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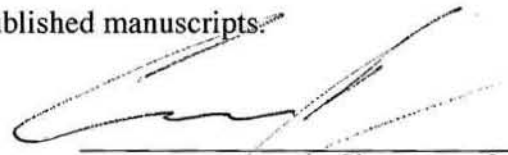
HIV-1 and Helminth Co-Infection in Adults in Kenya

Cameron Page



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Date

CORRELATION OF HELMINTH BURDEN AND SPECIES WITH CD4 COUNT IN HIV-1 INFECTED ADULTS IN NAIROBI, KENYA AND SURROUNDING AREA

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ABSTRACT PAGE

BACKGROUND: As part of rapidly expanding HIV-1 Care Programs in Sub Saharan Africa, many HIV-1 infected individuals are being identified who do not yet qualify for ART. These individuals may benefit from treatment of co-infections, such as soil-transmitted helminths. Treatment of helminth infection in HIV-1 infected individuals may improve health and nutrition and may slow progression to AIDS. In this setting, access to both medical care and diagnostic testing is limited. Determining correlates of helminth infection in HIV-1 infected individuals may allow clinicians to better target empiric anti-helminth therapy in HIV-1 infected individuals. METHODS: To describe the prevalence and correlates of helminth infection in HIV-1 infected individuals in Nairobi, Kenya, we screened stool samples of HIV-1 infected adults in Kenya. Stool samples were collected from consenting participants. Helminth infection was diagnosed by wet preparation, Kato-Katz technique, and Formol Ether Concentration. Sociodemographic data and medical history were collected using standardized questionnaires. RESULTS: 912 HIV-1 infected adults were screened. The median age was 34 years (IQR 28 - 40) and 74% of those screened were female. Most (75.6%) did not have access to adequate sanitation, as defined by a flush toilet and piped running water. Most participants had received primary education only (52.9%), followed by secondary schooling (32.4%), college (7.7%) and no education (6.2%). Dirt floor was reported by 35.8% of participants. The mean number of rooms in the home was 1.7 (SD+/- 1.1). The mean number of children per household was 1.6 (SD+/- 1.3). 10.7% reported current diarrhea,

and 6.3% current blood in stool. Mean CD4 count was 439 and median CD4 count 389 (range 4-1726). Among those screened, 16.1% had helminths detected in stool specimens. Hookworm was the most commonly found helminth, representing 56.5% of positive samples. All subjects had light helminth burden by WHO criteria except one patient who had moderate infection with ascaris. Helminth co-infection was associated with educational level. For every increase in level of education attained (none, primary, secondary, or college) there was a decrease in the odds of helminth infection (OR=0.41, CI 0.26 - 0.65, p=0.01). Helminth infection was also significantly associated with lack of flush toilet (OR 1.90, CI 1.07 - 3.39). Age, gender, having a dirt floor, number of children, current diarrhea, or blood in stool did not significantly correlate with the risk of helminth infection. In addition, there was no significant association between absolute CD4 count and presence of helminth infection. DISCUSSION: In this cohort of HIV-1 seropositive individuals, there was a lower prevalence of helminth co-infection (10-15%) than found in some other areas of Africa. Lower educational levels and lack of access to flush toilets were associated with helminth co-infection. These cofactors may be useful for identifying helminth co-infected HIV-1 infected individuals in urban centers to target for either stool testing or empiric anti-helminthics.

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INTRODUCTION

Of the 738 million people living in Africa, approximately 26 million (3.5%) are infected with HIV. As many as 50-90% of this population may be co-infected with soil-transmitted helminths such as enterobius vermicularis, ascaris lumbricoides, necator, ancylostoma, trichuris trichiura, hymenolepsis nana, and strongyloides stercoralis.[1 2]

Helminths are among the most common parasitic infections of the developing world. They require an environment of warmth and moisture, and are thus more commonly found in tropical and sub-tropical climates. In addition, with the exception of ascaris and trichuris trichiura, their life cycle requires direct contact between host feces and host epidermis, favoring communities of lower socioeconomic status with poor access to sanitation and footwear. Soil-transmitted helminths are more commonly found in rural areas than urban areas [3 4], although certain slums are ideal for transmission and maintenance of the life cycle. Soil-transmitted helminths, in particular hookworm infection, are more commonly found among farmers and others engaged in agricultural activities. Helminths with a snail intermediate host, such as schistosomes, are commonly found in areas near bodies of fresh water.

The life cycle of many soil-transmitted helminths begins with transmission through the skin into the microcirculation. Larval helminths, with the exception of trichuris trichiura, travel to the alveolar spaces, where the eggs are

expectorated into the trachea and swallowed. Mature worms develop upon reaching the small intestine, where mating between male and female worms occurs. Female worms then release eggs; detection of the eggs in stool serves as the primary method of diagnosing helminth infection. The eggs excreted in human feces develop into the larvae in the soil, where they continue the life cycle by making contact with the feet of another human host. Helminth infection causes persistent host immune stimulation as a result of the hundreds of thousands of eggs and secretory products excreted by the intestinal parasites. There is significant dysregulation of cytokine and chemokine profiles, especially among patients who are chronically infected. These patients show decreased levels of type 1 cytokines IL-12 and IFN-gamma, as well as decreased type 2 cytokines IL-5 and IL-13, compared with uninfected controls. The increased levels of TNF-alpha and IL-10 that are also seen in these patients is thought to be responsible for the immune dysregulation. [5 6]

This immune hyperactivation, which is characteristic of helminth infection, may play a crucial role in HIV-1 disease progression. Several studies have found an association between helminth co-infection and more rapid progression of HIV disease.

Among HIV-1 infected individuals, the level of immune activation correlates more closely with decline in CD4 count than HIV-1 RNA viral load.[7] There is evidence that an abnormal T-regulatory cell response in the setting of chronic immune activation, rather than a paucity of lymphocytes due to HIV, leads to the hyporesponsiveness and anergy seen in these patients.[8]

Among immunocompetent patients, chronic helminth infection induces several alterations similar to the changes seen in HIV. First, chronic helminth infection has been shown to cause a decrease in peripheral CD4+ lymphocytes.[9] Second, helminth infection has been found to lower the CD4/CD8 ratio in HIV-negative patients.[9] Third, helminth infection causes a significant decrease in the number of CD28+ CD8+ T cells, similar to the decreased expression of CD28 seen in HIV-1 infection.[10] [11] [12] Taken together, the data suggests that chronic helminth infection and HIV-1 may have a synergistic effect in altering host immune response.

Helminth infection in the developing world is common in children, many of whom live with the parasite into adulthood.[13] As such, there may be a large number of individuals who have chronic helminth infection at the time they are infected with HIV-1. There is evidence that extant helminth infection increases susceptibility to progression of HIV-1. First, helminth infection causes a significant increase in CD4+ memory cells and a decrease in naïve CD4+ cells, suggesting that helminth-infected individuals might be less able to respond effectively to HIV-1 infection.[9] Second, helminth infection shifts the immune system toward a Th2 response, characterized by eosinophilia and increased serum IgE. Although not conclusive, data suggest that activation of the Th2 immune subset facilitates more rapid HIV replication and progression. Some studies have also failed to find a correlation between helminth infection and HIV-1 viral load.[14]

Despite the large amount of immunological evidence, there is little epidemiological data on correlates of helminth infection in the developing world. The early studies of helminth-HIV-1 interaction were performed on recent immigrants from Africa to the developed world, and as such did not collect indicators of socioeconomic status.[7 10] A more recent study of 547 HIV-1 infected individuals in Uganda, which did collect demographic data, found that helminth infection was correlated significantly with male sex, no electricity at home, use of firewood as a primary cooking fuel, short walking time to Lake Victoria, and those who reported walking barefoot "often". Of note, this study did not show a significant correlation between helminth infection and any physical symptoms, including loose/watery stool. Data on education and access to sanitation was not collected in this study.

Another recent study of people co-infected with helminths and HIV-1 in Zambia focused on schistosomiasis.[15] This study found a significant correlation between male gender and helminth infection. However, like the Uganda study, this study did not collect data on education or access to sanitation.

Although there are studies of epidemiological correlates of helminth infection among immunocompetent persons, there is a dearth of epidemiological data for helminth infection in the HIV-1 infected population. To guide policy-makers and clinicians in deciding when and whether to empirically treat helminth infection in HIV-1 infected patients, more epidemiological data specific to this population is needed.

HYPOTHESIS

Among HIV-1 infected adults, helminth burden is correlated with markers of HIV-1 disease progression, in this case CD4 count.

GOAL

Determine whether degree of intestinal helminth burden in HIV-1 infected persons is correlated with epidemiological factors such as education, occupation, and other indicators of socioeconomic status among Kenyan adults.

SPECIFIC OBJECTIVES

1) Determine if sociodemographic factors are associated with the prevalence of helminth infection in patients with HIV-1.

2) Among Kenyan adults co-infected with HIV-1 and helminths, determine whether CD4 count is associated with degree of stool helminth burden (25 eggs/gram of stool versus greater than 25 eggs/gram of stool).

PREVIOUS RESEARCH

Information concerning stool helminth burden is not frequently reported in studies of helminths in HIV-infected individuals. While a number studies do report individual helminth species, very few correlate species with degree of immunosuppression.

Among the research that has addressed our first objective, a study by Wolday et al. showed a correlation between number of eggs excreted and plasma HIV-1 RNA viral load.[16] Clinically asymptomatic subjects not on anti-retroviral therapy were treated for helminth infection, and a significant decrease in viral load was seen after six months. This study did not find a correlation between stool helminth burden and CD4 decline. Another helminth study, performed in Kenya, found no correlation between eradication of helminths and plasma viral load.[17] That study, however, did not collect markers of immune activation—such as CD45, CD27, HLA-DR, and nuclear antigen Ki67—which have been shown to correlate more closely than HIV-1 RNA level with decline in CD4 count.[7] Further research is necessary to resolve these conflicting findings.

Regarding the secondary objective, few studies compare the presence of various species of helminth and correlate them with markers of HIV-1 progression. One study of 547 HIV-1 infected individuals in Uganda reported that subjects infected with helminthes had significantly higher HIV-1 RNA level than those who were helminth-negative (4.74 $\log_{10}$copies/ml vs 4.95 $\log_{10}$copies/ml, p=.03).[14] When regression analysis was performed by individual helminth species, however, it was discovered that patients with *Strongyloides stercoralis* constituted the bulk of those who had higher viral loads (4.79 $\log_{10}$copies/ml for uninfected subjects, versus 5.08 $\log_{10}$copies/ml for subjects with strongyloides, p=.02). The *Strongyloides* subset of patients were shown to have lower CD4 counts than the uninfected group, while every other helminth subset

(hookworm, *Schistosoma,* and *Mansonella*) was found to have higher CD4 counts compared with uninfected patients. These data emphasize the need for more species-specific helminth research.

APPLICATION OF RESULTS

There are 26 million HIV-1 infected individuals in sub-Saharan Africa. Approximately one-third of these people live in areas of known helminth endemicity. If helminth infections are found to adversely affect the natural history of HIV-1, antihelminthic treatment may have a significant impact on the health of HIV-1 infected persons. The goal of this work is to identify risk factors for helminth infections in HIV-1 infected individuals.

If the eradication of helminth infection in HIV infected individuals can lead to significant slowing of disease progression, the public health benefits would be enormous. A reduction in the rate of disease progression of even a very modest amount would lead to significant public health benefits by delaying the need for antiretroviral therapy for many individuals and improving the functional status of the group as a whole. Using this information, future efforts could also focus on eradicating helminthes in populations with high helminth burden.

The results of this research could impact the use of ARVs in low-resource countries. In many resource-poor settings, HIV treatment programs are being rapidly scaled up to provide ARV therapy to immunosuppressed HIV-1 infected individuals. Many of these countries are limited to a first- and second-line

regimen of ARV treatment, with no further options for patients who fail these therapies.[1] In an era of increasing HIV-1 viral resistance to nucleoside reverse transcriptase inhibitors, ensuring and maintaining the success of first-line therapy is a priority for patients commencing treatment. If HIV-1 progression can be slowed by significantly lessening the prevalence of helminth infection in co-infected individuals, it may be possible to delay ART for millions of individuals. This would lead to a clear reduction in the costs, adverse events and number of treatment failures seen with currently available antiretroviral therapy and possibly lessen the transmission of resistant HIV-1.

OPERATIONAL TERMS

Helminthes

- Nematodes
  - Strongyloides stercoralis
  - Enterobius vermicularis (pinworm)
  - Ancylostoma duodenale (hookworm)
  - Necator americanus (hookworm)
  - Ascaris lumbricoides (roundworm)
  - Trichuris trichiura
- Trematodes
  - Schistosoma mansoni
- Cestodes

Hymenolepsis nana

Immunosuppresion: For the purposes of this study, patients with CD4 < 250 were considered immunosuppressed.

Methods

Study Site

The data for this study was collected from five clinics in and around Nairobi, Kenya where we are currently conducting a randomized controlled trial of helminth treatment in HIV-1 infected individuals.

Kibera AMREF/CDC clinic: The African Medical Research Foundation is partnering with the Centers for Disease Control and Prevention to manage a clinic in the Kibera neighborhood of Nairobi. Kibera is the largest slum in East Africa, containing by some estimates over one million residents. The AMREF/CDC clinic provides HIV care and treatment for 3,000 to 4,000 HIV-1 infected individuals. Most patients live in Kibera.

Thika District Hospital: Thika is a town of approximately 90,000 residents, located 70 kilometers northeast of Nairobi city center. The hospital is located in an urban environment, but the catchment area is large and serves mainly rural residents, including farmers from the area surrounding Thika. There is a Del Monte Foods Corporation pineapple processing facility located in Thika, which employs many Thika residents.

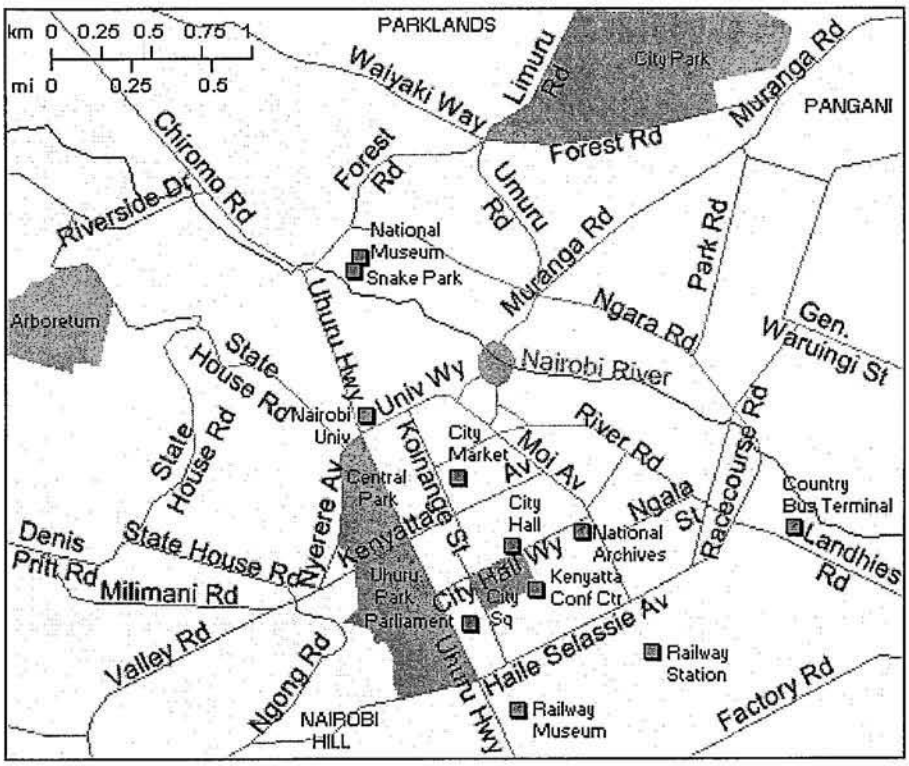

Figure 1: Map of Nairobi, Kenya

Kerugoya District Hospital: Kerugoya is a town of approximately 40,000 residents located 150 kilometers north of Nairobi, in the foothills of Mount Kenya. The hospital serves a combination of patients, including local farmers

living in rural environments, as well as mountain guides and porters. The hospital manages 2,000 to 3,000 HIV-1 infected individuals.

Coptic Hospital: The Coptic orthodox church, a Christian denomination based originally in Egypt, runs a mission hospital on Ngong Road in Nairobi. The 56-bed hospital is located one mile from Kenyatta National Hospital in Nairobi, the latter being the primary healthcare facility serving low-income residents. Coptic Hospital is the recipient of significant funding from the U.S. President's Emergency Plan for AIDS Relief (PEPFAR), and manages between 4,000 and 5,000 HIV-1 infected individuals.

Selection Criteria

Subjects from each site meeting the following criteria will be offered enrollment in the study: Adults 18 years or older, who are HIV-1 infected (as determined by lab result or self-report). Subjects must be antiretroviral treatment naïve, able and willing to participate and give written informed consent. They must not have received helminth treatment in past 6 months. In addition, subjects must not be pregnant at time of enrollment (as determined by urine HCG for all female patients of child-bearing age).

Ongoing Research Efforts

This research is a cross-sectional study that occurred in conjunction with an ongoing randomized controlled trial (RCT). We are currently conducting an

RCT to determine whether immediate or delayed therapy with albendazole in HIV seropositive individuals with documented soil-transmitted helminth infection is superior. This project is being performed as part of yet another study investigating the effect of antihelminthic therapy on progression of HIV-1. To test the hypothesis that helminth infection leads to decreased CD4 count and increased plasma HIV-1 RNA level. Subjects are HIV-positive individuals with CD4 counts of 250 cells/mm3 or greater and who have documented helminth infection by stool microscopy. At enrollment, CD4 count, viral load and various markers of immune activation (CD27, CD45, HLA-DR) are measured, and participants are randomized to receive either albendazole 400mg for three days or matching placebo. At 12 weeks, stool studies, CD4 count, viral load and immune activation markers are repeated. At the 12 week visit, all subjects with evidence of helminth infection are treated with a three day course of open-label albendazole (400mg per day for three days).

Clinical Procedures

This hypothesis-driven, cross-sectional study was performed concurrently with ongoing RCT described above. Subjects were recruited from the HIV clinics at the study sites. Patients with CD4 > 250 were approached for enrollment. A screening interview was performed to determine eligibility. Patients interested in participating were provided with a stool collection container and scheduled for a study appointment. Enrolled patients were instructed to return with a

stool sample collected within 24 hours preceding the scheduled study appointment.

When subjects returned for the study appointment, the study was explained in either English or Kiswahili, per individual preference, and written informed consent was obtained. Submitted stool specimens were screened for helminth infection using wet preparation (direct method) and Kato-Katz techniques, described in detail below. All patients were informed immediately of the results of their stool tests. Sociodemographic data were collected by survey from all screened individuals (Appendix 1).

Subjects found to be infected with helminthes but who declined to enroll and those who did not meet criteria for enrollment but were infected with helminthes were treated with open-label albendazole free-of-charge.

Those eligible who agreed to participate were consented for enrollment in this study. There were no follow-up visits.

Laboratory Procedures

Specimen

The minimum amount of stool required for adequate preparation and analysis was 60 g. For this study, Alpha-Tec Systems, Inc. ATS PARA-Set Collection/Transport Systems containers were used. This system consists of one empty vial and a vial containing Proto-Fix CLR preservative. The vials were

given to subjects in a clear plastic bag containing standard instructions (in both English and Kiswahili) for proper stool collection technique.

Routine Macroscopic Examination

All stool specimens were examined macroscopically prior to light microscopy. The following characteristics were noted for all specimens:

Specimen Quality - Has the specimen been correctly collected?

Specimen Quantity - Has a sufficient quantity of stool been collected (minimum 60g)?

Gross Appearance - Appearance of the specimen: formed, watery, semiformed, bloody, mucoid, etc.

Macroscopic Examination for Helminthes - Are there any visible eggs or helminthes (whole or sections).

Wet Preparation (Direct Method)

Preparation of Materials:

Iodine solution was distilled water containing 2 gram % of potassium iodine and 1 gram % of iodine crystals and was shaken to ensure components were completely dissolved. Normal saline consisted of a 0.85 gram % solution of sodium chloride and distilled water.

Procedure:

One drop of normal saline or iodine solution was placed on a glass slide. An applicator stick was used to emulsify a small portion of stool on the slide in the iodine solution or saline. A cover slip was applied, and the suspension was examined with X10 objective followed by X40 objective. Microscopy was performed systematically in order to ensure that the entire area of the slide was viewed. Findings were recorded immediately in stool findings log book.

Kato-Katz Method

Preparation of Materials:

1 ml of 3% aqueous malachite green or 3% methylene blue was added to 100ml of distilled water. Cellophane strips were soaked in the solution for at least 24hrs prior to use.

Procedure:

A small amount of fecal material was placed on a metal sieve using a small, flat-sided spatula. The spatula was scraped across the upper surface of the screen to collect the sieved feces from the lower surface. A plastic template was placed on the center of the microscope slide, and sieved feces from the spatula were added, so that the hole in the template was completely filled. Excess feces were removed by flattening with the side of the spatula. The template was then removed carefully so that the cylinder of feces was left on the slide. Fecal material was covered with the pre-soaked cellophane strip. The

microscope slide was inverted and the faecal sample was pressed firmly against the hydrophilic cellophane strip onto another slide to thin out the stool smear. The slide was then removed carefully by sliding it sideways to avoid separating the cellophane strip/stool/slide sandwich. The slide, with cellophane side up, was then left at room temperature for 60 minutes to allow evaporation of excess water. The slide was examined within 40 minutes to identify hookworm eggs. For all other eggs, the slide was kept for one or more hours at room temperature to clear the fecal material. The smear was examined by a parasitologist experienced in helminth identification. The smear was examined systematically and the number of each species was recorded as the total number of eggs per gram of feces (EPG) by multiplying by a factor of 24.

Formol-Ether Concentration

Procedure:

Formol saline solution was prepared by adding 4% formalin to 96mL of distilled water. One gram of stool was then emulsified in 7mL of 4% formol-saline solution. Using a funnel, the emulsification was filtered through the gauze into a centrifuge tube. Three mL of ether was added, and the rubber stopper was inserted. Emulsification was shaken vigorously for one minute. The tube containing the emulsification was centrifuged, with gradual acceleration for 2-3 minutes at 2000 rpm. The supernatant was decanted, and di-ether was added to the centrifuge tube containing the deposit. The deposit was placed on

the slide with a cover slip. The deposit was observed carefully under X 40 objective, by a trained parasitologist with experience identifying helminthes. The total number of eggs was recorded immediately.

Data Management and Statistical Analysis

Data was collected on preprinted forms and entered into a database (Access, Microsoft Corporation, Redmond, WA). Completeness and accuracy of all demographic and laboratory forms was double-checked by study staff on site at the end of every study day. Data forms were stored in locked file cabinets the investigator's office at KEMRI.

Continuous variables were analyzed using T-tests or non-parametric testing where the appropriate assumptions were met. Categorical variables were analyzed with Chi square tests or Fisher exact test where appropriate assumptions were met. (SPSS 14.0, SPSS, Inc., Chicago, IL).

Based on data from prior helminth studies, an equal distribution of subjects in each burden group (no infection, light, moderate, and heavy) was predicted. Assuming a power of 80% and alpha of 0.05, it was determined that 100 individuals would be needed in each group to detect a difference in CD4 count of 40 cells/mm^3 (SD of 200 cells/mm^3). Prevalence was estimated between 30-50%, which would require between 450 and 600 enrolled subjects.

Ethical Considerations

Risks: This study carries minimal risk to subjects. For helminth positive subjects, medication side effects included diarrhea, abdominal pain, nausea, and vomiting. There was a rare risk of rash, anemia, and elevated liver function enzymes. All enrolled subjects were given a card with the investigators contact information, and told to return to the clinic in the case of any adverse events.

Benefits: All subjects may have benefited from free stool examination. Subjects found to have helminths received free treatment. In addition, the public health impact of the study may benefit millions of HIV-infected individuals in low-resource settings.

Voluntary Participation: Consent forms were made available in English and Kiswahili. Subjects were given ample time to ask questions of the investigator prior to enrollment. Patients were informed that refusal to participate would not affect their care in any way.

Confidentiality: All screened subjects were given a unique study ID number at the time of enrollment. This number appeared in place of name on all study documents. The log book linking name with study ID number was kept locked in a secure file cabinet in the office of the investigators.

Justice: Subjects were enrolled consecutively, with no bias towards or against any particular patient. No preference or prejudice was given according to gender, age, race, ethnicity, or any other sociodemographic characteristic.

The study was approved by the Kenya Medical Research Institute Ethical Review Boards. The study was also approved by Institutional Review Board of the University of Washington at Seattle.

RESULTS

Between March 12, 2006 and November 16, 2006, 912 HIV-1 infected individuals were enrolled. 277 subjects were enrolled at the Kibera/CDC clinic site, 279 subjects were enrolled at the Thika site, 222 were enrolled at the Mbagathi District Hospital site, 78 at the Coptic Hospital site, and 56 at the Kerugoya site. 74.2% of those screened were female, and the median age was 35 for both men and women. HIV status was confirmed by blood test in 569 patients. Three clients were excluded for being HIV negative by blood test. The remaining clients' HIV status was confirmed by self-report or chart review.

61.7% lived in a single room. The median number of individuals in the home was 4 (IQR 3-5). The mean number of adults in the home was 2.7 (standard deviation 3.6). 75.7% of individuals lived with another adult in the home. The mean number of children in the home was 1.6 (sd 1.3). 77.0% of individuals lived with at least one child in the home. For all patients screened, the mean number of individuals of all ages living in the home was 4.3 (sd 3.8).

Table 1: Characteristics of 912 HIV-1 infected individuals screened for helminths in Kenya	
Characteristic	N=912
Helminths detected by microscopy	113 (12.4%)
Gender male	232 (25.4%)
Median Age (IQR)	35.0 (29.0-40.0)
Education	
None	56 (6.2%)
Primary	480 (52.9%)
Secondary	294 (32.4%)
College	70 (7.7%)
Housing	
Single room (not incl. bathroom)	533 (58.6%)
Earth floor	327 (35.8%)
Water Supply	
Piped into house	150 (16.4%)
Communal tap within housing unit	491 (53.6%)
River, lake, or pool	89 (9.7%)
Water tank in compound	59 (7.1%)
Communal water tank in vicinity	33 (3.6%)
No water source in vicinity	5 (0.5%)
Sanitation	
Private flush toilet in house	124 (13.6%)
Communal flush toilet	117 (12.8%)
Pit latrine for family only	220 (24.1%)
Communal pit latrine	446 (48.8%)
No toilet or pit latrine in vicinity	6 (0.7%)
Employment	
Jobless	291 (31.9%)
Casual worker	105 (11.5%)
Business	215 (23.5%)
Farmer	135 (14.8%)
All other occupations	167 (18.3%)
Family	
Median number of adults in home (IQR)	2 (2-3)
Median number of children in home (IQR)	1 (1-2)
3 or more children in home	219 (23.9%)
Any children in home	705 (77.0%)
Diarrhea currently	98 (10.7%)
Blood in stool currently	57 (6.3%)
CD4 count available	532 (58.1%)
Mean CD4 count (sd)	439 (242)
Median CD4 count (IQR)	389 (283 - 561)

37.4% reported having a dirt floor, with 63.7% reporting concrete. The most common wall material was stone and/or brick, reported by 38.4% of participants, followed by earth, reported by 26.3%. The most common roofing material was iron sheets, reported by 84.9% of participants, followed by cement (7.7%). 26.2% of participants had access to a flush toilet, either private or communal. Of the remaining participants, the majority (72.9%) used either a private or communal pit latrine. 70.0% had access to piped running water, and 9.0% obtained water from a river or lake. The most commonly reported occupation was unemployed, followed by businessperson, farmer, and casual worker. Among "other occupations" reported, the most common was security guard (14 participants), followed by driver (10 participants), student (7 participants), tailor (7 participants), and teacher (6 participants). There were no differences between helminth positive subjects and helminth negative subjects based on gender, age, occupation, housing material, water supply, number of adults or children in the home, or physical symptoms such as diarrhea or blood in stool.

There was a significant difference between helminth positive and helminth negative individuals based on education. Helminth-infected individuals were significantly more likely to have attended only primary school or have no education. Individuals without helminth infection were significantly more likely to have attended secondary school or college (Table 2). Those infected with helminthes were also much more likely to lack access to adequate sanitation, as defined by a private or communal flush toilet (Table 2, Table 5 for odds ratios).

Table 2: Correlates of helminth infection in 912 HIV-1 infected individuals in Kenya		
Characteristic	Helminth positive (n=113)	Helminth negative (n=795)
Gender male	21 (18.6%)	210 (26.4%)
Education		
None	13 (11.7%)	42 (5.3%)
Primary	72 (64.9%)	405 (51.3%)
Secondary	22 (19.8%)	269 (34.1%)
College	3 (2.7%)	67 (8.5%)
Housing		
Single room	71 (63.4%)	459 (58.0%)
Earth floor	43 (38.4%)	280 (35.3%)
Water supply		
Piped water	71 (62.9%)	566 (71.1%)
River, lake or pool	14 (12.4%)	75 (9.4%)
Communal tank	8 (7.0%)	52 (6.5%)
No water source	0 (0%)	5 (0.6%)
Sanitation		
Flush toilet	16 (14.3%)	224 (28.2%)
Pit latrine	95 (84.9%)	565 (71.1%)
None	1 (0.9%)	5 (0.6%)
Employment		
Jobless	36 (32.1%)	251 (31.6%)
Non-farm labor	55 (48.7%)	431 (54.2%)
Farmer	21 (18.8%)	112 (14.1%)
Family		
3 or more adults in home	40 (34.8%)	284 (35.7%)
3 or more children in home	24 (21.4%)	195 (24.5%)
Any children in home	88 (77.7%)	615 (77.4%)
Diarrhea currently	9 (8.0%)	88 (11.1%)
Blood in stool currently	9 (8.0%)	48 (6.1%)
CD4 count available	60 (53.1%)	470 (59.1%)
Mean CD4 count (sd)	450 (200)	435(246)
Median CD4 count (IQR)	436 (298 - 580)	383 (280 - 552)

121 helminths were found on stool examination from the 113 positive individuals: 63 hookworm (6.9%), 30 ascaris (3.3%), 11 schistosomes (1.2%), 10

hymenolepsis nana (1.1%), 6 trichuris (0.7%), and 1 strongyloides (0.1%). Several patients were infected with more than one helminth simultaneously. The most common co-infection, affecting 5 participants, was the presence of ascaris and hookworm simultaneously. Two patients were found to have both trichuris and hookworm. One patient was found to have each of the following co-infections: hookworm with schistosomiasis, ascaris with schistosomiasis, and ascaris with hymenolepsis nana.

Table 3: Helminth burden in immunosuppressed and immunocompetent subjects (n=52)				
	CD4 count			
Helminth burden	< 200	201-350	350-500	>500
<25 eggs/g	0	2	8	5
25-600 eggs/g	2	10	10	11
>600 eggs/g	1	0	0	1

Of the 113 subjects infected with helminthes, 70 were found to have greater than 25 helminth eggs per gram on stool examination, by either Kato Katz or Formol Ether Concentration techniques. The remaining 43 helminth-infected participants were found to have 25 or fewer helminth eggs/gram of stool, on either or both stool examination techniques.

By the wet preparation technique, only 50 of the 113 individuals (44.3%) were found to be positive. Of these individuals, all 50 were also found to have helminths on either kato katz technique or formol ether concentration technique.

Table 4: CD4 count and egg burden for four species of helminth in Nairobi, Kenya			
	1 egg	> 1 egg	All
Ascaris	8	9	17
Mean CD4	454 (172)	428 (197)	438 (177)
Trichuris	1	1	2
Mean CD4 count	580	335	458
Hookworm	11	20	29
Mean CD4	521 (197)	477 (210)	508 (210)
Schistosomiasis	2	6	8
Mean CD4	213 (217)	415 (194)	329 (175)
No helminth infection	N/A	N/A	471
Mean CD4 count			436 (247)

Of 2748 stool examinations performed (including all three examination techniques), a total of 203 positive samples were found. 50 unquantifiable helminth specimens were found on wet preparation, and 153 quantifiable specimens were found on either Kato Katz or Formol Ether Concentration. 81 helminth specimens were found on Kato Katz, and 72 specimens were found on Formol Ether Concentration. Of the 153 quantifiable helminth specimens found, 107 specimens had two or more helminth eggs on stool examination. A total of

46 specimens were found to have one helminth egg on stool examination. No significant associations between egg burden or helminth species and CD4 count were identified (Tables 3 and 4).

Table 5: Co-factors of helminth infection in adult HIV patients in Kenya

	Helminth infection		
	Positive (%) N=93	Negative (%) N=705	Odds Ratio (CI)
No secondary school education	21 (22.3%)	296 (41.3%)	2.42 (1.46 - 4.03)
No access to flush toilet	15 (16.%)	192 (26.8%)	1.90 (1.07 - 3.39)
No access to running water	58 (61.7%)	498 (69.5%)	1.41 (0.91 - 2.20)
Non-farm occupation	17 (18.5%)	104 (14.5%)	1.33 (0.76 - 2.35)
No current blood in stool	7 (7.5%)	45 (6.3%)	1.21 (0.58 - 2.76)
Currently employed	31 (33.7%)	236 (33.0%)	1.03 (0.65 - 1.64)
No earth floor in home	33 (35.9%)	263 (36.7%)	0.96 (0.61 - 1.51)
No current diarrhea	8 (8.6%)	80 (11.2%)	0.75 (0.35 - 1.60)

DISCUSSION

This study demonstrated that among individuals co-infected with both HIV-1 and helminthes, there was no significant association in either direction between a particular helminth species and CD4 count. This study also showed that the burden of helminth infection, as measured by number of eggs seen on Kato-Katz or Formol Ether microscopy, was not significantly correlated with CD4 count in either direction.

This study found a strong significant correlation between certain sociodemographic factors and helminth infection. Lack of secondary school education was the risk factor most highly correlated with helminth infection, followed by lack of access to a flush toilet.

One of the most interesting findings of this study was the complete absence of a correlation between helminth infection and either current diarrhea or current blood in stool. Many clinicians in Kenya use these two criteria, together with abdominal pain, in deciding whether to empirically treat HIV-1 infected individuals for helminths. These data bolster the data from previous work [14 15] showing no association between abdominal symptoms and helminth infection status.

If the findings of this and previous studies are borne out in larger studies, it would suggest that patient symptoms are not be a useful tool for clinicians to decide which patients to treat with antihelminthic therapy.

Multivariate analysis was not performed as part of this study. If such an analysis were performed, it might reveal significant co-linearity between

variables. Since education was the sociodemogaphic variable most strongly associated with helminth infection, this is a concern. Although the role of education on improving hygiene habits cannot be completely discounted, lack of education alone seems unlikely to cause increased helminth infection. Instead it seems more likely that education, while not directly causing helminth infection, serves as a proxy for other factors which do cause helminth infection. People with primary school education only, for example, are more likely to be unemployed than those with a college degree. In our future work, multivariable analysis will be performed to determine which specific variables remain significant and an assessment for co-linearity between variables will be performed.

Due to the low prevalence of helminths (12.4%) compared with other regions of sub-Saharan Africa[14], the sample size of this study was small. With relatively few helminth specimens to analyze, the study had less power than anticipated. An explicit determination of the power of the study has not yet been performed. The power will be measured and data generated from this study will be used to perform a sample size analysis for a definitive study.

Another limitation of this research is that, since this was a cross sectional study, causation is difficult to infer. Although several significant correlations were observed between sociodemographic factors and helminth infection, it is difficult to draw conclusions regarding the direction of causation between helminth infection and sociodemographic factors. For most of the variables assessed in this study, it is possible but unlikely that chronic helminth infection

is the causative factor for the demographic variables. Anemia and malnutrition are common effects of long-term helminth infection, and could contribute to increased sickness. Decreased general level of health could keep helminth-infected individuals at lower levels of socioeconomic status. This might happen because increased sick days could prevent helminth-infected individuals from maintaining a job, improving their housing situation and sanitation environment. Inability to escape from unsanitary and unsafe living conditions would also make it more difficult for these individuals to become helminth-free. Thus, it is possible that helminth infection as the initiating factor might also contribute to decreased socioeconomic status.

A more likely scenario is that individuals from lower levels of socioeconomic status are at greater risk of helminth infection. As described above, the helminth life cycle has been well elucidated. The transmission of larval stage helminthes via fecal matter has been well elucidated. Epidemiological studies have established the correlation between helminth infection and walking barefoot in housing conditions without adequate sewage. These conclusions were supported by the data in this study, which showed that individuals with access to a flush toilet had a significantly lower odds of helminth infection than those who only had access to a private or communal pit latrine.

In conclusion, this study found significant univariate association between helminth infection and several markers of socioeconomic status, including lack of education and lack of adequate santitation. This underpowered study did not

find significant correlation between helminth infection and either burden of infection or any particular helminth species.

FUTURE RESEARCH

Due to the low prevalence of helminthes in the study population, the power of the study was less than anticipated. In future work the exact power of this study will be determined, in order to assess how large a study would be necessary to answer definitively questions that could not be answered with this study. These questions include whether there is an association between CD4 count and either helminth burden and helminth species. Multivariate analysis will also be performed as part of future work, in order to assess co-linearity between sociodemographic variables.

It is known that intestinal worm infection is a chronic process that is highly linked to sociodemographic variables. Although subjects in this study were treated for helminth infection, re-infection is common and linked to factors such as education, income, and access to quality sanitation.[18] With additional resources, the infected and non-infected cohorts could be followed over the course of ARV therapy. Future studies to emerge from this work might include an analysis of the correlation between the robustness of immunological response to ARVs and helminth reinfection rates.

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