Prevalence of asymptomatic intestinal coccidian parasite infections among non-diarrhoeic HIV-positive children in Zaria, Nigeria

M. Aminu*‡ and Y. E. Yakubu*

OPPORTUNISTIC COCCIDIAN PARASITES, amongst other infections, frequently complicate human immunodeficiency virus (HIV) infection by causing chronic diarrhoea. The magnitude of these parasitic infections in HIV-positive patients requires careful attention in developing countries. There have been inadequate studies addressing this problem in Nigeria. The investigation reported here was conducted at Ahmadu Bello University Teaching Hospital, Zaria, Nigeria, with the objective of determining the prevalence of these parasitic infections among HIV-positive children. Eighty-eight stool samples were collected during the wet humid months of July and August 2006 from 60 non-diarrhoeic, HIV-positive and 28 HIV-negative children less than 10 years old. The samples were examined for intestinal coccidian parasites by microscopy and modified Kinyoun's acid fast staining methods. Coccidian parasites, *Cyclospora cayetanensis*, *Cryptosporidium parvum* and *Isospora belli*, were identified in 51% (45/88) of all the stool samples examined. The parasite oocysts were identified in 68% (41/60) of the HIV-positive patients presenting at the hospital and in 14% (4/28) of the controls (*P* < 0.01). We found an increase in parasite prevalence with age of the patient. This study indicates that coccidian parasites may be important opportunistic infection agents in non-diarrhoeic HIV-infected children. The prevalence of these parasites and their potential for compounding the health problems of HIV-infected patients suggest that the diagnosis and treatment of coccidian parasites should be a part of routine HIV care.

Introduction

The World Health Organization reported in 1998 that 33% of global deaths are a consequence of infectious and parasitic diseases, whereas the effect of mortality and morbidity are as a result of some parasitic infections.1 Parasitic infections caused by protozoan pathogens impose a substantial health and economic burden on tropical, poor countries where such infections are prevalent.2 Protozoan parasites constitute the largest group of parasites known to be associated with diarrhoea in humans. The incidence of protozoan pathogens with diarrhoea has been on the increase, following the advent of HIV infection and the acquired immune deficiency syndrome (AIDS) pandemic. Common diarrhoeal protozoan pathogens are *Giardia lamblia*, *Entamoeba histolytica* and *Balantidium coli* and, lately, coccidian parasites like *Isospora belli*, *Cryptosporidium parvum*, *Microsporidia* and *Cyclospora cayetanensis*.3–5

Coccidian parasites are protozoans belonging to the phylum Apicomplexa6 and were known historically to be pathogenic mainly to some animal species, including insects, birds and non-human primates.7,8 Today, these opportunistic parasites cause chronic diarrhoea in humans, especially in those who are immunocompromized.4,5,8,9 The parasites are transmitted to humans through contaminated drinking and recreational water, food, and contact with infected animals and persons.8

*Cryptosporidium*, *Cyclospora* and *Isospora* have been shown to complicate HIV infection by causing chronic diarrhoea. This facilitates progression to AIDS.9–14 Diarrhoea occurs in up to 80% of persons with HIV infection.15 The prevalence of coccidian parasitic infection is high and widely distributed in sub-Saharan Africa, where the majority of HIV cases are located.16 Few studies on the association of coccidian parasites and HIV infection have been carried out in Nigeria.17 Thus, there is need for further studies to examine the association of coccidian parasites with HIV infection, an association that can lead to chronic diarrhoea.

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and consequent weight loss, exacerbat-
ing the illness of HIV/AIDS individuals in the coun-
try.17

Materials and methods
See Appendix.

Results
Coccidian parasite oocysts were recorded in
45 (51%) of the 88 non-diarrhoeic stool
samples analysed in total. Oocysts were
recorded in 68% (41/60) of the HIV-positive
children and in 14% (4/28) of the control
children. The parasites were significantly
associated with HIV-positive children
(P < 0.01). The HIV patients were thirteen
times more likely to have been infected
(odds ratio = 13.0, 95% CI = 3.9–42.6).

*Cyclospora cayetanensis* was the most
prevalent parasite found in the study
(36%: 32/88), followed by *Cryptosporidium parvum* (25%: 22/88) (Fig. 1). *Cyclospora cayetanensis* and *C. parvum* were found
to be significantly associated with HIV
infection (P < 0.01), with *C. cayetanensis*
occurring with the highest frequency
(48%: 29/60). *Isospora belli* was detected in
only one HIV-positive child (Table 1).

*Cyclospora cayetanensis* and *C. parvum*
detected in three and one HIV-
negative stools, respectively. Dual infec-
tions comprising *C. cayetanensis* and *C. parvum* occurred in only 17% (10/60) of
HIV-positive patients.

Girls were found to have more (53%:
25/47) coccidian oocysts in their stools
than the boys (49%: 22/41); this was not
statistically significant (P > 0.05). The age
of the children ranged from six to 108
months. There was an increase in parasite
load with patient age; with the highest
prevalence in children in the age group
97–120 months (100%: 7/7) (Table 2).

Children of less than 24 months had
fewer stool oocysts, with none found in
children of less than 12 months. The only
*Isospora belli* oocyst detected was in the
stool of an 84-month-old child. No signifi-
cant association between patient age and
the detection of parasitic agents was
found (P > 0.05).

Discussion
This study shows that coccidian para-
sites are common in children in Zaria in
view of their rate of detection (51%) in
non-diarrhoeic stools. This prevalence is
higher than previously reported in a
previous study16 and even from studies
using diarrhoeic stools.4,9,11,17,19 The para-
site oocysts were found in 68% of the
HIV-positive and in 14% of the control
children. This indicates a correlation be-
 tween coccidian pathogens and HIV
infection and implies that infections by
these parasites increase during HIV infec-
tion as has already been suggested by
others.5,8 Detection in both patients and
controls could be a reflection of poor envi-
ronmental and personal hygiene prac-
tices that facilitate the parasites’ mode of
transmission.

Our study also shows *C. cayetanensis* to
be the most prevalent pathogenic coccidian
parasite found (36%). This is in contrast to
a previous report from Nigeria1 in which
*Cryptosporidium* was identified as the
most prevalent protozoan pathogen. In
agreement with other reports,5,11,12
*C. cayetanensis* and *C. parvum* were found
to be significantly associated with HIV
infection, implying that HIV predisposes
patients to these parasitic infections. The
oocysts of coccidian parasites have been
found more commonly in the stools of
older children, with all children within
the age group 97–120 months being
infected. We attribute this to the more
active nature of older children, and the
consequences of increased risk of infec-
tive contact with contaminated food and
water.

Combined coccidian parasitic infection
comprising *C. cayetanensis* and *C. parvum*
is shown to be common in HIV patients
and absent in the control group. Another
Nigerian study1 has drawn similar infer-
ences and indicates the facilitated infec-
tion by parasites in immunocompro-
mised patients. Co-infection with *Cyclo-
spora* or *Cryptosporidium* and *Isospora belli*
was not detected in this study, in contrast
to previous reports.15,17

The result of this and other studies14,17
shows a steady increase in the reported

<table>
<thead>
<tr>
<th>Patients</th>
<th>Coccidian parasites (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV-positive</td>
<td><em>Cyclospora cayetanensis</em></td>
</tr>
<tr>
<td>HIV-positive</td>
<td><em>Cryptosporidium parvum</em></td>
</tr>
<tr>
<td>HIV-positive</td>
<td><em>Isospora belli</em></td>
</tr>
<tr>
<td>HIV-negative</td>
<td><em>C. cayetanensis</em></td>
</tr>
<tr>
<td>HIV-negative</td>
<td><em>C. parvum</em></td>
</tr>
<tr>
<td>HIV-negative</td>
<td><em>I. belli</em></td>
</tr>
<tr>
<td>Total (n = 88)</td>
<td><em>C. cayetanensis</em></td>
</tr>
<tr>
<td>Total (n = 88)</td>
<td><em>C. parvum</em></td>
</tr>
<tr>
<td>Total (n = 88)</td>
<td><em>I. belli</em></td>
</tr>
</tbody>
</table>

*P = 0.001

Table 1. Prevalence of coccidian parasites detected in non-diarrhoeic stools of HIV-positive (n = 60) and HIV-negative (n = 28) children less than 10 years old in Zaria, Nigeria.

<table>
<thead>
<tr>
<th>Age group (months)</th>
<th>Study population</th>
<th>HIV-positive</th>
<th>HIV-negative</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total no. of samples</td>
<td>No. positive (%)</td>
<td>Total no. of samples</td>
</tr>
<tr>
<td>0–24</td>
<td>11</td>
<td>4 (36)</td>
<td>7</td>
</tr>
<tr>
<td>25–48</td>
<td>34</td>
<td>15 (44)</td>
<td>23</td>
</tr>
<tr>
<td>49–72</td>
<td>24</td>
<td>13 (54)</td>
<td>17</td>
</tr>
<tr>
<td>73–96</td>
<td>12</td>
<td>6 (50)</td>
<td>8</td>
</tr>
<tr>
<td>97–120</td>
<td>7</td>
<td>7 (100)</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>88</td>
<td>45 (51)</td>
<td>60</td>
</tr>
</tbody>
</table>

Fig. 1. Percentage prevalence of coccidian parasites detected in stool samples of non-diarrhoeic children less than 10 years old in Zaria, Nigeria.
incidence of Cryptosporidium and Cyclospora infections in Nigeria. This may have arisen from greater awareness of these pathogens among clinicians and laboratory scientists. Another reason could be the HIV/AIDS pandemic, which is claimed to increase coccidial parasite infection.11

In summary, our results make an important contribution to the detection and identification of coccidial parasites in non-diarrhoeic stool samples of HIV-positive children in Zaria, Nigeria. Our study also shows that Cyclospora cayetanensis is the most prevalent intestinal coccidial parasite in these patients. We have identified a need to collect more information on asymptomatic infections with these emerging pathogens and their relation to HIV/AIDS, and will continue investigations.

Our recommendations are that health practitioners should receive more intensive education on emerging diarrhoeal pathogens and the importance of targeting these common infections while treating HIV-positive patients for opportunistic infections. Asymptomatic infection with these parasites requires only time before symptomatic conditions develop, leading to chronic diarrhoea that may facilitate HIV infection and progression to clinical AIDS. Routine examination of stool samples for coccidial parasites will also benefit HIV-infected individuals significantly through reduction of morbidity and improving quality of life. The need for intervention measures at community level, with the purpose of reducing risk factors of acquiring coccidial diarrhoeal diseases, is emphasised through screening for the presence of parasites in both patients and controls.

We thank WN. Ogalla, O. Ogurundile, L.W. Umar, and the nurses at the Pediatric HIV Clinic of ABUTH for involving their patients in this study.


Appendix

Material and methods

The Ethical and Research Committee of the Ahmadu Bello University Teaching Hospital, Zaria, Nigeria, approved the study. Sixty asymptomatic HIV-positive children receiving antiretroviral (ARV) drugs at the hospital’s Pediatric HIV Clinic were selected as study participants, while controls were 28 HIV-negative children attending the same hospital. The children were aged <10 years and comprised 41 males and 47 females. Stool samples were collected according to WHO standard procedure.10 Non-diarrhoeic stool samples were placed in labelled leak-proof, screw-capped plastic containers during the wet humid months of July and August 2006. A single stool sample was collected from each child and transported to the laboratory of the Department of Microbiology, Faculty of Science, Ahmadu Bello University, for analysis within 1.5 hours of collection. Patient details such as age, sex and reason for visiting the hospital were provided by caregivers, who signed the consent forms.

Stool samples were first concentrated by the formalin-ether sedimentation method.50 Smears were stained by the modified Kinyoun carbol-fuchsin staining procedure11 and examined at ×400 magnification using a Nikon light microscope fitted with an eye-piece micrometer that had been calibrated with a stage micrometer. Oocyst sizes were thus measured under this magnification. Identification of the parasites was based on the size and shape of the oocysts. Cryptosporidium parvum (4–6 µm in diameter) is round in shape and contains 4 sporozoites within a thick-walled oocyst; Cyclospora cayetanensis (8–10 µm in diameter) is round to oval in shape, whereas Isospora belli (25–30 µm in diameter) is oval in shape. Coccidial species stained pinkish-red against a green background. Oocysts stained red and were easily recognized against a green background of yeast and faecal debris.

Data were analysed with the SPSS 11.0 version statistical package. Differences with P-values >0.05 were considered insignificant at 95% confidence intervals (CI).