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Plasmodium falciparum and helminth co-infection in a semi-urban population of pregnant women in Uganda

Stephen D. Hillier[*,†], **Mark Booth**[‡], **Lawrence Muhangi**[§], **Peter Nkurunziza**, **Macklyn Khihembo**[§], **Muhammad Kakande**[§], **Moses Sewankambo**[§], **Robert Kizindo**[§], **Moses Kizza**[§], **Moses Muwanga**[¶], **Mark Bambury**, and **Alison M. Elliott**[§,‖]

[*]The University of Birmingham Medical School, Birmingham, B15, 2TT, UK [†]University Hospital North Staffordshire, Hartshill Road, Stoke-on-Trent, ST4 7PA, UK [‡]Dept of Pathology, University of Cambridge, Cambridge CB2 1QP, UK [§]MRC/UVRI Uganda Research Unit on AIDS, P.O. Box 49, Entebbe, Uganda [¶]Entebbe Hospitals, P.O. Box 29, Entebbe, Uganda [‖]London School of Hygiene & Tropical Medicine, Keppel Street, London WC1E 7HT, UK

Abstract

Introduction—Helminth infections and malaria are widespread in the tropics. Recent studies suggest helminth infections may increase susceptibility to malaria. If confirmed, this could be particularly important during pregnancy-induced immunosuppression.

Aim—To evaluate the geographical distribution of *Plasmodium falciparum*-helminth co-infection, and associations between parasite species in pregnant women in Entebbe, Uganda.

Methods—A cross-sectional study was conducted at baseline in a trial of anti-helminthics during pregnancy. Helminth and *P.falciparum* infections were quantified in 2507 asymptomatic women; socio-demographic and geographical details were recorded.

Results—Hookworm and *Mansonella perstans* were associated with *P.falciparum* but the effect of hookworm was seen only in the absence of *M.perstans* (OR for *P.falciparum*, adjusted for age, tribe, socioeconomic status, HIV and location: hookworm without *M.perstans* 1.53 (95% CI 1.09-2.14); *M.perstans* without hookworm 2.33 (1.47-3.69), both hookworm and *M.perstans,* 1.85 (1.24-2.76)). No association was observed between *Schistosoma mansoni, Trichuris* or *Strongyloides* and *P.falciparum*.

Conclusions—Hookworm-*P.falciparum* and *M.perstans-P.falciparum* co-infection amongst pregnant women in Entebbe is more common than expected by chance. Further studies are needed to elucidate the mechanism of this association. Helminth-induced increased susceptibility to *P.falciparum* could have important consequences for pregnancy outcome and responses to malaria in infancy.

4. *Address for correspondence:* Stephen Hillier, c/o Alison Elliott, MRC/UVRI Uganda Research Unit on AIDS, P.O. Box 49, Entebbe, Uganda, sdhillier@doctors.org.uk Phone: +447900920290, (no fax),.

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Introduction

Parasitic infections represent a major cause of disease and morbidity in Africa[1]. The World Health Organization estimates that more than one billion people are chronically infected with soil-transmitted helminths, 200 million with schistosomes [2] and 150 million with filarial helminth infections [3], while mortality from malaria is estimated at two million deaths per year [4].

With helminths and malaria infections endemic through most of Africa, communities often endure infections with a number of different parasite species [5], and individuals are often 'co-infected' with combinations of helminths, and malaria parasites [6, 7]. Rates of co-infection may not only depend on chance, but also upon the spatial distribution of environmental conditions that favour transmission of multiple species [8], as well as upon immunological interactions and common factors in genetic susceptibility or host behaviour.

Immunological factors are expected to influence rates of co-infection because helminths modulate host immune responses both to themselves, and to concurrent infections [9-11]. With regard to malaria, murine models provide evidence of interactions manifesting in altered probability of morbidity or mortality[12-16]. For example, infection with the filarial nematode *Brugia pahangi* has been found to protect against development of cerebral malaria [14], while super-infection with *Schistosoma mansoni* delayed clearance and increased severity of *Plasmodium* infection [15]. Among humans, published studies have not led to any consensus [17-24]. Reports from Thailand suggest that hookworm, *Trichuris trichiura* and *Ascaris lumbricoides* infections may increase the incidence of *Plasmodium falciparum* infections [18] whilst also suggesting that *A. lumbricoides* infection protects against severe malaria [19, 20]. This agrees with an earlier report and a recent trial suggesting that the incidence of malaria attacks increased after treatment of severe *A. lumbricoides* infection[21, 25], but studies elsewhere have not supported these findings [16, 17, 22]. Inconsistent results have also been reported in studies of schistosome and malaria co-infections [23, 24]. These contrasting results leave important unanswered questions about the biological associations between malaria and helminths.

These contrasting results may be explained by a lack of assessment of spatial variation in exposure to parasitic infections [8]. Parasites are subject to micro-geographical variation in the risk of infection: for example, schistosomiasis is found around water contact sites, risk of malaria parasite infection is affected by distance to a larval breeding source [26], and *A. lumbricoides, T. Trichiura* and hookworm are influenced by environmental conditions [27]. As exposure to multiple species of parasitic infection may vary over small distances, there is a clear need for analyses to consider residential location as a confounder of the risk of co-infection: something that few previous studies have attempted [8].

Pregnant women are an under-studied group, but are more vulnerable to infections, due to suppression of the immune system during pregnancy [28, 29]. If helminths do have a biological effect on susceptibility to malaria, this may be particularly important in pregnancy, where malaria is associated with increased maternal mortality and anaemia, IUGR and foetal and perinatal death [30], and maternal immune responses influence offspring's immunity and response to malaria infection in infancy [31].

We therefore aimed to test the hypothesis that helminth infections increase susceptibility to malaria infection in the pregnant population; we examined whether helminth-*P.falciparum* co-infections among pregnant women are more common than expected by chance, and also explored the spatial stratification of co-infections to determine whether environmental factors were likely to explain any associations observed.

Methods

This cross-sectional study used data collected at baseline (before treatment) from the "Entebbe Mother and Baby Study" a randomised, double-blind, placebo-controlled trial of anti-helminthic treatment during pregnancy [ISRCTN32849447], conducted in Entebbe Municipality and the adjacent Katabi sub-county, Uganda. [32]. The area is a peninsula in Lake Victoria bounded by lake and swampland, and occupied by semi-urban, rural and fishing communities with a diverse tribal and socioeconomic make-up.

The study design and selection criteria have been described previously [32]. Women were enrolled at Entebbe General Hospital antenatal clinic between April 2003 and November 2005. They were eligible if they were pregnant at the time of enrolment, resident in Entebbe or Katabi, and in good health. They were excluded if they were severely anaemic (haemoglobin <8 g/dl), the pregnancy was not normal, they were unwilling to receive an HIV test result (as part of the hospital programme for Prevention of Mother To Child Transmission of HIV), or if they were unwell on the day of enrolment. Recruitment took place over two visits to the clinic; 'screening', and 'enrolment' visits.

At the screening visit, women gave blood for examination for malaria parasites and *M.perstans*. Socio-economic and demographic data were collected by questionnaire. Women returned with a stool sample for the enrolment visit. All samples were collected as part of the baseline survey before treatment was given.

Stool samples were examined using the Kato-Katz method [33], and charcoal culture for *Strongyloides stercoralis* [34]. Two Kato-Katz slides were prepared for each sample and examined within 30 minutes for hookworm, and the following day for other parasites. The intensity of hookworm infection was categorised as follows: light <1,000 eggs/gram of stool (epg), moderate 1,000-3,999 epg, high ≥4,000 epg. [35]

Blood was examined using a thick film for malaria parasites and modified Knott's method for *Mansonella perstans* with intensity estimated as malaria parasites per 200 white blood cells, and microfilariae per ml blood [36].

The residence of each participant was georeferenced using Garmin handheld Global Positioning System units, during a survey carried out in the first quarter of 2006. If the participant was found to have moved since enrolment, her address at enrolment was visited and georeferenced.

Approval for the study was given by of the Uganda Virus Research Institute Science and Ethics Committee; the Uganda National Council for Science and Technology; and the London School of Hygiene and Tropical Medicine.

Analyses were conducted using STATA version 7 (College Station, Texas, USA). ArcGIS Desktop (Environmental Systems Research Institute, California, USA) was used to assign subjects to geographically defined zones on the basis of their coordinates. The analysis was divided into two parts. First was the analysis of co-infection by helminth species and *P.falciparum*, adjusting for maternal demographic, socioeconomic and clinical confounding

factors, and second was a more detailed assessment of co-infection in relation to geographical area.

Simple univariate and adjusted analysis of association with *P.falciparum* was performed for each helminth species using logistic regression. Variables considered as potential confounding factors, and included in the initial model, were age, tribe, woman's socioeconomic status, household socioeconomic status, geographical zone and HIV status. Two scales for socio-economic status, each with six levels of scoring, were devised from the questionnaire. 'Woman's Socioeconomic Status' was determined by the woman's level of education, personal income and occupation. 'Household Socioeconomic Status' was determined by building materials, number of rooms and items owned [37].

Geographical zones were defined before analysis with the aim of stratifying the population in an attempt to acknowledge the reality of variations in environment across a large study area, and were guided by the location of geographical features such as coastline, forest, raised altitude and the location of settlements marked on the map (Figure 1). For example, zone 1 is predominantly coastal and exposed, zone 2 is the most urban environment in the study, and zones 3 and 4 are areas of 2-300m elevation above the lake: it is these varied conditions that necessitate stratification. Associations between helminths and *P.falciparum*, adjusted for potential confounding factors, were examined stratified by geographical zone to assess variations in the probability of co-infections over the geographical area. Finally, associations were examined adjusting for zone, in addition to other potential confounding factors.

Results

Enrolment and baseline characteristics have been described elsewhere [37]. Briefly, 11783 women were assessed to enrol a cohort of 2507 pregnant women. The chief reasons for exclusion were living outside the study area (6243), not wishing to have an HIV test (1186) or to participate in the study (874), and not returning for enrolment after screening (596). Enrolled women were aged between 14 and 47 years, mean age 23.7 years. More than six tribes were represented, the highest proportion of women being Baganda (49%). The prevalence of asymptomatic *Plasmodium falciparum* infection was 11 percent, and the geometric mean parasite count in infected individuals was 43 per 200 white blood cells. Sixty-eight percent of women were infected with one or more helminth. The dominant species were: hookworm, (45%); *Mansonella perstans*, (21%); *Schistosoma mansoni*, (18%); *Strongyloides stercoralis*, (12%); *Trichuris trichiuria,* (9%); and *Ascaris lumbricoides,* (2%). Among those with hookworm, infection intensity was low in 85%, moderate in 11% and heavy in 4%; among those with *M.perstans* the geometric mean parasite count was 57 microfilariae per ml.

Initial crude analyses showed a strong positive association between hookworm and malaria and between *M.perstans* and *P.falciparum*. There were no statistically significant associations between *S.mansoni, T.Trichiura* or *S.stercoralis* and *P.falciparum* (Table 1). The strength of the association increased with intensity of infection for hookworm (odds ratio (OR) for malaria compared to individuals without hookworm: 1.43 for light hookworm infections, 2.14 for moderate and 2.36 for heavy infections (test for trend, $p<0.001$)). No such trend was observed for intensity of *M.perstans* infection. Hookworm, *M.perstans* and *P.falciparum* infection were significantly associated with age and socioeconomic status; hookworm and *M.perstans* were associated with tribe; hookworm and *P.falciparum* were associated with HIV infection (data not shown). After adjusting for these potential confounding factors, the association between hookworm and *P.falciparum* was reduced. The association between *M.perstans* and *P.falciparum* was reduced, but remained strong.

There was also a statistically significant association between hookworm and *M.perstans* (OR 2.70 (95% confidence interval (CI) 2.20-3.31, p=<0.001)) and an interaction between hookworm and *M.perstans* in relation to their associations with *P.falciparum* (p=0.047): the prevalence of *P.falciparum* was 7.5% percent among participants with neither hookworm nor *M.perstans*, 11.5% among those with hookworm only, 18.8% among those with *M.perstans* only and 17.2% among those with both. Thus the hookworm-*P.falciparum* association was seen only in the absence of *M.perstans*. Adding the interaction term to the model increased the strength of the individual associations with *P.falciparum* for hookworm and *M.perstans* (aOR: for hookworm-*P.falciparum* in the absence of *M.perstans* 1.43 (95% CI 1.03-1.98; p=0.034); for *M.perstans*-*P.falciparum* in the absence of hookworm, 2.29 (95% CI 1.46-3.59; p<0.001) and for both hookworm and *M.perstans* with *P.falciparum*, 1.80 (95%CI 1.23-2.65; p=0.003).

The geographical distribution of women's homes and of zones is shown in the Figure and the distribution of infections by zone is indicated in table 2. There was an absence of *P.falciparum* infection in the area to the extreme southwest of the peninsula (zone one), and in the urban area of Entebbe town centre to the east of the airport runway (zone two). Two areas with increased density of infection were found to the northwest of the town; one at the northwest tip of the spur that reaches the inlet of the lake (zones three and four), and one on the edge of the adjacent swamp (zone three). Moving inland to the north was a band of more diffuse *P.falciparum* infection. Hookworm infection was spread throughout the study area, with little geographical clustering. *M.perstans* infection was spread across the study area with increased infection in the easternmost spur (zone 12). Moving north and west from this area were two further areas with increased density of infection.

In addition to prevalence of *P.falciparum*, hookworm and *M.perstans* infections, the adjusted ORs for co-infection, stratified by zone (defined in the Figure), are shown in Table 2. The adjusted ORs for hookworm-*P.falciparum* co-infection varied by geographical location: for example, in zone nine there was a particularly strong association, not observed in zones four, seven and eight. The association between *M.perstans* and *P.falciparum* infection was more consistent.

After adjusting for zone in addition to potential confounding factors, the odds ratios for both the hookworm-*P.falciparum* and *M.perstans*-*P.falciparum* associations increased slightly (aOR: hookworm-*P.falciparum* in the absence of *M.perstans,* 1.53 (CI 1.09-2.14; p=0.014); *M.perstans*-*P.falciparum* in the absence of hookworm, 2.33 (95% CI 1.47-3.69; p<0.001) and for both hookworm and *M.perstans* with *P.falciparum*, 1.85 (95%CI 1.24-2.76; p=0.002). As in the crude analysis, no associations between other helminth species and *P.falciparum* were observed in the adjusted model.

Discussion

This study offered a unique opportunity to examine helminth-malaria parasite co-infection in the neglected demographic stratum of pregnant women. The principal finding was a strong association between asymptomatic infection with *Plasmodium falciparum* and *Mansonella perstans*. A weaker association was observed between hookworm and *P.falciparum* infections, and there was an interaction between infections of the two helminths, such that the effect of hookworm was only seen in the absence of the stronger association with *M.perstans*. To our knowledge, this analysis provides the first report of an association between a filarial helminth infection and malaria parasites in humans. The results have implications for understanding of the host-parasite relationship, particularly in pregnancy, and for targeting treatment of co-infections among vulnerable groups.

This study focused on the issue that the geographical distribution of parasitic infections exhibit spatial dependency over small distances [27], and represents a step forward from studies conducted in one location that had assumed no spatial clustering, or had not measured residential location[18, 22, 38]. Geographical zones were defined based on simple geography, altitude, vegetation and location of settlements, as these have been found to correlate spatially with parasite infection [27, 39]. The zones provide a means to analyse the different environments of, for example an exposed costal location of zone 1 compared with the very different urban environment of zone 3 as separate entities, and to adjust for these differences in analysis. However, the stratification had limitations; while the aim was to provide a detailed stratification by environment, there was a need to strike a balance between achieving homogeneity within the zones without creating zones so small that the analysis is not sufficiently powered. With probability of infections known to exhibit spatial dependency over small distances [27], it may have been incorrect to assume homogenous parasite density within the study zones of up to four km in diameter. The use of zones in the analysis presented here enabled the description of variation between areas, but not within, areas..

Stratification by zone revealed considerable variation in the probability of co-infection with geographical location, particularly for hookworm-*P.falciparum*. This may be related, in part, to the observed variability in the prevalence of hookworm and *P.falciparum* infections. Associations were strongest in zones where the infection prevalence of both hookworm and *P.falciparum* were highest, consistent with a hypothesis that probability of infection with *P.falciparum* increases with intensity of hookworm transmission.

A considerable number of women seen at the antenatal clinic were excluded from this study; it is unlikely that the major reasons for exclusion created an important bias. The principal reason for exclusion, residence outside the study area, was appropriate to this analysis. A bias in relation to HIV status of those excluded is unlikely to have affected the results, since the principal effects showed no interaction with HIV among those analysed.

Women were included in this study only if they were well on the screening day, with no complaints (e.g. fever) and no gross evidence of severe, helminth-induced disease (such as anaemia, bloody diarrhoea, or overt liver disease). This analysis therefore addresses associations between helminth infections and asymptomatic *P.falciparum* parasitaemia, which may differ from associations between helminths and symptomatic *P.falciparum* infection [40]. Assuming that helminth infections are more often chronic and long-lived than infection with *P.falciparum*, the observed positive association between helminths and asymptomatic *P.falciparum* parasitaemia may imply an increased likelihood of *P.falciparum* infection, a reduced likelihood of clearing *P.falciparum*, and, or, a reduced likelihood of developing symptoms and seeking medication.

The first possibility, a helminth-*P.falciparum* association due to increased likelihood of infection with *P.falciparum* among women with helminth infections, could arise through behavioural or environmental factors, leading to increased exposure to both types of infection. Both *M.perstans* and *P.falciparum* are transmitted by flying insect vectors: *M.perstans* by *Culicoides* midges [41] and *P.falciparum* by *Anopheles* mosquitoes [26]. It is plausible that the distribution of these two vectors may be spatially correlated, as both require water sources for larval breeding [26, 42] though it is not clear whether the required conditions are exactly the same. Similarly, hookworm larvae flourish in damp soil and grass, which may be found close to stagnant water that is the breeding ground for malaria parasites. The slight reduction in the helminth-P.falciparum associations with adjustment for socio-demographic factors suggests a possible contribution of other behavioural effects; such as differences in the usage of antimalarial drugs during pregnancy, prior to enrolment

in the study; however, prior consumption of antimalarials showed no statistically significant association with helminth infection and adjustment for prior consumption of antimalarials did not alter the observed effects (data not shown). On the other hand, adjustment for geographical zone strengthened the associations, suggesting that, at zone-level, common environmental factors could not explain the effect. Thus the possibility that helminths may lead to a biological increase in susceptibility to infection, or to persistence of asymptomatic infection with *P.falciparum* remains plausible. This accords with previous studies suggesting associations between helminths and an increased incidence of *P.falciparum* infection [20], higher parasite counts and delayed parasite clearance [17], but a reduction in disease severity [21-24]. It is not possible to say whether the higher prevalence of *P.falciparum* among helminth-infected women resulted in increased incidence of disease events from the data in this cross sectional study.

Hookworm and *M.perstans* were the commonest helminth infections among participants in this study. The less common infections, *S.mansoni, Strongyloides, Trichuris* and *Ascaris*, showed no associations with *P.falciparum*, in conflict with some previous reports [20, 43-45], but in agreement with one other study from Uganda that reported no association [17]. For these species, a real association (if present) might not have been detected in this study because few women were infected (particularly with *Ascaris*), because the intensity of helminth infections was low, or because of misclassification of low-intensity infections as negative, resulting from the examination of a single stool sample (multiple samples are required for high sensitivity of detection) [46-48]. Such misclassification may also have contributed to the relatively weak observed effect of hookworm. By contrast, preliminary results suggest that the Knott's method used for assessment of *M.perstans* infection was particularly robust, with serial results in the same women showing 96% agreement for infection status and a correlation coefficient for microfilarial counts per millilitre of 0.88 ($p<0.001$) (AME, unpublished data).

This study does not explore potential biological mechanisms for the observed associations between helminths and *P.falciparum*. However, previously proposed mechanisms include the suggestion that the immuno-regulatory effects of helminths, which allow their own long-term survival in the host [9], "spill-over" to impair the immune response required to protect against or eliminate malaria parasites. *M.perstans* is a long-lived filarial worm that inhabits serosal body cavities and reproduces through microfilaria which circulate in the blood and are transmitted through biting midges [42]. Despite residence and migration through blood and tissues, *M.perstans* infection seldom causes detectable pathology, and this attests to its particularly potent immuno-modulating properties. It is thus interesting to speculate that *M.perstans* might produce a particularly strong immuno-modulating effect on the response to other pathogens, and that this might over-ride the effects of related helminths, such as hookworm, when both are present, perhaps accounting for the observed interaction between the two helminth species. This will be more comprehensively described in a future paper.

This study specifically examined co-infections in pregnant women. The unique environment that exists within the pregnant body means that one should be cautious in applying these results to the general population. This may be particularly important in relation to associations with *P.falciparum*, because parasites are sequestered within the placenta during pregnancy [30] and may be less readily detected in the peripheral blood (sampled in this study). Apparent biological associations might possibly reflect helminth effects on the sequestration of *P.falciparum* parasites in the placenta, rather than effects on the prevalence of infection.

In summary, this analysis has examined helminth-*P.falciparum* co-infection in pregnancy and attempted to address the influence of residential location on associations between these

environmentally dependent parasites. It provides evidence of an association between hookworm and *P.falciparum*, and the first report of an association between *M.perstans* and *P.falciparum* infection, effects not explained by measured social or geographical factors. Given the plausible hypothesis of a biological interaction between helminths and *P.falciparum*, and increasing advocacy for de-worming, there is a need for prospective studies of the effects of helminths and their treatment on *P.falciparum* and other malaria parasites, incorporating surveys of residential location, vector entomology and recording of malaria infection rates and illness events.

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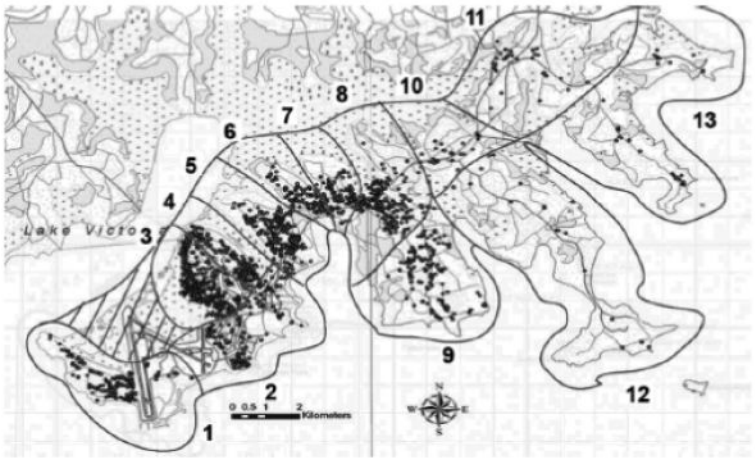

Figure 1.
Map of Entebbe and Katabi showing study area, location of women's residences and breakdown of geographical zones for spatial stratification.
Study area is situated 50km southwest of Kampala, Uganda's capital city

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Table 1

Associations (expressed as odds ratios – OR) between Helminth species and Malaria infection*

Helminths		Malaria Prevalence		Crude		Adjusted	
		Proportion	Percent	Crude OR	P Value	Adjusted OR†	P value
Hookworm	absent	118/1278	9.2%	1		1	
	present	138/1043	13.2%	1.50	0.002	1.28 (0.98-1.68)	0.072
M. perstans	absent	168/1829	9.2%	1		1	
	present	88/500	17.6%	2.11	<0.001	1.67 (1.25-2.24)	<0.001
S. stercoralis	absent	228/2031	11.2%	1		1	
	present	28/280	10.0%	0.88	0.53	0.78 (0.51-1.20)	0.24
S. mansoni	absent	214/1892	11.3%	1		1	
	present	42/429	9.8%	0.85	0.36	0.79 (0.55-1.13)	0.19
A. lumbricoides	absent	250/2267	11.0%	1		1	
	present	6/54	11.1%	1.01	0.98	0.85 (0.35-2.03)	0.70
T. Trichiura	absent	231/2110	11.0%	1		1	
	present	25/211	11.8%	1.09	0.69	0.91 (0.58-1.43)	0.68

*Data presented here do not include the full enrolment sample of 2507, as women were excluded from a calculation if any variable required for was not available.

†Adjusted for age, tribe, HIV status and woman's socioeconomic status

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Table 2

Helminth – Malaria prevalence and co-infection by geographical zone *

		Infection prevalence			Adjusted OR for combinations of co-infection			
Zone	**n**	**malaria**	**hookworm**	***M. perstans***	**Hookworm only and malaria**	***M. perstans* only and malaria**	**Hookworm, *M. perstans* and malaria**	**P Value**
1	267	4%	58%	23%	4.70	17.23	6.35	0.15
2	287	7%	40%	17%	0.96	2.16	2.43	0.49
3	502	14%	44%	20%	1.82	1.55	1.45	0.30
4	232	9%	33%	17%	0.77	5.21	0.68	0.24
5	366	11%	38%	19%	1.37	4.54	2.37	0.046
6	170	14%	44%	21%	1.57	0.60	2.67	0.42
7	151	10%	40%	16%	0.60	-†	1.76	0.50
8	198	11%	38%	29%	0.30	3.56	2.14	0.024
9	172	15%	64%	30%	13.87	2.98	4.16	0.002

*Zones 10-13 not included in this table because numbers of participants in these zones were small (≤41 participants).

†Adjusted odds ratio could not be computed: only seven participants in this zone had *M. perstans* without hookworm and none of these had malaria