ejpmr, 2020,7(1), 491-498

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

PREVALENCE OF *ENTAMOEBA HISTOLYTICA* AMONG PUPILS OF ALMAJIRI INTEGRATED MODERN BOARDING SCHOOL, SOKOTO, SOKOTO STATE-NIGERIA

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Article Received on 22/11/2019 **Article Revised on 13/12/2019** **Article Accepted on 02/01/2020**

ABSTRACT

Entamoeba histolytica is still considered a major public health problem and a major cause of morbidity and mortality in developing countries of the world because of lack of safe portable water and low level of hygiene due to high level of poverty. This study determined the prevalence of *Entamoeba histolytica* among pupils of Almajiri School Sokoto, sokoto State, Nigeria. A total of 120 stool samples were examined for *E. histolytica* cyst and trophozoites using microscopic formal ether sedimentation techniques. Sixty (50%) of the samples were found positive for the parasite. Male 60/120 (50%) were positive for amoebiasis since no female subjects were recruited for this research, but there was no statistical association between prevalence and gender ($P<0.05$). Pupils within age group 7-8 years old (63.83%) has the highest rate of infection. There was a positive association between prevalence and age ($P<0.05$). Insignificance highest prevalence of (85.00%) and (53.33%) was recorded in class one pupils and Fulani tribe ($P<0.05$). the highest prevalence of (60.94), (60.66) and (53.33) was recorded among pupils who drinking water fetched from river/stream, use bush as toilet and lives in rural areas ($P<0.05$). Improved sanitation, personal hygiene, health education and policy for regular de-worming of pupils by parents and government will decrease the rate of infections.

KEYWORDS: Prevalence, *Entamoeba histolytica*, Pupils, Almajiri, Sokoto.

1. INTRODUCTION

Amoebiasis is an infection caused by a protozoan parasite called *Entamoeba histolytica*.[37] *Entamoeba histolytica* is an intestinal protozoan of humans. Several species of the genus *Entamoeba* infect humans. These include: *Entamoeba histolytica*, *Entamoeba dispar*, *Entamoeba coli*, *Entamoeba hartmanni*, *Entamoeba polecki, and Entamoeba gingivalis.*[14] Among these, only *E. histolytica* is considered pathogenic and the disease it causes is called amoebiasis or amoebic dysentery.[27] Amoebiasis occurs worldwide, with approximately 50 million people infected annually, causing close to 100,000 deaths per year.[26]

Entamoeba histolytica is an enteric parasite that colonizes the human intestinal lumen and has the capacity to invade the epithelium. Amoebic dysentery occurs when *E. histolytica* trophozoites invade the walls of large intestines and multiply in the mucosa, forming ulcers. Most frequent manifestations of infection are dysentery, colitis, flatulent stomach, weight loss, fatigue and abdominal pain. A common outcome of invasion of the amoeba into tissues is liver abscesses which can be fatal. The pathogen secretes histolysin[29], which digest the gut of the infected individual hence the Latin name, histo (tissue) lytica (destruction).[29]

Ingestion of contaminated food or water containing infectious cysts leads to excystation in the intestine. Each cyst produces eight motile trophozoites which colonize the host's colon. In those cases where the infection is not self limiting, amoebic dysentery and liver abscesses formation can occur. Ninety percent of infections with *E. histolytica* are asymptomatic and self limiting.[27] Reported that an estimated 50 million cases of invasive infection occur annually.

Intestinal amoebiasis is frequently asymptomatic. Symptomatic cases vary from dysentery with fever, chills and bloody or mucoid diarrhoea alternating with periods of constipation. Also, invasive infection can cause severe amoebic dysentery.[5] Extra-intestinal amoebiasis occurs when the parasite invades other organs such as liver, lung or brain causing abscesses.[3] Only approximately 10% of *E. histolytica* infected individuals show clinical symptoms with intestinal and/or extra-intestinal pathology.[31]

Infection by the parasite can be acquired by the feacal-oral route either directly by person-to-person contact or indirectly by eating or drinking faecally contaminated food or water.[25] Studies have identified inadequately treated drinking water and ingestion of raw vegetables as

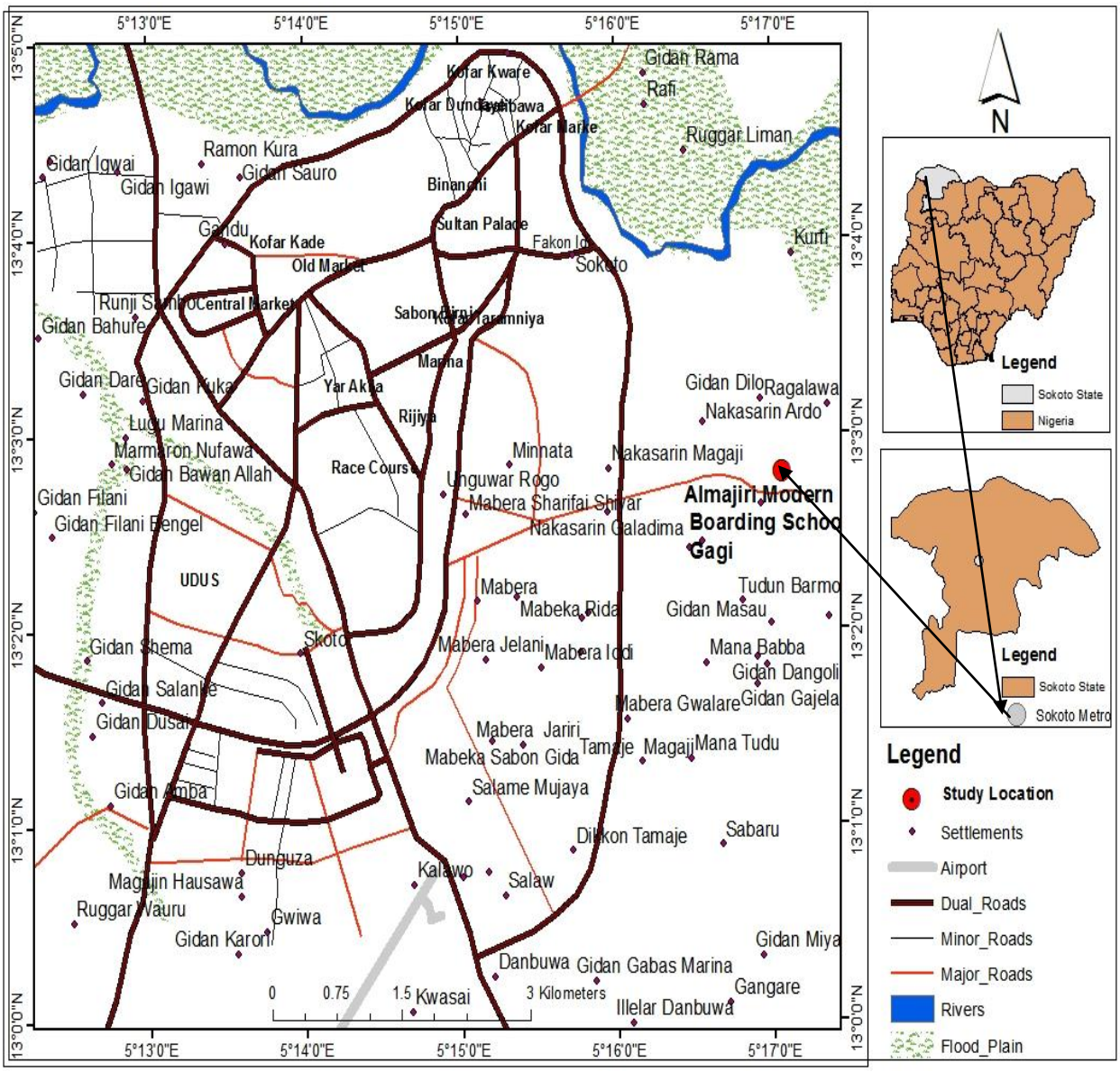
risk factors for infection.[8] generally, the risk of infection is highest in areas of poverty and in settings with poor sanitation where barriers between human faeces and water are inadequate.[29] *Entamoeba histolytica* is the pathogenic species of *Entamoeba* that causes amoebic dysentery or amoebic colitis and other invasive forms of the disease.[29]

Entamoeba histolytica is well recognized as a pathogenic amoeba associated with intestinal (amoebic colitis) and extra-intestinal (liver abscess) infections.[27-37] Amoebiasis is more common in areas of poor sanitation and nutrition particularly in the tropic.[26] Morbidity and mortality occurs in Africa, Asia, Central and South America.[25] Amoebiasis is ranked the third most important parasitic disease after malaria and second protozoan diseases to *schistosomiasis*, and that 100,000 deaths occur annually due to the disease.[36] The morbidity and mortality is primarily seen in the developing countries. The major cause of transmission is poor sanitation, particularly where food and water are concerned.[33] [36] Reported that *Entamoeba histolytica* affects approximately 500 million people worldwide, resulting in symptomatic diseases in 50 million and mortality in 100,000 persons.[18] About 80-90% of infections ware asymptomatic and are likely due to the nonpathogenic species *E. dispar* or *E. moshkovskii*. Therefore, the worldwide incidence of *E. histolytica* is more likely estimated to be 5 million cases annually, with global mortality still at 100.000 persons per annum.[37] More than any other causes, parasitic diseases are contributing significantly to the burden of illnesses, leading sometimes to death, and affecting people in developing and in developed world, even in regions that include high income countries.[37] This research work aimed at investigating the prevalence of *E. histolytica* among pupils of Almajiri School, Sokoto with a view to provide data about the disease and recommend the possible way of minimizing the infection.

2. MATERIALS AND METHODS

2.1 Study Area

This study was conducted at Almajiri integrated modern boarding school Gagi, Sokoto. The school is located in the township center of Sokoto South Local Government Area, opposite Minarat Academy School along Durbawa road Sokoto. It was established and commissioned in April, 10th 2012 by the then president Goodluck Ebeli Jonathan. The school was first of its kind build by the Federal Government of Nigeria to provide equal access to qualitative education for the Almajiri. Sokoto State is one of the Northwestern States of Nigeria. It is located 13^{0} 05'N 05^{0} 15'E as seen in figure 2 and cover the extensive geographical area of about 25, 973 square kilometer with a total population of over four million people.[23] Sokoto State shares border with Zamfara State, Kebbi State and Niger Republic. The State consist of 23 local government areas; with its head quarter in Sokoto and its peoples are predominantly Hausa – Fulani whose main occupation is farming and animal husbandry.[7]



Source: GIS Lab. Department of Geography UDUS 2019
Figure 2: Map of Sokoto and its Environs.

2.2 Study Population

The study population consists of two hundred (200) pupils class/form one to six, from Almajiri school, Sokoto, Northwestern, Nigeria.

2.3 Study Design

This is an observational cross section study aimed at determining the prevalence of *Entamoeba histolytca* among pupils of Almajiri School Sokoto. The research was conducted during the rainy season from March to November 2019. The procedure was explained to all participant and were each given the consent form to sign. Questionnaires were distributed to generate information on their bio-data.

2.4 Sample Size Determination

The sample size was determined by the method used by[16] and[35] for calculating sample size.

$$N = \frac{Z^2pq}{d^2}$$

Where:
N = sample size
Z = standard normal distribution
p = prevalence factor
q = complementary proportion of P (1-p)
d = tolerable margin of error

Thus:
N= ?
Z= 1.96
p= 0.0851 (Inovo *et al* 2000 and Wayne 2010)
q= 1- p = 1- 0.0851 = 0.9149
d= 5% (0.05)

Therefore

$$N = \frac{1.96^2 \times 0.0851 \times 0.9149}{0.05^2}$$

$$= \frac{3.84 \times 0.0779}{0.0025}$$

= 120

However, a total of one hundred and twenty (120) pupils of all ages from Almajiri Sokoto, form the subject of this study.

2.5 Ethical Clearance

An introduction letter to undertake the study was obtained from the Head of Department of Biological Sciences, Sokoto State University, Sokoto and submitted together with the research proposal to the management of Almajiri School Sokoto through the principal of the school. Ethical clearance and permission was granted by the management of the school which served as official approval for the research.

2.6 Administration of Questionnaire

A structured questionnaire was administered to the one hundred (120) students recruited in to study. Respondents who could not read or write in the English language were interviewed in Hausa by the researcher. The questionnaire was designed to provide information such as full name, age, sex, class, and risk factors such as source of drinking water and type of toilet used.

2.7 Samples Collection

Pupils of class/form one to six were recruited as subjects of the study. Stool sample were collected systematically from the 120 subjects. Specimens were collected into disinfected plastic specimen bottles (EDTA) at the various classes, with specific instruction given to the teachers/guardians of the students who collected the sample and submitted to the researcher. 2-3 drops of formalin were added to each samples and transported to Biology Laboratory of the Department of Biological Sciences Sokoto State University Sokoto within 1 hour, where they were assayed as described by.[21]

2.8 Laboratory Analysis of the Sample

This was done with the assistance of a qualified Laboratory technologist. The collected stool specimen was first observed physically for consistency, presence of blood stains and any macroscopic parasites as described by.[20] One to two grams (1-2g) of each specimen initially fixed with 2mls of 10% formal saline was emulsified in about 6mls of 10% formal saline by stirring with a clean glass rod in test tube. The suspension was filtered over two layers of surgical gauze into a 10mls-capacity centrifuge tubes. 2mls of diethyl-ether was then added into each tube and shaken vigorously. This was centrifuged using a centrifuge machine (KA-1000 Model SSU/BIO/CENT/01) at increasing speed to a maximum of 1000rpm for about 5mins. 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 binocular microscope at magnifications of ×10 and ×40 objectives for the presence of *E. histolytica* cysts and trophozoite as described by.[8]

2.9 Statistical Analysis

Data generated from this research were analyzed using the Statistical Package for Social Sciences (SPSS) Version 21.0. The results were presented in tables showing frequency distribution. Chi-square (χ^2) tests were conducted for analyses to determine the relationship between the *E. histolytica* prevalence and pupils age, class/form, tribe, risk factors and locality of the pupils at confidence interval (CI) of 95% as described by.[35]

RESULTS

The results of our findings showed that, a total of one hundred and twenty (120) pupils were examined for *E. histolytica* infection and are within aged range 7-15 years old. All the participants were from Sokoto and Zamfara states. Out of the number examined, sixty (60) pupils (50.00%) were to be infected with *E. histolytica.*

Table 1: Prevalence of *E. histolytica* in the Study Area

A total of one hundred (120) stool sample were collected and examined for *E. histolytica*, the result of *E. histolytica* prevalence in the study area showed that 60/120 were positive for *E. histolytica* infection which represent a total of 50%. All the 120 stool samples were male since there is no female subject in the study area as seen in Table1.

Table 1: Prevalence of *E. histolytica* in the Study Area.

Gender	No. Examined	No. Positive (+)	Prevalence (%)
Male	120	60	50
Female	-	-	-
Total	**120**	**60**	**50**

χ^2= 3.8415, df= 1, P= 0.0000

Table 2: Prevalence of *E. histolytica* According to Age Group of the Pupils

The result of *E. histolytica* prevalence according to the different age group of the pupils showed that age group of 7-8years 30/47 had 63.83% *E. histolytica* infection, age group of 9-10years 20/36 had 55.56% *E. histolytica* infection, then age group 11-12years 8/27 had 29.63% *E. histolytica* infection. While, age group >12year 2/10 had 20.00% *E. histolytica* infection as seen in Table 2.

Table 2: Prevalence of *E. histolytica* According to Age Group of the Pupils.

Age Group	No. Examined	No. positive (+)	Prevalence (%)
7-8	47	30	63.83
9-10	36	20	55.56
11-12	27	8	29.63
>12	10	2	20.00
Total	**120**	**60**	**42.26**

χ^2= 7.8147, df= 3, P= 0.0000

Table 3: Prevalence of *E. histolytica* According to Class/Form of the Pupils

The result of *E.histolytica* infection According to different classes of the subjects has indicated that out of the 60 samples that were positive for *E.histolytica*, class 1 17/20 had 80.00% *E.histolytica* infection, class 2 14/20 had 70.600% *E. histolytica* infection, class 3 12/20 had 60.00% *E.histolytica* infection, class 4 8/20 had 40.00% *E.histolytica* infection, class 5 6/20 30.00% *E. histolytica* infection and class 6 3/20 had 15.00% *E. histolytica* infection as seen in Table 3.

Table 3: Prevalence of *E. histolytica* According to Class/Form of the Pupils.

Class	No. Examined	No. positive (+)	Prevalence (%)
1	20	17	85.00
2	20	14	70.00
3	20	12	60.00
4	20	8	40.00
5	20	6	30.00
6	20	3	15.00
Total	**120**	**60**	**50.00**

χ^2= 11.0705, df= 5, P= 0.0169

Table 4: Prevalence of *E. histolytica* Based on Tribe of the Pupils

The prevalent of *E. histolytica* infection based on the tribe of the subjects indicated that subject with Hausa tribe 44/90 had 48.89% *E. histolytica* infection. While Fulani 16/30 had 53.33% *E. histolytica* infection as seen in Table 4.

Table 4: Prevalence of *E. histolytica* Based on Tribe of the Pupils.

Tribe	No. Examined	No. Positive (+)	Prevalence (%)
Hausa	90	44	48.89
Fulani	30	16	53.33
Total	**120**	**60**	**51.11**

χ^2= 3.8415, df= 1, P= 0.0003

Table 5: Prevalence of *E. histolytica* in Relation to Water Source of the Pupils

The prevalent of *E. histolytica* infection in relation to water source of the pupils indicated that, pupils that use River/Stream as their source of drinking water has the highest prevalence of 60.94% infections, followed by those that use well water for drinking with 47.22% infection. Pupils that use Tap/Borehole water as their drinking water has the least infection of 20.00% as seen in Table 5.

Table 5: Prevalence of *E. histolytica* in Relation to Water Source of the Pupils.

Water source	No. Examined	No. Positive (+)	Prevalence (%)
Tap/Borehole	20	4	20.00
Well	36	17	47.22
River/Steam	64	39	60.94
Total	**120**	**60**	**42.72**

χ^2= 5.9915 df= 2 P= 0.0000

Table 6: Prevalence of *E. histolytica* in Relation to Type of Toilet Use by the Pupils

The result of *E. histolytica* infection in relation to type of toilet use by the pupils indicated that, pupils that use bush for defecation has the highest prevalence of 60.66% infections, followed by those that use Pit latrine in their home with 46.34% infection. The least infection of 22.22% infection was recorded in those whose home use Water closet as seen in Table 7.

Table 6: Prevalence of *E. histolytica* in Relation to type of toilet use by the Pupils.

Type of Toilet	No. Examined	No. Positive (+)	Prevalence (%)
Water system	18	4	22.22
Pit latrine	41	19	46.34
Bush	61	37	60.66
Total	**120**	**60**	**43.07**

χ^2= 5.9915 df= 2 P= 0.0000

Table 7: Prevalence of *E. histolytica* in Relation to Locality of the Pupils

The prevalence of *E. histolytica* in relation to locality of the subjects showed that out of the 60 positive samples that rural areas 48/90 had 53.33% *E.histolytica* infection, and urban areas 12/30 had 40.00% *E.histolytica* infection as seen in table 7.

Table 7: Prevalence of *E. histolytica* in Relation to Locality of the Pupils.

Locality	No. Examined	No. positive (+)	Prevalence (%)
Urban	30	12	40.00
Rural	90	48	53.33
Total	**120**	**60**	**46.67**

χ^2= 3.8415, df= 1, P= 0.0000

DISCUSSION

The result of the present study showed that the overall prevalence of intestinal amoebiasis among pupils of Almajiri School Sokoto stood at 50%. This high prevalence infection was attributed to poor water source. The result was in agreement with the finding of[14] who reported a prevalence of 50% in children having serological evidence of exposure to *E. histolytica* infection by 5 years of age in Dhaka, Bangladesh. The high prevalence could also be linked to poor sanitary condition within both urban and rural settlement of the pupils. But the result was in contrast with the finding of[22] with 46.00% in Bosso Town Niger State North Central Nigeria,[2] with 30.80% among student in Katsina,[10] with 42.10% prevalence among children attending primary Schools in Kyuso District, Kenya and[13] with 42.00% among children age 1-5 years in Zaria. The high finding might be attributed to resistant of cyst of *E. histolytica* in the study area which can withstand adverse condition.

The prevalence obtained in this study was lower compared to study carried out by[21] with 56.90% among school-age children in Wamakko Local Government Area of Sokoto State North Western Nigeria,[15] with 72.00% in Abeokuta Ogun State South Western Nigeria,[9] with 80.90% in Konduga Borno State North Eastern Nigeria,[12] with 64.10% in Akonkwo Imo State South Eastern Nigeria and[28] with 67.63% in Akure Ondo State South Western Nigeria.

The result based on age group indicate that prevalence of *E. histolytica* among pupils of 7-8 years showed the highest infection rate 63.83%, this is not surprising and was attributed to the fact that children have been reported to be more exposed to infection with *E. histolytica* than adults. They are more susceptible to water-borne and food-borne infections, because, their playing and hygiene practices predispose them to infection than older, more so, their immune systems are not fully developed and their level of health education is not sufficient to distinguish the dangers of contaminations. This agreed with research carried out by[21] among school-age children in Wamakko Local Government Area, Sokoto State, which recorded highest prevalence in younger pupils than older ones also[28] and[4] reported high prevalence in younger pupils than adult in their separate researches in Akure and Benue among women and students attending primary schools. However, this finding is not in line with the prevalence rate of research carried out by[10] among primary school children in Kyuso district of Kenya, which recorded high prevalence infection in older age than younger ones. Similarly, similar situation happen to class of the subjects, but here it is attributed to their level of education and awareness.

The results based on water sources revealed that, the prevalence infection with *E. histolytica* showed there is significance differences, those that consume river/stream water has the highest prevalence infection of 60.94% this might be because human and domestic/wild animals use these (River/stream) as source of drinking water especially during dry season in local communities. A situation that could lead to contaminations with faeces and subsequent infection of exposed individuals, then followed by the those that consume well water with 47.22% this may be due to some well in the community are dug close to pit latrine and poor sanitation around the well enhances the transmission of the infection and those that consume borehole/tap water has the least prevalence infection. This agreed with the research carried out by[21] on prevalence of *E. histolytica* among school-age children in Wamakko, Sokoto State North Western Nigeria were they recorded highest prevalence infection among pupils that consume river/stream water 71.70% and[28] on intestinal amoebiasis among primary school children in Akure, ondo State South Western Nigeria, were they recorded highest prevalence infection among those pupils that consume stream water 94.30% followed by those that consume well water with 74.4% and those

that consume tap water has the least prevalence infection. Also our finding is in line with the research on intestinal amoebiasis carried out by[3] were they reported significance infection rate for laser of surface water and unprotected well water, while they recorded low prevalence infection for tap water and protected borehole water. However the finding of this study is not in line with report of[11] and[24] reported the prevalence *E. histolytica* was not associated with type of water supply but was seemingly influenced by storage of household supplies.

Based on our finding in this study, the prevalence of *E. histolytica* in relation to use of latrine indicated that those pupils that do not have latrine at home and use bush has a higher prevalence of infection 60.66% than those that have latrine at home having lower prevalence rate with slight differences, this could be due to poor sanitation which may encourage flies and cockroaches to spread the cysts of *E. histolytica*.[1] Also must of the people that do not have latrine at home involved in open defecation which can lead to washing away of the faeces contaminants into community water source during raining season, this practice could also enhance the transmission of cysts.[6] Findings based on open field defecation revealed that the prevalence of *E. histolytica*, shows that pupils who practice open field defecation toilet system has the highest prevalence of infection while those that do not practice open field defecation toilet system has the lower prevalence infection, similar observation were made among those that use open field defecation toilet system by.[32] This could be due to poor sanitation which might encourage flies and cockroaches to spreads the cysts of *E. histolytica*.[1]

5.2 CONCLUSIONS

The findings of this research work showed that *E. histolytica* is an endemic disease and of public health concern in Sokoto. Indeed almost half or more than half of the pupils were infected with this pathogenic intestinal protozoan. The following conclusions were therefore drawn: *Entamoeba histolytica* infections are very common in school children aged 7 - 12 years in Sokoto. This is indicated by the high prevalence (50%) observed. A significant association was observed between risk factors and prevalence where particularly the use of dry river bed wells and Earth dams and use of bush as latrine were significant. The parasite's prevalence decreases when tap water is used and increases when surface water is used, Age variables are relevant for prevalence of *E. histolytica* where some major variations occurred in the prevalence among the different age group. Improve sanitation, personal hygiene and policy for regular de-worming of school-age pupils by parents or government will decrease the rate of infections.

ACKNOWLEDGEMENTS

We gratefully acknowledge the contributions made by the management of Almajiri School Sokoto, most especially the principal, vice principal and entire staff, were very cooperative during the period of this study. We will also want to thank Ministry of Health and Ministry of Education sokoto state Nigeria for their generous cooperation during this study. We also thank the participant for their voluntary participation in this study. The authors also thank the entire staff of the Department of Biological Sciences, Faculty of Science, Sokoto State University, Sokoto Nigeria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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