ABSTRACT

A Cross-Sectional Study of Co-Infection with Helminths and Malaria: The Effect on Hemoglobin Levels among Luo Children in Rural Western Kenya

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Soil-transmitted helminths, also known as intestinal worms, are ubiquitous in Sub-Saharan Africa (SSA). In SSA, malaria accounts for 80-90% of all worldwide cases (WHO Malaria Report 2011), and there is significant geographical overlap between these parasites (Brooker et al 2004). In this study, the goal was to study the effect of co-infection of soil-transmitted helminths and malaria on hemoglobin concentrations, along with the modifying effects of acute inflammation and nutritional status among Luo children younger than 13 years. In a clinic sample of 227 children, 53% of the children were anemic. The majority of children had either malaria (49%) or a helminthic infection (42%) or both (21%). A multivariate regression analysis demonstrated that only malarial-helminth co-infection was significantly related to hemoglobin levels (overall model $p=0.0001$, $r2=0.2644$). Stratifying by gender and the presence of helminths, malarial infection was only statistically significant in boys with helminths ($p=0.0158$, $r2=0.2012$). Furthermore, acute inflammation played a significant role in the absence of a helminth infection ($p=0.0001$). Body-mass index, a surrogate measure of nutritional status, was negatively associated with anemia when tested alone ($p=0.005$), but was not significant in the multivariate regression models that explain the variance in hemoglobin. This cross sectional study portrays the complicated relationship of parasitic co-infections and the need for community research to address the long-term consequences on children.

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A CROSS-SECTIONAL STUDY OF CO-INFECTION WITH HELMINTHS AND MALARIA: THE EFFECT ON HEMOGLOBIN LEVELS AMONG LUO CHILDREN IN RURAL WESTERN KENYA

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CHAPTER ONE

Introduction

Sub-Saharan Africa (SSA) today bears the world's biggest burden in disease, poverty and other social injustices. As global efforts continue to alleviate the symptoms of the cycle that SSA and other similar places suffer, researchers must now be systematic and place their efforts on understanding the root of the problems that propagate these cycles. Scientific research from multiple countries has focused on health issues that feed the cycle of poverty. Huge resources have been devoted to combating HIV/AIDS, malaria, and maternal health problems. As attention has turned to chronic as well as acute diseases, problems such as helminthic infections have also gained attention. For children in SSA, the parasitic helminthic infections and the ubiquitous malaria comprise a large source of morbidity (WHO 2004; Hotez et al. 2007). Within Africa, children are among the most seriously affected (Brooker et al. 2007).

Malaria is caused by several plasmodium protozoan parasites, but the variant *plasmodium falciparum* is the main pathogen that causes symptoms of malaria in western Kenya. The Anopheles mosquito is the vector by which the protozoan is transmitted. The environmental conditions in sub-Saharan Africa favor the reproduction of the plasmodium falciparum parasite, and it has been difficult to develop long-term prevention and widespread treatment for malaria.

According to their 2010 data, the World Health Organization (WHO) estimates that there were about 219 million cases of malaria worldwide. Since malaria is heavily

concentrated in the tropical regions in the world, sub-Saharan Africa continues to share a disproportionate amount of the burden, having 80-90% of the cases (WHO Malaria Report 2011). The data from the WHO Malaria Report of 2012 also demonstrates an even higher number of cases per 1000 near Lake Victoria, suggesting that malaria is highly prevalent in rural areas of western Kenya.

In addition to malaria, other parasitic diseases are actually the most common infections among the world's poorest people. According to Hotez and colleagues, there are 13 parasitic infections that are responsible for significant morbidity but have not gotten the attention they deserve; thus, these diseases are now known in the literature as the "neglected tropical diseases" (NTD) (Hotez et al. 2007). Three of these diseases are soil-transmitted helminthic infections (ascariasis, hookworm, and trichuriasis). Others are lymphatic filariasis, onchocerciasis, dracunculiasis, schistosomiasis, Chagas' disease, human African trypanosomiasis, leishmaniasis, Buruli ulcer, leprosy, and trachoma (Hotez et al. 2007). The prevalence and consequences of the soil-transmitted helminthic diseases are devastating. Studies have demonstrated that these tropical infections are "diseases of the poor" by affecting productivity and economic growth (de Silva et al. 2003; Guyatt 2000; Stoltzfus et al. 2001; Sakti et al. 1999). The global prevalence of hookworm infection is about 576 million, with 3.2 billion of the world's people at risk for hookworm (de Silva et al. 2003). From the same study, SSA and China share the largest burden, with about 200 million cases each (de Silva et al. 2003). Although these numbers have been decreasing overall in the past decade, many rural areas of SSA are still at high-risk because they have no sanitation systems or dependable clean water. The Kenyan national school-based deworming program collectively summarized and analyzed a series

of pre and post-intervention cross-sectional surveys in Kenya to evaluate and monitor patterns of helminth infections in schoolchildren. According to their data, *Ascaris lumbricoides* is the most common helminth in the Nyanza province, followed by hookworms and *Trichuris trichiura;* with a sample size of 6908 children (younger than 21 years old) from the Nyanza province of Kenya, about 19.9% (95% CI 16.0-24.7) children were infected with *Ascaris lumbricoides*, followed by 11.8% (95% CI 9.8-14.1) with hookworms (Mwandawiro et al. 2013). Eradicating these diseases is essential to the economic development of poor countries.

One of the worst consequences of both malaria and helminthic parasitic disease is severe anemia. Long-term problems of anemia include stunted growth, cognitive function and even language development, which may further propagate the cycle of poverty (Walker et al. 2007 & Siegel et al. 2003). Low hemoglobin levels continue to be a large problem worldwide, especially in Africa where the heaviest burden is on pre-school age children and pregnant women. The estimated prevalence of anemia in these two groups in SSA is 67.6% (95% CI 64.3-71.0) and 57.1 (95% CI 52.8-61.23), respectively (Benoist et al. 2008). Anemia is multifactorial in origin, but WHO estimates that about half of anemia can be attributed to iron deficiency (WHO 2001). Several studies have found compelling evidence that malaria plays a role in the manifestation of anemia (Geerligs et al. 2003; Korenromp et al. 2004). A meta-analysis by Korenromp and colleagues found that across 29 intervention studies, the control of malaria helped increase hemoglobin levels. Using very different mechanisms, anemia is exacerbated with the presence of malaria (hemolysis) and helminthic infections (nutrition-stealing parasites). It is also important to note that non-infectious factors such as poverty and

malnutrition play a role in the development of anemia. Poverty leads to hunger and a decreasing amount of nourishment to children, who are the most vulnerable to its effects.

As helpful as it is to study the independent effects of these devastating conditions, they do not occur in isolation from each other. Though helminthic infection and malaria can each cause anemia, for example, there is little understanding about the complex interactions between these diseases. To further complicate the picture, poor nutrition is both a cause and a result of helminthic infection, compromises the immune response to infection in general, and also contributes to anemia. The focus of this study is the combined effect of helminths and malaria on anemia. The *Plasmodium falciparum* parasite attacking the red blood cells causes hemolysis, while the parasitic worm lodges within the small intestine and causes anemia over time, so it is reasonable to hypothesize that an additive or even a synergistic relationship exists between malaria and helminthic infections in their effect on hemoglobin levels.

One other way to advance our understanding of the effect of acute malarial illness on anemia is to take into account the severity of the infection. The C-reactive protein (CRP) is part of the innate immune response; it is an acute-phase protein with increased concentrations in response to a myriad of factors including infection, inflammation, tissue damage & combinations of these things. In healthy young adults, the median concentration of CRP usually lies around 0.8 mg/ L, but this level can increase up to 1000-fold in response to an acute inflammatory response (Hirschfield & Pepys 2003). Its non-specificity allows the measurement of CRP to be used as a management tool, like temperature, to monitor the severity of the disease (Hirschfield & Pepys 2003). However, studies have also shown that CRP concentrations in patients with malaria, in particular,

increase dramatically and can be especially useful in indicating the severity of the infection (Waller 2011; Paul et al. 2012; Hurt et al. 1994). When the malarial protozoan schizonts burst from the cell, monocytes and macrophages secrete pro-inflammatory cytokines that stimulate the production of CRP in hepatocytes (Karunaweera et al. 1992). Because CRP is an acute-phase reactant, it would not be expected to rise in relation to chronic helminthic infections. Therefore, CRP can be a useful indicator of acute infection as distinct from chronic infection in the study of co-infection and can be helpful in elucidating a possible interaction between severe malaria, helminthic infections and anemia.

The current study is aimed at elucidating the relationships between helminthic infections, malaria, and the C-reactive protein on hemoglobin levels in a high-prevalence, relatively homogeneous group of Luo children in rural Kenya.

CHAPTER TWO

Literature Review

Anemia: A public health problem of the world

Anemia is no stranger to the developing world. According to the World Health Organization's World Health Report, anemia in Africa accounted for 23% of nutrition-related Disability adjusted life years (DALY) (Rogers & Vaughan 2002). Anemia is characterized by a low erythrocyte count and hemoglobin concentration; it is of particular importance because of its wide range of deleterious effects. Its complex etiology makes it very difficult to prevent and treat in developing countries. Anemia manifests itself in various ages and locations around the world. A condition that is almost ubiquitous, it is not proportionally distributed, with developing countries bearing the greatest proportion of anemia. The World Health Organization (WHO) defines anemia with the following parameters:

Table 2.1: WHO's Anemia Parameters

Table 6. Haemoglobin and haematocrit levels below which anaemia is present in a population[a]

Age or gender group	Haemoglobin g/l	Haematocrit mmol/l	Haematocrit l/l
Children 6 months to 59 months	110	6.83	0.33
Children 5–11 years	115	7.13	0.34
Children 12–14 years	120	7.45	0.36
Non-pregnant women (above 15 years of age)	120	7.45	0.36
Pregnant women	110	6.83	0.33
Men (above 15 years of age)	130	8.07	0.39

Source: WHO 2001

According to the WHO's Global Anemia Prevalence database, it is unsettling that anemia affects about 1.62 billion people worldwide (95% CI: 1.50-1.74 billion) (Benoist et al. 2008). The table below demostrates global anemia prevalance among six age groups:

Table 2.2: Anemia Prevalence Among Different Age Groups

Table 3.2 ***Global anaemia prevalence and number of individuals affected***

Population group	Prevalence of anaemia		Population affected	
	Percent	95% CI	Number (million)	95% CI
Preschool-age children	47.4	45.7–49.1	293	283–303
School-age children	25.4	19.9–30.9	305	238–371
Pregnant women	41.8	39.9–43.8	56	54–59
Non-pregnant women	30.2	28.7–31.6	468	446–491
Men	12.7	8.6–16.9	260	175–345
Elderly	23.9	18.3–29.4	164	126–202
Total population	**24.8**	**22.9–26.7**	**1620**	**1500–1740**

Source: Benoist et al. 2008

In general, Table 2 makes it evident that pre-school children have the highest prevalence of anemia with 47.4% (95% CI: 45.7-49.1) while non-pregnant women have highest number affected at 468 million (95% CI: 446-491). These global estimates show that the most susceptible population groups for anemia in general include pre-school children, pregnant women, and non-pregnant women. In Sub-Saharan Africa (SSA), the prevalence of anemia among pre-school children is 67.6% (95% CI: 64.3-71.0), compared to the global prevalence of about 50% (Benoist et al. 2008). This alarming difference indicates that SSA bears a large burden of anemia across all ages and both genders (Benoist et al. 2008). In fact, anemia contributes to a quarter of Africa's nutrition-based Disability Adjusted Life Years (DALY) lost (WHO 2002).

Infectious diseases, malnutrition and genetic disorders are some causal factors of anemia. In anemia-endemic locations, more than one factor often plays a role in the

manifestation of anemia. In western Kenya, intestinal helminths and malaria are major influences on the severity of anemia. Long-term helminthic infections and short-term, but often repeated, malaria infections contribute to the chronic and harmful effects of anemia. For instance, it has been shown that low hemoglobin levels throughout childhood impair mental and physical performance, which leads to low education and productivity (Walker et al. 2007; Lopez et al. 2001). Anemia is a growing public health issue in several countries; in fact, WHO estimates that there about 69 countries, including Kenya, with national anemia estimates higher than 40% (Benoist et al. 2008).

Etiology of Anemia

Anemia is an especially difficult problem because there are a myriad of factors that cause it. In addition, these factors are tangled into a web of causation that complicates the prevention and control of anemia. In general, anemia is either caused by a low production of erythrocytes or by their destruction. Both the destruction of erythrocytes *and* the disruption of their production levels are seen in developing countries. For instance, a malarial infection plays a role in the destruction of erythrocytes while starvation disrupts the production of hemoglobin, the functional component of erythrocytes.

According to the World Health Organization, anemia is most commonly attributed to iron deficiency (WHO 2001). Oftentimes iron-deficiency anemia is part of or coupled with other etiologies such as the hemolysis of malaria, glucose-6-phosphate dehydrogenase deficiency, congenital hereditary defects in hemoglobin synthesis (e.g. sickle cell disease), and deficits in nutrients such as vitamin B12 and folic acid (WHO

Iron Deficiency Anemia, Prevention and Control 2001). It is interesting to note that a study done in the Nyando district in Kenya found a significant association between anemia and homozygous α-thalassemia (Foote et al. 2013), a hereditary disease that affects the alpha chains of the hemoglobin.

Another cited cause of anemia is helminthic infections. These parasites steal nutrition from the host, but they also promote blood loss, which leads to a deficiency in iron stores (Stoltzfus et al. 1997; Foote et al. 2013). With this in mind, iron-supplementation intervention programs are not enough to decrease the prevalence of iron-deficiency anemia. Treatment of the underlying infection is crucial.

Other causes of anemia include heavy menstrual cycles, cancers, and other problems such as HIV and tuberculosis infections. These infections are more common in developing countries, which also helps to explain why these parts of the world bear the largest burden of anemia.

Age and Gender Disparities in Anemia

Childhood anemia carries deleterious effects on the child's cognitive and motor functions (Sakti et al 1999; Walker et al. 2007). With this in mind, it is important to evaluate the reasons for anemia specific to children. In terms of gender differences in children, a study by the Partnership for Child Development reports that there is a general trend for the prevalence of anemia to be higher in boys than in girls, and the difference increases with age (Hall et al. 2001). In the study, the anemia thresholds were defined by UNICEF and WHO standards with gender-specific thresholds in three age groups (WHO 2001). This study estimated the prevalence of anemia in school children in eight countries

from Africa and Asia. Boys manifested a higher prevalence in each age class than girls, the ratio ranging from 1.07 to 1.30; the differences were statistically significant. For instance, for the age class of 7-11, the RR was 1.07 (95% 1.10-1.13) with a p-value of 0.019 (Hall et al. 2001). Although late adolescent girls had lower mean hemoglobin concentrations than late adolescent boys, it is important to note that late adolescent boys are classified as being anemic at a higher threshold than late adolescent girls (130 v. 120 g/L).

Another study demonstrated that the prevalence of anemia in Zanzibari schoolchildren is dependent on age and gender (Stotzfus et al. 1997). This study defined anemia as less than 110 g/L and severe anemia as less than 70 g/L. These data also demonstrated that boys in this region were more anemic than girls, with 65.6% of the boys being anemic compared to 58.8% . In addition, the study also estimated the prevalence of anemia in five age groups and found that the youngest age group had the highest prevalence of anemia and, specifically, of severe anemia. Though the sample size was small, the prevalence of severe anemia in the youngest age group was 19.6% while the 7 to 9-year old age group only had a severe anemia prevalence of 4.4%. If the prevalence of severe anemia is, in fact, higher in younger children, its consequences would be even more worrisome because of the crucial growth and development that occurs during this period.

A cross-sectional survey looking at the nutritional status of children in a rural area of Tanzania found that age and sex were major predictors for anemia (Lwambo et al. 2000). This large survey with a randomly selected sample (n=6801) utilized two thresholds (<120 g/L and <110 g/L) to classify anemia across all ages and gender. The researchers

found that young boys were more anemic than girls but that that changes on the onset of puberty. The multivariate analysis found that age, sex and hookworm infection were major predictors for hemoglobin concentrations. The most commonly studied African disease, malaria, is well known to cause anemia because of its mechanism of injury, with the parasite Plasmodium destroying erythrocytes. The effects of helminthic infections have received less attention.

Soil-transmitted Helminths: What are they?

In the developing world, intestinal worms are almost ubiquitous. These intestinal worms are also called soil-transmitted helminthic parasites. While they reproduce within the intestinal wall of their host, they deposit their eggs through the feces of the host. Since most of the developing world lacks adequate waste facilities, most individuals deposit their waste in the bushes or in small holes. As a result, the feces become part of the soil and the eggs are left waiting to mount onto their next host. With favorable environmental conditions, hookworm eggs, for example, can remain viable in the soil for several weeks. Other types, such as *ascaris lumbricoides* and *trichuris trichiura*, can survive even for several months (Brooker et al. 2007).

The life cycles of soil-transmitted helminthic parasites follow a general pattern. The adult parasites inhabit some part of the host intestine (*Ascaris lumbricoides* and hookworm in the small intestine; *trichuris trichiura* in the colon), reproduce sexually and produce eggs, which are passed in human feces and deposited in the external environment (Brooker et al. 2007). Parasite to human contact is usually through ingestion of raw food and even dirt (*Ascaris lumbricoides* and *trichuris trichiura*) or more unsettling,

penetration through the skin (hookworm). Through field studies, it is evident that ecological factors play a role in the establishment of these helminthic infections; specifically, soil-moisture and humidity are factors that have been widely studied (Nwosu & Anya 1980; Udonsi et al. 1980).

Ascaris lumbricoides is found in the small intestine, specifically in the jejunum. After ingestion by a human, the eggs pass to the duodenum where they hatch; the released larvae penetrate the intestinal mucosa, enter the lymphatic and portal system, and are carried to the liver, heart, and lungs (Cross 1996). After approximately eight to twelve weeks, the larvae become adults and live inside the jejunum. These worms start inflicting damage as migrating larvae; the severity depends upon the number of invading organisms, the sensitivity of the host, and the host's nutritional status (Cross 1996). By blocking the small intestine, these worms restrict major absorption that usually takes place in the small intestine. Specifically, these worms most likely hinder growth in children by impairing the digestion and absorption of proteins, fats and other essential nutrients.

Hookworms, also called *Necator americanus,* live within the small intestine as well. What is unsettling about hookworms is their form of transmission to humans. These helminthic parasites have the ability to penetrate skin. Once in the skin, they enter the venules and are carried to the heart and lungs where they grow and eventually break into the alveoli and pass up the respiratory tree. After they are swallowed, they attach to the intestinal mucosa and become sexually mature in 5 to 6 weeks. (Cross 1996). In the small intestine, worms attach to the mucosa by the buccal capsule, where they rob nutrients from their host. As a result, there is considerable blood loss; this pathology

usually manifests itself clinically through fever, anorexia, weight loss, nausea, vomiting and pica. Pica is the term given for the unusual appetite for dirt and clay; which accounts for some of the iron-deficiency that results from the theft of erythrocytes. Heavy and chronic presence of *N. americanus* in children often leads to growth and mental retardation; research is necessary to investigate school performance and the presence of these worms in children (Siegel et al. 2005).

Trichuris trichiura lives in the colon of humans. Also known as whipworms, these helminthic parasites are also transmitted via soil. As eggs, whipworms are often accidently ingested and find their way into the small intestine, where they hatch. Whipworms continue to develop until they eventually reach the colon and cause considerable damage. The pathogen causes petechial hemorrhage, edema, inflammation, and mucosal bleeding; in addition, small amounts of blood (0.005 ml per worm) are lost each day by seepage at the attachment site (Cross 1996). With this in mind, whipworms cause the same long-term effect on children – physical and mental retardation.

Malaria and Anemia

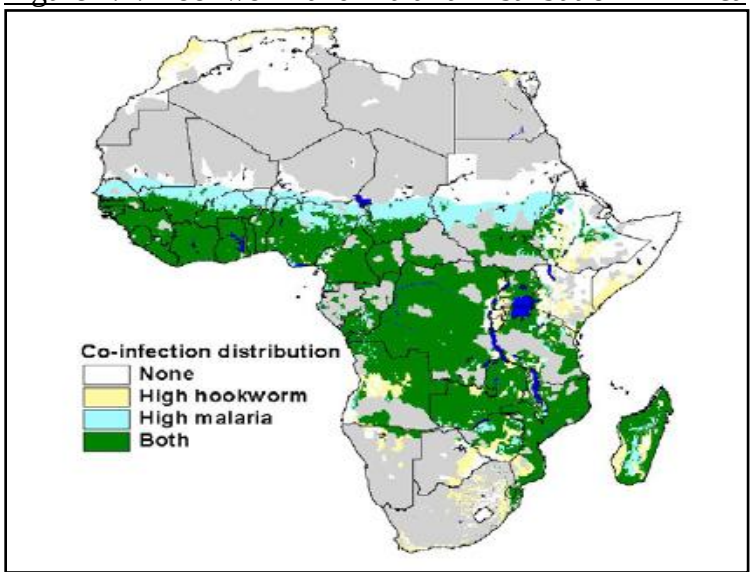
It is unsettling to know that today more than 2 billion people are at risk for malaria, in which one fourth of those who are at risk become infected and 1 million die from malaria each year (Greenwood et al. 2008). In addition, malaria remains the number one killer for young children with an estimated one million annual deaths (Hotez & Molyneux 2008). The children who survive have their lives limited due to anemia and cerebral complications.

The focus in the present study is on *Plasmodium falciparum*, the most deadly malarial species. The lifecycle of *P. falciparum* follows several stages of development in different tissues of the body. When a mosquito first bites an individual, the mosquito also injects the *P. falciparum* sporozoites into the bloodstream. However, the sporozoites do not directly lyse the red blood cells because they have not matured; instead, the sporozoites lodge themselves within hepatocytes to mature into merozoites (Greenwood et al. 2008). Once the sporozoites mature inside the hepatocytes, the infected liver cells rupture and release merozoites into the bloodstream as aggregates called merosomes, which propagate the cycle of erythrocyte invasions (Greenwood et al. 2008). Some of the mechanisms that cause anemia during the pathogenesis of malaria are more associated with the acute clinical state (such as hemolysis and cytokine disturbance); repeated or chronic malarial infections are more likely to involve dyserythropoiesis, which also propagates anemia (Menendez et al. 2000).

Anemia as a Result of Malarial and Helminthic Infections

Despite a different pathophysiology, both malaria and helminthic infections definitely both play a role in the clinical outcome of anemia. It is interesting to note the geographic distribution of helminthic infections and malaria. Since malaria is one of the most deadly acute infections of the developing world and helminthic infections are a very common chronic condition, there should be significant overlap. Below is the graph from the study by Brooke and colleagues showing the presence of both diseases in Africa (see Figure 2.1).

Figure 2.1: Hookworm and Malaria Distribution in Africa



Source: Brooke et al 2006

In addition, Figure 4 demonstrates that the highest concentration of co-infection occurs in Sub-Saharan Africa, an environment characterized by its moisture and heat. This overlap occurs especially near Lake Victoria, which is very near the area of interest in the present study, the Nyakach plateau of western Kenya. In fact, Brooker and colleagues estimate that 45.1 million (95% CI 43.9-46) school-aged children (5-14 years) in Sub-Saharan Africa are at coincidental risk of hookworm and malaria infection risk, a number that represents 25% of the population of this age group (Brooker et al. 2006). This raises questions as to whether co-infection with these two diseases provides a synergistic effect on the overall health of people and, specifically, on the presence and severity of anemia. It is generally accepted that parasitic diseases such as *P. falciparum* and helminthic parasites are major contributors individually to anemia in endemic countries (Brooker et al. 2007).

There is evidence of the cumulative effect on anemia of having several low-level parasites. A study conducted in a Philippines population that measured the impact of a

polyparasite infection, which included three geohelminth species (*Necator americanus, Ascaris lumbricoides, & Trichuris trichiura*) and *Schistosomiasis japonicum;* the study found that the odds of children having anemia with a low intensity polyparasite infection were nearly 5-fold at higher risk of developing anemia than children with no infection (p=0.052) (Ezeamama et al. 2005). However, there remains a "paucity of epidemiological investigation on the health consequences of co-infections with *Plasmodium falciparum* and hook worm" (Brooker et al. 2006), or, for that matter, co-infection with *Plasmodium falciparum* and any helminthic species.

The Pathophysiology Suggesting Synergy

Despite the diversity of helminthic parasites, the mammalian host immunologically responds to each in a consistent fashion (Maizels et al. 1993). Once helminths become established within the intestinal mucosa, they induce a type 2 immune response (T-helper 2 cytokine response), which includes the activation of interleukins and immunoglobulin E (IgE) (Maizels et al. 1993; Brooker et al. 2007). Despite these strong type 2 responses, helminthic worms can survive in the human host for decades (Hartgers & Yazdanbakhsh 2006). With this in mind, it is proposed that this immune type 2 reaction imposes the pro-inflammatory response of the initial malarial infection. That is, the antibodies used to combat malaria (cytophilic antibodies IgG1 and IgG3) are hindered (Geiger et al. 2002; Bouharoun-Tayoun & Druilhe 1992). This hypothesis would imply that chronic helminthic infections promote acute malarial infections by repressing protective antibodies. However, there are limited studies that confirm these underlying

immunological mechanisms in relation to malaria and hookworms (Druihle et al. 2005; Nacher 2004).

In addition to the lack of conclusive data, there exists some contradicting evidence of the effects of chronic helminth infections on host immune responses to malaria (Brooker et al. 2007; Mwangi et al. 2006). For example, two studies found that an *Ascaris lumbricoides,* a helminthic species, could be protective against malaria (Murray et al. 1977; Murray et al. 1978). Murray and colleagues' study of 1978 studied the relationship between malaria and severe ascariasis by measuring the physical symptoms of ascariasis and the presence of malaria after treating the ascariasis infection. With a total sample size of 112 children, aged 2 to 13 years old, the sample was stratified to four groups that differed in only geographical location; Murray and colleagues found that after treating for ascariasis, there was only one group that showed a significant number of children with malarial infections following ascariasis treatment (Murray et al. 1978). Following this hypothesis, a couple of studies from Nacher and colleagues indicate that *Ascaris lumbricoides* infection protect from cerebral malaria and acute renal failure (Nacher et al. 2000; Nacher et al. 2001; Nacher et al. 2002).

Conversely, there are also studies that demonstrate that helminth infections increase the risk for malaria infection. For instance, a study by Spiegel and colleagues analyzed the stool samples of 80 children (ages 1-14 years) and found that the risk of presenting with a clinical malarial attack increased in subjects with intestinal worms (RR= 1.54, p = 0.003) compared to those without a helminthic infection (Spiegel et al. 2003). Another study focused on the *Schistosoma mansori*, which found that the intensity load of *Schistosoma mansori* affects the susceptibility to malaria (Sokhna et al.

2004). Sokhna and colleagues analyzed a sample of 525 Senegalese children (aged 6-15 years) and found that the incidence rate of malarial attacks was higher among those with higher egg counts of *Schistosoma mansori*. In addition, adjusting for age, gender and egg counts of *Schistosoma mansori*, a logistical regression model demonstrated that those with the highest egg count (>1000 egg/ g of stool) were twice as likely to have a malarial attack than those without a Schistosoma mansori infection (95% CI 1.2-4.2; p=0.01) (Sokhna et al. 2004).

Additive and synergistic effects of co-infection (malaria and helminth infections) are not fully conclusive (Brooker et al. 2007). Brooker and colleagues reanalyze data from earlier studies to clarify the impact of co-infection on hemoglobin concentrations and anemia, especially among different age groups. Among preschool (n=460) and school children (n=392), the co-infection of hookworms and malaria is associated with lower hemoglobin concentrations than a single infection of malaria or hookworm, adjusting for age, sex, nutritional status, and socio-economic status (preschool children only); hemoglobin concentrations among school children were 4.2 g/L (95% CI: 3.1, 5.2 g/L) lower than those who were infected with either malaria or hookworm (Brooker et al. 2007; Stephenson et al. 1985; Brooker et al. 1996). Utilizing a Bayesian modeling approach, it was found that children aged 10 to 21 years old had lower hemoglobin concentrations than those with a single infection (Koukounari et al. 2008). Koukounari and colleagues demonstrated the need to establish the same relationships among younger age groups. With this in mind, two review articles suggest that more research would be needed to explore the relationship between the additive effect of co-infection of malaria and hookworms (Nacher 2004; Brooker et al. 2007).

The Role of Severe Inflammation

C - reactive protein is a non-specific, highly sensitive protein that combats cell injury. A serum protein from the family of acute-phase proteins (APP), CRP is particularly sensitive to inflammation and infection. CRP is produced by hepatocytes in response to an acute inflammatory attack, which is induced with the help of interleukin-6 (IL-6) and other cytokines such as IL-1, IL-8, interferon-γ, & tumor necrosis factor-α (Epstein et al. 1999). With a normal concentration range of 0-10 mg/L in healthy adults, it can increase a 1000-fold in an inflammatory response. CRP is able to recognize cells that are damaged in order to eliminate them from the system; this protein can activate the complement pathway via opsonization by binding with its ligand, which leads to phagocytosis of the damaged cells (Mold et al. 1999).

In the case of malaria, as the plasmodium ruptures out of the erythrocyte, it activates the monocytes and macrophages to secrete pro-inflammatory cytokines that stimulate the production of CRP (Karunaweera et al. 1992). With the complement mechanism activated, CRP molecules bind to the damaged erythrocytes for clearance, thus leading to hemolysis; with this in mind, the pathophysiology of malaria shows how the concentration of CRP plays a role in the acute phases of this disease (Ansar et al. 2006). These studies suggest an inverse relationship between hemoglobin levels and CRP concentrations in malaria; as CRP levels increase in response to an acute illness, hemoglobin levels subsequently decrease. In fact, CRP concentrations are sometimes used as a biomarker for disease management with malarial infections (Gillespie et al. 1991), and CRP concentrations have been used as markers for severity of disease (Waller 2011).

Since CRP is non-specific, meaning there are a wide range of cellular mechanisms that can increase its concentrations within a cell, high CRP concentrations may play a role in chronic infections as well by indicating periods of re-emergence or co-infection with other helminths. When a child is infected with an intestinal helminth, the parasite may repeatedly induce a variety of inflammatory mediators that produce harmful effects to protein metabolism and cause hemolysis. Helminthic infections may participate in the manifestation of anemia of inflammation (Weiss & Goodnough 2005; Kent et al 1994).

A study by Kung'u and colleagues (2009) suggests that early infection with some helminths is not associated with inflammation in infants, specifically 6 to 23 month-old children in Zanzibar. In this study, a sample of 2300 with a sub-sample of 690 children matched on age and helminth infection status; in addition to determining the type of helminth infection, this study also measured acute phase respondent markers such as alpha-1 glycoprotein (APO) and CRP concentrations. In general, the Kung'u study demonstrated that helminth infection intensities are not associated with either APO or CRP concentrations, each with a p-value greater than 0.05 in most comparisons. However, *Trichuris*-infected children had higher CRP values ($p=0.022$) than other children who were infected with other helminths (Kung'u et al 2009). Thus, in the context of the present study, the elevation of CRP is primarily an indicator of acute disease (except in the case of *Trichuris).* In a study of the co-infection of helminthes and plasmodium parasites, it would be helpful to include CRP to see how it interacts with malaria alone, helminthic infection alone, or co-infection with both.

Another study done in the Nyando district in western Kenya suggests that inflammation plays a large role in anemia (Foote et al. 2013). The purpose of the study was to determine the main determinants for anemia in a sample of 850 children between the ages 6-35 months. Overall, the main three determinants of anemia in the study were malaria, iron-deficiency and inflammation (Foote et al. 2013). A major characteristic associated with severe anemia (hemoglobin concentration less than 7 g/dL) was non-malarial inflammation (measured using CRP and α-1-acid glycoprotein) with a prevalence ratio of 6.7 (95% CI 2.3-14.3) and a p-value less than 0.001; in addition, children who did not have malaria but had severe anemia, 87% had inflammation (Foote et al. 2013). This tells us that there are other factors that play a role in the manifestation of anemia, such as multiple parasitic infections (Ezeamama et al. 2005).

Goal of Present Study

The current investigation was designed to explore some of the effects in children of co-infection with helminthes and the protozoan parasite that causes malaria, *Plasmodium falciparum.* One goal was to elucidate the relationship between helminthic infections and hemoglobin levels among children twelve years old and younger. In addition, it is reasonable to hypothesize that there exists a synergistic effect on hemoglobin when children are infected with both malaria and helminthes. While adjusting for age, gender, and CRP, along with clinical measures indicating severity of disease symptoms, the independent contributions of the co-infection to hemoglobin levels can be identified in a multivariate statistical analysis. Lastly, nutritional status (as

indicated by BMI) is an important characteristic of the host that may influence the impact of these other factors, so this variable was also included in the analysis.

CHAPTER THREE

Hypothesis

Research Questions

With the general objective of exploring the effects of helminthic infection and co-infection with malaria on hemoglobin concentration, along with the modifying effects of the severity of acute inflammation and nutritional status among Luo children, the following hypotheses were tested:

I. Research Question I

Is there a relationship between helminthic infection and hemoglobin levels in Luo children age 12 years old and younger?

Hypothesis:

a. There is a statistically significant relationship between helminthic infection and hemoglobin concentration in Luo children aged 12 and younger.

b. After adjusting for age, gender, CRP, malaria, severity of symptoms and BMI, there is an association between helminthic infection and hemoglobin levels in Luo children aged 12 and younger.

Null hypothesis:

a. There is no association between helminthic infection and hemoglobin concentration among Luo children aged 12 and younger.

b. After adjusting for age, gender, CRP, malaria, severity of symptoms and BMI, there is no association between helminthic infection and hemoglobin levels in Luo children aged 12 and younger.

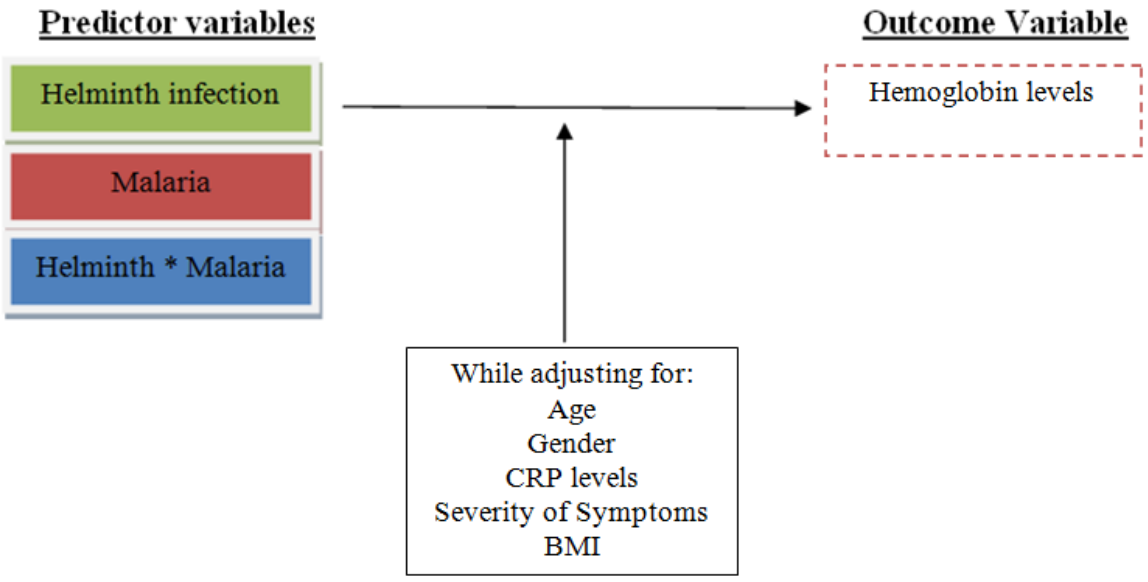
II. *Research Question II:*

Is the co-infection of malaria and helminthic parasites a greater predictor of lower hemoglobin concentration in Luo children aged 12 and younger than malaria or helminthic infection alone?

Hypothesis: Co-infection of malaria and helminthic parasites is a greater predictor for hemoglobin levels in children (age up to 12) than malaria or helminthic infection alone.

Null hypothesis: Co-infection with malaria and helminthic parasites is not a greater predictor for hemoglobin levels in children (age up to 12) than malaria or helminthic infection alone.

Figure 3.1: Study Design



Description of Study

The goal of this cross-sectional study was to elucidate a relationship between helminthic infection and co-infection with malaria as they affect hemoglobin concentration; in addition, the modifying effects of acute inflammation and nutritional status were explored among Luo children in western Kenya. This study utilized previously collected clinical data from a temporary clinic on the Nyakach Plateau in the Nyanza province and explored the relationship between malaria, C-reactive protein levels, and worm infections on hemoglobin levels among children aged 0 to 12 years.

CHAPTER FOUR

Methods

Setting

The original data for this study were previously collected during the last two weeks in May of 2010 from Luo patients who were seen in a temporary clinic located in the Upper Nyakach Division of western Kenya. This annual clinic is provided by members of the U.S.-based Straw to Bread non-profit organization for adults and children on this high, rocky plateau composed of very poor, subsistence farmers with a high prevalence of tropical diseases, HIV, and malnutrition. Luo is the second largest ethnic group in Kenya, and most of the population lives in the area near Lake Victoria.

Sample

The data for this study were abstracted from the medical records of 227 children 12 years of age and younger. Blood was drawn on all children who came to the clinic as patients in order to establish baseline prevalence for malaria, anemia, CRP, and a blood chemistry panel (not included in this analysis). Data from all subjects who met these criteria were included in the study.

Variable Measurements

The original measurements of the variables of interest were done by professional lab technicians and trained volunteers. In addition, patients were evaluated and treated by one U.S.-trained, board certified pediatrician.

Measurements include all the data collected from clinical observation notes, height and weight, and lab results. Each patient in the present sample had their blood collected, which was utilized for the analysis of CRP and a malaria blood smear. In this present study, the main outcome variable is hemoglobin concentration, which was measured from a chem-8 panel. The Chem-8 panel was completed using Abbott's i-STAT device. With this machine, hemoglobin was measured between 4.3 and 25.5 g/dL. The other measurements of the chem-8 panel were not utilized in this study.

Helminthic infection was diagnosed based on physical findings and subsequent clinical diagnosis by the physician as recorded on the progress note.

Severity of symptoms was defined with patients presenting the following observations in the physical exam: lethargic, wasted, malnourished, or show splenomegaly or hepatomegaly. In addition, severity of symptoms included patients who were diagnosed with seizures or dehydration or have temperatures greater than or equal to 38.5ºC (101.3ºF).

To study the role of inflammation, C-reactive protein was measured. CRP measurements were based on the blood sample from each patient. CRP was measured by QuikRead CRP, manufactured by Orion Diagnostica. This machine reads CRP values in a quantitative manner and is sensitive from 5mg/L to 180mg/L.

From the single venous blood sample, a malarial blood smear was analyzed by a single experienced lab technician using light microscopy. In this analysis, malaria is defined as a positive result on a rapid diagnostic test, positive blood smear with Field's stain, or a clinical diagnosis of malaria made by the physician and treatment with anti-malarial medication.

To study nutritional status, Body Mass Index (BMI) was utilized. On the statistical software SAS, BMI was calculated as mass (kg) / height (m^2). Weight was measured using a locally purchased floor scale, and height was measured using a tape measure with the children standing against a wall or lying down if the child was not yet walking. In addition, BMI quartiles were set up based on the BMI of the entire sample. The first quartile consisted of patients with a BMI in the lowest 25% and so forth.

Anemia was defined according to the World Health Organization's cutoffs (WHO 2011). Children 6 to 59 months of age and 5 to 11 years of age are considered anemic with a hemoglobin concentration lower than 11.0 g/dL and 11.5 g/dL respectively. The World Health Organization did not specify anemia cutoffs according to gender.

Statistical Analysis:

Data from patients were double-entered into Microsoft Excel and analyzed using the SAS 9.0 software. Alpha was set to 0.05.

Frequencies were run on all variables, and percentages were calculated for discrete variables. Descriptive statistics for continuous variables included the mean, median, standard deviation, and range. Bivariate analyses included Chi-square for

categorical variables and t-test for continuous variables. Multivariate analyses utilized multiple regression and analysis of variance (ANOVA).

IRB:

Since this study is analyzed using previously collected data gathered for clinical purposes, The Baylor University Institutional Review Board categorized it as exempt, and it was approved as such.

CHAPTER FIVE

Results

Descriptive Statistics

Table 5.1 gives the mean, standard deviation, and range for the continuous variables studied in this investigation. With a sample size of 227, the average hemoglobin level of the entire sample is 12.24 (95% CI 12.02-12.46) g/dL).

Table 5.1a: Univariate Statistics for Hemoglobin, Age, CRP, and BMI

n=227	Mean	SD	Range
Hgb (g/dL)	12.24 (12.02 - 12.46)	1.69	3.4-15.6
Age	5.61 (5.13 - 6.09)	3.66	0.17-12
CRP (mg/L)	24.28 (18.53 - 30.03)	44.02	0-180
BMI	14.82 (14.49 - 15.16)	2.5	5.74-26.98

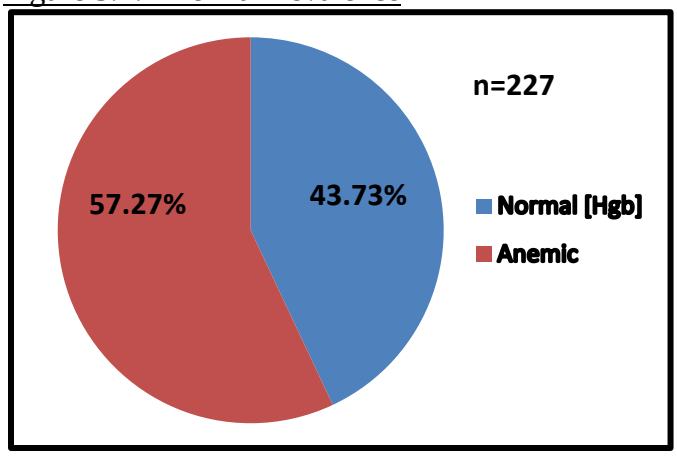
Parentheses indicate 95% Confidence intervals

Table 5.1b: Discrete Variables

n=227	%(n)
Malaria	49.78 (113)
Helminths	41.85 (95)
Severity of symptoms	26.87 (61)

As mentioned in the methods section, anemia was defined according to the World Health Organization's definition of anemia (Worldwide Prevalence of Anemia 1993-2005). In our sample, 57.27% of the children 12 years of age and under were anemic (Figure 5.1).

Figure 5.1: Anemia Prevalence



Figures 5.2 and 5.3 provide more detail about anemia. Subjects' values were plotted against normal values for hemoglobin levels for age and gender, which were obtained from the Harriett Lane Pediatric Growth Charts (Custer & Rau 2009). In addition, the minimum and maximum values for each age group demonstrate the wide range of hemoglobin levels seen in the clinic, especially with children older than 5 years old.

Figure 5.2: Average Hemoglobin Concentration in Boys v Age

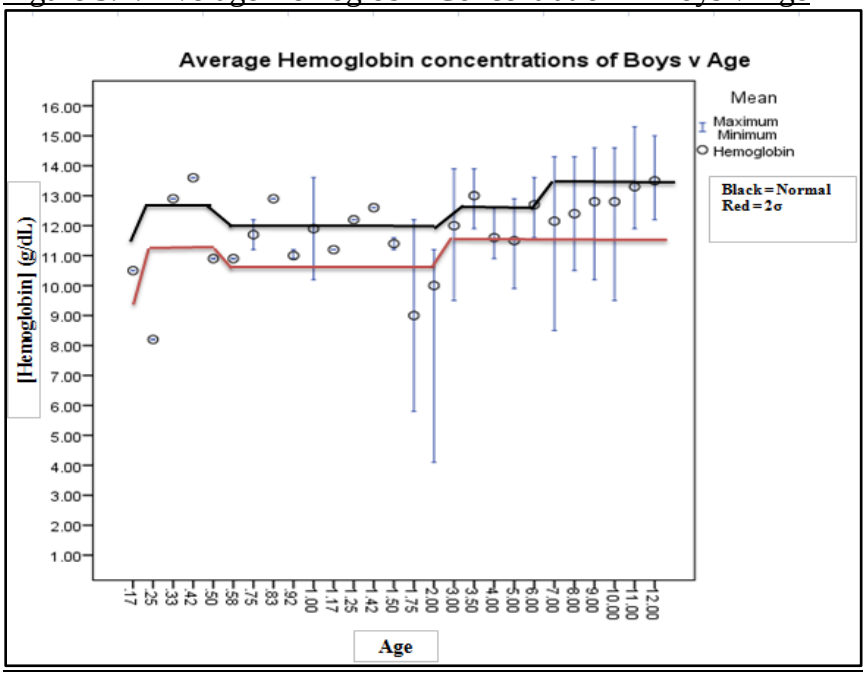
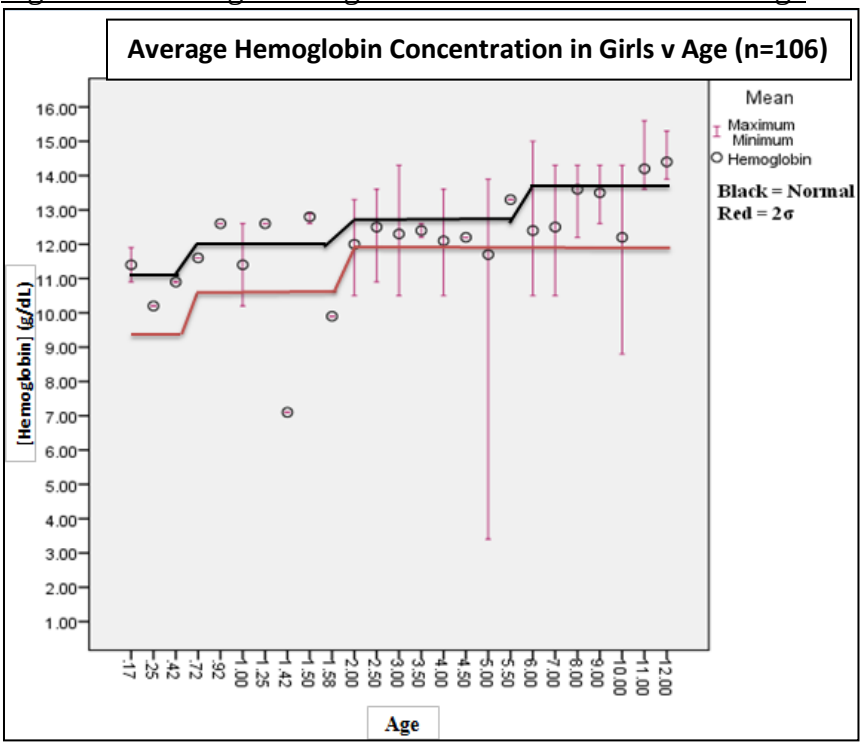


Figure 5.3: Average Hemoglobin Concentration in Girls v Age



Average hemoglobin levels are consistently lower than the normal values in both genders (Figure 5.2 & 5.3). In some age groups, we see that the average hemoglobin concentrations are more than two standard deviations below the normal values. In Figure 5.2, we can see that the average hemoglobin concentration for boys at age 4 is 11.6 g/dL. Though this value is only slightly above the WHO cut-off for anemia, this value is two standard deviations below the normal value of hemoglobin.

Table 5.2 stratifies the univariate data by gender. In this study, there was no significant difference between the hemoglobin levels of boys and girls (p=0.2505).

Table 5.2: Bivariate Statistics Stratified by Gender for Hemoglobin, Age, CRP, & BMI

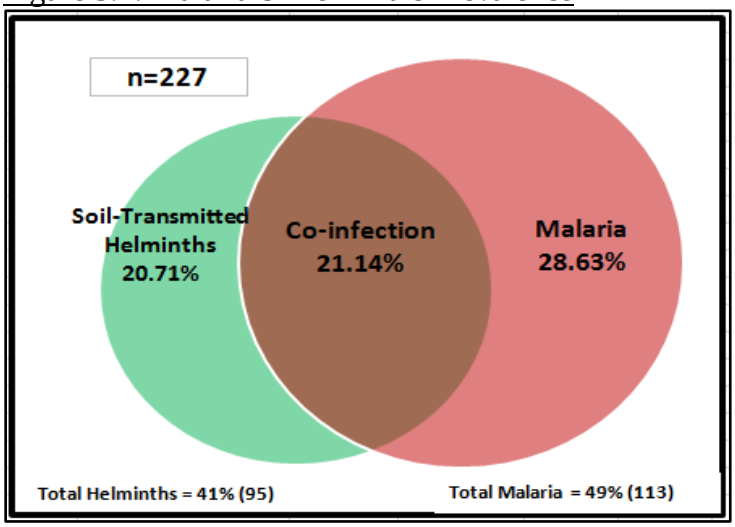
n=227	Boys			Girls				
	Mean	SD	Range	Mean	SD	Range	t	p
Hgb (g/dL)	12.13 (11.82 - 14.44)˜	1.68	4.1-15.3	12.39 (12.06 - 12.72)˜	1.71	3.4-15.6	-1.15	0.2505
Age	5.84 (5.13 - 6.55)˜	3.91	0.17-12	5.49 (4.84 - 6.15)˜	3.35	0.17-12	0.7	0.4873
CRP (mg/L)	27.64 (18.69 - 36.59)˜	49.3	0-180	20.88 (12.82 - 27.35)˜	27.4	0-180	1.28	0.2034
BMI	14.99 (14.48 -15.50)˜	2.72	5.74-26.98	14.56 (14.14 - 14.99) ˜	2.12	7.75-19.8	1.26	0.2104

˜: Parentheses indicate 95% Confidence intervals

Table 5.2b: Discrete Variables Stratified by Gender

Percentage of entire sample (n)		
n=227	**Boys**	**Girls**
Malaria	27.31 (62)	22.46 (51)
Helminths	21.14 (48)	20.71 (47)
Severity of symptoms	15.42 (35)	11.01 (25)
Helminth + Malaria	10.57 (24)	10.13 (23)
Helminth + Severity of symptoms	5.72(13)	2.20 (5)

Figure 5.4: Malaria & Helminths Prevalence



It is important to note that each category is not mutually exclusive. There were some children that were counted as having a helminthic infection and malaria, thus counted as having co-infection. In addition, the size of each circle in the Venn diagram is proportional to the frequency of that variable. Overall, the majority (69%) of the children within the sample had either malaria or a helminthic infection; children within the sample had either malaria (28%) or a helminthic infection (20%) alone. About 21% of the sample harbored a helminthic infection and malaria at the time of the study. It is also interesting to note that there were more children co-infected with malaria and helminths than helminths alone.

Inferential Statistics

First Hypothesis. The first hypothesis was that there would be a relationship between helminthic infection and hemoglobin levels in Luo children age 12 years old and younger. It was found that there is no statistically significant relationship between the two variables (p=0.7934).

Table 5.3: Hemoglobin Concentration Regressed on Helminths Infection

Overall model: n=227; F=0.07; p=0.7934 r^2=0.0003				
Variable	**Regression Coefficient**	**Standard Error**	**t**	**p**
Intercept	12.22	0.147	82.94	<0.0001
Helminths	0.059	0.228	0.26	0.7934

Hemoglobin level was then regressed on age, gender, CRP, malaria, symptom severity, BMI, and the presence of a helminthic infection. According to the data on Table 5.4, the overall model is statistically significant with a p value less than 0.0001. However, the presence of a helminthic infection was not a statistically significant variable.

However, age and CRP were very significant variables with a p value less than 0.0001. In Table 5.4, it is interesting to note the negative relationship between CRP and hemoglobin concentrations. As one would expect, lower CRP (acute inflammation) was associated with higher hemoglobin concentrations. Also, by adjusting for all the listed variables, we were able to explain almost 30% of the variance in hemoglobin (r^2=0.2979).

Table 5.4: Hemoglobin Concentrations Regressed On All Variables

Overall model: n=227; F=13.67; p<0.0001; r^2=0.2979				
Variable	**Regression Coefficient**	**Standard Error**	**t**	**p**
Intercept	11.121	0.707	15.72	<0.0001
Age	0.216	0.028	7.61	<0.0001
CRP	-0.012	0.003	-4.94	<0.0001
Gender	0.205	0.199	1.03	0.3052
Malaria	-0.351	0.221	-1.58	0.1148
Helminths	-0.117	0.205	-0.57	0.5674
Symptom severity	-0.025	0.246	-0.1	0.9178
BMI	0.023	0.041	0.58	0.5644

Second hypothesis. Despite there being no statistically significant association between the presence of a helminthic infection and hemoglobin concentrations, the co-infection of malaria and helminthic infection is the relationship of interest. The second hypothesis states that the co-infection of malaria and helminthic infections is a greater predictor for lower hemoglobin level than malaria or helminth alone with children 12 years old and younger. Adjusting for age, gender, severity of symptoms, BMI, and CRP concentration, three multivariate regression models were analyzed, each testing a pathogenic condition: malaria alone, helminth alone, and malarial-helminth co-infection. Table 5.5 summarizes the three models tested. In each multivariate analysis, the models were all statistically significant overall with a p value less than 0.0001, and they explain about 30% of the variance in hemoglobin. Malaria alone and helminthic infection alone were not significant contributors to their models, each with a p value of 0.09 and 0.45 respectively. However, malarial-helminthic co-infection played a statistically significant role in its respective regression model with a p value of 0.02. With this in mind, the data on Table 5 support the rejection of the null hypothesis. The data indicated that the

malarial-helminth co-infection was a greater predictor for lower hemoglobin concentrations than either infection alone.

Table 5.5: Three Multivariate Regression Models: Hemoglobin Regressed on Predictor Variables Stratified by Helminths, Malaria, and Co-Infection

Model n=227	Variable	Regression Coefficient	Standard Error	t	P
Helminths alone	**Intercept**	10.943	0.701	15.61	<0.0001
	Age	0.211	0.028	7.45	<0.0001
	Gender	0.204	0.2	1.02	0.3075
	CRP	-0.014	0.002	-5.69	<0.0001
	Helminths	-0.153	0.204	-0.75	0.4526
	Symptom severity	-0.241	0.241	-0.46	0.6459
	BMI	0.03	0.041	0.74	0.4616
	Overall model: F=15.41; p<0.0001: r^2=0.2927				
Malaria alone	**Intercept**	11.055	0.697	15.87	<0.0001
	Age	0.218	0.028	7.72	<0.0001
	Gender	0.201	0.199	1.01	0.3128
	CRP	-0.012	0.002	-4.92	<0.0001
	Malaria	-0.364	0.219	-1.66	0.0982
	Symptom severity	-0.005	0.243	-0.02	0.9832
	BMI	0.024	0.041	0.58	0.5596
	Overall model: F=15.94; p<0.0001: r^2=0.3002				
Helminths * Malaria	**Intercept**	11.394	0.982	11.6	<0.0001
	Age	0.206	0.035	5.75	<0.0001
	Gender	0.284	0.266	1.07	0.2888
	CRP	-0.006	0.004	-1.56	0.1218
	Helminths*Malaria	-0.652	0.275	-2.37	0.0196
	Symptom Severity	0.181	0.395	0.46	0.6472
	BMI	-0.008	0.058	-0.15	0.8837
	Overall model: F= 7.23; p<0.0001 r^2=0.2644				

The data on Table 5.6 confirmed the interaction of helminthic and malarial infections second hypothesis in another way. After stratifying the data by the presence or absence of helminthic infection, malaria plays a statistically significant role in the regression model only when a helminthic infection is present.

Table 5.6: Hemoglobin Regressed on Predictors Stratified by Presence of Helminths

With Helminths Infection: n=95; F=6.86; p<0.0001; r^2=0.2810				
Variable	**Regression Coefficient**	**Standard Error**	**t**	**p**
Intercept	11.505	0.972	11.84	<0.0001
Age	0.202	0.045	4.45	<0.0001
CRP	-0.002	0.005	-0.52	0.6039
Gender	0.5411	0.257	2.1	0.0384
Malaria	-0.986	0.264	-3.74	0.0003
Symptom severity	-0.219	0.339	-0.65	0.5201
BMI	-0.003	0.059	-0.04	0.9657
Without a Helminths Infection: n=132; F=11.65; p<0.0001; r^2=0.3514				
Variable	**Regression Coefficient**	**Standard Error**	**t**	**p**
Intercept	10.709	0.948	11.29	<0.0001
Age	0.221	0.036	6.11	<0.0001
CRP	-0.017	0.003	-5.4	<0.0001
Gender	-0.108	0.291	-0.37	0.7098
Malaria	0.194	0.348	0.56	0.5775
Symptom severity	0.007	0.361	0.02	0.9841
BMI	0.049	0.055	0.9	0.3677

Table 5.6 also demonstrates that among those with a helminthic infection, gender was a statistically significant variable in the model with a p value of 0.03. With this in mind, the data were furthered stratified by gender and the presence of helminths. The results of this analysis (see Table 5.7) further explain the role of helminths and malaria in these children. All multivariate regression models were statistically significant with the explained variance ranging from 0.29 to almost 0.50. The only regression model in which malaria was a statistically significant variable was in the model for boys with helminth infections, confirming an interaction between helminthes, malaria, and gender. Although it was suspected that malaria would also be a statistically significant in girls with helminths, only CRP and age were significant. This could be due to a sampling bias. The

difference in the variance is notable between models with and without the presence of helminths in boys. Having a helminthic infection, the model can explain about 20% of the variance in hemoglobin; without a helminthic infection, the explained variance increases to 43%.

Table 5.7: Hemoglobin Regressed on Predictors Stratified by Gender and Helminths

n=227		Boys				Girls			
Helminth (+)	Overall model: F=3.22; p=0.0158; r^2=0.2012; n=47					Overall model: F=5.07; p=0.0011; r2=0.3113; n=47			
	Variable	Regression Coefficient	Standard Error	t	p	Regression Coefficient	Standard Error	t	p
	Intercept	10.909	1.371	7.96	<0.0001	12.798	1.365	9.37	<0.0001
	Age	0.198	0.069	2.85	0.007	0.237	0.059	3.99	0.0003
	CRP	0.004	0.006	0.77	0.4441	-0.01	0.005	-1.87	0.0683
	Malaria	-1.337	0.422	-3.17	0.003	-0.63	0.329	-1.91	0.0627
	Symptom Severity	-0.421	0.446	-0.94	0.3525	0.112	0.565	0.2	0.8437
	BMI	0.046	0.083	0.56	0.5783	-0.073	0.088	-0.83	0.409
Helminth (-)	Overall model: F=10.80; p<0.0001; r^2=0.4298: n=72					Overall model: F=4.88; p=0.0011; r2=0.2718; n=57			
	Intercept	11.355	1.092	10.39	<0.0001	9.6	1.622	5.92	<0.0001
	Age	0.205	0.041	4.96	<0.0001	0.244	0.067	3.61	0.0007
	CRP	-0.013	0.003	-4.03	0.0002	-0.023	0.007	-3.54	0.0009
	Malaria	0.181	0.405	0.45	0.6567	0.278	0.622	0.45	0.6563
	Symptom Severity	-0.465	0.445	-1.05	0.3	0.473	0.597	0.79	0.4322
	BMI	0.015	0.062	0.25	0.807	0.106	0.104	1.01	0.3155

Additional Findings:

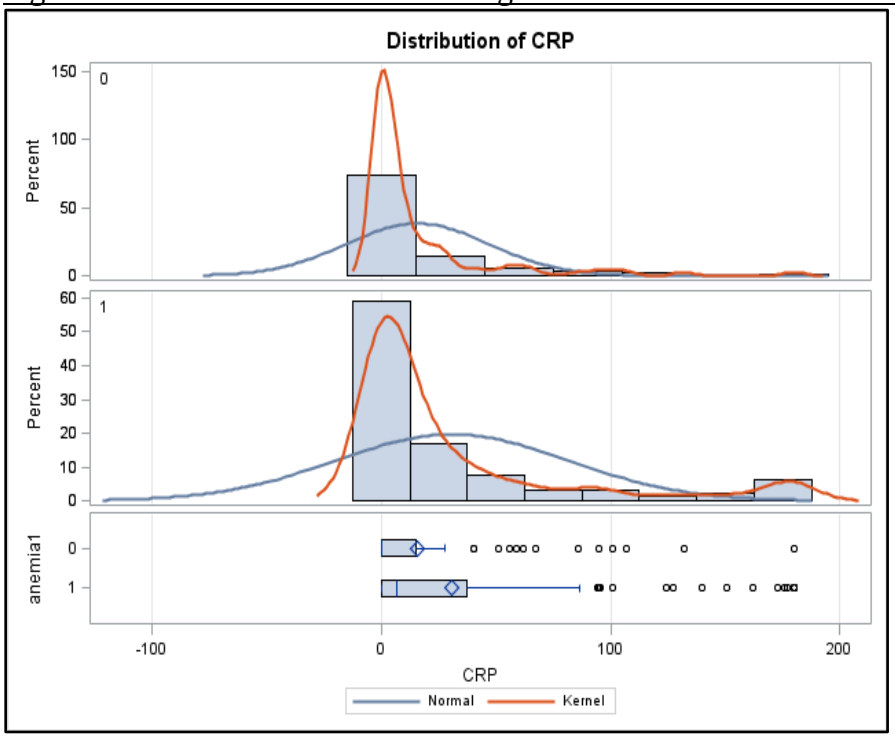
Acute inflammation (as measured by CRP) appears to play a somewhat complex role in the relationship of both of these infections and hemoglobin. A t-test demonstrates that children with anemia have significantly higher CRP concentrations than those

without anemia ($p = 0.0103$, $t=-2.59$). This finding is illustrated in Figure 5.5. Table 5.4 demonstrates that CRP is still a statistically significant variable when hemoglobin level is regressed on all of the predictor variables tested. However, the role of inflammation is elucidated when the models are stratified by the presence of helminthes. In Table 5.6, CRP is only statistically significant in the model without a helminthic infection, and malaria is only significant when helminthes are present. In other words, when helminthes are absent, acute inflammation is a significant predictor. However, the effects of malaria are the opposite, and, given that malaria is an acute infection, these relationships are not completely clear. Since CRP is high in malaria and in other acute infections, there may be some further distinctions to be made. However, it is not possible with the current sample size to determine whether non-malarial acute infections are independently impacting hemoglobin. It is more likely that the overlap between malaria and CRP and their particular distribution in this sample of boys and girls causes one or the other to be significant.

This relationship is further complicated by the fact that the same trend is present for boys and girls, but it is much more marked among boys. In fact, the presence or absence of helminthic infection makes a much bigger impact among boys in the ability of the variables to explain the variance in hemoglobin: r^2=.20 (presence) vs. r^2=.43 (absence) in boys compared to r^2 =.31 (presence) vs. r^2=.27 (absence) in girls.

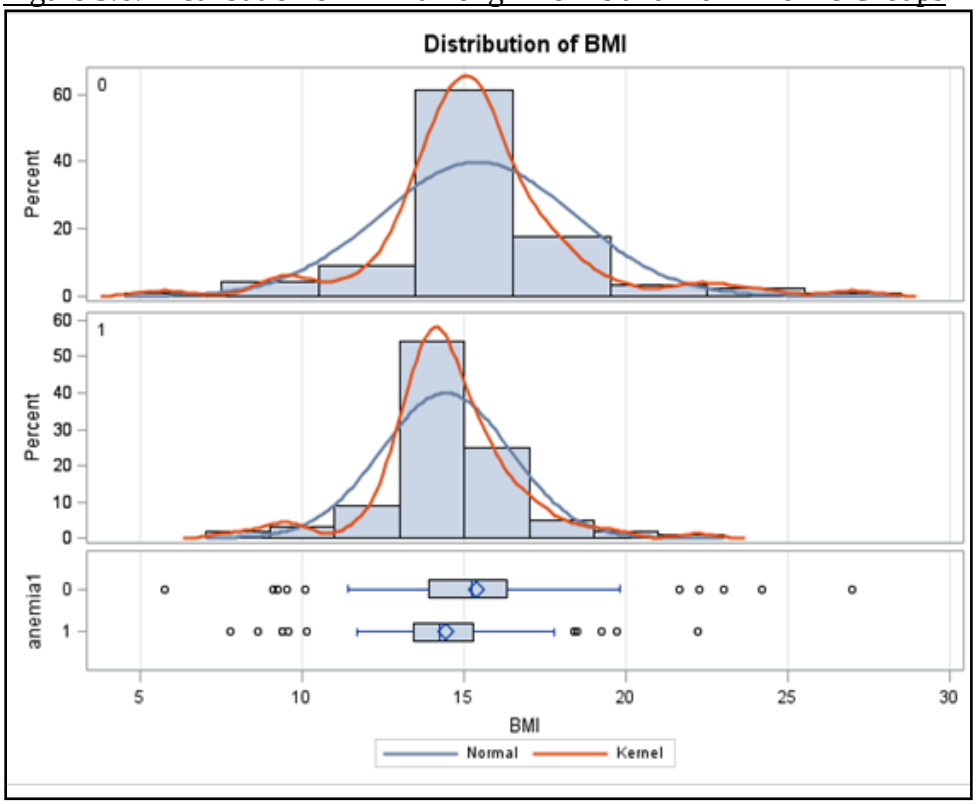
The other influence on hemoglobin levels in this sample is age, and it is the older children who have higher hemoglobin when controlling for all of the other predictors. This relationship remains robust throughout all the models regardless of stratification by helminthes and gender.

Figure 5.5: Distribution of CRP Among Non-Anemic and Anemic Groups



In this cross sectional study, body-mass index (BMI) was utilized as a surrogate measure for nutritional status. A t-test demonstrated that BMI is negatively associated with anemia when considered independently ($p = 0.005$, $t = 2.82$). However, BMI was not significant when it was combined with other variables using multiple regression to explain the variance in hemoglobin. Future analysis using standardized BMI scores for age and gender may clarify this relationship.

Figure 5.6: Distribution of BMI among Anemic and Non-Anemic Groups



CHAPTER SIX

Discussion

Anemia continues to be a problem on the Nyakach Plateau, especially with children. In this sample, about 57% of children 12 years of age and younger were anemic. The long-term consequences of anemia push the need for more research and community involvement to alleviate this condition. Infectious diseases play a role in the manifestation of anemia, especially malaria and helminthic infections, which are highly prevalent in sub-Saharan Africa. According to our data, about 21% of the children in the sample harbored both malarial and helminthic parasites. A comparison of three multivariate regression analyses, co-infection of malaria and helminthes was demonstrated to be a greater predictor for lower hemoglobin concentrations than either malaria or helminthic infection alone. Stratification by the presence of helminths showed that gender and CRP add another layer to the relationship between malaria-helminthic infections and hemoglobin concentrations. In a multivariate regression, it was shown that CRP is statistically significant in the absence of helminths, while gender is a statistically significant variable in the presence of helminths. Since CRP measures acute inflammation, it makes sense that CRP is not the main factor in the presence of a helminthic infection. In another multivariate hemoglobin regression, the results were stratified by both gender and the presence of helminths; it was found that malarial-helminth co-infection plays a larger role in boys. Furthermore, the presence of helminths had a greater impact on boys, and there was a larger difference in variance between genders.

Within this clinical sample of 227 children, the majority of children (69%) had either malaria or harbored a helminth parasite alone. It is alarming that 57.27% are anemic and 21.14% are co-infected with malaria and helminthes. These numbers may not be a representative sample of the general population of children, but the prevalence is still alarming. Field studies would be useful to determine an accurate prevalence of helminths and other parasites in the region.

Primary Hypothesis

According to our data, there was no statistically significant relationship between helminths alone and hemoglobin concentration (Table 5.3). Helminths in this study probably included a wide range of helminthic species, ranging from *Ascaris lumbricoides* to hookworms. It is plausible that some of the pathogens may have affected anemia more than others, so that the relationship was not as strong as it would have been otherwise. This lack of a precise diagnosis of which helminth is causing infection is a weakness in the validity of the helminth variable. Adjusting for age, gender, acute inflammation, nutritional status, and symptom severity made the overall relationship statistically significant; however, the presence of helminths itself was still not statistically significant (Table 5.4). In the regression model from Table 5.4, C-reactive protein was the only statistically significant variable, suggesting that acute inflammation plays a role in the absence of helminths. According to the model, as the C-reactive protein concentration increases, hemoglobin concentrations decrease, indicating more anemia with acute

inflammation. In the absence of chronic helminth infections, acute illnesses account for the majority of the variance in hemoglobin concentrations.

Secondary Hypothesis

The second hypothesis tested the effects of malarial-helminth co-infection. Malarial-helminth co-infection has a bigger impact on the variation in hemoglobin concentrations than malaria or helminths alone (overall model $p=0.0001$, $r^2=0.2644$). With this level of statistical significance, it is unlikely that this correlation is due to random error. In our sample, 26% of the variance in hemoglobin could be explained when considering the interaction of both pathogens. Stratifying the analysis by helminths also demonstrated that there is an interaction between the two infections in modifying hemoglobin concentrations. Malaria appears to be statistically significant only in the regression model in which helminths are present (Table 5.6). Although these two pathogens act independently, the data suggests that their effects are also synergistic in increasing the risk for anemia. Hypothesized mechanisms suggest that chronic helminth infections induce a T-helper 2 cell cytokine response, which may alter and hinder a pro-inflammatory response to an initial malarial infection, meaning that helminths help the *Plasmodium falciparum* parasite follow its course and lower hemoglobin levels (Geiger et al. 2002; Bouharoun-Tayoun & Druilhe 1992). It is also interesting to note that in these models, the variables account for about 30% of the variance in hemoglobin.

Despite the fact that in some things boys and girls were relatively the same, there were notable differences in the regression analysis for each gender when they were

considered separately. Stratifying by gender and the presence of helminths, malaria and age were the main factors among boys; however, malaria was not the statistically significant among girls with helminths. This finding suggests that boys may be at a higher risk for co-infection than girls in the Nyakach Plateau, although this pattern is not generally seen in the literature. Further studies should be done in order to elucidate disparities in infections between genders. Moreover, the presence of helminths made a larger impact on boys than in girls; the variance almost doubles in boys after helminthes are removed from the model. The variables in the equation for boys without helminths accounted for 43% of the variance in hemoglobin concentrations within the sample.

C - reactive protein, a biomarker for acute inflammation, is only statistically significant in the absence of helminths. In each regression model, (Table 5.6, 5.6 & 5.7), CRP is statistically significant in the absence of helminths. This pattern demonstrates the importance of acute inflammation when helminthic infection is absent. In addition, this finding demonstrates that chronic helminth infections may suppress an immunological response, thereby increasing the risk for other infections. With this in mind, it is essential to characterize the type of helminth species in order to draw precise relationships.

Limitations of the Study

As a cross-sectional study, our inference was restricted to correlation rather than the more robust conclusions that could be drawn from a longitudinal study. With a clinical sample of 227 children under 13 years old, it is difficult to generalize across other populations that may be infected with helminths and malaria. In addition, the results can

only be generalized across children under 13 years of age who live in tropical climates that favor the survival of these particular strains of parasites.

It is important to acknowledge the possibility of random error in our findings; however, most regression models yielded p values equal or less than 0.0001, except in the case of the relationship between helminths alone and hemoglobin concentrations. With this in mind, it is very unlikely that the main findings are due solely to chance. With alpha set to 0.05, malarial-helminth co-infection ($p=0.0196$) is a statistically significant variable in the hemoglobin regression model ($p<0.0001$).

A source for systematic error in the sample included the method of diagnosis of malaria and helminths. A subject was considered to have malaria by any one of several methods: the diagnosis was made clinically by a physician based on signs and symptoms, or a lab diagnosis was made using a rapid diagnostic blood test or by a single experienced lab technician using light microscopy. As a result, there is the possibility of misdiagnosis in the laboratory or in the clinic. The combination of these methods was used in the clinic because of the high volume of patients and the inadequacy of supplies that made it impossible to have independent verification by all three methods. This practice may have over- or under-diagnosed children with malaria, but the use of all three methods to identify malaria patients for the purpose of this study may have inflated the estimated prevalence. The diagnosis of helminths was based on the physician's physical findings and subsequent clinical diagnosis. A definitive diagnosis would have required stool samples and examination under a microscope after a time-intensive slide preparation.

This process was not realistic in a very busy, temporary clinic. As a result, it was impossible to determine the helminth species that afflicted each patient.

The main micronutrient deficiency contributing to anemia is iron, which was not measured in the current sample. To get an accurate measure of iron status, it is necessary to assess blood ferritin and transferrin in addition to iron. This is an expensive lab test that requires sophisticated equipment that was not available in our setting. In future investigations, iron status could provide a bigger picture of the variance of hemoglobin concentrations in children under 13 years old.

BMI was the surrogate measure for nutritional status. The BMI variable contained some systematic errors; for instance, there were several patients that had to be taken out of the sample due to errors in height and weight measurement. In retrospect, this may explain why the BMI variable was not statistically significant in the regression models.

Conclusion

The aim of this investigation was to elucidate the effect of malarial-helminth co-infection on hemoglobin concentrations among Luo children living on the Nyakach Plateau. By establishing these correlations, it can help guide the implementation of future interventions and studies that seek to alleviate the burden of the long term effects of anemia in children. Children who come into the clinic presenting with symptoms of malaria and helminths are at greater risk for lower hemoglobin concentrations. As a result, they are especially prone to the deleterious effects of anemia such as poor school performance and growth impediment (Sakti et al. 1999; Walker et al. 2007). In effect, it may hinder community development and create economic stagnation. In summary, it is

suggested that boys have the highest risk for co-infection overall; however, both genders would benefit from an integrated program that seeks to control both helminths and malaria.

BIBLIOGRAPHY

Akhwale, W. S. et al. "Anemia and Malaria at Different Altitudes in the Western Highlands of Kenya." *Acta Tropica* 91.2 (2004): 167–175. *ISI Web of Knowledge*. Web.

Alemu, Abebe et al. "Malaria Helminth Co-Infections and Their Contribution for Aneamia in Febrile Patients Attending Azzezo Health Center, Gondar, Northwest Ethiopia: A Cross Sectional Study." *Asian Pacific Journal of Tropical Medicine* 5.10 (2012): 803–809. *ISI Web of Knowledge*. Web.

Ansar, W. et al. "Role of C-Reactive Protein in Complement-Mediated Hemolysis in Malaria." *Glycoconjugate Journal* 23.3-4 (2006): 233–240. *ISI Web of Knowledge*. Web.

Bates, Imelda, Stephen McKew, and Faruk Sarkinfada. "Anaemia: A Useful Indicator of Neglected Disease Burden and Control." *Plos Medicine* 4.8 (2007): 1285–1290. *ISI Web of Knowledge*. Web.

Benoist, B. de et al. "Worldwide Prevalence of Anaemia 1993-2005: WHO Global Database on Anaemia." (2008): vi + 41 pp. Print.

Bouharountayoun, H., and P. Druilhe. "Plasmodium-Falciparum Malaria - Evidence for an Isotype Imbalance Which May Be Responsible for Delayed Acquisition of Protective Immunity." *Infection and Immunity* 60.4 (1992): 1473–1481. Print.

Bradman, A. et al. "Iron Deficiency Associated with Higher Blood Lead in Children Living in Contaminated Environments." *Environmental Health Perspectives* 109.10 (2001): 1079–1084. *ISI Web of Knowledge*. Web.

Brooker, S. et al. "The Epidemiology of Hookworm Infection and Its Contribution to Anaemia among Pre-School Children on the Kenyan Coast." *Transactions of the Royal Society of Tropical Medicine and Hygiene* 93.3 (1999): 240–246. *ISI Web of Knowledge*. Web.

Brooker, Simon, Willis Akhwale, et al. "Epidemiology of Plasmodium-Helminth Co-Infection in Africa: Populations at Risk, Potential Impact on Anemia, and Prospects for Combining Control." *American Journal of Tropical Medicine and Hygiene* 77.6 (2007): 88–98. Print.

Brooker, Simon, Archie C. A. Clements, et al. "The Co-Distribution of Plasmodium Falciparum and Hookworm among African Schoolchildren." *Malaria Journal* 5 (2006): n. pag. *ISI Web of Knowledge*. Web.

Brooker, Simon, Archie CA Clements, and Don AP Bundy. “Global Epidemiology, Ecology and Control of Soil-Transmitted Helminth Infections.” *Advances in parasitology* 62 (2006): 221–261. *PubMed Central*. Web. 10 Feb. 2013.

Custer, Jason W., and Rachel E. Rau. "Pediatric Growth Charts." *The Harriet Lane Handbook: A Manual for Pediatric House Officers*. Philadelphia, PA: Mosby/Elsevier, 2009. N. pag. Print.

De Silva, Nilanthi R et al. “Soil-Transmitted Helminth Infections: Updating the Global Picture.” *Trends in Parasitology* 19.12 (2003): 547–551. *ScienceDirect*. Web. 10 Feb. 2013.

Degarege, Abraham et al. “Malaria and Helminth Co-Infections in Outpatients of Alaba Kulito Health Center, Southern Ethiopia: A Cross Sectional Study.” *BMC Research Notes* 3.1 (2010): 143. *www.biomedcentral.com*. Web. 28 Oct. 2013.

Druilhe, P., A. Tall, and C. Sokhna. “Worms Can Worsen Malaria: Towards a New Means to Roll Back Malaria?” *Trends in Parasitology* 21.8 (2005): 359–362. *ISI Web of Knowledge*. Web.

Epstein, Franklin H., Cem Gabay, and Irving Kushner. “Acute-Phase Proteins and Other Systemic Responses to Inflammation.” *New England Journal of Medicine* 340.6 (1999): 448–454. *CrossRef*. Web. 22 Feb. 2014.

Ezeamama, A. E. et al. “Functional Significance of Low-Intensity Polyparasite Helminth Infections in Anemia.” *Journal of Infectious Diseases* 192.12 (2005): 2160–2170. *ISI Web of Knowledge*. Web.

Foote, Eric M. et al. “Determinants of Anemia among Preschool Children in Rural, Western Kenya.” *American Journal of Tropical Medicine and Hygiene* 88.4 (2013): 757–764. *ISI Web of Knowledge*. Web.

Friis, H. et al. “Serum Retinol Concentrations and Schistosoma Mansoni, Intestinal Helminths, and Malarial Parasitemia: A Cross-Sectional Study in Kenyan Preschool and Primary School Children.” *The American Journal of Clinical Nutrition* 66.3 (1997): 665–671. Print.

Geerligs, P. D. P., B. J. Brabin, and T. A. Eggelte. “Analysis of the Effects of Malaria Chemoprophylaxis in Children on Haematological Responses, Morbidity and Mortality.” *Bulletin of the World Health Organization* 81.3 (2003): 205–216. Print.

Geiger, S. M. et al. “Cellular Responses and Cytokine Profiles in Ascaris Lumbricoides and Trichuris Trichiura Infected Patients.” *Parasite Immunology* 24.11-12 (2002): 499–509. *ISI Web of Knowledge*. Web.

Gillespie, Sh et al. “Measurement of Acute Phase Proteins for Assessing Severity of Plasmodium-Falciparum Malaria.” *Journal of Clinical Pathology* 44.3 (1991): 228–231. *ISI Web of Knowledge*. Web.

Goodburn, Elizabeth A., David A. Ross, and World Health Organization Adolescent Health Programme. “A Picture of Health? : A Review and Annotated Bibliography of the Health of Young People in Developing Countries / Undertaken by Elizabeth A. Goodburn and David A. Ross.” N. p., 1995. Web. 8 Aug. 2013.

Greenwood, Brian M. et al. “Malaria: Progress, Perils, and Prospects for Eradication.” *Journal of Clinical Investigation* 118.4 (2008): 1266–1276. *ISI Web of Knowledge*. Web.

Guyatt, H. “Do Intestinal Nematodes Affect Productivity in Adulthood?” *Parasitology Today* 16.4 (2000): 153–158. *ISI Web of Knowledge*. Web.

Haghighi, Lotfali. “C-Reactive Protein in Malaria.” *Journal of Clinical Pathology* 22.4 (1969): 430–432. Print.

Hall, A et al. “Anaemia in Schoolchildren in Eight Countries in Africa and Asia.” *Public health nutrition* 4.3 (2001): 749–756. Print.

Hartgers, F. C., and M. Yazdanbakhsh. “Co-Infection of Helminths and Malaria: Modulation of the Immune Responses to Malaria.” *Parasite Immunology* 28.10 (2006): 497–506. *ISI Web of Knowledge*. Web.

Hay, S. I. et al. “The Global Distribution and Population at Risk of Malaria: Past, Present, and Future.” *Lancet Infectious Diseases* 4.6 (2004): 327–336. *ISI Web of Knowledge*. Web.

Hotez, Peter J., and Aruna Kamath. “Neglected Tropical Diseases in Sub-Saharan Africa: Review of Their Prevalence, Distribution, and Disease Burden.” *Plos Neglected Tropical Diseases* 3.8 (2009): n. pag. *ISI Web of Knowledge*. Web.

Hotez, Peter J., and David H. Molyneux. “Tropical Anemia: One of Africa’s Great Killers and a Rationale for Linking Malaria and Neglected Tropical Disease Control to Achieve a Common Goal.” *PLoS Neglected Tropical Diseases* 2.7 (2008): n. pag. *PubMed Central*. Web. 10 Feb. 2013.

Hotez, Peter J. et al. “Current Concepts - Control of Neglected Tropical Diseases.” *New England Journal of Medicine* 357.10 (2007): 1018–1027. *ISI Web of Knowledge*. Web.

Hurt, N et al. “Do High Levels of C-Reactive Protein in Tanzanian Children Indicate Malaria Morbidity.” *Clinical and Diagnostic Laboratory Immunology* 1.4 (1994): 437–444. Print.

Karunaweera, Nd et al. “Dynamics of Fever and Serum Levels of Tumor-Necrosis-Factor Are Closely Associated During Clinical Paroxysms in Plasmodium-Vivax Malaria.”

Proceedings of the National Academy of Sciences of the United States of America 89.8 (1992): 3200–3203. *ISI Web of Knowledge*. Web.

Kent, S., Ed Weinberg, and P. Stuartmacadam. "The Etiology of the Anemia of Chronic Disease and Infection." *Journal of Clinical Epidemiology* 47.1 (1994): 23–33. *ISI Web of Knowledge*. Web.

Korenromp, E. L. et al. "Impact of Malaria Control on Childhood Anaemia in Africa - a Quantitative Review." *Tropical Medicine & International Health* 9.10 (2004): 1050–1065. *ISI Web of Knowledge*. Web.

Koukounari, Artemis et al. "Relationships between Anaemia and Parasitic Infections in Kenyan Schoolchildren: A Bayesian Hierarchical Modelling Approach." *International Journal for Parasitology* 38.14 (2008): 1663–1671. *ScienceDirect*. Print.

Kung'u, Jacqueline K. et al. "Early Helminth Infections Are Inversely Related to Anemia, Malnutrition, and Malaria and Are Not Associated with Inflammation in 6- to 23-Month-Old Zanzibari Children." *The American Journal of Tropical Medicine and Hygiene* 81.6 (2009): 1062–1070. *www.ajtmh.org.ezproxy.baylor.edu*. Web. 27 Oct. 2013.

Lopez, Alan D. et al. "Global and Regional Burden of Disease and Risk Factors, 2001: Systematic Analysis of Population Health Data." *Lancet* n. pag. Print.

Lwambo, N. J. S. et al. "Age Patterns in Stunting and Anaemia in African Schoolchildren: A Cross-Sectional Study in Tanzania." *European Journal of Clinical Nutrition* 54.1 (2000): 36–40. *ISI Web of Knowledge*. Web.

Maizels, R. M. et al. "Helminth Parasites - Masters of Regulation." *Immunological Reviews* 201 (2004): 89–116. *ISI Web of Knowledge*. Web.

Maizels, Rick M. et al. "Immunological Modulation and Evasion by Helminth Parasites in Human Populations." *Nature* 365.6449 (1993): 797–805. *CrossRef*. Web. 27 Feb. 2014.

Mathers, Colin D., Alan D. Lopez, and Christopher J. L. Murray. "The Burden of Disease and Mortality by Condition: Data, Methods, and Results for 2001." *Global Burden of Disease and Risk Factors*. Ed. Alan D Lopez et al. Washington (DC): World Bank, 2006. *NCBI PubMed*. Web. 20 Nov. 2013.

Menendez, C., A. F. Fleming, and P. L. Alonso. "Malaria-Related Anaemia." *Parasitology Today* 16.11 (2000): 469–476. *ISI Web of Knowledge*. Web.

Mold, Carolyn, Henry Gewurz, and Terry W Du Clos. “Regulation of Complement Activation by C-Reactive Protein.” *Immunopharmacology* 42.1–3 (1999): 23–30. *ScienceDirect*. Web.

Murray, J. et al. “The Biological Suppression of Malaria: An Ecological and Nutritional Interrelationship of a Host and Two Parasites.” *The American Journal of Clinical Nutrition* 31.8 (1978): 1363–1366. Print.

Murray, Mj et al. “Parotid Enlargement, Forehead Edema, and Suppression of Malaria as Nutritional Consequences of Ascariasis.” *American Journal of Clinical Nutrition* 30.12 (1977): 2117–2121. Print.

Mwandawiro, Charles S. et al. “Monitoring and Evaluating the Impact of National School-Based Deworming in Kenya: Study Design and Baseline Results.” *Parasites & Vectors* 6.1 (2013): 198. *www.parasitesandvectors.com*. Web.

Mwangi, T. W., J. M. Bethony, and S. Brooker. “Malaria and Helminth Interactions in Humans: An Epidemiological Viewpoint.” *Annals of Tropical Medicine and Parasitology* 100.7 (2006): 551–570. *ISI Web of Knowledge*. Web.

Nacher, M., F. Gay, et al. “Ascaris Lumbricoides Infection Is Associated with Protection from Cerebral Malaria.” *Parasite Immunology* 22.3 (2000): 107–113. *ISI Web of Knowledge*. Web.

Nacher, M., P. Singhasivanon, U. Silachamroon, et al. “Helminth Infections Are Associated with Protection from Malaria-Related Acute Renal Failure and Jaundice in Thailand.” *American Journal of Tropical Medicine and Hygiene* 65.6 (2001): 834–836. Print.

Nacher, M. “Interactions between Worm Infections and Malaria.” *Clinical Reviews in Allergy & Immunology* 26.2 (2004): 85–92. *ISI Web of Knowledge*. Web.

Nacher, M., P. Singhasivanon, S. Treeprasertsuk, et al. “Intestinal Helminths and Malnutrition Are Independently Associated with Protection from Cerebral Malaria in Thailand.” *Annals of Tropical Medicine and Parasitology* 96.1 (2002): 5–13. Print.

Nwosu, Abc, and Ao Anya. “Seasonality in Human Hookworm Infection in an Endemic Area of Nigeria, and Its Relationship to Rainfall.” *Tropenmedizin Und Parasitologie* 31.2 (1980): 201–208. Print.

Paul, Rudrajit et al. “Study of C Reactive Protein as a Prognostic Marker in Malaria from Eastern India.” *Advanced Biomedical Research* 1 (2012): n. pag. *PubMed Central*. Web. 12 Oct. 2013.

Pepys, M. B., and G. M. Hirschfield. “C-Reactive Protein: A Critical Update.” *Journal of Clinical Investigation* 111.12 (2003): 1805–1812. *ISI Web of Knowledge*. Web.

Quinnell, R. J. et al. “Immune Responses in Human Necatoriasis: Association between Interleukin-5 Responses and Resistance to Reinfection.” *Journal of Infectious Diseases* 190.3 (2004): 430–438. *ISI Web of Knowledge*. Web.

Sakti, Hastaning et al. “Evidence for an Association between Hookworm Infection and Cognitive Function in Indonesian School Children.” *Tropical Medicine & International Health* 4.5 (1999): 322–334. *Wiley Online Library*. Web. 18 Nov. 2013.

Siegel, E. H. et al. “Growth Indices, Anemia, and Diet Independently Predict Motor Milestone Acquisition of Infants in South Central Nepal.” *Journal of Nutrition* 135.12 (2005): 2840–2844. Print.

Sokhna, C. et al. “Increase of Malaria Attacks among Children Presenting Concomitant Infection by Schistosoma Mansoni in Senegal.” *Malaria Journal* 3 (2004): 43. *ISI Web of Knowledge*. Web.

Spiegel, André et al. “Increased Frequency of Malaria Attacks in Subjects Co-Infected by Intestinal Worms and Plasmodium Falciparum Malaria.” *Transactions of The Royal Society of Tropical Medicine and Hygiene* 97.2 (2003): 198–199. *trstmh.oxfordjournals.org*. Web. 28 Feb. 2014.

Stephenson, Lani et al. “Relationships of Schistosoma Hematobium, Hookworm and Malarial Infections and Metrifonate Treatment to Hemoglobin Level in Kenyan School Children.” *American Journal of Tropical Medicine and Hygiene* 34.3 (1985): 519–28. Print.

Stoltzfus, R. J., J. D. Kvalsvig, et al. “Effects of Iron Supplementation and Anthelmintic Treatment on Motor and Language Development of Preschool Children in Zanzibar: Double Blind, Placebo Controlled Study.” *British Medical Journal* 323.7326 (2001): 1389–1393. *ISI Web of Knowledge*. Web.

Stoltzfus, R. J., H. M. Chwaya, et al. “Epidemiology of Iron Deficiency Anemia in Zanzibari Schoolchildren: The Importance of Hookworms.” *American Journal of Clinical Nutrition* 65.1 (1997): 153–159. Print.

“Table 2: Human Development Index Trends | Data | United Nations Development Programme.” *UNDP Open Data*. N. p., n.d. Web. 12 Mar. 2014.

Udonsi, Jk, Abc Nwosu, and Ao Anya. “Necator-Americanus - Population-Structure, Distribution, and Fluctuations in Population-Densities of Infective Larvae in Contaminated Farmlands.” *Zeitschrift Fur Parasitenkunde-Parasitology Research* 63.3 (1980): 251–259. *ISI Web of Knowledge*. Web.

Waller Andrew. “A Cross-Sectional Study of C-Reactive Protein as a Marker of Inflammation in Patients with Untreated Malaria in Rural Western Kenya.” *Baylor University Honors Thesis,* May 2011.

Walker, Susan P. et al. “Child Development in Developing Countries 2 - Child Development: Risk Factors for Adverse Outcomes in Developing Countries.” *Lancet* 369.9556 (2007): 145–157. *ISI Web of Knowledge*. Web.

Weiss, Guenter, and Lawrence T. Goodnough. “Anemia of Chronic Disease.” *New England Journal of Medicine* 352.10 (2005): 1011–1023. *Taylor and Francis+NEJM*. Web. 27 Oct. 2013.

“WHO | Iron Deficiency Anaemia: Assessment, Prevention and Control.” *WHO*. N. p., n.d. Web. 28 Oct. 2013

“WHO | The Global Burden of Disease: 2004 Update.” *WHO*. N. p., n.d. Web.

“WHO | The World Health Report 2002 - Reducing Risks, Promoting Healthy Life.” *WHO*. N. p., n.d. Web. 21 Nov. 2013.

“WHO | World Malaria Report 2010.” *WHO*. N. p., n.d. Web. 28 Oct. 2013.

“WHO | World Malaria Report 2011.” *WHO*. N. p., n.d. Web. 28 Oct. 2013.

“WHO | World Malaria Report 2012.” *WHO*. N. p., n.d. Web. 28 Oct. 2013.