South Asian Journal of Parasitology

6(2): 19-26, 2022; Article no.SAJP.85240

The Sero-Epidemiology and Risk Factors of *E. histolytica* Infection in Calabar, Nigeria

C. E. Ohanu [a], E. O. Onosakponome [a*], C. U. Nyenke [a] and A. E. Abah [b]

[a] Department of Medical Laboratory Science, Pamo University of Medical Sciences, Port Harcourt, Nigeria.
[b] Department of Animal and Environmental Biology, University of Port Harcourt, P.M.B. 5323, Port Harcourt 50001, Rivers State, Nigeria.

Authors' contributions

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

Article Information

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/85240

Original Research Article

Received 15 January 2022
Accepted 22 March 2022
Published 24 March 2022

ABSTRACT

Background: Amoebic infections is a prevalent infection in Nigeria. Currently, there is paucity of data on sero-epidemiology of *Entamoeba histolytica* in calabar. This research investigated the sero-epidemiology and risk-factors of *E. histolytica* infection among dysentery patients presenting at general hospital calabar, Cross river.
Methods: The Sero-epidemiology of *E. histolytica* were determined in three hundred and eighty-one subjects in calabar, Cross river state using an enzyme-linked immunoassay. In addition, sero-prevalence association with the socio-demographic and risk factors of the subjects studied was investigated.
Results: Forty five (45) out of the three hundred and eight one (381) samples were positive for *E.histolytica*, 45(11.8%). Subjects in the age group 1-10 years had the highest prevalence (32.8%).The research also discovered that *E. histolytica* infections was associated with age, educational status, occupational status, source of water, toilet facility, hand washing and contact with domestic animal/faecal matter($p<0.05$).Females were more infected (13.5%) than male (9.9%) but the difference was not statistically significant ($p> 0.05$). The infection rate was greater in the wet season (18.1%) than the dry season (4.9%). However, *E. histolytica* infection was statistically associated with season ($p<0.05$).
Conclusions: The sero prevalence of *E. histolytica* infection found in this study is moderate

**Corresponding author: E-mail: eonosakponome@pums.edu.ng, onosaeve@gmail.com;*

compared with those reported in other Nigeria populations. The data of *E. histolytica* sero-positivity found in the present study may be useful for the planning of optimal preventive measures against *E. histolytica* infection.

Keywords: E. histolytica; prevalence and risk factors.

1. INTRODUCTION

Entamoeba histolytica is a protozoan parasite that causes amoebiasis, High morbidity and mortality of amoebisis have been reported worldwide. Infections with *E. histolytica* are prevalent in the tropics and the major health problems in developing countries [1,2], Majority of the *E.histolytica* infection morbidity and mortality occurs in Africa, Central and South America and the Indian sub-continent.

Most of the *E.histolytica* infections occurs among the poor inhabitants in the tropical countries such Nigeria [2]. In some cases leading to high fatalities especially in children. There is insufficiency of data for public health intervention by government. The prevalence of amoebiasis due to *E. histolytica* has been difficult to establish because there is a probability to overestimate it in endemic areas, where cases of dysentery or bloody diarrhea are often misdiagnosed as amebiasis due to the non-pathogenic *E.dispar* [3].

In non-endemic areas with a low incidence of the disease, there is a tendency to underestimate, the prevalent of *E. histolytica* infection due other bacterial and viral pathogen causing dysentery and diarrhea [4] Studies in parts of Africa reported prevalence rates of 22% and 21% in South Africa and Egypt, respectively [5]. In Nigeria, prevalence rates of 21.6% in Enugu [6] and 13.7% in Ilesha have been reported [7]. The rate of infection by *E. histolytica* differs among countries, socio-economic and sanitary conditions and population's [5]. It is highly endemic throughout the tropics and subtropics [8]. Environmental, socio-economic, demographic and hygiene-related behavior is known to influence the transmission and distribution of intestinal parasitic infections. Humans are the host of *E. histolytica* and there are no other known animal reservoirs of this parasite [9].

Although ,E.histolytica parasite also infects non human primates such as cats and dogs but no cross transmission [10].

Most persons infected with *E. histolytica* are carrier [11]; infection with *E. histolytica*is responsible for most cases of prolonged diarrhea in travelers [12]. In addition, infection with *E. histolytica* may lead to the development of live-threatening abscess in liver, brain [13] or lungs [9], its becomes necessary to investigate the sero-epidemiology of *E.histolytica* parasite in the population. Transmission of *E. histolytica* occurs in areas with poor sanitation by contamination of drinking water or food with human feces [14]. Water-associated outbreaks of *E. histolytica* disease have been reported [15].

There is paucity of data on the sero-epidemiology of *E. histolytica* infection in calabar, Cross river state. Most of data are based on microscopic diagnosis of *E.histolytica* in stool which could be non-Pathogenic *E.dispar* 19],Enzyme linked Immunosorbent assay is specific *E. histolytica* Parasite [4], Furthermore, socio-demographic and Risk factors of the subjects associated with *E. histolytica* sero-prevalence were investigated for public health intervention.

2. MATERIALS AND METHODS

2.1 Study Area

Cross river state derives its name from the river which passes through the state. It is a coastal state located in the Niger Delta, and occupies 20,156 square kilometers. It shares boundaries with Benue state to the North, Ebonyi and Abia state to the west, its shares boundaries with Cameroun to the east and to the south west by Akwa Ibom State, Calabar is located in The South South geopolitical zone of Nigeria with a population of 3,737,517 inhabitants, Calabar is often described as the tourism capital of Nigeria [16].

2.2 Sample Size

The minimum sample size was determined by using the formula described by Naing et al, [17], therefore, to obtain a more reliable result, a total of 381 patients sample was collected.

2.3 Enrolment of Patients

All patients who presented to the general hospital in Calabar with acute and persistent diarrhoea or dysentery during the 12-month study period (January to December 2013) were enlisted after giving their consent and meeting the inclusion criteria, which included acute or persistent diarrhoea and dysenteric syndrome. Antimicrobial-treated patients with diarrhoea or dysentery were excluded. Patients who were admitted to the hospital for causes other than diarrhoea and who had not experienced a diarrhoeal sickness in the previous two weeks served as controls.

2.4 Data Collection

A well-Structured questionnaire was used to obtain information from each patient on the demographics and risk factors. Demographics include; Age,sex,Occupational status and education. Risk factors includes; Toilet facilities, Source of water and Contact with domestic animals/Faecal matter.

2.5 Specimen Collection and Processing

Three hundred and eighty one samples (381) blood specimens were aseptically collected, One hundred and eighty three (183) samples were collected during the dry season (November-March) and One hundred and ninety eight samples (198) collected during the wet season (April-October), respectively. The sera obtained from clotted blood and centrifuged to obtain the serum for each patient presenting with dysentery or diarrhoea at general hospital calabar, Cross river State.

2.6 Analysis of Samples

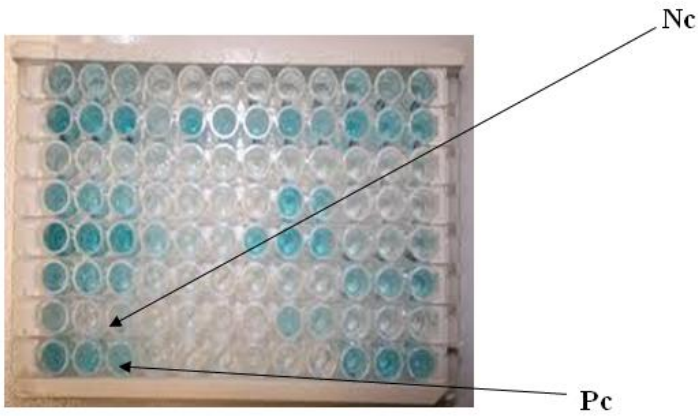
Detection of *E. Histolytica* antibody was carried out using the Enzyme linked Immunosorbent assay.

Table 1. Sero -Prevalence based on socio demographic factors among study population

Parameters/ factors	Number Examined	Number Positive (%)	x^2	df	p.value
Age group					
1 -10	61	20(32.8)	33.5	5	0.0012
11 -20	83	10(12.1)			
21 -30	104	5(4.8)			
31 -40	63	6(9.5)			
41 -50	40	2(5.0)			
>50	30	2(6.7)			
Sex					
Male	180	18(9.9)	1.2	1	0.283
Female	200	27(13.5)			
Occupational status					
Students	112	5(4.5)			
Unemployed	54	7(13.0)			
Farmers	43	14(32.6)			
Civil servants	63	3(4.8)			
Artisans	25	6(24.0)			
Business	84	10(11.9)			
Educational status					
Not educated	68	15(22.1)	11.6	2	0.003
Primary	70	5(7.1)			
Secondary	158	12(7.6)			
Tertiary	85	8(9.4)			

Table 2. Sero-Prevalence of *E. histolytica* among the study population based on risk factors

Risk Factors	Number Examined	Number Examined	x^2	df	p.value
Source of water					
Bore hole	201	28(13.9)	49.3	2	0.003
Tap water	164	7(4.3)			
Well	16	10(62.5)			
Toilet facility					
Pit	26	10(38.5)	19.0	1	0.0012
Water cistern	355	35(9.9)			
Hand washing /personal hygiene	281	25(8.9)	8.7	1	0.002
Washes hands with soap	100	20(20)			
Washes Hand without soap					
Contact with domestic animal/fecal matter	256	40(15.6)	10.9	1	0.001
Yes	125	5(4.0)			
No					
Season	198	36(18.1)	16.1	1	0.014
Dry	183	9(4.9)			
Wet					



Pc-Positive control Nc-Negative control

2.7 ELISA Antibody Detection Technique

The sera from the blood samples was tested using a TechLab Enzyme Linked Immunosorbent Assay (ELISA) (USA). The first well of the microplate was left blank (control), while the second and third wells were filled with 100ul of negative and positive controls, respectively. In the remaining wells, two drops of the diluted test samples were added and incubated for 10 minutes at room temperature (15-20oC). After that, the contents were shaken three times and rinsed three times with diluted buffer. Following washing, 2 drops of enzyme conjugate were added to each well and incubated for another 5 minutes at room temperature. After another shaking and washing with buffer, 2 drops of chromagen were added to each well and incubated at room temperature for another hour. Finally, 2 drops of the stop solution were put to each well and mixed with the strip holder by tapping it. The results were read with a micro plate reader machine set for bio chromatic readings at 450/650-620nm.Positive and negative control sample was included for each batch of sample assayed.

2.8 Data Analysis

The percentage of patients who tested positive for *E. histolytica* was used to establish the prevalence, and the Chi-square test was used to

determine the relationship between *E. histolytica* and the specified factors. The SPSS statistical software was used for all statistical analyses.

3. RESULTS

Out of the 381 subjects studied,45(11.8%) were sero-positive for *E.histolytica* infection, Based on Age, The age group 1-10 years of the study population had the highest prevalence 32.8% ,while those in age group 40-50 years had the lowest prevalence rate of 4.8%.Statistical analysis showed the difference was significant (P=0.0012)(Table 1).

E. histolytica infection among the subjects studied showed no significant association (P=0.283) with respect to gender in the study population. The female subjects had a prevalence rate of 13.5% and the male subjects a prevalence of 9.9% (Table 1). The subjects whose occupation were farmers, had the highest prevalence rate of *E.histolytica* infection of 32.6% while the subjects whose occupation were students had the lowest prevalence rate of 4.5%. This difference was statistically significant (P=0.002)(Table 1). Subjects not educated had the highest prevalence rate of *E,histolytica* infection of 22.1%, while subjects with primary education level had the lowest the lowest prevalence rate of 7.1%. *E.histolytica* infection was statistically associated with educational status (P=0.0014),(Table 1).

Subjects whose source of drinking water is wells had the highest prevalence rate of 62.5% of *E.histolytica* infection, while subjects whose source of drinking water is tap water and bore hole had the prevalence rates of 4.3% and 13.9%,respectively. This difference was significant (P=0.003),(Table 2). The highest prevalence rate of *E.histolytica* infection was recorded in subjects that use pit latrine. There was significant association between the type of toilet facility and *E.histolytica* infection (P=0.0012)(Table 2). Subject that washed their hands without soap had the highest prevalence rate of 20.0%, while those that washed their hands with soap had a prevalence rate of 8.9%.This difference was significant(P=0.002) (Table2). *E.histolytica* infection was associated with contact of domestic animal/FaecalMatter (P=0.001)(Table 2). The prevalence of *E.histolytica* infection among Participant in the study population was highest in the rainy season 18.1% than in the dry season 4.9%.The association of *E.histolytica* infection with seasons was significant (P=0.0014).(Table 2).

4. DISCUSSION

E. histolytica infection predominates in developing countries and represents a major public health problem in tropical countries [18]. This study established a low sero-prevalence rate of *E. histolytica* infection (11.8%) in the study population when compared with 22% and 21% reported in South Africa and Egypt respectively. In Nigeria, 21.6% in Enugu 14.3%, in Kaduna and 13.7% in Ilesa have been reported [5,6,7,19]. Although, slightly higher than 10 % reported by World health organization in developing countries [20]. The reasons for the disparity in the variation of prevalence rate of *E.histolytica* infection could be attributed togeographical, study design, seasonal, diagnostic methods, Patients selection and behavioral factors in the different study population.

The age group 1-10 years were the most infected with *E.histolytica* infection with a prevalence rate of 32.8% in agreement with Zahida et al. [21]. According to their findings, age is a significant risk factor for many infectious diseases, particularly those transmitted orally, such as E. histolytica [21,22]. The current findings are also consistent with earlier research conducted in Pakistan and Bangladesh, which found that E. histolytica infection is most common among young children who are prone to contact with infected material when crawling on the ground or playing outside games [9,22]. In addition, Children are less acquainted with hygiene habits which also makes them more vulnerable to infection [17]. The result from this study, found people from all age groups were infected with *E.histolytica*, Although, there is variation of prevalence rate among the age groups. This is consistent with the results by Zahida et al. [21] The association of *E. histolytica* infection with age is in agreement with Al-Harthi and Jamjoom, and Zahida et al, [9,4]. Who reported similar results.

The lack of significant association of *E. histolytica* infection with gender observed in this study is consistent with Dawah et al, [19], attributed to equal exposure of both sex in the study area to the risk factors of *E. histolytica* infection. Although, prevalence of 13.5% of *E. histolytica* infection in females was higher than male, it is inconsistence with other previous

studies on amoebiasis. Jamaiah and Shekhar and Stauffer et al. [5,23] reported higher prevalence in males.

The greater incidence rate seen in females could be linked to women being expected to do more domestic tasks than men. This could expose them to contaminated fruits, vegetables, and water, potentially promoting oral transmission of the disease through contaminated hands. This could explain why females have a slightly greater infection rate than males, as reported by Haque et al. [9]. Interestingly, occupation was associated with an increase risk increase of *E.histolytica* infection. Farmers had the highest prevalence rate 32.6% of *E.histolytica* infection. This is attributed to the nature of their occupation were they expose themselves with human and animal excreta especially when using it as manure.This findings from this study, is in consistent with Pham duc et al, [24]. In their study of the risk factors of *E.histolytica* in agricultural communities in Vietnam, They found a three-fold rise in amoebic infection among farmers.

The non-educated subject had highest number of *E. histolytic*a prevalence rate 22.1% when compared with primary, secondary and tertiary educated subjects, Education was significantly associated with *E.histolytica* infection from this study. Education is seen as one of the determinants of personal well-being, particularly in terms of increasing hygiene. According to Ross et al. (2003), people with higher levels of education are healthier than those with lower levels of education since they seek medical help sooner. In their studies [25,26,27], Espinosa-cantellano and Espinosa-cantellano,2000, and Karaman et al,2006, showed ignorance, toilet habit, and literacy level to be important risk factors for amoebiasis. This is consistence with our findings in this study.

The result from this study shows significant association of *E.histolytica* infection with source of drinking water. The association of *E. histolytica* with the source of drinking water of the patients agreed with the findings of Cox, Olsen et al*,* Ogunlesi et al*,* and Rinne et al, [7,28,29,30], all reported source of water as a risk factor for amoebiasis. Most of the subjects in this study obtained water from bore hole which are less likely to be contaminated with *E.histolytica* parasites when compared with well water. The subject who use well water as source of drinking water had the highest prevalence rate of *E.histolytica* infection. The majority of the wells in the study region were constructed manually, with no casing or covering. Surface run-off, which may be faecally contaminated, can sometimes pollute the well.

There was significant association between personal hygiene and *E.histolytica* infection, this is attributed to the route of transmission of *E.histolytica* infection which is faecal –oral transmited. This is in agreement with Espinosa-Cantellano and Espinosa-Cantellano; Ryan and Ray, which reported similar findings [26,31]. Close contact with domestic animals/faecal matter was associated with an important risk factor for *E.histolytica* infection in this study . The subject with the hisghest prevalence of 15.6% are respondent with contact domestic animals/faecal matter. it is well possible that cysts of *Entamoeba histolytica* deposited on the surface (fur) of the animals during close contact with humans and then later transmitted to a next person and facal matter with the parasite is transmitted faecal-orally.

The seasonal variation was observed in this study, there was association of *E. histolytica infection* with the season. higher prevalence rate of 18.1% *E. histolytica* infection was reported during the wet season in this study but slightly lower than 26.9% reported by Dawah *et al*,2010 in Kano [19], but consistent with some of the earlier studies of Park (2002) and Mawashi (2003), who reported similar prevalence rates in their studies [32,33]. Higher rate of fecal- oral contamination may be implicated during wet season and coincides with intensive farming activities. Low prevalence rate of parasite during the dry season is attributed high temperature and low humidity which is not favorable for parasite growth [34-38].

5. CONCLUSIONS

Surveillance methods and health education targeted at parents and guardians of children under the age of five years are needed to reduce the relatively high incidence of amoebiasis in Calabar, with the goal of early and proper treatment of the condition. The Ministry of Health should focus its health campaigns on improving hygiene behaviours at home, particularly among children under the age of five and their parents/guardians, particularly females. If the benefit of personal hygiene is to be realised, inhabitants in Calabar, Cross River State, must emphasise the use of safe water for all domestic duties.

CONSENT AND ETHICAL CONSIDERATION

Informed consent was obtained from each of the patient or their parents. The work was also approved by the Ethical Committee of the Ministry of Health of Cross River State. The general hospital Calabar used in this study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Ngui R, Ishak S, Chuen C S, Mahmud R and Lim YA. Prevalence and risk factors of intestinal parasitism in rural and remote West Malaysia. PLOS Negl. Trop. Dis. 2011;5(3):e974. Available:https://doi.org/10.1371/journal.pntd.0000974 PMID: 21390157
2. Lozano R, Naghavi M, Foreman K, Lim S, Shibuya K and Aboyans V. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: A systematic analysis for the Global Burden of Disease Study 2010. The lancet. 2012;380(9859):2095–2128.
3. Shimada A, Muraki Y, Awakura T, et al. Necrotic Colitis Associated with Entamoeba histolytica Infection in a Cat. J. lump. Path. 1992;106:195-199
4. Chacin-Bonilla L, An update on amebiasis. Rev. Med. Chil. 2013;141(5):609-615.
5. Cui Z, Li J, Chen Y and Zhang L. Molecular epidemiology, evolution, and phylogeny of Entamoeba spp. Infection Genetic and Evolution. 2019;75:104018. PMID: 31465857
6. Ozumba UC. Antimicrobial susceptibility pattern and sero-group distribution of Shigellaspecies at Enugu, Nigeria. Post-Graduate Medicine Journal. 1997;4:1-3.
7. Ogunlesi TA, Okeniyi JAO, Oyedeji OA, Oseni SBA, Oyelami OA, Njokonma OF. Childhood dysentery in Ilesa, Nigeria: the unusual role of E. histolytica. The Internet Journal of Tropical Medicine. 2005;2(2).
8. Norhayati M, Fatmah MS, Yusof S, Edariah AB. Intestinal parasitic infections in man: A review. Med. J. Malaysia. 2003;58:296-305.
9. Haque D, Duggal P, Kabir M, Roy S, Fair BM. Entamoeba histolytica infection in children and prevention from subsequent Amoebiasis. Infect Immun. 2006;74:904-909.
10. Oliveira FM, Neumann E, Gomes MA, Caliari MV. Entamoeba dispar: could it be pathogenic. Trop Parasitol. 2015;5(1):9–14.
11. Nair GV, Variyam EP. Noninvasive intestinal amebiasis: Entamoeba histolytica colonization without invasion. Curr.Opin Infect Dis. 2014;27(5):465-469.
12. Slack A. Parasitic causes of prolonged diarrhoea in travellers- diagnosis and management. Aust Fam Physician. 2012; 41(10):782-786.
13. Petri WA, Haque R. Entamoeba histolytica brain abscess .Handb .Clin Neurol. 2013; 114:147-152.
14. Singh A, Banerjee T, Kumar R,d Shukla SK. Prevalence of cases of amebic liver abscess in a tertiarycare centre in India: A study on risk factors, associated microflora and strain variation of Entamoeba histolytica. PloS one. 2019;14(4).
15. Karanis P, Kourenti C and Smith H. Waterborne transmission of protozoan parasites: A worldwide review of outbreaks and lessons learnt. J Water Health. 2007;5(1):1-38.
16. Sub-national HDI-Area Database, HDI global database; 2021. Retreived December 12,2021
17. Naing L, Winn T, Rusli B. Practical issues in calculating the sample size for prevalence studies. Journal of orofacial science. 2006;1:9-14.
18. Stanley SL. Amoebiasis. The Lancet. 2003;361:1025-1034
19. Dawah I, S, Inabo H, Jatua ED. Comparative study of microscopy with ELISA antibody based amoebiasis diagnosis in patient presenting with dysentery at government hospital in kaduna metropolis. Continental Journal of Biomedical sciences. 2010;4:43-49.
20. WHO. Estimates of the global burden of foodborne diseases: Foodborne disease burden epidemiology reference group 2007-2015; 2015. Retrieved December 21
21. Zahida T, Shabana K, Lashari MH. Prevalence of Entamoebahistolyticain humans, Pakistan. Pakistan Journal of Pharmaceutical Sciences. 2010;23:344-348.
22. Rinne S, Rodas EJ, Galer-until R, Glickman LT. Prevalence and Risk factors for protozoan and nematodes infections

among children in an Ecuadorian highland community. Transaction of the Royal Society of Tropical Medicine. 2005;99:585-92.

23. Jamaiah I, Shekhar KC. Amoebiasis: A 10 year retrospectivestudy at the University Hospital, Kuala Lumpur. Med J Malaysia. 1999;54:296- 302.
24. Pham Duc, Hung Nguyen-Viet, Jan Hattendorf, Jakob Zinsstag, Phung Dac Cam, Odermatt P. Risk factors for *E histolytica* infection in an agricultural community in Hanam province, Vietnam Parasites & Vectors, com/content/4/1/102; 2011.
25. Ross JT, Blair HS, Edwin R, Van T. Health and illness in the community Oxford University Press Inc. New York, USA. 2003;3-76.
26. Espinosa-Cantellano M, Espinosa-Cantellano A. Pathogenesis of intestinal amoebiasis: From molecules to disease. Journal of Clinical Microbiology. 2000;13:318-331.
27. Karaman U, Atambay M, Aycan O, Yologlu S, Daldal N. Incidence of intestinal parasites in municipal sanitary workers in Malatya. Turkish Society for parasitology. 2006;30:181-183.
28. Ajonina C, Buzie C, Moller J, Otterpohl R. The detection of Entamoeba histolytica and Toxoplasma gondii in wastewater. J. Toxicol. Environ. 2018;81(1–3):1–5.
29. Olsen A, Samuelson H, Onyango-Ouma. Study of risk factors for intestinal helminths and protozoan infection using epidemiological approaches. Journal Biosoc-Society. 2001;33(4):56 9-584.
30. Rinne S, Rodas EJ, Galer-until R, Glickman LT. Prevalence and risk factors for protozoan and nematodes infections among children in an Ecuadorian highland community. Transaction of the Royal Society of Tropical Medicine. 2005;99:85-92.
31. Kosar S, Afshan K, Salman M, Rizvi S, Naseem AA, Firasat S. Prevalence and risk factors associated with intestinal parasitic infections among schoolchildren in Punjab, Pakistan. Trop Biomed. 2017;770–780. PMID: 33592946
32. Mawashi KY. The prevalence of intestinal parasites in some parts of Katsina State Unpublished Masters'Thesis. Department of Microbiology, Ahmadu Bello University, Zaria. 2003;5-8529-9.
33. Park K. Amoebiasis. In Park K. (ed). Park's Textbook of preventive and Soc. Med. 17th Edition. M/S BarnasidasBhanor Publishers, Jabalpur, India. 2002;184-185.
34. Van Hal SJ, Stark DJ, Fotedar R, Marriott D, Ellis JT, Harkness JL. Amoebiasis: Current status in Australia. Med J Aust. 2007;186(8):412-416.
35. Choudhuri G, Rangan M. Amebic infection in humans. Indian J Gastroenterol. 2012;31(4):153-162.
36. Benetton ML, Goncalves AV, Meneghini M E, Silva EF, Carneiro M. Risk factors for infection by the *Entamoeba histolytica*/*E. dispar* complex: an epidemiological study conducted in outpatient clinics in the city of Manaus, Amazon Region, Brazil. Trans R Soc Trop Med Hyg. 2005;99:532-540.
37. Ali IKM, Roy S. A Real-Time PCR Assay for simultaneous detection and differentiation of four common Entamoeba species that infect humans. J. Clin. Microbiol. 2020;59(1):1986–20.
38. Al-Harthi SA, Jamjoom MB. Diagnosis and differentiation of entamoeba infection in makkah al mukarramah using microscopy and stool antigen detection kits. World Journal of Medical Sciences. 2007;2:15-20.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/85240