Impact of Helminth Diagnostic Test Performance on Estimation of Risk Factors and Outcomes in HIV-Positive Adults

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Abstract

**Background:** Traditional methods using microscopy for the detection of helminth infections have limited sensitivity. Polymerase chain reaction (PCR) assays enhance detection of helminths, particularly low burden infections. However, differences in test performance may modify the ability to detect associations between helminth infection, risk factors, and sequelae. We compared these associations using microscopy and PCR.

**Methods:** This cross-sectional study was nested within a randomized clinical trial conducted at 3 sites in Kenya. We performed microscopy and real-time multiplex PCR for the stool detection and quantification of *Ascaris lumbricoides*, *Necator americanus*, *Ancylostoma duodenale*, *Strongyloides stercoralis*, and *Schistosoma* species. We utilized regression to evaluate associations between potential risk factors or outcomes and infection as detected by either method.

**Results:** Of 153 HIV-positive adults surveyed, 55(36.0%) and 20(13.1%) were positive for one or more helminth species by PCR and microscopy, respectively (p<0.001). PCR-detected infections were associated with farming (Prevalence Ratio 1.57, 95% CI: 1.02, 2.40), communal water source (PR 3.80, 95% CI: 1.01, 14.27), and no primary education (PR 1.54, 95% CI: 1.14, 2.33), whereas microscopy-detected infections were not associated with any risk factors under investigation. Microscopy-detected infections were associated with significantly lower hematocrit and hemoglobin (means of -3.56% and -0.77 g/dl) and a 48% higher risk of anemia (PR 1.48, 95% CI: 1.17, 1.88) compared to uninfected. Such associations were absent for PCR-detected infections unless infection intensity was considered. Infections diagnosed with either method were associated with increased risk of eosinophilia (PCR PR 2.42, 95% CI: 1.02, 5.76; microscopy PR 2.92, 95% CI: 1.29, 6.60).

**Conclusion:** Newer diagnostic methods, including PCR, improve the detection of helminth infections. This heightened sensitivity may improve the identification of risk factors for infection while reducing ability to discriminate infections associated with adverse clinical outcomes. Quantitative assays can be used to differentiate infection loads and discriminate infections associated with sequelae.
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 occurring in Sub-Saharan Africa[1]. Helminth infections, including schistosomiasis, are a significant source of morbidity; contributing to iron deficiency/anemia[2], growth and cognitive deficiencies,[3], impaired Vitamin A absorption[4], and reduced economic productivity[5,6].

Helminth infections have traditionally been detected using stool microscopy techniques, which have high specificity but limited sensitivity[7-10], especially in populations where infection intensity (based on egg excretion) is low. Newer assays, including polymerase chain reaction (PCR), have higher sensitivity and high specificity and enhance detection of helminth infections[11-13]. As these newer diagnostic assays become more available and are more widely used, it is important to determine the impact that such testing will have on our understanding of the risk factors and consequences of helminth infection.

Studies using microscopy to evaluate risk factors associated with helminth infections have found associations with age[14-20], gender[16,17], education[15,19,21,22], farming occupation[22,23], rural habitation[22,24,25] and poor hygiene practices[16]. In addition, microscopy-identified helminth infections have also been associated with anemia[2,26-28], micronutrient deficiency[4], reduced physical fitness and worker productivity[5,6]. Real-time PCR detected A. duodenale infections have also been associated with iron deficiency and severe anemia in pre-school children[29].

In the present study we investigated differences in factors associated with helminth infections detected by microscopy versus 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 influenced by diagnostic method. As HIV-infection may alter excretion of parasite eggs in stool due to immunodysregulation[30], we also assessed the impact of immune status (as measured by CD4 count level) on the ability of these assays to detect helminth infection.

Methods

Ethics statement

The UW Human Subjects Review Committee and the KEMRI Ethical Review Committee approved the study protocol, including the use of oral consent, and the trial was registered (ClinicalTrials.gov, number NCT00507221). Participants provided 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.

Study design

The study utilized a cross-sectional design nested within a 2-year randomized clinical trial, the Helminth Eradication to delay ART Trial (HEAT) study. HEAT compared an anthelmintic regimen consisting of single dose albendazole (400 mg) every three months and praziquantel (25 mg/kg) given annually to standard care among ART (antiretroviral therapy) naïve, HIV infected adults in Kenya. The methods and results of this clinical trial have been previously published[31].

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, anthelmintic use in the previous 6 months, or prior ART use (except for the prevention of mother-to-child transmission).

Data collection

Socioeconomic and demographic information was collected from individuals at enrollment. At enrollment and every 6 month study visit, all individuals in the RCT had full blood counts with differential assessed at study site, CD4 counts assessed by FACS Caltibur at the KEMRI/UW Flow Laboratory at the Centre for Clinical Research in Nairobi, Kenya, and HIV-1 RNA levels quantified at the KEMRI/CDC.

A single stool sample was provided by 740 participants at the final 24 month study visit and was evaluated by technicians with training and certification in the differentiation and quantification of stool helminth species using a combination of wet preparation, Kato-Katz technique (including quantitative assessment of egg counts), and formol-ether concentration within 20 minutes of preparation. Slides were prepared from each sample and were evaluated using each method. For this study, we randomly selected (using a computer-generated algorithm) a subset of 155 individuals with 24 month stool samples from the standard of care arm in the original trial (Figure 1) to receive additional PCR analysis for helminth infection. One gram of unprocessed stool was gathered from each sample within 24 hours of production, sieved, labeled, and stored at -20°C or -80°C in either Greiner 146361 tube or cryo-tube (Corning430659). Stool samples underwent real-time multiplex PCR assessment at the University of Leiden (Leiden, Netherlands) to detect hookworm (A. duodenale, N. americanus), A. lumbricoides, S. stercoralis, Schistosoma-genus[13,32,33] using procedures that have been detailed previously[32,33]. Phocin Herpes Virus 1 (PhHV-1) -specific primers and probe[34] were used as positive controls.

Data analysis

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

Diagnostic performance

We calculated sensitivity using PCR and microscopy results; however patients who had taken anthelmintic agents during the past year were excluded (n=2). 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.

Figure 1. Study flow diagram.
doi: 10.1371/journal.pone.0081915.g001

**Helminth infection intensity**
Fifty PCR amplification cycles were run per sample, and the 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 species-specific DNA load in the stool samples. Infections were grouped based on DNA load into intensity categories[35] of low (Ct >35), moderate (Ct 30-35), and high (Ct ≤30). Individuals
with mixed infections were categorized based on the highest intensity infection.

**Risk factor and outcome analysis**

Baseline risk factors and month 24 clinical outcomes were compared in those with any helminth infection at 24 months 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. The species-specific microscopy results were combined into a pooled variable in order to enhance helminth detection in the absence of multiple samples[7,10]. Relative risk regression was used to identify univariate risk factors for helminth infection at 24 months, 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. Relative risk regression was used to identify associations between helminth infection at 24 months and dichotomous clinical outcomes gathered at 24 months: diarrhea in the prior 3 months, anemia, and eosinophilia. The coefficients from relative risk regression should be considered prevalence ratios (PR), as we do not know the baseline infection status of patients and the clinical measure analysis was cross-sectional. In this paper we consider the inference from relative risks as a description of associations between variables; therefore the term “risk factor” should be interpreted as a positive association, without implying prediction. Linear regression was used to examine associations between month 24 infection and continuous clinical/laboratory outcomes measured at that visit; including CD4 count, HIV plasma viral load, hemoglobin, hematocrit, eosinophil count, and BMI. Robust standard errors were specified, as values were unlikely to be normally distributed. Anemia status was determined based on standard clinical cutoffs[36]: males were considered anemic if hematocrit $<41.0$ percent and/or hemoglobin $<13.5$ g/dl, and females if hematocrit $<36.0$ percent and/or hemoglobin $<12$ g/dl[36]. Patients were categorized as having eosinophilia if their eosinophil count was $>500$ cells/µl[37]. In cases where multicollinearity was a concern, a multivariate regression was run, which included exposure and suspected co-linear variable, and the standard error of the exposure of interest was compared to that of the univariate analysis. Standard errors differing by 10 percent or more were considered suggestive of multicollinearity.

**Immune status and diagnostic accuracy**

We used relative risk regression to evaluate the association between month 24 CD4 count $>350$ cells/mm$^3$ and helminth detection by PCR and microscopy.

**Helminth intensity and outcomes**

We investigated the effect of PCR-based infection intensity category on select clinical outcomes. Linear regression was used to investigate eosinophil count, hematocrit percentage, and hemoglobin, and relative risk regression was used for the binary outcomes of anemia and eosinophilia. Additional species-specific intensity analyses were run for schistosomiasis and hookworm infections. Individuals infected with other species were dropped from such analyses. As schistosomiasis-hookworm co-infection was common, species-specific analyses included the second infection intensity (as a continuous Ct value) as a covariate.

**Results**

**Patient characteristics**

Baseline demographic, socioeconomic, and clinical data from the 153 individuals were summarized and are provided in Table 1. Most participants were women (81.5%) and the median age was 35 years (IQR: 27-41). Over one-third of the cohort had less than a primary education (38.6%) and many subjects (42.8%) reported monthly household incomes of less than 2,000 Kenyan shillings ($23.50). The majority of households utilized a communal water source (55.6%) and most reported using latrines (88.2%) rather than flush toilets (5.9%) or the bush (5.9%). At the time of stool collection (24 month visit of the RCT), the median CD4 count was 365.2 cells/mm$^3$.

**Diagnostic performance**

The prevalence of helminth infection in the study was 13.1% as detected using microscopy methods and 36.0% by PCR. Table 2 summarizes the species-specific prevalence and performance of pooled microscopy and PCR. PCR was more sensitive (98.2%) than microscopy (35.7%) (p<0.001). Comparing PCR and pooled microscopy, Cohen's Kappa statistic was 0.39. All hookworm infections identified by PCR were positive for *N. americanus* and negative for *A. duodenale*.

**Helminth infection intensity**

Table 3 summarizes the helminth infection intensity classified by PCR. Among the 55 helminth-infected individuals there were a total of 62 infections: 24 of low intensity, 11 of moderate intensity and 27 of high intensity. There were six individuals infected with both hookworm and schistosomiasis, and one individual infected with hookworm and *S. stercoralis*. The Kato-Katz method identified half (10) of the helminth infections identified by microscopy and therefore egg count information has not been presented.

Infection intensity was positively associated with diagnosis by microscopy. Sixty percent of high intensity infections were detected by microscopy, as compared to only 12% of low intensity infections. The risk of being microscopy-positive for an individual of low, moderate, or high intensity infection was 12.0, 22.7, and 61.0 times that of an individual who was considered uninfected by PCR respectively, with a significant trend test (p<0.001).

**Risk factor analysis**

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, PCR diagnosis yielded significant associations between helminth infection and...
Table 1. Characteristics of the study participants at baseline (unless otherwise noted) and helminth prevalence at 24 months (N=153).

| Characteristics                                      | n (%) or median (IQR) |
|------------------------------------------------------|-----------------------|
| Sex                                                  |                       |
| Female                                               | 124 (81.5)            |
| Age                                                  | 35 (27-41)            |
| Married                                              | 86 (56.2)             |
| Education                                            |                       |
| Less than primary                                    | 59 (38.6)             |
| Primary school                                       | 38 (25.5)             |
| Secondary or higher                                  | 55 (35.9)             |
| Occupation                                           |                       |
| None                                                 | 35 (22.9)             |
| Farmer                                               | 34 (22.2)             |
| Other                                                | 84 (54.9)             |
| Monthly income (Ksh)                                 |                       |
| <2,000                                               | 65 (42.8)             |
| 2,000-4,999                                          | 42 (27.6)             |
| >5,000                                               | 45 (29.6)             |
| Number of household residents                        |                       |
| Number of household residents ages 5-14             | 4 (3-6)               |
| Number of household residents ages 5-14             | 1 (0-2)               |
| Water source                                         |                       |
| Piped water in the house                             | 17 (11.1)             |
| Communal water source                                | 85 (55.6)             |
| Environmental water source                           | 17 (11.1)             |
| Outside water source for household only              | 34 (22.2)             |
| Sanitation                                           |                       |
| Bush toilet                                          | 9 (5.9)               |
| Flush toilet                                         | 9 (5.9)               |
| Pit latrine                                          | 135 (88.2)            |
| Immune status                                        |                       |
| Month 0 CD4 T-cell Count (cells/mm³)                 | 409.9 (321.5-538.7)   |
| Month 24 CD4 T-cell Count (cells/mm³)                | 365.2 (267.8-489.5)   |
| Helminth prevalence                                  |                       |
| Microscopy                                           | 20 (13.1)             |
| PCR                                                  | 55 (36.0)             |

doi: 10.1371/journal.pone.0081915.t001

Table 2. Comparison of PCR with pooled microscopy and PCR for species diagnoses (N=153).

| Helminth Species          | PCR performance 1 | Pooled microscopy performance 1 |
|---------------------------|-------------------|----------------------------------|
|                           | Positive n (%)    | Sensitivity MIC and/or PCR+       | Positive n (%) | Sensitivity MIC and/or PCR+ | Kappa2 |
| Any species3              | 55 (36.0)         | 98.2                             | 20 (13.1)      | 35.7                         | 0.3896 |
| A. lumbricoides           | 6 (3.9)           | 85.7                             | 5 (3.3)        | 71.4                         | 0.7172 |
| Hookworm                  | 31 (20.3)         | 96.9                             | 10 (6.5)       | 31.3                         | 0.3775 |
| S. stercoralis            | 5 (3.3)           | 83.3                             | 1 (0.7)        | 16.7                         | -0.0110 |
| Schistosoma spp.          | 20 (13.1)         | 95.2                             | 6 (3.9)        | 28.6                         | 0.3451 |

1 Specificity was 100% because each diagnostic method was contained within the “gold-standard”.
2 Kappa calculated in comparison to PCR detection S. stercoralis (1), counted singly
3 7 individuals co-infected with hookworm and schistosomiasis (6) or S. stercoralis (1), counted singly

doi: 10.1371/journal.pone.0081915.t002

farming occupation (PR 1.57, 95% CI: 1.02, 2.40), communal water source (PR 3.80, 95% CI: 1.01, 14.27), and with lack of primary education (PR 1.54, 95% CI: 1.14, 2.33).

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 those using microscopy. In analyses comparing individuals with microscopy-diagnosed infections versus without microscopy-detected infection, those with infection had significantly lower hematocrit (mean of -3.56%, 95% CI: -5.85, -1.27), lower hemoglobin (mean of -0.77g/dl, 95% CI: -1.47, -0.07), and 48% higher risk of anemia (PR 1.48, 95% CI: 1.17, 1.88) as compared to individuals considered uninfected. These associations were not observed in analyses comparing infected individuals to those without based on PCR. However, individuals with high intensity (Ct≤30) infections detected by
PCR had significantly lower hematocrit than those without high intensity infection (mean of -2.37%, 95% CI: -4.34, -0.40). Significant associations between helminth infection and eosinophilia were also detected in analyses using microscopy (PR 2.92, 95% CI: 1.29, 6.60) and PCR (PR 2.42, 95% CI: 1.02, 5.76). The association was strengthened when individuals with high intensity infections were compared to those without such infections (PR 2.96, 95% CI: 1.32, 6.65).

**Immune status and diagnostic accuracy**

There was no association between lowered month 24 CD4 count (less than 350 cells/mm$^3$) and helminth infection detected by PCR or microscopy (Table 4). The average CD4 count was not significantly different between helminth infected and uninfected individuals using either detection method (Table 5).

**Helminth intensity and outcomes**

Helminth infection intensity and hematocrit were significantly associated, as individuals with moderate intensity infection had hematocrit values that were 2.36% lower (95% CI: -4.69, -0.03; p= 0.047) than those who were uninfected, with a significant trend test when treated as a grouped-linear term (p= 0.039). Infection intensity was not significantly associated with hemoglobin or risk of anemia.

While there was no association between infection intensity and eosinophil count, the risk of eosinophilia was associated with infection intensity, as the risk of eosinophilia among individuals with high intensity infection was 3.4 times that in the uninfected group (PR 3.41, 95% CI: 1.39, 8.37).

**Species-specific intensity and clinical outcomes**

Controlling for schistosomiasis intensity, hookworm intensity was associated with decreased hematocrit (2.42% lower among individuals with moderate infection compared with uninfected, 95% CI: 4.73, -0.13; p=0.039) and possibly hemoglobin (-0.04 g/dl, 95%: -0.07, 0.00; p=0.052). When controlling for hookworm infection intensity, schistosomiasis intensity was associated with a higher risk of eosinophilia; the odds of eosinophilia in those with high intensity schistosomiasis were 9.7 times that in those uninfected (OR 9.70, 95% CI: 1.42, 66.21; p=0.020). There was an association between schistosomiasis intensity and eosinophil count; however this association was with moderate intensity schistosomiasis, a
category which contained only one individual. Hookworm infection intensity was not found to be associated with raised eosinophil count or risk of eosinophilia.

**Discussion**

PCR 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 microscopy when interpreted as dichotomous, higher than microscopy methods, and PCR requires levels of laboratory infrastructure and training which are not readily available in many settings. These factors pose challenges to the incremental costs of PCR to microscopy.

The observed sensitivity for PCR assays and microscopy are consistent with those described previously in other studies[11,13,38,39]. We found that helminths detected by PCR were associated with previously described risk factors for helminth infection, including agricultural occupation[22,23], lack of piped water[22], and low educational level[15,19,21,22]. Importantly, we failed to detect these associations when infection status was determined by microscopy. In previous larger cohorts, microscopy detected risk factors similar to those we detected with PCR assays. However, the use of PCR in this study enabled the detection of risk factors with a smaller sample size, suggesting that sensitive diagnostics improve ability to identify risk factors. We had lower power to detect risk factors with microscopy due to the smaller number of infections.

Microscopy-identified infections were associated with anemia and eosinophilia, suggesting that only higher intensity cases were associated with hematologic and immune dysregulation. This finding is supported by the observed relationship between the risk of eosinophilia and intensity of infection, as the risk of eosinophilia in individuals with high intensity helminth infection was 3.4 times that of uninfected individuals. In addition, among those with high intensity schistosomiasis infection, the odds of eosinophilia were 9.7 times that of uninfected individuals. The significantly lower hematocrit percentages and hemoglobin levels observed in subjects with microscopy-diagnosed infections is consistent with previous studies documenting associations between hookworm infection and anemia[2,27]. However, both moderate and high intensity *A. duodenale* infections detected using real time PCR have previously been shown to be associated with severe anemia among pre-school children in Malawi (adjusted odds ratio: 2.49 and 9.04 respectively)[29].

The implications of the data suggest that PCR detects infections of such low intensity that they may be undetectable by traditional methods. While this increased sensitivity may have ramifications for helminth elimination and control efforts, there are several considerations which factor into potential scale-up of PCR diagnostics. The cost of PCR is considerably higher than microscopy methods, and PCR requires levels of laboratory infrastructure and training which are not readily available in many settings. These factors pose challenges which limit current PCR diagnostics to research rather than broader clinical application. Future research should compare the incremental costs of PCR to microscopy.

**Strengths of this study include the multi-site sample and parallel comparison of helminth detection techniques.** It is the first report to compare the risk factors and outcomes of helminth infection using the two different diagnostic methods, and to assess performance of PCR for helminth infection among HIV-positive adults. The PCR methods used included an internal control to determine efficiency of the PCR and

### Table 5. Comparison of clinical outcomes at month 24, month 24 helminth infection; cases ascertained by microscopy vs. PCR vs. high intensity PCR (Ct≤30).

|                        | Helminth-infected by microscopy (n=20) | Helminth-infected by PCR (n=55) | High intensity helminth-infected (n=25) |
|------------------------|---------------------------------------|---------------------------------|-----------------------------------------|
|                        | Regression coefficient (95% CI)        | Regression coefficient (95% CI) | Regression coefficient (95% CI)         |
| Hemoglobin (g/dl)      | -0.77 (-1.47, -0.07)                  | -0.24 (-0.85, -0.38)           | -0.60 (-1.28, 0.08)                     |
| Anemia*                | 1.48 (0.95, 1.58)                     | 1.22 (0.95, 1.58)              | 1.26 (0.95, 1.68)                       |
| CD4 T-cell Count (cells/mm³)* | 3.38 (97.17, 103.93)               | -21.05 (-82.30, 40.20)        | -33.40 (-100.24, 33.46)                 |
| Log Viral Load*        | 0.21 (-0.21, 0.65)                    | 0.15 (-0.15, 0.46)            | -0.33 (-0.08, 0.74)                     |
| Eosinophil count (cells/µl) | 2.92 (1.29, 6.60)                   | 2.42 (1.02, 5.76)             | 2.96 (1.32, 6.65)                       |
| Diarrhea in the past 3 months* | 1.91 (0.49, 7.40)                  | 0.38 (0.09, 1.62)             | 0.57 (0.08, 4.08)                       |
| 2-year change in BMI  | -0.22 (-1.07, -0.62)                  | -0.20 (-0.96, 0.56)           | -0.41 (-1.20, -0.37)                    |

* Significant (p<0.05)

* Relative risk regression for binary outcome

* Adjusted for ART use

doi: 10.1371/journal.pone.0081915.l005
detect inhibition of nucleic acid replication in the sample as reported previously[40].

While the study was conducted at several sites in Kenya, there are features 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. The helminth infection burdens were bi-modally distributed, in contrast to the more common overdispersed distribution, in which most individuals harbor a small number of parasites and a small proportion carry the bulk of the parasites[41,42]. Infection status was also not gathered at enrollment, limiting the use of baseline laboratory measurements to control for intra-individual variation. Budget limitations in performing the assays restricted the PCR to a subsample of the patients enrolled in the RCT, half of which were enrolled in the treatment arm (but were excluded from these analyses). While pre-study sample size calculations were performed for the RCT aims, post-hoc power calculations were performed for this secondary analysis. This secondary analysis achieved 80% power to detect a 0.75 g/dl difference in hemoglobin between helminth infected and uninfected subjects, and to detect a prevalence ratio of 1.76 for helminth infection for subjects whose baseline CD4 count was less than the median compared to those above the median.

Another significant limitation of this study was the microscopy method used to detect parasites. The objective of the parent trial was to evaluate the impact of empiric deworming on HIV progression, and stool testing was only performed to confirm that rates of infection were lower in the treatment arm. As a result, in the parent trial all participants provided a single stool sample at the final study visit which was evaluated using three microscopy methods as described above. These samples were read on site by trained lab technicians but were not subject to verification from a second reader. Given the high variability in day-to-day shedding of eggs, standard stool diagnosis requires testing of multiple stool samples with triplicate Kato-Katz being an optimal microscopy method for helminth diagnosis[8,43]. In a previous study, a single Kato-Katz test had a sensitivity of 17.7% in comparison to repeated collection for detection of S. mansoni and hookworm species[8]. The use of a single stool sample for diagnosis may have substantially underestimated the ability of microscopy to detect infection and may have affected the analyses presented here. Because only 50% of the infections identified by microscopy were from the Kato-Katz technique, we could not evaluate the association between infection intensity determined by PCR Ct and traditional infection intensity expressed as eggs per gram of stool. A standard curve has not been created for such an analysis; however the correlation between hookworm egg count and PCR Ct has been estimated by a previous study ($p = -0.76; P < 0.001$)[13].

In summary, our findings suggest that while helminth infections of all intensities are underdiagnosed by microscopy methods, the use of the more sensitive PCR diagnostic test may lead to the detection of a higher proportion of low intensity infections which may be less clinically relevant. PCR can accurately diagnose helminth infections, including low burden infections, and may be useful to discern risk factors and transmission epidemiology. However, the increased diagnostic sensitivity provided by PCR may capture large proportion of lower-intensity infections, which may pose less health risk. Further validation of the quantitative PCR output in different endemic settings is therefore needed. Our study suggests that interpretation of studies on risk factors and outcomes of helminth infection should note the influence of the diagnostic method used to detect infection status.

**Acknowledgements**

We would like to thank all of the participants and the clinics and organizations caring for persons living with HIV/AIDS who participated in this study. We would also like to acknowledge the staff of the University of Washington (UW)/ Kenya Medical Research Institute (KEMRI) collaboration and Eric A.T Brienen of Leiden University Medical Center for performing the PCR assays.

**Author Contributions**

Conceived and designed the experiments: JLW JJV LV BS JMN. Performed the experiments: JJV LV LWM. Analyzed the data: MBA. Contributed reagents/materials/analysis tools: JLW JJV LV BAR LRS. Wrote the manuscript: MBA JLV GJ.

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