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Molecular Epidemiology Of Hookworm Infection In The Ashanti Region, Ghana.

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I. Abstract

INTRODUCTION: Parasitic helminth infections have persisted in resource-limited settings around the world, leading to greater numbers of people experiencing sequelae including malnutrition, anemia, and impaired growth and cognitive function among children. Despite periodic deworming efforts, a high burden of disease remains in sub-Saharan Africa. Establishing baseline prevalence of these infections can inform local and national deworming campaign objectives as well as tailor additional interventions accordingly. Here we describe our efforts to determine baseline prevalence and analyze potential risk factors for infection in Ashanti Region, Ghana.

OBJECTIVES: The primary objective of this study was to characterize the molecular epidemiology of hookworm and schistosomiasis co-infections among communities surrounding Lake Bosumtwe in Ashanti Region, Ghana.

METHODS: This was a cross-sectional epidemiological analysis in which participants (n= 907) were recruited from four rural communities. Qualitative demographic surveys were issued to identify possible risk-factors for infection along with quantitative collection of fecal samples and urine samples for helminth analysis. A single community, Abono (n= 406), was used for post-hoc analyses.

RESULTS: Total hookworm prevalence was 5.6% and varied by community (3.1-7.3%). Age (AOR= 1.05, p=0.0004), wealth (AOR= 5.94, p= 0.02), and daily shoe use (AOR=0.13, p=0.01) were independently and significantly associated hookworm status. In comparison to hookworm infection, incidence of the whipworm, *Trichuris trichiura,* infection was observed in two communities. There was a total of two schistosomiasis infections across all communities.

CONCLUSION: Hookworm prevalence in each community was lower than previously reported findings. This suggests either a penetrance of mass drug administration campaigns or a strong mechanism of protective immunity. The independently associated risk factors of age, wealth, and daily shoe use are consistent with other studies. Interestingly, age had a positive correlation with hookworm infection, indicating that previously established patterns of immunity in Sub-Saharan Africa may not exist in these communities. Significant association with the poorest wealth quartile suggests a celling effect of wealth for protection against hookworm infection. Our findings highlight need for more robust community-based monitoring of MDA programs in order to detect local variability in performance as well as lay the framework for investigating diagnostic sensitivity and treatment response.

Acknowledgements

This work would not have been possible without the generous mentorship and support of many individuals. I am grateful to Dr. Michael Cappello for his guidance at each step of the research process. I am also grateful to my colleagues from NMIMR for welcoming me into their country, home, and parasitological research. I extend a special thank you to Professor Michael Wilson for his support throughout the field study as well as Dickson Osabutey for his leadership in the field. This work would not have been completed without the incredible members of the research team, including Lydia Okyere, Joseph Quartey, Frances McCarthy, Samson Otoo, and Mavis Konama.

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Table of Contents

<u>List of Figures</u>

<u>List of Tables</u>

II. Introduction

Global Context of Human Hookworm Infection

Neglected tropical diseases (NTDs) persist in many resource-limited settings that lack sanitation, adequate hygiene practices, and infrastructure. Of the NTD infections, nearly 85% are the result of parasitic helminth (worm) infections, affecting over 1 billion people worldwide [1]. The most common soil-transmitted helminth (STH) infections are hookworms (*Necator americanus* and *Ancylostoma duodenale*), roundworms (*Ascaris lumbricoidedes*), and whipworms (*Trichuris trichiura*), which frequently occur in populations that are co-endemic for blood trematodes (*Schistosoma mansoni, S. japonicum*, *S. haematobium*) [2]. *N. americanus* is the predominate hookworm of Sub-Saharn Africa, Southeast Asia, and the Americas while *Ancylostma duodenale* is endemic in China and India [3]. There has been little reduction in the prevalence rates of STH infections since the 1990s [4]. The rise in population coupled with a stagnant prevalence rate has led to an increase in the total number of STH infections globally, which contribute to 22.1 million disability-adjusted life years annually [5].

A high burden of disease is associated with communities in Sub-Saharan Africa (SSA) that are located near lakes, rivers, and streams, which can be populated with the intermediate parasite reservoir hosts [1]. As endemic regions typically overlap in tropical environments, people are commonly affected by more than one species of parasitic helminths. Climate and soil structure in these regions appear to be crucial determinants of hookworm infection, with the high temperatures and moist environments ideal for larvae growth outside a host [2]. While helminth mono-infection has been long studied, little is known regarding the effects of polyparasitism on health, a gap in knowledge that potentially reduces efficacy of treatment and prevention measures.

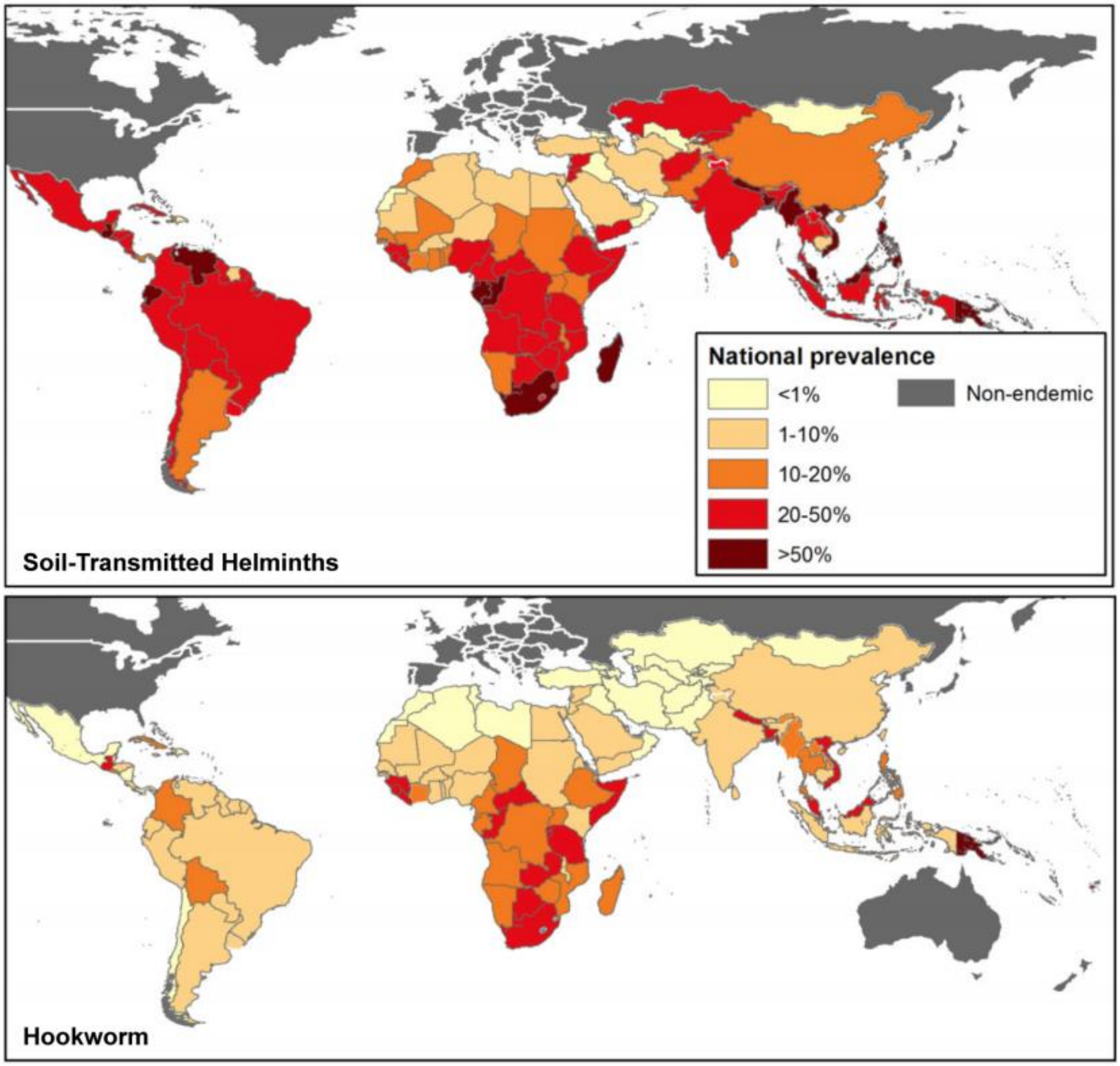

Figure 1. Global Distribution of STH infection and Hookworm in 2010. Top: National-level prevalence data for STH infections. Bottom: National-level prevalence of Hookworm. Maps courtesy of Pullan et al. [6].

Hookworm and Schistosomiasis Parasite Biology

Over 1.5 billion people, or 24% of the world's population, experience infections from soil-transmitted helminths [7]. Humans can be exposed to STHs in a variety of ways. In areas that lack adequate sanitation, there is increased risk of exposure through multiple pathways. STH eggs can attach to fruits and vegetables and are ingested when the food goes unwashed and is consumed. Exposure through contaminated soil is common in areas of barefoot behavior and eggs can be ingested from contaminated water sources. There is no intermediate host for any species of hookworm.

Hookworms are nematodes belonging to the family Ancylostomatidae. The two genera that most affect humans are *Necator americanus* and *Ancylostoma duodenale [8]*. These parasites are characterized by the presence of teeth or cutting plates lining the buccal capsule of adults. Pigs have been suggested as transport hosts for *N. americanus* [9]. The adult worms are roughly 1 cm long and attach to the host's mucosa where they feed on blood, up to roughly 30 μL per day per worm [10]. A female worm can produce up to 10,000 eggs per day. Anticoagulation processes and degradation of blood proteins facilitate the feeding of adult hookworms.

Human infection by helminths is caused by contact with their eggs or larvae, which thrive in moist, soil-enriched climates [11]. Rural communities located around bodies of water are particularly vulnerable to infection as the areas can be well populated with the intermediate host of schistosomes- the snail. Additional exposure to fecal matter can result from the close-proximity of these communities to farming pastures. After penetrating the epithelial barrier, hookworms attach to a host's intestinal mucosa, using sharp teeth to lacerate small blood vessels for feeding [2]. Schistosome parasites reside in the host's hepatic veins (*Schistosoma mansoni* and *Schistosoma japonicum*) or bladder (*Schistosoma haematobium*), where the helminth eggs induce an inflammatory response that causes tissue destruction [1]. Hookworm larvae mature in moist soil prior to host infection and schistosomes are transported by a snail intermediate host until human exposure. Due to the nature of their life cycles, STH parasitemia has been associated with residence location (urban or rural) [11].

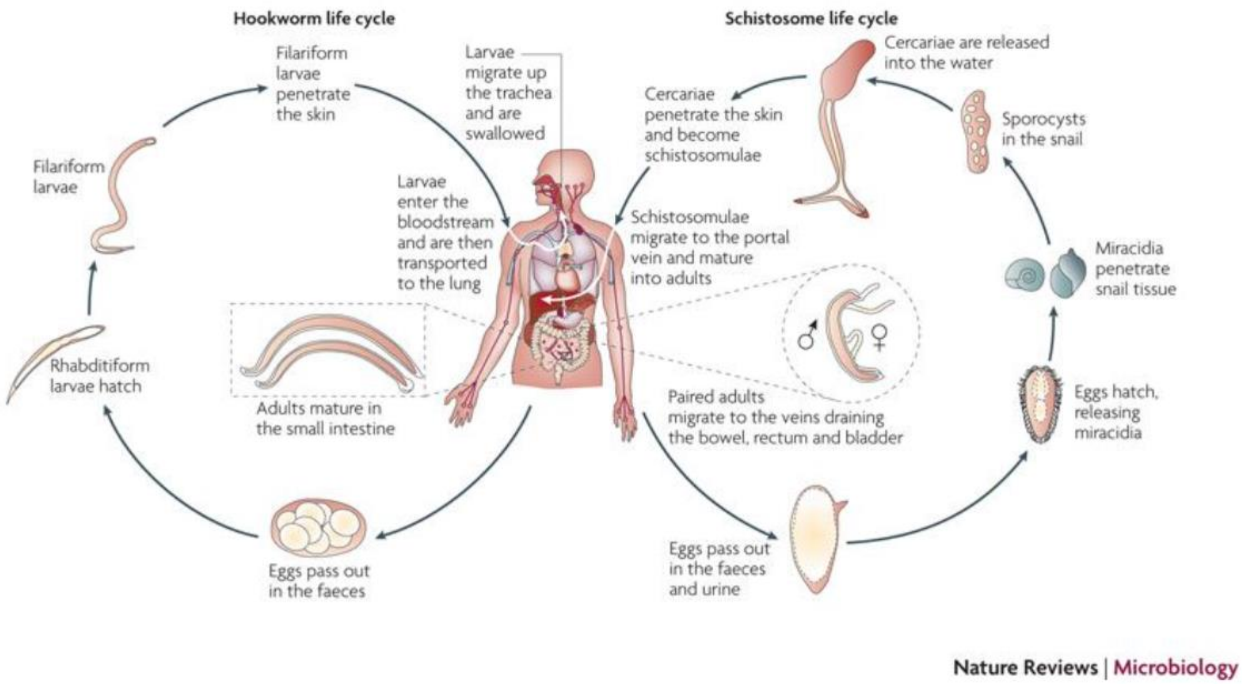

Figure 2. Life cycle of hookworm (left) and schistosome parasite (right). Courtesy of Hotez et al. 2010 [12].

Hookworm eggs are released by females in the host's small intestine, passed in the feces, and hatch into first stage larvae (L1) in the soil (see Figure 2) [13]. The larval stage at which hookworms can infect humans is known as the third-stage, or L3. This occurs when the larvae living in the soil penetrate the skin (*N. Americas* and *A. duodenale*) or are ingested (*A. duodenale*) [8]. The L3 stage hookworms can survive in the soil for weeks at optimal conditions until their lipid metabolic reserves are depleted. Once it enters the host, the larvae encounter physiochemical signaling complexes that result in their sexual maturation in the gastrointestinal tract (L4 stage) [13]. The mechanisms by which this transition occurs are unclear. There have been several invasion factors reported from hookworm larva analysis and excretory/secretory products. The enzymes detected from these analyses affect skin and tissue penetration through degradation. Feeding responses of L3 suggest a receptor-mediated mechanism of activation in response to host-specific stimuli. Signaling pathways from the host serum allow the L3 to continue their maturation and migrate through the host's vasculature. Following their migration to and from pulmonary vasculature, the L3 are coughed, swallowed, and enter the gastrointestinal tract. It is here they reach sexual maturation and mate.

Hookworm and Schistosomiasis Co-infections

Helminths are able to establish chronic infections through pattern recognition receptors (PRRs) such as toll-like receptors, C-type lectin receptors, and inflammasomes [14]. The association between schistosome and soil-transmitted helminth polyparasitism has been found to be dependent on the socio-economic status of an individual, the altitude of the study area, and household decision-making processes [15]. As elevation can serve as a proxy for the flow velocity of rivers, this factor can be used to infer exposure to schistosome-infected host snails. The ability of a host to maintain the parasite infection may depend on host factors like age. Older hosts tend to sustain larger colonies of parasites than children; however, colony size varies even within demographic groups [16]. The TH2 response is able to limit helminthic colonization through cytokines such as interleukin-4, interleukin-5, and interleukin-13. Experimental studies have shown that helminths may be able to induce hyporesponsiveness in mucosal T-cells that can mitigate unwanted immune responses and may even reverse the inflammatory process.

The effects of the aforementioned mechanisms can be grouped accordingly: disabling, degrading, and dislodging [17]. Disabling effects hinder the parasites' ability to grow and mobilize through innate defensin-like molecules such as RELM-b. Larval development can be additionally delayed through amino acid (arginine) deprivation. Processes such as the elevation of mast cells or eosinophils coupled with the release of nitric oxide from macrophages can result in cumulative degradation of the parasites. In the GI tract, parasites are able to be dislodged through the immune response. In response to infection, IL-4Ra-mediated signaling activates smooth muscle cells and stimulate Th2-promoting cytokine production from muscle cells [17]. Additionally, goblet cells can produce mucus that helps expel the helminths [18].

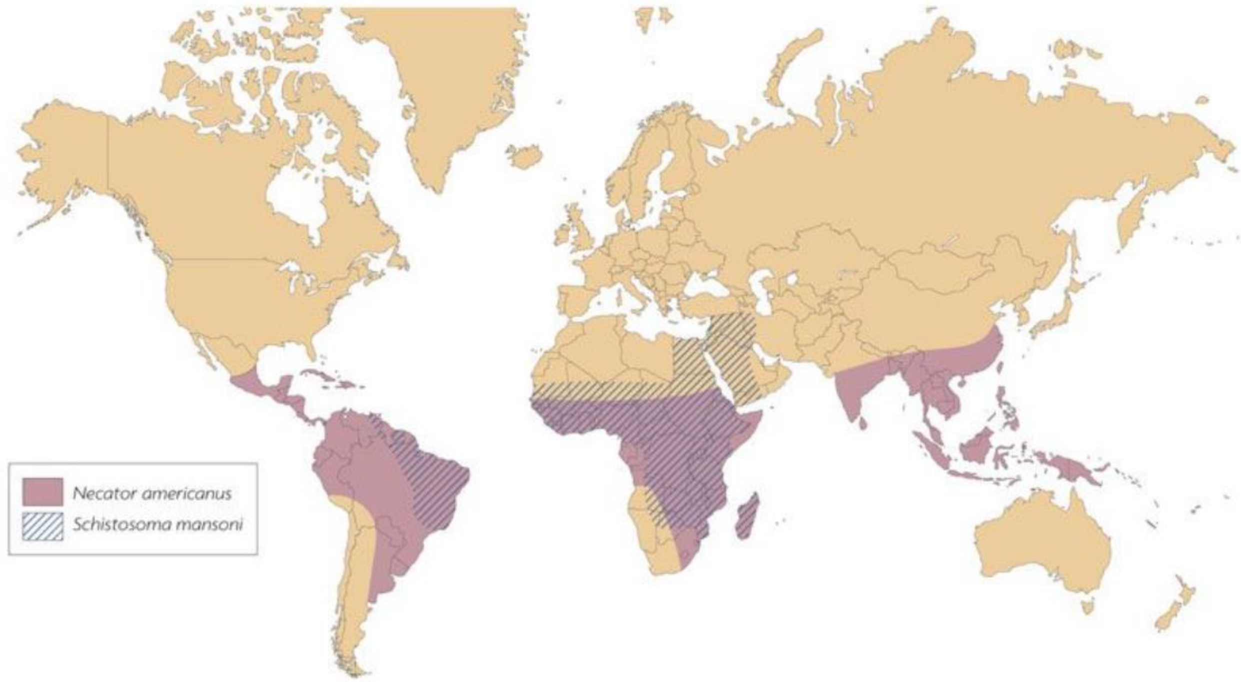

Figure 3. Global distribution of hookworm and *S. mansoni*. The majority of infections with *Necator americanus* and/or *Schistosoma mansoni* occur throughout the global South. Courtesy of Hotez et al. 2010 [12].

Hookworm Clinical Disease and Risk Groups

Over 610 million school-aged children (SAC) are at risk for STH-associated morbidity. [19] The cascade of sequelae from childhood helminthiasis include malnutrition, anemia, and impaired growth and cognitive function. Pregnant women are also at high risk for these infections, the effects of which can be compounded by low-iron diets and blood loss from childbirth [2]. People residing in low-income nations are particularly susceptible to STH infections as they can lack adequate water and sanitation, may live in crowded conditions, and can have poor access to health care [4]. There are numerous links between the prevalence of hookworm infection and socio-economic status, defined by purchasing power-adjusted average per capita income and the human development index (see Figure 4).

Although not all individuals with schistosomiasis experience morbidity, there are symptoms associated with infection. Those who suffer from *S. haematobium* infection can experience hematuria, dysuria, and hydronephrosis. Infection with *S. mansoni* has been associated with diarrhea, bloody stool, and hepatomegaly. Secondary symptoms of these illnesses can be slowed growth, impaired cognitive development, and reduced physical fitness [20]. Exposure to infective larvae of *N. americanus* or *A. duodenale* produces a papular rash, also known as "ground itch" [3]. This occurs mostly on the hands and feet as they are typically the entry points for the larvae. In some cases, pulmonary hookworm infection and hookworm pneumonitis may occur. Wakana disease can result from oral infection with *A. duodenale*.

Intestinal blood loss occurs when worms attach themselves to intestinal mucosa, sucking a plug of tissue into their buccal capsules. Chronic intestinal blood loss results in clinical manifestations of hookworm disease such as iron-deficiency anemia and hypoalbuminemia. There has been some debate as to how intensity of infection affects the onset of anemia, with some reports that anemia can be caused by low-intensity infections. The physical signs of hookworm infection reflect the symptoms of iron-deficiency anemia, such as edema of the face, waxy skin, and a yellowish color. As young women and children have the lowest iron stores, they are the most vulnerable to chronic blood loss regardless of exposure levels [3]. Due to the physiological demands for iron during pregnancy, it has been estimated that more than half the pregnant women in low-income nations experience problems due to iron-deficiency anemia. Vertical transmission has been suggested as one route of infection among neonates in China, with some researchers hypothesizing that *A. duodenale* is transferred through the passage of infective larvae into the milk and colostrum [21].

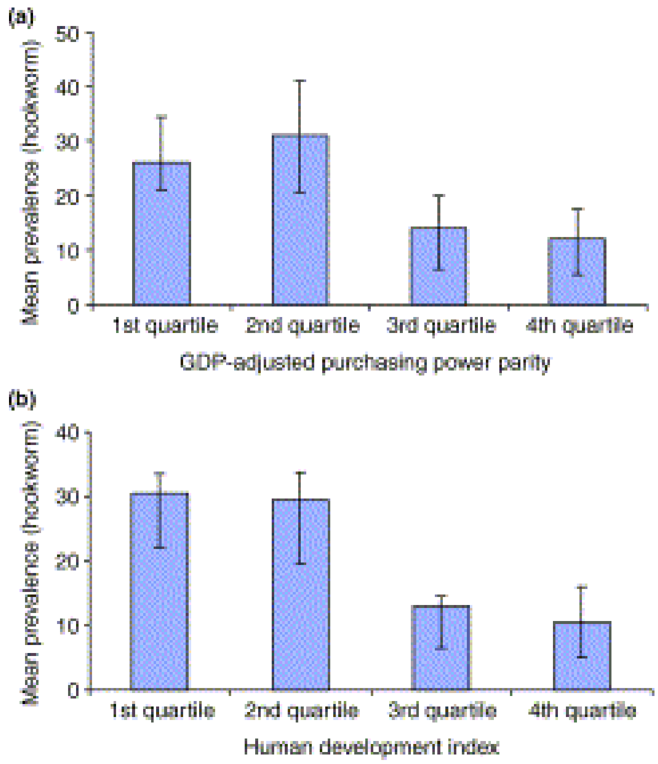

Figure 4. Trends in wealth and hookworm infection. Socioeconomic status of 94 countries were assessed according to UN Population Program standards. Quartiles were divided into most poor (1st), very poor (2nd), poor (3rd), and least poor (4th). The highest proportion of hookworm infections occur among the most and very poor quartiles. Courtesy of de Silva et al 2003 [4].

Although there is a variation in helminth burden by age according to geographic region, there is thought to be a positive correlation between the intensity of infection with *N. Americanus* and age in humans [16]. While this pattern has been demonstrated in China, the age trend in Sub-Saharan Africa is less clear as high burden of hookworm infection has been observed in early childhood studies [22]. This is in contrast to the prevalence and intensity of infection of the *Ascaris* and *Trichuris* species which peak during middle and late childhood. The age distribution of hookworm infection appears to diverge from other soil transmitted helminths. This may result from the longer lifespan of hookworms, their superior immune evasion mechanisms, and potential immunological tolerance by humans. This suggests antihelminthic chemotherapy programs should be tailored to the specific age of peak mean infection by helminth group.

Children and pregnant women remain high risk groups affected by hookworm infection. Prevention and control of hookworm infection has been neglected on the global scale as those with light to moderate infections are typically asymptomatic and can be easily ignored. Although school-based deworming programs can target children, they miss potential adult cases. One study found that incidence of hookworm infection in children and adults did not significantly differ [23]. This can be explained by a shared environment and shared health behaviors. Both children and adults can be exposed to infected soil by walking barefoot.

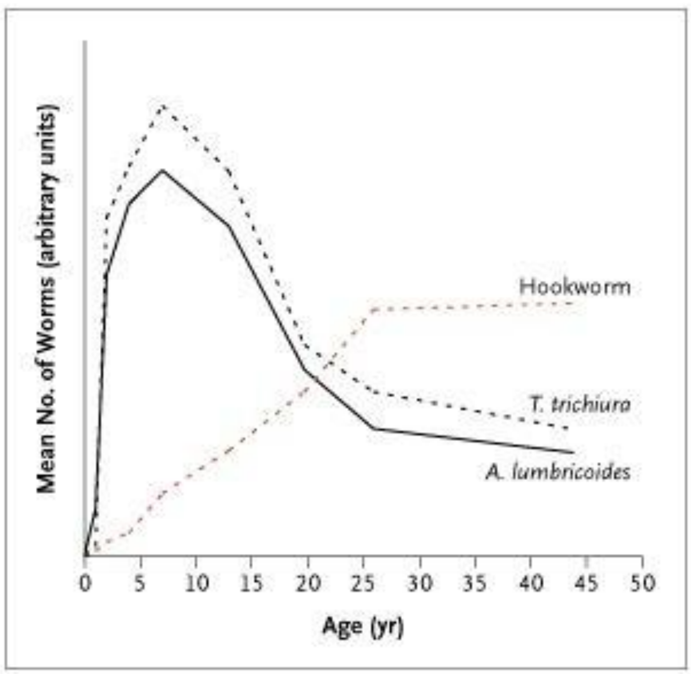

Figure 5. Relationship between age and intensity of hookworm infections. The mean number of infections with *A. lumbricoides* and *T. trichiura* peak around age 10, decreasing with each additional year. Infection with Hookworm is shown to increase steadily until with age. This may be the result of longer hookworm lifespan and increased immunological tolerance [3].

Hookworm Treatment and Public Health Control Efforts

The World Health Organization currently lists four anti-helminthic drugs for treatment of STH infections: albendazole, mebendazole, levamisole, and pyrantel pamoate [24]. A systematic review found albendazole to be the most efficacious single-dose drug at reducing the prevalence of hookworm infection [25]. A 3-day mebendazole therapy has been recommended as an alternative to single-dose therapy when possible. Single-dose oral albendazole, mebendazole, and pyrantel pamoate produce high enough cure rates against *A. lumbricoides* and warrant recommendation. New anti-helminthic drugs are required to treat *T. trichiuria* as the cure rates observed in previous studies are considered insufficiently low [25]. Praziquantel is used against schistosomiasis to reduce morbidity and prevent chronic infections [26].

Although anti-helminthic drugs can be effective in eliminating adult parasites from the body, reinfection can occur soon after treatment [27]. For this reason, current control strategies are focused on reducing the infection load rather than achieve total elimination. Periodic deworming regimens have been recommended by the World Health Organization (WHO), usually through the form of mass drug administration (MDA) campaigns, and are considered the main control strategy for STH infections. These methods have not been successful in eradicating the helminths in low-resource settings as the lack of sanitation infrastructure significantly increases the risk of reinfection. Many of these programs, however, only target children who are officially enrolled in and attend school. This methodology does not reach those who cannot attend school and potential adult reservoirs in communities. To target schistosomiasis, MDA campaigns conducted with praziquantel and snail control are recommended as effective control methods [28].

Although previous data has shown that MDA can reduce the intensity of infection, they do not significantly decrease the prevalence of helminth infections over time [29]. The preventive chemotherapy drugs most commonly used in these deworming regimens are albendazole and mebendazole. Concerns regarding long-term effectiveness and potential for emergence of antimicrobial resistance have led to research on other methods of prevention like improvements to water, sanitation, and hygiene (WASH) access and practices. [30] The most successful elimination campaign will likely require a combination of preventive practices with newer treatment strategies.

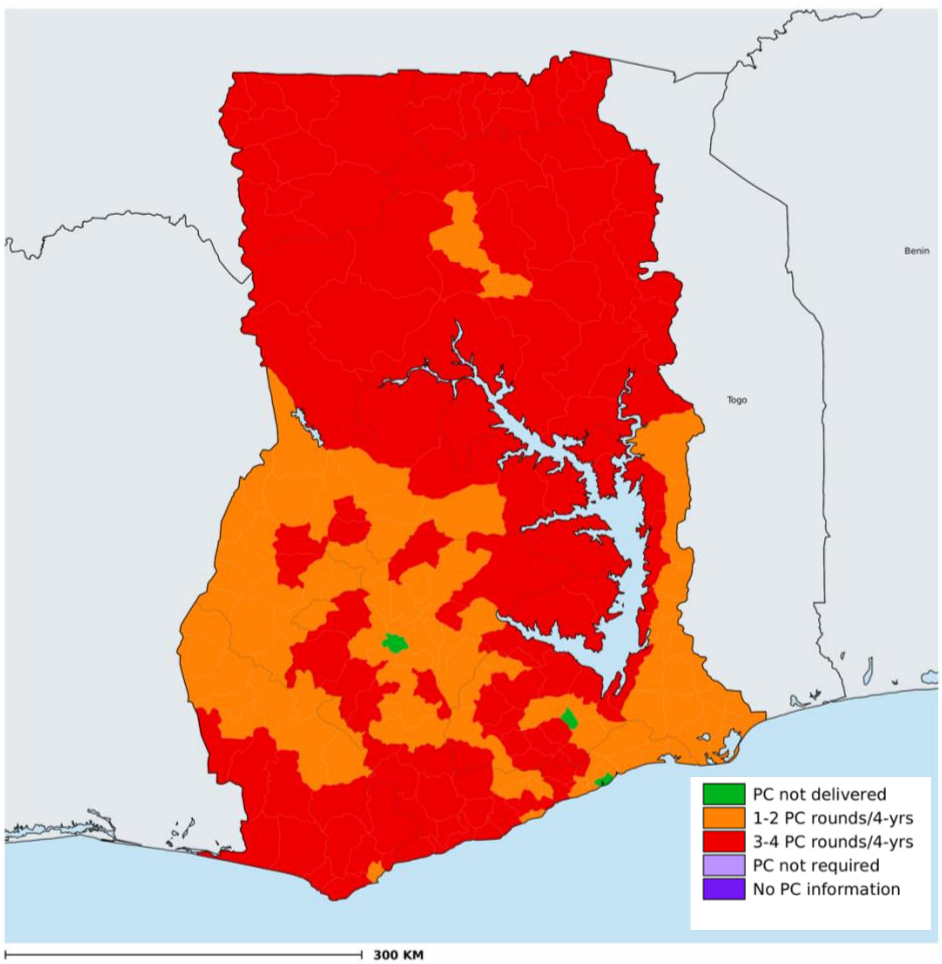

Figure 6. Previous MDA rounds for STH infections in Ghana (2013-2016) [31]. A large portion of Ghana has received between 1-2 rounds (orange) or 3-4 rounds (red). Courtesy of Health Ministries & ESPEN partnership.

The current lack of epidemiological data regarding these infections obstructs development of a successful preventative campaign. Without a clear understanding of the prevalence and reinfection risks associated with these helminths, public health and medical practitioners will be limited in their ability to effectively decrease infections in resource-limited settings. Further, there is increased concern with respect to emerging anthelmintic resistance associated with the periodic deworming programs. These topics must be addressed in the context of larger epidemiological studies before implementing a new intervention. Communities that are subjected to long-term MDAs may experience neither control nor elimination if the past data translates to legitimate future concerns.

The Human Hookworm Vaccine Initiative (HHVI) was established in 2000 to create recombinant hookworm vaccines in the non-profit sector, with the goal to develop the vaccine with resource-limited settings in mind [32]. The current collaborating partners in Ghanaian NTD control and elimination include the Ghana Health Service NTD Control Program, USAID, Sight Savers, ANESVAD, and the American Leprosy Mission [31].

Current Challenges in Hookworm Control and Research

The immune response induced by a single hookworm infection fails to protect the host against subsequent infections, as is true for other helminths. This failure of the host's system to establish immunological memory makes it challenging to develop long-term protective measures, such as a vaccine. In endemic areas, it can be nearly impossible to find hookworm-naïve individuals for longitudinal studies and determine a "correlate of protection" [33]. In the laboratory, it has become challenging to maintain the hookworm life cycle in vitro and within an animal model. The absence of a laboratory animal that is permissive to a human hookworm and can reproduce the human disease (such as anemia) has become a challenge for researchers.

When the life cycle has been established within an animal model, there remains doubt as whether the infection is reproduced as it occurs in humans [34]. The need for a valid animal model has been driven by new anthelminthic therapies as well as vaccine developments [27].

Canines and hamster models are typically used for experimental hookworm infections as dogs are permissive to *A. ceylanicum* and *A. caninum* while hamsters can be infected with *A. ceylanicum* and *N. americanus* larvae. Canines may represent the most suitable animal model as they are natural hosts of *A. ceylanicum* and *A. caninum* [34]. Infection with *A. caninum* reflects human hookworm infection in method of entry (oral ingestion or skin penetration) and maturation within the host [35]. Canines infected with *A. ceylanicum* have been shown to develop chronic infections that present similarly to those of humans [36]. Due to the lack of immunological reagents available for dogs, the majority of experimental canine model studies have been limited to administration of infection larvae. Syrian Golden hamsters are also used to study *A. ceylanicum*, reproducing the route of infection and blood loss seen in human infections with *A. duodenale* [34]. The hookworm *N. americanus* has also been adapted to hamsters. The maintenance of the hookworm life cycle has been established in adult hamsters, a promising turn for drug and vaccine development [37].

Both canine and hamster models, however, have been shown to develop natural resistance to third-stage infective larvae after 20 weeks of infection. As neither of these animals easily reproduce the clinical endpoints as seen with human hookworm, observing the traditional intestinal blood loss in these models is difficult [33]. Less than 20% of *N. americanus* larvae develop into adults in the gastrointestinal tract of a hamster model [32]. Non-sterilizing immunity to *A. caninum* in dogs after repeated exposure has been shown to result in reduced egg counts and lower numbers of adult worms in the intestine regardless of administration technique

[38]. This inefficacy creates a costly barrier to studying helminth infections using laboratory animal models. Direct comparison of human infections and animal models is not possible. Alternative experimental models have been explored using rabbits, chickens, chimpanzees, and mice [34]. None of these models allowed for the maturation of infective larvae to blood-feeding adults.

History and Significance of Present Study

Disparities in sanitary conditions, access to health care, and learned health behaviors may affect the persistence of helminthiasis infections among specific populations, particularly school-age children. Current studies in Ghana lack the epidemiological data required for assessing exposure and health risks of the STH infections among children. There is a need for survey data regarding household demographics, socioeconomic status, and water, sanitation, and hygiene (WASH) practices, all of which can reduce the burden of disease. [2] Ghana has successfully eliminated NTDs like guinea worm and trachoma and may be able to use similar control strategies once the epidemiological landscape of helminth infections is established. According to GBD 2017, hookworm infection and schistosomiasis remain the two most prevalent helminth infections in Ghana. [39] Though Ghana has seen a recent economic boom that has been associated with a decline of total NTDs nationwide, rural areas throughout Ghana carry a high burden of helminth infections. [40] Although MDAs have significantly reduced the presence of these NTDs over the past decade, complete elimination has not been achieved. Reduced albendazole susceptibility among hookworm isolates may contribute to its persistence, though malnutrition among children likely plays a role in the treatment outcomes. [41] Future case detection in these increasingly low-endemic settings may require more sensitive diagnostic techniques and treatments.

As the prevalence of parasitic worm infections decrease in endemic areas due to improvements in sanitation practices, public health practitioners will need a more sensitive diagnostic test for hookworm detection. Both helminth infections are typically diagnosed through egg detection in fecal or urine samples.[42] The Kato-Katz technique has long been considered the gold standard for intestinal helminth epidemiological surveys [43]. Kato-Katz microscopy is the most commonly used diagnostic test for detecting eggs in fecal matter or urine (eggs per grams of feces). While this method can be done in the field, it is both labor intensive and requires expertise in microscopy. Additionally, this technique has poor sensitivity for detecting light-intensity infections and includes large variations in readings. As MDA programs reduce the intensity of STH infections in endemic regions, there will be a need for new, more sensitive diagnostic tools.

Prior research suggests that polymerase chain reaction (PCR) may be successful in molecular analysis of helminth infections. [44] Although the cost of developing and implementing this technology in the field will be high, the greater sensitivity and reduction of labor requirements offers a more attractive alternative to Kato-Katz for diagnosing and quantifying helminth infections. Using higher sensitivity tools as prevalence/intensity declines will allow for more selective treatment strategies in high-risk areas. This can reduce cost, decrease potential anthelmintic drug resistance, and provide better quality treatment for STH infections. To accomplish the validation of this tool in the context of low-endemic areas, defining the epidemiological landscape of the Ashanti region is necessary.

III. Methods

Between June and August 2019, fieldwork was conducted in partnership with the Noguchi Memorial Institute for Medical Research (NMIMR) in four communities within the

Ashanti Region. As described below, this effort gathered cross-sectional epidemiological data on helminth infections in these communities and collected human hookworm specimens for further research. The present thesis will use these primary data as collected and analyzed by the methodology below.

Ethical Approval

This study was approved by both the Yale University HIC (Protocol #2000020831) and Noguchi Memorial Medical Research Institute. Local community stakeholders, including village assemblymen and district health officers, were approached prior to the study and participated in its planning and implementation.

Study Site Description

Lake Bosomtwe is located in the Ashanti Region of Ghana, enclosed in two administrative districts, Bosomtwe and Bosome-Freho [45]. It is the sole endorheic lake in both Ghana and Africa, considered as a tourist and recreational center for the public. According to the 2010 population census, the catchment area around Lake Bosomtwe contains 154,307 people [46]. There are approximately 23 communities surrounding the lake, the majority of whom identify as Akan.

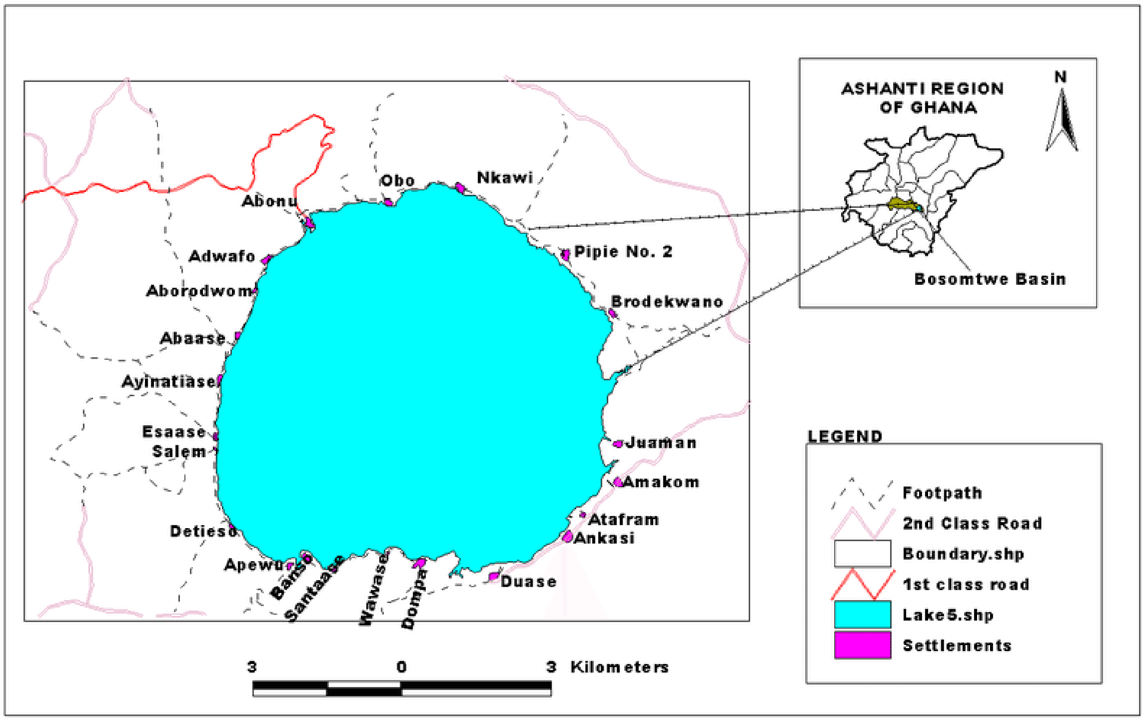

Figure 7. Map of Study Site- Lake Bosumtwe. The first community included in the study, Abono, can be seen at Northern tip of the lake. The following two communities, Abrodwum and Duase, can be viewed at the Southern region of the lake. Not pictured: Konkoma [45].

Participant Enrollment: Lake Bosumtwe

Adults and children more than 6 years old were invited to participate in the study from four rural communities in the Ashanti Region: Abono, Konkoma, Abrodwum, and Duase. Participants were recruited according to a geographically-stratified convenience sampling method and informed of the study aims and procedures. A total of 907 subjects were enrolled from across 228 households. All participants provided formal written consent or, in the case of children under 18 years old, written assent along with the consent of a parent or guardian.

Fecal Sample Collection and Processing

Enrolled participants were asked to provide a 24-hour fecal sample. Samples were collected from households in the early morning and transported to a field laboratory for testing. Two NMIMR microscopists independently examined each fecal sample, diagnosing helminth infections by the Kato-Katz method as per WHO standard protocols. Nearly all samples were evaluated within 90 minutes of collection. Reported infections included the following: hookworm, *Trichuris trichiura*, *Ascaris lumbricoides*, *Hymenolepis nana*, and *Taenia solium*. Infection intensity, recorded as eggs per gram (epg) of stool, was assessed as the average of two independent egg counts. Hookworm eggs were purified from all hookworm-positive samples according to previously published methods. Purified eggs were preserved in RNAlater solution according to manufacturer protocol and stored at 0°C.

Treatment and Post-Treatment Sample Collection

All individuals with an identified helminth infection were provided directly supervised single-dose 400mg albendazole (ABZ) treatment. Three individuals with a diagnosed infection were not treated due to medical contraindications or refusal of treatment. All treated individuals were then asked to provide a second stool sample for evaluation 10-14 days after treatment. These post-treatment stool samples were reviewed by microscopists as described previously, and individuals with a persistent or newly acquired infection were referred to the Ghana Health Service for continuing care. Hookworm eggs were also purified from all hookworm-positive post-treatment stool samples as described above.

Urine Sample Collection and Processing

Enrolled participants were asked to provide a 24-hour urine sample. Participants were asked to visit the local healthcare center for sample allocation. Two NMIMR microscopists independently examined each urine sample, diagnosing schistosome infections by urine filtration method as per WHO standard protocols. The samples were assessed for the following: visible hematuria, *S. haematobium*, and *S. mansoni*. All individuals identified with schistosomiasis were referred for treatment at the local healthcare center.

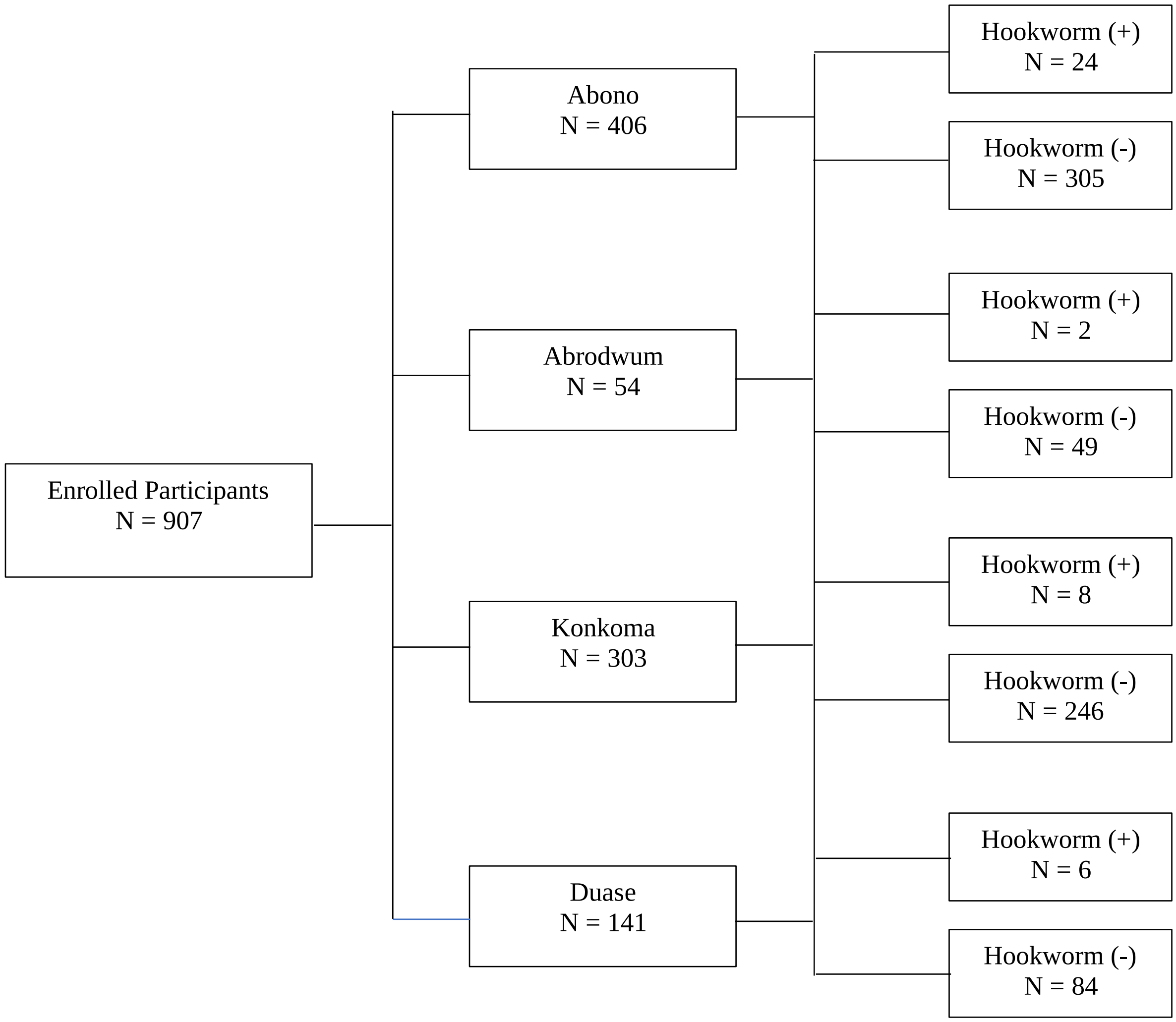

Figure 8. Schematic of Study Design. 907 participants were enrolled in the study and 724 provided a stool sample for analysis. Among those who provided a stool sample, 44 tested positive for a hookworm infection. Those who tested positive received one therapeutic dose of Albdendazole.122 people lost to follow-up. 61 asked to be excluded.

Household and Individual Questionnaires

The survey instrument was adapted from the Demographic and Health Surveys (DHS) household questionnaire. Study participants were asked to complete a questionnaire assessing sociodemographic characteristics and individual helminth-related exposures. Heads of households were asked to complete an additional component of the questionnaire assessing household SES and WASH practices. The survey instrument was adapted from locally validated questionnaires including the Demographic and Health Surveys. Local community volunteers from the District Health Management Team of the regional hospital in Kutanase, Ghana assisted in translating and administering the questionnaire in the main languages of the region (Twi). Translators were extensively trained, regularly and randomly audited, and routinely accompanied by a research team member to ensure adherence to study protocol.

Anthropometric Measurement

All study participants were measured for body weight, height, and mid-upper arm circumference (MUAC). Measurements were conducted in duplicate by trained research personnel from the local healthcare center. A digital scale, stadiometer, and tape measure were used for duplicate measurements of weight, height, and MUAC.

Data Analysis

Initial data entry and cleaning was performed in Microsoft Excel. Figures and descriptive statistics were prepared in RStudio (v1.0.136) using open-source libraries. Linear modeling and calculation of odds ratios was performed in SAS (v9.4), with variable selection guided by a conceptual framework for hookworm infection. Software for anthropometric data analyses included the z-score AnthroPlus software provided by the World Health Organization and the MUACz r-package created by Mramba et al [47-49].

A codebook of relevant variables to these analyses is separately available. Initial data analysis will examine the baseline epidemiological profile of hookworm and schistosomiasis infections in the Ashanti Region study group. This will include quantification of hookworm prevalence and intensity of infection in the four communities under study as well as assessment of variation at the individual and household levels. Controlling for SES, age, and sex, a multiple linear regression model will be used to identify risk factors for continuous dependent outcomes (e.g. EPG). Similar multiple logistic regression models will be used to assess correlates of categorical outcome variables (e.g. infection status). A continuous SES control variable will be constructed from relevant component variables of the household survey by principal component analysis as described previously [50, 51].

A second phase of data analysis assessed response to treatment among hookworm-positive individuals. Risk factors were assessed against outcome variables, controlling for confounders as above. To control for potential household-level clustering of infection, odds ratios for the dichotomous outcome variables of interest were calculated by logistic regression using a generalized estimating equation (GEE) model and robust standard error estimation.

Wealth Index

An absolute wealth index was constructed in the manner of Filmer and Pritchett (2001) from principal components analysis of a household asset index of 21 binary variables [50]. The variables included in the model included the following categories: ownership of household assets (electricity, radio, television, telephone, refrigerator, DVD/VCR player); ownership of a vehicle (bicycle, motorcycle, car); ownership of a savings account (at a bank, at a cooperative); ownership of agricultural land; access to a latrine toilet; use of a cook-fuel other than wood; exclusive use of an improved drinking water source; and ownership of large animal (cows, goats, sheep, pigs). The wealth index assumes that household long-run wealth explains the maximum variance (and covariance) in the asset variables [50].

IV. Results

Baseline Epidemiology of Helminth Infections in Study Population

A total of 907 individuals were enrolled in the study and 724 provided a stool sample for analysis. At baseline, 6.0% of participants were diagnosed with one or more intestinal helminth infections (Table 2). Hookworm was the most commonly observed infection, with 33 individuals

(4.6%) testing positive. Other observed helminth infections included *T. trichiura, S. haematobium*, and *S. mansoni*, though these were relatively rare (prevalence < 2%).

Table 1- Prevalence of Helminth Infections at Baseline

	Community Prevalence				Total Prevalence N= 724
Helminth	Abono N= 329	Abrodwum N= 51	Konkoma N= 254	Duase N= 90	
Hookworm	24	2	8	6	40
T. trichiura	7	0	5	1	13
S. haematobium	1	0	2	0	3
S. mansoni	3	0	0	1	4

Diagnosed infections included cases of poly-parasitism (Table 6). Though dual infections were rare, the most common cases were infections with Hookworm and *T. trichiura* (0.6%).

Table 2- Cases of Coinfection

Infection Type	Cases %
Mono-infections	
Hookworm only	33 (4.6)
Trichuris trichiura only	7 (1.0)
S. haematobium only	3 (0.4)
Dual infections	
Hookworm + *Trichuris trichiura*	4 (0.6)
Hookworm + *S. mansoni*	2 (0.3)
Hookworm + *S. haematobium*	1 (0.1)
Trichuris trichiura + S. mansoni	2 (0.3)

Small differences in prevalence were observed within the study cohort according to community (Figure 10). The community of Abono constituted the largest majority of observed caseload with a prevalence of infection about 7.3%.

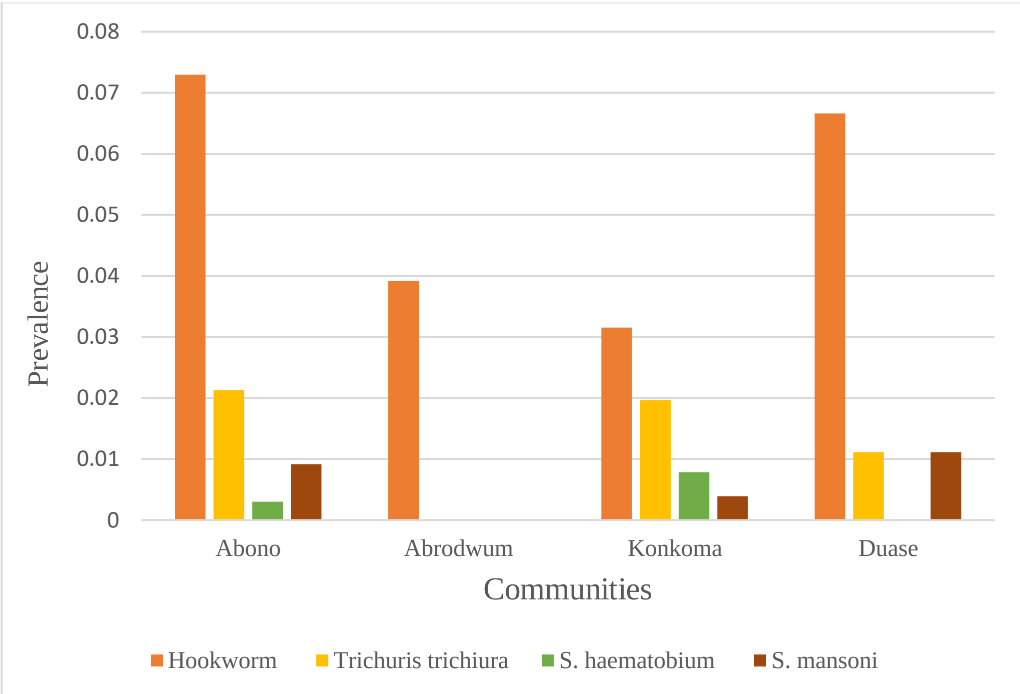

Figure 9. Variation in Helminth Infection by Community. Hookworm prevalence varied slightly by community, with the highest prevalence in Abono (7.3%). Prevalence of *Trichuris trichiura* was noted in Abono, Konkoma, and Duase. Other helminthiases included *S. haematobium* and *S. mansoni*.

The age distribution of hookworm infected individuals in childhood was concentrated around age 11. Among adults, hookworm infections appeared distributed evenly between ages 30-70 years.

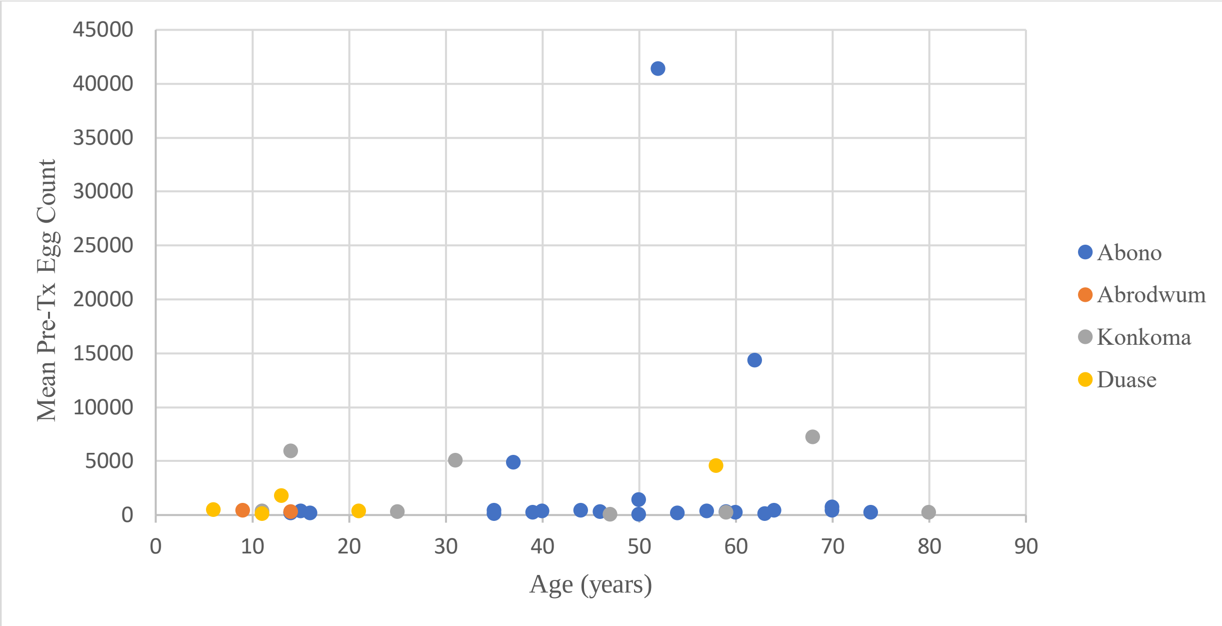

Figure 10. Age-Specific Burden of Hookworm. The mean pre-treatment egg count (in eggs per gram) according to age in Abono, Abrodwum, Konkoma, and Duase in the Ashanti Region, Ghana.

Hookworm Infection: Summary Characteristics

A total of 40 individuals (5.6%) were diagnosed with a hookworm infection at baseline. Summary measures of hookworm infection intensity and egg counts are provided in Table 3. The majority of observed infections (82.5%) were classified as "light" intensity infections according to WHO guidelines (1-1999 EPG) while 17.5% of infections were categorized as "heavy" infections (≥4,000 EPG). There were no "moderate" infections in the study population. Mean baseline fecal egg count was 131.3 EPG (± 1693.4 SD) and varied across the communities (14.1-205.6).

Table 3- Hookworm Infection Characteristics by Community					
Characteristic	Abono	Abrodwum	Konkoma	Duase	Total
Baseline infections	24 (7.3)	2 (3.9)	8 (3.1)	6 (6.7)	40 (5.6)
Cases by infection intensity					
Light	21 (87.5)	2 (100.0)	5 (62.5)	5 (83.3)	33 (82.5)
Moderate	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Heavy	3 (12.5)	0 (0.0)	3 (37.5)	1 (16.7)	7 (17.5)
Mean egg count (epg)					
Pre-treatment	205.6 ± 2426.9	14.1 ± 72.0	76.1 ± 518.0	80.8 ± 662.9	131.3 ± 1693.4
Post-treatment	37.3 ± 97.6	0.0	32.0 ± 55.4	N/A	33.4 ± 88.1

Mean egg counts were calculated only over positive cases. Counts and proportions may not sum to total due to missing values. Mean values are arithmetic means ± standard deviation.

Description of Abono Study Population According to Hookworm Infection Status

For the purposes of demographic characteristics, the following analyses were limited to the Abono study population. Sociodemographic information was assessed in relation to baseline hookworm infection status in the Abono study population. Descriptive statistics can be found in Table 4. The study population was 59.5% female and average participant age was 36.6 years old. Among the adults, the most common occupation was farming. The majority of children self-identified as students. The average self-reported household size was 6.33 members.

The descriptive characteristics showed several patterns. Females in the study population were more likely to be infected with hookworm than males. Additionally, the average age of those infected with hookworm (46.7 years old) was significantly higher than the mean age of those uninfected (26.6 years of age). A decreasing trend was observed in rates of infection with increased level of education. Farmers had the highest proportion of infected individuals among the occupations.

Medical history and risk behaviors for hookworm infection were also assessed according to baseline hookworm infection status (Table 5). Individuals who had not received anti-parasitic treatment in the last year were infected with hookworm at a slighter higher rate than those who had received treatment. Additionally, individuals who self-reported daily sandal wear were infected at a lower rate than those who did not wear sandals daily.

Table 4- Description of Study Demographics by Hookworm Infection Status

	Baseline Hookworm Status	
Characteristic	Uninfected (N%)	Infected (N%)
Age (Mean + SD)	26.6 (19.3)	46.7 (18.3)
Absolute wealth quartile		
1 Poorest	57 (86.4)	9 (13.6)
2 Above-median	55 (90.2)	6 (9.8)
3 Below-median	57 (93.4)	4 (6.6)
4 Richest	63 (95.4)	3 (4.6)
Sex		
Female	177 (91.3)	17 (8.8)
Male	125 (94.7)	7 (5.3)
Education level		
None	14 (82.4)	3 (17.7)
Primary	109 (92.4)	9 (7.6)
Junior High	94 (91.3)	9 (8.4)
Senior High	15 (100.0)	0 (0.0)
Tertiary	0 (0.0)	0 (0.0)
Post-grad	2 (100.0)	0 (0.0)
Occupation		
Farmer	56 (82.4)	12 (17.7)
Fisher	0 (0.0)	1 (100.0)
Small Trader	31 (93.9)	2 (6.1)

Student	121 (96.0)	5 (3.8)
Unemployed	9 (90.0)	1 (10.0)
Other	19 (100.0)	0 (0.0)
Pig Ownership		
No	301 (92.9)	23 (7.1)
Yes	3 (75.0)	1 (25.0)

Table 5- Description of Health & Sanitation Behaviors by Hookworm Infection Status

	Baseline Hookworm Status	
Characteristic	Uninfected (N%)	Infected (N%)
Anti-parasitic treatment in the last year		
No	77 (90.6)	8 (9.4)
Yes	161 (92.5)	13 (7.5)
Daily Sandal Use		
No	19 (82.6)	4 (17.4)
Yes	214 (92.6)	17 (7.4)
Household drinking water source		
Unimproved only	124 (89.9)	14 (10.1)
Mixed	93 (94.9)	5 (5.1)
Improved only	3 (60.00)	2 (40.0)

An analysis of child anthropometry according to hookworm status was included in the data analysis (Table 6). All children (=< 19 as per WHO guidelines) were included. There were no hookworm-positive individuals outside of normal range for weight, height, mid upper-arm circumference, and body mass index.

Table 6- Description of Child Anthropometry by Hookworm Status		
	Baseline Hookworm Status	
Measurement	Uninfected (N%)	Infected (N%)
Weight for age (N= 74)		
Normal range (67)	67 (100.0)	0 (0.0)
Moderate or severe malnutrition (7)	7 (100.0)	0 (0.0)
Height for age (N= 155)		
Normal range (133)	128 (96.2)	5 (3.8)
Moderate or severe stunting (22)_	22 (100.0)	0 (0.0)
MUAC for age (N= 155)		
Normal range (150)	145 (96.7)	5 (3.3)
Moderate or severe malnutrition (5)	5 (100.0)	0 (0.0)
BMI for age (N= 155)		
Normal range (149)	144 (96.6)	5 (3.4)
Moderate or severe thinness (6)	6 (100.0)	0 (0.0)
Z-score cutoffs of WAZ, HAZ, MUACZ and BAZ < -2 for moderate or severe categories. Numbers or percentages may not sum to total due to missing or censored values.		

Risk Factors for Baseline Hookworm Infection

To identify potential risk factors for hookworm infections, a logistic regression model was used to calculate the significance of association between hookworm infection and various predictor variables while controlling for correlation in the dependent variable (e.g. clustering of hookworm infection at the household level).

Table 7- Unadjusted Associations with Baseline Hookworm Infection			
Characteristic	Unadjusted OR	95% CI	p-value
Sociodemographic & SES			
Age	1.05	(1.03, 1.07)	<.0001
Sex			
Male	1.00	-	-
Female	1.72	(0.69, 4.26)	0.25
Wealth Quartile			
4 Richest	1.00	-	-
3 Above-median	1.48	(0.32, 6.89)	0.62
2 Below-median	2.34	(0.56, 9.81)	0.25
1 Poorest	3.58	(0.92, 13.90)	0.07
Occupation			
None	1.00	-	-
Student	0.61	(0.16, 2.35)	0.47
Farmer	3.16	(0.96, 10.38)	0.06
Medical History			
Anti-parasitic treatment in last year	0.78	(0.31, 1.95)	0.59
Water, Sanitation, and Hygiene			
Daily shoe use	0.38	(0.12, 1.24)	0.11
High quality housing materials	0.84	(0.36, 1.97)	0.68
Open defecation or urination	1.65	(0.67, 4.05)	0.28
Improved drinking water source	0.93	(0.40 2.15)	0.86
Bivariate GEE logistic regression			

Table 8- Adjusted Associations with Baseline Hookworm Infection			
Characteristic	Adjusted OR	95% CI	p-value
Sociodemographics & SES			
Age	1.05	(1.02, 1.07)	0.0004
Wealth Quartile			
4 Richest	1.00	-	-
3 Above-median	1.34	(0.22, 8.18)	0.75
2 Below-median	2.62	(0.54, 12.88)	0.23
1 Poorest	5.94	(1.26, 28.03)	0.02
Water, Sanitation, and Hygiene			
Daily shoe use	0.13	(0.03, 0.69)	0.01
Open defecation or urination	2.56	(0.88, 7.45)	0.08
Multivariate GEE logistic regression *Adjusted for all other variables listed.			

V. Discussion

Burden of Hookworm Infection

Previous studies in the middle-belt of Ghana have reported higher prevalence of intestinal helminth infections (19.3%) than found in this work [52]. The prevalence of hookworm infections (*Ancylostoma duodenale* and *Necator americanus*) specifically was lower at 12.1%.

The overall prevalence of baseline hookworm infections in this study (5.6%) was much lower. Abono had the highest prevalence of hookworm burden (7.3%), followed by Duase (6.7%), then Abrodwum (3.9%), and finally Konkoma (3.1%). Abono also experienced the highest prevalence of *T. trichiura* infections. The prevalence of hookworm infections in each community was substantially lower than the Ghanaian national averages found in previous studies (30%). The low prevalence of hookworm infections in the Ashanti Region could be explained by numerous factors. There may be low re-infection rates as people regularly take anti-parasitic treatment. The low infection rates could also suggest high MDA penetration from previous national campaigns. Additionally, as noted in previous analyses, the Kato-Katz test results can exhibit day-to-day variation that may not accurately represent true burden of STH infections [53].

The majority of cases occurred in adults over the age of 30 years, and the distribution of cases by age was found to be similar between communities included in this study. The average age of uninfected individuals was 26.6 years while the average age of infected participants was 46.7 years. This result has not previously been observed in studies, as most research has shown the highest distribution of infections centered around age 11 [3]. The newly observed age pattern of infection suggests that cumulative infections over time may not result in protective immunity. Additionally, the previously considered exposures among children and adults may not differ accordingly.

Variation in Hookworm Burden

The four communities around Lake Bosumtwe displayed concordance in their baseline prevalence (3.1-7.3%). They did, however, differ in their pre-treatment mean infection intensity (14.1-205.6 EPG) with Abono experiencing the highest intensity. Although the demographic data for the other three communities was not included in data analysis, the variation in infection intensity is surprising given the proximity of the communities' location and similarity of characteristics. Local stakeholders and district health officers confirmed that the communities did not differ in deworming history. According to information provided by these informants, annual community-based administration of albendazole and ivermectin through the Ghana Lymphatic Filariasis Elimination Program (GLFEP) and school-based deworming of children for STHs had not occurred in any community for the past two years. As this was uniform throughout all four communities, it was surprising to see variation in STH distribution.

This heterogeneity of local deworming program impact may suggest that re-infection rates differ across the communities. It is also possible that resistance genes are circulating among a portion of these communities rather a homogenous distribution. As the majority of infections throughout the study were of low intensity, the low sensitivity of Kato-Katz may have not accurately portrayed the true dispersion of cases.

Predictors of Infection

Health and sanitation behaviors according to hookworm infection status revealed few patterns. A higher percentage of individuals who experienced hookworm infections did not wear sandals (17.4%) than those who did (7.4%). Anti-parasitic treatment in the last year was not indicative of infections. Local school and health officials in the Lake Bosumtwe area reported no deworming programs in the previous two years. As anti-parasitic treatment was then subject to purchase of over-the-counter treatments following a diagnostic test or self-report, the reliability of this survey question may low.

All children who experienced infections were classified in the normal age-adjusted range for weight, height, MUAC, and BMI. This lack of association mirrors that of previous studies conducted in Ghana examining the effect of nutritional status on risk of infection [41]. Although there were few cases of hookworm infections in Abono children under the age of 18, it is still important to examine the anthropometric data according to hookworm status for determination of infection predictors.

The unadjusted associations with baseline hookworm infections in Abono showed age to be the only significant risk factor for hookworm infection. Every additional year is associated with a 1.05 increased risk of hookworm infection. Although not statistically significant, the odds of infection among females were 1.72 the odds of males. This result diverges from previous findings from agricultural communities suggesting that the risk of infection is higher among males [54]. The observed association between age and prevalence for hookworm infection shown to increase with age suggests geographic heterogeneity in parasite and host factors [22]. Ownership of one or more pigs has been shown to increase individual likelihood of hookworm infection [51]. While there was a larger observed percentage of infected individuals (25.0%) who owned pigs, there were not enough total observations of pig ownership to examine potential associations.

Age, wealth, daily shoe use, and open defecation or urination are independently associated with risk of hookworm infection. After adjustment for other variables in the multivariate regression model, age remained the most significant predictor of hookworm infection (AOR= 1.05, p= 0.0004). While the age-prevalence association is discordant from previous studies, the adjusted analyses of wealth, shoe use, and open defecation or urination practices with risk of hookworm infection have been previously observed.

Daily shoe use was found to be a protective factor against hookworm infection, in concordance with the results from previous studies [55, 56]. It is important to note that the definition of shoe can vary in physical protection from open-toe to closed-toe shoes. This may alter how the shoe is works against the route of transmission through the sole of the foot.

The adjusted regression model suggests that there is a ceiling effect of wealth on infection, in which additional wealth accumulation outside of the poorest category (AOR= 5.94, p= 0.02) does not significantly alter protective benefits against hookworm infection. This phenomenon can be observed among the richest, above-median, and below-median quartiles. While previous work has found no association between wealth and hookworm infections, this study reports that belonging to the poorest wealth quartile can be considered a significant risk factor for infection [41]. This finding can allow for better targeted interventions for those who are most vulnerable to STH infections.

Study Limitations

This study has several limitations. Although household clustering effects were adjusted for in all bivariate and multivariate analyses, the covariance in observations makes statistical interpretation more challenging than other study designs such as random sampling. Additional challenges are highlighted below.

Although Kato-Katz microscopy is regarded as the best and most practical technique for field detection of helminth infections, numerous studies have questioned its sensitivity in comparison to newer methods. While the Kato-Katz technique is dependent on egg distribution within the stool sample and can miss light intensity infections, PCR analysis of these samples can detect trace amounts of helminth eggs [53, 57]. In an area with light intensity infections like Lake Bosumtwe, the low sensitivity of Kato-Katz can prove an insurmountable challenge for detection.

Kato-Katz is also susceptible to rapid degeneration of hookworm eggs within the timeframe for detection. Any time delays from stool donation to slide preparation may have hindered the ability to detect eggs.

Additionally, the low hookworm infection rate makes statistically determining unadjusted and adjusted relationships more challenging. The overall low sample size may have not provided enough participants with the risk factor to be observed in the analysis. The variables used in this questionnaire are susceptible to inaccuracies in their proxy measurements for exposure, such as the level of protection actually provided by the type of shoe worn by the participant.

Future Directions

The positive association between age and hookworm infection has not been described in this geographic region. Future studies should seek to replicate this result and determine how intrinsic and extrinsic host factors contribute to the etiology of this association.

As MDAs reduce the prevalence of STH infections in endemic regions, there will be a need for new, more sensitive diagnostic tools. Prior research suggests that quantitative PCR may be successful in molecular analysis of helminth infections [44]. Although the cost of developing and implementing this technology in the field will be high, the greater specificity and reduction of labor requirements offers a more attractive alternative to Kato-Katz for diagnosing and quantifying helminth infections. Using higher specificity tools as prevalence declines will allow for more selective treatment strategies in high-risk areas. This can reduce cost, decrease potential anthelmintic drug resistance, and provide better quality treatment for STH infections.

Although we used proxy variables to determine nutritional status, future studies should include specific food security survey questions for direct measurement. As many questionnaires probe only macronutrient intake, it could be useful to include assessment of micronutrient deficiencies in relation to infection status [58].

Additional studies should investigate the treatment response rate as other research groups have noticed a decline in cure rates of anthelminthic drugs as selective pressures shift with the ongoing preventative and treatment programs [59]. Molecular genomics can be used to identify the presence of resistance genes, which would elucidate possible mechanisms responsible for declining cure rates.

VI. Conclusion

In this study, we have conducted analyses of soil-transmitted helminths around Lake Bosumtwe in the Ashanti Region of Ghana to define local prevalence and identify potential risk factors for hookworm infection. The data presented in this study will be used for meaningful comparisons of other sites within the country as well contribute to the development of future molecular diagnostic techniques for hookworm.

Notably, we found an overall hookworm prevalence (5.6%) that is significantly lower than the national average (30%), suggesting that there is some success in local deworming efforts. Additionally, we found a positive relationship between age and infection that has not been previously observed in the literature for this region.

Together, these findings highlight the need for more robust community-based monitoring of MDA programs to detect local variability in performance and design interventions accordingly. Moreover, the unexpected patterns of exposure and infection presented here suggest that important gaps still exist in our understanding of STH epidemiology.

References

1. Hotez, P.J. and A. Kamath, *Neglected tropical diseases in sub-saharan Africa: review of their prevalence, distribution, and disease burden.* PLoS neglected tropical diseases, 2009. **3**(8): p. e412-e412.
2. Loukas, A., et al., *Hookworm infection.* Nature Reviews Disease Primers, 2016. **2**(1): p. 16088.
3. Hotez, P.J., et al., *Hookworm Infection.* New England Journal of Medicine, 2004. **351**(8): p. 799-807.
4. de Silva, N.R., et al., *Soil-transmitted helminth infections: updating the global picture.* Trends in Parasitology, 2003. **19**(12): p. 547-551.
5. Chan, M.S., *The global burden of intestinal nematode infections — Fifty years on.* Parasitology Today, 1997. **13**(11): p. 438-443.
6. Pullan, R.L., et al., *Global numbers of infection and disease burden of soil transmitted helminth infections in 2010.* Parasites & Vectors, 2014. **7**(1): p. 37.
7. Organization, W.H. *Soil-transmitted helminth infections* Fact Sheets 2019; Available from: https://www.who.int/news-room/fact-sheets/detail/soil-transmitted-helminth-infections.
8. Brooker, S., J. Bethony, and P.J. Hotez, *Human hookworm infection in the 21st century.* Advances in parasitology, 2004. **58**: p. 197-288.
9. Steenhard, N.R., et al., *The role of pigs as transport hosts of the human helminths Oesophagostomum bifurcum and Necator americanus.* Acta Tropica, 2000. **76**(2): p. 125-130.
10. Tang, Y.T., et al., *Genome of the human hookworm Necator americanus.* Nature Genetics, 2014. **46**(3): p. 261-269.
11. Colley, D.G., T.S. Andros, and C.H. Campbell, Jr., *Schistosomiasis is more prevalent than previously thought: what does it mean for public health goals, policies, strategies, guidelines and intervention programs?* Infectious diseases of poverty, 2017. **6**(1): p. 63-63.
12. Hotez, P.J., et al., *Developing vaccines to combat hookworm infection and intestinal schistosomiasis.* Nature Reviews Microbiology, 2010. **8**(11): p. 814-826.
13. Hawdon, J.M. and P.J. Hotez, *Hookworm: developmental biology of the infectious process.* Current Opinion in Genetics & Development, 1996. **6**(5): p. 618-623.
14. Zakeri, A., et al., *Immunomodulation by Helminths: Intracellular Pathways and Extracellular Vesicles.* Frontiers in Immunology, 2018. **9**(2349).
15. Raso, G., et al., *An integrated approach for risk profiling and spatial prediction of Schistosoma mansoni-hookworm coinfection.* Proceedings of the National Academy of Sciences of the United States of America, 2006. **103**(18): p. 6934-6939.
16. Bethony, J., et al., *Emerging Patterns of Hookworm Infection: Influence of Aging on the Intensity of Necator Infection in Hainan Province, People's Republic of China.* Clinical Infectious Diseases, 2002. **35**(11): p. 1336-1344.
17. Maizels, R.M., J.P. Hewitson, and K.A. Smith, *Susceptibility and immunity to helminth parasites.* Current opinion in immunology, 2012. **24**(4): p. 459-466.
18. Inclan-Rico, J.M. and M.C. Siracusa, *First Responders: Innate Immunity to Helminths.* Trends in parasitology, 2018. **34**(10): p. 861-880.

19. Strunz, E.C., et al., *Water, sanitation, hygiene, and soil-transmitted helminth infection: a systematic review and meta-analysis.* PLoS medicine, 2014. **11**(3): p. e1001620-e1001620.
20. van der Werf, M.J., et al., *Quantification of clinical morbidity associated with schistosome infection in sub-Saharan Africa.* Acta Tropica, 2003. **86**(2): p. 125-139.
21. Yu, S.-H., Z.-X. Jiang, and L.-Q. Xu, *Infantile hookworm disease in China. A review.* Acta Tropica, 1995. **59**(4): p. 265-270.
22. Brooker, S., et al., *The epidemiology of hookworm infection and its contribution to anaemia among pre-school children on the Kenyan coast.* Transactions of The Royal Society of Tropical Medicine and Hygiene, 1999. **93**(3): p. 240-246.
23. Jiraanankul, V., et al., *Incidence and risk factors of hookworm infection in a rural community of central Thailand.* The American journal of tropical medicine and hygiene, 2011. **84**(4): p. 594-598.
24. Organization, W.H., *The use of Essential Drugs: Model List of Essential Drugs.* Genva, Switzerland: World Health Organization, 1997.
25. Keiser, J. and J. Utzinger, *Efficacy of Current Drugs Against Soil-Transmitted Helminth Infections: Systematic Review and Meta-analysis.* JAMA, 2008. **299**(16): p. 1937-1948.
26. Savioli, L., et al., *Schistosomiasis and soil-transmitted helminth infections: forging control efforts.* Transactions of the Royal Society of Tropical Medicine and Hygiene, 2002. **96**(6): p. 577-579.
27. Albonico, M., et al., *Rate of reinfection with intestinal nematodes after treatment of children with mebendazole or albendazole in a highly endemic area.* Transactions of the Royal Society of Tropical Medicine and Hygiene, 1995. **89**(5): p. 538-541.
28. Sokolow, S.H., et al., *Global Assessment of Schistosomiasis Control Over the Past Century Shows Targeting the Snail Intermediate Host Works Best.* PLoS neglected tropical diseases, 2016. **10**(7): p. e0004794-e0004794.
29. Taylor-Robinson, D.C., et al., *Public health deworming programmes for soil-transmitted helminths in children living in endemic areas.* Cochrane Database of Systematic Reviews, 2019(9).
30. Tandoh MA, M.-R.F., Wilson MD, Anderson AK, *Disparities of Sanitary Conditions/Habits and Helminthiasis Prevalence between School-Age-Children Living in Fishing and Farming Communities in Ghana: A Cross Sectional Study.* J Infec Dis Treat., 2018. **Vol.4 No. 2:6.**
31. *Ghana*. 2019; Available from: http://espen.afro.who.int/countries/ghana.
32. Xiao, S., et al., *The evaluation of recombinant hookworm antigens as vaccines in hamsters (Mesocricetus auratus) challenged with human hookworm, Necator americanus.* Experimental Parasitology, 2008. **118**(1): p. 32-40.
33. Schneider, B., et al., *A history of hookworm vaccine development.* Human vaccines, 2011. **7**(11): p. 1234-1244.
34. Fujiwara, R.T., et al., *Comparative immunology of human and animal models of hookworm infection.* Parasite Immunology, 2006. **28**(7): p. 285-293.
35. Hoagland, K.E. and G.A. Schad, *Necator americanus and Ancylostoma duodenale: Life history parameters and epidemiological implications of two sympatric hookworms of humans.* Experimental Parasitology, 1978. **44**(1): p. 36-49.
36. Carroll, S.M. and D.I. Grove, *Parasitological, Hematologic, and Immunologic Responses in Acute and Chronic Infections of Dogs with Ancylostoma ceylanicum: A Model of*

Human Hookworm Infection. The Journal of Infectious Diseases, 1984. **150**(2): p. 284-294.
37. Jian, X., et al., *Necator americanus: maintenance through one hundred generations in golden hamsters (Mesocricetus auratus). II. Morphological development of the adult and its comparison with humans.* Experimental Parasitology, 2003. **105**(3): p. 192-200.
38. McCOY, O.R., *IMMUNITY REACTIONS OF THE DOG AGAINST HOOKWORM (ANCYLOSTOMA CANINUM) UNDER CONDITIONS OF REPEATED INFECTION*.* American Journal of Epidemiology, 1931. **14**(2): p. 268-303.
39. *GHDX, Global Health Data Exchange*. 2018; Available from: http://ghdx.healthdata.org/gbd-results-tool.
40. Hotez, P.J., et al., *Ghana: Accelerating neglected tropical disease control in a setting of economic development.* PLOS Neglected Tropical Diseases, 2019. **13**(1): p. e0007005.
41. Humphries, D., et al., *Effectiveness of Albendazole for Hookworm Varies Widely by Community and Correlates with Nutritional Factors: A Cross-Sectional Study of School-Age Children in Ghana.* The American journal of tropical medicine and hygiene, 2017. **96**(2): p. 347-354.
42. Mirisho, R., M.L. Neizer, and B. Sarfo, *Prevalence of Intestinal Helminths Infestation in Children Attending Princess Marie Louise Children's Hospital in Accra, Ghana.* Journal of parasitology research, 2017. **2017**: p. 8524985-8524985.
43. Turner, H.C., et al., *Economic Considerations for Moving beyond the Kato-Katz Technique for Diagnosing Intestinal Parasites As We Move Towards Elimination.* Trends in parasitology, 2017. **33**(6): p. 435-443.
44. Albonico, M., D. Engels, and L. Savioli, *Monitoring drug efficacy and early detection of drug resistance in human soil-transmitted nematodes: a pressing public health agenda for helminth control.* International Journal for Parasitology, 2004. **34**(11): p. 1205-1210.
45. Amu-Mensah, M., et al., *Significance of Lake Bosomtwe as a freshwater resource in Ghana; communities' perception.* International Journal of Development and Sustainability, 2017. **66**: p. 2186-8662.
46. *2010 Population and Housing Census.* Ghana Statistical Services GSS (2012).
47. Mramba, L., et al., *A growth reference for mid upper arm circumference for age among school age children and adolescents, and validation for mortality: growth curve construction and longitudinal cohort study.* BMJ, 2017. **358**: p. j3423.
48. Organization, W.H., *WHO AnthroPlus software*. 2007.
49. Mramba, L., *MUACz: Generate MUAC z-Scores for School Children Aged 5-19 Years Old*
. 2019.
50. Filmer, D.P., L.H. , *Estimating Wealth Effects Without Expenditure Data—Or Tears: An Application To Educational Enrollments In States Of India*.* Demography, 2001. **38**(1): p. 115–132.
51. Humphries, D., et al., *Hookworm infection among school age children in Kintampo north municipality, Ghana: nutritional risk factors and response to albendazole treatment.* The American journal of tropical medicine and hygiene, 2013. **89**(3): p. 540-548.
52. Adu-Gyasi, D., et al., *Epidemiology of soil transmitted Helminth infections in the middle-belt of Ghana, Africa.* Parasite epidemiology and control, 2018. **3**(3): p. e00071-e00071.
53. Tarafder, M.R., et al., *Estimating the sensitivity and specificity of Kato-Katz stool examination technique for detection of hookworms, Ascaris lumbricoides and Trichuris*

trichiura infections in humans in the absence of a 'gold standard'. International journal for parasitology, 2010. **40**(4): p. 399-404.
54. Matthys, B., et al., *Risk factors for Schistosoma mansoni and hookworm in urban farming communities in western Côte d'Ivoire.* Tropical Medicine & International Health, 2007. **12**(6): p. 709-723.
55. Hailu, T., et al., *Multivariate analysis of factors associated with Schistosoma mansoni and hookworm infection among primary school children in rural Bahir Dar, Northwest Ethiopia.* Tropical diseases, travel medicine and vaccines, 2018. **4**: p. 4-4.
56. Tomczyk, S., et al., *Association between footwear use and neglected tropical diseases: a systematic review and meta-analysis.* PLoS neglected tropical diseases, 2014. **8**(11): p. e3285-e3285.
57. Krauth, S.J., et al., *An in-depth analysis of a piece of shit: distribution of Schistosoma mansoni and hookworm eggs in human stool.* PLoS neglected tropical diseases, 2012. **6**(12): p. e1969-e1969.
58. Rajagopal, S., P.J. Hotez, and D.A.P. Bundy, *Micronutrient Supplementation and Deworming in Children with Geohelminth Infections*. PLOS Neglected Tropical Diseases, 2014. **8**(8): p. e2920.
59. Diawara, A., et al., *Association between response to albendazole treatment and β-tubulin genotype frequencies in soil-transmitted helminths.* PLoS neglected tropical diseases, 2013. **7**(5): p. e2247-e2247.