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The Effects of Host Nutritional Status and Dietary Factors on the Treatment Efficacy of Albendazole in School-Age Children Infected with Hookworm in the Kintampo North District of Ghana

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Abstract

Background: In endemic settings unable to support economic growth, inexpensive anthelmintics, such as albendazole, are the cornerstone for hookworm infection treatment and control. However, the overall efficacy of albendazole against hookworm may be reduced after periodic chemotherapy. (Hotez *et al.*, 2004) In addition, there are few studies exploring the role of nutritional status as a potential host factor influencing drug response and the constituents of diet has often been overlooked when determining anthelmintic efficacy, despite previous accounts of nutrient content of a meal or consumed food item affecting drug metabolism. Evaluating nutritional status as a potential host factor may partly explain the wide variability of drug efficacy. (Vercruysse *et al.*, 2011)

Methodology: Ghanaian school children that met inclusion criteria were selected from five communities previously identified as having high prevalence of hookworm infection. After enrolling 141 eligible school-age children for baseline assessment, those positive for hookworm infection were treated with single-dose 400 mg albendazole. Nutritional status and dietary data were assessed to identify modifiable host factors affecting treatment response.

Principal Findings: Our results showed that the efficacy of single-dose oral albendazole for curing hookworm infection was significantly reduced for children with lower dietary diversity and protein intake on treatment day. In addition, children with higher dietary diversity scores within the same reference period were 3.08 times more likely than those with lower scores to experience the highest egg reduction rates (p-value 0.0430).

Conclusion: The cross-sectional study provides new data on the nutritional status and dietary patterns of children in the Kintampo North Municipality and elucidates the potential role of modifiable host factors in affecting response to treatment.

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Sara A. Nguyen

Date

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Introduction

A Brief Historical Context of Hookworm Disease

The effective control of parasitic soil-transmitted helminth infections in humans has seen equal amounts of success and failure. Public health initiatives and the engines of socioeconomic development led to the near eradication of hookworm disease in the early 20th century America, but such achievement has proven to be extremely difficult in developing countries afflicted with chronic poverty and poor sanitation. (Hotez *et al.*, 2005) As one of the most widespread neglected tropical diseases in the world, hookworm not only impacts the health of affected populations, but also plays a negative role in education and rural poverty. (Gillespie, 2001) Furthermore, moderate to heavy hookworm infection in children results in severe deficits— anemia, iron deficiency, and retarded physical and cognitive growth.

(Dreyfuss *et al.*, 2000; Hotez *et al.*, 2004; Hotez, 2006)

In 2001, the 54th World Health Assembly declared helminth control as a top priority in the global health arena. As children and women of reproductive age are at a more heightened risk for hookworm-related anemia, hookworm has been increasingly targeted by maternal and child health programs. In addition, ongoing prevention and control efforts include the promotion of large-scale treatment programs in at-risk settings (e.g., school-based deworming) and the development of anthelmintic drugs and vaccines. (Brooker *et al.*, 2004)

Hookworm Disease Biology

Hookworms are nematode endoparasites from the family Ancylostomatidae—with the species *Ancylostoma duodenale* and (predominant) *Necator americanus* causing human infection after skin exposure to fecally contaminated soil. (Brooker *et al.*, 2004) Primarily affecting impoverished regions of the tropics and subtropics, the combination of warm, shaded climates (ideally 20–30 degrees Celsius) and moist soils provide the optimum environment for hookworm larvae. Although hookworm may enter any part of the human body, the resulting burning rash or localized “ground itch” is often found on the hands and feet of an infected host—the most vulnerable sites for parasite entry. (Hotez *et al.*, 2004; Hotez, 2006)

Developmental Processes of the Hookworm Life Cycle

Before entering human hosts, the infective larvae live in fecally contaminated soil where warm temperatures and moisture provide a safe haven from desiccation (Figure 1). During their time in an external environment, hookworm eggs hatch into first-stage (L1) rhabditiform larva and eventually molt twice to transform into the infective larval stage—third-stage larva (L3). Hookworm infection develops after filariform larva penetrates the skin of a susceptible host. After entering the host through skin invasion and the production of proteolytic enzymes, the hookworm larvae continue to grow and migrate toward the lungs and other pulmonary vasculature. (Gillespie, 2001; Hotez, 2006) After the unknowing host coughs up and swallows the larvae, they enter the gastrointestinal tract and ultimately mature into adult-stage worms (L4) in the small intestine. It is here where sexually mature adult worms mechanically attach themselves to the intestinal wall with their buccal capsules and cutting plates and cause intestinal

blood loss in the gut. (Gillespie, 2001) The consequences of infection and chronic blood loss will be discussed later in further detail.

The next life stage of the hookworm involves mating and sexual reproduction—a single female has the ability to produce between 10,000 and 20,000 eggs per day, which ultimately leave the body through feces. (Hotez *et al.*, 2004) A hookworm egg is described as “thin-shelled, hyaline, and ovoid, measuring approximately 60 x 40 μm ”. (Hotez, 2006) After fecal excretion by the host into the environment, fresh hookworm eggs are then able to take the necessary step to develop and repeat the parent life cycle. (Hotez *et al.*, 2004)

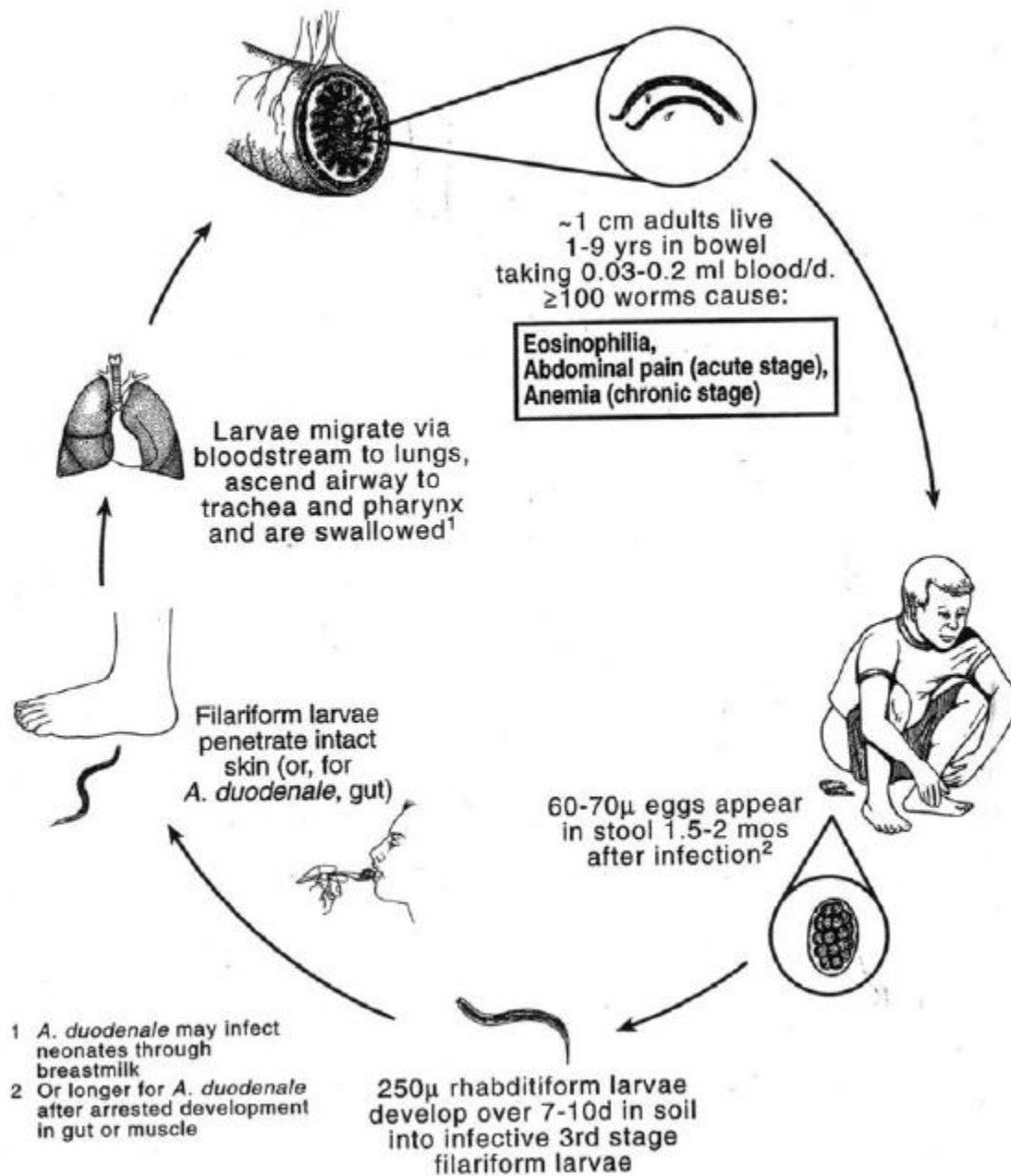


Figure 1. Hookworm life cycle

Hookworm Pathogenesis – The Disease

Moderate to severe hookworm infections are often accompanied by abdominal pain (from intestinal inflammation), nausea, fatigue, diarrhea, pallor, and heart murmurs. During the pivotal stage of the hookworm life cycle, the developing worm will mechanically “hook” onto the wall of the small intestine with its mouthparts and cause host blood loss from feeding on blood and mucosa (e.g., destruction of mucosal capillaries and secretion of anticoagulant peptides). (Hotez, 2006) Intestinal blood loss from *Necator americanus* and *Ancylostoma duodenale* has been estimated to be as high as 0.03 mL and 0.15 ml per worm per day, respectively. (Hotez *et al.*, 2004) Some of the most severe consequences of chronic intestinal blood loss include iron deficiency anemia and hypoalbuminemia.

(Brooker *et al.*, 2004; Gillespie *et al.*, 2001; Hotez *et al.*, 2004)

Because of their increased iron needs, children and pregnant women are especially at risk for the deleterious effects of hookworm-related anemia. (Hotez, 2006) In childhood, severe hookworm disease has led to considerable developmental delays in physical and mental growth. (Hotez, 2006) For example, significant loss of iron and plasma proteins affects the regulation of enzymatic systems with an iron prosthetic group (e.g., neurotransmitters)—resulting in infection-associated cognitive and intellectual delays. (Gillespie, 2001) For women of reproductive age and pregnant mothers, hookworm-associated anemia has been reported as a risk factor for increased maternal mortality and low birth weight among newborns. (Brooker *et al.*, 2004; Hotez, 2006)

There is a non-linear association between infection and morbidity—infection is not always sufficient for hookworm disease and acute symptoms are usually only seen in individuals harboring large worm burdens. Similar to other intestinal nematode species, hookworm infection

intensity follows a non-random pattern of worm distribution in humans. Most infected individuals suffer from light burdens, while a small minority is heavily infected with a large disproportionate number of worms. (Brooker *et al.*, 2004; Gillespie, 2001) The predisposition of “wormy individuals” to heavy patterns of infection remains poorly understood.

Although hookworm infection does not follow a normal distribution of infection by age, young children with low iron stores are most vulnerable to blood loss. Hookworm infection can occur in children as young as six months and increase with age until reaching a plateau in adulthood. (Brooker *et al.*, 2004; Gillespie, 2001) As previously mentioned, when children are continuously infected with hookworm, chronic iron deficiency can lead to delays in physical fitness and mental development. (Brooker *et al.*, 2004; Hotez *et al.*, 2004)

Human Immune Responses to Hookworm Infection

Although protective immunological responses have been difficult to describe in both human and animal models, a consistent observation from infected individuals has been the distinct T helper type 2-activated production of interleukins (4, 5, 6, 9, and 10), circulation of eosinophilia, and antibody isotypes (IgG1, IgG4, and IgE). (Brooker *et al.*, 2004) As reported by Jarrett *et al.*, blood serum levels of IgE are elevated by 100-fold in patients with hookworm infection. In an experimental human hookworm infection study, antigen specific IgE increased during primary infection and were significantly boosted following secondary infection. (Wright and Bickle, 2005) In addition, the subsequent secretion and release of toxic granules and reactive oxygen intermediates from these immune cells target different stages of the hookworm parasite with toxic activity. Despite their roles being poorly understood, IgG1 and IgG4 levels are also increased and upregulated during infection. (Brooker *et al.*, 2004) The final observed immune response is peaked eosinophil counts during the course of infection. (Loukas and Prociv, 2001; Wright and Bickle, 2005) Following experimental human infection, eosinophilia has been confirmed to be involved in the inflammatory responses to hookworm L3 in submucosal tissues. (Wright and Bickle, 2005) Although adult hookworms have been reported to stimulate the production of secretory IgE, IgG, and even IgM in humans, there is no marked IgA response to infection. Its absence may be due to the secretion of hookworm IgA-proteases. (Loukas and Prociv, 2001)

As evidenced by overlapping distributions of infection with other pathogens, hookworms may exhibit immunosuppressive properties which allow for increased susceptibility to polyparasitic infections, such as malaria, tuberculosis, and HIV/AIDS. (Brooker *et al.*, 2004; Hotez, 2006) As an example of adversely influencing host immune response, the hookworm-

induced activation of eosinophilia may result in the upregulation of CD4+ molecules on host cell surfaces, thus supporting progression of HIV infection. (Brooker *et al.*, 2004) The influence of helminthes on susceptibility and progression of bacterial and viral diseases warrants further study.

Present Epidemiology

With an estimated 740 million cases worldwide and an additional 4.5 billion individuals at risk, hookworm is one of the most common parasitic infections in tropical and subtropical regions. (Hotez *et al.*, 2004; Keiser and Utzinger, 2008; Saathoff *et al.*, 2005) The life cycle of the hookworm is closely associated with the rural poverty found in developing countries where poor sanitation, inadequate housing, and lack of healthcare access allow the parasitic disease to become stubbornly endemic. (Hotez *et al.*, 2004) Not surprisingly, hookworm is seldom seen in urban cities, but rather, occurs mostly in sandy coastal regions—perfect living environments for the survival and development of larvae.

Global Distribution and Burden of Disease

The highest prevalence and intensity of hookworm infection is found in Sub-Saharan Africa (29%), with China (16%), South and East Asia (16% and 26%, respectively), and the Central and South Americas (10%) following closely behind (Figure 2). (Brooker *et al.*, 2004; Hotez, 2006) Although *Necator Americanus* is the predominant infective species in many parts of the world, *Ancylostoma duodenale* infections are also commonly seen in Africa, India, and China. (Hotez, 2006) Decreases in infections have been observed in Asia and the Americas due to vast improvements in socioeconomic status and control programs. (Brooker *et al.*, 2004) For

instance, overall hookworm prevalence in Thailand drastically decreased from 40.6% in 1982 to 11.4% in 2001. (Anantaphruti *et al.*, 2007)

By adding the number of years lost to premature death and years of life lived with a disability or ill-health, the disability-adjusted life year (DALY) measure offers an estimate of the overall disease burden in a population. According to the most recent Global Burden of Disease study, hookworm was responsible for the worldwide loss of 1.8 million DALYs in 2001—with 23% of the total lost in Sub-Saharan Africa alone. However, as hookworm disease may contribute to iron deficiency anemia (12 million DALYS) in endemic settings, the hookworm-attributed DALY is almost certainly underestimated. (Brooker *et al.*, 2004)

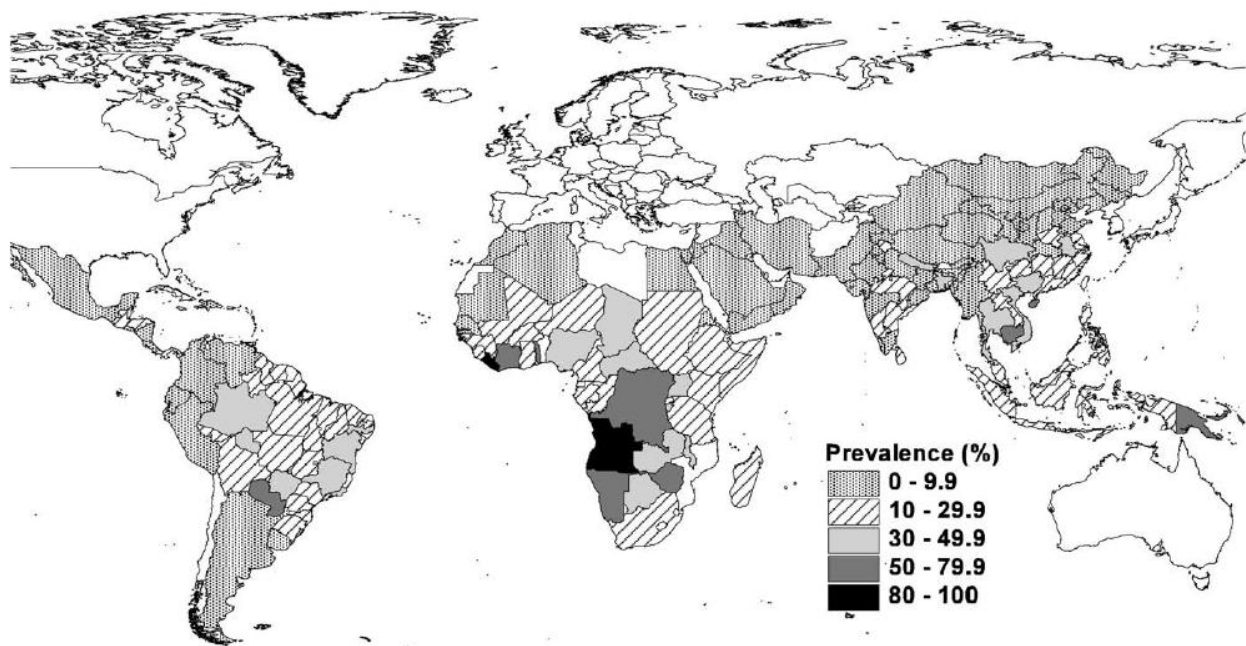


Figure 2. The global distribution of hookworm (WHO)

Disease Occurrence in At-Risk Settings – Ghana

Sub-Saharan Africa has the highest prevalence of hookworm with approximately 200 million infections. (Saathoff *et al.*, 2005) There has been little change in steadfast prevalence rates across the continent due to serious political, economic, and social challenges. With a population of over 24 million people, Ghana has seen prevalence rates exceed 50% in certain parts of the country—from 45% in the Kintampo North District to 87% in rural northern Ghana. (Humphries *et al.*, 2011; Zeim *et al.*, 2006)

Prevention Strategies and Control Methods

Due to lack of socioeconomic mechanisms and health infrastructures, hookworm control has been extremely difficult in high transmission areas throughout Sub-Saharan Africa. Instead of total eradication, control methods for this disease have focused on large-scale chemotherapy programs and improved sanitation and water services. Although needed for long-term control of parasitic diseases, sanitation (e.g., access to clean latrine facilities) and behavior change (e.g., use of footwear) must be complemented with chemotherapeutic measures to effectively interrupt hookworm transmission in rural communities. (Brooker *et al.*, 2004)

Current Concepts in Treatment – Mass Drug Administration

In hookworm endemic settings unable to support economic growth, inexpensive benzimidazole anthelmintics are the cornerstone for infection treatment and morbidity control. (Brooker *et al.*, 2004) Periodic mass drug administration programs are one of the most cost-effective health interventions in developing countries and often organized in local schools and health clinics as part of an integrated national control effort. (Knopp *et al.*, 2010) Deworming

treatments are based on either a single dose of albendazole or mebendazole. As broad-spectrum benzimidazole anthelmintics, both albendazole and mebendazole are affordable, effective, and easy to administer in large communities. (Gillespie, 2001) From a public health perspective, numerous reviews have confirmed the reduction of hookworm-associated morbidity and improved health outcomes with regular chemotherapy. (Brooker *et al.*, 2004) For example, a semi-annual treatment program in Zanzibar resulted in a 55% reduction of severe anemia among school-age children. (Stoltzfus *et al.*, 1998)

Chemotherapeutic Treatment of Intestinal Hookworms

The range of single-dose anthelmintics available for hookworm treatment, as endorsed by the World Health Organization, include: albendazole (400 mg), levamisole (2.5 mg/kg), mebendazole (500 mg), and pyrantel pamoate (10 mg/kg). (Savioli *et al.*, 2004) Anthelmintics act on metabolic pathways or the nervous system of nematodes. (Coles *et al.*, 1975) With the aim to cut down worm burden and transmission potential, periodic treatments with anthelmintics have been shown to drastically reduce chronic morbidity in high risk settings. (Albonico *et al.*, 2004) Despite enduring levels of hookworm transmission and large numbers of reinfections, anthelmintics remain integral in control programs against soil-transmitted helminths due to its low cost and accessibility through community-based programs. (Gillespie, 2001; Savioli *et al.*, 2004)

Mebendazole

Most helminth control programs exclusively use benzimidazole treatments due to its single-dosage form and ease of administration in children. (Albonico, 2003) Although systematic reviews have shown that albendazole is more effective for curing hookworm infection than mebendazole, the latter continues to be used for safe treatment of roundworm, pinworm, whipworm, and other helminth infections in developing countries. (Keiser and Utzinger, 2008; Miller *et al.*, 1974) For a hookworm-infected individual (regardless of age and weight), a single dose of mebendazole twice a day for three consecutive days is recommended for clearance. (Hotez, 2006) Its primary mode of action involves the selective inhibition of microtubule formation (and subsequent glucose depletion) in worms. The efficacy of mebendazole varies considerably due to factors such as preexisting diarrhea and gastrointestinal transit time, intensity of infection, and strain diversity in helminth parasites. (Miller *et al.*, 1974; Munst *et al.*, 1980)

Albendazole

Albendazole is a more potent benzimidazole; even at low dosages, it is rapidly absorbed from the small intestine resulting in increased concentrations in tissue. (Cook, 1990) In comparison with other benzimidazole compounds, its better potency may be due to its stronger bond at the drug-receptor binding site (nematode β -tubulin). (Theorides *et al.*, 1975) As the drug of choice for treatment against hookworm, it may also be used to treat roundworms, tapeworms, and pinworms. Although evidence from published literature indicates that albendazole is satisfactory for the treatment of hookworm, varying levels of efficacy in endemic settings has also been reported. (Albonico, 2003; Vercruyse *et al.*, 2011) A clinical evaluation of albendazole will be discussed with more depth in the following section.

Levamisole

Although levamisole has been used in previous control efforts, it is less effective than benzimidazoles against human infections from *Necator americanus*. (Albonico, 2003) However, it is still used as a veterinary anthelmintic to treat worm infestations in livestock. (Gillespie, 2001) As a synthetic imidazothiazole derivative, its mechanism of action involves nicotinic agonists at acetylcholine receptors and interference with neuromuscular transmission in nematodes resulting in worm contractions and paralysis. (Coles *et al.*, 1975)

Pyrantel Pamoate

While chemically different from previous anthelmintic agents, pyrantel pamoate is efficient against a variety of intestinal parasites. Similar to levamisole, pyrantel pamoate causes depolarization of acetylcholine receptors and ensuing paralysis of the intestinal helminth. The affected worms exit the host in the excretion of stool. Although well tolerated in humans, pyrantel is not often used for periodic therapy due to poor absorption in the gastrointestinal tract. (Gillespie, 2001)

A Clinical Evaluation of Albendazole

As an active and equipotent benzimidazole derivative, single-dose albendazole is inexpensive, safe, and highly effective in treatment of soil-transmitted helminth infections. Although mebendazole is also safe and well tolerated, albendazole is the preferred treatment regimen for various mass drug administration programs. (Jongsuksuntigul *et al.*, 1993) In addition, it provides major advantages over previous anthelmintics due to its unique broad spectrum of activity for use in at-risk settings, efficacy against immature larval stages, and low toxicity in the host. (Campbell, 1990; Cook, 1990)

Conducted by Keiser *et al.*, a recent meta-analysis of overall cure rates from the recommended dosage of albendazole demonstrates its broad anthelmintic efficacy and safety in humans: hookworm (72%), *Ascaris lumbricoides* (88%), and *Trichuris trichura* (28%). In comparison, treatment of hookworm with mebendazole resulted in 15% cure rate in the same review. Similar to the general spectrum of activity of the benzimidazole drug family, albendazole acts against the parasite by disruption of metabolic pathways and energy production. (Keiser and Utzinger, 2008)

Drug Metabolism and Mechanism of Action

A single dose of 400 mg is recommended for clearance of hookworm infection, regardless of age and weight of the individual. After albendazole is absorbed from the gastrointestinal tract, the drug passes through the liver and returns to the rumen and small intestine by way of the bile duct and blood. The compound and its metabolites are distributed throughout the body and extensive metabolic processes begin expulsion of the intestinal parasite.

(Campbell, 1990) Albendazole has been reported to have moderate to high drug potency against both arrested and developing larval stages and adult worms in the gut.

(Campbell, 1990; Horton, 2000)

Albendazole negatively selects against helminth tubulin in mammalian hosts—previous studies have confirmed nematode β -tubulin to be the site of benzimidazole drug action. (Lacey, 1990) The selective attachment of albendazole to tubulin receptors within the parasite inhibits formation of cytoplasmic microtubules—ultimately disrupting cell division. Other noted mechanistic effects of albendazole are the reduction of glucose uptake and depletion of adenosine triphosphate storage in the parasite. (McKellar and Scott, 1990; Lacey, 1990)

Literature Review of Effects of Nutritional Status and Host-Related Factors on Treatment Response

Although albendazole has been shown to cure infection and reduce worm burden with higher efficacy than mebendazole, both benzimidazole carbamates have demonstrated low cure rates and varied efficacy against hookworm. (Anantaphruti *et al.*, 2007; Hotez *et al.*, 2005; Soukhathammavong *et al.*, 2012; Steinmann *et al.*, 2011; Vercruyse *et al.*, 2011) In parts of Africa where hookworm infection by *Necator americanus* is prevalent, varying cure rates have been reported across different regions after treatment with single-dose albendazole.

(Anantaphruti *et al.*, 2007)

As specific host factors may affect hookworm disease transmission, they may also play an influential role in the efficacy of anthelmintics. A number of modifiable host factors could be responsible for variations in drug efficacy, particularly nutritional state and availability of nutrients during treatment. Surprisingly, there are few descriptions of the influence of nutritional

status on drug response, especially since malnutrition may negatively alter the fate of the anthelmintic drug as it moves from the site of administration to the site of infection.

(Krishnaswamy, 1978) Similarly, the influence of diet has often been overlooked when determining anthelmintic efficacy, despite previous accounts of nutrient content of a meal or consumed food item affecting drug metabolism. (Santos and Boullata, 2005)

Proteins, carbohydrates, fats, and vitamins and minerals are essential for sufficient nutritional status. However, hookworm disease is often prevalent in areas where individual diets lack these nutritional components. In a milestone study, Duncombe *et al.* demonstrated that anthelmintic therapy (with mebendazole) was notably less effective in combined iron and protein deficient rats than in their nutritionally sufficient counterparts. Previous findings also confirm the authors' conclusion that iron and protein deficiency may significantly delay normal worm expulsion in animal models. Anthelmintic treatment was significantly less effective in iron and protein deficient rats when compared with sufficient animals (p-value < 0.01). Furthermore, drug efficacy was improved in animals after restoring sufficient nutrition and protein. (Bolin *et al.*, 1977) Preliminary extrapolation of animal studies to humans suggest that it may be fruitful to explore the effects of iron and protein deficiency in potentiating helminth infections and altering response to benzimidazole treatment.

The anti-parasitic activity of albendazole is dependent on drug affinity for parasite β -tubulin and increased drug concentration at the localized site of tissue infection. (Lacey, 1990) The oral administration of albendazole introduces several uncertainties related to its absorption, bioavailability, and transformation in the body. Bioavailability describes the rate and extent of drug absorption—equally important properties for identifying the response to drug dose. Bioavailability following orally administered albendazole treatment may vary due to either host-

related or dosage-form-related factors. However, lower bioavailability during chemotherapeutic treatment may permit higher concentrations of drug to remain in the gut environment—a potentially favorable effect for improved worm expulsion. Other host factors include the nature and timing of meals, age, disease, genetic traits, and gastrointestinal physiology. (Krishnaswamy, 1978)

According to Munst *et al.*, the bioavailability and absorption of mebendazole in the host subject was significantly enhanced if the drug was given together with a fatty meal.

Concomitant food intake with mebendazole increased plasma concentrations of 17 nmol/L to 134 nmol/L in human subjects. In another albendazole drug study, systemic availability, as measured by mean plasma concentrations, increased five-fold when taken with a fatty meal (estimated fat content 40 g) at breakfast time. The previous results strongly conclude that enhanced systemic availability of benzimidazole may require the drug to be taken with meals, but oral administration on an empty stomach (fasting) may be more effective for intestinal infections due to increased intraluminal drug concentrations. (Lange *et al.*, 1988) In a separate study evaluating different fasting intervals in calves, the availability of albendazole and its metabolites in gastrointestinal mucosa and fluids were observed to be greater in fasted calves than those who fed freely. (Sanchez *et al.*, 1997)

In addition to the pH of the intestinal lumen, gastric emptying time, intestinal blood flow, gut bacteria, digestive diseases (in the gastrointestinal tract), malnutrition, and immunodeficiency may also play supporting roles in lowered anthelmintic efficacy.

(Krishnaswamy, 1978) Other host factors include differences in gastrointestinal transit time (which is accelerated by diarrhea) and diet which affects the rate of intestinal transit, thus decreasing the duration of exposure of parasites to albendazole and reducing its drug efficacy.

(Nagy *et al.*, 2002; Sanchez *et al.*, 1997) In addition, the results of another study by Kohri *et al.* suggest that albendazole absorption and bioavailability is influenced by gastric acidity—rabbits with low gastric pH (around pH 1) experienced a three-fold increase in bioavailability of albendazole compared to those with relatively greater gastric pH (pH 5 or higher).

Assessment of Treatment Efficacy in Public Health

Comparative studies on the evaluation of albendazole treatment using a standardized protocol in Africa, Asia, and South America has shown promising, but varying therapeutic efficacy. (Vercruysse *et al.*, 2011) In another systematic review, albendazole (72% cure rate) was more effective in clearing hookworm infection than mebendazole and pamoate (15% and 31% cure rate, respectively). (Keiser and Utzinger, 2008) Despite the current therapeutic advantage of albendazole, variations in drug efficacy in endemic areas shine a spotlight on potential drug resistance. Equally unsettling, in rural areas with high transmission potential, hookworm-positive children often become reinfected within a year after receiving chemotherapeutic treatment. (Hotez, 2006) Even in regions with lower rates of transmission, hookworm reinfection has been frequently observed in populations. (Hotez *et al.*, 2004; Hotez, 2006) Because of frequent treatments needed to target reinfections, efficacy of benzimidazole anthelmintics may diminish with periodic therapy. (Hotez *et al.*, 2004)

After many years of large-scale treatment of livestock and farm animals, the presence of drug resistance has been frequently reported in different parts of the world. (Albonico *et al.*, 2004) Livestock populations with high levels of resistance to benzimidazole have arisen in Africa, South America, and Europe. (Albonico *et al.*, 2003; Albonico *et al.*, 2004) With such alarming reports and troubling lessons from livestock, there is a rising concern that targeted mass

treatment with the same albendazole regimen will ultimately result in a selection of worms with resistant genotypes. (Albonico *et al.*, 2004) More importantly, small-scale studies have detailed mebendazole and pyrantel drug failure in the treatment of human hookworm infections. (Albonico *et al.*, 2004; Savioli *et al.*, 2004) However, the threat of emerging resistance should be approached cautiously and with an open mind—low cure rates in humans may have other underlying mechanisms and there is not yet any evidence of genetically-transmitted drug resistance in the field. (Albonico *et al.*, 2004)

A resolution passed by the World Health Organization (2001) called for regular chemotherapeutic treatments for at least 75 percent of all school-age children at risk for hookworm. (Hotez *et al.*, 2004; Albonico *et al.*, 2004) Although chemotherapy-based public health interventions are important control measures, evidence of reduced drug efficacy and potential for drug resistance requires that improved strategies and methods be implemented to sustain lasting progress in the worldwide reduction of hookworm disease. (Albonico *et al.*, 2003) Recommended control approaches, as it relates to the findings of this study, will be discussed later in greater detail.

Study Purpose and Rationale

In evaluating response to albendazole treatment, it is important to consider a variety of host-related factors that could affect response—with dietary intake and nutritional status of individuals being potential factors. Already prevalent in young children across Sub-Saharan Africa, malnutrition may have the ability of altering drug metabolism and disposition. According to reports by Mao *et al.*, the capacity and affinity of cytochrome P450 enzymes were impaired by protein deficiency and calorie malnutrition in rats—diminishing drug metabolism.

In another animal study, reduced substrate binding was displayed in young rats fed a reduced protein diet similar in low protein value of diets consumed by children with malnutrition. (Anthony, 1973) The deleterious effect of malnutrition have also been observed in previous human studies—poor nutrition was associated with compromised metabolic drug activity in Sudanese school-age children. Notably, after restoring proper nutrition, reduction of metabolism was reversed. Many host factors may have contributed to the improved outcome, particularly sufficient nutritional status. (Homeida *et al.*, 1979)

In a more recent study, the authors analyzed the negative impact of malnutrition on the incidence of malaria and on the drug efficacy of sulfadoxine-pyrimethamine (an antimalarial drug) in a cohort study in northern Ghana. Compared to nutritionally sufficient children, incidence for malaria was higher in malnourished children. Equally important, the protective efficacy of sulfadoxine-pyrimethamine was roughly half or even less in the same malnourished cohort. (Danquah *et al.*, 2009)

Nutrient-drug interactions in humans depend on a wide variety of external and internal factors. Key pathophysiological and pharmacokinetic attributes may be altered by both macro- and micro-nutrient deficiencies. Earlier studies in both laboratory animals and malnourished human populations have suggested that host nutritional status and dietary factors significantly influence absorption, tissue uptake, plasma protein bonding, drug distribution, biotransformation, and rate of excretion from the body. (Krishnaswamy, 2003) If poor nutritional status and host diet affects anthelmintic efficacy, such findings could be extremely relevant to hookworm control efforts in resource-limited settings. In regions where malnutrition is prevalent, albendazole may not achieve its maximum effect.

Study Objectives

Diet and nutritional status of individuals have been shown to affect response to benzimidazoles in animal models and may also be principal factors in humans. The effects of low dietary energy, protein deficiency, and malnutritional state may modify drug metabolism and thus negatively influence intensity and duration of drug action. It has been shown that the anthelmintic efficacy of mebendazole is decreased in combined iron and protein deficiency states—these results raise the possibility that mebendazole acts synergistically with the immune system. (Duncombe *et al.*, 1977) As protein and iron deficiency often coexist with heavy worm infestations, it may be that these nutrient deficiencies potentiate the infestation, thus explaining the lack of success of some control programs. With an in-depth focus on modifiable host factors affecting anthelmintic efficacy, the objectives of the study include:

1. Describing the epidemiology of hookworm infection and responses to albendazole treatment in the Kintampo North District
2. Evaluating the relationship of nutritional status and diet as they influence treatment efficacy
3. Identifying modifiable host factors affecting response to treatment

Methods

Ethical Review and Oversight

Both field and laboratory components of the epidemiology project were approved by the Human Investigation Committee (HIC) at Yale University (protocol number 07050022669) and institutional review boards within the Ghana Health Service, the Noguchi Memorial Institute for Medical Research (NMIMR), and the Kintampo Health Research Centre (KHRC). The study protocol used the following inclusion criteria: 1) an enrolled student at the respective primary school, 2) residing within the study area, 3) between the ages of 6 and 13, and 4) willing and able to give informed consent. Exclusion criteria included participation in a hookworm disease study within the past two years.

Preliminary steps for subject recruitment in the Kintampo North Municipality included meetings with the Kintampo office of the Ministry of Education to discuss the study protocol. Previously identified as having high prevalence of hookworm infection, five villages along the Techiman-Tamale Road were chosen to participate in the survey (Figure 3). Further meetings with local community leaders were held to address the specific aims of the study, and following their approval, community meetings were organized with eligible school-age children and their parents/guardians. Written consent forms in both English and Twi were provided for any prospective subject. All consent forms and survey instruments (e.g., questionnaire) were translated into Twi and back-translated into English to ensure validity.

Study Design

Setting – Study Area and Population

The study presented here was conducted from June to August 2011 in the Kintampo North Municipality of the Brong Ahafo Region (Figure 3). The region is mostly rural plains with low hills and few rivers. Most of the population is involved in agriculture and subsistence farming, clean water and sanitation infrastructure are lacking in communities, and helminth infections are prevalent among children. In each village along the Techiman-Tamale Road, there was a primary school with pupils within different grade levels. Intestinal helminth infections are dominated by hookworms in the study area.

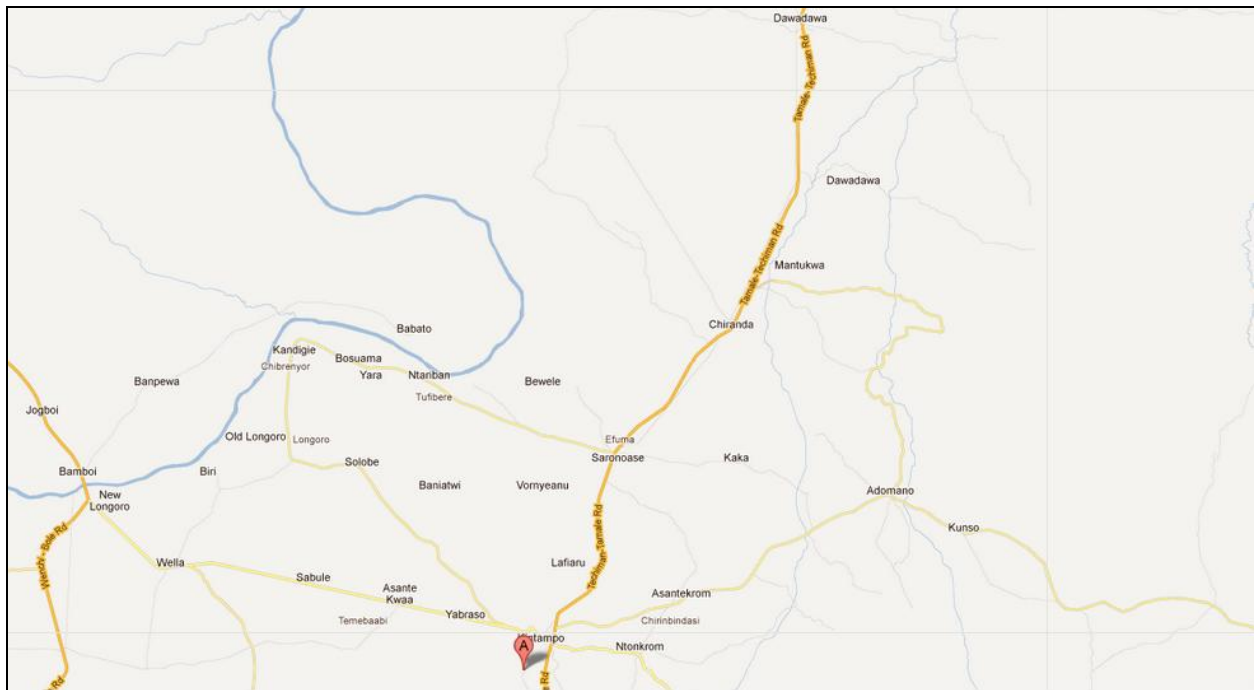


Figure 3. Techiman-Tamale Road, north of Kintampo, Ghana

Subjects

The study population consisted of children 6- to 13-years-old selected from five communities (north to most southern): Atta, Chiranda, Jato, Mahama, and Tahiru. Given the available resources, sample size calculations determined that 125 children must be included to provide 80 percent power. However, the sample size was increased to 150 children to allow for losses at follow-up and missing data. The final sample size was 141 eligible child participants.

Surveying Instruments

Parasitological Diagnosis and Treatment

After preliminary consent meetings and laboratory preparations, the baseline parasitological survey was conducted in mid-June 2011. At enrollment, 500 mL plastic containers labeled with unique identification codes were distributed to study participants. They were asked to collect and deposit large, fresh portions of their morning stools into the containers, which were then sealed and brought to school. Containers were collected in front of the school building by the research team and transported back to the central laboratory at the health research center for immediate processing.

At the laboratory, collected stool samples were examined to determine the presence of hookworm infection. Individual stools were analyzed by the Kato-Katz fecal thick smear technique (Katz *et al.*, 1972) for estimation of eggs per gram (EPG) of feces. After preparation of thick smears, they were counted under light microscopes by laboratory personnel. Egg counts were multiplied by a factor of 24 to express the number of eggs per gram of stool sample. Each child was asked to provide another fecal sample the following day. A single slide was prepared

and read from each day's fresh stool sample—for a total of two slides presented as the mean egg count for each child.

Following microscopic examination of the stools and diagnosis, hookworm-infected children received albendazole, orally administered, at a single dose of 400 mg. Treatment was supervised by a local pharmacist from the Kintampo Municipal Hospital. Approximately two weeks after albendazole had been administered to hookworm-infected study participants, two stool specimens were again collected with the same field and laboratory procedures as described above. At the end of the follow-up survey, children who remained infected with hookworm eggs were treated with another dose of albendazole.

Anthropometry

Anthropometric measurements of each study participant were obtained at a central screening site. Weight was measured to the nearest 0.1 kg using an electronic balance and height was measured to the nearest 0.1 cm using a portable fixed stadiometer. From the height and weight measurements, body mass index (BMI) was calculated as $[\text{weight (kg)}] / [\text{height (m)}]^2$ and converted to BMI-for-age z-scores with WHO AnthroPlus software. The measurement of mid-upper arm circumference (MUAC) is a simple screening method to determine nutritional status and it is a useful indicator for estimating prevalence of malnutrition of a population. (WHO, 2009) MUAC was measured to nearest 0.1 cm at the midpoint of the left arm with a standardized tape ribbon. According to the World Health Organization, children with a MUAC less than 115 mm have a highly elevated risk of morbidity and mortality compared to those who are above. Measures below 115 mm are indicative of severe wasting. (WHO, 2009)

Evaluation of Hemoglobin Levels

Iron status was assessed by hemoglobin (Hb) and serum ferritin (SF), currently considered as the most efficient indicators of iron status. Hb can be used to diagnose anemia, a specific condition where red blood cells provide inadequate amounts of oxygen to tissues, which can be caused by nutritional iron deficiency and anemia arising from parasitic infections.

(Mei *et al.*, 2005) Anemia was defined as Hb levels falling below 11.0 g/dL. (WHO, 2008)

Laboratory technicians from the Kintampo Municipal Hospital collected venous blood from each study participant in separate 5 mL tubes at a central location. Participants were provided a sugar biscuit and a can of condensed milk after blood draw. Initial blood tests were performed at KHRC laboratories. Malaria antigen detection tests and thick and thin blood smears were also used to examine for the presence of *Plasmodium falciparum*. Unused serum samples were frozen for further analyses.

Household Survey

Two individual field teams of researchers and translators conducted home interviews between June and August 2011. A pre-tested standardized questionnaire was used to gather socioeconomic information, including types of household construction, sources of water and sanitation, level of parental education and occupation, and ownership of select household assets (e.g., land, automobiles, and livestock). In addition, measures of food security, dietary diversity, and details of medical history were collected as part of the individual survey.

Cross-sectional food recalls were used to determine dietary diversity scores and describe dietary patterns among study participants—with two 24-hour dietary recalls on two separate occasions, spaced approximately four weeks apart. Although recalling two days does not cover all day-to-day variations of dietary intake, using two non-consecutive days over a period of four weeks significantly enhances the food recall coverage. Food recall questionnaires were the ideal instruments for population survey and were culturally appropriate, reliable, and valid in obtaining dietary data. Examples of food items were modified to reflect food items unique to the region. During each household visit, both the child and parent/guardian were asked to respond to the questionnaire to improve accuracy of the child’s dietary recall. To be counted as a “consumer” of any category, the child had to have consumed at least a half-serving of the food group at any time during the previous 24 hours.

Methodology and Assessment of Nutritional Status and Dietary Factors

Isolating the effect of host nutritional status on efficacy of albendazole required the precise measurement of nutrient intake. An individual dietary diversity score is a standard measure and functional proxy of dietary quality. (Arimond and Ruel, 2004) To calculate a dietary diversity score, the number of different groups consumed is summed for a total. For example, a sum of five different food groups implies a diet that offers some diversity in both macro- and micro-nutrients.

A diversified diet is highly correlated with factors, such as caloric and protein adequacy, percentage of high-quality protein, and improved food consumption. Dietary diversity can also serve as a marker of physical growth, cognitive development, and health outcomes. (Arimond and Ruel, 2004) The following set of 18 food groups was derived from the Food and Nutrition

Technical Assistance Project and was used to calculate individual dietary diversity score within the study population:

- | | |
|---------------------------------------|------------------------------------|
| <i>A. Milk</i> | <i>J. Organ Meats</i> |
| <i>B. Tea, Coffee, Cocoa</i> | <i>K. Meats</i> |
| <i>C. Other Liquids</i> | <i>L. Eggs</i> |
| <i>D. Grains</i> | <i>M. Fish</i> |
| <i>E. Orange or Yellow Vegetables</i> | <i>N. Legumes</i> |
| <i>F. Tubers</i> | <i>O. Cheese or Yogurt</i> |
| <i>G. Green Vegetables</i> | <i>P. Red Palm Oil-based Foods</i> |
| <i>H. Vitamin A-rich Fruits</i> | <i>Q. Butter and Oils</i> |
| <i>I. Other Fruits and Vegetables</i> | <i>R. Sweets</i> |

The total number of consumed food groups are then added together to indicate a score between 0 and 18:

$$\text{Sum} (A + B + C + D + E + F + G + H + I + J + K + L + M + N + O + P + Q + R)$$

A score of 0 indicates that the child had eaten nothing from any of the 18 food groups whereas a value of 18 indicates all food groups had been consumed within the reference period. Data for the dietary diversity score was collected by asking the respondent a series of yes-no questions using the previous 24 hours as a reference period to minimize recall limitations.

Data Analysis

Only those study participants with complete data sets were retained for full data analysis. Data was entered into Microsoft Excel and analyzed by SAS software (version 9.2). Univariate analyses were performed for descriptive purposes and summaries of baseline and post-treatment populations. Chi-squared tests were used to test for differences between populations and groups. Ordered logistic regression was also performed to identify predictors of egg reduction rate.

The response to treatment of soil-transmitted helminth infections is expressed by two standard indicators: the egg reduction rate and the cure rate. (Vercruysse *et al.*, 2011) The egg reduction rate is described as the percentage decrease from pre-treatment to post-treatment levels. (Anantaphruti *et al.*, 2007) The egg reduction rate is calculated as follows:

$$\frac{\text{Mean EPG before treatment} - \text{Mean EPG after treatment}}{\text{Mean EPG before treatment}} * 100\%$$

Similarly, the cure rate for the study population was calculated as the percentage or prevalence of children with EPG counts greater than zero before treatment and zero EPG counts after treatment:

$$\frac{\text{Prevalence before treatment} - \text{Prevalence after treatment}}{\text{Prevalence before treatment}} * 100\%$$

Ordered logistic regression was used for predicting increasing levels of egg reduction rate with both continuous and categorical variables. The final model included variables of statistical significance (p-value < 0.05).

Results

Study Population Characteristics

The study population included 141 eligible school-age children, ranging from 6- to 13-years-old from 5 different villages along the Techiman-Tamale Road (Figure 4).

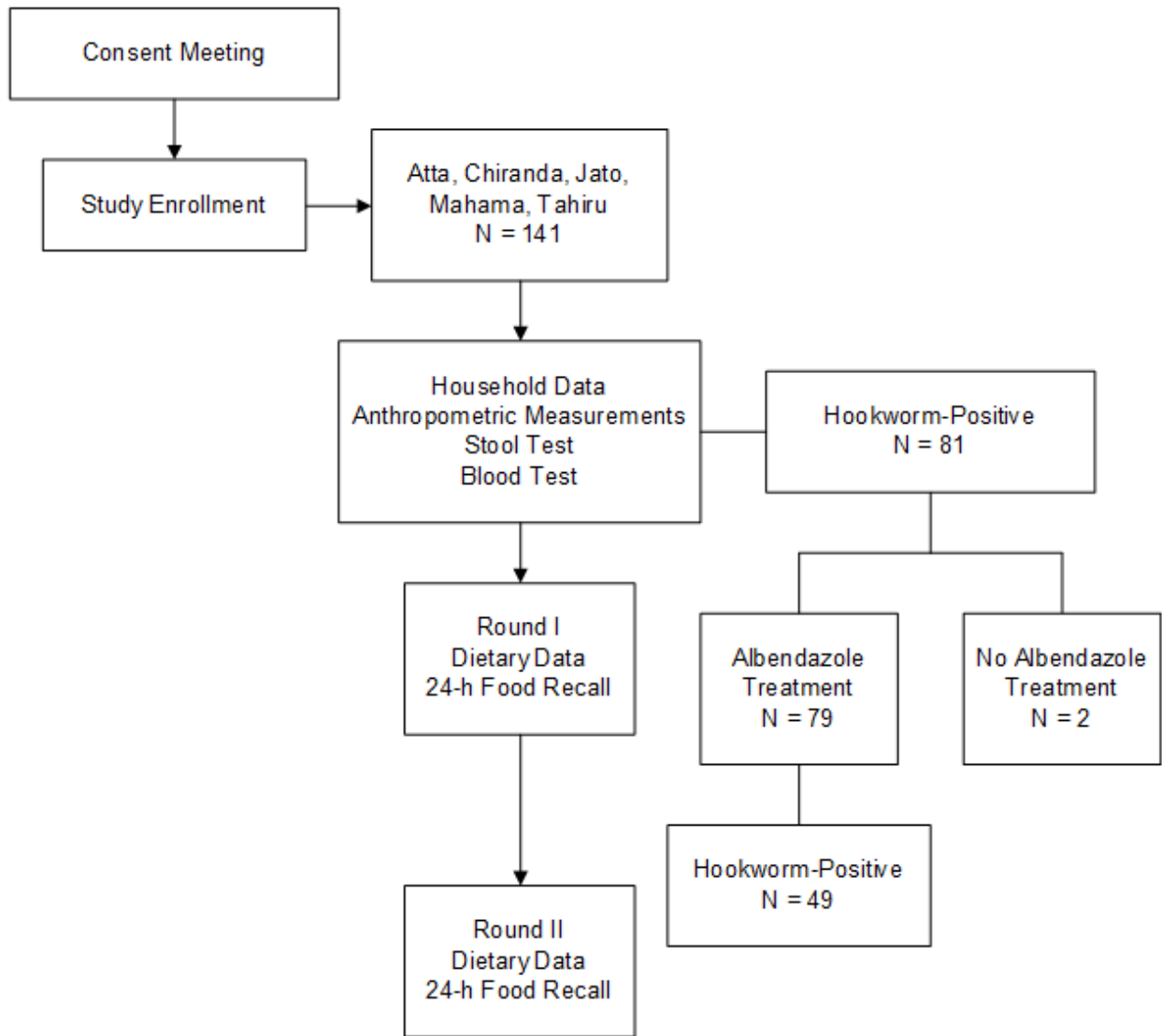


Figure 4. Study population characteristics

The mean age of study participants was 9.6 years. There was a nearly even distribution of males (48.2%) and females (51.8%). From the participating households, 96.4% owned agricultural land and 31.4% reported owning any type of savings account. As a reliable measure of a socioeconomic status, an absolute wealth index was constructed based on asset ownership (e.g., automobile, agricultural land, livestock) for each household. (Brooker *et al.*, 2007) Based on a maximum index of 18 units, the mean absolute wealth index for the study population was 6.8. Atta, the most northern school, had the lowest mean absolute wealth index (5.9) and Tahiru, the most southern school, had the highest mean of the study population (7.5) (Figure 5).

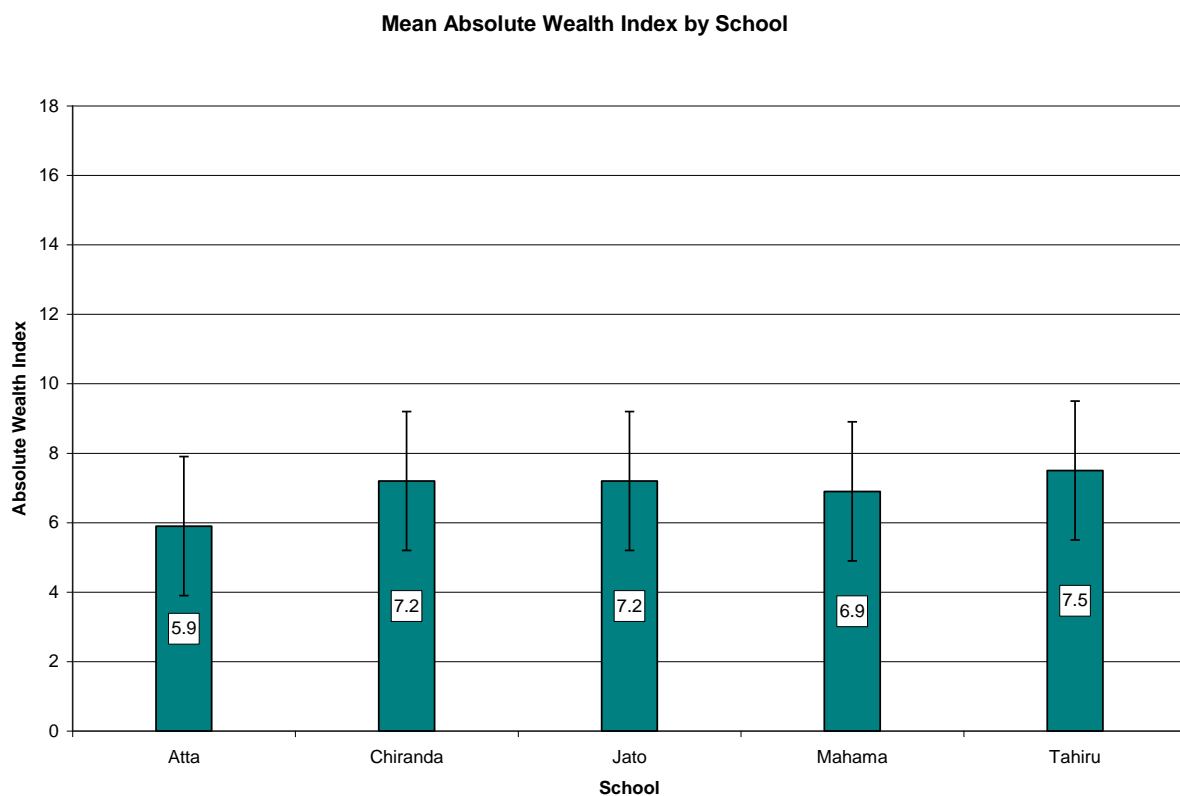


Figure 5. Mean absolute wealth index by school (from north to south)

Across the study population, 82.3% of households treated their drinking water and only 31.9% had access to a pit latrine. Of those owning mosquito nets (91.5%), 93.8% of index children reported sleeping under the net the previous night. The main occupation of head of households was agricultural farming (88.9%).

Nutritional Profile of the Study Population

Preliminary findings indicate many study participants consume low-quality roots and tubers—a monotonous and bulky diet is the hallmark of poverty and poor nutrition. Diets were sometimes supplemented with vegetables and legumes, but rarely with animal-source protein (Figure 6). With respect to weekly consumption, only 41% of respondents reported consuming meats—and less than 21% consumed organ meats, orange and yellow vegetables, and vitamin A-rich fruits.

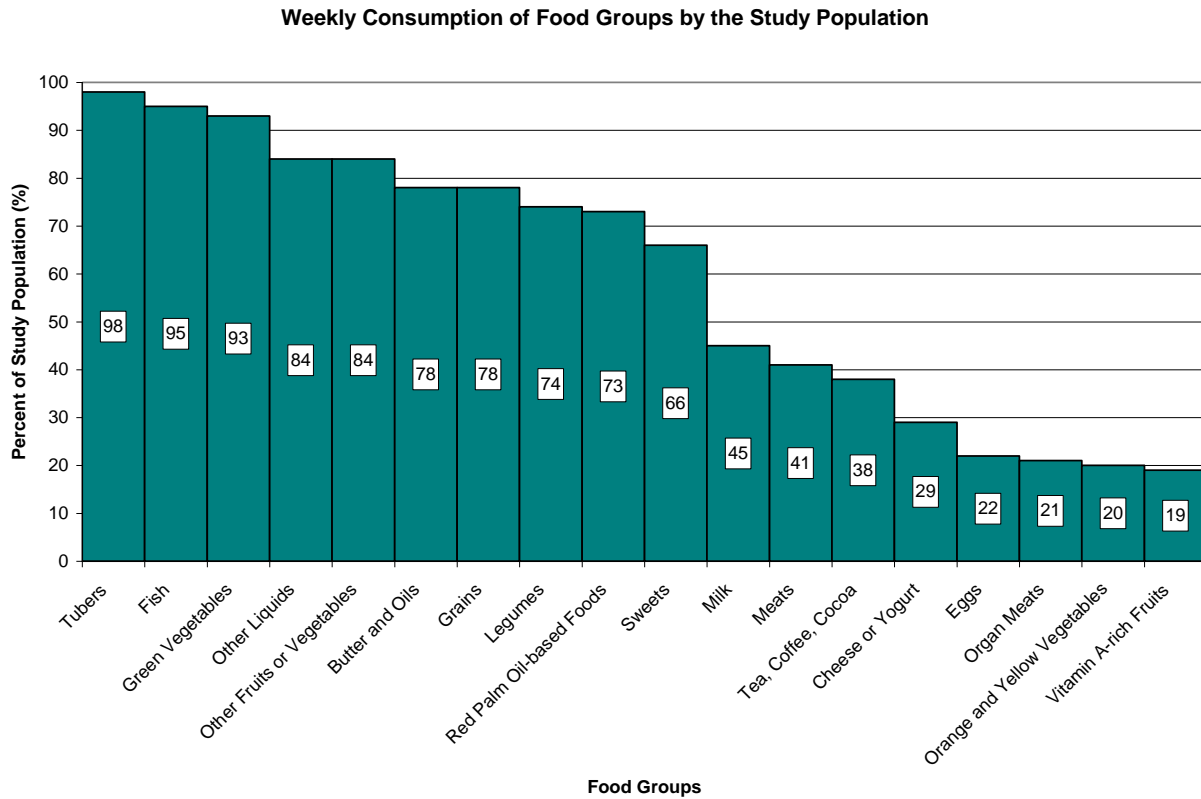


Figure 6. Weekly consumption of food groups by the study population

Independent of socioeconomic status, dietary diversity has been associated with child nutritional status and often serves as a functional proxy of dietary intake. (Arimond and Ruel, 2004) The mean weekly dietary diversity score across the study population was 10.5—Jato had the lowest mean (8.9) whereas Atta had the highest mean (11.4) (Figure 7 and Figure 8).

Weekly Dietary Diversity Scores of the Study Population

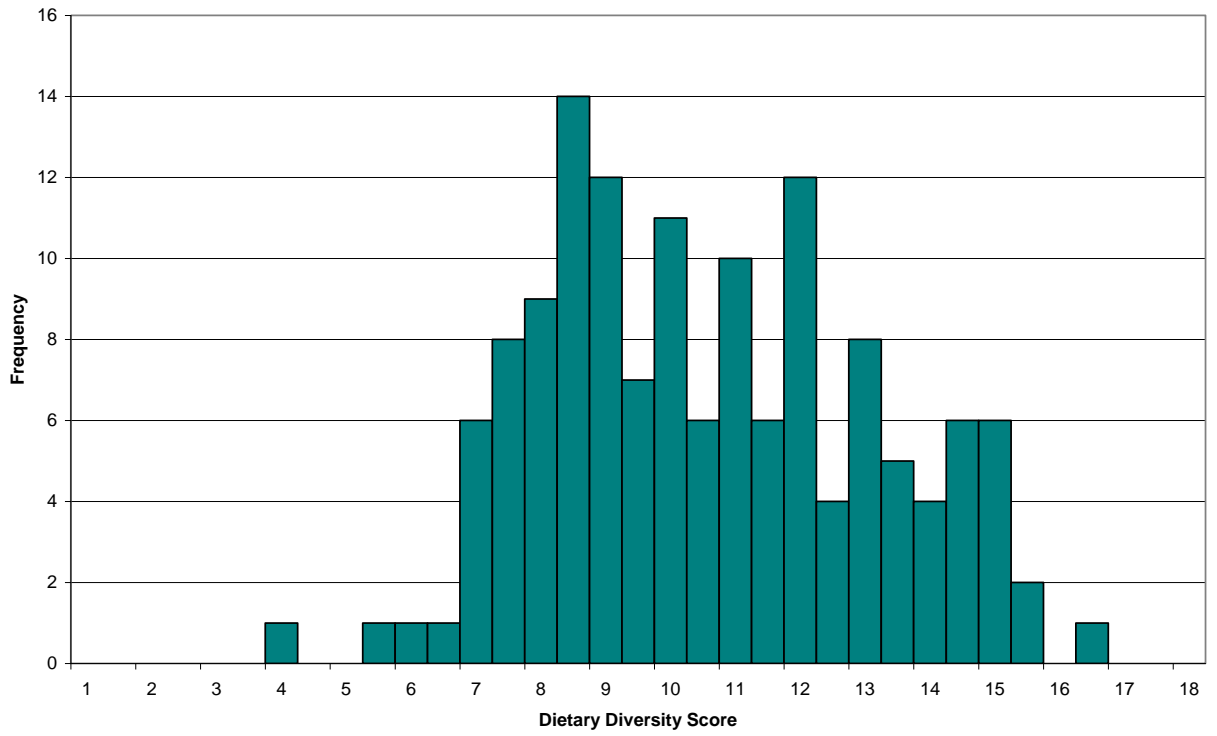


Figure 7. Weekly dietary diversity scores of the study population

Mean Weekly Dietary Diversity Score by School

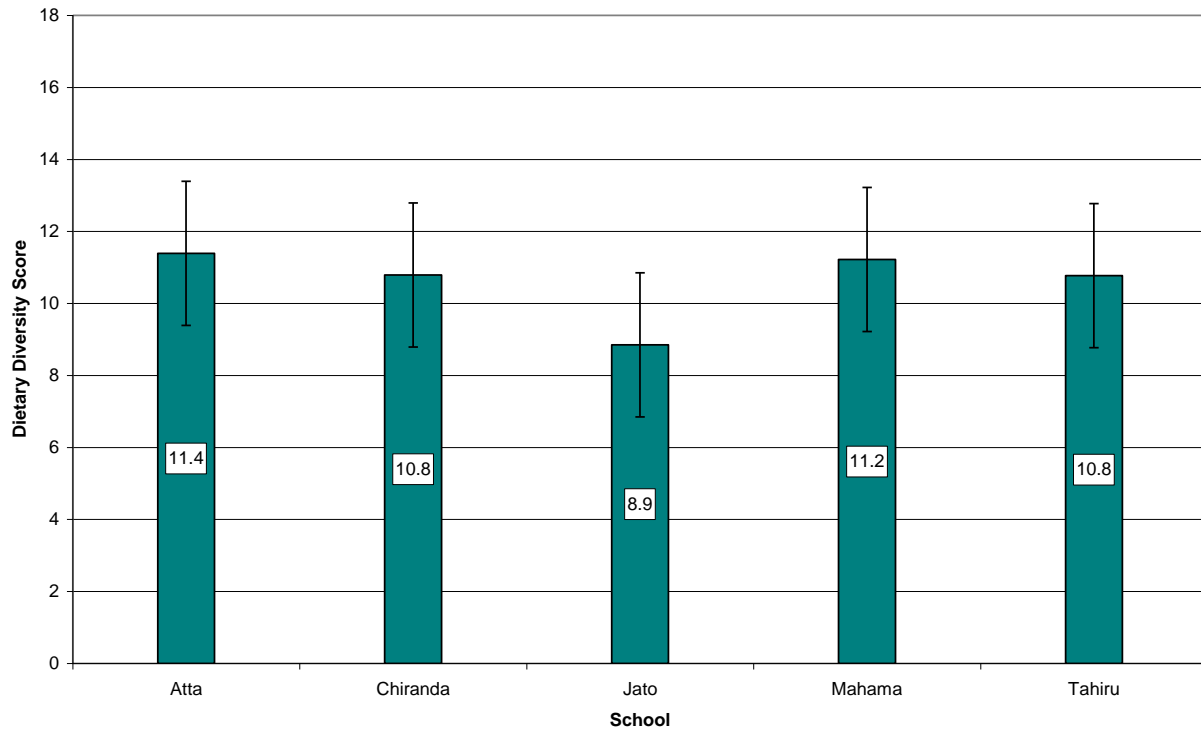


Figure 8. Mean weekly dietary diversity score by school

Although the study population heavily consumed carbohydrate staples, such as yams and cassava, they were limited in dietary protein and iron. The mean weekly dietary protein intake of the study population was only 3.3 out of a maximum 7 protein-rich food groups (Figure 9).

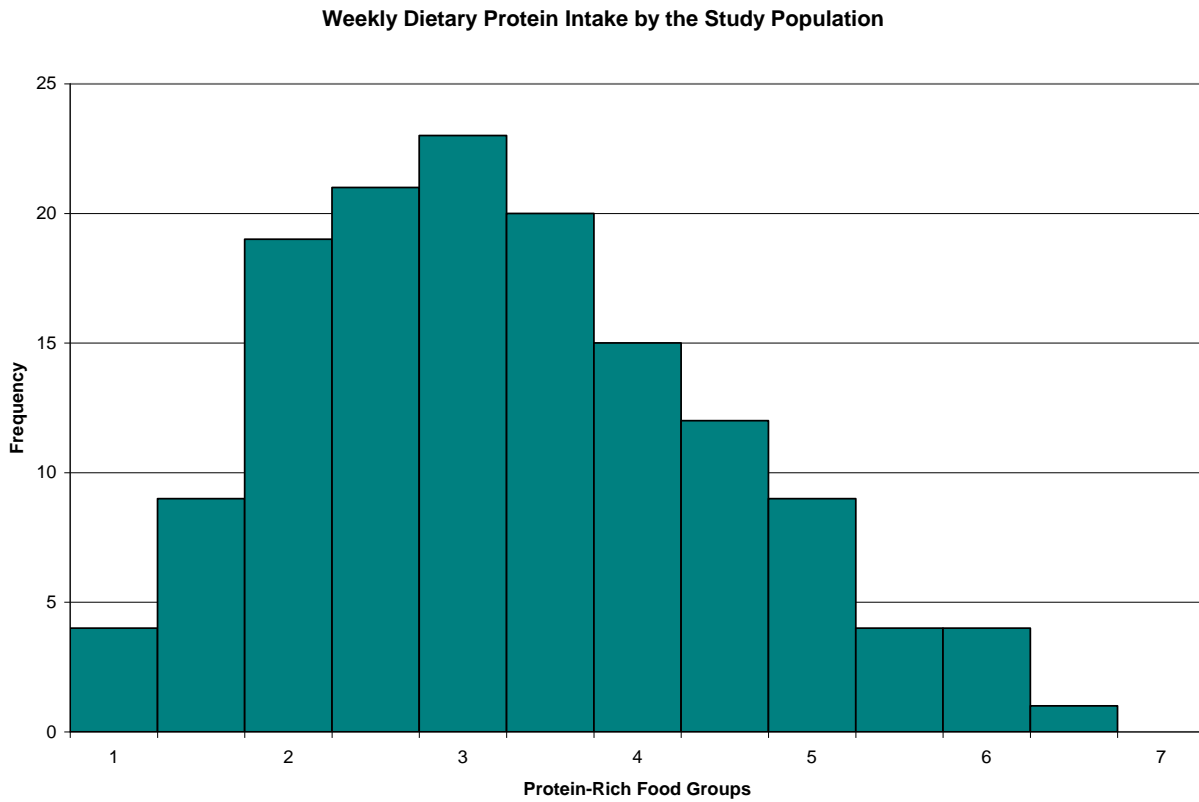


Figure 9. Weekly dietary protein intake by the study population

Again, Jato had the lowest mean of weekly dietary protein intake (2.8) (Figure 10).

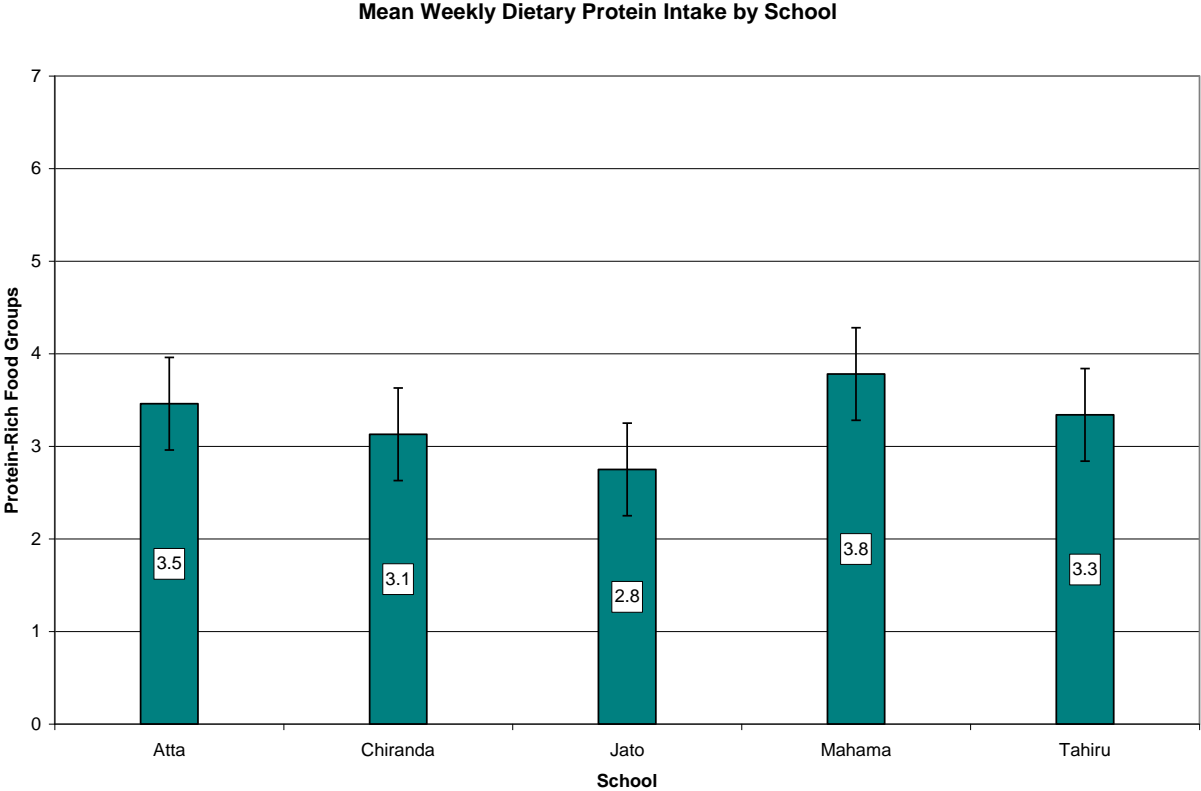


Figure 10. Mean weekly dietary protein intake by school

Survey Results

At baseline, the overall prevalence for hookworm infection was 57.4%. By school, rates of infection ranged from 40.9% in Tahiru to 77.8% in Mahama (Figure 11).

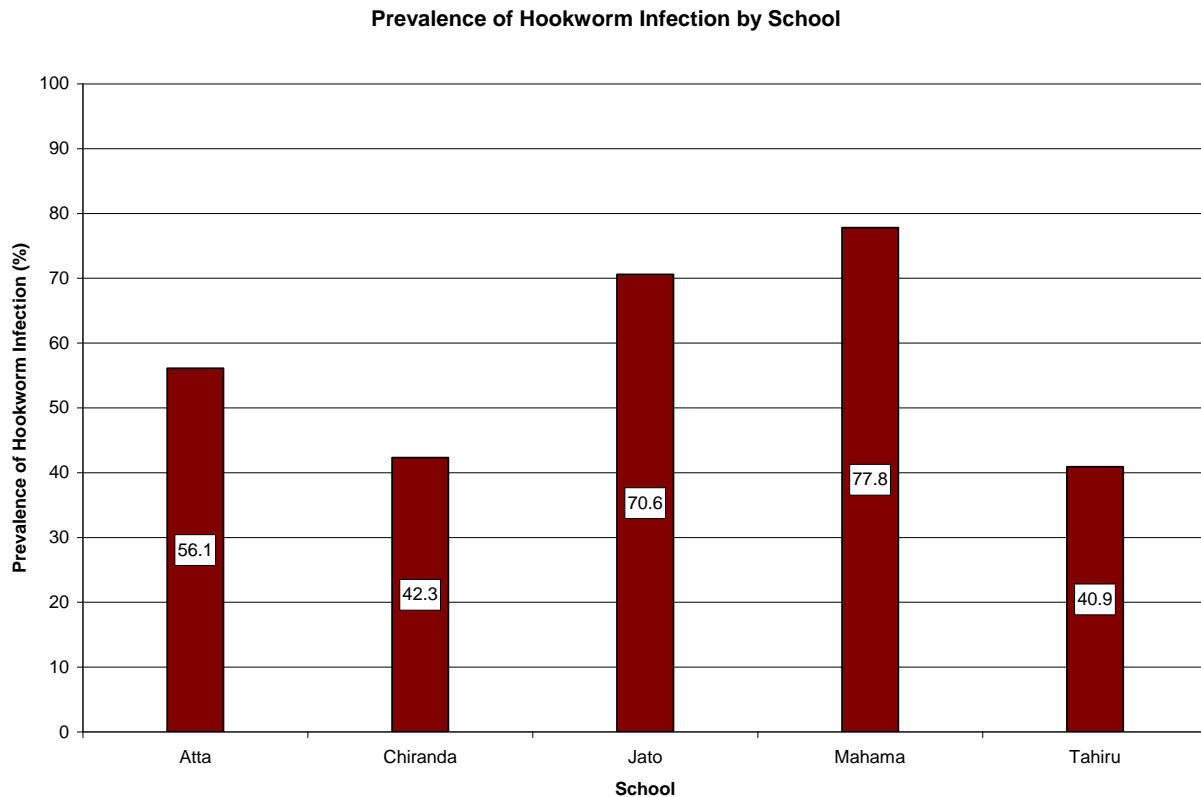


Figure 11. Prevalence of hookworm infection by school

The arithmetic mean fecal egg count was 429.4 (\pm 640.8) with counts ranging between 12 EPG to 3,216 EPG in infected children. According to the World Health Organization's classification of level of intensity, 96.2% of infected children had light infections (1 – 1,999 EPG) whereas only 3.8% children had moderate infections (2,000 – 3,999 EPG). (Montresor *et al.*) There were no heavy infections (> 4,000 EPG) in the study population at baseline (Table 1).

Table 1. Intensity of Hookworm Infection

| Classification ^a | N (%) ^b |
|-----------------------------|--------------------|
| Light | 77 (96) |
| Moderate | 3 (4) |
| Heavy | 0 (0) |

^aBased upon WHO criteria (Light infection: 1 – 1,999 EPG; moderate infection: 2,000 – 3,999 EPG; heavy infection: > 4,000 EPG)

^bNumbers may not sum to total due to missing data

The prevalence of hookworm and malaria co-infection in the study population was 54.6% and, by school, ranged from 34.6% in Chiranda to 77.8% in Mahama (Figure 12). Hookworm and malaria co-infection may produce compounded deficits in hemoglobin. (Hotez, 2006) In addition, the overlapping distribution of malaria infection with hookworm may adversely affect host immune response. (Brooker *et al.*, 2004)

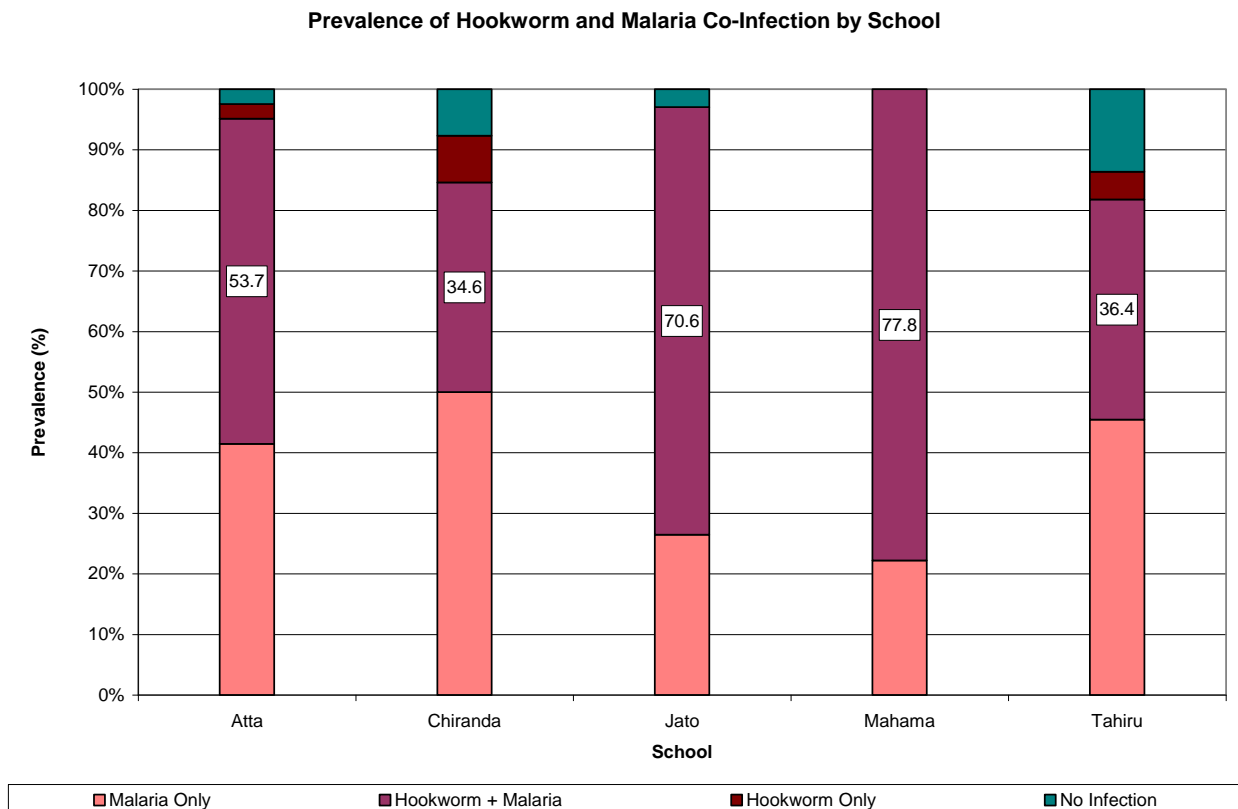


Figure 12. Prevalence of hookworm and malaria co-infection by school

Parasitic co-infection remained highly endemic in the region—only 5% of the entire study population was negative for both hookworm and malaria infection. As determined by hemoglobin levels falling below 11.0 g/dL, 28.1% of study participants were anemic.

Characterization of the Study Population as Defined by Hookworm Infection Status at Baseline

Univariate analysis was conducted to determine risk factors of acquiring hookworm infection. Statistically significant associations between study characteristics and hookworm infection (p-value < 0.05) are described in the following tables. Comparing anthropometric indicators and nutritional status of the study population, age, sex, and MUAC were associated with hookworm infection at baseline (Table 2).

Table 2. Anthropometric Indicators and Nutritional Status of the Study Population by Hookworm Infection Status at Baseline^a

| Characteristic | Hookworm Infection | | p ^c |
|----------------------------------|--------------------------------|--------------------------------|----------------|
| | Positive (N = 81) ^b | Negative (N = 60) ^b | |
| Age (years) | 10.1 ± 1.6 | 9.4 ± 1.6 | 0.0163 |
| Sex | | | 0.043 |
| Male | 55.6 (45) | 38.3 (23) | |
| Female | 44.4 (36) | 61.7 (37) | |
| Household Food Insecurity | | | 0.088 |
| Low | 25.9 (21) | 35 (21) | |
| Moderate | 37.0 (30) | 45 (27) | |
| High | 37.0 (30) | 20 (12) | |
| Weekly Dietary Diversity | | | 0.505 |
| Low | 28.4 (23) | 30 (18) | |
| Moderate | 40.7 (33) | 31.7 (19) | |
| High | 30.9 (25) | 38.3 (23) | |
| Weekly Dietary Protein | | | 0.535 |
| Low | 40.7 (33) | 33.3 (20) | |
| Moderate | 30.9 (25) | 30 (18) | |
| High | 28.4 (23) | 36.7 (22) | |
| BMI-for-age Z-score | -0.58 ± 2.0 | -0.89 ± 0.7 | 0.264 |
| MUAC | 19 ± 1.5 | 18.4 ± 1.5 | 0.0221 |

^aTable values are mean ± SD for continuous variables and column % (N) for categorical variables

^bNumbers may not sum to total due to missing data and percentages may not sum to 100% due to rounding

^cp-value is for t-test (continuous variables) or χ^2 -test (categorical variables)

Demographic and socioeconomic indicators associated with hookworm infection at baseline were location of village, daily shoe usage, and waste on property (Table 3). Waste on property was assessed by the interviewer during the first round of household visits. Without involving the respondent, the interviewer personally answered the yes-no question, “Is there noticeable garbage around the household?” by observing for physical waste on the property. Pit latrine usage was not a statistically significant association.

Table 3. Demographic and Socioeconomic Indicators of the Study Population by Hookworm Infection Status at Baseline^a

| Characteristic | Hookworm Infection | | p ^c |
|--------------------------|--------------------------------|--------------------------------|----------------|
| | Positive (N = 81) ^b | Negative (N = 60) ^b | |
| Village | | | 0.0346 |
| Atta | 28.4 (23) | 30 (18) | |
| Chiranda | 13.6 (11) | 25 (15) | |
| Jato | 29.6 (24) | 16.7 (10) | |
| Mahama | 17.3 (14) | 6.7 (4) | |
| Tahiru | 11.1 (9) | 21.7 (13) | |
| Absolute Wealth | | | 0.986 |
| Low | 27.2 (22) | 28.3 (17) | |
| Moderate | 39.5 (32) | 38.3 (23) | |
| High | 33.3 (27) | 33.3 (20) | |
| Daily Shoe Usage | | | 0.0229 |
| Yes | 45.7 (37) | 65 (39) | |
| No | 54.3 (44) | 35 (21) | |
| Pit Latrine Usage | | | 0.0763 |
| Yes | 25.9 (21) | 40 (24) | |
| No | 74.1 (60) | 60 (36) | |
| Waste on Property | | | 0.0253 |
| Yes | 40 (32) | 22 (13) | |
| No | 60 (48) | 78 (46) | |

^aTable values are mean ± SD for continuous variables and column % (N) for categorical variables

^bNumbers may not sum to total due to missing data and percentages may not sum to 100% due to rounding

^cp-value is for t-test (continuous variables) or χ^2 -test (categorical variables)

Anemia (Hb < 11.0 g/dL) was associated with hookworm infection at baseline (Table 4).

Healthcare access and anthelmintic treatment within the past year were not significant associations.

Table 4. Health Indicators of the Study Population by Hookworm Infection Status at Baseline^a

| Characteristic | Hookworm Infection | | p ^c |
|---------------------------------|--------------------------------|--------------------------------|----------------|
| | Positive (N = 81) ^b | Negative (N = 60) ^b | |
| Healthcare Access | | | 0.199 |
| Within one year | 41.8 (33) | 55.9 (33) | |
| More than one year | 54.4 (43) | 39 (23) | |
| Never | 3.8 (3) | 5.1 (3) | |
| Malaria Infection Status | | | 0.141 |
| Positive | 95.1 (77) | 88.3 (53) | |
| Negative | 4.9 (4) | 11.7 (7) | |
| Hemoglobin Levels (g/dL) | | | 0.0375 |
| ≥ 11.0 | 21.3 (17) | 37.3 (22) | |
| < 11.0 | 78.8 (63) | 62.7 (37) | |

^aTable values are mean ± SD for continuous variables and column % (N) for categorical variables

^bNumbers may not sum to total due to missing data and percentages may not sum to 100% due to rounding

^cp-value is for t-test (continuous variables) or χ^2 -test (categorical variables)

Study participants reporting moderate and high household food insecurity had a greater prevalence of hookworm infection at baseline than those reporting lower household food insecurity (Figure 13).

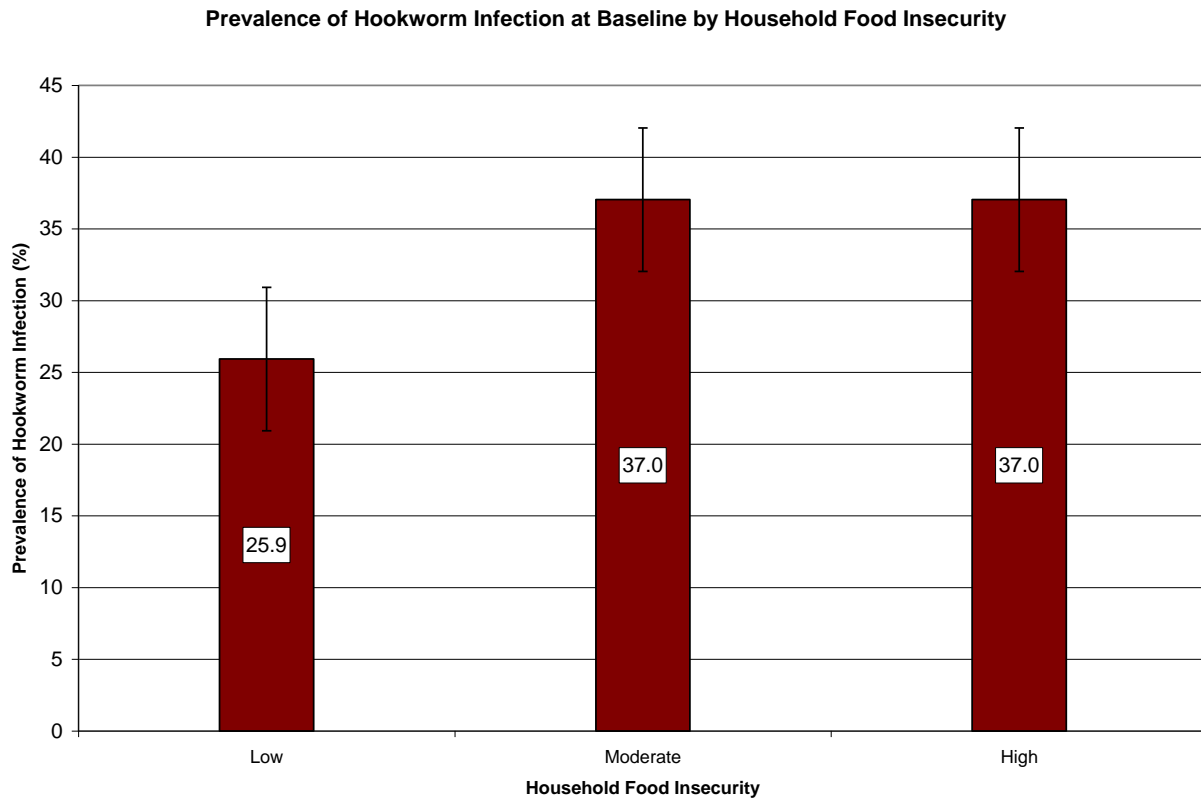


Figure 13. Prevalence of hookworm infection at baseline by tertiles of household food insecurity

The median weekly dietary diversity score of the study population at baseline was 10.0. Children falling below the median had higher rates of hookworm infection than those above the median (Figure 14). Similarly, participants below the study population median of weekly dietary protein intake (3.0) experienced higher rates of hookworm than those above the median (Figure 15).

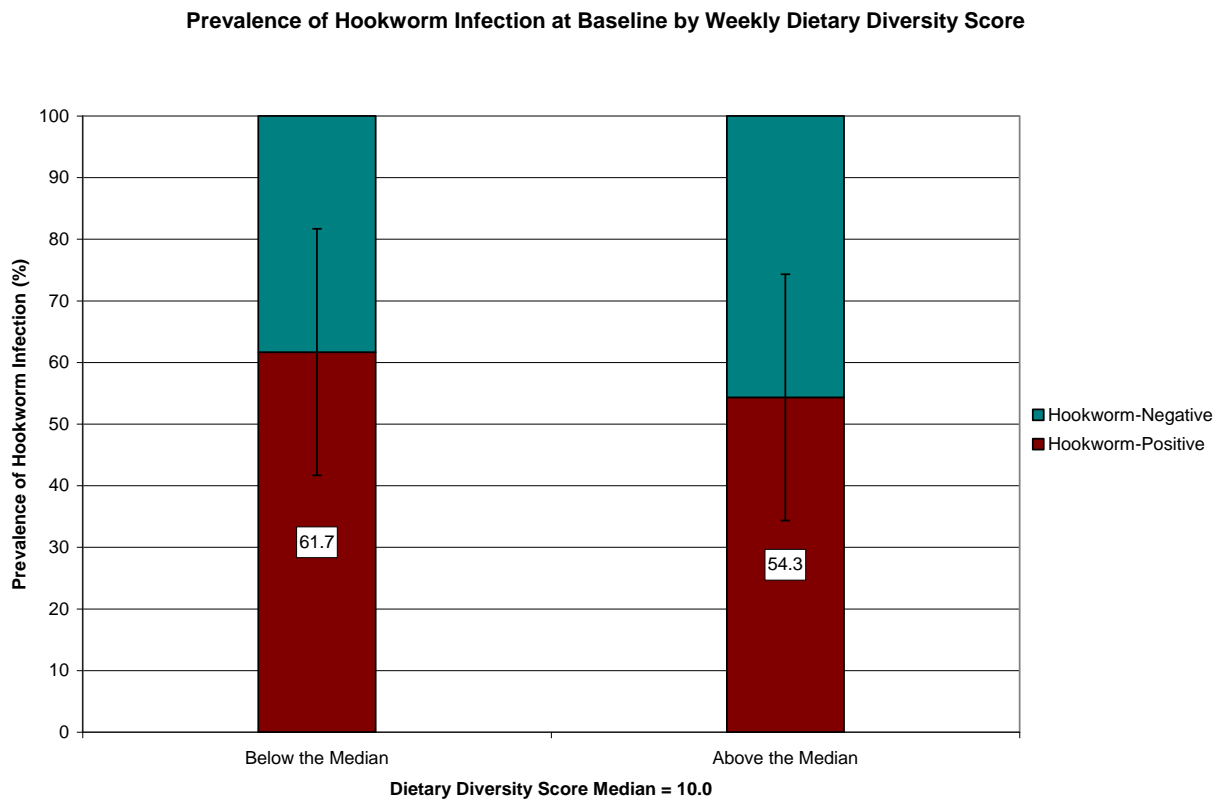


Figure 14. Prevalence of hookworm infection at baseline by weekly dietary diversity score (as defined by the median)

Prevalence of Hookworm Infection at Baseline by Weekly Dietary Protein Intake

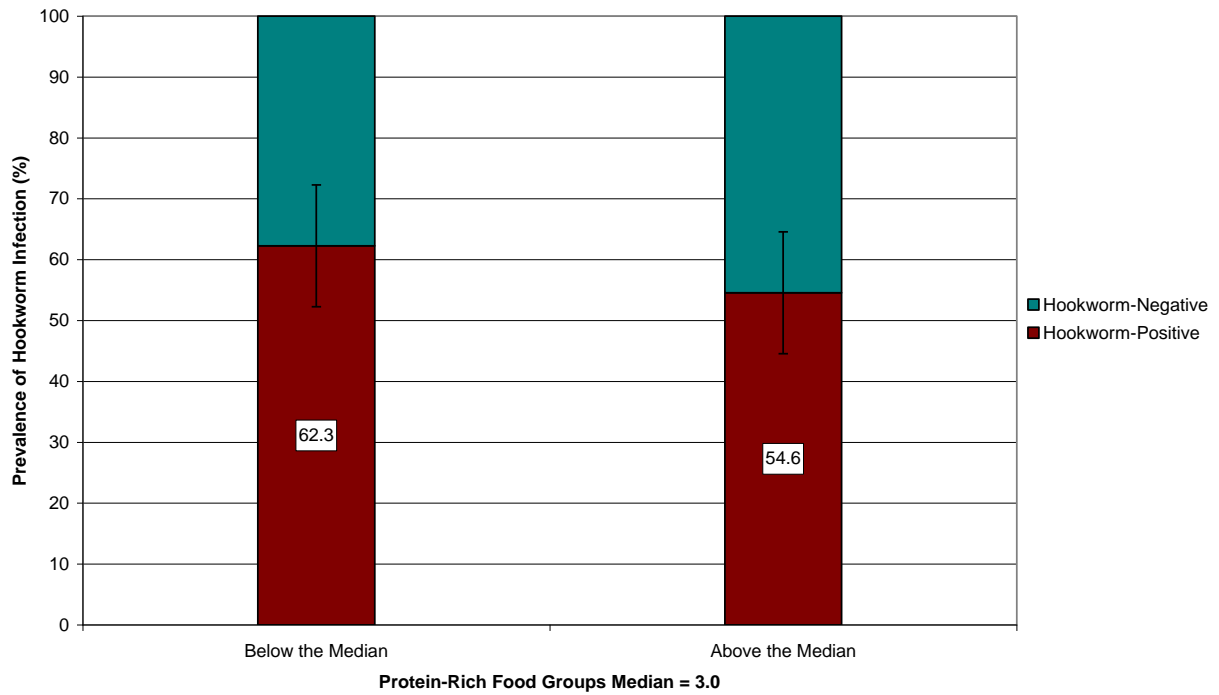


Figure 15. Prevalence of hookworm infection at baseline by weekly dietary protein intake (as defined by the median)

Low protein consumption in the study population may be an important predictor of hookworm infection. The dose-response effect was characterized by protein consumption expressed in tertiles, such that subjects in the lowest tertile had higher rates of infection than those in upper tertiles (Figure 16).

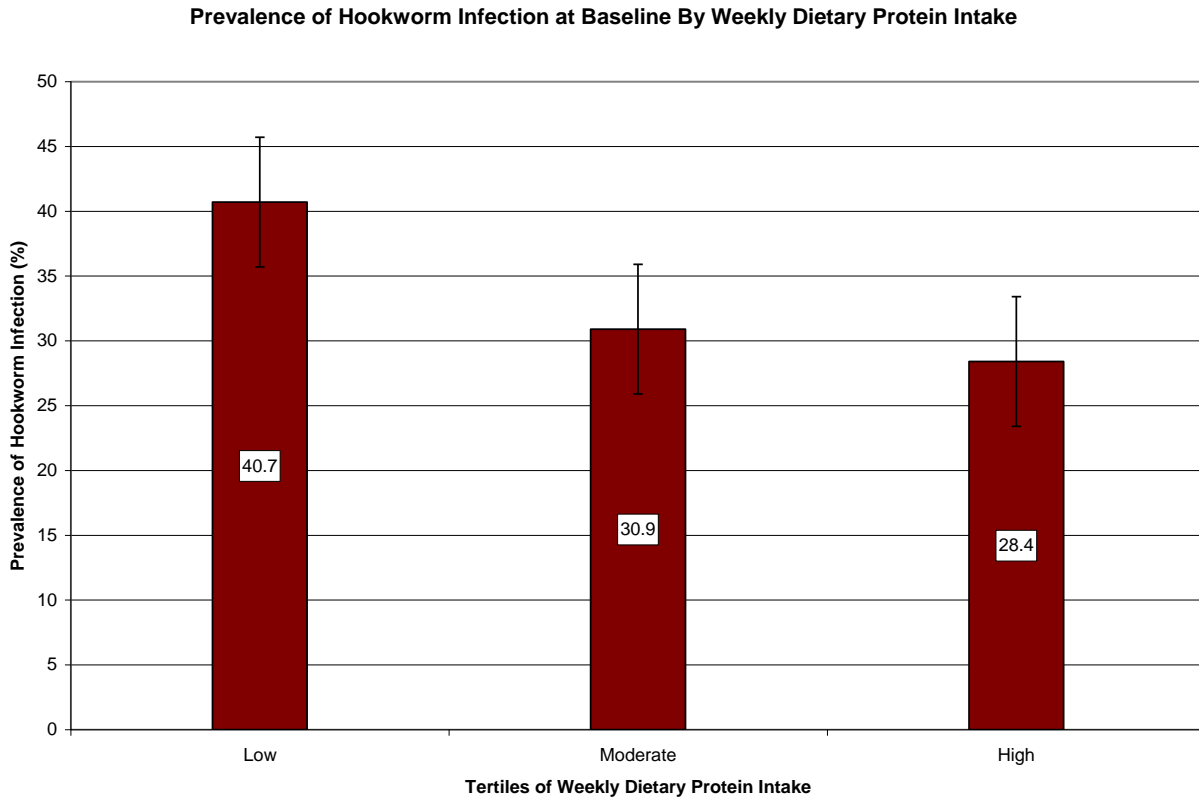


Table 16. Prevalence of hookworm infection at baseline by tertiles of weekly dietary protein intake

Characterization of the Study Population as Defined by Hookworm Infection

Status Post-Treatment

For post-treatment analysis, univariate statistics again described associations between study population characteristics and hookworm infection status after administration of albendazole. Weekly dietary diversity was the only statistically significant association seen between nutritional status and post-treatment hookworm infection status (Table 5).

Table 5. Anthropometric Indicators and Nutritional Status of the Treatment Population by Post-Treatment Hookworm Infection Status^a

| Characteristic | Hookworm Infection | | p ^c |
|----------------------------------|--------------------------------|--------------------------------|----------------|
| | Positive (N = 49) ^b | Negative (N = 29) ^b | |
| Age (years) | 9.9 ± 1.7 | 10.4 ± 1.5 | 0.221 |
| Sex | | | 0.265 |
| Male | 61.2 (30) | 48.3 (14) | |
| Female | 38.8 (19) | 51.7 (15) | |
| Household Food Insecurity | | | 0.868 |
| Low | 28.6 (14) | 24.1 (7) | |
| Moderate | 32.7 (16) | 37.9 (11) | |
| High | 38.8 (19) | 37.9 (11) | |
| Weekly Dietary Diversity | | | 0.0111 |
| Low | 40.8 (20) | 10.3 (3) | |
| Moderate | 36.7 (18) | 44.8 (13) | |
| High | 22.5 (11) | 44.8 (13) | |
| Weekly Dietary Protein | | | 0.559 |
| Low | 46.9 (23) | 34.5 (10) | |
| Moderate | 28.6 (14) | 34.5 (10) | |
| High | 24.5 (12) | 31 (9) | |
| BMI-for-age Z-score | -0.43 ± 2.5 | -0.85 ± 0.56 | 0.398 |
| MUAC | 18.7 ± 1.4 | 19.3 ± 1.6 | 0.069 |

^aTable values are mean ± SD for continuous variables and column % (N) for categorical variables

^bNumbers may not sum to total due to missing data and percentages may not sum to 100% due to rounding

^cp-value is for t-test (continuous variables) or χ^2 -test (categorical variables)

As demographic and socioeconomic indicators of the treatment population, location of village and waste on property were significantly associated with post-treatment hookworm infection (Table 6).

Table 6. Demographic and Socioeconomic Indicators of the Treatment Population by Post-Treatment Hookworm Infection Status^a

| Characteristic | Hookworm Infection | | p ^c |
|--------------------------|--------------------------------|--------------------------------|----------------|
| | Positive (N = 49) ^b | Negative (N = 29) ^b | |
| Village | | | < .0001 |
| Atta | 12.2 (6) | 55.2 (16) | |
| Chiranda | 4.1 (2) | 31 (9) | |
| Jato | 49 (24) | 0 (0) | |
| Mahama | 20.4 (10) | 10.3 (3) | |
| Tahiru | 14.3 (7) | 3.5 (1) | |
| Absolute Wealth | | | 0.913 |
| Low | 28.6 (14) | 27.6 (8) | |
| Moderate | 36.7 (18) | 41.4 (12) | |
| High | 34.7 (17) | 31 (9) | |
| Daily Shoe Usage | | | 0.642 |
| Yes | 42.9 (21) | 48.3 (14) | |
| No | 57.1 (28) | 51.7 (15) | |
| Pit Latrine Usage | | | 0.0558 |
| Yes | 18.4 (9) | 38 (11) | |
| No | 81.6 (40) | 62.1 (18) | |
| Waste on Property | | | 0.0253 |
| Yes | 43.8 (21) | 37.9 (11) | |
| No | 56.3 (27) | 62.1 (18) | |

^aTable values are mean \pm SD for continuous variables and column % (N) for categorical variables

^bNumbers may not sum to total due to missing data and percentages may not sum to 100% due to rounding

^cp-value is for t-test (continuous variables) or χ^2 -test (categorical variables)

With respect to surveyed health indicators, only malaria infection status was significantly associated with post-treatment hookworm infection (Table 7). Again, healthcare access and anthelmintic treatment within the past year were not associated with post-treatment hookworm infection status.

Table 7. Health Indicators of the Treatment Population by Post-Treatment Hookworm Infection Status^a

| Characteristic | Hookworm Infection | | p ^c |
|---------------------------------|--------------------------------|--------------------------------|----------------|
| | Positive (N = 49) ^b | Negative (N = 29) ^b | |
| Healthcare Access | | | 0.332 |
| Within one year | 36.2 (17) | 44.8 (13) | |
| More than one year | 57.5 (27) | 55.2 (16) | |
| Never | 6.4 (3) | 0 (0) | |
| Malaria Infection Status | | | 0.0217 |
| Positive | 100 (49) | 89.7 (26) | |
| Negative | 0 (0) | 10.3 (3) | |
| Hemoglobin Levels (g/dL) | | | 0.454 |
| ≥ 11.0 | 24.5 (12) | 17.2 (5) | |
| < 11.0 | 75.5 (37) | 82.8 (24) | |

^aTable values are mean ± SD for continuous variables and column % (N) for categorical variables

^bNumbers may not sum to total due to missing data and percentages may not sum to 100% due to rounding

^cp-value is for t-test (continuous variables) or χ^2 -test (categorical variables)

Study participants residing in households with high household food insecurity experienced greater rates of post-treatment infection than those in both moderate and low reporting households (Figure 17).

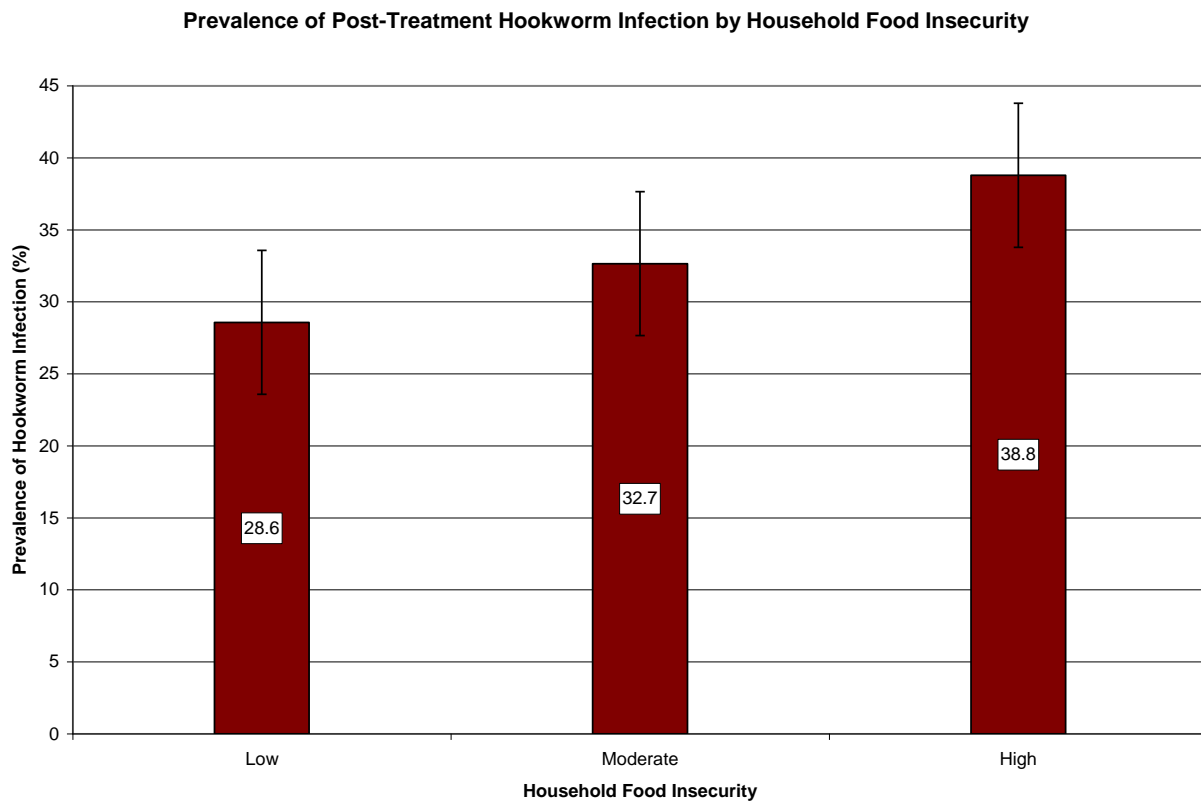


Figure 17. Prevalence of post-treatment hookworm infection by tertiles of household food insecurity

Overall Drug Efficacy

Cure Rate and Egg Reduction Rate

Only hookworm-infected cases were assessed for the curative efficacy of albendazole. The egg reduction rate was also analyzed. Administration of albendazole produced substantial cure rates and reduction of hookworm egg counts, although there were a wide variety of results (Figure 18). Out of those identified as hookworm-positive at baseline and received treatment (N = 79), 49 children remained infected—a cure rate of 37.2% (Table 8).

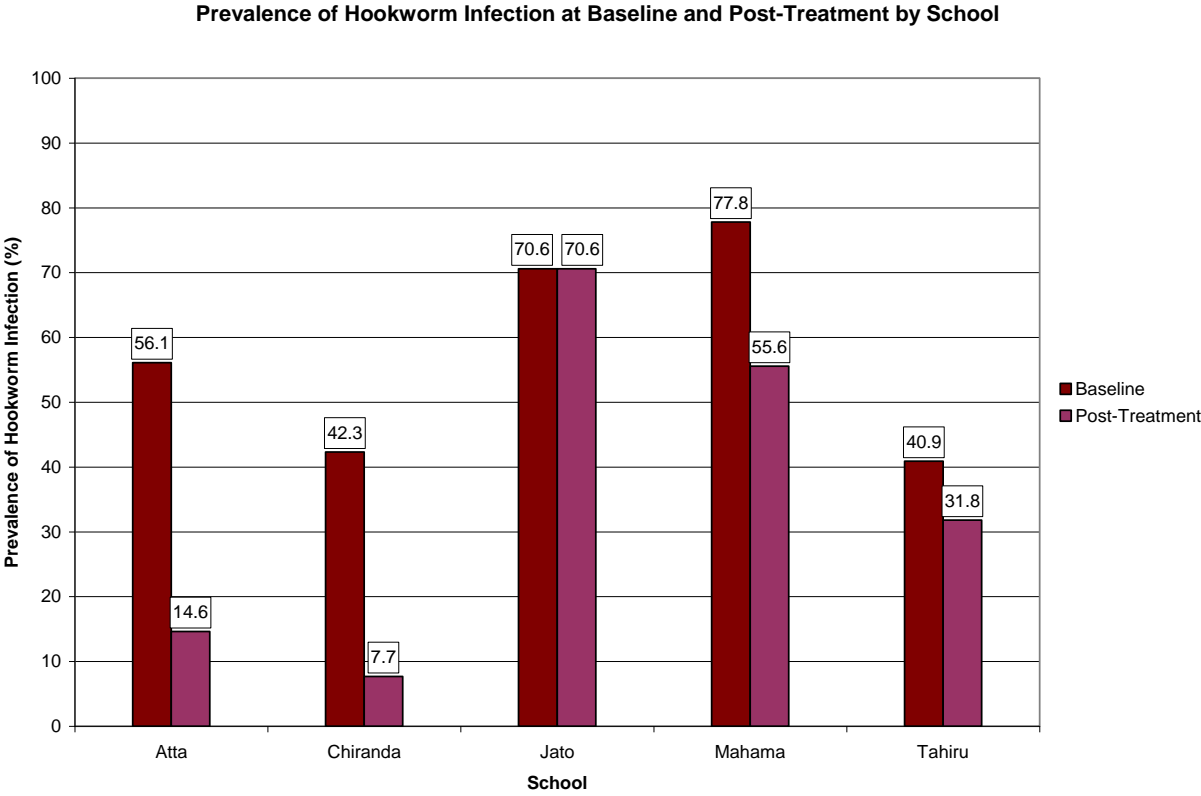


Figure 18. Prevalence of hookworm infection at baseline and post-treatment by school

Jato, a community previously identified with the lowest mean dietary diversity and protein intake in the study population, not only experienced a zero cure rate, but had extremely poor mean egg reduction rates after treatment with albendazole (Figure 19). Atta and Chiranda had mean egg reduction rates of 94.3% and 96.3%, respectively, but in contrast, Jato had a mean egg reduction rate of only 1.2%.

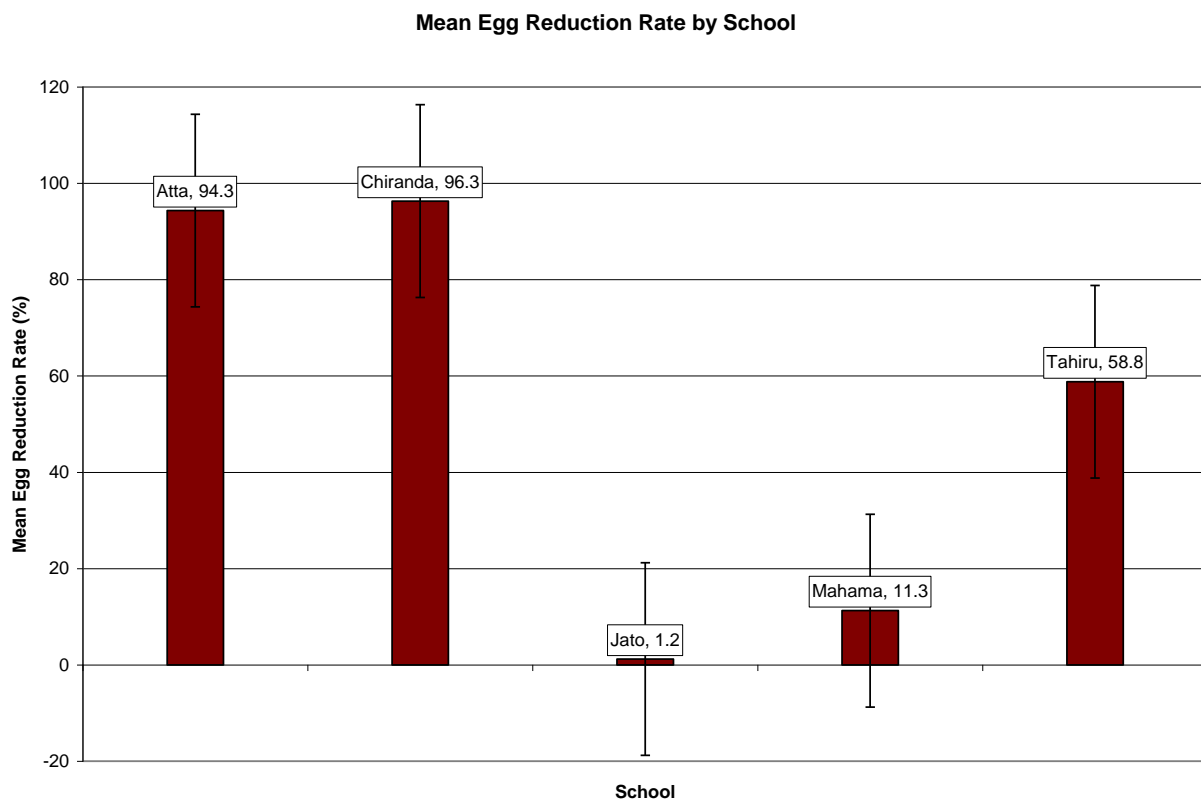


Figure 19. Mean egg reduction rate by school

Although 81 children were positive for hookworm infection at baseline, 79 received albendazole treatment with 2 lost at follow-up. Compared to 429.4 (\pm 640.8) at baseline, the arithmetic mean fecal egg count after treatment was 170.2 (\pm 434.6)—an egg reduction rate of 60.4% (Table 8).

Table 8. Hookworm Cure Rate and Egg Reduction Rate of the Treatment Group

| | |
|--|-------------------|
| Prevalence (%) | |
| Baseline | 100 |
| Post-Treatment | 62.8 |
| Cure Rate (%) | |
| | 37.2 |
| Arithmetic Mean Egg Count^a | |
| Baseline | 429.4 \pm 640.8 |
| Post-Treatment | 170.2 \pm 434.6 |
| Egg Reduction Rate (%) | |
| | 60.4 |

^aEggs per gram

Assessment of Nutritional Status and Host Factors on Treatment Efficacy

The following assessment reviews the treatment population’s dietary intake and host-related factors as surveyed on the day albendazole administration. Throughout the remainder of the study, dietary intake on “treatment day” refers explicitly to the 24 hour reference period prior to receiving albendazole. Although previous reports have shown that protein and calorie malnutrition reduce drug efficacy, the magnitude of dietary diversity and protein intake on days of treatment have not been well studied. (Mao *et al.*, 2006) Identifying risk factors responsible for low cure rate and egg reduction rates may provide clearer insight into how host nutritional status and dietary factors can improve response to treatment.

With respect to post-treatment hookworm infection status, negative cases consumed a greater number of food groups than positive cases (Figure 20). A larger proportion of hookworm-negative cases ate more amounts of different food groups than hookworm-positive

cases. It was notable that zero post-treatment hookworm-positive cases consumed organ meats, vitamin-A rich fruits, and cheese and yogurt during the reference period.

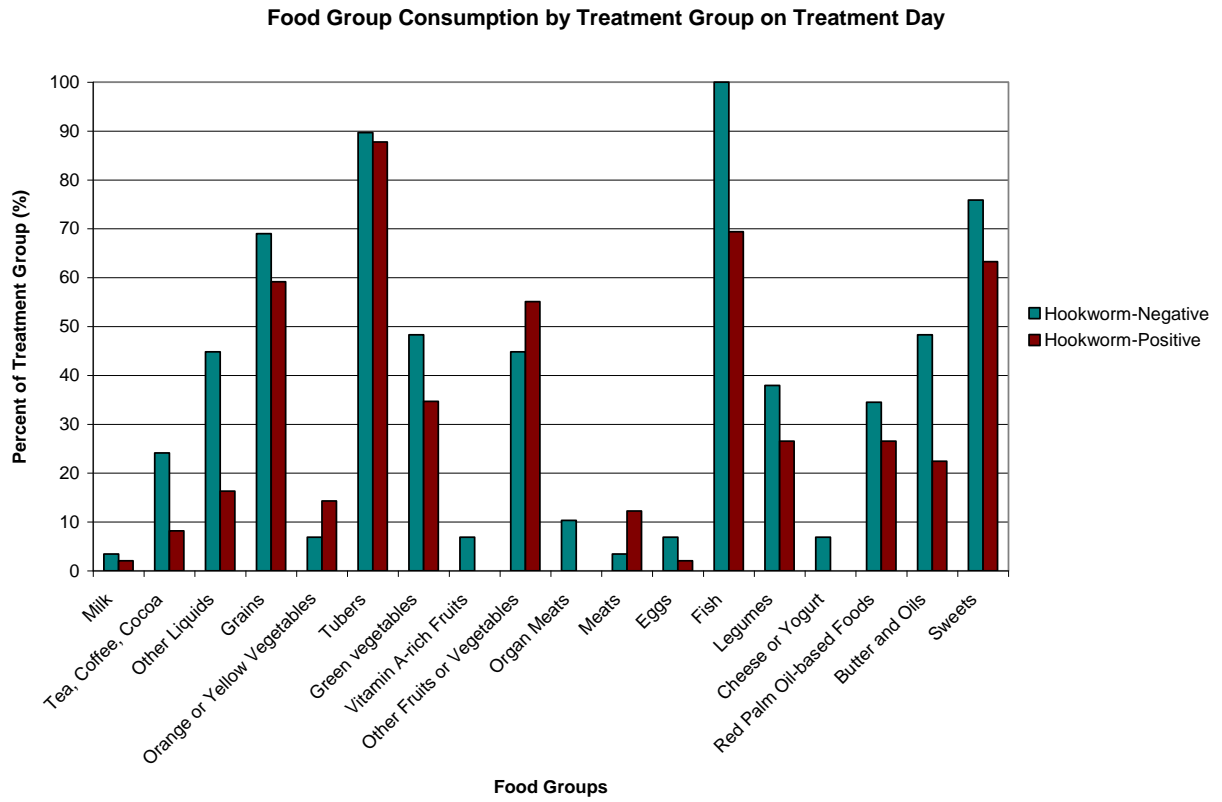


Figure 20. Food group consumption by treatment group on treatment day

Dietary diversity scores of post-treatment hookworm-positive cases ranged from 2 to 9 on treatment day whereas scores of post-treatment hookworm-negative cases ranged from 3 to 16 (Figure 21). Hookworm-infected children’s diets during the reference period were less diverse than those of uninfected children (p-value 0.0016). Dietary diversity may be a significant host factor contributing to increased morbidity and poorer cure rate in the study population.

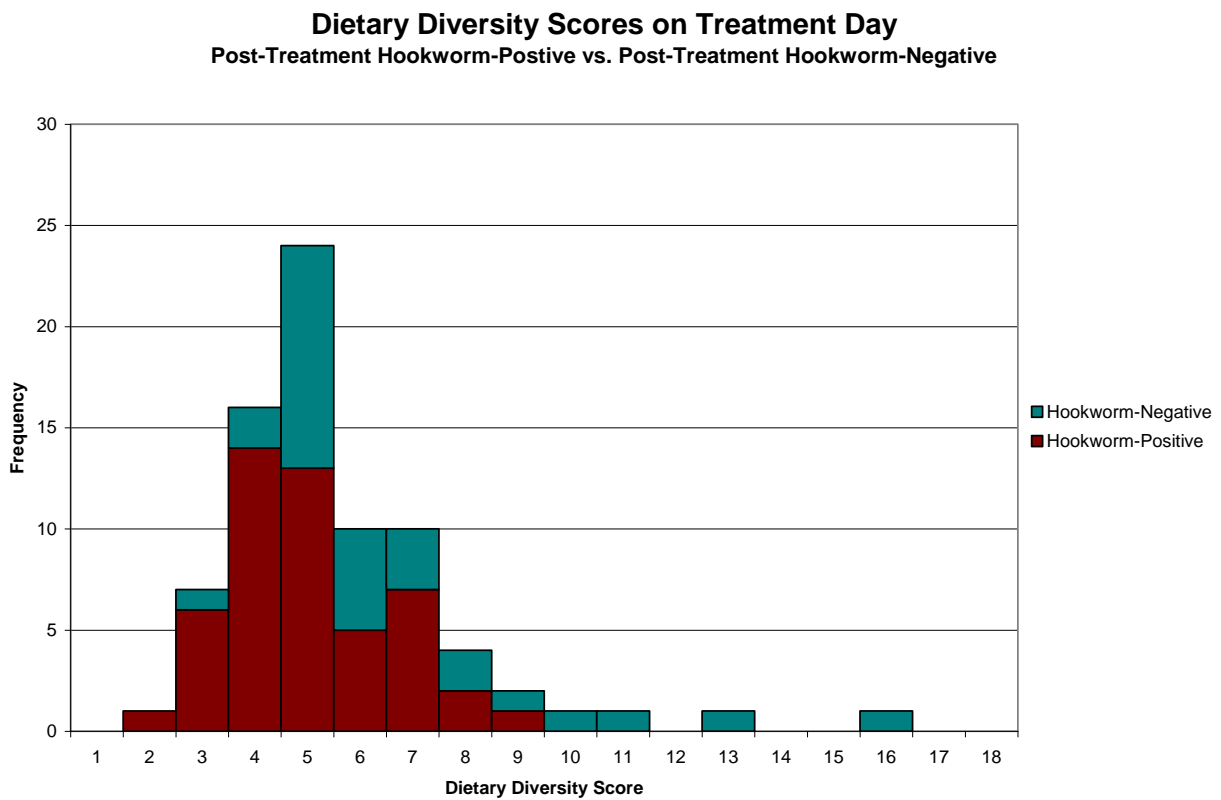


Figure 21. Dietary diversity scores on treatment day—post-treatment hookworm-positive cases versus post-treatment hookworm-negative cases

Similar to food group consumption on treatment day, hookworm-negative cases consumed a larger proportion of protein-rich food groups than hookworm-positive cases (Figure 22). Those who consumed organ meats and fish during the reference period experienced a statistically significantly higher rate of successful deworming on treatment day.

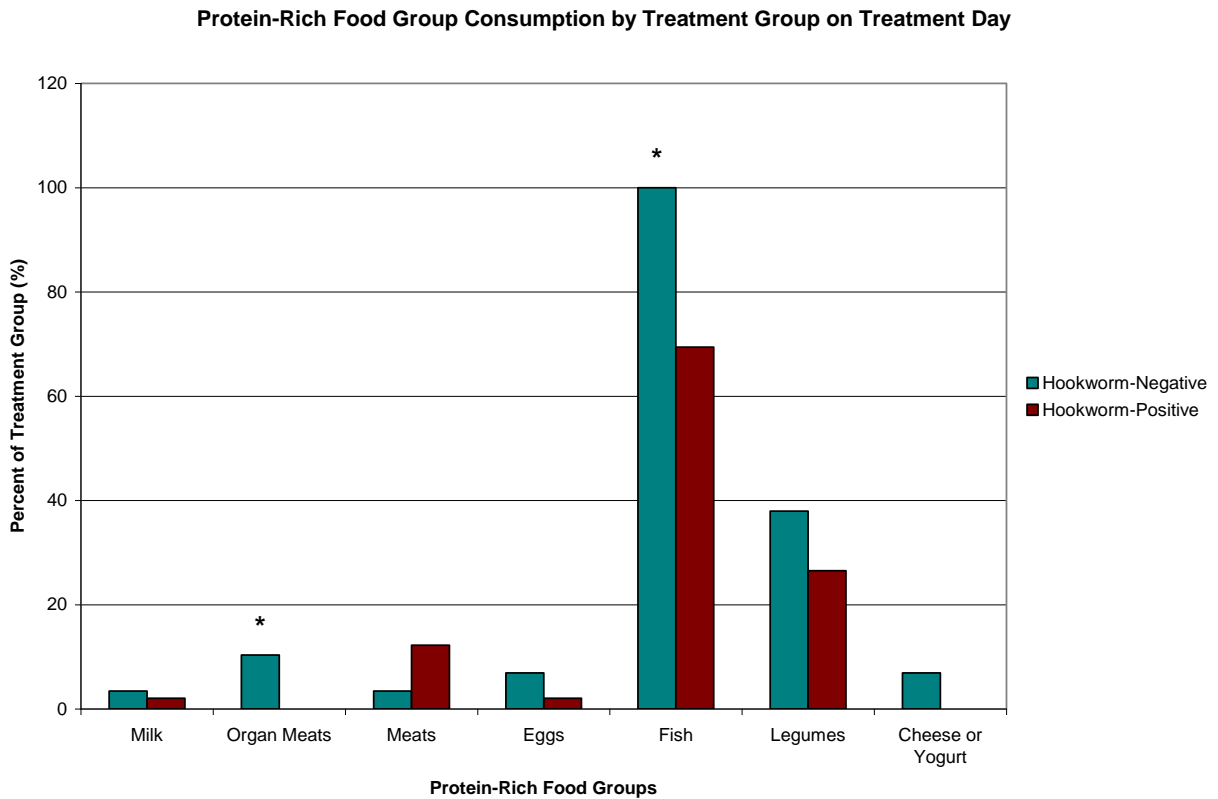


Figure 22. Protein-rich food group consumption by treatment group on treatment day

Dietary protein intake on treatment day ranged from 0 to 3 protein-rich food groups for hookworm-positive cases and 1 to 5 for hookworm-negative cases. Nine children from the treatment population consumed zero protein-rich food groups prior to treatment—and all nine remained hookworm-positive after administration of albendazole (Figure 23).

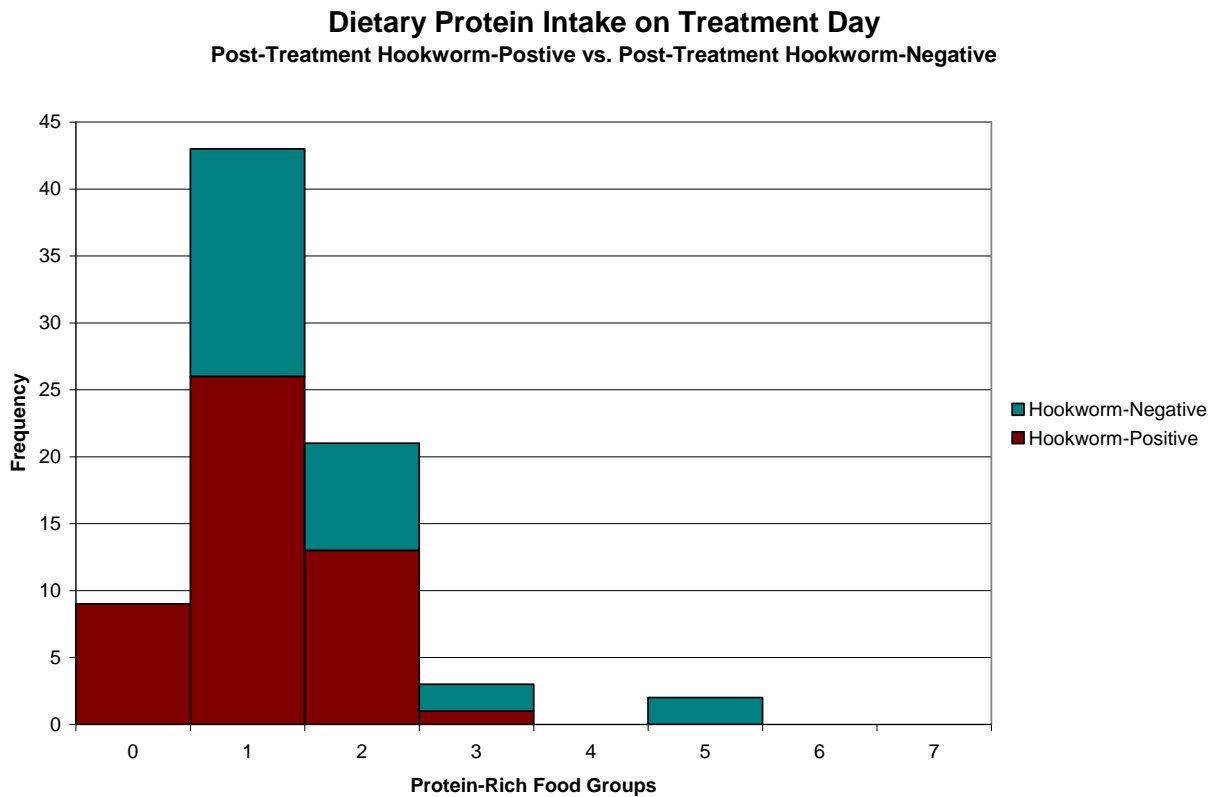


Figure 23. Dietary protein intake on treatment day—post-treatment hookworm-positive cases versus post-treatment hookworm-negative cases

As seen in Figure 24, dietary protein intake patterns notably decrease from the entire study population to hookworm-positive infection status at baseline and finally to post-treatment cases. The effects of low dietary protein intake may play a role not only in susceptibility to hookworm infection, but also treatment response.

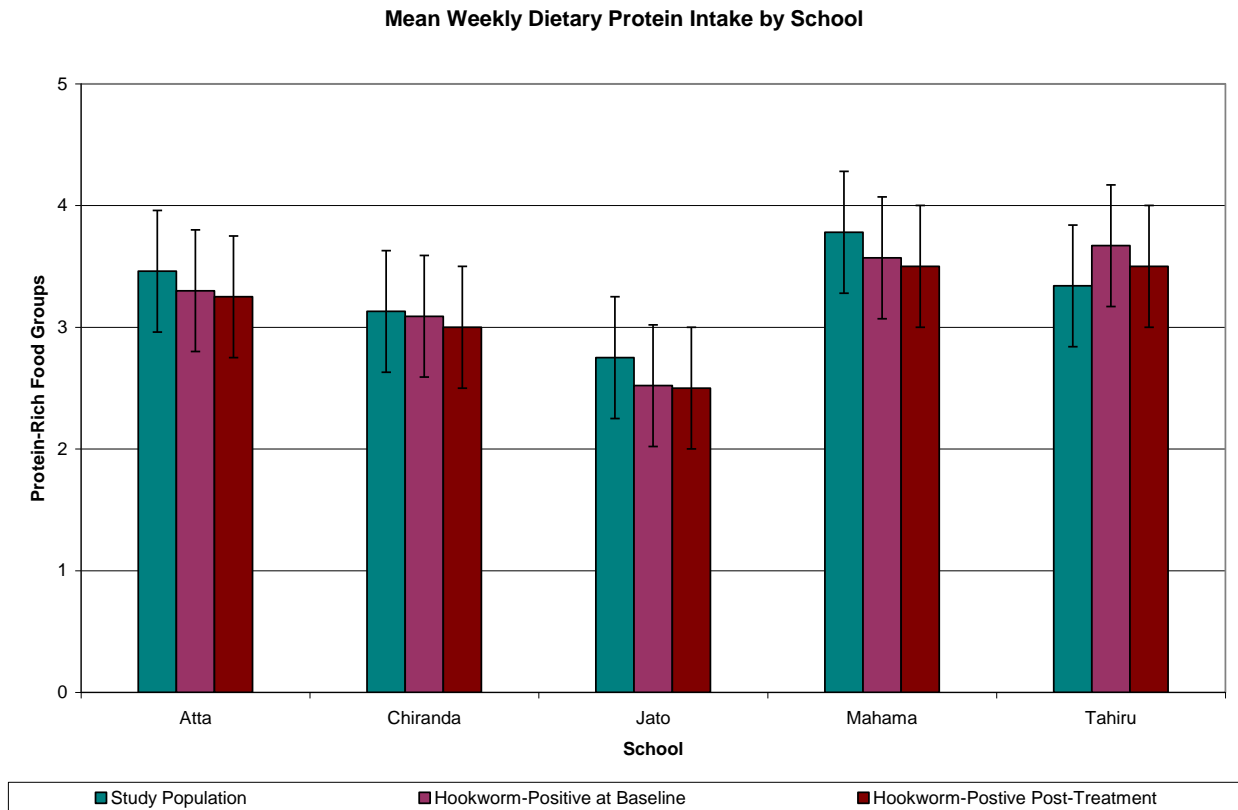


Figure 24. Mean weekly dietary protein intake of the study population, hookworm-positive cases at baseline, and hookworm-positive cases post-treatment (left to right)

Weekly dietary diversity, as an indicator of nutritional status, was significantly associated with post-treatment hookworm infection status (p-value 0.0111). The median weekly dietary diversity score of the treatment population was 10.0—those falling below the median had higher rates of post-treatment hookworm infection than those above the median (Figure 25).

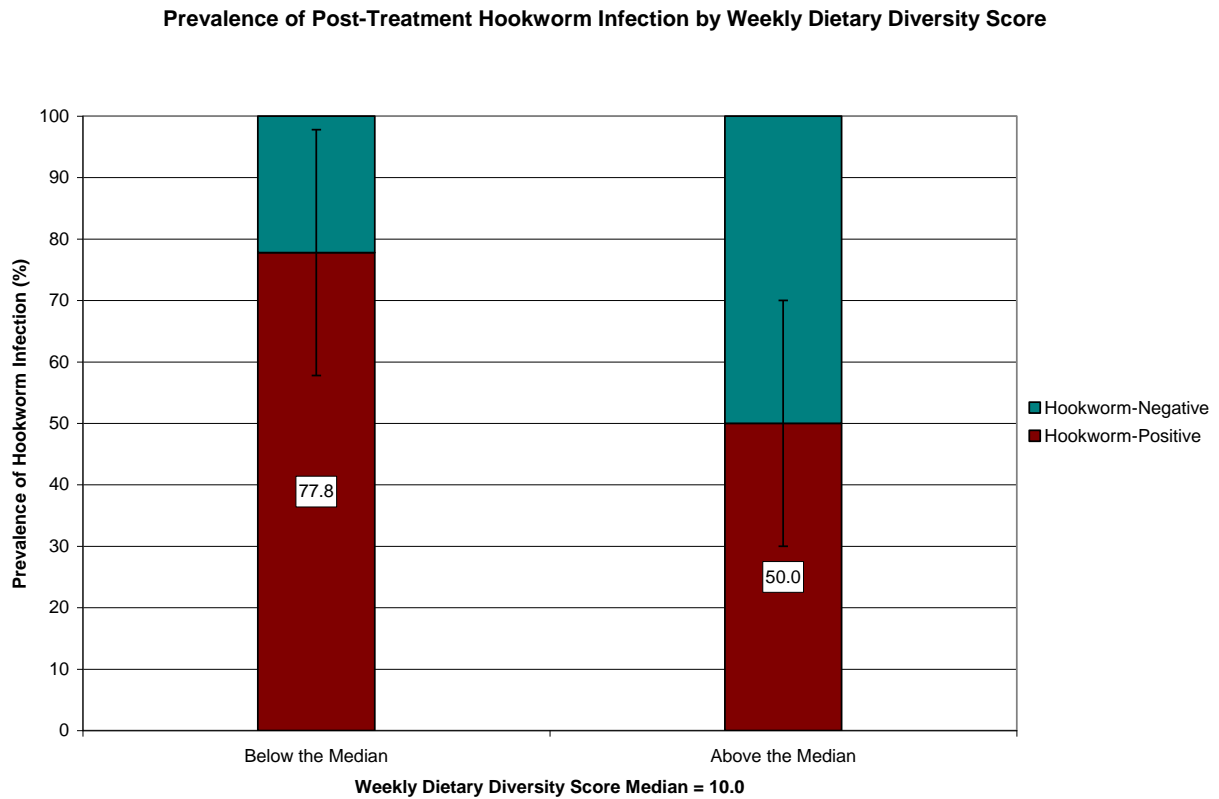


Figure 25. Prevalence of post-treatment hookworm infection by weekly dietary diversity score

The association between weekly dietary diversity and post-treatment hookworm infection status was also seen as a dose-response relationship between tertiles—infection clearance was more successful among children in the highest tertile of dietary diversity (Figure 26).

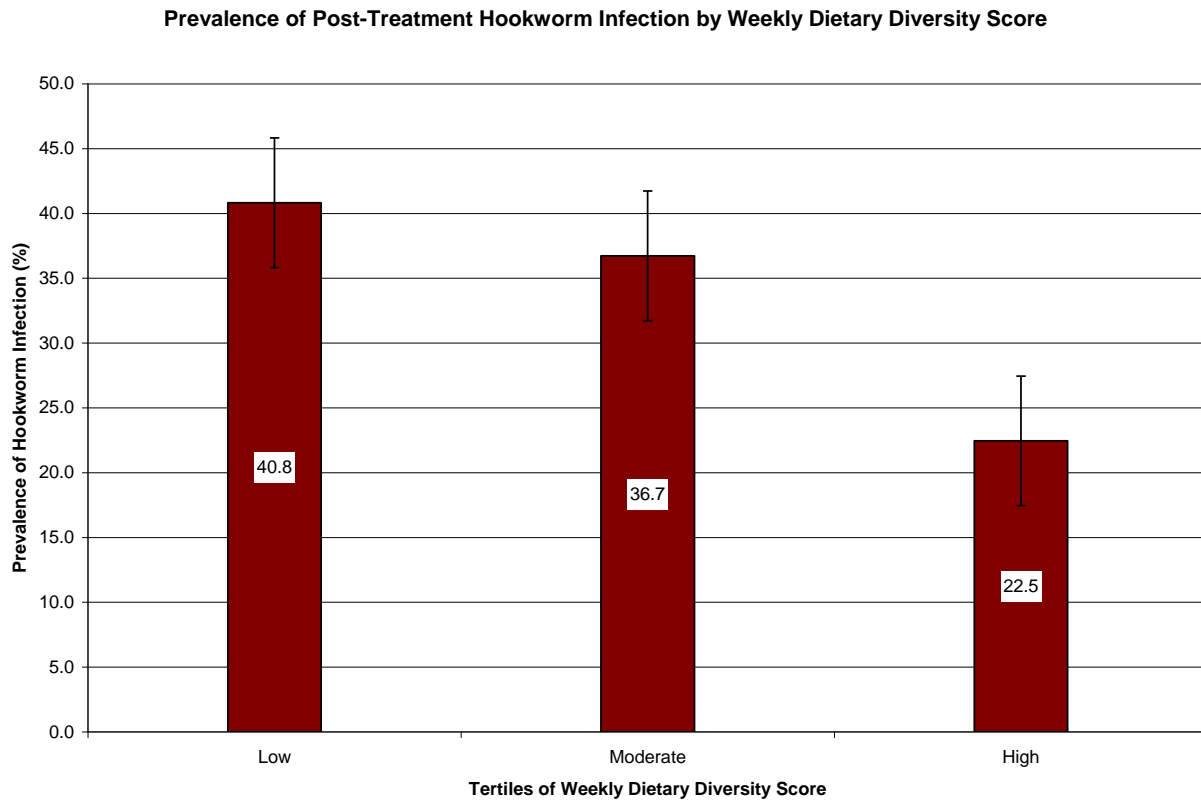


Figure 26. Prevalence of post-treatment hookworm infection by tertiles of weekly dietary diversity score

To further investigate the role of dietary diversity on treatment response, scores were tabulated with respect to the previous 24 hours prior to administration of albendazole. The median dietary diversity score of the reference period prior to treatment was 5.0 food groups—children following below the median had considerably lower rates of hookworm clearance than those above the median (Figure 27). Out of 24 children falling below the median, 21 remained positive for hookworm infection after treatment. Cases with dietary diversity scores above the median had better responses to drug therapy and a higher a cure rate.

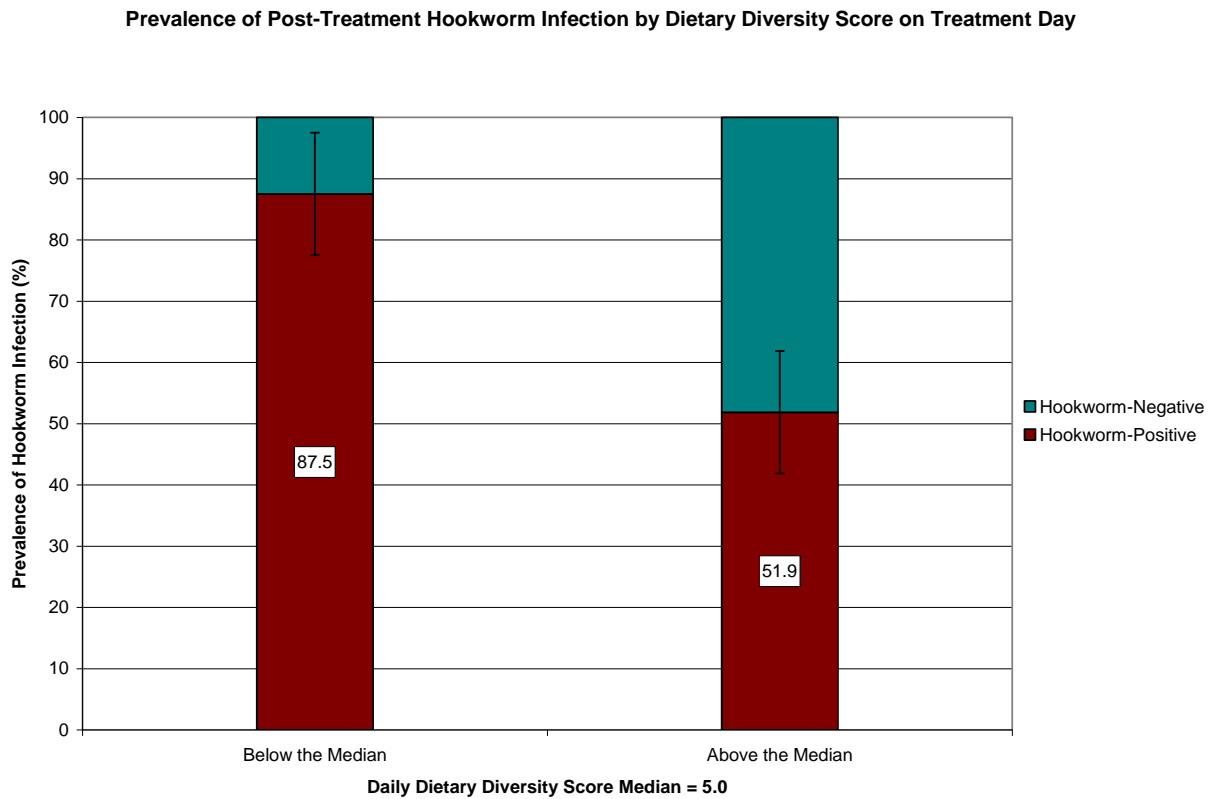


Figure 27. Prevalence of post-treatment hookworm infection by dietary diversity score on treatment day

The median protein-rich food groups consumed on treatment day was 1.0—all treatment participants falling below the median remained positive for hookworm (Figure 28). Those above the median showed a better response to albendazole and had a higher cure rate.

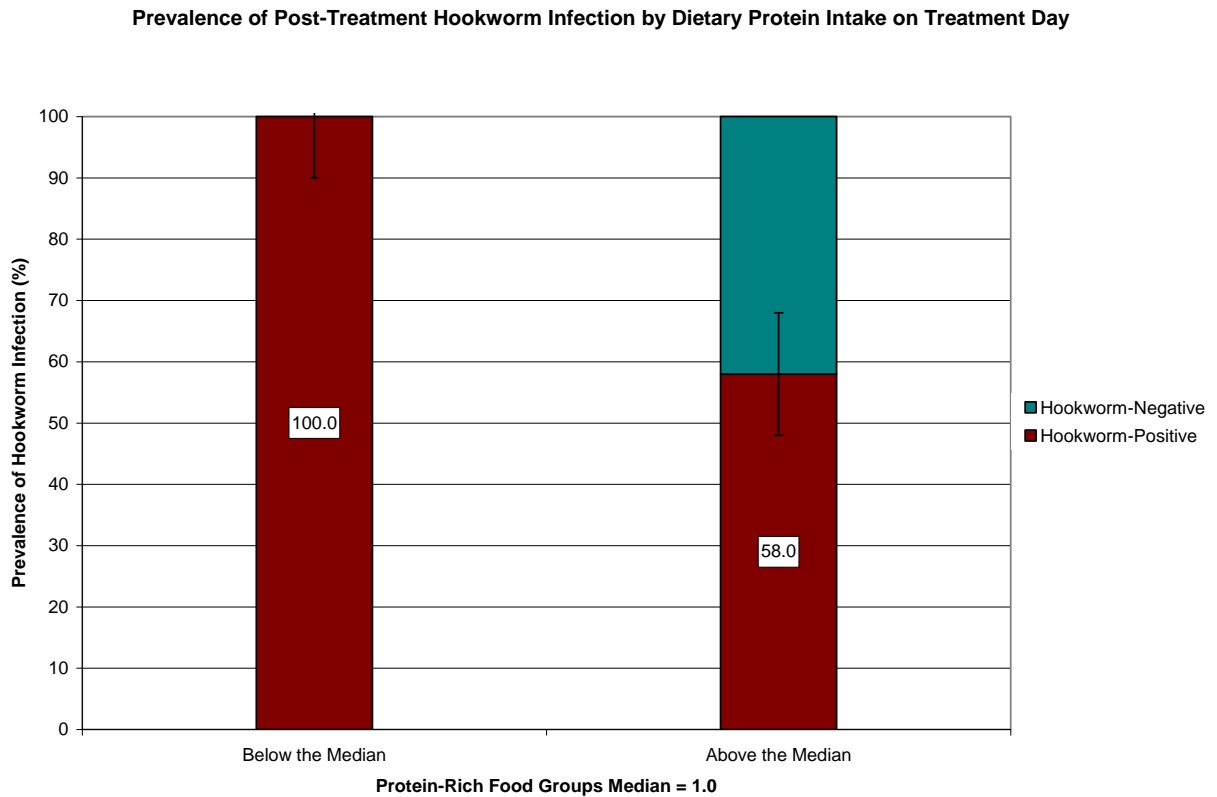


Figure 28. Prevalence of post-treatment hookworm infection by dietary protein intake on treatment day

Study characteristics surveyed on treatment day included dietary diversity score, dietary protein intake, the number of bowel movements within the past 24 hours, presence of diarrhea or loose stools within the past 24 hours, and time since last meal (Table 9). Dietary diversity score and dietary protein intake, characterized by tertiles, within the reference period were significantly associated with post-treatment hookworm infection status. In addition, time since last meal was associated with presence of remaining infection after treatment—those who did not eat for more than six hours prior to receiving albendazole had better response to treatment (p-value 0.0016).

Table 9. Nutritional Status and Dietary Factors of the Treatment Population by Post-Treatment Hookworm Infection Status^a

| Characteristic | Hookworm Infection | | p ^c |
|---|--------------------------------|--------------------------------|----------------|
| | Positive (N = 49) ^b | Negative (N = 29) ^b | |
| Dietary Diversity Score on Treatment Day | | | 0.0104 |
| Low | 42.9 (21) | 10.3 (3) | |
| Moderate | 26.5 (13) | 37.9 (11) | |
| High | 30.6 (15) | 51.7 (15) | |
| Dietary Protein Intake on Treatment Day | | | 0.0423 |
| Low | 18.4 (9) | 0 (0) | |
| Moderate | 53.1 (26) | 58.6 (17) | |
| High | 28.6 (14) | 41.4 (12) | |
| Diarrhea | | | 0.0558 |
| Yes | 18.4 (9) | 37.9 (11) | |
| No | 81.6 (40) | 62.1 (18) | |
| Number of Bowel Movements within 24 Hours | | | 0.0344 |
| | 1.06 ± 0.69 | 1.52 ± 1.18 | |
| Did Not Eat for More than 6 Hours Prior to Treatment | | | 0.0016 |
| Yes | 10.4 (5) | 41.4 (12) | |
| No | 89.6 (43) | 58.6 (17) | |

^aTable values are mean ± SD for continuous variables and column % (N) for categorical variables

^bNumbers may not sum to total due to missing data and percentages may not sum to 100% due to rounding

^cp-value is for t-test (continuous variables) or χ^2 -test (categorical variables)

To perform an ordered logistic regression analysis, egg reduction rate was ordered into increasing levels:

No Reduction (0% egg reduction)

Low Reduction (1% to 50% egg reduction)

Moderate Reduction (51% to 75% egg reduction)

High Reduction (76% to 100% egg reduction)

Children with higher dietary diversity scores on treatment day were 3.08 times more likely than those with lower scores to experience the highest egg reduction rates (p-value 0.0430)

(Table 10).

Table 10. Potential Host Factors Predicting Increasing Egg Reduction Rate with Treatment^a

| Characteristic | Adjusted Odds Ratio | 95% CI | p^c |
|--|----------------------------|---------------|----------------------|
| Sex | | | |
| Male | 1.00 | | |
| Female | 0.65 | (0.26, 1.61) | 0.353 |
| Time Since Last Meal | | | |
| Ate Within 6 Hours Prior to Treatment | 1.00 | | |
| Did Not Eat for More than 6 Hours Prior to Treatment | 4.95 | (1.24, 19.80) | 0.0239 |
| Diarrhea | | | |
| Yes | 1.00 | | |
| No | 0.32 | | 0.0523 |
| Dietary Diversity Score on Treatment Day | | | |
| Low | 1.00 | | |
| Moderate | 1.42 | (0.48, 4.22) | 0.530 |
| High | 3.08 | (1.04, 9.15) | 0.0430 |
| Dietary Protein Intake on Treatment Day | | | |
| Low | 1.00 | | |
| Moderate | 1.70 | (0.43, 6.71) | 0.448 |
| High | 2.62 | (0.60, 11.38) | 0.200 |

^aAdjusted for sex, age, and absolute wealth index

A Potential Fasting-Induced Effect to Albendazole Treatment

On treatment day, each study participant reported time since last meal which was then calculated into hours by the interviewer. Those who had not eaten for more than six hours prior to treatment displayed a better response to albendazole than those who ate within six hours prior to receiving albendazole (Figure 29). A possible hypothesis to explain the potential effect of fasting may be that albendazole is more effective in a fasting individual due to resultant higher drug concentration in the gut. The influence of fasting on the bioavailability and pharmacokinetics of albendazole warrants further investigation.

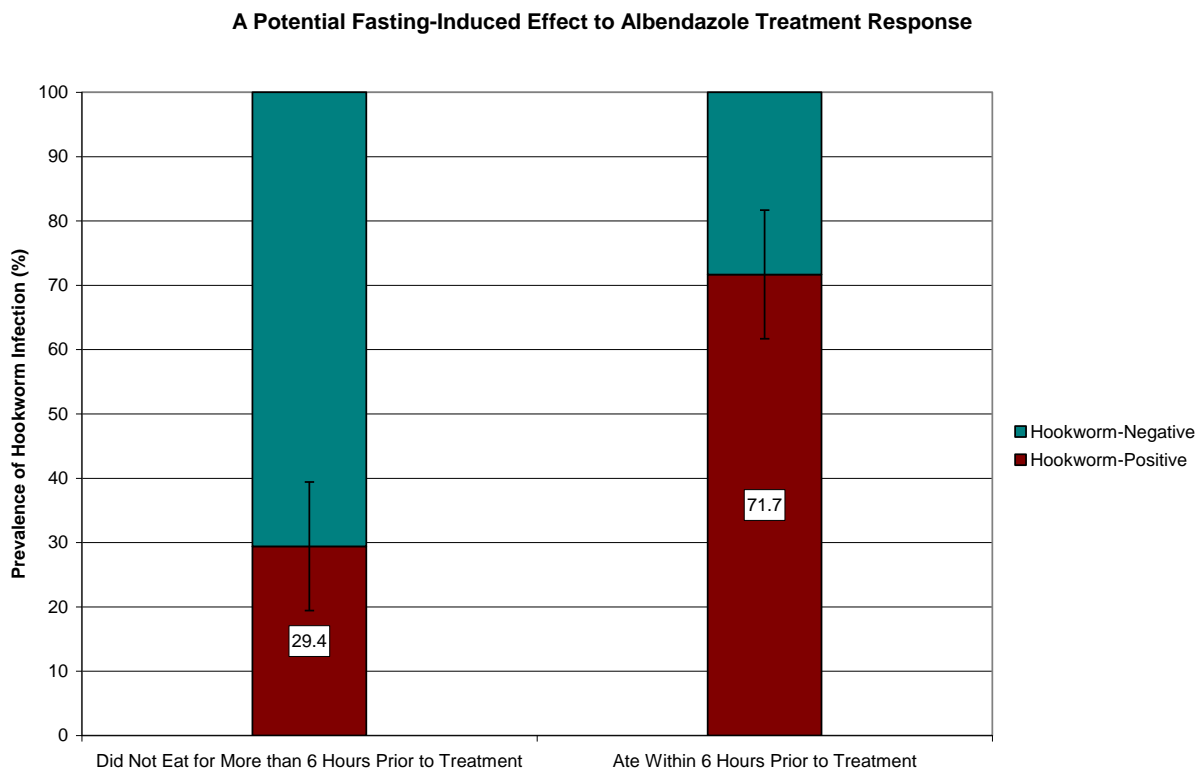


Figure 29. A potential fasting-induced effect to albendazole treatment response

Discussion

Nutritional Status of the Treatment Population

Providing sufficient food and nutrition to meet people's basic needs for health, growth, and development has been a long-standing challenge for African countries. Dietary diversity has shown to be positively linked to nutrient adequacy and improved health status in individuals. The traditional rural Ghanaian diet disproportionately comprises of staple foods at the expense of dietary diversity. In addition to placing young children at risk for chronic malnutrition and nutrient deficiencies, high consumption of cereal-based diets and low consumption of protein-rich foods may lead to reduced effectiveness of chemotherapeutic schemes for hookworm. Previous literature has identified an association between host nutritional status and susceptibility to hookworm infection, but the same association with anthelmintic efficacy of albendazole remains unclear.

Weekly consumption of food groups by the study population indicates participating children consume a limited diet of cereals and tubers also lacking in protein-rich foods. A wide range of foods is the best source of essential nutrients—less than half of the children consumed milk, meats, cheese or yogurt, eggs, organ meats, orange and yellow vegetables, and vitamin A-rich fruits. In addition to reporting both the lowest mean weekly dietary diversity and protein intake of the study population, Jato had the second highest prevalence of hookworm infection at baseline (70.6%) and more disturbingly, exhibited a zero cure rate and extremely low mean egg reduction rate (1.2%) after treatment.

Similar to hookworm cases at baseline, those in the highest tertile of household food insecurity had the highest prevalence of post-treatment hookworm infection (38.8%). Low food security indicates chronic hunger and reduced food intake in individuals, which adversely affect

health outcomes and physiological function. The association between weekly dietary diversity and post-treatment hookworm infection was also displayed as dose-response relationship—hookworm clearance was more successful among children falling in the highest tertile of dietary diversity (Figure 26). The difference in means of consumed protein-rich food groups on treatment day was statistically significant (p-value 0.0077) between post-treatment hookworm-positive (1.12) and hookworm-negative children (1.69). Likewise, the difference in means of dietary diversity score between post-treatment hookworm-positive (5.0) and hookworm-negative (6.6) was statistically significant (p-value 0.0016).

It is surprising how seldom host nutritional status and dietary factors are described in treatment regimens, especially for drugs used for infectious disease where malnutrition is widely prevalent. As specific nutrients are involved in microsomal enzyme systems, capacity for drug metabolism is reduced in deficient states of nutrition and protein intake. In addition, drug distribution and clearance are likely to be influenced by malnutrition. (Santos *et al.*, 2005) A variety of mechanisms may be responsible for the role of reduced efficacy in nutrition deficient subjects—poor status may affect drug absorption, drug metabolism, and drug uptake by the intestinal parasite.

The Effects of Host Nutritional Status and Dietary Factors on Treatment Efficacy as Defined by Egg Reduction Rate

Intestinal nematode infections, particularly hookworm, are of major public health importance in developing countries due to its toll on morbidity—the overall burden of hookworm disease, as assessed by DALYs, currently outranks African trypanosomiasis, dengue, Chagas' disease, schistosomiasis, and leprosy. (Hotez *et al.*, 2004) Individuals harboring large numbers of adult worms may suffer from poor iron status and iron deficiency anemia—the hallmark of hookworm disease (Hotez, 2006) Although large-scale deworming efforts may offer better health outcomes, such as improvements in physical growth and reduced helminth transmission, reinfection often occurs within only a few months after treatment. In addition, periodic chemotherapy may increase occurrence of genetically-mediated drug resistance. (Albonico *et al.*, 2004) The identification of new tools and approaches are needed for the successful control of hookworm.

Based on previous literature reviewing iron and protein deficiency as modifiers of worm expulsion in animal models, the primary objective of the study was to investigate whether host nutritional status and dietary factors did affect response to albendazole in a population where low cure rates have been reported. (Duncombe *et al.*, 1977; Duncombe *et al.*, 1979) More broadly, we aimed to review the elements that may play a role in response to treatment: biological, physical, and chemical factors which are directly and indirectly influenced by host nutrition and diet (Figure 30). Bacterial populations in the intestine (biological), gut motility and function (physical), and the effects of food in the gut (chemical) are some of the participating mechanisms in response to treatment, but they are all ultimately affected by host nutritional status and dietary parameters.

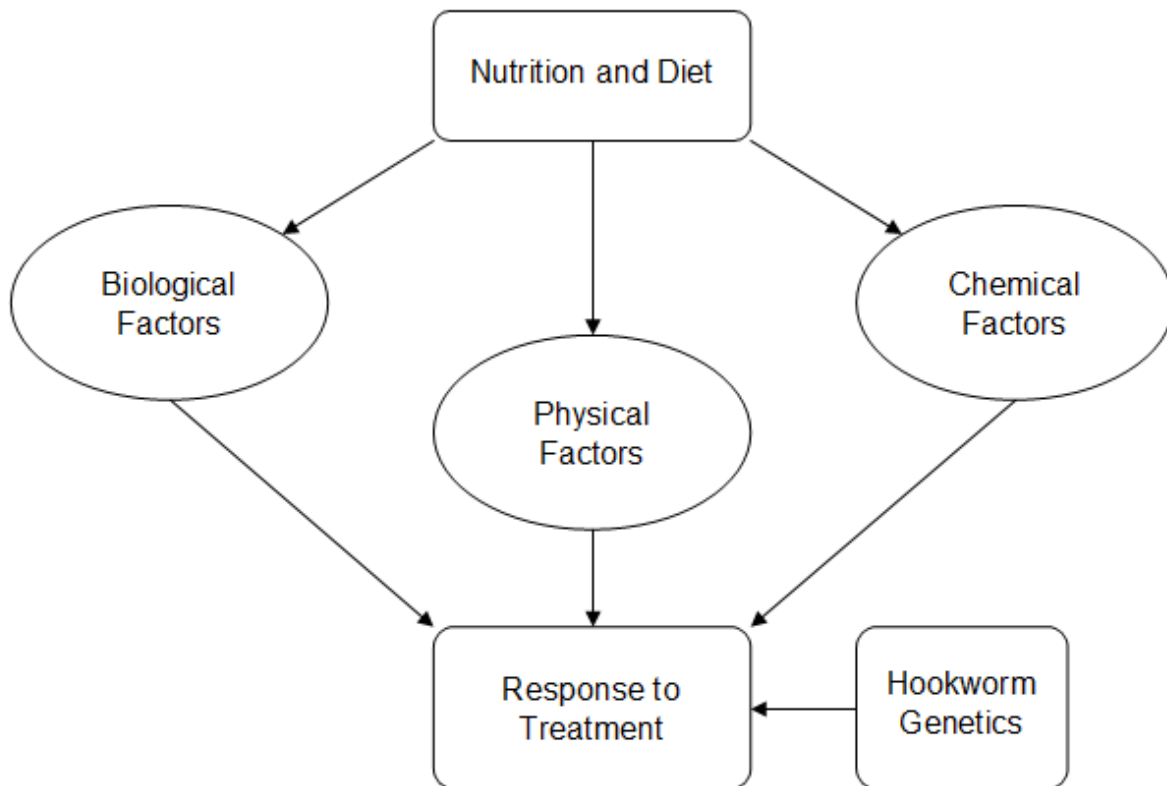


Figure 30. Influences on treatment response

In addition to the consistent epidemiology of hookworm infection in the study population with previous studies, the results and findings described in our study provide evidence to support further research into the effect of nutritional status and dietary factors on treatment efficacy of albendazole. The nutritional status of an individual and the availability of nutrients may be essential to the regulation of albendazole drug action. In a previous study by Bidlack *et al.*, they demonstrated nutritional factors, specifically the lack of nutrients including protein, carbohydrates, fats, and specific vitamins and minerals, altered drug metabolism and efficiency in human subjects. Nutritional factors were reported to affect the regulation of detoxification systems of the liver (e.g., cytochrome P-450-dependent mixed function oxidase). Restricted

diets with deficiencies in calories, protein, and other nutritional components may affect drug mechanisms and its components in unique ways not yet explored at length.

The present study potentially indicates the complex interactions between nutrient deficiency and anthelmintic efficacy. As shown in Figures 27 and 28, children falling below the treatment population median of both dietary diversity and protein intake on days of treatment had poorer responses to albendazole. In the group of hookworm-positive children falling below the population median of dietary diversity on treatment day, 21 out of 24 children (87.5%) remained positive for infection after treatment. Those in the group above the median of dietary diversity had higher rates of clearance—only 28 out of 54 children remained positive (51.9%). Consuming more food groups on treatment day, as indicated by higher dietary diversity scores, was notably associated with better rates of hookworm clearance after treatment as compared to those consuming less food groups (p-value 0.0026). These principal findings, never before described in literature, strongly merit further research.

Prevalence of post-treatment hookworm infection was higher for those with low dietary protein intake on treatment day— all children falling below the population median of dietary protein intake remained infected with hookworm following treatment. Those with higher dietary protein intake (above the population median) had improved response to treatment—42% successfully cleared intestinal worms with albendazole. Having higher dietary protein intake on day of treatment, as indicated by the consumption of a larger number of protein-rich food groups within the 24-hour reference period, was also significantly associated with greater treatment efficacy compared to those consuming a lesser amount of dietary protein (p-value 0.0141). The role of protein as a dietary factor stimulating drug metabolism has been previously investigated and confirmed in experimental studies using human subjects. (Krishnaswamy, 1978) If low

dietary diversity and protein intake negatively influence response to albendazole then they would be relevant host factors for treatment efficacy.

In addition to dietary diversity score and protein intake on treatment day, time since last meal was significantly associated with post-treatment hookworm infection status—those who did not eat for more than six hours prior to administration of albendazole had better drug response (p-value 0.0016). “Fasting” prior to treatment may be a physiological and/or biochemical factor affecting treatment efficacy of albendazole. The effect of not eating for more than six hours prior to treatment in the population produced improved rates of hookworm clearance—it is possible that fasting produces fundamental physiologic and biochemical changes within the gut environment that affect the rate and/or extent of intestinal absorption or permit increased concentrations of the drug. The presence of food in the stomach may result in competition for binding sites with the drug. Furthermore, aside from the physical influence of food, the nutrient content of a meal can profoundly affect drug metabolism. (Santos *et al.*, 2005) Additional studies are needed to determine the underlying effect of fasting on response to albendazole.

Potential Predictors of Egg Reduction Rate

Albendazole produced substantial reductions in hookworm burdens, but a wide variety of results occurred across the study population. Low egg reduction rates against hookworm may be due to a number of factors, such as genetic variations in hookworm subpopulations in endemic settings. Although there may be some concern about emerging resistance to benzimidazole anthelmintics, there has been no evidence attributing poor anthelmintic efficacy to drug resistance in the human populations. (Brooker *et al.*, 2004) There have been reports of low efficacy of benzimidazole anthelmintics against hookworm in humans, but resistance in

intestinal nematodes of humans has not been confirmed. (Prichard *et al.*, 2007) Therefore, identification of modifiable host factors is urgently needed to provide evidence of variation in drug efficacy and to improve anthelmintic programs and for the development of novel control approaches against hookworm.

This study indicates that in the treatment population, low dietary diversity and protein intake significantly reduced the efficacy of albendazole. These results are consistent with studies conducted by Duncombe *et al.* in which the authors demonstrated a decreased efficacy of mebendazole against *Nippostrongylus brasiliensis* infections in rats consuming a protein deficient diet. Of note, their findings provided the working hypothesis for our field study investigating treatment efficacy.

After adjusting for sex, age, and wealth, an ordered logistic regression model identified potential host factors associated with increasing egg reduction rate in the treatment population—time since last meal and dietary diversity. Those who did not eat for more than six hours prior to treatment were 4.95 times more likely to experience higher egg reduction rates than those who ate prior to treatment (p-value 0.0239). Another host factor was dietary diversity score on treatment day (within the 24-hour reference period)—those with high dietary diversity were 3.08 times more likely to produce the highest egg reduction rate than those with lower dietary diversity (p-value 0.0430). The contribution of time since last meal and dietary diversity to increasing egg reduction rates of albendazole may be biologically relevant host-related factors for the improved treatment of hookworm.

Study Limitations

A potential limitation of the present study was the implementation of questionnaires and its related data collection. Although any recall method is susceptible to bias, collection of dietary data by trained interviewers and translators reduced recall bias. All responses were clearly documented to ensure accurate interpretation during data analysis. Another limitation of this study has to do with the possibility of omitted variables and measurement error. There may be relevant variables at the individual level that are not included in our study or are not measured adequately.

The Kato-Katz method is another potential area of concern. To correctly assess the efficacy of albendazole, the type of diagnostic test is a critical factor due to rapid egg deterioration. Stool samples were transported to the central laboratory and prepared for readings immediately after collection from school sites. Despite low negative-predictive power and variability, the Kato-Katz method is appropriate for the epidemiology survey of hookworm due to its relative simplicity, low cost, and rapidity. (Katz *et al.*, 1972; Montresor *et al.*)

Future Directions and Strategies for Control

In the experiments outlined above, there was a statistically significant association between relatively low dietary diversity and protein intake on treatment day and response to albendazole. Pathophysiological changes encountered in malnutrition are diverse and complicated due to parasitic infections with hookworm. A more in-depth focus is needed to evaluate the influence of nutritional status and food components on treatment regimens. An imbalance of nutrient intake may have direct clinical relevance such that deficiencies can interfere with drug processes in the body resulting in altered response.

Approximately 70% of children who did not eat more than six hours prior to treatment displayed a better response to albendazole and cleared hookworm infection. The underlying mechanism of a potential fasting-induced effect demands a closer examination. Fasting prior to treatment may notably affect the bioavailability and disposition kinetics of albendazole. In a pharmacological study conducted by Sanchez *et al.*, the authors observed the effects of fasting on albendazole kinetics in calves—fasting the animals prior to treatment resulted in enhanced gastrointestinal absorption of the drug compared to fed calves. The authors also report that the bioavailability and disposition kinetics of albendazole and its metabolites in calves were affected by fasting. In a separate study, different authors reported that administration of albendazole on an empty stomach may better elicit intraluminal effects against intestinal parasites. (Lange *et al.*, 1988) The findings of the present study combined with those by Sanchez *et al.* and Lange *et al.* hold practical implications that warrant further investigation.

Developed countries such as those in North America, Europe, and parts of Asia have been able to control endemic hookworm, but the same level of success has yet to be witnessed in at-risk settings (Sub-Saharan Africa) lacking the same engines of economic development. Although resistance has not yet threatened anthelmintic-based interventions, methods to sustain drug efficacy, such as nutritional supplementation with iron and protein, are needed to improve long-term control. According to Koski *et al.*, iron deficiency increased larval growth and adult survival of *Nippostrongylus brasiliensis* in animal models, but repletion of iron accelerated worm expulsion. Effective management of helminth control programs require not only evaluation of drug quality and combination of products, but a greater awareness of the effect of nutritional deficiencies and host factors on treatment efficacy.

The design and implementation of chemotherapy programs in rural communities where poverty and poor sanitation exist may need to be supported by nutritional intervention during treatment periods for improved efficacy against hookworm infection. Low dietary diversity and protein intake can potentially reduce the success of drug interventions and the decreased efficacy of treatment may promote selection of resistant hookworms. Furthermore, combining chemotherapy with nutritional intervention may allow for maximal drug effectiveness. The effects of nutritional supplementation on response to treatment have yet to be fully established and will require extensive longitudinal studies.

Conclusion

Some determinants of observed low drug efficacy may include reduced absorption and bioavailability, host-parasite relationship (intensity of infection), and genetic variations between parasite strains resulting in poor drug susceptibility and tolerance. (Savioli *et al.*, 2004) However, a growing understanding of modifiable host factors affecting treatment efficacy is needed to guide control objectives and novel strategies. Nutritional status and dietary intake have a great potential for altering drug metabolism and disposition—a tremendously important clinical implication. Our results showed that the efficacy of single-dose oral albendazole for curing hookworm infection was reduced for children with lower dietary diversity and protein intake on treatment day. To our knowledge, our findings are the first to address host nutritional status and dietary factors potentially affecting treatment efficacy, as measured by cure rate and egg reduction rate.

Dietary diversity is a powerful source of better nutrition and thus improved health outcomes. There is an urgent need to explore ways to improve individual nutritional status through dietary diversity as nutritional deficiencies and low dietary protein intake may delay normal worm expulsion and perpetuate reinfection. Modifiable host factors may play an important role for maximizing efficacy of existing drug compounds and improving treatment response. Single-dose administration of albendazole cured infection and reduced intensity of infection with differing levels of efficacy across the study population—a promising hypothesis is the influential role of host nutritional status and dietary factors in affecting response to albendazole as seen in the present study. The results show that the anthelmintic treatment was much less effective in subjects with lower dietary diversity and protein intake. By evaluating response to albendazole treatment among hookworm infected participants and identifying modifiable host factors affecting resultant efficacy (as defined by egg reduction rate), we were able to highlight potential biological, chemical, and physical factors important for the correction and optimization of future helminth control.

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