*Full Length Research Paper*

# Intestinal parasites and human immunodeficiency virus (HIV) status of children in Jos, Nigeria

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Intestinal parasitic infestations (IPI) are not uncommon in immunocompetent individuals. However, human immunodeficiency virus (HIV)-infected individuals with depleted immunity have an abnormally high susceptibility to infections. This study therefore, examines children with intestinal parasites according to HIV status and degree of immunosuppression. Consecutively consenting patients aged 1 to 15 years attending the Paediatric Clinic of acquired immune deficiency syndrome (AIDS) Prevention Initiative, Nigeria, were recruited as cases, while age and sex matched HIV negative controls were recruited from Out Patient Department of Jos University Teaching Hospital. Stool samples were examined for parasites by direct wet mount, formol-ether and modified Ziehl-Neelsen technique. Levels of immunosuppression were assessed amongst HIV-positive subjects. Five hundred and ten children aged 1 to 15 years equally divided between the two cohorts were enrolled for the study. Seventy-nine had IPI, giving a prevalence rate of 15.5%; 44 (8.6%) HIV positive and 35 (6.9%) HIV-negative children. The most prevalent extracellular parasite was *Giardia lamblia,* however HIV positives had significantly higher rate of *G. lamblia* infestation. Among the intracellular parasites, the infection rate in HIV-positive subjects (5.9%) was three times that in HIV-negative subjects (2.0%). HIV positive children with advanced and severe immunosuppression had significantly higher intracellular parasites. HIV status did not significantly predict the overall risk of having extracellular intestinal parasites however, it was noted that *G. lamblia* infection was significantly higher in HIV positive children. HIV positive children had higher risk of having intracellular parasites especially if they have advanced or severe immunosuppression. Therefore, the policy of screening children for intestinal parasites should continue irrespective of their HIV status. Those that are HIV positive children should specifically be screened for intracellular parasites.

**Key words:** Intestinal parasites, HIV, Children, Prevalence, Immunosuppression

## INTRODUCTION

Intestinal parasitic infestations (IPI) are not uncommon in immunocompetent individuals. However, human

immunodeficiency virus (HIV)-infected individuals with depleted immunity has an abnormally high susceptibility to infections with even minimally pathogenic organisms (Garcia et al., 1997). Globally about 40 million of people are infected with HIV/acquired immune deficiency syndrome (AIDS) including adults and children < 15 years, and out of this, about 2.5 million are children < 15 years. More than 90% of these infections are in sub-Saharan Africa.

Nigeria accounts for 30% of the global burden of mother-to-child transmission (MTCT) of HIV and 10% of Paediatric HIV/AIDS. HIV accounts for 3% of deaths in under-5 years children in Nigeria and most of the deaths are from opportunistic infections (World Health Organisation, 2007). Studies (Wiwanitkit, 2001; Sadraei et al., 2005; Cotte et al., 1993) have shown that there is an increased risk of opportunistic intestinal protozoans in HIV-infected than HIV-uninfected individuals. This is because HIV causes a progressive decline of the mucosal immunological defence mechanisms and altera-tions of production of IgA antibodies, thus increasing the susceptibility to various intracellular intestinal opportu-nistic agents, such as *Cryptosporidium parvum, Isospora belli* and *Microsporidium species* (Cimerman et al., 1999).

Children may suffer from other non-opportunistic extracellular intestinal parasitic infestations such as *Entamoeba histolytica, Giardia lamblia, Trichuris trichiura, Ascaris lumbricoides* and *Strongyloides stercoralis* (Sadraei et al., 2005). These enteric infections frequently cause severe diarrhoea especially in immunocompro-mised children than immunocompetent and sometimes lead to death (Cimerman et al., 1999). They also cause impairment of growth and development, reduce physical activity, impaired learning ability, recurrent abdominal pains, anaemia, intestinal obstruction, and under nutrition (Pensa et al., 2000; Kucik et al, 2004). Factors influencing the transmission of parasites are geographical and ecological conditions. Improper food processing also contributes to increase risks of parasitic infestations (Ikpeme et al., 1999).

HIV and IPI contribute to childhood morbidity and mortality in developing countries and therefore deserve a high degree of priority (Sadraei et al., 2005). It is estimated that approximately 70% of the disease burden on the whole population can be prevented in high prevalence communities by treating school children alone (Nematian et al., 2004). In Jos, Ibrahim (2004) found the prevalence of IPI in HIV infected adults with chronic diarrhoea as 20.8%. In view of the paucity of data on Paediatric HIV and IPI co-infection in this part of the country, the current study was undertaken to determine the prevalence and pattern of intestinal parasites (extracellular and intracellular) in HIV-infected and HIV-uninfected children and relate pattern of IPI to the severity of immunosuppression in HIV-infected children in

Jos University Teaching Hospital (JUTH), Jos, Plateau State.

## MATERIALS AND METHODS

### Study design

The study was cross-sectional involving HIV positive and HIV negative children.

### Study area

The study was carried out in Jos University Teaching Hospital (JUTH), a tertiary health institution. JUTH is located in Jos metropolis, the capital city of Plateau State, Nigeria. Plateau state has a size of 26,899 km$^2$, with a population of 2,959,588 (Plateau State, 2009). It lies at latitude 9° 55' N and longitude 8° 53' E. The high lands rises from 1,200 m above sea level at the low lands to a peak of 1,829 m above sea level. It has a near temperate climate with an approximate mean high temperature of 22°C and mean low temperature of 18°C. The mean annual rainfall varies from 131.8 to 146 cm (Plateau State, 2009).

### Study population

All consecutively consenting patients aged 1 to 15 years, who have been confirmed HIV positive by polymerase chain reaction (PCR) or Western blot and were attending the Paediatric Infectious Disease Clinic of AIDS Prevention Initiative Nigeria (APIN), JUTH, were recruited as cases. Age and sex-matched children from Paediatric Out Patient Department (POPD), JUTH were used as controls. The controls were screened for HIV using rapid diagnostic kit (determine). The PCR was done with a commercially available kit for qualitative DNA polymerase chain reaction assay using Roche Amplicor HIV-1 DNA test, Version 1.5, manufactured by Roche Diagnostics, 9115 Hague Road Indianapolis, IN 46250-0457, USA. The Western blot kits were supplied by Immunetics Company, USA. Data obtained from the patients using structured questionnaire were age, sex, drug history, CD4 count/CD4%, family and social history and nutritional history including breastfeeding and use of breast milk substitute. A general physical examination was also carried out including the anthropometric measurements. Clinical staging was done using WHO clinical staging system (World Health Organisation, 2007). WHO HIV immunological staging using CD4% for children < 5 years and CD4 count for children > 5 years was done (World Health Organisation, 2007). The data were analysed with EpiInfo version 3.5.1.

### Laboratory sample collection and analyses

Blood samples were taken from the controls and tested for HIV-1 and 2 for recruitment into the study. Fresh stool samples were taken from all the study population and were examined for parasite using the following 3 methods: direct wet mount, formol-ether and modified Ziehl-Neelsen technique (Blacklock et al., 1973; Chessbrough, 1999). Those with positive parasites in their stool were treated accordingly except those with *Cryptosporidium*.

**Ethical considerations**

Ethical approval was obtained from the Health Research Ethical Committee (HREC) of JUTH. Participation in the study was voluntary and written informed consent was obtained from all the parents/guardians. The information obtained from the study was kept confidential.

**Data analysis**

The data was analysed using EpiInfo version 3.5.1. Student t-test, Wilcoxon two-sample test and analysis of variance (ANOVA) were used to compare means of variables while Chi square test was used for categorical variables. P-value < 0.05 was considered statistically significant.

## RESULTS

### Characteristics of study population

Five hundred and ten children aged 1 to 15 years were studied. Two hundred and fifty-five HIV positive children (mean age 5.50 ± 3.18) and 255 HIV negative children (5.55 ± 3.22) were examined for intestinal parasitic infestations. The socio-demographic characteristics of the study population are presented in Table 1. There was no statistical significance in the sex, socioeconomic status and the mother's educational level in the 2 groups (p > 0.05).

### Prevalence of intestinal parasitic infestations in HIV positive subjects and HIV negative controls

Seventy nine children had intestinal parasites, giving a prevalence rate of 15.5% in the study population, with 44 (8.6%) being HIV positive and 35 of them (6.9%) being HIV-negative children (p > 0.05). Among the study groups, ages 1 to 5 years were 155 HIV positive children and 154 HIV negative children while in ages 6 to 10 years, there were 80 and 79 children, respectively. In ages 11 to 15, there were 21 children in each group. Fifty five (17.7%) in ages 1 to 5 years had IPI, 21 (13.0%) in ages 6 to 10 and 5 (11.6%) ages 11 to 15 had IPI. These differences did not show any statistical significance. Table 2 shows relative frequency of intestinal parasites in both groups. There was no significant difference statistically in the overall prevalence of extracellular parasites between the HIV positive and HIV negative children (p > 0.05). The most prevalent extracellular parasite was *G. lamblia* with a prevalence rate of 5.9%. *G. lamblia* infection rate in HIV positive children (9.0%) was about three times that in HIV negative children (2.7%). This difference was statistically significant (p < 0.05). The second commonest extracellular parasite was *E. histolytica* which was twice as common in HIV negative as in HIV positive (p <0.05). However, amongst the HIV-negative children, the extracellular parasite most commonly isolated was *E.histolytica.* Fifteen (5.9%) HIV-positive subjects were infected with intracellular parasites (*Cryptosporidium* and *Isospora*). This was three times the infection rate of 2.0% in HIV-negative children (p < 0.05). Seven (2.8%) and 2 (0.8%) of HIV positive and HIV negative children had multiple parasites, respectively (p > 0.05).

### Stage of HIV disease and IPI

The prevalence of IPI (both extra and intracellular) was not statistically significant to the clinical stage of the disease (p > 0.05). There was also no significant relationship between WHO clinical staging and acquisition of extracellular parasites. However, the frequency of intracellular intestinal parasitaemia tends to increase with clinical severity of disease. However, this was not statistically significant (Table 3). The prevalence of extracellular IPI did not vary with immunological status (Table 4). However, those with advanced and severe immunosuppression had a significantly higher prevalence of intracellular intestinal parasitic infestation than those with mild immunosuppression.

## DISCUSSION

The overall prevalence rate (15.5%) of intestinal parasites in this study is lower than previous studies carried out on children from other parts of Nigeria (Holland et al., 1989; Enekwechi et al., 1994; Agi, 1995). The prevalence in these areas ranged from (20.8 to 67.2%). The lower prevalence rates in this study could be due to the fact that Jos is at a higher altitude and as such it has a lower environmental temperature compared to other parts of Nigeria. This lower temperature (mean high temperature of 22°C and mean minimum low temperature of 18°C may not be favourable for the survival of larvae/ova of these parasites which require temperature of 24 to 37°C for optimal survival (WHO Technical Report series, 1991). Furthermore, the prevalence rate of 15.5% obtained in this study is lower than earlier findings reported from Jos (Zoakah et al., 1999; Ighoboja et al., 1997). This could be explained by the fact that the present study was a hospital based study and that could have affected the overall prevalence of IPI in the children studied. The other two previous studies were carried out in a rural community among malnourished children in Jos (Zoakah et al., 1999; Ighoboja et al., 1997; Ogbonna et al, 2004). Globally, the prevalence of IPI ranges between 20 to 90% which is higher than the overall prevalence in this study. The differences in prevalence rates in different parts of the world where the studies were carried out may be as a

**Table 1.** Socio-demographic characteristics of HIV positive and HIV negative children in Jos.

| Parameter | HIV positive (n=255) | HIV negative (n=255) | Statistical test value | p value |
|---|---|---|---|---|
| **Mean age** | 5.50±3.18 | 5.55±3.22 | 0.19^ | 0.85 |
| **Sex** | | | | |
| Male | 113 (44.3%) | 116 (45.1%) | 0.07^^^ | 0.78 |
| Female | 142 (55.7%) | 139 (54.9%) | | |
| **Social class** | | | | |
| Lower | 122 | 137 | | |
| Middle | 98 | 98 | 4.96^^^ | 0.08 |
| Upper | 35 | 20 | | |
| **Maternal educational** | **Status** | | | |
| No formal education | 19 | 33 | | |
| Primary | 48 | 45 | 7.19^^^ | 0.07 |
| Secondary | 100 | 110 | | |
| Tertiary | 88 | 67 | | |

^^^chi square, ^ = T-test.

**Table 2.** Relative frequencies of intestinal parasites among children in Jos.

| Intestinal parasite | HIV positive n (%) | HIV negative n (%) | Total (%) | $x^2$ | *P* value |
|---|---|---|---|---|---|
| *A. lumbricoides* | 1 (0.4) | 3 (1.2) | 4 (0.8) | 0.25 (0.65) | |
| *E. histolytica* | 8 (3.1) | 19 (7.5) | 27 (5.3) | 4.73 (0.03*) | - |
| *G. lamblia* | 23 (9.0) | 7 (2.7) | 30 (5.9) | 9.07 (0.003*) | |
| *A. duodenale* | 1 (0.4) | 0(0) | 1 (0.2) | 0.00 (1.000) | - |
| *S. mansoni* | 0 (0) | 3 (1.2) | 3 (0.6) | 1.34 1.000 | |
| *Taenia spp* | 0 (0) | 1 (0.4) | 1 (0.2) | 0.00 (0.25) | - |
| *Cryptosporidium spp* | 12 (4.7) | 5 (2.0) | 17 (3.3) | 2.98 (0.08) | - |
| *Isospora spp* | 3 (1.2) | 0 (0) | 3 (0.6) | 1.34 (0.25) | |
| *Cyclospora* | 0 (0) | 0 (0) | 0 (0) | 0.00 (0.00) | - |
| No parasites | 214 (83.5) | 219 (86.3) | 433 (84.9) | - | - |
| Total | 262 | 257 | 519 | - | - |

Some of the children had multiple parasites. Extracellular parasites- *E. histolytica*, *G. lamblia* , *A. lumbricoides and S. mansoni, A. duodenale and Taenia spp;* Intracellular parasites- *Cryptosporidium spp* and *Isospora belli.*

result of variations in study populations, methods, environmental and geographical factors (Whitefield, 1982).

This study shows that there is no significant difference in intestinal infestation amongst HIV positive and HIV negative children. This is similar to the report by Meamar

**Table 3.** Relationship between clinical staging and intestinal parasites among children in Jos.

| Clinical Staging (WHO) | HIV positive | Extracellular parasite n (%) | Intracellular parasite n (%) |
|---|---|---|---|
| 1 | 57 | 7(12.28) | 2(3.51) |
| 2 | 61 | 11(18.03) | 4(6.56) |
| 3 | 126 | 14(11.11) | 8(6.35) |
| 4 | 11 | 1(9.09) ($\chi^2$=8.89, p value=0.71) | 1(9.09) ($\chi^2$=0.84, p value=0.84) |

**Table 4.** Relationship between immune status and intestinal parasites among children in Jos.

| Immunological staging (WHO) | HIV positive (N) | Extracellular parasite n (%) | Intracellular parasite n (%) |
|---|---|---|---|
| Not Significant | 103 | 14 (13.6 ) | 2 (1.9) |
| Mild | 70 | 10 (14.3 ) | 1 (1.4) |
| Advanced | 40 | 4 (10.0) | 5 (12.5) |
| Severe | 42 | 5(12.0) ($\chi^2$=1.37, p value= 0.71) | 7(16.7) ($\chi^2$=6.38, p value=0.04) |

et al. (2007) which showed that the overall prevalence of intestinal parasites in HIV positive and HIV negative individuals were similar. Meamar et al. (2007) did not give a full description of the patients studied; however, the present study was carried out in HIV positive children who have been receiving care, treatment and support. That could have lowered the overall prevalence of IPI in these children. Mbae et al. (2013) in Kenya showed an overall prevalence of 25.6% in HIV positive versus HIV negative children < 5 years who had diarrhoea and were either being managed as outpatients or inpatients. A study in HIV positive and HIV negative patients conducted in Zaria, Nigeria by Inabo et al. (2012) showed a prevalence of 70.6% while Okodua et al. (2003) in Abeokuta, Nigeria showed an overall prevalence of 28.4% which are both higher than what was found in this study. Inabo (2012) and Okodua (2003) studies were conducted in adults with diarrhoea while Mbae (2013) study was on children with diarrhoea, and thus could have increased the parasite yield.

The prevalence rates of the extracellular parasites were similar in the HIV-infected and their age and sex-matched controls. In both groups, 33 of the 255 patients (12.9%) were infected with extracellular parasites. This was similar to studies by Lindo et al. (1998) and Meamar et al. (2007) who reported similar rates in both HIV infected and uninfected individuals studied. HIV-induced enteropathy does not favour the establishment of extracellular parasites (Lindo et al., 1998). Secondly, Th2 CD4 lymphocytes which are highly necessary to protect the host against such parasites remain less affected than Th1 in HIV seropositive patients (Meamar et al., 2007).

Even though extracellular parasite rates were similar in HIV positive and HIV negative, this study showed a significantly higher *G. lamblia* infection rate in HIV positive patients than HIV negative children. Okodua et al. (2003) in Abeokuta, Nigeria found a statistically higher infection with *G. lamblia* and *Cryptosporidium* in HIV positive compared to HIV negative individuals. Similarly, Babatunde et al. (2010) in Ilorin, Nigeria, found *G. lamblia* and *Strongyloides stercoralis* to be four times higher and *Cryptosporidium* to be five times higher in HIV positive compared to HIV negative adults, and these were related to level of immunosuppression. These findings were similar to this current study though the Okodua et al. (2003) and Babatunde et al. (2010) studies were conducted in adults. The relatively high prevalence rate of *G. lamblia* infection in HIV positive compared to HIV negative individuals could be as a result of depressed hosts' humoral immunity as observed by Robinson et al. (1990). Heavier parasites load of non-opportunistic parasite could also accumulate in HIV positive individuals who are severely immunocompromised as they may have delayed clearance of these parasites (Awole et al., 2003).

Meamar et al. (2007) in Iran did not find any difference in *G. lamblia* infection rate between HIV positive and HIV negative individuals. However, contrary to the present study, Lindo et al. (1998) reported a higher infection rate in HIV negative than HIV positive individuals. The difference in prevalence of *G. lamblia* infection between Lindo et al. (1998) study and that of Meamar et al. (2007), and the current could be because the study by Lindo et al. (1998) had a smaller sample size compared with the other two studies. The second commonest

extracellular parasite in this study was *E. histolytica* which was two times commoner in HIV negative than in HIV positive children and the difference was statistically significant. Lindo et al. (1998) had suggested that pathological changes in gut epithelium caused by HIV itself do not favour the extracellular parasites like *E. histolytica* to thrive well in HIV-infected persons. The prevalence rate of intracellular parasite was significantly higher in HIV positive than in HIV negative control. This is because intracellular parasites thrive when there is depressed T cell activity (Lindo et al., 1998; Farthing, 2003; Okodua, 2003; Inabo et al., 2012; Mbae et al., 2013).

*Isospora belli* was found exclusively in HIV positive children. This was consistent with the report of Meamar et al. (2007) in Iran. *I. belli* is an intracellular parasite which thrives when there is depressed T cell activity. However, the prevalence rate of *I. belli* in this study is lower than what was reported by Meamar et al. (2007) probably because most of the HIV positive children at PEPFAR, JUTH are routinely placed on cotrimoxazole for prophylaxis against pneumocystis jirovecii pneumonia (PCP). Cotrimaxazole has some activity against *I. belli* (Garcia, 1997; Johnson, 1997).

There was a significant relationship between immune status and intracellular parasitic infestation but there was no relationship between immune status and extracellular intestinal parasites. The fact that intracellular parasitic infestation occurred more in children with advanced and severe immunosuppression than mild immunosuppression is consistent with previous reports (Cimerman et al., 1999; Flynn, 2000). This can be explained by the nature of the immunological disturbances in HIV/AIDS patients in whom immune deficiency is most related to T-cell sub-populations and lymphokines. This is characterized by depletion of CD4 T-cells and compromised cellular immune response (Th1) that is considered protective against intracellular protozoan infection. On the other hand, clinical staging of HIV infection did not show any relationship with any particular parasite. This may be due to the fact that the parameters used in the clinical staging may not really increase the risk of having intestinal parasites as an HIV-infected child with clinical stage I may have severe immunosuppression.

There was no statistically significant difference in multiple parasitic infestations between the cases and controls. This finding differs from the earlier report by Hailemariam et al. (2004) in Ethiopia who found significantly higher multiple parasites among HIV positive children and adults than their controls. Though the Hailemariam et al. (2004) study was conducted in a tertiary health care centre like the present study, there was no indication as to whether the patients were on any form of care or treatment. However, the subjects in this present study had already been recruited into a programme and they are cared for through health education, nutritional advice and supplement, treatment and prophylaxis of opportunistic infections and general advice on healthy living. These could have affected the actual prevalence observed.

The age group of 1 to 5 years was mostly affected with intestinal parasites, followed by 6 to 10 years. This age group preponderance is in contrast to some other reports (Ikpeme, 1999; Jalo, 1999; Johnson, 1997) which showed that most infestations occur at 6 to 12 years age category, followed by 1 to 5 years age group. This observation might be because children go to school much earlier now than before and as such, they are exposed to environmental factors much earlier. The drop in prevalence rates as the children approach adulthood could be due to increasing awareness of personal hygiene as the child grows. The sex distribution of intestinal parasitic infestation showed no significant discrimination in prevalence of infestation. This finding compares with a previous report (Jalo, 1999) but contradicts other reports which showed higher infestation rates in males than females (Ikpeme, 1999; Silverman et al, 1983). The lack of difference in infestation rates could be that both sexes were exposed to almost the same environmental conditions.

## Conclusion

HIV status did not significantly predict the overall risk of having extracellular intestinal parasites however, it was noted that *G. lamblia* infection was significantly higher in HIV positive children. HIV positive children had higher risk of having intracellular parasites especially if they have advanced or severe immunosuppression. Therefore, the policy of screening children for intestinal parasites should continue irrespective of their HIV status. Those that are HIV positive should specifically be screened for intracellular parasites.

This study has some limitations. In this study, only one stool sample was analysed and this could have affected the yield of intestinal parasites. Also, *Microsporidium* was not studied because of unavailability of the reagent. Most of the HIV positive patients in the Paediatric Infectious Disease Clinic have received some form of treatment, care and support prior to recruitment into the study. Therefore, this might have affected the prevalence and spectrum of intestinal parasites.

## Conflict of Interests

The author(s) have not declared any conflict of interests.

## ACKNOWLEDGEMENT

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