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The Relationship Between *Ascaris lumbricoides* and Malaria in Children Aged 1-4 Years

Patrick Kirwan

A thesis submitted in the fulfillment for the Degree of Doctor in Philosophy to Trinity College, University of Dublin.

May 2008

DECLARATION

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Patrick Kirwan

SUMMARY

Soil-transmitted helminths (STH) are among the most prevalent of chronic human infections worldwide. More than 1.2 billion people are thought to be infected with one or more species of STH (de Silva *et al.*, 2003). In nature, concomitant infections are the rule rather than the exception (Cox, 2001). The interaction between helminths and malaria has gained considerable attention from the scientific community (Basavaraju and Schantz, 2006; Mwangi *et al.*, 2007). Animal and human studies have shown that there is an association between helminths and malaria (Spiegel *et al.*, 2003; Helmby *et al.*, 1998). The findings from studies investigating these co-infections are contradictory: some studies have shown that co-infection increases parasitaemia and clinical malaria (Briand *et al.*, 2005), whereas others have shown co-infection to have the opposite effect (Brutus *et al.*, 2006). Most of the human studies are observational in nature i.e. cross-sectional surveys (Tshikuka *et al.*, 1996; Briand *et al.*, 2005), case-control studies (Nacher *et al.*, 2000; Nacher *et al.*, 2001c) or longitudinal studies that monitor disease trends (Spiegel *et al.*, 2003; Sokhna *et al.*, 2004) and therefore fail to demonstrate causality. The thesis presented here represents an investigation of the relationship between soil-transmitted helminths and malaria in preschool children.

The study was conducted in four semi-urban villages, outside Ile-Ife, Osun State, Nigeria. Phase I, an epidemiological survey of geohelminths, intestinal protozoa and *Plasmodium falciparum* malaria in children aged 0-25 months, was conducted in May/June 2005. The results from this study showed a high prevalence of *Ascaris lumbricoides* (24.7%) and *P. falciparum* malaria (81.5%) in children aged 1-2 years.

Phase II, a double-blind placebo-controlled randomised trial of anthelminthic treatment, took place over 16 months, beginning in May 2006 and terminating in August 2007, in preschool children aged 1-4 years. This intervention trial investigated the effectiveness of four-monthly anthelminthic treatments on the prevalence and intensity of *A. lumbricoides* and examined the relationship between *A. lumbricoides* and malaria by comparing the acquisition of malaria and susceptibility to malaria attacks in the treatment and placebo groups.

Results from this study showed that *A. lumbricoides* was the most prevalent geohelminth in this particular region of Nigeria, infecting 47.7% of children aged 1-4 years. Four-monthly anthelminthic treatments were successful in reducing prevalence and intensity of *A. lumbricoides* infections in preschool children. At the end of the follow-up period, the prevalence and intensity of *Ascaris* in the treatment and placebo groups were 14.2% and 147 mean epg and 43.2% and 1460 mean epg respectively. Given the difference in the prevalence and intensity of *A. lumbricoides* between treatment and placebo groups, and the high endemicity of *A. lumbricoides*, these children represent a formidable cohort to investigate the relationship between helminth-malaria co-infections.

To our knowledge, we performed the first double-blind placebo-controlled randomised trial of anthelminthic treatment in children aged 1-4 years. The results presented here show that while there is no interaction between *A. lumbricoides* and *Plasmodium* spp. in children aged 1-3 years, *A. lumbricoides* has a protective affect against malaria in children aged 4 years. The results have important implications for public health. Large-scale deworming programmes could significantly increase malaria morbidity in children aged 4 years and possibly older children and adults in areas where *Ascaris* in endemic.

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1.1 Introduction

Soil-transmitted helminths (STH) are among the most prevalent of chronic human infections worldwide. More than 1.2 billion people are thought to be infected with one or more species of STH (de Silva *et al.*, 2003). Growth stunting, iron-deficiency anaemia, rectal prolapse and chronic dysentery are features of STH infections; these parasitic infections can also adversely affect cognitive development in childhood (Crompton and Nesheim, 2002). There is mounting evidence in the literature that helminth infections can interact with other diseases such as HIV/AIDS and TB (Fincham *et al.*, 2003).

In nature, concomitant infections are the rule rather than the exception (Cox, 2001). Synergistic and competitive interactions can occur between parasite species, which can influence the likelihood of their successful transmission to other hosts and increase or decrease their overall pathogenic impact (Petney and Andrews, 1998). Animal and human studies have shown that there is an association between helminths and malaria (Spiegel *et al.*, 2003; Helmby *et al.*, 1998). The interactions between helminths and malaria have gained considerable attention from the scientific community (Basavaraju and Schantz, 2006; Mwangi *et al.*, 2007). It has been speculated that helminth infections may alter susceptibility to clinical malaria and therefore deworming could offer an alternative to roll back malaria (Druilhe *et al.*, 2005). The findings from studies investigating these co-infections are contradictory: some studies have shown that co-infection increases parasitaemia and clinical malaria (Briand *et al.*, 2005), whereas others have shown co-infection to have the opposite effect (Brutus *et al.*, 2006). Most of the human studies are observational in nature i.e. cross-sectional surveys (Tshikuka *et al.*, 1996; Briand *et al.*, 2005), case-control studies (Nacher *et al.*, 2000; Nacher *et al.*, 2001c) or longitudinal studies that monitor disease trends (Spiegel *et al.*, 2003; Sokhna *et al.*, 2004) and therefore fail to demonstrate causality. Therefore, it is imperative that well-designed intervention studies are undertaken to investigate the relationship between these two micro- and macro-parasites.

In light of the above, the main objective of the study was to conduct a double-blind placebo-controlled randomised trial of anthelminthic treatment in preschool children. However, first a pilot survey was undertaken in three villages outside Ile-Ife, Nigeria, to establish a good

location to conduct this study. An additional aim of this pilot study was to examine the prevalence and intensity of STH infections in children aged 0-2 years, as there is a lack of information in the literature on this age group; especially when compared with data available on school-age children (WHO, 2003). The main aims of the intervention study were:

- To study the effect of four-monthly anthelminthic treatments on the prevalence and intensity of STHs in preschool children.

- To investigate the relationship between STHs and the incidence of malaria and malaria attacks. This will be achieved by conducting a double-blind placebo-controlled randomised trial of anthelminthic treatment and comparing the acquisition of malaria and susceptibility to malaria attacks in the treatment and placebo groups.

1.2 Literature Review

1.2.1 Soil-transmitted helminths

The preceding literature review focused on STH infections and their impact on malaria. This review will provide a detailed overview of the epidemiology of STHs, a brief introduction to malaria and an evaluation of helminth-malaria co-infection studies to date. The geohelminths are soil-transmitted parasitic nematodes, so defined because a part of their life-cycle occurs in the soil (i.e. the development of eggs or larvae prior to their ingestion by or penetration of the definitive host). They are prevalent in areas of poverty in the tropics and subtropics with more than one billion people thought to be infected with at least one species (de Silva *et al.*, 2003). The STHs include the roundworm *Ascaris lumbricoides*, the whipworm *Trichuris trichiura*, the hookworms, *Ancylostoma duodenale* and *Necator americanus*, and *Strongyloides stercoralis*. These nematodes are an important cause of morbidity, particularly in school-age children who harbour the highest intensity of worm infestation (O' Lorcain and Holland, 2000). The morbidity attributed to STH infection includes malnutrition, growth retardation, anaemia, vitamin A deficiency and impaired cognitive development (WHO, 2005a).

Despite their educational, economic and public-health importance, STHs remain largely neglected by the medical and international community. Bethony *et al.* (2006) attribute this neglect to three features: first, the people most affected are the world's most impoverished; second, the infections cause chronic ill health and have insidious clinical presentation; and third, quantification of the effect of soil-transmitted helminth infections on economic development and education is difficult. STHs have been included in the list of the world's neglected tropical diseases (NTD) along with the other three most prevalent parasitic infections: schistosomiasis, lymphatic filariasis, and onchocerciasis. The common features of the NTDs include high endemicity in rural and in impoverished urban areas of low-income countries, an ability to impair childhood growth, intellectual development and education, as well as worker productivity and affected populations are frequently polyparasitised (Hotez *et al.*, 2007). The burden of disease resulting from the NTDs is vast. They cause approximately 534,000 deaths annually, with five diseases – schistosomiasis, hookworm, ascariasis, leishmaniasis and human African trypanosomiasis – accounting for more than 504,000 deaths (Hotez *el al.*, 2006c).

In recent years, the wider community has begun to recognise the importance of STHs acknowledging that their disease burden is as great as those of malaria or tuberculosis (Bethony *et al.*, 2006). In addition to this, such infections might also increase the hosts susceptibility to other important diseases such as malaria, tuberculosis and HIV (Fincham *et al.*, 2003; Spiegel *et al.*, 2003). In 2001, the World Health Assembly passed a resolution urging member states to control the morbidity of STH infections through large-scale use of anthelminthic drugs for school-aged children in less developed countries (WHO, 2005a). Efforts to tackle NTDs have been stepped up (Senior, 2005). Just recently an initiative was announced by the United States president, George W. Bush, to vastly increase funding for the integrated treatment of NTDs including STHs (http://www.who.int/neglected_diseases/NTD_Bush_announcement.pdf).

1.2.1.1 Parasite biology and lifecycle

Ascaris roundworms inhabit the middle part of the small intestine, the jejunum, while adult *Trichuris* whipworms live in the large intestine, particularly the caecum, and adult hookworms parasitise the upper part of the human small intestine (Makidono, 1956; Stephenson and Holland, 1987). Human beings are regarded as the only definitive hosts for these parasites. The life span of *A. lumbricoides* and *T. trichiura* in the host is short compared to that of hookworm, being one year, 1.5-2 years and 5-7 years, respectively (Hobo, 1956; Miller, 1979; Pawlowski, 1984). Studies have demonstrated that infection with *N. americanus* can last for 17 and 18 years (Beaver 1988; Palmer, 1955). The STHs vary greatly in size, *Ascaris* is the largest (15-35cm) followed by *Trichuris* (3-5cm) and hookworms (0.7 to 1.3cm); female worms are usually larger than males. After mating, female worms can produce thousands of eggs per day. An *Ascaris* female worm can potentially produce over 200,000 eggs per day (Sinniah, 1982), whereas *Trichuris* (5,000) and hookworms (30,000) produce fewer eggs per day (Stephenson and Holland, 1987).

STH eggs are passed in the unembryonated state. Egg survival, once infective, is variable up to a period of 14 years (Muller, 1953; Krasnosos, 1978) but most are thought to be destroyed soon after passage. Nevertheless, many will embryonate to produce second stage larvae if provided with adequate moisture, oxygen and shade (Beaver *et al.*, 1984). Humans become

infected with *A. lumbricoides* and *T. trichiura* when they ingest fully developed eggs. After ingestion of *Trichuris* eggs, the released larvae moult and travel to the colon where they burrow into the epithelia and develop into adult whipworms within about 12 weeks. *Ascaris* larvae penetrate the intestinal mucosa and undergo an extra-intestinal migration, where they enter the liver, then the lungs, before being swallowed to re-enter the gastrointestinal tract and develop into egg-laying adult worms about 9-11 weeks after egg injestion (Stephenson and Holland, 1987; Figure 1.1).

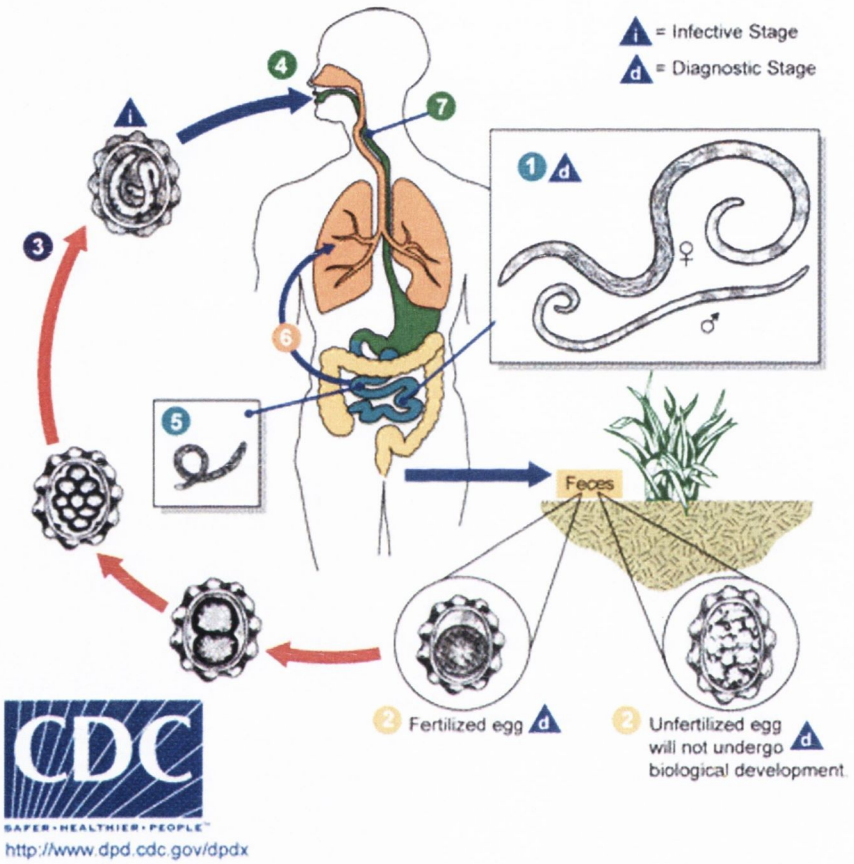

Figure 1.1 Adult worms[1] live in the lumen of the small intestine. A female produces eggs which are passed in the faeces[2]. Unfertilised eggs may be ingested but are not infective. Fertile eggs embryonate and become infective after 18 days to several weeks[3], depending on the environmental conditions. After infective eggs are swallowed[4], the larvae hatch[5], invade the intestinal mucosa, and are carried via the portal, then systematic circulation to the lungs[6]. The larvae mature further in the lungs (10 to 14 days), penetrate the alveolar walls, ascend the bronchial tree to the throat, and are swallowed[7]. Upon reaching the small intestine, they develop into adult worms[1]. Between two and three months are required from ingestion of the infective eggs to oviposition by the adult female. Adult worms can live for one to two years.

Unlike *Ascaris* and *Trichuris*, the hookworm eggs hatch out in the soil, the larvae moult twice to become infective third-stage larvae, which are motile organisms that seek out higher ground to improve the chance of contact with human skin (Gilman, 1982). The hookworm larvae penetrate exposed skin, are carried by the circulation to the lungs, penetrate the alveolar walls and progress up the trachea, where they are swallowed and carried to the small intestine. Infection by *A. duodenale* may occur by the oral or transmammary route. *N. americanus*, however, requires a transpulmonary migration phase. The adults attach themselves to the mucosa with their buccal cavities, and after 3-6 weeks the females begin to produce eggs, which are passed out in the faeces (Schad and Banwell, 1984). STHs do not reproduce within the host. This is important to note for understanding of the epidemiology and clinical features of STHs, as well as the approaches to their control (Anderson and May, 1979).

1.2.1.2 Diagnosis

Intensity of STH infection is defined as the mean number of worms or eggs per person. This is best measured by directly counting the number of worms passed in the stool after anthelminthic treatment (Croll and Ghadirian, 1981). However, this is an arduous process and is not suitable for many epidemiology studies. Therefore, an indirect measure of worm burden is used and can be achieved by counting the number of worm eggs per gram (epg) of faeces (WHO, 1991). The Kato-Katz and formol-ether concentration technique are used to determine epg (WHO, 1991). In the Kato-Katz technique, a template with a hole is used to obtain a known volume of faeces. The preserved faecal material is placed directly in the hole and the sample is cleared using a cellophane coverslip impregnated with glycerine (Denham and Suswillo, 1995). In the formol-ether concentration technique, the faecal material is sieved to remove debris and the parasites are concentrated into a pellet while the fats are removed. The relatively clean deposit, and enhanced visibility of the structural details of the eggs obtained by formol-ether concentration justify its use as a diagnostic procedure (Denham and Suswillo, 1995). A decreased sensitivity for the detection of hookworm has been shown with both methods when preservation or refrigeration was delayed by more than three hours (Dacombe *et al.*, 2007).

Using epg as an indirect measure of worm burden involves a number of assumptions, for example, only females produce eggs, so an even sex ratio is assumed. The reliability of egg

counts depends on several factors such as the amount of stool passed, concentration of eggs in the stool sample, daily egg output per worm, worm load, and method used for estimating epg (Sinniah, 1982). Nevertheless, when data are aggregated for a reasonable sample of subjects, the mean concentration of eggs in faeces is generally considered to be representative of the worm burden (WHO, 1987). The intensity of infection can be quantified into different classes: light-intensity infections, moderate-intensity infections, and heavy-intensity infections (WHO, 2002). Intensity is vital for understanding the population biology and transmission dynamics of STHs (Anderson and May, 1979); for evaluating the public health significance of the infections, since morbidity and mortality increase as worm burden increases (Pawlowski and Davis, 1989; Brooker *et al.*, 1999; Stephenson et al., 2000a); and for implementing and monitoring control programmes (WHO, 1987).

1.2.1.3 Epidemiology and burden of disease

In 1947, Norman Stoll estimated global infections with *A. lumbricoides*, *T. trichiura,* and hookworm to be 644 million (30% prevalence), 355 million (16%) and 457 million (21%), respectively (Stoll, 1999). The proportions of the world population infected with each of these parasites remain virtually unchanged (Chan, 1997). More recent estimates show that > 1 billion people are infected with at least one of these species (de Silva *et al.*, 2003). Infections with all three STHs are widespread in the tropics and subtropics. The highest rates of *Ascaris* infection occur in China and Southeast Asia, in the coastal regions of West Africa and in Central Africa. *Trichuris* infections reach their highest prevalences in Central Africa, Southern India and Southeast Asia. Hookworm infections are common throughout much of sub-Saharan Africa, in addition to South China and Southeast Asia (de Silva *et al.*, 2003). Ascariasis remains common with more than 1.2 billion infections globally. Almost 50% of these infections are in China, which still has the highest prevalence. *Trichuris* and hookworm infections amount to approximately 700 to 800 million infections each (de Silva *et al.*, 2003). The poorest people in the world suffer the greatest burden of infectious disease (Gwatkin and Guillot, 1999).

It is estimated that over 35.4 million African school-aged children are infected with *A. lumbricoides*, 40.1 million with *T. trichiura*, and 41.1 million with hookworm. In Africa,

forty four percent of the infections are congregated in just four countries: in descending order of magnitude these are Nigeria, the Democratic Republic of Congo, South Africa and Tanzania (Brooker *et al.*, 2006). Climate, poverty, inadequate water supplies and sanitation are important determinant of transmission of STH infections (de Silva, 2003; Brooker *et al.*, 2006). In these conditions, STH species are commonly co-endemic.

The most intense infections with *T. trichiura* and *A. lumbricoides* occur in children aged 5-15 years, with a decline in intensity and frequency in adulthood (Bundy *et al.*, 1985; Bundy *et al.*, 1988; Crompton, 1994). In contrast, hookworm infections are not as prevalent in childhood (Holland *et al.*, 1989), but remain high in adulthood (Bethony *et al.*, 2002). The decline of *T. trichiura* and *A. lumbricoides* in adults suggest that there may be an age-related reduction in either the rate of establishment of infection (due to acquired immunity) or the rate of exposure to infection (due to changes in behaviour) or a combination of both processes (Crompton, 1994; Anderson and May, 1985).

The distribution of STH intensity is characteristically overdispersed or aggregated. This means that most individuals harbour few or no worms, but a small proportion harbour heavy burdens (Anderson, 1986). Studies on reinfection have shown that individuals are predisposed to heavy or light infections with *Ascaris*, *Trichuris* and hookworm (Schad and Anderson, 1985; Elkins *et al.*, 1986; Thein-Hlaing *et al.*, 1987; Holland *et al.*, 1989). While the mechanisms of predisposition are currently not understood it is believed that host genetics may play a role (Holland *et al.*, 1989; Holland *et al.*, 1992; Williams-Blangero *et al.*, 2002). There is also evidence of household (Forrester *et al.*, 1988) and familial aggregation of infections (Chan *et al.*, 1994). Behavioural, immunological and physiological factors are also thought to play key roles in causing predisposition (McSharry *et al.*, 1999; Holland and Boes, 2002).

Geohelminth infections represent a significant public health issue causing an estimated 714 million disease cases and 135, 000 annual deaths (Crompton and Nesheim, 2002). STHs present an enormous infectious burden upon poor people in developing countries. Infections due to STHs cause more disability than death and are therefore assessed by disability adjusted life years (DALYs), which is a measure of the burden of disease. It is the sum of years of life lost due to premature mortality and the years of life lived with a disability (Bundy and Guyatt,

1996). The global burden of disease caused by these three intestinal nematodes is an estimated 22.1 million DALYs lost to hookworm, 10.5 million to *A. lumbricoides*, 6.4 million to *T. trichiura*, giving a combined total of 39.0 million life years (Chan, 1997). The combined DALYs lost to STHs are similar to that of malaria (46.5m DALYs lost) and tuberculosis (34.7 DALYs lost) (Hotez *et al.*, 2006c). 16.7 million DALYs were lost in children ages 5-14 years due to STHs; this represents 11.3% of the total burden in this age group. It has been estimated that 70% of the total burden of disease due to STH infections can be prevented in high-prevalence communities by treating only school-age children (WHO, 2002). Hence school-age children have been the major targets for anthelminthic treatment (Bethony *et al.*, 2006).

1.2.1.4 Clinical features of disease

The clinical features of STH infections can be classified into the acute manifestations associated with larval migration through the skin and viscera, and the acute and chronic manifestations resulting from parasitism of the gastrointestinal tract by adult worms (Table 1.1; Bethony *et al.*, 2006). The manifestations of severe disease include fatal intestinal obstruction or pulmonary allergic reactions in ascariasis, severe anaemia in hookworm infections and chronic dysentery and rectal prolapse in trichuriasis. Furthermore, evidence has accumulated that moderate infections of geohelminths can interfere with growth, appetite, physical fitness, physical activity, work capacity, cognitive development and school performance in malnourished populations (Stephenson *et al.*, 1990; Adams *et al.*, 1994; Brooker *et al.*, 1999; O' Lorcain and Holland, 2000; Stephenson et al., 2000a; Montressor *et al.*, 2003).

Many of the adverse effects of geohelminth infection concern nutrition. Children infected with ascariasis and trichuriasis are frequently observed to suffer from reduced food intake, impaired digestion, malabsorption, and poor growth rate (Thein-Hlaing, 1993; Stephenson et al., 2000b). Poor iron status and iron deficiency anaemia are indicative of hookworm disease (Crompton, 2000). Knowledge of the impacts of helminth infections are well documented in a series of reviews (Thein-Hlaing, 1993; Crompton, 2000; O'Lorcain and Holland, 2000; Stephenson et al., 2000a; Crompton and Nesheim, 2002).

Table 1.1 Specific or general features or syndromes of the soil-transmitted helminth infections of major medical importance (adapted from Bethony *et al.*, 2006).

	Specific clinical features/syndromes		**General features**
	Larval migration	Adult gastrointestinal parasitism	
Ascariasis	Verminous pneumonia	Lactose intolerance	Impaired growth
		Vitamin A malabsorption	Impaired physical fitness
		Intestinal obstruction	Impaired cognition
		Hepatopancreatic ascariasis	Reductions in school attendance and performance
Trichuriasis	None	Colitis	
		Trichuris dysentery syndrome	
		Rectal prolapse	
Hookworm	Ground itch	Intestinal blood loss	
	Cough	Iron-deficiency anaemia	
	Wakana disease	Protein malnutrition	

By influencing nutritional status intestinal helminths also indirectly effect a person's physical, cognitive, educational and overall societal development (Stephenson et al., 2000a). Energy intake, according to Stephenson *et al.* (2000c) is the most important and commonly compromised nutritional variable in children. It is difficult to prove that helminths decrease food intake in children because food intake is difficult to measure in human populations. However, several studies suggest that appetite is improved when children are dewormed (Crompton and Nesheim, 2002). Children with combined infections of *Ascaris* and *Trichuris* were found to eat significantly more of a mid-day meal of a local porridge following treatment, compared with untreated children. These children also reported that their appetites improved when asked to rate their appetite on a five point scale (Hadju *et al.*, 1996; 1998). Malnutrition is considered to be an underlying cause of more than 50% of all childhood deaths in the world (Pelletier *et al.*, 1995). Malnutrition diminishes the ability of all systems of the body to perform properly, with particular grave consequences in young children (Caufield *et al.*, 2004). Numerous studies have demonstrated that children ranging in age from 5-15 years are particularly at risk from infection and in the past they have been the focus of intervention studies and age targeted strategies for chemotherapy (Asaolu *et al.*, 1991; 1992; Holland *et al.*, 1996a).

1.2.1.5 STH in children aged less than 24 months

Children aged 24 months and less make up between 5 and 10% of the 3.5 billion people either infected with or at risk of infection from geohelminths (WHO, 2003b). Twenty one million preschool children defined as aged less than five years are infected with hookworm, 122 million are infected with *A. lumbricoides* and 86 million are infected with *T. trichiura* (de Silva *et al.,* 2003). In the past, these children have been excluded from universal chemotherapy programmes in endemic countries. Accumulating evidence demonstrates that geohelminth infection can be common in these very young children and that their impact is potentially substantial (Montressor *et al.*, 2003). Evidence suggests that soil-transmitted helminthiasis has a potential effect on growth and development of children under 24 months (Awasthi *et al.*, 2000; Awasthi and Pande, 2001). For this reason in 2002, WHO organised an informal consultation to assess the current recommendations which are to avoid the use of mebendazole (MBZ) and albendazole (ABZ) in children under two years of age. The consultation concluded that although there is little published information about the use of anthelminthic drugs in this age group, such data as exist offer no obvious reason for excluding children of this age group from treatment (WHO, 2003b). The report recommended treatment for children with soil-transmitted helminthiasis aged 12 to 24 months. However children aged less than one year should be seen on a case by case basis by their physician (WHO, 2003b).

Despite the numbers of surveys undertaken to assess the distribution and abundance of STHs in communities worldwide, comparatively few provide detailed information about the infections specifically in children under 24 months (Montresor *et al.*, 2003). Generally results are expressed as prevalence or intensity in relation to host age, with host age being displayed in classes of 0-4 years, 5-9 years, 10-14 years and so on (Kloetzel and TMJ, 1982; Saldiva *et al.*, 1999; Awasthi *et al.*, 2000; Stoltzfus *et al.*, 2001). Thus it is not possible to extract the data on children under 24 months from these studies. Montresor *et al.* (2003) published results from surveys based on stool examinations of this age group; these results are presented in table 1.2. Montresor noted that the comments in the publications indicate that the infections are usually acquired in children older than 12 months.

Table 1.2 Summary of available epidemiological data on prevalence of soil-transmitted helminthiasis in children under 24 months (adapted from Montresor *et al.*, 2003)

Country	Sample Size	Age-range (months)	Parasites[a]	Prevalence (%) 13-24 months
China	329	0-48	A/T/H	80/20/0
Sierra Leone	191	0-12	A	2
Philippines	544	1-24	A+T	25-75
Nicaragua	372	0-24	A/T	23/12
Laos	24	0-24	A/T/H	37/12/4
Indonesia	30	6-60	A/T/H	73/63/6
Philippines	91	0-48	A/T/H	77/77/14
Malaysia	37	0-12	A+T+H	68
Sri Lanka	63	0-48	A/T/H	62/50/7
Kenya	100	8-24	A/T/H	62/52/39
Zaire	100	9-23	A/T/H	66/48/7
Tanzania	42	6-24	A/T/H	54/81/64
Tanzania	317	6-24	A/T/H	49/69/41

[a] A = *A. lumbricoides*, T = *T. trichiura*, H = hookworms

/ = prevalence given for each species

+ = prevalence given for species combined

Studies concerned with the effect of soil-transmitted helminthiasis on the health of children aged less than 24 months have shown that deworming is accompanied by the following benefits: improved nutritional status (Pamba *et al.*, 1989), reduced risk of stunting (Awasthi and Pande, 2001; Awasthi *et al.*, 2000), reduced wasting (Stoltzfus *et al.*, 2004), reduced moderate anaemia (Stoltzfus *et al.*, 2004) and increases in motor and language development (Stoltzfus *et al*, 2001).

1.2.1.6 Treatment

The treatment goal for STH infections is to remove the adult worms from the gastrointestinal tract. The most commonly used drugs used to treat STHs are ABZ, levamisole, and MBZ (Urbani and Albonico, 2003). ABZ is the best broad spectrum drug, being effective against *A. lumbricoides, T. trichiura* and hookworm (WHO, 2002). ABZ is more effective against hookworm infections than MBZ but has lower cure rates for *T. trichiura* (Bartoloni *et al.*,

1993). ABZ is available in pharmaceutical form as chewable tablets (200 and 400mg) and as an oral suspension (100mg/5ml). A single dose of 400mg is highly effective against ascariasis. ABZ, like other benzimidazoles, exerts its action by selectively binding to nematode tubulin, inhibiting tubulin polymerase, which prevents the formulation of microtubules and so impedes cell division. The drug also impairs the uptake of glucose, thereby increasing glycogen depletion, and hampering the formation of ATP which is used as an energy source by the worms (Lacey, 1990). Systemic toxic effects, such as those on the liver and bone marrow, are rare for the benzimidazole anthelminthic drugs in the doses used to treat soil-transmitted helminth infections. However, transient abdominal pain, diarrhoea, nausea, dizziness and headache commonly occur (Dayan, 2003).

Previous concerns about the use of anthelminthics in children aged less than 24 months precluded children of this age group from chemotherapy programmes (Montresor *et al.*, 2003). A review of benzimidazole anthelminthic drugs in children aged 12-24 months concluded that they can be used "if local circumstances show that relief from ascariasis and trichuriasis is justified" (Montresor, *et al.*, 2003). It is recommended that children aged 1-2 years receive half a tablet (200mg) of ABZ or one tablet (500mg) of MBZ (WHO, 2007a).

1.2.1.7 Control of STH

The 54th World Health Assembly, which met in 2001, urged member states to ensure access to essential drugs for schistosomiasis and STH infections in endemic areas for the treatment of clinical cases and groups at high risk of morbidity (WHO, 2002). Their targets, aimed at reducing morbidity by 80%, included: (a) access to adequate diagnosis and essential anthelminthic drugs in all health services in all endemic areas, even at peripheral levels, for the treatment of symptomatic cases and of children, women, and other groups at high risk of morbidity; (b) regular administration of chemotherapy to at least 75% of all school-age children at risk for morbidity by 2010; and (c) sustained, community-based efforts to improve sanitation, clean water supplies, and health education. The evidence for substantial public-health benefits and reductions in global burden of disease from deworming is overwhelming (WHO, 2005a). It is recognised that helminth control could contribute to achieving seven of the eight Millennium Development Goals (Editorial, 2004).

1.2.1.7.1 Types of intervention

There are three major interventions for STHs; anthelminthic drug treatment (deworming), sanitation, and health education (Hotez *et al.*, 2006b). Deworming is aimed at reducing morbidity by decreasing worm burden. Early and regular administration of single-dose anthelminthic drugs recommended by WHO reduces the occurance, extent, severity and long-term consequences of morbidity, and in certain epidemiological conditions contributes to sustained reduction in transmission (WHO, 2006b). Periodic deworming is common place in areas where infections are intensely transmitted, resources for disease control are limited, and funding for sanitation is lacking. Regular treatment is necessary because of high rates of reinfection in endemic regions. After community-wide treatment, rates of hookworm infection reach 80% of pretreatment rates within 30-36 months (Quinnell *et al.*, 1993). *A. lumbricoides* infections reached 55% of pretreatment rates within 11 months (Elkins *et al.*, 1988) and *T. trichiura* infection reached 44% of pretreatment rates within 17 months (Chan *et al.*, 1994). Drug treatment can be administered in the community using different strategies: Universal treatment, the entire community is treated; Targeted treatment, targets population groups which may be defined by age, sex or other social characteristics, irrespective of infection status; Selective treatment, targets individual-level application of anthelminthic drugs, which is selected on the basis of either diagnosis or a suspicion of current infection (Asaolu *et al.*, 1991). The recommended strategy for helminth control is a population-based approach, in which individuals in targeted communities are treated irrespective of their infection status (WHO, 2002). To control morbidity in areas of intense transmission (prevalence greater than 70% and more than 10% of moderate to heavy infections), WHO (2002) recommends treatment two or three times a year for STH infections. In areas with a lower intensity of transmission (prevalence between 40% and 60% and less than 10% of moderate to heavy intensity infection), intervention once a year is recommended (WHO, 2002).

In addition to regular treatment, prevention of transmission has to be tackled by the provision of safe water supplies, sanitation facilities, and promotion of hygiene measures, such as handwashing, use of latrines, and encouraging footwear. In the long term, sanitation is the only definitive intervention to eliminate STH infections but the investment needed to provide access to adequate sanitation is beyond the resources of low-income countries (Hotez *et al.*,

2006b). In order to be effective, the coverage of well built, used and maintained sanitation has to be higher than 90% to have any effects on worm transmission (Asaolu and Ofoezie, 2003). To install a modern sewage system for the population of a large urban centre, for example, Lagos, Nigeria, could cost in the region of a billion US dollars (Albonico *et al.*, 2006). The resources needed to improve hygienic standards are clearly vast and require the cooperation of several sectors of society (Asaolu and Ofoezie, 2003). In order to correctly evaluate the advantage of such investments, one must take into account the consequences for other health indicators and for economic development. Currently, no cost-effective analysis estimates exist for sanitation in this context (Hotez *et al.*, 2006b). A proper sanitation infrastructure removes the underlying cause of most poverty-related communicable diseases and so supports the economic development of a country (Albonico *et al.*, 2006).

The aim of health education is to reduce transmission and reinfection by encouraging healthy behaviour. Health-education and health promotion in combination with chemotherapy have been successful in reducing reinfection rates among inhabitants of *S. japonicum*-endemic villages (Hu *et al.*, 2005). In addition, health education increases compliance with regard to chemotherapy (Hu *et al.*, 2005). Health intervention is significantly better in reducing the prevalence and intensity rate of helminths when combined with chemotherapy. Health education sustains control outcomes over long periods of time, however education alone may not achieve rapid rates of reduction (Asaolu and Ofoezie, 2003). Health education should be included as a component in all helminth control programmes (Hotez *et al.*, 2006b).

1.2.1.7.2 High risk groups

The groups identified as being at risk from STH infection are preschool children, school-age children, and women of child-bearing age. Children aged between one and five years are particularly vulnerable to disease caused by STH infections (Carrera *et al.*, 1984; Oberhelman *et al.*, 1998; Crompton and Nesheim, 2002). Although, children of these age groups are less likely to harbour heavy infections, their worm burdens are housed in smaller bodies, and therefore they are at a higher risk of anaemia and wasting malnutrition (Awasthi and Pande, 2001).

School-age children typically have the highest intensity of worm infection by *A. lumbricoides* and *T. trichiura* of any age group. Children of this age group are at an important stage of physical and cognitive development and benefit most from deworming in terms of growth and school performance (Crompton and Nesheim, 2002). Schools offer an ideal setting to administer anthelminthics to this age group, as they have a widespread distribution and a skilled workforce that is in close contact with the community (Hotez *et al.*, 2006b). This can offer benefits to the wider community as studies have shown that chemotherapy targeted at children aged 2-15 years was effective in reducing intensity in untreated adults (Asaolu *et al.*, 1991). Furthermore, by reducing transmission of *Ascaris* and *Trichuris* infections, deworming improves the health and school participation of both treated and untreated children, both in treatment schools and in neighbouring schools (Bundy *et al.*, 1990; Miguel and Kremer, 2004). School-based interventions against STHs can even reach non-enrolled school children by adopting a system of having 'treatment days' at school, where non-enrolled children are invited for treatment (Olsen, 2003).

Women of reproductive age, between the ages of 15 to 49 years, are susceptible to iron deficiency anaemia because of blood loss during menstruation and increased nutritional needs during pregnancy (Nurdia *et al.*, 2001; Torlesse and Hodges, 2001). This problem is aggravated by hookworm infection, which reaches a peak in this age group and causes iron deficiency through blood loss (Bundy *et al.*, 1995). The use of anthelminthic drugs is not licensed for use in pregnancy (WHO, 2006b). However, in areas where schistosomiasis and soil-transmitted helminthiasis are endemic, deworming out weighs the health risk for mothers and their babies (WHO, 2006b). For soil-transmitted helminthiasis it is recommended that ABZ or MBZ be offered to pregnant women in the second or third trimesters of pregnancy (WHO, 2006b).

1.2.1.7.3 Anthelminthic drug resistance

The well documented occurrence of resistance to anthelminthic drugs in nematodes of animal importance raises concern that the frequent treatments used in chemotherapy-based programmes to control human disease could select for resistant nematodes in man (Albonico *et al.*, 2004; Beiser, 2007). However, there are as of yet only few reports that suggest resistance in nematodes of human importance (De Clercq *et al.*, 1997; Reynoldson *et al*, 1997;

Albonico *et al.*, 2003). The development of sensitive methods for the early detection of anthelminthic resistance are part of the research agenda (Albonico *et al.*, 2004). The available parasitological methods to monitor efficacy of anthelminthic drugs are the egg reduction rate and the egg hatch assay (WHO, 1999). Although the faecal egg count reduction rate is a suitable method for detecting resistance in the field, tests that are more sensitive to low levels of resistance are required (Martin *et al.*, 1989). Molecular biological methods for the detection of resistance are being developed (Kwa *et al.*, 1995).

Concerns about the sustainability of periodic deworming with benzimidazole anthelminthic drugs and the emergence of resistance with widespread use have motivated scientists to develop and test new control methods, for example, combination therapy, existing compounds with anthelminthic properties, and development of a hookworm vaccine. Combined treatments with two drugs with different modes of action is one method used to safeguard efficacy and to delay the possible emergence of drug resistance. Combined treatment with mebendazole and levamisole has been proved safe and more effective than either drug alone (Albonico *et al.*, 2003). Tribendimidine is a promising new anthelminthic drug that has no toxicity and also has broad spectrum activity against the STHs. In randomised studies in China, tribendimidine was found to be comparable to ABZ and MBZ for the treatment of *Ascaris*, *Trichuris* and hookworm infections and even better than these drugs for *N. americanus* infection (Xiao *et al.*, 2005). Nitazoxanide, used in children with giardiasis and cryptosporoidiosis, is being explored as a broad-spectrum anthelminthic (Gilles and Hoffman, 2002). In addition to chemotherapy, the development of a hookworm vaccine is underway and undergoing clinical development in human beings (Hotez *et al.*, 2006a).

1.2.1.7.4 Integrated control

The World Health Assembly met in 2001 and passed a resolution to combine the approach to morbidity control in both schistosomiasis and STHs (WHO, 2002). A joint initiative from the United Nations Educational, Scientific and Cutural Organisation (UNESCO), the United Nations Children's Fund (UNICEF), the World Health Organisation (WHO) and the World Bank, called Focusing Resources on Effective School Health (FRESH) has also endorsed this integrated control approach (Kabatereine *et al.*, 2006). An integrated approach is sensible considering the fact that the tools and target groups are similar for these two parasitic groups

(WHO, 2002). The approaches to control programs for STHs and schistosomiasis in Uganda, Tanzania, and Zambia have been assessed (Kabaterine *et al.*, 2006). In Zambia, deworming and health education were combined; deworming accompanied health education on other topics for example, HIV/AIDS, water and sanitation and gender issues. Other than Zambia, the control programmes received all their support from the Schistosomiasis Control Initiative, which received all its funding from the Bill and Melinda Gates Foundation.

The successful control of STH and schistosomiasis in Japan can be accredited to their effective schemes for parasite control (Ohta and Waikagul, 2007). The Japanese Association of Parasite Control (JAPC) implemented a school-based approach, in which teachers had the main role in health education and deworming that was backed by the Japanese Government. STH control was also implemented with family planning and nutritional improvement. Japanese scientists have sought to build a close relationship with researchers in other Asian countries; this will help to strengthen the training aspects needed for parasite control, which are based on the lessons learned during previous success in Japan. In 2000, the Asian Center of International Parasite Control (ACIPAC) was established which carries out activities to link deworming with health-promoting school programs in Cambodia, Laos, Myanmar, Thailand and Vietnam. The ACIPAC has also conducted an integrated school-health-based program, including deworming and malaria education, under the umbrella of the health-promoting school initiative.

1.2.1.7.5 Neglected Tropical Disease (NTD)

The NTDs (Table 1.3) share a number of similar features, which prompted a rationale for linking vertical NTD control programs with a preventative chemotherapy package of up to four drugs (Molyneux *et al.*, 2005). The NTDs are responsible for approximately 534,000 deaths annually (Molyneux *et al.*, 2005) and 56.6 million DALYs lost (Hotez *et al.*, 2006c). The geographic overlap of NTDs emphasises that co-infections are widespread (Molyneux *et al.*, 2005). Integrating interventions for multiple diseases makes sense especially when these interventions share a common technical approach, a common target population, and a collectively high disease burden (Utzinger and de Savigny, 2006). Hotez *et al.*, (2006c) proposed a pro-poor health policy, which suggests the large-scale deployment of just four safe, efficacious drugs i.e. ABZ, azithromycin, ivermectin and praziquantal. This will target over

90% of the neglected disease burden. It is estimated that the integrated control of seven of the major NTDs could have a rapid impact on morbidity, blindness, and skin disease, at a minimal cost of US$0.40 per person per year (Fenwick *et al.*, 2005).

Table 1.3 The thirteen neglected tropical diseases in Africa and their major etiologic agents (Molyneux *et al*, 2005)

Protozoan Infections	
African trypanomiasis	*Trypanosoma gambiense, T. rhodesiense*
Kala-azar (visceral leishmaniasis)	*Leishmania donovani*
Helminth infections	
STH infections	
Ascariasis	*Ascaris lumbricoides*
Trichuriasis	*Trichuris trichiura*
Hookworm infection	*Necator americanus*
Schistosomiasis	
Urinary schistosomiasis	*Schistosoma haematobium*
Hepatobiliary schistosomiasis	*Schistosoma mansoni*
Lymphatic filariasis	*Wuchereria bancrofti*
Onchocerciasis	*Onchocerca volvulus*
Dracunculiasis	*Dracunculus medinensis*
Bacterial Infections	
Trachoma	*Chlamydia trachomitis*
Leprosy	*Mycobacterium leprae*
Buruli ulcer	*Mycobacterium ulcerans*

Integrated programs at some level are underway in Burkina Faso, Ghana, Mali, Niger, Nigeria, Togo and Uganda (Hotez *et al.*, 2007). The implementation challenges to integration cited by Hotez *et al.* (2007) include: inadequate access to drugs; piecemeal and unpredictable funding sources from the national and international level; issues related to different guidelines for the different medicines in treatment of young children; the need for simplified surveillance tools; the need for simplified data collection; management and reporting mechanisms; lack of consistent policy on providing incentives for drug distributors; working in resource-poor

health systems; working in post conflict countries; and the reporting of serious adverse events in large populations distributed over a wide geographical area. Overall, the experience in sub-Saharan Africa shows that integration appears to have multiple benefits and strengthens health systems by helping communities, empowering national health teams, and bringing a new workforce into the health system, such as schoolteachers and community drug distributors.

1.2.2 Malaria

Malaria is a vector-borne infectious disease caused by protozoan parasites. It has infected humans for over 50,000 years, and may have been a human pathogen for the entire history of our species (Joy *et al.*, 2003). Human malaria is caused by four species: *Plasmodium falciparum*, *P. malariae*, *P. vivax*, and *P. ovale*. The most serious forms of the disease are caused by *P. falciparum* (Hommel and Gilles, 2005). Malaria is a major cause of morbidity, anaemia, and mortality worldwide (Shankar, 2000). Every year, up to 2.7 million people die from malaria, over 75% of them African children (Breman, 2001). Recent estimates that have combined epidemiological, geographical and demographic data indicate that there were 515 (range 300-660) million episodes of clinical *P. falciparum* malaria in 2002 (Snow *et al.*, 2005). Anaemia, hypoglycemia, acute respiratory distress and cerebral malaria dominate the pathology (Breman, 2004). Malaria has a strong association with poverty and has been estimated to cost Africa more than $12 billion US every year (Sheppard *et al.*, 1991). Studies in sub-Saharan Africa have demonstrated that episodes of malaria in children have a huge economic impact on poor families both directly in terms of treatment costs and indirectly by preventing adult caregivers from carrying out work (Ettling *et al.*, 1994; Asenso-Okyere and Dzator, 1997). The control of malaria parasites is hampered by the emergence and spread of parasite resistance to the majority of antimalarial drugs (Woodrow *et al.*, 2005) and mosquitoes to insecticides (Weill *et al.*, 2003).

1.2.2.1 Epidemiology of malaria

Malaria has a worldwide distribution, and is found in tropical areas, particularly in sub-Saharan Africa and to a lesser extent in South Africa, South East Asia, the Pacific islands, India, and Central and South America (Ashley *et al.*, 2006). *P. falciparum* is the predominant species in most endemic countries with the exceptions of India and South America where *P. vivax* is common. *P. vivax* accounts for over half of all malaria infections outside Africa

(Sina, 2002). *P. malariae* infections are most common in sub-Saharan Africa and the southwest Pacific. This species has also been infrequently detected in malaria-endemic regions of Asia, the Middle East, South America and Central America (Mueller *et al.*, 2007). *P. ovale* is relatively uncommon and its prevalence rarely exceeds 3-5% (Mueller *et al.*, 2007). Its distribution was thought to be limited to tropical Africa, New Guinea, the eastern parts of Indonesia and the Philippines but infections have been reported in the Middle East, the Indian subcontinent and different parts of Southeast Asia (Mueller *et al.*, 2007).

The development of natural acquired immunity to malaria is a slow process and it may take years of repeated exposure to malaria parasites to produce a level of control of but not complete elimination of parasitaemia (Baird, 1998). The prevalence of *P. falciparum* has been shown to peak in children aged 2-4 years and steadily decrease in older children and adults. The intensity of *P. falciparum* has also been shown to peak in children aged less than two years but decreases steeply to children aged six years remaining at lower levels throughout life (Wilson, 1936). As immunity is related to exposure to the malaria parasites, it is logical that the level of malaria transmission is an important determinant of morbidity and mortality. In areas where malaria is endemic (stable transmission) the prevalence of infection may be sufficiently high to engender a substantial level of clinical immunity within a population, which usually results in many asymptomatic infections. In contrast, unstable malaria transmission settings, characterised by spatial and temporal variability, are associated with lower levels of clinical immunity within a population, the consequence of which is a higher rate of severe disease (Vinetz and Gilman, 2002). The age-groups at highest risk are determined by the intensity of malarial transmission. In areas where the transmission is intense, clinical disease is most common in young children. When transmission is low, clinical disease will affect all age groups (Lalloo *et al.* 2006). In all endemic areas, children and pregnant women are at higher risk of malaria and are more susceptible to severe disease (WHO, 2006a).

Malaria transmission is not homogenous throughout an endemic area and depends on two primary factors: location of mosquito breeding sites, and the clustering of human habitations where people serve as reservoirs of parasites for mosquito infection (Carter *et al.*, 2000). The habitats that support breeding by anopheline vectors include marshlands and other areas of poor drainage, silted rivers, and ponds (Thompson *et al.*, 1997; Rejmankova *et al.*, 1998). The

incidence of malaria cases may be clustered in some households more than others. The risk factors for this include location, for example, an increased number of malaria cases occur in houses near larval habitats (Roberts *et al.*, 1999), as well as houses with particular structural features, for example the risk of getting malaria has been demonstrated to be greater for habitants of the poorest type of house construction (incomplete, mud or palm walls, palm thatched roofs) compared to houses built of concrete brick and plaster walls and tiled roofs (Gamage-Mendis *et al.*, 1991), and the economic, cultural and genetic characteristics of the inhabitants (Mackinnon *et al.*, 2000).

Studies have shown that genetic characteristics of the host are major determinants of the outcome of a malarial infection (Mckinnon *et al.*, 2000). The historic burden of malaria has resulted in the selection of genetic variants that confer some degree of protection against death from the disease (Mackinnon *et al.*, 2005). Some of these malaria-protective red blood cell polymorphisms include: duffy antigens, sickle-cell, thalassaemias, and glucose-six-phosphate dehydrogenase deficiency (Williams, 2006). The duffy antigen is the main reason why *P. vivax* is absent from West Africa. The duffy antigens are antigens that are expressed on red blood cells. The expression of duffy antigens on blood cells is encoded by Fy genes. *P. vivax* uses the duffy antigen determinants (Fya or Fyb) on the erythrocyte surface to enter blood cells and this is absent in indigenous populations of West Africa and sub-Saharan Africa where the duffy antigen is not expressed (Miller *et al.*, 1976). The sickle-cell anomaly is caused by a single mutant gene which is responsible for the production of a type of haemoglobin differing in several important aspects from normal adult haemoglobin. Carriers of the sickle-cell trait who are heterozygous for the sickle-cell gene have a mixture of relatively insoluble haemoglobin and normal haemoglobin in their blood; hence their erythrocytes do not sickle *in vivo*, whereas all of the homozygotes, who have a much greater proportion of sickle-cell haemoglobin, have sickle-cells in the blood with inevitable haemolysis and a severe, often fatal, haemolytic anaemia (Allison, 1954). Humans with the sickle-cell trait are afforded a degree of protection from malaria. When individuals with sickle cell haemoglobin are infected with malaria, the infected cells tend to sickle and are selectively destroyed by the body's immune system (Balgir, 2006). Hence, there is considerable overlap in the worldwide distribution of malaria and the sickle-cell trait. Other red blood cell polymorphisms are reviewed by Kwiatkowski (2005) and Williams (2006).

Infants, preschool children and pregnant women are at high risk from malaria (WHO, 2006a). Malaria's most significant impact is in sub-Saharan Africa, where it is responsible for at least 20% of the mortality in young children, or approximately 3,000 deaths per day (Breman 2001; WHO, 2005b). The manifestations of a malaria attack in non-immune children are variable and can consist of any of the following symptoms: restlessness or drowsiness, refusal to eat or to suck, headache, vomiting, loose stools and cough. Febrile convulsions, with high temperatures (40°C) are common (Summer *et al.*, 2005). Many of the clinical features of severe malaria occur in children. The commonest and most important complications are cerebral malaria and severe anaemia. In holo-endemic areas of malaria, cerebral malaria occurs in children aged between six months and five years. Children admitted with cerebral malaria present with a 1-3 day history of fever, anorexia and vomiting. The main neurological features are coma, seizures and brainstem disease (Idro *et al.*, 2005). Anaemia is particularly common in children aged between six months and two years and is usually the result of repeated untreated episodes of uncomplicated malaria. Children with severe anaemia may present with tachycardia, dyspnea, respiratory distress, confusion, restlessness, coma, retinal hemorrhages, cardiac failure and pulmonary edema (Menedez *et al.*, 2000). Malaria in infants can be less frequent and less severe when compared to older children (Snow *et al.*, 1998). Infants appear to be protected from clinical malaria, and from severe consequences of malaria infection, for the first three to six months of life (Brabin, 1990; Snow *et al.*, 1998). The duration of this protection seems to be inversely related to the intensity of malaria transmission such that in hyperendemic areas of Tanzania, the protection lasts less than three months (Kitua, 1996 cited by Riley *et al.*, 2001), whereas in an area of low to moderate transmission in coastal Ghana, the risk of clinical attack of malaria remains low throughout the first six months of life (McGuinness *et al.*, 1998). The mechanism of this protection has been debated and could be attributed to passive immunity from maternally derived antibodies (Riley *et al.*, 2001).

Malaria infection during pregnancy is an enormous public health problem, with substantial risk for the mother, her fetus and the neonate (WHO, 2007c). In areas of low transmission of *P. falciparum*, women are susceptible to episodes of severe malaria, which can result in still births, spontaneous abortion or lead to the death of the mother (Luxemberger *et al.*, 1997). In areas of high transmission of *P. falciparum*, where levels of acquired immunity tend to be high, women are susceptible to asymptomatic infection. This can result in maternal anaemia

and placental parasitaemia, both of which can subsequently lead to low birth weight (Steketee *et al.*, 1996). Low birth weight is an important contributor to infant mortality (McDermot *et al.*, 1996) and it has been estimated that malaria during pregnancy is responsible for 5-12% of all low birth weight (Steketee *et al.*, 1996).

1.2.2.2 Malaria lifecycle

The malaria lifecycle has been well described and is summarised here from Markell *et al.* (1999) and Hommel and Gilles (2005). Malaria parasites undergo many stages of development, and their complete life cycle occurs in both humans and mosquitoes (Figure 1.2). The parasites are transmitted to humans by the female *Anopheles* mosquitoes, which are the definitive hosts. Malaria in humans develops via two phases: an exoerythrocytic (hepatic) and an erythrocytic phase. Malaria infection starts when a female mosquito, that carries *Plasmodium* sporozoites in its salivary glands, bites a human. The sporozoites are injected into the blood stream and rapidly make their way to the liver, where they invade hepatocytes. Within the hepatocytes, they undergo a period of differentiation and asexual multiplication to produce the pre-erythrocytic schizont, containing thousands of merozoites. The duration of schizogony varies considerably from one species to another and can be a minimum to 5.5 days for *P. falciparum* and 15 days for *P. malariae*.

When the merozoites are released from the hepatocytes they enter the blood stream and invade new erythrocytes. Here they start a periodic phase of differentiation and asexual multiplication leading to the erythrocytic schizont. This contains a small number of merozoites. At the end of the schizogonic cycle, the infected red blood cells rupture, liberating merozoites, which in turn invade new erythrocytes. When erythrocytes lyse, symptoms of malaria can occur. The length of the intra-erythrocytic development varies, being 48 hours for *P. falciparum*, *P. vivax*, and *P. ovale* and 72 hours for *P. malariae*. The metabolism of the malaria parasite is largely dependent on the digestion of red cell haemoglobin, which is transformed into malaria pigment.

At some stage, some merozoites undergo sexual differentiation and gametocytegenesis. The factors which induce sexual differentiation, rather than schizogonic development, are essentially unknown. Maturation of *P. falciparum* gametocytes takes much longer than that of

the other species, 10 days compared to three or four days. Transmission of malaria occurs when a new biting mosquito picks up the male and female gametocytes in the peripheral blood.

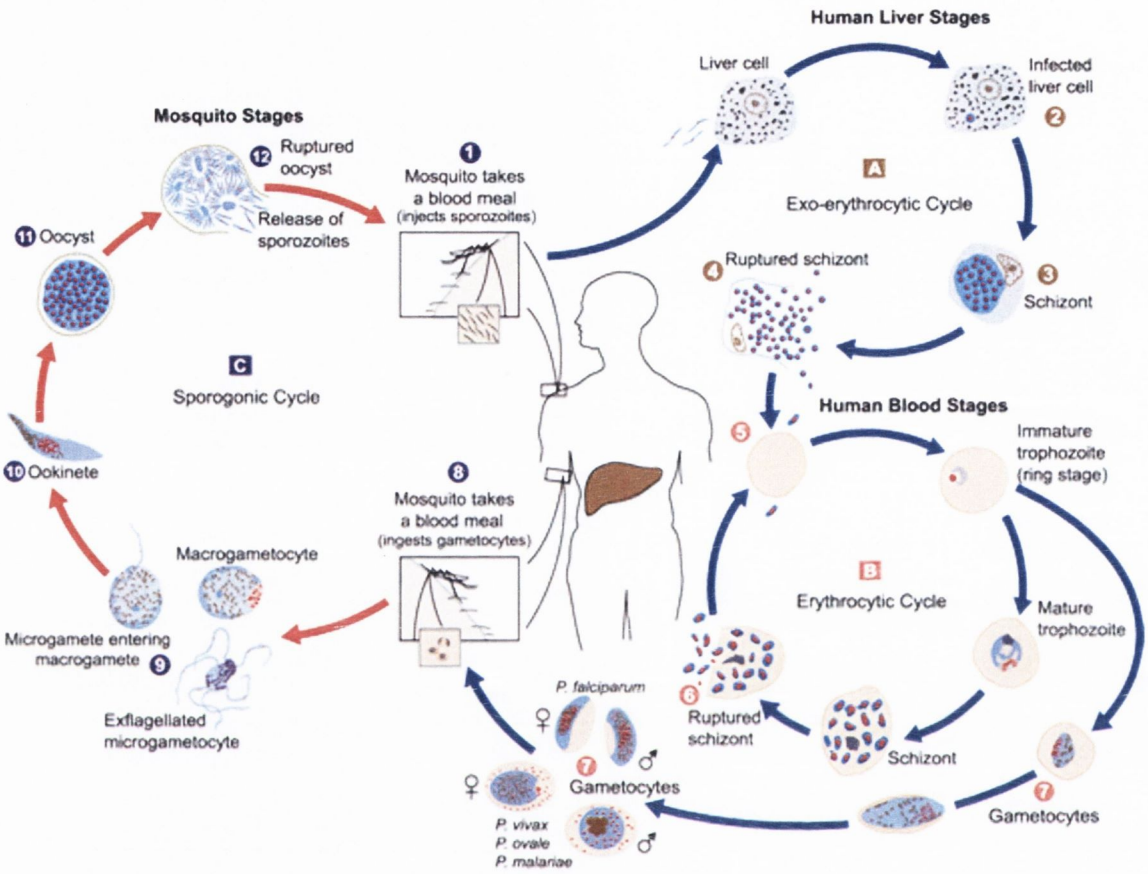

Figure 1.2 The malaria parasite life cycle involves two hosts. During a blood meal, a malaria-infected female *Anopheles* mosquito inoculates sporozoites into the human host[1]. Sporozoites infect liver cells[2] and mature into schizonts[3], which rupture and release merozoites[4]. After this initial replication in the liver (exo-erythrocytic schizogony[A]), the parasites undergo asexual multiplication in the erythrocytes (erythrocytic schizogony[B]). Merozoites infect red blood cells[5]. The ring stage trophozoites mature into schizonts, which rupture releasing merozoites[6]. Some parasites differentiate into sexual erythrocytic stages (gametocytes)[7]. Blood stage parasites are responsible for the clinical manifestations of the disease.

The gametocytes, male (microgametocytes) and female (macrogametocytes), are injested by an *Anopheles* mosquito during a blood meal[8]. The parasite's multiplication in the mosquito is known as the sporogonic cycle[C]. While in the mosquito's stomach, the microgametes penetrate the macrogametes generating zygotes[9]. The zygotes in turn become motile and elongated (ookinetes)[10] which invade the midgut wall of the mosquito where they develop into oocysts[11]. The oocysts grow, rupture, and release sporozoites[12], which make their way to the mosquito's salivary glands. Inoculation of the sporozoites into a new human host perpetuates the malaria life cycle[1]. Source: CDC (http://www.dpd.cdc.gov.dpx)

The gametocytes are released into the acidic, low temperature environment of the insect midgut, and transform into gametes, which fertilise to form first a zygote, then an ookinete. The motile ookinete crosses the epithelial wall of the midgut before transforming into an oocyst. The oocyst divides by schizogony to produce thousands of sporozoites. These undergo a final differentiation into their infective forms while migrating to the salivary glands of the insect where they will be transferred to a human host with the next bite, thus completing the life cycle.

1.2.2.2.1 Recrudescence and relapses

When merozoites infect red blood cells, there is a first wave of parasitaemia that is responsible for the primary attack of malaria. This can last for a few weeks to a few months in a non-immune individual. There are cases where waves of parasitaemia may occur a few weeks, months or years after the primary attack, in the absence of reinfection. These relapses and recrudescences occur for specific malaria species. Relapses can occur in *P. vivax* and *P. ovale* infections; these malaria species produce hyponozoites, parasites that can remain dormant in the liver for periods ranging from several months to as long as three years. After a period of dormancy, they can reactivate and produce merozoites. Hyponozoites can be responsible for a series of waves of parasitaemia or relapses (Cogswell, 1992). Recrudescences occur in *P. falciparum* and *P. malariae* infections which can produce secondary waves of parasitaemia either by the long term survival of the erythrocytic stages or the continuation of erythrocytic schizogony in their peripheral blood stream at low or undetectable levels.

1.2.2.2.2 Sequestration

In the case of *P. falciparum* infections, parasites may be removed from the peripheral circulation and be sequestered in various host tissues. This sequestration is due to adhesive proteins on the surface of the infected red blood cells that cause the blood cells to adhere to capillary endothelial cells (Chen *et al.*, 2000). Through this mechanism, vital organs such as the brain and kidneys may suffer impaired oxygen and nutrient exchange (Dondrop *et al.*, 2004). This can result in clinical symptoms such as impaired consciousness, respiratory distress and renal dysfunction (Idro *et al.*, 2005).

1.2.2.3 Symptoms of the disease

Malaria infections can be categorised as asymptomatic, uncomplicated, and severe malaria. Asymptomatic infection describes an individual who has malaria parasites in their blood but shows no signs of clinical symptoms (Njama-Meya *et al.*, 2004). Asymptomatic *P. falciparum* infections are commonplace and widespread in Africa (Smith *et al.*, 1993; Trape *et al.*, 1994; Bottius *et al.*, 1996). The immune response of individuals with asymptomatic infections produces control but not complete elimination of parasitaemia, this clinical immunity to malaria is termed premonition and has already been mentioned.

Patients with uncomplicated malaria have malaria parasites and clinical symptoms of the disease. Clinical features of malaria include fever, chills, rigors, headache and muscle pain. Initially the symptoms in patients resemble flu-like illness and can then develop into fever paroxysms, a sudden rise and fall in temperature, that tends to occur at regular intervals of time. If the infection is left untreated, the fever recurs every third day in *P. falciparum*, *P. vivax* and *P. ovale* infections establishing a two-day cycle (tertian) and every three days (quartan) in *P. malariae* infections, establishing a four-day cycle (Hommel and Gilles, 2005). Gastrointestinal and respiratory symptoms are also features of malaria, particularly in *P. falciparum* infections in children. A Nigerian study, in children aged six months to 15 years, with uncomplicated *P. falciparum* infections, recorded gastrointestinal symptoms of vomiting, abdominal pain and diarrhoea (Sowunmi *et al.*, 2000).

When the malaria infection progresses untreated, the patient can suffer from severe malaria. Severe malaria can result from *P. falciparum* infections and can include one or more of the following clinical features: cerebral malaria (unrousable coma), severe anaemia (haemoglobin <5gd/l), renal failure, pulmonary odema or adult respiratory distress syndrome, hypoglycaemia, circulatory collapse or shock, repeated generalized convulsions and haemoglobinuria (WHO, 1990). The host's immunological response is an important factor in the pathophysiology of disease (Clark and Cowden, 2003; Kaestli *et al.*, 2004; Pouniotis *et al.*, 2004). *P. falciparum* infection induces the release of inflammatory mediators, such as cytokines including tumor necrosis factor, which contribute to the pathology (Malaguarnera and Musumeci, 2002). There are many host and parasite epidemiologic factors associated

with the expression of the disease. Host factors, such as age, pregnancy, pre-existing hemoglobinopathies and host antigenic variation influence disease susceptibility.

1.2.2.4 Malaria and anaemia

Anaemia is a leading cause of morbidity and mortality worldwide (WHO, 1996a). Severe malarial anaemia is an important and common life threatening complication of *P. falciparum* infection, especially in young children (WHO, 1986). *P. falciparum* infection contributes to the cause and severity of anaemia through a number of mechanisms, including the direct destruction of parasitised red blood cells, immune mechanisms and dyserythropoiesis (Weatherall and Abdalla, 2002). Malaria may also contribute to iron-deficiency by reducing iron absorption during acute episodes and through sequestration of iron in malaria pigment (Menendez *et al.*, 1997). Malaria chemoprophylaxis has been shown to lower severe anaemia and malaria in infants (Menendez *et al.*, 1997). Children with severe anaemia may present with tachycardia, dyspnea, respiratory distress, confusion, restlessness, coma, retinal hemorrhages, cardiac failure and pulmonary odema (Hommel and Gilles, 2005).

1.2.2.5 Malaria and under-nutrition

It has long been acknowledged that populations residing in malarious areas generally live under conditions that lead to poor nutritional status (Shankar, 2000). The nutritional status of the host is important to parasite growth and the outcome of malaria infections. For example, vitamin A supplementation reduced the frequency of *P. falciparum* episodes by 30% among preschool children in Papua New Guinea (Shankar *et al.*, 1999), and riboflavin (vitamin B2) deficiency confers a degree of protection against malaria infection (Das *et al.*, 1988). It has been thought that malnutrition reduces susceptibility to malaria and when nutritional status improves, for example, in famine relief situations, outbreaks of clinical malaria occur (Murray *et al.*, 1978). Shankar (2000) has challenged this concept after a re-analysis of published studies. Shankar showed that malnutrition was actually more often associated with an increased risk of malaria. A Nigerian study in children aged 1-5 years showed that poor outcomes of cerebral malaria (death or recovery with neurological deficits) were commoner in the malnourished group than the well nourished group (Olumese *et al.*, 1997). Four other studies conducted in Madagascar (Randriamiharisoa *et al.*, 1993), Chad (Renaudin, 1997), The Gambia (Man *et al.*, 1998), and Senegal (Faye *et al.*, 1998) indicate that malnourished patients

are 1.3-3.5 times more likely to die or have permanent neurologic sequelae than normally nourished malaria patients.

1.2.2.6 Diagnosis of malaria infection

There are a number of ways to diagnose malaria infections including microscopic examination of peripheral blood smears; antigen-capture assays which detect and differentiate *P. falciparum* and non-*falciparum* malaria; polymerase chain reaction (PCR), which amplifies parasite DNA or mRNA; and the quantitative buffy coat (QBC) technique, which uses a special lens to detect acridine orange-stained parasite DNA or RNA (Haditsch, 2004; Summer *et al.*, 2005)

Microscopic examination is the most widely available clinical test and remains the gold standard for malaria diagnosis (WHO, 1991). There is not always a clear and straightforward relationship between clinical severity of malaria and the density of parasitaemia. This may be due in part to the sequestration of parasites during *P. falciparum* infections. Nevertheless, high parasitaemia is, in general, more likely to cause illness than low parasitaemia (Greenwood, 1997). Thus, the determination of malaria parasite density is useful both in the assessment of an individual subject with symptoms and malaria parasitaemia and in epidemiological surveys. Thick blood smears are used to detect the presence of malaria parasites, while the thin blood smears can be used for identifying the different species of malaria. The thick and thin blood smear can be made on the same slide which reduces preparation and staining time. Giemsa staining is recommended most frequently but Field's stain is also a good option (Lema *et al.*, 1999). There are different methods for determining malaria parasite density (Trape, 1985; Molineaux and Gramicca, 1980). Accurate determination of parasite density requires a red blood cell (RBC) count and accurate measurement of the level of RBC infection as assessed by examining the thin blood film (Greenwood and Armstrong, 1991). However, this method is time-consuming and is not suitable for large-scale field studies. Instead, the number of parasites per white blood cell (WBC) in a thick blood film is recorded and parasite density is determined by multiplying this figure by 8000, an average WBC count per μl (Trape, 1985).

The development of rapid diagnostic tests (RDTs) has offered the potential for the extension of accurate diagnosis to remote and poorly-resourced areas that are beyond the reach of high quality microscopic services (WHO, 2003a). The RDTs are immunochromatographic tests that are based on the detection either of the histidine-rich-protein-2 (HRP-2) from *P. falciparum* or the parasite-specific lactate dehydrogenase (pLDH) from the parasite glycolytic pathway found in all species (Moody, 2002). The RDTs based on HRP-2 antigen have a high sensitivity, 84-94% and specificity, 81-99% (WHO, 1996b) but they are not without their limitations. The performance of the tests decreases at low parasitaemias, <100/µl (Beadle *et al.*, 1994; WHO, 1996b). There is an occasional failure to detect high parasitaemias, >500-1000/µl (Beadle *et al.*, 1994; Kodisinghe *et al.*, 1997). After successful treatment the HRP-2 antigen can remain in the blood for up to 7-14 days, which can result in false positives (WHO, 1996b; Kodisinghe *et al.*, 1997). Rheumatoid fever is another reason for potential false positive results (Laferi *et al.*, 1997). Tests using pLDH are only positive when viable parasites are present. These tests have sensitivities and specificities that are similar to the HRP-2 tests, with similar limitations at lower parasitaemias (John *et al.*, 1998). Even with these limitations, RDT are important in the control and treatment of malaria (WHO, 2000). They are simpler to perform and to interpret than microscopy. They may detect *P. falciparum* antigens even when the parasites are sequestered in the deep vasculature of tissues and thus remain undetectable by microscopic examination of a peripheral blood smear (Leke *et al.*, 1999). These and other methods of diagnosing malaria are discussed in detail in two reviews (Hanscheid, 1999; Bell *et al.*, 2006).

In many malaria-endemic areas where there is limited access to medical care and often lack of adequate laboratory facilities and skilled personnel. Treatment is often administered based on clinical signs or symptoms and this usually involves fever (WHO, 2000). In non-endemic areas, the diagnosis of clinical malaria may be made on the basis of fever and a positive blood film. In endemic areas, asymptomatic parasitaemia is very common and assuming that a child who presents with fever and parasitaemia is ill from malaria will result in over diagnosis (Schellenberg *et al.*, 1994). Febrile temperatures in young children are frequently the result of causes such as respiratory infections and are not necessarily a consequence of malaria even when the patient is parasitemic. It is possible to estimate the proportion of fever cases that are malaria-attributable using parasitaemia data, assuming that the risk of symptomatic malaria

increases with parasite density (Smith *et al.*, 1995). The malaria attributable fraction is the proportion of the morbidity that would be removed if malaria were eliminated.

1.2.2.7 Treatment and control

The treatment objectives vary for uncomplicated malaria and severe malaria (WHO, 2006a). The objective of treating uncomplicated malaria is to cure the infection. This will prevent the progression to severe disease and prevent additional morbidity associated with treatment failure. Cure of the infection means eradicating malaria from the body, reducing transmission and preventing the emergence and spread of resistance to antimalarials. The primary objective of antimalarial treatment in severe malaria is to prevent death. Prevention of recrudescence and avoidance of minor adverse effects are secondary.

A list of available medications and treatment regimes for malaria are given in Summer *et al.*, (2005). Malarial control in Africa deteriorated markedly in the 1990s, largely due to the emergence of strains of *P. falciparum* resistant to chloroquine and sulphadoxine-pyrimethamine (SP), the mainstays of treatment (Greenwood, 2008). Resistance has arisen to all classes of antimalarials except, as yet, to the artemisinin derivatives (WHO, 2006a). The discovery and development of the artemisinin derivatives in China have provided a new class of highly effective antimalarials. Artemisin-based drugs are rapid acting, well tolerated, safe and effective (Adjuik *et al.* 2004). They act on all blood stages of the parasite and also damage gametocytes. To contain the risk of resistance to artemisinin monotherapies, WHO recommends the use of artemisinin-based combination therapies (ACTs) in order to ensure high cure rates of *P. falciparum* malaria (WHO, 2007b). Artemisinins are more expensive to produce than chloroquine or SP but the price is falling. Treating an adult for malaria with chloroquine costs $0.10 while artemisinin-based treatments are more expensive at $2.50 (WHO, 2003c).

The malaria control strategy now being promoted by WHO focuses on the reduction of malarial disease and recognises four elements: (1) to provide early diagnosis and prompt treatment; (2) to plan and implement selective and sustainable preventative measures against the parasite, as well as the vector; (3) to detect early, contain or prevent epidemics; and (4) to reassess regularly a country's malaria situation, in particular the ecological, social, and

economic determinants of disease (Hommel and Gilles, 2005). In 1998, Roll Back Malaria (RBM) was launched and was a catalyst for renewed global commitment to tackle a disease that affects 3.2 billion people (WHO, 2005b). Appropriate malaria control strategies vary with local malaria endemicity. The key strategies advocated by the RBM for different epidemiological settings are outlined in table 1.4.

Intermittent preventative treatment in infants (IPTi) is a promising new alternative for malaria control (O'Meara *et al.*, 2005). Treatment doses with SP are delivered to infants irrespective of infection status, at the time of routine vaccination during the first year of life. A trial in Tanzanian infants found that IPTi had a 50% protective efficacy against severe anaemia (Schellenberg *et al.*, 2001). Studies in West Africa have shown that seasonal IPTi could be an effective malaria preventive strategy for children less than 5 years in areas of seasonal transmission (Cisse *et al.*, 2006; Sagara *et al.*, 2006) but it is yet to be determined whether or not this is a practical approach to malaria control (Greenwood, 2006). There is huge potential for the education sector to address the impact of malaria on school-age children (Bundy *et al.*, 2000). A Tanzanian study showed that teachers were effective in recognising and treating malaria in school children (Magnussen *et al.*, 2001). Another study in Malawi showed the effectiveness of presumptive treatment in reducing malaria-related mortality among schoolchildren where teachers were trained to use "first aid kits" and to dispense SP treatment to symptomatic children (Pasha *et al.*, 2003). Currently, the recommended control strategy for malaria during pregnancy in areas of stable transmission is IPT with SP. SP is safe in the second and third trimester, and can be given as a single dose (Menendez *et al.*, 2007). This reduces rates of maternal anaemia (Shulman *et al.*, 1999) and morbidity (Rogerson *et al.*, 2000).

Table 1.4 Priority malaria control strategies, by epidemiological setting (WHO, 2005b)

Epidemiological setting	Control strategy
Stable endemic malaria	Prevention
Examples: large parts of East, Central and West Africa, Papua New Guinea, Solomon Islands and Vanuata	- ITNs[a] for children under 5 years of age, pregnant women and people living with HIV/AIDS - IRS[b], where appropriate - IPT[c] in pregnancy
	Treatment
	- Early and effective case management including presumptive treatment for suspected cases and home management where appropriate
Unstable malaria	Prevention
Examples: parts of Southern Africa, Transcaucasia, Central Asia and the Americas: highland and desert fringe areas, plantations, irrigation schemes	- IRS - Larviciding - Environmental management - ITNs
	Treatment
	- Early and effective case management in suspected cases - Diagnostics to confirm cases, if possible before treatment
Free of malaria	Prevention
Examples: parts of Southern and North Africa, Ethiopian and Eritrean highlands and Transcaucasia	- For travellers going to malarious areas, chemoprophylaxis and personal protective measures against mosquitoes
	Treatment
	- Early and effective case management in suspected cases -Diagnostics to confirm cases, if possible before treatment

[a]Insecticide Treated Net
[b]Indoor Residual Spraying
[c]Intermittant preventative treatment

Other methods of control include the use of insecticide treated nets (ITNs), indoor residual spraying (IRS) and vaccination. Vector control in Africa has primarily relied on ITNs. The use of ITNs has been shown in well conducted trials to reduce overall mortality in African children by about 20% (Lengeler, 2004). The recent development of long-lasting insecticide treatment of nets (LLINs) provides a solution for the need to regularly re-treat nets. Although LLINs are more expensive than ITNs, the cost of maintaining coverage is lower, since they remain effective for 4-5 years. LLINs are currently promoted among high risk groups, especially young children and pregnant women (WHO, 2005b). The WHO also recommends that LLINs should be used by everyone including school children. Currently there is a very low level of use in African school children, if coverage is to increase, LLINs may need to be

provided free to schoolchildren (Brooker *et al.*, 2008). IRS is a highly effective method for malaria vector control and is particularly useful for achieving a rapid reduction in transmission during epidemics and other emergency situations (WHO, 2005b). The initial malaria eradication campaign relied heavily on indoor household spraying, usually with DDT. Spraying is being undertaken in parts of Africa where IRS has not been used before or not for many decades. DDT and pyrethoids are being used (Greenwood, 2007). The development of a malaria vaccine has been underway for some time (Menais, 1991). The RTS,S/AS02A is one of the most advanced malaria vaccine candidates in development (Ballou *et al.*, 2004). The vaccine specifically targets the pre-erythrocytic stage of *P. falciparum* and confers protection against infection by *P. falciparum* sporozoites (Alonso *et al.*, 2004). A recent clinical trial in Mozambique, showed that the malaria vaccine was safe, well tolerated, and immunogenic in young infants (Aponte *et al.*, 2007). In the vaccinated group, there were 22 cases of infection with *P. falciparum*, over a six-month study period compared with 46 cases in the control group. Overall, the vaccine was roughly 66% effective. Recently, deworming has been considered to be an alternative approach to malaria control (Druilhe *et al.*, 2005). It is speculated that helminth infections may alter the susceptibility to clinical malaria and therefore deworming could offer an alternative to roll back malaria (Druilhe *et al.*, 2005; Nacher, 2006).

1.2.3 Helminth and malaria co-infections

Humans are subject to infections with a range of parasite species. Ashford (1991) estimated that there are about 270 species of helminths, protozoa, and anthropods which can infect humans. Infection with multiple parasite species is the rule rather than the exception (Cox, 2001). Synergistic and competitive interactions can occur between parasite species, which can influence the likelihood of their successful transmission to other hosts and increase or decrease their overall pathogenic effect (Petney and Andrews, 1998). Observations suggest that geohelminth infection modifies the host's immune response to HIV and TB which increases the susceptibility and spread of these diseases in the developing world (Bundy *et al.*, 2000).

It is estimated that over a third of the world's population are infected with helminths (de Silva *et al.*, 2003) or one or more of the *Plasmodium* species (Snow *et al.*, 2005). Populations infected with species of helminths and malaria are mainly confined to the tropics and

subtropics and this results in high rates of co-infection (Petney and Andrews, 1998). The interactions between helminths and malaria have gained considerable attention from the scientific community in recent years (Basavaraju and Schantz, 2006; Mwangi *et al.*, 2006). It is speculated that helminth infections may alter the susceptibility to clinical malaria (Druilhe *et al.*, 2005; Nacher, 2006). This interaction has been studied in animal models (Lewinsohn, 1975; Helmby *et al.*, 1998; Yoshida *et al.*, 2000; Legesse *et al.*, 2004) and humans (Nacher *et al.*, 2000; Spiegel *et al.*, 2003; Sokhna *et al.*, 2004; Shapiro *et al.*, 2005). The findings are contrasting: some studies have shown that co-infection increases parasitaemia (Spiegel *et al.*, 2003) and clinical malaria, whereas others have shown co-infection to have the opposite effect (Lyke *et al.*, 2005). These observations raise the serious question of whether anthelminthic drugs would increase or decrease the population burden of diseases like malaria. The mechanism underlying this interaction is thought to be determined by the immune response, particularly the Th1/Th2 dichotomy (Specht and Hoerauf, 2007). The need for well-designed longitudinal intervention studies to further investigate this relationship between helminths and malaria is recognised (Mwangi *et al.*, 2006). The association between these two micro- and macro-parasites needs to be defined in order to implement effective control strategies.

1.2.3.1 Patterns of STHs/malaria co-infections

Malaria and STHs are widely co-endemic and are largely a burden of the poor. The worldwide rates of co-infection with malaria and helminths are expected to be high (Petney and Andrews, 1998). In absolute numbers, most infections with plasmodia and helminth species occur in Asia, but the largest clinical disease burden due to these infections is carried by populations living in sub-Saharan Africa (Mwangi *et al.*, 2006). In Africa, in areas of stable endemic malaria transmission between 17.9 and 32.1 million children aged 5-14 years are estimated to be at risk of co-infection with *P. falciparum* and different STH species, with the greatest risk for hookworm. Between 5.8 and 9.6 million and 1.6 and 3.4 million children are at risk of co-infection in areas of acute marginal seasonal transmission and marginal transmission, respectively (Brooker *et al.*, 2007). Climate is the main determinant of the geographical distribution of malaria and helminths with both parasitic species being prevalent in the tropics and sub tropics (Brooker and Michael, 2000; Hay *et al.*, 2000).

STHs and malaria share similar risk factors for disease occurance and also have similar high risk age groups. Risk factors that contribute to the co-endemicity of malaria and helminths include: inadequate sanitation, open sewers, crowded living conditions, lower socio-economic status and lack of access to health care. People living in poorer households, who have low levels of education, may not have the monetary resources or knowledge that might afford them protection against malaria (Worral *et al.*, 2003) and may also live in unsanitary conditions that leave them more exposed to helminth infection (Asaolu and Ofoezie, 2003).

Children aged less than five years are a high risk group for both malaria and helminths. While the intensity of STH infections is greatest among children aged 5-15 years (Bundy *et al.*, 1985; 1988; Crompton, 1994), STHs have been shown to induce morbidity in children aged less than five years who generally have light intensity infections (Stoltzfus *et al.*, 2001; 2004). Age patterns of malaria morbidity are dependent on the level of transmission in a community (Snow *et al.*, 1997). In areas of high malaria transmission, severe malaria is restricted to children under two years of age, whereas in areas of moderate transmission, it is restricted to children < 5 years of age. Severe malaria is rare among school-age children in endemic areas, asymptomatic *P. falciparum* infections are highest in this age group (Kimbi *et al.*, 2005).

1.2.3.2 Animal models

Four species of murine parasites are available to model human malaria: *P. chabaudi*, *P. berghei*, *P. yoelii* and *P. vinckei*. *P. chabaudi* provides an excellent experimental tool with many similarities to *P. falciparum* and *P. berghei* is a useful experimental model of cerebral malaria (Hernandez-Valladares *et al.*, 2005). Three of these parasites have been used in murine experiments to explore the relationship between helminths and malaria. Twelve studies, that show contrasting results, are listed in table 1.5. Five of the studies use the trematode, *Schistosoma mansoni* and the other six studies use the nematodes, *Brugia pahangi*, *Litomosoides sigmodontis*, *Trichinella spiralis*, *Nippostrongylus brasilensis*, *Heligmosomoides bakeri* and the trematode, *Echinostoma caproni* as the helminth infection.

1.2.3.2.1 *S. mansoni*/malaria co-infections

A study examining *S. mansoni* and malaria infections in Swiss TO mice showed that there was no difference in malaria parasitaemia or the development of anaemia in mice infected with both *S. mansoni* (3 or 5 weeks after infection) and *P. yoelii* (Lewinsohn, 1975). A later study showed that C57BL/6 mice co-infected with *S. mansoni* and *P. chabaudi* had higher malaria parasitaemia and mortality than mice infected with only *P. chabaudi* (Helmby *et al.*, 1998). The *S. mansoni* infection was initiated eight weeks before the *P. chabaudi* infection. *P. chabaudi* interferon gamma responses (IFN-γ) were unchanged but tumor necrosis factor (TNF-α) production was significantly lower in co-infected mice. In concurrently infected mice, Th2 responses to schistosome antigens were suppressed up to one month after the malaria infection, which suggests that malarial parasites may also be able to modulate responses to helminth co-infections. Mice with concomitant infections showed significantly reduced red blood cell (RBC) counts during the acute phase of malaria when compared to mice with *P. chabaudi* only. Likewise, the same result was found in Swiss albino mice co-infected with *S. mansoni* and a lethal strain of *P. berghei* (ANKA strain), where injection of infected erythrocytes took place seven weeks after infection with *S. mansoni*, resulting in increased parasitaemia and mortality (Legesse *et al.*, 2004). After treatment with chloroquine, co-infected mice showed a delay in parasite reduction/clearance in parasitaemia, which suggests that co-infections with schistosomes and malaria parasites would aggravate malaria severity and prolong parasite reduction or clearance after chemotherapy in humans.

Table 1.5 Animal models of co-infection (adapted from Hartgers & Yazdanbakhsh, 2006)

Background mouse	Malaria strain	Helminth type	Outcome for malaria disease	Reference
Swiss TO	*P. yoelii*	*S. mansoni*	No differences for parasitaemia or anaemia	Lewinsohn, 1975
CBA	*P. chabaudi*	*S. mansoni*	Decreased parasitaemia	Lwin *et al.*, 1982
CBA	*P. yoelii*	*S. mansoni*	No differences for parasitaemia	Lwin *et al.*, 1982
CD1	*P. berghei*	*T. spiralis* (larvae)	Decreased parasitaemia, decreased mortality	Ngwenya. 1982
CD1	*P. berghei*	*T. spiralis* (larvae)	No difference for mortality rate	Ngwenya. 1982
C57BL/6	*P. berghei*	*T. spiralis* (larvae)	Decreased mortality rate	Ngwenya. 1982
Mus musculus	*P. berghei*	*N. brasiliensis*	Prolonged helminth patent periods	Modric and Mayberry, 1994
CBA/J	*P. berghei*	*B. pahangi* (irradiated larvae)	Increased anaemia; decreased cerebral malaria	Yan *et al.*, 1997
C57BL/6	*P. chabaudi*	*S. mansoni*	Increased parasitaemia and mortality; lower TNF-α responses	Helmby *et al.*, 1998
A/J	*P. chabaudi*	*S. mansoni*	Decreased mortality; higher IFN-γ responses	Yoshida *et al.*, 2000
C57BL/6	*P. chabaudi*	*S. mansoni*	Increased parasitaemia and mortality	Yoshida *et al.*, 2000
Swiss albino	*P. berghei* (ANKA)	*S. mansoni*	Increased parasitaemia and mortality, delayed parasite clearance following chloroquine treatment	Legesse *et al.*, 2004
BALB/c	*P. chabaudi*	*L. sigmodontis* (no microfilaria)	Increased anaemia; higher IFN-γ responses	Graham *et al.*, 2005
BALB/c	*P. chabaudi*	*L. sigmodontis* (with microfilaria)	No differences for anaemia	Graham *et al.*, 2005
BALB/c	*P. yoelii*	*E. caproni*	Increased parasitaemia, increased mortality, exacerbation of malaria reversible by anthelmintic treatment	Noland *et al.*, 2005
C57BL/6	*P. chabaudi*	*H. bakeri*	Increased parasitaemia and mortality; lower IFN- γ responses	Su *et al.*, 2005
-	*P. yoelii*	*E. caproni*	Higher rate of *Anopheles stephens* mosquito infectivity	Noland *et al.*, 2007

- = Abstract with no details for background of mouse

In contrast to these last two studies, A/J mice that were co-infected with *S. mansoni* and *P. chabaudi* could clear malaria parasites and had decreased mortality when compared to A/J mice that were infected with only *P. chabaudi* (Yoshida *et al.*, 2000). The co-infected mice also showed enhanced production of IFN-γ, which was shown to be responsible for the resistance to malaria. This indicates that the effect of helminth infections on malaria differ in the strain of mouse used. Interestingly, mice in this study were infected with 25 *S. mansoni* cercariae unlike Helmby's and Legesse's study where mice were infected with 50 *S. mansoni* cercariae. Yoshida and colleagues repeated the experiment using C57BL/6 mice and showed

that mice co-infected with schistosomes and malaria had increased parasitaemia and mortality; these results were concurrent with findings of Helmby *et al.* (1998). This discounts the idea that the dose of *S. mansoni* might have altered the results in A/J mice. A study by Lwin *et al.* (1982) showed that CBA mice infected for 8-12 weeks with *S. mansoni*, when co-infected with *P. chabaudi*, showed lower parasitaemia compared with mice not infected with *S. mansoni*. This finding contrasts with Helmby's study which showed a reverse effect in C57Bl/6 mice with the same malaria and helminth species. Lwin and colleagues also indicated that the outcome of co-infection may also depend on the malaria parasite strain used. There was no effect of *S. mansoni* infection on the parasitaemia caused by *P. yoelii*, unless a more virulent *P. yoelii* strain was used. It was suggested that egg deposition may be important for modulation of the responses to malaria as male *S. mansoni* worms had little inhibitory effect on malaria in co-infected mice (Lwin *et al.*, 1982).

1.2.3.2.2 Other malaria/helminth co-infections

A study with CD1 mice showed that mice co-infected with *Trichinella spiralis* and *P. berghei* had decreased parasitaemia and mortality when compared with control mice only infected with *P. berghei* (Ngwenya, 1982). In the same study, Ngwenya tried to substantiate these results in CD1 and C57BL/6 mice, that *Trichinella* infection is associated with prolonged survival and subdued parasitaemia. Mice were infected with *P. berghei* 65 days after *Trichinella* infection (compared to 10 and 30 days in the previous experiment). The results showed no marked difference between the mortality rate of control and experimental CD1 mice. However, C57BL/6 mice similarly infected and challenged, outlived the control mice showing a decreased mortality rate. This suggests genetic differences between the two different strains of mice, inbred C57BL/6 being more resistant to *P. berghei* infection than outbred CD1 mice. In contrast to this, another study with C57BL/6 mice showed that chronic infection with the nematode for two, three or five weeks before *P. chabaudi* (AS strain) infection severely impaired the ability of mice to control malaria, as demonstrated by severe mortality and significantly increased malaria peak parasitaemia levels (Su *et al.*, 2005). Co-infected mice produced significantly lower levels of IFN- γ during *P. chabaudi* (AS) infection than mice infected with malaria alone. Anthelminthic treatment before *P. chabaudi* (AS) infection restored immunity to malaria, which suggests that nematode-induced immunosuppression is not permanent and requires the presence of a chronic nematode infection.

Yan *et al.* (1997) investigated the course of *P. berghei* malaria infection in CBA/J mice inoculated with irradiated attenuated third-stage larvae of *Brugia pahangi.* Results showed that the filarial antigen induced a Th2 cell predominance in co-infected mice which protected them against the development of cerebral malaria. The survival of co-infected mice was significantly prolonged when compared with the control mice after *P. berghei* infection. Co-infected mice also suffered from increased anaemia. BALB/c mice co-infected with *Litomosoides sigmodontis* and *P. chabaudi* (non-cerebral malaria) that did not have microfilaremia had more severe anaemia and loss of body mass than did mice with malaria alone (Graham *et al.*, 2005). Microfilaremia concerns patent, transmissible infection. Malaria was more severe in co-infected mice that did not have microfilaremia and this was associated with increased IFN-γ responsiveness. Malaria infections were also exacerbated in BALB/c co-infected with *Echinostoma caproni* and *P. yoelii* (Nolland *et al.*, 2005). Co-infected mice displayed significantly increased malaria parasitaemia, extended patency of malaria, and increased fatality compared to mice infected only with *P. yoelii.* Clearance of *E. caproni* worms with praziquantel treatment significantly reduced parasitaemia and prevented mortality. Another study by Nolland *et al.* (2007) examined the effect of co-infection with *E. caproni* and *P. yoelii* on malaria transmission and found that *Anopheles* mosquitoes had a higher rate of infectivity when exposed to co-infected mice than mice infected with malaria alone. Modric and Mayberry (1994) reported no effect of a pre-existing *N. brasilensis* infection on *P. berghei* infections. Instead they found that co-infected mice had prolonged helminth patent periods which indicated that *Nippostronglyus* self-cure in concurrently infected mice was suppressed.

The studies on helminth-malaria interactions in murine models show different results depending on the genetic background of the mouse, the type and stage of helminth infection and the species or strain of malaria that is used. *P. chabaudi* and *P. berghei* might best be used to study immunology and pathogenesis of malaria as these are models for *P. falciparum* and cerebral malaria, respectively. Animal models are important for improving our understanding of the immunological mechanisms underlying these interactions and also examining the affect of co-infections on morbidity. Furthermore, when conducting these experiments in rodent models, the timing of infection can be controlled and therefore we can

also use these models to look at the effect of existing malaria infections on helminth infections which has not been studied.

In order to draw accurate conclusions, comparisons need to made between studies where all factors are standardized except for one, the point of interest. Only three studies can be compared to examine the effect of helminth species on malaria-helminth interactions (Helmby *et al.*, 1998; Yoshida *et al.*, 2000; Su *et al.*, 2005). These studies use the same mouse strain (C57BL/6), and malaria species (*P. chabaudi*), while two of the studies use the same helminth species (*S. mansoni*; Helmby *et al.*, 1998; Yoshida *et al.*, 2000) and the other study uses a different helminth species (*H. polygyrus*; Su *et al.*, 2005). When comparing these studies, we can conclude that different helminth species have the same interaction with malaria i.e. they increase malaria parasitaemia and mortality in co-infected mice. However, this is a big assumption to make based on the results of three studies that allow for a comparison of only two species of helminth. Future research should build on the existing studies to investigate whether or not different helminth species show the same interaction with malaria, using the same species of malaria in the same strain of mouse. Only one study examined the effect of different species of malaria keeping all other factors constant (Lwin *et al.*, 1982), thus it is evident that the effect of malaria species on helminth-malaria interactions also needs further investigation.

1.2.3.3 Human studies

There are three possible outcomes for helminth-malaria interactions: (1) helminths may exacerbate malaria infections by increasing parasitaemia, disease symptoms and pathology; (2) helminth infections might suppress malaria disease symptoms and pathology or (3) helminths may have no negative or positive effect on malaria infections. All of these outcomes have been shown in human studies (Shaprio *et al.*, 2005; Sokhna *et al.*, 2005; Brutus *et al.*, 2006). It is important to keep in mind when comparing results from these human studies that differences may arise due to study design. The study designs used include: monitoring disease trends, cross-sectional surveys, case-control, longitudinal and intervention studies. The disadvantages and advantages of each study design are listed in table 1.6. Mwangi and colleagues (2006) noted that most of the studies have been conducted by two groups: Pierre Druilhe of the Pasteur Institute and his Senegalese collaborators whose studies

have focused mainly on uncomplicated malaria and Mathieu Nacher and his Thai collaborators at the Mahidol University whose studies have focused on severe malaria with the exception of one study which concerned uncomplicated malaria. The human studies that have investigated the effect of helminths on uncomplicated malaria and severe malaria are summarised in tables 1.7 and 1.8. The studies on uncomplicated malaria and severe malaria (Section 1.2.2.3) will be discussed separately.

Table 1.6. Advantages and disadvantages of study designs to investigate interactions between helminth infections and malaria (Mwangi *et al.*, 2006).

Study Type	Methodology	Advantages	Disadvantages
Ecological	Association between helminth and malaria infection/disease investigated in different communities	Uses existing data Cheap and easy to perform Avoids measurement and design limitations of individual-level studies	Individual level associations lost in some studies Failure of ecological effects estimated to reflect biological effects (ecologic bias) Collinearity in analysis Migration may occur across groups over time
Cross-sectional	Association between helminth and malaria infection/disease investigated at a single time point in randomly selected individuals	Simple to perform Ideal for common conditions	Cannot determine temporal associations For rare conditions, large sample size required Re-call bias for confounders Current information may not be aetiologically relevant
Case-control	Individuals with helminths or malaria (cases) compared to uninfected individuals (controls)	Ideal for rare conditions Can be hospital-based and therefore low cost	Selection of controls problematic Temporal sequence of events difficult to elucidate
Longitudinal or cohort	Follow-up for individuals with none or one of the infections for a period of time	Clear associations between conditions Temporal sequence clear	Expensive Time-consuming Drop-outs if long-term follow-up Repeated samplings (especially of blood) problematic
Intervention	Intervene against one infection (easier for helminths) and investigate the effect on the other infection (malaria)	Direct means of determining effect of one condition over the other Can show small/moderate effects	Expensive Time-consuming Ethical issues in untreated group

Table 1.7. Summary of key features and results of human studies that investigated interactions between helminth infections and uncomplicated malaria (adapted from Mwangi *et al.* 2006)

Location	Study design	Age group (years)	Sample size	Malaria prevalence	Helminth prevalence	Relative risk, odds ratio*, associations and conclusions.	Reference
Comoro Islands	Cross-sectional	0-14	869	1.7% & 23%	As: 93% & 24%	As infection protect against malaria	Murray *et al.* (1977)
Comoro Islands	Intervention with longitudinal follow-up (20days)	2-14	122 (in 4 groups)	1.7%	As: 93%	51% malaria attacks in those treated vs. 0% in non-treated.	Murray *et al.* (1978)
Zaire	Cross-sectional survey	All ages	1,100	61%	STH	The presence of mixed infections does not increase or reduce the clinical presentations of parasites	Tshikuka *et al.* (1996)
Thailand	Longitudinal (1 year)	All ages	731	-	STH: 62%	Pf: AOR=2.24 (1.4-3.6) and Pv: AOR=1.1 (0.6-2) STH increases incidence of P.f non-severe malaria	Nacher *et al.* (2002c)
Senegal	Daily malaria surveillance and cross-section helminth survey	1-14	80	86%	STH: 16%	STH: RR=1.54 (p=0.003).	Spiegel *et al.* (2003)
Senegal	Passive malaria surveillance & multiple cross-sectional helminth surveys	5-15	511	5-11%	Sm: 67%	Heavy Sm (>1000epg): RR=2.24 (p=0.01) Heavy Sm infection increases risk	Sokhna *et al.*, (2004)
Uganda	Weekly active malaria surveillance & single cross-sectional helminth survey	All ages	435	-	As 17% Hk: 32% Tr: 8% Any: 47%	As: AOR=1.14 (0.59-2.2) Hk: AOR=1.03 (0.56-1.91) Tr: AOR=1.24 (0.36-4.24) Any: AOR=1.08 (0.59-1.95) No association	Shapiro *et al.* (2005)
Senegal	Four cross-sectional malariometric surveys and single helminth survey	3-15	523	50%-56%	Sh: 67% STH: 31%	Children lightly infected with Sh had lower Pf densities. No association between STH and Pf. Sh may be protective against Pf	Briand *et al.* (2005)
Senegal	Weekly active surveillance	4-14	654	-	Sh: 25%	Sh: RR=0.76 (0.59-0.97)	Lyke *et al.* (2005)
Madagascar	Controlled randomised trial of 2-monthly anthelminthic treatment (levamisole)	All ages	350	26.6%	As: 26.9% Sm: 8.6% Hk: 9.4%	Subjects in the treatment group >5yrs had a statistically significant increase in Pf densities compared to controls.	Brutus *et al.*, (2006)

RR = Relative risk, AOR = Adjusted Odds Ratio

Malaria prevalence refers to *P. falciparum* parasite prevalence within the studied populations

*95% confidence intervals in parentheses. Sh = *S. haematobium*, Sm = *S. mansoni*, As = *A. lumbricoides*, Hk = Hookworm, Tr = *Trichuris trichiura*, STH = all STH species, Pf = *P. falciparum*, Pv = *P. vivax*

- = Data not recorded

Table 1.8 Summary of key features and results of the studies that investigated interactions between helminth infections and severe malaria in Thailand[a] (adapted from Mwangi *et al.*, 2006)

Study design	Sample size	Malaria prevalence (%)	Helminth prevalence	Relative risk, odds ratios*, associations and conclusions	Author
Case control[b]	537	24-93	As	Cerebral malaria: AOR=0.25(0.009-0.67) Pulmonary oedema: AOR=0.34(0.04-2.65) Acute renal failure: AOR=0.46(0.16-1.31)	Nacher *et al.*, (2000)
Case-control[c]	336	93	STH, Sm	AOR=0.28(p=0.03) STH protective against malaria-related renal failure	Nacher *et al.*, (2001c)
Cross-sectional case records[d]	307	61.2	STH, Sm	STH associated with increased gametocyte carriage	Nacher *et al.*, (2001b)
Hospital survey	200	86.2	Hk	Hk associated with low body temperature among mild malaria cases	Nacher *et al.*, (2001d)
Case-control[b]	98	-	STH, Sm	Cerebral malaria: AOR=0.24(0.07-0.78) STH protective against cerebral malaria after controlling for SES and nutritional status	Nacher *et al.*, (2002b)
Case-control[b]	284	5-11.2	STH Sm	Cerebral malaria: AOR=0.36 (0.19-0.7) STH protective against cerebral malaria	Nacher *et al.*, (2002a)
Hospital survey and community controls	210	-	As: 50% Sh: 8%	As: AOR = 9.95 (3.03-32.7) Sh: AOR = 3.47 (0.95-6.38) As predisposes to severe malaria	Le Hesran *et al.*, (2004)

Malaria prevalence refers to *P. falciparum* parasite prevalence within the studies populations.

[a]All studies conducted in the Hospital of Tropical Diseases in Bangkok except the study by Le Hesran *et al.* (2004) and all studies were conducted in adults (≥15years) except Le Hesran *et al.* (2004) in which children were recruited.

[b]Individuals with severe malaria compared to controls with mild malaria accompanied by high parasite biomass or circulating schizonts,

[c]Individuals with malaria-related renal failure compared to controls with mild malaria accompanied by high parasite biomass or circulating schizonts,

[d]Individuals with increased gametocyte carriage during mild malaria compared to controls with normal carriage.

*95% confidence intervals in parentheses. Sh = *S. haematobium*, Sm = *S. mansoni*, As = *A. lumbricoides* , Hk = hookworm,, STH = all STH species, AOR = adjusted odds ratio, - = Data not recorded

though the drive to explore the interactions in helminth-malaria co-infections has increased dramatically in recent years, the first study to examine this relationship was conducted approximately 30 years ago. An ecological study in the Comorro islands in the Indian Ocean focused on two groups of children aged 2-14 years on neighbouring islands, Anjouan and Grande Comore and observed an association between *A. lumbricoides* and malaria (Murray *et al.*, 1977). Children on Anjouan had a high prevalence of *A. lumbricoides* (93%) and low prevalence of malaria (1.7%) and enlarged spleen (1.1%) whereas children on Grande Comorre had a lower prevalence of *A. lumbricoides* (24%) and a high prevalence of malaria (23%) and enlarged spleen (18%). This finding led to a subsequent intervention study involving 112 children aged 2-14 years divided into four groups: group one had heavy infestations with *Ascaris*, no malaria and had enlarged parotids (salivary glands) and forehead edema who received a deworming drug; group two had similar findings and received a placebo; group three had 'minimal ascariasis', defined as light infections, no malaria and normal parotids and no forehead edema, these children received a deworming drug and group four had similar findings to group three and were treated with placebo (Murray *et al.*, 1978). After 20 days, 54% of group one suffered malaria attacks, 97% had parotid regression and all children had edema regression. Thus, *A. lumbricoides* was shown to have a protective effect against malaria. This study has been ignored by some authors because the children studied were severely malnourished (Druilhe, 2006). The study also has other limitations owing to the small sample size (112 divided into four treatment groups) and short follow-up (Mwangi *et al.*, 2006).

Eighteen years later a cross-sectional study in Zaire provided evidence for co-occurrence of *Plasmodium* and *A. lumbricoides* (Tshikuka *et al.*, 1996). In this study *Ascaris* and *Plasmodium* were the most frequent double-species infection, with a significant positive association between these two micro- and macro-parasites. Other subsequent studies investigated the interactions in helminth-malaria co-infections. In Thailand, a longitudinal study demonstrated that intestinal helminth infections are associated with an increased incidence of *P. falciparum* (Nacher *et al.*, 2002c). This study took place in a mountainous area, in the Suan Phung district, with low malaria transmission. 731 villagers of all age groups were screened for helminths at the beginning of the study and monitored for malaria for up to one year. Disease was defined as the presence of malaria symptoms with a positive malaria slide. 62% of the population were infected with helminths, the predominant infections being

hookworm and *T. trichiura*. Similar findings were found in a case-control study in Senegal involving 80 children aged 1-14 years (Spiegel *et al.*, 2003). The study showed an increased risk of presenting with clinical malaria attacks in subjects with intestinal worms compared with helminth-free children. The study was conducted over a year with a fever threshold of parasite density, characteristic of each age group to distinguish malaria from other fevers. The sample size was small and only 13 of the children were infected with one of the STHs. A cross-sectional examination of stools in the middle of the study was used to identify individuals that had STHs through the follow-up period.

In another Senegalese study, 511 children aged 5-15 years were monitored for malaria for eight months (Sokhna *et al.*, 2004). Children infected with only *S. mansoni* infections were included in the analysis. Children were screened at the beginning of the study and four months into the study for intestinal helminths. 67.5% of the children were infected with *S. mansoni*. Malaria attacks were defined as children presenting with a body temperature above 38°C and with a parasite density ≥ 5,000 parasites/μl of blood. Again the results were similar to the two previous studies, the incidence rate of malaria attacks was significantly higher among *S. mansoni*-infected individuals, particularly those carrying the highest worm loads, as compared to uninfected subjects. In a Ugandan study, no association was shown between helminth infection and clinical malaria (Shapiro *et al.*, 2004). Households were monitored weekly for malaria over 18 months and a cross-sectional survey was undertaken at the end of the study to screen subjects for helminths. 47% of people were infected with at least one helminth species. Malaria attacks were defined as individuals presenting with fever ≥37.5 °C and parasitaemia. Household clustering and socioeconomic status was taken into account in the analysis and even when the analysis was restricted to the last six months, no association was observed. The authors noted that they were not sure if individuals were infected with helminths at the time of their malaria attacks.

In 2005, Briand *et al.* examined the relationship between *S. haematobium* and *P. falciparum* in 523 Senegalese children aged 3-15 years. This study found that children lightly infected with *S. haematobium* had lower *P. falciparum* densities than those not infected. The study was conducted over six months, subjects were examined for malaria on four occasions and subjects were screened for helminths at the end of the study and the assumption was made that subjects who were positive for *S. haematobium* were so for the previous six months. 31% were

infected with other helminths and these were not excluded from the analyses. This study suggests that *S. haematobium* has a protective effect against *P. falciparum*. Another study in Senegal also found *S. haematobium* to have a protective effect against *P. falciparum* malaria (Lyke *et al.*, 2005). This was a case-control study with 654 children aged 4-14 years being matched for age, sex and residence in an area of intense seasonal malaria transmission. Clinical malaria attacks were defined as children presenting with parasitaemia and an auxillary temperature $\geq$37.5 ^{o}C. Children were screened for helminths at the beginning of the study and treated for intestinal infections regardless of helminth status. Subjects were followed-up for a 25-week period and screened for helminths at the end of the study. Children infected with schistosomiasis had less malaria, longer disease-free intervals until the first clinical episode, and reduced parasitaemias during that episode. This was the first study to show an age dependent effect as this result was only seen in children aged 4-8 years.

In 2006, Brutus *et al.* published the first controlled randomised trial of anthelminthic treatment in children and adults aged six months to $\geq$ 15 years. Subjects more than five years of age, treated with levamisole had a significant increase in their *P. falciparum* densities compared with controls, whereas there was no effect of anthelminthic treatment on children six months to four years of age. The study took place over a period of 17 months in Madagascar in a highland village where malaria is mesoendemic. Levamisole was given to the treatment group and a multivitamin treatment was given to the control group every two months and children were screened for malaria and helminths at each of these time points. 350 subjects were included in the analysis. *A. lumbricoides* was demonstrated to have a protective effect against malaria. The analysis was based on malaria parasitaemia, no measure of clinical malaria was taken. Levamisole is an immune response regulator, modulating the immune function at a dose of 2-5 mg kg^{-1} body weight (Sajid *et al.*, 2005). The dose of levamisole administered in the Brutus study was 3 mg kg^{-1} for children and 150 mg in adults. It has been demonstrated that levamisole can induce production of natural killer cells and also cause a reduction in suppressor T cell function. Therefore it is possible that levamisole could influence the susceptibility to malaria infections and thus should not be used as the anthelminthic of choice in future studies investigating interactions between helminths and malaria.

The results from these studies indicate that the outcome of helminth-malaria interactions may depend on the species of helminth involved, the age of the population studied, and the

intensity of the helminth infection. Comparing these studies is difficult due to differences in study design such as: the case definition for malaria, the malaria transmission setting, and the method of monitoring helminth infections. Some studies examine the effect of helminths on parasitaemia but fail to examine clinical malaria. Only one study included a malaria fever-attributable fraction in their case definition for clinical malaria. Animal models have shown that the outcome of helminth-malaria interactions can depend on the species of malaria involved. Identifying malaria to species is difficult and time-consuming and is not possible for some large-scale epidemiological studies (Shapiro *et al.*, 2004), therefore some studies do not differentiate between malaria species (Spiegel *et al.*, 2003; Sohkna *et al.*, 2004). Different malaria transmission settings have different effects on malaria morbidity in different age groups and populations; this has already been discussed and could also contribute to the differences shown in these studies. Four studies have used cross-sectional surveys to examine helminths at one or two time points (Spiegel *et al.*, 2003; Shapiro *et al.*, 2004; Briand *et al.*, 2005; Lyke *et al.*, 2005). Assuming that subjects were infected with helminths for the entire duration of the study based on one or two cross-sectional surveys may introduce error and makes interpretation of the results difficult. It is interesting that when the results of these studies on different species of helminths are compared, they show contrasting results but when studies on different helminth species are considered separately, trends are noticeable. *S. haematobium* seems to protect against malaria (Briand *et al.*, 2005; Lyke *et al.*, 2005), while *S. mansoni* increases the incidence of malaria (Sokhna *et al.*, 2004). The two intervention studies have also shown that *A. lumbricoides* protects against clinical malaria (Murray *et al.*, 1978; Brutus *et al.*, 2006). Therefore, it may not useful to compare studies that involve different species as it may lead to confusion.

Nacher and colleagues have produced a series of publications examining the interactions between helminths and severe malaria in adults. These case-control studies have taken place in the Hospital of Tropical Diseases, in Bangkok, Thailand. Nacher's definition of cases are based on WHO criteria for severe malaria (Nacher *et al.*, 2000). When comparing cerebral malaria with mild controls, *A. lumbricoides* was associated with protection from cerebral malaria (Nacher *et al.*, 2000). This protection ranged from 40% to 70% depending on whether exposure to *Ascaris* was measured qualitatively or quantitatively. In a subsequent study helminths were shown to be associated with protection from renal failure (Nacher *et al.*, 2001c) and helminth-infected controls were less likely to have jaundice. These findings

suggest that pre-existing helminth infections may have been protective by influencing sequestration and obstructive jaundice. The authors noted that the proportion of sequestered parasites in helminth-infected controls seemed to be lower than in controls without helminths i.e. the patients were more tolerant to high burdens of malaria parasites. In another study, helminth infections were associated with presence of gametocytes (Nacher *et al.*, 2001b). However, when the authors adjusted for haemoglobin concentration the significance of the association between helminths and gametocytes disappeared. This suggests that pre-existing helminth infections may increase the severity of malarial anaemia and therefore increase the likelihood of carrying gametocytes. A later study also found that *A. lumbricoides* was associated with a protective, dose-dependent effect against cerebral malaria and was found to hold for all helminths even after controlling for nutritional status and personal protection measures against mosquito biting (Nacher *et al.*, 2002a). Helminth-infected controls had higher reactive nitrogen intermediates (RNI) than those without helminths. An increase of RNI could be both protective and pathogenic depending on the concentration of sCD23. Nacher *et al.* (2002b) showed that intestinal helminths and malnutrition were independently associated with protection from cerebral malaria and thus, this apparent association is not a result of socio-economic or nutritional confounders. The risk of mixed plasmodia infections was suggested to be increased in individuals infected with helminths compared to uninfected individuals (Nacher *et al.*, 2001a). Results from Nacher's Thailand studies suggest that while helminths may increase the risk of uncomplicated malaria they may also protect against cerebral malaria.

Le Hesran (2004) carried out a field-based, case-control study, involving children in a rural zone of Senegal where 105 presumptive severe malaria attacks were studied. Presumptive severe malaria was defined as children presenting fever and repeated vomiting and/or coma and/or convulsions and/or temperature >40°C. The study showed, in contrast to Nacher's work, that the risk of *A. lumbricoides* infection increased in the case of severe malaria i.e. *Ascaris* was not associated with protection from severe malaria. It is important to note that the latter study was a field-based study involving children as opposed to Nacher's (2000) study, which was hospital-based involving adults, in a very different transmission setting (Nacher, 2005). The case definitions for malaria were different in Nacher's studies (Nacher *et al.*, 2000; Nacher *et al.*, 2002a) and Le Hesran's (2004) study. About 40% of the initial diagnoses

in Le Hesran's (2004) study were revealed to be non-severe malaria, which suggest the diagnostic accuracy was poor (Nacher, 2005).

It is difficult to directly compare Nacher's studies on severe malaria with those studies investigating uncomplicated malaria because of differences in study population and design. *Plasmodium* species (mixed *P. falciparum* and *P. vivax* in Thailand versus predominately *P. falciparum* in Africa; age groups (adults in Thailand and children in Africa); study population (hospital-based in Thailand and community-based in Africa) and the choice of controls (individuals hospitalised with malaria and high parasite biomass with circulating schizonts but no clinical signs of severe malaria in Thailand and children without severe malaria in Africa) differed between the studies (Mwangi *et al.*, 2006). It has been suggested that helminths may increase the susceptibility to uncomplicated malaria disease but reduce the risk of hosts developing cerebral malaria (Nacher, 2006). Different helminths and protozoa have synergistic and antagonistic interactions (Christensen *et al.*, 1987). This has been demonstrated in human studies where *S. haematobium* and *S. mansoni* had different interactions with malaria (Sokhna *et al.*, 2004; Briand *et al.*, 2005). It may be more useful to view the studies that have already been conducted as 'adding a piece to the puzzle' rather than 'contrasting' or 'conflicting' with each other as it is difficult to believe that worms and malaria have black and white interactions.

1.2.3.5 Mechanism of interaction

1.2.3.5.1 Immunological response to infection

The immune response can be divided into two components: innate immunity and adaptive immunity. The innate immune response, involving phagocytes, mast cells and dendritic cells, is the first line of defense against infection, rapidly responding to pathogens that have penetrated the host's external defenses. Although it has some pathogen-specificity, it has no memory, affording the host no long-term protection. Adaptive immunity is a stronger, longer lasting immunity involving T- and B-cells. It is an antigen-specific response and leads to a more rapid expulsion of the pathogen on its second or third entry (Goldsby *et al.*, 2003).

One of the main subsets of T cells involved in driving the adaptive immune response are known as CD4+ T-helper cells (Th). They have no cytotoxic or phagocytic activity and are

involved in activating and directing other immune cells. They can further differentiate into Th1 and Th2 cells (as well as regulatory T-cells (Tregs) or Th17 cells), depending on how they are activated (Goldsby *et al.*, 2003). The differentiation of Th cells into their particular subset will determine the type of immune response elicited by the host. Th1 cells predominantly induce a cell-mediated response which generally occurs in response to intracellular pathogens, such as malaria, while Th2 cells induce a more humoral response with the production of antibodies, mast cells and eosinophils and is commonly associated with the control of extracellular pathogens such as helminths. The balance between the two main arms of the immune response, Th1 and Th2, is controlled by small secreted proteins known as cytokines, which act as intracellular messengers (Figure 1.3; Goldsby et al, 2003). Each subset of Th cell has its own distinct pattern of cytokines - Th1 cells secrete interferon-γ (IFN-γ) and are involved in monocyte/macrophage-mediated inflammatory responses, while Th2 derived cytokines (interleukin-4 (IL-4), IL-5, and IL-13) encourage antibody production (including IgE responses) and promote mast cell and eosinophil proliferation and function (Muraille and Leo, 1998). The production of these cytokines and the effector mechanisms they drive are crucial in the control of parasite infections.

1.2.3.5.2 Immunological response to malaria and helminth infection

The immune response induced by malaria is complex and depends on the species and stage of infection. T cells are crucial to malaria immunity (Weidanz and Long, 1988). The parasites trigger the production of IFN-γ from these cells, thus stimulating the production of IL-1 and tumor necrosis factor-α (TNF-α) by macrophages, and nitric oxide by neutrophils and macrophages – all important in the control of the parasite. As the infection progresses the response develops a typical Th1 response (including IFN-γ, TNF-α, IL-12, and IL-18) (Torre *et al.*, 2001). After repeated infection the immune response changes to a Th2/Treg type response, with the production of anti-inflammatory cytokines (IL-4, IL-10, transforming growth factor (TGF)- α), which down regulates the inflammatory events leading to pathology (Druilhe *et al.*, 2005).

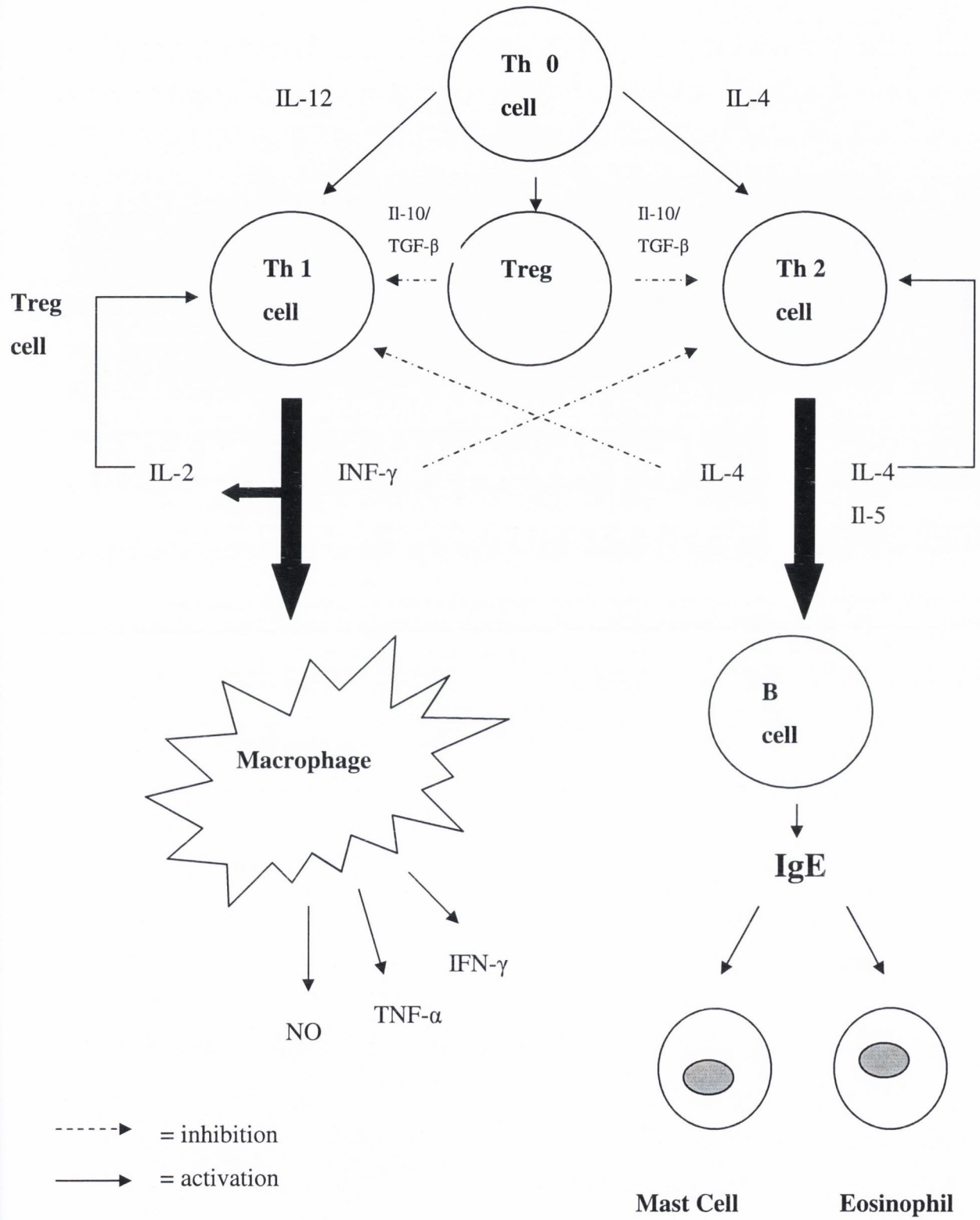

Figure 1.3 Th1 versus Th2 immune response during infection (adapted from Hamilton, 2006).

Helminth infections are associated with polarisation towards a Th2 response. This is characterised by high levels of cytokines such as IL-4, IL-5, IL-13 and high serum levels of immunoglobulin E (IgE) (van Riet *et al.*, 2007). Despite these strong Th2 responses, adult worms often survive in the host for long periods of time. The survival of helminths in this environment is thought to be facilitated by the induction of immunoregulatory mechanisms (Hartgers and Yazdanbakhsh, 2006). These mechanisms include the induction of Tregs and modulation of cells of the innate immune system, such as macrophages and dendritic cells, which results in an anti-inflammatory environment characterised by increased levels of IL-10 and TGF-β. Immunomodulation is thought to be beneficial to both the human host and the parasite, as it can protect helminths from being expelled, and at the same time protect the host from excessive pro-inflammatory responses that may lead to organ damage. This suppression of the immune response is evident in chronic infections. The modulation of the immune response might also affect the immune reaction to concomitant infections.

1.2.3.5.3 Mechanisms of immunological interaction between helminths and malaria

Many papers have discussed the possible immunological mechanism of action that underlies helminth/malaria interactions (Druilhe *et al.*, 2005; Druilhe *et al.*, 2006; Hartgers and Yazdanbakhsh *et al.*, 2006; Helmby, 2007; Specht and Hoerauf, 2007; van Riet *et al.*, 2007). So far, the literature has focused on the hypothesis that helminths make the host more susceptible to uncomplicated malaria (Druilhe *et al.*, 2005). It is thought that the Th2 cytokine response induced by the helminth infection predominates, resulting in down-modulation of Th1 cytokines, thus exacerbating malaria infections (Basavaraju and Schantz, 2006). This has been shown in animal models where mice co-infected with *H. polygyrus* and *P. chabaudi* produced significantly lower levels of IFN-γ than mice infected with malaria alone (Su *et al.*, 2005). Graham (2008) conducted a meta-analysis of data from 54 experiments that compared microparasite densities in laboratory mice with and without helminth infection. The analysis showed that the greater the helminth-induced suppression of IFN-γ, the greater the increase in microparasite density. The role of IFN-γ is considered to be crucial in the control of malaria in the early stage of infection. Peripheral blood mono-nuclear cells taken from African children with mild malaria are significantly better at producing IFN-γ in response to malaria antigens *in vitro*, than cells taken from children with severe disease (Luty *et al.*, 1999).

The acquisition of immunity to malaria is slow and increases with age in human populations. This is attributed to a switch of non-cytophilic (IgG2, IgG4, and IgM) towards cytophilic classes of antibodies (IgG1 and IgG3) (Bouharoun-Tayoun and Druilhe, 1992). Helminth infections are known to drive the immune response towards the production of non-cytophilic subclasses (IgG2, IgG4 and IgM), and therefore it is possible that helminths may exacerbate malarial attacks by contributing to this slow acquisition of immunity (Druilhe *et al.*, 2005).

The other side of the argument is that helminth infection protects against malaria. One theory that explains why helminths may protect the host from cerebral malaria focuses around the mechanism of nitric oxide (NO) (Anstey *et al.*, 1996; Hobbs *et al.*, 2002). Nacher (2002c) put forward a suggestion that the IgE cellular receptor, CD23, has an important role to play. This receptor is found on a variety of blood cells and activates an intracellular response that can lead to elevated endogenous NO levels (Dugas *et al.*, 1995). In helminth-infected hosts, the Th2 response elevates IgE concentrations; this activates binding CD23 and results in subsequent reduction of soluble CD23 and production of NO. The increased NO levels are suggested to be associated with parasite sequestration and the down-modulation of cerebral malaria (Hobbs *et al.*, 2002). Red blood cell (RBC) availability may also play a role in protection. Graham (2008) demonstrated that helminths that cause anaemia-imposed resource limitation on RBC-dependant microparasites (i.e. malaria) and thereby reduced their population size compared with what was observed during microparasite infection alone. This suggests that some protection maybe conferred to hosts co-infected with helminths that cause anaemia, (hookworm and schistosomes) and malaria.

Studies by Brutus *et al.* (2006) and Murray *et al.*, (1978) have shown that *A. lumbricoides* is protective against uncomplicated malaria, with host malnutrition being suggested as a potential mechanism for this interaction (Murray *et al.*, 1978). Murray suggested that *Ascaris* may deprive the *Plasmodium* of a nutrient essential for its growth and by doing so may permit optimal co-survival of the host and its parasites. This view has been discounted as malnutrition is more often associated with increased risk of malaria (Shankar, 2000). The discovery of cross-reactive antigens may provide another possible mechanism for this interaction. Naus *et al.*, (2003) showed that individuals living in malaria-endemic areas with no schistosomiasis had anti-*P. falciparum* antibodies in serum that reacted with *S. mansoni*

worm and egg antigens and, conversely, anti-*S. mansoni* antibodies from individuals living in schistosomiasis-endemic areas that are free from malaria reacted with *P. falciparum* antigens. Pierrot *et al.* (2006) has described a cross-reactive antigen that is shared between *S. mansoni* and *P. falciparum*, SmLRR. It is quite possible that cross-reactive antigens exist for *A. lumbricoides* and malaria that could confer protection from malaria in hosts co-infected with these parasites. The immunological mechanisms underlying the interactions in hosts co-infected with helminths and malaria are still unclear. The theories put forward are biologically plausible but have yet to be proven.

1.2.3.6 Integrated Control

The relationship between helminths and malaria is still unclear. Caution is needed, as if helminths suppress malaria symptoms (Murray *et al.*, 1978; Nacher *et al.*, 2000; Briand *et al.*, 2005; Lyke *et al.*, 2005; Brutus *et al.*, 2006) then deworming programmes could increase malaria morbidity. Thus, a combined approach to STH and malaria control would seem appropriate. Mass chemotherapy with anthelminthics, delivered through school systems, is the basis for helminth control (Brooker *et al.*, 2007). Malaria control focuses on accurate diagnosis, prompt treatment, IPT treatment for infants and pregnant women as well as vector control (Greenwood *et al.*, 2005). The opportunities for combined control of malaria and helminths have been recognised (Hotez *et al.*, 2006c). The increasing political and financial support for malaria control and the renewed emphasis on population-based anthelminthic treatment (WHO, 2006a) provides an encouraging milieu for integrated control of these two parasitic infections. As school-based deworming programs are already in place, this setting may also provide a possible avenue for combined delivery of IPT and anthelminthics. School-health-based malaria control programs have proven to be successful in Japan where schoolchildren disseminate health messages to their community (Kobayashi *et al.*, 2007). This would be a cost effective approach as resources are shared.

HIV/AIDS, tuberculosis, and malaria account for 5.6 million deaths and the loss of 166 million DALYs annually and are referred to together as the "big three" (WHO, 2004). Many prominent partnerships and initiatives are devoted to the big three including: The joint UN programme on HIV/AID, Roll Back Malaria, and Stop Tuberculosis (Hotez *et al.* 2007). The geographical overlap of the big three is extensive in many parts of the developing world,

particularly in sub-Saharan Africa (van Eijk *et al.*, 2002; Maher *et al.*, 2005). There is a high degree of overlap with the big three and the NTDs (Raso *et al.*, 2004; Druilhe *et al.*, 2005; Shapiro *et al.*, 2005). Integrating NTD control into the big three partnership programmes could dramatically reduce the number of life years lost from premature disability and death in Africa (Molyneux *et al.*, 2005). Integrated programs are already underway at some level in Burkina Faso, Ghana, Mali, Niger, Nigeria, Togo, and Uganda (Hotez *et al.*, 2007). In Nigeria, ITN distribution was integrated with the 2004 Lymphatic Filariasis/onchocerciasis mass drug administration in Central Nigeria. Community volunteers distributed 38, 600 ITNs, while simultaneously treating 150, 800 persons with ivermectin and albendazole, which also treats STH infections (Blackburn *et al.*, 2006). Although there are great opportunities for integrating the control of NTDs with the 'big three', there are also enormous challenges both in implementation and research, for example, there is a lack of adequate safety information on the optimal way to combine preventative chemotherapy drugs (Hotez *et al.*, 2007). In the discussion of integrated control, the control of helminths has been suggested as a means to facilitate the control of the 'big three' (Molyneux *et al.*, 2005), yet much is still unknown about these interactions.

Investigations of helminth-malaria interactions in humans and animal models have suggested that the outcome of these co-infections may depend on a number of factors including the helminth and malaria species involved and malaria transmission settings. Depending on these factors, helminths may protect against malaria or exacerbate malaria infections in co-infected hosts. This is vastly important as deworming control programmes could either roll back malaria or significantly increase malaria morbidity. The relationship between these coinfections is highly complex and it is clear that further detailed and integrated studies are required to substantiate and to investigate helminth-malaria interactions. Well-designed, randomised placebo-controlled clinical trials provide the most robust study design to investigate the association between malaria and helminth infections and are needed to answer a number of remaining questions: Does anthelminthic treatment reduce the incidence of malaria and what is the effect of age and malaria transmission intensity on the interactions between malaria and helminths? Future studies should use anthelminthics that do not have immunomodulatory properties and also take into account the effect of different helminth and malaria species on the outcomes of these co-infections. In particular, attention should focus on high risk age groups for both infections, for example, preschool children. Clearly there is

deficit in our knowledge in relation to co-infection. It is important that we grasp the opportunity and undertake well-conducted longitudinal studies to understand these complex interactions as there are huge implications for public health. The study described in this thesis aims to fill the gap in our knowledge.

CHAPTER 2: General Materials and Methods and Discussion

Figure **Page**

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2.1 General Materials and Methods

2.1.1 Study site and population

The study was conducted in four semi-urban villages, Moro (altitude 227m), Edunabon (234m), Ipetumodu (226m) and Akinlalu (223m). These villages fall under the catchment area of the Ife North Local Government (INLG), Ile-Ife, Osun State, Nigeria (Plate 2.1). This local government area comprises seven towns and some smaller villages with Ipetumodu as its headquarters. A census undertaken in 1991 showed the population size in the Ife North Local Government Area to be 129,996. Ipetumodu and Edunabon are situated approximately 2km either side of Moro, while Akinlalu is located 6km south-west of the three other villages. All four villages are 12-15km west of Ife town.

The climate is characterised by a high uniform temperature, moderate to heavy seasonal rainfall, and high relative humidity. The dry season extends from November to March while the rainy season occurs from April to October. The rainfall is marked by a dual maxima (July and September) as well as a dual minima (August and January). Annual rainfall in the region ranges from 1000 to 4000mm (Asaolu *et al.*, 1991). The average maximum and minimum daily temperatures are 32 ^{o}C and 20 ^{o}C respectively and the vegetation is rainforest (Asaolu *et al.*, 1991).

The inhabitants of these communities are a mixture of people from different ethnic groups, although the majority are Yoruba-speaking. The population is agricultural-based with yam, cassava, corn, citrus and cashew crops produced in commercial quantities, cocoa, kolanut and palm oil are also produced. Houses in the villages are predominately built of concrete floors and walls and roofed with corrugated galvanised iron sheets (Plate 2.2). There is no organised sewage disposal system and refuse and human faeces are dumped in the bush or burned. The main source of water is from shared community taps and/or wells located in each village. Health care is provided in health centers located in each village, these are inadequately equipped and lack essential supplies and qualified staff. STHs and malaria are endemic in this region (Holland *et al.*, 1989; Salako *et al.*, 1990; Asaolu *et al.*, 1992). Malaria transmission is intense, occurring all year round, with a major peak during the rainy season (Salako *et al.*, 1990).

A

B

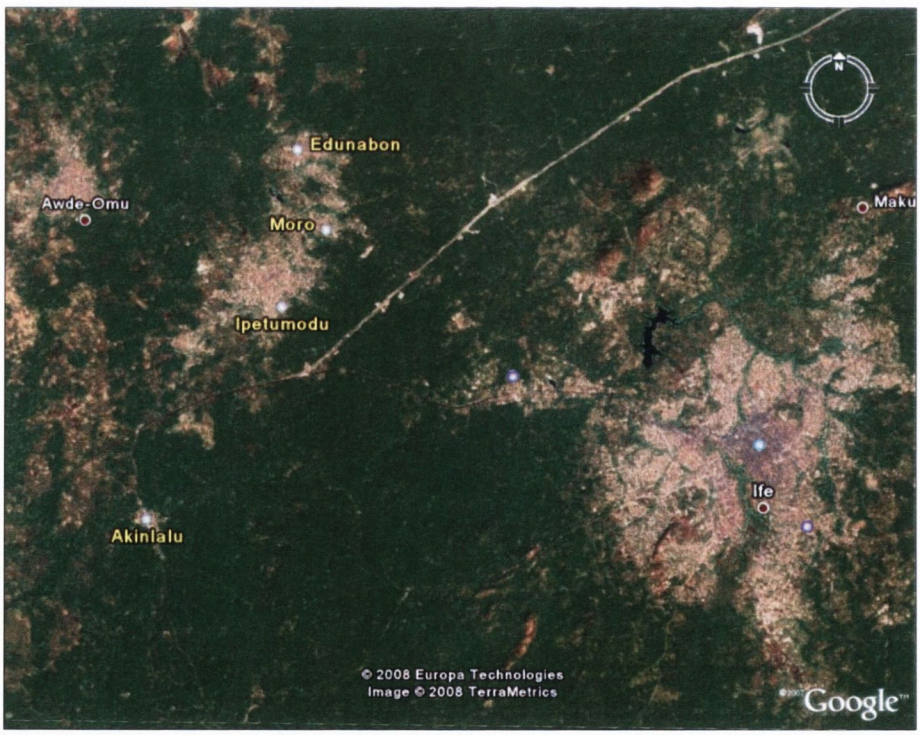


Plate 2.1 (A) Map of Nigeria (Source: www.thecommonwealth.org) (B) Location of Akinlalu, Ipetumodu, Moro and Edunabon. The distance from Ipetumodu to Moro is 2km. (Source: Google Earth)

Plate 2.2 Typical village house with concrete walls and corrugated iron roof (Photo by: Patrick Kirwan)

2.1.2 Phase I: An epidemiological survey of geohelminths, intestinal protozoa and *P. falciparum* malaria in children from 0-24 months.

2.1.2.1 Study design

Phase I was undertaken in three villages, Ipetumodu, Moro, and Edunabon. Meetings were held in April and May 2005 with the INLG and the Obas of each village to gain their support for the study. The Oba is the traditional head of a Yoruba village. He holds no political power but is sought for counsel by the residents of the village. It was explained that Phase I, an epidemiological survey (geohelminths, malaria and intestinal protozoa) in children aged 0-24 months, would take place during May and June 2005. Both the INLG and Obas were also informed that Phase II, an intervention study, would occur in 2006. Introductory meetings were held in each village to explain the purpose of the study, introduced as the 'Child Health project', to as many inhabitants as possible (Plate 2.3). The people who attended the meeting were asked to disseminate this information to the rest of the community. A call was made for

children from 0-24 months to attend temporary clinics for assessments on specified dates arranged during the meeting. It was taken into account that being an agricultural-based community, the parents who have farms spend the majority of their time working on their farms for three to four weeks every month and return to the villages once at the end of every month to attend markets. Therefore the assessments took place two weeks before and after the end of each month. The Obas permitted the study to take place in their palaces which were known to everyone in the community, geographically central and were easily accessible. Local government health officers, Obas and fieldworkers mobilised the mothers for field assessments. The aim of the mobilisation was to ensure that all mothers were aware of the project, the dates of the assessments and to provide encouragement for attendance. The local government health officers and Obas mobilised in churches and mosques in each village. The fieldworkers mobilised by paying house-to-house visits and going to markets.

Participation in the study was voluntary. The study was explained to each mother on the day of the assessment and they were asked to sign or finger print the consent form to enroll their child. The consent forms were provided in English and Yoruba. Each child was given an identification card with an ID number. Mothers were interviewed using a questionnaire by a trained fieldworker who collected data on age and gender of the child, socio-economic status, access to drinkable water, latrine availability, animal ownership, parental education and occupation and history of their child's illnesses (Plate 2.4; Appendix 1).

2.1.2.2 Collection of stool samples

Mothers were supplied with a flexible weigh boat, a 50ml centrifuge tube and an applicator stick to help them collect their child's stool and bring the sample back to the field station the same or following days. The child was instructed to defecate into the weigh boat and the mother could then use the applicator stick to fill the centrifuge tube three quarters full with faeces.

Each stool sample was mixed thoroughly with an applicator stick. One gram of stool was weighed and stored in a 2ml Eppendorf tube and fixed in 10% formalin (one volume of 40% formaldehyde diluted with nine volume distilled water). Stool consistency was recorded as formed, unformed or watery with mucus or blood.

Plate 2.3 Introductory meeting in Edunabon (Photo by: Patrick Kirwan)

Plate 2.4 Fieldworker interviewing mother (Photo by: Patrick Kirwan)

2.1.2.3 Formol-Ether Concentration

Stool samples were examined for geohelminths (Plate 2.5) and intestinal protozoa. Samples were examined in the laboratory by means of the modified formol-ether-concentration technique (Allen and Ridley, 1970). This method concentrates the helminth ova and protozoan cysts while removing partly digested food particles which can mask the presence of parasites in the film examined. The contents of the Eppendorf were placed in a clean 15ml graduated centrifuge tube. 7ml of 10% formalin were added to the centrifuge tube. The sample was thoroughly broken up and emulsified with an applicator stick. The resulting suspension was filtered through a 425 μm aperture sieve which sat neatly on a 50ml beaker. The filtrate was poured back into the same tube. The debris trapped on the sieve was discarded. Both the sieve and the beaker were washed thoroughly in running tap water between each sample. 3 ml of diethyl ether was added to the formalinised solution, to aid the removal of fats and oils, and the cap was screwed tightly back onto the centrifuge tube. The solution was shaken vigorously for 30 seconds. The centrifuge tube was inverted a few times during this procedure and the pressure which developed was released by unscrewing the cap. The tube was centrifuged at 2400rpm for two minutes. The fatty plug was loosened with a wooden applicator stick by passing the stick between the inner wall of the tube and the plug. The plug and the fluid above and below were decanted off allowing only the last one or two drops to fall back into the tube. The pellet was then re-suspended by agitation. All of the re-suspended pellet was placed onto a microscope slide using a 3ml Pasteur pipette. A coverslip was applied and the slide was examined under × 4 objective lens using a light microscope. An indirect measure of helminth intensity was obtained by counting eggs (epg). The intensity of infection can be quantified into different classes: light-intensity infections, moderate-intensity infections, and heavy-intensity infections (Table 2.1). In this thesis figures for intensity include all subjects, both those who are infected and uninfected. Prevalence of geohelminths was expressed as the number of egg positive patients over the total number of patients.

A B C

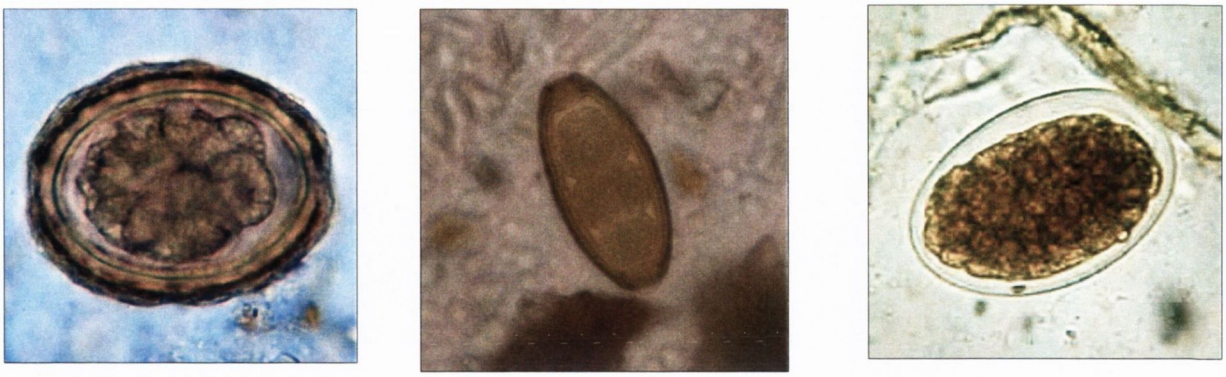


Plate 2.5 Geohelminth eggs. (A) *A. lumbricoides* egg* measuring approx. 55-75µm by 35-50µm; (B) *T. trichiura* egg measuring approx. 50-55µm by 22-24µm (Photo by: Patrick Kirwan); (C) hookworm egg* measuring approx. 60-75µm by 36-40µm. * Source: CDC (http://www.cdc.gov/healthypets/diseases)

Table 2.1 Classes of intensity for soil-transmitted helminth infections, by stool examination (WHO, 2002)

Organism	Light-intensity Infections	Moderate-intensity infections	Heavy-intensity infections
A. lumbricoides	1-4999 epg[a]	5000 – 49 999 epg	≥ 50 000 epg
T. trichiura	1- 999 epg	1000 – 9999 epg	≥ 10 000 epg
Hookworms	1- 1999 epg	2000 – 3999 epg	≥ 4000 epg

[a] epg: eggs per gram (of faeces)

2.1.2.4 Auramine Phenol

Two staining methods were used to detect *Cryptosporidium* oocysts. The first method used carbol fuchsin as a counter stain to auramine phenol; these stool smears were difficult to examine owing to a bright background fluorescence and a residual film that remained on the smears after staining. Thus, the remaining stool smears were stained using a different method which made the smear easier to examine.

Once stool smears were examined for geohelminths and intestinal protozoa, the coverslip was removed and they were air-dried. 55 stool smears were stained with auramine phenol and counter stained with carbol fuchsin using the method of Casemore *et al.* (1985). The smear was fixed in methanol (3 min) and then immersed in auramine-phenol stain (5 min). The slides were rinsed in tap water to remove excess stain and counterstained with carbol fuchsin (10 seconds). Finally the slides were rinsed in tap water to remove excess stain and air-dried.

The smears were examined for the presence of oocysts using a fluorescence microscope equipped with FITC filters. The slides were scanned using x 20 objective lens and the presence of oocysts was confirmed under the x40 objective lens. *Cryptosporidium* oocysts appeared doughnut-shaped (4-6 μm dia.) and exhibit a characteristically bright fluorescence against a dark red background (Plate 2.6). The following staining procedure was used for the remaining 314 stool smears. The smear was fixed in methanol (10 min) and then immersed in auramine-phenol stain (10 min). The slides were rinsed in tap water to remove excess stain and decolourised in acid-alcohol (5 min), 4.5ml acetic acid in 95.5ml of 90% ethanol. After rinsing in tap water the slides were counterstained with 0.1% potassium permanganate (30 secs). The slides were rinsed in tap water and air-dried. *Cryptosporidium* sp. infections were categorised into light (1-4 parasites per field of view), medium (5-10 parasites per field of view) and heavy (10+ parasites per field of view) infections.

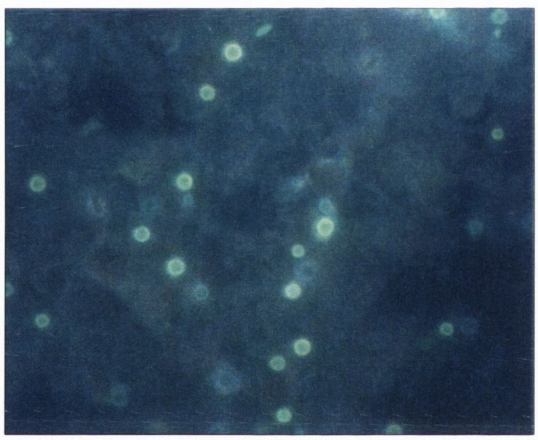


Plate 2.6 Cryptospordium oocysts under a fluorescent microscope (4-6 μm) (Photo by: Patrick Kirwan)

2.1.2.5 Paracheck malaria rapid diagnostic test

Rapid diagnostic tests (RDT) were utilised to identify the presence of *P. falciparum* parasites in the children. The Paracheck™ test (Orchid Biomedical System, Goa, India) is based on the detection of the *P. falciparum* specific histidine-rich protein-2 (HRP-2). The procedure for carrying out the test is detailed in Chapter 5 (Section 5.2.1.1).

2.1.2.6 Blood smears

Peripheral thick and thin blood smears were prepared on different slides using the method described in the WHO Basic Laboratory Methods in Medical Parasitology (WHO, 1991). The

blood smear was left to dry after preparation. Once dry, the thin smear was dipped in a 50ml beaker-containing methanol to fix the thin smear being careful to avoid exposure of the thick film to methanol or methanol vapour.

2.1.2.7 Preparation of phosphate buffer solution

Two stock solutions, A and B were prepared. Stock solution A was made by dissolving 9.5g of Na_2HPO_4 in 1L of distilled water. 9.2g of $NaH_2PO_4H_2O$ was dissolved in 1L of distilled water to make stock solution B. 72ml of Solution A, 28ml of Solution B and 900ml of distilled water were mixed together to get pH 7.2.

The thick and thin blood smears were stained using Giemsa (WHO, 1991). The slides were placed in a staining rack. A 3% Giemsa solution in buffered, distilled water, pH7.2, was prepared in sufficient quantity to fill the staining trough. The stain was mixed well. The slides were immersed in the stain for 50 minutes. After staining, tap water was poured gently into the dish to float off the iridescent scum on the surface of the stain. The rack was removed from the trough and rinsed quickly in a new trough of clean water. Before removing the rack from the trough, clean water was poured in the side of the trough to remove the residual iridescent scum on the surface of the water. The slides were removed from the slide rack and allowed to drain and dry.

2.1.2.8 Anthropometry

Anthropometric measurements (weight and height) were taken. Three anthropometrists were trained, one measurer, one recorder and an assistant. Children were weighed without their clothes on scales to the nearest 0.1kg using a SECA electronic scale. For infants, the mother and child were weighed simultaneously (Plate 2.7). The mother was asked to stand on the scale. The scale was then zeroed and the child was handed to the mother. The measurer called the weight to the recorder. To reduce intra-observer error, each anthropometric measurement was performed twice and the mean value was used for analyses. Height was measured to the nearest millimeter using a Shorr infant/child height/length measuring board. Length was taken for children less than 24 months (Plate 2.8) and height was taken for children more than 24 months.

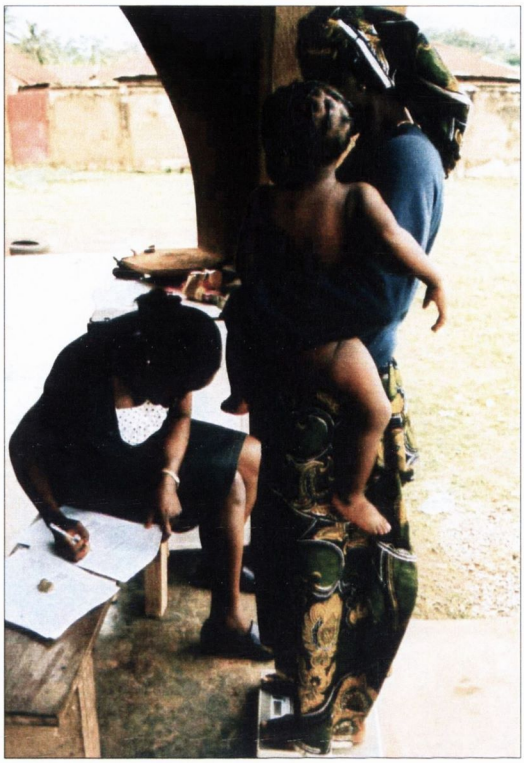


Plate 2.7 Mother and infant being weighed (Photo by: Patrick Kirwan)

2.1.2.8.1 Child length measurement

The measuring board was placed on a table (Plate 2.8). The child was gently placed on the board. If the child was distressed the mother was asked to help keep the child calm. The assistant placed his/her hands on the child's heels, which were flat against the base of the board. The measurer placed one hand on the child's head making sure that the neck was extended and the other hand on the sliding panel. Before taking the reading it was ensured that the child was flat and in the center of the board. The measurer called the measurement to the recorder and the procedure was repeated.

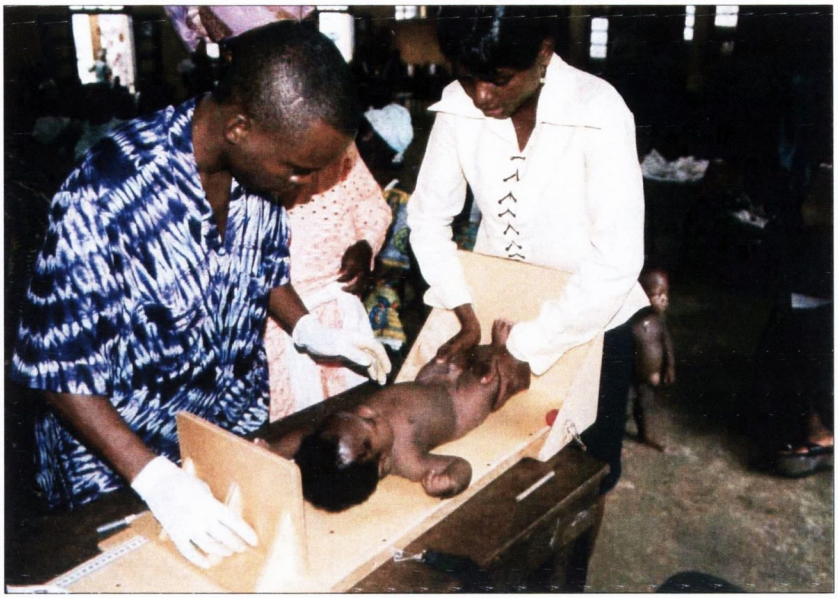


Plate 2.8 Measuring length of an infant (Photo by: Patrick Kirwan)

2.1.2.8.2 Child height measurement

The measuring board was placed on a hard flat surface against a wall. The child was asked to stand on the board. The child's feet were placed flat and together in the centre of and against the back and base of the board. The assistant kneeled down in front of the child placing one of their hands above the child's ankles on the shins and his/her other hand on the child's knees. The assistant made sure the child's legs were straight and the heels and calves were against the board. The child was asked to look straight at the measurer. The measurer placed their left hand under the child's chin while their right hand moved the sliding board. When the child's shoulders were level, his/her hands were at their sides, and the head, shoulder blades and buttocks were against the board/wall the headpiece was lowered on top of the child's head making sure to push through the hair and the measure was taken and called out to the recorder. This procedure was repeated.

The anthropometric measurements of the children were compared with standard values from CDC/WHO (1978) and were expressed as Z scores. Stunting was defined as height-for-age (HAZ) Z scores below -2, underweight was defined as weight-for-age (WAH) Z scores below

-2, and wasting was defined weight-for-height (WHZ) Z scores below -2 (Stoltzfus *et al.*, 2004). Anthropometric scores were computed using Epi info software (2002).

2.1.2.9 Treatment for parasitic infections

Children >1yr were treated with the anthelminthic, albendazole (ABZ) on presenting a stool sample. Children <1yr were treated on a case-by-case basis if they were egg positive (WHO, 2003b). Coartem (artemether-lumefantrine) was used to treat malaria in accordance with the Nigerian Ministry of Health guidelines (Federal Ministry of Health, 2005a). Ethical clearance was granted by the Ethics and Research Committee, Obafemi Awolowo University Teaching Hospitals' Complex, Ile-Ife, Nigeria.

2.1.3 Phase II: Double-blind, placebo-controlled randomised trial of anthelminthic treatment

2.1.3.1 Study design

Phase II was undertaken in the four villages, Ipetumodu, Moro, Edunabon and Akinlalu. Meetings were held with the Obas and Ife North Local Government in May 2006 to explain the aims of Phase II. The Obas made a call for children aged 12-60 months to attend temporary clinics for assessments on specified dates. As recruitment was extended to a new village, Akinlalu, an introductory meeting was held to introduce the local people to the study and dates were arranged for the baseline assessment.

The sample size needed to detect a difference in malaria attacks between the groups was calculated to be 824 (Appendix 2). The intervention study took place over 16 months, beginning in May 2006 and terminating in August 2007. Children were recruited into the study in May/June 2006 and Sept./Oct. 2006 and were followed up thereafter every four months (time points 8, 12 and 14) (Figure 2.1). Recruitment stopped after the second follow-up which took place in Sept./Oct. 2006. The children were randomly allocated to treatment and placebo group. Children in either group received the appropriate tablet at 0, 4, 8 and 12 months. Questionnaires (Appendix 3) and a consent form (Appendix 4) explaining the aims of the study were issued to the mothers when their child was recruited into the study, anthropometric measurements were also taken at this time. Each child was also given a 'Child Health Project' identification card, which they had to present at each follow up. This card was

printed in a different colour to the previous card given out in Phase I. A stool sample and a finger-prick blood sample were taken from each child during the assessments. Anthropometric measurements were also taken during the final assessment. Children were screened out of the study if: they suffered from severe protein-energy malnutrition; their blood haemoglobin was below 5.0 g/dl, and/or they suffered from severe malaria. A schematic representation of the progression of fieldwork is given in figure 2.2.

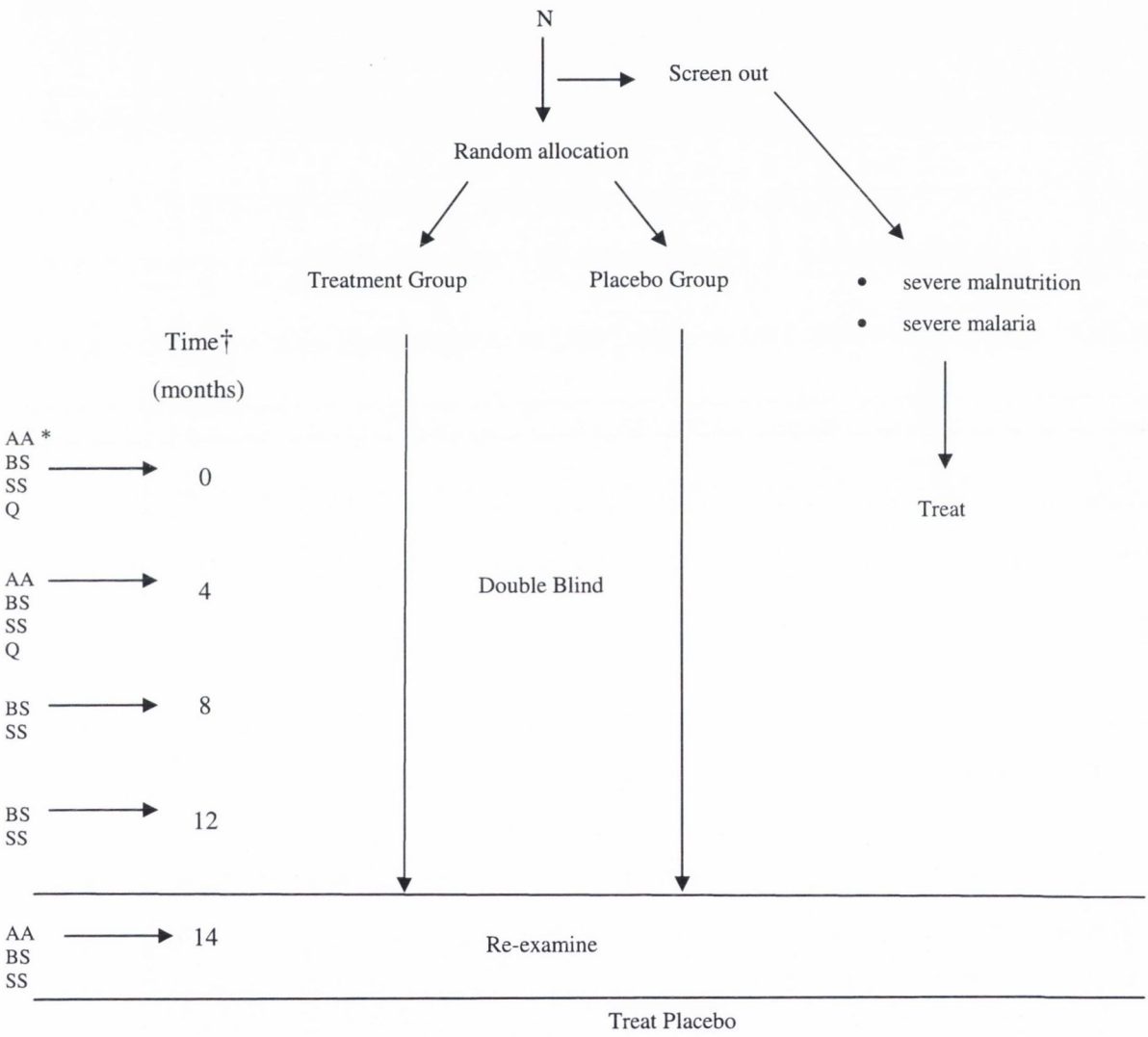
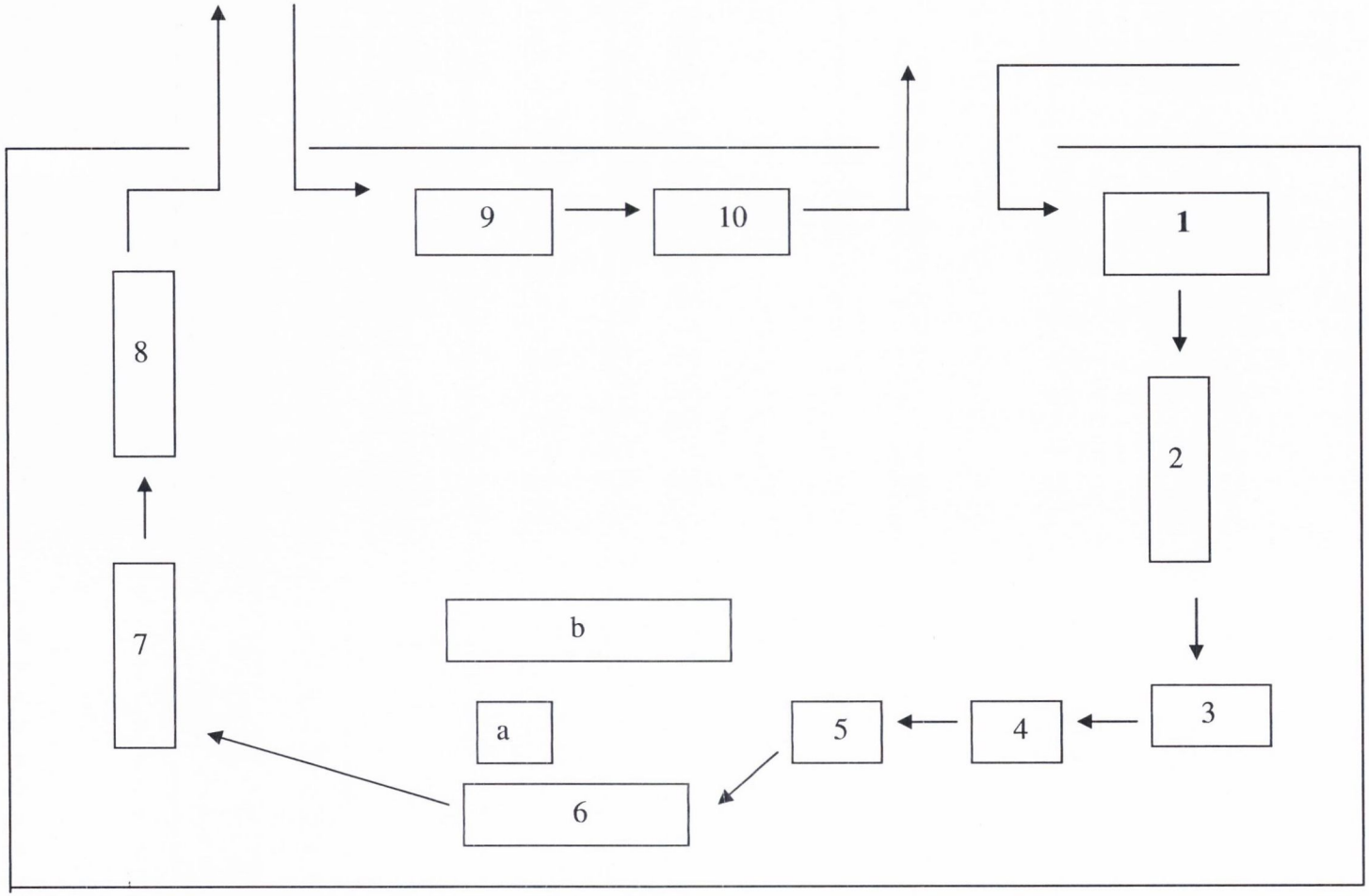


Figure 2.1 Double blind placebo controlled randomised trial of anthelminthic treatment

Adapted from Stephenson (1984).

*AA = Anthropometric assessments; BS = Blood samples; SS = Stool sample; Q = Questionnaire.

† 0 = May/June '06, 4 = Sept./Oct '06, 8 = Feb. '07, 12 = May/June '07, 14 = Aug. = '07.



1 = Registration and consent form, 2 = Interview with questionnaire, 3 = Collection of bottles for stool samples, 4 = General talk and time for questions, 5 = Temperature, 6 = Anthropometrics, 7 = Malaria diagnostics (blood smears, RDT, hemoglobin, and blood spots on filter paper), 8 = Malaria diagnosis and treatment, 9 = Collection of stool samples, 10 = Treatment or placebo tablets, a = weighing scales, b = height/length board

Figure 2.2 Schematic representation of the progression of fieldwork

2.1.3.2 Collection of stool samples

Mothers were instructed to collect their children's faeces and bring these samples back to the field stations the same or following days. In Phase I, compliance to return stool specimens was moderate (56%). To increase compliance, mothers were offered an incentive for example, a scarf, plastic bucket or towel, to return their child's stool sample. Fieldworkers who distributed the stool bottles explained the purpose of giving a faecal sample to each mother. Once stool samples were collected, they were fixed (Chapter 2, section 2.2.2), processed by formol-ether concentration (Chapter 2, section 2.2.3) and examined for geohelminths.

2.1.3.3 Randomisation

Albendazole and Placebo tablets were placed in containers labelled either A or B. The fieldworkers and all study participants (mothers and children) were blinded to which tablets were the active drug and placebo (double blind). On returning a faecal sample, each alternate child received tablet B. Albendazole was given in 200mg tablets. Glaxo Smith Kline recommended that children <2yrs should be given one 200mg albendazole tablet and children ≥2yrs should be given two 200mg tablets. Therefore children less than 2 yrs received one tablet of either A or B and children over two years received two tablets of either A or B. The principal investigators in this study were Prof. S. O. Asaolu and Prof. C.V. Holland. Prof. Asaolu was responsible for placing the albendazole and placebo tablets in the labelled containers, while Prof. Holland was responsible for reporting serious adverse events and the number of children treated with anthelminthic drugs to GlaxoSmithKline.

2.1.3.4 Clinical indicators for malaria

At each time point the children were assessed for temperature, haemoglobin, and spleen enlargement. Auxillary temperature was taken by a nurse with a digital thermometer. A fever was defined as temperature > 37.5 ^{o}C. Hemoglobin (g/dl) was measured with a hemocue (Accuscience, Ireland) using a finger prick blood sample. The hemocue was calibrated daily before fieldwork was undertaken. Each child was seen by a medical doctor who assessed the

child for spleen enlargement, diarrhoea, and clinical signs and symptoms of malaria (Appendix 5). A malarial attack was defined as a positive RDT and a fever (>37.5 °C). If a child was suffering from a malaria attack he/she was treated with Coartem (artemether-lumefantrine). All children received 10ml of multivitamins (over 2 days) as an incentive at each time point. Each 5 ml of multivitamin contained: Vitamin A 3000 IU, Vitamin B2 2.0 mg, Nicotinamide 15.0 mg, Vitamin B1 1.5mg, Vitamin B6 2.0mg, Vitamin D2 400 IU, D panthenol 1.0 mg.

2.1.3.5 Malaria attacks

Malaria can be categorised into severe malaria and uncomplicated malaria (Chapter 1, section 1.2.2.3). Any children found suffering from severe malaria were removed from the study and treated. The presentation of uncomplicated malaria is variable and mimics that of many other diseases (Schellenberg *et al.*, 1994). Patients commonly complain of fever, headache and aches and pains in the body, and occasionally of abdominal pain and diarrhoea. In a young child there may be irritability, refusal to eat and vomiting. On physical examination fever may be the only sign of a malarial attack. To reduce the number of fevers related to illnesses other than malaria, a malaria-attributable parasitaemia threshold will be calculated from enrolment clinical surveillance data (Smith *et al.*, 1994). In combination with clinical symptoms, this parasitaemia threshold will be used to retrospectively define a clinical malaria case for the purposes of the analysis.

2.1.3.6 Daily malaria clinics

From the baseline assessment, mothers were invited to bring their children to their local health centre if their child was suffering from a fever. Each nurse was trained by a medical doctor to diagnose malaria. A malaria technician who carried out the RDT and made blood smears travelled between health centres when a patient needed care. Following negative feedback from the mothers concerning the poor attitudes of the nurses in the local Primary Health Care Centers, the venue for the daily malaria clinics moved to the Obas palace or town hall. Four nurses, a medical doctor and a malaria technician were hired to run the daily malaria clinics. The attendance of the daily malaria clinics is discussed in chapter 5, section 5.4.2.

Ethical clearance was granted by the Ethics and Research Committee, Obafemi Awolowo University Teaching Hospitals' Complex, Ile-Ife, Nigeria.

2.1.3.7 Malaria diagnosis

Malaria was diagnosed using peripheral thick and thin blood smears and Parascreen rapid diagnostic tests (RDTs; Zephyr Biomedicals, Verna Industrial Estate, Verna Goa, India). The thick and thin blood smears were made on the same slide (WHO, 1991) and were stained using the same procedure outlined in section 2.2.6 and 2.2.7 of this chapter. The RDT used detected *P. falciparum* and other species of malaria; the procedure for using this test is outlined in Chapter 5, section 5.2.1.1.

2.1.3.8 Preparation of filter papers containing *Plasmodium* spp. infected human blood

Blood was 'spotted' onto filter paper for PCR analysis. Whatman 3mm chromatography paper was cut into 21×100mm strips using clean scissors that had not come into contact with any *Plasmodium* spp. DNA. The strip was folded in half and at one end cuts of 30mm were made lengthways down the paper to make three prongs of paper about 7mm wide. A finger prick blood sample was spotted onto the filter paper prongs. The folded filter paper was allowed to air dry for at least 30 minutes. After drying the filter papers were placed into an individual snap-seal plastic bag. A small hole was made in the bags by cutting the corner off; the bags were then placed in a larger snap-seal bag with silica gel and stored at room temperature.

2.2 Discussion

This section focuses on the methodological issues that ensued while setting up fieldwork in the four project villages. The issues presented encompass: (1) community involvement; (2) mobilisation; (3) malaria diagnosis and (4) collection of faecal samples.

2.2.1 Community involvement

When planning any epidemiological study it is vital to choose the correct point of entry into a community. In Nigerian communities, support from Local Government officials and Obas is essential for community approval and mobilisation (Korpela *et al.*, 1998; Mafiana *et al.*, 2003). Before fieldwork began in Phase I, an introductory meeting was held with Dr. Oyeniyi Kolapo, the chairman of Ife North Local Government, who subsequently organised meetings with each of the Obas in the four project villages. At the beginning of Phase II, Dr. Oyeniyi was no longer employed by the local government. His successor was not as cooperative and therefore some distance was created between the study and the local government. This change of leadership in the local government did not hinder compliance as the study was already supported by the Obas. However, no 'manpower' was provided by the local government for mobilisation throughout Phase II which could have increased the number of children that presented at registration.

In addition to contacting the village leaders, another Nigerian study also included local health workers, male and female opinion leaders, market women, teachers, and youth leaders (Olaniran *et al.*, 1997). Including these influential members of the community in the planning of epidemiological studies may improve study participation by illuminating community beliefs and behaviours that could act as obstacles to community involvement. Furthermore, such contacts could provide a basis for dissemination of information in a more positive manner to the broader community.

2.2.2 Mobilisation

The mobilisation for Phase II was carried out by the Obas and the 'mobilisation team'. When mobilising, the Obas sent their Chiefs (deputies) on house-to-house visits to disseminate information about the clinics. The level of enthusiasm shown by each Oba varied and it was difficult to ascertain how active the Chiefs were in mobilising and if they were providing the correct information. Thus, in addition to these mobilisation efforts a 'mobilisation team' was created, which consisted of a group of field workers employed by the 'Child Health Project' to mobilise the community for field assessments; some of these fieldworkers were indigenous to the communities.

For the baseline assessment, flyers (translated into Yoruba and English) were distributed in churches, mosques and markets by the mobilisation team. Mobilisation through flyers and local markets was ineffective owing to a high level of illiteracy and markets drawing people from other localities and states. Radio is the primary communication medium for reaching large sections of the population in Nigeria (Falobi *et al.*, 2002). Mobilising through radio was considered to be problematic because it might attract people from other communities to come for treatment preventing locals from participating in the study.

During the second and subsequent assessments the mobilisation team paid house-to-house visits to the people registered in the study. Initially, a lack of an organised house numbering system, poorly detailed addresses and community suspicion impeded the location of participant's households. To relieve suspicion of locals the mobilisation team wore 'Child Health Project' t-shirts, which had a logo printed on them and were easily recognised by the mothers during the assessment and around the village. On seeing the t-shirts locals were more inclined to help field workers locate households. Therefore, future studies should include similar t-shirts and logos at the beginning of fieldwork.

Mobilisation in churches and mosques was an effective method to disseminate information on assessment dates to participants of the study. The distribution of flyers to churches was ceased; instead a manager attended the churches and mosques from the second assessment onwards taking time to address and answer questions posed by the congregation during the Sunday service. Only the main churches and mosques were visited in this way in each village

as there were many. In cases where no formal talk took place during the service, pastors were asked to pass on information to the congregation.

2.2.3 Preparation and reading of malaria blood smears

The preparation and reading of thick and thin peripheral blood smears during Phase I was not satisfactory. Two 'qualified technicians', who worked in Obafemi Awolowo University and a local hospital, were employed during the pilot study to make thick and thin blood smears on separate slides. The preparation and reading of the smears were brought into question after certain laboratory practices were observed. The preparation of the blood smears was not satisfactory and the ability of the technicians to identify malaria parasites was questionable. The need for uniform training of malaria technicians and quality assurance programmes has been recognised (Durrhelm *et al.*, 1997). A study in Zambia has shown that the sensitivity and specificity for malaria microscopy varied from 25% - 100% and 59% - 100% respectively between health centers (Barat *et al.*, 1999). This was thought to be due to a lack of technical supervision, poor condition of microscopes, and a high volume of blood smear requests.

The WHO provided a contact for a reputable malaria research laboratory in the University College Hospital, Ibadan. This laboratory deemed the blood smears, that were made by the technicians during Phase I, to be of poor quality and of no use in determining malaria parasitaemia. Consequently, a malaria technician who was trained by the Ibadan malaria research group in the preparation and staining of blood smears was employed temporarily to work during Phase II. This malaria technician provided training in the preparation and staining of blood smears to field personnel and monitored their progress until he was satisfied that they were competent. Further malaria diagnostic issues concerning the reading of malaria parasitaemia and identifying malaria to species are described in Chapter 5, section 5.2.1 and 5.4.1.

2.2.4 Collection of stool samples

Two issues were highlighted during the collection of stool samples, namely, local suspicion concerning the motivation behind the collection of stool samples and the offering of incentives. Although the mothers were educated on the reasons why stool samples were being collected, some still remained suspicious believing that the study had intentions of witchcraft

or satanism. Fears, misconceptions, and religious beliefs are common factors inhibiting the collection of human blood or tissues in Nigerians and other African communities (Okapara, 1989; Ottong *et al.*, 1997; Hassell *et al.*, 2008). During phase I compliance to return stool samples was moderate. Many of the mothers felt that worms are common and are not harmful to the body. A study carried out in Ogun state, Nigeria showed that the majority of food vendors believed that worms are useful in the body (Idowu and Rowland, 2006). The general consensus among the women, that worms are part of the human body, may have decreased compliance to return stool samples. The ability to recognise illness can determine treatment seeking behaviour. A study in the rural Ibarapa Central Local Government Area of Oyo State, Nigeria, showed that people viewed malaria and febrile convulsions as completely separate conditions with the former caused by heat and the later caused by cold (Ramakrishna, 1988). Malaria was perceived as a less serious condition, while convulsions prompted an immediate treatment response.

During Phase II, an incentive was offered to increase the return of stool samples. The introduction of the incentives increased compliance but also increased suspicion among some mothers. A rumour circulated around the villages stating that the incentives were given by 'antichrists who would steal the light of the children'. The rumour began in a church in Moro. A meeting was arranged with the pastor of the church in question to explain the motivation behind the collection the stool samples. It was difficult to know whether or not the pastor accepted the explanation.

The methodological problems experienced during this study show that an understanding of the communities' beliefs and behaviour is crucial to the planning and success of epidemiological studies. Studies have shown that local community knowledge can act as a barrier to community interventions for malaria (Klein *et al.*, 1995; Adongo *et al.*, 2005). Despite the considerable fieldwork experience gained in this region of Nigeria (Holland *et al.*, 1989; Asaolu *et al.*, 1991; Holland *et al.*, 1996a, b; Asaolu *et al.*, 2002), the multiple meetings with the INLG and Obas, and the efforts to educate mothers during the assessments these issues still ensued. This suggests that a more comprehensive approach is needed to establish links with communities before epidemiological studies are undertaken. This approach should include the involvement of all members of the community in the planning of the study in

conjunction with educational workshops to raise awareness and increase treatment-seeking behaviour for helminths and malaria.

CHAPTER 3: Risk factors for *A. lumbricoides* and *Cryptosporidium* sp. in Nigerian children aged 0-25 months

3.1 Introduction

Geohelminth infections have a significant public health impact causing an estimated 714 million disease cases and 135,000 annual deaths (Crompton and Nesheim, 2002). *A. lumbricoides* infects in the order of 1471 million people (over a quarter of the world's population), *Trichuris trichiura* 1049 million and hookworm 1277 million (Holland and Kennedy, 2002). The manifestations of severe disease include fatal intestinal obstruction or pulmonary allergic reactions in the case of ascariasis, severe anaemia in hookworm infections and chronic dysentery and rectal prolapse in trichuriasis (Holland and Kennedy, 2002). Numerous studies have demonstrated that children ranging in age from 5-15 years are particularly at risk from infection and they have been the focus of intervention studies and age-targeted strategies for chemotherapy (Asaolu *et al.*, 1991; 1992; Holland *et al.*, 1996a).

Children aged 24 months and less make up between 5 and 10% of the 3.5 billion people either infected with, or at risk of, infection from geohelminths (WHO, 2003b). Recent evidence suggests that soil-transmitted helminthiasis has a potential effect on growth and development of children under 24 months (Awasthi *et al.*, 2000; Awasthi and Pande, 2001). For this reason in 2002, WHO organised an informal consultation to assess the current recommendations to avoid the use of anthelminthic drugs in children less than two years of age. The consultation concluded that although there is little published information about the use of anthelminthic drugs in this age group, the data that exist offer no obvious reason for excluding children of this age group from treatment (WHO, 2003b). Furthermore, the consultation identified a lack of specific studies on this age group and highlighted a paucity of epidemiological data particularly parasite intensity.

Cryptosporidium species, Apicomplexan protozoan parasites, are the causative agents of acute, self-limiting diarrhoea in immunocompetent patients worldwide. However, in immunocompromised patients (especially those with HIV/AIDS and the malnourished) the disease can be severe, protracted and debilitating possibly leading to chronic dehydration and even death (Laurent *et al.*, 1999). Transmission is by the faecal-oral route, with person-to-person, zoonotic, waterborne and foodborne routes well recognised (Huang *et al.*, 2004). Recently, attention has been focused upon *Cryptosporidium* sp. in young children in

developing countries. It has been suggested that the diarrhoeal disease they suffer as a consequence of their *Cryptosporidium* sp. infection can have long-term deleterious effects upon their growth and cognitive development (Molbak *et al.*, 1997; Checkley *et al.*, 1998; Guerrant *et al.* 1999). This study aims to determine the prevalence and intensity of geohelminths and intestinal protozoa in children aged 0-25 months and to identify the associated risk factors for *A. lumbricoides* and *Cryptosporidium* sp. infections.

3.2 Materials and methods

Details of the study location and methodology are described in Chapter 2, section 2.1.1 and 2.1.2.

3.2.1 Statistical analysis

Statistical analysis was performed using SPSS 14. The characteristics of children who did and did not submit a stool sample were tested to see if they differed in terms of age, sex, and socio-economic status (SES). Chi-squared analysis was used to test the difference in proportions of age groups and sex between children who did and did not submit stool samples, while a 2-sample t-test was used to test the difference in the SES between the groups. The SES index was created by adding up the number of possessions in the subjects' household. These possessions included a generator, fridge, and toilet. Toilet was categorised and ranked as: 1 = bush toilet, 2 = pit latrine, and 3 = flush toilet. As the parents knew the purpose of the study, and provision of treatment, compliance may be related to the suspected or known presence of helminths. Therefore, Chi-squared analysis was used to test the difference in the proportion of children who had or had not previously excreted or vomited a worm and the children who did and did not submit a stool sample.

Chi-squared analysis was used to determine if the prevalence of *A. lumbricoides* and *Cryptosporidium* sp. varied significantly among villages, and between age and sex. The epg data for *A. lumbricoides* was not normally distributed and log-transforming these data could not correct the distribution asymmetry. A Mann-Whitney U test was used to test the difference in epg between sex, while Kruskal-Wallis tests were used to test the difference in epg among age groups and villages.

Chi-squared analysis was used to undertake a series of univariate analyses to examine associations between various demographic, sociological, and behavioural factors and the prevalence of *A. lumbricoides* and *Cryptosporidium* sp. The variables that were highlighted as potential risk factors for either *A. lumbricoides* or *Cryptosporidium* sp. were entered into a forward stepwise logistic regression model with interactions to see if they still remained

significantly correlated with the outcome variable in the presence of other predictor variables. Variables with a P-value < 0.2 were selected for the model (Hosmer and Lemeshow, 2000).

As the feeding behaviour of children aged < 6 months and ≥ 6 months was recorded in a questionnaire, the logistic regression model analysis was carried out on two subsets of data: a subset for children aged < 6 months and a subset of data for children aged ≥ 6 months. However, logistic regression model for *A. lumbricoides* was based on a subset of children aged ≥ 6 months. No analysis was carried out for children aged < 6 months owing to the low prevalence of *A. lumbricoides* in this age group. Age and father's occupation were coded as categorical variables with indicator coding having children aged 7-11 months and farming as the reference categories.

For the logistic regression analysis for *Cryptosporidium* sp. on children aged < 6 months village was coded as a categorical variable with deviation coding and Moro as the reference category. Father's occupation was coded as a categorical variable with indicator coding and farmer as the reference category.

In the logistic regression analysis for *Cryptosporidium* sp. in children aged ≥ 6 months, age was coded as a categorical variable with indicator coding and children aged 7-11 months as the reference category. Father's education and occupation were coded as categorical variables with deviation coding and primary school education and farmer as the reference categories.

3.3 Results

655 children visited the clinics during the epidemiological survey. 652 children stayed for the assessments after the questionnaires were given to the mothers. Compliance to return stool specimens was moderate; 369 children (56%) provided stool samples for appraisal of geohelminths and intestinal protozoan infections. There was no significant difference in the age ($\chi^2 = 0.697$, df = 1, P = 0.404), sex ($\chi^2 < 0.001$, df = 1, P = 0.995), or SES (t = 0.531, df = 651, P = 0.595) of children who did or did not submit a faecal sample. Similarly there was no significant difference between children who had or had not previously excreted or vomited a worm and children who did or did not submit a faecal sample for analysis ($\chi^2 = 0.130$, df = 1, P = 0.719). The sample population examined consisted of 182 males and 187 females, with a mean age of 11 ± S.D. 6.93 months. 128 children harboured a single parasite species, 29 harboured more than one species, many of whom (26) had double infections. *Cryptosporidium* sp. (27.1%) and *A. lumbricoides* (12.2%) were the dominant infections (Table 3.1). The prevalence of *A. lumbricoides*, *T. trichiura*, *Cryptosporidium* sp., *P. falciparum* and *Entamoeba hartmanni* were significantly associated with age (Table 3.1).

Table 3.1. Age-related differences in the prevalence of geohelminths, intestinal protozoa, and *Plasmodium falciparum* among inhabitants of Moro, Edunabon, and Ipetumodu, Osun state, Nigeria

	% prevalence				
Intestinal Parasite	Overall (n = 369)	< 12mo (n = 207)	≥ 12 mo (n = 162)	χ^2	P-value*
Geohelminths					
Ascaris lumbricoides	12.5 112.39±718.73†	2.9 17.48±139.49	24.7 233.66±1062.76	39.55	<0.001
Trichuris trichiura	1.4 0.25±3.96	0	3.1 0.56±5.97	6.48	0.016‡
Intestinal Protozoa					
Cryptosporidium sp.	27.1	20.8	35.2	9.56	0.002
Entamoeba histolytica/dispar §	4.3	4.3	4.3	0	0.99
Entamoeba hartmanni	3.8	5.8	1.2	5.18	0.023
Blastocystis hominis	1.1	1	1.2	0.061	1.0‡
Entamoeba coli	0.5	1	0	1.57	0.50‡
Endolimax nana	0.5	1	0	1.57	0.51‡
Iodamoeba butschlii	0.5	1	0	0.21	0.51‡
Plasmodium falciparum	64.2	50.7	81.5	37.42	<0.001

*The association between the prevalence of geohelminth and protozoan infections and age were tested using chi-square (χ^2) analysis
†Mean eggs per gram of faeces (epg) ± S.D.
‡Tested using Fisher's Exact
§ *Entamoeba histolytica* and *Entamoeba dispar* are morphologically identical but genetically distinct species. Here the two species are regarded as one morphological complex, named *Entamoeba histolytica/dispar*.

Prevalence of *A. lumbricoides* and *Cryptosporidium* sp. increases significantly with age (Table 3.1). *A. lumbricoides* is more common in older children with a sharp increase at 12 months. The youngest child to be infected with *A. lumbricoides* was 5 months old. There was a steep increase in the mean intensity of *A. lumbricoides* after the 16-19 month age category (Figure 3.1). *Cryptosporidium* sp. is common in children from 0-3 months and the prevalence begins to rise in the 8-11 month age category. The prevalence of *A. lumbricoides* ($\chi^2 = 0.283$, df = 1, P = 0.595)or *Cryptosporidium* sp. ($\chi^2 = 2.194$, df = 1, P = 0.139) did not differ significantly between sexes. Intensity of *A. lumbricoides* was significantly different among age groups (Z= -6.234, P < 0.001) but not significantly different between sexes (Z= -0.703, P = 0.482).

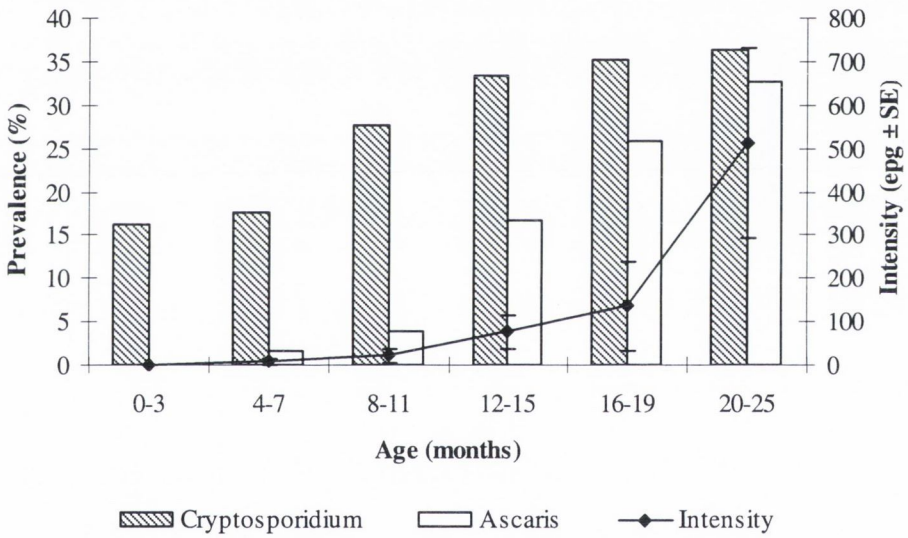


Figure 3.1. The relationship between the prevalence and mean intensity of *A. lumbricoides* and prevalence of *Cryptosporidium* among children aged 0-25 months

No significant difference was found in the prevalence of *A. lumbricoides* ($\chi^2 = 2.685$, df = 2, P = 0.261) and *Cryptosporidium* sp. ($\chi^2 = 1.719$, df = 2, P = 0.423) infections among the villages. Similarly, there was no significant difference in the intensity of *A. lumbricoides* among the villages (Kruskal-Wallis; $\chi^2 = 2.865$, df = 2, P = 0.239) . Three children aged ≥18 months had moderate intensity geohelminth infections, all other geohelminth infections were of light intensity. The majority (88) of *Cryptosporidium* sp. infections were light. Few

children harboured medium (7) or heavy (5) *Cryptosporidium* sp. infections. No significant difference was found in the prevalence of *Cryptosporidium* sp. in relation to stool consistency.

22.5% of the children had low HAZ, 12.5% had low WAZ, and 12.9% had low WHZ. There were no statistically significant associations between the prevalence of either *A. lumbricoides* and low WAZ (χ^2 = 0.389, df = 1, P = 0..533) , HAZ (χ^2 = 0.123, df = 2, P = 0.726) and WHZ (χ^2 = 0.956, df = 1, P = 0.328) or *Cryptosporidium* sp. and low WAZ (χ^2 = 1.598, df = 1, P = 0.206) , HAZ (χ^2 = 0.765, df = 1, P = 0.382) and WHZ (χ^2 = 0.194, df = 1, P = 0.660; Chapter 2, section 2.1.2.8.2.).

Table 3.2 presents the results of the chi-square analysis examining the associations between various demographic, sociological, behavioural factors and the prevalence of *A. lumbricoides* in children aged ≥ 6 months. The variables age, father's occupation, dog or cat ownership, care at a private hospital, feeding a child semi-solid food or family food and still breast feeding after the age of 6 months were significantly (P < 0.2) associated with *A. lumbricoides* infection. Age and the interactions of father's occupation×still breastfeeding, and attending a private hospital×dog ownership were the variables that were still significantly correlated with the occurance of *A. lumbricoides* in the logistic regression model (Table 3.3). Children aged 12-17 months and 18-25 months were 8.6 and 14.9 times more likely to be infected with *Ascaris* than children aged 6-11 months respectively. If a child was not being breastfed their odds of infection with *A. lumbricoides* were significantly decreased 0.5 times those whose fathers were businessmen when compared to those whose fathers were farmers. Children whose families did not attend a private hospital and who owned a dog were 2 times more likely to harbour *Ascaris* than children whose families attended a private hospital and who did not own a dog.

Table 3.2. Chi Square (χ^2) analysis for factors significantly associated with *Ascaris lumbricoides* infections among children aged ≥ 6 months

Variable	Group	N	Prevalence (%)[a]	χ^2	P-value
Village	Moro	66	18.2	1.93	0.382
	Edun-abon	94	20.2		
	Ipetumodu	107	13.1		
Sex	Male	139	14.4	1.26	0.262
	Female	128	19.5		
Number of people living in Household	1-5	184	16.8	1.59	0.452
	6-10	75	18.7		
	>10	7	0		
Age	7-11 months	106	3.8	23.78	P < 0.001
	12-17 months	77	20.8		
	18 -25 months	84	29.8		
The house is connected to Electricity supply	Yes	250	17.6	1.56	0.321 [b]
	No	17	5.9		
The house is connected to a generator	Yes	45	11.1	1.35	0.245
	No	219	18.3		
Working Fridge	Yes	83	19.3	0.51	0.477
	No	184	15.8		
Type of Toilet	Flush Toilet	68	16.2	1.27	0.529
	Pit latrine	153	15.7		
	Bush Toilet	29	24.1		
Dispose of rubbish by open dumping	Yes	130	15.4	0.27	0.600
	No	135	17.8		
Dispose of rubbish by burning	Yes	137	17.5	0.17	0.680
	No	128	15.6		
Mother's education	Primary	70	18.6	0.16	0.925
	Secondary	170	16.5		
	Tertiary	23	17.4		
Father's education	Primary	26	19.2	0.10	0.950
	Secondary	173	16.8		
	Tertiary	60	16.7		
Father's occupation	Farmer	89	27	9.32	0.009
	Business man	114	11.4		
	Professional	60	13.3		
Cultivate food	Yes	175	18.3	0.74	0.390
	No	92	14.1		
Domestic animal ownership	Yes	212	16.5	0.22	0.640
	No	52	19.2		
Dog ownership	Yes	37	32.4	7.44	0.006
	No	230	14.3		
Goat ownership	Yes	140	18.6	0.62	0.431
	No	127	15		
Cat ownership	Yes	33	33.3	7.30	0.007
	No	234	14.5		
Chicken Ownership	Yes	195	16.9	0.002	0.960
	No	72	16.7		

[a]The prevalence of *A. lumbricoides* positive cases, to show the direction of association
[b]Fisher's exact test

Table 3.2 continued

Variable	Group	N	Prevalence (%)[a]	χ^2	P-value
Visit local health clinic	Yes	216	17.6	0.31	0.578
	No	49	14.3		
Visit private hospital	Yes	84	11.9	2.25	0.134
	No	181	19.3		
Self medicate	Yes	123	16.3	0.09	0.771
	No	142	17.6		
Possession of window net	Yes	161	17.4	0.05	0.825
	No	104	16.3		
Possession of door net	Yes	70	17.1	0.00	0.993
	No	193	17.1		
Possession of bed net	Yes	58	12.1	1.30	0.254
	No	206	18.4		
Feeding behaviour for children aged > 6 months					
Herbal concoctions	Yes	106	17.9	0.01	0.941
	No	148	17.6		
Infant formula	Yes	112	19.6	0.51	0.475
	No	142	16.2		
Pap	Yes	199	18.1	0.12	0.726
	No	56	16.1		
Semi-solid food	Yes	189	20.1	2.89	0.089
	No	65	10.8		
Family foods	Yes	178	22.5	9.23	0.002
	No	76	6.6		
Still breast feeding	Yes	187	13.4	8.07	0.004
	No	66	28.8		
HAZ	Normal	232	17.2	0.19	0.663
	Underweight	35	14.3		
WAZ	Normal	190	17.4	0.12	0.724
	Stunted	77	15.6		
WHZ	Normal	221	16.7	0.01	0.915
	Wasted	46	17.4		
Condition of Stool					
Mucoid	Yes	31	22.6	0.84	0.358
	No	225	16		
Watery	Yes	64	17.2	0.009	0.923
	No	192	16.7		
Formed	Yes	75	20	0.78	0.378
	No	181	15.5		
Unformed	Yes	132	15.2	0.53	0.467
	No	124	18.5		
Blood	Yes	14	28.6	1.45	0.264[b]
	No	241	16.2		

[a]The prevalence of *A. lumbricoides* positive cases, to show the direction of association
[b]Fisher's exact test

Table 3.3 Risk factors for *Ascaris lumbricoides* in children aged ≥ 6 months

Variable	Odds ratio	(95% CI)	P-value
Age			
7-11 months (reference category)	1		
12-17 months	8.566	(2.330- 31.497)	0.001
18-25 months	14.875	(4.161- 53.176)	<0.001
Father's occupation×not breast feeding			
Farmer× not breast feeding	1		
Businessman× not breast feeding	0.466	(0.252- 0.865)	0.015
Professional× not breast feeding	0.644	(0.363- 1.143)	0.132
Dog ownership×visit private health clinic (vs no dog ownership × doesn't visit private health clinic)	2.041	(1.250 – 3.334)	0.004

Table 3.4 presents the results of the chi-square analysis examining the associations between various demographic, sociological, behavioural factors and the prevalence of *Cryptospordium* sp. in children aged < 6 months. The variables village, drinking water from a river/stream, boiling or filtering water before drinking, father's occupation, animal ownership, goat or chicken ownership, possession of a window or bed net, having a watery or formed stool were significantly associated (P≤ 0.2) with *Cryptosporidium* sp. infection. The interactions of filtering water before drinking×village and bed net ownership×father's occupation were significantly correlated with the occurance of *Cryptosporidium* sp in the logistic regression model (Table 3.5). If the children's water was filtered before drinking their odds of harbouring *Cryptosporidium* sp. were 0.3 times less likely if they were from Edunabon when compared to those that were from Moro. In contrast, the odds of infection with *Cryptosporidium* sp. were 3.5 times more likely if the children's water was filtered and they lived in Ipetumodu when compared to those that lived in Moro. Children whose fathers were professionals and whose families did not own a bed net were 1.5 times more likely to be infected with *Cryptosporidium* sp. than children whose fathers were farmers and did not own a bed net.

Table 3.4 Chi Square (χ^2) analysis for factors significantly associated with *Cryptosporidium* sp. infections for children aged < 6 months

Variable	Group	N	Prevalence (%)[a]	χ^2	P-value
Village	Moro	29	6.9	5.04	0.08
	Edun-abon	28	14.3		
	Ipetumodu	45	26.7		
Sex	Male	43	16.3	0.10	0.757
	Female	59	18.6		
The house is connected to Electricity supply	Yes	95	17.9	1.08	0.585[b]
	No	5	0		
The house is connected to a generator	Yes	11	0	2.68	0.206[b]
	No	90	20		
Working Fridge	Yes	30	13.3	0.54	0.461
	No	72	19.4		
Tap inside house	Yes	16	12.5	0.35	0.731[b]
	No	86	18.6		
Tap outside house	Yes	24	8.3	1.87	0.229
	No	78	20.5		
Shared or community tap	Yes	60	20	0.56	0.456
	No	42	14.3		
Protected or covered well	Yes	30	16.7	0.03	0.867
	No	72	18.1		
Unprotected or uncovered well	Yes	2	50	1.47	0.323[b]
	No	100	17		
Bore Hole	Yes	9	0	2.12	0.355
	No	63	19.4		
River or Stream	Yes	8	37.5	2.354	0.146
	No	94	16		
Boil water	Yes	46	28.3	6.29	0.012
	No	55	9.1		
Filter Water	Yes	16	56.3	19.17	$P \leq 0.001$[b]
	No	85	10.6		
Chlorination	Yes	2	50	1.44	0.326[b]
	No	99	17.2		
Allow to settle	Yes	27	11.1	1.13	0.385[b]
	No	74	20.3		
Dispose of rubbish by open dumping	Yes	54	18.5	0.04	0.845
	No	47	17		
Dispose of rubbish by burning	Yes	50	16	0.22	0.636
	No	51	19.6		
Father's occupation	Farmer	35	8.6	5.57	0.062
	Business man	37	16.2		
	Professional	29	31		
Cultivate food	Yes	69	15.9	0.43	0.514
	No	33	21.2		
Domestic animal ownership	Yes	78	12.8	5.85	0.027[b]
	No	23	34.8		

[a]The prevalence of *Cryptosporidium sp* positive cases, to show the direction of association
[b]Fisher's exact test

Table 3.4 continued

Variable	Group	N	Prevalence (%)[a]	χ^2	P-value
Dog ownership	Yes	19	21.1	0.19	0.740[b]
	No	83	16.9		
Goat ownership	Yes	44	6.8	6.24	0.012
	No	58	25.9		
Cat ownership	Yes	8	0	1.86	0.173[b]
	No	94	19.1		
Chicken Ownership	Yes	71	12.7	3.97	0.046
	No	31	29		
Visit general hospital	Yes	24	25	1.17	0.358[b]
	No	78	15.4		
Visit local health clinic	Yes	80	18.8	0.31	0.577[b]
	No	22	13.6		
Visit private hospital	Yes	28	25	1.44	0.231[b]
	No	74	14.9		
Self medicate	Yes	46	17.4	0.00	0.951
	No	56	17.9		
Possession of window net	Yes	65	23.1	3.64	0.057
	No	37	8.1		
Possession of door net	Yes	26	15.4	0.12	1[b]
	No	76	18.4		
Possession of bed net	Yes	42	23.8	1.87	0.17
	No	60	13.3		
Feeding behaviour for children aged > 6 months					
Water	Yes	77	19.5	0.61	0.435[b]
	No	24	12.5		
Herbal concoctions	Yes	42	16.7	0.07	0.798
	No	59	18.6		
Infant formula	Yes	13	30.8	1.71	0.241[b]
	No	88	15.9		
Pap	Yes	22	18.2	0.00	1[b]
	No	79	17.7		
Exclusively breast feeding	Yes	50	20	0.32	0.571
	No	51	15.7		
HAZ	Normal	91	17.6	0.00	1[b]
	Underweight	11	18.2		
WAZ	Normal	96	17.7	0.00	1[b]
	Stunted	6	16.7		
WHZ	Normal	97	17.5	021	1[b]
	Wasted	1	0		
Condition of Stool					
Mucoid	Yes	13	23.1	0.45	0.450[b]
	No	83	15.7		
Watery	Yes	47	23.4	3.01	0.083
	No	49	10.2		

[a]The prevalence of *Cryptosporidium* sp. positive cases, to show the direction of association
[b] Fisher's exact test

Table 3.4 continued

Variable	Group	N	Prevalence (%)[a]	χ^2	P-value
Formed	Yes	9	0	1.99	0.159
	No	87	18.4		
Unformed	Yes	48	12.5	1.20	0.273
	No	48	20.8		
Blood	Yes	1	0	0.20	1[b]
	No	95	16.8		

[a]The prevalence of *Cryptosporidium* sp. positive cases, to show the direction of association
[b] Fisher's exact test

Table 3.5 Risk factors for *Cryptosporidium* sp. in children aged < 6 months

Variable	Odds ratio	(95% CI)	P-value
Filtering water×village			
Filtering water×Moro	1		
Filtering water×Edunabon	0.255	(0.077 – 0.838)	0.024
Filtering water×Ipetumodu	3.503	(1.432 – 8.572)	0.006
Bed net×Father's job			
Bed net×Farmer	1		
Bed net×Business man	0.819	(0.598 – 1.123)	0.215
Bed net×Professional	1.482	(1.061 – 2.069)	0.021

Table 3.6 presents the results of the chi-square analysis examining the associations between various demographic, sociological, behaviour factors and the presence of *Cryptospordium* sp. in children aged ≥ 6 months. The variables sex, age, drinking water from a bore hole, adding chlorine to water before drinking, settling water before drinking, disposing of rubbish by open dumping or burning, father's education or occupation, feeding a child infant formula or semi-solid food, still breast feeding after a child reaches the age of 6 months and having a stool with mucus were significantly associated ($P \leq 0.2$) with *Cryptosporidium* sp. infection.

Father's education and interactions with sex×settling water before drinking, obtaining water from a bore hole×open dumping of rubbish and feeding a child semi-solid food×still breast feeding were significantly correlated with the occurance of *Cryptosporidium* sp in the model (Table 3.7). Children whose fathers had secondary education were 0.6 times less likely to harbour *Cryptosporidium* sp. when compared to children whose father's had primary education. Children who were female and whose water was not settled were 1.4 times more likely to be infected with *Crytposporidium* sp. than those who were male and whose water was

settled. The odds of infection with *Cryptosporidium* sp. were 0.6 times lees likely for children whose families did not obtain water from a bore hole and who did not dispose of their refuse by open dumping. Children who ate semi-solid food and who were not being breastfed were 2 times more likely to harbour *Cryptosporidium* sp. when compared to children who were not eating semi-solid food and who were being breastfed.

Table 3.6 Chi Square (χ^2) analysis for factors significantly associated with *Cryptosporidium* sp. infections for children aged ≥ 6 months

Variable	Group	N	Prevalence (%)[a]	X^2	P-value
Village	Moro	66	28.8	0.18	0.914
	Edun-abon	94	31.9		
	Ipetumodu	107	30.8		
Sex	Male	139	25.9	3.16	0.076
	Female	128	35.9		
Age	7-11 months	106	24.5	3.33	0.189
	12-17 months	77	36.4		
	18 -25 months	84	33.3		
The house is connected to Electricity supply	Yes	250	30.4	0.18	0.672
	No	17	35.3		
The house is connected to a generator	Yes	45	31.1	0.01	0.945
	No	219	30.6		
Working Fridge	Yes	83	30.1	0.02	0.888
	No	184	31		
Type of Toilet	Flush Toilet	68	22.1	3.09	0.214
	Pit latrine	153	33.3		
	Bush Toilet	29	34.5		
Tap inside house	Yes	44	31.8	0.03	0.862
	No	223	30.5		
Tap outside house	Yes	84	33.3	0.40	0.529
	No	183	29.5		
Shared or community tap	Yes	156	28.2	1.11	0.293
	No	111	34.2		
Protected or covered well	Yes	64	25	1.29	0.256
	No	203	32.5		
Unprotected or uncovered well	Yes	6	50	1.07	0.38[b]
	No	261	30.3		
Bore Hole	Yes	25	48	3.87	0.049
	No	242	28.9		
River or Stream	Yes	38	34.2	0.26	0.614
	No	229	30.1		
Boil water	Yes	111	31.5	0.16	0.686
	No	154	29.2		
Filter Water	Yes	37	30.2	0.00	1
	No	148	30.2		

[a]The prevalence of *Crytposporidium* sp. positive cases, to show the direction of association
[b] Fisher's exact test

Table 3.6 continued

Variable	Group	N	Prevalence (%)[a]	X^2	P-value
Chlorination	Yes	12	8.3	2.849	0.114[b]
	No	253	31.2		
Allow to settle	Yes	80	23.8	2.25	0.133
	No	185	33		
Dispose of rubbish by open dumping	Yes	130	34.6	1.97	0.160
	No	135	26.7		
Dispose of rubbish by burning	Yes	137	27	1.69	0.193
	No	128	34.4		
Mother's education	Primary	70	34.3	0.55	0.758
	Secondary	170	29.4		
	Tertiary	23	30.4		
Father's education	Primary	26	34.6	8.18	0.017
	Secondary	173	25.4		
	Tertiary	60	45		
Father's occupation	Farmer	89	32.6	7.20	0.027
	Business man	114	23.7		
	Professional	60	43.3		
Cultivate food	Yes	175	31.4	0.12	0.726
	No	92	29.3		
Domestic animal ownership	Yes	212	29.7	0.91	0.341
	No	52	36.5		
Dog ownership	Yes	37	24.3	0.82	0.364
	No	230	31.7		
Goat ownership	Yes	140	28.6	0.63	0.426
	No	127	33.1		
Cat ownership	Yes	33	21.2	1.60	0.206
	No	234	32.1		
Chicken Ownership	Yes	195	30.3	0.07	0.791
	No	72	31.9		
Visit general hospital	Yes	76	26.3	1.07	0.301
	No	189	32.8		
Visit local health clinic	Yes	216	31.9	0.55	0.459
	No	49	26.5		
Visit private hospital	Yes	84	29.8	0.08	0.777
	No	181	31.5		
Self medicate	Yes	123	30.9	0.00	0.987
	No	142	31		
Possession of window net	Yes	161	29.8	0.25	0.621
	No	104	32.7		
Possession of door net	Yes	70	27.1	0.72	0.40
	No	193	32.6		
Possession of bed net	Yes	58	32.8	0.10	0.752
	No	206	30.6		

[a]The prevalence of *Crytposporidium* sp. positive cases, to show the direction of association
[b] Fisher's exact test

Table 3.6 continued

Variable	Group	N	Prevalence (%)[a]	X^2	P-value
Feeding behaviour for children aged > 6 months					
Herbal concoctions	Yes	106	32.1	0.03	0.866
	No	148	31.1		
Infant formula	Yes	112	35.7	1.65	0.199
	No	142	28.2		
Pap	Yes	199	31.7	0.03	0.853
	No	56	30.4		
Semi-solid food	Yes	189	35.4	5.35	0.021
	No	65	20		
Family foods	Yes	178	33.1	0.75	0.386
	No	76	27.6		
Still breast feeding	Yes	187	28.3	3.56	0.059
	No	66	40.9		
HAZ	Normal	232	31.9	1.17	0.280
	Underweight	35	22.9		
WAZ	Normal	190	29.5	0.47	0.491
	Stunted	77	33.8		
WHZ	Normal	221	30.8	0.00	0.964
	Wasted	46	30.4		
Condition of Stool					
Mucoid	Yes	31	16.1	3.75	0.053
	No	225	33.3		
Watery	Yes	64	29.7	0.10	0.755
	No	192	31.8		
Formed	Yes	75	30.7	0.17	0.897
	No	181	31.5		
Unformed	Yes	132	33.3	0.55	0.458
	No	124	29		
Blood	Yes	14	42.9	0.91	0.379
	No	241	30.7		

[a]The prevalence of *Cryptosporidium* sp. positive cases, to show the direction of association

Table 3.7 Risk factors for *Cryptosporidium* sp. in children aged > 6 months

Variable	Odds ratio	(95% CI)	P-value
Father's education			
Primary (reference category)	1		
Secondary	0.611	(0.388-0.960)	0.033
Tertiary	1.649	(0.980-2.773)	0.059
Sex×settling water	1.397	(1.042-1.872)	0.025
Bore hole×open dumping	0.630	(0.465-0.854)	0.003
Semi-solid food×still breast feeding	1.979	(1.251-3.131)	0.004

3.4 Discussion

Previous to the WHO consultation in 2002, children less than 24 months were excluded from large-scale intervention programmes for geohelminths in endemic countries. The high prevalence of *A. lumbricoides* (24.7%) in children aged 12-25 months found in our epidemiological survey supports the recommendation of WHO to enrol children from 1-2 yrs in control programmes for geohelminths. *A. lumbricoides* was the dominant geohelminth infection in this population. Few children harboured *T. trichiura.* *A. lumbricoides* and *T. trichiura* infections are more important in younger children as opposed to hookworm which is found in older age groups (Asaolu *et al.*, 2002), this may explain why no hookworm infections were present in these children. Age was one of the most significant risk factors for *A. lumbricoides* in the logistic regression model. The prevalence of *A. lumbricoides* gradually increased when children reached the age of 4-7 months, reaching a steep incline from 16-19 months. Weaning from breast milk to solid foods and better mobility of children aged >10 months may explain the increase in the prevalence of *A. lumbricoides* in older children as the exposure to infective eggs increases (Montresor *et al.*, 2003). Results from studies on children less than 24 months show the prevalence of *A. lumbricoides* ranged from 6.2% in Nicaragua (Oberhelman *et al.*, 1998) to 66% in Zaire (Mbendi *et al.*, 1988). The low prevalence of *A. lumbricoides* (2.9%) in children less than one year compares well with studies undertaken in Sierra Leone (2%) (Montresor *et al.*, 2003), Rio de Janeiro (4.3%) (Costa-Macedo and Rey, 2000), and Nigeria (7.6%) (Asaolu *et al.*, 2002).

In the present study, an interaction with father's occupation and breast feeding was significant in the logistic regression model. The risk of being infected with *A. lumbricoides* was less likely if the children were not being breastfed and their fathers were businessmen compared to those whose fathers were farmers. Children who were still being breast fed after they reached six months of age had a lower prevalence of *A. lumbricoides*. However, this association did not remain significant in the logistic regression model. The protective association found between children that were still being breast fed after six months and *A. lumbricoides* has been supported in other studies (Khan et al., 1983; Gendrel et al., 1988). Despite this potential protective effect of breast feeding, the interaction demonstrated in this present study may suggest that children whose fathers are business men are at lower risk of acquiring *Ascaris*

infections owing to socio-economic factors associated with such occupations for example, better living conditions. Farmers who use nightsoil (human faeces and urine) as fertiliser for crops may be at more risk of STH infection as nightsoil has previously been demonstrated as a risk factor for hookworm infection in Vietnam (Van der Hoek et al. 2003). In the present study, the use of human faeces as fertiliser is not well practiced in this region; 2.5 % of the mothers interviewed stated that they used human faeces as a fertiliser, hence the high prevalence of *A. lumbricoides* in this group may be attributed to other socio-economic or behavioural factors. Naish et al. (2004) revealed that children whose parents' occupation was fishing had a higher prevalence and greater intensity of *A. lumbricoides* infection. The authors suggested that this finding could be attributed to lack of nearby latrines, which may also be a contributing factor to our finding.

A significant interaction was demonstrated between dog ownership and not attending a private hospital in the logistic regression model. Traub *et al.* (2002) have shown that dogs were significant disseminators and environmental contaminators of *A. lumbricoides* in communities where promiscuous defecation by humans occurs. In another Indian study by Traub *et al.* (2004), pig ownership was recognised as a potential risk factor for the prevalence and intensity of all three geohelminths and also remained in the final multivariate model for intensities of *A. lumbricoides*. The interaction revealed here in this present study between dog ownership and not attending a private hospital may suggest that families who can afford to attend private hospitals have better hygiene and sanitation practices and are therefore not affected by the potential increase in transmission of *A. lumbricoides* caused by dogs. The association between dog ownership and *A. lumbricoides* suggest that there may be a significant health risk for young children in similar communities where dogs are present.

The high prevalence of *Cryptosporidium* sp. demonstrated in our epidemiological survey suggest that *Cryptosporidosis* may pose a significant public health concern for young children in this region. Many studies have been undertaken on *Cryptosporidium* sp. in children aged less than five years (Chacin-Bonilla *et al.*, 1997; Iqbal *et al.*, 1999a; Perch *et al.*, 2001; Adjei *et al.*, 2004). In sub-Saharan Africa the prevalence of *Cryptospordium* sp. in children less than five years ranges from 3.8% to 26% (Simwa *et al.*, 1989; Amadi *et al.*, 2001). Age is an important factor in disease manifestation of childhood cryptosporidiosis. Cryptosporidiosis is

more common in infants aged less than two years than older children and adults (Smith and Van den Ende, 1986; Fripp *et al.*, 1991; Moodley *et al.*, 1991; Molbak *et al.*, 1993; Agnew *et al.*, 1998; Perch *et al.*, 2001; Adjei *et al.*, 2004). The prevalence of *Cryptosporidium* sp. (27.1%) shown here is higher than that found in South Africa, Haiti and Pakistan (Smith and Van den Ende, 1986; Pape *et al.*, 1987; Iqbal *et al.*, 1999a). *Cryptosporidium* sp. was more common in children greater than six months of age; this finding has been supported in other studies (Aidara *et al.*, 1993).

The logistic regression model identified a significant interaction between filtering water before drinking and village. The odds of a child harbouring *Cryptosporidium* sp. varied according to the village they lived in, if the child was from Edunabon they were 0.3 times less likely to become infected if their drinking water was filtered whereas if they were from Ipetumodu they were 3.5 times more likely to become infected if their drinking water was filtered compared to those that lived in Moro. Transmission of *Cryptosporidium* sp. through drinking water is well documented (Smith *et al.*, 2006). It has been accepted that infectious oocysts are likely to be present in conventionally treated (i.e. filtration, coagulation, sedimentation, and disinfection) drinking water due to ineffective water treatment processes (Smith *et al.*, 2006). This result may suggest that filtering water could increase or decrease transmission depending on location where inhabitants may have different ways of filtering their water. Filtering water could possibly concentrate infectious oocysts if an individual is not using a filter correctly. Similarly it is also plausible that in areas where there is a greater density of people for example, Ipetumodu, transmission of *Cryptosporidium* sp. is higher.

In this study, children aged < 6 months were more likely to be infected with *Cryptosporidium* sp. if their families did not own a bed net and their fathers were professionals compared to those whose fathers were farmers. This interaction between father's occupation and owning a bed net was significant in the logistic regression model. This finding was surprising as one would expect that children whose fathers are farmers would be at more risk of becoming infected with *Cryptosporidium* sp. since farm animals are a known source of infection (Stantic-Pavlinic *et al.,* 2003). Cattle act as a reservoir for *C. parvum* which can also infect humans (Hunter and Thompson, 2005). Less than 1% of the families in this study had cattle, many families had chickens (72.1%) and goats (50%); fewer families had dogs (15.2%) and cats (11.1%). The result found in this study may indicate that children whose fathers are

professionals spend more time in a semi-urban environment where there is indiscriminate defecation and no organised sewage disposal system which might potentially increase water contamination that may leave them more exposed to infection. Apart from livestock and cattle genotypes of *C. parvum*, the role of other mammals and birds in the zoonotic transmission of *Cryptosporidium* sp. is uncertain (Monis and Thompson, 2003). *Cryptosporidium* sp. infection has been demonstrated in a wide range of wild animals (Sturdee *et al.*, 1999) including wild mice (Chalmers *et al.*, 1997) and muskrats (Perz and Le Blancq, 2001). Rodents are particularly common in urban environments and could also be a source of infection for humans (Gatei *et al.*, 2003). In the present study, the interaction with bed net ownership and father's occupation may signify that children whose father's are professionals with lower SES, as indicated by having no bed nets, are at higher risk of *Cryptosporidium* sp. infection.

Paternal education was a significant risk factor in the logistic regression model for children aged $\geq$ 6 months. Children were significantly less likely to be infected with *Cryptosporidium* sp. when their fathers had a second level education. Parents with a higher level of education may exhibit behaviours that would reduce exposure to *Cryptosporidium* sp. infections in children. Few studies have identified parental education as a risk factor for *Cryptosporidium* sp. Leach *et al.* (2000) found there was an association between mothers having less secondary education and *C. parvum* infection in children but this did not remain significant in their logistic regression model.

The odds of being infected with *Cryptosporidium* sp. were 1.4 times more likely for females whose water was not settled in the logistic regression model for children aged $\geq$ 6 months. A study in Guatemala demonstrated that males aged 2-5 years had fewer *Cryptosporidium* sp. infections than females but since parents treated both sexes of this age group the same, making their risk factors similar, the authors could provide no obvious reason for the differences observed (Laubach *et al.*, 2004). Similarly, it cannot be explained why females are more exposed to untreated water than males. Sedimentation has previously been shown to reduce the concentration of *Cryptosporidium* (oo)cysts in water samples (Hsu and Yeh, 2003). Furthermore, ultraviolet light has been used as a disinfectant to inactivate *Cryptosporidium* (oo)cysts (Lorenzo-Lorenzo *et al.*, 1993) and therefore it is plausible that if the water is left to settle outside and is exposed to ultraviolet light this could further reduce the concentration of

(oo)cysts in water samples. This practice is a simple measure that could be undertaken (in conjunction with other preventative measures) to reduce transmission and risk of infection in these young children.

The interaction between disposing of refuse by open dumping and obtaining water from a bore hole was also significant in the logistic regression model for children aged ≥ 6 months. The risk of harbouring *Cryptosporidium* sp. was lower for children whose families did not obtain water from a bore hole and who did not dispose of their refuse by open dumping. Waterborne transmission of *Cryptosporidium* sp. remains one of the most prominent public health concerns worldwide (Rose *et al.*, 2000). The water source is one of the key environmental factors involved in the potential risk for waterborne transmission of cryptosporidosis. Data on drinking water outbreaks in the USA from 1971 to 1994 from all infectious agents demonstrate a statistical association with extreme precipitation (Rose *et al.*, 2000). This epidemiological survey was undertaken at the beginning of the rainy season. It is thought that the rains exacerbate the washing of human and animal faecal matter into open bathing and drinking water sources after which they are consumed. Seasonality, a risk factor for *Cryptosporidium* sp. (Newman *et al.*, 1999, Banwat *et al.*, 2003) may explain the high prevalence exhibited in this epidemiological survey. Trends indicate an increase in the incidence of Crytposporidiosis during the rainy seasons in South Africa (Fripp *et al.*, 1991, Moodley *et al.*, 1991). In the present analysis, there was an interaction between dispoing of refuse by open dumping and use of a bore hole. It is possible that open dumping of refuse can increase environmental contamination, for example water obtained from bore holes and transmission of *Cryptosporidium* sp..

The logistic regression model for children aged ≥ 6 months demonstrated a significant interaction between feeding a child semi-solid food and still breast feeding after the child has reached the age of 6 months. Children were more likely to be infected with *Cryptosporidium* sp. if they ate semi-solid food and were not being breastfed. Other studies have shown that children under 6 months, particularly those who are exclusively breastfed, lack *Crytposporidium* sp. infection (Adjei et al., 2004; Zhu, 1991). A study in Guinea-Bissau, West Africa, demonstrated that the benefits of breast feeding were not restricted to infancy (Molbak et al. 1994). Their study revealed that the incidence of diarrhoea was higher in children who were weaned when compared to children that were partially breast fed, both in 1

year olds (relative risk 1.41) and in 2 year olds (relative risk 1.67). Weaning children from breast milk to solid foods is an important risk factor for *Cryptosporidium* sp. that has been documented in other studies (Cruz et al., 1988; Sterling et al., 1991) . Food borne outbreaks of *Cryptosporidium* sp. have occurred and given the low infectious doses for *Cryptosporidium* sp., surface contamination of produce that receives minimal heat treatment before ingestion may pose an important source of transmission (Smith *et al.*, 2006). The findings in the present study suggest that although children aged ≥ 6 months may consume other foods which could increase the risk of becoming infected with *Cryptosporidium* sp. partially breast fed children have a lower risk of contracting the parasite and thus mothers should be encouraged to breast feed their children for up to 2 years and beyond as recommended by WHO (2003d).

Unlike *Ascaris* infections which are more important in older children from the age of 12 months, there is a high prevalence of *Cryptosporidium* sp. in infants in this region and the associated morbidity with this parasitic infection may be considerable in children aged 1-2 years. At present, there is no effective treatment for cryptosporidosis and therefore efforts should be concentrated on preventative measures, especially during the rainy season in tropical countries when prevalences can increase dramatically (Fripp *et al.*, 1991; Moodley *et al.*, 1991). Large-scale studies are required to define more clearly the overall role of intestinal cryptosporidosis as a cause of morbidity and mortality in infants (Current and Garcia, 1991), where those less than 2 years old have the greatest prevalence (Fayer and Ungar, 1986). Molecular identification of *Cryptosporidium* sp. species should be included in future epidemiological studies to identify causative agents of disease and possible sources of transmission.

The analysis of risk factors in this study was based on the presence or absence of parasitism, rather than the intensity of infection. This analysis does not control for potential differences in health outcomes based on intensity of infection. However, the majority of infections in these young children were light and therefore may be expected to have similar health outcomes. Although we cannot interpret a cause and effect relationship between risk factors and parasitic infection, most of the risk factors identified in this study are biologically plausible and important for planning parasite prevention in Ile-Ife, Nigeria. The findings from our study suggest that many of these young children, who are at a critical stage of development, are infected with *Ascaris* and *Cryptosporidium* sp. and that the rate of infection with these

parasites increases with age. Health care for children aged 1-2 years in this region needs to be improved by establishing an effective deworming control programme, providing adequate supply of treated water for household use and health education to improve the standard of hygiene.

CHAPTER 4: The effect of repeated four-monthly anthelminthic treatment on the prevalence and intensity of *A. lumbricoides* infection

Figure **Page**

Table **Page**

4.1 Introduction

Soil-transmitted helminths (STH) are considered to be the most prevalent infections of humankind. The extraordinary numbers of STH infections, which approach two billion, are a reflection of a remarkably successful adaptation to parasitism by *Ascaris*, *Trichuris* and hookworm nematodes (de Silva *et al.,* 2003). 1221 million people are estimated to be infected with *A. lumbricoides*, with 10% of these infections residing in children aged 0-4 years (de Silva *et al.,* 2003). *A. lumbricoides* is a remarkably infectious and persistent parasite. In areas where *A. lumbricoides* is endemic, there is a rapid rate of reinfection, with children under five years being most frequently reinfected (Thein-Hlaing *et al.,* 1987). High prevalences of *A. lumbricoides* are indicative of poor socio-economic conditions (O'Lorcain and Holland, 2000). Infections thrive and persist in communities in need of better housing, clean water, appropriate sanitation, better access to health care and education (Crompton, 1999). In the long term, sustained relief from *A. lumbricoides* may be realised by provision of safe water supplies, sanitation facilities and promotion of hygienic measures, such as hand washing, use of latrines and encouraging footwear. However, in the short term regular chemotherapy is needed to reduce morbidity associated with infection. WHO recommends regular treatment of high risk groups, including school-age children, preschool children, pregnant women and special occupational groups (WHO, 2002).

Many studies have examined reinfection with *A. lumbricoides* after anthelminthic treatment (Farid *et al.*, 1966; Arfaa and Ghadirian, 1977; Thein-Hlaing, *et al.*, 1984; 1987; Elkins *et al.*, 1988; Holland *et al.*, 1989; Thein-Hlaing *et al.*, 1991; Holland *et al.*, 1996a; Hall *et al.*, 1999). Egg output can reattain pre-treatment levels by six months of reinfection (Elkins *et al.*, 1988). Prevalence, according to egg positivity, can approach initial levels by six months after treatment (Elkins *et al.*, 1988) and in children under 10 years has been shown to be even higher than pre-treatment values after six-monthly treatments (Thein-Hlaing *et al.*, 1987). Thus, it is recommended that treatment should be applied at intervals of six months or less (Elkins *et al.*, 1988). The present study design was based on a paper by Holland *et al.* (1996a) where four-monthly treatments with levamisole, compared with six monthly and one yearly treatments, significantly reduced the intensity of *A. lumbricoides* infection and prevalence by 54% in children aged 5-15 years.

The overall objective of this study was to describe the relationship between *A. lumbricoides* and uncomplicated malaria attacks. In order to achieve this aim a double-blind, placebo-controlled randomised trial of anthelminthic treatment was conducted. This chapter focuses on the effectiveness of four-monthly treatments in reducing the prevalence and intensity of *A. lumbricoides* infection in children aged 12-59 months.

4.2 Materials and methods

Please refer to Chapter 2, for a description of the study location (Section 2.1.1) and materials and methods (Section 2.1.3).

4.2.1 Data management and analysis

The assessments took place in May/June 2006, Sept./Oct. 2006, Feb. 2007, May/June 2007 and Aug. 2007 (Chapter 2, section 2.1.3.1), these assessments will be referred to as time points 0, 1, 2, 3 and 4, respectively. The analysis included children aged between 12-59 months and was carried out on four subsets of data, each of which will be referred to as the first, second, third, and fourth data set for ease of discussion. The first data set included children who were recruited at time 0 and 1 and submitted a stool sample at any of the time points 0, 1, 2, 3 and 4. This analysis examined the effect of anthelminthic treatment at the population level i.e. individuals who received treatment one or more times over the five time points. The second data set included children who were recruited at time 0 and submitted a stool sample at time points 0, 1, 2, 3 and 4. This analysis examined the effect of anthelminthic treatment at the individual level i.e. individuals who received treatment at all time points. The third data set included children who were recruited into study at time 0 and 1 and submitted a stool sample at time points 1, 2, 3 and 4. A second recruitment at time 1 increased the sample size, therefore the analysis could be undertaken with a larger population over four time points which increased the probability of the study detecting an effect of anthelminthic treatment on malaria attacks. The fourth data set includes children who were recruited at time 1 and submitted stool samples at time points 1, 2, 3 and 4. The analysis on this data set will provide a comparison for the second data set.

Chi-square analysis was used to examine village compliance, i.e. the proportion of people that complied with all assessments in each village for subjects that were recruited at time 0 and time 1. The data on epg were assessed for normality visually and statistically. The epg values did not confirm to a normal distribution and therefore the epg data were log transformed (epg +1) for the purposes of statistical analysis. All statistics were carried out in SPSS 14.

A chi-square analysis was used to test the difference in the prevalence of *Ascaris* among age groups, villages and between the sexes. The influence of factors (age, village and sex) on epg were analysed by analysis of variance (ANOVA), using 3-way ANOVAs. In some cases least squares difference (LSD) *post-hoc* tests were applied to tease out the major sources of variation within factors.

The individuals in the treatment and placebo groups were compared on the basis of age, sex, village, prevalence and intensity of *Ascaris*, and SES at inclusion into the study. This was not carried out for the first data set because subjects did not comply with all assessments. Chi-square analysis was used to test differences in proportions and a 2-sample t-test was used to detect differences in epg and SES between groups.

Mean epg was measured over five time points from the same individuals. Since the data were not independent they were analysed by repeated measures rmANOVA (General Linear Model) with the different time points as a within-subject factor. Group, village, age and sex were chosen as the between-subject factors; these factors were entered into the model if they were significant in the 3-way ANOVA. Since the data did not meet the requirements of sphericity, the Huynh-Feldt adjustment to the degrees of freedom was used to interpret the output on the side of caution. A rmANOVA was not carried out for the first data set because this data set included subjects who did not comply with all time points.

4.3 Results

1451 children aged between 12-59 months were recruited into the study in May/June 2006. 1239 (85.3%) children provided stool samples for the detection of geohelminths. A further 703 children aged 12-59 months were recruited in Sept./Oct. 2006. 573 (81.5%) of these children submitted stool samples for analysis. The number of stool samples submitted by the subjects recruited in May/June 2006 and Sept./Oct. 2006 increased by 29% and 25.2%, respectively, when compared with compliance to return stool samples in Phase I.

26.7% (388) of the children recruited in May/June 2006 submitted stool samples at all time points 0, 1, 2, 3 and 4. Compliance varied significantly among villages (χ^2 = 21.539, d.f. = 3, P < 0.001). Compliance was lowest in Moro (21.9%) and highest in Akinlalu (39%). 32.8% (231) of the children recruited in Sept./Oct. 2006 submitted stool samples at the time points 1, 2, 3 and 4. Again compliance varied significantly among villages (χ^2 = 13.189, d.f. = 3, P = 0.004). For this group, compliance was lowest in Edunabon (25.6%) and highest again in Akinlalu (45%).

4.3.1. First data set: children who were recruited at time 0 and 1 and submitted a stool sample at any of the time points 0, 1, 2, 3 and 4

4.3.1.1 Baseline data

A. lumbricoides was the most prevalent geohelminth infection in the population (Table 4.1). 86% of the *A. lumbricoides* infections were of light intensity while 14% were of moderate intensity. Few children harboured *Trichuris trichiura* (3.7%) or hookworm infection (4.3%). *Schistosoma haematobium* (based on faecal samples) was detected in 7.5% of the children in Akinlalu. There were 556 single helminth infections, 62 double infections and 8 triple infections.

The prevalence of *A. lumbricoides* increased in children aged 1-3 years and decreased thereafter, and prevalence was significantly associated with age (Figure 4.1; χ^2 = 30.183, d.f. = 3, P < 0.001). No significant difference in the prevalence of *A. lumbricoides* was detected among villages (χ^2 = 7.188, d.f. = 3, P = 0.066) and between the sexes (χ^2 = 2.241, d.f. = 1, P = 0.134).

Table 4.1 Prevalence of helminths among the habitants of Akinlalu, Ipetumodu, Moro, and Edunabon, Ile-Ife, Osun State, Nigeria

	% prevalence of infection				
	Overall (N=1239)	Akinlalu (N=187)	Ipetumodu (N=537)	Moro (N=223)	Edunabon (N=292)
Ascaris lumbricoides	47.7 1071.03±2739.98[a]	49.2 1108.7±2711.8	43.6 918.4±2349.1	49.8 1428±3665.2	52.7 1055±2585.
Trichuris trichiura	3.7[b]	6.4	1.5	1.8	7.5
Hookworm	4.3[b]	9.1	2.2	2.7	6.2
Schistosoma haematobium	1.1[b]	7.5			

[a] Mean eggs per gram of faeces (epg) ± S.D.
[b] Few helminth eggs were counted, hence no intensity is given

The mean epg increased from children aged one year to children aged three years and decreased thereafter, and this was statistically significant (Figure 4.1; 3-way ANOVA with village, sex, and age as factors, model $R^2_{adj} = 0.045$, main effect of age, $F_{3, 1191} = 15.838$, $P < 0.001$). Males had a lower mean epg (938 ± S.E. 106.5) than females (1220.4 ± S.E. 115.4) (main effect of sex, $F_{1, 1191} = 6.398$, $P = 0.012$). The mean epg for subjects in Moro was higher than for other villages (Table 4.1), but this was not statistically significant. There were no statistically significant two- or three-way interactions. The results of a LSD *post-hoc* test showed a significant difference in epg between children aged one year and two ($P < 0.001$), three ($P < 0.001$) and four ($P = 0.015$) years; children aged two and four years ($P = 0.008$); and children aged three and four years ($P = 0.002$).

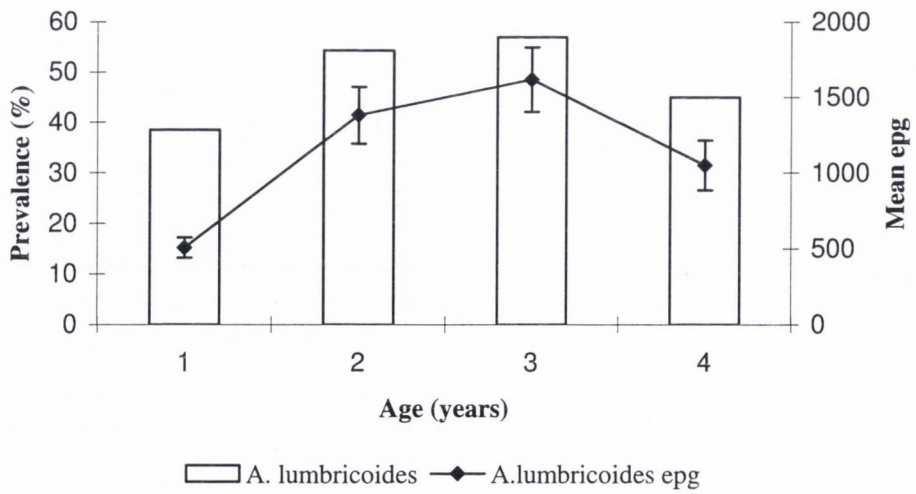


Figure 4.1. The relationship between the prevalence and mean intensity ± S.E of *A. lumbricoides* and age among children aged 1-4 years

4.3.1.2 Prevalence and intensity of *A. lumbricoides* in the treatment and placebo group

The prevalence of *A. lumbricoides* remained high in the placebo group throughout the study period with a slight peak in February whereas the prevalence in the treatment group dropped suddenly after February (Figure 4.2A). Mean epg remained persistently high throughout the follow-up period in the placebo group. Unlike prevalence, mean epg dropped after two treatments in the treated group (Figure 4.2B).

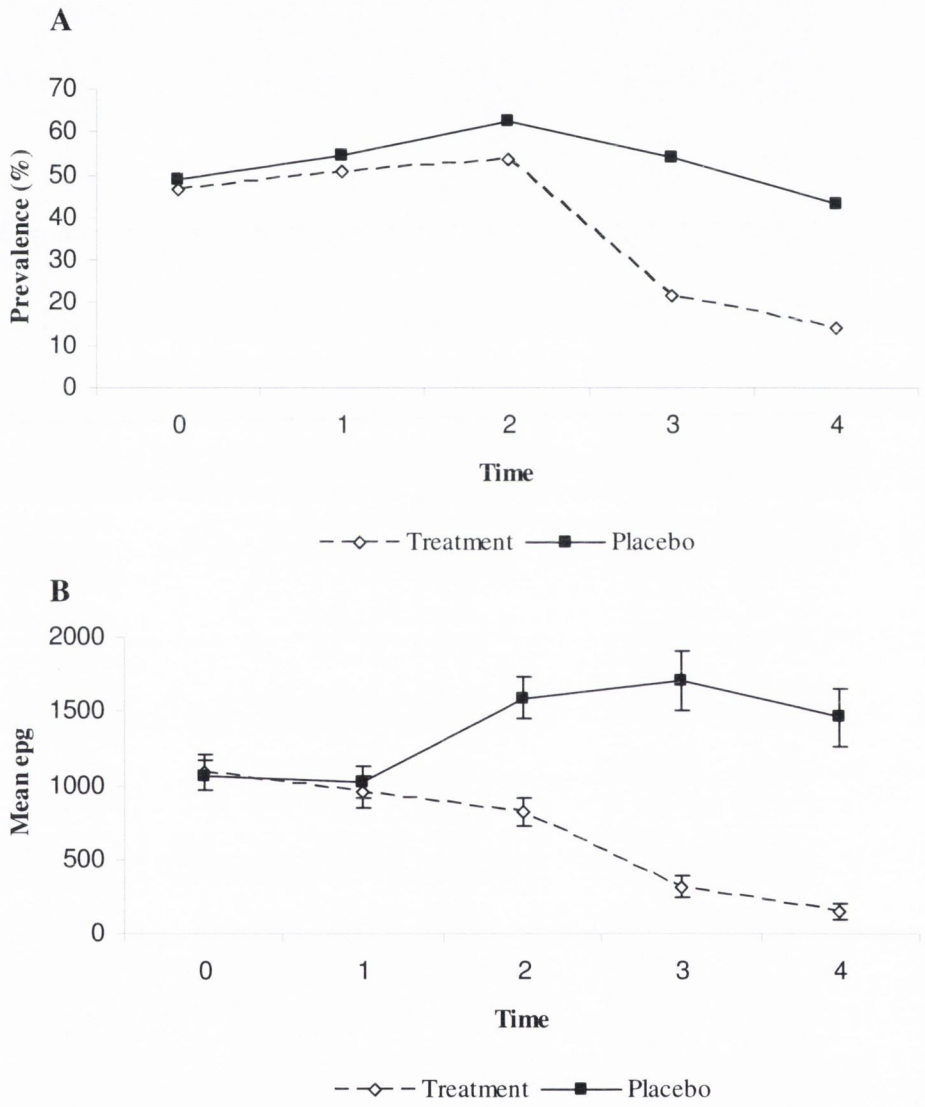


Figure 4.2 (A) Prevalence rates (*A. lumbricoides*) and mean epg in treated and placebo groups during the follow-up period. (B) Mean epg ± S.E. 0 = May/June 2006 (N = 1239), 1 = Sept./Oct. 2006 (N= 1355), 2 = Feb. 2007 (N = 1252), 3 = May/June 2007 (N = 1059), 4 = Aug. 2007 (N = 732).

All age groups followed the same trend for prevalence of *A. lumbricoides* within both treatment and placebo groups (Figure 4.3A). After time 2, prevalence of *A. lumbricoides* was lower for all age classes within the treatment group when compared to the age classes within placebo group. The mean epg for all age classes within the placebo and treatment groups generally followed the same trend. Unlike prevalence, there was a more pronounced variation in mean epg between the age classes at different time points. Mean epg for the placebo one year age group was lowest among the age categories but remained higher than the treatment group after time 2. Mean epg was higher for the placebo three year age group at times 2 to 4 than the other age groups. Mean epg remained lower for the age categories within the treatment group when compared to the age categories within the placebo group after time 2 (Figure 4.3B).

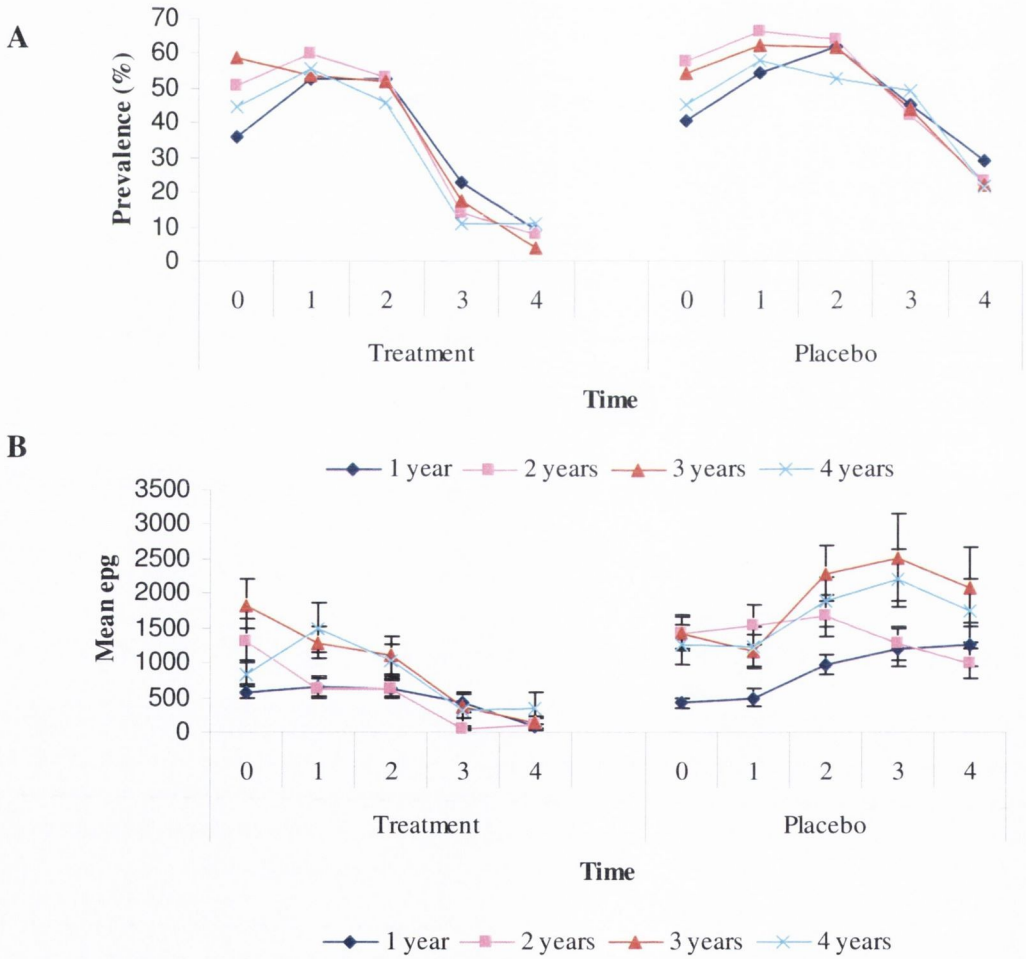


Figure 4.3 (A) Prevalence rates of *A. lumbricoides* and mean epg in treated and placebo groups for each age group during the follow-up period. (B) Mean epg ± S.E. 0 = May/June 2006, 1 = Sept./Oct. 2006 2 = Feb. 2007, 3 =May/June 2007, 4 = Aug. 2007

Similarly, all villages within the placebo and treatment groups showed the same pattern for prevalence of *A. lumbricoides* (Figure 4.4A). However, there was a more pronounced peak in the prevalence of *A. lumbricoides* in the Akinlalu treatment group at time 2. The prevalence in the villages within the placebo group remained higher than the prevalence in the villages within the treatment group after time 2. Villages within both experimental groups followed the same trend for mean epg over the follow-up period, albeit the intensity varied more among the villages within the placebo group at different time points. The mean epg in the villages within the placebo group remained higher than the mean epg in the villages within the treatment group after time 2 (Figure 4.4B).

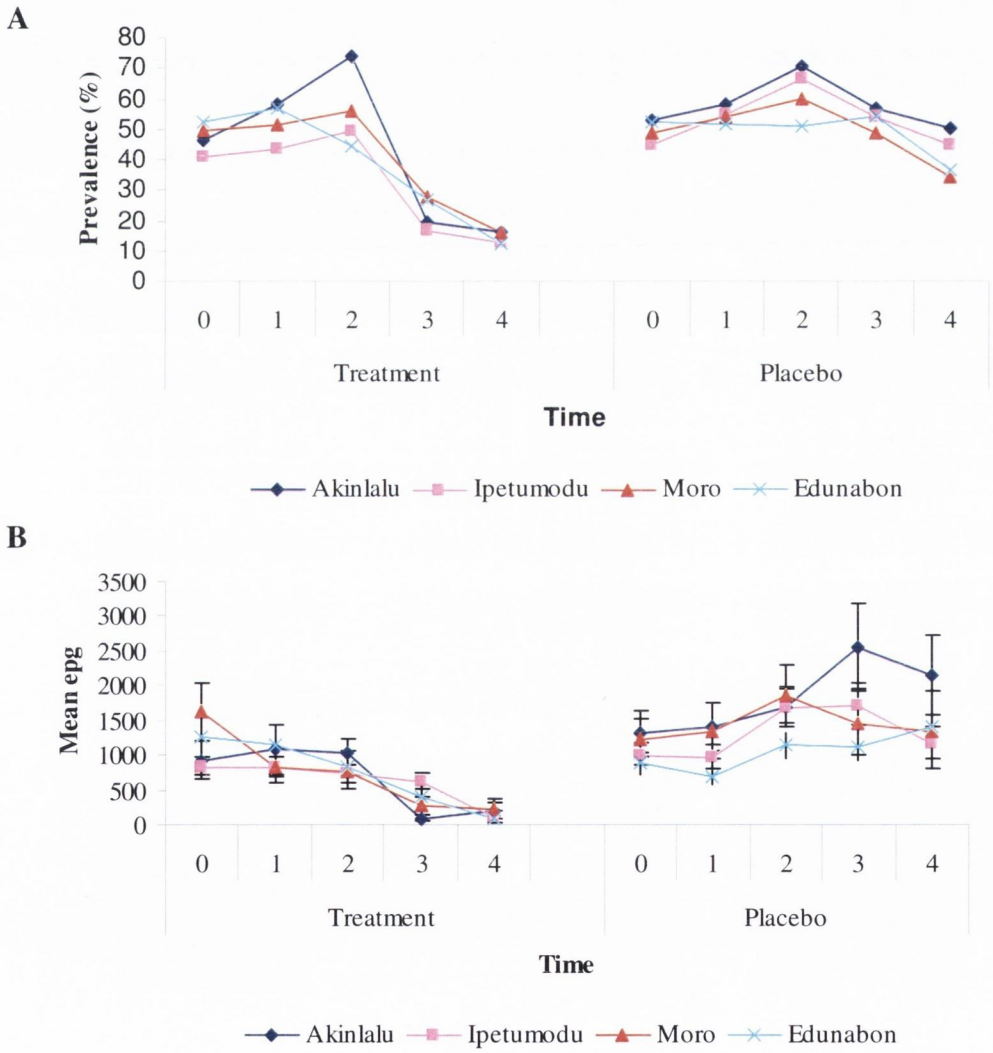


Figure 4.4 (A) Prevalence rates of *A. lumbricoides* and mean epg in treated and placebo groups for each village during the follow-up period. (B) Mean epg ± S.E. 0 = May/June 2006, 1 = Sept./Oct. 2006 2 = Feb. 2007, 3 =May/June 2007, 4 = Aug. 2007

4.3.2 Second data set: Children who were recruited at time 0 and submitted a stool sample at all time points 0, 1, 2, 3 and 4

4.3.2.1 Baseline data

388 children aged between 12-59 months submitted a stool sample at all time points 0, 1, 2, 3 and 4. This sample population examined consisted of 194 males and 194 females, with a mean age of 32 months ± S.D. 13.7 months.

Table 4.2 Prevalence of helminths among the habitants of Akinlalu, Ipetumodu, Moro, and Edunabon, Ile-Ife, Osun State, Nigeria

	% prevalence of infection				
	Overall (N=388)	Akinlalu (N=86)	Ipetumodu (N=157)	Moro (N=59)	Edunabon (N=86)
Ascaris lumbricoides	45.4 1111±2945.32[a]	40.7 952±2381.62	38.9 818.6±2035.6	55.9 2526.3±5763.2	54.7 832±1379.5
Trichuris trichiura	3.1[b]	4.7	0	1.7	8.1
Hookworm	3.8[b]	9.3	1.3	1.7	4.7
Schistosoma haematobium	1.8[b]	8.1			

[a]Mean eggs per gram of faeces (epg) ± S.D.
[b]Few helminth eggs were counted, hence no intensity is given

A. lumbricoides was the predominant geohelminth infection in this sample population (Table 4.2). 84.7% of *A. lumbricoides* infections were of light intensity and 15.3% were of moderate intensity. The prevalence of *A. lumbricoides* was higher in Moro and Edunabon (Table 4.2; $\chi^2 = 9.092$, d.f. = 3, P = 0.028). There was no statistically significant difference in the prevalence of *A. lumbricoides* among age groups (Figure 4.5; $\chi^2 = 1.531$, d.f. = 3, P = 0.675) or between sexes ($\chi^2 = 2.662$, d.f. = 1, P = 0.103).

Mean epg increased from children aged one year to children aged three years and decreased thereafter, this was statistically significant (Figure 4.5; 3-way ANOVA with village, age and sex as factors, model $R^2_{adj} = 0.101$, main effect of age, $F_{3, 356} = 4.210$, P = 0.006). Moro had the highest mean epg, and epg varied significantly among villages (Table 4.2; main effect of village, $F_{3, 356} = 5.269$, P = 0.001). Mean epg was lower for males (1067.4 ± S.E. 244.9) than

for females (1154.6 ± S.E. 176.4), and this was statistically significant (main effect of sex, $F_{1, 356} = 4.924$, P = 0.027). The mean epg for children aged two and three years was higher for Moro when compared to the other villages (2-way interaction (village*age); $F_{9, 356,} = 2.165$, P = 0.024). In Moro, males (7493 ± S.E. 4458) aged four years had a higher mean epg when compared to females (3749 ± S.E. 1682). In contrast, females (2340 ± S.E. 1023) aged four years in Akinlalu had a higher mean epg than males (230 ± S.E. 230; 3-way interaction (village*age*sex); $F_{9, 356} = 3.612$, P < 0.001). The LSD *post-hoc* analysis confirmed that mean epg was significantly different between Moro and Ipetumodu (P = 0.019) and between Ipetumodu and Edunabon (P = 0.020). The analysis also showed that mean epg differed significantly between children in the one year age category and children in the two year category (P = 0.042).

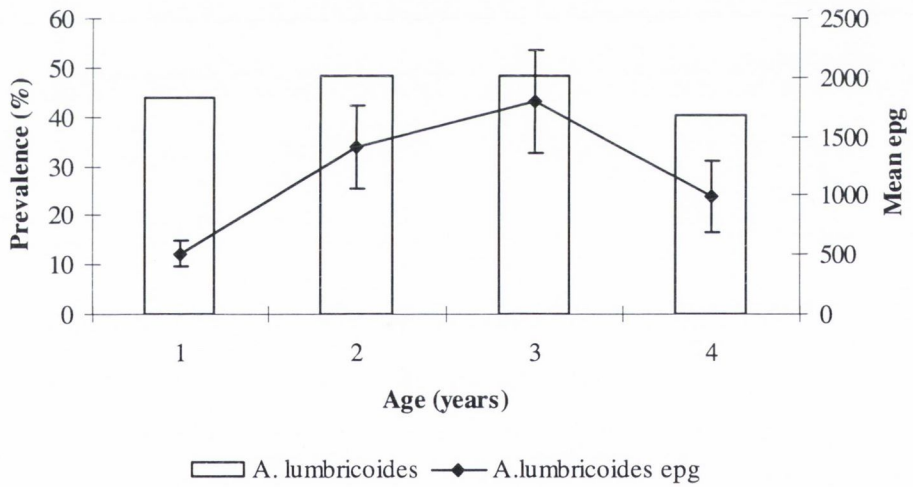


Figure 4.5 The relationship between the prevalence and mean intensity ± S.E of *A. lumbricoides* and age among children aged 1-4 years

Table 4.3 Characteristics of treatment and placebo group at inclusion (before anthelminthic treatment)

	Treatment Group (N = 194)	Placebo Group (N = 194)	P
Age (years)			
1	72	63	0.313[a]
2	46	55	
3	38	47	
4	38	29	
Sex			
Male	103	91	0.223[a]
Female	91	103	
Village			
Akinlalu	43	43	0.562[a]
Ipetumodu	80	77	
Moro	33	26	
Edun-abon	38	48	
Ascaris lumbricoides			
No. infected subjects	88	88	
Prevalence rate	45.4%	45.4%	1[a]
Mean epg ± S.E.	1095.2±237	1126.8±182	0.802[b]
SES			
Mean ± S.E.	6.27 ± 0.12	6.53 ± 0.14	0.176[b]

[a] χ^2 test
[b] 2-sample t-test

The two groups were compared on the basis of age, sex, village, prevalence and intensity of *A. lumbricoides* infection and SES. Table 4.3 shows that, on entering the study, no significant difference was recorded between the groups for any of these variables.

4.3.2.2 Prevalence and intensity of *A. lumbricoides* in the treatment and placebo group

The prevalence of *A. lumbricoides* in the placebo group remained persistently higher throughout the follow-up when compared to the treatment group. The prevalence in both groups peaked in February and decreased thereafter through to August. It took three treatments before the prevalence collapsed in the treatment group (Figure 4.6A). Unlike prevalence, there was a steep drop in the mean epg after one treatment in the treatment group. The intensity in the placebo groups remained at high levels whereas the intensity in the treatment group remained at low levels throughout the study (Figure 4.6B).

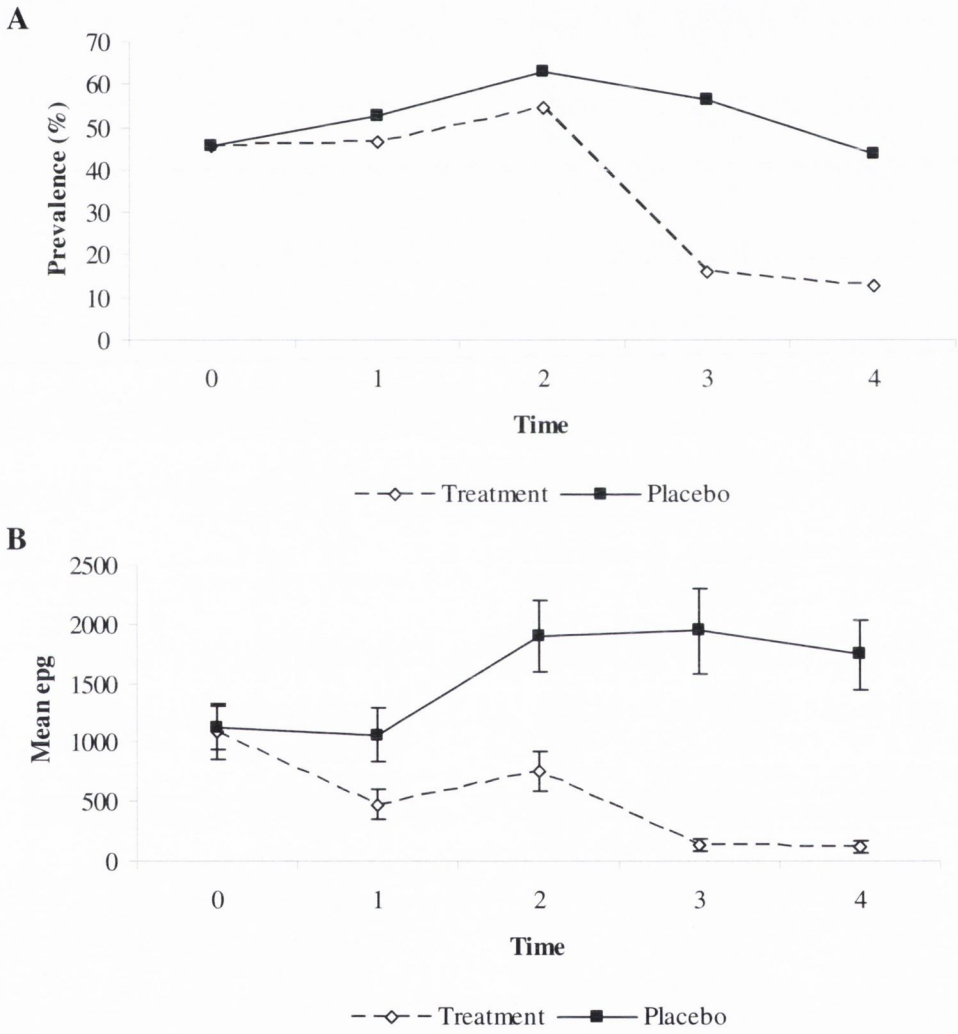


Figure 4.6 (A) Prevalence rates (*A. lumbricoides*) and mean epg in treated and placebo groups during the follow-up period. (B) Mean epg ± S.E. 0 = May/June 2006, 1 = Sept./Oct. 2006, 2 = Feb. 2007, 3 = May/June 2007, 4 = Aug. 2007.

The prevalence of *A. lumbricoides* in the age categories within the placebo group was higher than the prevalence demonstrated in the age categories within the treatment group after time point 2 (Figure 4.7A). The mean epg was lower for the placebo one year age group at time 0 to 2. The mean epg was higher for all age categories within the placebo group when compared to the mean epg for the age categories within the treatment group after time 2 (Figure 4.7B). There was more variation in the mean epg of the age categories within the placebo group when compared to the mean epg for the age categories within the treatment group.

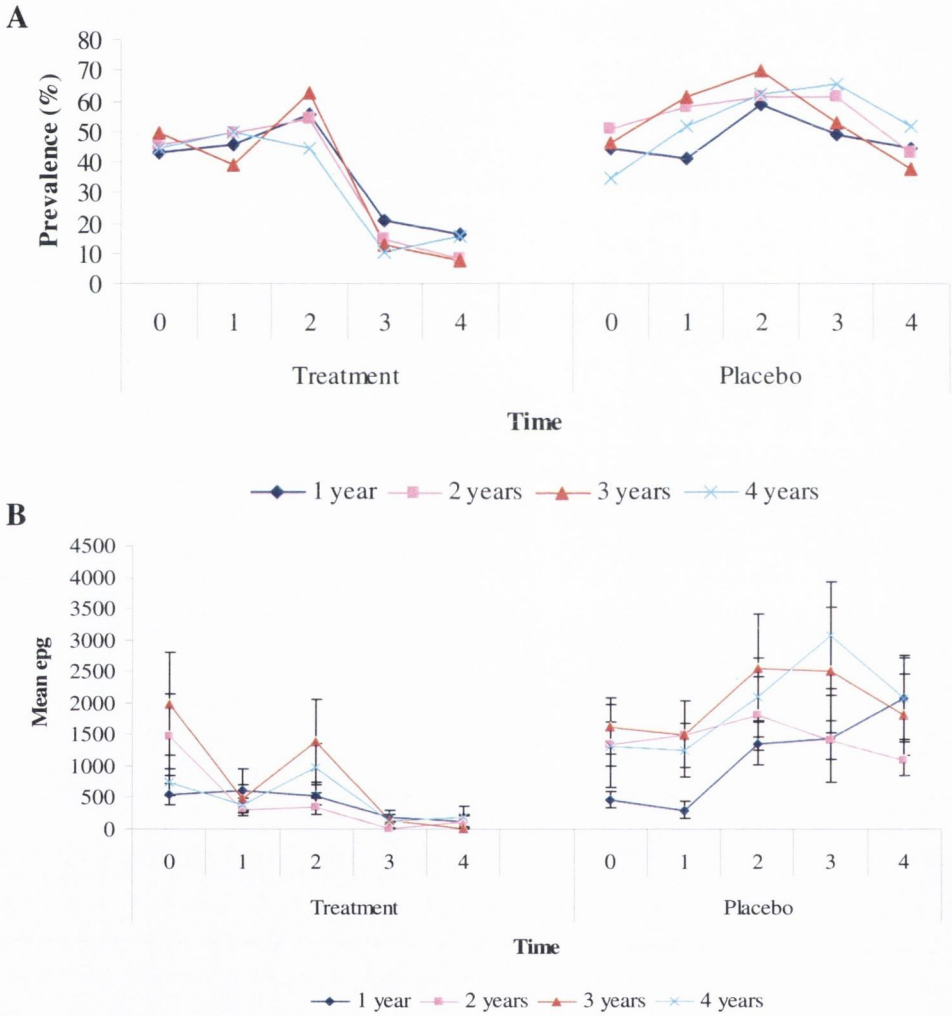


Figure 4.7 (A) Prevalence rates of *A. lumbricoides* and mean epg in treated and placebo groups for each age group during the follow-up period. (B) Mean epg ± S.E. 0 = May/June 2006, 1 = Sept./Oct. 2006 2 = Feb. 2007, 3 =May/June 2007, 4 = Aug. 2007

The prevalence of *A. lumbricoides* in the treatment and placebo group Akinlalu was higher than the other villages at time 3. The prevalence decreased in all villages within the treatment group after time 3. The prevalence in the villages within the placebo group was greater than the prevalence in the villages within the treatment group at times 4 and 5 (Figure 4.8A).

Mean epg in the placebo Akinlalu was higher at time points 3 to 5 than the other villages within the placebo group (Figure 4.8B). Epg was lowest in the Edunabon placebo than the other villages within the placebo group at times 1, 2 and 3. Mean epg was higher in the villages within the placebo group when compared to the mean epg in the villages within the treatment group after time 2. Epg varied more in the villages within the placebo group at different time points when compared to the villages within the treatment group.

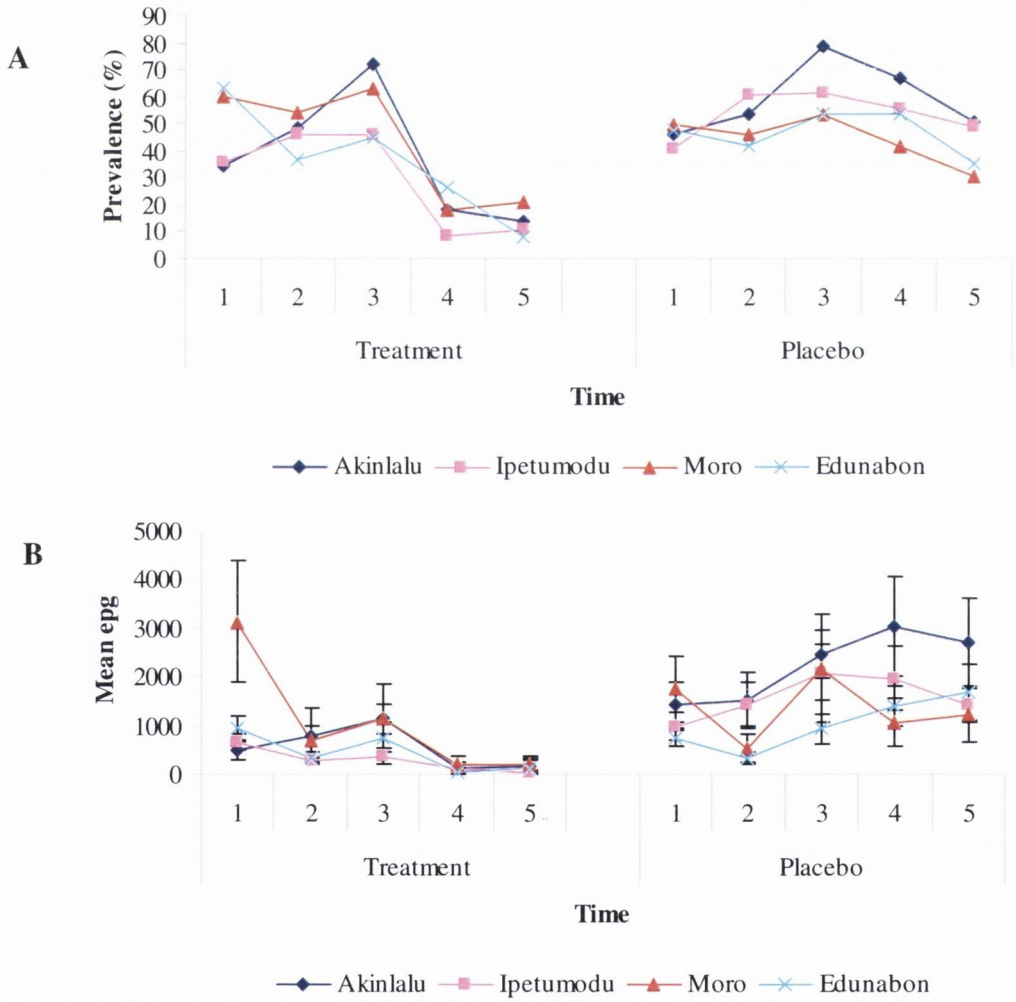


Figure 4.8 (A) Prevalence rates of *A. lumbricoides* and mean epg in treated and placebo groups for each village during the follow-up period. (B) Mean epg ± S.E. 0 = May/June 2006, 1 = Sept./Oct. 2006 2 = Feb. 2007, 3 =May/June 2007, 4 = Aug. 2007

Table 4.4 Test of within-subject effects from rmANOVA analysis on log-transformed epg in treatment and placebo groups over the study period

Source	Df	F	Significance
Time	4	19.946	< 0.001
Time*Group	4	15.258	< 0.001
Time*Village	12	3.321	< 0.001
Time*Sex	4	0.671	0.612
Time*Age	12	2.104	0.014
Time*Group*Village	12	0.311	0.988
Time*Group*Sex	4	0.670	0.613
Time*Village*Sex	12	1.125	0.335
Time*Group*Village*Sex	12	0.995	0.451
Time*Group*Age	12	0.633	0.816
Time*Village*Age	36	1.268	0.135
Time*Group*Village*Age	36	0.970	0.521
Time*Sex*Age	12	0.946	0.499
Time*Group*Sex*Age	12	0.537	0.892
Time*Village*Sex*Age	36	1.510	0.028
Time*Group*Village*Sex*Age	28	0.880	0.647
Error	1304		

Table 4.4 and 4.5 present the results for within-subject effects and between-subject effects, respectively, from the rmANOVA analysis on epg in the treatment and placebo groups over time. The epg varied significantly over time (Table 4.4). This is highlighted in figure 4.6B where mean epg increased at time 2 in the placebo group and mean epg decreased after time 0 in the treatment group. There was a significant time*group interaction, therefore the pattern of epg across time was significantly different for the placebo and treatment group. Mean epg of both groups diverged at time 1 (Figure 4.6B). The villages varied in their epg over time but the time*group*village interaction was not significant. Therefore, the pattern of epg was similar in the villages within the experimental groups over time. Figure 4.8B showed that the mean epg for the villages within the treatment group were similar; although there was more variation in the mean epg for villages within the placebo group. Generally, there was a reduction in mean epg in the villages within the treatment group over time while the mean epg for villages within the placebo group increased over time. The age groups varied in their epg

over time but the time*group*age interaction was not significant. Therefore, the pattern of epg was similar among age categories within the groups over time. The mean epg was similar in the age categories within the treatment group over time with the exception of times 0 and 2 where there was some variation in epg between the age categories (Figure 4.7B). The mean epg showed more variation in the age classes within the placebo group (Figure 4.7B). The age categories within the treatment group showed a reduction in mean epg over time while the mean epg for the age categories within the placebo group increased over time.

Table 4.5 Test of between-subject effects from rmANOVA analysis on epg in treatment and placebo groups over the study period

Source	Df	F	Significance
Group	1	22.314	< 0.001
Village	3	1.730	0.161
Sex	1	7.634	0.006
Age	3	1.352	0.258
Group*Village	3	5.168	0.002
Group*Sex	1	1.058	0.304
Village*Sex	3	0.395	0.757
Group*Village*Sex	3	0.222	0.881
Group*Age	3	0.831	0.477
Village*Age	9	1.062	0.390
Group*Village*Age	9	1.154	0.324
Sex*Age	3	1.007	0.390
Group*Sex*Age	3	0.492	0.688
Village*Sex*Age	9	2.180	0.023
Group*Village*Sex*Age	7	1.023	0.415
Error	326		

There was a vastly significant effect of group so the groups varied in their epg (Table 4.5); mean epg was higher in the placebo group throughout the study period when compared to the treatment group (Figure 4.6). There was also a slightly less significant main effect of sex so the sexes varied in their epg. The mean epg for females decreased at time 1 in the treatment group and remained at low levels throughout the follow-up period when compared to the placebo group where mean epg remained higher with a sharp increase at time 1. In contrast to females, although mean epg for males decreased after time 1 in the treatment group, the mean epg for both treatment and placebo groups does not diverge until time 2 i.e. after three rounds

of treatments. There was no significant main effect of village or age, so the epg did not vary in villages or age groups. The group*village interaction indicates that the groups showed a different pattern of change in epg among villages. The LSD *post-hoc* analysis confirmed that epg was significantly different between Akinlalu and Ipetumodu (Figure 4.8; P = 0.018).

4.3.3 Third data set: children who registered at time 0 and 1 and submitted a stool sample at time points 1, 2, 3 and 4

4.3.3.1 Baseline data

598 children who registered at time 0 and time 1, aged between 12-59 months submitted a stool sample at time points 1, 2, 3 and 4. This sample population examined consisted of 298 males and 300 females, with a mean age of 32.1 months ± S.D. 13.7 months. *A. lumbricoides* was the dominant infection in this population (Table 4.6). 90.9% of *A. lumbricoides* infections were of light intensity and 9.1% were of moderate intensity.

Table 4.6. Prevalence of helminths among the habitants of Akinlalu, Ipetumodu, Moro, and Edunabon, Ile-Ife, Osun State, Nigeria

	% prevalence of infection				
	Overall (N=598)	Akinlalu (N=126)	Ipetumodu (N=260)	Moro (N=77)	Edunabon (N=135)
Ascaris lumbricoides	53 918±2733.2[a]	57.1 1256.5±3669.83	54.2 862.4±2562.3	58.4 1207.6±3201.6	43.7 547.1±1384.2
Trichuris trichiura	2.8[b]	4	1.2		6.7
Hookworm	5.2[b]	9.5	1.5	6.5	7.4
Schistosoma haematobium	0.7[b]	3.2			

[a]Mean eggs per gram of faeces (epg) ± S.D.
[b]Few helminth eggs were counted, hence no intensity is given

There was no difference in the prevalence of *A. lumbricoides* among villages even though there was a lower prevalence in children from Edunabon (Table 4.6; $\chi^2 = 6.625$, d.f. = 1, P = 0.085). Males had a higher prevalence (58.4%) of *A. lumbricoides* than females (47.7%) and this was statistically significant ($\chi^2 = 6.515$, d.f. = 1, P = 0.011). The prevalence of *A. lumbricoides* increased from children aged one year to children aged three years and then decreased thereafter, and prevalence was significantly associated with age (Figure 4.9; $\chi^2 = 9.696$, d.f. = 1, P = 0.021).

Mean epg increased from children aged one year peaking in the three year age category (Figure 4.9; 3-way ANOVA with village, age, and sex as factors, model $R^2_{adj} = 0.041$, main effect of age, $F_{3, 558} = 6.799$, $P < 0.001$). Mean epg was highest in Akinlalu and Moro (Table 4.6; main effect of village, $F_{3, 558} = 3.192$, P = 0.006). There was no significant difference in mean epg between the sexes (main effect of sex, $F_{1, 558} = 2.504$, P = 0.114). The LSD *post-hoc* analysis showed that epg for children aged one year were significantly different from epg of children aged three and four years. The intensity was also significantly different for children

aged two years and children aged three years. In this *post-hoc* analyses mean epg significantly differed between children living in Edunabon and children living in Moro and Akinlalu.

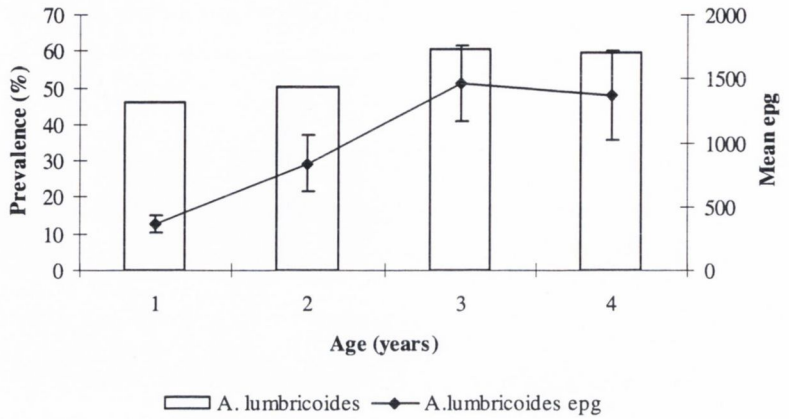


Figure 4.9 The relationship between the prevalence and mean intensity ± S.E of *A. lumbricoides* and age among children aged 1-4 years

Table 4.7 Characteristics of treatment and placebo group at inclusion (before anthelminthic treatment)

	Treatment Group (N = 293)	**Placebo Group (N = 301)**	**P**
Age (months)			
12-23	101	103	0.678[a]
24-35	70	76	
36-47	66	75	
48-59	56	47	
Sex			
Male	151	145	0.412[a]
Female	142	156	
Village			
Akinlalu	59	66	0.354[a]
Ipetumodu	129	130	
Moro	44	32	
Edun-abon	61	73	
Ascaris lumbricoides			
No. infected subjects	151	163	
Prevalence rate	51.5%	54.2%	0.523[a]
Mean epg ± S.E	794.1± 140	1049 ± 174	0.360[b]
SES			
Mean ± S.E	6.15 ± 0.12	6.31± 0.12	0.362[b]

[a] χ^2 test [b] 2-sample t-test

The subjects in the treatment and placebo groups were compared on the basis of age, sex, village, prevalence and intensity of *A. lumbricoides* infection and SES. Table 4.7 shows that, on entering the study, no significant difference was recorded between the groups for any of these variables.

4.3.3.2 Prevalence and intensity of *A. lumbricoides* in the treatment and placebo group

The prevalence of *A. lumbricoides* in the placebo group remained higher than the prevalence in the treatment group throughout the study. From February onwards, after two treatments, there was a steep decrease in prevalence in the treatment group. The decrease in prevalence in the placebo group from February onwards was much less pronounced (Figure 4.10A). Similarly, the mean epg in the placebo group remained persistently higher than the treatment group throughout the follow-up period. However, unlike prevalence, mean epg dropped significantly after one treatment. The placebo group also showed a very slight decrease in epg from February onwards (Figure 4.10B).

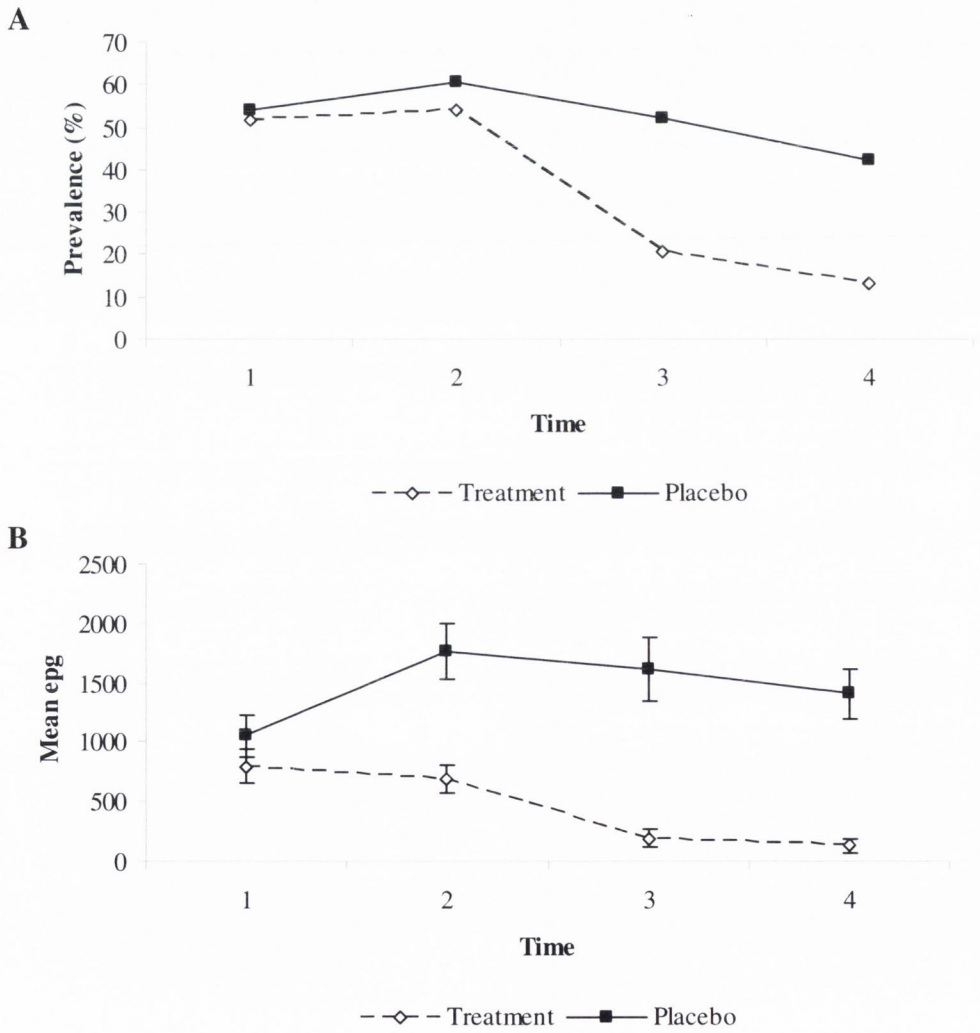


Figure 4.10 (A) Prevalence rates (*A. lumbricoides*) and mean epg in treated and placebo groups during the follow-up period. (B) Mean epg ± S.E. 1 = Sept./Oct. 2006, 2 = Feb. 2007, 3 = May/June 2007, 4 = Aug. 2007.

The prevalence of *A. lumbricoides* showed the same trend in all age categories within the treatment and placebo groups throughout the study period, albeit the prevalence seemed to vary between age classes within the placebo group. Prevalence in the age categories within the placebo group was higher than the age categories within the treatment group at times 3 and 4 (Figure 4.11A). Similarly, the same trend was shown for mean epg in all age classes within the placebo and treatment groups over the follow-up period. However, the mean epg varied more among the age classes within the placebo group. The mean epg was lower for the one and two year age groups than the four and five year age groups (Figure 4.11B).

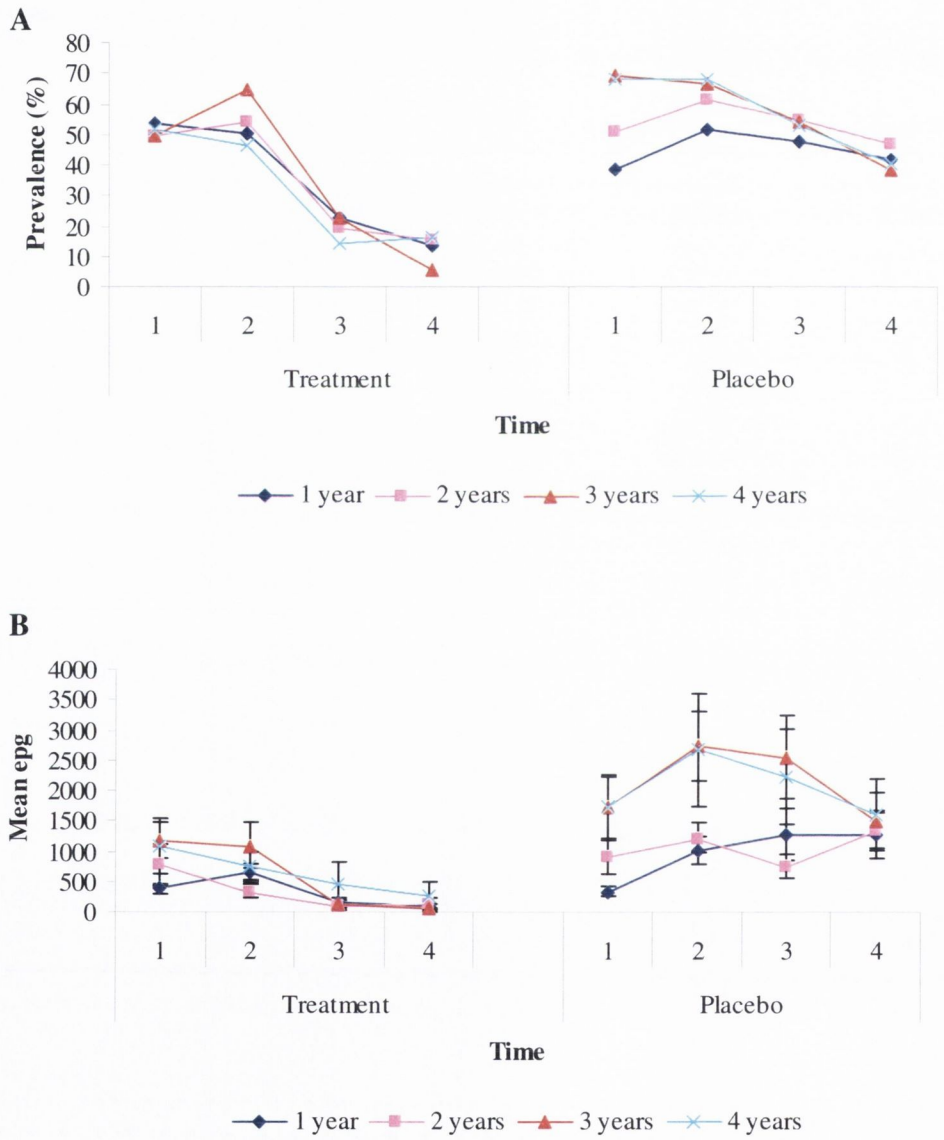


Figure 4.11 (A) Prevalence rates of *A. lumbricoides* and mean epg in treated and placebo groups for each age group during the follow-up period. (B) Mean epg ± S.E. 1 = Sept./Oct. 2006 2 = Feb. 2007, 3 =May/June 2007, 4 = Aug. 2007

The prevalence of *A. lumbricoides* showed the same trend for all villages within both placebo and treatment groups, albeit the prevalence did vary between villages. The prevalence of *A. lumbricoides* in the treatment group was particularly high for Akinlalu compared with the other villages at time point 2. Prevalence in the villages within the placebo group slightly decreased after time 2 but remained higher than the prevalence demonstrated in villages within the treatment group (Figure 4.12A). Similarly the mean epg in all age classes also showed the same pattern over the follow-up period. The mean epg was higher in the placebo group from time 2 onwards.

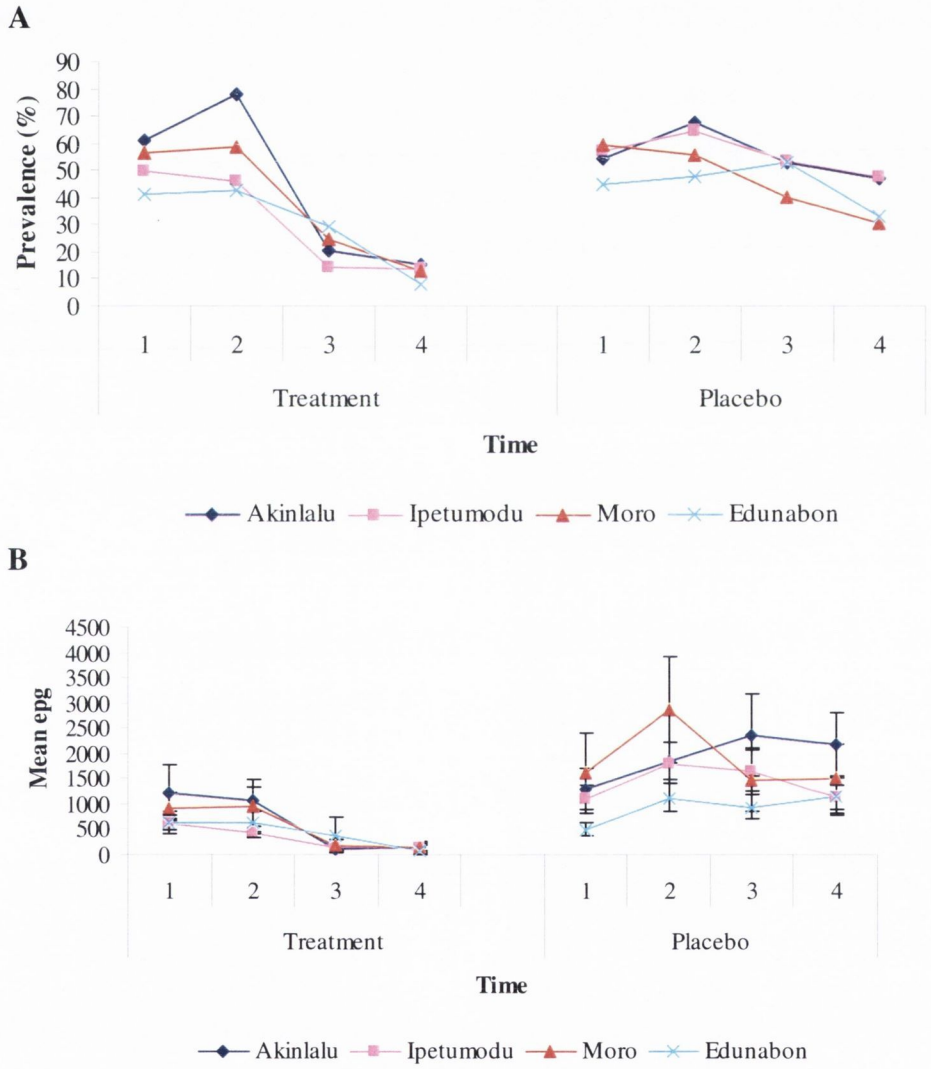


Figure 4.12 (A) Prevalence rates of *A. lumbricoides* and mean epg in treated and placebo groups for each village during the follow-up period. (B) Mean epg ± S.E. 1 = Sept./Oct. 2006 2 = Feb. 2007, 3 =May/June 2007, 4 = Aug. 2007

Tables 4.8 and 4.9 present the results for within-subject effects and between-subject effects, respectively, from the rmANOVA analysis on epg in the treatment and placebo groups over time. Mean epg varied significantly over time (Table 4.8). There was an 84.1% reduction in the mean epg from time 1 to time 4 in the treatment group as compared with a 33% increase in the placebo group (Figure 4.10B). The pattern of epg varied across time and was significantly different among the treatment and placebo groups. The groups diverged in their epg at time 2 (Figure 4.10B). Epg varied among age groups over time. Overall mean epg was lower for children aged one and two when compared to children aged three and four through the study period. There was no significant three-way interaction with time*group*age, therefore the pattern of epg did not vary among age categories within groups over time. The age classes within the treatment group were similar in their mean epg throughout the study while the mean epg varied more in the age classes within the placebo group (Figure 4.11B). The mean epg in the age categories within the treatment group reduced over time while the age categories within the placebo group increased over time.

Table 4.8 Test of within-subject effects from rmANOVA analysis on epg in the treatment and placebo group over the study period

Source	Df	F	Significance
Time	3	46.684	<0.001
Time*Group	3	12.715	<0.001
Time*Village	9	1.564	0.121
Time*Age	9	2.784	0.003
Time*Group*Village	9	0.883	0.540
Time*Group*Age	9	1.349	0.207
Time*Village*Age	27	0.734	0.837
Time*Group*Village*Age	27	1.006	0.455
Error	1683		

There was a vastly significant effect of group, thus the groups varied in epg (Table 4.9); mean epg was higher in the placebo group when compared to the treatment group (Figure 4.10). There was also a significant main effect of village so epg varied among villages; mean epg was generally lower for children in Edunabon in the placebo group when compared to the other villages (Figure 4.12). The main effect of age was slightly less significant so epg varied between age groups; mean epg was higher for children aged three and four years when compared to children aged one and two years in the placebo group (Figure 4.11).

The LSD *post-hoc* analysis showed that epg was significantly different for children aged one year and children aged three (P<0.001) and four years (P = 0.037). The intensity was also significantly different between Akinlalu and Ipetumodu (P=0.006), Edunabon (P<0.001) and Moro (P=0.032).

Table 4.9 Test of between-subject effects from rmANOVA analysis on epg in the treatment and placebo group over the study period

Source	Df	F	Significance
Group	1	60.442	P<0.001
Village	3	7.452	P<0.001
Age	3	3.195	0.023
Group*Village	3	1.471	0.221
Group*Age	3	1.162	0.323
Village*Age	9	1.887	0.051
Group*Village*Age	9	1.253	0.260
Error	562		

4.3.4. Fourth data set: Children who were recruited at time 1 and submitted stool samples at time points 1, 2, 3 and 4

4.3.4.1 Baseline data

235 children aged between 12-59 months recruited in Sept./Oct. submitted a stool sample at time points 1, 2, 3 and 4. This sample population examined consisted of 118 males and 117 females, with a mean age of 29.3 months ± S.D. 15.2 months.

Table 4.10 Prevalence of helminths among the habitants of Akinlalu, Ipetumodu, Moro, and Edunabon, Ile-Ife, Osun State, Nigeria

	% prevalence of infection				
	Overall (N=235)	Akinlalu (N=49)	Ipetumodu (N=111)	Moro (N=23)	Edunabon (N=52)
Ascaris lumbricoides	57 1144.5±2845.8[a]	61.2 1405.8±511.2	55 856.2±1984.7	73.9 2577.3±5206.4	50 879.8±1916.9
Trichuris trichiura	3.8[b]	6.1	1.8	0	7.7
Hookworm	6.8[b]	8.2	1.8	8.7	1.5
Schistosoma haematobium	1.7[b]	8.2			

[a]Mean eggs per gram of faeces (epg) ± S.D.
[b]Few helminth eggs were counted, hence no intensity is given

A. lumbricoides was the dominant infection (Table 4.10). 88.8% of *A. lumbricoides* infections were of light intensity and 11.2% were of moderate intensity. The prevalence of *A. lumbricoides* increased slightly from children aged one year and peaked in children aged four years and this was statistically significant (Figure 4.13; χ^2 = 10.040, d.f. = 3, P = 0.018). Males had a higher prevalence (66.1%) than females (47.9%) and this was also statistically significant (χ^2 = 7.906, d.f. = 1, P = 0.005). No significant difference was detected for the prevalence of *A. lumbricoides* among villages (χ^2 = 4.271, d.f. = 3, P = 0.234).

Mean epg increased steadily from children aged one year to children aged four years and this was statistically significant (Figure 4.13; 3-way ANOVA with village, age and sex as factors, model R^2_{adj} = 0.086, main effect of age, $F_{3, 200,}$ = 4.379, P = 0.005). Even though Moro had a much higher mean epg than the other villages this was not statistically significant (main effect of village, $F_{3, 200,}$ = 0.676, P = 0.568). There was also no significant difference in mean epg between sexes (main effect of sex, $F_{1, 200,}$ = 2.504, P = 0.078). The *post-hoc* analysis showed that epg was significantly different for children aged one year and children in the three (P = 0.005) and four year category (P<0.001).

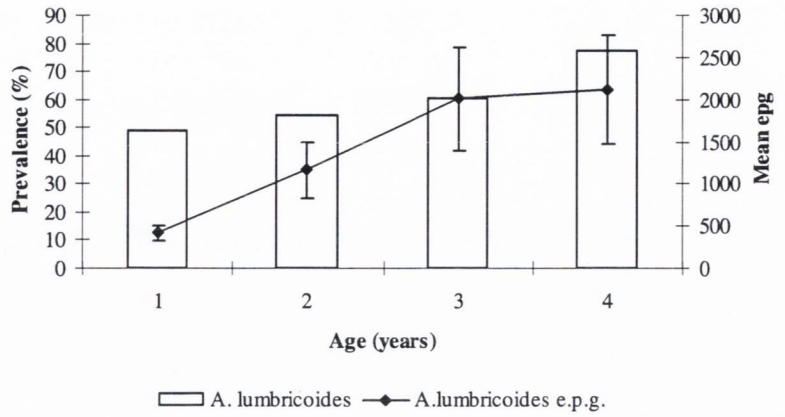


Figure 4.13 The relationship between the prevalence and mean intensity ± S.E of *A. lumbricoides* and age among children aged 1-4 years

Table 4.11 Characteristics of treatment and placebo group at inclusion (before antihelminthic treatment)

	Treatment Group (N =112)	Placebo Group (N = 119)	P
Age (months)			
12-23	51	61	0.711[a]
24-35	18	16	
36-47	25	21	
48-59	18	21	
Sex			
Male	55	61	0.744[a]
Female	57	58	
Village			
Akinlalu	21	27	0.687[a]
Ipetumodu	54	56	
Moro	13	9	
Edun-abon	24	27	
Ascaris lumbricoides			
No. infected subjects	66	65	
Prevalence rate	58.9%	54.6%	0.509[a]
Mean epg ± S.E.	1297 ± 282	1031.5 ± 252	0.620[b]
SES			
Mean ± S.E.	6.25 ± 0.20	6.28 ± 0.18	0.923[b]

[a] χ^2 test
[b] 2-sample t-test

The subjects in the treatment and placebo groups were compared on the basis of age, sex, village, prevalence and intensity of *A. lumbricoides* infection, and SES. Table 4.11 shows that, on entering the study, no significant difference was recorded between the groups for any of these variables.

4.3.4.2 Prevalence and intensity of *A. lumbricoides* in the treatment and placebo group

The prevalence of *A. lumbricoides* was lower in the treatment group when compared to the placebo group after two treatments (Figure 4.14A). Unlike prevalence, the mean epg showed a more pronounced drop in the treatment groups after the first treatment. The mean epg remained higher in the placebo group when compared to the treatment group after time 1 (Figure 4.14B).

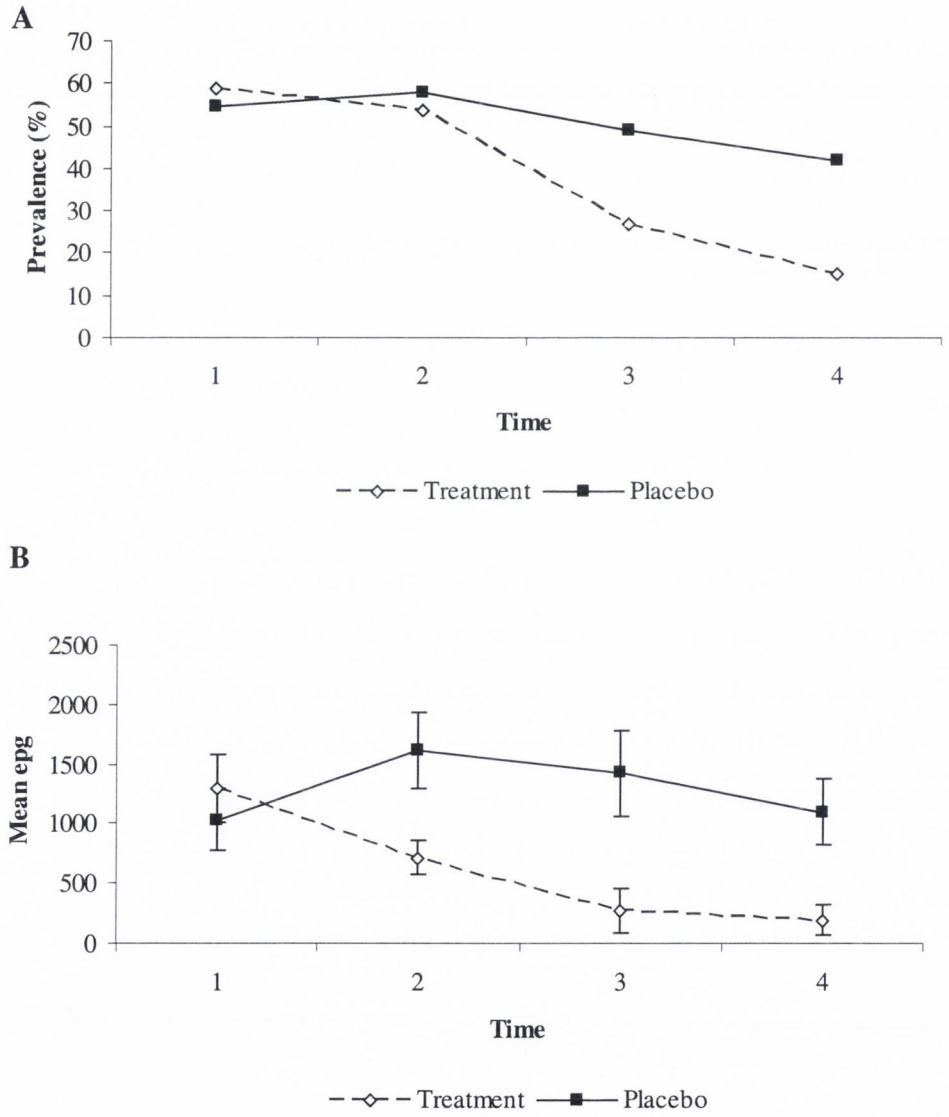


Figure 4.14 (A) Prevalence rates (*A. lumbricoides*) and mean epg in treated and placebo groups during the follow-up period. (B) Mean epg ± S.E.. 1 = Sept./Oct. 2006, 2 = Feb. 2007, 3 = May/June 2007, 4 = Aug. 2007.

The prevalence of *A. lumbricoides* showed the same pattern for all age groups over time within each group (Figure 4.15A). The prevalence varied more among age classes within the placebo group being lowest for the one year age and highest for the four year age group at time 1. The mean epg varied considerably for all age classes within the placebo group over time with the three year age category having the highest intensity (Figure 4.15B). There was a drop in the mean intensity for the two year age group at time 3. The mean epg for all age classes within the treatment group followed the same general trend, although the mean epg for the four year age category increased at time 3 and remained slightly higher than the other age classes within the treatment group (Figure 4.15B).

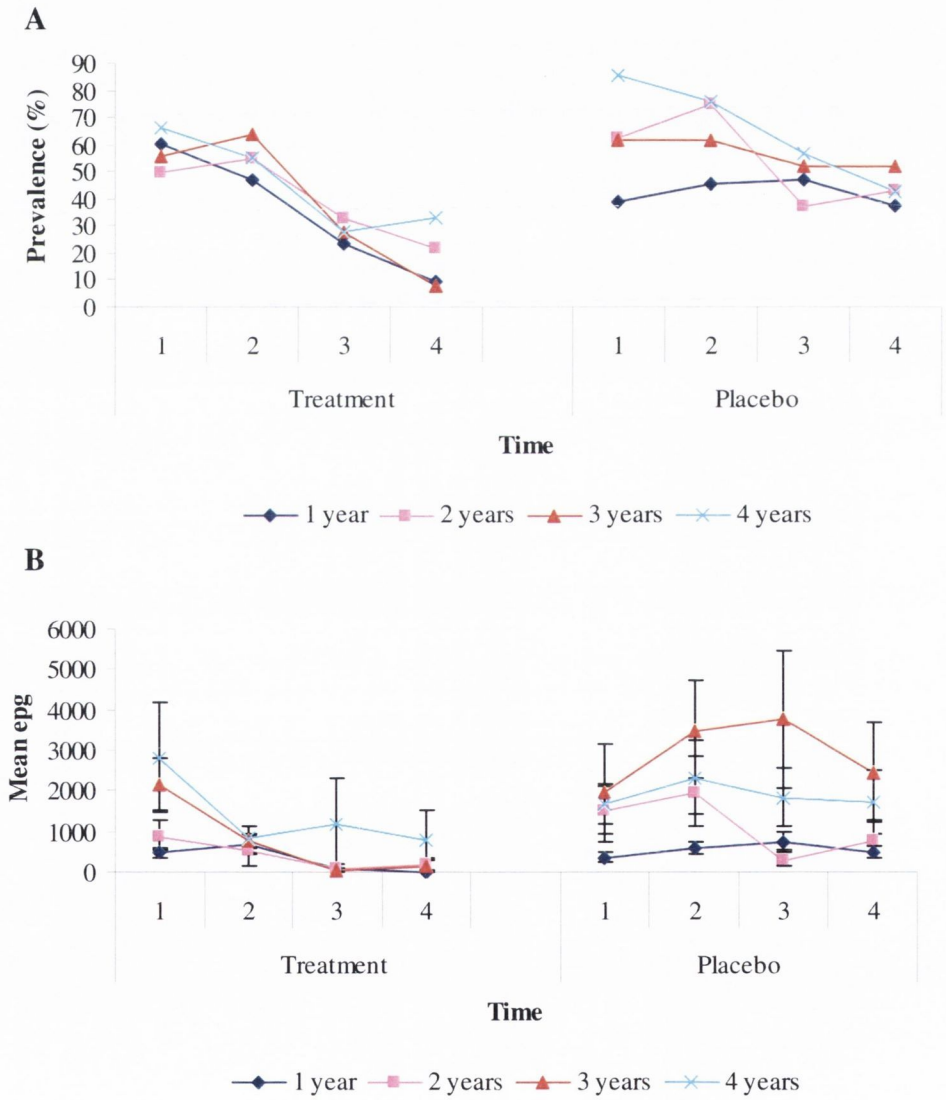


Figure 4.15. (A) Prevalence rates of *A. lumbricoides* and mean epg in treated and placebo groups for each age group during the follow-up period. (B) Mean epg ± S.E. 1 = Sept./Oct. 2006 2 = Feb. 2007, 3 =May/June 2007, 4 = Aug. 2007

The prevalence of *A. lumbricoides* generally followed the same pattern over time for all villages within placebo and treatment groups (Figure 4.16A). The prevalence in Akinlalu in the treatment group peaked at time 2. The mean epg showed the same trend for all villages within placebo and treatment groups over the study period (Figure 14.6B). However, the mean epg is particularly high for Moro at times 1 and 2 in the placebo group.

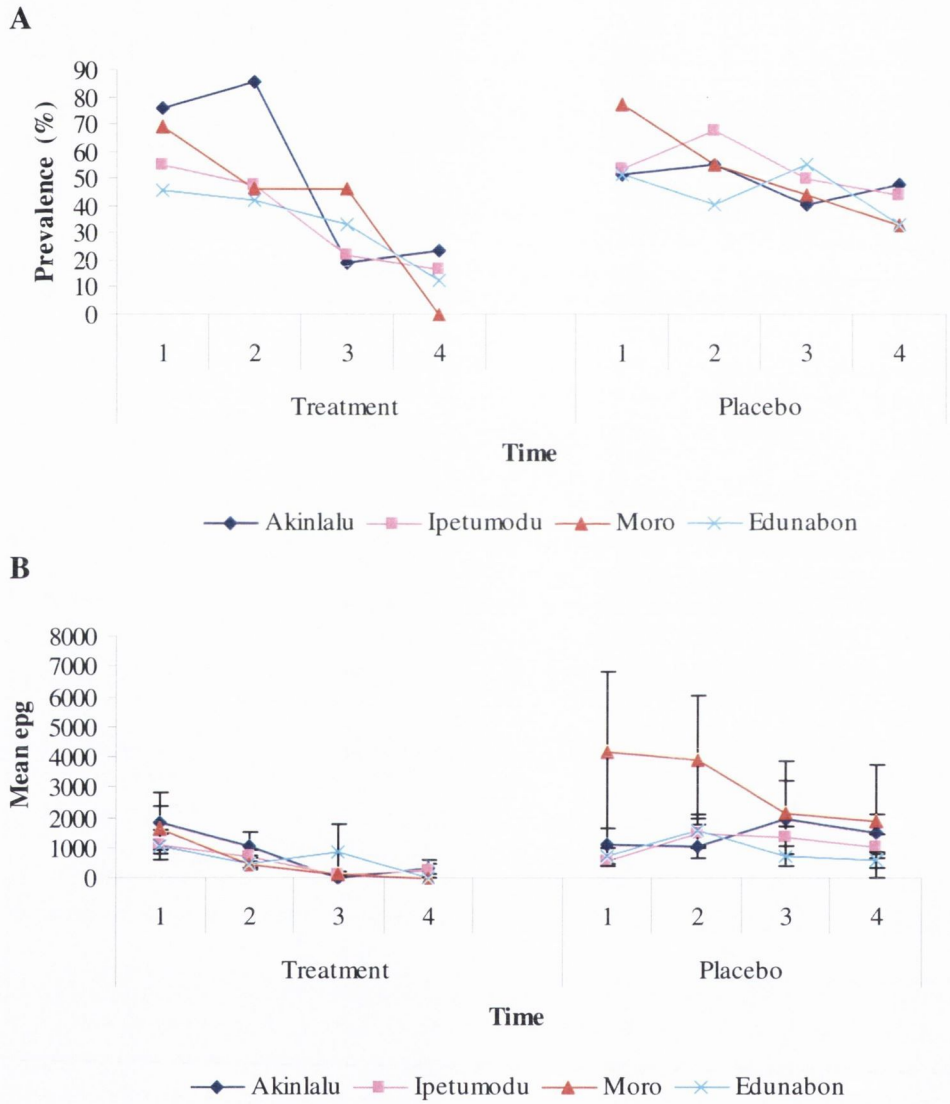


Figure 4.16 (A) Prevalence rates of *A. lumbricoides* and mean epg in treated and placebo groups for each village during the follow-up period. (B) Mean epg ± S.E.. 1 = Sept./Oct. 2006 2 = Feb. 2007, 3 =May/June 2007, 4 = Aug. 2007

Tables 4.12 and 4.13 present the results for within-subject effects and between-subject effects, respectively, from the rmANOVA analysis on epg in the treatment and placebo groups over time. The intensity of *A. lumbricoides* infection varied significantly over time (Table 4.12). By time 4, there was an 85.3% reduction in epg in the treatment group as opposed to a 6.4% increase in the placebo group. There was a more pronounced increase (55.7%) in epg in the placebo group from times 1 to 2. There was a significant time*group interaction, therefore the pattern of epg was significantly different for the treatment and placebo groups over time.

Table 4.12 Test of within-subject effects from rmANOVA analysis on epg in the treatment and placebo group over the study period

Source	Df	F	Significance
Time	3	23.392	P<0.001
Time*Group	3	3.185	0.026
Time*Age	8	0.835	0.578
Time*Group*Age	8	1.399	0.189
Error	630		

There was a vastly significant effect of group so the groups varied in their epg (Table 4.13); Mean epg was higher in the placebo group throughout the study period when compared to the treatment group (Figure 4.14). At the end of the study period, the mean epg in the placebo group (1098) was much higher than the treatment group (189). There was also a significant main effect of age so the epg varied among age groups; mean epg was higher in children aged three and four years when compared to children aged one and two years (Figure 4.15). There was no significant group*age interaction, so both groups showed the same pattern of change in epg over age groups, albeit at different levels of infection.

In the LSD post hoc analysis there was a statistically significant difference between the children age one year and three (P=0.003) and four years (P<0.001). There was also a statistically significant difference between children aged two and four years (P=0.043).

Table 4.13 Test of between-subject effects from rmANOVA analysis on epg in the treatment and placebo group over the study period

Source	Df	F	Significance
Group	1	21.567	P<0.001
Age	3	7.316	P<0.001
Group*Age	3	0.75	0.523
Error	223		

4.4 Discussion

Most longitudinal studies investigating the effects of anthelminthic treatment on intestinal helminths centre on the community and school-age children (Seo and Chai, 1980; Thein-Hlaing *et al.*, 1984; Asaolu *et al.*, 1991; Thein-Hlaing *et al.*, 1991; Chan *et al.*, 1992; Hall *et al.*, 1992; Holland *et al.*, 1996a; Zani *et al.*, 2004; de Rochars *et al.*, 2004; Saathoff *et al.*, 2005). In the studies that concern solely preschool children, the focus has been the impact of anthelminthic treatment on growth, nutritional status and cognitive development, not the effect of anthelminthic treatment on reducing the prevalence and intensity of helminth infections (Gupta and Urrutia, 1982; Rousham and Mascie-Taylor, 1994; Northtrop-Cleves *et al.*, 2001; Stoltzfus *et al.*, 2001; 2004). These studies on preschool children have used two- and three-monthly repeated anthelminthic treatments with mebendazole, piperazine, and metronidazole. The present study showed that repeated four-monthly anthelminthic treatments were successful in reducing prevalence and intensity of *A. lumbricoides* infections in children aged 1-4 years. At the end of the follow-up period, the prevalence and intensity of *Ascaris* in the treatment and placebo groups was 14.2% and 147 mean epg and 43.2% and 1460 mean epg respectively, at the population level (first data set).

The compliance varied significantly among villages, being higher for Akinlalu. In comparison to the other villages, Akinlalu is less widely dispersed, which might explain why the inhabitants of this village complied more with stool samples at all time points. Despite this, there was no significant difference in the characteristics of subjects between the treatment and placebo groups at inclusion into the study and therefore the varying compliance between villages should not have any important implications for the results presented here.

A. lumbricoides was the predominant geohelminth infection in children aged 1-4 years (47.7%; first data set). Lower prevalences of *T. trichiura* (3.7%), hookworm (4.3%) and *S. haematobium* (1.1%) were detected in this population. The prevalence of *A. lumbricoides* was significantly associated with age; children aged 1 year had lower prevalences than older children. Maximum prevalence values for *A. lumbricoides* are usually observed when children are 5-10 years old (Crompton, 1994). A previous study in this region of Nigeria showed the prevalence of *A. lumbricoides* to be 88.5% in school children aged 5-15 years (Holland *et al.*,

1989). Nevertheless, higher prevalences of *Ascaris* in studies on children aged 0-48 months, have been found in China (80%) (Yu *et al.*, 1989), the Philippines (77%) (Cabrera *et al.*, 1989), and Sri Lanka (62%) (Ismail *et al.*, 1993). Prevalence can vary geographically, lower prevalences have been found in children aged 12-48 months in Zanzibar (41.5%) (Stoltzfus *et al.*, 2001), and children aged 12-47 months in Nigeria (20.5%) (Asaolu *et al.*, 2002).

The prevalence of *A. lumbricoides* peaked in February (dry season) for both treatment and placebo groups. This is a curious finding as *Ascaris* infection is associated with climate, having a low prevalence in dry areas and high prevalence in wet tropical climates (Ratard *et al.*, 1991). Seasonal changes in transmission of *A. lumbricoides* have previously been recorded (Chia-Tung Pan *et al*, 1954; Seo *et al.*, 1979). Since *Ascaris* eggs also occur in the air and household dust, it is possible that eggs could be inhaled or swallowed (Crompton, 1994). The risk of swallowing eggs may be increased during Harmattan, north-easterly winds blowing south occurring from December to February bringing higher temperatures, lower humidity and dust-laden air. Seo *et al.* (1979) conducted a study of reinfection with *A. lumbricoides* in rural people in South Korea following mass chemotherapy. In this study, peaks in prevalence were related to earlier periods of seasonal peak transmission. Seo and colleagues stated that the seasonal effect may be indirect; seasonal transmission could have been associated with the traditional consumption of pickles at certain times in the year, which were a likely source of infection.

The intensity of *A. lumbricoides* infections in this study was predominately light (86%), and only a few children harboured moderate intensity infections (14%). The intensity of infection increased with age, children aged 1 year had a lower mean epg than older children. Children aged 5-15 years usually have heavier worm burdens (O'Lorcain and Holland, 2000). A Nigerian study that examined strategies for community control of *Ascaris*, showed that intensity peaked in children aged 10-14 years (mean epg >20,000) and declined thereafter in older children and adults (mean epg <10,000) (Asaolu *et al.*, 1991). In the present study, although males were found to have a higher prevalence of *Ascaris* (third and fourth data set), females were more likely to have higher mean epg. Other studies have also demonstrated that females have heavier worm burdens (Arfaa and Ghadirian, 1977; Holland *et al.*, 1989; Kightlinger *et al.*, 1998) but the cause of this is as yet unknown (Crompton, 1994) and could be related to behavioural differences between males and females. A study in South African

school children showed that female children were more likely to be infected with *Ascaris* and also had a higher incidence of soil-eating than males (Saathoff *et al.*, 2002).

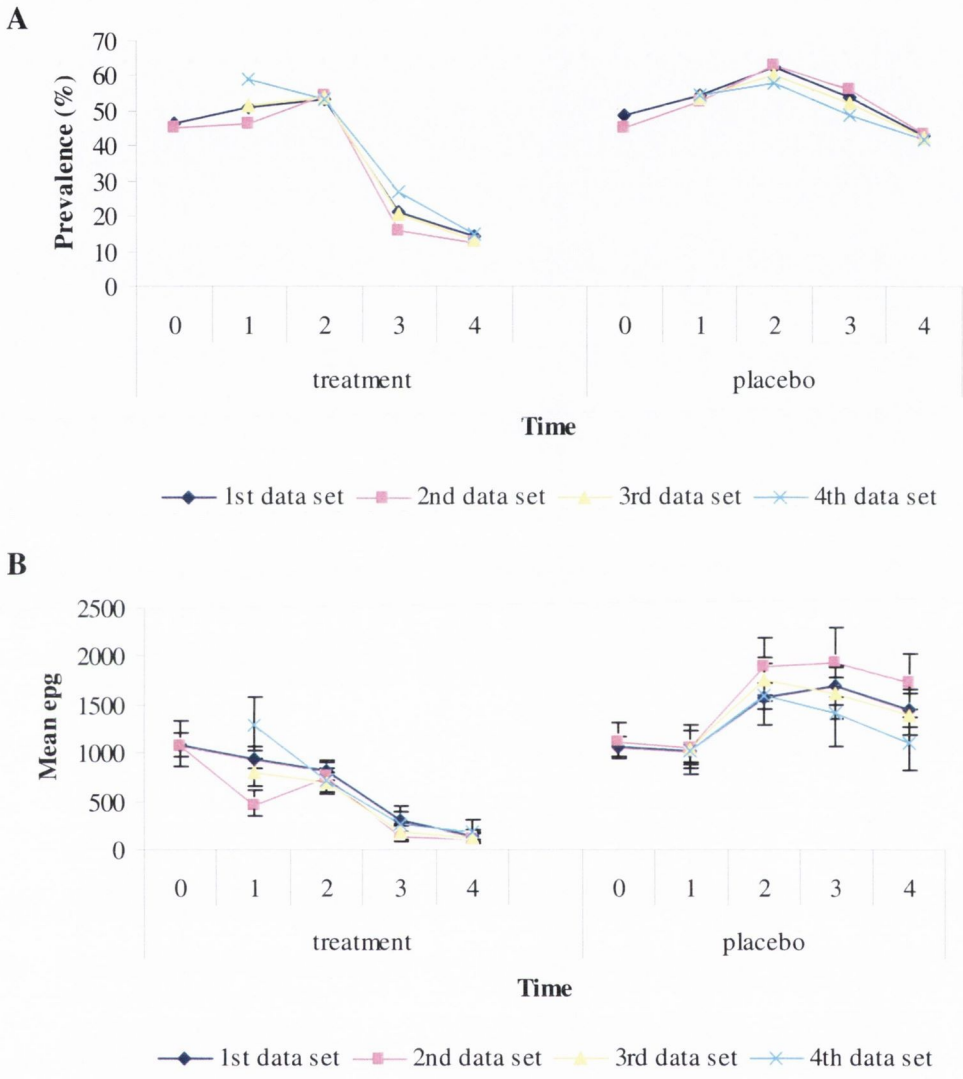


Figure 4.17 (A) Prevalence rates of *A. lumbricoides* and mean epg in treated and placebo groups for data set during the follow-up period. (B) Mean epg. 1 = Sept./Oct. '06 2 = Feb. '07, 3 =May/June '07, 4 = Aug. '07

Four-monthly repeated anthelminthic treatments were successful in reducing the prevalence of *Ascaris*. In all data sets, the difference in the prevalence between treatment and placebo groups at the end of the follow-up ranged from 29% to 30.9% (Figure 4.17A). The first data set shows that although most of the subjects only received treatments on one or more

occasions throughout the study period, the reduction in prevalence was similar to that of other subjects who received anthelminthic treatment on all occasions (second, third, and fourth data set). Interestingly, in the first and second data set, the prevalence of *A. lumbricoides* in the treatment group dropped after three rounds of anthelminthic treatment, where as in the third and fourth data set, the prevalence dropped after only two rounds of anthelminthic treatment when compared to the placebo groups. This result might have been expected in the third data set as most of the children (64.7%) would have already received one round of anthelminthic treatment (at time 0), however, the same pattern in the drop of prevalence after two rounds of treatment ensued for the fourth data set; all of these children were recruited at time 1 and received no prior anthelminthic treatment in the study. Thus, the seasonal transmission of *Ascaris* in February could have masked the effect of anthelminthic treatment on prevalence in the first and second data set. It is possible that conditions for embryonation were optimal after the cessation of the rainy season in October, increasing transmission thereafter. Therefore despite a reduction in intensity, the prevalence of infection remained high because of increased transmission.

Four-monthly anthelminthic treatments significantly reduced the intensity of *A. lumbricoides* infections in the treatment group when compared to the placebo group. The rmANOVA analysis on epg in the second, third, and fourth data set showed that there was a statistically significant difference in epg between treatment and placebo groups, irrespective of varying sample size in each data set. Similar to prevalence, the first data set shows that although most of the subjects only received treatments on one or more occasions throughout the study period, the reduction in intensity was similar to that of other subjects who received anthelminthic treatment on all occasions (second, third, and fourth data set). Treating children who complied at all time points may have reduced the mean epg in the overall population. Holland and colleagues (1996a) showed that four-monthly targeted chemotherapy of children aged 5-15 years, was found to be effective in the treated children but also caused a reduction in intensity of *Ascaris* infections in untreated adults. Unlike prevalence, the intensity of *A. lumbricoides* infections dropped after two rounds of treatment in the first data set whereas in the second and subsequent data sets the drop in intensity occurs after one round of treatment (Figure 4.17B). The second recruitment of new study participants, who had not received treatment before, may have increased the mean epg in the sample population in Sept./Oct. 2006. This may explain why there is no drop in intensity after the first round of treatment in

the first data set. Prevalence is regarded as a relatively insensitive measure of reinfection because of the aggregated distribution of worms per child (Holland *et al.*, 1989). It has been well documented that marked changes in intensity may be accompanied by only small changes in prevalence (Anderson and May, 1985; Thein-Hlaing *et al.*, 1987; Chan *et al.*, 1992).

Holland *et al.*, (1996a) showed that four-monthly anthelminthic treatments with levamisole were better than six-monthly or one-yearly treatments in reducing prevalence and intensity of *A. lumbricoides* infection in children aged 5-15 years. The prevalence fell from 71% to 17% among treated children. The drop in prevalence (30.9%) of *Ascaris* between the treatment and placebo groups at the end of the follow-up (second data set), is lower to the result shown by Holland *et al.* (1996a). The significant reduction of mean epg (93%) between treatment and placebo groups at the end of the study period was also comparable to the reduction of *Ascaris* intensity shown in Holland *et al.*'s study (97%; taken from graph). A study in an urban community in Malaysia showed that 6-monthly treatments with albendazole were effective in reducing prevalence and intensity of *A. lumbricoides* infection (Chan *et al.*, 1992). After 12 months (two 6-monthly treatments) the prevalence was reduced by 12% in treated children aged 2-6 years (prevalences were taken from a graph in Chan *et al.* (1992). Therefore, four-monthly treatments are better in reducing prevalence of *Ascaris* in a similar age group. Mean worm burden was reduced from 3.2 to 1.9 worms in the 2-6 year olds. Since the analysis for this present study is based on an indirect measure of worm burden, epg, it is difficult to compare these results.

A study examining the effect of iron supplementation and anthelminthic treatment on motor and language development of preschool children aged 0.5 -5 years in Zanzibar used repeated three-monthly treatments with mebendazole (Stoltzfus *et al.*, 2001). After 12 months, prevalence of *Ascaris* in the treatment group was reduced by 12.7% and prevalence in the placebo group increased by 5.4%. Results from the present study showed that four-monthly treatments with albendazole reduced the prevalence of *A. lumbricoides* by 34% in the treatment group after 12 months and thus was more effective than three-monthly treatments with mebendazole. Three-monthly treatments significantly reduced *Ascaris* intensity (Stoltzfus *et al.*, 2001); comparing these data with results presented here is difficult as the intensity data is presented in geometric means. In contrast to the work carried out by Stoltzfus *et al.* (2001) a study by Thein-Hlaing *et al.* (1991) showed that three-monthly anthelminthic

treatments were better than four-monthly treatments at reducing the prevalence of *A. lumbricoides*, however this Burmese study was undertaken in children and adults. The pre-treatment prevalences for all ages and children under 15 years were 83.6% and 77.1%, respectively. After two years of three-monthly treatments the corresponding prevalences fell to 21% and 5% respectively.

In contrast to the results presented here, a study in 117 Bangladeshi preschool children aged 2-6 years showed that two-monthly anthelminthic treatments were better than four-monthly treatments in reducing the prevalence of *A. lumbricoides* infections (Northrop-Clewes *et al.*, 2001). After one round of treatment, prevalence in the treatment group dropped from 78% to 8% and remained high in the placebo group increasing from 71% to 74%. After 12 months, the mean worm burden in the placebo group was 4.1 (maximum: 40) for infected children. An examination of the nine infected children in the treatment group, yielded five children with small immature worms and four children with one adult worm. Another study in Bangladesh, used two-monthly repeated treatments with albendazole in 1402 children aged 2-6 years old (Rousham and Mascie-Taylor, 1994). When assessing the effectiveness of anthelminthic treatment on prevalence of STH infections, a random sub-sample of children were screened eight months after the initial treatment. Prevalence of *Ascaris* in the treatment group fell by 73% while prevalence in the placebo group fell by 2%. Similarly, a study in Madagascar, examining helminth-malaria co-infections in children and adults used two-monthly treatments with levamisole (Brutus *et al.*, 2006). Prevalence and intensity of *A. lumbricoides* collapsed immediately in the treatment group after the first round of treatment. Prevalence and intensity remained high in the placebo group throughout the study period. After 12 months of treatment there was a slight peak in the prevalence and intensity of *Ascaris*, which was thought to be due to seasonal transmission. Nevertheless, shorter treatment intervals would not have been feasible for this present study and may have had a further negative impact on compliance.

Results from this study show that *A. lumbricoides* was the most prevalent geohelminth in this particular region of Nigeria, infecting 47.7% of children aged 1-4 years. While shorter deworming treatment intervals of two and three months are better than four-monthly intervals at reducing prevalence and intensity of *Ascaris*, our results demonstrate that deworming children at four-monthly intervals proves just as effective in the immediate reduction of intensity and prevalence after two rounds of anthelminthic treatment. Given the difference in

the prevalence and intensity of *A. lumbricoides* between treatment and placebo groups, and the high endemicity of *A. lumbricoides*, these children represent a formidable cohort to investigate the relationship between helminth-malaria co-infections.

CHAPTER 5: The effect of anthelminthic treatment on *Plasmodium* spp. infections in children aged 1-4 years

Figure **Page**

Table **Page**

5.1 INTRODUCTION

Multiple parasitic species infections are common in nature (McKenzie, 2005), particularly in developing countries where overall rates of parasitism are high (Petney and Andrews, 1998; Tchuem Tchuente *et al.*, 2003). Despite the widespread acceptance that hosts can harbour a number of parasitic species, and that these species can, and do, interact with one another directly or indirectly (Christensen *et al.* 1987), parasitologists seldom consider more than one single organism that directly concerns them (Cox, 2001). Nevertheless, because of mounting evidence, in animal and human studies, demonstrating that concurrent infections by two or more parasite species can affect the pathogenesis of each other (Cox, 2001; Fincham *et al.*, 2003; Brutus *et al.*, 2006), more studies are needed to investigate the interactions in co-infections, particularly since the implications of co-infections on host morbidity could be quite substantial (Brooker *et al.*, 2007).

Across Africa, soil-transmitted helminths (STH: *Ascaris lumbricoides*, *Trichuris trichiura*, and hookworms) share the same spatial distribution as *Plasmodium falciparum* (Brooker *et al.*, 2007) and this results in high rates of co-infection (Petney and Andrews, 1998). Over 30 years ago, studies by Murray *et al.* (1977; 1978) suggested that infection with *A. lumbricoides* was associated with the suppression of malaria symptoms and that anthelminthic treatment led to a recrudescence of malaria. Little interest in the interaction between parasite species has been shown since then until recently. Animal models have explored the interactions between helminths and malaria and produced conflicting results (Helmby *et al.*, 1998; Yoshida *et al.*, 2000). It has been hypothesised that helminth infections may alter susceptibility to clinical malaria (Druilhe *et al.*, 2005) and studies are now trying to elucidate the consequences of co-infection in humans (Nacher *et al.*, 2000; Spiegel *et al.*, 2003; Briand *et al.*, 2005; Brutus *et al.*, 2006; review by Mwangi *et al.*, 2006).

Studies examining the associations between helminths and malaria among adults admitted to the Hospital of Tropical Diseases in Bangkok, Thailand, have suggested that helminths protect against severe *P. falciparum* malaria (Nacher *et al.*, 2000; 2001c; 2002a). In contrast, studies in Senegal investigating associations between helminth infection and clinical malaria or *P. falciparum* infections among children of different age groups have shown that helminths

increase the risk of clinical malaria (Spiegel *et al.*, 2003) and that *A. lumbricoides* increased the risk of severe malaria (Le Hesran *et al.*, 2004). Studies comparing the relationship between schistosomes and *P. falciparum* infections have found that the incidence of malaria was significantly higher in children infected with *Schistosoma mansoni* than those who were uninfected (Sokhna *et al.*, 2004). In contrast Lyke *et al.* (2005) reported that *S. haematobium* was protective against clinical malaria. Most of these studies are observational, i.e. they do not demonstrate causality, except for the intervention study by Murray *et al.* (1978), that had its limitations owing to the small sample size, short follow-up (20 days) and the fact that the study was undertaken among malnourished individuals (Mwangi *et al.*, 2006). To date, there is only one other published intervention study by Brutus *et al.* (2006) which showed that subjects, more than five years of age, infected with *A. lumbricoides* and treated with levamisole had a significant increase in their *P. falciparum* densities compared with controls. There was no effect of anthelminthic treatment on children aged six months to four years. Brutus and colleagues used levamisole as their anthelminthic which is well recognised as having immunomodulatory properties (review by Sajid *et al.*, 2005) and therefore the data should be interpreted with caution.

Observational studies are subject to unmeasured bias such as temporal associations, and more well designed intervention studies are needed to provide robust epidemiological evidence of the interactions between helminth infections and malaria (Mwangi *et al.* 2006). The high endemicity of *Plasmodium*, particularly *P. falciparum* (Cooper *et. al.*, 2002), and *A. lumbricoides* (Holland *et al.*, 1989; 1996a) in Ile-Ife, Osun state, West Nigeria makes this location an ideal setting for an intervention trial of this nature. A double-blind placebo-controlled randomised trial of four-monthly anthelminthic treatment, with albendazole, was conducted from May 2006 to August 2007. To our knowledge, this is the first intervention trial to focus solely on children aged 1-4 years. The main objective of this chapter was to evaluate the effect of *Ascaris*-reduced prevalence and intensity on the prevalence of uncomplicated malaria and malaria attacks in children of a high risk age group.

5.2 MATERIALS AND METHODS

See Chapter 2 for a description of the study location (2.1.1), study design (2.1.3.1), and general materials and methods (2.1.3).

5.2.1 Malaria diagnosis

Malaria was diagnosed using Parascreen rapid diagnostic tests (RDTs; Zephyr Biomedicals, Verna Industrial Estate, Verna Goa, India; section 5.2.1.1) and peripheral thick and thin blood smears. After the initial field work was completed in May/June 2006 (time 0) blood smears were sent to the College of Medicine, University of Ibadan, Ibadan, Oyo state, Nigeria to be analysed for malaria parasitaemia including *P. falciparum* asexual and gametocyte density, *P. malariae*, *P. ovale*, and *P. vivax*. This analysis was not conducted as requested; instead the blood smears were read for malaria parasite density assuming predominance of *P. falciparum* species.

The blood smears made at time 0 were then sent to the Kenya Medical Research Institute (KEMRI), Nairobi, Kenya to be reread as originally requested. Similar problems were encountered with the KEMRI laboratory as parasite density was read for *P. falciparum* single species infections only. In addition to this, the analysis from KEMRI diagnosed many of the blood smears with *P. vivax* - a result which was queried given the rarity of *P. vivax* in West Africa. In West Africa, indigenous populations rarely have the Duffy group antigen (which *P. vivax* needs to gain entry into the host's red blood cells) on their red cell surface. This genetic variance accounts for the very low percentage of *P. vivax* found in West Africa (Summer *et al.*, 2005). For quality control purposes, a small number of slides from time zero were analysed blind for malaria parasites by another Kenyan laboratory (AMREF). In addition to this, polymerase chain reaction (PCR) assay was used to identify *Plasmodium* species using blood spotted onto filter papers (Chapter 2 section 2.1.3.8) for the same samples from time 0. The PCR analysis was carried out by the division of Infection and Immunity, University of Glasgow, United Kingdom. A comparison of the PCR and Kenyan laboratories results is provided in section 5.3.1. Although microscopy is the gold standard diagnostic test for

malaria, the analysis in this chapter will be based upon the results from the RDTs owing to the continued prolonged delay in attaining satisfactory results from the blood smears.

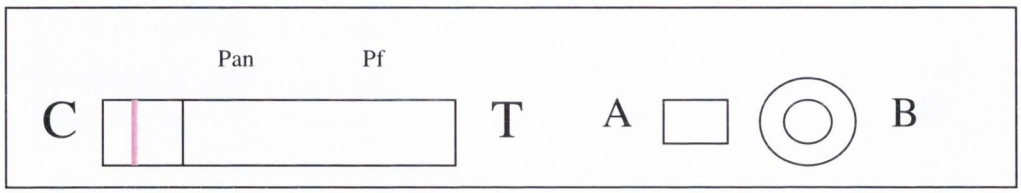
5.2.1.1 Parascreen RDT

The Parascreen RDT is based on the detection of the *P. falciparum* specific histidine-rich protein-2 (HRP-2) and other *plasmodium* species (PAN)-specific parasite lactate dehydrogenase (pLDH) (Moody, 2002). Parascreen utilises the principle of immunochromatography. HRP-2 is a water soluble, heat-stable antigen that is present in the cytoplasm and membrane of infected erythrocytes, and is also produced by young (but not mature) gametocytes of *P. falciparum*. pLDH is an enzyme in the glycolytic pathway of malaria parasites and is abundant and soluble in the cytoplasm of the parasite (Bell *et al.*, 2006). pLDH is also found in mature gametocytes.

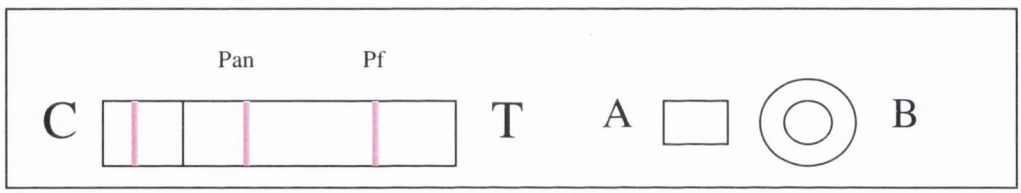
Each Parascreen kit contains a test device: membrane assembly predispensed with monoclonal anti HRP-2-colloidal gold conjugate, monoclonal anti pan specific pLDH-collodial gold conjugate, rabbit globulin-collodial gold conjugate, monoclonal anti *P. falciparum*, HRP-2 antibody, monoclonal anti pan-specific pLDH antibody and anti-rabbit antibody at the respective regions, and clearing buffer in a dropper bottle.

The test was performed according to the manufacturer's instructions. After the subject was pricked with a lancet, a heparinised capillary tube was used to transfer approximately 5 µl of blood onto the sample pad in sample port 'A' (Figure 5.1). Four drops of the clearing buffer were dispensed into port 'B' (Figure 5.1) and the results were read at the end of 15 minutes as follows: If only one pink-purple band appeared in the control window 'C' the test was negative for malaria (Figure 5.1A); if in addition to the control band, two pink-purple bands appeared at regions 'Pf' and 'Pan' in the test window 'T' (Figure 5.1B) the test was positive for *P. falciparum* or mixed infection; if in addition to the control band, one pink-purple band appeared only at the region 'Pan' in the test window 'T' (Figure 5.1C) the test was positive for other species (non *P. falciparum*). The test was considered invalid if no bands appeared on the device. In this case the test was repeated again with a new test device.

(A) Negative for malaria



(B) Positive for malaria: *Plasmodium falciparum* or mixed infection



(C) Positive for malaria: Other species (non *Plasmodium falciparum)*

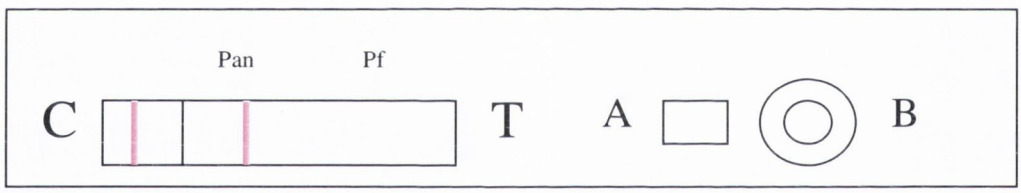


Figure 5.1. Illustration of Parascreen rapid diagnostic tests. (A) Negative for malaria: only one pink-purple band appears in the control window 'C'. (B) Positive for malaria: *Plasmodium falciparum* or mixed infection: In addition to the control band, two pink-purple bands appear at the regions 'Pf' and 'Pan' in the test window 'T'. (C) Positive for malaria: Other species (non *Plasmodium falciparum*): In addition to the control band, one pink-purple band appears at the region 'Pan' in the test window 'T'.

5.2.2 Data management and analysis

In this chapter the analyses will be based on two of the four subsets of data described in Chapter 4, section 4.2. The subsets of data will be referred to as the first and second subset for ease of discussion throughout the text. The first subset of data includes subjects who were recruited at time 0, and submitted stool samples and complied with malaria assessments at times 0, 1, 2, 3 and 4. The second subset of data includes subjects who were recruited at times 0 and 1 and submitted stool samples and complied with malaria assessments at times 1, 2, 3 and 4. The results for the first and second data set will be compared to examine whether or not a longer follow-up is needed to detect an effect of anthelminthic treatment on incidence of malaria and malaria attacks. All statistical analysis was carried out in SPSS 14.0.1.

The malaria daily clinic was set up to monitor children for their malaria attacks (Chapter 2, section 2.1.3.6). The data generated in these clinics were not used in the overall analysis because the data were thought to be biased. A descriptive analysis of the attendance at the daily clinic is provided in the results section of this chapter. Chi-square analysis was used to test the difference in proportions for the subjects that attended the daily clinics in each village. A 2-sample t-test was used to test the difference in the socio-economic status (SES) of subjects who did and did not attend the daily clinics. The SES index was created by adding up the number of possessions in the subjects' household such as a generator, fridge, television, radio, mobile phone, and toilet. Mobile phone was categorised and ranked as: 0 = having no mobile phone, 1 = either the wife or husband has a mobile phone, and 2 = both the wife and husband have a mobile phone. Toilet was categorised and ranked as: 1 = bush toilet, 2 = pit latrine, and 3 = flush toilet. Household income was added to the index after it was categorised and ranked as: 0 = no income, 1 = < 5000 Naira, 2 = 5000-10000 Naira, and 3 = > 10000 Naira.

The same analysis was carried out for the first and second subset of data. When undertaking analysis for the second data set, age was adjusted for children who were recruited in time 0 as they were four months older. The analysis for each data set was carried out as follows: SES was investigated for subjects that complied for all time points; associations were examined between the prevalence of malaria and age, sex and village; characteristics (age, sex, village,

prevalence of parasitic infections and SES) of subjects in the treatment and placebo groups were assessed at inclusion into the study; the prevalence of malaria and malaria attacks were examined between groups over time; and a mixed-effects model analysis was used to predict the significant variables (group, sex and village) that affect the incidence of malaria and prevalence of malaria attacks.

A 2-sample t-test was used to test the difference in the SES index for children who did and did not comply for all time points for that particular data set. A chi-square analysis was used to test the difference in the prevalence of malaria among age groups, sex, and villages. The drug used to treat malaria infections was Coartem (artemether-lumefantrine). To rule out the effects of other treatments (other than anthelminthic treatment), the difference in the total intake of Coartem between two groups was tested using a Mann-Whitney U test. The data for Coartem were not normal, even when log transformed, and therefore parametric tests could not be used for the analysis. The characteristics of subjects in the treatment and placebo groups at inclusion into the study were assessed using chi-square analysis to test proportions or Fisher's exact test when more than 20% of the cells had expected counts of less than five, and 2-sample t-tests to test epg and SES. The distribution of epg was not normal and therefore the epg data were log transformed (epg +1) for the purposes of statistical analysis.

The prevalence of malaria and malaria attacks were examined in the treatment and placebo groups over time, in subjects with light and moderate *A. lumbricoides* infections. The categories of light infections were defined differently for the first data and second data set because subjects in the first data set were followed up over a longer period of time. In the first data set, subjects were defined as having light intensity *Ascaris* infections if they presented more than two times with light infections during the study period. In the second data set, subjects were defined as having light infections if they presented more than one time with light infections during the study period. Moderate infections were defined as subjects presenting one or more times with moderate infections for all data sets during the study period.

The malaria prevalence data and malaria attack data were analysed using a linear mixed effects model using a logit function with the assumption that the observed data were binomially distributed. The analyses were run in R v2.6.2 (R Development Core Team, 2007) using the function *lmer* contained in the package *lme4*. A random effect for each individual

was included to take account of the nested data structure (multiple observations made on each child). In order to determine the proportional change in *odds* referred to in the text, we calculate exp(log(*odds*)). The effect of treatment (treatment or placebo), village and sex were included as fixed factors. Data were analysed initially for all age groups combined (1-4 year olds) and then identical analyses were performed on each separate age group (one, two, three, four years) as interactions between effects and age group may be non-linear.

5.3 RESULTS

5.3.1 Comparison of malaria diagnosis using RDT, PCR and microscopy by KEMRI and AMREF

Table 5.1 Comparison of malaria diagnosis based on RDT, PCR and microscopy analysis by two Kenyan laboratories.

	Number of samples (N = 9) diagnosed with *Plasmodium* spp.				
	Negative	*P. falciparum*	*P. vivax*	*P. ovale*	*P. malariae*
RDT[a]	1	8	-	-	-
PCR	1	8	0	2	2
KEMRI[b]	2	5[c]	5	0	2[d]
AMREF[b]	2	5[c]	2	0	0

[a]Comparison made for *P. falciparum* only.

[b]Microscopy analysis undertaken by two different Kenyan laboratories

[c] KEMRI and AMREF results were concordant for four blood smears

[d] KEMRI and PCR results were concordant for one blood smear

The PCR and RDT analyses were concordant for *P. falciparum* diagnosis (Table 5.1). KEMRI and AMREF misdiagnosed two *P. falciparum* infections as *P. vivax* and no *P. vivax* species was present in the blood smears in the PCR analysis. Both laboratories misdiagnosed one blood smear as negative for *P. falciparum* when it was positive in the PCR analysis. KEMRI and AMREF failed to diagnose *P. ovale* and AMREF failed to diagnose *P. malariae*.

5.3.2 Malaria Daily Clinics

Of the 1451 children that registered in May 2006, 19.7% attended the malaria daily clinics one or more times. The proportion of children that attended the clinics once, twice, three, four, five or six times were 68.6%, 21.6%, 6.3%, 1.7%, 1.4% and 0.3% respectively. Fewer children attended the malaria daily clinics in Ipetumodu (15.4%) when compared to Akinlalu (21.9%), Moro (21.9%), or Edunabon (19.7%) and this was statistically significant ($\chi^2 = 8.65$, d.f. = 3, P = 0.034). The daily clinic was attended by subjects on 421 occasions, and 77.9% presented with malaria attacks. The mean SES index was significantly higher for subjects

who attended the daily clinics when compared to children who did not attend the daily clinics (6.55 versus 6.22; t = -8.874, d.f. = 1450, P < 0.001).

765 children registered for the study in Sept./Oct. 2006. When examining the data on subjects from Sept. 2006 onwards (both subjects registered in May and Sept. 2006) it was shown that 16.4% of 2261 children attended the malaria daily clinics one or more times. The proportion of children that attended the clinics once, twice, three, four, five or six times were 72.9%, 19.7%, 4.7%, 1.8%, 0.5% and 0.3% respectively. There was no statistically significant difference in the proportion of children who attended the malaria daily clinics among the villages. The daily clinic was attended by subjects on 526 occasions, and 76.8% presented with malaria attacks. Although the mean SES was higher for children who attended the daily clinics than for children who did not attend the daily clinics, it was not statistically significant.

When examining the attendance of the malaria daily clinic for the subjects in the first and second data set the compliance decreased from 36.9% to 31.9% respectively.

5.3.2 First Dataset: Children who were recruited at time 0 and submitted a stool sample and complied with all malaria assessments at time points 0, 1, 2, 3 and 4

5.3.2.1 Baseline data

388 children submitted stool samples for analysis at time points 0, 1, 2, 3 and 4. 14 of these children did not complete a full malaria assessment and therefore 374 children were included in this analysis. The mean SES index was significantly higher for the subjects that complied for all time points when compared to the subjects that did not comply for all time points (6.47 versus 6.18 mean; t = 2.243, d.f. = 1450, P = 0.025).

Of the 374 children analysed, 78.3% tested positive for malaria with the prevalence of malaria increasing from children aged one year, peaking in children aged two years (Figure 5.2). Even though prevalence seems to vary across age groups, it was not statistically significant (χ^2 = 5.810, d.f. = 3, P = 0.121). There was also no statistically significant difference in the prevalence of malaria between the sexes (χ^2 = 0.935, d.f. = 1, P = 0.334). The prevalence of malaria in Akinlalu, Ipetumodu, Moro, and Edunabon was 86.9%, 70.7%, 82.8% and 80%

respectively. Prevalence was significantly different among the villages (χ^2 = 9.430, d.f. = 3, P = 0.024). 187 children had one or more malaria attacks. In total, 224 malaria attacks were detected. There was no significant difference in the intake of Coartem between treatment and placebo groups (132 versus 132; U = 16864, Z = -0.645, P = 0.519).

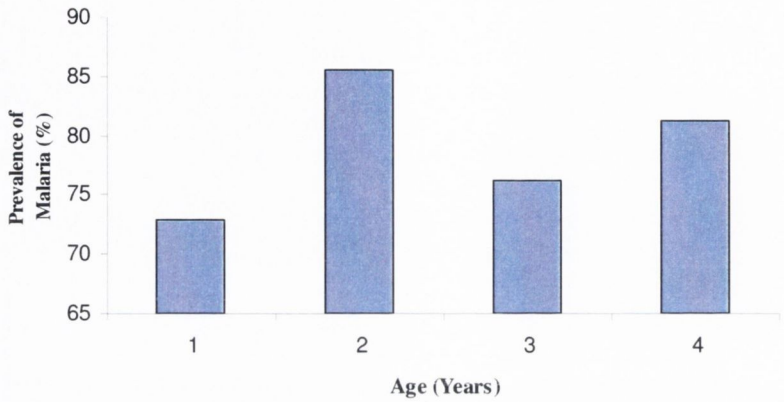


Figure 5.2 Baseline prevalence of Malaria for children aged 1-4 years at time point 0

Table 5.2 Characteristics of treatment and placebo group at inclusion into the study for the first data set

	Treatment Group (N = 183)	**Placebo Group (N = 191)**	**P**
Age (months)			
12-23	68	61	0.351 [a]
24-35	42	55	
36-47	38	46	
48-59	35	29	
Sex			
Male	100	90	0.146 [a]
Female	83	101	
Village			
Akinlalu	41	43	0.587 [a]
Ipetumodu	73	74	
Moro	32	26	
Edun-abon	37	48	
Ascaris lumbricoides			
No. infected subjects	83	86	
Prevalence rate	45.4	45	0.949 [a]
Mean epg ± S.E.	1098 ± 248	1110 ± 183	0.855 [b]

[a] χ^2 test

[b] t-test

Table 5.2 continued

	Treatment Group	Placebo Group	P
Plasmodium spp.			
No. infected subjects	142	151	
Prevalence rate	77.6	79.1	0.732 [a]
Trichuris trichiura			
No. infected subjects	5	7	
Prevalence rate	2.7	3.7	0.609 [a]
Mean epg ± S.E.	0.86 ± 0.49	2.63 ± 2.09	0.615 [c]
Hookworm			
No. infected subjects	5	10	
Prevalence rate	2.7	5.2	0.217 [a]
Mean epg ± S.E.	3.8 ± 3	3.3 ± 2	0.227 [c]
Schistosoma haematobium			
No. infected subjects	2	4	
Prevalence rate	1.1	2.1	0.685 [d]
Mean epg ± S.E.	0.04 ± 0.03	0.06 ± 0.03	0.446 [c]
Socio-economic status index			
Mean ± S.E.	12.22 ± 0.13	12.51 ± 0.14	0.339 [b]

[a] χ^2 test

[b] t-test

[c] Mann-Whitney U test

[d] Fisher's exact test

Both treatment and placebo groups were compared on the basis of age, sex, village and prevalence of *A. lumbricoides*, *T. trichiura*, hookworm, and *S. haematobium* infections, and SES. Table 5.2 shows that, on entering the study, no significant difference was recorded between the groups and any of these variables.

5.3.2.2 Prevalence of malaria in treatment and placebo groups

The prevalence of malaria was comparably high in both treatment and placebo groups during the follow-up period (Figure 5.3). Unlike the placebo group, the prevalence in the treatment group increased slightly from time point 0 to time point 1. The prevalence decreased in both groups after time point 1 and increased after time point 3. The prevalence of malaria in the villages in both treatment and placebo groups showed similar trends (Figure 5.4), although, the prevalence in the Moro treatment group was higher than the placebo group from time points 0

to 2. Malaria prevalence showed similar trends between treatment and placebo groups in 1, 2 and three year olds (Figure 5.5). However, the prevalence in the four year old treatment group was markedly higher than the placebo group at times 1, 2 and 4. Overall treatment and placebo groups showed the same trend for the prevalence of malaria in subjects defined as having light and moderate intensity *A. lumbricoides* infections (Figure 5.6). Malaria prevalence of the light intensity treatment group was slightly higher than the placebo group at times 0, 1, 2 and 4. Similarly, the prevalence of malaria in the moderate treatment group was somewhat higher than the placebo group at times 0, 1 and 2.

Table 5.3 demonstrates the percentage of children who had malaria attacks ≥ 1 times in the treatment and placebo groups over the study period. Overall, the percentage of children who had malaria one or more times was higher in the treatment group when compared to the placebo group. This trend ensues for children aged 1, 2, and 4 years with a vast increase in the percentage of children who had malaria one or more times in the treatment group for children aged 4 years (48.6% vs 17.2%).

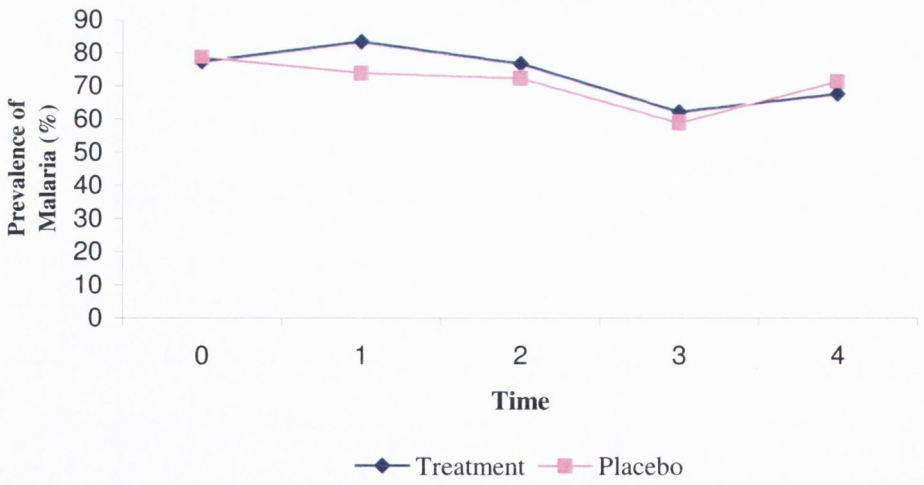


Figure 5.3 Prevalence of malaria in treatment (N = 183) and placebo (N = 191) groups over the follow-up period. 0 = May/June 2006, 1 = Sept./Oct. 2006, 2 = Feb. 2007, 3 =May/June 2007, 4 = Aug. 2007

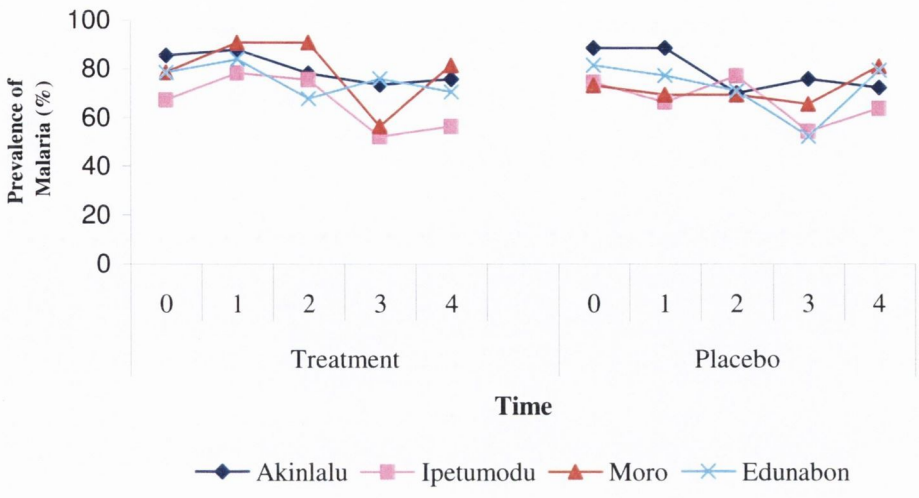


Figure 5.4 Prevalence of malaria in treatment and placebo groups for each village during the follow-up period. 0 = May/June 2006, 1 = Sept./Oct. 2006, 2 = Feb. 2007, 3 =May/June 2007, 4 = Aug. 2007

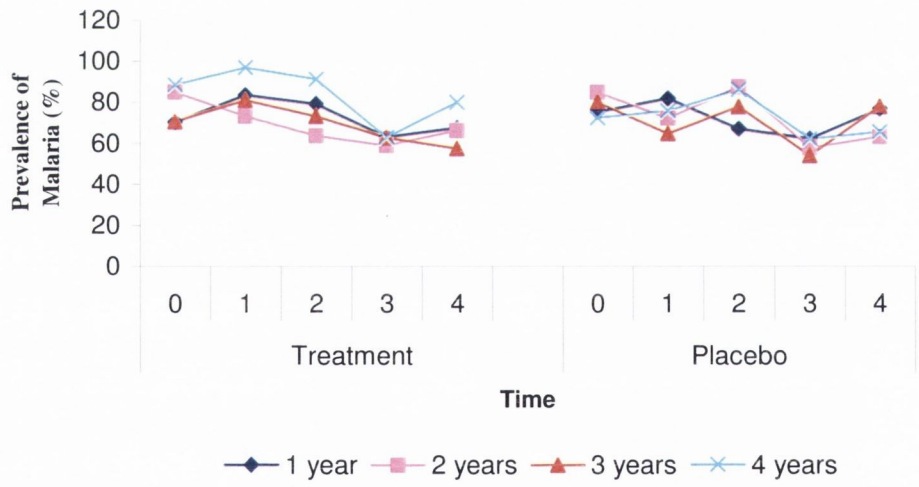


Figure 5.5 Prevalence of malaria in treatment and placebo groups for each age group during the follow-up period. 0 = May/June 2006, 1 = Sept./Oct. 2006, 2 = Feb. 2007, 3 =May/June 2007, 4 = Aug. 2007

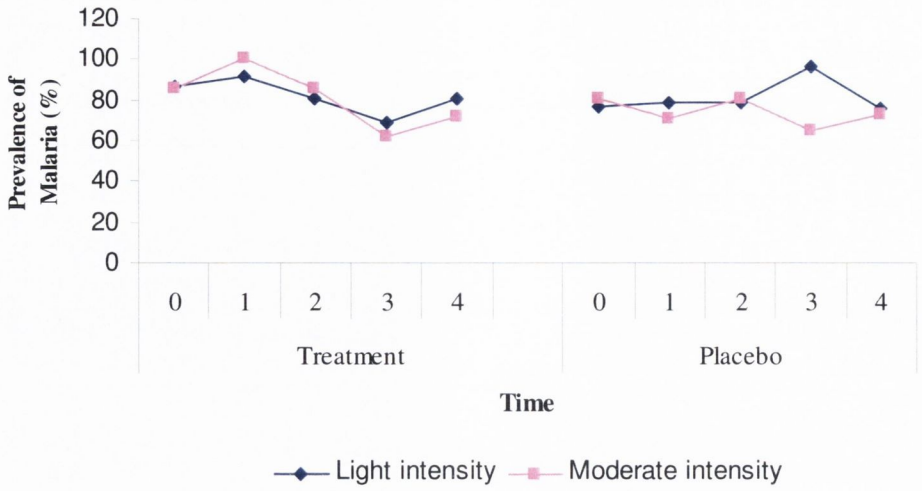


Figure 5.6 Prevalence of malaria in treatment and placebo groups for subjects with light and moderate intensity *A. lumbricoides* infections during the follow up-period. 0 = May/June 2006, 1 = Sept./Oct. 2006, 2 = Feb. 2007, 3 =May/June 2007, 4 = Aug. 2007

Table 5.3 Percentage of children who had malaria ≥ 1 times throughout the follow-up period in the Treatment (T) and Placebo (P) groups

Age (years)	Group	% of children with malaria ≥ 1 times
1-4	T	54%
	P	45.5%
1	T	64.7%
	P	60.6%
2	T	59.5%
	P	47.3%
3	T	36.8%
	P	41.3%
4	T	48.6%
	P	17.2%

In the linear mixed effects model analysis for all age groups, children in Ipetumodu were 0.4 times less likely to have malaria than children in Akinlalu (P<0.001). The prevalence of malaria was generally higher in Akinlalu when compared to the other villages in both placebo and treatment groups (Figure 5.4) When the analysis was undertaken for children aged 1 year, subjects in Ipetumodu (P<0.001) and Edunabon (P<0.001) were 0.3 times less likely to have malaria when compared to subjects in Akinlalu. The analysis carried out on children aged four years showed that children in the treatment group were 2.5 times more likely to have malaria when compared to children in the placebo group (Table 5.4;P = 0.02). Figure 5.5 and Table 5.3 highlight the increase in the prevalence of malaria in the children aged four years in the treatment group.

Table 5.4 Linear mixed effects model for the incidence of *Plasmodium sp.* in children aged 4 years, with parameter estimates (expressed as log-odds) and associated standard error of the estimate and p-values.

Coefficient	**Estimate**	**Std. Error**	**P-value**
Intercept	0.605	0.473	0.201
Group			
(Treatment vs Placebo)	0.915	0.40	0.021
Father's occupation			
Akinlalu	Reference	-	-
Ipetumodu	-0.207	0.500	0.679
Moro	1.120	0.644	0.082
Edunabon	0.142	0.608	0.815
Sex			
(Female vs Male)	0.640	0.411	0.120

5.3.2.3 Prevalence of malaria attacks in treatment and placebo groups

Overall, the prevalence of malaria attacks in both treatment and placebo groups were low and showed no general trend over the follow-up period (Figure 5.7). 100 children suffered a malaria attack in the treatment group, while 87 had a malaria attack in the placebo group during the study period.

Prevalence of malaria attacks in subjects in the villages within the treatment and placebo groups showed the same general trend (Figure 5.8). Subjects in the Akinlalu treatment group had a higher prevalence of malaria attacks than subjects in the Akinlalu placebo group at times 1, 2 and 3. Malaria attacks in the Ipetumodu groups showed no consistent trend. Prevalence of malaria attacks in the Moro treatment group remained higher than the placebo group from time 1 onwards. No general trend can be observed from the Edunabon treatment groups.

Prevalence of malaria attacks in the age classes within the experimental groups showed no trend (Figure 5.9). The placebo age groups showed a similar trend, albeit the prevalence of malaria attacks in the 1 year age group remained higher throughout the study period. There was no discernable pattern to the prevalence of malaria attacks in the treatment and placebo age groups. The 2 year age group was the only group where the treatment group remained higher than the placebo group for three consistent time points, namely 1, 2 and 3.

Malaria attacks showed a similar trend in the subjects defined as having light and moderate intensity infections between treatment and placebo groups (Figure 5.10). The prevalence of malaria attacks in the placebo light intensity group increased and decreased throughout the study period but remained higher than the placebo group at times 0, 2 and 4. The same pattern ensued for the moderate intensity groups.

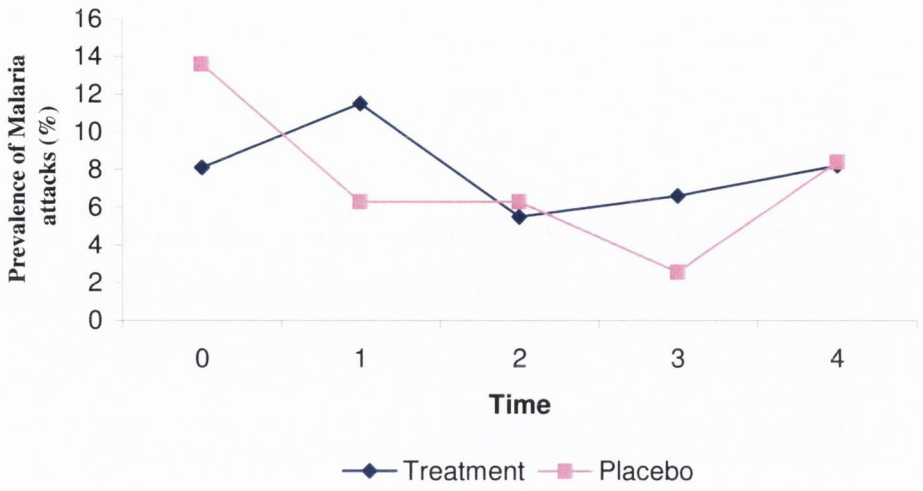


Figure 5.7 Prevalence of malaria attacks in treatment (N = 183) and placebo (N = 191) groups over the follow-up period. 0 = May/June 2006, 1 = Sept./Oct. 2006, 2 = Feb. 2007, 3 =May/June 2007, 4 = Aug. 2007

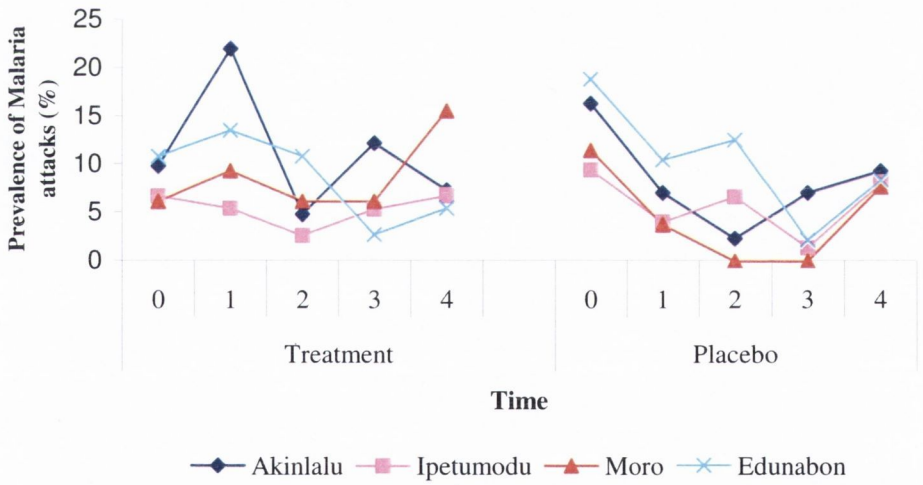


Figure 5.8 Prevalence of malaria attacks in treatment and placebo groups for each village during the follow-up period. 0 = May/June 2006, 1 = Sept./Oct. 2006, 2 = Feb. 2007, 3 =May/June 2007, 4 = Aug. 2007

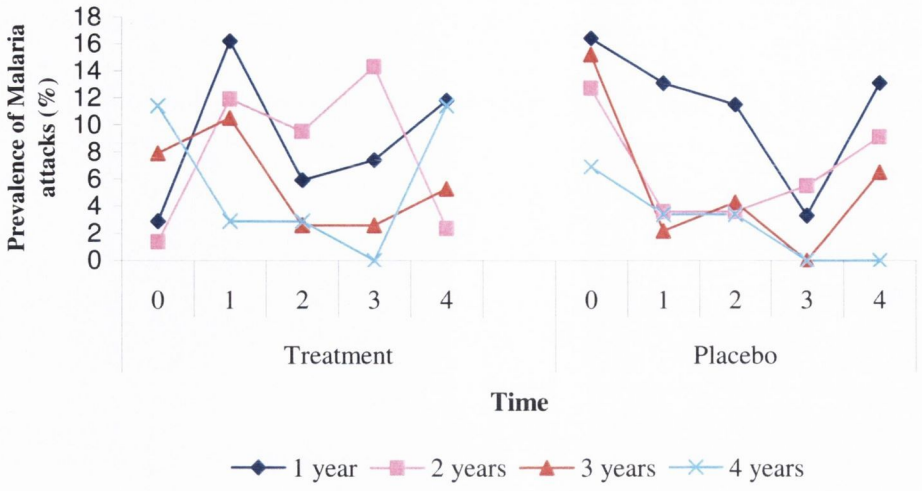


Figure 5.9 Prevalence of malaria in treatment and placebo groups for each age group during the follow-up period. 0 = May/June 2006, 1 = Sept./Oct. 2006, 2 = Feb. 2007, 3 =May/June 2007, 4 = Aug. 2007

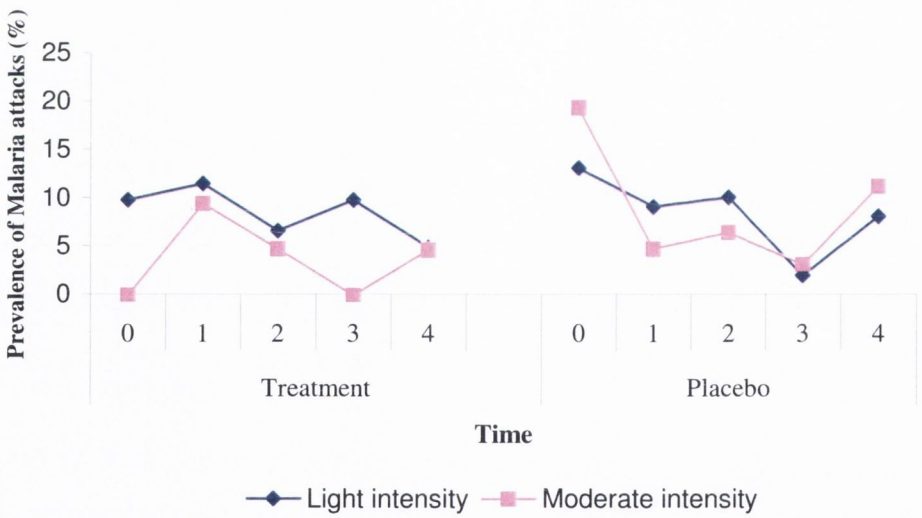


Figure 5.10 Prevalence of malaria attacks in treatment and placebo groups for subjects with light and moderate intensity *A. lumbricoides* infections during the follow-up period. 0 = May/June 2006, 1 = Sept./Oct. 2006, 2 = Feb. 2007, 3 =May/June 2007, 4 = Aug. 2007

In the linear mixed effects model analysis for all age groups, children in Ipetumodu were 0.6 times less likely to have malaria attacks than children in Akinlalu (P = 0.017). Figure 5.8 shows that the prevalence of malaria attacks was generally lower for children in Ipetumodu than children in Akinlalu. When the analysis was undertaken for children aged one year, subjects in Ipetumodu (P<0.003) were 0.4 times less likely to have malaria attacks when compared to subjects in Akinlalu.

5.3.3. Second dataset: Children who were recruited at time 1 and submitted stool samples and complied with malaria assessments at time points 1, 2, 3 and 4

5.3.3.1 Baseline data

598 children submitted faecal samples for analysis at time points 1, 2, 3 and 4. 34 of these children did not complete a full malaria assessment and therefore 564 children were included in this analysis. The mean SES index was significantly higher for the subjects that complied for all time points when compared to the subjects that did not comply for all time points (6.47 versus 6.17; t = 3.137, d.f. = 2305, P = 0.002).

Of the 564 children analysed, 79.1% of the children tested positive for malaria. Children aged two years had the highest prevalence of malaria, although there was no statistically significant difference in the prevalence between the age groups (Figure 5.11). There was also no statistically significant difference in the prevalence of malaria between the sexes. The prevalence of malaria in Akinlalu, Ipetumodu, Moro and Edunabon was 86.1%, 72.9%, 82.4% and 82.2% respectively. There was a significant difference in the prevalence of malaria between villages (χ^2 = 10.369, df = 3, P = 0.016). 231 children had one or more malaria attacks. In total 252 malaria attacks were detected. There was no significant difference in the intake of Coartem between treatment and placebo groups (186 versus 179; U = 37684.5, Z = -1.188, P = 0.235).

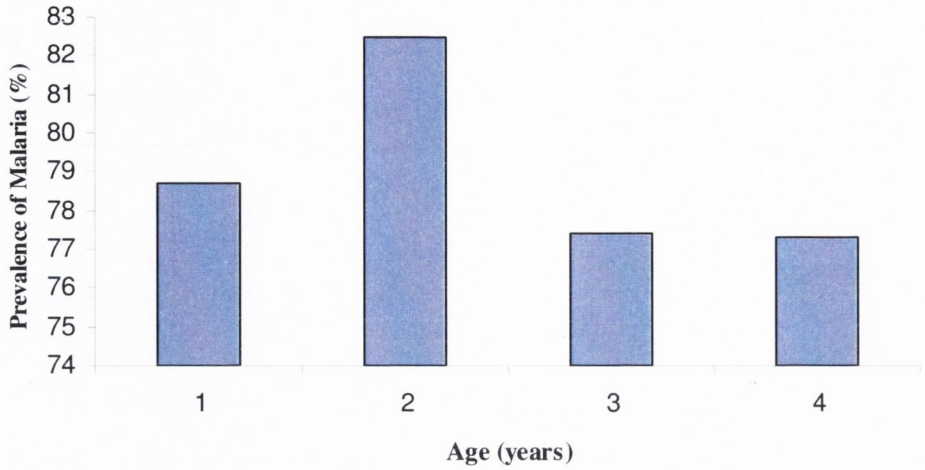


Figure 5.11 Baseline prevalence of malaria for children aged 1-4 years at time point 1.

Table 5.5 Characteristics of treatment and placebo groups at inclusion into the study for the second data set.

	Treatment Group (N = 273)	**Placebo Group (N = 291)**	**P**
Age (months)			
12-23	91	97	0.812 [a]
24-35	68	75	
36-47	63	73	
48-59	51	46	
Sex			
Male	140	140	0.451 [a]
Female	133	151	
Village			
Akinlalu	57	65	0.420 [a]
Ipetumodu	116	123	
Moro	42	32	
Edun-abon	58	71	
Ascaris lumbricoides			
No. infected subjects	137	157	
Prevalence rate	50.2	54	0.371 [a]
Mean epg ± S.E.	803.8 ± 149	1014.4 ± 177	0.224 [b]
Plasmodium spp.			
No. infected subjects	222	224	
Prevalence rate	81.3	77	0.205 [a]

[a] χ^2 test

[b] t-test

Table 5.5 continued

	Treatment Group	Placebo Group	P
Trichuris trichiura			
No. infected subjects	6	11	
Prevalence rate	2.2	3.8	0.272 [a]
Mean epg ± S.E.	2.4 ± 1.31	1.8 ± 1.41	0.290 [c]
Hookworm			
No. infected subjects	15	12	
Prevalence rate	5.5	4.1	0.446 [a]
Mean epg ± S.E.	4.6 ± 2.24	5.2 ± 3.38	0.435 [c]
Schistosoma haematobium			
No. infected subjects	1	1	
Prevalence rate	.4	1	0.624 [d]
Mean epg ± S.E.	0.014 ± 0.01	0.08 ± 0.05	0.345 [c]
Socio-economic status index			
Mean ± S.E.	6.40 ± 0.10	6.52 ± 0.11	0.407 [b]

[a] χ^2 test

[b] t-test

[c] Mann-Whitney U test

[d] Fisher's exact test

Both treatment and placebo groups were compared on the basis of age, sex, village and prevalence of *A. lumbricoides*, *T. trichiura*, Hookworm, and *S. haematobium* infections, and SES index. Table 5.5 shows that, on entering the study, no statistically significant difference was recorded between the groups and any of these variables.

5.3.3.2 Prevalence of malaria in treatment and placebo groups

Both the treatment and placebo groups show the same pattern for prevalence of malaria over the follow-up period (Figure 5.12). The prevalence of malaria peaks in time 1, decreasing thereafter to time 3.

Both the villages within the treatment and placebo groups followed the same general trend for the prevalence of malaria (Figure 5.13). Towards the end of the follow-up (times 3 and 4) the prevalence of malaria in the Akinlalu treatment group was slightly lower than the placebo. The same trend was shown for Ipetumodu and Moro. Prevalence of malaria in the treatment

Edunabon was lower than the placebo at times 1, 2 and 4 but markedly higher than the placebo at time 3.

The pattern of malaria prevalence for the age groups within the treatment and placebo groups showed a comparable trend (Figure 5.14). The prevalence of malaria in the treatment group for children aged one year was slightly higher than the placebo group. Prevalence of malaria in the two year treatment group was higher than the placebo at time 2 and lower than placebo at times 3 and 4. The three year treatment group was lower than the placebo group at times 2 and 4 for the prevalence of malaria. There was no consistent pattern in the prevalence of malaria when comparing treatment and placebo groups for the four year old children.

Table 5.6 demonstrates the percentage of children who had malaria attacks $\geq$ 1 times in the treatment and placebo groups over the study period. Overall, the percentage of children who had malaria one or more times was higher in the treatment group when compared to the placebo group. This trend ensues for children aged 1, 2, 3, and 4 years with a bigger increase in the percentage of children who had malaria in the treatment group for children aged 4 years (39.2% vs 21.7%).

The prevalence of malaria in the treatment light intensity group remained slightly higher than the placebo group throughout the follow-up, except for time 3 where the prevalences were the same (Figure 5.15). There was no consistent trend of malaria prevalence in the moderate intensity groups. The prevalence in the treatment and placebo groups increased and decreased alternately during the study period.

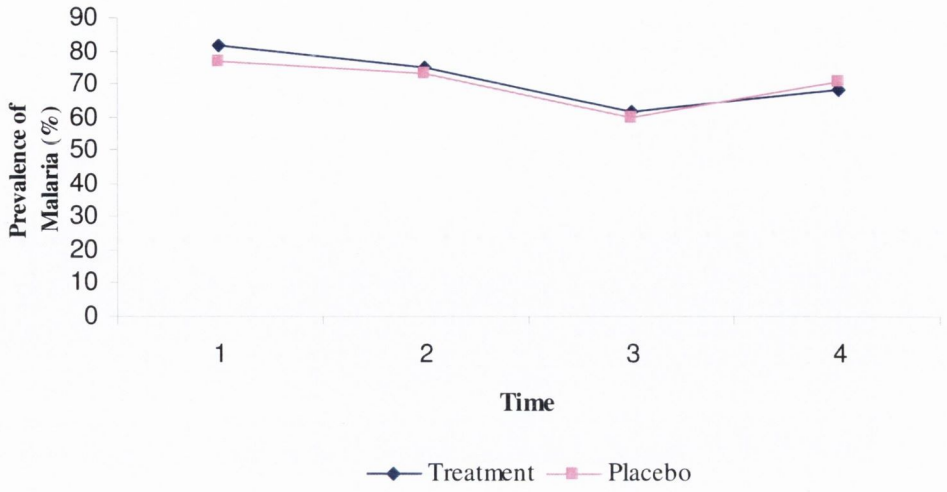


Figure 5.12 Prevalence of malaria in treatment (N = 273) and placebo (N = 291) groups over the follow-up period. 1 = Sept./Oct. 2006, 2 = Feb. 2007, 3 =May/June 2007, 4 = Aug. 2007

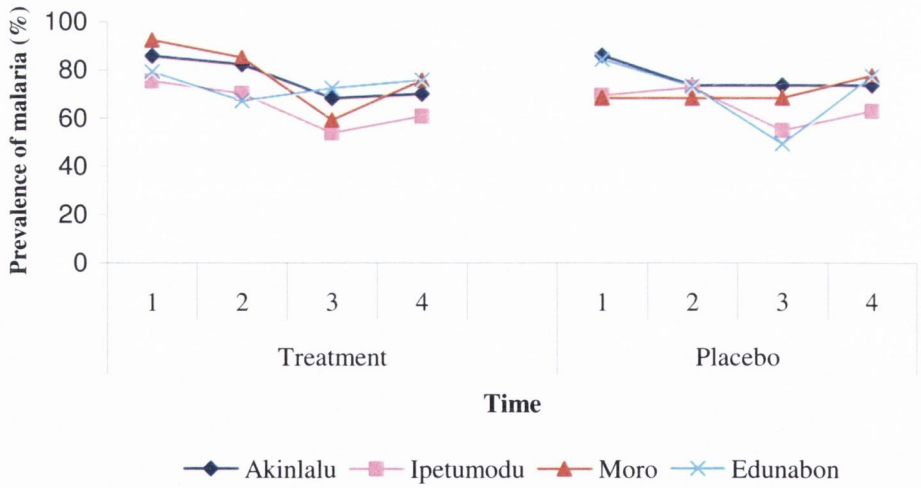


Figure 5.13 Prevalence of malaria in treatment and placebo groups for each village during the follow-up period. 1 = Sept./Oct. 2006, 2 = Feb. 2007, 3 =May/June 2007, 4 = Aug. 2007.

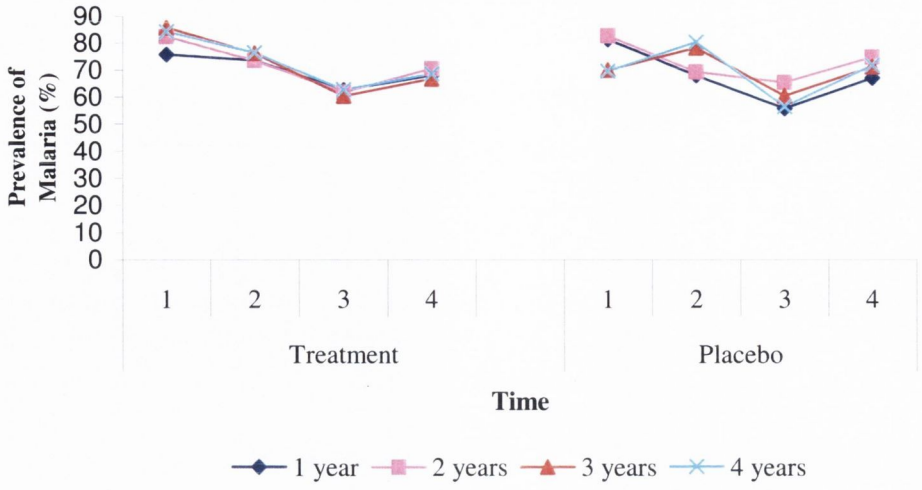


Figure 5.14 Prevalence of malaria in treatment and placebo groups for each age group during the follow-up period. 1 = Sept./Oct. 2006, 2 = Feb. 2007, 3 =May/June 2007, 4 = Aug. 2007

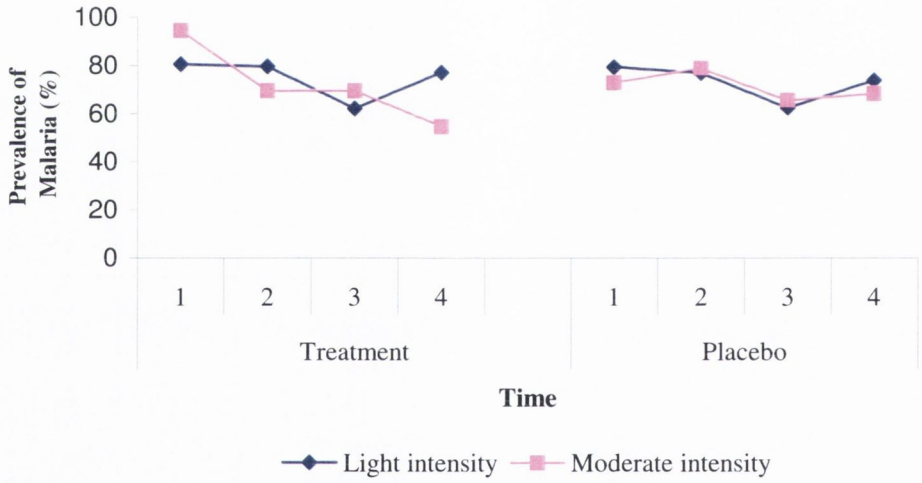


Figure 5.15 Prevalence of malaria in treatment and placebo groups for subjects with light and moderate intensity *A. lumbricoides* infections during the follow-up period. 1 = Sept./Oct. 2006, 2 = Feb. 2007, 3 =May/June 2007, 4 = Aug. 2007

Table 5.6 Percentage of children who had malaria ≥ 1 times throughout the follow-up period in the Treatment (T) and Placebo (P) groups

Age (years)	Group	% of children with malaria ≥ 1 times
1-4	T	44.3%
	P	37.8%
1	T	50.5%
	P	44.3%
2	T	47%
	P	41.3%
3	T	36.5%
	P	35%
4	T	39.2%
	P	21.7%

In the linear mixed effects model analysis for all age groups, children in Ipetumodu were 0.5 times less likely to have malaria than children in Akinlalu ($P<0.001$). Figure 5.13 shows that the prevalence of malaria was higher for Akinlalu when compared to Ipetumodu, and this was more pronounced in the treatment group. When the analysis was undertaken for children aged one year, subjects in Ipetumodu ($P<0.001$) were 0.5 times less likely to have malaria when compared to subjects in Akinlalu. In the analysis for subjects aged three years, children in Ipetumodu ($P = 0.006$), Moro ($P = 0.007$), and Edunabon ($P = 0.012$) were 0.4, 0.3 and 0.4 times less likely to have malaria, respectively, when compared to children in Akinlalu.

5.3.3.3 Prevalence of malaria attacks in treatment and placebo groups

Overall, the percentage of children suffering from malaria attacks was low in both treatment and placebo groups (Figure 5.16). The prevalence of malaria attacks in the treatment group remained higher than the placebo group from times 1 to 3. For time 4 the prevalence of malaria attacks was greater in the placebo group. 121 children suffered a malaria attack in the treatment group, while 110 had a malaria attack in the placebo group during the study period.

The prevalence of malaria attacks in both treatment and placebo groups, although generally low, varied between villages (Figure 5.17). Both Akinlalu treatment and placebo groups exhibited a similar pattern in malaria attacks to that seen in the overall population (Figure 5.18). The prevalence of malaria attacks in the Ipetumodu treatment group was slightly lower than the placebo group from times 1 to 4. The Moro treatment group showed a higher prevalence of malaria attacks when compared to the placebo group throughout the entire study period. The Edunabon treatment and placebo groups exhibited the same trend as the Ipetumodu groups where the prevalence of attacks were higher in the treatment group from times 1 to 3 but then became lower than the placebo group for time 4.

The prevalence of malaria attacks varied in the age classes within the treatment groups and followed no particular trend, whereas the age groups within the placebo groups also varied in prevalence but followed the same trend (Figure 5.18). The prevalence of malaria attacks were alternately lower in the one year old treatment group when compared to the placebo group. In the two year old groups the prevalence of malaria attacks were higher in the treatment group from time 1 to 3 than the placebo group and then decreased thereafter. The same pattern was seen in the three year old groups. The prevalence of malaria attacks in the 4 year old treatment and placebo groups were similar for times 2 and 3, and the prevalence increased in the treatment group at time 4.

The prevalence of malaria attacks were higher in the light intensity treatment group when compared to the placebo group from times 1 to 3, but the prevalence in the treatment group decreased thereafter (Figure 5.19). In the moderate intensity treatment group malaria attack

prevalence was lower than the placebo group for times 2 and 3 and higher than the placebo group for time 4.

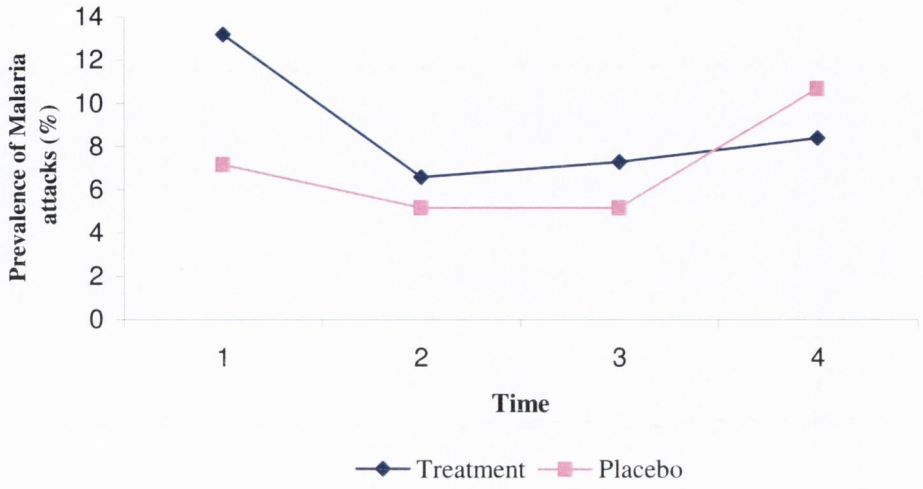


Figure 5.16 Prevalence of malaria attacks in treatment (N = 273) and placebo (N = 291) groups over the follow-up period. 1 = Sept./Oct. 2006, 2 = Feb. 2007, 3 =May/June 2007, 4 = Aug. 2007

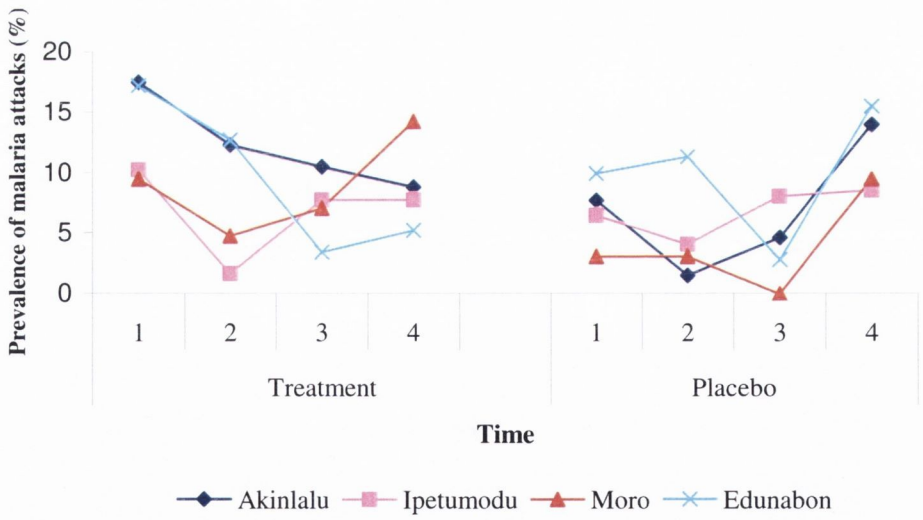


Figure 5.17 Prevalence of malaria attacks in treatment and placebo groups for each village during the follow-up period. 1 = Sept./Oct. 2006, 2 = Feb. 2007, 3 =May/June 2007, 4 = Aug. 2007

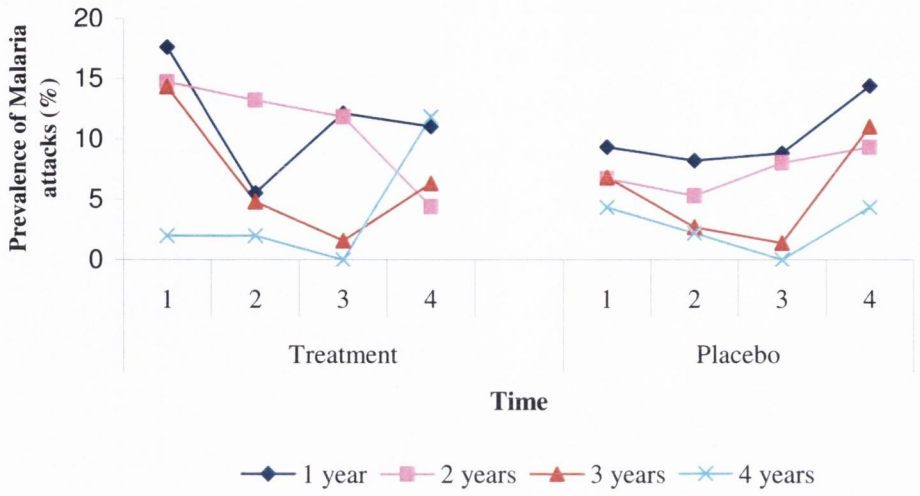


Figure 5.18 Prevalence of malaria attacks in treatment and placebo groups for each age group during the follow-up period. 1 = Sept./Oct. 2006, 2 = Feb. 2007, 3 =May/June 2007, 4 = Aug. 2007

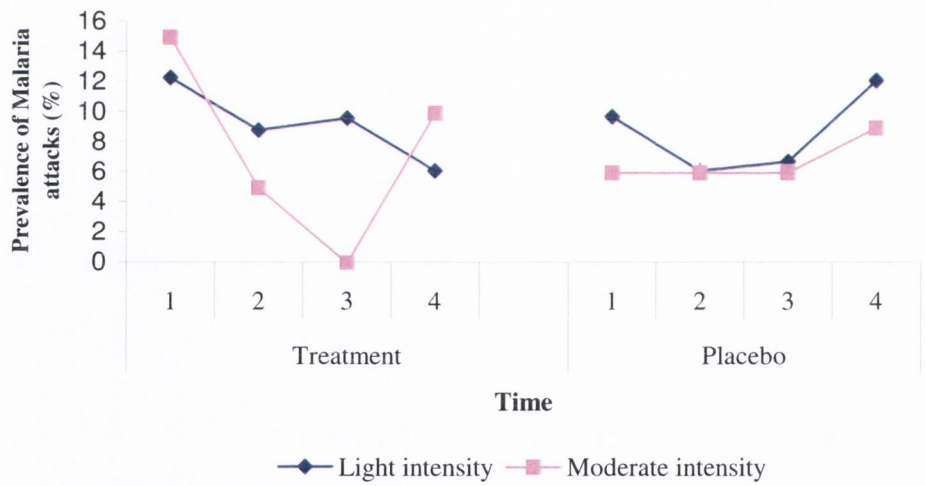


Figure 5.19 Prevalence of malaria attacks in treatment and placebo groups for subjects with light and moderate intensity *A. lumbricoides* infections during the follow-up period. 1 = Sept./Oct. 2006, 2 = Feb. 2007, 3 =May/June 2007, 4 = Aug. 2007

No variables were significant in the linear mixed effects model analysis for malaria attacks.

5.4 DISCUSSION

5.4.1 Malaria diagnosis

The PCR analysis confirmed that none of the subjects were infected with *P. vivax*. This was expected as *P. vivax* is not found in much of sub-Saharan Africa (Summer *et al.*, 2005). The comparison in malaria diagnosis using RDT, PCR and microscopy was based on nine subjects. Although the sample size was small, it allowed the *P. vivax* diagnoses made by KEMRI and AMREF to be nullified. Both laboratories noted that the quality of some smears were poor and indicated that this was the reason for the spurious *P. vivax* diagnoses. However, the *P. vivax* was also diagnosed in blood smears that were deemed to be of good quality by the laboratories. It is possible that the technicians from both laboratories may be more familiar with diagnosing *P. vivax* infections, as *P. vivax* is more common in East Africa (Mendis *et al.*, 2001), and are therefore misdiasnosing *P. falciparum* as *P. vivax*. The Parascreen RDT used in this study was equally effective as PCR in diagnosing *P. falciparum* infections. Both RDT and the PCR tests were better at diagnosing *P. falciparum* than microscopy. When infected red blood cells are sequestered in the deep vasculature of tissues (Bell *et al.*, 2006), there may be no infected red erythrocytes in the peripheral circulation, however HRP-2 antigen will still be in circulation and this may explain why microscopy did not diagnose *P. falciparum* infections as well as the RDTs. Microscopy is generally accepted as the gold standard for diagnosing malaria; however, PCR has a higher detection and identification rate of parasites when compared to microscopic tests (Haditsch, 2004). PCR tests have the best sensitivity and specificity but are not as rapid as other tests and due to costs are not feasible in some locations (Hanscheid and Grobusch, 2002). The malaria analysis for this chapter was based on results from RDTs as slide data were not yet available (Chapter 5, section 5.2.1).

The Parascreen RDT was based on HRP-2 and pLDH detection. Tests based on HRP-2 detection have been shown to have a high sensitivity (how many samples are positive/negative with a specific test as compared to the gold standard test i.e. out of all confirmed positive/negative samples) for the diagnosis of *P. falciparum* malaria, usually over 90% (Lema *et al.*, 1999; Ricci *et al.*, 2000; Singh *et al.*, 2000; Proux *et al.*, 2001; Huong *et al.*, 2002).

Optimal™ and similar tests based on pLDH have given more variable results in different clinical settings, with sensitivity of detection as low as 50% (Huong *et al.* 2002). Although, RDTs are quick and easy to use in a field study setting, they are not without their limitations. Both tests based on HRP-2 and pLDH detection perform worse with low parasitaemias (<500 parasites/ul blood) resulting in more false negatives (Iqbal *et al.*, 1999b; Fryauff *et al.*, 2000; Ricci *et al.*, 2000). It has been suggested that a less sensitive RDT with a higher limit of detection could be used in areas of very high prevalence (some areas of sub-Saharan Africa). Using a less sensitive RDT would be equivalent to setting a threshold density of parasitaemia in the clinical case definition of malaria i.e. increasing the fraction of fevers attributable to malaria. HRP-2 antigen can persist for several weeks after successful treatment (Shiff *et al.*, 1993; Labbe *et al.*, 2001; Proux *et al.*, 2001; Wongsrichanalai, 2001; Toma *et al.*, 2003), whereas pLDH is more rapidly cleared from the blood following parasite clearance (Palmer *et al.* 1999; Moody *et al.* 2000). This is known to increase the number of false positive tests (Tjitra *et al.*, 2001). The number of false positives may have been reduced in this analysis as every subject in this analysis was only screened once for malaria at each time point. Malaria treatment was given after the child was screened if it was necessary. Every child was screened at four-monthly intervals, thus providing enough time for the clearance of the HRP-2 and pLDH antigen. Attendance at the malaria daily clinics increased during the two weeks after all the children were screened at each time point (personal observation). This coupled with the low attendance at the daily malaria clinic may have reduced the number of false positive results.

Gametocytes are able to produce detectable levels of pLDH and HRP-2 (Oduola *et al.*, 1997; Bell *et al.*, 2006). As gametocytes do not contribute to morbidity, their carriage in the blood would not be associated with any clinical symptoms. Gametocytes can be found in the blood for 2-3 weeks following clearance of asexual parasitaemia, and would therefore contribute to a positive RDT test following clearance of asexual parasitaemia, if the drug used is not gametocytocidal (Tjitra and Anstey, 2001). However the treatment given for acute malaria in this study (and in Nigeria) is Coartem (artemether-lumefantrine), which is gametocytocidal. Therefore any HRP-2 or pLDH detected is likely to be associated with asexual parasitaemia only.

In our study, RDTs were needed to identify the presence of *Plasmodium* spp. parasites, particularly in children who presented with fever symptoms, as microscopy was not available under field conditions. WHO supports the use of RDTs in the management of malaria but does not recommend or certify any specific malaria RDT brand or product (WHO, 2003a). The results presented in this study indicate that the Parascreen RDT is equally effective as PCR and better than microscopy at diagnosing *P. falciparum* infections. In most malaria-epidemic situations, RDTs have been calculated to reduce over treatment considerably, for only a moderate increase in the costs of over presumptive diagnosis (Rolland *et al.*, 2006).

5.4.2 Malaria Daily Clinics

The malaria daily clinics were not well attended by the subjects registered in the study. The majority of subjects (68.6% for children that registered in May 2006 and 72.9% for subjects who came from September 2006 onwards) that attended the clinics only visited the clinics once. Subjects who attended the clinics had a higher SES index. The turn out at the daily clinics increased somewhat during the two weeks after all the children were screened at each time point. Once this period had passed, very few mothers would bring their children to the daily clinics, which resulted in periods of two or three weeks without anyone using this service.

When subjects were screened, mothers were educated about the malaria daily clinic service that was available to them. The primary health care centers in each village were highly inadequate being poorly equipped, lacking essential supplies and qualified staff. Health care workers extract informal fees from the mothers for malaria medication (personal observation) - this is not uncommon in sub-Saharan Africa where low paid health workers have to supplement their income (McCoy *et al.*, 2008). In addition to this, Chloroquine is the medication used to treat malaria, which can be ineffective due to increasing resistance of the parasites. Many studies describe wide dissatisfaction with public facilities, the key issues contributing to this dissatisfaction include: poor staff attitudes (Gilson *et al.*, 1994; Dondi *et al.*, 1998); cost, both direct and indirect (Jenkins 1998; Geissler *et al.*, 2000); and consistent lack of drugs and equipment (Osei and Commey, 1994; Massele *et al.*, 1998). Thus, it was not surprising that the daily malaria clinics were unsuccessful as every child had access to free

medical care provided by qualified nurses and doctors. The low attendance may have been due to a combination of factors including: distance from the daily clinics, mother's ignorance to illness caused by malaria, opening hours of the daily clinics, perceptions of primary health care services and lack of incentives other than free medical care.

The study participants were widely dispersed in each village. The daily malaria clinics were held in the Oba's palace, or the town hall in Akinlalu, which were located in the centre of each village. Mothers who lived further away from the centre of the village may have found it difficult to gain access to the malaria daily clinics on a regular basis and this may explain why some subjects did not attend the daily clinics or indeed why the majority of subjects who did attend the clinics only came once. Ease of access to health care has been shown to affect treatment seeking behaviour in hospitals (Lindblade *et al.*, 2000). A study in Uganda found that easy access to health facilities was associated with a 50% and 60% increase in the odds of attending a clinic or hospital within one day of the onset of symptoms for adults and children. The daily clinics in the present study were open from 9am to 3pm, Monday to Friday. This may have also contributed to the lack of attendance as mothers who were constrained by work or indeed away from the village at their farms would not have been able to attend the clinics with febrile children until the evening or at the weekend.

Throughout the study, mothers were educated about the importance of taking their child to the daily clinic if the child had a fever. Despite this, mothers still remained ignorant to the serious nature of malaria infections, equating malaria with the 'common cold'. Studies have shown that illness recognition can determine treatment responses (Ramakrishna *et al.*, 1988; Salako *et al.*, 2001). A study in Oyo state, Nigeria, showed that people viewed malaria and febrile convulsions as separate conditions. Malaria was perceived as a less serious condition caused by heat and sun, while convulsions caused by the cold prompted an immediate treatment response (Ramakrihsna *et al.*, 1988). In this present study, mothers frequently gave paracetamol to their children as treatment for malaria. One mother reported that she gave her child paracetamol everyday to prevent the child from getting malaria. Without an understanding of such local perceptions, health education to improve malaria illness and treatment behaviours may fall on deaf ears (Salako *et al.*, 2001).

A general lack of participation in the primary health care service by the locals was observed in each village. This is not uncommon in sub-Saharan Africa (Jenkins, 1998; Geissler *et al.*, 2000). In the present study, indigenous fieldworkers reported that mothers found health care workers in the primary health care centres to be unhelpful and unmotivated. Poor staff attitudes appear to play a role in discouraging caregivers from seeking clinic care (Gilson *et al.*, 1994; Baume *et al.*, 2000). This perception of primary health care service providers may have prevented mothers from bringing their child to the daily malaria clinics even though mothers were informed of the distinction between our services and the one that is provided locally.

Generally the mothers may have found it easier to comply with the screening at each 4-monthly time point using this as a health check up for their children. The incentives, multivitamins and head scarves, which were given out to mothers when all children were screened at each time point helped to increase compliance particularly with the return of stool samples (Chapter 2, section 2.2.4). The lack of incentives other than free good quality medical care for malaria may have been another contributing factor to the poor turnout at the daily clinics. The people who came to the clinics had a higher socio-economic index and this may be indicative of mothers who could afford transport to the daily clinics, afford housing closer to the center of the village or indeed have more of an awareness of the complications of malarial illness. A study on treatment seeking behaviour in ethnic communities of Assam, North-eastern India, has shown that the time lag between onset of fever and presentation of more than 2 days was due to low education levels and poor socio-economic conditions (Dev *et al.*, 2006).

While active case detection would improve the pick-up rate of clinical attacks, it was totally unfeasible with the resources available and in such a widely dispersed urban population. Active case detection would be better suited to studies based in smaller rural villages or schools where the subjects of study are children. Although passive case detection is widely used in malaria studies (Nacher *et al.*, 2002c; Spiegel *et al.*, 2003; Aponte *et al.*, 2007), it was not successful in this study and using this data in the analysis may have provided biased results.

5.4.3 The effect of anthelminthic treatment on *Plasmodium spp.* infections

Recent papers discussing the control of malaria and the NTDs have suggested that the control of helminth infections could offer a means to roll back malaria (Druilhe *et al.*, 2005; Molyneux *et al.*, 2005; Hotez *et al.*, 2006c). Previous human studies on helminth-malaria interactions have shown contrasting results (Nacher *et al.*, 2000; Spiegel *et al.*, 2003; Sokhna *et al.*, 2004; Briand *et al.*, 2005; Shapiro *et al.*, 2005). Therefore, the scientific community should be cautious when making such statements as 'worms can worsen malaria' (Druilhe *et al.*, 2005) and should resist the temptation to generalise (Nacher *et al.*, 2006). Some studies have shown that helminths can protect against malaria (Nacher *et al.*, 2000; Briand *et al.*, 2005) while others have shown that helminths can make the host more susceptible to malaria (Spiegel *et al.*, 2003; Sokhna *et al.*, 2004). To our knowledge, we performed the first double-blind placebo-controlled randomised trial of anthelminthic treatment in children aged 1-4 years. The results presented here show that while there is no interaction between *A. lumbricoides* and *Plasmodium* spp. in children aged 1-3 years, *A. lumbricoides* has a protective effect against malaria in children aged four years.

The linear mixed effects model analysis showed that: (1) children in the treatment group aged four years were more susceptible to malaria and (2) children in Akinlalu suffered more from malaria and malaria attacks than the other villages, particularly Ipetumodu. The significant finding that children aged four years were more susceptible to malaria when treated with anthelminthics was shown in the first data set only. Children in the first data set were followed up for 16 months, whereas children in the second data set were followed up for 12 months. It is possible that three rounds of anthelminthic treatment may not be enough to show an effect on the frequency of children presenting with malaria or malaria attacks. This is an important finding that, despite a larger sample size in the second data set, a longer follow-up may be critical to detecting an effect of anthelminthic treatment on malaria, therefore future studies should take this into account. There was no significant difference in the frequency of malaria attacks between treatment and placebo groups for any age category. The use of RDTs in the helminth-malaria analysis precluded the ability to calculate a malaria attributable fraction for fevers which is calculated using parasitaemia data. This may have resulted in the

overestimation of malaria fevers and dilution of the effect of anthelminthic treatment on malaria attacks. The malaria attributable fraction will be incorporated at a later stage when microscopy results become available.

Village was identified as a significant factor in the linear mixed effects model analysis for all data sets. Children in Akinlalu were more susceptible to malaria when compared to children living in Ipetumodu, Moro, and Edunabon. In the previous chapter on anthelmintic treatment, compliance was also shown to be higher in Akinlalu than for the other villages. As mentioned in Chapter 4, Akinlalu is less widely dispersed than the other three villages. In addition to this, the presence of a river on entry into the village is unique to Akinlalu. Location has been identified as a risk factor for malaria (van de Hoek *et al.,* 2003) and this may explain the higher prevalence of malaria found in Akinlalu. Studies in Sri Lanka (van de Hoek *et al.*, 2003) and Uganda (Staeke *et al.*, 2003) have shown that the distance of households from swamps, and streams (mosquito breeding sites) is related to the incidence of malaria. Despite this, a significant effect of group was still shown in the present study in children aged four years with village as a fixed factor in the linear mixed effects model analysis.

The characteristics of subjects in the treatment and placebo group on entry into the study did not significantly differ for the first and second dataset. SES index was significantly higher for subjects that complied for all time points in the first and second dataset. SES index was not significantly different between subjects in the placebo and treatment groups and therefore should have no impact on the results. I do not believe that the intake of Coartem had any effect on the results. There was no significant difference in the intake of Coartem between the two groups.

The negative impact of anthelminthic treatment on malaria infections in children aged four years compares well with other studies on *A. lumbricoides* and malarial infections (Murray *et al.*, 1978, Nacher *et al.*, 2000; Brutus *et al.*, 2006). It is important to keep in mind that our results are based on malaria rapid test prevalence data which do not differentiate between *Plasmodium* species when comparing this study with other studies. Two interventions have been undertaken in the Comoro islands, located between Madagascar and the African continent (Murray *et al.*, 1978), and Madagascar (Brutus *et al.*, 2006); both these studies have included subjects with a wide age range, 2-14yrs and 0 - >15yrs, respectively. The children on

the Comoro islands showed an increase in clinical malaria cases with anthelminthic treatment. This study compared children with severe ascariasis and those with so-called 'minimal' ascariasis. The study had many limitations and has been criticised for its small sample size (112), short follow-up (20 days) and severely malnourished study population (Druilhe, 2006; Mwangi *et al.*, 2006).

The Madagascar study showed that subjects more than five years of age, treated with levamisole, had a significant increase in their *P. falciparum* parasitaemia compared with controls. The Brutus study was both impressive and ambitious with its controlled randomised longitudinal (17 months) design coupled with the two-monthly anthelminthic treatments. Nevertheless, the study had some drawbacks. The immune response to malaria builds up progressively in children and can be markedly different in adults (Druilhe *et al.*, 2005). Brutus and colleagues controlled for this in the analysis by defining three age groups (6 months to 4 years, 5-14 years, $\geq$ 15 years). The sample size in the six months to four year age group (67) may have been too small to detect an effect of anthelminthic treatment on *P. falciparum* parasitaemia. Furthermore, the prevalence of *A. lumbricoides* in the treatment (26.2%) and placebo (27.4%) groups was moderate at entry into the study in comparison to the prevalence shown here in these Nigerian children. No breakdown of prevalence for each age group was indicated in the paper and therefore it is difficult to know how robust the results for the 0-4 year age groups are as the prevalence of *A. lumbricoides* may have been much lower in this age category. Levamisole is an immune response regulator, modulating the immune function at a dose of 2-5 mg kg^{-1} body weight (Sajid *et al.*, 2005). The dose of levamisole administered in the Brutus study was 3 mg kg^{-1} for children and 150 mg in adults and thus could have modulated the immune response and effected the results of the study.

An observational field-based, case-control study in rural Senegal investigated the relationship between *A. lumbricoides* and severe malaria in children (Le Hesran *et al.*, 2004) demonstrating that children infected with *A. lumbricoides* had an increased risk of severe malaria. This contrasts with the protective effect of *A. lumbricoides* on uncomplicated malaria found in our study. Results from hospital-based studies in Thailand show similar protective effects of *A. lumbricoides* on malaria (Nacher *et al.*, 2000; 2001c; 2002b). Through a retrospective case-control study of individuals with or without cerebral malaria, Nacher *et al.* (2000) showed that *A. lumbricoides* was associated with a protective, dose-dependent effect against cerebral

malaria among an adult population. The protection associated with *Ascaris* ranged from 40% to 70% depending on whether exposure to *A. lumbricoides* infection was measured qualitatively or quantitatively. In contrast, this Nigerian study observed no dose-dependant effect; Figures 5.6 and 5.15 demonstrate that the prevalence of malaria was similar for subjects with light or moderate infections with *A. lumbricoides*. It is important to note that in Nacher's study the majority of *Ascaris* infections were of light intensity; only three adults had moderate infections and one adult had a heavy infection. A subsequent study by Nacher and colleagues showed a protective association of helminths, with a dose-effect trend, against cerebral malaria even after controlling for nutritional status and personal protection measures against mosquito biting (Nacher *et al.*, 2002a). The helminth species included *A. lumbricoides*, *T. trichiura*, *S. stercoralis*, and hookworm.

In contrast to Nacher's previous studies on severe malaria, a study on uncomplicated malaria showed that helminths led to an increased risk of *P. falciparum* in Thai adults (Nacher *et al.*, 2002c). Unlike most of Nacher's studies that have been hospital-based, and undertaken in Thai adults with severe malaria, this study was field-based in a mountainous region bordering Myanamar in an area of low malaria transmission. A similar finding was shown in a Senegalese case-control study by Spiegel *et al.* (2003) where children, aged 1-14 years, infected with soil-transmitted helminths, had an increased risk of clinical malaria compared to those uninfected. The contrasting results between these studies and ours may reflect the difference between single helminth species infections and multiple helminth species infection on malaria infections. Differences in malaria immunity, owing to different transmission settings, in the Nigerian and Thai population may also have contributed to the contrasting results.

Observational studies examining the relationship between malaria and other helminth species, notably schistosomes show contrasting results. In comparison to our study, Lyke *et al.* (2005) showed an age-dependent protective effect of *S. haematobium* on malaria. Malian children aged 4-8 years who were positive for schistosomiasis demonstrated delayed time to first clinical malaria infection, fewer number of malaria episodes, and lower geometric mean parasite densities at first infection when compared with schistosomiasis-negative children. No association between schistosomiasis and *P. falciparum* malaria was observed in children 9-14 years of age. In contrast, a Senegalese study showed that the incidence of clinical malaria was

significantly higher in children, aged 6-15 years, infected with *Schistosoma mansoni* than those uninfected (Sokhna *et al.*, 2004). The incidence rate of malaria attacks was higher for children with either a high or low helminth load. Conversely, a lower attack rate was observed in children presenting with a medium egg load, although this opposite trend did not reach significance. Similar to the results of the study by Lyke and colleagues (2005), a Senegalese study showed that children, aged 3-15 years, lightly infected with *S. haematobium*, had lower *P. falciparum* densities, although this was not statistically significant (Briand *et al.*, 2005).

The present double-blind placebo-controlled trial of anthelminthic treatment focused on a high risk age group. The results show an age-dependent protection of *A. lumbricoides* against malaria in children aged four years. The two other intervention studies (Murray *et al.*, 1978; Brutus *et al.*, 2006) showed a similarly protective effect of *A. lumbricoides* on malaria even though the studies differed in study design, age of subjects, geographical location and malaria transmission setting. These results have potentially important implications for public health. Based on the results shown here and results presented by Brutus and colleagues (2006), large scale deworming programmes could potentially increase malaria morbidity in children aged 4 years, and possibly older children and adults in areas where *Ascaris* in endemic.

Recently, the potential opportunities for combined control of malaria and helminths have been recognised (Hotez *et al.*, 2006c). Efforts are already underway in Central Nigeria where the distribution of insecticide-treated bed nets has been combined with mass drug administration for lymphatic filariasis and STHs (Blackburn *et al.*, 2006). This integrated management of malaria and helminths could avoid possible deleterious effects of mass distribution of anthelminthic drugs on malaria. Nevertheless, there is still much we do not know about helminth-malaria interactions. While the results of this study illuminate interactions between *A. lumbricoides* and malaria in children aged 1-4 years, we do not know what the outcome of these interactions will be in populations of different geographical settings in areas of low malaria transmission. In addition to this, observational studies with other helminth species have shown contrasting results, which are indeed quite plausible as different helminths and protozoa have synergistic and antagonistic interactions (Christensen *et al.*, 1987).

CHAPTER 6: General discussion

6.1 General Discussion

The overall aim of this study was to investigate the relationship between STHs and malaria in preschool children. In order to achieve this objective, a pilot study was carried out in three semi-urban villages in Ile-Ife, Osun state, Nigeria, to determine a good location to study these interactions. Following this pilot study, a double-blind placebo-controlled randomised trial of anthelminthic treatment was conducted in children aged 1-4 years. Previous studies examining the relationship between helminth and malaria co-infections have utilised various study designs which do not demonstrate causality. Out of the two intervention studies that have taken place, one study has been discounted owing to poor study design (Murray *et al.*, 1978) and the second study used levamisole as the choice of anthelminthic which has immunomodulatory properties (Brutus *et al.*, 2006). To my knowledge, no study has yet examined the effect of anthelminthic treatment in preschool children.

The pilot investigation of intestinal parasites in children aged 0-2 years revealed a high prevalence of *A. lumbricoides* (24.7%) in children aged 1-2 years and a high prevalence of *Cryptosporidium* sp. (27.1%) in children aged 0-2 years. Although children aged 1-2 years are now considered to be eligible for anthelminthic treatment (WHO, 2003b), there is much need to develop strategies on how best to deliver treatment to these children. In addition to this, more research should be undertaken to establish the optimal dosages of anthelminthics in order to maximise the efficacy of these drugs in children under 24 months (WHO, 2003b).

Overall, Phase I and II have highlighted several important issues concerning the conduct of longitudinal epidemiological field studies including: (1) the choice of study location; (2) compliance and cultural issues; (3) passive surveillance, and (4) the availability of technical malaria expertise. When choosing a location to study the relationship between helminth and malaria co-infections it is important that the prevalence of both these micro- and macro-parasite infections is high. This increases the rate of co-infection and the probability of detecting an effect of anthelminthic treatment. Results from the pilot study demonstrated that the prevalence of malaria and STH infection was high in children aged 1-2 years in this region. In addition, the predominant STH infection was *A. lumbricoides* which provided the

opportunity to study the effect of one STH species on malaria, discounting other species of STH which may have a different interaction with malaria.

As no previous work was carried out in these villages, the pilot study was essential as a precursor to the longitudinal study to build relationships with the Obas (village leaders) and the mothers as well as providing a platform to work out the logistics of setting up a field-based study. This pilot study also provided the opportunity to preempt the issue of poor compliance with stool samples by providing incentives. However, the introduction of these incentives caused some mothers to be suspicious and may have affected overall compliance. If the link with the villages had been well established for many years, the risk of such a situation occurring would have been greatly reduced as relationships would have been well developed. The Dielmo Project in Senegal is a good example of an established project. It was initiated in 1990 and consisted of long-term investigations on malaria immunity in a Senegalese village (Trape *et al.*, 1994). In such an established setting, fieldworkers would be more familiar with the culture of the people and routines of the community which would improve mobilisation of mothers and children.

In the current study, compliance was poor, the percentage of children that complied with all time points was low and there was also a lack of attendance at the malaria daily clinics. Loss to follow-up is a recognised problem of longitudinal studies (Holland *et al.*, 1996a). Moreover, the people's perception of malaria and worms as insignificant diseases may also have contributed to this lack of compliance. Perceptions of parasitic diseases, such as malaria, in the local community are important to the success of control programmes (Klein *et al.*, 1995; Adongo *et al.*, 2005). Before the fieldwork began, the objectives of the study were relayed to the community to ensure that everyone understood the reasons for the study. In addition to this, mothers were also educated on the rationale for collecting stool samples, taking finger prick blood samples, and taking anthropometric measurements, on entry into study and every time point thereafter. This task was given to a particular fieldworker who answered any questions posed by mothers. Notwithstanding, our efforts to inform the mothers may not have been sufficient. The low attendance at the malaria daily clinics underlines the ineffectiveness of passive surveillance in a widely dispersed community. Other factors that contributed to poor compliance, besides perceptions of disease, include: (1) access to daily clinics, (2) perception of primary health care services in the locality and (3) lack of incentives other than

free medical care. This emphasises the need to better understand the role that cultural perceptions of disease play in the control of parasitic infections. When future studies are conducted in 'new' communities, a social scientist should be employed to identify factors that may hinder compliance and devise methods to overcome these issues (Williams and Jones *et al.*, 2004).

Study participants were not randomly selected from the community. Achieving a random sample of study participants in this field setting would prove very difficult owing to the widely dispersed nature of these semi-urban communities, and to the restricted age group being studied. Nevertheless, this is common in epidemiological studies (Spiegel *et al.*, 2003; Sokhna *et al.*, 2004; Briand *et al.*, 2005; Shapiro *et al.*, 2005; Brutus *et al.*, 2006) and the large sample size, small age range and randomised design make this a formidable study. When dealing with older school-age children it would be feasible and better to consider a randomised school-based design as this might overcome the issue of a non-random sample and should improve compliance considerably (Cooper *et al.*, 2006).

The diagnosis of malaria, using peripheral blood smears, has not been a straightforward process in relation to both the preparation and reading of the smears. The issues raised during this present study concerning the malaria technicians hired during Phase I and the reading of blood smears by Nigerian and Kenyan laboratories stress the need for quality control in malaria diagnosis in endemic regions. PCR analysis has the best sensitivity and specificity for malaria parasites when compared to other diagnostic methods including microscopy (Hanscheid and Grobusch, 2002; Haditsch, 2004). Although PCR tests are expensive and time consuming, they should be a prerequisite in this type of epidemiological study providing an important back up for microscopic analysis. Furthermore, the methodological issues presented here draw attention to the lack of malaria expertise on the ground when the field work was being conducted. Similar studies in the future should include a collaboration with an indigenous malaria research facility; this may ameliorate some of the issues experienced throughout the present study. The current study also clearly reveals the usefulness of the RDT in areas where microscopy and other diagnostic techniques are unavailable.

The investigation of deworming showed that repeated four-monthly anthelminthic treatments were successful in reducing the prevalence and intensity of *A. lumbricoides* infections in

children aged 1-4 years. Epg was used as an indirect measure of prevalence and intensity of helminth infections. Getting a true measure of worm burden by expelling the worms from the gut is very difficult, time-consuming work and is not feasible for such large-scale studies. The interpretation of faecal egg counts (FEC) in helminth infections and the quantification of the effect of control strategies have been debated (Montresor, 2007; Morgan and Coles, 2008). The use of geometric means (GM) in interpreting FEC normalises the variance of the data, allowing the use of standardised statistical tests to estimate the significance of the result obtained. The use of GM does not adequately capture changes in FEC occurring in the group with high egg counts (Montresor, 2008). Individuals with higher egg counts are of particular interest because they are at a higher risk of disease, thus, the objective of control programs is to reduce morbidity by decreasing worm burden (WHO, 2005a). Montresor suggests that a proper evaluation of control strategies should involve arithmetic mean (AM) and an evaluation of the changes in the class of intensities (i.e. light, moderate, and heavy). Morgan and Coles (2008) suggest using two alternative methods: bootstrapping and replacement and generalised linear models. The results for FEC in this study were presented in AM and the rmANOVA analysis was conducted with log-transformed FEC data. The 90% reduction in the mean epg in the treatment group when compared to the placebo group at the end of the study period clearly demonstrates the effectiveness of repeated four-monthly anthelmintic treatments. The change in class of helminth intensity after treatment was not examined in this study because the majority of the infections in these young children were of light intensity.

The results from this double blind placebo-controlled randomised trial of anthelminthic treatment indicate that *A. lumbricoides* has a potentially protective effect against malaria in children aged four years. While *Ascaris* may make the host less susceptible to malaria we were not able to prove that this protection extended to malaria attacks. This result was found in the first data set concerning children that were followed up for 16 months which suggests that four rounds of anthelminthic treatment are needed to show an effect on the frequency of children presenting with malaria. It is clear from figure 5.5 and table 5.3 that overall the prevalence of malaria was higher in the children aged four years than other age groups. This was also demonstrated in the second data set concerning children who were followed-up for 12 months, Table 5.6 shows that the percentage of children who had malaria one or more times was higher in the treatment group when compared to the placebo group for children aged four years, although this was not statistically significant. The issues surrounding malaria

diagnosis preclude the opportunity to support this result with parasitaemia data at this time. Despite the fact that the significant effect of anthelminthic treatment on the incidence of malaria was shown only for children who were followed-up for 14 months, a robust linear mixed effects model analysis, which controlled for village and sex, still highlighted that children aged four years in the treatment group were 2.5 times more likely to have malaria when compared with children in the placebo group. Caution should be exercised when making inferences about the implications of anthelminthic treatment in children aged four years and its potential effect on malaria morbidity.

No observable trend was detected in the prevalence of malaria in subjects defined as having light or moderate intensity *A. lumbricoides* infections (Figures 5.6; and 5.15). However, when examining the prevalence of malaria attacks in subjects with light and heavy *Ascaris* infections, subjects with light intensity infections generally have a higher prevalence of malaria attacks when compared to subjects with moderate intensity infections (Figures 5.10 and 5.19). This trend may be an artefact and due to small sample size of subjects defined as having moderate intensity infections.

The protective effect of *Ascaris* against malaria was age-dependent; no protection from malaria was shown in children aged 1-3 years. The reason underlying the activation of this *Ascaris* associated protective response from malaria in children aged four years may be explained by the slow development of immunity to malaria (Baird, 1998) rather than the higher intensity of *A. lumbricoides* infections in children aged four years. The rmANOVA showed that there was no main effect of age or group*age interaction in the between-subjects effects analysis for the groups of children that complied with five assessments. Therefore, epg did not vary among age groups. However, when groups of children that complied with only four assessments were examined, the analysis showed that children aged three and four years had a higher epg than children aged one year. If the protection afforded by *Ascaris* from malaria was caused by the increase in intensity of *Ascaris* infections in children aged four years then it would be reasonable to expect that this protection would also be afforded to children aged three years.

The results shown in this present study suggest that children in this region of Nigeria develop a stable immune response to malaria at the age of four years. It is possible that an equilibrium

develops between these two micro- and macro-parasites in children aged four years and that the anthelminthic treatment may have disrupted this equilibrium. The 'slow' acquirement of immunity to malaria refers to the consistent pattern of age-related prevalence and density of parasitaemia i.e. the prevalence and density of parasitaemia gradually diminishes throughout life beyond 3-10 years of age (Baird, 1995). In West Africa, studies have shown that the percentages of death as a result of malaria gradually increase from birth until age 4 ½ years, and then drops sharply (Greenwood *et al.*, 1987). A study on the epidemiology of malaria infections in Kenya showed that as children enter their third year, they begin to develop an ability to significantly reduce the occurrence of high density malaria infection (Bloland *et al.*, 1999). The average monthly parasite prevalence peaked in the 2-3 year age group and decreased thereafter. The age-dependent result in our double-blind placebo-controlled randomised trial compares well with the study by Brutus and colleagues (2006) who demonstrated that subjects more than four years of age, treated with levamisole had a significant increase in their *P. falciparum* densities compared with controls, whereas there was no effect of anthelminthic treatment on children aged six months to four years. It is interesting to note that Brutus *et al.* used repeated two-monthly intervals of anthelminthic treatment versus four-monthly intervals of anthelminthic treatment in our study. Therefore, longer frequencies of anthelminthic treatment can also have a significant effect on malaria infections. Four-monthly treatments are also a better representation of the delivery frequency of anthelminthics in deworming control programmes (WHO, 2002).

In conclusion, this investigation clearly shows a negative interaction between *Ascaris* and malaria in children aged four years and suggests that large-scale deworming programs could potentially increase malaria morbidity in this age group. This key finding indicates that the protection afforded by *Ascaris* is activated at four years, which is very important considering that children aged four years are just about to enter primary school and are more likely to receive anthelminthic treatment. The benefits of anthelminthic treatment, such as improved health and growth status of preschool children (Crompton and Nesheim, 2002), could be undermined in Nigeria and other regions where both malaria and *A. lumbricoides* are endemic. The public health significance of these results is particularly important for Nigeria, which has a quarter of Africa's population (133 million), 45% of which comprises children less than 15 years of age (Federal Ministry of Health, 2005b). Malaria constitutes a serious public health problem in Nigeria. In 2005, from January to June, 239 600 malaria disease cases were

recorded in Osun state alone.

In 2001, the World Health Assembly passed a resolution urging member states to control the morbidity of STH infections through large-scale use of anthelminthic drugs for school-aged children in less developed countries (WHO, 2005a). It is fortunate that in recent years STHs are now beginning to receive attention as one of the world's neglected tropical diseases (NTDs) (Senior, 2005). It has been recognised that the burden of disease resulting from NTDs is similar to that of malaria, HIV/AIDS, and tuberculosis (Hotez *et al.*, 2006c) and that there are opportunities for combined control of malaria and helminths (Hotez *et al.*, 2006c). Integrating control strategies for helminths and malaria would not only reduce the disease burden of these infections but would also reduce or prevent a possible potential increase in malaria morbidity imposed by deworming programs in areas where *A. lumbricoides* is endemic. Although this study has made a significant contribution to the discussion on helminth-malaria interactions it has also highlighted a number of issues that concern the conduct of future studies in this field of research. It is important that future intervention studies, investigating the relationship between malaria and helminths, should have a long follow-up of at least 16 months. Future studies should also focus on the age at which this *Ascaris* associated protective effect is activated using a bigger sample size and also incorporating the collection of blood samples throughout the follow-up for immunological analysis. In addition, malaria diagnosis should be more comprehensive, using parasitaemia from blood smears for prevalence and intensity of malaria (supported with PCR analysis) and fevers with a malaria attributable fraction. Furthermore, while *Ascaris* is associated with protection from malaria it is not known if this protection is afforded by other species of helminth. It is crucial that this topic receives more attention, in light of a renewed emphasis on deworming and the fact that integrated control programs for malaria and helminths have yet to be put into policy.

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APPENDIX 1: Phase I Questionnaire

Obafemi Awolowo University/University of Dublin
Child Health Project

I. Complete Pre-Interview Questions

(a) Date: _____

(b) Name of child: _____

(e) Name of mother:_____

(f) Household address:_____

(g) ID#: _____

II Complete Demographic Information

1. a. How old is your child now? _____(in months) b. DOB(DD/MM/YY)__/__/____
2. Sex of child: (1) Male ☐ (2)Female ☐
3. How many people live in your household? _____
4. Position of child in the family? (e.g. 2nd child born out of 5 children)_____
5. Is your house connected to the national electricity supply? (1) Yes (2) No
6. Is your house connected to a generator? (1) Yes (2) No
7. Does your house have a working fridge?
   (1)Yes
   (2) No If no: where do you store perishable food items (e.g. liquid milk)?
   _____
   _____
8. Where do you obtain drinking water for use at home? *(Tick all that apply)*

(1) Tap inside the house
(2) Tap outside the house (on property)
(3) Shared or community tap (including fetching water on campus and at water works)
(4) Protected or covered well
(5) Unprotected or uncovered well
(6) Bore hole
(7) River, stream
(8) Other (specify)

8.1 How do you prepare water at home Before drinking? *(Tick all that apply)*

1.	Boil
2.	Filter
3.	Chlorination
4.	Alum
5.	Allow to settle
6.	Nothing
7.	Other (specify)
99.	Don't know

9. What type of toilet do you use in the child's household? *(Tick all that apply)*

1.Flush toilet
2. Pit latrine
3. Ventilated pit latrine

4. None
91. Other (specify) (eg. Bush)
99. Don't know

10. How do you dispose of your refuse? (1) Open dumping ☐ (2) Burning ☐

11. How is your house constructed?

Floor		Walls		Roof	
1	Dirt	1	Concrete	1	Corrugated
2	Tiles	2	Mud	2	Tiles
3	Concrete	3	Wood	3	Thatch
4	Other	4	Other	4	Other

12. How many bedrooms are in your house? _____

13. Age of Mother_____ (years)

14. Are you married?

(a) No (go to question 16)

(b)Yes

15. If yes to 14, does your husband have any other wives (1) yes (2) No

15.1 If yes to 15, what is your position among the wives? _____

16. What is your religion?

(1) Christian
(2) Muslim
(3) Traditional
(4) Other (please specify)

17. What is the highest level of education of (child's mother)?*(One response only)*

1.	None
2.	Some Primary school (or modern 1)
3.	Completed Primary school
4.	Some 2° school (or modern 3)
5.	Completed 2° school exit exams
6.	Post-secondary

18. What is the mother's main occupation? *(One response only)*

1.	House/family work *(non-income generating)*	8.	Street vendor
2.	Farmer	9.	Artisan
3.	Business woman	10.	Civil servant
4.	Professional (please specify)	11.	Teacher
5.	Clerk *e.g. secretarial*	91.	Other(pls specify)_____
6.	Manual worker	93.	No response
7.	Student		

19. What is the highest level of education the child's father have achieved?*(One response only)*

1.	None
2.	Some Primary school (or modern 1)
3.	Completed Primary school
4.	Some 2° school (or modern 3)
5.	Completed 2° school exit exams
6.	Post-secondary

20. What is the father's main occupation? *(One response only)*

1.	House/family work *(non-income generating)*	8.	Street vendor
2.	Farmer	9.	Artisan
3.	Business man	10.	Civil servant
4.	Professional (please specify)	11.	Teacher
5.	Clerk *e.g. secretarial*	91.	Other
6.	Manual worker	93.	No response
7.	Student		

21. Do you cultivate food/have a farm? (1) Yes (2) No (If No. go to Q 24)
22. What do you cultivate (plant) list all?

_____

23. Do you use human faeces as a fertilizer? (1) Yes (2) No
24. Do you keep domesticated animals? (1) Yes (2) No
25. What animals do you keep?

1	Dog
2	Goat
3	Cow
4	Cat
5	Chicken
6	Other(s)

III Health Care

26. Do you seek health care when you or members of your family are ill? (1) Yes (2) No
27. Where do you go for health care?

1	General Hospital
2	Local health clinic
3	Private Hospital
4	Self medication
5	Chemist
6	Other

28. How often do you give your child medicine for worms?

1	Once a month
2	Every 3 months
3	Every six months
4	Once a year
5	Never given (go to Q 33)
6.	Can't remember
7	Other (pls specify)

29. When was the last time your child received treatment for worms? _____(in months)

30. What medicine did you give your child for worms the last time?
(Mother should describe medicine and dose if she does not know name and dose)
_____

31. Has your child suffered from any side effects from this medicine?
(1) Yes
(2) No (if no go to question 33)

32. What were the side effects? _____

33. Has your child ever excreted or vomited a worm? (1) Yes (2) No

34. How do children get worms?
_____
_____

35. What can be done to prevent children from getting worms?
_____
_____

36. Use of Mosquito Nets?

	(1)Yes	(2)No
Window net		
Door Net		
Bed Net		

37. Do you use insecticide in your home? (1) Yes (2) No

38. Has your child ever had a fever? (1) Yes (2) No

39. When was the last time your child had a fever? _____days/weeks/months

40. Did the fever follow immunisation? (1) Yes (2) No

41. Did your child receive any treatment for this fever? (1) Yes (2) No

42. Where did your child receive treatment?

1	General Hospital
2	Local health clinic
3	Private Hospital
4	Self Medication
5	Chemist
6	Other

43. What treatment was prescribed for your child?
(Drugs/injections)? _____

44. If self medication which drugs did you buy (list all)? _____

45. Has your child ever had blood transfusion? (1) Yes (2) No

46. If yes to 45, why was he transfused? _____

IV Infant feeding
(For children 6 months and above)

47. How did you feed your baby between 0-6 months? (Probe to get the most truthful answer)

Item	yes	no	Age commenced
(1) Breast milk			
(2) Water			
(3) Herbal concoctions			
(4) Infant formula			
(5) Pap			
(6) Pre-Packaged cereal (e.g. cerelac, nutrend)			
(7) Others (please specify)_____			

48. How did you feed your child from age 6 months?

Item	yes	no	Age commenced
(1) Breast milk			
(2) Herbal concoctions			
(3) Infant formula			
(4) Pap			
(5) Pre-Packaged cereal (e.g. cerelac, nutrend) Pap			
(6) Semi-solid food			
(7) Family food			
(8) Others (please specify)_____			

49. Is your child still breast feeding? (1) Yes (2) No

50. If no what was your child's age when you stopped breast feeding? _____(in months)

51. Did you ever breast feed exclusively? (1) Yes (2) No 51a. If yes, how long?_____(weeks/months)

(For children less than 6 months old)

52. How are you feeding your baby? (Probe to get the most truthful answer)

Item	Yes	no	Age commenced
(1) Breast milk			
(2) Water			
(3) Herbal concoctions			
(4) Infant formula			
(5) Pap			
(6) Pre-Packaged cereal (e.g. cerelac, nutrend)			
(7) Others (please specify)_____			

53. Did you ever breastfeed your child exclusively? 1. Yes 2. No

54. If yes to 50, how long_____ (weeks/months)?

APPENDIX 2: Sample Size Calculation for Phase II

The sample size calculation was based on estimates from Phase I. This formula was used to determine what sample size was needed to detect a statistically significant difference at 5% with a power of 90% between the groups. Where d is the smallest difference considered to be of scientific importance. F was taken from the table 1 below.

Table 1. Taken from 'A guide to calculating sample size, Research and development office'. Great Ormond Street Hospital for Children NHS trust and the institute of child health.

		Power required:			
		80%	90%	95%	99%
Significance	0.100	6.18	8.56	10.82	15.77
Level required	0.050	7.85	10.51	12.99	18.37
(P-value):	0.025	9.51	12.41	15.10	20.86
	0.010	11.68	14.88	17.81	24.03

Difference between two percentages

$$n > \frac{F[p_1^{\%}(100- p_1^{\%}) + p_2^{\%}(100- p_2^{\%})]}{d^2}$$

The aim was to estimate a sample size to detect the percentage change in malaria attacks for patients with *Ascaris* treated with albendazole. 11.1% of patients who were infected with *A. lumbricoides* suffered malaria attacks. This percentage was reduced by 7.1% (to 4%) in patients who were not infected with *A. lumbricoides* ($p_1^{\%} = 11.1, p_2^{\%} = 4, d = 7.1, F = 10.51$)

$$n > \frac{10.51[11.1(100- 11.1) + 4(100- 4)]}{7.1^2}$$

$n = 286$

The total sample size needed was 572 (286 in each group).

1.4% of the population in Phase I were severely malnourished; taking into account that severely malnourished children would be screened out of the study, the sample size was increased to 580. 42% of the participants did not return faecal samples in Phase I. Therefore the sample size was further increased by 244 to reach a figure of 824.

APPENDIX 3: Phase II Questionnaire

Obafemi Awolowo University/University of Dublin
Child Health Project

I. Complete Pre-Interview Questions

(a) Date: _____

(b) Name of child: _____

(e) Name of mother:_____

(f) Household address:_____

(g) ID#: _____

(h) Mother/Father's mobile number: _____

II Complete Demographic Information

55. a. How old is your child now? _____(in months) b. DOB(DD/MM/YY)__/__/____

56. Sex of child: (1) Male ☐ (2)Female ☐

IIa Socioeconomic status

57. Is your house connected to a generator? (1) Yes (2) No

58. Does your house have a working fridge? (1) Yes (2) No

59. Does your house have a television? (1) Yes (2) No

60. Does your house have a radio? (1) Yes (2) No

61. What type of fuel do you use in your home for cooking most of the time? *Please tick one only*

(1) Gas
(2) Firewood/coal
(3) Kerosene
(4) Electricity
(5) Other, Please specify

62. Mobile phone?

(1) self
(2) husband
(3) both
(4) None

63. Which of these sources is the main source of household income (i.e. by which source does most of your money come?) *Please tick one only*

(1) Pension
(2) Wages/salaries from job
(3) Earnings from selling, trading or hawking
(4) Income from rental property
(5) Others, Please specify

64. Approximately how much money is available for your household in one month?
N.....

65. What type of toilet do you use in the child's household? *(Tick all that apply)*

1.Flush toilet
2. Pit latrine
3. Ventilated pit latrine
4. Bush
5. Other (specify)

66. Where do you obtain drinking water for use at home? *(Tick all that apply)*

(1) Tap inside the house
(2) Tap outside the house (on property)
(3) Shared or community tap (including fetching water on campus and at water works)
(4) Protected or covered well
(5) Unprotected or uncovered well
(6) Bore hole
(7) River, stream
(8) Other (specify)

12.1 How do you prepare water at home Before drinking? *(Tick all that apply)*

8.	Boil
9.	Filter
10.	Chlorination
11.	Alum
12.	Allow to settle
13.	Nothing
14.	Other (specify)

67. Age of Mother_____ (years)

68. What is the highest level of education of (child's mother)?*(One response only)*

1.	None
2.	Some Primary school (or modern 1)
3.	Completed Primary school
4.	Some 2° school (or modern 3)
5.	Completed 2° school exit exams
6.	Post-secondary

69. What is the mother's main occupation? *(One response only)*

1.	House/family work *(non-income generating)*	8.	Street vendor
2.	Farmer	9.	Craftsperson (carpenter, Fashion designer)
3.	Business woman	10.	Civil servant
4.	Professional (please specify)	11.	Teacher
5.	Clerk *e.g. secretarial*	12.	Other(pls specify)_____
6.	Manual worker	99.	No response
7.	Student		

70. What is the highest level of education the child's father has achieved?*(One response only)*

1.	None
2.	Some Primary school (or modern 1)
3.	Completed Primary school
4.	Some 2° school (or modern 3)
5.	Completed 2° school exit exams
6.	Post-secondary

71. What is the father's main occupation? *(One response only)*

1.	House/family work *(non-income generating)*	8.	Street vendor
2.	Farmer	9.	Craftsperson (carpenter, Fashion designer)
3.	Business man	10.	Civil servant
4.	Professional (please specify)	11.	Teacher
5.	Clerk *e.g. secretarial*	12.	Driver
6.	Manual worker	13.	Other(pls specify)_____
7.	Student	99.	No response

72. Do you cultivate food/have a farm? (1) Yes (2) No

73. Do you keep domesticated animals? (1) Yes (2) No

74. What animals do you keep?

1	Dog
2	Goat
3	Cow
4	Cat
5	Chicken
6	Other(s), please specify

III Health Care

75. When was the last time your child received treatment for worms? _____(in months) (if never, go to question 23)

76. What medicine did you give your child for worms the last time?
(Mother should describe medicine and dose if she does not know name) _____

77. Has your child ever excreted or vomited a worm? (1) Yes (2) No

78. Use of Mosquito Nets?

	(1)Yes	(2)No		
Window net				
Door Net			Ordinary	Insecticide treated nets
Bed Net				

79. Do you use insecticide in your home? (1) Yes (2) No

IV History of Illness

80. When was the last time your child had a fever? _____days/weeks/months (if never, go to question 28)

81. Did the fever follow immunisation? (1) Yes (2) No

82. Has your child ever had blood transfusion? (1) Yes (2) No (if no go to question 30)

83. If yes to 28, why was he transfused? _____

84. When was the last time your child received treatment for malaria? _____days/weeks/months (if never, you do not answer question 31)

85. What medicine did your child receive for malaria the last time?
(Mother should describe medicine and dose if she does not know name and dose)

_____

APPENDIX 4: Consent Form (in English and Yoruba)

Obafemi Awolowo University, Ile-Ife, Nigeria

Consent to Take Part in a Study titled: Geohelminth infection in children under 24 months: epidemiology, impact and strategies for control

Subject Information Sheet and Consent form for Legal guardian of Children

Instructions for Person Obtaining Consent:

1. Please read each section of the subject information sheet and consent form aloud to the legal guardian. Ask the legal guardian if he/she has any questions.

2. If the legal guardian agrees that his/her child should participate in the study, ask him/her to sign on the last page of the consent form on the line that says "signature of research subject." Ask the legal guardian to provide the date and print him/her name on the line that says "printed name of research subject."

   If the legal guardian is unable to sign his/her name, ask him/her to sign with a personal mark or place an X on the line that says "signature of research subject's legal guardian." Then, you will print his/her name on the line that says, "printed name of research subject's legal guardian."

3. Sign your name on the line that says "signature of person obtaining consent," provide the date, and print your name on the line that says, "printed name of person obtaining consent."

4. Hand the legal guardian of the participant his/her copy of the consent form. (This is the consent form that doesn't include a cover page.)

5. Take the signed copy of the consent form with you.

6. Tear off the first page (the cover page) of the signed consent form. Give the signed copy to any of the investigators. Throw away the first page.

Title of Study: Geohelminth and Malaria infection in children aged 12-60 months: epidemiology, impact and strategies for control

Principal Investigator (Name, Qualifications and address): Paddy Kirwan, B.A. (mod) Natural Sciences, Zoology; 1st Class, Department of Zoology, Trinity College Dublin, Dublin 2, Ireland.

Phone number: 002348033963156

Co-Investigators (Names and Qualifications): Prof. Samuel Ore Asaolu, Dr. Titilayo Abiona (medical doctor), and Prof. Celia V. Holland

Sponsor (If any)**:** Irish Research Council of Science, Engineering, and Technology and the Irish National Children's Hospital

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Your child is being invited to take part in an intervention study to treat helminths. The investigators listed above are in charge of the study; other professional persons may help them or act for them.

What are some general things you should know about research studies?

Research studies are designed to gain scientific knowledge that may help other people in the future. You may not receive any direct benefit from taking part. There may also be risks associated with taking part in research studies.

Your participation is voluntary. You may refuse to take part, or may withdraw your consent to take part in any study at any time, and for any reason. This will not affect your future care at any time and you will not suffer any disadvantages for refusing to participate. If you are a patient with an illness, you do not have to take part in research in order to receive treatment.

Details about this study are discussed below. It is important that you understand this information so that you can decide in a free and informed manner whether you want your child to take part. You will be given a copy of this consent form. You are urged to ask the investigators named above, or staff members who may assist them, any questions you have about this study at any time.

What is the purpose of this study?

The purpose of this research study is to assess the impact of treating young children with anti-helminthic drugs and to see if this will also have an effect on malaria. The information obtained from

this study will be used to show that treating young children for their helminth infections will also help them to cope with their malaria infections. Your child is being asked to part because he/she is aged between 12-60 months.

How many subjects will take part in this study?

If you decide to take part, your child will be one of about 2000 participants in this research study.

How long will your participation last?

Your participation in this study will last for 14 months.

What will happen if you take part in the study?

If you agree to take part, you will be required to provide information about your child at 0, 4, 8 and 12 months. Your child will also receive a tablet at 0, 4, 8 and 12 months. The tablet will contain either a drug which has effect on intestinal helminths or an inert substance which contains no active drug against helminths. Neither you nor the investigators will be able to tell which kind of tablet your child will receive. In addition to the above, your child will have his height and weight taken on each occasion. Anytime your child has a fever, you will report at the health centre where your child will have some investigations done (blood and stool examination) and be treated for the fever.

Are there any reasons you should not take part?

You do not have to take part in this study if you feel uncomfortable about your child receiving the tablets.

What are the possible risks or discomforts?

There are no physical risks to you if you take part in the interview. However, your child will experience some pain any time blood is being drawn from his finger. In addition, he/she may also suffer some disturbances like vomiting, diarrhoea when he/she takes the tablets.

If any side effects occur as a result of the tablets received during this study, your child will treated by a medical doctor without any cost.

What are the possible benefits?

By taking part in this study, your child will have the chance to be treated for helminths either every four months for one year if he/she is taking the active drug or at the end of 12 months if he is taking the inactive drug. He/she will also have his blood examined for malaria parasite and his stool for worms. The results of the study will be used to recommend whether children between 12-60 months should be routinely treated for worms. The results will also be shared with the State and Federal Ministries of

Health. Remember that what you tell us and the information we get from the tests we will do for your child will remain private and can never be linked back to you, as we explain in the next section.

How will your privacy be protected?

The interview, blood and faecal sample collection will take place in your village. You will not be identified in any report or publication about this study. All the information you give us from this study will be held private. This information will be accessible only to research personnel, who will review the information and use these for research purposes only.

Will it cost you anything to take part?

There will be no costs to you for taking part in this study.

Who is sponsoring this study?

Irish Research Council of Science, Engineering, and Technology and the Irish National Children's Hospital

What if you want to stop before your part in the study is complete?

You may stop and leave the interview at any time, without penalty.

What if you have questions about this study?

You have the right to ask, and have answered, any questions you may have about this research. If you have further questions you should contact

- Prof. S.O. Asaolu, Dept. of Zoology, Obafemi Awolowo University Ile-Ife, Mobile no: 08033963156
- Dr. Titi Abiona, Obafemi Awolowo University Teaching Hospitals Ile-Ife. Mobile No: 08037149725, 08034453466.

Subject's Agreement:

I have read the information provided above, or it has been read to me. I have had the opportunity to ask questions about it and any questions I have asked have been answered to my satisfaction. I consent voluntarily to participate as a participant in this study and understand that I have the right to withdraw from the study at any time.

_____ _____

Signature of Research Subject's legal guardian Date

_____

Printed Name of Research Subject's legal guardian

_____ _____

Signature of Person Obtaining Consent Date

_____

Printed Name of Person Obtaining Consent

Subject's Agreement:

I have read the information provided above, or it has been read to me. I have had the opportunity to ask questions about it and any questions I have asked have been answered to my satisfaction. I consent voluntarily to participate as a participant in this study and understand that I have the right to withdraw from the study at any time.

_____ _____

Signature of Research Subject's legal guardian Date

_____

Printed Name of Research Subject's legal guardian

_____ _____

Signature of Person Obtaining Consent Date

_____

Printed Name of Person Obtaining Consent

Fasiti Obafemi Awolowo, Ile-Ife, Naijiria

Yiyonda omo lati kopa ninu ise iwadii ti akole re n je: Aisan aran-inu ninu awon omode ti ojo ori won ko ti i pe odun meji pelu ona ati kapa re

<u>Ifinimona ati foomu fun eni to lase lori itoju omo</u>

Itosona fun eni to n gbase lodo olutoju omo:

1. Ka isori kookan ninu iwe ifinimona yii seti eni to lase lori itoju omo naa, ki o si beere lowo re bo ba ni ibeere lati bi o lori oro naa.

2. Bi olutoju naa ba yonda omo naa fun kikopa ninu akitiyan iwadii ohun, je ko kowo bowe loju-ewe to keyin nibi ti a ko "ikowobowe eni ti a n ye wo" si.

   Bi olutoju ohun ko ba le koruko ara re, je ko se ami x sibi ti a ko "ikowobowe olutoju eni ti a n ye wo" si. Leyin naa, ko akoja oruko re sibi ti a ko "akoja oruko" akoja oruko olutoju eni ti a n wo" si.

3. fowo si iwe naa nibi ti a a ko "ikowobowe eni to n wa iyonda" si, fi deeti si i, si ko akoja oruko araa re sibi ti a ko "akoja oruko eni to n wa iyonda" si

4. Fi eda iwe iyonda naa le eni to lase lori omo naa lowo. (Iwe iyonda naa ko ni ni eepo eyin ninu).

5. Mu edaa foomu igbayonda naa dani leyin ti won ba ti kowo bowe.

6. Ya oju-ewe akoko (eepo eyin iwe) akosile ti won ti kowo bo kuro. Mu foomu ti won ti kowo bo naa le eni kan lowo ninu awon ti yoo se ibeere fun iwadii. So oju-ewe akoko ti o ti fa ya nu.

Akole ise iwadii: Aisan aran-inu ati ibaa (Malaria) lara awon omo odun kan si odun marun-un: bo se n tan kale, bo se n gbile to, ati ogbon ti a fi n kapa won.

Olori Ise Iwadii: (Oruko, Eri ikunju-iwon, ati adiresi): Paddy Kirwan, B.A. (mod) Natural Sciences, Zoology; Onipo Kinni, Eka Ekoo Zoology, Trinity College Dublin, Dublin 2 , Ireland.

Nombaa foonu: 002348033963156

Awon Oniwadii yooku (Oruko ati eri imo): Ojogbon Samuel Ore Asaolu, Omowe (onisegun) Titilayo Abiona ati Ojogbon Celia V. Holland

Onigbowo (bo ba wa)**:** Irish Research Council of Science, Engineering, and Technology and the Irish National Children's Hospital

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A n ke gbajare pe ki omo re kopa ninu iwadii lori ona ti a fi n wo arun-un ara-inu. Awon oluwadii to n sakoso iwadii naa ni a ti daruko soke yen sugbon awon miran le ran won lowo tabi ki won sele de won.

<u>Kin lo ye ki o mo nipa iwadii oni-mo ijinle?</u>

Idi ti a fi n se iwadii onimo ijinle ni lati le ran araye lowo lojo iwaju. Eni to n kopa ninu iru iwadii yii le ma ri anfaani kan kan gunmo je lakooko iwadii ju pe oun pelu won lo jo n sise iwadii naa. O tile se e se ki akopa fara gba lakooko iwadii.

Dandan ko ni kikopa. O le ko lati kopa, o si le ba won bere, ki o waa yowo nigba to ba wu o ohunkohun to wu ko mu o yowo. Eyi ko di itoju re lowo lojo iwaju, ko si si akoba kan kan ti yoo se fun o lati je o niya pe o yowo. Bi aisan kan ba n se o, ki i se pe o gbodo kopa ninu ise iwadii yii ki a too le toju re.

Itupale ise iwadii yii wa nisale akosile yii. O ni lati mo idi iwadii ki o le fi tinutedo pinnu boya o fe tabi o ko ki omo re kopa nibe. A oo fun o ni edaa foomu ti oo fowo si bi o ba gba lati kopa. Bi ohunkohun ko ba ye o nibe, beere lowo awon oluwadii. Ko si igba ti o ko le se ibeere naa, bo tile se pe awon oluranlowo oluwadii lo beere lowo won.

<u>Kin ni afojusun iwadii yii?</u>

Iwadii yii fee ri aridaju ona itoju awon omode ti a n lo egboogi aisan aran-inu, boya itoju naa yoo tun wulo fun ikapa aisan ibaa (malaria). Abayo ti a ba ri ninu iwadii yii ni yoo je ka mo boya bi a ba lo

oogun ikapaa ara-inu a o le fi bee kapa ibaa (malaria) naa. B o le ti je pe ojo ori omo re ti le ni odun meji, ko si ti i pe odun marun-un, konge lo se fun atikopa ninu iwadii yii.

Omo meloo ni a o ye wo ninu iwadii yii?

Bi o ba gba lati kopa, omo re yoo je okan ninu egbaa (2,000) akopa ninu iwadii naa.

Bawo ni asiko ise iwadii naa yoo ti pe to?

Osu merinla (14) ni ise iwadii naa yoo gba o bi o ba kopa.

Kin ni yoo sele bi o ba kopa ninu ise iwadii naa?

A o foro wa o lenu wo lori omo re nigba to je omo jojolo, omo osu meriu, omo osu mejo, ati omo odun kan. Omo re yoo le gba oogun ni jojolo, lomo osu merin, lomo osu mejo, ati lomo odun kan. Oogun onihoro ni a oo maa fun omo naa. Oogun naa le je eyi ti yoo sise lori aran inu ifun, tabi eyi ti ko ni ise kan kan ti yoo se nibikibi debii pe yoo sise kan kan lori ara-inu. Ko seni ti yoo mo iru oogun onikoro naa ti a o maa fun omo re, bee naa si ni awon oluranwo oluwadii naa ko si ni mo. Bi a ba ti n fun omo ni oogun onihoro yi i la o maa won on bo ti ga si ati bo ti tewon to. Bi iba kan kan ba omo re, loo so fun won ni ile-iwonsan, won o si se ayewo eje ati igbonse omo naa, a o si se iwosan iba to mu un.

Se idi kan kan wa ti ko fi ye ki o kopa?

Tipatipa ko ni fun o lati kopa ninu ise iwadii yii, bi okan re ba n mi nipa ki omo re lo oogun naa.

Ewu tabi ijamba wo lo le su yo?

Ko sewu rara bi o ba kopa ninu iforo-wani-lenu-wo. Sa o, yoo dun omo naa die nigba ti a ba fabere gun un nika, nigba ti a ba n gba eje. O si se e se ki eebi o le omo naa, tabi ko yagbe gbuuru nigba to ba n loogun onihoro naa. Bi lilo oogun naa ba fa idaamu kan kan, dokita yoo setoju re lofee.

Ifa wo lo le ri nibe?

Osu merin merin ni a o maa lo oogun itoju aisan aran-inu fun omo to ba kopa ninu ise iwadii yii, fun odidi odun kan, a o maa lo oogun onihoro to kapaa aran-inu fun un. Bakan naa la o maa sayewo ejee re boya o ni kokoro aisan ibaa (malaria), bi a ti n sayewo igbonsee re fun aran. Abajade ayewo ti a ba se yoo je ka mo boya ka maa lo oogun aran fun omo olodun kan si marun-un. A oo tun fi abajade iwadii naa han Ile-ise Eto Ilera ti Ijoba Ipinle ati ti Ijoba Apapo, ki won o le mu un lo. Ranti o, gbogbo ohun ti o ba so fun wa nipa omoo re, ati ohun ti a ba ri ninu ayewo ti a n se, asiri patapata ni yoo je, ko si enikeni ti yoo mo pe latodo omoo re la ti ri i. A oo tun salaye siwaju si i nipa eyi.

Bawo ni a o se pa asiri naa mo?

Ni ileto yin la o ti se iforo-wani-lenu-wo, ibe naa la o si ti gba eje ati igbonse omo. Akosile ti a o se ko ni da omo kan kan yato. Asiri abonipon ni gbogbo ohun ti a ba gbo tabi ti a ba ri, awon osise iwadii nikan ni yoo si ri abajade iwadii naa ka, lati le mu un lo fun ise iwadii nikan.

Kin ni yoo na o lati kopa?

Ko si owo kan kan ti o o san ki o le kopa ninu iwadii naa.

Ta ni onigbowo ise Iwadii yi i ?

Awon omgbowo ni Irish Research Council of Science, Engineering, ati Technology ati the Irish National Children's Hospital

Bi kikopa ba su o, ti o fee yowo laipari iwadii n ko?

Igbakigba to ba wu o lo le yowo ninu ise iwadii naa, elemuu kan ko si, ko si si nnkan kan ti o o padanu.

Bi o ba fee beere nnkan kan nipa ise iwadii naa n ko?

Eto re ni lati beere, ati lati gba idahun, lori ohunkohun to ba ru o loju nipa ise iwadii yii. Bi o ba fee beere ohunkohun, kan si.

- Ojogbon S.O. Asaolu, Dept. of Zoology, Fasiti Obafemi Awolowo Ile-Ife. Ero ibanisoro alagbeeka: 08033963156
- Omowe Titi Abiona, Obafemi Awolowo University Teaching Hospitals Ile-Ife. Ero ibanisoro: 08037149725, 08034453466.

Iwee Mo-gba-lati-kopa:

Mo ti ka alaye lori ise iwadii yii, tabi won ka a si mi leti. Mo ti ni anfaaru lati beere ibeere lori re, won si ti dahun awon ibeere ti mo bi won, o si ye mi yekeyeke. Mo finnufedo gba pe n oo kopa ninu ise iwadii yii gege bi awon ti a o lo fun iwadii. Mo mo pe igbakigba to ba w mi ni mo le so pe n ko se mo.

_____ _____

Ikowobowe Eletoo lori Omo Deeti

_____

Akoja oruko Eletoo Lori Omo

_____ _____

Ikowobowe Eni to n gbase lowo Eletoo Deeti

_____

Akoja oruko Eni to n gbase

.....

Iwee Mo-gba-lati-kopa:

Mo ti ka alaye lori ise iwadii yii, tabi won ka a si mi leti. Mo ti ni anfaarni lati beere ibeere lori re, won si ti dahun awon ibeere ti mo bi won, o si ye mi yekeyeke. Mo finrufedo gba pe n oo kopa ninu ise iwadii yii gege bi awon ti a o lo fun iwadii. Mo mo pe igbakigba to ba w mi ni mo le so pe n ko se mo.

_____ _____

Ikowobowe Eletoo lori Omo Deet

_____

Akoja oruko Eletoo Lori Omo

_____ _____

Ikowobowe Eni to n gbase lowo Eletoo Deeti

_____

Akoja oruko Eni to n gbase

APPENDIX 5: Record sheet

Anthropometric and Parasitological Record Sheet

Date:_____

Name:_____ **ID number** _____**Age:** _____**(months)**

Body weight (to the nearest .1kg)**(1)**_____**(2)**_____**Mean** _____

Body Height (to the nearest .1cm)**(1)**_____**(2)**_____**Mean** _____

	Percentile	Z score
HAZ		
WAZ		
WHZ		

Temperature _____

Is the spleen enlarged? Yes ☐ **No**☐ **If yes** _____ **cm**

Haemoglobin _____**g/dl** (Screen out children below 5g/dl)

Malaria Rapid Test *P. falciparum* ☐ *P. falciparum* and other species ☐ Other species ☐ Negative ☐

Is the child suffering from Diarrhoea? **Yes** ☐ **No** ☐
(3 loose stools in the last 24 hours)
How many times has the child suffered from diarrhoea in the last 4 weeks?_____

Is the child presently suffering from any of the following symptoms of uncomplicated malaria?

Symptom	**Yes**	**No**
Fever		
Chills (feeling cold) & Rigors (shaking of the body)		
Headache		
Joint weakness/tiredness		
Pallor (paleness)		

Is the child presently suffering from any of the following symptoms of severe malaria?

Symptoms of severe malaria	**Yes**	**No**
Weakness		
Impaired consciousness		
Respiratory distress		
Multiple convulsions *(>2 seizures in 24 hrs with regaining of consciousness)*		
Severe anaemia *(Hb <5 gm/dl)*		
Circulatory collapse *(shock)*		
Abnormal bleeding *(disseminated intravascular coagulopathy)*		
Jaundice *(yellow discoloration of the eyes)*		
Haemoglobinuria *(Coca-Cola coloured urine)*		
Renal failure *(Urine output of less than 400 ml in 24 hours or <12ml/kg per 24 hours in children and a serum creatinine of more than 265 μ mol/l (> 3.0 mg/dl), failing to improve after rehydration)*		

Is the child suffering from severe malaria? Yes ☐ No ☐

Is the child suffering from an uncomplicated malaria attack? Yes ☐ No ☐

Coartem prescribed? Yes ☐ No ☐