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Original Article

Epidemiological Prevalence of *Entamoeba histolytica* Infections Among the Patients Attending Nyanza District Hospital, Rwanda in 2018

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Abstract

Background and aims: *Entamoeba histolytica* is an intestinal parasite and a causative agent of amoebiasis which is a common life-threatening parasitic disease. This study was intended to determine the prevalence of *E. histolytica* and to provide the primary data about its infections among the patients attending Nyanza District Hospital in Rwanda.

Methods: Bottles were used to collect 138 stool specimens from patients. All the samples were physically analyzed based on their colors, states, and the presence of blood or mucus. The wet preparation method and zinc sulphate floatation technique were used to concentrate the parasites. Microscopic analysis was done to examine the presence of cysts and trophozoites. Data were statistically analyzed by SPSS using chi-square test and independent *t* test.

Results: The prevalence of *E. histolytica* was 15.94%. The sex distribution of infections revealed that males (21.54%) were more infected than females (10.95%). The highest prevalence of *E. histolytica* was found in the age group of 1-19 years old (27.11%) and the lowest prevalence was observed in the age group of 20-39 years old (6.89%). A prevalence of 33.33% was reported among the people who directly drink tap water and 40% of infections among the patients who did not wash their hands before eating and after using latrines.

Conclusion: Overall, *E. histolytica* is still considered as a health burden in Nyanza District Hospital. Therefore, it is vital to control direct exposure to its risk factors for mitigating the occurrence of amoebiasis.

Keywords: *Entamoeba histolytica*, Prevalence, Amoebiasis, Rwanda

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Introduction

Parasitic infections are currently affecting a large number of people of all races and demographic areas. Besides malaria and schistosomiasis, amoebiasis is also considered as the leading cause of mortality and morbidity worldwide.[1] It is caused by *Entamoeba histolytica* which is also the main cause of diarrhea, colitis and amoebic liver abscesses.[2] It is mainly transmitted through fecal-oral route by eating or drinking faecally-contaminated food or water. It is also transmitted through soil, fresh vegetables, direct contact between individuals, subjection in endemic places, and swimming in contaminated water.[3]

Entamoeba histolytica is a threat in tropical and subtropical regions worldwide with serious health burdens in developing countries due to inadequate health services, poor personal hygiene, and environmental conditions such as poor sewage disposal and low level of sanitation. It is commonly known to affect children under 15 years of age with a prominent increase in children aged between 5 to 9 years old.[4] The virulence of *E. histolytica* is attributed to its capability to release proteases and enzymatic chemicals. These enzymes allow it to invade the colon via the perforation of columnar cells and interfere with the host of antibody-mediated response.[5] It lives as cysts and trophozoites. Cysts are non-motile and infective forms of parasite and are excreted in the feces, whereas trophozoites are typically the motile forms found in diarrheal stool.[2,6] It has the ability to colonize human intestinal lumen and

its trophozoites invade the walls of the large intestine. It proliferates in the mucosa and induces severe ulcers. The trophozoites of this parasite remain in the intestinal lumen of individuals who are asymptomatic carriers, passing the cysts in their faeces. Any physical contact with these stools leads to infection.[7]

Amoebiasis is estimated to affect 3.5 billion people, causing 450 million health problems annually.[8] The prevalence is as high as 50% in different developing countries, especially African countries [9], due to inaccessibility to clean water, illiteracy, socioeconomic conditions, and lack of sanitation products.[10] Its high prevalence is observed in stunted, malnourished, and immunocompromised people such as HIV patients and gestating women in Uganda.[11] In Nigeria, the prevalence of amoebiasis is high among the families who ate with fingers, shared the same plates, and ate away from home in different food trucks or street restaurants. The low percentage of infections is observed in families which have water closets and modern toilets.[12] In Egypt, the prevalence of asymptomatic *E. histolytica* is high, with more than 21%, while the rates in South Africa and Côte d'Ivoire range between 0 and 2%.[13] Considering all research done in different parts of the world, there are no published ones on the prevalence of *E. histolytica* in Nyanza district to the best of my knowledge. The current study is specifically aimed to assess the prevalence of *E. histolytica* and to provide the first data on this protozoa infection by stool examinations among the patients who attended Nyanza district hospital in Rwanda.

Materials and Methods

Description of Study Area

This cross-sectional study was conducted in the period of 4 months (May-August 2018) at Nyanza District Hospital located in Nyanza district, Southern province, Rwanda. This district is located at -2°20'12.91" S and 29°47'40.24" E with a surface area of 672 km². The monthly mean temperature of this area is above 18°C with a relative humidity of 67% and a wind speed of 8 km/h. The estimated number of population is 323 588 inhabitants, of which 96 236 are under the age of 10 and the population density is 480 people/km² (1200/sq mi). This rural area is inhabited by low and middle-income families who are mostly uneducated and their predominant occupation is agriculture. This community lacks proper waste disposal, modern latrines, hygiene and sanitation, adequate health services, sewage, and drainage systems.

Sample Size and Specimen Collection

The biological specimens were taken from the patients by following the guidelines of the ethical clearance for research. The study population was composed of 138 consented patients who complained about diarrhea, abdominal cramps, fatigue, excessive gas, and unintentional weight loss. The inclusion criteria considered all the patients who attended Bacteriology department at Nyanza District Hospital including children and adults who were referred for stool examination by a medical doctor. The study population was divided into 4 status groups based on their personal hygiene habits (washing or not washing hands before eating and after using toilets), drinking water (boiled or not), age (1-19, 20-39, 40- ≥50) and gender (male and female)

The specimens were collected in sterile containers and labeled appropriately with patients' names, sex, time and date of collection. A form was also used to record patients' age, occupation, full address, socio-economic level, latrine system, sewage disposal, household sanitary, and education level. For the children, the consent forms were voluntarily signed by the parents or guardians. In the process of testing, the author performed physical examinations to assess each stool sample in terms of its color, state, and presence of blood or mucus at room temperature within 30 minutes after its receipt for analysis.

Preparation of Faecal Smears and Examination

As soon as the stool samples were collected, the researcher used the wet preparation method to prepare the smears.[14] A small amount of each stool sample was suspended in normal saline-Lugol and the zinc sulphate floatation technique was used to quantify and concentrate parasite for better identification.[15] Small portions of the formed stool samples were placed on microscope slides and covered with glass cover slips. The slides were eventually examined under the light microscope to assess the presence of eggs, cysts, damaged red blood cells, and trophozoites using the 10X objective lens and then switched to high magnification for detailed morphology. To maximize the chances of detecting parasites, tests were done in triplicate. Both macroscopic and microscopic examinations were done according to NCCLS recommendations[16] and the results were carefully recorded in tables.

Statistical Analysis

The obtained data were analyzed by SPSS version 23.0 (SPSS Inc., Chicago, IL, USA) and by using chi-square test, and independent *t* test was used to compute the exact probabilities and check the significance of obtained results in accordance with the risk factors. The confidence interval of 95% ($\alpha = 0.05$) and the *P* values less than 0.05 were considered statistically significant.

Results

Sterile bottles were used to collect 138 stool samples composed of 52.9% females and 47.1% males. Cysts, eggs, trophozoites or damaged red blood cells were observed under microscope. The analysis of 138 examined stool samples revealed that 22 (15.94%) were positive and 116 (84.1%) were free of any kind of intestinal parasites.

Frequency of Occurrence

Table 1. The Prevalence of *Entamoeba histolytica* in Relation to Gender

Gender	Positive	Negative	Total	*T* Test	*P* Value
Female	8 (10.95%)	65 (89.05%)	73		
Male	14 (21.54%)	51 (78.46 %)	65	8.286	0.076
Total	22 (15.94%)	116 (84.06%)	138		

Table 2. The Prevalence of *Entamoeba histolytica* in Relation to Age

Age Group	Positive	Negative	Total	*T* Test	*P* alue
1-19	16 (27.11%)	43 (72.89%)	59		
20-39	4 (6.89%)	54 (93.10%)	58	3.742	0.065
≥ 40	2 (9.52%)	19 (90.48%)	21		
Total	22 (15.94%)	116 (84.06%)	138		

In the present study, sex distribution of the infections demonstrated that males are more infected than females, as it is shown by the results presented in Table 1.

The results of microscopic examination of *E. histolytica* in relation to age are presented in Table 2, which indicate a high rate of infections in children aged between 1-19 years old.

A high rate of parasitic infections was observed in patients who did not boil drinking water, as it is expressed by the findings presented in Table 3.

Table 4 shows the elevated prevalence of *E. histolytica* among the patients who never kept their hands clean.

Discussion

This study was conducted to determine the epidemiological prevalence of *E. histolytica* and to provide the first data on this protozoa infection by stool examinations among the patients attending Nyanza District Hospital in Rwanda. The findings of this study showed that males were highly infected more than females. A high prevalence of infections was also observed among the children aged between 1 and 19 years old, including the ones who directly drank tap water and the patients who did not keep their hands clean.

In the present investigation, the prevalence of *E. histolytica* was 15.94%. This prevalence is low compared to other similar research conducted in other regions of Rwanda. For instance, Gahamanyi et al[17] in their study reported a prevalence of 25.2% at Muhondo Health Center. Niyizurugero et al[18] also confirmed a prevalence of 54.5% in students at Kigali Institute of Education in Kigali, Rwanda. The reason why the mentioned reports found a higher prevalence of *E. histolytica* compared to the current study is that perhaps because they screened mainly the patients who lived in crowded places and mostly used street restaurants and shared latrines.

As the sex distribution of *E. histolytica* was not statistically significant ($P=0.076$), gender was not a determinant in the prevalence of intestinal infections. It means there was no relationship between the observed infections and gender. This study showed that 21.54% of males are positively infected. This finding is absolutely in agreement with the results of the study of Obadiah[12] and Rine et al[19] who reported higher prevalence of *E. histolytica* in males compared to females. The higher prevalence of *E. histolytica* in males is due to the fact that they are more susceptible to infections compared to females.[20] The revealed sex difference is attributed to the variability in endocrine-immune interactions and the sex steroids such as androgens and estrogens which modulate several aspects of host immunity through the control of cytokines, Toll-like receptors expressions, and antibody productions.[21] The obtained results are supported by the findings of Brelet[22] who reported a high prevalence in boys due to socio-cultural lifestyles. It is explained by the fact that males are more active and likely to interact with contaminated environments. The findings of this study are in contrast to the results of Ejaz et al[23] who reported a higher prevalence of infection in females (31.5%) than in males (19.6%) due to their direct exposure to garbage and contaminated materials while doing the domestic chores.

People of all ages in developing countries are at risk of amoebiasis infection although prevalence among the ages varies greatly.[1,24] The age distribution was not statistically significant ($P=0.065$) in this evaluation and it was as well considered as a determinant of *E. histolytica* infections. It means that the observed infections were not absolutely correlated with the age. Among the three age groups represented in this study, a high prevalence (27.11%) of intestinal infection was found in the patients aged between 1 and 19 years old. This result is in tandem with the research done by Tasawar et al[25] and Ouattara et al[26] who reported the elevated prevalence of infections by *E. histolytica* in children because they are less acquainted with hygienic habits and cleanliness. The findings of the current study

Table 3. The Prevalence of *Entamoeba histolytica* in Relation to Drinking Water

Drinking Boiled Water	Positive	Negative	Total	*T* Test	*P* Value
Yes	8 (12.12%)	58 (87.88%)	66		
Sometimes	7 (13.73%)	44 (86.27%	51	2.979	0.097
No	7 (33.33%)	14 (66.67%)	21		
Total	22 (15.94%)	116 (84.06%)	138		

Table 4. The Prevalence of *Entamoeba histolytica* in Relation to Personal Hygiene

Washing Hands After Using Toilets and Before Eating	Positive	Negative	Total	*T* Test	*P* Value
Yes	2 (3.39%)	57 (96.61%)	59		
Sometimes	4 (10.26%)	35 (89.74%)	39	3.986	0.05
No	16 (40%)	24 (60%)	40		
Total	22 (15.94%)	116 (84.06%)	138		

are also in consistence with previous investigations done in Pakistan and Bangladesh which reported that *E. histolytica* is mostly prevalent in children who are likely to come into contact with infected material as they crawl on the ground and likely to put the play items in their mouths.[3] The high prevalence of infections in children is also allocated to their defense systems which are not well developed and are insufficiently exposed to the antigens. All those facts make children more sensitive to parasites than mid-adults.[27] The lowest prevalence (6.89%) was recorded in the age range of 20-39 years old. This is attributed to the fact that these patients are knowledgeable and relatively maintain their best personal hygiene. As a large number of patients in this age range were students, the observed low occurrence of infection in this group suggests the street restaurants and college canteens as the main sources of contamination.[21]

The difference in the prevalence of infections related to drinking water was not statistically significant (P=0.097). This probability showed that there was no positive correlation between drinking water and the observed rate of infections. The outrageous prevalence of *E. histolytica* was found in patients who did not boil drinking water (33.3%). This finding agrees with the study done in Mexico which reported a high prevalence of amoebiasis in rural areas where the people consume water directly from taps, dams, and rivers.[6] It is also in a positive correlation with the results of Alyousefi et al[28] who reported that drinking untreated water increases the risk of amoebiasis by 11.5 times due to contaminated buckets/jerrycans and direct exposure to animal faeces that contain parasites.

Hand washing habits of the tested patients was acknowledged as one of the critical parameters in personal hygiene. The prevalence of *E. histolytica* in relation to this parameter was statistically significant (P=0.05). This probability showed that there was a strong relationship between the observed infections and the hand washing habit as the risk factor of the occurrence of *E. histolytica*. This study revealed that 40% of infections were observed in patients who did not keep their hands clean. It is in agreement with the research done by Feachem et al[29] who reported high risks of infections in farmers who handled animal wastes and finally ate without washing their hands with soaps.

Limitations of the Study

This study included only one hospital in Nyanza district; it would have been better if it could include more hospitals and health centers in various places to be a multicenter research. However, the number of admitted patients to this hospital demonstrated a quite sufficient representative sample of the population in this area.

Conclusion

To sum up, *E. histolytica* is still a public health concern among the children aged between 1 and 19 years old in Nyanza district. This investigation concludes that household sanitary, socio-cultural lifestyle, and inadequate health services are among the risk factors of the occurrence of *E. histolytica*. As *E. histolytica* is transmitted via fecal-oral route and easily spreads from one person to another, the sustainable preventive measures are highly recommended to reduce the prevalence rate of amoebiasis.

Conflict of Interest Disclosures

There is no conflict of interest for this study.

Ethical Approval

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