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Epidemiology Of Malaria Infection Among School Aged Children In Kintampo North District, Ghana

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Epidemiology of Malaria Infection Among School Aged Children in Kintampo North District, Ghana: An Evaluation of Behavior, Nutritional Status, Hookworm Co-Infection, and Antibody Responses

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ABSTRACT

In June 2010 286 children from 16 participating schools were enrolled, with no more than one child from each household. Serum samples, fecal samples, and household surveys were used to assess the associations between the presence or density of malaria parasites and risk factors including nutritional status, hookworm infection, household risk prevention behaviors, and serum measures of parasite-specific immunoglobulin G (IgG).

Anthropometric and nutritional indicators were not associated with either outcome, nor was total malaria IgG. The primary risk factors for presence of infection included the house being sprayed in the past year (OR=0.04, p<0.001), child having a health care visit in the past year (OR=0.39, p<0.001), household malaria in the past year (OR=0.37, p=0.001), hookworm antibody density, with higher quartiles associated with elevated risk, greater household food insecurity, and geographic location.

Primary risk factors for elevated parasite density included the house being sprayed in the past year (OR=9.83, p<0.001), higher proportion of the household using a bednet the previous night, household and child history of malaria in the past year (OR=2.80, p=0.039; OR=0.15, p<0.001, respectively), hookworm antibody density, with the highest quartile associated with reduced parasite density, frequency of consumption of protein-rich food groups, with the middle tertile associated with elevated risk (OR=4.72, p=0.001), and geographic location. In addition, presence of hookworm infection increased both the risk of malaria infection and the risk of higher density of malaria parasites among those infected (OR=2.65, p=0.010; OR=2.81, p=0.001, respectively).

These risk factors highlight areas of programmatic interest, particularly the elevated risk of both any malaria infection and higher density of parasites among those infected with

hookworm. Further research should focus on elucidating the mechanism of this interaction, and health prevention and treatment measures should focus on reducing the burden of hookworm infection, especially among malaria infected children.

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Jennifer Du Mond

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Date

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INTRODUCTION

Global Burden of Malaria Infection

Malaria, a parasitic disease caused by several species of the genus *Plasmodium*, infects half a billion people annually worldwide.[1,2] Transmission occurs almost entirely in the tropics (Figure 1), and approximately 2.6 billion people live in regions of *Plasmodium falciparum* transmission, about one-third of the world population.[3] The African continent bears over half of the burden, and in Ghana there are 3.2 million cases recorded annually with estimates as high as 12.4 million clinical cases each year.[2,4] Estimates of up to 1 million deaths are due to malaria worldwide each year,[4] with about 38,000 deaths in Ghana.[5]

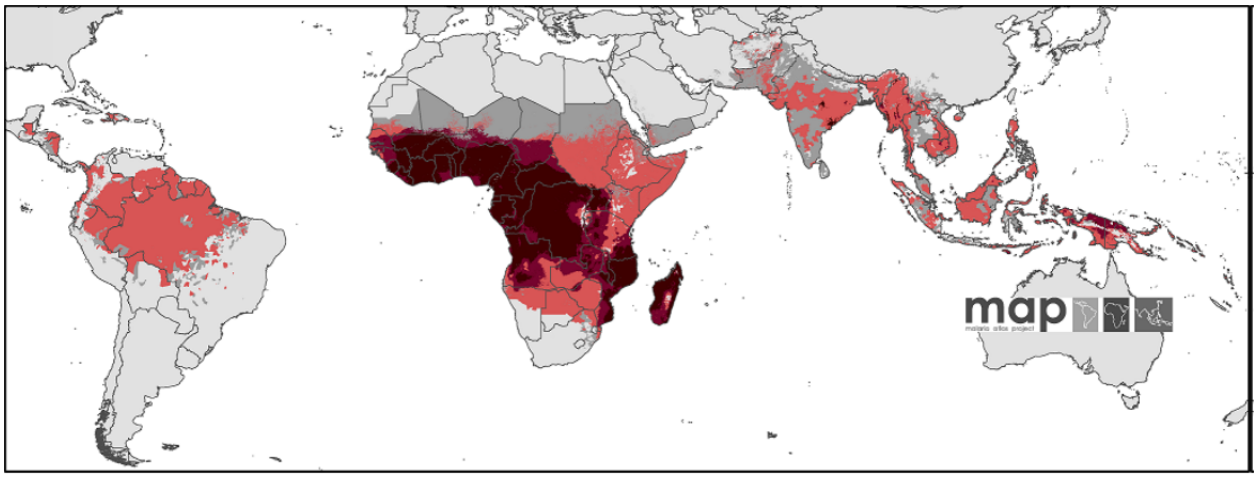

Figure 1: Global Distribution of malaria transmission risk. Light grey = no risk, dark grey = unstable risk, light red = low risk, medium red = intermediate risk, dark red = high risk.[3]

Malaria Life Cycle

Plasmodium parasites have human and mosquito-specific life forms. A full transmission cycle requires development in both hosts, and is detailed in Figure 2. Sexual reproduction occurs within the mosquito while asexual reproduction and gametogenesis occur within the human. The parasite is passed between the human host and mosquito vector when the mosquito takes a blood meal. An infective mosquito injects sporozoites into the blood stream, which enter hepatocytes and progress through the exo-erythrocytic cycle.[6] Development of symptoms typically occurs

between 7 and 30 days after an infectious bite, with shorter periods associated with *P. falciparum*.[7] Fully developed schizonts release merozoites into the blood stream and attack red blood cells, beginning the erythrocytic cycle. Periodic rupturing of infected blood cells, causing more severe symptoms and cyclical fever, occurs until infection is controlled, either by the

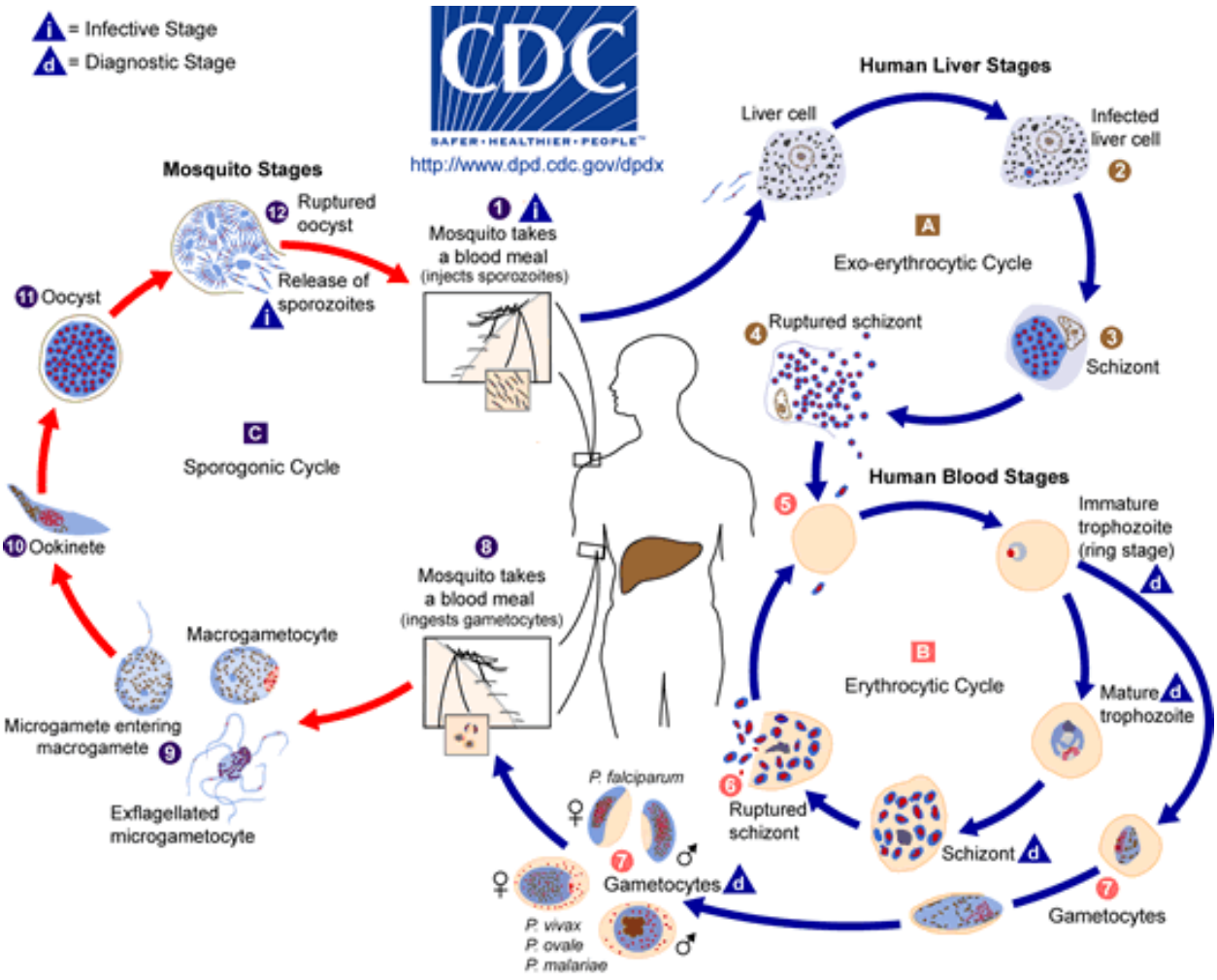

Figure 2: The lifecycle of *Plasmodium* parasites in humans and mosquitos. (CDC Website)
Three distinct cycles. The exo-erythrocytic cycle (A) begins when the mosquito takes a blood meal and injects infectious sporozoites into the human blood stream (1). The sporozoites travel to the liver and invade hepatocytes (2), reproducing asexually and forming a schizont (3). The schizont bursts and releases merozoites back into the blood stream (4). The erythrocytic cycle (B) begins with invasion of erythrocytes by merozoites (5). Once inside an erythrocyte, merozoites differentiate into ring form trophozoites. Trophozoites then either undergo additional asexual reproduction within the erythrocyte, leading to formation of another schizont and release of additional merozoites (6) or differentiate into gametocytes (7) which are taken up by a mosquito during the next blood meal (8), beginning the sporogonic cycle (C). Once inside the mosquito midgut, micro- and macrogametocytes fuse (9) and form an ookinete (10), which penetrates the gut wall and develops into an oocyst (11). Within the oocyte, sporozoites develop, and when the oocyte ruptures (12), the sporozoites migrate to the mosquito salivary glands for release during the next blood meal (1).

immune system or through medication.[8] Some of the released merozoites will morph into gametocytes, which are then picked up by another mosquito. Sexual reproduction occurs within the mosquito gut, and sporozoites migrate to the salivary gland for further transmission. Blood stage infection is associated with significant anemia and immune inflammatory action due to erythrocyte rupturing, and the majority of morbidity and mortality is due to this phase of infection.[9]

Pathogenesis and Clinical Features

Clinical malaria may be caused by one of five *Plasmodium* species: *falciparum, vivax, malariae, ovale, and knowlesii*. Of these five species, *P. falciparum* is both most prevalent worldwide and typically causes the most severe clinical disease, although the emerging *P. knowlesii* may also be fatal, as can *P. vivax* in rare cases.[7,10] Malaria infection may be associated with asymptomatic parasitemia; mild symptoms including fever, chills, headache, nausea, vomiting, diarrhea, jaundice, and anemia; or severe symptoms including coma, pulmonary edema, renal failure, spontaneous bleeding, convulsions, and death.[10] The full range of clinical symptoms is typically only seen in children, although immunologically naïve adults may also experience severe symptoms or death.[8,10]

Severe malarial disease, as described above, is treatable when addressed promptly. Symptoms typically begin with a fever, and treatment within the first 24 hours generally leads to recovery. Cases of malaria involving severe anemia, cerebral malaria, and respiratory distress are most severe and most likely to lead to death. Given the multiple systems involved, it is unlikely that a single mechanism or pathway is responsible for all cases of severe malaria, but proper treatment of parasitic infection can greatly reduce the risk of complications.[8]

Episodes of fever and associated clinical symptoms coincide with peaks of higher blood parasitemia as erythrocytes burst and merozoites are released.[8] However, the relationship between disease severity and density of blood parasitemia is poorly elucidated, as parasitemia depends on the stage and synchronicity of infection.[10] In addition, symptom-inducing parasite densities may be different for subjects of different ages, races, body mass, sex, or other host factors. Repeated exposure leads to almost complete immunity to severe complications and death.[8]

As immunity develops, even mild clinical symptoms may cease, although low grade parasitemia may persist.[6] Asymptomatic malaria may still be associated with cellular abnormalities such as reduced platelet counts.[10] Challenges for determining an asymptomatic infection include detection of parasite (in some cases, subjects may be smear negative but PCR positive) and establishing temporality. Detected asymptomatic infection may in fact be pre-symptomatic and still in the incubation phase. It is difficult to assess the extent of asymptomatic infection, as persons without symptoms are unlikely to submit for testing, so estimates of the prevalence of asymptomatic infection must be based on cross-sectional population studies. For the infected individual, asymptomatic infections likely do not pose a health risk, but these infections are estimated to be 4-5 times as prevalent and infective for longer periods compared to treated symptomatic infection, posing a significant public health issue.[10] Detection and treatment of these infections is important for reducing the burden of disease. However, in endemic areas, up to two-thirds of blood smear negative subjects may have subpatent infections only detectable by PCR.[11] Extensive testing for subpatent infection is costly, and many facilities may not have the necessary resources to conduct the tests, but even subpatent infections have been shown to be transmissible.[10]

Immunology and Immunoepidemiology

In endemic areas, malaria immunity often develops by mid-childhood.[8,10,12-14] Immunity develops after repeated exposure, and effective immune protection has been attributed to the cytophilic antibodies IgG1 and IgG3.[15-17] Early antibody development to malaria is usually IgG2 and IgG4, which have not been associated with any enhanced protection.[10] Protective antibodies are usually against the merozoite stages of the parasite.[8] The slow development of immunity is likely due to the large variety of malaria antigens.[6] Previous studies have indicated that total IgG concentration is associated with ability to neutralize malaria antigens, but this has been specifically attributed to the subclass IgG1 when both have been measured.[18-21]

The role of other antibody classes in immunity to malaria remains poorly elucidated. Elevated IgE levels have been associated with increased risk of cerebral malaria,[10] but also with decreased risk of infection and severe disease.[22] Some studies have shown a possible protective effect of IgM, although the statistical significance for most comparisons is lost in adjusting for covariates,[17] while others suggest that IgM offers no protection and may in fact inhibit the protective action of other antibodies.[23,24] Limited investigations of the role of IgA have been undertaken, but those that have studied this class of antibodies have found a potential protective effect of elevated levels.[22,25] IgD is even less well described, but limited reports suggest no involvement in malaria protection.[26]

Epidemiology of Hookworm Co-infection

Soil-transmitted (intestinal) helminths, in general, have a geographic range similar to malaria.[27] In many parts of sub-Saharan Africa, including Ghana, there is a high prevalence of both hookworm and malaria (Figure 3).[28] The high prevalence of both infections means that there is a high likelihood of co-infection, and over 25% of schoolchildren are at risk for co-

infection.[28] Among the soil-transmitted helminthes, risk of co-infection is greatest with hookworms.[29] In addition, prevalence of hookworm infection increases with increasing age,[30] and malaria infection is most prevalent in young children, with a steep reduction in incidence in the teen years due to progressively developing immunity. Studies examining the clinical impacts of co-infection are inconsistent. A previous study in the same area of Ghana indicates that anemia risk is higher among those infected with malaria alone than among those infected with hookworm alone or co-infected,[31] and a study in Côte d'Ivoire found lower odds of both anemia and cellular iron deficiency in children age 6-8 with hookworm and malaria co-infection compared to *P. falciparum* alone.[30] However, studies examining the anemic impact of these infections in Ethiopia and Kenya found opposite results - reduced hemoglobin levels, higher prevalence of malaria, and an adjusted odds ratio of anemia of 2.58 among people co-infected with hookworm and malaria compared to malaria alone.[27,29]

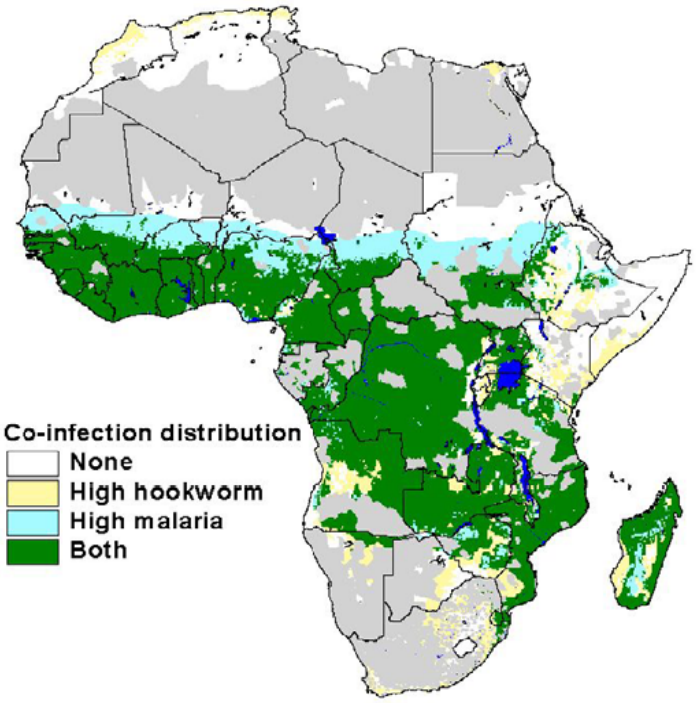

Figure 3. Distribution map of malaria and hookworm endemicity, showing overlapping ranges for co-infection.[28]

Co-infection rates may be even higher than expected by chance alone. A Kenyan study found that 35.2% of school-age children were heavily co-infected, a dramatically higher figure than the 3.5% of preschoolers found to be co-infected by the same study.[29] This age discrepancy is partially mediated by the mechanism of exposure for hookworm, leading to increasing likelihood of hookworm infection with age. In conjunction, although school-age children in endemic regions generally have some immunity to malaria, the pre-existing presence of a hookworm infection may alter the child's susceptibility by disrupting the Th1/Th2 balance and affecting immunoglobulin production.[10]

Nutrition and Immune Responses in the Context of Malaria Infection

In Ghana, a large number of children are undernourished. A survey conducted by the Ghana Health Service in 2008 found that 28% of children under 5 are stunted, 10% severely stunted, 9% and 2% are moderately and severely wasted, respectively, and 14% and 3% are moderately and severely underweight, respectively.[32] Reduced nutritional status, as indicated by anthropometric measures such as those given above or by dietary quality and quantity measures, may be associated with numerous immune deficiencies. Significant underweight is indicative of inadequate absorption of key nutrients, either because of infection or insufficient consumption, which in turn limits the body's capacity to respond to infection. In particular, limited intake of animal source foods is often associated with insufficient iron intake, which can exacerbate the effects of disease associated anemia. Persistent intestinal parasite infection, especially with concomitant malaria infection, may be a contributing factor to the reduced nutritional status of children in Ghana.[33] Hookworm and malaria infection both contribute to malnutrition through several pathways. Hookworms can induce pathophysiological reactions and reduce food intake, contribute to blood loss, and cause intestinal inflammation and reduced absorption.[27] Malaria

induces inflammatory cytokines that increase the likelihood of anorexia and a catabolic response, leading to degradation of stored body fuels and tissues.[27]

Protein has been particularly implicated as an essential energy source; farmers and mud workers in Iraq given protein supplements recovered more quickly from anemia than those not given supplements.[34] Protein from animal sources is generally accepted as the most efficiently absorbed source of iron,[35,36] and malnutrition and iron-deficiency anemia are often associated among people with low intake of animal source protein and low dietary diversity.[37] Malnourished individuals are also often infected with soil-transmitted helminths and malaria, an association related to nutritionally derived reduced immune functionality.[37]

Malaria Control Efforts

The primary methods of malaria prevention are vector control via indoor residual spraying (IRS), prevention of transmission from infected human to mosquito through various antimalarial drug treatments, and prevention of transmission from an infected mosquito to a susceptible human using bed nets. The combination of these three interventions has shown a reduction of over 75% in the number of slide-positive malaria cases in all age groups.[10]

Vector control, which is primarily undertaken through IRS and the use of insecticide treated nets (ITNs) and long-lasting insecticidal nets (LLINs), reduces the frequency with which humans interact with the intermediate host by eliminating mosquitoes in the immediate environment.[38-40] The Global Malaria Eradication Program, started in 1955 but abandoned in 1969, was highly effective at reducing the prevalence of malaria, both using IRS and targeted therapy for systematically detected cases.[41] Depending on the chemical that is used and the surface it is sprayed onto, spraying may be effective for up to 6 months, during which time mosquitoes that enter the home and land on the sprayed surfaces will be killed.[42] A meta-analysis

of thirteen studies examining the effect of IRS on malaria prevalence, all of which had individual protective effects, found a pooled relative risk of 0.38, or a 62% reduction in prevalence of malaria.[43] A Cochrane review of IRS also provided fairly consistent evidence for a protective effect across a variety of settings and age groups.[44]

Insecticide-treated bed nets (ITNs) are another approach to reducing malaria infection. ITNs combine a physical barrier to contact with mosquitoes as well as insecticidal action for any mosquitoes that land on the net. Many nets have to be re-treated after several months, because the concentration of the insecticidal chemical will diminish over time, especially if the net is washed. More recently, long-lasting insecticidal nets (LLINs) have been developed and distributed. Such nets are factory impregnated with insecticide and maintain their insecticidal activity for 3 to 5 years.[45] ITNs and LLINs provide a strong protective effect of 50.3% compared to no net use, and a less strong protective effect of 24.3% compared to non-insecticide treated nets in quality-score adjusted pooled analysis.[46] In addition to preventing the user from acquiring malaria parasites from an infected mosquito, ITNs and LLINs prevent mosquitoes from picking up gametocytes from infected humans, breaking the transmission cycle in two places.[4]

The final method of malaria control is antimalarial drug therapy. The preferred antimalarial drug class is artemisinin-based combination therapy (ACT).[47] Many circulating parasite strains have developed resistance to the original drugs developed, such as chloroquine, rendering these drugs ineffective. ACTs, however, act quickly and have less associated resistance, although concern is growing about increasing resistance to these drugs as well. Use of ACTs is typically limited to patients that present with clinical malaria (or anything that looks like it), but alternative measures would be to provide mass drug administration or mass screening to identify those infected and eliminate infection in the many subclinical cases.[41]

Geospatial clustering of Infection

Malaria requires, at a minimum, sufficiently close interaction of an infected host and a susceptible host, as well as a mosquito population to facilitate the transmission from infected to susceptible. Localized malaria prevalence and density of mosquito population are important factors in determining the likelihood of transmission from infected to susceptible host.[48] Lower prevalence of malaria in a community means a smaller percentage of mosquitoes will pick up transmissible parasites, and a smaller density of mosquitoes means a reduced likelihood of any bites.

In addition, environmental factors and host genetic and socioeconomic factors are more likely to be similar among people in a defined geographic region than between people from different areas.[48] Climate impacts the rate of mosquito development and mosquito lifespan, and a shorter lifespan leaves less opportunity for transmission of the parasite.[49] Host genetic factors, including race, and blood characteristics, alter the immune response to infection, and are more likely to be similar within a specified region, especially if immigration and emigration are infrequent.[50,51] Socioeconomic factors such as occupation and household absolute wealth index are also likely to be similar within villages, and any previously introduced interventions would have taken place at the community level.

Study Rationale

A previous examination of this data assessed the relationship between hookworm infection, nutritional status, and treatment failure, with malaria infection investigated only as a confounding factor in elucidating these relationships.[52] However, considerable data from this study is available for examining the factors associated with malaria infection, and previous work (by other researchers) has suggested, but failed to conclusively define, the association between

infection risk and nutritional status, socio-economic status, prevention behaviors, and the density of antibodies to both diseases. This study aims to determine the effect of these factors within this population and add to the evidence for previously examined malaria risk factors.

Study Objectives

The following are the specific objectives of this examination of malaria risk factors among children school age children in Kintampo North District, Brong Ahafo Region, Ghana:

1. Assess appropriateness of the use of a malaria parasitemia measure defined per 200 white blood cells.
2. Determine the relative utility of two age-independent, unstandardized measures of nutritional status, weight-for-height and BMI.
3. Characterize the nutritional and socioeconomic status, as well as prevention behaviors, of the study population by malaria infection status.
4. Identify the risk factors associated with malaria infection and increased parasite density.

METHODS

Ethical Approval and Participant Enrollment

This project was approved by the Yale University Human Investigations Committee (HIC) in June 2010 (protocol number 0705002669). Approval from IRBs of the Noguchi Memorial Institute for Medical Research (NMIMR), the Ghana Health Service, and the Scientific Review Committee and the Institutional Ethics Committee of the Kintampo Health Research Center (KHRC) were also received in June 2010.

Following IRB approval, a tiered consent and contact system, as previously described,[52,53] was used to identify 16 schools, where children ages 6-11 were screened and considered eligible if their height-for-age Z-score (HAZ) was below -1.80 or above -0.10. No more than one child per household was enrolled, and community meetings were held in the local language to ensure parental and child consent and understanding.

Sample Collection and Processing

Fecal samples for assessment of hookworm infection were collected by distribution of two sample cups per participant, and analyzed for hookworm ova using the Kato-Katz method, described by the World Health Organization.[54] Approximately two weeks after collection of fecal samples, 5 mL of blood were collected from each participant. Aliquots of 1 mL were taken from each sample and combined with EDTA to prevent coagulation. One drop of the 1 mL sample was used for malaria rapid diagnostic testing, and one drop each was used for thick and thin blood smears. The remainder of the 1 mL aliquot was used for a complete blood count. The remainder of the original 5 mL sample was centrifuged and serum separated and stored for additional analysis.

Plasmodium falciparum was cultured for antigen preparation by incubating parasites (3D7, BEI Resources Laboratory, NIAID, NIH, Bethesda, MD) with human blood cells (American Red Cross). Infection cultures and uninfected control cells were subjected to the same conditions and incubated overnight in 5 mL of RPMI (Sigma-Aldrich) containing 10% human serum and 25 μg/ml gentimicin at 37°C in an atmosphere of 5% O_2, 5% CO_2, and 90% N_2. Cultures were then briefly centrifuged at 500 x *g*, washed at a concentration of 40% in RPMI, and incubated for an additional 6 hr at 37°C. Following the second incubation, cells were pelleted and immediately resuspended in a protease inhibitor mixture (Sigma, St. Louis, MO) containing 0.12 mM N-tosyl-L-lysyl chloromethylketone, 0.25 mM N-tosyl-L-phenylalanyl chloromethylketone, 2 mM phenylmethylsulfonyl fluoride, 0.23 U/mg of aprotinin, 50 mg/ml of chymostatin, 50 mg/ml of leupeptin, and 1 mM EDTA. After gentle mixing, saponin was added to a final concentration of 0.1% to lyse cells. Parasites were separated by centrifugation and stored until use. Solubilized parasite antigen was prepared by lysing parasites in 100mM NaCl, 1 M EDTA, 50 mM Tris HCL, and 1% triton X-100. Total protein concentration was measured using a BCA kit (Thermo Fisher Scientific, Rockford, IL).

Malaria-specific IgG in human serum was measured by enzyme linked immunosorbent assay (ELISA). Prepared *P. falciparum* antigen diluted in PBS to 2μg/ml was added to flat-bottomed 96-well immulon 1HB plates (Dynex Technology Inc., Chantilly, VA) and incubated overnight at 4°C. Plates were washed with a mixture of 0.05% tween-20 in PBS (PBST) and blocked with 5% nonfat dry milk diluted in PBST for 1 hr at room temperature. Participant serum samples were diluted 1:50 in PBS with 0.05% tween-20 and 0.1% non-fat dry milk, added to duplicate wells and incubated at 37°C for 2 hr. After incubation and several more washes with PBST, malaria specific antibodies were detected using peroxidase conjugated goat anti-human

IgG (KPL, Gaithersburg, MD), which was added to the plate and incubated for an additional 2 hr at 37°C. Plates were washed again and bounded secondary substrate was detected using 100 µl ABTS [2,2=-azinobis (3-ethylbenzthiazolinesulfonicacid)] substrate (Sigma, St. Louis, MO) added to each well. Color development was measured after 60 minutes using a microplate reader (Molecular Devices, Inc., Sunnyvale, CA) at an optical density of 405nm. Plates were normalized to a pooled positive control run on all plates, and endeminc and non-endemic negative controls were used to measure non-specific signal interference. Plate blanks did not contain primary serum antibody reagents and were subtracted from all values.

Hookworm-specific IgG antibodies to *A ceylancicum* excretory-secretory (ES) proteins were measured using a similar process of antigen preparation and ELISA testing, as described previously.[31,53,55] Serum reactivity to each antigen was categorized by quartiles for statistical analysis.

Questionnaire Data Collection

The household questionnaire (Appendix) was adapted from the Demographic and Health Surveys.[56] Six Ghanaian public health students from the Ghana Rural Health Training School in Kintampo were trained to ensure understandability and cultural sensitivity of the questionnaire, and their ability to translate the questionnaire into Twi and ask the questions in a consistent way. Students were paired for administration of the surveys at participants' homes.

Statistical Analysis

The data in this study was collected and organized in Microsoft Excel (2010). Analysis was carried out using SAS Software version 9.3 (SAS Institute, Cary, NC, 2012). Bivariate statistical analysis used t-tests for continuous variables and chi-square tests for categorical variables. Outcome variables for binary and multivariate analyses were a binary measure of the

presence of malaria infection and high or low parasitemia. Chi-square tests were used to identify variables for use in multivariate models. Adjusted multivariate models were determined using logistic regression with generalized estimating equations, using categorized geographic region as the clustering variable. Stepwise backward elimination and the Quasi-Akaike Information Criterion (QIC) and QICu, which takes into account the number of parameters in the model, were used to select the best model for each examined binary outcome. The final models contain only the adjustment variables of age, sex, and absolute wealth index, variables of statistical significance ($p<0.05$), and any variable whose removal would diminish the overall fit.

RESULTS

Serum and Anthropometric Indicators

Malaria parasitemia may be measured using parasite density per a specified number of red or white blood cells. The white blood cell (WBC) count approach generates a measure of the number of parasites per microliter of blood, using the white blood cell count per microliter to convert parasites per 200 WBC to a per microliter value. The red blood cell count approach measures percentage of infected red blood cells. This data set contains parasitemia determined using the white blood cell method, so the correlation between parasite density per microliter and several serum measures that are commonly associated with clinical malaria were examined: the white blood cell count, red blood cell count, and hemoglobin.

For each of the examined blood measures, no association was found with parasite density. Across the four categories of parasitemia examined (no parasites, 1-499 parasites, 500-1999 parasites, and 2000 parasites or more), average white blood cell counts, red blood cell counts, and hemoglobin were very similar, with no apparent trends. Sex and age were also investigated as potential confounders, and although females made up a higher proportion of the study population in the no parasite and lowest parasite density groups, neither factor was statistically associated with parasite density (p-value for sex = 0.174, p-value for age = 0.854).

Table 1: Demographic and Blood Measures by Parasite Density[a]

Characteristic	no parasites (n = 49)	1-499 parasites/uL (n = 80)	500-1999 parasites/uL (n = 60)	2000+ parasites/uL (n = 60)	p[c]
Female, n (%)	29 (59.2)	45 (57.0)	25 (41.7)	28 (46.7)	0.174
Age in years, mean ± SD	8.7 ± 1.8	9.1 ± 1.8	8.9 ± 1.5	8.7 ± 1.7	0.854
WBC, mean ± SD	6.8 ± 2.1	7.0 ± 2.2	7.2 ± 1.7	6.7 ± 1.8	0.690
RBC, mean ± SD	3.9 ± 0.6	4.1 ± 0.4	4.1 ± 0.5	4.0 ± 0.6	0.713
Hgb, mean ± SD	10.2 ± 1.6	10.7 ± 1.0	10.6 ± 1.0	10.4 ± 1.2	0.729

[a] Table values are mean ± SD for continuous variables or n (column %) for categorical variables
[c] P-value is for t-test (continuous variables) or χ^2 test (categorical variables)

Participant ages and birthdates were collected both from school administrators and household interviews. However, this information was not reported from all interviews, and when available from both sources, there were many inconsistencies, which reduced the utility of WHO age-standardized anthropometric measures. To maintain the inclusion of nutritional status as a factor in subsequent analysis, while minimizing confounding by age, two age-unstandardized measures of nutritional status were examined: weight-for-height and BMI. Linear regression, using age as a continuous variable, as well as ordinal logistic regression, using age tertiles, revealed significant associations with both BMI and weight-for-height (p-value for all <0.0001). BMI had smaller R^2 values in both regression analyses, indicating that it is a less age-sensitive measure of nutritional status. Sex was examined as a possible confounder of these two measures of nutritional status, but no statistically significant association was found.

Descriptive Statistics According to Malaria Infection Status and Parasite Density

Bivariate analysis was used to assess the nutritional, demographic and socioeconomic, household exposure, prevention behavior, and health indicators of the population. Characteristics were examined against both presence or absence of malaria parasites and high or low burden of parasites (above or below the median) among those positive. Anthropometric and nutritional indicators are described in Table 2. Sex was borderline statistically significantly associated with lower parasite burden (p=0.056), and low parasitemia was more common among older children than younger children (p=0.016). None of the examined food intake or anthropometry measures were significantly associated with either presence of parasitemia or density of parasitemia.

Demographic and socioeconomic characteristics by malaria infection status are described in Table 3. Head of household occupation was significantly associated with being infected (p=0.013) but not parasite density, while a greater number of children under the age of 5 in the

Table 2: Anthropometry and Nutritional Indicators by Malaria Infection Status[a]						
Characteristic	**Malaria negative (n = 49)[b]**	**Malaria positive (n = 199)[b]**	**p[c]**	**Low parasitemia (n=100)[b]**	**High parasitemia (n=99)[b]**	**p[c]**
Female	29 (59.2)	98 (49.3)	0.213	56 (56.0)	42 (42.4)	0.056
Under 8yrs of age	20 (40.8)	61 (30.7)	0.370	27 (27.0)	34 (34.3)	0.016
8-9yrs	15 (30.6)	77 (38.7)		33 (33.0)	44 (44.4)	
10yrs and up	14 (28.6)	61 (30.7)		40 (40.0)	21 (21.1)	
Lowest BMI quartile	14 (28.6)	48 (24.1)	0.594	19 (19.0)	29 (29.3)	0.300
Quartile 2	10 (20.4)	56 (28.1)		30 (30.0)	26 (26.3)	
Quartile 3	11 (22.5)	50 (25.1)		29 (29.0)	21 (21.2)	
Quartile 4	14 (28.6)	45 (22.6)		22 (22.0)	23 (23.2)	
Weekly protein food groups, lowest tertile	16 (34.8)	51 (29.8)	0.547	29 (33.7)	22 (25.9)	0.515
Middle tertile	15 (32.6)	49 (28.7)		24 (27.9)	25 (29.4)	
Highest tertile	15 (32.6)	71 (41.5)		33 (38.4)	38 (44.7)	
0 or 1 Animal source foods, weekly	13 (27.1)	42 (24.0)	0.519	22 (25.3)	20 (22.7)	0.505
2	18 (37.5)	55 (31.4)		30 (34.5)	25 (28.4)	
3 or more	17 (35.4)	78 (44.6)		35 (40.2)	43 (48.9)	
Any household hunger	25 (51.0)	80 (40.2)	0.170	43 (43.0)	37 (37.4)	0.418
Household Food insecurity			0.126			0.832
None	3 (7.1)	42 (22.6)		24 (25.3)	18 (19.8)	
Some (1-5)	23 (54.8)	76 (40.9)		38 (40.0)	38 (41.8)	
Moderate (6-10)	9 (21.4)	40 (21.5)		19 (20.0)	21 (23.1)	
Severe (11-15)	7 (16.7)	28 (15.1)		14 (14.7)	14 (15.4)	

[a] Table values are n (column %)

[b] Numbers may not sum to total due to missing data, and percentages may not sum to 100% due to rounding

[c] P-values are for χ^2 test

Table 3: Demographics and Socioeconomic Indicators by Malaria status[a]						
Characteristic	**Malaria negative (n = 49)[b]**	**Malaria positive (n = 199)[b]**	**p[c]**	**Low parasitemia (n=100)[b]**	**High parasitemia (n=99)[b]**	**p[c]**
Head of Household education			0.742			0.161
Some	13 (27.7)	59 (30.1)		34 (34.7)	25 (25.5)	
None	34 (72.3)	137 (69.9)		64 (65.3)	73 (74.5)	
Head of Household occupation			0.013			0.105
None	3 (6.1)	10 (5.1)		8 (8.1)	2 (2.0)	
Farmer	33 (67.4)	167 (84.3)		79 (79.8)	88 (88.9)	
Small trader / Other	13 (26.5)	21 (10.6)		12 (12.1)	9 (9.1)	
Maternal caregiver education			0.729			0.969
Some	8 (19.1)	38 (21.5)		19 (21.4)	19 (21.6)	
None	34 (81.0)	139 (78.5)		70 (78.7)	69 (78.4)	
Maternal caregiver occupation						0.184
None	5 (10.6)	17 (8.6)	0.349	11 (11.1)	6 (6.1)	
Farmer	26 (55.3)	131 (66.5)		60 (60.6)	71 (72.5)	
Other	16 (34.0)	49 (24.9)		28 (28.3)	21 (21.4)	
No. of children in the house <5yrs	1.36 1.17	1.40 1.16	0.852	1.23 ± 1.16	1.58 ± 1.14	0.036
Household size	8.10 ± 4.83	8.01 ± 3.74	0.901*	7.53 ± 3.01	8.49 ± 4.31	0.069*
Absolute Wealth Index			0.772			0.524
Below Median	26 (53.1)	101 (50.8)		53 (53.0)	48 (48.5)	
Above Median	23 (46.9)	98 (49.3)		47 (47.0)	51 (51.5)	
Geographic Location			<0.001			0.088
South	8 (16.3)	54 (21.8)		31 (31.0)	23 (23.2)	
Middle South	10 (20.4)	83 (41.7)		39 (39.0)	44 (44.4)	
Middle North	12 (24.5)	41 (20.6)		24 (24.0)	17 (17.2)	
North	19 (38.8)	21 (10.6)		6 (6.0)	15 (15.2)	

[a] Table values are mean ± SD for continuous variables and n (column %) for categorical variables

[b] Numbers may not sum to total due to missing data, and percentages may not sum to 100% due to rounding

[c] P-values are for χ^2 test unless otherwise indicated

*Satterthwaite method for testing difference in means, for unequal variances

house and a greater number of total household members were associated with higher parasite density (p=0.036, p=0.069, respectively) but not presence or absence of infection. Geographic region was associated with both presence of infection (p<0.001) and higher parasitemia (p=0.088). No other demographic or socioeconomic factors were significantly associated with either the presence of malaria infection or the density of infection.

Malaria prevention behaviors and infection history are described in Table 4. Fewer children from houses that were sprayed in the past 12 months had malaria parasites compared to children from houses that were not sprayed (p=0.064), but among those infected, parasite density was higher among children from houses that had been sprayed (p=0.037). The child's history of malaria infection in the past year was also associated with infection status; malaria diagnosis in the past year was more common in malaria negative children (p=0.029) and in children with lower parasite density (p=0.025). The child's history of fever in the past month and a household member's history of malaria infection in the past twelve months were only associated with presence of infection. Higher proportions of malaria negative children had recent fever history (p=0.051) and recent household malaria (p=0.009). Household and child bed net usage, deworming in the past year, and household possession of at least one treated net were not associated with either presence or intensity of infection.

Health indicators, including use of the healthcare system, antibody levels for malaria and hookworm, and hookworm infection status are described in Table 5. Hookworm positive fecal sample and having a health card (an indication that the child has ever attended a hospital or clinic) were significantly associated both with presence of malaria infection (p=0.029 and p=0.044, respectively) and higher density of parasites (p=0.043 and p=0.028, respectively). Any history of vaccination was also marginally more common among those with higher parasitemia

Table 4: Malaria Prevention Behavior and Household Infection History by Malaria Infection Status[a]						
Characteristic	**Malaria negative (n = 49)[b]**	**Malaria positive (n = 200)[b]**	**p[c]**	**Low parasitemia (n=100)[b]**	**High parasitemia (n=99)[b]**	**p[c]**
House was sprayed in the past 12 months	8 (17.0)	16 (8.1)	0.064	4 (4.04)	12 (12.1)	0.037
Household bednet use previous night			0.700			0.174
None	16 (32.7)	53 (26.6)		32 (32.0)	21 (21.2)	
Some	10 (20.4)	45 (22.6)		23 (23.0)	22 (22.2)	
All	23 (46.9)	101 (50.8)		45 (45.0)	56 (56.6)	
Index Child used Bednet last night	4 (44.4)	31 (64.0)	0.222	14 (58.3)	17 (73.9)	0.260
House has at least one treated net	17 (34.7)	82 (41.4)	0.390	38 (38.4)	44 (44.4)	0.387
Anyone in the house dewormed in the past year	19 (38.8)	66 (33.7)	0.502	27 (27.3)	39 (40.2)	0.055
Anyone in the house had malaria in the past year	37 (77.1)	111 (56.6)	0.009	55 (55.6)	56 (57.3)	0.759
Index Child had fever in past month	31 (63.3)	94 (47.7)	0.051	48 (48.5)	46 (46.9)	0.828
Index Child dewormed in past year	9 (18.4)	29 (15.1)	0.576	11 (11.3)	18 (19.0)	0.141
Index Child had malaria in past year	22 (46.8)	59 (30.1)	0.029	37 (37.4)	22 (22.7)	0.025

[a] Table values are n (column %) for categorical variables

[b] Numbers may not sum to total due to missing data, and percentages may not sum to 100% due to rounding

[c] P-values are for χ^2 test

Table 5: Health indicators by Malaria Infection Status[a]						
Characteristic	**Malaria negative (n = 49)[b]**	**Malaria positive (n = 200)[b]**	**p[c]**	**Low parasitemia (n=100)[b]**	**High parasitemia (n=99)[b]**	**p[c]**
Child has health card	17 (34.7)	101 (50.8)	0.044	43 (43.0)	58 (58.6)	0.028
Last health care access			0.103			0.930
Within the last year	30 (61.2)	89 (48.1)		46 (48.4)	43 (47.8)	
More than one year ago or never	19 (38.8)	96 (51.9)		49 (51.6)	47 (52.2)	
Where child goes for health care			0.139			0.564
Hospital	16 (34.0)	87 (45.3)		41 (42.7)	46 (47.9)	
Local Clinic	18 (38.3)	44 (22.9)		21 (21.9)	23 (24.0)	
Pharmacist	10 (21.3)	39 (20.3)		20 (20.8)	19 (19.8)	
Other including family	3 (6.4)	22 (11.5)		14 (14.6)	8 (8.3)	
Where child goes for medication			0.609			0.533
Hospital	10 (20.8)	45 (23.1)		21 (21.2)	24 (25.0)	
Local Clinic	9 (18.8)	23 (11.8)		12 (12.1)	11 (11.5)	
Pharmacist	20 (41.7)	82 (42.1)		39 (39.4)	43 (44.8)	
Drugstore or other	9 (18.8)	45 (23.1)		27 (27.3)	18 (18.8)	
Child has had any vaccination	37 (86.1)	160 (89.9)	0.468	77 (85.6)	83 (94.3)	0.053
Current hookworm infection			0.029			0.043
Negative	37 (75.5)	116 (58.6)		65 (65.7)	51 (51.5)	
Positive	12 (24.5)	82 (41.4)		34 (34.3)	48 (48.5)	
Lowest Quartile of Malaria IgG Antibodies	6 (14.6)	48 (27.4)	0.200	23 (26.1)	25 (28.7)	0.179
Quartile 2	11 (26.8)	42 (24.0)		17 (19.3)	25 (28.7)	
Quartile 3	9 (22.0)	44 (25.1)		28 (31.8)	16 (18.4)	
Quartile 4	15 (36.6)	41 (23.4)		20 (22.7)	21 (24.1)	
Lowest Quartile of Hookworm IgG Antibodies	15 (31.3)	46 (24.2)	0.115	20 (20.6)	26 (28.0)	0.478
Quartile 2	15 (31.3)	42 (22.1)		23 (23.7)	19 (20.4)	
Quartile 3	12 (25.0)	48 (25.3)		28 (28.9)	20 (21.5)	
Quartile 4	6 (12.5)	54 (28.4)		26 (26.8)	28 (30.1)	

[a] Table values are n (column %) for categorical variables

[b] Numbers may not sum to total due to missing data, and percentages may not sum to 100% due to rounding

[c] P-values are for χ^2 test

(although greater than 85% of children fell into this category across all subdivisions). Last health care access more than one year ago appeared to be more common among those with malaria infection compared to those without, but this result was not statistically significant. No apparent trends were observed for where the child goes for treatment or medications or measures of blood antibodies to either malaria or hookworm.

Risk Factors for Malaria Infection and Parasite Density

Tables 6 and 7 contain the results of the generalized estimating equation logistic regression models for any malaria infection compared to none and high malaria parasitemia compared to low parasitemia, respectively. The level of parasitemia was examined in this way because ordinal logistic regression using a multilevel response variable including no parasitemia and three levels of parasite density failed to meet the proportional odds assumption.

Table 6 describes the model predicting any malaria infection. Geographic clustering was accounted for with repeated subject analysis at the level of geographic location, which was also included as a model covariate. The model is adjusted for the covariates of age, sex, and absolute wealth index. The final model had the best fit diagnostics as well as almost all explanatory variables at a statistically significant level. Of note is the remaining variable for whether or not the index child had malaria in the past year, which is not statistically significant but dramatically affected the fit of the model if removed. The largest effect size was due to indoor residual spraying. Children from houses that had not been sprayed were 25 times as likely as those from houses that had been sprayed to have malaria infection ($p<0.001$). Current hookworm infection ($p=0.010$), as well as hookworm antibodies levels in the 3rd (OR=3.82, $p=0.006$) or 4th (OR=3.07, $p=0.012$) quartiles were associated with increased odds of having malaria. The remaining statistically significant covariates were associated with reduced likelihood of having

malaria infection. Some food insecurity (OR=0.05, p<0.001) and high food insecurity (OR=0.04, p<0.001) were significantly associated with reduced likelihood of malaria infection after controlling for age, sex, and absolute wealth index. Moderate food insecurity was also associated with reduced likelihood of infection (OR=0.21), but this result was not statistically significant.

Table 6: Logistic Regression Model predicting Malaria Infection[a]

Characteristic	Adjusted Odds Ratio[b]	95% Confidence Interval	p
House was sprayed in the past year	0.04	0.02, 0.08	<0.001
Health care visit in the past year	0.39	0.25, 0.61	<0.001
Anyone in the house had malaria in the past year	0.37	0.20, 0.68	0.001
Index child had malaria in the past year	1.47	0.58, 3.72	0.412
Current hookworm infection	2.65	1.26, 5.57	0.010
Hookworm antibodies, lowest quartile (reference)	1.00		---
Quartile 2	0.95	0.71, 1.26	0.706
Quartile 3	3.82	1.47, 9.94	0.006
Quartile 4	3.07	1.28, 7.38	0.012
No Household Food Insecurity (reference)	1.00		---
Some (1-5)	0.05	0.02, 0.12	<0.001
Moderate (6-10)	0.21	0.03, 1.31	0.095
High (11-15)	0.04	0.02, 0.11	<0.001
Geographic Location			
South	0.15	0.11, 0.20	<0.001
Mid-south	1.78	0.94, 3.34	0.075
Mid-north	0.92	0.66, 1.28	0.603
North (reference)	1.00		---

[a] For adjusted model, N=151
[b] Adjusted for age, sex, and household absolute wealth index

Table 7 describes the model predicting higher levels of parasite burden. The final model is adjusted for age, sex, and absolute wealth index. Both of the examined behavioral interventions for reducing malaria infection rates, indoor residual spraying and household bed net use, were significantly associated with a higher likelihood of high parasite density. Children whose houses had been sprayed in the past year were 9.83 times as likely to have a high parasite density as those whose houses had not been sprayed (p<0.001). Some household bed net use was associated with 4.57 times the likelihood of high parasite density (p=0.029), and if all members

of the household slept under a bed net, the likelihood of high parasite density was 9.56 times that of a child from a house where no one slept under a net (p=0.025). An episode of malaria in any household member was associated with an increased likelihood of high parasite density (OR=2.80, p=0.039) while a malaria episode in the index child was associate with significantly reduced likelihood of high parasite density (OR=0.15, p<0.001). Current hookworm infection in the index child, conversely, was associated with 2.81 times the likelihood of high parasite density (p=0.001). Only the highest quartile of hookworm antibodies was significantly associated with reduced likelihood of high parasite density (OR=0.26, p=0.007), although the second and third quartiles also had odds ratios suggestive of a protective effect (OR=0.46 and OR=0.20, respectively). Finally, those who consumed higher numbers of protein food groups in a week were more likely to have higher parasite density. This was statistically significant for the middle tertile of protein consumption, such that those who consumed 3 protein food groups per week, compared to those who consumed fewer, were 4.72 times as likely to have high parasite density (p=0.001).

Several covariates remained significant in both the model predicting malaria infection and the model predicting parasite density. Indoor residual spraying in the past year was protective for predicting malaria infection but indicated a higher probability of high parasite density. Similarly, an episode of malaria in the house in the past year was associated with reduced likelihood of malaria infection, but was associated with a higher parasite density among those infected. Current hookworm infection was associated with increased risk of malaria infection and increased parasite density. Hookworm antibodies displayed the opposite trend as spraying and household malaria infection, such that high antibody levels were associated with increased likelihood of infection but decreased likelihood of high parasite density.

Table 7: Logistic Regression Model predicting High Malaria Parasite Density[a]			
Characteristic	**Adjusted Odds Ratio[b]**	**95% Confidence Interval**	**p**
House was sprayed in the past year	9.83	3.54, 27.27	<0.001
Household bednet use previous night			
None (reference)	1.00		---
Some	4.57	1.17, 17.83	0.029
All	9.56	1.32, 69.05	0.025
Anyone in the house had malaria in the past year	2.80	1.05, 7.44	0.039
Index child had malaria in the past year	0.15	0.05, 0.42	<0.001
Current hookworm infection	2.81	1.53, 5.18	0.001
Hookworm antibodies, lowest quartile (reference)	1.00		---
Quartile 2	0.46	0.18, 1.20	0.113
Quartile 3	0.20	0.02, 1.76	0.146
Quartile 4	0.26	0.10, 0.69	0.007
Weekly protein food groups			
Lowest tertile, 0, 1, or 2 (reference)	1.00		---
Middle tertile, 3	4.72	1.96, 11.35	0.001
Highest tertile, 4, 5, or 6	2.68	0.67, 10.78	0.164
Geographic Location			
South	4.36	1.54, 12.40	0.006
Mid-south	0.48	0.27, 0.85	0.012
Mid-north	0.54	0.32, 0.92	0.024
North (reference)	1.00		---

[a] For adjusted model, N=128

[b] Adjusted for age, sex, and household absolute wealth index

DISCUSSION

Comparison of Anthropometric Measures

School children were considered eligible for participation if, in the initial screening, height-for-age Z-score was either below -1.80 or above -0.10, using birthdates obtained from the schools where they were screened. However, birthdates obtained during subsequent household interviews did not necessarily match those given by schools, limiting the utility of the age-standardized measure of low nutritional status. To account for nutritional status in subsequent analyses while reducing the influence of age, the correlations of two age-unstandardized measures of nutritional status were assessed – weight-for-height and BMI. Weight for height is nearly independent of age for children between 1 and 10 years old,[57] and BMI was examined because it similarly incorporates weight and height measurements without an inherent age component.

Linear regressions, examining age with either weight-for-height or BMI, all as continuous variables, were used to determine which of these two measures was more robust to age variation. Although both were significantly correlated with age, the R^2-values were low, and BMI had a lower R^2-value. BMI was thus considered a more robust indicator of age-independent nutritional status and used in subsequent analysis. To further minimize the impact of potentially inaccurate age reporting, BMI was categorized into quartiles, reducing the impact of incorrectly ordered data points.

Nutritional Status and Risk of Malaria Infection

Nutritional status, as assessed by BMI quartiles within the study population, was not associated with either risk of infection or high parasitemia in either bivariate or multivariate analyses. Nutritional status was also examined from the perspective of food intake using the

number of weekly animal source foods and number of weekly protein-rich foods. Neither measure of dietary intake was associated with either outcome in bivariate analysis.

However, because nutritional intake has previously been associated with malaria infection and outcomes, both measures were separately examined as contributing risk factors by sequential examination of one or the other in multivariate analysis. Using this method, the number of protein-rich food groups emerged as significantly associated with high parasitemia but not with overall risk of infection. This is consistent with previous studies, which have shown more severe malaria outcomes among children who are well-nourished or receiving iron supplements,[58,59] and protective effects seen in children with reduced iron status,[60] because protein-rich food groups generally have higher quantities of bioavailable iron.[36,37,61]

Demographic and Socioeconomic Risk Factors

Bivariate analysis did not reveal a significant association between risk of malaria infection and level of household food insecurity, but in multivariate analysis, higher levels of food insecurity were strongly associated with reduced likelihood of infection, in contrast with the results of a previous study in Haiti that examined this risk factor.[62] It is not clear what the mechanism is of this association, but further examination of the prevalence of malaria infection by geographic location and food insecurity category reveals a lower percentage of infected children in the higher food insecurity categories. Examination of the data in this way also reveals, however, that there is an overall low number of malaria negative participants that can be included in this analysis, so further examination of this risk factor is necessary to determine if it is actually a result of low numbers and chance.

Geographic location was used as the cluster variable and also found to be statistically significant. No distinct north-to-south trend emerged, but there were strikingly different risks for

malaria infection and for higher parasite density between the four regions. The region with the highest risk of any infection had the lowest risk of high parasite density and vice versa; this trend emerges for many of the examined risk factors and suggests that the risk factors that reduce the likelihood of infection are preventing the development of immunity and effective control of infection.

Prevention behaviors

Household bed net use was found to be significantly protective for malaria infection overall, but along with indoor residual spraying, was associated with increased likelihood of higher parasite density. A couple of mechanisms are possible for this association. First it is not known if the protective behaviors have been well established in the household or if they reflect recent behavioral changes. Malaria infection may have been established prior to use of these protective measures.

Alternatively, and perhaps more plausibly, the families that use these measures may be less likely to have frequent episodes of malaria, and thus the children would have fewer immunity-inducing infections. This idea is supported by the reduced likelihood of infection in the households where indoor residual spraying is used. Fewer infections and reduced immunity would then preclude the child to have higher parasite density, because he or she would have less immune control of the infection. A third possibility is that there is an expected level of protection from the use of bed nets and indoor residual spraying, and thus families that use these protective measures might be less likely to expect malaria infection and take children for treatment if they experience mild symptoms.

Other infections

Children who had been diagnosed with malaria in the past year were significantly less likely to have higher parasite density in both the bivariate and multivariate analysis. Risk of any infection was elevated, but non-significantly, in multivariate analysis but significantly lower in bivariate analysis. Thus, a recent malaria infection in the child cannot predict current infection after adjustment for other co-factors, but the results do indicate that there is a protective effect for the density of malaria parasites in the current infection. Recent malaria exposure would likely boost the immune response to subsequent infection, although circulating antibody levels may rapidly diminish.[9,63,64]

A diagnosis for any other household member in the past year was associated with a reduced risk of any malaria infection and an increased risk of higher parasite density in multivariate analysis. It may be that households that had any episodes of malaria undertook additional protective measures that were not measured in this study and subsequently led to reduced incidence of malaria in the index child.

Infection with hookworm was associated with increased likelihood of malaria infection and increased likelihood of high parasite density. This is consistent with several previous studies, which have found increased risk of malaria infection.[30,65-68] The most widely promoted mechanism for this association is the cytokine profile induced by hookworm infection has been associated in previous studies with increased risk for malaria infection. [6,69,70]

Antibody levels

In bivariate analysis, neither malaria nor hookworm antibody levels were associated with malaria infection or with higher parasite density, a result that was consistent in multivariate analysis for the malaria antibodies. Malaria antibodies measured for this study were total IgG,

without subclass measurements. Although total IgG has been associated with reduced risk of malaria infection in some studies,[71] this is inconsistent and generally attributed to the cytophilic subclasses IgG1 and IgG3, which have been more strongly associated with protection from and control of infection.[21]

In contrast, after adjustment, higher levels of hookworm antibodies were found to be associated with greater risk of malaria infection but reduced risk of high parasite density in multivariate analysis. The association with increased likelihood of infection is expected; hookworm infection induces higher levels of anti-hookworm IgG[72] as well as a Th2 cytokine response that diminishes the production of the cytophilic antibody classes associated with malaria protection.[69,73] The mechanism of the association of higher levels of hookworm antibodies and lower malaria parasite density, however, is unclear. Further research exploring this association is needed to determine if the presence of hookworm antibodies alongside malaria parasites induces differential modulation of the immune response to malaria.

Study Limitations

The primary study limitations are potentially inaccurate diagnosis of asymptomatic malaria, limited specificity of IgG measures, and a lack of information about earlier childhood exposures to malaria.

Malaria diagnostic tests were carried out using Rapid Diagnostic Testing, followed by thick and thin blood smears for children who tested positive by RDT. However, children who tested negative by RDT were not further assessed for malaria infection, and the accuracy of the test is not 100%. In addition, thick and thin blood smears have a limit of detection of parasites, and it is possible that several children who did have circulating parasite were deemed malaria negative by this test. No further testing was used to determine if these children, or those that

tested negative by RDT, were truly negative, and thus the "malaria negative" group may have had a few children who were actually infected. This would make the two groups more similar for analysis of risk factors and would thus reduce the significance of the examined risk factors. Thus, although this study may not have identified all of the associated risk factors, we can be confident that the associations found are likely to be accurate.

Immunoglobulin levels assessed as risk factors in this study are against whole parasite and a combination of all subclasses. As discussed in the background of this study, not all subclasses and antigens are equally associated with risk of infection, so these may be oversimplifications of the role of IgG antibodies. In line with this suggestion, malaria IgG was not found to be associated with risk of infection or with higher parasite density, indicating that it may be useful to examine specific subclasses in the future.

Finally, the household survey does not ask about earlier childhood exposures to malaria or total number of malarial episodes, both of which may be associated with risk of infection. The questions about both child and household episodes of malaria in the past year are reliant on accurate self-report, and none are confirmed by any medical records, nor is there any information about how these results were determined. There may be a high number of non-malaria cases of fever, which is often assumed to be malaria, included in these counts. This would change the nature of the associations seen – other febrile illnesses may also increase the risk of malaria, creating an artificially strong association between these measures if cases of other illness are misclassified. These concerns are somewhat mitigated by the question about fever in the past month, which many more respondents answered affirmatively, indicating that not all febrile illness is considered malaria.

Implications for Research and Practice

The results of this study confirm previous work indicating an increased risk of malaria infection among children infected with hookworm, as well as an increased parasite density among those that are malaria positive. These results, combined with the extensive overlap in susceptibility range, highlight the importance of identifying and treating cases of hookworm infection, especially among children diagnosed with malaria. Although this study dealt exclusively with children who were asymptomatic, if similar results are found among a broader population of malaria infected children, treatment of hookworm infection may be used as a means of reducing the risk of severe malaria.

Effective control measures such as use of insecticide treated bed nets and indoor residual spraying should be given greater emphasis. Less than 10% of the participants lived in houses that had been sprayed in the past year, and more than a quarter reported no bed net use. We can reasonably expect that reported use is higher than actual use, so there is likely a large proportion of the population that is not protected by either measure. Educational campaigns or incentives for the use of these two control measures may help significantly reduce the high burden of malaria in the area.

Conclusion

In summary, this study characterized the association between malaria infection and parasite density with nutritional indicators, socioeconomic status, serum indicators of hookworm and malaria infection, and behaviors that are protective for malaria infection, as well as identified the prominent risk factors for both malaria infection and higher levels of parasitemia. Associated risk factors for malaria infection and higher malaria parasitemia are similar, but in many cases the direction of association is opposite for any malaria infection and density of infection.

Presence of hookworm infection is the exception, and is associated both with increased risk of any infection and increased risk of high parasite density. The strong and consistent association between these two infections suggests a valuable point of collaboration for prevention and treatment. No association was seen between concentration of malaria IgG and either presence or density of infection, although this may be due to the limitations of the available antibody data. Further research on this study area is necessary to fully evaluate the role of various classes of malaria IgG in malaria infection.

REFERENCES

1 Mohandas, N. & An, X. Malaria nad human red blood cells. *Med Microbiol Immunol* **201**, 593-598 (2012).

2 Hay, S. I. *et al.* Estimating the Global Clinical Burden of Plasmodium falciparum Malaria in 2007. *PLoS Medicine* **7** (2010).

3 Gething, P. W. *et al.* A new world malaria map: Plasmodium falciparum endemicity in 2010. *Malaria journal* **10**, 378 (2011).

4 Bousema, T. & Drakeley, C. Epidemiology and Infectivity of Plasmodium falciparum and Plasmodium vivax Gametocytes in Relation to Malaria Control and Elimination. *Clin Microbiol Rev* **24**, 377 (2011).

5 Ageyman-Budu, A. *et al.* Trends in multiplicity of Plasmodium falciparum infections among asymptomatic residents in the middle belt of Ghana. *Malaria journal* **12** (2013).

6 Hartgers, F. C. & Yazdanbakhsh, M. Co-infection of helminths and malaria: modulation of the immune responses to malaria. *Parasite Immunology* **28**, 497-506 (2006).

7 Garcia, L. S. Malaria. *Clin Lab Med* **30**, 93-129 (2010).

8 Langhorne, J., Ndungu, F. M., Anee-Marit, S. & Marsh, K. Immunity to malaria: more questions than answers. *Nature Immunology* **9**, 725-732 (2008).

9 Garraud, O., Mahanty, S. & Perraut, R. Malaria-specific antibody subclasses in immune individuals: a key source of information for vaccine design. *TRENDS in Immunology* **24**, 30-35 (2003).

10 Laishram, D. D. *et al.* The complexities of malaria disease manifestations with a focus on asymptomatic malaria. *Malaria journal* **11** (2012).

11 Bottius, E. *et al.* Malaria: even more chronic in nature than previously thought; evidence for subpatent parasitaemia detectable by the polymerase chain reaction. *Transaction of the Royal Society of Tropical Medicine and Hygiene* **90**, 15-19 (1996).

12 Bejon, P. *et al.* Helminth infection asd eosinophilia and the risk of Plasmodium falciparum malaria in 1- to 6-year-old children in a malaria endemic area. *PLoS Neglected Tropical Diseases* **2**, e164 (2008).

13 Rogier, C. *et al.* Plasmodium falciparum clinical malaria: lessons from longitudinal studies in Senegal. *Parassitologia* **41**, 255-259 (1999).

14 Baird, J. K. Host Age as a Determinant of Naturally Acquired Immunity to Plasmodium falciparum. *Parasitology Today* **11**, 105-111 (1995).

15 Piper, K. P., Roberts, D. J. & Day, K. P. Plasmodium falciparum: Analysis of the Antibody Specificity to the Surface of the Trophozoite-Infected Erythrocyte. *Experimental Parasitology* **91**, 161-169 (1999).

16 Scopel, K. K. G., Fontes, C. J. F., Ferreira, M. U. & Braga, E. M. Factors Associated with Immunoglubulin G Subclass Polarization in Naturally Acquired Antibodies to Plasmodium falciparum Merozoite Surface Proteins: a Cross-Sectional Survey in Brazilian Amazonia. *Clinical and Vaccine Immunology* **13**, 810-813 (2006).

17 Richards, J. S. *et al.* Association between Naturally Acquired Antibodies to Erythrocyte-Binding Antigens of Plasmodium falciparum and Protection from Malaria and High-Density Parasitemia. *Clinical Infectious Diseases* **51**, e50-e60 (2010).

18 Omosun, Y. *et al.* Total immunoglobulin G and IgG1 subclass levels specific for the MSP-1,19 of Plasmodium falciparum are different in individuals with either processing-inhibitory, blocking or neutral antibodies. *African Health Sciences* **10**, 106-110 (2010).

19 Nwuba, R. I. *et al.* The Human Immune Response to Plasmodium falciparum Includes Both Antibodies That Inhibit Merozoite Surface Protein 1 Secondary Processing and Blocking Antibodies. *Infection and Immunity* **70**, 5328-5331 (2002).
20 Courtin, D. *et al.* The Quantity and Quality of African Children's IgG Responses to Merozoite Surface Antigens Reflect Protection against Plasmodium falciparum Malaria. *PloS one* **4**, e7590 (2009).
21 Dobano, C. *et al.* Age-Dependent IgG Subclass Responses to Plasmodium falciparum EBA-175 Are Differentially Associated with Incidence of Malaria in Mazambican Children. *Clinical and Vaccine Immunology* **19**, 157-166 (2012).
22 Nmorsi, O. P. G., Ukwandu, N. C. D., Isaac, C., Ekoma, N. E. & Asibor, V. Immunoglobulin profile of Nigerian children with Plasmodium falciparum infection. *African Journal of Biotechnology* **7**, 77-80 (2008).
23 Tangteerawatana, P. *et al.* Differential regulation of IgG subclasses and IgE antimalarial antibody responses in complicated and uncomplicated Plasmodium falciparum malaria. *Parasite Immunology* **29**, 475-483 (2007).
24 Bouharoun-Tayoun, H. & Druilhe, P. Plasmodium falciparum Malaria: Evidence for an Isotype Imbalance Which May Be Responsible for Delayed Acquisition of Protective Immunity. *Infection and Immunity* **60**, 1473-1481 (1992).
25 Mibei, E. K., Otieno, W. O., Orago, A. S. S. & Stoute, J. A. Distinct pattern of class and subclass antibodies in immune complexes of children with cerebral malaria and severe malarial anaemia. *Parasite Immunology* **30**, 334-341 (2008).
26 Kaay, H. J. V. d., Klein, F., Hagenaar-de Weerdt, M. & Meuwissen, J. H. E. T. Immuno-epidemiology of malaria. *Bulletin of the World Health Organization* **49**, 267-274 (1973).
27 Degarege, A., Animut, A., Legesse, M. & Erko, B. Malaria and helminth co-infections in outpatients of Alaba Kulito Health Center, southern Ethiopia: a cross sectional study. *BMC Research Notes* **3** (2010).
28 Brooker, S. *et al.* The co-distribution of Plasmodium falciparum and hookworm among African schoolchildren. *Malaria journal* **5** (2006).
29 Brooker, S. *et al.* Epidemiology of Plasmodium-helminth co-infection in Africa: populations and risk, potential impact on anemia, and prospects for combining control. *American Journal of Tropical Medicine and Hygiene* **77**, 88-98 (2007).
30 Righetti, A. A. *et al.* Interactions and potential implications of Plasmodium falciparum-hookworm coinfection in different age groups in South-Central Cote d'Ivoire. *PLoS Neglected Tropical Diseases* **6**, e1889 (2012).
31 Humphries, D. *et al.* Epidemiology of hookworm infection in Kintampo North municipality, Ghana: patterns of malaria coinfection, anemia, and albendazole treatment failure. *American Journal of Tropical Medicine and Hygiene* **84**, 792-800 (2011).
32 (GSS), G. S. S., (GHS), G. H. S. & Macro, I. Ghana Demographic and Health Survey 2008. (Accra, Ghana, 2009).
33 Anderson, A. K., Bignell, W., Winful, S., Soyiri, I. & Steiner-Asiedu, M. Risk factors for malnutrition among children 5-years and younger in the Akuapim-North District in the Eastern Region of Ghana. *Current Research Journal of Biological Sciences* **2**, 183-188 (2010).
34 Gilgen, D. & Mascie-Taylor, C. G. N. The effect of anthelmintic treatment on helminth infection and anemia. *Parasitology* **122**, 105-110 (2000).

35 Murphy, S. P. & Allen, L. H. Animal Source Foods to Improve Micronutrient Nutrition and Human Function in Developing Countries. *American Society for Nutritional Sciences* **133**, 3932S-3935S (2003).
36 Dror, D. K. & Allen, L. H. The importance of milk and other animal-source foods for children in low-income countries. *Food and nutrition bulletin* **32**, 227-243 (2011).
37 Ngui, R., Lim, Y. A. L., Kin, L. C., Chuen, C. S. & Jaffar, S. Association between anaemia, iron deficiency anaemia, neglected parasitic infections and socioeconomic factors in rural children of West Malaysia. *PLoS Neglected Tropical Diseases* **6**, e1550 (2012).
38 Okumu, F. O. & Moore, S. J. Combining indoor residual spraying and insecticide-treated nets for malaria control in Africa: a review of possible outcomes and an outline of suggestions for the future. *Malaria journal* **10** (2011).
39 Mathanga, D. P. *et al.* Malaria control in Malawi: Current status and directions for the future. *Acta tropica* **121**, 212-217 (2012).
40 Raghavendra, K. & Barik, T. K. Malaria vector control: from past to future. *Parasitology Res* **108**, 757-779 (2011).
41 Feachem, R. G. A. *et al.* Shrinking the malaria map: progress and prospects. *The Lancet* **376**, 1566-1578 (2010).
42 Najera, J. A. & Zaim, M. Malaria Vector Control: Insecticides for Indoor Residual Spraying. (World Health Organization, 2001).
43 Kim, D., Fedak, K. & Kramer, R. Reduction of Malaria Prevalence by Indoor Residual Spraying: A Meta-Regression Analysis. *American Journal of Tropical Medicine and Hygiene* **87**, 117-124 (2012).
44 Pluess, B., Tanser, F. C., Lengeler, C. & Sharp, B. L. Indoor residual spraying for preventing malaria. *Cochrane Database of Systematic Reviews*, doi:10.1002/14651858.CD006657.pub2 (2010).
45 Renshaw, M., Daudrumey, A. & Bilous, J. (ed World Health Organization) (World Health Organization, 2007).
46 Choi, H. W. *et al.* The Effectiveness of Insecticide-Impregnated Bed Nets in Reducing Cases of Malaria Infection: A Meta-Analysis of Published Results. *American Journal of Tropical Medicine and Hygiene* **52**, 377-382 (1995).
47 Malm, K. L. *et al.* Implementation of the Affordable Medicines for Malaria Facility (AMFm) in Ghana: Processes, challenges and achievements. *Journal of Public Health Policy* (2013).
48 Olotu, A. *et al.* Estimating Individual Exposure to Malaria Using Local Prevalence of Malaria Infeciton in the Field. *PloS one* **7**, e32929 (2012).
49 Kaga, T. & Ohta, S. Ecophysiological and Climatological Effects on Distribution of Vector Species and Malaria Incidence in India. *Int J Environ Res Public Health* **9**, 4704-4714 (2012).
50 Achidi, E. A. *et al.* Severe and uncomplicated falciparum malaria in children from three regions and three ethnic groups in Cameroon: a prospective study. *Malaria journal* **11** (2012).
51 Aucan, C., Traore, Y., Fumoux, F. & Rihet, P. Familial Correlation of Immunoglobulin G Subclass Responses to Plasmodium falciparum Antigens in Burkina Faso. *Infection and Immunity* **69**, 996-1001 (2001).

52 Simms, B. T. *Epidemiology of hookworm infection among school aged children in the Kintampo North District: an evaluation of nutritional status, albendazole efficacy, and implications for future control efforts* Master of Public Health thesis, Yale School of Public Health, (2011).
53 Humphries, D. *et al.* Hookworm infection among school age children in Kintampo North Municipality, Ghana: Nutritional risk factors and response to albendazole treatment. (Manuscript submitted for publication).
54 Organization, W. H. Basic Laboratory Methods in Medical Parasitology. 25-29 (World Health Organization, 1991).
55 Richard D. Bungiro, J., Green, J., Kruglov, E. & Cappello, M. Mitigation of Hookworm Disease by Immunization with Soluble Extracts of Ancylostoma ceylanicum. *Journal of Infectious Diseases* **183**, 1380-1387 (2001).
56 DHS, M. *Demographic and Health Survey (DHS)*.
57 McMurray, C. Cross-sectional anthropometry: what can it tell us about the health of young children? *Health Transition Review* **6**, 147-168 (1996).
58 Schumann, K., Ettle, T., Szegner, B., Elsenhans, B. & Solomons, N. W. On risks and benefits of iron supplementation recommendations for iron intake revisited. *Journal of Trace Elements in Medicine and Biology* **21**, 147-168 (2007).
59 Lepowsky, M. in *Child Survival* Vol. 11 *Culture, Illness and Healing* (ed Nancy Scheper-Hughes) Ch. 4, 71-92 (Springer Netherlands, 1987).
60 Nyakeriga, A. M. *et al.* Iron Deficiency and Malaria among Children Living on the Coast of Kenya. *Journal of Infectious Diseases* **190**, 439-447 (2004).
61 Murray, J., Murray, A., Murray, M. & Murray, C. The biological suppression of malalria: an ecological and nutritional interrelationship of a host and two parasites. *The American journal of clinical nutrition* **31**, 1363-1366 (1978).
62 Perez-Escamilla, R. *et al.* Houshold Food Insecurity Is Associated with Childhood Malaria in Rural Haiti. *The Journal of nutrition* **139**, 2132-2138 (2009).
63 Kinyanjui, S. M., Bejon, P., Osier, F. H., Bull, P. C. & Marsh, K. What you see is not what you get: implications of the brevity of antibody responses to malaria antigens and transmission heterogeneity in longitudinal studies of malaria immunity. *Malaria journal* **8** (2009).
64 Fonjungo, P. N. *et al.* A Longitudinal Study of Human Antibody Responses to Plasmodium falciparum Rhoptry-Associated Protein 1 in a Region of Seasonal and Unstable Malaria Transmission. *Infection and Immunity* **67**, 2975-2985 (1999).
65 Nacher, M. *et al.* Intestinal helmith infections are associated with increased incidence of Plasmodium falciparum malaria in Thailand. *Journal of Parasitology* **88**, 55-58 (2002).
66 Midzi, N. *et al.* The burden of polyparasitism among primary schoolchildren in rural and farming areas in Zimbabwe. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **102**, 1039-1045 (2008).
67 Boel, M. *et al.* Complex interactions between soil-transmitted helminths and malaria in pregnant women on the Thai-Burmese border. *PLoS Neglected Tropical Diseases* **4**, e887 (2010).
68 Pullan, R. L., Kabatereine, N. B., Bukirwa, H., Staedke, S. G. & Brooker, S. Heterogeneities and consequences of Plasmodium species and hookworm coinfection: a population based study in Uganda. *The Journal of Infectious Disease* **203**, 406-417 (2010).

69 Mwangi, T. W., Bethony, J. & Brooker, S. Malaria and helminth interactions in humans: an epidemiological viewpoint. *Annals of tropical medicine and parasitology* **100**, 551-570 (2007).
70 Shapiro, A. E. *et al.* Epidemiology of helminth infection and their relationship to clinical malaria in southwest Uganda. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **99**, 18-24 (2005).
71 Fowkes, F. J. I., Richards, J. S., Simpson, J. A. & Beeson, J. G. The Relationship between Anti-merozoite Antibodies and Incidence of Plasmodium falciparum Malaria: a Systematic Review and Meta-analysis. *PLoS Medicine* **7**, e1000218 (2010).
72 Blount, D. *et al.* Immunologic Profiles of Presons Recruited for a Randomized, Placebo-Controlled Clinical Trial of Hookworm Infection. *American Journal of Tropical Medicine and Hygiene* **81**, 911-916 (2009).
73 Druilhe, P., Tall, A. & Sokhna, C. Worms can worsen malaria: towards a new means to roll back malaria? *Trends in parasitology* **21**, 359-362 (2005).

IDENTIFICATION	
CHILD NAME _____ SCHOOL/CLASS_____ CHILD ID #_____ HEAD OF HOUSEHOLD _____ RESPONDENT NAME_____ RELATIONSHIP OF RESPONDENT TO CHILD_____ HOUSE NUMBER……...…….. COMMUNITY…….……..... GPS LATITUDE AND LONGITUDE…… INTERVIEWER NAME _____ NUMBER _____ INTERVIEWER NAME _____ NUMBER _____ QUESTIONNAIRE ANSWERS REVIEWED _____ (Date) _____ (Initials)	

1. SOCIOECONOMIC INDICATORS			
NO.	QUESTIONS AND FILTERS	CODING CATEGORIES	ENTER #
1.1	What is the main material of the floor?	NATURAL FLOOR……1 CEMENT FLOOR……2 TILE FLOOR……3	
1.2	What type of fuel does the household mainly use for cooking?	ELECTRICITY……1 NATURAL GAS……2 BIOGAS……3 KEROSENE……4 CHARCOAL ……5 FIREWOOD/STRAW ……6 DUNG……7 OTHER_____66 (SPECIFY)	
1.3	Does your household have:	YES NO ELECTRICITY……1 2 RADIO……1 2 TELEVISION……1 2 TELEPHONE……1 2 REFRIGERATOR……1 2 OTHER _____1 2	
1.4	Does any member of the household own:	YES NO BICYCLE……1 2 MOTORCYCLE/SCOOTER……1 2 CAR/TRUCK……1 2	
1.5	Does any member of the household own agricultural land?	YES……1 NO……2 DON'T KNOW……88	
1.6	Does any member of the household own at least one:	YES NO COW……1 2 HORSE……1 2 DONKEY ……1 2 GOAT……1 2 SHEEP……1 2 POULTRY……1 2 DOG……1 2 PIG……1 2	
1.7	Does anyone in the household own a savings account?	YES NO DK BANK……1 2 88 CO-OPERATIVE……1 2 88	
1.8	How far is the household from the nearest health facility?	LESS THAN 1KM……1 BETWEEN 1 AND 5KM……2 BETWEEN 5 AND 10KM……3 GREATER THAN 10KM……4 DON'T KNOW……88	

1.9	How many people in the household?	Total number _____ ≤ 5 yrs _____ 6-11 yrs _____ 12-15 yrs _____ Women > 15 _____ Men > 15 _____	

2. FOOD SECURITY SCALE (ELCSA)			
Now we want to ask you some questions about some experiences you or members of your household may have had around food. Please answer the following questions referring to the entire household.			
NO.	DURING THE LAST 3 MONTHS, BECAUSE OF LACK OF MONEY OR OTHER RESOURCES…	CODING CATEGORIES	ENTER #
2.1	Were you worried about running out of food?	YES……...1 NO……2 DON'T REMEMBER ....88 REFUSED……77	
2.2	Did your household run out of food at any time?	YES……...1 NO……2 DON'T REMEMBER…..88 REFUSED……77	
2.3	Were you or any other adult in your household unable to eat the kinds of nutritious foods that make people healthy?	YES……...1 NO……2 DON'T REMEMBER.....88 REFUSED……77	
2.4	Did you or any other adult in your household usually have to eat the same foods almost every day?	YES……...1 NO……2 DON'T REMEMBER.....88 REFUSED……77	
2.5	Was there any day that you or any other adult in your household skipped a meal because of lack of food?	YES……...1 NO……2 DON'T REMEMBER…..88 REFUSED……77	
2.6	Did any adult in your household eat less food than what they needed because there wasn't enough food?	YES……...1 NO……2 DON'T REMEMBER…..88 REFUSED……77	
2.7	Was there any day when you or any other adult in your household felt hungry but did not eat because there wasn't enough food?	YES……...1 NO……2 DON'T REMEMBER…..88 REFUSED……77	
2.8	Was there any day when you or any other adult in your household didn't eat for a whole day because there wasn't enough food?	YES……...1 NO……2 DON'T REMEMBER.....88 REFUSED……77	
2.9	Did you do things that you would have preferred not to do, such as begging or sending children to work, to get food?	YES……...1 NO……2 DON'T REMEMBER…..88 REFUSED……77	

The following questions refer to children under 15 years old in the household			
NO.	DURING THE LAST 3 MONTHS, BECAUSE OF LACK OF MONEY OR OTHER RESOURCES…	CODING CATEGORIES	ENTER #
2.10	Were you unable to provide the children in your household with the kinds of nutritious foods they need to be healthy?	YES……...1 NO……2 DON'T REMEMBER…..88 REFUSED……77	
2.11	Did any children in your household usually have to eat the same foods almost every day?	YES……...1 NO……2 DON'T REMEMBER…..88 REFUSED……77	
2.12	Did any child in your household eat less food than what s/he needed because there wasn't enough food?	YES……...1 NO……2 DON'T REMEMBER…..88 REFUSED……77	
2.13	Did you have to serve less food to any child because there wasn't enough food?	YES……...1 NO……2 DON'T REMEMBER…..88 REFUSED……77	
2.14	Was there any day when any child in your household felt hungry but could not be fed because there wasn't enough food?	YES……...1 NO……2 DON'T REMEMBER…..88 REFUSED……77	
2.15	Was there any day when any child in your household didn't eat for a whole day because there wasn't enough food?	YES……...1 NO……2 DON'T REMEMBER…..88 REFUSED……77	
2.16	Did any child in your household go to bed hungry in any day during the past week because of lack of food?	YES……...1 NO……2 DON'T REMEMBER…..88 REFUSED……77	

3. HOUSEHOLD WATER SOURCES			
NO.	QUESTIONS AND FILTERS	CODING CATEGORIES	ENTER #
3.1	What is the main source of drinking water for members of your household?	PIPED WATER……..……..1 BOREHOLE……..…..……2 DUG WELL……..……...3 WATER FROM A SPRING...…...…..……...4 RAINWATER……..5 SURFACE WATER……..…..6 OTHER _____ 66 (SPECIFY)	
3.2	What is the main source of water for bathing in the household?	PIPED WATER……..……..1 BOREHOLE……...……2 DUG WELL……..……...3 WATER FROM A SPRING...…...…..……...4 RAINWATER……..5 SURFACE WATER……..…..6 OTHER _____ 66 (SPECIFY)	
3.3	What is the main source of water for cooking in the household?	PIPED WATER……..……..1 BOREHOLE……...……2 DUG WELL……..……...3 WATER FROM A SPRING...…...…..……...4 RAINWATER……..5 SURFACE WATER……..…..6 OTHER _____ 66 (SPECIFY)	
3.4	Where is the water source located?	IN OWN HOUSE…...……1 < 50 METERS FROM HOUSE….……..2 ≥ 50 METERS FROM HOUSE….……..3	
3.5	Is the water source shared?	YES……..1 NO……SKIP to Q3.7 ……..……2 DON'T KNOW……88	
3.6	How many people share the water source?	Enter number (00 if > 50) _____ Enter "88" for "DON'T KNOW"	
3.7	Do you do anything to the water to make it safer before drinking it?	YES……..……..1 NO……SKIP to Q4.1 ……..……2 DON'T KNOW……88	
3.8	What do you do to the water to make it safer before drinking it?	BOIL……1 ADD ALUM……2 STRAIN THROUGH CLOTH……3 FILTER……4 LET IT SIT AND SETTLE……..5 OTHER _____66 (SPECIFY)	

4. TOILET FACILITIES AND GARBAGE			
NO.	QUESTIONS AND FILTERS	CODING CATEGORIES	ENTER #
4.1	What kind of toilet facility do members of the household use?	FLUSH OR POUR……..…1 PIT LATRINE…….....2 COMPOST…….....3 BUCKET…….....4 BUSH OR FIELD…….....5 OTHER _____66 (SPECIFY)	
4.2	Is this a public toilet facility?	YES……(SKIP TO Q4.4)…..……..1 NO……2 DON'T KNOW……88	
4.3	How many people use this facility?	ENTER # _____ DON'T KNOW ……..88	
4.4	Method of garbage disposal for the household?	ORGANIZED PICK UP……...1 BURNING……2 BURYING……3 RIVER……...4 BUSH/FIELD……...…….5 OTHER _____66 (SPECIFY)	
4.5	Is there noticeable garbage around the household?	YES……...1 NO……2	

5. EXPOSURE/DISEASE PREVENTION			
NO.	QUESTIONS AND FILTERS	CODING CATEGORIES	ENTER #
5.1	At any time in the past 12 months, has anyone sprayed the interior walls of your dwelling against mosquitoes?	YES……..1 NO……SKIP to Q5.4..……2 DON'T KNOW……88	
5.2	How many months ago was the house sprayed? IF LESS THAN ONE MONTH, RECORD '00' MONTHS AGO.	MONTHS AGO…… ____ DON'T KNOW/DON'T REMEMBER ……. 88	
5.3	Who sprayed the house?	GOVT. PROGRAM/WORKER…….1 PRIVATE COMPANY…….…..2 HOUSEHOLD MEMBER……...3 OTHER _____ 66 (SPECIFY) DON'T KNOW……...88	
5.4	Does your household have any mosquito nets that can be used while sleeping?	YES…….……..…1 NO……SKIP to Q5.11……...2	

5.5	How many mosquito nets does your household have? IF 7 OR MORE NETS, RECORD '7'.	NUMBER OF NETS……	
5.6	How long ago did you obtain the mosquito net? IF MORE THAN 3 YEARS AGO, ENTER 55	MONTHS AGO……... DON'T KNOW……...……....88	
5.7	When you got the net, was it already factory-repelled with an insecticide to kill or repel mosquitoes?	YES……1 NO..……2 DON'T KNOW……...……....88	
5.8	Since you got the mosquito net, was it ever soaked or dipped in a liquid to kill or repel mosquitoes?	YES……1 NO..……SKIP TO Q5.10..……2 DON'T KNOW……...……....88	
5.9	How long ago was it last soaked or dipped? IF MORE THAN 3 YEARS AGO, ENTER 55	MONTHS AGO……... DON'T KNOW……...……....88	
5.10	Did anyone sleep under the net last night?	YES……1 NO..……2 DON'T KNOW……...……....88	
5.11	Has any member of your household had deworming medication in the past year?	YES…….……1 NO..……..2 DON'T KNOW…….…....88	
5.12	Has any member of your household had a fever in the last month?	YES…….……1 NO..…….….2 DON'T KNOW……...……....88	
5.13	Has any member of your household had malaria in the past year?	YES…….…1 NO..…….….2 DON'T KNOW……...……....88	

6. HOUSEHOLD LISTING. Please identify other people in the household.
If more than 7, select in the following order (1) children 6-11 yrs, (2) children ≤ 5 yrs, (3) women > 15-45 yrs, (4) teens 12-15 yrs, (5) men > 15

LINE NO.	RELATIONSHIP TO INDEX CHILD	SEX	RESIDENCE	AGE	EDUCATION LEVEL	OCCUPATION	SHOES	BED NET USAGE
One line per person living or usually present in the household	What is the relationship of (#) to the index child?	Is (#) male or female?	Does (#) usually live here?	How old is (#)? IN YEARS	None...1 Primary…2 Jr High...3 Sr High...4 Vocational...5 Tertiary…6 Post Grad…7	SELF-DESCRIBED Farmer...1 Small trader...2 Student…3 None…4 Other (specify)	OWNS SHOES?	SLEPT UNDER BED NET LAST NIGHT?
(1)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
Head of Household		M F 1 2	YES NO 1 2				YES NO 1 2	YES NO 1 2
Mother or Caregiver		1 2	1 2				1 2	1 2
03		1 2	1 2				1 2	1 2
04		1 2	1 2				1 2	1 2
05		1 2	1 2				1 2	1 2
06		1 2	1 2				1 2	1 2
07		1 2	1 2				1 2	1 2

CODES FOR RELATIONSHP TO INDEX CHILD:
01 = PARENT
02 = BROTHER/SISTER
03 = HALF SISTER/HALF BROTHER
04 = AUNT/UNCLE
05 = GRANDPARENT
06 = OTHER RELATIVE
07 = NOT RELATED
88 = DON'T KNOW

7. PARASITE TREATMENT									
LINE NO.	ANTI-PARASITIC HISTORY	ANTIPARASITIC TREATMENT	TREATMENT SOURCE	FEVER HISTORY	FEVER TREATMENT	TREATMENT SOURCE	MALARIA HISTORY	MALARIA TREATMENT	TREATMENT SOURCE
	Antiparasitic treatment in the past year?	See treatment codes below	See treatment source codes below	Fever in past MONTH?	See treatment codes below	See treatment source codes below	Malaria in the past YEAR?	See treatment codes below	See treatment source codes below
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
	YES NO			YES NO			YES NO		
Index Child	1 2			1 2			1 2		
Head of Household	1 2			1 2			1 2		
Mother or Caregiver	1 2			1 2			1 2		
03 FROM TABLE 6	1 2			1 2			1 2		
04 FROM TABLE 6	1 2			1 2			1 2		
05 FROM TABLE 6	1 2			1 2			1 2		
06 FROM TABLE 6	1 2			1 2			1 2		
07 FROM TABLE 6	1 2			1 2			1 2		

TREATMENT CODES	
ALBENDAZOLE……1	MEBENDAZOLE……2
PYRANTEL……3	OTHER ANTI-PARASITIC_____4
DON'T KNOW.....88	(SPECIFY)
ASPIRIN……..…..5	ACETOMINOPHEN/PARACETAMOL…..6
IBUPROFEN……..7	OTHER ANTI-PYRETIC_____8
DON'T KNOW.....88	(SPECIFY)
SP/FANSIDAR……9	CHLOROQUINE……10
AMODIAQUINE……11	QUININE……...12
ACT……13	OTHER ANTIMALARIAL_____14
DON'T KNOW.....88	(SPECIFY)

TREATMENT SOURCE CODES	
HOSPITAL…….....1	LOCAL CLINIC…..... 2
PHARMACIST…..…..3	LOCAL HEALER….....4
FAMILY MEMBER…..5	DRUG STORE…..…..6
OTHER_____ 66	DON'T KNOW……88
(SPECIFY)	

8. DIETARY DIVERSITY SCORE (INDEX CHILD ONLY)							
NO.	DID THE PARTICIPATING CHILD EAT THE FOLLOWING FOODS DURING THE DAY OR AT NIGHT?	YESTERDAY			IN THE PREVIOUS WEEK		
		YES	NO	DK	YES	NO	DK
8.1	Bread, noodles, biscuits, or any other foods made from millet, sorghum, maize, rice, or wheat	1	2	88	1	2	88
8.2	Potatoes, yams, cassava or any other foods made from roots or tubers	1	2	88	1	2	88
8.3	Vegetables	1	2	88	1	2	88
8.4	Fruits	1	2	88	1	2	88
8.5	Beef, pork, lamb, goat, rabbit, wild game, chicken, duck, or other birds	1	2	88	1	2	88
8.6	Liver, kidney, heart, or other organ meats	1	2	88	1	2	88
8.7	Eggs	1	2	88	1	2	88
8.8	Fresh or dried fish or shellfish	1	2	88	1	2	88
8.9	Foods made from beans, peas, lentils, soybeans, or nuts	1	2	88	1	2	88
8.10	Cheese, yogurt, milk or other milk products	1	2	88	1	2	88
8.11	Foods made with oil, fat, or butter	1	2	88	1	2	88
8.12	Sugar or honey	1	2	88	1	2	88
8.13	Other foods such as condiments, coffee, or tea	1	2	88	1	2	88

9. CHILD HEALTH INDICATORS (INDEX CHILD ONLY)			
NO.	QUESTIONS AND FILTERS	CODING CATEGORIES	ENTER
9.1	When was the last time the INDEX CHILD consulted a healthcare worker?	IN THE LAST WEEK……..1 IN THE LAST MONTH……...2 IN THE LAST YEAR……..3 MORE THAN ONE YEAR……..4 NEVER……5 DON'T KNOW……88	
9.2	Where does the child get medical care if he/she is sick?	HOSPITAL……....1 LOCAL CLINIC..……..2 PHARMACIST…….3 LOCAL HEALER……..4 FAMILY MEMBER……..5 DON'T KNOW……88 OTHER_____66 (SPECIFY)	
9.3	Where does the child get medications if he/she needs them?	HOSPITAL……....1 LOCAL CLINIC..……..2 PHARMACIST…….3 LOCAL HEALER……..4 FAMILY MEMBER……..5 DON'T KNOW……88 OTHER_____66 (SPECIFY)	
9.4	Does the child have a health card?	YES……..1 NO……2 DON'T KNOW……88	
9.5	Has the child ever received a vaccine?	YES……1 NO…(SKIP REMAINING QUESTIONS)……2 DON'T KNOW……88	
9.6	If so, against what disease(s) was he/she vaccinated?	TETANUS……...1 TYPHOID……....2 POLIO……3 DIPTHERIA……..…...4 YELLOW FEVER……..…….5 TUBERCULOSIS (BCG)……..…...6 RABIES……..…..7 MUMPS……..…..8 MEASLES……..…..9 RUBELLA……10 DON'T KNOW……..88 OTHER _____66 (SPECIFY)	
9.7	Vaccinations confirmed on health card?	YES……1 NO..……2	

9.8	Where did the child get the vaccinations?	HOSPITAL……....1 LOCAL CLINIC..……..2 PHARMACIST…….3 LOCAL HEALER……..4 FAMILY MEMBER……...5 DON'T KNOW…….….88 OTHER_____66 (SPECIFY)	

THANK YOU for all of your help. We are very grateful for your time.