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Original Article

Paediatrics Section

Intestinal Parasitoses in HIV Infected Children in a Nigerian Tertiary Hospital

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ABSTRACT

Background: Intestinal parasitoses are common amongst people living in developing countries. They may impact negatively on the growth and health of immune competent children. There is paucity of information on the association between HIV and intestinal parasitoses in African children.

Objective: To identify the intestinal infections responsible for infections in HIV infected children and document characteristics of HIV infected children at a Nigerian teaching hospital.

Materials and Methods: Consecutive children attending a Paediatric anti-retroviral clinic were studied. Information such as socio-demographics and clinical characteristics elicited from clinical examination were recorded in the proforma. Stool samples of the children were obtained and examined for intestinal parasites. Data was analysed with the SPSS 18 software.

Results: A total 52 children were studied and their age ranged between 6 months and 14 years, with a mean of 6.5 years ± 3.93. The 52 were made up of 27 boys and 25 girls, giving a male: female ratio of 1.1:1. 10 (19.2%) of the 52 children were infected with *cryptosporidium* spp, while 1(1.9%) had *Ascaris lumbricoides* infestation. Anti-helminthics had previously been administered to 86.5% of children studied. Those who previously received anti-helminthics had lower prevalence estimates of *cryptosporidium* infections. (p<0.01, RR = 0.42, 95%CI = 0.20 – 0.90). Children on co-trimoxazole prophylaxis had lower prevalence estimates of *cryptosporidium* infections. (P<0.01, RR = 0.35, 95%CI = 0.14 – 0.91). Use of highly active antiretroviral drugs was also associated with lower prevalence estimates of intestinal cryptosporidium. (p=0.04, RR = 0.58, 95%CI = 0.31 – 1.10). Eight of the 10 children infected with *cryptosporidium* had recurrent abdominal pain in comparison with the six with recurrent abdominal pain amongst the 42 without cryptosporidial infections. (p<0.01, RR=5.6, 95%CI= 2.51 – 12.1).

Conclusion: Cryptosporidial infection is the most common intestinal parasitoses among HIV infected children in this study, while intestinal helminthiasis are not so common. Anti-helminthics, Co-trimoxazole prophylaxis and highly active anti-retroviral therapy have a protective effect against intestinal cryptosporidium. Screening for intestinal *cryptosporidium* is suggested in HIV infected children with recurrent abdominal pain, because of the statistically association.

Keywords: Gut, Helminths, Immunodeficiency, Infections and paediatrics

INTRODUCTION

Human immunodeficiency virus is a common cause of infection globally, accounting for an estimated 33.8 million infections in the year 2008 [1]. Sub-Saharan Africa was disproportionately affected, accounting for 91% of new paediatric infections in the year 2008 [1]. Nigeria being the most populous African nation has also had its own share of these pandemic recording 220,000 infections in the year 2007, thus making Nigeria the second largest country in Africa with paediatric infections [2].

People living in the tropics are also unfortunately over-burdened with other diseases such as soil transmitted helminths (STH) apart from HIV [3]. STH easily thrive in the tropics because of the poverty, poor levels of hygiene and sanitation coupled with the tropical weather which favour transmission of these infections. Most of the STH are parasites of the human intestine and they also pose a major health challenge for people living in the tropics. *Ascaris lumbricoides*, and hook worm are notable examples of common STH infesting children in developing countries, while *cryptosporidium* is also a common protozoan and intestinal parasite [4]. These parasites have been documented to cause co-morbidities such as malnutrition, delayed growth, anaemia and diarrhoea [3,4].

Similarities in the geographical predilection by both intestinal parasites and HIV for resource constrained settings are likely to favour the occurrence of co-infections in such settings. Available information on the extent to which both diseases co-occur in African children is however scanty [5,6]. Intestinal parasitoses in HIV infected Nigerian children has also been under researched [6]. This informed our decision to conduct this study among, HIV infected children attending the paediatric ARV clinic of the Obafemi Awolowo University Teaching Hospitals Complex, Ile-Ife, Osun State, Nigeria.

The Obafemi Awolowo University Teaching Hospitals Complex is the only federal government owned tertiary hospital providing free care to individuals infected with HIV in Osun state. This state was estimated to have a population of 3.2 million in 2006 [7]. People from the neighbouring states of Ondo, Kwara and Ekiti also patronize the facility. The hospital is supported by the government of Nigeria and the United States President's emergency plan for AIDS relief program.

MATERIALS AND METHODS

This is a prospective study of HIV infected children attending the paediatric anti-retroviral clinic of Obafemi Awolowo University Teaching Hospital, Ile - Ife, Nigeria between May, 2011 and July 2011. Subjects studied were consenting consecutive HIV infected children aged 3 months to 14 years, attending the clinic in the stated period. Children and care givers who declined from taking part in the study and those who had been treated with anti-helminthics at least three months prior to the study were excluded.

Ethical approval was obtained from the research and ethics committee of the Obafemi Awolowo University Teaching Hospital Complex, Ile Ife, Nigeria. Diagnosis of HIV in this clinic was based on a positive ELISA reaction and confirmed by a Western blot in children aged 18 months and older. Infections in children aged less than 18 months were established using the HIV DNA polymerase chain reaction kit.

Information was obtained from the recruited cases by means of a proforma. Details obtained include age, sex, clinical presentation, occupation and educational attainments of both the recruits and their parents. The weight was taken with minimal clothing and without shoes using a weighing balance in children who could not stand and a bathroom scale adjusting the scale for zero error and checking for precision from time to time with known weights. A stadiometer and infantometer were used to obtain the height and full length respectively in those who could not stand.

A note of anti-retroviral administered was recorded. Children on Highly Active Anti-retroviral Therapy were usually on Zidovudine, Lamivudine and Nevirapine or Efavirenz as indicated by the national policy [8]. Stool samples were collected from the patients in a clean bottle. The freshly collected stools were processed through a faecal parasite concentrator in order to concentrate the parasites and then examined for ova, eggs or parasites of helminths and other parasites such as protozoans. There after stool examinations were conducted by the direct method and formal ether concentration technique as described by Cheesbough [9]. The Modified Ziehl-Neelsen stain was also used to stain the concentrated stool smears in order to identify *cryptosporidium* and other intestinal protozoa using the technique described by Casemore [10]. The blood was also taken for complete blood counts and CD4 counts.

The data obtained was analysed with PASW statistics version 18 using simple descriptive statistics such as range, mean and percentages for continuous variables. Tests for significance for associations computed for categorical variables were based on the chi–square and values less than 0.05 were regarded as statistical significant.

RESULTS

Population Studied

Of the 210 total HIV infected children enrolled for care at the paediatric HIV in the year 2011, 150 attended the clinic during the period. The 52 children studied represent 60 percent of the HIV infected children and 30 percent of the total enrolled patients for HIV care.

Age and Sex Distribution

The age of the children studied ranged between 6 months and 14 years. Of the 52 children studied 32(61.5%) were 5 years or above, 16(38.4%) were aged below 5 years. Four of the subjects were aged between 4 and below 1 year of age. The mean age of the boys was 5.9 years and girls 7.2 years. Concerning distribution of sexes, the 27 boys and 25 girls studied gave a 1.1:1 male to female ratio. [Table/Fig-1] shows the age distribution of the children studied.

None of the four infants in this study had *cryptosporidium* infection. Of the 16 children aged between 1 and 5 years, 2 (11.1%) had *cryptosporidium* infections, while 8 (25.0%) of the 32 children age above 5 years were infected. The mean age of the children infected with *cryptosporidium* was 6.3 years, while it was 7.6 years in the uninfected.

Intestinal Parasitoses in the Studied Children

Ten of the 52 children studied were infected with intestinal parasites giving a prevalence of 19.2%. *Cryptosporidium* was the most common enteric parasite detected and ten children were infected with this parasite. The other parasites were helminths. *Ascaris lumbricoides* and *Enteriobius vermicularis* were both present in one child. The child with helminthiasis was also infected with Cryptosporidium, thus giving rise to polyparasitism. Other helminths such as *Strongyloides stercoralis, Trichuris trichiura* or hookworm were not detected in this study.

Mode of HIV Transmission Among Studied Children and Association with Intestinal Parasitoses

Of the 52 children studied the mode of transmission of HIV was presumed to be vertical in 48(92.3%) and horizontal in 4(7.7%). None of the children with presumed horizontal infections were infected with cryptosporidium, while 10 of the 48 children with presumed vertical infections were infected with cryptosporidium.

HIV Clinical Staging and Clinical Features of Children with Co-infections

None of the HIV infected children had AIDS. Of the 52 children 9(17.3%) were from clinical stage I, 30(57.7%) from stage II and 13(25.0%) from stage III. Of the 9 children in clinical stage I, 3(33.3%) had *cryptosporidium* cysts in their stools, while 4(13.3%) of the 30 children in stage II had *cryptosporidium* cysts in their stools and 3(23.1%) had *cryptosporidium* cysts in their stools amongst the 13 children in stage III.

Recurrent abdominal pain were more common in the infected compared to uninfected children. This association was statistically significant. p<0.01, RR=5.6, 95%C.I=2.51-12.5. The other clinical related associations between infected and non-infected children are shown in [Table/Fig-2].

Nutritional Status of Infected and Non-infected Children

Of the 52 children 42 (80.8%) were well fed and 10 (19.2%) were malnourished based on Z scores. Amongst the 20 children aged 5years and less, failure to thrive was recorded in 3 (15.0%). Marasmus and underweight malnutrition were recorded in 8 and 2 children respectively and none of the children had kwashiorkor or Marasmic kwashiorkor according to Wellcome's classification. Stunting was recorded in 10 children,

All the four infants were breast fed exclusively for 6 months. None of the 52 children studied had severe malnutrition. Amongst the 42 well nourished HIV infected children 7 (16.7%) had cryptosporidial infections, compared with 3 (30.0%) cryptosporidial infected of the 10 HIV infected children with moderate malnutrition. (p = 0.61, RR = 0.84, 95% C.I = 0.55 – 1.29).

Laboratory Characteristics of Infected Children

A differential eosinophil count above 3% was taken to indicate eosinophilia while, counts less than 3% were regarded as normal. Of the total 52 children studied 45(86.5%) had eosinophilia. Nine (90.0%) of the 10 *cryptosporidium* infected children had eosinophilia, while 36(85.7%) of the remaining 42 children uninfected with *cryptosporidium* had eosinophilia. This is not a statistically significant difference between these two groups. p= 0.87, RR=1.40, 95%C.I= 0.21–9.42.

Of the total 52, 49 (94.2%) had normal haematocrit values (>30%). The mean haematocrit of the children without *cryptosporidium* was 33.9% while it was 33% for those with cryptosporidium.

A mean CD4 count of 991cells/µl was recorded in children with intestinal *cryptosporidium* while it was 1240 cells/µl in children without cryptosporidium. CD4 counts <15% were considered as profound immunosuppression, while CD4 counts above 15% were regarded as mild or insignificant immunosuppression. [Table/Fig-3] shows the association between HIV infected children with and without *cryptosporidium* infections and eosinophil counts, packed cell volume and CD4 counts.

Cryptosporidium Infection and Use of Anti-retrovirals, Co-trimoxazole and Previous Use of Anti-helminthics

Co-trimoxazole prophylaxis (CPZ) was associated with fewer *cryptosporidium* infections. Three (7.7%) of the 39 children on CPZ prophylaxis had *cryptosporidium* infections compared with seven (53.8%) infected children of the 13 not on CPZ prophylaxis. The

difference between these two categories was statistically significant. p<0.01, RR = 0.35, 95%CI = 0.14 – 0.91. [Table/Fig-4] shows the association between, co-trimoxazole and *cryptosporidium* infections. The same table also shows the association between anti-retrovirals and *cryptosporidium* infection and previously used anti-helminthics and *cryptosporidium* infections.

Concerning anti-helminthic use among the 52 children, 44 had taken anti-helminthics before at least once outside 3 months prior to the study. Of the studied population 2(3.8%), 3(5.8%) and 4(7.7%) used mebendazole, pyrantel pamoate and albendazole respectively. Thirty five (67.3%) could not recall the names of the drug taken and eight (15.4%) took nothing.

Clinical Characteristics of the Single Child Infected with Ascariasis

Of the 52 children studied only one had a helminth infection with ascariasis and none had *Strongyloides stercoralis* or hookworm infection. This gave a prevalence of 1.9%. This 8 year old girl had both eggs of *Ascaris lumbricoides* and *Enteriobius vermicularis* detected in the stool sample. The presenting complaints were recurrent abdominal pain and occasional anal prolapse while defecating. The child also complained of passage of intestinal worms. There was no associated vomiting or diarrhoea or other clinical symptoms. The child was classified as HIV class group 1, because she was asymptomatic for HIV/AIDS infection. She was well nourished with a Z score above 2 standard deviations for weight and height. A haematocrit of 33% was recorded. Total white blood cell count was within the normal range (4,300/mm^3). The differential count for the neutrophils, lymphocytes and eosinophils was, 54%, 32% and 14% respectively indicating an eosinophilia. The CD4 count was 360cells/µl, which is 26% of the lymphocyte count. The child was from the middle socio-economic class and her father was a school teacher while the mother was a trader and was uneducated. The household had piped water and the animals bred included goats, rabbits and pigs.

Characteristics	Number	Percentage
Mean age (years); (Range)	6.5 (0.5–14)	
Sex		
Male	27	51.9
Female	25	48.1
Location		
Urban	39	75.0
Rural	13	25.0
HIV clinical staging		
I	9	17.3
II	30	57.7
III	13	25.0
IV	0	0.0
Nutritional status		
Well nourished > 2S.D	42	80.8
Moderate malnutrition < 2 S.D	10	19.2
Severe malnutrition < 3 S.D	0	0.0

[Table/fig-1]: Subject characteristics

Demographic characteristics	Infected n=10(%)	Non-infected n=42(%)	p-value	Relative risk	95% CI
Age(mean ± S.D)	6.3 ± 3.9	7.6± 3.7			
Sex					
Male (N=27)	3(30.0)	24 (57.1)	0.23	0.53	0.20 – 1.40
Female (25)	7 (70.0)	18 (42.9)			
Age					
0 -5 years	2 (20.0)	18 (42.9)	0.33	0.47	0.13 – 1.69
>5 – 14 years	8 (80.0)	24 (57.1)			
Recurrent abdominal pain					
Yes	8(80.0)	6(14.3)	<0.01	5.6	2.51 – 12.5
No	2 (20.0)	36(85.7)			
Diarrhoea					
Yes	1(10.0)	0 (0.0)	0.19#	*	*
No	9 (90.0)	42(100.0)			
HIV clinical staging (%)					
I	3(30.0)	6(14.3)	0.47	2.10	0.63 – 6.99
II	4(40.0)	26(61.9)	0.37	0.65	0.29 – 1.43
III	3(30.0)	10(23.8)	1.00	1.26	0.42 – 3.75
IV	0(0.0)	0(0.0)	*	*	*

[Table/Fig-2]: Clinical features of children infected with and without cryptosporidium
*Cannot be determined
P-value with Yate's correction

Immunologic and laboratory characteristics	Infected n = 10 (%)	Non-infected n = 42 (%)	p-value	Relative risk	95% CI
Eosinophilia (differential eosinophil count>3%)	9 (90.0)	36(85.7)	0.87	1.40	0.21–9.42
Differential eosinophil count <3%	1(10.0)	6(14.3)			
Packed cell volume <30%	0(0.0)	3(7.1)	*	*	*
Packed cell volume >30%	10(100.0)	39(92.9)			
CD4 count >15%	9(90.0)	37(88.1)	0.70	1.02	0.81–1.29
CD4 count <15%	1(10.0)	5(11.9)			

[Table/Fig-3]: Distribution of immunologic and haematologic characteristics in relation to stool cryptosporidial cysts
*Cannot be determined

Drugs Administered	Infected n = 10 (%)	Non-infected n = 42 (%)	p-value	Relative risk	95%CI
Co-trimoxazole					
Yes	3(30.0)	36(85.7)	<0.01	0.35	0.14 – 0.91
No	7(70.0)	6(14.3)			
Anti-retrovirals					
Yes	5(50.0)	36(85.7)	0.04	0.58	0.31 - 1.10
No	5(50.0)	6(14.3)			
Anti-helminthics					
Yes	4 (40.0)	40(95.2)	<0.01	0.42	0.20 – 0.90
No	6 (60.0)	2(4.8)			

[Table/Fig-4]: *Cryptosporidium* infection and use of anti-retroviral, Co-trimoxazole and anti-helminthics

DISCUSSION

The present study shows that intestinal parasitoses are not uncommon among HIV infected children with parasitoses occurring close to five in one subjects. Much higher prevalence estimates of 52% and 59.5% were recorded among Indonesian and Cameroonian HIV infected patients respectively [11,12]. The very high prevalence estimates among the Indonesian children can be explained by the fact that they were diarrheal, while the risk factor for higher infection rates in the Cameroon study could have been the age of the participants, who were all adults. Exposure to infection is likely to be higher among adults by reason of greater interaction and adventure with the environment. No helminths were however discovered in the Indonesian study while, *Trichuris trichiura*, *Teania* spp and *Strongyloides stercoralis* were identified in 0.25% cases each of the Cameroonian population studied [11,12]. This paucity

of soil transmitted helminths in the latter two studies are consistent with the findings in the present study.

Amongst other reasons, the paucity of helminths recorded in this study may be due to the presumptive administration of anti-helminthics by parents and other caregivers to children for gastro-intestinal related symptoms, which is a common practice in the study location environ. Easy accessibility to anti-helminthics, by purchase over the counter without a prescription in our setting, may also contribute to over use or abuse of anti-helminthics. Thus it was not surprising that majority of the population studied had taken anti-helminthics before. Another factor that may be responsible for the low rates of parasitic co-infections amongst HIV infected patients receiving care in a tertiary hospital setting is the health education received at the paediatric anti-retroviral clinics.

The finding of polyparasitism by *Ascaris lumbricoides*, *Enterobius vermicularis* and *Cryptosporidium spp* recorded in a child is similar to the two cases of polyparasitism recorded among 42 HIV infected patients in Cameroon by Nkenfou et al., [12]. A higher polyparasitism rate of 12% was however obtained among HIV infected Kenyan children and *cryptosporidium spp* was the most common parasite implicated in intestinal parasitoses [13]. *Cryptosporidium* was also the most common parasite identified in Nkenfou et al., study accounting for 19% of the population studied, which is also the exact percentage of children infected with *cryptosporidium* in the present study. The high rates of cryptosporidial infection in the present study may probably be due to difficulties in isolating the aetiologic pathogen which is surmised to untowardly impact negatively on disease management. Cheap and effective drugs for the management of cryptosporidiosis are also scarce [14,15].

Intestinal parasitoses was common among those older than five years, however it did not occur among infants and was uncommon among the under fives in the present study. This finding depicts a direct relationship between intestinal parasitoses and age. This relationship is not unexpected as the chances of acquiring an infection increases with age as a result of exposure from interaction or adventure, which is usually the rule with growing up. Furthermore immunity eventually wanes with increasing age in HIV infected individuals and this may predispose infected children to more and severe infections. Comparison of our results with other studies is however difficult because of a scarcity of similar studies. The available studies excluded under fives either on ethical grounds or for other unstated reasons [16,17].

Almost all of the patients in the present series acquired HIV by vertical transmission. This observation is similar to findings in previous Nigerian studies thus highlighting the need for more effective prevention of mother to child transmission of HIV programs [18-20]. Most of the patients in the present study presented at the symptomatic and advanced clinical stages of HIV in which co-infections are common. *Cryptosporidium* has been previously noted to present with recurrent abdominal pains and cramps among HIV infected individuals [14,15]. This lends credence to the significant association between abdominal pain and intestinal *cryptosporidium* in the present study. However, only one case of acute diarrhoea was reported in the present study. Chronic diarrhoea was not noted in this case series probably because there was no child with AIDS amongst them. Chronic diarrhoea is however very common amongst AIDS patients [15,21,22].

A common abnormal haematologic finding in the children studied was eosinophilia. More than 85% of children studied with and without intestinal helminths had eosinophilia thus the differences in these two groups were not statistically significant. The results obtained from this study were consistent with other studies and they also suggest the possibility of concurrent infections with helminths [23,24]. The low prevalence of helminths in the present study however suggested that the aetiology of eosinophilia in HIV infected individuals might be due to other aetiologies than helminths.

The mean haematocrit of children with *cryptosporidium* was slightly lower than those without, therefore it was surprising that all the infected children with *cryptosporidium* had normal haematocrits, while less than 10% of the uninfected children had anaemia. These differences were not statistically significant. The results in the present study differ from a previous report which associated *cryptosporidium* with anaemia in HIV infected patients [25]. The haematocrit in the child with helminth co-infection was normal although lower than the mean for the other children. Previous studies also showed a similar trend of higher haematocrit among children without helminths [26,27].

A minor proportion of the population studied had profound immune suppression based on CD4 counts. Similar rates of infection with *cryptosporidium* were recorded amongst those with and without profound immune suppression, unlike other studies which reported more infections among the severely immune deficient [28,29]. The absence of children with AIDS in the present study, may account for fewer infections among those with profound immunodeficiency. Individuals with AIDS would be more likely to be susceptible to new infections and lack the ability to clear established infections.

The present study showed that HIV infected children on Highly Active Anti-retroviral Therapy had lower prevalence of infection with cryptosporidium. This association was statistically significant however the range of confidence intervals reduces the strength of this association. HIV infected children on co-trimoxazole however had statistically significant lower prevalence estimates of infection compared to those not on prophylaxis. This association has not been previously documented and it may be due to co-trimoxazole's broad range of antimicrobial activity. Co-trimoxazole anti-microbial activity against *Isospora belli, Plasmodium falciparum, pneumococcus, non-typhoidal salmonella* etc. Protection from pathogens and parasites sensitive to the co-trimoxazole is expected to improve the immune system thus indirectly preventing infections from cryptosporidium. Co-trimoxazole's broad anti-infective properties on protozoans also gives room for speculation that it might be have some activity on cryptosporidium. Drug trial studies, evaluating the effect of co-trimoxazole on *cryptosporidium* are needed to resolve this issue.

Previous use of anti-helminthics among HIV infected children was also associated with statistically significant reduced infection with *cryptosporidium* in this study. It is suggested that the eradication of co-parasitism using anti-helminthics may boost immune function thus protecting against *cryptosporidium* infections. The greater majority of the population studied who used anti-helminthics may also have better health seeking behaviour and this may account for the reduced rates of *cryptosporidium* infection among this group. It is speculated that some anti-helminthics may have anti-*cryptosporidium* activities. It is also possible that levamisole was administered in some of the cases were the anti-helminthics taken could not be re-called. Levamisole has an immune stimulant action [30].

LIMITATION

The short period of the study was a limitation and it could not be extended beyond this period because it was the time available for an overseas research. This factor might be responsible for the limited subjects co-opted. A no so robust sample size also determines the extent to which statistical computation and generalisations can be made.

CONCLUSION

The prevalence of the intestinal parasite, *cryptosporidium* in this study population was high, while that of *Ascaris lumbricoides* and *Enteriobius vermicularis* were low. *Cryptosporidium* should be suspected in HIV infected patient with recurrent abdominal pains because of the statistically significant associations between this symptom and infection. Eosinophilia was not characteristic

of intestinal cryptosporidium. Rational use of anti-helminthics and co-trimoxazole in HIV infected patients may be protective against intestinal *cryptosporidium* in HIV infected children. Regular de-worming of patients is also advised in order to keep the burden of helminthiasis as low as possible in HIV infected children. De-worming is also expected to be beneficial in *cryptosporidium* infected patients.

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