Cystoisosporiasis and associated risk factors in HIV-positive malaria patients in Minna, Niger State, Nigeria

ABSTRACT

In immunocompromised people, particularly those with HIV/AIDS, *Cystoisospora belli* infection is common. *Cystoisospora belli* was diagnosed using concentration techniques. The improved kiyoun staining procedures were used to process 375 samples from HIV-positive patients and 50 samples from HIV-negative individuals. Flow cytometry and the Giemsa staining technique were also used to test the patient's blood samples for malaria and CD4 cells. The overall coccidian prevalence was 14.93 percent, with a strong correlation between HIV-positive patients and non-HIV participants ($p< 0.05$). There was no discernible difference in gender or age groupings ($p< 0.05$). In HIV-positive patients, there was a substantial link between Cystoisosporiasis and CD4 cell levels ($p< 0.05$). The prevalence of *C. belli* infection was not substantially affected by risk variables such as degree of education, swimming ability, or occupation among HIV patients ($p>0.05$), but the source of water and contact with animals were. Other risk factors, such as hand washing and vegetable washing, revealed substantial differences. *C. belli* infection is common in General Hospital Minna, Niger State, Nigeria, according to this study, which could increase the burden on HIV-infected patients.

Keywords: **Cystoisosporiasis, Giemsa, Minna, CD4 cell, Cytometry**

Background

Cystoisosporiasis, formerly known as Isosporiasis, is a rare diarrheal infection caused by the protozoan *Cystoisospora belli* (formerly known as *Isospora belli*). Virchow initially described *Cystoisospora belli* in 1980. Cryptosporidium, Cyclospora, and Toxoplasma are all closely related to the genus Cystoisospora. *Cystoisospora belli* infection, on the other hand, is less common than Cryptosporidium and Toxoplasma infections. The only known hosts of *C. belli*, which has no known reservoir, are humans. This protozoan parasite is opportunistic in immune-compromised human hosts [1]. It is found mostly in the epithelial cells of the small intestine and grows in the cytoplasm of the cells [2]. This coccidian parasite has a global distribution but is primarily found in tropical and subtropical regions of the world, such as the Caribbean, Central America, and South America. India, Africa, and Southeast Asia are three of the world's most populous Regions. It is commonly connected with HIV infection in the United States [3].

Cystoisosporiasis can be found all over the world, however it is more common in tropical and subtropical locations. Infection is common among immunocompromised individuals, particularly

in patients with the Human Immunodeficiency Virus (HIV) or Acquired Immunodeficiency Syndrome (AIDS), and outbreaks in institutionalized groups in the United States have been documented. In 1915, the first case was documented. The etiological agent belonged to the genus Isospora until 2005. It was assigned to the genus Cystoisospora in 2005. These genuses are divided into several families. Isospora belongs to the Eimeriidae family, while Cystoisospora belongs to the Sarcocystidae family. Both families are members of the Eimeriorina suborder (order Eucoccidiorida of the phylum Apicomplexa).

C. belli, a protozoan belonging to the subclass Coccidia in the phylum Apicomplexa, is the pathogen that causes cystoisosporiasis. The faecal-oral method of transmission for Cytoisosporiasis is food or water contaminated with human feces. *C. belli* infection causes a self-limited diarrheal disease in immunocompetent people. It can induce chronic, life-threatening Diarrhoea and dehydration in people with weakened immune systems. Africa, Australia, the Caribbean islands, Latin America, and Southeast Asia are among the endemic locations of cystoisosporiasis [4]. According to one study, up to 15% of AIDS-infected Haitians had positive examination findings. Infected people account for 8–40% of AIDS patients in poor countries. In about 2-3 percent of patients with AIDS from Africa, cystoisosporiasis is the first AIDS-defining infection. Cystoisosporiasis is seen in 10% of AIDS patients from South America who has persistent diarrhoea.

Cystoisosporiasis affects 7–20 percent of AIDS patients from Haiti and Africa who have persistent diarrhea. *C. belli* infection affects people of all ages, although it is particularly dangerous in infants and small children, presumably due to the population's risk of dehydration. In babies, *C. belli* can cause severe diarrhoea. Apart from the gender distribution of patients with AIDS and the risk factor most usually connected with this disease, a gender propensity for infection has been observed. Except for the racial distribution of patients with AIDS in the United States, no racial preference for Cystoisosporiasis has been recorded.

Cystoisosporiasis is normally a brief, self-limiting condition in immunocompetent persons, although it can occasionally lead to a chronic diarrheal illness. In immunocompetent patients, cystoisosporiasis has also been implicated as a cause of malabsorption syndrome [5]. Infection with the intestinal protozoa Cystoisospora belli is linked to chronic and severe diarrhoea, especially in AIDS patients and other immune-compromised people [6]. Children and visitors to

tropical locations are also susceptible to infection [7, 8, 9]. Although symptoms in immune-competent individuals are self-limiting, early identification and treatment can significantly reduce the duration of intestinal symptoms.

Malaria is a lethal infectious disease that is one of the most serious health issues in Sub-Saharan Africa (SSA) and Asia. Globally, 3.4 billion individuals are at risk of new malaria infections, with one million people dying each year (WHO). *Plasmodium falciparum, Plasmodium vivax, Plasmodium malariae, Plasmodium ovale, and Plasmodium knowlesi* parasites infect humans under normal conditions [10], with *Plasmodium falciparum* and *Plasmodium vivax* being the most common species that cause morbidity and mortality in children under the age of five, pregnant women, and travelers from non-malarious areas [11].

Despite the lack of a malaria vaccine, the emergence of parasite resistance to available anti-malarial drugs, the Anopheles mosquito's resistance to insecticide residual spraying, and a poor socio-economic situation that makes malaria control and management difficult, malaria morbidity and mortality are decreasing in Sub-Saharan Africa. Limited understanding of the underlying cellular and molecular mechanisms of host-parasite interactions during co-infection and polyparasitism stymies medication discovery and vaccine development [12].

Communities residing in poor parts of developing countries bear the greatest burden of malaria infections, according to epidemiological studies [13]. Co-infections, multi-parasitism, and poly-parasitism are all possible outcomes [14]. Several researches have been conducted over the last three decades to determine the nature of the interaction between intestinal parasites and malaria during co-infection scenarios.

Over 40 million individuals worldwide are infected with HIV/AIDS, with the majority (over 25 million) living in Sub-Saharan Africa. In 2005, up to 2.4 million deaths were reported worldwide [15]. People with advanced HIV infection are susceptible to secondary infections and cancers known as "opportunistic illnesses," which take advantage of the chance provided by a compromised immune system [16]. Chronic diarrhoea and wasting related to enteric infection are the most common clinical manifestations in HIV-positive patients [17]. As a result, the goal of this study is to look at cystoisosporiasis and associated risk factors in HIV-infected malaria patients in Minna, Niger state.

MATERIALS AND METHODS

Study Area and Population

The research was carried out at Minna, Niger state, which is located between latitudes 3.200E and 8.30N. The state covers an area of 76,363 square kilometers. The state is situated in Nigeria's northern central region. It has a population of approximately 3,950,249 persons [18]. A total of 425 samples were examined, comprising 375 HIV-infected malaria patients and 375 non-HIV-infected individuals [17].

Obtaining Samples

Each participant had their blood sample taken. Venous blood samples were taken using a dry, sterile syringe and needle, and blood was pulled from a suitable vein in the arm with little stasis. The blood was gently discharged and thoroughly mixed into an ethylene diamine tetra-acetic acid (EDTA) container [15]. Clean, wide-mouthed containers were used to collect stools. Prior to the collection of samples, patients and volunteers were given questionnaires.

Blood Analyses

1. **Screening for HIV**

The patients' HIV status was confirmed using the HIV/AIDS test strip method for detecting HIV 1 and HIV 2 antibodies in whole blood. On the sample pad, a volume of 50 mL of sample (precision pipette) was applied (marked by the arrow symbol). After one minute, one drop of buffer was put to the sample pad. After a minimum of 15 min, the result was read [17].

2. **Malaria Test**

A stained thick blood film was used to diagnose a malaria infection. Each blood sample was converted into thick blood films and allowed to air dry. Slides were stained for 30 min with 3% Giemsa stain, washed with tap water, and left to air dry. Microscopy using an oil immersion objective lens was used to look for malaria parasites on the stained films (x100). Each field had a total of 200 fields [15, 17].

3. **CD4 Count Examination**

Flow cytometry (Partec Gmbh, Münster, Germany) was used to estimate CD4+ T lymphocyte cells in the blood samples. In a Partec test tube, 20 l of CD4 PE antibody was added, along with 20 l of well-mixed whole EDTA blood; the contents were gently mixed and incubated in the dark for 15 min at room temperature. During the incubation period, this mixture was stirred every 5 min. After that, 800 µl of CD4 buffer was gently mixed into the antibody and sample mixture. This was then used for counting purposes [17].

Stool Examination

The concentration technique and the Kiyoun staining procedure were used to process the freshly void stool samples. Protozoan cysts were recovered using the concentration process. The concentration method was used to prepare the newly voided stool specimens, which were then tested microscopically for *C. belli* and other intestinal parasites [19, 17].

One (1) gram of feces was emulsified and stirred in 4 mL of formalin. The finished product was sieved. In the centrifuge, the mixture was spun at 2,000 rpm for four minutes. With the help of an applicator stick, the faecal matter on the tube's side was removed, and the supernatant was disposed. A concentrated smear was formed and stained from this on a grease-free slide. An even amount of fecal material was distributed on the slide using a homogenized fecal sample. The fecal stain was fixed in methanol for 3 min before being air dried. Basic Fuschin (1%) was applied for 30 min, and then the slide was cleaned with tap water and decolorized for 1 min with 1 percent acid alcohol. The counter stain was treated with 0.5 percent malachite green for 2 min before being rinsed with water and air dried [17, 19].

The number of oocysts on the slide was counted using low and high power objectives, and the number of oocysts was counted in various fields of the slide. The stained smears were checked for *Cystoisospora belli* oocysts [20].

Data Analyses

The information gathered was organized into a table. The variables were compared using a Chi square (x2) analysis. The significance level was set at p=0.05.

RESULTS

Prevalence of *Cystoisospora belli* infection among HIV/AIDS Patients with Malaria in General hospital Minna

According to the findings, 56 (14.93%) of the 375 HIV/AIDS patient samples tested positive for *Cystoisospora belli*. A total of 50 non HIV/AIDS subjects were also tested and all of them tested negative for *Cystoisospora belli* infection. As a result, the prevalence of *Cystoisospora belli* was 14.93% among HIV patients and 0.00 percent among non-HIV subjects, respectively. Infection with *Cystoisospora belli* was a significant ($p < 0.05$) determinant in the development of Cystoisospora infection (Table 1).

Table **1: Prevalence of *Cystoisospora belli* infection among HIV/AIDS Patients with Malaria in General hospital Minna**

Samples	**No. Examined**	**No. Positive (%)**
HIV/AIDS +ve	375	56 (14.93)
HIV/AIDS –ve	**50**	0 (0.00)
Total	**425**	**56 (13.18)**

$\chi 2$ cal=8.59; χ 2; tab= 3.841; df=1 $p<0.05$

Prevalence of *Cystoisospora belli* infection among Sex group in HIV/AIDS Patients with malaria in General hospital Minna

Table 2 demonstrates the prevalence of *Cystoisospora belli* infection among HIV/AIDS patients in relation to sex. The results showed that 12 of the 90 male samples tested were positive, while 44 of the 285 female samples tested were positive. As a result, among HIV/AIDS patients, the prevalence of *Cystoisospora belli* infection is 13.33% for males and 15.33% for females, respectively. There was no statistically significant difference in terms of gender ($p > 0.05$).

Table 2: Prevalence of *Cystoisospora belli* infection among Sex group in HIV/AIDS Patients with malaria in General hospital Minna

Sex	**No. Examined**	**No. Positive (%)**
Male	90	12 (13.33)
Female	**285**	44 (15.44)
Total	**375**	**56 (14.93)**

$\chi 2$ cal=0.21; χ 2; tab= 3.841; df=1; $p>0.05$

Prevalence of *Cystoisospora belli* infection among Age group in HIV/AIDS Patients with Malaria in General Hospital Minna

The highest prevalence (20.00%) was found in the 25-29 year age group, the lowest prevalence (11.86%) was found in the 40-44 year age group, the highest prevalence (15.512%) was found in the age group 24 years, and the lowest prevalence (12.67%) was found in the age group between 35 and 44 years. Infection levels did not differ significantly by age group (P > 0.05) (Table 3).

Table 3: Prevalence of *Cystoisospora belli* infection among Age group in HIV/AIDS Patients with malaria in General Hospital, Minna.

Age group (yrs)	No. Examined	No. Positive (%)
<24	**58**	**9(15.51)**
25 – 29	**65**	**13(20.0)**
30 – 34	**59**	**7(11.86)**
35 – 39	**71**	**9(12.67)**
40 – 44	**42**	**7(16.66)**
45 – 49	**49**	**6(12.24)**
>50	**31**	**5(16.12)**
Total	**375**	**56(14.93)**

$\chi 2$ cal = 2.42; $\chi 2$ tab = 12.592; df = 6; p>0.05

***Cystoisospora belli* infection in HIV/AIDS Patients with Malaria in relation to CD4 cell Count in General Hospital, Minna**

Cystoisospora belli infection in HIV/AIDS patients with malaria who had CD4 cell counts below 200 had the highest prevalence rate (20.51%), whereas those with CD4 cell counts above 200 had the lowest prevalence rate (3.00 percent). The CD4 cell count and the infection had a significant difference (p< 0.05) (Table 4).

Table 4: *Cystoisospora belli* infection in HIV/AIDS Patients with malaria in relation to CD4 cell Count in General Hospital, Minna

CD4 Count	No. Examined	No +ve (%)
< 200	**78**	**16 (20.51)**
200 – 299	**88**	**16(18.18)**

300 – 82	**12**	**12(14.63)**
400 – 499	**77**	**9(11.68)**
>500	**50**	**3(6.00)**
Total	**375**	**56(14.93)**

$\chi 2$ cal = 58.74, $\chi 2$ tab = 9.488; df = 4; p<0.05

Risk factors associated with *Cystoisospora belli* infection in General Hospital, Minna

The findings for evaluating *Cystoisospora belli* risk variables, as well as environmental and personal hygiene factors, are reported. Among the variables studied, source of water, swimming, and animal contact were all found to be significantly associated with infection ($p< 0.05$), whereas educational status, occupation, hand washing before eating, and washing of vegetables and fruits were not ($p > 0.05$). Patients who drink untreated (well, river, rain) water have the highest prevalence (34.95%), while patients who wash their fruits and vegetables before eating have the lowest prevalence (3.46 %) (Table 5).

Table 5: **Risk factors associated with *Cystoisospora belli* infection in General Hospital, Minna**

Risk Factors	**Yes**	**No +ve (%)**
Educational status	301	41(13.62)
No formal education	68	15(22.05)
Washing of hands		
Yes	370	46(12.43)
No	0	0(0.00)
Washing of fruits/vegetables		
Yes	289	10(3.46)
No	50	2(4.00)
Swimming		
Yes	100	4(4.0)
No	250	40(16.0)
Occupation		
Farmers	27	4(14.81)

Artisans	100	18(18.0)
Govt. Employed	85	28(17.77)
Students	85	6(7.05)
Contact with domestic animals		
Yes	88	13(14.77)
No	261	5(1.91)
Sources of water		
Untreated water (Stream/River/Well/Rain)	103	36(34.95)
Treated pipe water	272	20(7.35)

Discussion

The prevalence of *C. belli* infection and associated risk factors in HIV-infected patients with malaria in General Hospital Minna, Niger State, Nigeria, is the first study of its kind.

The findings of this study could help researchers better understand cystoisosporiasis in HIV-positive patients in the future. The 14.93 percent overall prevalence rate is higher than that seen in several previous studies. In Edo state, Akinbo et *al*. [15] found a prevalence rate of 3.1 percent. In Benin City, Edo State, Oguntibeju, Akinbo et *al*. [20] found a prevalence rate of 7.8%. In Lesotho, South Africa, Assefa et *al*. [???] found a prevalence rate of 3.3%. In Gujarat, India, [22] found a prevalence rate of 7.72 %. Improved sanitation and HIV medicine treatment may have contributed to the low prevalence rate. The prevalence percentage achieved, on the other hand, is lower than that reported by Guptal et *al*. [23], who found a prevalence rate of 17.24% in Gujarat, India. The high prevalence rate in the samples used could be due to high rates of diarrhea infection [23]. In HIV-infected patients with malaria, the prevalence of cystoisosporiasis was shown to be greatest (20.00%) in proportion to age group.

The lowest prevalence (11.86%) was reported in the 25-29 year old group, followed by the 30-34 year old group. There was no statistically significant relationship between infection and age group. Because the age groups are sexually active, the total prevalence (20.00%) seen in patients between the ages of 25 and 29 is higher than that found by Akinbo et *al*. who found a prevalence of 10.3%in the age group of 31–40 [15].

It's also likely that using a diagnostic real-time PCR assay for *C. belli*, which is considered the gold standard for diagnosing Cystoisospora, would result in a higher prevalence. It has been found that once these patients begin HAART treatment, a reduced prevalence of HIV may be detected. *C. belli* infection rates appeared to differ significantly between HIV-infected individuals and non-HIV-infected subjects. This aligns with the findings of Akinbo et *al*. [15]. This could be attributed to a change in the subjects' immunological status.

The prevalence of *Cystoisospora belli* infection was 13.33 and 15.33%(male and female, respectively) among HIV/AIDS patients with malaria, which is higher than the 3.30and 3.40% (male and female, respectively) found by Akinbo et *al*. [15, 20]. Females, on the other hand, were shown to have a high prevalence of the illness in both investigations. Cystoisosporiasis and sex had no statistically significant relationship.

C. belli infection was more likely in HIV patients with CD4+ T cell levels of 200 cells/L. This greater *C. belli* infection rate could be due to these individuals' weakened immune systems, which exposes them to opportunistic infections. This is in line with Akinbo et al prior's findings [15, 24, 25]. HIV destroys CD4 cells, which are important for a person's immunity, resulting in a weakened immune system. In these patients, cystoisosporiasis can cause an increase in morbidity and mortality.

Cystoisospora belli risk factors, as well as environmental and personal hygiene concerns, are discussed. The prevalence of *Cystoisospora belli* infection was unaffected by the HIV-infected patients' educational backgrounds, with those with no formal education having the highest prevalence (22.05%). This could be related to a lack of understanding of the infection as well as poor personal cleanliness. The prevalence of *Cystoisospora belli* infection was unaffected by the HIV-infected people's occupation, with craftspeople having the highest prevalence (18.00%). While working, artisans are more prone to consume food and drink water from questionable sources. They're also more likely to have a bad educational background and, to a big extent, poor hygiene. This could be the cause for the group's high predominance.

In HIV-infected patients, the source of water had a substantial impact on the prevalence of *Cystoisospora belli* infections, with untreated (stream/rivers, well, rain, tap) water having the highest prevalence (34.95%). Streams and rivers are not highly sanitary sources of water for

home usage because they are known to host a variety of activities such as bathing, defecating, and washing. In this investigation, contact with animals was a significant risk factor for *Cystoisospora belli* infection, with the highest prevalence (14.77%).

Conclusion

This study found that *Cystoisospora belli* infection is common in General Hospital Minna, Niger State, Nigeria, with a 14.93% prevalence rate, potentially increasing the burden on HIV-positive patients. The infection was also found to be more common in girls than in males in this investigation. The research also raises awareness of the common opportunistic parasite and restricts comprehensive evaluation and nonspecific treatment of diarrhoea in HIV patients. Early and correct diagnosis of infection will benefit not only in the implementation of specialized treatment and prophylaxis (chemoprophylaxis as needed) to avoid infection relapse/recurrence in HIV patients, but also in the implementation of different preventative measures. This will not only extend HIV-infected people's lives, but will also improve their quality of life.

Ethical Approval and Consent

This study was approved by the ethics committee of the general hospital in Minna, Niger State. Participants' or subjects' verbal informed consent was collected and used in this investigation.

Recommendations

In many tertiary hospitals, routine investigation of *Cystoisospora belli* infection should be recommended in order to improve the management of HIV-infected patients. To prevent and minimize the rate of protozoan infection, a public education program on personal cleanliness, correct toilet use, and improved sanitation should be offered. Treatment with HAART is also indicated. It is recommended that a more thorough examination employing the real-time PCR method be carried out to determine the advanced detection of *Cystroisospora belli* in the area.

Contributions of the authors

The tests were conceptualized and designed by IOR, ESS, and OICJ. Authors ESS, IOR, and AMO were in charge of the experiments. The manuscript was reviewed and edited by OICJ and ESS. The manuscript's original draft was written by authors IOR and UYH. The writers ESS and NCI analyzed the data. The manuscript's final condition was agreed upon by all writers.

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Declarations

Consent for publication

Not applicable.

Competing interests

The authors declared that they have no competing interests.

References

1. World health Organization (2018). Body Mass Index. UncityMarmorrey DK – 2100 Copnhagen ǿ Denmark. Available at http://www.euro.who.int/en/health-topics/disease-prevention/nutrition/a-healthy-lifestyle/body-mass-index-bmi. Retrieved on the 7th of February, 2018.

2. Centre for Disease Control and Prevention (2012b). Laboratory Procedure Manual: Complete Blood Count with 5 parts differentials in whole blood using NHANES. Pp. 1-195. Available at https://www.cdc.gov/nchs/data/nhanes/nhanes_11_12/cbc_g_met_he.pdf.
3. Auwaerter, *P. (2015). Cystoisospora belli. Johns Hopkins Guides. Johns Hopkins Medicine*. Retrieved 20 April 2015.

4. Desalegn, A. (2013). Effects of Intestinal Parasitic Infection and Nutritional Status on Academic performance of school children. *World Journal of Arts, Commerce and Science*, 1(2), 01-07.

5. Centre for Disease Control and Prevention (2012a). Parasites – Cystoisosporiasis (formerly known as Isosporiasis). Available at https://www.cdc.gov/parasites/cystoisospora/index.html. Retrieved on 18/04/2015.

6. Atambay, M., Bayraktar, M. R., Kayabas, U., Yilmaz, S., Bayindir, Y. (2007). A rare diarrheic parasite in a liver transplant patient: *Isospora belli*, 39, 1693-1695.

7. Din, U. N., Torka, P., Hutchison, R. E., Riddell, W., & Gajra, A. (2012). Case severe *Isospora* (*Cystoisospora*) *belli* diarrhoea preceeding the diagnosis of Human T-cell-leukemia-virus-1- Associated T-cell lymphoma. *Case reports in infectious Diseases*. 2012(2012), 4p.

8. Djieyep, A. C. N., Djieyep, F. D., Pokam, B. T., David, D. L., & Kamga, H. L. F. (2014). The prevalence of intestinal coccidian parasites burden in HIV/AIDs patients on antiretroviral therapy in HIV centres in Mubi, Nigeria. *African Journal of Clinical and Experimental Microbiology.* 15(3), 165-172.

9. Sanad, T., Al-Olayan, A., Al-Hammaad, A., & Mohamed, (2014). Opportunistic Coccidian Parasites among Saudi Cancer Patients Presenting with Diarrhea: Prevalence and Immune Status. *Research Journal of Parasitology*, 9, 55-63.

10. Hayakwa, T., Culleton, R., Otan, H., Horii, T. & Tanabe, K. (2008). Big bang in the evolution of extent malaria parasites. *Molecular Biology Evolution,* 28: 2233-9.

11. Woon, S. A., Yang, R., Ryan, U., Boan, P., & Prentice, D. (2016). Chronic *Cystoisospora belli* infection in an immunocompetent Myanmar refugee–microscopy is not sensitive enough. *Journal of BioMedical Centre and Infectious Diseases*, 16(1), 221.

12. Vignesh, R., Balakrishnan, P., Shankar, E. M., Murugavel, K. G., Hanas, S., & Cecilia, A. J. (2017). High proportions of Isosporiasis among HIV infected patients with diarrhoea in Southern India. *American Journal of Tropical Medicine and Hygiene*, 77(5), 823-824.

13. Brooker, S., Akhwale, W., Pullan, R., Estambale, B., Clarke, S. E. & Snow, R. E. (2007). Epidermiology of *Plasmodium*-helminth co-infection in African: population at risk, potential impacts in anemia and prospects for combining control. *American Journal Tropical Medical Hygiene,* 77, 88-98.

14. Neha, G., Sumeetaq, K., Aman, S., & Rakesh, S. (2014). Isosporiasis in a tertiary care centre of North India. *Indian Journal of Pathology and Microbiology*, 57(2), 272-274.

15. Akinbo, F. O., Okaka, C. E., Omoregie, R. & Igbinumwen, O. (2009). Prevalence of Malaria &Anemia among HIV-infected patients in Benin City Nigeria. *New Zealand Journal Medical LaboratoryScience,* 63: 78-80.

16. Omalu, I. C. J., Yako, A. B., Duhlinska, D. D., Anyanwu, G. I., Pam, V. A. &Inyama, P. U. (2005). First detection of intestinal microsporidia in Nigeria. *Online Journal of Health All Science.* 3: 4.

17. Cheesbrough, M. (2000). Parasitological tests. District laboratory practices in tropical countries, Cambridge University Press; P. 220-221.

18. National Population Commission (NPC) (2006). *Access my library.com,* NgEx.com.

19. Cheesbrough, M. (1999). Parasitological Tests. Cambridge University Press, P: 178-308p.

20. Akinbo, F. O., Okaka, C. E. & Machado, R. L. D. (2010).Isospariasis in HIV/AIDS patient in Edo State, Nigeria. *Malaysian Journal of Medical Science,* 16: 43-46.

21. Baker, F. J., Silverton R. E., Pallister C. J. (2001). Introduction to Medical Laboratory Technology, 7th Edition. Ibadan: Bounty Press 348 p.

22. Assefa, S., Erko, B., Medhin, G., Assafa, Z., & Shimelis, T. (2012). Intestinal parasitic infections in relation to HIV/AIDs status, diarrhoea and CD4 T-cell count. *Journal of Biomedical Central Infectious Disease,* 9,155.

23. Guptal, S., Narang S., Nunavath V. & Singh S. (2008). Chronic diarrhea in HIV patients: prevalence of coccidian parasites. *Indian Journal of Medical Microbiology,* 26: 172–5.

24. Ana, P. A., Begoria, M. M., Marta, D. M., Francesca, N., Jose, A. P., & Rogelio, L. V. (2010). Self – limited Travellers' Diarrhoea by *I. belli* in a Patient with Dengue Fever. *Journal of Society of Travel Medicine,* 18 (3), 212-213.

25. Oguntibeju, O. O. (2006). Prevalence of intestinal parasites in HIV-positive/AIDS patients. *Malaysian Journal of Medical Science,* 13: 68–73