

Cystoisosporiasis in Apparently Healthy subjects and HIV/AIDS patients in Minna, Niger State

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ABSTRACT: A random sampling and cross sectional study was carried out in General Hospital, Minna, Niger State, Nigeria using parasitological techniques to screen faecal samples for *Cystoisospora belli*. Blood samples of participants were also screened to determine the CD4 counts of infected subjects while Body Mass Index of subjects was determined to describe their nutritional status. A total of 783 individuals consisting of 317 apparently healthy subjects and 466 HIV/AIDS patients were screened for cystoisosporiasis. Out of the 783 subjects screened, 81 (10.34%) were positive for *Cystoisospora belli*. The infection was significantly higher ($P < 0.05$) in HIV/AIDS patients (12.45%) than in apparently healthy subjects (2.84%). The infection was more prevalent (6.69%) in males than in females (6.40%), ($P < 0.05$). The rate of infection in relation to age group was highest in subjects who were ≤ 10 years old (11.90%) and least in subjects who were 11 to 20 years old (4.24%). A significant difference in infection rate ($P < 0.05$) was found between the categories of subjects screened and age groups. The infection rate was highest (22.64%) in subjects who were nutritionally deficient and least (13.12%) in subjects with normal body mass index. Subjects with CD4 cell counts < 200 cells/ μ l had the highest infection rate (38.24%) while those with CD4 cell counts ≥ 500 cells/ μ l had the least infection rate (2.78%). Chi – square analysis showed significant difference ($P < 0.05$) in infection rates between the categories of subjects screened and CD4 counts.

Key words: Apparently healthy, CD4 cell counts, Cystoisosporiasis, HIV/AIDS, prevalence.

INTRODUCTION

Cystoisosporiasis, 'an opportunistic infection', has been classified among "Neglected Tropical Diseases" with public health importance in immunocompromised patients, especially in HIV/AIDS patients. The disease is associated with an uncommon diarrhoea illness and it is responsible for the phenomenon known as "traveller's diarrhoea". The causative agent of the disease was formerly known as *Isospora belli* (CDC, 2012a), but presently it is called "*Cystoisospora belli*". The genus *Isospora* is closely related to the genera; *Cryptosporidia*, *Cyclospora* and *Toxoplasma*. Little or no attention is paid to this salient but devastating infection in immunocompromised and immunosuppressed patients in second and third world countries such as Nigeria, Malaysia, China, India, Ethiopia, Haiti and Brazil.

Three species of *Isospora* that are pathogenic to man

identified by Rattan and Rajesh (2005) include; *Isospora belli* (now called *Cystoisospora belli*, the only important species), *Isospora natalensis* (this is rarely isolated) and *Isospora hominis* (now called sarcocystosis). *Cystoisospora belli* parasite infection occurs alongside other opportunistic parasitic diseases and parasites of gastro-intestinal tract. The combination of cystosporiasis and other helminthic infection is usually detrimental to apparently healthy people, HIV positive patients as well as other immune-compromised patients (Akinbo et al., 2009; Indrani et al., 2013; Sauda et al., 1993). The sites of infection of the parasite in man include small intestine where it causes diarrhoea and mal-absorption; gall bladder and biliary tree where it causes acalculous cholecystitis, cholangitis; in spleen and in liver where it causes rare dissemination, and in rheumatological site where reactive

arthritis may occur (Paul, 2012). The prevalence of *Cystoisospora belli* recorded is high in HIV/AIDS patients. However, few cases have been documented in immunocompetent and other immunocompromised individuals including those with renal transplant, lymphoma and leukaemia patients, cancer patients, malnourished children and sickle-cell anaemia patients (Inabo et al., 2012; Mahdi and Ali, 2002; Neha et al., 2014; Resiere and Chachaty, 2003; Sanad et al., 2014).

The parasite is said to be rare or absent in immunocompetent or apparently healthy individuals because their immune system could deal with the infection. However, recent reports documented in Nigeria and other parts of the world suggest that the notion of rare or absence of *Cystoisosporiasis* parasite in immunocompetent or apparently healthy subjects due to its non – symptoms nature in this group may no longer be true as the parasite has been detected in this group of people (Woon et al., 2016; Pravean et al., 2017). Clinical symptoms associated with this infection ranges from of stomach upset, abdominal pain, weight loss (Vignesh et al., 2017), profuse diarrhoea, acalculouscholecystitis, tissue inversion and dissemination, reactive arthritis, (Bialek et al., 2011; Neha et al., 2014; Rattan and Rajesh, 2005), impair cognitive abilities, physical and mental growth (Drake et al., 2000; Guyatt, 2000), decrease in life expectancy and increase in mortality rates especially in tropical Africa and other subtropical regions in the world where safe drinking water and sanitation facilities are inadequate (Amuta and Mker, 2009; Cappello, 2004; Savioli and Albonico, 2004). The parasite thrives well in unhygienic conditions and infection is acquired mostly through faecal-oral route in contaminated food and water. However, transmission through oral-anal route is also possible (CDC, 2012a). Socio-cultural and socio-economic challenges of this parasitic infection ranges from school absenteeism and drop outs, absenteeism from work and from business, thwart in educational achievement, waste of money, low productivity, increase in poverty to hindering of economic development (Edward and Michael, 2004; Conteh et al., 2010; Desalegn, 2013).

Far from declining, this infection is increasing throughout the world. The increase in infection rate results from climatic changes induced by global warming, poor sanitation status that accompanies war as well as socio-cultural life patterns of the people. Globally, infection rates reported for this parasite in different parts of the world is such that in Nigeria, the rate of infection rose from 3.1% in 2008 to 9.8% in 2013 (Akinbo et al., 2009; Inabo et al., 2012). From Ethiopia, the infection rate reported was 5% (Assefa et al., 2012) while the infection rate in New Delhi in 2008 was 50% (Gupta et al., 2008). In Chennai, the infection rate rose from 18.6% in 2002 to 26.1% in 2007 (Kuma et al., 2002; Vignesh et al., 2017). Generally, this infection is associated with poor sanitary habits, lack of access to safe drinking water and improper hygiene, lack of access to health care, lack of basic health knowledge and the health implications of the infection especially in

countries with high rates of poverty (Steketee, 2003). The degree of each risk factor, prevalence and intensity of parasitic infections vary from one region to another (Ogbe and Odudu, 1990). Most studies on *cystoisosporiasis* involve relatively small number of participants from small socio-economic groups and from ecological system involving other groups of intestinal parasites. Little is known about the salient and devastating effects of the parasite on apparently healthy/ immunocompetent and in immunocompromised HIV/AIDS patients in Minna, Niger State, Nigeria. The study therefore sought to evaluate *Cystoisosporiasis* in Apparently Healthy subjects and HIV/AIDS patients in Minna, Niger state, Nigeria.

MATERIALS AND METHODS

Study area

The study was carried out in General Hospital Minna, Niger State, Nigeria. Niger State is known as the power state, a state in North-Central Nigeria with the largest land mass in the country. Niger state is located on latitude 3.20¹ East and longitude 8 and 11.3¹ North. It covers an area of 76,363 km² with a population of about 3,950,249 (NPC, 2006). The state is bordered to the north by Sokoto state, west by Kebbi State, south by Kogi state and southwest by Kwara state, northeast by Kaduna state and southeast by the Federal Capital Territory. The state has common boundary with The Republic of Benin along New Bussa, Agwara and Wushishi local government area, given rise to common inter-border trade between Nigeria and The Republic of Benin (Ministry of Land and Survey, 2014).

Study design

A cross-sectional descriptive study and random sampling was carried out to determine the prevalence and risk factors associated with *Cystoisospora belli* infection and its relationship with haematological status of subjects. Questionnaires were administered to patients to determine their demographic data and risk factors for transmission of the infection among population within the study area.

Study population

The study population in this research were made up of apparently healthy subjects and HIV/AIDS patients.

Ethical clearance and approval

A letter from the Head, Department of Biological Sciences, Federal University of Technology, Minna, Niger State was used to obtain an ethical clearance from the ethical committee of General Hospital, Minna, Niger State, Nigeria. Subjects consent was sought before the commencement of the study.

Sample collection and analysis

Modified Kinyoun Acid fast staining for stool sampling

A 1 g of faeces was homogenized in 10 ml of 10% formalin in a test tube. The homogenate was filtered through a 300 µm mesh sieve into a centrifuge tube. 100 µl of the supernatant was removed by pipette and the sediment washed twice in distilled water by alternate centrifugation for 2 minutes in a micro-centrifuge at 6500 rpm. The final sediment after decanting was re-suspended in 150 µl distilled water and centrifuged in a cytopspin centrifuge at 1000 rpm for 2 minutes.

Smear from the sediments were made on slides and allowed to dry. Slides were fixed in methanol for 3 minutes. The fixed slides were stained in cold carbolfuchsin for 15 to 20 minutes, washed in tap water, decolorized in 1% acid alcohol for 3 to 4 minutes and rinsed thoroughly in tap water, counter stained with 0.4% Malachite green for 30 seconds, dried and examined at x 100 (oil immersion) for oocyst of *C. belli* (Sauda et al., 1993; Akinbo et al., 2009; Indrani et al., 2013).

Procedure for CD4 T-cell counts

A 5 mL whole blood was collected and analysed using Cyflow Counter analyser (Sysmex Partec, Germany). A 20 µl of CD4 PE antibody was mixed with 20 µl whole blood from an EDTA bottle in a partec test tube. The mixture was incubated in the dark for 15 minutes at room temperature. Thereafter, 800 µl of CD4 buffer was added to the mixture and homogenized. The homogenate was placed in the Partec Cyflow counter analyser to measure the CD4 cell counts (CDC, 2012b).

Anthropometric method for determination of the nutritional status of participants

Nutritional status of each individual was determined by measuring the anthropometric parameters, which were used in determining the Body Mass Index (quetelet index) of each individual. The BMI is a measure for indicating nutritional status of individuals (WHO, 2018). Weight and height were measured from each participant, except in pregnant women, wheelchair bound individuals, or persons who have difficulty standing steady. Self-reported weights and heights were not accepted. Weight was measured using balanced beam scale/bathroom weighing scale; height was measured using flexible but non-stretchable measuring tape/insertion tape. The body mass index (BMI) for each participant was determined using the following formula below.

$$\text{BMI} = \frac{m \text{ (kg)}}{h^2 \text{ (m}^2\text{)}}$$

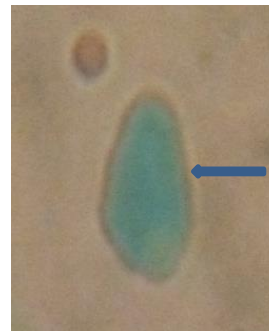


Plate 1. Micrograph of *C. belli* from Modified Kinyoun's acid fast stain.

BMI = Body Mass Index, m = mass of the body (weight) of respondent in kilogram (kg) and h = height of respondent in meters (WHO, 2018).

Data analysis

Statistical analyses were performed using version 20.0 of the Statistical Package for the Social Sciences (SPSS). Chi-square (χ^2) test was used to relate data with the observed probabilities and applied at 5% level of significance. Mean and percentages were used to analyse data with simple proportional comparison.

RESULTS

A total of 783 subjects were screened for *Cystoisospora belli* infections. Out of these, 317 subjects were Apparently Healthy while 466 were HIV/AIDs patients. Of the 317 apparently healthy subjects, 6 (4.41%) males and 9 (2.84%) females were positive for *Cystoisospora belli* (Plate 1) infection while out of the 466 HIV/AIDs patients, 14 (8.59%) males and 58 (19.14%) females were positive for *C. belli* infection (Table 1). There was a significant difference ($P < 0.05$) in infection rates between males and females.

The age group 21 to 30 years old had the highest (18.18%) infection rates while 11 to 20 years old had the least infection rates (4.24%). The high infection rates documented in 21 to 30 years old could be due to exposure to the parasite and weak immune status. The low prevalence in 11 to 20 years old could have resulted from less exposure to infection. There was a significant difference in infection rates between age groups and categories of subjects ($P < 0.05$) (Table 2).

The prevalence of the infection was highest (22.64%) in subjects who are wasting and least (13.12%) in those with normal body weight (Table 3). There was however, no significant difference in infection rates between wasting and the categories of subjects ($P > 0.05$).

Table 1. Sex related Prevalence of *Cystoisospora belli* in Apparently Healthy subjects and HIV/AIDs patients in Minna.

Sex	Males		Females		Total	
	No Exam	No +ve (%)	No Exam	No +ve (%)	No Exam	No +ve (%)
App. Healthy	136	6 (4.41)	181	3 (1.66)	317	9 (2.84)
HIV/AIDs	163	14 (8.59)	303	58 (19.14)	466	72 (12.45)
Total	299	20 (6.69)	484	31 (6.40)	783	81 (10.34)

$X^2_{crit} = 39.84$, $X^2_{cal} = 39.84$, $df = 1$, $P < 0.05$.

Table 2. Age group related Prevalence of *Cystoisospora belli* in Apparently Healthy subjects and HIV/AIDs patients in Minna.

Age group	App. Healthy		HIV/AIDs		Total	
	No Exam	No +ve (%)	No Exam	No +ve (%)	No Exam	No +ve (%)
≤ 10	26	3 (11.54)	16	2 (12.50)	42	5 (11.90)
11 – 20	41	0 (0.00)	77	5 (6.49)	118	5 (4.24)
21 – 30	72	1 (1.39)	126	35 (27.78)	198	36 (18.18)
31 – 40	89	2 (2.25)	137	17 (12.41)	226	19 (8.41)
41 – 50	56	2 (3.57)	65	7 (10.77)	121	9 (7.44)
≥ 51	33	1 (3.03)	45	6 (13.33)	78	7 (8.97)
Total	317	9 (2.84)	466	72 (15.45)	783	81 (10.34)

$X^2_{crit} = 12.59$, $X^2_{cal} = 16.34$, $df = 5$, $P < 0.05$.

Table 3. Prevalence of *Cystoisospora belli* in relation to Nutritional Status in Apparently Healthy subjects and HIV/AIDs patients in Minna

BMI	Normal (18.5 – 24.9 kg/m ²)		Wasting (< 18.5 kg/m ²)		Obese (> 25.0 kg/m ²)		Total(Kg/m ²)	
	No Exam	No +ve (%)	No Exam	No +ve (%)	No Exam	No +ve (%)	No Exam	No +ve (%)
App. Healthy	156	2 (1.28)	75	5 (6.67)	86	2 (2.33)	317	9 (2.84)
HIV/AIDs	315	41 (13.02)	106	24 (22.64)	45	7 (15.53)	466	72 (15.45)
Total	471	43 (9.13)	181	29 (16.02)	131	9 (6.87)	783	81 (10.34)

$X^2_{crit} = 5.99$, $X^2_{cal} = 4.05$, $df = 2$, $P > 0.05$.

Table 4. CD4 count related Prevalence of *Cystoisospora belli* in Apparently Healthy subjects and HIV/AIDs patients in Minna.

CD 4 count (Cells/μL)	App. Healthy		HIV/AIDs		Total	
	No Exam	No +ve (%)	No Exam	No +ve (%)	No Exam	No +ve (%)
< 200	-	-	102	39 (38.24)	102	39 (38.24)
200 – 499	150	6 (4.00)	243	28 (11.52)	393	34 (8.65)
≥ 500	167	3 (1.80)	121	5 (4.13)	288	8 (2.78)
Total	317	9 (2.84)	466	72 (15.45)	783	81 (10.34)

$X^2_{crit} = 5.99$, $X^2_{cal} = 8.0$, $df = 2$, $P < 0.05$.

Subjects whose CD4 cell counts were < 200 cells/μl had the highest (38.24%) infection rate while the least infection rate (2.78%) was found in subjects with CD4 cell counts ≥ 500 cells/μl. There was significant difference in infection rates between CD4 cell counts and categories of subjects ($P < 0.05$) (Table 4).

DISCUSSION

This study confirmed the presence of cystoisosporiasis in Minna, Niger State, Nigeria with an overall prevalence of 10.34%. The prevalence in this study was found to be higher than the report documented by Akinbo et al. (2009)

in Edo State, Nigeria. Despite the high prevalence recorded in this study, the prevalence was however lower than 31% recorded by Djieyep et al. (2014) in Mubi and 22% documented by Indrani et al. (2013) in Odishi among HIV patients. The low prevalence recorded in this study could be due to low circulation of the parasite among the population, loss of some of the parasite in the course of stool screening and less exposure to the parasite. The highest prevalence was recorded in subjects who are wasting is an indication of the inability of the body to withstand the infection due to weak immune status. Nutritional status determines the ability of the body to withstand infection because proper nutrition helps increase the immune system of the body.

Age is one of the risk factors associated with parasite infection and its establishment because of the varying nature of immune system. This was observed in significance in infection rate between age group and the categories of subjects screened in this study. The highest infection rate recorded in 21 to 30 years old could be as a result of decline in immune status since depreciation in immune system in subjects make them susceptible to opportunistic infections than those whose immune system are strong to withstand infection. The highest prevalence among the 21 to 30 years old in this study differs from the report of Dawit (2013) who reported the highest prevalence among patients who were ≥ 48 years old.

Subjects who are wasting in this study had the highest infection rate compared to those without weight loss among the categories of subjects screened. The susceptibility of this group to the infection could have resulted from depletion of body immune system due to loss of useful body nutrients. This finding is in line with the report of Mekonnen et al. (2014) on patients who are on HAART in Ethiopia. Other researchers also documented loss of weight in patients infected with *C. belli* (Ana et al., 2010; Min et al., 2013; Din et al., 2012).

The infection rate was highest in subjects with CD4 counts < 200 cells/ μ L. This could be an indication of depreciating immune system. A CD4 cell plays very important role in maintenance of health. However, depletion of CD4 cell counts often lead to increased susceptibility to opportunistic parasites. The result in this study agrees with the work of Djieyep et al. (2014), Fredrick et al. (2011) and Gupta et al. (2008). This study thus, confirmed that Cystoisosporiasis is prevalent in Minna, Niger State, Nigeria.

Conclusion and recommendations

Cystoisospora belli is prevalent in Apparently Healthy subjects and HIV/AIDS patients in Minna, Niger State, Nigeria and the infection rate is higher in HIV/AIDS patients than in Apparently Healthy subjects.

Therefore, it is important to carry out routine screening for *C. belli* parasite in individuals who are immune deficient, malnourished or may have suffered prolong

diarrhoea symptom. *Cystoisospora belli* infected individuals should be screened for HIV because it a marker for HIV infection.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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