Impact of Increased Helminth Diagnostic Sensitivity on Estimation of Risk Factors and Outcomes

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Abstract

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Background: Traditional methods utilizing microscopy for the detection of helminth infections have limited sensitivity to detect infection, particularly in populations with lower helminth prevalence and burden. Newer polymerase chain reaction (PCR) assays may enhance detection of helminth infections and improve identification of risk factors for infection. However, these methods may detect low level helminth infections with limited impact on clinical outcomes.

Methods: This cross-sectional study was nested within a randomized clinical trial (RCT) conducted at 3 HIV Care sites in Kenya. We performed three microscopy methods and real-time multiplex PCR for the detection of *Ascaris lumbricoides*, hookworm spp., *Strongyloides stercoralis*, and *Schistosoma spp.* in stool. Sensitivity for each diagnostic modality to detect helminths was calculated using positive results from microscopy and PCR as the gold standard positive. PCR cycle threshold is the amplification cycle in which the fluorescent probe signal level exceeds background fluorescence, and correlates with parasite DNA load. We utilized relative risk regression and linear regression models to evaluate the association between helminth infection as detected by either microscopy or PCR and potential risk factors or outcomes.

Results: Of 307 adults surveyed, 61 (19.1%) and 21 (6.8%) were positive for one or more helminth species by PCR and microscopy, respectively ($p<0.0001$). PCR was markedly more sensitive (96.8%) than the microscopy techniques (33.3%) ($p<0.0001$), and had higher negative predictive values (99.2% vs. 85.3%). Increased infection intensity based on Ct was associated with diagnosis by microscopy (OR=1.24; 95% CI: 1.14, 1.36).

PCR-identified helminth infections were significantly associated with farming occupation (RR 1.57, 95% CI: 1.02, 2.40), piped water source (RR 0.26, 95% CI: 0.07, 0.99), and with completion of primary education (RR 0.65, 95% CI: 0.43, 0.99). Microscopy-detected helminth infection was not significantly associated with any of these risk factors.

Microscopy-detected helminth infections were significantly associated with lower hematocrit (mean difference of -3.56, 95% CI: -6.27, -0.85) and higher eosinophil counts (+630.92, 95% CI: 121.52, 1140.33). In contrast, these associations were not observed for PCR-detected infections, however infections diagnosed with either PCR (RR=2.42, 95% CI: 1.02, 5.76) or microscopy (RR=2.92, 95% CI: 1.29, 6.60) were associated with increased risk of eosinophilia (>500 cells/µl).

Conclusion: PCR detection of helminths had greater sensitivity and NPVs compared to microscopy. PCR assays enabled identification of more potential risk factors for infection while microscopy was better than PCR in discriminating infections with clinical outcomes, such as anemia and eosinophilia due to its specific detection of higher burden infections.

INTRODUCTION

The burden of soil-transmitted helminth infections and schistosomiasis is considerable; there are over a billion infections globally, with more than half of these infections (533 million) occurring in sub Saharan Africa[1]. Helminth infections are a significant source of morbidity; contributing to iron deficiency/anemia, as well as growth and cognitive deficiencies in school-age children[2]. In adults, helminth infections have been associated with reduced Vitamin A absorption[3], economic productivity, and higher rates of anemia [4].

Traditionally, helminth and schistosoma infections have been detected using a variety of stool microscopy techniques (Kato-Katz, Formol-ether, and Wet-prep technique), which have high specificity in ruling out infection in those uninfected, but have limited sensitivity[5-8] especially in populations where infection intensity (based on egg excretion) is low. New polymerase chain reaction (PCR) assays enhance detection of helminth infections, having higher sensitivity and high specificity[9-11].

There is significant geographic overlap in areas in which HIV and helminth infections are prevalent [12]. Infection with HIV may alter excretion of parasite eggs in stool due to HIV-associated immunodysregulation[13], potentially influencing the diagnostic accuracy of microscopy. Because PCR-based diagnostic methods do not depend on egg excretion alone for the detection of infection, they may be useful in detecting infections in HIV-infected individuals.

Previous studies have used microscopy diagnostic methods to identify several social and environmental factors associated with helminth infection in adults. Age, education status, gender and poor hygiene practices have all been associated with higher risk of helminth infection. The prevalence and intensity of helminth infection is generally higher among younger adults and

correlates with age[14-20]. Lower education has been associated with increased risk of helminth and *Schistosoma* infection [15,19,21,22]. Male gender has been associated with Schistosoma infection[16,17]; but the association has not been observed consistently[23]. Environmental factors, such as rural habitation or visitation have been associated with higher risk of helminth infection[22,24,25]. Factors related to specific helminths include distance to large bodies of water[19,20] or water contact[26] for *Schistosoma spp.* and contact with soil for hookworms, as both farming occupation and lack of shoes are associated with higher risk[22,27]. Good hygiene practices, such as hand-washing after defecation and latrine use, are also associated with lower risk of helminth infections[16,21,22,24,27,28], which may account for the associations between lower socioeconomic status and helminth infection [16,29].

Clinical consequences of microscopy-detected helminth infection in adults include reduced Vitamin A absorption[3] and reductions in physical fitness and worker productivity[30,31]. Hookworm infections of moderate to high intensity have been associated with lower hemoglobin levels and anemia in school-age children and adults[4,32,33], and with iron deficiency anemia in pregnant women[34]. A 2010 systematic review found that hemoglobin in non-pregnant adults decreased progressively with hookworm infection intensity[33], and a 2012 study using real-time PCR demonstrated a load-dependent relationship between *A. duodenale* infection and iron deficiency and anemia in school children[35]. A recent review described mixed findings regarding the relationship between schistosomiasis and anemia[36].

In the present study we compared the helminth diagnostic performance of microscopy to that of PCR in a cohort of HIV-positive Kenyan adults. We hypothesized that identification of risk factors and clinical outcomes associated with helminth infection would be substantially influenced by helminth diagnostic method. We explored whether the use of PCR for diagnoses

altered our ability to detect risk factors and outcomes of helminth infection by conducting parallel correlates analyses: one using PCR and the other using microscopy for helminth diagnosis.

METHODS

Study design: The study utilized a cross-sectional design nested in a 2-year randomized clinical trial (RCT)-the Helminth Eradication to delay ART Trial (HEAT) study (ClinicalTrials.gov, number NCT0050722), which compared an antihelminthic regimen consisting of single dose albendazole (400mg) every three months and praziquantel (25 mg/kg) given annually to standard care among ART naïve, HIV infected adults in Kenya. The methods and results of this clinical trial have been previously reported in detail[37].

Study subjects: HIV-positive individuals were recruited from clinics at three sites in Kenya (Kisii Provincial Hospital, Kisumu District Hospital, and Kilifi District Hospital) between February 2008, and June 2010. Individuals were included if they were aged 18 years or older, were HIV seropositive, and did not meet WHO criteria for ART initiation (on the basis of disease stage and CD4 cell count). Exclusion criteria included pregnancy at enrolment, anthelminthic use in the previous 6 months, or prior ART use (except for the prevention of mother-to-child transmission). Participants gave written consent in their preferred language (Kiswahili, Kisii, Luo, Giriama, or English) or if illiterate, gave oral consent in the presence of a witness and confirmed by thumbprint.

The University of Washington Human Subjects Review Committee and the Kenya Medical Research Institute Ethical Review Committee approved the study protocol.

Data collection: For this study, among 877 individuals completing the RCT we randomly selected (using a computer-generated algorithm) a subset of 309 individuals, 154 and 155 participants from the treatment and the standard of care arm (figure 1), respectively, to receive additional PCR analysis for helminth infection. Stool was evaluated for infection with the following helminths: *A. lumbricoides*, Hookworm, *S. stercoralis*, and *Schistosoma spp. Microscopy* was performed by technicians with training and certification in the differentiation and quantification of stool helminth species. Stool was prepared for examination by wet preparation, Kato-Katz technique and formol-ether concentration within 20 minutes of collection. Both qualitative and quantitative diagnoses were made for all helminths detected in stool. Stool samples underwent real-time multiplex PCR assessment at the University of Leiden (Leiden, Netherlands) to detect *A. duodenale, N. americanus, A. lumbricoides, Strongyloides stercoralis, Schistosoma haematobium*, and *Schistosoma mansoni*[9,11,38].

Multiplex real-time Stool PCR: One gram of unpreserved stool was gathered from select samples within 24 hours of production, sieved to remove debris, and stored in either Greiner 146361 tube or cryo-tube (Corning430659). If stool was very dry, one small drop of distilled water was added and mixed thoroughly. Tubes were clearly marked and stored at -20◦C or -80◦C. Tubes were transported by a staff member to Leiden University Medical Center, in Amsterdam, Netherlands where PCR assessment was performed by trained scientists using species-specific target sequences listed in appendix 1. Phocin Herpes Virus 1 (PhHV-1) -specific primers and probe[39] were used as positive controls. Procedures used have been detailed previously[9,38].

PCR output was expressed as the cycle threshold (Ct), meaning the amplification cycle in which the level of fluorescent signal exceeds background fluorescence. The Ct reflects parasite species-specific DNA load in the stool samples. We ran 50 PCR amplification cycles for each stool

sample. Infection intensity categories were defined by Verweij and colleagues[40]: low (>35 cycles), medium (30-35 cycles), high (≤30 cycles). Individuals with more than one infection were assigned the highest intensity category of the two infections.

Laboratory measures included hemoglobin levels, CD4, counts, and eosinophilia, and HIV-1 RNA levels. *CD4 measurements* were assessed by FACSCalibur at the KEMRI/University of Washington Flow Laboratory at the Centre for Clinical Research in Nairobi, Kenya. *Full Blood Counts with Differential* were assessed at each individual study site. *HIV-1 RNA levels* (8 mL) were quantified at the Kenya Medical Research Institute/Centers for Disease Control.

Data analysis: Data analysis was performed using STATA version 12.1 IC (College Station, Texas, USA).

In analyses comparing sensitivity and negative predictive values (NPV), we compared PCR and microscopy in all individuals with stool helminth testing (307 of the 309 with valid stool tests). Socio-demographic and laboratory data from the parent RCT were used in comparison analyses. As there is no true gold standard for helminth diagnosis, individuals identified as positive by PCR or any microscopy method were considered as "true" positives. Sensitivity was calculated for PCR, pooled microscopy, and each microscopy method. Inter-method agreement between microscopy methods and PCR was measured using Cohen's Kappa statistics. A continuous measure of PCR infection intensity was created for the purpose of exploring an association between intensity and detection by microscopy methods. As a total of 50 cycles were run per each sample, this variable was created by subtracting the Ct from 50, and therefore a 1 unit increase in intensity was the same as a 1 unit decrease in Ct.

Risk factors and clinical outcomes were compared in those with helminth infection versus those without in two series of analyses – first using microscopy detection as the basis for classifying individuals as helminth-infected and second using PCR detection as the basis for classifying individuals as helminth infected. Risk factor and outcome analyses were conducted only in the 153 individuals from the standard of care group, due to near complete eradication of helminths in the serially treated group. The species-specific microscopy results were combined into a pooled-microscopy variable indicating positivity by any microscopy method to enhance helminth detection in the absence of multiple samples[5,8].Analyses were conducted in parallel: one using PCR for helminth diagnosis and the other using microscopy. Our choice of potential risk factors was informed by a review of studies published within the past 5 years (appendix 2). Relative risk regression was used to identify univariate risk factors for helminth infection, and focused on variables gathered at baseline: CD4 count, HIV plasma viral load, age, number of children in household, education, housing materials, water source, and rural setting. Linear regression was used to examine associations between infection and clinical/laboratory outcomes measured at month 24; including CD4 count, HIV plasma viral load, hemoglobin, hematocrit, eosinophil count, and BMI. Patients' anemia status was determined based on standard clinical cutoffs [41]: males were considered anemic if hematocrit <41.0 percent and/or hemoglobin <13.5g/dl, and females if hematocrit <36.0 percent and/or hemoglobin <12g/dl[41]. Patients were categorized as having eosinophilia if their eosinophil count was >500 cells/µl[42].

RESULTS

Patient characteristics: Overall summaries for 307 individuals from both RCT arms with both PCR and microscopy at exit are provided in table 1, and were used for sensitivity and NPV estimates. Participants had a median age of 34 years (IQR: 28-40) and were predominantly

women (78.8%). Just over half were married (57.0%), and nearly 40% of participants had less than a primary education (37.8%). Less than a quarter (22.2%) was unemployed and 19.2% listed farming as their primary occupation. Income levels among the cohort were generally low; 41.0% of subjects reported monthly household incomes of less than 2,000 Kenyan shillings ($23.50), and only 30.8% reported monthly household incomes over 5,000 shillings ($58.75). On average, households contained 4.5 members, with 1.4 members between the age of 5 and 14. The majority of households (55.4%) utilized a communal water source, and 4.9% reported using bush toilets (the environment) rather than latrines (89.2%) or flush toilets (5.9%). These variables were obtained at enrollment in the RCT two years prior to the time of the stool evaluation and were assumed to remain fairly stable. At the time of the stool helminth study (last visit of the RCT), median CD4 counts were 356.2 cells/mm^3.

Prevalence and infection intensity: The prevalence of helminth infection in the overall sample of 307 participants was 6.8% as detected using microscopy and 19.9% by PCR. Among the untreated standard of care group, infection prevalence was considerably higher (13.1% by microscopy and 36.0% by PCR). PCR was more sensitive (96.8%) than microscopy (33.3%) ($p<0.0001$). The NPV of PCR was significantly higher (99.2%) than microscopy (85.3%) ($p<0.0001$). Comparing PCR and pooled microscopy, Cohen's Kappa statistic was 0.40 (Table 2).

Table 3 summarizes the helminth infection intensity classified by PCR. In the overall sample there were 28 low-intensity infections, 12 moderate intensity infections and 28 high intensity infections. 7 hookworm infected individuals were also infected with Schistosoma (6) or *S. stercoralis* (1). Infection intensity was positively associated with diagnosis by microscopy; a 1 Ct difference in PCR was associated with 24% higher odds of microscopy detection (1.16, 1.32).

This association remained significant, in analyses restricted to the standard of care arm. Over half (57%) of high intensity infections were detected by microscopy, compared to only 9.8% of low intensity infections. The likelihood of being microscopy-positive for an individual of low, medium, or high intensity infection was 12, 22.65, and 60.95 times that of an individual who was considered uninfected by PCR, with a significant trend test ($p<0.001$).

Risk factor analysis: Because those in the treatment arm received comprehensive deworming over a 2-year period, analyses of risk factors and outcomes was restricted to patients in the standard of care arm who had not antihelminthic agents during the past year (n=153). Baseline characteristics of individuals in the standard of care arm are summarized in and did not differ considerably from the overall sample unless otherwise noted (table 1). Table 4 summarizes risk factors for helminth infection identified by diagnosis with PCR compared to risk factors identified by microscopy. Using microscopy, no significant associations between helminth infection and the examined risk factors were detected in the cohort. However, using PCR yielded significant associations between helminth infection and farming occupation (RR 1.57, 95% CI: 1.02, 2.40), piped water source (RR 0.26, 95% CI: 0.07, 0.99), and with completion of primary education (RR 0.65, 95% CI: 0.43, 0.99).

Outcome analysis: Table 5 summarizes the analysis of potential clinical and laboratory outcomes from helminth infection, comparing associations in analyses using helminth diagnosis based on PCR to analyses using microscopy. In analyses comparing individuals with microscopy-diagnosed infections versus without microscopy-detected infection, those with infection had significantly lower hematocrit levels (Mean difference of -3.56, 95% CI: -6.27, -0.85), higher eosinophil counts (mean difference of 630.9 cells/µl, 95% CI: 121.5, 1140.3), and 48% higher risk of anemia (RR=1.48, 95% CI: 1.17, 1.88). In contrast, these associations were not observed

in analyses comparing individuals with PCR-detected infections versus without. However, significant associations between helminth infection and eosinophilia (>500 cells/μl) were detected in analyses using PCR-detection (RR=2.50, 95% CI: 1.06, 5.92) and in those using microscopy-detection (RR=2.88, 95% CI: 1.28, 6.51). Inclusion of ART use as a covariate did not alter the findings.

Helminth intensity and outcomes: While the association between infection intensity and eosinophil count was not significant (18.49 cells/μl per 1 unit increase, 95% CI: -1.01, 37.99), the risk of eosinophilia was associated with infection intensity using both continuous (RR for 1 unit increase= 1.061, 95% CI: 1.017, 1.106) and categorical methods. The risk of eosinophilia in the group with high-intensity infections was 3.4 times that in the uninfected group (RR=3.41, 95% CI: 1.39, 8.37).

Infection intensity and hematocrit were not significantly associated (-.08 per 1 unit increase, 95% CI: -0.18, 0.03, p=0.141), there were trends for significance in the association between infection intensity and risk of anemia using both continuous (RR for 1 unit increase= 1.01, 95% CI: 1.00, 1.02; p=0.083) and categorical methods. The risk of anemia was 29% higher among individuals with high-intensity infections compared to uninfected individuals (RR=1.29, 95% CI: 0.96, 1.74).

DISCUSSION

In this study, we found that PCR helminth detection methods markedly increased detection of helminth infections when compared with traditional microscopy in a cohort of HIV-infected adults. The increased sensitivity of the PCR assay resulted in greater power to detect several risk factors significantly associated with helminth infection in the cohort which were not detected in

analyses of the same cohort using stool microscopy. In contrast, less sensitive stool microscopy, which preferentially detected higher intensity infections, detected infections associated with clinically relevant outcomes. PCR-detected infections were not associated with as many significant clinical outcomes, likely because the PCR assay detected many low-burden infections with minimal clinical impact. Overall, our study illustrates several potential benefits and limitations of each of these helminth detection methods.

Our findings regarding relative benefits of the diagnostic methods, though derived from an HIV-positive cohort, likely extend to general adult populations in other settings. Past studies have shown that correlates of helminth infection in HIV-positive populations are consistent with those observed in the general population[22,25]. As patients with low baseline CD4 counts were excluded from the study, it is unlikely that immune responses to helminth infection differed markedly in this study from that of HIV-negative populations.

The observed differences in the sensitivity of PCR assays and microscopy are consistent with those described previously in other cohorts[9,11,43,44]. The observed sensitivities for individual microscopy methods were similar to the sensitivity of 17.7% observed for a single Kato-Katz test in comparison to repeated collection and detection for detection of S. mansoni plus hookworms[6]. As the NPV of microscopy was 85%, a negative microscopy results does not rule out helminth infection as well as PCR(99.2%) in this setting. Past studies have found that Ct value and egg counts derived from microscopy methods are correlated[9,11], which supports our observation of an association between PCR Ct value and detection by microscopy.

Infections detected by PCR were associated known risk factors for helminth infection; agricultural occupation[22,27], lack of piped water[22], and decreased education[15,19,21,22].

Given that detection by microscopy was not associated with these risk factors, microscopy likely missed many infections, resulting in inadequate power to observe these associations. In previous larger cohorts, microscopy did detect risk factors similar to the ones we detected with PCR assays. However, the use of PCR in this study enabled the detection of risk factors using a much smaller sample size, suggesting that these more sensitive diagnostics improve the ability to identify risk factors.

While PCR and microscopy-identified infections were both associated with elevated risk of eosinophilia, the greater risk increase of anemia and eosinophilia observed in cases diagnosed by microscopy suggests that these higher intensity cases may be associated with higher levels of hematologic and immune dysregulation. This was supported by the observed relationship between the risk of eosinophilia and intensity of infection, as the risk of eosinophilia in individuals with high-intensity infection was 3.4 times that of uninfected individuals(RR=3.41, 95% CI: 1.39, 8.37). The significantly lower hematocrit levels observed in subjects with microscopy-diagnosed infections is consistent with previous studies documenting associations between hookworm infection and anemia[4,33]. However, we did not detect an association between helminth infection as determined by PCR and hematocrit. This is consistent with data from another recent study using PCR, which did not find associations between low-intensity hookworm infection and hematocrit or iron status[35]. While we did detect a trend towards a significant association between infection intensity and risk of anemia using both continuous and categorical methods, our sample may have been underpowered for this analysis.

Strengths of this study include the multi-site sample and the rigorous lab and specimen handling practices. It is the first report to compare the risk factors and potential outcomes of helminth infection using the two different diagnostic methods. It is also the first study to assess

performance of PCR for helminth infection among HIV-positive adults. Our PCR methods included an internal control to determine efficiency of the PCR and detect inhibition of nucleic acid replication in the sample which has been reported previously[45]. While the study was conducted at two sites in Kenya and included populations with a range of socio-demographic and other characteristics, there are several features of this population that affect the potential external generalizability. The study only included HIV infected adults and was conducted in an area of moderate to low baseline helminth prevalence. In addition, the two study sites were geographically similar. The helminth infection burdens described by PCR were bi-modally distributed, which was unusual in that helminth infections are generally overdispersed, with most individuals harboring a small number of parasites and a small proportion carry the bulk of the parasites[46,47]. The pattern observed may suggest that while there is high correlation between PCR and egg count from microscopy, it is not a perfect measure of infection intensity. Infection status was not gathered at enrollment, limiting the use of baseline laboratory measurements to control for intra-individual variation. There is high variability in day-to-day shedding of eggs and a multi-day stool sampling scheme could have considerably increased the number of microscopy-identified cases, as has been shown in low-intensity settings[6]. Triplicate Kato-Katz is the standard test for helminth diagnosis[48]; therefore our use of Cohen's Kappa to compare each microscopy method to PCR may be of limited value. As few infections were identified in the treatment arm, the correlates analysis was restricted to the standard of care arm; which decreased sample size and decreased our power to detect associations. Though species-specific correlates analyses would have provided interesting additional data, the relatively small sample size limited the power of this study for these analyses. Finally, the hematocrit and hemoglobin

cutoffs utilized for anemia are based on a population distribution[41] which may differ from that of Kenya; therefore the cutoffs may not be optimal in our study population.

In conclusion, our findings suggest that while helminth infections (particularly hookworm and *Schistosoma spp*.) may be underdiagnosed by microscopy methods, the use of the more sensitive PCR diagnostic test may lead to the detection of more low-intensity infections; which may be less clinically relevant. PCR can quickly and accurately diagnose helminth infections and may be useful to discern risk factors and transmission epidemiology. However, in resource-limited settings, the increased diagnostic sensitivity provided by PCR may capture many infections which pose little health risk and which may not contribute substantially to transmission. Our study suggests that interpretation of studies on risk factors and outcomes of helminth infection should note the marked impact of the method used to detect helminths.

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Figure 1. Study Flow Chart

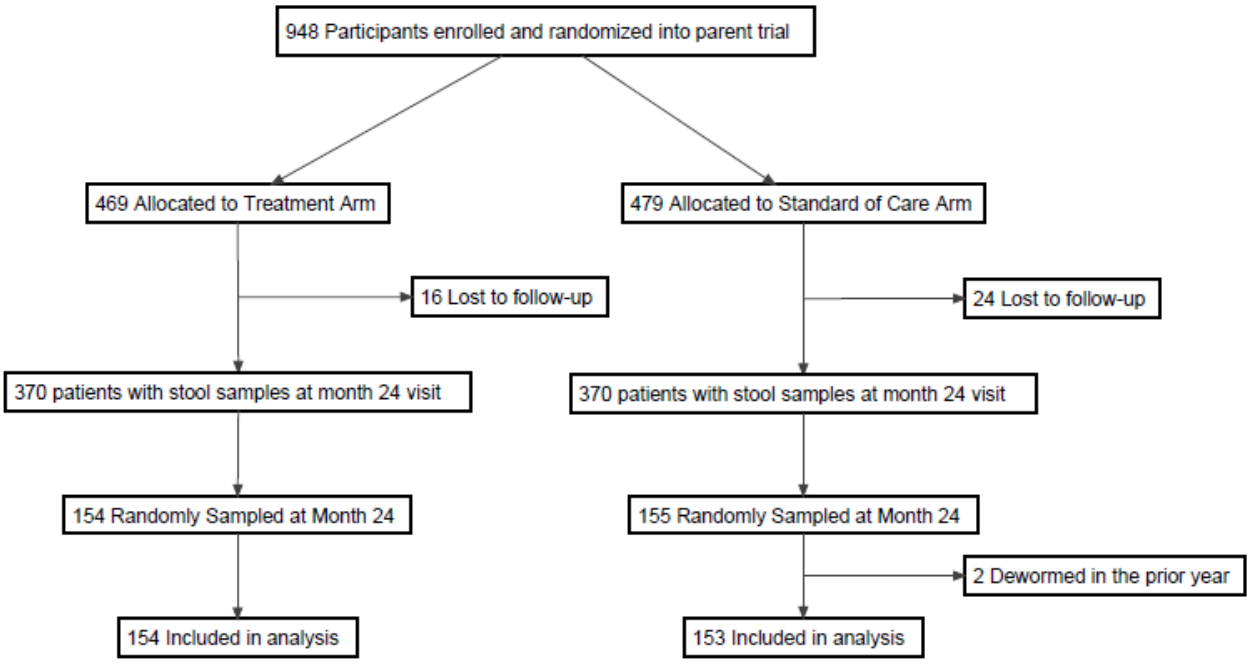

Table 1: Characteristics of the study participants at baseline (unless otherwise noted) and helminth prevalence at 24 months:

Variable	**Standard of care arm n= 153 n (%) or median (IQR)**	**Overall sample N= 307 N (%) or median (IQR)**
Female gender	124 (81.5)	242 (78.8)
Age	35 (27-41)	34 (28-40)
Married	86 (56.2)	175 (57.0)
Education (highest completed)		
Less than primary	59 (38.6)	116 (37.8)
Primary school	38 (25.5)	93 (30.3)
Secondary or higher	55 (35.9)	98 (31.9)
Income generating occupation		
None	35 (22.9)	68 (22.2)
Farmer	34 (22.2)	59 (19.2)
Other	84 (54.9)	180 (58.6)
Estimated monthly income (Ksh.)		
<2,000	65 (42.8)	125 (41.0)
2,000-4,999	42 (27.6)	86 (28.2)
>5,000	45 (29.6)	94 (30.8)
No. HH residents; mean (SD)	4.5 (2.4)	4.5 (2.2)
No. HH residents ages 5-14; mean (SD)	1.3 (1.1)	1.4 (1.2)
Water source		
Piped water in the house	17 (11.1)	36 (11.7)
Communal water source	85 (55.6)	170 (55.4)
Environmental water source	17 (11.1)	35 (11.4)
Well/Water source outside house for HH only	34 (22.2)	66 (21.5)
Bush toilet	9 (5.9)	15 (4.9)

M0 CD4 T-cell Count (cells/mm^3)	409.9 (321.5-538.7)	421.9 (308.5-558.6)
M24 CD4 T-cell Count (cells/mm^3)	365.2 (267.8-489.5)	373.5 (270.6-511.9)
Prevalence of helminth infection		
By microscopy	20 (13.1)	21 (6.8)
By PCR	55 (36.0)	61 (19.9)

Table 2: Comparison of PCR with pooled microscopy + PCR for any helminth diagnoses (N=307)

Diagnostic method[1]	**Positive n (%)**	**Sensitivity**[2] $\frac{Test+}{MIC\ and/or\ PCR+}$	**Kappa**
PCR	61(19.9)	96.8	-
Pooled microscopy	21(6.8)	33.3	0.4026
Kato-Katz	10(3.3)	15.9	0.2391
Formol-Ether	10(3.3)	15.9	0.2093
Wet Preparation	9(2.9)	14.3	0.2171

Table 3: Intensity of infection based on PCR DNA load (N=307)

Intensity based on PCR Cycle threshold (Ct)	*A. lumbricoides*	Hookworm	*S. stercoralis*	*Schistosoma* spp.	Any helminth
Low (Ct>35)	-	12	2	14	21
Moderate (30<Ct<35)	3	6	2	1	10
High (Ct<30)	4	14	1	9	26

[1] Some individuals were infected with multiple species, only counted once as infected.
[2] Specificity was 100% because each method was contained within the "gold-standard"

Table 4: Comparison of risk factors, cases ascertained by PCR vs. microscopy

Category	Variable	Subjects in analysis	Risk of helminth infection by PCR RR (95% CI)	Risk of helminth infection by microscopy RR (95% CI)
	Age (continuous, per 10 year increase)	153	0.83 (0.66, 1.05)	0.63 (0.38, 1.04)
	Completed primary education	153	0.65* (0.43, 0.99)	0.41 (0.23, 1.16)
	Farming occupation	153	1.57* (1.02, 2.40)	1.88 (0.82, 4.35)
Water source	Communal water source	85	1.00	1.00
	Environmental water source	17	0.53 (0.22, 1.28)	1.15 (0.37, 3.62)
	Piped water in the house	17	0.26* (0.07, 0.99)	----
	Well/Water source outside house for HH only	34	0.72 (0.42, 1.24)	0.77 (0.27, 2.19)
	Bush toilet use	153	0.92 (0.36, 2.38)	-----
	Baseline CD4 count<median	153	1.27 (0.83, 1.96)	0.99 (0.44, 2.23)
	HH with at least 1 child between ages 5-14	153	1.11 (0.70, 1.78)	1.07 (0.44, 2.61)
	HH with at least 1 child under age 5	153	1.19 (0.78, 1.82)	1.30 (0.57, 2.97)

Table 5: Comparison of potential outcomes at month 24, cases ascertained by PCR vs. microscopy (all standard of care subjects)

Variable	**Subjects in analysis**	**Helminth-infected by PCR** **Regression coefficient (95% CI)**	**Helminth-infected by microscopy** **Regression Coefficient (95% CI)**
Hematocrit (%)	144	-1.39 (-3.37, 0.59)	-3.56* (-6.27, -0.85)
Hemoglobin (g/dl)	144	-0.24 (-0.91, 0.44)	-.77 (-1.70, 0.16)
Anemia[3]	144	1.22 (0.95, 1.58)	1.48* (1.17, 1.88)
CD4 T-cell Count (cells/mm^3)	153	-26.83 (-91.74, 38.08)	0.92 (-91.69, 93.52)
Log Viral Load	153	0.06 (-0.30, 0.41)	0.18 (-0.33, 0.68)
Eosinophil count (cells/µl)	89	314.01 (-77.44, 705.46)	630.92* (121.52, 1140.33)
Eosinophilia (count<500)[6]	89	2.42* (1.02, 5.76)	2.92* (1.29, 6.60)
Diarrhea in the past 3 months[4]	95	-0.10 (-0.24, 0.04)	0.11 (-0.12, 0.33)
2-year change in BMI	147	-0.20 (-0.93, -0.53)	-0.22 (-1.25, 0.802)

[3] Relative risk regression for binary outcome
[4] Logistic regression for binary outcome
* Significant ($p<0.05$)

Appendix 1: Species-specific oligonucleotide PCR targets and GenBank accession numbers[9,38]

Organism	Gene target	Oligonucleotide name	GenBank accession number
S. mansoni	COX1	Smcyt748F Smcyt847R Smcyt785T	NC 002545
S. haematobium	COX1	Sh307F Sh447R Shaem377MGB	AY157209
A. lumbricoides	ITS1	Alum96F Alum183R Alum124T	AJ000895
S. stercoralis	18S	Stro18S-1530F Stro18S-1630R Stro18S-1586T	AF279916
A. duodenale	ITS2	Ad125F Ad195R Ad155MGB	AJ001594
N. americanus	ITS2	Na58F Na158R Na81MGB	AJ001599

Appendix 2: Risk factors for helminth infection among adults (or mixed ages)

Study	Population	Association OR or RR (95% CI)	Method of diagnosis	HIV status
[15]	292 pregnant women at a hospital in Geizera state, Sudan	***S. mansoni:*** • Maternal age less than 20 years OR= 9.8 (1.5, 16.3) • No education OR= 6.2 (2.8, 12.9)	Formol-ether and duplicate Kato-Katz slide examination	Not reported
[27]	132 adults (age>15) from 62 households (HH) in Kintampo North, Ghana	**Hookworm:** • Poor nutritional status (BMI≤23) OR= 0.7 (0.60, 0.85) • Not using a latrine OR= 6.10 (2.09, 17.54) • Not wearing shoes OR= 4.06 (1.57, 10.53) • Occupation (farming) OR= 4.89 (1.90, 12.61)	Single Kato-Katz slide examination	Not reported
[14]	457 women living in eight rural villages in northwest Tanzania	***S. haematobium*:** • HIV OR= 4.0 (1.2–13.5) • Younger age OR= 5.5 (1.2, 26.3 for ages < 25 yrs compared with 35+) OR =8.2 (1.7, 38.4) for ages 25–29 yrs compared with age 35+)	Urine microscopy	Mixed-5.9% positive
[23]	Cross-sectional study of 295 villagers in Lowveld, Swaziland.	***S. haematobium*:** • Female gender: OR = 8.2 (1.8, 36.2) • Younger age OR= 0.1 (0.02, 1.09) for ages 13-18 compared with age <5 OR= 0.2 (0.04, 0.57) for ages 19+ compared with age <5	Urine microscopy	Not reported
[49]	Hospital-based cross-sectional study of 1,090 diarrhea patients from 5 hospitals in Ibadan, Nigeria. 65.1% were adults	***S. stercoralis:*** • Open field or bush for toilet OR= 2.83 (1.40, 5.76) (calculated from 2x2 in table 2)	Formol-ether concentration method, Wet-preparation concentration method	Mixed-5.2% positive
[26]	290 villagers (age 5-66) from Booma, Uganda	***S. mansoni*:** • Male gender OR= 2.17 (1.14, 4.13) • Water contact exposure OR= 4.19 (2.50, 7.04) (per SD increase in exposure) • Cercarial exposure OR= 4.80 (2.89, 7.95) (per SD increase in exposure)	Duplicate Kato-Katz slide examination	Not reported

[50]	Population-based cross-sectional study of 1023 villagers in Osun State, Nigeria. Ages 2-78, with median age of 12.	***S. haematobium:*** • Family income of <$500/month OR= 3.73 (2.66, 5.21) • Number of children aged 10-15 in household OR= 1.6 (1.24, 2.06) • Literate family head OR= 0.28 (0.19, 0.42) • HH close to river OR= 1.38 (1.00, 1.89) • Being single OR= 4.27 (2.43, 7.50) • Not living with biological parents OR= 1.93 (1.29, 2.88)	Urine microscopy	Not reported
[16]	454 villagers from a community-based cross-sectional study in Zanzibar, TZ	***A. lumbricoides:*** • Male OR= 1.94 (1.03, 3.65) • Age OR= 0.98 (0.96, 0.99) • Eating raw vegetables or salad OR= 2.54 (1.27, 5.10) **Hookworm:** • Recent travel history OR= 5.06 (1.21, 21.06) • Very poor OR= 0.11 (0.02, 0.58) • Least poor OR= 0.12 (0.04, 0.42) • Eating unpeeled fruits OR= 0.28 (0.11, 0.73) • Male OR= 2.25 (1.23, 4.12) ***S. stercoralis*:** • Male OR= 4.11 (1.21, 13.90) • Washing hands after defection OR= 0.29 (0.09, 0.96) • Recent travel history OR= 5.43 (1.08, 27.27) • Age OR= 0.97 (0.94, 1.00) ***S. haematobium*:** • Age OR= 0.97 (0.95, 1.00)	Kato-Katz slide examination, Baermann method, and Koga agar plate method.	Not reported
[51]	500 villagers from two different villages in Niger (Lossa and Tara)	***S. haematobium*:** • Age OR= 3.4 (2.1, 5.7) for moderate or heavy infection (versus no infection for ages 7-15 compared with 2-6)	Urine microscopy	Not reported
[24]	908 mothers from Butajira, Ethiopia, 1 year after giving birth	**Pooled:** • Infrequent use of soap by the mother OR= 1.40 (1.04-1.88) OR= 1.66 (0.92-2.99) for use at least once a week and less frequent than once a week respectively relative to daily use; p for trend =0.018) • Urban place of residence OR= 0.45 (0.28-0.73)	Formol-ether concentration method.	Not reported

[17]	Cross-sectional survey involving 890 subjects in Kano, Nigeria	***S. haematobium:*** • Male gender (OR not reported, p≤ 0.01) • Age (OR not reported, 10 to 14 years recorded the highest rate of infection (P < 0.05)	Urine-microscopy	Not reported
[19]	2,507 pregnant women in Entebbe, Uganda	**Hookworm:** • Age (continuous, per year) OR= 0.94 (0.93–0.96) • Education (continuous, per stage) OR= 0.65 (0.57–0.73) • HIV positive OR= 0.72 (0.55–0.94) • CD4 count (continuous, per 100 cell increase, HIV+ only) OR= 1.16 (1.02–1.32) ***S. mansoni*:** • Age (continuous, per year) OR= 0.98 (0.96–1.00) • Education (continuous, per stage) OR= 0.77 (0.66–0.90) • Ever swims/bathes in lake OR= 2.39 (1.85–3.09)	Duplicate Kato-Katz slide examination	Mixed-11.9% positive
[29]	Cross-sectional study of 463 individuals aged 10–20 years in Walukuba district, Kenya	***S. mansoni*:** • Wealth index OR= 10.42 (3.38–32.36) from lowest level to highest.. • Intensity of infection decreased with wealth index: Geometric egg count for those in the lowest wealth index was 230 (95% CI 199–279) vs. 114 (95% CI 80–162) for the highest wealth index.	Duplicate Kato-Katz slide examination	Not reported
[52]	Cross-sectional study of 390 pregnant women in Rural Western Kenya.	**Hookworm:** • Age<30 OR= 1.79 (1.13–2.84) • Married OR= 0.42 (0.23–0.77) • *T. trichiura* infection OR= 2.11 (1.33–3.34) ***A. lumbricoides:*** • Age<30 OR= 0.52 (0.33–0.82) • *T. trichiura* infection OR= 2.41 (1.50–3.87)	Formol-ether concentration technique, Kato-Katz slide examination	Not reported
[53]	971 mothers in Dar-es-Salaam, Tanzania	***A. lumbricoides*:** • Geophagy (soil-eating) OR= 1.81 (1.37, 2.40)	Wet-preparation technique followed by Formol-ether sedimentation quantification	HIV positive
[28]	322 mothers from Ungunga, Zanzibar	**Pooled:** • Access to a HH latrine OR= 0.56 (0.32, 0.99) • Having an infected family member OR= 3.72 (2.22, 6.26)	Kato-Katz slide examination	Not reported

[20]	766 villagers in Northern Cameroon ages 1 and older (62% >age 30)	***S. haematobium*** • Age: Risk highest among 10-19 yr. olds and declined in each subsequent age group OR= 4.2 (.3.5-4.9) compared with 1-9 year olds • High water contact in comparison to average contact OR= 1.3 (1.2, 1.4)	Urine microscopy and sedimentation	Not reported
[21][5]	752 individuals from 233 HHs within 121 compounds in Kisumu district, Kenya	**Hookworm:** • Age above 15 years increased risk (OR not calculated, p=0.009) • Low educational level of the HH head (OR not calculated, p=0.002) • Distance from lake (OR not calculated, p=0.003) • Absence of children of 5 years and under OR= 3.77 (2.00–7.09) • HH crowding OR= 3.17 (1.71–5.86) • Lack of latrines OR= 1.93 (1.19-3.18) ***A. lumbricoides*:** • Absence of soap in HH OR= 2.56 (1.14–5.75) • Presence of children ≤5 years OR= 2.68, 1.27–5.67) (HH level) • HH crowding (OR not calculated)	Duplicate Kato-Katz slide examination.	Not reported
[22]	1,541 HIV-1 infected adults from 10 sites across Kenya	**Pooled:** • Age RR= 0.88 (0.78–1.00) • No education vs. secondary education RR= 1.90 (1.34, 2.69) • Primary education vs. secondary education RR= 1.29 (0.99–1.68) • Farming as an occupation RR=1.59 (1.22, 2.06) • No toilet or latrine vs. flush RR=3.39 (2.26, 5.08) • Lake, river, or pool water source vs. piped water RR=2.43 (1.55, 3.81) • Rural residence RR= 1.85 (1.51–2.26) • Higher CD4 count (≥350 cells/mm^3) RR=1.40 (1.12, 1.76)	Wet preparation, Kato-Katz slide examination, and formol-ether technique	HIV Positive
[25][5]	297 HIV positive individuals, WHO stage 1 or 2. ART-naïve and not dewormed in the prior 3 months	**Pooled:** • Food shortage OR= 0.5 (0.3-0.8) • Visit to rural area OR= 2.2 (1.0- 4.6) • Recent helminth infection OR= 0.5 (0.3-1.0)	Formol–ether concentration technique for diagnosis, Kato-Katz for quantification of positive samples (done once)	HIV Positive

Search terms: ("Adult"[MeSH Terms] AND Africa AND "Helminthiasis/epidemiology"[MAJR]) AND "Risk Factors"[MeSH Terms] AND ("2007/07/16"[PDat] : "2012/07/13"[PDat]) **n=37, 17 relevant**

[5] Outside of date range, but referenced in Walson, Stewart et al. 2010