereby get exposed to pathogens including *E. histolytica*. The association observed with respect to these two areas suggests that other risk factors may be at play as reported by Haque et al. [24].

The prevalence of the infection was highest among parents/guardians of children with the lowest level of education and lowest among those with a tertiary educational qualification ($p > 0.05$). Education has been acknowledged to be of advantage in various facets of life. It helps in making informed decision and also sourcing for useful information regarding health concerns [25].

In this study source of water was not a factor responsible for infection by *E. histolytica* ($p > 0.05$). Many communities in North Central Nigeria depends on well and stream water for drinking and other domestic uses; these sources of water are usually left uncovered and are subject to contamination with cysts of *E. histolytica*, which are the infective stage, from various types of wastes including human and animal faeces. Cysts are known to persist in water for weeks or months and in the dry season, are known to withstand dessication and survive for a long period in the environment [26,2,27]. Water, irrespective of its source can easily be contaminated during handling, especially where sanitation and personal hygiene of caregivers are generally poor [26].

The type of toilet facilities used in homes of the children similarly showed no significant influence on infection. The relative safety of the water closet system depends on the availability of water. Tap water is increasingly becoming scarce commodity in Keffi and thus most homes do not actually have the luxury and safety of water closet system. This is why infection was found in most homes irrespective of the toilet facility in use.

A significant high prevalence of infection among children that suck finger (32.9%) and those that do not suck finger (22.4%) was observed in this study ($p \leq 0.05$). The habit of sucking finger is developed by children, it is a reflex action and is done without the consciousness that the finger may be dirty or harbour infectious agents. Finger sucking has the potential to aid direct transmission which was revealed in this study. In other words, a child that suck his/her finger is vulnerable to contract the infection in the study area especially in populations with poor personal hygiene.

A further study that requires detection of adhesin in the positive stools examined by microscopy was carried out. The investigation represents the first time research to use commercially adhesin

detection kit to investigate the prevalence of *E. histolytica* infections in Nasarawa State, Central Nigeria in addition to microscopy.

The TechLab *E. histolytica* II test revealed that out of the 88 school children screened by microscopy, only 85 (96.6%) were *E. histolytica* positive, this indicates that the remaining 3 (3.4%) stool samples were negative to the parasite. This is in contrary with works of Saeed and Manal, [16]; Lebbad and Svard, [28].

The study also indicates that *E. histolytica* specific ELISA was able to detect the antigens of the parasite and some new cases had not developed antibodies against the infection. Undoubtedly, microscopic examination is inexpensive compared to antigen detection method. However, identification of amoebae parasites mostly rely on the proficiency of the microscopist performance and interpretation. Results of microscopic examination therefore vary between studies based on staff experience and techniques used for diagnosis owed to the difficulty in differentiating *E. histolytica* infections. Many laboratories also suffer from the decrease number of well expertise staff. Over diagnosis *E. histolytica* infection and microscopical false positives have been reported [16]. It may be attributed to the load of the parasite in the faecal sample; stools with a low quantity of cysts, according to the manufacturer of the ELISA some sample may react weakly and therefore are inconclusive which may be due to factors such as the presence of binding substances and inactivating enzymes in the faeces. Under such conditions, a fresh specimen should be tested [27].

Entamoeba histolytica infection is one of the Neglected Tropical Infections (NTI's) that affects most rural communities in developing countries that possess great problems on human development. This has an adverse effect on school children who are the hardest hit.

5. CONCLUSION

This study concludes that the infection has a relatively high prevalence rate among school children in Keffi with potential health consequences. Children of both sexes and different ages were infected and the infection was higher in males than females and in older school aged children (6-10 years). The ELISA test for detection of *E. histolytica* antigen in stool samples had 85 (96.6%) positive cases for the

presence of the antigens and thus some school children are asymptomatic to the infection therefore serve as a big source of transmission of the organism to new ones.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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