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Prevalence of *Entamoeba histolytica* Among HIV/AIDS Positive and Negative Patients Attending Two Healthcare Facilities in Nasarawa State, Nigeria

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Abstract
Entamoeba histolyticainfection remains an important source of morbidity and mortality particularly in developing countries of the world. In this study, direct smears and formal ether concentration techniques were used to identify E. histolytica while the automated Flow Cytometry method was employed in CD_4 count estimation. The overall prevalence of 90 (22.5%) was recorded, co-infection (HIV/AIDS and E. histolytica) recorded 53 (26.5%) prevalence with significant association ($p<0.05$). In relation to CD_4 counts, 83 (15.7%) of HIV/AIDS positive patients with co-infection had increase in their CD_4 counts and 117 (34.2%) patients on ART had low CD_4 counts with a significant ($p<0.05$) association between E.histolytica and HIV/AIDS infection with CD_4 counts variation. Despite the fact that even after the introduction of free anti-retroviral drugs, opportunistic intestinal infections are still a threat.However, subjecting HIV/AIDS positive patients with low CD_4 count below 200 and presenting diarrhoea to routine stool examination for E. histolyticawould significantly benefit them in curbing the prevalence of the parasite.

Keywords: Entamoeba histolytica, HIV/AIDS positive, HIV negative, CD_4 counts

Introduction
The global implications of intestinal parasites linked with Human Immunodeficiency Syndrome (HIV) and Acquired Immunodeficiency Syndrome (AIDS) are well documented (Aiemjoy*et al*., 2017). In fact, *Entamoeba histolytica* and other intestinal parasites are endemic where patients with HIV and AIDS are more prevalent and in nations tagged developing countries (Miressa and Dufera, 2021). As a result of limited resources in tropical, sub-tropical regions and sub-Saharan African, intestinal parasites after malaria and schistosomiasis have

been a major cause of morbidity and mortality in the world (Ameen and Arzek, 2023). In fact, an estimated 10% of the world population is affected with *E. histolytica* and *Entamoebadispar* and estimated 90% of the infections are asymptomatic (Ameen and Arzek, 2023). Amebiasis caused by parasitic infection result to 110,000 fatalities every year and the spread of the infections is promoted when fresh foods and beverages are contaminated by the cysts of *E. histolytica* and in endemic areas with poor sanitation while people with low socio-economic status are at risk of the infection (Abozahra *et al*., 2020).

Human Immunodeficiency Virus (HIV) is the virus responsible for Acquired Immunodeficiency Syndrome (AIDS). JUNAIDS (2022) reported that the estimated population of people living with HIV in the year 2022 has reached 39 million while the people that were newly infected were 1.3 million. AIDS in 2022 also claimed the lives of 630,000. Due to the fatality of AIDS which break down the immune system of the host and making it vulnerable to parasitic infections and other opportunistic infections (JUNAIDS, 2022; Nagai *et al*., 2023).

Ifeoma *et al*. (2022) reported that apart from the complexity of HIV, HIV/AIDS patients infected with intestinal parasites increase the risk of gastroenteritis. Previous reports showed thatthe interactions between the HIV/AIDS, intestinal parasites and gastroenteritis are contributory factors resulting to HIV replication and progression of AIDS in Africa. Ifeoma *et al*. (2022) further captured in their study that cluster of differentiation (CD_4) cell count less than 100 make HIV/AIDS patients more vulnerable to parasitic infections and it has been suggested that HIV positive patients should be screened for the presence of intestinal parasites.

Furthermore, a prevalence of 30% (180/600) out of 600 stool samples screened in the study conducted by Ifeoma *et al*. (2022) on the prevalence of intestinal parasites in HIV infected patients in Eastern Cape, South Africa was obtained while the cross-sectional study carried out by Miressa and Dufera (2021) among HIV positive and HIV negative patients between April and August, 2020 in Nekemte Specialized Hospital, Western Ethiopia showed that intestinal parasitic infections was higher (73.3%) among the HIV positive patients than the HIV negative patients with 22.7%. More so, the rate of infection caused by intestinal parasites was higher in patients whose CD_4 cell count was less than 200 cells/µL while *E. histolytica* (16%) closely followed *Giardia lamblia* (35%) were recorded in HIV positive patients (Miressa and Dufera, 2021). In the study carried out by Teklemariam *et al*. (2013) on the prevalence of intestinal parasites among HIV positive individuals who are naive and who are on antiretroviral treatment (ART) in Hiwot Fana Specialized University Hospital, Eastern Ethiopia, a prevalence of 33.7% of intestinal parasitic infections was obtained and it was significant among patients on ART.

Nigeria has the largest HIV burden in Sub-Saharan Africa with over 1.9 million people currently living with HIV (NACA, 2019b). AIDS related deaths in Nigeria have been estimated to be around 49,000 across all age groups in the year 2020 alone (JUNAIDS, 2021).

In addition, intestinal parasitic prevalence rate of 50.8% was obtained from HIV/AIDS patients in the cross-sectional study carried out in Kenya by Kipyegen *et al*. (2012) while Bamba *et al*. (2017) recorded a prevalence of 24.73% in the comparable study conducted in Burkina Faso.

Diarrhoea is a common clinical presentation of these infections. In HIV patients, Cluster of differentiation 4 (CD_4) count is important in order to measures the number of T cells expressing CD_4. This in turn determines the vulnerability of the patients to other opportunistic infections (Sarfati *et al.,* 2006). The CD_4 in molecular biology is a glycoprotein found on the surface of immune cells such as T helper cells, monocytes, macrophages and dentritic cells. CD_4 and T helper cells are white blood cells that are essential part of human immune system. They are often referred to as CD_4 cells or T_4 cells. They are called helper cells because one of the main roles is to send signals to other types of immune cells including CD_8 killer cells, which then destroy the infectious particles including virus, bacteria and parasite (Lehman *et al*., 2012). If CD_4 cells become depleted, for instance in untreated HIV infection, the body is left vulnerable to a wide range of infections that it would otherwise have been able to fight.

Materials and Methods

Study Area

The study was conducted in Keffi and Karu Medical Centres in Nasarawa state, Nigeria respectively. Karu is about 15 km and Keffi is approximately 68 km, East of Abuja the Federal Capital Territory and 128 km from Lafia the capital of Nasarawa state. The state is bounded on the North by Kaduna state, on the South by Benue state, East by Plateau state and on the West by the Federal Capital Territory. Both Karu and Keffi lies between 7°45' and 925'N of the equator and latitude7 and 937' E of the Greenwich Meridian and located on the latitude of 850M above the sea level. Keffi which is a fenced settlement shares common boarders with Karu and Kokona in the South East and North respectively occupying an area of 3,019 Km with a population of 242,764 (Akwa *et al*., 2007). Karu on the other hand, is stockade settlement shares boarders with Abuja Municipal council Area (AMAC) in the West, Keffi in the East and Jema'a LGA in Kaduna state in the North respectively, occupying an area of 3,145 Km with a population of 33,569 (Akwa *et al*., 2007). Both are fertile lands with Agriculture as the main stay of the economy and subsistence of peasant farming is greatly enhanced among the people and rearing of domestic animals. Trading is also important economic activity in the community (Ombugadu *et al*., 2011).

Sample Collection and Laboratory Analysis

About 20 g of faecal samples was collected from each of the 400 participants. A clean dry sample container was given to each person for their faecal specimen. The sample collected was then taken to Microbiology/Parasitology unit of the Laboratory Department of Federal Medical Centre Keffi and Mararaba Medical Centre, Karu respectively within 45 minutes where they were assayed. One to two gram (1-2 g) of each stool sample was fixed in two millilitres (2 ml) of 10% formal saline and another iodine preparation was made for examination by microscopy. For the microscopic examination, both saline and iodine preparation of the stools were made and examined. The former was used for the identification of the trophozoites while the later for the identification of cysts. Wet preparation using 3% iodine was the method of choice because the nucleus of *E. histolytica* retains the dye and thus allows easy identification of the cyst if present. A little portion of the formed stool sample was fetched and emulsified with the dye to form a smear. This was covered with a cover slip and viewed under the microscope using 10 and 40 objectives for examination and identification of the parasite respectively (Oti *et al.,* 2017).

Furthermore, another stool specimen was also prepared using a drop of physiological saline. A cover slip was applied before examining the preparation microscopically. The presence of ingested erythrocyte and characteristic directional movement are diagnostic of *E. histolytica* cyst of the parasite was identified based on the diagnostic characters described by Cheesbrough (2009).

Formalin/ether concentration technique was adopted to concentrate cysts in the stool samples based on the principle of the force of gravity. Four millilitre of formal water was placed in the screw capped test tube and the applicator stick was used to collect 1 gram of the same from the container and was mixed with the water in the tube. The tube was capped and mixed vigorously after which it was sieved with gauze into the beaker as suspension. The suspension was harvested into another tube, 3ml of ether was added and content was mixed properly for about 5 minutes. The tube was uncapped gradually and the preparation was then centrifuged at 3000 rpm for 2 minutes. An applicator stick was used to loosen the thick layer of faecal debris at the side of the tube and it was inverted to discard the suspended particles and solutions with the sediment remaining at the bottom of the tube. The tube was turned upright with the droplet of water on the side draining back into the tube and tapped at the base to re-suspend the sedimented parasites. A Pasteur pipette was used to harvest and transfer the sediment onto a clean glass slide. The preparation was viewed with the 10 and 40 objectives for the parasites. A drop of iodine solution was placed under the cover slip to enhance the identification of the parasites (Cheesbrough, 2009).

Statistical Analysis

Chi Square ($\div^2$) analyses were used to test for significance using Smith Statistical package (SSP) version 2.8. The statistical significance was determined at 0.5% probability. Also, data were pre-coded and double entered for accuracy in univariate and multivariate regression logistic model to determine the relationship between CD_4 countand *E. histolytica* infection.

Results

The Prevalence of *Entamoeba histolytica* Infection

Data were obtained from the stool samples collected from HIV and non HIV patients and analyzed but HIV-positive had co-infection with *E. histolytica* with variation in CD_4 count. Table 1 shows the distribution of *Entamoeba histolytica* in relation to socio-demographic factors among HIV positive patients in two study areas.

Table 1: The Prevalence of *Entamoeba histolytica* in Relation to Socio-Demographic Factors among HIV Positive Patients at Federal Medical Centre Keffi and Mararaba Medical Centre Karu LGAs Nasarawa State, Nigeria

Socio-demographic Factors	No. Examined	No. Positive (%)	(χ^2)	p-value
Gender				
Male	172	32(18.6)	0.9401	0.124
Female	228	58 (25.4)		
	400	**90 (7.5)**		
AGE				
18-27	189	66 (34.9)		
28-37	102	17 (16.7)	15.515	0.002
38-47	74	7 (9.5)		
48-57	33	0 (0.0)		
59+	2	0 (0.0)		
	400	**90 (22.5)**		
MARITAL				
Singles	98	25 (25.5)	2.928	0.105
Married	38	15 (39.5)		
Divorced	71	11 (15.5)		
Widow	193	39 (20.2)		
	400	**90 (7.5)**		
EDUCATION				
Primary	82	8 (27.6)	7.261	0.01
Secondary	73	21 (28.8)		
Tertiary	29	26 (31.7)		
Illiterates	216	35 (16.2)		
	400	**90 (7.5)**		
OCCUPATION	61	8 (13.1)	0.730	0.04
Students	105	30 (28.6)		
Farmers	90	23 (25.6)		
Unemployed	75	19 (25.3)		
Artisans	69	10 (14.5)		
C/servants	**400**	**90 (7.5)**		
LOCATION	203	47 (23.2)	0.632	0.426
FMC	197	43 (21.8)		
MRB	**400**	**90 (7.5)**		

Key: FMC- Federal Medical Centre, MRB- Mararaba Medical Centre, % - Percentage

Prevalence of *E. histolytica* in Relation to CD_4 Status of Patients

Table 2 shows the prevalence of *E. histolytica* inrelation to CD_4 status of patients. The result shows that the higher CD_4 value (>350), the lower the frequency of *E. histolytica* infection (15.70%). Out of the 200 samples examined, prevalence of 26.5% was obtained (Table 2).

Table 2: **Prevalence of *E. histolytica* in Relation to CD_4 Status of Patients**

CD_4	Number of Samples Examined	Number (%) Infected
≤200	70	30 (42.90)
200-350	47	10 (21.30)
>350	13	13 (21.30)
Total	200	53 (26.50)

Relationship between CD_4 Count and *Entamoeba histolytica*Co-Infection

Table 3 shows relationship between CD_4 count and *Entamoeba histolytica*Co-Infection. Using the univariate logistic regression model subjects with lower abdominal pain were three times more likely to have a CD_4 count below 200 than those without lower abdominal pain. This was statistically significant with a P-value of 0.003 (Table 3). Subjects who showed amoebic dysentery were two times more likely to have CD_4 count below 200 than those without amoebic dysentery. There was significant difference at P-value of 0.001. The subjects with fever were three times more likely to have a CD_4 value below 200 than those without fever and this was statistically significant at P-value of 0.0001. Subjects with nausea during the study were two times more likely to have a CD_4 value below 200 than those without nausea. There was a significant difference at P-value of 0.022 (Table 3).

Table 3: Relationship between CD_4 Count and *Entamoeba histolytica*Co-Infection (CD_4 Count of Patients on ARV, n=200)

Symptoms	Responses	No. of *E. histolytica*	Univariate Regression	Odd ratio	Multivariate regression	Odd ratio	P- value
Fever	Yes	38	1.62; 11.59	3.33	0.18;3.2 21	0.75	0.698
	No	13	1		1		
Amoebic Dysentery	Yes	56	2.32; 7.83	2.27	1.52; 6.38	3.11	0.002
	No	7	1		1		
Lower abdominal pain	Yes	42	1.20; 10.80	3.27	2.77; 26.58	8.58	0.001
	No	19	1		1		
Nausea	Yes	18	3.04; 9. 49	2.37	0.44; 6.08	1.64	0.458
	No	7	1		1		

*** There is a significant association in the univariate logistic regression model on lower abdominal pain and amoebic dysentery.

Discussion

Amoebiasis/amoebic dysentery is one of the major causes of lower abdominal pains, amoebic ulcer, liver brain abscesses among HIV infected patients. The finding of this study revealed that *E. histolytica* is prevalent among HIV/AIDS positive than the HIV negative patients. Lower abdominal pains, amoebic dysentery, fever and nausea using univariate and multivariate regression model had a significant relationship between co-infection and amoebic dysentery and lower abdominal pains among patients with HIV/AIDS.

The current study indicated the prevalence of 22.5% which is lower than the result (30.6%) obtained in a study conducted at Nnewi South Eastern Nigeria by Ekejindu *et al.* (2010). Similarly, the prevalence results (56.7% and 42.1%) obtained by Oguntibeju *et al.* (2006) and David (2014)

respectively disagree with the result obtained in this study. The comparatively low level recorded in this study may be attributed to improved sanitation habits by majority of the patients regardingsafe drinking water and waste disposal methods. Most were urban dwellers and were educated. Variation in the prevalence of parasites from different communities could be related to several factors including level of education, standard of personal/environmental hygiene and perhaps social habits.

Furthermore, with respect to age, prevalence of. *histolytica* was observed to decrease with increase in age. This is seen among the patients aged 18-27 years with 34.9% which had the highest prevalence. However, there was a significant association between age and *E. histolytica* prevalence among the HIV/AIDS positive and HIV negative patients ($P<0.05$). This is an indication that younger patients are vulnerable to *E. histolytica* infection compared to patients above 58 years who do not have the infection. This may be attributed to juvenile delinquency and being active which made them to engage in work such as farming in a contaminated environment, fishing and swimming in a polluted water and desire for variety of foodstuffs such as vegetables and raw groundnut.

E. histolytica prevalence differs in both males and females and there is no significant association between sex and prevalence of *E. histolytica* among HIV/AIDS positive and HIV negative patients ($P>0.05$). This might be associated with cultural restriction as women are being saddled with specific task in this part of the society which may include fetching water for domestic use, searching for vegetables for cooking and washing of toilets which naturally exposed them and make vulnerable to *E. histolytica* infection. This agreeswith the findings of Emem *et al*., (2008) who recorded similar result which revealed 20% and 24% of *E. histolytica* prevalence for male and female respectively.

In relation to occupation, *E. histolytica* prevalence recorded highest among patients who were farmers with 28.6%. This may be attributed to improper faecal disposal which directly contaminate the farmlands and poor personal hygiene. However, there was a significant association between occupation and *E. histolytica* prevalence among HIV/AIDS positive and HIV negative patients ($P<0.05$). However, David (2014) recorded 42.1% among school children. The variation can be attributed to the differences in age and primary school pupils who are always in contact with contaminated environment.

In Nigeria, the informal economy represents a growing economic sector and source of employment, but farming is one the largest sector of the informal economy and men generally make up majority of the farmers. However, in most cases in Africa the informal economy is associated with high level of poor environmental setting and social inequality. These factors translate to high rates of *E. histolytica* (Dibua *et al.*, 2010). Policy approach to the informal sector has marginalized farming and heightened their vulnerability to parasitic and HIV/AIDS infection through several possibilities which include: Lack of access roads to transport farm produce to markets; lack of knowledge of legal rights and inaccessible procedures to register businesses; lack of access to credit and finance services availability for farming inputs, such as fertilizer and pesticides; lack of access to vocational skills training, education, information and business support measures to move beyond survivalist activity; lack of access to affordable healthcare services and health education and lack of clear communication channels with informal and limited attention to building the capacity of this sector.

Meanwhile, some farmers could be tempted of applying even human manure in their farms which will predispose them to protozoan infection and further expose them to contaminated water, soil, faeces and food which are basic vehicles for transmission of the parasites while selling their farm produce which may involve long distances out of their matrimonial homes could also tempt them to engage in unprotected sexual escapades possibly for financial gain.

The least (16.2%) prevalence of *E. histolytica* was recorded among patients without formal education and the highest (31.7%) among patients with primary level of education although there was a diminishing trend of prevalence with increased in educational level from primary to tertiary. The low percentage (16.2%) among patients who are illiterates may be attributed to lack of awareness and frequent contact with contaminated water; food and soil as farming remain their major occupation. Educational attainment seems to lower the prevalence rate of *E.histolytica* infection as observed in this study with majority of the patient's primary education and it diminishes among patients with secondary and tertiary education. However,

there was a significant association between education and *E. histolytica* prevalence among HIV/AIDS positive and HIV negative patients (P<0.05).

Based on marital status, *E. histolytica* prevalence is associated with domestic activities among patients. This is obvious as married patients with 39.5% had the highest prevalence. This agrees with previous studies obtained among the married by Emem *et al.* (2008) who recorded 51.7%. Married patients forming majority of patients in this study may be linked with the higher tasks and responsibilities attached to married patients in a quest to meet up to the needs of the family which may involve farming in a large quantity for both grains and vegetables, use of animal excreta as fertilizer and sometimes use of irrigation as a source of water during dry season while frequent contact with children's faecal products and sometimes dirty toilet and untreated drinking water are observed among large family. However, there was no significant association between marital status and *E. histolytica* prevalence among HIV/AIDS positive and HIV negative patients (P<0.05).

The locations of this study, Federal Medical Centre Keffi and Mararaba Medical Centre Karu had variation in prevalence among the patients within the facilities. This study recorded 23.2% prevalence of patients who were from Federal Medical Centre while Mararaba Medical Centre had the least 21.8% prevalence. This agrees with the findings of Bennttonn *et al.* (2005) who recorded 37.3% and 28.9% from two different locations. This variation may be becausethe study was carried out in an old existing town Keffi and newly emerging settlement Mararaba. In this study however, there was no statistical significance between location and prevalence of *E. histolytica* among HIV/AIDS positive and HIV negative patients (P>0.05). Adoption of practices that may predispose an individual to infectious agent can promote the spread of parasitic amongst human Kinuthia *et al.,* (2012). These include but not limited to consumption of contaminated water and general sanitation negligence. In Federal Medical Centre Keffi and Mararaba Medical Centre Karu, it was observed that such practices are common among patients to increased prevalence *of E. histolytica* among HIV/AIDS positive and HIV negative patients.

Meanwhile, keeping long fingernails were considered as one of the risk factors associated with *E.histolytica* infection. This is because *E. histolytica* cysts can be found in formed stool, while trophozoites can be present in the watery stool. Patients who keep long fingernailscan retain *E.histolytica* cysts in the nails which cause infection. The result indicated 26.8% and 21.9% of patients at Federal Medical Centre (FMC), Keffi and Mararaba Medica Centre (MRB), Karu who keep long fingernails while 73.2% and 78.1% at FMC and MRB who do not keep long finger nails can be attributed to improve in personal hygiene and may be due to religion inclination. However, there was no significant difference between keeping of long finger nails and E. histolytica among HIV/AIDS positive and HIV negative patients. (P>0.05).

E. histolytica infection is orally transmitted, eating of unwashed vegetables predispose patients to the disease Though there was no significant association between eating of unwashed vegetables and *E. histolytica* prevalence among HIV/AIDS positive and HIV negative patients (P>0.05). This was evidence on the percentages recorded in this study as patients who do not eat unwashed vegetables recorded the highest prevalence with 86.1% and 86% at FMC and MRB respectively. This is slightly higher than the findings of Mojtaba *et al*., (2009) and Tamirat *et al*., (2014). This result reflectshigh level of awareness, increased in the level of education, improved in personal hygiene and use of safe drinking water that translate into employing better techniques of washing vegetables products before eating.

HIV- infected men who have sex with men were at significantly higher risk of amoebiasis than patients from other risk groups in a study conducted by Chien-chung *et al.* (2005). In this study, those who engaged in anal sex are quite low. This in sharp contrast with the findings of Chien-chung *et al.* (2005) who recorded higher anal sex practice among the patients. However, there was no difference between anal sex and *E. histolytica* prevalence among HIV/AIDS positive and HIV negative patients (P>0.05). This is an indication that most of the patients do not engage in anal sex which may be due to religion factor that frown at it or the stigmatization associated with the practice in these areas.

Use of water that is likely to be contaminated was one of such factors. Bore holes, tap and well are the main water sources in Keffi and Mararaba. There was however no significant association between the Bore holes, tap and well and prevalence of *E. histolytica* among HIV/AIDS positive and HIV negative patients (P=0.257). This finding is in consistent with that of Mail *et al.* (2011) in a study to determine the factors associated with high prevalence of intestinal parasites, identified the use of well water and bore hole as a predictor of *E. histolytica* infections, with no significant association between the use of water and *E. histolytica* prevalence (P>0.05).

In Federal Medical Centre and Mararaba Medical Centre Karu, significant association was found between the type of toilet used and prevalence of *E. histolytica* (P < 0.02). This may be attributed to the fact that those who had no toilet often defecated in the bushes and this led to contamination of water sources and foodstuffs such as vegetables. Most of the toilets had very low standards of cleanliness leading to many flies which occasional settle on foodstuffs. This agrees with the work reported by Kinuthia *et al.* (2012) in a study to determine the factors associated with high prevalence of *E, histolytica* indicated a prevalence of 22.9% among members of an ethnic group who drank untreated water.

In this study, *E. histolytica* as an opportunistic infection recorded highest number of HIV patients with low CD_4 count than those with high CD_4 count. However, there was a significant association between CD_4 count and *E.histolytica* prevalence (P<0.05). This was in sharp contrast with the findings of Buyukbaba *et al.* (2004), Brandonisio *et al.* (1999) and Kuniawan *et al.* (2009) that recorded that recorded 58.3% patients with CD_4 count less than 200 and 41.7% of those CD_4 count of 200 and above with a significant difference (P<0.05). This explains the fact that *E. histolytica* was common and strongly associated with patients with low CD_4 cells counts.

Conclusion

The study showed that *Entamoeba histolytica*, an enteric parasite though found in HIV/AIDS negative patients, but the prevalence was higher in HIV/AIDS positive patients than HIV/AIDS negative patients.

Recommendations

Based on the results obtained in this study, the following recommendations are made:

(i) HIV/AIDS positive patients with low CD_4 count below 200 and presenting diarrhea should be screened for the presence of *Entamoeba histolytica.*

(ii) Provision of quality drinking water by government will help to reduce the adverse effect of amoebiasis among people that are immunocompromised and immunocompetent.

(iii) Further studies are recommended to increase knowledge and awareness in the eradication of *E. histolytica* and three months deworming interval should be maintained as recommended by World Health Organization.

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