

(RESEARCH ARTICLE)



Pattern of *E. histolytica* infection and its correlates among patients in a general practice clinic: A four-year retrospective evaluation

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Abstract

Background: Overcrowding, poor hygiene and scarcity of portable water are common features of population growth in a resource poor nation especially with rising cost of living from inflation. This is the often the state of many underdeveloped nations such as Nigeria. Such environments encourage the spread of communicable diseases. This study assess the pattern, trend, and at-risk population of *Entamoeba histolytica* (*E. histolytica*), implicated in intestinal Amoebiasis in South-South Nigeria.

Method: This retrospective study was conducted using hospital data obtained from the laboratory department of a general hospital clinic in Warri and covered a period of four years from January 2015 - December 2018.

The record of 4,169 laboratory results of stool samples examined by direct smear and concentration technique, were recovered from a designed computer database. Trend of infection over the period as well as variations in distribution patterns between sex, age and seasons were determined using appropriate statistical tools.

Results: Out of the 4,169 stool samples 365 (8.8%) had *E. histolytica* with decreasing trend of infection from 2015 – 2018 as 135 of 1210 (11%); 118 of 1114 (10.6%); 57 of 792 (7.2%); 55 of 1053 (5.2%) respectively. Prevalence of infections was higher in females, 57.5% (95CI%, 51.7-59.7, n= 210) than males (n=155, 42.5%, 95%CI, 40.3-48.6). The prevalence of infections was highest among Age brackets 1-5years, 6-15 years and 35+ years; but lowest in the 26-35 years with peak season of infections at first and last quarters of each year.

Conclusions: Children are more vulnerable to *E. histolytica* infestation and the dry seasons of the year place them at risk. This information underscores the need for local surveillance to evaluate the effectiveness of control and preventive health measures with a view of reducing the prevalence and morbidity of parasitic infections in our locality.

Keywords: *E. histolytica*; Infection; Direct smear; Concentration technique; Stool

1. Introduction

Rising cost of living in the face of uncontrolled population growth in a resource poor nation as Nigeria is greeted with poverty and its attendant problems of overcrowding, poor hygiene and scarcity of portable water. Factors of course that are favourable to enhanced spread of communicable diseases. Intestinal amoebiasis is one of such communicable diseases, caused by infection with the pseudopod-forming, non-flagellated protozoan parasite, with a world-wide distribution known as *Entamoeba histolytica* ^[1].

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Transmission of *E. histolytica* occurs after the ingestion of the infectious cyst. This most commonly arises from fecally contaminated hands, food, or water, but there is also the risk of infection among men who have sex with men (MSM) due to increased chances of oro-anal sex [2]. It has an estimated prevalence of about 500 million infections worldwide as a result of current techniques showing other serotype –*E. dispar* [3], and about 40,000 to 110,000 deaths annually [4].

E. histolytica infection exhibits a variety of clinical presentations, from asymptomatic intestinal colonization to invasive amebic dysentery and extra-intestinal amoebiasis [5]. Intestinal amoebiasis is frequently asymptomatic with only approximately 10% of *E. histolytica* infected individuals showing clinical symptoms with intestinal and/or extra-intestinal pathology [6].

The prevalence of infection by *E. histolytica* vary among countries, study design, targeted sample type, sample size, incubation, symptom severity, and the sensitivity of the diagnostic modality used. Globally it ranges from as low as 1% to as high as 42% [1]. In many developing countries, the burden of parasitic diseases are a major source of concern, especially in the younger age group, which are often malnourished pregnant women, those on corticosteroids and immunosuppressed [4, 6]. However, in industrialized nations, on the other hand, populations at greatest risk for severe intestinal protozoan infections include immune-compromised patients [7, 8].

Several epidemiological studies have indicated a high prevalence of intestinal parasitic infections among Nigerian children [9 -12]. Nonetheless, most of the published works were either age specific, or school based. This study was undertaken to assess the pattern, trend of *Entamoeba histolytica*, and at-risk group across the studied population of varied aged groups.

2. Material and methods

2.1 Patient selection

This is a retrospective evaluation, of 4,169 laboratory results of stool samples examined by direct smear and concentration method in computer database of the laboratory department of the General Hospital Warri, within a 4-year period, from January 2015 and December 2018,

The population comprise of all patients Infants, children and adults from birth to 60 years of age either symptomatic by presenting with acute gastroenteritis (GE) that necessitated hospitalization or Emergency Department (ED) management or asymptomatic as in those categories for routine screening (pre-school, pre-employment for food handlers etc.) were enrolled for the study. This study was approved by the research and ethical committee of the hospital institutional review board.

2.2 Exclusion criteria

All patients above 60 years, those with chronic diarrhea, those with immunodeficiency and those having any extra-intestinal infection at the time of presentation, were excluded from the study. Patients with diarrheal illness whose medical record showed had received antibiotics in the preceding 1 month that might be related to bacterial infection with more than one organism were also excluded from the study.

2.3 Methodology

All the report of stool samples sent to the laboratory for clinical and routine screening examination were extracted from the data base along with their case file numbers. Clinical notes and drug history were reviewed to exclude those not eligible. The following data were recorded for all the patients, Age, gender, residence and period of presentation.

2.4 Method and Sample types analyzed by the laboratory of the hospital

Most samples received as documented were fresh stool samples collected in sterile containers and sent within a maximum period of 2h to the hospital laboratory and were examined for intestinal parasites within 30 min of reaching the laboratory using a concentration technique according to the manufacturer's instructions (Fecal Parasite Concentrator (FPC), Evergreen Scientific, Los Angeles, CA, USA). However, “watery stool” were examined microscopically without concentration looking for trophozoites of parasites.

Concentration method was done by adding by one spoonful of stool specimen to 9 ml of 10% formalin in a 15 ml sterile tube. After fixing the mixture at room temperature for 30 min, three drops of Triton X-100 (surfactant) were added, followed by 3 ml of ethyl acetate to dissolve fat and reduce the bulk of stool.

The mixture was transferred to a 15 ml centrifuge tube through the FPC strainer attached to the tube. This FPC strainer has a precision molded filter matrix (0.6 × 0.6 mm² holes) that allows helminthes eggs and larvae, protozoan cysts, and coccidian oocytes to pass but will retain the coarse particulate matter (excess fecal debris). After completing this filtration step, the tube was capped and centrifuged at 2000 ppm for 10 min. The supernatant was decanted, and 3 drops of 10 ml of 10% formalin were added and mixed with the sediment. Part of the latter was transferred to a slide and examined for parasites under the light microscope.

2.5 Statistical analysis

Analysis was done using statistical package for social science program version 19. Data were reported as mean standard deviation (SD), or number of patients (percentage). T-test (two-tailed) and Chi-square test were used to assess any significant difference between the groups. P < 0.05 were considered statistically significant.

3. Results

Out of the 4,169 stool samples 365 (8.8%) had *E. histolytica* as shown in figure 1, with decreasing trend of infection from 2015 – 2018 as 135 of 1210(11%); 118 of 1114 (10.6%); 57 of 792 (7.2%); 55 of 1053 (5.2%) as shown in figure 2. respectively.

Also, Prevalence of infections was higher in females, 57.5% (95CI%, 51.7-59.7, n= 210) than males (n=155, 42.5%, 95%CI, 40.3-48.6) in figure 3.

Furthermore figure 4 and 5 showed that the prevalence of infections was highest among Age brackets 1-5years, 6-15 years and 35+ years; but lowest in the 26-35 years with peak season of infections at first and last quarters of each year.

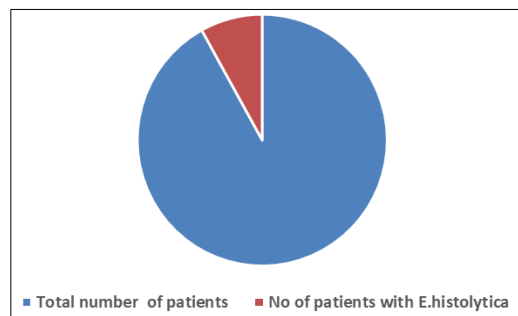


Figure 1 Prevalence of *E. histolytica* in the study population

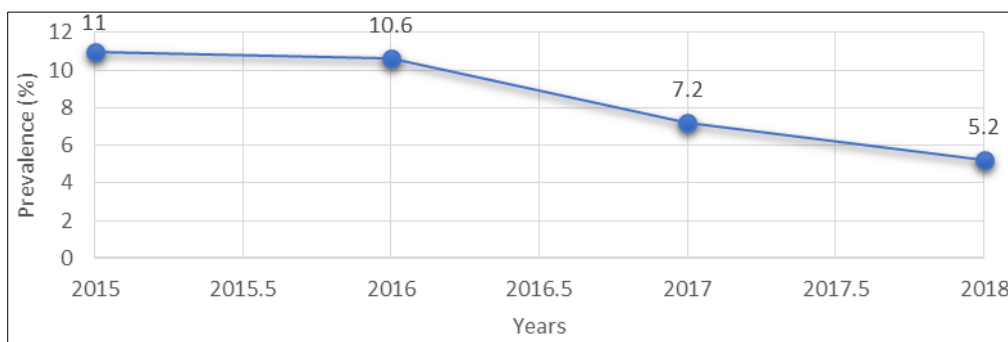


Figure 2 Yearly trend of prevalence of *E. histolytica*

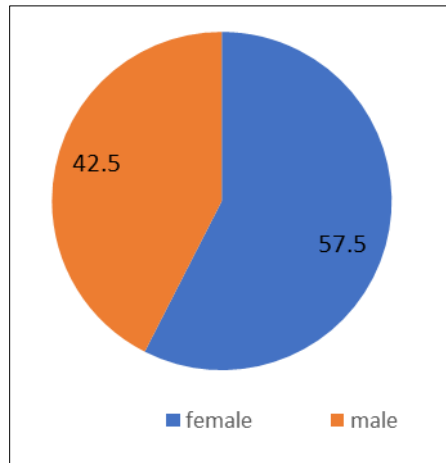


Figure 3 Sex distribution of infected subjects

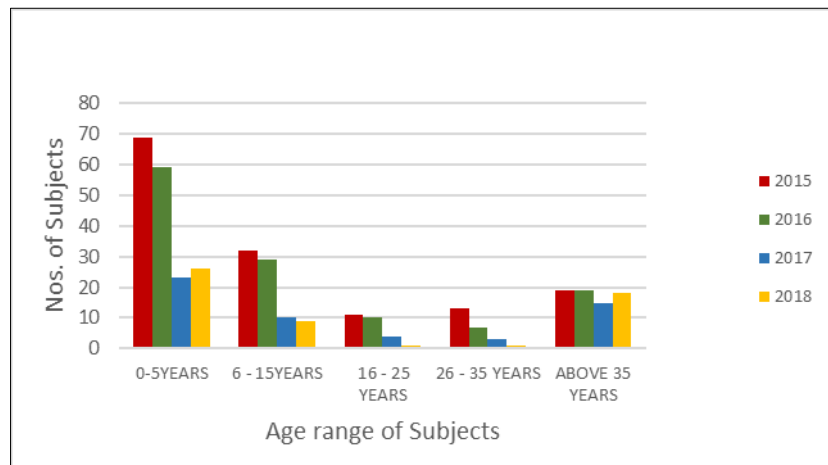


Figure 4 Age distribution of infected subjects

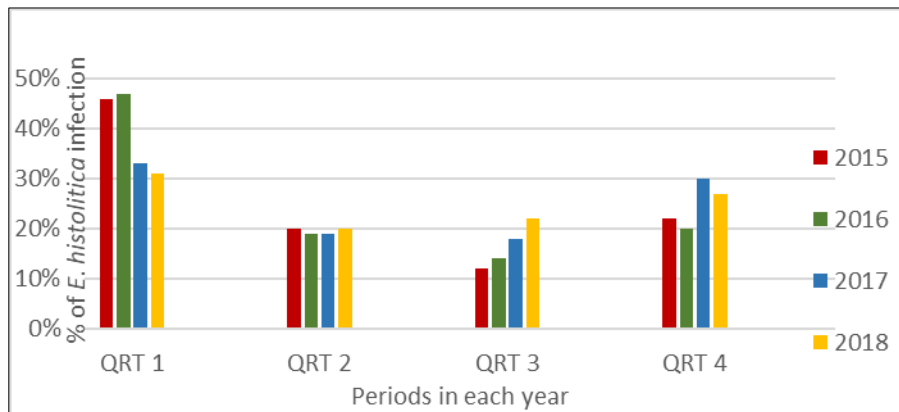


Figure 5 Quarterly prevalence of *E. histolytica* in each year

4. Discussion

The diagnosis of *E. histolytica* infection in this study was based on detection of *E. histolytica* trophozoites or cysts in several fresh stool samples using Fecal Parasite Concentrator, which is an effective device to concentrate parasites and protozoa [13].

The 8.8% average prevalence of *E. histolytica* over the four years period recorded in the study was much lower than the 22.3% from other studies, reported in Asia. However, it was similar to the 8.5% reported by Renay et al in South Africa [14]. Other countries with higher prevalence were 20.0% in Saudi Arabia [4], 17.1% in Yemen [15], 25.9% in Tajikistan [16], and 18% in Tanzania [17]. In Nigeria several studies have reported prevalence ranging from as low as 0.1% to values as high as 67.6% [11, 12, 18, 19]. The difference between most of these studies and ours is the fact that most of them were based on a population subset, and some included clinical manifestation unlike ours that reviewed the parasitology records of an undifferentiated hospital population. Also, most of the increased prevalence of *E. histolytica* infection was reported among the inpatient pediatric cases which may be attributed to the fact that this group is more at risk of manifesting the disease. One common inference drawn by all these studies, is the fact that differences in prevalence is attributable to associated risk factors which include, poverty, poor sanitation, cultural and religious issues, individual personal hygiene practices, unhygienic methods of waste disposal, poor clean or pipe borne water supply, immune status and level of enlightenment about the infection in the community. Also, as majority of the studies with higher prevalence were done mostly in the rural communities unlike our urban study evaluation. These communities are most likely poorer than the urban as well as more disadvantageous in public health campaigns and proper sewage disposal system.

Reassuringly, apart from a lower prevalence, our study also showed that *E. histolytica* prevalence had decreasing trend of infection from 2015 – 2018 with a decline from the (11%) in 2015 through; (10.6%), (7.2%) and (5.2%) respectively. A possible indicator of improved hygienic practices among study population where public enlightenment may have in recent times been heightened on appropriate waste disposal as *E. histolytica* is usually transmitted via fecal oral route with contaminated food and water. This is similar to the findings by Araj *et al* when looking the trends and prevalence of intestinal parasites in Lebanon over a decade [20].

Gender preponderance was also noticed in this study. The prevalence of infections was higher in females, 57.5% (95CI%, 51.7-59.7, n= 210) than males (n=155, 42.5%, 95%CI, 40.3-48.6). The increased prevalence among females may be attributed to the fact that women are more prone to hygienic driven activities like food preparation and handling as transmission is via fecal oral route with contaminated food and water.

Furthermore, the prevalence of infection was highest among Age brackets 1-5years, 6-15 years and 35+ years; but lowest in the 26-35 years with peak season of infections at first and last quarters of each year. This increased frequency of *E. histolytica* infection in this small age group, may be linked to the formula feeding procedures associated with such age group as well as the fact that children in that age group are mostly exposed to activities such as open defaecation and playing on sand that predispose them to infection than other age groups. In addition, immunity among the elderly may not be the same as those in the very active age bracket hence infection may thrive more among those age groups. This finding collaborates that Naous et al [21] who also recorded high prevalence among those age groups. Although, (16) found a considerable number of inpatients under 1 year infected with *E. histolytica* (80/311, 25.7%), which is an unusual presentation in this age group because *E. histolytica* is usually transmitted via fecal oral route with contaminated food and water, so young infants are less likely to develop intestinal amoebiasis very often [8].

The peak season of infections seen in our study population are the first and last quarters of each year. These periods tally with when most children who are among the most hit age group are in school session and may not be too compliant with hygienic practices when outside their homes. Also, these are periods of dry season and harmattan which can encourage the spread of the cysts. This finding was similar to the study by Naous et al [21] who though reported that *E. histolytica* was prevalent all over the year, but it seems to have its highest prevalence during summer and autumn seasons. The knowledge of the peak season of infection in different locality will help to narrow differential diagnosis for acute GE among children depending on the season.

5. Conclusion

Although the prevalence of *E. histolytica* infection in this study was high and the dry seasons of the year was found to be the period of peak, there was a decreasing trend over the four-year period with children more vulnerable to this infestation. Provision of portable pipe-borne water along with health education continues to be a reliable policy implementation path to stem this disease while efforts towards vaccine development is on-going.

Limitation of the study

This was a retrospective study, however, there were incomplete data and so we had to rely on every available documentation in the patients' medical charts. Microscopy has been the most widely used but has limited diagnostic utility compared to the gold standard which is the stool PCR, which unfortunately was not readily available to us. It would have been better if study was multi-centered to provide more informative data regarding the prevalence and nature of *E. histolytica* infection.

Compliance with ethical standards

Acknowledgment

We thank the staff of the Medical Laboratory dept. of general Hospital Warri for assisting us with the records used in this study.

Disclosure of conflict of interest

The author declares there is no conflict of interest.

Statement of ethical approval

This study proposal was discussed with the Medical Director of the practice, and was reviewed by the research and ethical review committee of the hospital. Approval was received on December 15th 2014.

Statement of informed consent

Consent to use clinical information from the hospital records without names as well laboratory results were all part of the approval obtained.

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