The effects of maternal helminth and malaria infections on mother-to-child HIV transmission

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Objective: To investigate the effect of helminth and/or malaria infection on the risk of HIV infection in pregnant women and its transmission to their offspring.

Design: A retrospective cohort study of pregnant Kenyan women and their offspring from term, uncomplicated vaginal deliveries (n = 936) with a nested case–control study.

Methods: We determined the presence of HIV, malaria, schistosomiasis, lymphatic filariasis, and intestinal helminthes in mothers and tested for HIV antibodies in 12-24 month-old offspring of HIV-positive women. We related these findings to the presence of cord blood lymphocyte activation and cytokine production in response to helminth antigens.

Results: HIV-positive women (n = 83, 8.9% of all women tested) were 2-fold more likely to have peripheral blood and/or placental malaria (P < 0.025) and a 2.1-fold greater likelihood of lymphatic filariasis infection (P < 0.001) compared to location- and-parity matched HIV-negative women. Women with HIV and malaria tended to show an increased risk for mother-to-child-transmission (MTCT) of HIV, although this difference was not significant. MTCT of HIV, however, was significantly higher in women co-infected with one or more helminthes (48%) verses women without helminth infections (10%, P < 0.01; adjusted odds ratio, 7.3; 95% confidence interval, 2.4–33.7). This increased risk for MTCT of HIV correlated with cord blood lymphocytes production of interleukin-5/interleukin-13 in response to helminth antigens (P < 0.001).

Conclusion: Helminth co-infection is associated with increased risk for MTCT of HIV, possibly by a mechanism in which parasite antigens activates lymphocytes in utero. Treatment of helminthic infections during pregnancy may reduce the risk of MTCT of HIV.

Introduction

Mother-to-child transmission (MTCT) of HIV occurs in an estimated 500,000 newborns and infants each year in sub-Saharan Africa [1]. Pregnant women infected with HIV are often co-infected with malaria, and various blood-borne and intestinal helminth infections in sub-Saharan Africa. These co-infections may increase the risk

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for MTCT of HIV. Thus the eradication of these co-infections in pregnant women could reduce MTCT of HIV.

Several observations have implicated malaria as a potential risk factor for MTCT of HIV. Malaria infections can increase HIV loads in peripheral blood [2,3] and greater viral loads enhance the risk for MTCT of HIV [4]. Co-infection with HIV and malaria during pregnancy doubles the risk that women will have placental malaria [5]. Placental malaria stimulates increased CCR5 expression on placental macrophages [6], making those macrophages more susceptible to HIV infection that can potentially increase viral loads in the placenta [6]. Placental malaria can also damage the placenta which may facilitate transplacental passage of HIV to the fetus. Yet the few epidemiological studies that have examined the association of placental malaria with the risk of MTCT have produced conflicting results. Some studies show malaria increases risk for MTCT [7], others do not [8]. Other studies show a variable effect; low levels of placental parasitemia actually protect against HIV transmission, and high placental parasitemia increases the risk for MTCT of HIV [9].

Concomitant helminth infections have also been postulated to increase the risk for HIV infections [10]. The hallmark of persistent helminth infections are expanded populations of Th2-type lymphocytes [11,12] that are more susceptible to HIV infection and propagation [13,14]. Chronic helminth infections can lead to a state of persistent immune activation [15], with increased expression of CCR5 and CXCR4 on peripheral blood CD4 T cells and monocytes [16], thus increasing their susceptibility to HIV infection. Helminth co-infections may also increase the risk of MTCT of HIV by releasing antigens intravascularly that can cross the placenta and stimulate an immune response in the fetus [17]. These chronically activated lymphocytes may increase a neonate’s susceptibility to HIV infection in utero, perinatally or post-natally (breast-feeding). Urinary schistosomiasis can produce lesions in the cervix or vagina that may increase viral shedding thereby enhancing the infant’s risk for HIV infection [18]. The presence of chronic helminth infections has not been previously examined as to whether it increases risk of MTCT of HIV. Here we test the hypotheses that women with chronic intravascular parasite infections are more likely to be HIV infected and that HIV-positive, pregnant women co-infected with malaria or helminthes have an increased risk for MTCT of HIV in a Kenyan population where urinary schistosomiasis, lymphatic filariasis, malaria, intestinal helminthes and HIV are endemic.

**Methods**

This retrospective cohort study examined the presence of HIV and parasitic coinfections in 936 Kenyan mother and infant pairs recruited between 1996 and 2002 at the Msambweni District Hospital and Vanga Health Center in Kwale District, Coast Province of Kenya, as part of a birth cohort study examining schistosomiasis and lymphatic filariasis. Plasma samples were cryopreserved at −80°C prior to examination. Fresh cord blood lymphocytes were previously examined for the capacity to produce interleukin (IL)-2, IL-5, IL-13, and interferon (IFN)-γ to crude soluble antigens prepared from adult *Schistosoma haematobium* and *Wuchereria bancrofti* worms and *W. bancrofti* microfilaria as previously described [17]. Plasma was obtained every 6 months until 2 years of age and annually thereafter from infants as part of the birth cohort study beginning in 1996. Ethical approval to conduct this retrospective study was obtained from Institutional Review Boards at Case Western Reserve University and the Kenyan Institute for Medical Research. All samples were collected prior to institution of a program involving treatment with anti-retroviral drugs perinatally (HIV testing, counseling, and peripartum anti-retroviral treatment began in the antenatal clinic at Msambweni District Hospital in June 2002). All women included in the retrospective cohort had healthy, term, uncomplicated, vaginal deliveries. The overall sample ascertainment and study design is shown in Fig. 1.

![Fig. 1. Diagram of the study design and analysis (in bold).](image-url)

One HIV-positive woman had twins. Therefore of 43 HIV-positive women, 44 offspring were examined for the presence of HIV infection during infancy.
HIV-ELISA tests were performed on the frozen, stored samples collected from women at delivery and from their infants at 12 months and repeated at 18–24 months of age. A child was considered infected if both samples were HIV positive by serology. If a sample was unavailable at 18–24 months of age and was found to be serologically positive at 12 months (n = 2), an HIV serology was performed on the 6-month sample and a positive test was scored only if the antibody titer was similar or higher in the sample collected at age 12 months to exclude the possibility that HIV antibodies may have persisted from birth. Testing was conducted anonymously such that the HIV status of a sample could not be linked to a particular individual, and only relevant data was extracted from the database. We measured the presence of anti-HIV antibodies with the Genetic Systems HIV-1 assay (Bio-Rad Laboratory Virus Division, Redmond, Washington, USA) that uses a highly purified lysate of the LAV strain of HIV-1 and recombinant HIV-1 envelope protein gp41. A positive sample had to have at least an antigen unit (AU, optical density of test sample/control) of > 2 which was repeated twice to confirm positivity. Samples with AU between 1 and 2 or that showed discordant results in the repeated ELISA assays were confirmed by Western Blot.

Pregnant women in the study were screened for helminth and malaria infections in the antenatal clinics and/or at delivery. Urine samples for detecting schistosomiasis were not available for 64 women, stools samples were not collected for 103 women, and plasma samples for detecting for lymphatic filariasis were missing for 14 individuals. In addition, insufficient or missing intervillous placental blood samples made it not possible to test for malaria for 109 deliveries. The proportion of missing samples was equally distributed between HIV-positive and -negative women (P > 0.2).

To examine for the presence of malaria, thin and thick blood smears were performed on maternal peripheral blood and intervillous placental blood; smears were stained with Geimsa, and 200 high-powered fields with at least one polymorphonuclear cell were examined for malaria parasites. The presence of malaria was also assessed by real-time quantitative PCR which [19] amplifies the gene encoding the small subunit ribosome of Plasmodium falciparum using 2.5 μl of DNA prepared from 200 μl of the red cell pellet obtained after ficoll–lympho preparation of peripheral blood mononuclear cells. DNA was also extracted from 200 μl of whole intervillous placental blood obtained by careful cannulation of the intervillous space, after clearing away excess blood. Fresh stool samples were concentrated and examined for intestinal helminthes by the Kato–Katz method. The presence of S. haematobium eggs was measured by Nucleopore (Pleasanton, California, USA) polycarbonate filtration of 10 ml of freshly collected urine. Infection by W. bancrofti or lymphatic filariasis (LF) was assessed by detection of circulating antigen in plasma using the Og4C3 mAb assay (Trop Biomed, Townsville, Australia).

Univariate analysis was performed by chi-square or Fisher’s exact test (i.e., for cross-tabulations with an expected value in any cell ≤ 5) comparing proportions for categorical variables among those individuals HIV positive and negative. Differences in cytokine production were compared using the Mann–Whitney U test. To evaluate the independent associations of co-infections and risk of HIV maternal infection, a conditional logistic regression analysis was performed using SPSS 11.0 (SPSS Inc., Chicago, Illinois, USA). Odds ratios (OR) and confidence intervals (CI) were calculated for infections with malaria and lymphatic filariasis. We used a logistic regression model (SPSS, 11.0) to estimate the risk of MTCT between helminth infected and uninfected HIV-infected women and examined the potential interaction of malaria. Odds ratios and their respective CI were calculated for maternal malaria and helminth infection with respect to MTCT.

Results

Relationship of malaria, lymphatic filariasis, schistosomiasis and intestinal helminthes to the presence of HIV infection

To determine whether HIV infected women have more co-infections with helminthes and malaria, we compared HIV-positive with HIV-negative women matched according to date of recruitment, parity, and location. Out of 936 women screened for anti-HIV antibodies, 83 were identified as positive (8.9%). Each HIV-positive individual was matched with two controls with respect to age (± 2 years), parity, residence, level of education (highest grade completed) and date of enrollment (± 1 month, n = 166, Fig. 1, Table 1). HIV-positive women were twofold more likely to be infected with malaria. HIV-positive women were also more likely to be infected with LF. There was no association between HIV positivity and infection with schistosomiasis or intestinal helminth infections, which were predominantly hookworm (91%). Infection with LF and malaria were independent risk factors for the presence of HIV infection. The adjusted OR of HIV infection in pregnant women with malaria was 2.9 (95% CI, 1.3–6.5; P = 0.007) and for lymphatic filariasis it was 3.5 (95% CI, 1.9–7.2; P = 0.003). Addition of co-infection with schistosomiasis or intestinal helminthes into the model did not alter the observed association between presence of HIV and co-infection with LF or malaria in pregnant women.

The effect of parasite infection on vertical HIV transmission

To determine whether the presence of helminth infections or malaria affected the probability that a
HIV-positive woman would infect their offspring with HIV perinatally, plasma samples were collected from infants at 12, 18 and/or 24 months of age and examined for the presence of HIV antibodies. Of the 83 HIV-positive women initially identified, plasma samples were available from offspring for 43 women (52%). A total of 44 infant samples (one woman had twins) were examined: 13 HIV positive and the remaining 31 HIV negative (MTCT rate of 29.5%, Table 2). HIV-positive women who transmitted HIV infection to their infants were more likely to be co-infected with malaria; however this difference was not significant (Table 2). Since primigravid or secundagravid women are more likely to be infected with malaria and have placental malaria during gestation [20], this serves as a surrogate for increased risk for malaria throughout pregnancy. We found no association with gravidity and whether an infant became HIV infected or not. In contrast, HIV-positive women co-infected with one or more helminthes were more likely to have children infected with HIV at 1 year of age (Table 2). The presence of maternal LF infection was most strongly associated with MTCT, although similar trends were observed for the other helminth infections. Twenty-three HIV-infected pregnant women were also infected with one or more helminth infections and 11 transmitted HIV to their offspring (48% MTCT transmission). Two of 20 HIV infected women who were not co-infected with helminth infections transmitted HIV to their offspring (10% MTCT, $P < 0.01$ compared to infected women). Controlling for a potential confounding effect of concurrent malaria, HIV-positive women with helminth infections were sevenfold more likely to transmit HIV to their offspring (Table 2).

**Relationship of cord blood cytokine production to helminth antigens and risk for MTCT of HIV**

To investigate whether activation of fetal lymphocytes in utero by helminth antigens contributes to the observed association of helminth infection with MTCT of HIV, we examined helminth antigen-induced cytokine responses in available cord blood lymphocytes from 34 of 44 newborns (77%) born to HIV-positive mothers. Seventeen of 34 (50%) samples had IL-5, IL-13, IFN-γ and/or IL-2 production in culture supernatants based on previously reported criteria [17].

<table>
<thead>
<tr>
<th>Table 1. Relationship of parasitic infections with HIV seropositivity among women attending antenatal clinics in Kenya.</th>
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<tbody>
<tr>
<td>Women HIV status</td>
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<tr>
<td>Presence of maternal malaria$^b$</td>
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<tr>
<td>Peripheral blood (blood smear)</td>
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<tr>
<td>Peripheral blood (PCR)</td>
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<td>Placental intervillous blood (PCR)</td>
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<tr>
<td>Lymphatic filariasis$^c$</td>
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<tr>
<td>Schistosomiasis</td>
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<tr>
<td>Intestinal helminths$^d$</td>
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</tbody>
</table>

$^a$Chi-square test; significance taken as $P < 0.05$.
$^b$Presence of malaria determined by blood smear and/or PCR in peripheral circulation and/or placental intervillous blood.
$^c$Presence of lymphatic filariasis assessed by detection of circulating antigen in plasma using the monoclonal antibody Og43C.
$^d$91% of intestinal helminthes were hookworm; many of these patients were co-infected with Trichuris trichuris.

<table>
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<tr>
<th>Table 2. The relationship of maternal malaria or helminth infections among HIV-positive women with vertical transmission of HIV to their newborns.</th>
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<tbody>
<tr>
<td>Vertical transmission of HIV</td>
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<tr>
<td>Malaria infection in mothers$^b$</td>
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<tr>
<td>Overall malaria infection in mother$^b$</td>
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<tr>
<td>Peripheral blood (blood smear)</td>
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<tr>
<td>Peripheral blood (PCR)</td>
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<tr>
<td>Placental intervillous blood (PCR)</td>
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<td>Maternal gravid status</td>
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<td>1</td>
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<td>2</td>
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<td>3+</td>
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<tr>
<td>Helminths</td>
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<td>Any helminth infection$^c$</td>
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<td>Lymphatic filariasis</td>
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<td>Hookworm</td>
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<tr>
<td>Schistosomiasis</td>
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</tbody>
</table>

$^a$One-tailed Fisher’s exact test.
$^b$Presence of malaria determined by blood smear and/or RTQ–PCR in maternal peripheral or placenta intervillous blood.
$^c$Lymphatic filariasis, schistosomiasis and/or hookworm.
$^dP = 0.02$. OR, Odds ratio; CI, confidence interval.
blood lymphocytes from 9 of 12 (75%) HIV-positive newborns produced IL-5 and/or IL-13, in response to helminth antigens, compared with 4 of 22 (18%) newborns who did not become HIV infected \( (P < 0.01) \). The overall amount of helminth antigen-driven IL-5/IL-13 by infants who became infected with HIV was significantly higher compared to HIV-negative infants \((P < 0.001)\). Although infants that became infected with HIV tended to produce more helminth Ag-driven IFN-\( \gamma \) and/or IL-2 \( (7 \text{ of } 12, 58\%) \) compared to HIV-negative infants \( (7 \text{ of } 22, 32\%), \text{Fig. 1 right panel} \) these differences were not significant.

### Discussion

These results show that HIV infected women were more likely to be co-infected with LF. This association was not observed with other helminth infections. It is unlikely that LF-infected women display behaviors that put them at increased risk of HIV compared to LF-negative women, e.g., blood transfusions (none of the study subjects had records of blood transfusions) or increased sexual activity, since women with HIV were matched for age, parity, residence, level of education, and date of recruitment to women without HIV. It is also unlikely that HIV infection predisposes to LF because most women acquire LF during childhood and early adolescence before becoming sexually active [21]. A more likely explanation is that LF infection increases a women’s susceptibility to HIV if exposed. This explanation is supported by the observation that HIV infected women co-infected with LF or other helminths is associated with sevenfold increased odds for transmitting HIV to their offspring.

Potential mechanisms by which helminth co-infections may affect cellular susceptibility to HIV infection in women and newborns include immune activation of lymphocytes and monocytes [22], expansion of Th2-type cells [13], differential expression of chemokine receptors that also serve as co-receptors for viral entry into cells [23,24] or impaired host immunity to HIV because of immune modulation generated by helminth infection [25]. Lymphatic filariasis is a persistent intravascular infection with chronic activation of lymphocytes and strong skewing toward Th2-type phenotype [11]. Helminth infections have been shown to increase the density of CCR5 and CXCR4, chemokine co-receptors for viral entry on surfaces of CD4 T cells and monocytes [16,26]. It has been shown that peripheral blood lymphocytes from an LF-infected subjects show increased susceptibility to HIV infection in vitro compared to the lymphocytes from the same individual following antifilarial treatment [27]. Why LF differs from other helminth infections in their association with HIV infection in pregnant women is unclear. Intestinal helminths may produce less strong immune activation and Th2 skewing because of their localization in gut mucosa compared to tissue dwelling or intravascular parasites such as LF or schistosomiasis. The sample sizes were also smaller for evaluation of schistosomiasis and intestinal helminthes and thus may have insufficient power to observe an association. Other studies, however, have also failed to associate schistosomiasis infection with increased probability of HIV infection, higher viral loads, or accelerated progression of HIV to AIDS [28,29].

One circumstance where helminth infections may affect susceptibility to HIV infection is by vertical transmission of HIV \textit{in utero}, peri-partum or post-partum as has been suggested in the current study. We postulate some of the same mechanisms that may enhance adults’ susceptibility to HIV may also operate in the fetus, newborns and infants. The fetus is exposed to soluble parasite antigens resulting in immune activation \textit{in utero} and strong skewing to a Th2-type cytokine responses [15,17]. We found that newborns whose cord blood cells generated a predominantly Th2-type cytokine response to helminth antigens were at the greatest risk for infection. Consistent with these observations are previous reports showing that activated lymphocytes with a Th2-type phenotype in other species or \textit{in vitro} (e.g. schistosome infections in rhesus macaques) are more susceptible to HIV infection [13,15,30–33]. Peripheral blood lymphocytes from individuals with LF and/or schistosomiasis produce more IL-10 [12,34,35], especially in the fetus [36]. Interleukin-10, in turn, stimulates increased CCR5 expression by fetal
monocytes [37,38], which is typically low in cord blood [39], and may thus increase the fetus or newborn’s susceptibility to HIV infection.

Co-infections with malaria were more common among HIV-positive pregnant women as compared to HIV-negative women, and this is similar to findings noted in previous reports [5,40]. Presumably this results from impaired cellular and humoral immunity, particularly in the placenta [40]. There was trend that malaria co-infection increased vertical transmission of HIV to their newborns, although this difference was not significant which might be due to the small sample size. This contrasts with some studies that show malaria as a risk factor for MTCT of HIV [7,9], although other studies do not support this finding [8].

There are several weaknesses in the study. We did not report HIV viral load, an important variable in MTCT transmission. This was a retrospective study using stored frozen samples where partial RNA degradation may have resulted in unreliable viral load measurements. It is unlikely that co-infection with helminths increased MTCT via a mechanism of increased viral load because helminth co-infections appear not to affect HIV viral loads [28,29,41]. Also co-infections with malaria, which have been shown to affect viral loads [2], was not significantly associated with increased MTCT of HIV in the current study. Additional variables that could also increase the risk for vertical HIV transmission at delivery (such as episiotomy, perineal tears, prolonged rupture of membranes and presence of other sexually transmitted diseases) were not consistently recorded for this retrospective cohort. However these variables are unlikely to bias the results for several reasons. First, only women with term pregnancies and uncomplicated deliveries were selected for the study, i.e., women with prolonged rupture of membranes were excluded. Second, we recorded these variables in a more recent cohort and found no association between helminth or malaria infections in pregnant women and the presence of perineal tears, episiotomies or sexually transmitted diseases. Sexually transmitted diseases occurred in < 7% of study subjects. Finally we determined HIV infection in infants by serology and not by detection of viral nucleic acid. It is unlikely that anti-HIV antibodies in maternal milk persist in the infant at 12–18 months of age and most infants were evaluated twice. We were also unable to distinguish whether HIV transmission occurred in utero, peri-partum, or post-partum (breast-feeding).

In summary this study indicates that in pregnant women, co-infection with helminths increases the chance that offspring will be infected with HIV. Recently, mass drug treatment for LF and intestinal helminth, and impending mass control programs for schistosomiasis has been started on the Kenyan Coast. This may provide an opportunity to assess whether the rate of HIV spread in high risk populations can be reduced in areas where integrated control programs are implemented.

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Note: MG and IM contributed equally to the study.

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