Epidemiological Study of Entamoeba Histolytica Among People Living In Rural Area Nkombo Sector, Rusizi- Rwanda

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Abstract

Objective: *The aim of the study was to assess the prevalence and risk factors associated to E. histolytica in patients attended Nkombo health center using direct microscopy examination (wet mount) for the identification of intestinal parasitic infections.*

Method: *All samples were tested with wet mount technique using light microscope (in saline water and iodine smears) for the determination of E. histolytica infection.*

Results: *The prevalence of E. histolytica by the help ofwet mount test was 44.89%. The prevalence of infection in males was 47/66 (31.97%), and was higher than that of females 19/66 (12.92%). The lowest prevalence of E. histolytica was detected in the extremities of age groups infants and elders with 13% and the highest prevalence of E. histolytica was observed in group of age 30-39 years old (21.21%). A prevalence of 19.69% was declared in participants usually practising fishing work and findings were significant (P < 0.005). Street food consumption and drinking of non-treated water were significantly the main source of infections with (P < 0.05).*

Conclusion: *Generally Entamoebahistolytica is quite taken into account as a public health problem at Nkombo health center. Moreover it is very important to conduct more studies to yield data on the source and risk factors to ameliorate the education and prevent public health threats to secure Nkombo people from all gastric intestinal parasites starting from E. histolytica.*

Keywords: *Amebiasis, Patients, Parasite, Entamoebahystolytica, Nkombo*

Introduction

Around the world, approximately 50 million cases of invasive *E. histolytica* disease occur each year, resulting in up to 100,000 deaths. This is the tip of the iceberg, as only 10% to 20% of infected individuals become symptomatic. The incidence of amebiasis is higher in developing countries. Amoebiasis is the second leading cause of death due to parasitic diseases, killing around 40,000 to 100,000 people a year worldwide [1-2].

The rate of infection with *E. histolytica* differs according to countries, socio-economic and health conditions and populations. It is very endemic in poor and socio-economically disadvantaged communities in tropical and subtropical regions. Environmental, socio-economic, demographic and hygiene behaviors are known to influence the transmission and distribution of intestinal parasitic infections[3]. As transmission is frequently associated with contaminated food and water, young infants should not develop amebiasis very often. More serious illness is associated with young age, malnutrition and immunosuppression. Intestinal parasitic infection remains a common and significant public health problem among children in Saudi Arabia [4].

E.histolytica can live in the form of cysts and trophozoites. Cysts are usually found in formed faeces while trophozoites are usually found in diarrheal stools. Infection with *Entamoebahistolytica* occurs through ingestion of mature cysts in food, water or hands contaminated with feces. Excystation

occurs in the small intestine and trophozoites are released, which migrate to the large intestine [5]. Trophozoites passed in the stool are quickly destroyed once outside the host and, if ingested, would not survive gastric exposure. In many cases, trophozoites remain confined to the intestinal lumen of individuals who are asymptomatic carriers, and the cysts passe through their stool [6]. Trophozoites and cysts are located in the intestinal lumen, but only trophozoites are able to invade tissue. In animals, exhaustion of intestinal mucus, diffuse inflammation and disruption of the epithelial barrier occur before the trophozoites actually come into contact with the colonic mucosa. The trophozoites attach to colonic mucus and epithelial cells by a galactose inhibiting lectin [7].

Amoebiasis is endemic in the developing regions of Central and South America, Africa and Asia. In the United States, the incidence of amoebiasis is low, although deaths from amoebiasis still occur routinely, accounting for at least 5 deaths per year. In the United States, amoebiasis is observed mainly in return travelers or immigrants from endemic countries [8]. The pathogenesis of E. Histolytica can be characterized by 3 events: the death of host cells, inflammation and the invasion of parasites. Contact with host cells is necessary for parasite-induced death. Adhesion is mediated by the parasitic adhesion molecule Gal / GalNAclectin. Trophozoites are able to kill host cells by several different mechanisms, including the induction of programmed cell death, phagocytosis and trogocytosis[9].

E.hystolytica is a protozoa which causes amoebic dysentery and disese is commonly known as amibiasis. It can invade the intestinal mucosa and subsequently reaching the other organs [10].*E. histolytica*is the major invasive formand possesses many virulence factors that play role in the pathogenesis of intestinal as well as extraintestinalamoebiasis. Trophozoites invade the colonic mucosa producing characteristic ulcerative lesions and profuse bloody diarrhea (amoebic dysentery). Males and females are affected equally with a ratio of 1:1. Intestinal amibiasis can lead to different complications such as amoebic colitis, Intestinal perforation and amoebic peritonitis. Following 1–3 months of intestinal amoebiasis, about 5–10% of patients develop extraintestinalamoebiasis [11]. Liver is the most common site causing *Amoebic liver abscess* if not treated it may progress resulting to skin troubles like granuloma cutis or rupture into lungs (pulmonary amoebiasis with trophozoites in sputum) or into the right pleura (amoebic pleuritis), left sided liver abscess may rupture into stomach or left pleura or pericardial cavity resulting to amoebic pericarditis[11]. Around the world, approximately 50 million cases of invasive *E. histolytica* disease occur each year, resulting in up to 100,000 deaths [1] The prevalence of E. histolytica in rwanda, the data show that 26 (25.2%) were positive, for E. histolytica, 17 (16.5%) and 9 (8.7%) came from males and females respectively. In addition, of these 26 positive samples for E. histolytica, 15 (14.6%) and 11 (10.7%) were from people ≤ 15 years old and> 15 years old respectively in the stool samples [10].

MATERIALS AND METHODS

Study area

The study was conducted at Nkombo health center located in Bigoga cell, Nyaweya village Nkombo sector, Rusizi district, Western Province of Rwanda Nkombo Island is approximately 11 Kilometres from Kamembe town and 679 metres away from the shores of Lake Kivu. Nkombo is the Island, which is one of the 18 sectors that make up Rusizi District, covers an area of 21 square kilometres and is inhabited by about 17,000 residents. The study was oriented in the field of clinical microbiology.

Study design

This study was a cross sectional prospective study and was carried out from June to December 2018. Stool samples were given by the patients whose health center care givers suspected to have amoebic dysentery at Nkombo health center in the period of the study.

Study population and sample size

The study population was patients having symptoms such as loose stool, stomach pain, stomach cramping, Bloody stool and fever. 147 stool samples were collected in appropriate conditions and processed for parasite diagnosis; the containers containing stool samples were first of all stored in the fridge; a sealed plastic bag and wet mount techniquewas performed in clinical microbiology laboratory at Kibogora Polytechnic.

Survey questionnaire

A structured questionnaire was framed in English and then converted into Kinyarwanda language (the national language of Rwanda). The interview was given to participants in person, and the questions regarding their demographics (ie, age, gender), socioeconomic background (ie, occupation such as

fishing work), behavioral risks (ie personal hygiene such as hand hygiene washing and food consumption), environmental hygiene conditions and life (i.e. types of previous chronic diseases like diabetes, HIV/AIDS, alcoholism, type of water supply, wastewater system, presence of pond water and pets). Participants were also asked if they had any sign such as diarrhea and symptoms of gastroenteritis (ie, vomiting, nausea, abdominal pain, loose stools, and blood or mucous stools and discomfort mood). For infants, the interview was provided by their parents or other family member who accompanied them.

Stool sample collection

Stool samples were collected following the appropriate conditions to reduce chances for sample rejection. The clean and dry universal containers were used for poop collection. Containers were tightly sealed to avoid any kind of contamination, time, date, labelling were taken into consideration in order to avoid criteria for sample rejection which could result to wrong results. Transportation of the stool samples was done using transport vials filled with human faeces for stool testing.

LABORATORY DIAGNOSIS

Visual examination

Stool samples should be assessed macroscopically in terms of odor, quantity, color, and consistency form of the specimen; bloody, watery, loose, soft or formed, fresh blood, digested blood, presence of mucus. A small amount of mucus in stool is normal. However, the presence of copious mucus or bloody mucus is abnormal. Observation is a paramount test which may also show the presence of adult worms, proglottids, scoleces and other abnormal conditions. The normal color is light brown due to the presence of bilirubin and bile.

STOOL MICROSCOPY

DIRECT FAECAL SMEARS

Saline and iodine wet mount preparations

These procedures are mainly used to detect motile trophozoites and larvae, red blood cells, leukocytes, Charcot–Leyden crystals (saline preparation) and cysts of protozoa (iodine preparation, Lugol). When examining diarrhoeic or liquid faeces containing mucus, both preparations should be applied to the mucous part of the stools [13].

Procedure

One drop was placed in a solution of 0.85% NaCl on the left side of the slide and one drop of iodine (working solution) on the right side of the slide. A small amount of faecal specimen was taken and thoroughly the stool has been emulsified in saline and iodine using an applicator stick. The sample was spread thinly enough that newsprint can barely be read when the slide is placed on top of text. A 22mm cover slip was applied at an angle into the edge of the emulsified faecal drop. The cover slip was pushed across the drop before allowing it to fall into place. Systematically the entire 22mm cover slip was scanned with overlapping fields with the 10x objective. Then high dry (40X objective) was used for more detailed study of any suspect eggs or protozoa [13].

Data analysis

The data were analysed using Microsoft Excel program. The results were presented in frequency tables, percentages using descriptiveStatistics.

Ethical Consideration

The written permission to carry out the study obtained from Kibogora Polytechnic and Nkombohealth center committees. The participants were first of all informed about the purpose and the benefit of the study. The participation in the study was voluntary. Personal information was treated with maximum confidentiality. Before the interviews and submission of samples, the selected persons, were given a brief explanation by the survey leaders on the aim of the survey, questionnaire, and survey in general.

RESULTS

Table 1: Socio-demographic characteristics of the participants

Gender	Persons with disease		persons without disease	Total	X^2	*df*	P-value
	Number	%					
Males	47(46.21)	31.97	56(56.75)	103	9.92	1	0.001635
Females	19(19.75)	12.92	25(24.24)	44	0.04	1	0.841481
Total	**66**(65.96)	**44.89**	**81**(80.99)	**147**			
Age groups							
0-9	9(8.97)	13.63	11(11.02)	20	4.62	1	0.031601
10-19	9(11.22)	13.63	16(13.77)	25	0.79	1	0.3741
20-29	12(14.36)	18.18	20(17.63)	32	0.39	1	0.406164
30-39	14(14.36)	21.21	18(17.63)	32	16.78	1	0.00049
40-49	13(8.08)	19.69	5(9.91)	18	3.58	1	0.019907
50-59	9(8.97)	13.66	11(11.02)	20	3.43	1	0.064022
Total	**66**(65.69)		**81**(80.98)	**147**			

Table 2 shows sex distributionof infections the lowest prevalence was found in females with (12.92%) and the high prevalence 31.97% of males having *E. hystolytica* in participants with statistical significance of (**x^2**= 9.92, *df*=1, P= 0.001635). It also figures that the lower extremities of age and middle group of ages are most vulnerable group of ages to *E. hystolytica*. It reveals a significance in children with (x^2= 4.62, *df*=1, P= 0.031601<0.05). The group of age (40-49) predominated the group of low extremity in having *E. hystolytica*as it is the generation of fishermen with (x^2 = 3.58, *df*=1, P= 0.019907). 30-39 was the most predominant group suffering from *E. hystolytica* as it shows significance with (x^2 = 16.78, *df*=1, P= 0.00049 <0.05).

Table 2: Epidemiological risk factors associated with *Entamoebahistolytica*among patients

	People with disease		People without disease	Total	x^2	*df*	*P- value*
Risk Factors	**Number**	**%**					
Contact with kivu lake during fishing work	13(8.08)	19.69	5(9.91)	18	5.42	1	0.019907
Drinking of unsafe water	9 (8.97)	13.63	11 (11.02)	20	4.62	1	0.031601
Close contact with domestic animals in household	2(3.59)	3.03	6(4.40)	8	1.28	1	0.256786
Eating leftover food	8(9.42)	12.12	13(11.57)	21	0.38	1	0.537603
Street food consumption	5(10.32)	7.57	18(12.67)	23	4.98	1	0.025642
Poor individual hygiene	10(8.53)	15.15	9(10.46)	19	0.45	1	0.502335
Farming work	7(8.08)	10.60	11(9.91)	18	0.25	1	0.617075
Patients with immunosuppression	12(8.97)	18.18	8(11.02)	20	1.84	1	0.174951

Total	**66(**65.96**)**		**81(**80.96**)**	**147**			

Table 2: Epidemiological risk factors associated with *Entamoebahistolytica* among participants. As indicated in table2, the risk factor which was the most prevalent to be the source of infection to *E. hystolytica* was people in contact with Lake Kivu water with significance of (x^2 = 5.42, *df* =1, P= 0.019907<0.05). The other side street food consumption was the second probable source of the infection showing significance of x^2 = 4.98, *df* =1, P = 0.025642<0.05).The least risk factor was drinking water from the ponds which shows significance of (x^2 = 4.62, *df*=1, P= 0.031601 <0.05).

DISCUSSION

Socio-demographic characteristics of the participants

The present study was conducted inrural area to identify E. hystolytica infection in patients living nearest the pond water of lake kivu attended Nkombo health center presenting gastrointestinal troubles. Figure 1 shows that males 47/66 (31.97%) were significantly (x^2 = 9.92, *df* =1, P= 0.001635< 0.05) more infected in having*E. histolytica* infection than the females 19/66(12.92).However contrary to the studies carried out by Sadaga and Kassem (2007) [15] reported no significant difference in the prevalence of gastro intestinal infections among boys and girls. The difference in results is probably because a big number of people exerting fishingworks are males also have a habit of taking street foods. Additionally males consume more alcohol than females. The same hypothesis has been confirmied in the study done by Rajasuriya (2009); De Silva (1994) [16-17], about all of the positive cases were men (approximately between 31 and 50 years of age) with a previous history of alcohol consumption. Surprisingly, the high prevalence of consumption happened during desiccated season, which was associated to the peak alcohol sales in the North region. The same result has also been reported by Singh et al. (2019) [18]demonstrated that among 115 cases of *E.hystolytica* in India, 93% were found in men significant with (x^2 =5.49, *df* = 1, p = 0.0191) [20].

Epidemiological risk factors associated with *Entamoebahistolytica* among patients.

Table 2 denotes the hihg prevalence of *E. histolytica* among participants who usually like to be in contact with kivu lake water and has been significant (x^2 = 5.42, *df* =1, P= 0.019907< 0.05) to be risk factors associated with *Entamoeba hystolytica* infection. Another risk factor associated toparasitic infection was the consumption of unsafe water significant with (x^2 = 4.98, *df* =1, P= 0.025642 < 0.05). The other source of the parasitic infection was the consuption of street food and has been sgnificant with (x^2 = 4.62, *df* =1, P= 0.031601< 0.05).

The present study shows a prevalence of 44.89% which is high compared to other research carried out in other places of Rwanda. For example, Gahamanyi*et al*. [19] in similar study found a high prevalence of men 25.2% at Muhondo Health Center. Niyizurugero et al. [20] also demonstrated a prevalence of 54.5% higher than our findings in students at Kigali Institute of Education in Kigali, Rwanda. The reason why the mentioned findings denoted a higher prevalence of *E. histolytica* compared to the present study is that probably because they recruited patients who were in slum places and mostly used street food and use the same latrines.

The current study drinking unsafe water play a significant risk factor for gastrointestinal infection with a prevalence of (13.63%) significant (x^2 = 4.62, *df* =1, P= 0.031601< 0.05). This finding agrees with the study done in Mexico by Espinosa et al. (2000) [21] which reported a high prevalence of amoebiasis in rural areas 49.07% where the people consume water directly from taps, dams, and rivers. It is also in a positive relationship with the findings of Alyousefi et al. 49.01% [22] who announced that drinking untreated water enhances the risk of amoebiasis by 11.5 times due to contaminated buckets/jerrycans and direct exposure to animal faeces that contain parasites.

A habit of patient hand washing was known as one of the criteria in personal hygiene. The frequency of *E. histolytica* was not statistically significant (x^2 = 0.45, *df* =1, P= 0.502335 >0.05) hence no relationship between hand wash and E. histolytica infection. It is in disagreement with the study carried out by Feachem et al. [23] who presented high risks of infections in farmers who handled animals'wastes

and took their meals without cleaning their hands with soaps.Infectious diseases are accountable for 45% deaths in the countries where economy is low, poor hygiene and sanitation are responsible to the spread of parasitic infections [24].

The present study has found the intestinal parasitee in patients who have eaten a street food, the prevalence of *E. hylytica* was 7.57% and it is statistically significant with (x^2 = 4.98, *df* =1, P= 0.025642< 0.05) this shows the relationship between the isolated *E. hystolytica* infection and eating street food. Costa-Cruz et al. [25] carried out a similar study in the city of Uberlandia in Brazil and found a high prevalence rate of 60% among food vendors, and *Entamoeba coli* was equally the most prevalent parasite (14.0%), in discordance of another research in Abeokuta, Nigeria (2004)[26], in which *E. coli* was absent and the most prevalent parasite was *E. histolytica.*

The current study showed that farming practice implies humanfaeces and animal manure manipulationis not responsible for the spread. Statistical analysis was not significant (x^2= 0.25, df= 1, P= 0.617075> 0.05) and no association between farming practices and infection with *E. histolytica* probably because cysts of *E. histolytica* are fully resistant, they get damaged in human excreta within a short period of time in compost as they cannotunlikely resist for 10 days in desiccation. In concordance with findings in the study carried out by Pham et al. (2011) [2] in their study farming was not the factor testing exposure to human and animal excreta such as composting excreta in the household or using excreta as fertilisers in the field resulted in an increased risk *E. histolytica* infection. In the present study risk factor such as immunosuppression in patients attended Nkombo health center, statistical findings (x^2= 1.84, *df*= 1, P= 0.174951 > 0.05) showed no association with *E.hystolytica* infection. Similar findings were observed by Okamoto et al. (2005) [27] comparing prevalence and risk factors between ameobic colitis patients with and without HIV infection. An incidence of 0.1% (4/5,193) has been reported in studies of HIV-negative patients with positive results for occult blood in feces and our results (18%) were different. Although, in present study, no relationship found between *E. histolytica* infection and Living with domestic animal (x^2= 1.28, *df*= 1, P= 0.256786 > 0.05). In study carried out by Pham et al. (2011) [2] surprisingly, reported that living together with domestic animals was not a parameter associated with a crucial risk increase of *E.histolytica* infection.

CONCLUSION

Briefly *E. histolytica* is still a common health concern among people living in rural areas especially those whose practice requests to be in contact with pond water, fisher men, people who frequently take street food and children, people aged between 20 and 49 years old, people with a history of immunosuppression and alcohol consumption in Nkombo sector are at high risk of developing amebias is, which is aggravated by lack of knowledge related to hygiene and sanitation, its transmission route, and broad ignorance of proper faecal disposal places. As an eradicable disease, this requires to be undertaken by public healthcare system and considerable occasion for amelioration quite exists to meet this healthcare imbalance.

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References

[1] John CC, Salata RA. Amebiasis. In: Kliegman RM, Stanton B, St. Geme J, Schor N, Behrman RE, editors. Nelson textbook of pediatrics. Philadelphia: Saunders; 2010. p. 1123-5.

[2] Pham Duc P, Nguyen-Viet H, Hattendorf J, Zinsstag J, Dac Cam P, Odermatt P. Risk factors for *Entamoebahistolytica* infection in an agricultural community in Hanam province, Vietnam. Parasit Vectors. 2011. P. 4-102.

[3] Al-Harthi S, Jamjoom M. Diagnosis and differentiation of entamoeba infection in Makhah Al Mukarramah using microscopy and stool antigen detection kits. *World J Med Sci*. 2007. Vol. 2, 15-20.

[4] Ilikkan DY, Ilikkan B, Vural M. Amebiasis in infancy in the middle-high socioeconomic class in Istanbul, Turkey. *Pediatr Infect Dis J*. 2005, vol. 24, p. 929-30.

[5] Haque R., Mondal D, Duggal P, Kabir M, Roy S, Farr B.M, Sack R.B, and Petri W.A.

[6] Ryan, K.J, Ray, C.G. Sherris Medical Microbiology, An Introduction to infectious diseases. McGraw-Hill Medical Publishing Division, New York, 2004; 4:261-270.

[7] Seydel K.B., Stanley S.L. Jr. *Entamoebahistolytica* induces host cell death in amebicliver abscess by a non-Fas-dependent, nontumornecrosis factor alpha-dependent pathway of apoptosis. *Infect Immun*. 199

[8] Gunther J, Shafir S, Bristow B, Sorvillo F. Short report: amebiasis-related mortality among United States residents,

1990–2007. *Am J Trop Med Hyg* 2011.vol. 85, p. 1038–40.

[9] Ralston KS, Solga MD, Mackey-Lawrence NM, et al.. Trogocytosis by *Entamoebahistolytica* contributes to cell killing and tissue invasion. Nature. 2014. P. 508–30.

[10] Gahamanyi, Noel, Mugabo Jean De Dieu, Bayingana Claude, (2016). Prevalence Of*EntamoebaHistolytica*In Stool Specimens At Muhondo Health Center, Rwanda. *Afr. J. Cln. Exper.Microbiol.*17 (2): 83-87.

[11] Apurba S. S. (2014).Essentials of Medical Parasitology 1st Edition.Jaypee Brothers Medical Publishers.

[12] World Health Organization (2019). Bench aids for the diagnosis of intestinal parasites 2nd edition.

[13] World Health Organization. Basic Laboratory Methods in Parasitology. (1991): 25-28.

[14] Paniker, J. (2018). Textbook of medical parasitology 8th edition, Jaypee Brothers Medical Publishers (P) Ltd 4838/24, Ansari Road, Daryaganj.

[15] Sadaga GA, Kassem HH. Prevalence of intestinal parasites among primary schoolchildren in Derna District, Libya.*J Egypt SocParasitol* 2007;37:205-14.

[16] Rajasuriya K, Nagaratnam N. Hepatic amoebiasis in Ceylon. *J Trop Med Hyg*. 2009;65(7):165–78.

[17] De Silva N, De Silva H, Jayapani V. Intestinal parasitoses in the Kandy area, Sri Lanka. *Southeast Asian J Trop Med Public Health*.1994; 25:469.

[18] Singh A, Banerjee T, Kumar R, Shukla SK. Prevalence of cases of amebic liver abscess in a tertiary care Centre in India: a study on risk factors, associated microflora and strain variation of Entamoebahistolytica. *PLoS One*. 2019;14(4):e0214880.

[19] Gahamanyi N, Mugabo JD, Bayingana C. Prevalence of *Entamoebahistolytica* in stool specimens at Muhondo Health Center, Rwanda. *African Journal of Clinical and Experimental Microbiology*.2016;17(2):83-7. doi: 10.4314/ajcem.v17i2.2.

[20] Niyizurugero E, Ndayanze JB, Bernard K. Prevalence of intestinal parasitic infections and associated risk factors among Kigali Institute of Education students in Kigali, Rwanda. *Trop Biomed*. 2013;30(4):718-26.

[21] Espinosa-Cantellano M, Martinez-Palomo A. Pathogenesis of intestinal amebiasis: from molecules to disease. *ClinMicrobiol Rev*. 2000;13(2):318-31. doi: 10.1128/cmr.13.2.318- 331.2000.

[22] Alyousefi NA, Mahdy MA, Mahmud R, Lim YA. Factors associated with high prevalence of intestinal protozoan infections among patients in Sana'a City, Yemen. *PLoS One*. 2011; 6 (7):e22044. doi: 10.1371/journal.pone.0022044.

[23] Feachem RG, Bradley DJ, Garelick H, Mara DD. Sanitation and Disease: Health Aspects of Excreta and Wastewater Management. New York: John Wiley & Sons; 1983.

[24] Cavalini LT, Ponce de Leon ACM. Morbidity and mortality in Brazilian municipalities: a multilevel study of the association between socioeconomic and healthcare indicators. *International Journal of Epidemiology*. 2008; 37:775–785.

[25] Costa-Cruz JM, Cardoso ML, Marques DE. Intestinal parasites in school food handlers in the City of Uberlandia (Minas Gerais), Brazil. Rev Institute Medical Tropical Soa Paulo. 1995;37:191–196.

[26] Idowu OA, Rowland SA. Oral fecal parasites and personal hygiene of food handlers in Abeokuta, Nigeria. *African Health Science*. 2006;6:160–164.

[27] Okamoto, M, T, Ohata K, Togo G, Hada T, Katamoto T, Amebic colitis in asymptomatic subjects with positive fecal occult blood test results: clinical features different from symptomatic cases. *Am J Trop Med Hyg*. 2005; 73:934–5.