INTESTINAL PARASITIC INFECTIONS IN HIV-INFECTED ADULT PATIENTS WITH CHRONIC DIARRHOEA AT KENYATTA NATIONAL HOSPITAL

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JUNE 2006
DECLARATION

I certify that this dissertation is my own original work and has not been presented for a degree at any other university.

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To all people living with HIV/AIDS
ACKNOWLEDGMENTS

To my supervisors for their undivided commitment and guidance from protocol development to writing of this report.

To all the laboratory staff of the department of parasitology, U.O.N, and in particular Eric M. Mbithi (chief acting technologist), Felista W. Muthini (Laboratory technologist), Patricia J. Korir (Senior laboratory technologists) and Joel F. Kilonzo (Senior laboratory technologists) for the crucial role in processing the stool specimens.

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To all the doctor’s (in particular Dr. Agnes Obita) and Nurses of KNH medical wards and comprehensive care clinic.

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LIST OF ABBREVIATIONS

KNH  Kenyatta National Hospital
CD   Chronic diarrhoea
AIDS Acquired immunodeficiency syndrome
HIV  Human immunodeficiency virus
WHO  World Health Organization
CD4  CD4+ T lymphocytes
Tho  T suppressor lymphocytes
Th1  T regulator lymphocytes
Th2  T cytotoxic lymphocytes
IL-4 Interleukin 4
CCC  Comprehensive care clinic
ELISA Enzyme-linked-immunosorbent assay
UON  University of Nairobi
ABSTRACT

Introduction: Infestations caused by intestinal parasites are common and occur in up to 50% of patients with HIV infection and chronic diarrhea. They are a major health problem and contribute greatly to the morbidity and mortality of these patients. Data on the pattern of intestinal parasite infestations is lacking locally. Such information can optimize the care of patients with HIV infection and chronic diarrhea.

Study site: It was conducted at KNH (tertiary referral hospital in Kenya) between August and December 2005.

Study Design: This was a cross-sectional survey.

Sampling: Three hundred and ninety nine adult patients with HIV infection and chronic diarrhea were consecutively recruited after signing an informed consent.

Materials and Methods: A blood sample and a stool specimen were taken from each patient for determination the CD4 counts and intestinal parasite infestations respectively.

Results: The prevalence of intestinal parasite infestation was found to be 36.8%. C. Parvum was the commonest opportunistic parasite isolated with a prevalence of 11%. Other parasites detected included E. coli (7.5%), hookworm (4.3%), S. mansoni (2.0%) and T. trichura (0.8%). A mean CD4 count of 194.58 cells/cc was found with a median of 152 cells/cc. The mean CD4 count in the patients who had at least one parasite was 152 as compared to 219 for those who did not have any parasite in their stool. This difference was statistically significant (P= 0.001).

Conclusion: Intestinal parasitic infestations are common in HIV infected patients with chronic diarrhea. Low CD4 cell counts are associated with occurrence of intestinal opportunistic infections.
LITERATURE REVIEW

Infestations caused by intestinal parasites are widespread (1). Current assessments suggest that at least 25% of the world's population is chronically infected by enteric parasites, with most of the patients living in the developing countries (2).

Since the first described cases of acquired immunodeficiency syndrome (3), a high prevalence of gastrointestinal infections has been reported, including diarrhoea associated with parasitoses. Thus, infections in the gastrointestinal tract play a fundamental role in the morbidity and mortality of AIDS. The incidence of these infections is 50% in the developed countries, whereas it reaches up to 95% in the developing countries (4, 5). Diarrhoea caused by parasites is prominent in patients with AIDS. The occurrence of opportunistic infections caused by enteric parasites varies according to the geographic area and endemicity of each region (1).

Intestinal parasites are among the most common infections in developing countries (6). They cause considerable morbidity contributing greatly to societal ill health in developing countries. In the background of HIV epidemic, intestinal parasites are now known to cause major morbidity and mortality further undermining the economic development of developing countries. Indeed, Goodgame and et al have noted that since the onset of the AIDS epidemic, the number of intestinal parasitic pathogens recognized and the frequency with which they are encountered in clinical practice have increased(2).

According to WHO estimates, at least 2 billion people in the world suffer from helminthiasis and of these approximately 300 million suffer associated severe morbidity. These infections represent more than 40% of the disease burden due to all tropical diseases, excluding malaria, and that 40%- 60% of patients with AIDS in developing countries have chronic diarrhoea (7, 8, 9).
In Kenya, as in many other African countries, intestinal parasites constitute a major public health problem. Prevalences are high in both urban/suburban areas and in rural villages where they are accompanied by malnutrition (10).

Gastrointestinal involvement in HIV/AIDS is almost universal and significant disease occurs in 50-96% of patients (5). Diarrhoea can be a presenting manifestation or a life threatening complication of infection with HIV sometimes during the course of the disease (1). Some parasites do not cause diarrhoea; others cause systemic upsets, while others are largely asymptomatic (11).

Overall, infectious causes of diarrhoea have been found in 30-80% of patients with HIV infection depending on the extent of the study and characteristics of the study population. Chronic diarrhoea occurs in up to 70% of patients infected with HIV in Africa. The aetiology for such diarrhoea could be either parasitic, bacterial, fungal, enteric viruses or HIV itself (12, 13). In Ethiopia, one study found that 51% of patients with chronic diarrhea and HIV infection had one or more intestinal parasitic infection(s) (12). Such pathogens include opportunistic agents that consistently cause severe, chronic, or frequent gastrointestinal disease and non-opportunistic agents that usually cause acute treatable diarrhoeal illnesses (14, 15, 16, 17). In addition to microbes, other factors such as medication, immune dysregulation, autonomic dysfunction and nutritional supplementation play substantial role in diarrhoea of HIV / AIDS patients (13, 17, 18).

Protozoa (amoeba, flagellates, ciliates, coccidia and microsporidia) and helminths (trematodes, cestodes and nematodes) are common pathogens causing disease in HIV infected persons (11). Several species of protozoa have been associated with acute and chronic diarrhea in HIV disease. These include; Cryptosporidium parvum, Isospora belli, microsporidia species, Giardia intestinalis, Entamoeba histolytica, cyclospora species, Blastocystis hominis and Dientamoeba fragilis (12). Strongyloides stercoralis, a ubiquitous parasite in the tropics and subtropics, can cause diarrhoea and overwhelming infestation (hyperinfection syndrome) in patients with profound immunosuppression (18, 19). Previous studies have shown that Cryptosporidium parvum and Isospora belli...
are frequently associated with diarrhoea in HIV-infected patients in Africa (13,20). However, the role of emerging pathogens such as microsporidia and cyclospora in HIV-associated diarrhoea in Africa has not been extensively examined (13). Studies on HIV infected patients show that no etiological agent is found in 15-50% of patients with chronic diarrhoea (12).

The two most commonly (up to 60%) diagnosed pathogens were *C. Parvum* and *I. Belli*. *G. intestinalis* and *E. histolytica*, organism often associated with diarrhea in the tropics, were uncommon in AIDS patients (21, 22). Strongyloidiasis was found in 17% of patients in Ivory Coast (18, 23).

Cyclospora, a newly (7) recognized human protozoan parasite that causes prolonged watery diarrhoea in travelers and HIV/AIDS patients, has limited epidemiological relevance especially in association with HIV infection. A study done in Thailand shows a prevalence of 2.2% in HIV infected patients (24).

Parasites cause chronic activation T cells and can increase rate of HIV infection and/or progression to AIDS. Furthermore, they damage the intestinal wall causing malabsorption and eventual malnutrition, further weakening the body and the immune system. The idea that intestinal parasites act as cofactors in the development of AIDS in HIV infected patients has recently gained much support (25).

Intestinal helminths induce immunological alterations that favour the progression from HIV sero-conversion to AIDS. After HIV has spread to the systemic circulation its replication is limited by the fact that usually few activated lymphocytes and differentiated macrophages are present in the blood stream and that resting T cells and undifferentiated monocytes are not susceptible to HIV infection. However, in patients infected with intestinal helminths the number of activated T cells expressing human leucocyte antigen (HLA) - DR and HIV co-receptors is elevated. Secondly, HIV replicates preferentially in Th2 and Th0 type clones, and Th2 cells are usually abundant in individuals infected with
helminths. Thirdly, peripheral blood mononuclear cells of patients with helminthic infection are significantly more susceptible to infection with HIV than those of uninfected controls. Finally, elevated IL-4 levels, characteristic of the Th2 type of immune response in helminthic infections, down-regulate Th1 differentiation and function (26).

The assumption that immune dysregulation associated with chronic helminthic infections alters the natural history of HIV infection in an unfavourable manner is sustained by results from a field study in Ethiopia: HIV viral load was significantly higher in individuals with various helminthic infections than in individuals without helminthes and correlated positively with the parasite load. Furthermore the viral load decreased after elimination of the worms by antiparasitic treatment. (25)

The optimal laboratory diagnosis of intestinal protozoa generally requires the examination of at least 3 stool specimens collected over several days. This gives about an 80% chance of finding parasites if they are there (25). Recent studies, however, suggest that one or two stool samples will detect up to 90% of the protozoa present (27).

COMMON INTESTINAL PARASITES

STRONGYLOIDES STERCORALIS

*Strongyloides stercoralis* is a faecally transmitted parasite. Host immune factors seem to play a role in determining the outcome of infection with this helminth. Immunosuppressive therapy may produce a condition known as the hyperinfection syndrome (34). Although most infected individuals are symptom free, once immunity is disturbed the parasite can internally autoinfect and the host may suffer overwhelming strongyloidiasis. This is defined as an opportunistic infection. *Strongyloides* is recognized as being a homosexually transmitted parasite, but in such patients it is uncommon even in men with high rates of intestinal protozoal infections (34).
Strongyloides does not feature in earlier gastroenterological studies of African patients with AIDS (36). A high incidence of HTLV I antibody in carriers of *strongyloides stercoralis* has been reported by Nakada et al (37). The relative contributions of cellular, humoral and local immunity in protection against strongyloides are not clear (36).

**OTHER GUT HELMINTHS**

There is no evidence of increases AIDS-related infection with Ascaris, Enterobius, hookworms, cestodes or trematodes. The effect of the immune defect in AIDS on the susceptibility to infection by these parasites is not clear (34).

**CRYPTOSPORIDIOSIS**

**Epidemiology:** The first case of human disease caused by this veterinary pathogen was reported in 1979 (38). Disease outbreaks in day care centres, hospitals and urban groups indicated that most human infections result from person to person transmission rather than zoonotic spread. Human disease is more common in the young. Asymptomatic carriage is uncommon. Other enteric pathogens, especially *Giadlia lamblia*, are recovered in a significant minority of infected patients. The principal route of transmission is faecooral. Other modes may be direct as in care centres and between male homosexuals: or indirect via contaminated food, water and formites (41).

A study done in Uganda in 1985 showed that 48% of 23 patients with AIDS had evidence of cryptosporidiosis. These patients had the triad of diarrhoea, weight loss and oral thrush. In Haiti, cryptosporidiosis has been reported in 38% of AIDS cases (20).

**Pathogenesis**

Cryptosporidiosis has particular importance in AIDS because it is in this disease more than any other immunodeficiency state that cryptosporidiosis is likely to cause persistent
debilitating diarrhea. In the immunocompetent the diarrhea is a mild, self-limiting process (38). The exact pathophysiology of the diarrhea that accompanies cryptosporidiosis is unknown but the host’s immune status plays a big role in the pathogenesis (41).

**ISOSPORA BELLI INFECTION**

Like cryptosporidium species, Isospora belli is a coccidian protozoan that is associated with severe, persistent enteritis in patients with AIDS. Isospora belli was first described as an opportunistic enteric pathogen in 15% of 131 Haitian patients with AIDS (39).

In Uganda, the findings on histology and faecal smears showed that 13% of 23 patients had isosporiasis (20). A study on patients with chronic diarrhoea in Kinshasa showed that 19% had *I. Belli* oocysts (40).

**GIARDIASIS**

Giardiasis is more common in hospital patients, especially those with gastrointestinal disorders or bacterial infections. Among adult hospital patients giardiasis was shown to be 1.5 times more frequent than in healthy controls (34). Low levels of *G. lamblia* antibody in patients with AIDS who had acute giardiasis have been noted indicating the presence of an impaired humoral immune response to this enteric infection (23).

**AMOEBIASIS**

Entamoeba histolytica may be asymptomatic and prevalence rates have ranged between 20% to 45%. Other forms of amoebiasis are the acute intestinal amoebiasis, fulminating forms of intestinal amoebiasis, protracted intestinal amoebiasis and extra-intestinal forms of amoebiasis. The immune effects produced in AIDS do not seem to favour amoebic invasion (36).
STUDY JUSTIFICATION

- Chronic diarrhea is common in HIV infected patients.
- Infective causes of chronic diarrhea in HIV infected patients are difficult to diagnose although most of them are treatable. Demonstrating intestinal parasites, majority of which are curable, in this group of patients can help unravel the cause of the chronic diarrhea.
- Intestinal parasites are associated with immune dysregulation and rapid progression to AIDS. Therefore, early detection and treatment of these parasites can retard the rapid progression to AIDS.
- The prevalence and types of intestinal parasites vary from country to country and from region to region in the same country. The prevalence and types of intestinal parasites afflicting HIV infected patients with chronic diarrhea at KNH is not known.
- The commonest opportunistic intestinal parasites have not been determined in this region. Prophylaxis guidelines are, therefore, non-existent. Demonstrating the commonest intestinal parasites infesting patients with severe immunosuppression and chronic diarrhea may help in designing prophylactic regimens.
MAIN OBJECTIVE
To determine the pattern of intestinal parasitic infestations at various stages of HIV disease in patients with chronic diarrhoea at KNH.

SPECIFIC OBJECTIVES
1. To determine the prevalence and types of intestinal parasitic infestations in HIV seropositive patients with chronic diarrhoea at K.N.H
2. To determine the demographic characteristics of patients with HIV infection and chronic diarrhoea at KNH
3. To determine the clinical manifestations of HIV seropositive patients with chronic diarrhoea at KNH.
4. To determine the CD4+ T cell counts in HIV seropositive patients with chronic diarrhea at KNH
5. To determine the relationship between CD4 levels and parasitic infestations in HIV seropositive patients with chronic diarrhoea at KNH.

METHODOLOGY
STUDY DESIGN AND AREA
This was a cross-sectional survey conducted at Kenyatta National Hospital (KNH) between August 2 and December 2005. It was a hospital-based study; conducted at medical wards and comprehensive clinic (CCC) of KNH. KNH is a tertiary referral located in Nairobi; the political and business capital of Kenya. Though most patients attended to at KNH live in Nairobi, a good proportion is also referred from the district and provincial hospitals countrywide. The CCC of KNH is among the few HIV clinics, in public hospitals, that care specifically for HIV infected patients. General medical wards cater for all medical inpatients. Upto 60% of the patients in the medical wards are HIV positive. Most patients seen at KNH are of low socio-economic status. The population is representative of almost all ethnic groups in Kenyan.
SAMPLE SIZE ESTIMATION:
The sample size was calculated using formula below: (28)

\[
N = \frac{(Z1 - \alpha)^2 \cdot P \cdot (1-P)}{d^2}
\]

- \(N\) = Minimum sample size
- \(\alpha\) = Level of significance = 5%
- \(P\) = Prevalence of intestinal parasites in HIV infected patients with chronic diarrhea
- \(d\) = Degree of precision ± 5%

\((Z1 - \alpha)^2 = 1.96\) (from tables of standard normal distribution) corresponds to 95% confidence interval

The prevalence of intestinal parasites in HIV infected patients with chronic diarrhea in this region ranges from 48% to 50%. Forty eight percent has been used to calculate the sample size in this study.

\[
1.96^2 \times 0.48 \times 0.52 = 384
\]

The minimum sample size required was 384 patients.

STUDY POPULATION
Patients aged 13 or more years with chronic diarrhea and HIV infection who utilized health services at KNH between August and December 2005.

CASE DEFINITION
CD was defined as three or more unusually loose stool passed daily for a period of two or more weeks (17). HIV infection was defined as a positive HIV serological test (ENZYGNOST, BEHRING – GERMAN)
PATIENT SELECTION

INCLUSION CRITERIA
1. Adults (>13 years) with HIV and chronic diarrhoea
2. Informed consent

EXCLUSION CRITERIA
1. Use of antiparasitic drugs in the preceding two months.

SAMPLING PROCEDURE
Eligible patients were consecutively recruited into the study until the sample size was achieved. Patients from the medical wards were interviewed and recruited from Monday to Thursday every week. Recruitment was done between two pm and nine pm. Patients from the CCC were interviewed and recruited from Monday to Friday every week from nine am to twelve pm.

CLINICAL PROCEDURE
Patients with chronic diarrhea in the medical wards and CCC were reviewed. Those who fulfilled the inclusion criteria and signed consent were recruited consecutively. Recruited patients had a thorough history and physical examination. A stool specimen and 2ml of blood were taken from each patient for parasitological examination and CD4 counts respectively.

HISTORY
Demographic data such as name, age, sex, and area of residence, marital status, occupation and level of education was entered as per the study proforma (Appendix 1). History of the diarrhea was taken; its duration, stool frequency, consistency, colour and presence of blood. History of associated abdominal discomfort and weight loss was also taken. Previous and/or current illness (es) was/were recorded e.g. recurrent upper respiratory tract infections (URTI), Pneumonia, Tuberculosis, Herpes simplex, Malignancy, Toxoplasmosis and Cryptococcosis (Appendix I, II).
PHYSICAL EXAMINATION

A thorough physical examination was done. Oral thrush, oral hairy leukoplakia and minor mucocutaneous manifestations as per WHO clinical staging of HIV was noted. Other parameters such as herpes zoster and lymphadenopathy were noted (Appendix II).

LABORATORY METHODS

Stool samples (one sample per patient) were collected in plastic polypot containers provided to the patient. The samples were taken to the parasitology laboratory of the Department of Parasitology U.O.N within 1 hour of collection.

Stool examination (22, 23): fresh stool specimens were examined as saline wet mounts to detect motile trophozoites (Appendix III). Formo-ether concentration technique was performed and the sediments examined as iodine wet mounts to detect ova and cysts (Appendix IV).

Air-dried smears from the sediments were stained by modified Ziehl Nelson stain to detect coccidia oocysts (Appendix V). Trichrome staining was used to detect microsporidium (Appendix VI). Two independent observers performed the microscopy and compared results. Whenever there was a disagreement of results, the microscopy was repeated and the two observers discussed the results to arrive at uniform conclusion.

CD4 Count

Two milliliters (mls) of blood was taken from the antecubital vein for CD4 count using five mls syringes and gauge 21 needles. Blood was transported to the laboratory in EDTA bottles and reached there within one hour of collection. CD4 counts were determined by the automated flow cytometry analyzer, Fascount (benedict dick, USA). All subjects were categorized by their immune status according to the CDC 1993-revised classification system for HIV infection by CD4-T cell categories. (Appendix VII, VIII).
STUDY VARIABLES
1. Parasite
2. Type of parasite
3. CD4 count

DATA MANAGEMENT AND ANALYSIS
Data was coded (open ended questions) and entered into a microcomputer using SPSS /PC + Version 12 programme. Data validation was done before analysis. Analysis involved descriptive statistics such as means, medians and standard deviations for continuous variables and proportions and frequency distributions for categorical variables. Study population was described in terms of age, gender, residence, level of education, occupation, WHO clinical stage, CDC CD4 category and types of parasite found. Point prevalence was determined as percentages of the study population. To relate infection to immunosuppression, chi-square test and Kruskal-Wallis tests were used. Associations were measured and considered statistically significant at a p-value of 0.05 or less. Summarised data was presented in form of tables, pie charts and graphs.

ETHICAL CONSIDERATIONS
This study was carried out after ethical approval from the Kenyatta National Hospital Ethics and Review committee.
The intention, benefits and risks of the study were fully explained to eligible subjects (appendix ix)
Only those subjects who consented and signed the consent form were recruited.
RESULTS

Three hundred and ninety nine patients were enrolled in the study between August and December 2005. The male to female ratio was 1:1.75, with 145 (36.3%) males and 254 (63.7%) females (figure 1).

Figure 1: Gender distribution of the study population

N = 399
The mean age of the study subjects was 33.53 years (CI 95% 32.59 – 34.47) with a range of 13 – 70 years and median 32.00 years (figure 2).

Figure 2: Age distribution of the study population

Mean: 33.53 years (CI 95% 32.54-34.47), median: 32 years
Three hundred and thirty eight (84.8%) of the patients recruited resided in Nairobi (urban area) while 61 (15.2%) came from the rural area (figure 3).
Of the 399 patients recruited, 243 (60.9%) were married, 113 (28.4%) were single, 28 (7.1%) were widowed while 14 (3.6%) were divorced or separated (table 1).

Table 1: Marital status and occupation of the 399 recruited patients

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<th>Percentage</th>
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<td>113</td>
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<td>Married</td>
<td>243</td>
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<tr>
<td>Divorced/Separated</td>
<td>14</td>
<td>3.5</td>
</tr>
<tr>
<td>Occupation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unemployed</td>
<td>219</td>
<td>54.9</td>
</tr>
<tr>
<td>Civil servant</td>
<td>22</td>
<td>5.5</td>
</tr>
<tr>
<td>Small Businessperson</td>
<td>67</td>
<td>16.8</td>
</tr>
<tr>
<td>Casual labourer</td>
<td>50</td>
<td>12.5</td>
</tr>
<tr>
<td>Peasant Farmer</td>
<td>5</td>
<td>1.3</td>
</tr>
<tr>
<td>Private sector employee</td>
<td>36</td>
<td>9.0</td>
</tr>
</tbody>
</table>

Two hundred and fourteen (54.3%) patients were unemployed. Those employed; small businessmen/women were 67 (17%) while casual laborers were 50 (12.5%). The remainder were private sector employees, civil servants or peasant farmers. (table1).
One hundred and seventy six (44.6%) of the patients studied had up to primary level education, 153 (38.7%) up to secondary level education, 39 (9.9%) up to tertiary (higher) education while 27 (6.8%) had no formal education (figure 4).

Figure 4: Level of education of the study subjects
Three hundred and twenty three (81%) patients complained of weight loss, 301 (75.4%) general malaise and 274 (68.7%) poor appetite. Other symptoms were fever (29.1%), mouth sores (10.5%), dysphagia (7%) and odynophagia (6.3%) (table 2).

**Table 2: Clinical symptoms in the 399 patients with HIV infection and chronic diarrhea at KNII**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No. of Patients</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight loss</td>
<td>323</td>
<td>81.0</td>
</tr>
<tr>
<td>General malaise</td>
<td>301</td>
<td>75.4</td>
</tr>
<tr>
<td>Poor appetite</td>
<td>274</td>
<td>68.7</td>
</tr>
<tr>
<td>Body hotness</td>
<td>116</td>
<td>29.1</td>
</tr>
<tr>
<td>Mouth sores</td>
<td>42</td>
<td>10.5</td>
</tr>
<tr>
<td>Dysphagia</td>
<td>28</td>
<td>7.0</td>
</tr>
<tr>
<td>Odynophagia</td>
<td>25</td>
<td>6.3</td>
</tr>
<tr>
<td>Abdominal pains</td>
<td>221</td>
<td>55.3</td>
</tr>
<tr>
<td>Flatulence</td>
<td>56</td>
<td>14.0</td>
</tr>
<tr>
<td>Watery diarrhoea</td>
<td>165</td>
<td>41.4</td>
</tr>
<tr>
<td>Bloody stool</td>
<td>99</td>
<td>24.8</td>
</tr>
<tr>
<td>Mucoid stool</td>
<td>135</td>
<td>33.8</td>
</tr>
</tbody>
</table>
On physical examination, of the 399 patients recruited, 321 (80.5%) were wasted, 175 (43.9%) had oral thrush and 167 (41.9%) had generalized lymphadenopathy. Fever was found in 165 (41.4%) patients while pallor, dehydration and current/old herpes zoster were also frequent (table 3).

Table 3: Clinical signs in the 399 patients with HIV infection and chronic diarrhea at KNH

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No. of Patients</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wasting</td>
<td>321</td>
<td>80.5</td>
</tr>
<tr>
<td>Fever</td>
<td>165</td>
<td>41.4</td>
</tr>
<tr>
<td>Pallor</td>
<td>143</td>
<td>35.8</td>
</tr>
<tr>
<td>Dehydration</td>
<td>138</td>
<td>34.6</td>
</tr>
<tr>
<td>Oral thrush</td>
<td>175</td>
<td>43.9</td>
</tr>
<tr>
<td>Lymphadenopathy</td>
<td>167</td>
<td>41.9</td>
</tr>
<tr>
<td>Herpes Zoster</td>
<td>33</td>
<td>8.3</td>
</tr>
</tbody>
</table>
One hundred and ninety five (48.9%) patients were in WHO HIV clinical stage III. Twenty two (5.5%), 44 (11%) and 130 (34.6%) were in stages I, II and IV respectively (figure 5a). Similarly, majority of the patients had CD4 counts of less than 200 cells/cc (figure 5b).

Figure 5a: WHO HIV clinical stages of the study subjects

Majority (48.9%) of the patients were in WHO HIV clinical stage III
Figure 5b: Distribution of the study subjects in various CD4 categories

Mean: 194.58 cells/cc, IQR: 40 – 300 cells/cc, Median: 152 cells/cc
One hundred and forty seven (36.8%) patients had one or more parasite(s) in their stool. Two hundred and fifty two (63.2%) patients had no detectable parasite in their stools (figure 6).

Figure 6: Prevalence of stool parasites in the 399 patients with HIV infection and chronic diarrhea at KNH
Cryptosporidium parvum was the commonest parasite found with a prevalence of 11%. The non-pathogenic protozoan, Entamoeba coli was found in 7.5% of the patients while Entamoeba histolytica was found in 7% of the patients (table 4). Hookworms were found in 4.5%, Endolimax nana in 3.5%, Giardia lamblia in 3.5%, Ascaris lumbricoides in 3.3% and Blastocystis hominis in 2.0% of the patients (table 4).

Table 4: Prevalence of specific intestinal parasites among HIV infected Patients with chronic diarrhoea at KNH (N= 399)

<table>
<thead>
<tr>
<th>PARASITE</th>
<th>No.</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cryptosporidium Parvum</td>
<td>44</td>
<td>11.0</td>
</tr>
<tr>
<td>Entamoeba Coli (cysts)</td>
<td>30</td>
<td>7.5</td>
</tr>
<tr>
<td>Entamoeba histolytica (cysts)</td>
<td>28</td>
<td>7.0</td>
</tr>
<tr>
<td>Hookworm</td>
<td>18</td>
<td>4.5</td>
</tr>
<tr>
<td>Endolimax Nana</td>
<td>14</td>
<td>3.5</td>
</tr>
<tr>
<td>Giardia Lamblia</td>
<td>14</td>
<td>3.5</td>
</tr>
<tr>
<td>Ascaris Lumbricoides</td>
<td>13</td>
<td>3.3</td>
</tr>
<tr>
<td>Blastocystis Hominis</td>
<td>12</td>
<td>3.0</td>
</tr>
<tr>
<td>Schistosoma Mansoni</td>
<td>8</td>
<td>2.0</td>
</tr>
<tr>
<td>Chilomastix Mesnili</td>
<td>7</td>
<td>1.8</td>
</tr>
<tr>
<td>Strongyloides stercoralis</td>
<td>5</td>
<td>1.2</td>
</tr>
<tr>
<td>Trichuris Trichura</td>
<td>3</td>
<td>0.8</td>
</tr>
<tr>
<td>Heminolepis nana</td>
<td>2</td>
<td>0.5</td>
</tr>
<tr>
<td>Taenia species</td>
<td>0.2</td>
<td>0.5</td>
</tr>
</tbody>
</table>
Schistosoma mansoni was found in 2.0% of the patients while Chilomastix mesnili, Trichuris trichura, Heminolepis nana and Taenia species were found in 1.8%, 0.8%, 0.5% and 0.5% of the patients respectively. Strongyloides stercoralis was found in 1.2% of the patients.

Figure 7: Number of patients with stool parasite(s) in various WHO HIV clinical stages

The total number of patients with parasites in each WHO clinical stage increased progressively from stage I to stage IV (Figure 7).
The same trend was seen in CDC CD4 staging system with majority (26.1%) of the patients with parasites concentrated in the category with CD4 counts less than 200 cells/cc (Figure 8).

**Figure 8: Number of patients with stool parasite(s) in Various CD4 categories**

Of the 147 patients who had parasites, 95 (64.6%) had one parasite species, 44 (29.9%) had two while 8 (5.4%) had 3 parasites species (table 5).
Table 5: Distribution of parasite(s) among the 147 patients who had at least 1 or more parasite species in their stool

<table>
<thead>
<tr>
<th>No. of Parasite species</th>
<th>No. Of Patients</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>95</td>
<td>64.6</td>
</tr>
<tr>
<td>2</td>
<td>44</td>
<td>29.9</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>5.4</td>
</tr>
</tbody>
</table>

Figure 9: Number of patients with stool parasites at various CD4 counts

\[ P = 0.001 \text{ (Patients with 2 parasite species)} \]
There was no significant difference between males and females who had parasites (p = 0.378). The same was noted in relation to the area of residence (p = 0.891), and level of education (p = 0.225). Similarly, there was no significant difference between various occupational activities and the likelihood of having parasites (table 6).

Table 6: Distribution of parasite(s) in various demographic characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>P+ No (%)</th>
<th>P- No%</th>
<th>P value (chi-square tests)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male N=145</td>
<td>56 (38.6%)</td>
<td>89 (61.4%)</td>
<td>0.578</td>
</tr>
<tr>
<td>Female N=254</td>
<td>91 (35.8%)</td>
<td>163 (64.2%)</td>
<td></td>
</tr>
<tr>
<td>Residence</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urban N=338</td>
<td>125 (36.8%)</td>
<td>213 (63.0%)</td>
<td>0.891</td>
</tr>
<tr>
<td>Rural N=61</td>
<td>22 (36.8%)</td>
<td>39 (63.9%)</td>
<td></td>
</tr>
<tr>
<td>Level of education</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None N=31</td>
<td>13 (41.9%)</td>
<td>18 (58.1%)</td>
<td>0.225</td>
</tr>
<tr>
<td>Primary N=176</td>
<td>73 (41.5%)</td>
<td>103 (58.5%)</td>
<td></td>
</tr>
<tr>
<td>Secondary N=153</td>
<td>50 (32.7%)</td>
<td>103 (67.3%)</td>
<td></td>
</tr>
<tr>
<td>College N=39</td>
<td>11 (28.2%)</td>
<td>28 (71.8%)</td>
<td></td>
</tr>
<tr>
<td>Occupation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unemployed N=219</td>
<td>82 (37.4%)</td>
<td>137 (62.7%)</td>
<td>0.826</td>
</tr>
<tr>
<td>Civil Servant N=22</td>
<td>9 (40.9%)</td>
<td>13 (59.1%)</td>
<td></td>
</tr>
<tr>
<td>Businessperson</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(small) N=67</td>
<td>23 (34.3%)</td>
<td>44 (65.7%)</td>
<td></td>
</tr>
<tr>
<td>Private s/employment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N=36</td>
<td>11 (30.6%)</td>
<td>25 (69.4%)</td>
<td></td>
</tr>
<tr>
<td>Farmer N=5</td>
<td>3 (60.0%)</td>
<td>2 (40.0%)</td>
<td></td>
</tr>
<tr>
<td>Casual labourer N=50</td>
<td>19 (38.0%)</td>
<td>31 (62.0%)</td>
<td></td>
</tr>
</tbody>
</table>
The mean CD4 count was 194.58 cells/cc (CI 95% 176.14 – 213.03) with an interquartile range (IQR) of 40 – 300 cells/cc and median of 152 cells/cc (figure 5b).

The mean CD4 count was progressively smaller from patients without to those with 1, 2, or 3 parasite species. It was lowest in those patients with 3 parasite species. There was significant difference (p= 0.001) between those patients with 2 parasites as compared with the other groups (table 7, figure 9).

Table 7: Mean CD4 counts in various groups of patients with or without parasites

<table>
<thead>
<tr>
<th>No. of Parasites Species</th>
<th>No. of Patients</th>
<th>Mean CD4 count</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>252</td>
<td>215</td>
</tr>
<tr>
<td>1</td>
<td>95</td>
<td>187</td>
</tr>
<tr>
<td>2</td>
<td>44</td>
<td>148</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>144</td>
</tr>
</tbody>
</table>

P= 0.001 (Kruskal – Wallis test)

The mean CD4 count in the patients who had at least one parasite species was 152 cells/cc as compared to 219 cells/cc for those who did not have any parasite in their stool. This difference was statistically significant (P= 0.001) (table 8). The mean CD4 counts in patients with opportunistic parasites were below 200 cells/cc. Patients with *C. parvum* had a mean count of 75 cells/cc while those with *S. stercoralis* had a mean CD4 count of 139 cells/cc. There was a statistically significant difference between the median CD4 counts of patients with opportunistic parasites and those with other parasites (table 9).
Table 8: Mean CD4 counts in patients with or without parasites

<table>
<thead>
<tr>
<th>Parasite</th>
<th>No. Of Patients</th>
<th>Mean CD4 count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present</td>
<td>147</td>
<td>152</td>
</tr>
<tr>
<td>Absent</td>
<td>252</td>
<td>219</td>
</tr>
</tbody>
</table>

P = 0.001 (t-test).

Table 9: The WHO clinical stage, mean CD4 count, median CD4 count and CD4 count interquartile range (IQR) of patients with opportunistic parasitic infections

<table>
<thead>
<tr>
<th>Parasites</th>
<th>No. of patients (%)</th>
<th>WHO Clinical Stage</th>
<th>Mean CD4 count (cells/cc)</th>
<th>Median CD4 count (cells/cc)</th>
<th>IQR (cells/cc)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. parvum</td>
<td>1 (2%)</td>
<td>III</td>
<td>75</td>
<td>30</td>
<td>18 – 92</td>
</tr>
<tr>
<td>N = 44</td>
<td>43 (98%)</td>
<td>IV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I. stercoralis</td>
<td>2 (40%)</td>
<td>III</td>
<td>139</td>
<td>53</td>
<td>19 – 304</td>
</tr>
<tr>
<td>N = 5</td>
<td>3 (60%)</td>
<td>IV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td>194</td>
<td>152</td>
<td>40 – 300</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N = 103</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

P = 0.000    P = 0.001

There is a significant difference between the means (P = 0.000) and medians (P = 0.001) of opportunistic parasites and that of other parasites.
Discussion

In HIV infected patients, chronic diarrhea is a common problem. Its usually associated with advanced HIV disease (12). In this study, most patients were adults in the reproductive age group. Majority of them were females; corresponding to current epidemiological profile of HIV infected patients in Kenya (35). Many of them had low levels of education, were unemployed and resided in Nairobi province. This is typical of majority of patients seen at KNH; a public referral hospital that largely serves patients from low social economic strata. Majority (83.5%) of the patients had AIDS and presented with clinical features of severe immunosuppression.

Intestinal parasite infections are an important problem in HIV-infected patients with chronic diarrhoea. Many of these patients had severely depressed immunity from the long-standing effects of HIV disease. In our study, the prevalence of intestinal parasitic infestations in patients with HIV infection and chronic diarrhoea was 36.8%. In northeastern Tanzania, Tarimo found a prevalence of 35% (8). Awole found a prevalence of 44.8% in Ethiopia (32). In a Thai study, a prevalence of 50% was found. In all these studies the subjects were HIV infected with or without diarrhoea.

The prevalence of parasites differs from region to region and indeed our prevalence may reflect the prevalence of intestinal parasitic infestations in patients with HIV infection and chronic diarrhoea in our referral hospital. Thiongo, while studying immunocompetent school going children in a rural set up in Kenya found a prevalence of intestinal parasites to be 50% (6). It is thought that in such a setting, the prevalence of intestinal parasites in the adult population is lower than that of children (10).

Multiple stool samples improve the yield of detecting parasite in stool by upto 12% (33). However recent studies show that if meticulously examined, the yield from one stool can be up to 90% (27). We used one stool sample in this study. Thus, we may have underestimated the prevalence of intestinal parasites in the study population.
Among opportunistic parasites, *C. parvum* was the most prevalent (11%). Patients with *C. parvum* were severely immunosuppressed with a mean CD4 count of 75 cells/cc. This is consistent with the study done by Awole in Ethiopia. In Tanzania the prevalence was found to 3.3% (8, 32) and in Zimbabwe it was 9% (13). *C. Parvum* is an opportunistic infection that has been found consistently in adults with AIDS and chronic diarrhea. Its thought to be the most likely cause of such diarrhea (37). In two local studies the prevalence of *C. parvum* was found to be 4% and 14.5% in adults and children with diarrhoea respectively (unpublished data). The HIV status of these patients was unknown and many of them may have been immunocompetent. Other opportunistic protozoa such as *I. Belli*, *C. Catayenesis* and microsporidium were not found in this study.

*S. Stercolaris*, that can cause hyperinfection syndrome in HIV/AIDS patients, had a prevalence of 1.2% (32). In a Thai study, the prevalence was 3.3% (31).

A strong association has been shown to exist between HIV infection and parasitologically proven *S. stercoralis* (36). However, cases of hyperinfection have been noted to be very rare (37). Disseminated malignant strongyloides is reported to be more frequent in patients infected by human T- cell lymphotropic virus type I (HTLV-1) which, for unknown reasons, seems to facilitate disseminated strongyloides more than HIV (38).

The other protozoa (*G. lamblia* and *E. histolytica*) have not been found to be opportunistic in HIV infected patients. In one local study of immunocompetent patients with diarrheoa, the prevalence of *G. lamblia* was 3% and that of *E. histolytica* was 2%. In this study we found a prevalence of 3.5% and 7% for *G. lamblia* and *E. histolytica* respectively. The mean CD4 count of patients with these parasites was below 200 cells/cc. These organisms are likely to occur independently of the immune status. Helminthes such as hookworm and Ascarisiasis were common regardless of the immune status. This reaffirms the importance of common intestinal parasite infections in our society (6).

Majority of patients with at least 1 or more parasite(s) had low CD4 counts. The lower the CD4 count the more likely that the patient would have a parasite found in stool. This
finding was statistically significant. The mean CD4 count of patients with 1 or more parasite species was significantly lower than that of patients without parasites. Opportunistic parasites were much more common in low CD4 counts as compared to the other parasite species. The total number of patients with parasites in each WHO clinical stage increased progressively from stage I to stage IV. The same trend was seen in CDC CD4 staging system with majority (26.1%) of the patients with parasites concentrated in the category with CD4 count less than 200 cells/cc. This finding was consistent with other studies done in Brazil and Thailand (1). However, this applied to only opportunistic parasites. Our study shows this to be the case for both opportunistic and non-opportunistic parasites. This study demonstrates that patients with advanced HIV disease and chronic diarrhea are likely to have parasites in their stool. Opportunistic intestinal parasites were detected mainly in patients with severe immunosuppression. At CD4 counts less than 100 cells/cc, opportunistic intestinal parasites manifest explaining the more parasite species noted with severe immune deficiency (23). Whether these parasites are the major aetiological factors of the chronic diarrhea cannot be expressly inferred from this study. However, intestinal parasites and in particular protozoa, are known to be a major cause of diarrheal illnesses in the tropics (17).

Severely immunosuppressed patients have higher prevalence of both opportunistic and pathogenic intestinal parasites. Gastrointestinal immunity is important in preventing opportunistic parasites from becoming invasive. Breakdown of these defenses as occurs in advanced HIV diseases is associated with overgrowth and invasiveness of otherwise non-pathogenic parasites (23).

Intestinal parasites have been known to chronically activate T cells leading to immune dysregulation. In the presence of HIV infection, this scenario results in rapid progression to AIDS. Thus, these parasites appear to act as cofactors in the development of AIDS in HIV infected patients (25). This implies that those infected with HIV and harbor intestinal parasites progress faster to advanced HIV disease. This may explain the high
prevalence of pathogenic parasites in patients with advanced HIV disease and chronic diarrhea. This supposition needs to be investigated further.

Conclusion
From this study, the following conclusions can be drawn;

- Intestinal parasitic infections were common in HIV infected patients with chronic diarrhea and multiple infections occurred frequently.
- CD4 cell counts of less than 150 cells/cc were related to multiple intestinal parasitic infections and occurrence of opportunistic intestinal parasitic infections.

Limitations

1. Only one stool sample was taken. This may have lead to underestimation of the prevalence of parasites.
2. This is a hospital based study and its findings may not be generalized to the community.

Recommendation
From this study, we recommend the following;

- Stool examination for opportunistic intestinal parasitic infections should routinely be done in HIV infected patients with chronic diarrhea.
- A study to assess the parasite load in a similar population needs to be carried out.
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APPENDIX I

STUDY PROFORMA

DATE OF INTERVIEW
PATIENT NUMBER
STUDY NUMBER

1. NAME..........................
2. SEX 1) Male 2) Female
3. AGE IN YEARS
4. RESIDENCE 1) Rural 2) Urban
5. MARITAL STATUS 1) Single 2) Married
3) Widowed 4) Divorced/separated
6. LEVEL OF EDUCATION 1) primary 2) secondary 3) college
7. OCCUPATION..........................
8. HISTORY 1) Yes 2) No
   Weight loss
   Fatigue/lassitude
   Anorexia
   Body hotness
   Mouth sores
   Dysphagia
   Odynophagia
   Abdominal pains
   Flatulence

   Stool characteristics
   Watery
   Gross blood in stool
   Mucoid
   Semiformed
9. PHYSICAL EXAMINATION
Wasting
Fever
Pallor
Dehydration
Oral thrush
Lymphadenopathy
Herpes zoster
Weight............
Height.............
BMI.................
Systemic Exam........................................

10. A. Clinical stage of HIV
B. ART use
   1) Yes   2) No   Initial CD4   Current CD4
D. CDC staging
E. Parasite(s) found
APPENDIX II

WHO staging system for HIV infection and disease in adults and adolescents

Clinical stage I

- Asymptomatic
- Primary HIV infection
- Persistent generalized lymphadenopathy

_Please note:_ Performance scale 1: asymptomatic, normal activity

Clinical stage II

- Weight loss, <10% of body weight
- Minor mucocutaneous manifestations (seborrheic dermatitis, papular pruritic eruption, fungal nail infections, recurrent oral ulcerations, angular cheilitis)
Herpes zoster within the last five years (uncomplicated)

Recurrent upper respiratory tract infections (i.e. bacterial sinusitis, otitis media) in past 12 months

Thrombocytopenia not responsive to steroids

*And/or performance scale 2: symptomatic, normal activity*

---

**Clinical stage III**

Weight loss, >10% of body weight and/or BMI <18.5, unexplained.

Unexplained chronic diarrhoea, >1 month

Unexplained prolonged fever (intermittent or constant), >1 month

Oral candidiasis (thrush)

Oral hairy leukoplakia

*Pulmonary tuberculosis* within the past year

Severe bacterial infections (i.e. pneumonia, pyomyositis, bacterial meningitis, bacteraemia)

Bacillary angiomatosis

Herpes zoster: complicated (recurrent, disseminated, multidermatomal)

*And/or performance scale 3: bedridden <50% of the day during the last month*

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**Clinical stage IV**

HIV wasting syndrome, as defined by the Centers for Disease Control and Prevention

*Pneumocystis carinii* pneumonia

Toxoplasmosis of the brain

Cryptosporidiosis, Isosporiasis, Microsporidiosis with diarrhoea >1 month

Cryptococcosis, extrapulmonary
Cytomegalovirus disease of an organ other than liver, spleen or lymph nodes
Herpes simplex virus infection, mucocutaneous >1 month, or visceral any duration
Progressive multifocal leukoencephalopathy
Any disseminated endemic mycosis (i.e. histoplasmosis, coccidioidomycosis, Penicilliosis)
Candidiasis of the oesophagus, trachea, bronchi or lungs
Non-tuberculous mycobacteriosis, disseminated
Non-typhoid *Salmonella* septicaemia
Extrapulmonary tuberculosis
Lymphoma
Kaposi’s sarcoma
HIV encephalopathy, as defined by the Centers for Disease Control and Prevention.b
Invasive cervical carcinoma
American trypanosomiasis-reactivation
Major aphthous ulceration: ulcers of GI tract >5mm and for >1 month
Nephropathy
Cardiomyopathy, unexplained
Visceral leishmaniasis
Strongyloides hyperinfection syndrome

*And/or performance scale 4: bedridden >50% of the day during the last month*

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a. HIV wasting syndrome: weight loss of >10% of body weight, plus either unexplained chronic diarrhoea
   (>1 month) or chronic weakness and unexplained prolonged fever (>1 month).

b. HIV encephalopathy: clinical findings of disabling cognitive and/or motor dysfunction interfering with activities of daily living, progressing over weeks to months, in the absence of a concurrent illness or condition other than HIV infection which could explain the findings.
DIRECT EXAMINATION OF FEACES FOR INTESTINAL PARASITES

1. Report appearance of stool specimen and identify any parasitic worms or tapeworm segments.
2. Examine stool specimen microscopically for motile parasites, helminth eggs and cysts and oocysts of intestinal protozoa and/or coccidia.

Tools
1. Stool
2. Coverslips
3. Slides

Reporting the appearance of faecal specimens:
- Colour of stool specimen
- Consistency (formed, semifomed, unformed, watery)
- Presence of blood
- Presence of worms or tape worm segments

Microscopical examination of faecal specimens:

a. Dysenteric and unformed specimen
   - Using a wire loop or piece of stick, place a small amount of specimen on one end of a slide. Cover with a cover glass and press gently using a tissue.
- Place a drop of eosin reagent on the other end of the slide. Mix a small amount of the specimen with the eosin and cover with a cover glass.
- Examine immediately the preparations microscopically, first use 10 x objective with the condenser iris closed sufficiently to give good contrast; then use the 40x objective to identify motile trophozoites.
- Records down all parasites noted.

b. Semiformed and formed stool / faeces
- Place a drop of fresh physiological saline on one end of a slide and a drop of iodine on the other end
- Using a wire loop or piece of stick, mix a small amount of specimen, about 2mg, (matchstick head) with the saline and a similar amount with the iodine. Make smooth thin preparations. Cover each preparation with cover glass.
- Examine systematically the entire saline preparation for larvae, ciliates, helminth eggs, cysts and oocysts. Use the 10x objective with condenser iris closed sufficiently to give good contrast. Use the 40x objective to assist in detection and identification of eggs, cysts, and oocysts. Examine several microscope fields with this objective (40x) before reporting no parasites found.
- Use the iodine preparation to assist in the identification of cysts.
- Reports the number and type of parasites seen in the entire saline preparation.
APPENDIX IV

FORMOL – ETHER CONCENTRATION TECHNIQUE FOR FAECAL PARASITES

REAGENTS
10% formalin (1 volume of 40% formaldehyde diluted with 9 volumes distilled water);
Diethyl ether

APPARATUS: Centrifuge, centrifuge tubes (conical), with 15ml capacity, swab sticks long enough to reach the bottom of the centrifuge tube, copper wire gauge (mesh 40 to the inch, 15 pen centimeter), evaporating basin.

PROCEDURE
1) Take specimen of faeces about the size of a pea on the end of a swab stick and emulsify it in 7ml of formalin in centrifuge tube.
2) Sieve by pouring whole contents of the tube through wire gauze in to an evaporating basin. Wash out the tube.
3) Return the fluid in the evaporating basin to the centrifuge tube. Add 3ml of ether. Shake vigorously for a full 30 seconds.
4) Centrifuge. Set the regular or the centrifuge to a mark corresponding to 3000 rpm: switch off after exactly 60 seconds. If the centrifuge is only capable of a speed of 2000 rpm spin for one and a half minutes.
5) A layer of debris will have accumulated at the interphase between the two liquids: loosen it by passing a swab stick gently round the inner circumference of the tube. Pour the contents of the tube down the sink. Allowing only the last one or two drops to return to the bottom, where there will be a small deposit.
6) Shake up the deposit. Pour the whole or most of it on to a slide and examine under a coverslip. If the inside of the tube is dirty, wipe with cotton wool, or alternatively transfer the deposit to the slide with a Pasteur pipette.
APPENDIX V

MODIFIED ZIEL NELSON STAIN FOR FAECAL SMEARS

PREPARATION OF SMEAR

It is important that the smears are not too thick. With watery faeces, the sample is touched with a swab and a thin smear is spread on a slide. With firm or tacky faeces, mixing with saline to produce a more liquid emulsion is recommended. Smears should be air-dried.

REAGENT

Basic fuchsin
Absolute alcohol with 3% HCL
5% phenol in distilled water
Malachite green 0.25%

Dissolve the basic fuchsin in the alcohol, then mix with the phenol solution and filter.

METHOD

1. Stain with cold carbol-fuchsin for 5-10 minutes
2. Differentiate in 3% HCL in 95% ethanol until colour ceases to flood out.
3. Rinse in tap water.
4. Counter-stain with 0.25% malachite green for 0.5 minutes.
5. Rinse in tap water.
6. Blot dry and examine using oil immersion objective for identification and detection of oocysts.
MODIFIED TRICHROME STAIN

SPECIMENS
The specimen can be fresh stool or stool that has been preserved in
5 or 10% Formalin.

MATERIALS
Reagents:

a. Modified trichrome (Ryan-Blue formulation)
   i. Chromotrope 2R...........................6.0 g
   ii. Aniline blue.............................0.5 g
   iii. Phosphotungstic acid..............0.25 g
   iv. Acetic acid (glacial)..............3.0 ml
   v. Distilled water.......................100.0 ml

Prepare the stain by adding 3.0 ml of acetic acid to the dry
ingredients. Allow the mixture to stand (ripen) for 30 min at room
temperature. Add 100 ml of distilled water and adjust the pH to 2.5
with 1.0 M HCl. Properly prepared stain will be dark purple in color.
The staining solution should be protected from light. Store in a
glass or plastic bottle at room temperature. The shelf life is at least
24 months.

b. Acid-alcohol
   i. 90% ethyl alcohol..................995.5 ml
   ii. Acetic acid (glacial).............4.5 ml

Prepare by combining the two solutions

2 Modified Trichrome (Ryan-Blue) Protocol
PROCEDURE

A. Using a 10-μl aliquot of concentrated (formalin ethyl-acetate sedimentation concentration; 500 X g for 10 min centrifugation), preserved liquid stool (5 or 10% formalin or SAF), prepare the smear by spreading the material over an area of 45 by 25 mm.

B. Allow the smear to air dry.

C. Place the smear in absolute methanol for 5 or 10 min.

D. Allow the smear to air dry.

E. Place in trichrome stain for 90 min.

F. Rinse in acid-alcohol for no more than 10 s (1 to 3 s).

G. Briefly rinse; dip slides several times in 95% alcohol. Use this step as a rinse (no more than 10 s).

H. Place in 95% alcohol for 5 min.

I. Place in 95% alcohol for 5 min.

J. Place in 100% alcohol for 10 min.

K. Place in xylene substitute for 10 min.

L. Mount with coverslip (no. 1 thickness), using mounting medium (this step is optional).

M. Examine smears under oil immersion (1,000 x) and read at least 100 fields; the examination time will probably be at least 10 min per slide.

APPENDIX VII

Fascount Machine for CD4 Count (Benedict Dick USA)

Procedure

1. Take blood in a EDTA K₃ sterile bottle
2. Mix the blood not to avoid clotting
3. Take 50μl of the blood in to already made reagents (monoclonal
Antibodies) for CD4 and CD8 counts.

5. Incubate for 1-2 hours to allow staining to take place
6. Fix the already stained samples with fixative.
7. Read with the machine within 30min.

APPENDIX VIII
CDC Classification system for HIV infected adolescents and adults

<table>
<thead>
<tr>
<th>CD4+ T Cell categories</th>
<th>A asymptomatic Acute (primary) HIV or PGL</th>
<th>B Symptomatic, Not A or C Conditions</th>
<th>C AIDS – Indicator Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt; 500/μL</td>
<td>A1</td>
<td>B1</td>
<td></td>
</tr>
<tr>
<td>200 – 499/μL</td>
<td>A2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Clinical categories of HIV infection**

<table>
<thead>
<tr>
<th>Category A</th>
<th>Consists of one or more of the conditions listed below in an adolescent or adult (&gt;13 years) with documented HIV infection. Condition listed in categories B and C must not have occurred.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Asymptomatic HIV infection</td>
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<tr>
<td></td>
<td>Persistent generalized lymphadenopathy</td>
</tr>
<tr>
<td></td>
<td>Acute (primary) HIV infection with accompanying illnesses or history of acute HIV infection</td>
</tr>
</tbody>
</table>
| Category B | Consists of symptomatic conditions in an HIV-infected adolescent or adult that are not included among conditions listed in clinical category C and that meet at least one of the following criteria: (1) The conditions are attributed to HIV infection or are indicative of a defect in cell-mediated immunity; or (2) the conditions are considered by physicians to have a clinical course that require management that is complicated by HIV infection. Examples include, but are not limited to the following:

- Bacillary angiomatosis
- Candidiasis, oropharyngeal (thrush)
- Candidiasis, vulvovaginal; persistent, frequent, or poorly responsive to therapy
- Cervical dysplasia (moderate or severe / cervical carcinoma in situ
- Constitutional symptoms, such as fever (38.5°C) or diarrhea lasting >1 month.
- Hairy leukoplakia, oral
- Herpes zoster (shingles), involving at least two distinct episodes or more than one dermatome.
- Idiopathic thrombocytopenic purpura
- Listeriosis
- Pelvic inflammatory disease, particularly if complicated by tuboovarian absence
- Peripheral neuropathy

| Category C | Conditions listed in the AIDS surveillance case definitions.

- Candidiasis, of bronchi, trachea, or lungs
- Candidiasis, esophageal
- Cervical cancer, invasive
- Coccidioidomycosis, disseminated or extrapulmonary
- Cryptococcosis, extrapulmonary
- Cryptosporidiosis, chronic intestinal (>1 month’s duration)
- Cytomegalovirus disease (other than liver, spleen, or nodes)
- Cytomegalovirus retinitis (with loss of vision)
- Encephalopathy, HIV-related
<table>
<thead>
<tr>
<th>Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Herpes simplex: chronic ulcers(s) (&gt; 1 month’s duration); or bronchitis,</td>
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<tr>
<td>pneumonia, or esophagitis</td>
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<tr>
<td>Histoplasmosis, disseminated or extrapulmonary</td>
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<tr>
<td>Isosporiasis, chronic intestinal (&gt; 1 month’s duration)</td>
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<tr>
<td>Kaposi’s sarcoma</td>
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<tr>
<td>Lymphoma, Burkitt’s (or equivalent term)</td>
</tr>
<tr>
<td>Lymphoma, primary, or brain</td>
</tr>
<tr>
<td><em>Mycobacterium avium</em> complex or <em>M. kansasii</em>, disseminated or extrapulmonary</td>
</tr>
<tr>
<td><em>Mycobacterium tuberculosis</em>, any site (pulmonary or extrapulmonary)</td>
</tr>
<tr>
<td>Mycobacterium, other species or unidentified species, disseminated or</td>
</tr>
<tr>
<td>extrapulmonary</td>
</tr>
<tr>
<td><em>Pneumocystis carinii</em> pneumonia</td>
</tr>
<tr>
<td>Pneumonia, recurrent</td>
</tr>
<tr>
<td>Progressive multifocal leukoencephalopathy</td>
</tr>
<tr>
<td>Salmonella septicemia, recurrent</td>
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<tr>
<td>Toxoplasmosis of brain</td>
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<tr>
<td>Wasting syndrome due to HIV</td>
</tr>
</tbody>
</table>
CONSENT EXPLANATION

Introduction  My name is Dr. Moturi M. George. I am a postgraduate student pursuing a master's degree in internal medicine, University of Nairobi. I am in the second year of study. The curriculum requires that I write a thesis, which entails research collecting and analyzing data on various aspects of diseases. My research is on the patterns of intestinal parasites infesting patients with HIV infection and chronic diarrhoea at KNII.
To do this I will require a thorough history and physical examination from each participant. A stool sample and blood sample will be required. A container will be provided for the stool specimen. I will draw 2mls (about a table spoonful) of blood for CD4 count. Decency will be maintained at all stages of history taking and physical examination. The extent of discomfort (minor) during venepuncture will be explained and fears allayed.

Benefits  This study is intended to establish the pattern of intestinal parasites in patients with chronic diarrhoea and HIV infection. This will help in planning the care of patients with HIV infection in the community that utilize KNH. Participants found to be having parasites will be treated and referred to their primary physician for further follow-up.

Risks  History taking, physical examination and stool collection have no risks. Taking venous blood for CD 4 count has some level of minor discomfort. Use of sterilized needles, and syringes with proper aseptic techniques during blood sampling will ensure no risk to the participants.

Participation  Participation in this study is purely voluntary. All information collected will be confidential. A written consent will be required. Participants have a right to withdrawal from the study at any stage without jeopardy to current treatment the patient is on. Patient has a right to know results of all tests done.

IN CASE OF ANY QUESTIONS, PLEASE CONTACT DR MOTURI ON 0722737470 OR 0733953964
CONSENT

Consent by patients / next of kin for participation in the study.

I.......................................................................... Of .....................................................................

Hereby consent to participate in this study / research, the nature of which has been fully explained to me by: DR. / MR..............................................................................................................

I am required to give one stool specimen for parasitological analysis and 5ml venous blood for CD4 counts. I understand that Dr. Moturi shall use the results of these tests for research work only.

Date ........................................................ Signed ...................................................

I confirm that I have explained to the patient the nature of the study and tests to be done.

Date ........................................................ Signed ...................................................