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Schistosomiasis, malaria, and co-infection: Contributions to anemia in Kenyan schoolchildren

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B.S., University of Michigan, 2014

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Abstract

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By Emily Valice

We assessed the impact of schistosomiasis drug treatment strategies and the interactive effect of schistosomiasis and malaria on anemia in schoolchildren in western Kenya. Using a cohort nested within a 5-year longitudinal study that evaluated different treatment approaches on *Schistosoma mansoni* infection levels, we ran a village-clustered, multilevel model examining the crude association of schistosomiasis treatment regimen on hemoglobin levels. A separate village-clustered, multilevel model was run assessing the impact of and interaction between categorized schistosome infection and malaria on hemoglobin level from baseline to year 5. Mean hemoglobin level in the study decreased from baseline to year 5; correspondingly, anemia prevalence increased from baseline to year 5. Treatment arms comparing annual community-based treatment and biennial school-based treatment were not significantly associated with the change in hemoglobin level. There was also no significant interactive effect of schistosomiasis and malaria on hemoglobin level. The overall negative temporal trend of hemoglobin levels as well as the lack of heterogeneity in hemoglobin levels between treatment arms indicates that the health benefit of schistosomiasis drug treatment with respect to anemia requires further evaluation. If no differences are found between other key health indicators by treatment arm, public health programs may be able to treat schoolchildren rather than full communities every other year rather than annually with no loss of public health impact. Though our research did not find significant interaction between schistosomiasis and malaria on hemoglobin level, it may have been underpowered; further studies are needed to better elucidate the nature of this relationship.

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Literature Review:

Anemia as a Public Health Problem

Anemia is characterized by an insufficient mass of circulating red cells in the blood (1). It is commonly diagnosed after quantifying the concentration of hemoglobin in a blood sample (1). The cutoff level of hemoglobin that defines anemia is dependent on age, sex, altitude, smoking, pregnancy status, and other factors (2). Two recent estimates of the global burden of anemia reported that prevalence is 43% in children, 38% in pregnant women, and 29% in nonpregnant women, with an overall burden of 32.9% (3, 4). However, the burden of disease is not distributed evenly throughout the world; it is highest in South Asia and sub-Saharan Africa (4).

Causes of anemia are diverse and often multifactorial, and include nutritional, genetic, and infectious etiologies (5). Among the infectious etiologies are parasitic diseases such as malaria, hookworm, and schistosomiasis; bacterial infections such as tuberculosis and *Helicobacter pylori*; and viral infections such as infection with human immunodeficiency virus (HIV) (6-10). However, different infections may cause different types of anemia. For example, hookworm can cause iron deficiency anemia (IDA) through blood loss (11). Once inside a host, the parasite attaches to the intestinal mucosa and releases coagulases that lead to ongoing loss of blood in the stool. The development of hookworm-related IDA depends on the species of hookworm and the intensity of infection, as well as an individual's iron stores (12, 13). By contrast, infection with HIV has been shown to cause anemia of inflammation (AI), formerly known as anemia of chronic disease (12). This type of anemia is characterized by sufficient iron in storage even as iron levels in the blood are decreased, and is classically attributed to the body's

reaction to inflammation. Briefly, AI is induced by the body itself as a mechanism to limit the iron available in the blood for the growth, development, and reproduction of disease-causing organisms (14). IDA is the most common type of anemia, representing about half of all anemia worldwide, but AI is also a significant contributor the global burden (4).

Generally, anemia has been associated with a variety of negative health-related outcomes, including reduced work productivity, lower intelligence or cognitive capacity, and various other morbidities (15). Young children and pregnant or postpartum women are the most commonly and severely affected by anemia due to the high iron demands of growth and pregnancy, respectively (16). Many studies have found associations between IDA and poor cognitive and motor development as well as behavioral problems (17). Additionally, it has been consistently observed that infants who suffer from IDA continue to have poorer cognition, lower school achievement, and more behavioral problems into middle childhood (17). Though both IDA and AI will ultimately lead to iron insufficiency, it is likely that health outcomes associated with each type differ (18). Though there is a lack of research on the health impacts associated specifically with AI, recent advances in understanding of the mechanisms behind different types of anemia, along with improvements in type-specific diagnostic techniques, should help to better differentiate the health impacts of different anemias.

The type of anemia presenting in an individual or in a population dictates the best treatment practices. In settings where IDA is the most prevalent type of anemia, increasing access to iron-rich foods and/or oral iron supplementation may be the most appropriate course of action (16). However, if the anemia present in the population is AI,

treatment with oral iron supplementation should be considered with caution, as this may not result in a positive health outcome (19). When possible, infections contributing to anemia should be treated (19). If anemia persists after clearance of infection, further assessment and treatment may be necessary.

Schistosomiasis as a Public Health Problem

One common infectious cause of anemia is schistosomiasis, or bilharzia, which is caused by trematode parasites of the genus *Schistosoma* (20). The main schistosomes that infect humans are *S. mansoni*, *S. haematobium*, and *S. japonicum*, with *S. intercalatum* and *S. mekongi* of only limited importance (21). The life cycle of schistosomes is complex, and is summarized below [see reference (22)]. Schistosomes have separate sexes, and both male and female adult worms live in blood vessels where they mate and produce fertilized eggs. The eggs either become trapped in host tissues or are shed into the environment through urine (*S. haematobium*) or feces (other species). Upon contact with freshwater, eggs hatch and release miricidia that then infect an intermediate snail host. Asexual reproduction occurs in the snail, after which infectious cercariae are released back into the water. Schistosomes are acquired when cercariae penetrate the skin of people exposed to infested freshwater through swimming, bathing, or wading. Cercariae transform into young worms, or schistosomulae, in the skin, migrate through the lungs and the liver, mature to adulthood in the blood vessels around the bladder (*S. haematobium*) or intestines (*S. mansoni and S. japonicum*), and mate, completing the life cycle. Theoretically, one mature schistosome pair may potentially produce up to 600 billion offspring (21).

Morbidity induced by schistosomiasis is caused by schistosome eggs, rather than the adult worms (22). Eggs that are not excreted by the body become lodged in the bladder and urogenital system (*S. haematobium*) or in the intestines or liver (*S. mansoni* and *S. japonicum*), inducing inflammation which leads to further disease manifestations (22). Without treatment, infection becomes chronic and symptoms may start to include blood in the urine, increased urinary frequency, burning sensation during urination, abdominal discomfort, kidney damage, and in women, pain and issues with reproductive health (*S. haematobium*) or non-specific intermittent abdominal pain, diarrhea, rectal bleeding, liver or spleen organomegaly, periportal fibrosis, ascites, collateral circulation, and esophageal varices (*S. mansoni* and *S. japonicum*) (22, 23). Frequency of symptoms may be linked with intensity of infection (24). Chronic schistosomiasis can also cause non-specific morbidities including anemia (both IDA and/or AI), growth stunting, caloric undernutrition, impaired cognitive development, fatigue, poor exercise tolerance, increased susceptibility to, and subsequent enhanced disease from, co-infections, and more (23). Unexpected morbidities may also occur infrequently, such as parasites or eggs migrating to the central nervous system, causing cerebral schistosomiasis and potentially leading to seizures, paralysis, or spinal cord inflammation. However, it is the so-called 'subtle' morbidities, such as anemia, that comprise the bulk of the disease's negative health impact (22, 25).

Control of schistosomiasis has historically included interventions targeting both the adult parasite within the human host and the intermediate snail host. The first documented attempts of these control measures occurred in the early 20th century in Egypt and Japan (26). Efforts to control this disease have not halted, and remain ongoing

today. The World Health Organization (WHO) has recognized the relevance of schistosomiasis in public health since its establishment in 1948, and currently provides guidance and support to countries in their disease control efforts (26). With the development of safe and effective drugs in the 1970s, simple treatment of infected individuals is now possible (27). The primary WHO strategy for schistosomiasis control is mass-drug administration of praziquantel (28). Additionally, tools to combat the snail intermediate host include molluscicides and snail habitat modification guidelines (26). Nevertheless, the current global prevalence of schistosomiasis is conservatively estimated to be greater than 230 million people (29). It remains a significant public health issue, especially in many African countries.

Malaria as a Public Health Problem

Another parasitic infection associated with anemia is malaria, which is caused by *Plasmodium spp*. The main species that infect humans are *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae*, and *P. knowlesi* (30). Some other species can, under rare circumstances, infect humans (30). The life cycle of malaria parasites is complex, and summarized below [see reference (30)]. Malaria is transmitted between humans through the bite of female mosquitoes. These mosquitoes take blood meals from humans, and acquire the parasite if the individual that they feed off of is infected. The parasite develops inside the mosquito, then, if the mosquito feeds again, it can infect another human. Parasites will develop through a liver stage to blood stage, after which some will differentiate into the stage that infects mosquitoes. Transmission is dependent on a variety of factors, including climate and human immunity (30).

Clinical malaria symptoms are caused by the blood stage, or asexual erythrocytic stage, of the parasite (30). Symptoms of uncomplicated malaria include fever, sweats, nausea and vomiting, chills, headaches, body aches, and general malaise (31). If the disease progresses and serious organ failures or blood or metabolism abnormalities occur, severe malaria may present (30). Manifestations of severe malaria include cerebral malaria, severe anemia, hemoglobin in the urine, acute respiratory distress syndrome, abnormal blood coagulation, low blood pressure, acute kidney failure, hyperparasitemia, metabolic acidosis, and hypoglycemia (31). Other severe manifestations, as well as death, may occur (30, 31).

Malaria is a public health problem of immense scale that continues to plague many countries today, especially those located on the African continent. The WHO estimated that 212 million cases of malaria occurred in 2015, with approximately 191 million (90%) of those occurring in the WHO African Region (32). *P. falciparum* and *P. vivax* pose the greatest threat to humans; *P. falciparum* is the most prevalent species on the African continent and is responsible for the most malaria-related deaths globally (32). *P. vivax* is the more common species in most countries outside of sub-Saharan Africa. In 2015, an estimated 429,000 deaths globally were due to malaria, with 92% of those occurring in the WHO African Region (32). Large-scale action is required to achieve long-lasting reduction of these numbers, and this action must be grounded in scientific evidence.

Interaction of Schistosomiasis and Malaria on Anemia

In many areas, individuals may be at risk for multiple parasitic diseases. For example, helminths and *Plasmodium* parasites share much of the same geographic

distribution (33). Because both schistosome and *Plasmodium* infections are associated with anemia, co-infection with these two organisms is of particular interest to scientists and public health officials as it has important implications for treatment, control, and elimination of both diseases. Currently, this interaction and its potential mechanism is poorly understood.

Though several studies have been conducted to investigate the interactions that may occur during schistosome and *Plasmodium* co-infection, many of these focus solely on the risk of acquiring infection. The reported results are highly variable. Some studies report an association between *S. mansoni* infection and increased susceptibility to malaria; a similar number of studies report the opposite [reviewed in (33)]. Conversely, infection with *S. haematobium* has a protective effect against manifestation of malaria in some studies [reviewed in (33)]. One meta-analysis found that *S. mansoni* or *S. haematobium* and *P. falciparum* co-infection may be associated with increased prevalence of asymptomatic or uncomplicated malaria in children, but may protect against high density *P. falciparum* infection (34). Factors that may contribute to the variable nature of results from different studies include study methodology, environment/study setting, host factors/study population, and parasitic species and intensity of infection (35). Additionally, it appears that the nature of the association between schistosomiasis and malaria varies with both the age of the individuals and the intensity of schistosomiasis (35). Further research in this area is needed when planning public health interventions to maximize health benefits.

The specific effect on morbidities generally and anemia specifically in individuals co-infected with schistosomes and *Plasmodium* parasites is less studied. One review

reports that while some studies show decreased malaria prevalence, incidence, parasitemia, disease severity, and associated splenomegaly, others show increased prevalence or risk of malaria and density of the parasite or low hemoglobin level and enlarged spleens among individuals infected with *S. haematobium* [reviewed in (35)]. A meta-analysis found that *S. mansoni* or *S. haematobium* and *P. falciparum* co-infection may protect against reduction in hemoglobin levels resulting from high density *Plasmodium* infections (34). However, other studies show no relationship between malaria prevalence or parasitemia with hemoglobin level and *S. haematobium* infection in children [reviewed in (35)]. Work done in the Philippines has shown an interactive effect between hookworm and *S. japonicum* on anemia, suggesting that in helminth-endemic regions, integrated treatment programs could lead to greater than additive gains in anemia reduction (36). An opposite relationship was observed in Brazil with some helminths other than schistosomes; children with *Ascaris lumbricoides*, hookworm, or *Trichuris trichiura* infections showed no significant difference in hemoglobin levels during *P. vivax* episodes compared to the children with malaria who did not have helminth co-infections (37). Further research is needed to examine the relationship between schistosomiasis and malaria co-infection on morbidities such as anemia.

Need, Goals, and Aims

Parasitic infections are important drivers of anemia, especially with respect to the growth and development of children. Kenya is among the many African countries in which individuals may be at risk for infection with both schistosome and malaria parasites. Lake Victoria in western Kenya is a known reservoir for the snail species that serves as intermediate hosts for schistosomes. Children who attend schools within 5 km

of the lake have a high prevalence and intensity of *S. mansoni* infection (38, 39). Additionally, malaria is holoendemic in this area (40). As both of these infections may separately cause anemia and its associated deleterious health outcomes, the effects of co-infection with *Schistosoma* and *Plasmodium*, especially in children still undergoing rapid growth and development, requires further elucidation. Equipped with a better understanding of the dynamics of this co-infection, we will be able to design safer and more effective public health interventions.

We attempted to assess two questions using data from a longitudinal cohort study of Kenyan schoolchildren in villages along the shores of Lake Victoria. First, what is the effectiveness of different preventative chemotherapy strategies for schistosomiasis treatment on anemia? Second, is there an interactive effect of schistosomiasis and malaria co-infection on anemia, and if so, what is the strength of that interaction?

Significance

As described above, non-specific morbidities caused by schistosomiasis are not well understood. By investigating the impact of different preventative chemotherapy delivery strategies for schistosomiasis on hemoglobin levels, we can begin to better formulate methods to help reduce morbidities caused by the infection. The preventative chemotherapy strategies considered in this study also vary in frequency of administration and target population (school-based or community-wide treatment), leading to large differences in cost of implementation. If more frequent, less targeted administration does not lead to better population health outcomes than less frequent, more targeted administration, resources may be allocated over a wider timespan or population with

similar impact. Reduction in these morbidities may also have wide-reaching implications for the individuals and communities affected.

The impact of a demonstrated interactive effect between schistosome and malaria infections include better evidence-based guidance for schistosomiasis, malaria, and anemia public health program planning and execution. In an ideal world, schistosomiasis and malaria programs would treat all infected individuals and maintain an infection-free population. However, real-world resources are always limited. If a greater than additive interaction between schistosomiasis and malaria on anemia were demonstrated, a program may be able to target a single infection over a wider population and achieve significant reduction in morbidity.

Methods:

Study Context

The Schistosomiasis Consortium for Operational Research and Evaluation (SCORE) was established in 2008 through a five-year grant from the Bill & Melinda Gates Foundation to the University of Georgia (UGA) Research Foundation. The focus of SCORE is to conduct operational research that will answer strategic questions about schistosomiasis control and elimination in order to enhance effectiveness of public health programs in these areas (41). One way to help answer these questions are field studies investigating multi-year strategies of delivering preventive chemotherapy for gaining and sustaining control of schistosomiasis in moderate and high prevalence areas. SCORE supported study teams as they implemented cluster randomized trials with different preventive chemotherapy schemes in five African countries (42). The data analyzed here were collected during one such study to gain control of *Schistosoma mansoni* in the Kisumu region of western Kenya, bordering Lake Victoria. This study was led by the Center for Global Health Research at the Kenya Medical Research Institute (KEMRI) and the Centers for Disease Control and Prevention (CDC) (42). The objective was to determine the impact of different praziquantel (an anti-helminthic drug) treatment arms, varying by population targeted (school-based versus community-wide) and frequency (annual or with skipped years), on schistosomiasis prevalence and intensity. To evaluate the impact of treatment on morbidity reduction, a five-year nested longitudinal cohort study collected additional morbidity variables from children attending schools in two of the six treatment arms. Children enrolled in this nested cohort were followed over five years.

Institutional Review Boards

The Institutional Review Boards of both CDC and UGA reviewed the parent study protocol, which described the nested cohort study as well, and deferred to the KEMRI Ethical Clearance Committee, which approved it. The subset of data obtained for this analysis does not meet the definition of research with human subjects as set forth in Emory policies and procedures, as the dataset is de-identified (see Appendix A for official Emory Institutional Review Board letter).

Study Area and Population

Full descriptions of the SCORE parent study and nested morbidity cohort study designs, study populations, and baseline measurements are published elsewhere (42, 43). Briefly, the parent study population was drawn from communities where schistosomiasis prevalence had previously been identified as ≥25%. One hundred fifty communities were selected for inclusion and were assigned randomly to one of six different drug treatment arms after which they were followed for five years. For the nested cohort, 20 communities were selected for participation based on community size and proximity to CDC/KEMRI laboratory facilities. Of these 20 communities, 10 belonged to the study arm with the highest drug pressure (Figure 1, Arm A) and 10 belonged to the study arm with the lowest drug pressure (Figure 1, Arm B). From each set of 10 communities, six schools within 5km of Lake Victoria were randomly selected to participate. A total of 12 schools were selected from 12 of the eligible communities (Figure 2). Children aged 7 or 8 years without obvious physical disabilities who assented and also had parental or guardian consent were eligible for inclusion. Ultimately, a total of 802 children were consented and enrolled into the nested cohort at baseline. Children were assessed for a

variety of metrics at baseline, year 3, and year 5. For this analysis, only data from baseline and year 5 were analyzed.

Data Collection

Demographic information: Age and gender were obtained from school registries and parent or guardian report.

Anthropometric measurements: Height was measured after children removed shoes and dressing on their heads. Each child stood on the base of a stadiometer with both feet together and their head in contact with the vertical board. Examiners asked each child to stand as tall as possible and take a deep breath. A ruler was placed on their head, and a precise height was measured in centimeters. Weight was measured after children removed shoes and any excess clothing. They stood on the center of the scale and examiners recorded the result in kilograms with one decimal place. The scale was tared each morning or at every school visited, whichever was more frequent. Two readings for height and weight were taken for each child, and the mean of those results was calculated. Upper arm circumference was also measured (43).

Stool examination: Children were asked to provide a fresh stool sample on three consecutive days. Samples were collected in stool containers and transported to the CDC/KEMRI laboratory. There, they were processed and examined by the Kato-Katz technique for detection of parasite eggs, with two slides prepared per stool. The presence of *S. mansoni, Ascaris lumbricoides, Trichuris trichiura,* and hookworm eggs was recorded. Egg count quantification was performed for only *S. mansoni*. The arithmetic mean of egg counts was calculated from the total slides per child (maximum 6 slides per measurement occasion), and expressed as eggs per gram (epg). Infection intensity was

categorized as light (1-99 epg), moderate (100-399 epg), or heavy (>400 epg) (26). Stool evaluations were performed blinded to prior stool results (43).

Blood collection and processing: A 5mL venous blood sample was collected from each child. Hemoglobin concentration was calculated with HemoCue (Ängelholm, Sweden) in g/dL. For this cohort of children, we defined anemia as a hemoglobin level below 11.5 g/dL (44). *Plasmodium falciparum* parasitemia was determined through blood smear examination by experienced microscopists (43).

Data Handling and Analysis

Demographic and anthropometric data were collected on smartphones and uploaded to a central server maintained database [EpiCollect (45)]. Laboratory (stool and blood) data were collected on paper forms and entered into the same database via a secure, web-based portal. Data were analyzed using SAS version 9.4 (SAS Institute, Inc., Cary, NC). Statistical significance was set at the 5% level. Univariate analyses were conducted on baseline and year 5 data separately, with statistical testing to compare differences between treatment arms at each measurement occasion (Tables 1a and 1b). The Rao-Scott chi-square test was used to account for clustering by village when testing for associations between categorical variables (46). A two sample t-test using Taylor series linearization was used to test for differences with approximately normally distributed continuous variables (47). For the continuous but highly skewed *S. mansoni* epg measurement, the Mann-Whitney U/Wilcoxon sum-rank test was used. Raw change in mean village hemoglobin over time was plotted by treatment arm along with mean overall change by treatment arm (with error bars representing ± one standard error) using Microsoft Excel 2016 (Microsoft Corp., Redmond, WA).

This cohort experienced loss to follow up. To assess potential bias arising from loss to follow up, univariate analyses were conducted on baseline demographics by dropout status, with statistical testing to compare differences between those who were measured at both baseline and year 5 and those who were measured only at baseline (Table 2). Testing was conducted similarly to that in Tables 1a and 1b.

To assess predictors alone as well as in groups, multilevel modeling was performed (48). Attempts at incorporating both person- and village-level clustering did not achieve convergence. Therefore, all models only control for clustering at the village-level. The correlation structure was assumed to be compound symmetry. Multilevel models examining the crude impacts of treatment arm, schistosomiasis (both dichotomous and categorized by infection intensity), and malaria on hemoglobin levels over time were run separately. These models followed the form:

$$\text{Hemoglobin}_{ij} = \pi_{0i} + \pi_{1i}\,(\text{Study Year}_{ij}) + \varepsilon_{ij}, \text{ where}$$

$$\pi_{0i} = \gamma_{00} + \gamma_{01}(\text{Variable of Interest}) + \zeta_{0i}, \text{ and}$$

$$\pi_{1i} = \gamma_{10}. \qquad [1]$$

A subscript of *i* refers to a village mean, and a subscript of *j* refers to measurement occasion (year).

To compare the simple prevalence of anemia (as it is clinically defined) between baseline and year 5, we used a generalized linear mixed model with a logit link function (49). The outcome of anemia prevalence was defined as the proportion of individuals with a blood hemoglobin level less than 11.5 g/dL (44). Again, including both person and village clustering was attempted but unsuccessful due to a failure to converge. Therefore, this model included only village clustering. Though this model examines the data at a less

granular level (i.e. anemia is a dichotomization of hemoglobin level), this is the most relevant clinical assessment of the outcome.

A separate multilevel model examining the impact of and interaction between schistosomiasis infection (categorized by intensity, reference group zero eggs per gram) and malaria infection (dichotomized as infected or uninfected) on hemoglobin levels was constructed. All possible two and three-way interactions were initially included in the model; however, all but the interaction of interest (schistosomiasis x malaria) were ultimately removed due to a failure to achieve convergence. The final version of the model controlled for available confounders and followed the form:

$$\text{Hemoglobin}_{ij} = \pi_{0i} + \pi_{1i}(\text{Study Year}_{ij}) + \pi_{2i}(\text{Light Schistosomiasis}_{ij}) +$$

$$\pi_{3i}(\text{Moderate Schistosomiasis}_{ij}) + \pi_{4i}(\text{Heavy Schistosomiasis}_{ij}) + \pi 5i(\text{Malaria}_{ij}) +$$

$$\pi_{6i}(\text{Light Schistosomiasis*Malaria}_{ij}) + \pi_{7i}(\text{Moderate Schistosomiasis *Malaria}_{ij}) +$$

$$\pi_{8i}(\text{Heavy Schistosomiasis *Malaria}_{ij}) + \varepsilon_{ij}, \text{ where}$$

$$\pi_{0i} = \gamma_{00} + \gamma_{01}(\text{Study Arm}) + \gamma_{02}(\text{Male}) + \zeta_{0i}, \text{ and}$$

$$\pi_{xi} = \gamma_{x0} \text{ when } 1 \leq x \leq 8. \qquad [2]$$

As with model [1], a subscript of *i* refers to a village mean, and a subscript of *j* refers to a measurement occasion (year). The correlation structure of this model was assumed to be compound symmetry. Fit statistics such as AIC, AICC, and BIC were slightly lower with this model than a model also including the soil-transmitted helminth measurements available (*Ascaris spp.* and *Trichuris trichiura*). Model [2] was additionally run on the subset of the cohort excluding those who were lost to follow up (measured only at baseline).

Results:

The goal of this thesis is to further our understanding of the possible causal relationship of selected parasitic diseases to anemia in children. We first assess the impact of two longitudinal praziquantel treatment strategies on anemia in Kenyan children living in an area where schistosomiasis prevalence is high. Next, we examine the potential interaction between schistosomiasis and malaria on anemia in the aforementioned population, as malaria is also endemic in the area.

Impact of Treatment Arms

To assess the impact of the two treatment arms on anemia, we first compared baseline demographic and health measurements between schools assigned to each of those treatment arms (Table 1a). Possibly due to the selection of these schools (they were primarily selected for proximity to laboratory facilities), there were statistically significant differences in subject characteristics between Arms A and B. Weight (Arm A mean ± SD = 20.0 kg ± 2.8 kg; Arm B mean ± SD = 21.0 kg ± 3.0 kg), mid-upper arm circumference (Arm A mean ± SD = 17.1 cm ± 1.3 cm; Arm B mean ± SD = 17.7 cm ± 1.3 cm), continuous schistosomiasis eggs per gram (Arm A mean = 153.3 epg, range = 0 to 1008 epg; Arm B mean = 112.8 epg, range = 0 to 1008 epg), malaria prevalence (Arm A prevalence = 6.3%; Arm B prevalence = 9.2%), ascariasis prevalence (Arm A prevalence = 7.1%; Arm B prevalence = 14.3%), whipworm prevalence (Arm A prevalence = 9.1%; Arm B prevalence = 20.7%), and hookworm prevalence (Arm A prevalence = 1.7%; Arm B prevalence = 8.8%) demonstrated evidence of a difference between arms ($p < 0.05$). However, as seen from the absolute values of the differences, they may not be clinically significant. For example, the difference in mean weight

between treatment arms is statistically significant (Arm A mean ± SD = 20.0 kg ± 2.8 kg; Arm B mean ± SD = 21.0 kg ± 3.0 kg; $p < 0.0001$), but the difference between arms is only 1 kg. Note that there was a statistically significant difference in mean schistosome eggs per gram between arms (Arm A mean = 153.3 epg, range = 0 to 1008 epg; Arm B mean = 112.8 epg, range = 0 to 1008 epg; $p < 0.0001$), but no statistically significant difference in prevalence or categorized intensity levels. This between-arm comparison was replicated using the data collected in year 5 (Table 1b). From baseline to year 5, height and moderate intensity infection (compared to no infection) transitioned from not significantly different to significantly different, while arm circumference, malaria infection, and ascariasis demonstrated the reverse pattern. The inferences about the differences between treatment arm, hemoglobin level, anemia prevalence, or other variables at the year 5 measurement did not change.

This cohort experienced substantial loss to follow up. Sample size decreased by 61%, from 802 individuals at baseline to 313 individuals in year 5. A comparison of individuals not lost to follow up (those who were assessed in year 5) to those lost to follow up (those who were not assessed in year 5) can be seen in Table 2. Statistically significant differences between these two groups of individuals were found for height (Not Lost to FU mean ± SD = 122.3 cm ± 6.5 cm; Lost to FU mean ± SD = 121.2 cm ± 6.4 cm; $p = 0.02$), light intensity schistosomiasis infection (prevalence in Not Lost to FU = 27.1%; Lost to FU = 35.2%; $p = 0.03$), schistosomiasis prevalence (prevalence in Not Lost to FU = 60.2%; Lost to FU = 68.3%; $p = 0.02$), and hookworm prevalence (prevalence in Not Lost to FU = 6.7%; Lost to FU = 4.0%; $p = 0.01$). No other

differences between those not lost to follow up and those lost to follow up were statistically significant.

A visual representation of hemoglobin measurements is shown in Figure 3, which includes two plots of the decline in mean hemoglobin over time by study arm. Changes in village mean over time are shown in addition to the overall treatment arm mean, which is shown with error bars representing ± one standard error. Though the general temporal trend is a decrease, there is noticeable variation between individual villages. In each arm, at least one village shows a clear increase in hemoglobin over time.

Model [1], using treatment arm as a predictor for hemoglobin levels over time and incorporating village-level clustering, confirmed that in this population treatment arm was not significantly associated with change in hemoglobin level. We found that enrollment in Arm B compared to Arm A was associated with an average decrease of 0.05 g/dL of hemoglobin (SE = 0.28 g/dL; p = 0.87) (Table 3).

Our crude model comparing the prevalence of anemia between baseline and year 5, accounting for village-level clustering, indicated that there was a significant increase in anemia prevalence in year 5 compared to baseline. We found that the unadjusted odds of being anemic in year 5 were 1.5 times the unadjusted odds of being anemic at baseline (95% confidence interval: 1.2 to 1.9; p = 0.0003) [data not shown].

Interaction between Schistosomiasis and Malaria

Before exploring the potential interaction between schistosomiasis and malaria we ran model [1] using each of these infections (representing schistosomiasis as both dichotomous and categorical) separately as a predictor of hemoglobin over time, clustered by village (Table 3). Dichotomous infection as well as each intensity of

schistosomiasis were associated with slight drops in hemoglobin level when compared to no infection in their respective models, though none of these associations were statistically significant. Any intensity of schistosome infection was associated with a decrease of 0.20 g/dL hemoglobin (SE = 0.17 g/dL; $p = 0.22$) when compared to no infection. A light intensity schistosome infection was associated with a decrease of 0.18 g/dL of hemoglobin (SE = 0.18 g/dL; $p = 0.33$), a moderate intensity schistosome infection was associated with a decrease of 0.20 g/dL (SE = 0.22 g/dL; $p = 0.37$), and a heavy intensity schistosome infection was associated with a decrease of 0.43 g/dL hemoglobin (SE = 0.29 g/dL; $p = 0.14$), all when compared to no infection. Similarly, malaria was associated with a non-statistically significant decrease of 0.18 g/dL of hemoglobin (SE = 0.23 g/dL; $p = 0.41$). In all of these models, time was a significant predictor of hemoglobin level decrease ($p < 0.0001$).

Model [2] explored the interaction between schistosomiasis and malaria over time and controlled for treatment arm and sex, as well as village-level clustering (Table 4). The interaction between malaria and a light schistosomiasis infection demonstrated a negative effect on hemoglobin level (-0.49 g/dL; SE = 0.56 g/dL) whereas malaria and a moderate or heavy schistosomiasis demonstrated a positive effect on hemoglobin level (0.36 g/dL and SE = 0.73 g/dL; 0.09 g/dL and SE = 0.87 g/dL, respectively). However, these interactive effects were not statistically significant ($p \geq 0.38$). Similar to all variations of model [1] (Table 3), time was significant in the multivariable model ($p < 0.0001$), demonstrating a reduction in hemoglobin levels in year 5 compared to baseline. Treatment arm and sex were not strong confounders, but were included in the model because of study design and biological plausibility, respectively.

In addressing the potential bias caused by loss to follow up, we also ran model [2] on the subset of individuals who were followed through year 5 (excluding those who were only measured at baseline). Though there were some slight changes in p-values compared to the model that used all possible measurements, model estimates did not change meaningfully and there were no changes in the statistical inferences.

Discussion:

Impact of Treatment Arms

We evaluated the impact of two longitudinal praziquantel treatment strategies on anemia in Kenyan children living in an area where schistosomiasis prevalence is high. Though treatment may be effective in reducing schistosomiasis prevalence, it is also desirable to see a decrease in morbidity among the target population. Hemoglobin levels are one such marker of morbidity. Each individual in the cohort experienced one of two schedules of drug treatment (Arm A or Arm B, see Figure 1). At baseline, there existed some statistically significant differences in subject characteristics, such as weight, between subjects in Arm A vs. those in Arm B. We chose to move forward with the analysis as the characteristics that differed statistically between arms were small and not likely to be clinically significant. One particularly relevant example of this was prevalence, intensity, and eggs per gram schistosome infection between arms. Though there was a statistically significant difference in mean schistosome egg per gram between arms, there was no statistically significant difference in prevalence or categorized intensity levels. This was likely due to the highly skewed distribution of individual egg per gram values, and potentially also due to a loss of power following categorization (50). Additionally, though measurements were collected using a systematic protocol, the possibility of measurement error exists, especially for the demographic and anthropomorphic data.

Malaria, a major driver of anemia, was found in 6.3% (Arm A) and 9.2% (Arm B) of children while schistosomiasis, another driver, was even more prevalent, with 73.5% (Arm A) and 56.2% infected (Arm B) (Table 1a). The prevalence of anemia within these

communities was substantial at baseline, at roughly 40% (Table 1a). Though these infections are far from the only plausible causes of anemia, they are potential contributors to the widespread anemia in the region (16, 51). Other risk factors for anemia in this region may include iron deficiency due to low intake or poor absorption of iron, other acute and chronic infections, and the presence of other micronutrient deficiencies (16). Unfortunately, measurements of these other risk factors were not taken in our study population.

Our assessment of potential loss to follow up bias indicated that prevalence of schistosomiasis at baseline was significantly higher in those who dropped out than those who remained in the study. Since the individuals lost to follow up were more likely to have schistosomiasis, they may also be more likely to experience negative health impacts due to infection. If this is true, then further treatment of infection may have benefitted this group (perhaps even increasing their hemoglobin levels) more than the group that remained enrolled until year 5. However, the categorical breakdown of schistosomiasis infection intensity indicates that the only significant difference between those lost to follow up versus those not lost to follow up was in those with a light intensity infection. Light intensity infections generally cause less morbidity than moderate or heavy intensity infections, so it is possible that the difference in light intensity infection prevalence, and thus general prevalence, is not meaningful (35).

The implementation of praziquantel treatment strategies resulted in differential reduction in prevalence of schistosomiasis by study arm (Arm A exhibited a 23.7% decrease and Arm B exhibited a 0.9% decrease) during the 5-year study. Though the WHO reports an observed parasitological cure rate between 70% and 100%, reinfection

is possible and common (26). For example, one study reported only a 37% reduction in prevalence in Nigerian schoolchildren just one year after a school-based praziquantel administration program similar to what was done in this study (52). With the ever-present possibility of reinfection for the subjects of this study, it is a success to achieve a reduction in prevalence after five years, especially of the magnitude seen in Arm A. However, mean hemoglobin levels did not increase in either arm accompanying this reduction in schistosomiasis prevalence; in contrast, mean hemoglobin levels *decreased* in both arms over time (Figure 3). This reduction in hemoglobin levels resulted in a significantly increased prevalence of anemia in both arms over time (data not shown). One possible explanation for this phenomenon is an unrelated factor (such as nutrition) driving hemoglobin levels in the region (16). If iron intake in the area is generally low, individuals may become anemic regardless of malaria or schistosomiasis status. Further assessment is required to determine the direct health benefits of schistosomiasis treatment programs in different settings. In studying these relationships, researchers must remain cognizant of other possible, non-infectious causes of the morbidities that are associated with schistosomiasis.

Though mean hemoglobin levels decreased over time, we see some heterogeneity in this trend (Figure 3). Three of the 12 villages show an increase in mean hemoglobin from baseline to year 5. Heterogeneity was also seen in the reduction of schistosomiasis prevalence. The parent study determined that a cluster of villages located in Siaya County (see Figure 2) fell inside a geographic area of increased schistosomiasis prevalence and intensity (“hotspot”) for schistosomiasis transmission, with these villages experiencing a higher than average rate of reinfection and infection intensity. The nested cohort study

included villages inside, sometimes inside (the hotspot area shifted slightly each year), and outside of the hotspot. However, it was determined, by comparing location and crude change in hemoglobin as well as including categorized location (inside, sometimes inside, or outside of the hotspot) in a multilevel model, that village location relative to the hotspot was not statistically significantly related to the temporal change in hemoglobin levels during the study.

In model [1], treatment arm was not significantly associated with hemoglobin levels (Table 3). This finding is novel, as other studies either do not use both a school-based and a community-based treatment model in the same region or do not measure blood hemoglobin levels post-treatment. If hemoglobin levels in the area are being driven by some other factor, then this non-differential effect of treatment arm follows logically. However, in an area where schistosomiasis drives hemoglobin levels, this has important public health programming implications. Within this design, Arm A was more resource intensive than Arm B, due to both the population targeted (community-wide versus school-wide treatment) and the frequency of treatment (every year versus every other year). Current WHO guidelines advocate for mass drug administration at intervals specific to community risk level, but do not necessarily differentiate between community-wide and school-wide treatment (28). If neither treatment arm proves better at reducing morbidity, the strategy employed in Arm B may be appropriate for future public health interventions, and could help stretch resources over a wider population or for a longer time span than the strategy employed in Arm A.

The clinical assessment of this cohort in which anemia is defined as hemoglobin levels lower than 11.5 g/dL indicated that the prevalence of anemia increased over time.

However, as seen in Figure 3, mean hemoglobin levels in both arms were between 11 g/dL and 12 g/dL at both measurement occasions. As such, this change in population anemia prevalence does not actually reflect a large change in mean population hemoglobin levels. Additionally, the cutoffs for clinical anemia are somewhat subjective, further decreasing the precision of "anemia" as a health outcome. Here, we used general guidelines provided with the WHO [see reference (44)], but with multiple factors influencing what "normal" hemoglobin levels are, cutoffs may vary depending on expert opinion and setting. Future studies that are only able to obtain dichotomous anemia status should consider this same possibility in their analyses and be aware of which anemia cutoff guidelines were used.

Interaction between Schistosomiasis and Malaria

As seen in Table 3, both infection with schistosomes (overall, as well as categorical) and infection with malaria were associated with a decrease in hemoglobin over time, though this association was not statistically significant. This lack of significant association contradicts the large body of literature that has demonstrated both of these associations (7, 26). This is possibly due to the lack of power in this study. It is also possible that an unmeasured factor is driving hemoglobin levels in this region (16). In the adjusted model that included the interaction of interest (schistosomiasis x malaria) there was a similar lack of statistical significance of the two diseases' joint effect on hemoglobin levels. The magnitude of the effect estimates of the interaction terms, though they are not significant, show an interesting pattern. The model indicates that when individuals are lightly infected with schistosomes and also have a malaria infection, their hemoglobin levels are on average slightly lower than individuals who are infected with

either parasite alone. However, those who are moderately or heavily infected with schistosomes and also have a malaria infection have hemoglobin levels that are slightly higher than those infected with either parasite alone. It is not appropriate to draw strong conclusions from estimates that are not statistically significant, but it does raise interesting questions about the relationship between schistosomiasis and malaria. Further research would be useful to explore whether different intensities of schistosome infection interact differently with malaria on anemia in children in this region.

Strengths

This study examines the impact of anti-helminthic drug strategies longitudinally, which is a design less common in this research area than cross-sectional studies. With longitudinal observation of individuals, we were able to examine the temporal association between treatment or disease(s) with morbidity. Additionally, we were able to assess differences in morbidity for two treatment arms that differed both temporally (treatment occurred every year in Arm A and every other year in Arm B) and in population of implementation (treatment was community-wide in Arm A and school-based in Arm B, see Figure 1).

Weaknesses

Measurement error is a potential weakness of this analysis. Stool examination is known to have limited sensitivity for detecting helminth infections, so some infections may have been missed. Though we assessed potential loss to follow up bias and did not find evidence of extreme bias (see Figure 2), it is possible that loss to follow up caused different results than would have been found with no loss to follow up. Additionally, with the method of modeling used, the analysis was run on complete cases only. Imputation of

data may have helped us to avoid excluding cases that were not fully complete from our models. Furthermore, since only 12 clusters of data were available, the analysis' power to detect true differences may have been reduced.

Conclusion

The goal of this thesis was to assess the effectiveness of different preventative chemotherapy strategies for schistosomiasis on anemia and to explore the potential interactive effect of schistosomiasis and malaria co-infection on anemia in a 5-year nested cohort of schoolchildren in western Kenya. We found no significant difference in the change in hemoglobin levels between treatment arms, though it should be noted that there was an overall decline in hemoglobin levels during the study period. This potentially indicates that hemoglobin levels in the region are being driven by other factors. Further study is needed to determine if differences in health outcomes between different treatment strategies exist. Additionally, we did not find a significant interaction between schistosomiasis and malaria on hemoglobin levels. Though not significant, our results indicate that there may be a differential relationship between malaria and different intensities of schistosome infection on anemia. If this relationship were true, it could impact public health programs that target either of these diseases as well as those that are intended to reduce anemia.

Future Directions:

- Further investigation is required to determine the comprehensive health impacts of different drug treatment strategies for schistosomiasis control. Here, we examined the impact on anemia and failed to detect a statistically significant relationship, but other morbidities attributed to schistosomiasis may or may not show significant improvement when the infection is cleared.
- Though this study was longitudinal, it would be beneficial for measurement occasions to occur more frequently. Though praziquantel is very effective in eliminating or at least reducing parasite burden, reinfection may occur shortly afterwards. With more frequent measurement, researchers may find benefits or shortcomings between different treatment strategies that could have been obscured here due to the 5-year gap between assessments.
- It is possible that the anemia caused by these infections is iron deficiency anemia rather than anemia of inflammation. However, only testing hemoglobin levels does not provide that information. More specific testing, such as serum ferritin levels, would help to contextualize the anemia present in this study (53).
- Further elucidation of the drivers of anemia in this area may be necessary before exploring the relationships between the parasitic infections. If most of the anemia in this region is attributable to nutritional deficiencies, then subtle relationships between schistosomiasis and malaria and their effect on anemia may be masked.

Tables and Figures:

Table 1a. Baseline demographic information for subjects enrolled in a nested SCORE cohort by treatment arm.

	Arm A (n= 416)[a]			Arm B (n= 386)[b]			Comparison	
	N Miss.	N or Mean	%, SD, or Range	N Miss.	N or Mean	%, SD, or Range	Difference	p-value[c]
Male	0	197	47.4	0	198	51.3	3.9%	0.26
Age (years)	0	7.5	0.5	0	7.6	0.5	0.1	0.15
Height (cm)	12	121.2	6.9	29	122.1	6.0	0.9	0.053
Weight (kg)	19	20.0	2.8	35	21.0	3.0	1.0	<0.0001
Mid-upper arm circumference (cm)	85	17.1	1.3	118	17.7	1.3	0.7	<0.0001
Hemoglobin (g/dL)	16	11.8	2.7	38	11.9	2.6	0.0	0.88
Anemia prevalence[d]	16	160.0	40.0	38	144.0	41.4	1.4%	0.84
Schistosoma mansoni (schistosomiasis) eggs per gram[e]	9	153.3	0-1008	14	112.8	0-1008	40.5	<0.0001
Light intensity infection, 1-100 eggs per gram		139	34.2		112	30.1	4.0%	0.65
Moderate intensity infection, 101-300 eggs per gram		114	28.0		61	16.4	11.6%	0.17
Heavy intensity infection, >400 eggs per gram		46	11.3		36	9.7	1.4%	0.82
Schistosoma mansoni (schistosomiasis) prevalence[f]	9	299	73.5	14	209	56.2	17.3%	0.23
Plasmodium spp. (malaria) prevalence[g]	16	25	6.3	37	32	9.2	2.9%	0.04
Ascaris spp. (ascariasis) prevalence[f]	9	29	7.1	14	53	14.3	7.1%	0.02
Trichuris trichiura (whipworm) prevalence[f]	9	37	9.1	14	77	20.7	11.6%	<0.0001
Hookworm prevalence[f]	13	7	1.7	34	31	8.8	7.1%	<0.0001

[a] Arm A received community-wide drug treatment with praziquantel in years 1, 2, 3, and 4.

[b] Arm B received school-based drug treatment with praziquantel in years 1 and 3 (drug holidays in years 2 and 4).

[c] P-values correspond to a village-clustered Rao-Scott chi-square test for categorical variables. P-values correspond to a village-clustered two sample t-test for continuous variables with the exception of *Schistosoma mansoni* eggs per gram, which was tested using the Mann-Whitney U/Wilcoxon sum-rank test.

[d] Anemia is defined as hemoglobin <11.5 g/dL.

[e] Intensity of schistosomiasis infection (in eggs per gram of stool) was calculated from stool egg counts averaged over 3 consecutive days (when available).

[f] Prevalence of this infection was defined as presence of eggs in any of three consecutive days (when available) of stool samples.

[g] Presence of malaria was determined via blood smear microscopy.

Table 1b. Demographic information at year 5 for subjects enrolled in a nested SCORE cohort by treatment arm. Note that hookworm measurements were not taken in year 5.

	Arm A (n= 277)[a]			Arm B (n= 235)[b]			Comparison	
	N Miss.	N or Mean	%, SD, or Range	N Miss.	N or Mean	%, SD, or Range	Difference	p-value[c]
Male	0	133	48.0	0	128	54.5	6.5%	0.15
Age (years)	12	11.6	0.6	3	11.7	0.6	0.1	0.06
Height (cm)	32	141.9	7.4	14	143.7	7.9	1.9	0.01
Weight (kg)	32	32.9	5.2	14	34.4	5.8	1.5	0.005
Mid-upper arm circumference (cm)	32	18.8	1.6	14	19.1	1.8	0.3	0.09
Hemoglobin (g/dL)	29	11.2	1.8	24	11.2	1.8	0.04	0.78
Anemia prevalence[d]	29	128	51.6	24	109	51.7	0.05%	0.995
Schistosoma mansoni (schistosomiasis) eggs per gram[e]	44	58.8	0-2980	29	119.8	0-2064	61.0	0.0039
Light intensity infection, 1-100 eggs per gram		95	40.8		61	29.6	11.2%	0.2692
Moderate intensity infection, 101-300 eggs per gram		15	6.4		39	18.9	12.5%	0.0054
Heavy intensity infection, >400 eggs per gram		6	2.6		14	6.8	4.2%	0.2768
Schistosoma mansoni (schistosomiasis) prevalence[f]	44	116	49.8	29	114	55.3	5.6%	0.7746
Plasmodium spp. (malaria) prevalence[g]	29	38	15.3	24	30	14.2	1.1%	0.8410
Ascaris spp. (ascariasis) prevalence[f]	44	6	2.6	29	8	3.9	1.3%	0.3815
Trichuris trichiura (whipworm) prevalence[f]	44	8	3.3	29	22	10.7	7.3%	0.0027

[a] Arm A received community-wide drug treatment with praziquantel in years 1, 2, 3, and 4.

[b] Arm B received school-based drug treatment with praziquantel in years 1 and 3 (drug holidays in years 2 and 4).

[c] P-values correspond to a village-clustered Rao-Scott chi-square test for categorical variables with the exception of whipworm, which was tested using a chi-square test with no clustering due to convergence issues. P-values correspond to a village-clustered two sample t-test for continuous variables with the exception of Schistosoma mansoni eggs per gram, which was tested using the Mann-Whitney U/Wilcoxon sum-rank test.

[d] Anemia is defined as hemoglobin <11.5 g/dL.

[e] Intensity of schistosomiasis infection (in eggs per gram of stool) was calculated from stool egg counts averaged over 3 consecutive days (when available).

[f] Prevalence of this infection was defined as presence of eggs in any of three consecutive days (when available) of stool samples.

[g] Presence of malaria was determined via blood smear microscopy.

Table 2. Baseline demographic information for subjects enrolled in a nested SCORE cohort by loss to follow up status.

	Not Lost to FU (n= 313)[a]			Lost to FU (n= 489)[b]			Comparison	
	N Miss.	N or Mean	%, SD, or Range	N Miss.	N or Mean	%, SD, or Range	Difference	p-value[c]
Male	0	164	52.4	0	243	49.7	2.7%	0.65
Age (years)	0	7.6	0.5	0	7.5	0.5	0.01	0.72
Height (cm)	30	122.3	6.5	11	121.2	6.4	1.2	0.02
Weight (kg)	34	20.6	3.0	20	20.3	2.9	0.3	0.16
Mid-upper arm circumference (cm)	110	17.5	1.4	93	17.3	1.3	0.2	0.15
Hemoglobin (g/dL)	27	11.9	2.6	27	11.8	2.7	0.01	0.98
Anemia prevalence[d]	27	103	36.0	27	201	43.5	7.5%	0.14
Schistosoma mansoni (schistosomiasis) eggs per gram[e]	14	143.9	0-1008	9	127.8	0-1008	16.1	0.36
Light intensity infection, 1-100 eggs per gram		81	27.1		169	35.2	8.1%	0.03
Moderate intensity infection, 101-300 eggs per gram		60	20.1		113	23.5	3.5%	0.40
Heavy intensity infection, >400 eggs per gram		39	13.0		46	9.6	3.5%	0.17
Schistosoma mansoni (schistosomiasis) prevalence[f]	14	180	60.2	9	382	68.3	8.1%	0.02
Plasmodium spp. (malaria) prevalence[g]	29	20	7.0	24	37	8.0	0.9%	0.69
Ascaris spp. (ascariasis) prevalence[f]	14	34	11.4	9	48	10.0	1.4%	0.48
Trichuris trichiura (whipworm) prevalence[f]	14	46	15.4	9	68	14.2	1.2%	0.20
Hookworm prevalence[f]	29	19	6.7	18	19	4.0	2.7%	0.01

[a] Those not lost to follow-up were measured at both baseline and year 5.

[b] Those lost to follow-up were measured only at baseline.

[c] P-values correspond to a village-clustered Rao-Scott chi-square test for categorical variables. P-values correspond to a village-clustered two sample t-test for continuous variables with the exception of *Schistosoma mansoni* eggs per gram, which was tested using the Mann-Whitney U/Wilcoxon sum-rank test.

[d] Anemia is defined as hemoglobin <11.5 g/dL.

[e] Intensity of schistosomiasis infection (in eggs per gram of stool) was calculated from stool egg counts averaged over 3 consecutive days (when available).

[f] Prevalence of this infection was defined as presence of eggs in any of three consecutive days (when available) of stool samples.

[g] Presence of malaria was determined via blood smear microscopy.

Table 3. Crude estimated effects of treatment arm, schistosomiasis, schistosomiasis intensity, and malaria (separately) on hemoglobin levels in a nested SCORE cohort, clustered by village (see model [1]). Time (study year 5 compared to baseline), as well as the intercept, was statistically significant in each model ($p<0.0001$) [results not displayed here].

Parameter	Estimate	Standard Error	p-value
Model of Treatment Arm			
Arm B vs Arm A	-0.05	0.2842	0.87
Model of Dichotomous Schistosomiasis			
Schistosomiasis	-0.20	0.1663	0.22
Model of Categorical Schistosomiasis[a]			
Light schistosomiasis	-0.18	0.1804	0.33
Moderate schistosomiasis	-0.20	0.2205	0.37
Heavy schistosomiasis	-0.43	0.2910	0.14
Model of Malaria			
Malaria	-0.18	0.2251	0.41

[a] The reference group for categorical schistosomiasis is no infection (0 epg).

Table 4. Effects of and interaction between schistosomiasis intensity and malaria on hemoglobin levels in a nested SCORE cohort, clustered by village (see model [2]). Time (study year 5 compared to baseline), as well as the intercept, was statistically significant ($p<0.0001$) [results not displayed here].

Parameter	Estimate	Standard Error	p-value
Treatment Arm	-0.0004	0.2748	0.999
Male	0.07	0.1445	0.61
Schistosomiasis[a]			
Light schistosomiasis infection	-0.12	0.1913	0.53
Moderate schistosomiasis infection	-0.22	0.2298	0.34
Heavy schistosomiasis infection	-0.43	0.3086	0.17
Malaria	-0.03	0.4000	0.94
Interaction Between Schistosomiasis and Malaria[a]			
Malaria x light schisotosmiasis infection	-0.49	0.5584	0.38
Malaria x moderate schistosomiasis infection	0.36	0.7277	0.62
Malaria x heavy schistosomiasis infection	0.09	0.8658	0.92

[a] The reference group for categorical schistosomiasis is no infection (0 epg).

Figure 1. Nested SCORE cohort study design. Communities in each treatment arm were chosen based on location and size. CWT = community-wide treatment; SBT = school-based treatment; Holiday = no drug administration.

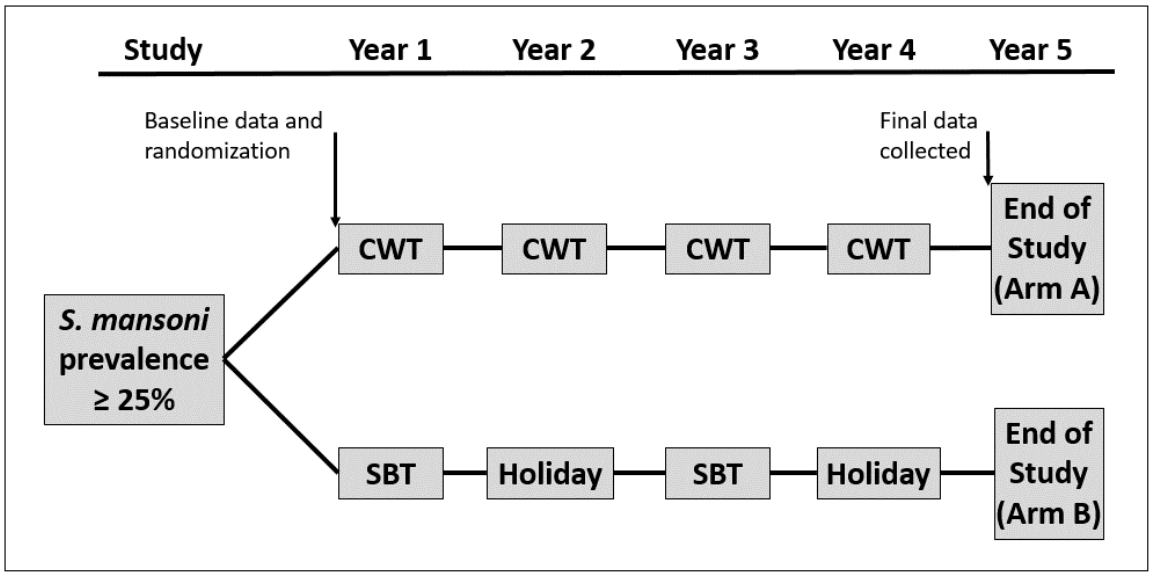
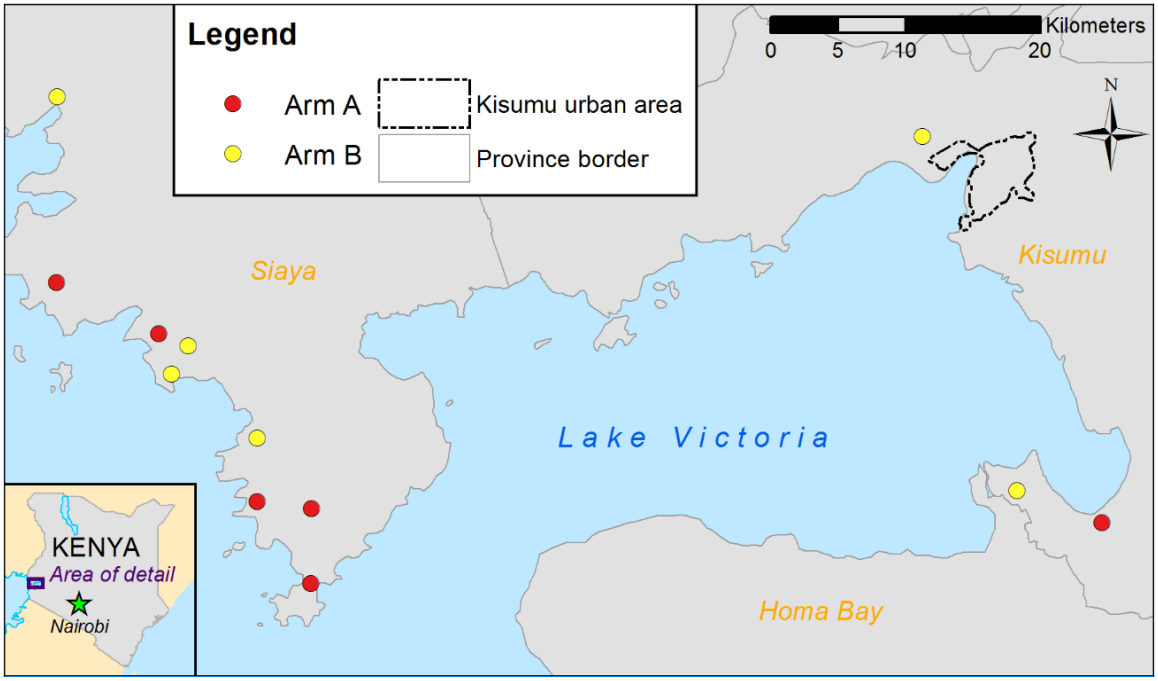


Figure 2. Map of schools included in a nested SCORE cohort by treatment arm.



Map courtesy of Ryan Wiegand; created with ArcGIS version 10.3 (Esri, Redlands, CA).

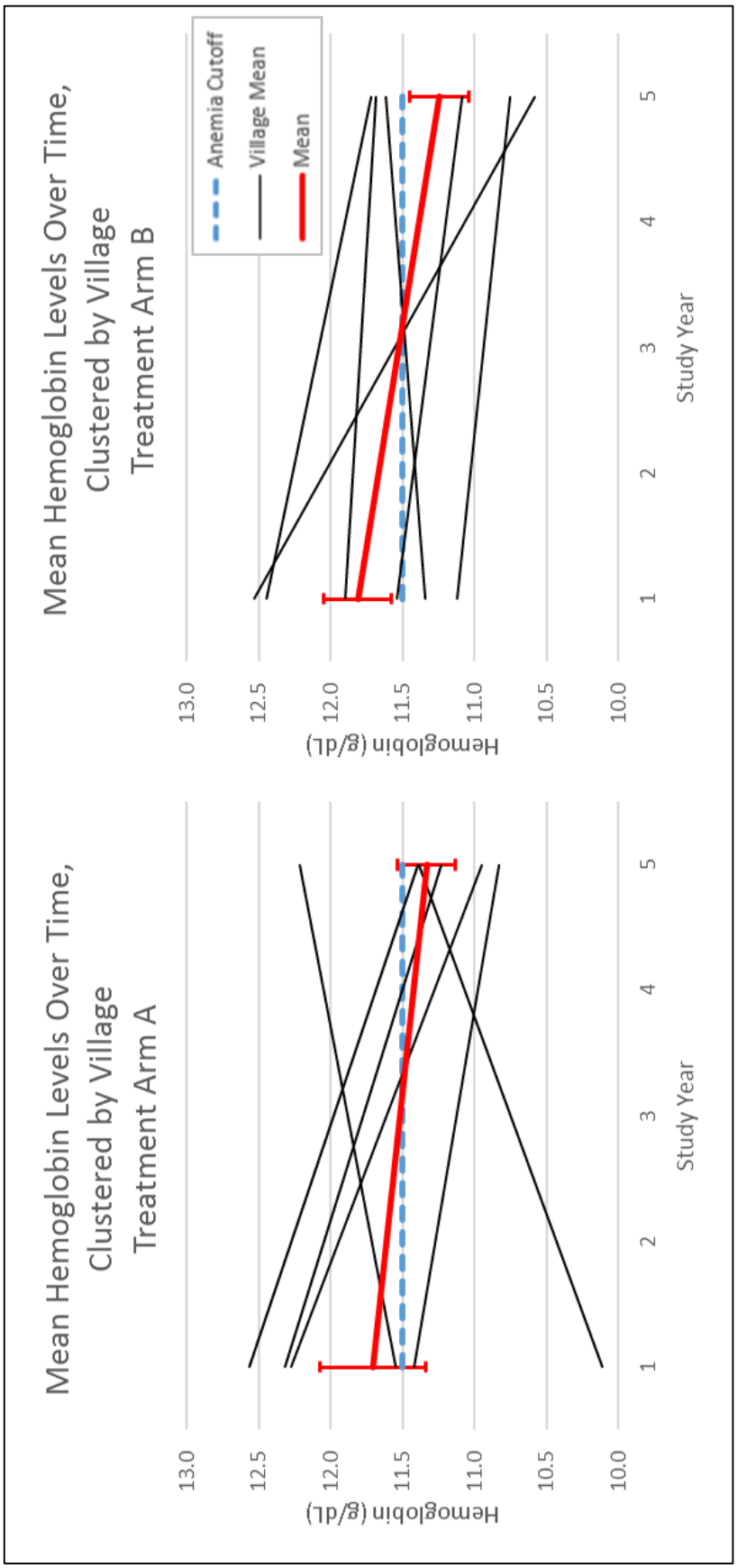


Figure 3. Plot of mean village hemoglobin and mean hemoglobin from year 1 to year 5, paneled by treatment arm. Thin lines represent the change in a single village's mean hemoglobin over time. Thick lines represent the change in the treatment arm mean hemoglobin over time (with error bars showing ±SE). Dashed lines represent World Health Organization definition hemoglobin cutoff levels for anemia.

Appendix A:

EMORY UNIVERSITY

Institutional Review Board

January 3, 2017

Emily Valice
MPH Candidate 2017: Epidemiology
Rollins School of Public Health: Emory University

RE: Determination: No IRB Review Required
Title: Effect of Schistosomiasis on Anemia
PI: Emily Valice

Dear Ms. Valice:

Thank you for requesting a determination from our office about the above-referenced project. Based on our review of the materials you provided, we have determined that it does not require IRB review because it does not meet the definition of research with "human subjects" as set forth in Emory policies and procedures and federal rules, if applicable. Specifically, in this project, you will examine the relationship between schistosomiasis and anemia in schoolchildren using a **de-identified** subset of data from a cohort of Kenyan schoolchildren. The subset of data was obtained a larger Schistosomiasis Consortium for Operational Research and Evaluation (SCORE) project and the data used for this project will not contain any of the 18 HIPAA identifiers.

Please note that this determination does not mean that you cannot publish the results. This determination could be affected by substantive changes in the study design, subject populations, or identifiability of data. If the project changes in any substantive way, please contact our office for clarification.

Thank you for consulting the IRB.

Sincerely,

Jackson E. Parker

Jackson Parker, CIP
Research Protocol Analyst
Emory University Institutional Review Board

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