The epidemiology of soil-transmitted helminths in Bungoma, Kenya, with an emphasis on immuno-epidemiology in a community receiving anthelmintic treatment

by

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ABSTRACT

Soil-transmitted helminth (STH) infections are endemic in Kenya, where preventive anthelminthic treatment has been provided to school-age children annually, through a school-based deworming (SBD) programme, since 2012. The aim of this thesis was to investigate the epidemiology and immuno-epidemiology of STH infections in a whole community with an ongoing SBD programme to assess the impact of control on the prevalence and intensity of infection, plus the distribution of remaining infection in treated communities.

This study was conducted in four rural villages near Bungoma, western Kenya. Samples were collected from 2,273 individuals between 2 and 93 years of age during two cross-sectional surveys in Mar-Apr and Aug-Sep 2014. Stool samples were analysed using Kato-Katz, blood samples were analysed using enzyme-linked immunosorbent assays (ELISA). 400mg albendazole was administered between the cross-sectional surveys to simulate a round of mass drug administration (MDA).

At study baseline, *Ascaris lumbricoides* and *Necator americanus* infections had 9.8% and 6.7% prevalence, which were reduced to 1.8% and 1.4%, respectively, three months post-MDA. STH infection was highly aggregated in the population, and clustering at household and village levels was observed for *A. lumbricoides* infection. Young age and poor sanitation were risk factors for *A. lumbricoides* infection, while old age, farming, and poverty-associated factors increased the risk for *N. americanus* infection. The MDA had 93.9% coverage, 98.9% and 91.5% cure rates for *A. lumbricoides* and *N. americanus*, respectively, and was particularly effective in reducing hookworm infection in the older age groups of the population. A positive correlation was observed between hookworm age-prevalence and *Necator*-specific IgG4 antibody prevalence. No significant changes in seroprevalence were found post-treatment. There was evidence of cross-reactivity between antigens. This study highlights the need for improved control strategies and diagnostic tools for STH infections, particularly as SBD programmes bring us closer to the “endgame” of transmission elimination.

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LIST OF ABBREVIATIONS

Ab	Antibody
AlExt	*Ascaris lumbricoides* PBS extract
AsHb	*Ascaris suum* haemoglobin
CHWs	Community Health Workers
CI	Confidence interval
CIFF	Child Investment Fund Foundation
CR	Cure rate
DALYs	Disability-adjusted life years
DEC	Diethylcarbamazine citrate
DOT	Directly observed treatment
ELISA	Enzyme-linked immunosorbent assay
EPG	Eggs per gram
ERR	Egg reduction rate
ES	Excretory/secretory (antigen)
ESACIPAC	Eastern and Southern Africa Centre of International Parasite Control
FEC	Faecal egg count
GAHI	Global Atlas of Helminth Infections
GPS	Global Positioning System
Hb	Haemoglobin
IFN-γ	Interferon γ
Ig	Immunoglobulin
IL	Interleukin
IQR	Inter-quartile range
IRR	Incidence-rate ratio
KEMRI	Kenya Medical Research Institute
LAMP	Loop-mediated isothermal amplification
LSHTM	London School of Hygiene & Tropical Medicine
M&E	Monitoring & Evaluation
MDA	Mass drug administration
Na-ASP-2	*Necator americanus Ancylostoma* secreted protein 2
Na-SAA-2	*Necator americanus* surface-associated antigen 2
NGO	Non-governmental organisation
OR	Odds ratio
PBL	Peripheral blood lymphocytes

PCA	Principal component analysis
PCD	Partnership for Child Development
POC-CCA	Point-of-care circulating cathodic antigen
QGIS ™	Quantum Geographic Information System
qPCR	Quantitative polymerase chain reaction
RDT	Rapid diagnostic test
SAC	School age children
SBD	School-based deworming
SD	Standard deviation
SES	Socio-economic status
SSC	Scientific Steering Committee
STH	Soil-transmitted helminths
TGF-β	Transforming growth factor β
VIP	Ventilated improved latrines
WASH	Water, sanitation and hygiene
WHO	World Health Organization

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Author's contributions

The author was involved in all stages of the work presented in this thesis. The study design was defined in collaboration with both supervisors and Alice Easton. The fieldwork and related activities were carried out equally by the author and Alice Easton, including writing the project proposals, preparing and managing the budget, recruiting, training and supervising the teams, day-to-day planning and preparation of materials, assisting the teams in the field and in the lab, data entry and data checks. The immunology lab work was entirely carried out by the author, with contributions from the individuals mentioned in the acknowledgments. The statistical analyses were carried out by the author, with guidance provided by colleagues and both supervisors. The writing of the thesis, including preparation of figures and maps, was carried out by the author, with revisions and suggestions from supervisors.

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Ethics Statement

Ethical approval for this project was obtained from the Kenya Medical Research Institute (KEMRI Scientific Steering Committee [SSC] no. 2687), the Kenyan National Ethics Review Committee, and the Imperial College London Research Ethics Committee (ICREC 13_3_10). Written or oral informed consent was obtained from all study participants and the parents/guardians of young children previous to participation in the study. All data collected were coded and treated confidentially.

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DECLARATION OF ORIGINALITY

I declare that the work in this thesis is the result of my own work and any quotation from, or description of the work of others is acknowledged herein by reference to the sources.

CHAPTER 1. INTRODUCTION

1.1. Helminth infections

Soil-transmitted helminth (STH) infections remain a significant public health problem, and affect mostly the poor rural communities, and urban slums, of the tropical and subtropical world. The most recent estimates indicated that in 2010, at least 1.45 billion people were infected with one or more STH species (1), the large majority living in Asia (Figure 1.1). The most common STH are the roundworm (*Ascaris lumbricoides*), hookworms (*Necator americanus* and *Ancylostoma duodenale*), and the whipworm (*Trichuris trichiura*). These species are generally regarded as a single neglected tropical disease (NTD) due to the extensive overlap in their endemic regions and populations affected, similarities in their life cycles, as well as sharing common diagnostic and treatment methods. As we shall see in this thesis, differences between the species might in fact warrant different approaches in terms of diagnostics and control, particularly when considering elimination.

STHs can cause chronic infections associated with abdominal pain, diarrhoea, general weakness and anaemia (mostly associated with hookworm), and may impair physical and intellectual development in children (2). The global burden of disease attributed to STH infections is estimated at 5.18 million disability-adjusted life years (DALYs) (1).

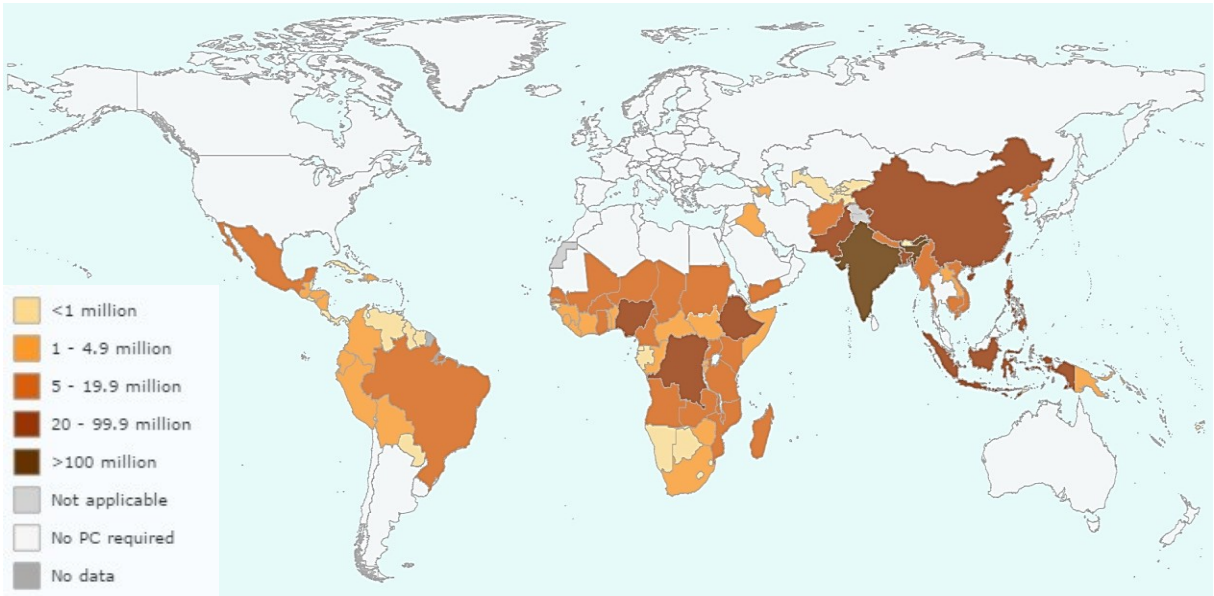


Figure 1.1 Global distribution of soil-transmitted helminths in 2014. Number of children (1-14 years old) requiring preventive chemotherapy for soil-transmitted helminthiases in 2014. Adapted from (3).

1.1.1. Life cycle and biology of soil-transmitted helminths

STH are a group of intestinal nematode parasites which are present in areas with suitable environmental conditions (e.g. warm temperatures and rainfall), poor hygiene and inadequate sanitation (4). Soil plays a central role in the transmission of all these infections, since it is necessary for the maturation of *A. lumbricoides* and *T. trichiura*'s eggs and the hatching of free-living hookworm larvae. Humans become infected through either ingestion of parasite eggs (e.g. in contaminated food or water, through direct or indirect contact with contaminated soil) or by the penetration of infectious hookworm larvae (L_3) through the skin (Figure 1.2).

Following ingestion, *A. lumbricoides* and *T. trichiura* eggs hatch in the host's intestine. *A. lumbricoides* larvae penetrate the mucosa of the small intestine and travel through the vascular system to the liver, heart, and lungs, where they ascend to the trachea, are swallowed, return to the small intestine and develop into adults. This pre-patent development period between ingestion of eggs and production of eggs by mature worms requires 10-12 weeks, and adult worms have a lifespan of 1-2 years (5,6). Adult *Ascaris* females are larger than the males (20-35 cm and 15-25 cm, respectively) and can produce up to 200,000 eggs/day (7). Fertilized *Ascaris* eggs excreted in stool become infectious after 2-8 weeks, depending on the environmental conditions, but are thought to be able to survive for up to 15 years (6–8). *T. trichiura* eggs, meanwhile, hatch and moult entirely in the large intestine, and mature worms burrow into the mucosal epithelium of the caecum or colon. The pre-patent period is estimated at 30-90 days (9). Adult *Trichuris* measure 3-5 cm, with females larger than males, and have a lifespan of 1-3 years (9,10). Mating *Trichuris* females produce 3,000-20,000 eggs/day, which have an embryonation period in the soil of 11-30 days in ideal conditions (10).

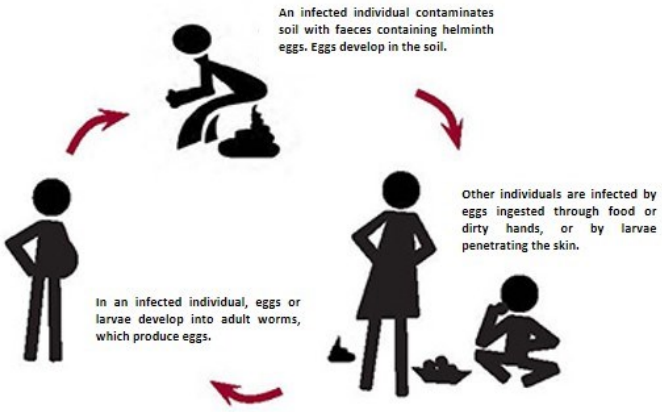


Figure 1.2. Transmission cycle of soil-transmitted helminths.
Source: http://www.who.int/intestinal_worms/disease/en/, Accessed: 15 Mar 2017

Hookworm eggs hatch in moist soil 1-2 days after being passed in faeces, and the resulting free living larvae mature into the infectious L3 stage in 5-10 days (11). L3 larvae can survive up to 4 weeks under favourable environmental circumstances, searching for high ground and even climbing vegetation, to penetrate the skin of people passing by (11). They then enter the blood stream and follow a lifecycle similar to *Ascaris,* involving heart and lung passage before reaching the small intestine. Infectious *Ancylostoma* larvae can also be ingested, penetrating the oral mucosa to initiate their migration (11,12). L3 larvae of both species develop into mature egg-laying adults 6-8 weeks after skin penetration, and have an average lifespan of one year for *A. duodenale* and 3-5 years for *N. americanus* (12,13). Mature *N. americanus* are smaller than *A. duodenale* (females: 9-11 mm vs. 10-13 mm, respectively), and the fecundity rate of *N. americanus* females is also smaller than that of *A. duodenale* (3,000-6,000 eggs/day vs. 10,000-20,000 eggs/day) (12,13).

1.1.2. Morbidity and burden of soil-transmitted helminths

When left untreated, STH can cause chronic debilitating disease, particularly in children and women of childbearing age (14). In 2010, 439 million people were estimated to be infected with hookworm, 819 million with *A. lumbricoides* and 465 million with *T. trichiura*, the majority in Asia, followed by Sub-Saharan Africa and Latin America and the Caribbean islands (Figure 1.1) (1). Since STH infections cause more disability than mortality, the burden of disease is best measured in DALYs. The most recent estimates attribute 5.18 million DALYs lost to STH, with 1.31 million due to *A. lumbricoides* infection, 3.23 to hookworm and 0.64 to *T. trichiura* (1). STH are generally co-endemic, and co-infections are very common (15) .

Morbidity is positively related to an individual's worm burden; while individuals carrying light infections are generally asymptomatic, those carrying larger numbers of worms can develop a range of conditions (16,17). Heavy *Ascaris* infection can cause vitamin A deficiency, acute intestinal obstruction (responsible for close to 10,000 annual deaths), and hepatobiliary and pancreatic ascariasis (5). Heavy *Trichuris* infection is specifically associated with finger-clubbing and "*Trichuris* dysentery syndrome", whose symptoms include rectal bleeding and rectal prolapse (10). Heavy hookworm infection is mostly associated with anaemia, protein malnutrition and poor birth outcomes, including low birth weight and infant mortality (11).

Anaemia is caused by the worms' direct disruption of the intestinal epithelia and capillaries to feed on the host's blood, as well as their competition for host resources in very heavy infections. *A. duodenale* is responsible for greater blood loss than *N. americanus*, and it is thus associated with higher prevalence of iron deficiency anaemia (11). However, because hookworm infection is generally co-

endemic with malaria and malnutrition, which are underlying causes of anaemia, it is difficult to attribute specific anaemia morbidity to hookworm infection.

All STH infections are also associated with abdominal pain, diarrhoea, malnutrition, and general weakness. In children, they are also associated with impaired growth and physical fitness, school absenteeism and poor cognitive performance, while longitudinal studies have also found associations with low productivity and reduced earnings in adult life, compromising the social and economic development of the population and effectively sustaining poverty (18,19). The extent of STH-derived morbidity in children – as the reasoning behind the advocacy for mass drug administration – has been (and remains) under debate in the so-called "worm wars". A systematic review of randomised control trials published by the Cochrane Collaboration in 2015 found "little or no effect" of mass deworming programmes in improving children's weight, height, haemoglobin levels and cognition, as well as school attendance (20). Recent replication studies of Miguel and Kremer's study conducted in Kenya in the 1990s could not replicate the finding of a spill-over effect of increased school attendance after deworming all children in nearby schools (21–23). However, deworming advocates and researchers have highlighted how longitudinal studies measuring the long-term effects of STH infection and treatment are often not long enough to find evidence of morbidity reversion, and generally fail to adequately take into account the intensity of infection as the key driver of morbidity (24,25). Also highlighted is the cost-effectiveness of mass drug administration programmes, and its capacity to reach lightly infected children which might be missed in a test-and-treat programme due to low diagnostic sensitivity (26,27). The choice of studies to include in systematic reviews, the statistical analyses applied and the divisions or groups under comparison all affect study outcomes but, as de Silva et al. put it, "lack of evidence to support effectiveness cannot be considered as evidence of ineffectiveness", and the debate continues as do national deworming campaigns the world over (24,25,28).

1.1.3. Diagnosis of soil-transmitted helminths

Adult female worms produce thousands of eggs per day, which are excreted in stool. The microscopic identification of eggs in faecal samples is the most common diagnostic method for STH infection. It assumes that the number of eggs detected in stool are proportional to the number of (female) worms harboured by the host, which can only otherwise be measured directly in expulsion studies (17). The Kato-Katz thick-smear is the most widely used egg-counting technique since its advent in the 1970s (29), and remains the method recommended by WHO guidelines (30). It consists in clearing with glycerol a standardized portion of stool (10 to 50mg, depending on the template used) to allow

visualization of eggs stained with methylene blue or malachite green in a glass slide under the microscope (31). The egg count is then multiplied by a given number (e.g. 41.7 mg stool x 24) to obtain an estimate of eggs-per-gram (EPG) of stool, used as the estimate of intensity of infection or worm burden. The wide use of Kato-Katz, particularly in field settings, reflects its low cost and ease of use, requiring only microscopes and reusable plastic kits (30). However, Kato-Katz has some limitations; the glycerol used in its preparation desiccates the outer layer of hookworm eggs in 30-60 minutes, imposing time constraints for accurate detection. The very small amounts of stool used, as well as their inconsistent solidity, reduce its sensitivity in low intensity infections (32–34). However, repeated Kato-Katz slides (from multiple stool samples or multiple sub-samples of the same stool) have been shown to increase the sensitivity of this method (32,35).

Other microscope-based techniques can be used, including the qualitative formalin-ether and diethyl-acetate concentration methods, the quantitative McMaster flotation method and more recently the FLOTAC and mini-FLOTAC techniques (36). These are often more sensitive, but require higher logistical input. Highly sensitive but more expensive molecular-based methods of DNA detection can also be used; genetic sequencing can be used as a qualitative tool, while multiplex quantitative polymerase chain reaction (qPCR) and loop-mediated isothermal amplification (LAMP) are still being validated as quantitative diagnostic methods (37–40). Molecular methods are generally more sensitive than microscope-based methods, but their use in field studies is limited (to date) because they require expensive equipment and solid infrastructure. Furthermore, serological assays such as the enzyme-linked immunosorbent assay (ELISA) are also being tested as possible diagnostic tools (41,42). They too are more sensitive than microscope-based methods but also require good laboratory infrastructure and the collection of more invasive blood samples. Besides, cross-reactivity between antigens of different STH species is common, which reduces the specificity of the test (43,44). Although not suitable for a national control level, or field-based diagnostic technique, ELISAs can help detect parasite exposure in very low intensity infections. In addition, they might provide information, following repeated treatments at a community level, on how exposure might be changing, particularly in young children and people migrating into endemic areas, under a national control programme which may be reducing transmission.

1.1.4. Control of soil-transmitted helminths

As previously mentioned, soil is a vital element in the lifecycle of STH. It is effectively the vehicle of transmission, thus any successful strategy designed to control STH infection must 1) prevent

contamination of soil with eggs passed in stool, and 2) prevent contact with contaminated soil, as well as contaminated water sources.

Water, sanitation and hygiene (WASH) is a collective term of interdependent issues associated with STH infections and other water- and sanitation-related diseases. It is used in the context of public health because the three sectors are dependent on each other, e.g. good sanitation is necessary to avoid contamination of water, and clean water is necessary for good hygiene practices (45). Soil (and water) contamination can be prevented by the construction and correct usage of improved sanitation facilities. Improved sanitation comprises 1) flush toilets or latrines connected to a piped sewer system, septic tank or pit, 2) ventilated improved pit (VIP) latrines, and 3) pit latrines with slab or cement floor. When built correctly, these sanitation facilities fulfil the requirements of separating human stool from human contact, as well as avoiding faecal contamination of surrounding soil and groundwater. Health education helps promote good hygiene practices essential to prevent STH infection. These include 1) handwashing with soap after using the toilet, after farming and before eating, 2) washing, peeling or cooking vegetables and fruit, 3) wearing shoes, and 4) not practicing open defecation. The water safety component of WASH consists in constructing improved drinking water sources, such as piped water into households, public taps, boreholes, protected wells or springs, and rainwater collection. When these are not available, or even in combination, boiling, filtering or treating water with disinfectant chemicals such as chlorine will also help prevent transmission of STH infection.

All these WASH-related issues are associated with poor socio-economic status, and are generally difficult to overcome in afflicted areas due to limited resources. In countries where improvements in WASH occurred naturally as an effect of economic growth (e.g. Japan, the USA, Italy), STH infections were consistently brought under control (46,47). In developing countries however, economic growth is slower and unequally distributed, and the financial resources and political effort directed at building and maintaining efficient improved sanitation and water facilities in endemic rural areas are limited. In these settings, control of STH infection through periodic deworming is thought to be the best strategy for morbidity and transmission control.

The World Health Organization (WHO) recommends regular deworming, through preventive chemotherapy, of high-risk sectors of the population in endemic countries with the goal of eliminating STH infections as a public health problem (i.e. reducing morbidity due to STH infection) (Figure 1.3) (14,30). Preventive chemotherapy consists in administrating anthelminthic drugs at regular intervals to endemic populations, which are defined through a population-based diagnostic survey (6). The main goal for the year 2020 is that at least 75% of school-age children (SAC, 5-14 years old) and preschool-age children (pre-SAC, 1-4 years old) in need of deworming worldwide receive treatment (48). The evidence base of this deworming strategy includes the fact that children generally harbour

the peak worm burden in the population, and that heavy-intensity infections are the major source of STH morbidity. Treating this high-risk section of the population should have a significant impact in both morbidity and transmission of STH infections. Additionally, since without change in environmental and behavioural conditions the prevalence of STH infection tends to return to pre-treatment (equilibrium) levels, this deworming treatment should be provided repeatedly over a period of time (6).

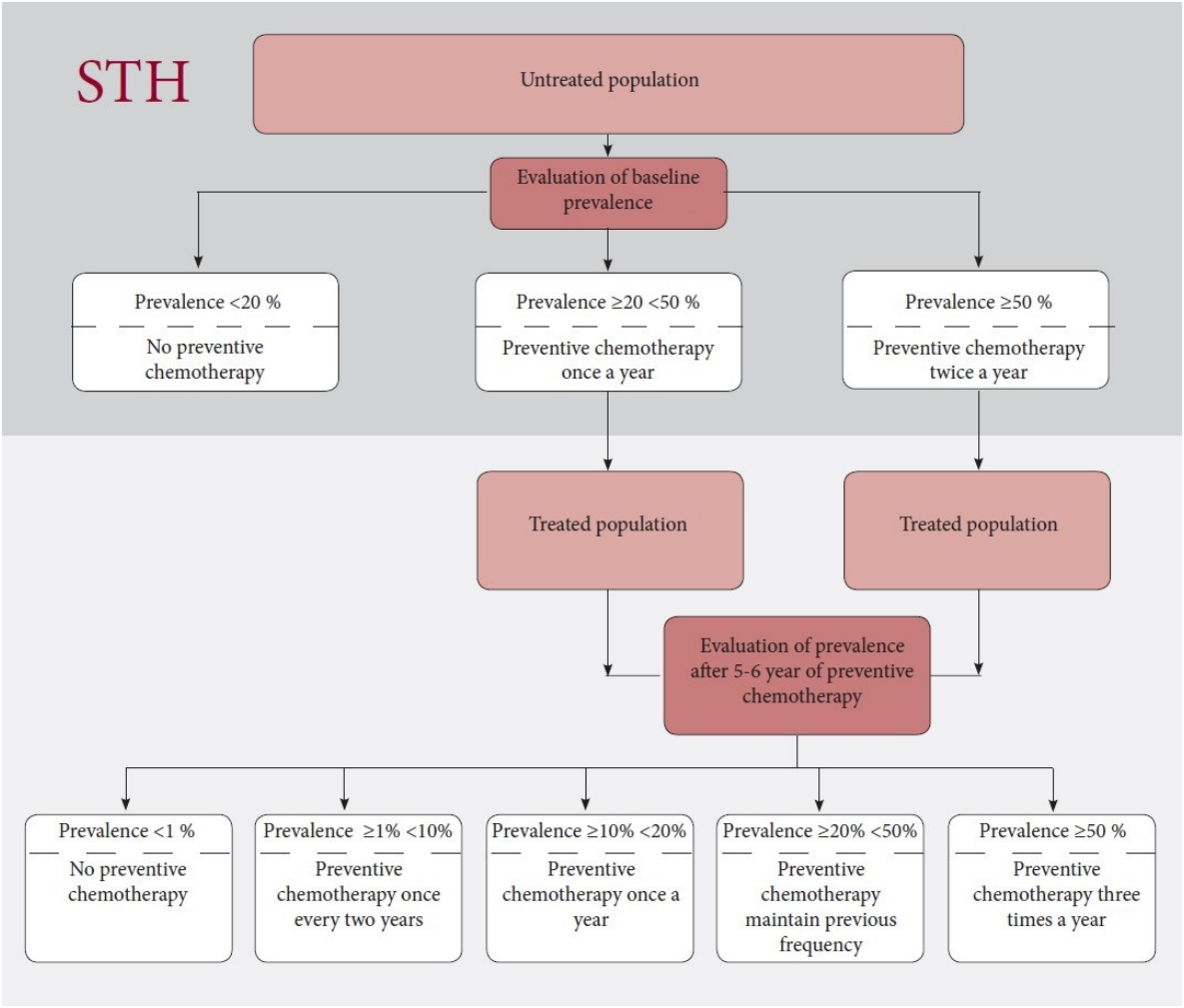


Figure 1.3. WHO decision tree for preventive chemotherapy programmes for STH infections. Adapted from (30).

Preventive chemotherapy interventions are possible because the deworming drugs used are safe, donated for free from large pharmaceutical companies via WHO, and can be easily administered by trained teachers and community health workers (CHWs) in schools in a very cost-effective manner, using a school-based deworming (SBD) strategy (49). In 2015, 66 countries reported delivering

preventive chemotherapy to over 400 million SAC, while 56 countries also dewormed just over 150 million pre-SAC (50).

The available drugs against STH, which may be administered alone or in combination, include the benzimidazoles (e.g. albendazole and mebendazole), levamisole and pyrantel (51). Albendazole and mebendazole are most commonly used in preventive chemotherapy, and are provided free of charge by GlaxoSmithKline and Johnson & Johnson, respectively, for use in STH control programmes worldwide (52,53). These two drugs were first approved for human use in the 1970s-80s and have very low toxicity, with most commonly reported mild and transient side effects such as abdominal pain (54). They both act by binding to the nematode tubulin, disrupting the formation of microtubules and inhibiting cell division, leading to worm death. The dying adult worms are expelled in stool, and larvae and eggs are also killed by albendazole and possibly mebendazole.

The efficacy of the drugs (treatment efficacy) can be measured by their cure rate (CR) – reduction in number of infected vs. non-infected individuals – and egg reduction rate (ERR) – reduction in faecal egg counts (55). Albendazole is generally highly effective against *A. lumbricoides* (CR=98.2%) and hookworm (CR=87.8%), but not as much against *T. trichiura* (CR=46.6%) (55). Mebendazole is also highly effective against *A. lumbricoides* (CR=96.5%), but has low efficacy against hookworm and *T. trichiura* (CR=22.9% and CR=23.0%, respectively) (56). Both albendazole and mebendazole can be administered in a single dose of 400 mg and 500 mg tablets, respectively, to adults and children from 2 years old. Pregnant women should not be treated, as there is not enough safety information from human studies and some teratogenic effects were observed in animal studies (57,58). Children between 1-2 years of age can receive a smaller dosage; a paediatric liquid formula is available and tablets can also be crushed and mixed with water when administering to small children (see Appendix II).

Lymphatic filariasis preventive chemotherapy control programmes have been running since the launch of the Global Programme to Eliminate Lymphatic Filariasis (GPELF) by the WHO in 2000, co-administering ivermectin and albendazole across whole endemic communities. As the lymphatic filariasis programmes reach their WHO goals and start to be scaled back, it is imperative that demand and drive are maintained for continued albendazole distribution in these communities, so that the fight against STHs does not start to slow down.

1.1.5. Transmission dynamics of soil-transmitted helminths

Soil-transmitted helminths display a characteristic aggregation of worms in the host population. This is best observed in expulsion studies, when worms expelled from a population of hosts following treatment are counted and individual worm burdens can be measured directly (59). The distribution of worms among the hosts generally follows the Pareto principle, where approximately 20% of the population harbours close to 80% of the total worm burden, while a substantial proportion of individuals carries only few or no worms (Figure 1.4a).

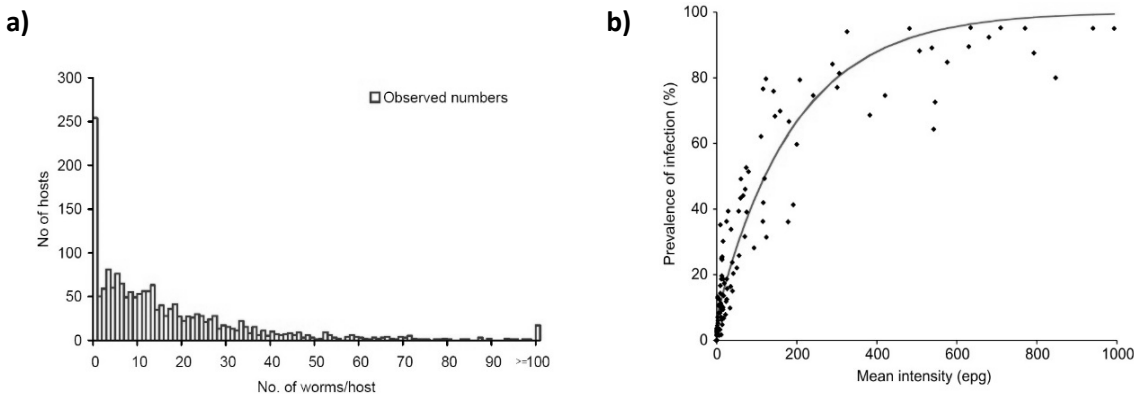


Figure 1.4. **Aggregation of parasite population creates density-dependent worm fecundity. a)** Frequency distribution of *A. lumbricoides* worms expelled post-treatment in 1,765 people in Bangladesh, showing aggregation of the parasite in the host population (60). **b)** Curve obtained by plotting the prevalence of *S. mansoni* infection and its associated average intensity (EPG) in a series of primary schools, showing the non-linear relation between prevalence and intensity of infection (61).

This distribution can be well described by the negative binomial probability distribution, where the parameter *k* provides an inverse measure of the degree of worm aggregation in the host population, i.e. smaller *k* values indicate higher worm aggregation. This aggregation is reflected in the prevalence of infection, where the degree of aggregation increases when the prevalence of infection decreases. As defined by the negative binomial distribution, prevalence *P* as a proportion is given by the equation $P=1-[1+M/k]^{-k}$, where *M* is the mean intensity of infection and *k* is the aggregation parameter (17).

Two mechanisms are thought to account for most of the worm aggregation in the population: host predisposition to heavy or light infection and/or heterogeneous environmental contamination. Individual predisposition can be described as a consistency in the pattern of reinfection after several chemotherapeutic treatments, when some individuals are consistently found to carry heavier worm burdens (62). These differences can be due to genetic, immunological and/or behavioural factors, which cause some individuals to be particularly “wormy” and thus be more likely to suffer STH-related

morbidity and mortality, as well as to make a greater contribution to transmission of infection. The clumping of infectious material in the environment can also contribute towards aggregation of infection, which can be manifested in various types of spatial clustering of infection, including at a household level (63,64).

The aggregation of the parasite population amplifies the density-dependent processes which regulate the parasite's sexual reproduction and fecundity. A series of worm expulsion studies have observed a reduction in the number of eggs produced per female worm when the number of worms per host increases, which is likely to derive from competition for nutrients and space in the intestine (65). This process is responsible for the non-linear efficacy of mass drug administration (MDA) programmes, where large reductions in average intensity of infection not always correlate with large reductions in prevalence or, in some cases, egg output (Figure 1.4b). When worm burdens are low, there is typically a reduction in transmission of infection due to an increase in the number of unfertilized eggs. Transmission of infection occurs only through ingestion of fertilized eggs, which requires sexual reproduction between female and male worms in the host. Due to aggregation of infection, the probability of male and female worms establishing themselves in the same host is reduced in very low prevalence settings. Indeed, mathematical models show the possibility of reaching a breakpoint in transmission through MDA, if the number of worms per host is reduced below a critical level (66,67).

A well-known feature of STH infections is the different patterns observed in the epidemiological profiles of infection prevalence and intensity with age between species (Figure 1.5) (17). Regardless of endemicity, *Ascaris* and *Trichuris* generally show convex epidemiological curves, which peak in children and decrease in adults. Hookworm prevalence and intensity profiles, on the other hand, are generally low in children, increasing continually or reaching a plateau in adults. These differences in the age-profiles of infection are significant in terms of effectiveness of SAC-targeted MDA programmes. Depending on the age structure of the population, the level of school attendance and the local endemic species, SAC-targeted programmes may have a direct effect on as low as 15% of hookworms to as high as 50% of the *A. lumbricoides* worms harboured by a population (68,69).

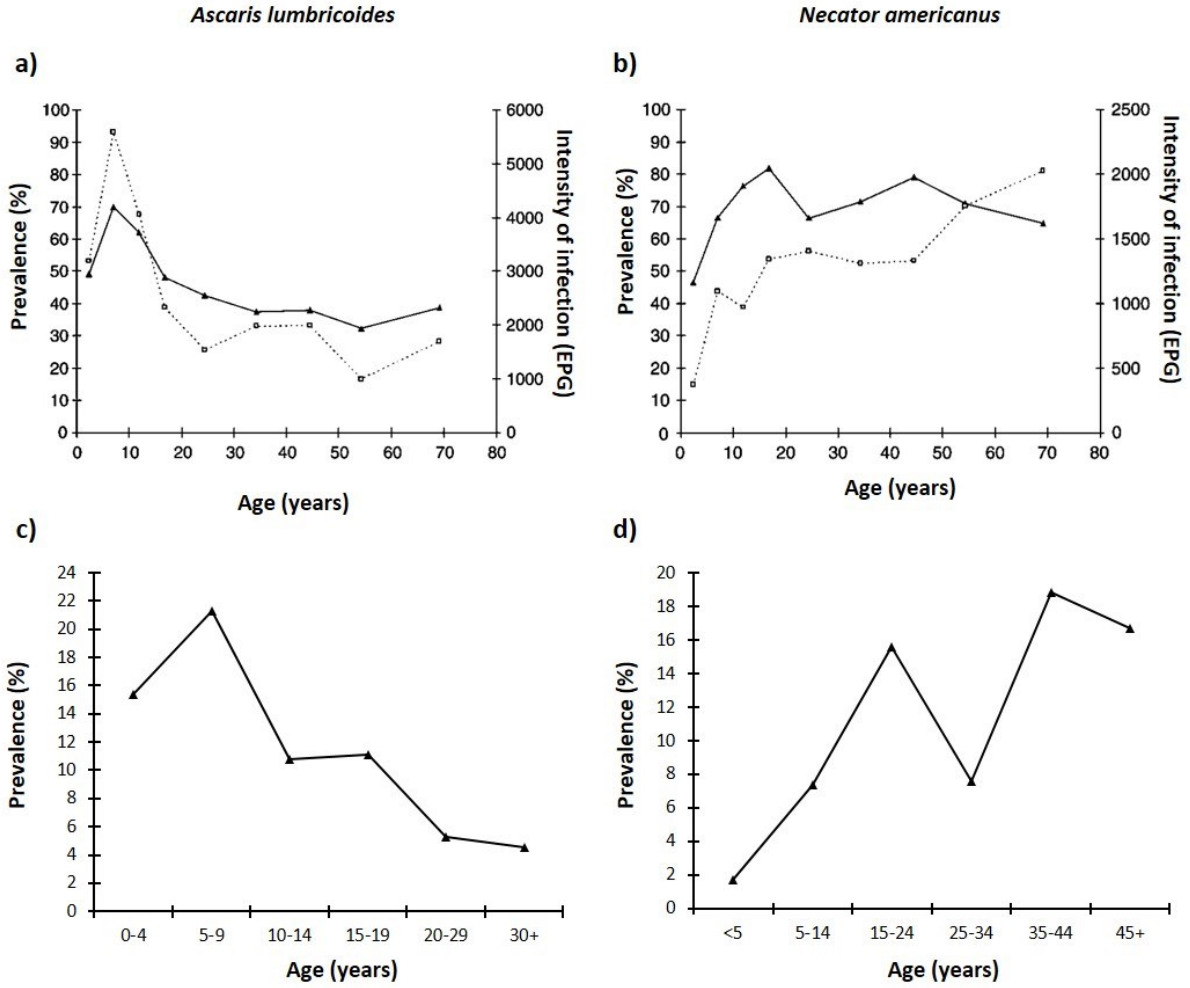


Figure 1.5. Age-prevalence and age-intensity patterns of endemic populations. a) Age-distribution of *A. lumbricoides* infection in highly endemic community (Brazil, adapted from (70)); **b)** Age-distribution of *N. americanus* infection in highly endemic community (Brazil, adapted from (70)); **c)** Age-distribution of *A. lumbricoides* infection in moderately endemic community (Kenya, adapted from (71)); **d)** Age-distribution of *N. americanus* infection in moderately endemic community (Kenya, adapted from (72)). Solid lines represent prevalence (left *y* axis) and dashed lines represent arithmetic mean intensity of infection (right *y* axis). Maximum scale values vary between graphs.

These patterns must result from age-dependent factors, which mainly alter the exposure to infection and/or the acquired immunity to infection (17). Several studies have investigated a range of environmental and behavioural risk factors which can be involved in increasing exposure to infection (73–75). Together with the lack of an effective protective immunity (and in the absence of WASH improvements), reinfection happens quickly after treatment and can be observed in prevalence and intensity profiles bouncing back from very low levels immediately post-treatment to near pre-treatment (equilibrium) levels in a short amount of time, perhaps of the order of one year for *Ascaris* to 2-3 years for hookworm. Repeated rounds of treatment are necessary to maintain low levels of infection, as well as ensuring high coverage of treatment in the population (at least 75%, as recommended by the WHO) and ensuring compliance over repeated rounds of MDA, particularly of "wormy" people (76).

1.2. Immuno-epidemiology of soil-transmitted helminths

1.2.1. Basic immunology

The immune system is a collection of tissues, cells and molecules which protects the body from all types of pathogenic threats. It can be fundamentally divided into two categories: innate immunity and adaptive immunity (77). The innate immunity is always present and ready to respond to infection, and encompasses 1) physical barriers, including the skin and the intestinal mucosa, 2) phagocytic cells (e.g. macrophages), dendritic cells and natural killer cells, and 3) the complement system plasma proteins. The adaptive immunity is usually dormant, and is only activated when the threats overcome the innate immune system. It is composed of two types: humoral immunity, processed by antibodies (produced by B lymphocytes), and cell-mediated immunity, processed by T lymphocytes. When activated, these components rapidly adapt to the specific infectious agents, multiply and generate potent mechanisms which neutralize or eliminate the threat.

Focusing on humoral immunity, mature B lymphocytes have extremely specific receptors on their surface (the B-cell receptors or BCRs) which are composed of an outer variable antigen-binding region and an inner constant "tail" region. When a B lymphocyte finds its antigen match (e.g. a protein produced by a parasite), it starts multiplying, producing close to 20,000 clones with BCRs on their surface which recognize that same antigen. Following this proliferation, B cells become plasma cells, which start releasing large amounts of antibody molecules, which are identical to the BCR but are not anchored to the cell by the constant region (78) (Figure 1.6).

Antibodies have two main purposes: neutralization of the threat, by binding to the antigen and inhibiting its action, or recruitment of other immune system cells or molecules, which will then proceed to eliminate the threat. There are several isotypes of antibodies, depending on the constant region they display (77). When B cells are first activated the antibodies produced are immunoglobulins M (IgM). IgM are large antibodies specialised in activating the complement system and assisting innate immune cells. During proliferation, B cells can undergo class switching by snipping part of their DNA and start producing IgG, IgA or IgE. IgG antibodies have a series of subclasses; IgG1 is a good opsonizer, binding to antigens and leading them to phagocytic cells. IgG3 are good complement fixers and can build bridges between virus-infected cells and natural killer cells. IgG4 antibodies do not activate the complement and have reduced effector functions; they are generally produced after long-term exposure to a particular antigen and are thought to regulate inflammatory responses (79). All IgG antibodies can cross the placenta and are the first line of immune defence in infants. IgA antibodies are the most abundant class in the human body, lining all the mucosal surfaces. They are best at neutralizing invading pathogens and are secreted in human milk, protecting the baby's intestinal mucosa. Finally, IgE antibodies specialise in binding to mast cells. These are long-lived white blood cells stationed beneath surface tissues, which are particularly effective against parasites. When a mast cell comes across a parasite it degranulates, releasing numerous toxic chemicals which aim to kill the parasite. On a second exposure to the same antigen, IgE antibodies already bound to mast cells create a faster, more potent response.

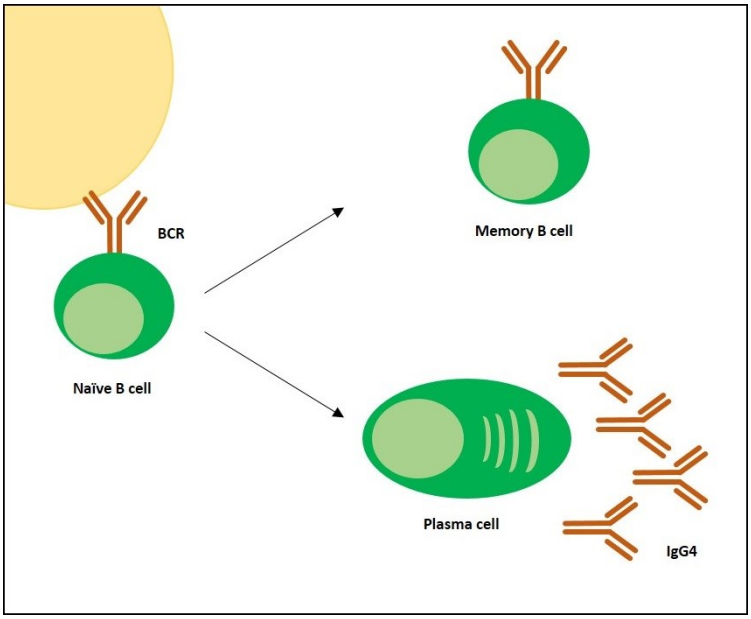


Figure 1.6. B cell differentiation. Following activation (through contact of BCR with its activating antigen), naïve B cells differentiate into antibody-secreting plasma cells (in this case, secreting IgG4 antibodies) or memory cells.

There are three types of T lymphocytes: cytotoxic killer T cells (Tc), helper T cells (Th) and regulatory T cells (Treg). Like B lymphocytes, T cells also have very specific receptors at their surface which must recognise one foreign antigen to be activated. Tc cells are particularly good at destroying virus-infected cells, while Th cells specialise in producing cytokines. Depending on the subset of cytokines Th cells secrete, they can be divided into Th1 and Th2. Th1 cytokines include interleukin-2 (IL-2), interferon-γ (IFN-γ) and tumour necrosis factor (TNF), and are associated with inflammatory responses, usually against virus and bacteria. Th2 cytokines include IL-4, IL-5 and IL-10, which stimulate growth of B cells and class switching to IgA and IgE, producing antibodies to protect against parasites and mucosal infections. The main function of Treg cells is to "turn off" immune responses by secreting cytokines such as IL-10 and transforming growth factor-β (TGF-β), which reduce cell proliferation and activation. They help deactivating the immune system at the end of an inflammatory process, as well as avoiding immune overreaction in chronic infections (80).

Finally, an important feature of the immune system is its memory. Following activation, some B and T lymphocytes become memory cells instead of antibody-producing plasma cells or effector T cells (Figure 1.6). Memory cells are long-lived and maintain the memory of the first exposure to an antigen. Memory B cells carry class-switched, high-affinity BCRs on their surface, ready to become plasma cells and release antibodies at any moment. When exposed to the same antigen, the abundant memory cells proliferate quickly and mount faster and stronger responses against the pathogen.

1.2.2. Helminth immunology

The most striking feature of helminth immunology is the capacity of worm populations to bounce back following treatment (81,82). The fact that hosts can harbour chronic infections and are quickly reinfected post-treatment seems to indicate an at best partial acquired resistance to reinfection. The age-profiles of prevalence and intensity of *A. lumbricoides* or *T. trichiura* infection, which peak in children and then decrease with age could be partly explained by slowly increasing acquired immunity with age, but might also be determined by age-specific exposure to infection. A feature of these age groups is the peak shift – the peak prevalence of infection is higher and in a younger age in a population with overall high prevalence of infection, and it shifts to a lower peak prevalence at a slightly older age if the prevalence of infection in the population is reduced. This pattern is consistent with gradually acquired protective immunity and supported by experimental studies in animals (83,84). Indeed, a study comparing humoral responses in three countries with different STH endemicity found evidence for a peak shift in total IgE, where the populations with the highest total

IgE levels had earlier and higher IgE peaks, as well as higher prevalence of both *Ascaris* and hookworm infection (85).

STH infections do elicit strong immune responses, which are still not fully understood in terms of their effect on established infections or new exposures. Many studies have tried to identify immunological components associated with immune protection in helminth infection in humans (86). IgE and IgG4 antibodies appear to play opposing roles, with IgE being associated with inflammatory responses to helminth infection and IgG4 being associated with regulatory, tolerant responses to infection. A study in Nigeria comparing individuals infected or not infected with *Ascaris* in the same highly endemic community found high levels of inflammatory markers in peripheral blood and elevated specific IgE in individuals putatively immune to *Ascaris* (87). A study in Cameroon showed a correlation between the decline of *Ascaris* prevalence and an increase in Th2 cytokines produced by peripheral blood lymphocytes (PBL) with increasing age (88). Another study in Cameroon showed elevated Th2 cytokine production by PBL associated with resistance to reinfection with *Ascaris* (89). On the other hand, several studies have found significant positive associations between the intensity of *N. americanus* infection and IgG4, as well as elevated IgG4 in infected and reinfected individuals in comparison with non-infected and cured individuals (90–92). The IgE/IgG4 ratio has also been recognised as pending towards IgG4 when STH infections are asymptomatic, in a regulatory process probably intended to prevent IgE overreaction during heavy and persistent infections (93).

The fact that helminth parasites have a relatively long lifespan (see 1.1.1) shows their capacity to evade and modulate the host's immune responses (93,94). This immunomodulation is likely also responsible for the apparent lack of immune memory that enables frequent reinfections to take place in endemic communities, and which can be observed as a bounce back of prevalence of infection shortly after deworming campaigns (95). Several studies have reported a rapid reduction in antibody titres following anthelminthic treatment. In a study in India, hookworm infected patients had elevated specific IgE antibodies, which decreased to levels seen in uninfected people soon after treatment (96). In another study in India, the titre of *Ascaris*-specific IgG4 was shown to decay relatively quickly (becoming negative within six months) following successful monthly treatment with pyrantel pamoate (97). IgG4 levels were also found to decrease significantly two months post-albendazole treatment in *N. americanus* infected Ugandan school children, with greater reductions being observed in heavily infected children (98). These rapid decreases in antibody titres post-treatment open the possibility of using immunological techniques such as enzyme-linked immunosorbent assays (ELISA) as diagnostic methods in the context of STH control (and possibly elimination) programmes.

1.3. Identified research needs

There are currently 102 countries around the globe where STH infections are endemic, and for which the WHO recommends preventive chemotherapy as a strategy to eliminate STH as a public health problem (50). More than half of those countries, including Kenya, have introduced school-based STH control programmes which, as recommended, deliver albendazole or mebendazole, annually or biannually, to SAC and pre-SAC living in areas where STH infections are endemic. Close monitoring and evaluation (M&E) of these programmes is of utmost importance to ensure their objectives are accomplished, but to date such M&E programmes have not been well implemented in many countries. Of particular importance has been the poor recording of individual compliance to treatment over multiple rounds of MDA (76). The epidemiology of STH, associated morbidities such as anaemia, and the risk factors associated with infection need to be regularly reassessed in settings undergoing STH control programmes, such as Bungoma in Kenya, the site of the field epidemiological research reported in this thesis. Monitoring drug coverage and efficacy, detecting clustering of infection and predisposition to heavy infection, as well as identifying risk factors associated with residual infection or rapid reinfection post-treatment are equally important to ensure the successful delivery of the programme and to detect emerging drug resistance. In areas where prevalence and intensity of STH infections are decreasing as a result of the ongoing control interventions, there is an opportunity to push for elimination of transmission. To fully consider this strategy, the impact of current SBD treatment programmes needs to be analysed at the entire population level, and the possible epidemiological benefits of a transition to community-wide MDA strategies need to be considered. Furthermore, a deeper scientific understanding of the immuno-epidemiology of STH infections in whole endemic populations and of the protective roles of anti-STH antibodies is required, as the end of STH deworming programmes without accompanying WASH and socio-economic improvements might result in worse outcomes due to loss of acquired immunity to infection. Reaching the "endgame" for STH infections will also require new diagnostic methods able to detect low intensity infections, and immunoassays such as ELISA, coupled with species-specific antigens, may provide possible avenues of measuring continued transmission in highly treated populations. Finally, while STH control programmes apply a universal strategy in terms of drug delivery and population targeted, the effect of these interventions on the different STH species instead of "all STH infections" needs to be thoroughly investigated, as their distinct dynamics might influence programmatic outcomes. This is particularly important for *Trichuris* infections, for which albendazole and mebendazole are known to have low efficacy (55,99), and for hookworm infections, which tend to be highly prevalent in adults in endemic populations (70,100).

1.3.1. Goals

The three main goals of this PhD thesis were 1) to determine the current epidemiology and risk factors of STH infections in a set of villages in Bungoma, Kenya to help inform on current control programme success, 2) to examine the delivery and impact of one round of community-wide deworming treatment with albendazole to establish current levels of treatment success and potentially highlight any low clearance issues, and 3) to describe the antibody seroprofiles of a population under deworming pressure and the possible use of ELISA tests as a diagnostic tool for continued infection in low prevalence communities. These goals can be subdivided into the following specific objectives.

1.3.1.1 Specific objectives

Current epidemiology and risk factors of STH infection in Bungoma:

- To assess the full age-profiles of prevalence and intensity of the distinct species causing STH infections in four rural villages in the context of the Kenyan SBD programme.
- To identify environmental and sociological risk factors associated with each STH infection in this setting.
- To assess the various levels of aggregation of infection, from individual worm aggregation to spatial clustering in villages, schools and households.
- To determine the full population profile of anaemia, as a measure of STH-associated morbidity, and compare it against the recorded STH infection profiles.

Evaluation of one round of community-wide deworming treatment:

- To compare the full age-profiles of prevalence and intensity of each species of STH infection before and after one round of community-wide deworming treatment, to study the transmission dynamics of each STH infection under MDA.
- To evaluate the coverage of deworming treatment in a round of MDA and to measure the current efficacy of albendazole against STH infections in Bungoma, Kenya.
- To assess levels of predisposition to heavy, moderate and light intensity of STH infections and to identify factors associated with residual infection and reinfection post-treatment.

Antibody seroprofiles of STH infections in a SBD-targeted population:

- To determine the full age-profiles of anti-STH antibody titres in the context of the Kenyan SBD programme.

- To evaluate the ability of IgG4 antibody titres to reflect intensity of STH infection and epidemiological events such as deworming treatment and reinfection.
- To assess the adequacy of using ELISA as an alternative diagnostic test, and the suitability of four STH antigens for use in this test.

The results reported in this thesis provide a detailed epidemiological and immuno-epidemiological assessment of a whole community under SBD pressure, providing insights into a possible transition towards community-wide deworming strategies and the use of ELISA as a diagnostic tool in the endgame. Lessons learnt from this rural Kenyan community can be used to inform the current and future STH control strategies in Kenya, and potentially in other similar endemicity areas in sub-Saharan African countries and beyond.

1.4. Study site

1.4.1. Study site and population

The studies described in this PhD thesis were carried out in four rural villages in Bungoma District, in the Western Province of Kenya (Figure 1.7). This area lies approximately 1,300m above sea level, 35km east of the border with Uganda and 70km north of the equator, in the fertile grasslands between Mount Elgon and Lake Victoria. The villages – Siangwe, Siaka, Sangalo and Nasimbo – are all within 5 km of each other and 10 km away from Bungoma Town, along the Kakamega-Bungoma dirt road.

In the Western Province of Kenya there are two main seasons, the long rainy season with “long rains” in March-June and “short rains” in September-November, interspersed with short dry seasons. The average minimum and maximum temperatures in the region are 17^{o}C and 30^{o}C, respectively, and the annual rainfall is around 1800 mm. Kenya had an estimated population of 45 million in 2014 (101). Life expectancy at birth is 63.8 years (102) and the population annual growth rate has been declining since the 1980s, but is still high at 2.2% in 2013-2014 (101). The age-specific population size in Kenya decreases with age. There is a high proportion of children in the total population in contrast to Western European and North American countries, with 42.1% of people aged 15 or less in 2014 (101). This trend is more noticeable in rural areas; in Bungoma District, SAC and pre-SAC accounted for 57.7% of the population in 2009 (103).

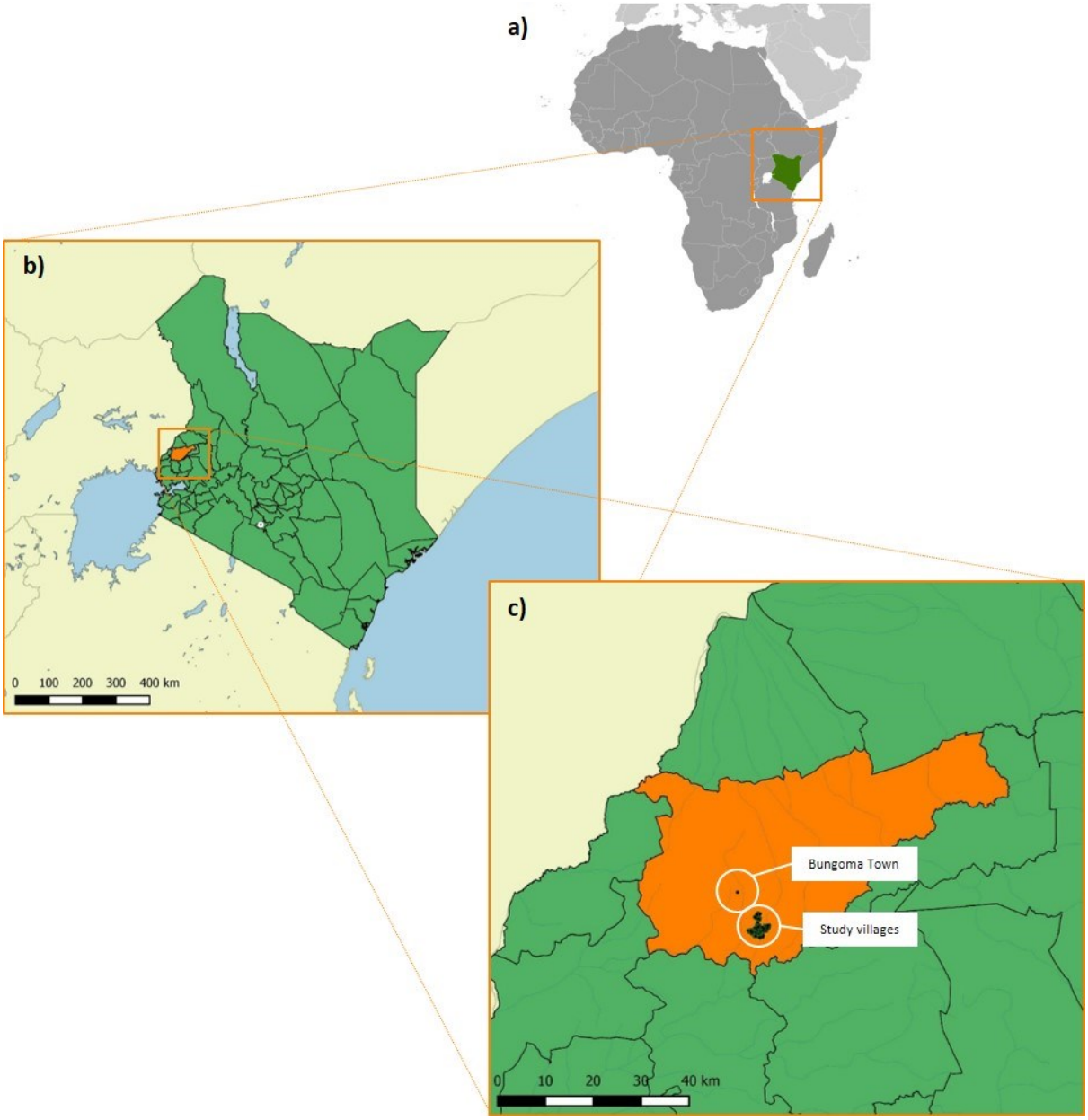


Figure 1.7. Maps of study location. a) Map of the Africa continent showcasing Kenya in green, adapted from (102)**. b)** Kenya in green, with Bungoma District highlighted in orange. **c)** Bungoma District with Bungoma Town and the study villages represented in black. Maps **b)** and **c)** were made using Quantum Geographic Information System (QGIS™) and the global positioning system (GPS) coordinates gathered during study recruitment in November-December 2013.

1.4.2. Soil-transmitted helminths in Bungoma

STH infections have historically been highly prevalent in the Western Province of Kenya. A study published by Pullan *et al.* in 2011 assembled survey data collected between 1974 and 2009 and revealed a predicted infection prevalence of any STH infection between 30-50% in 1989 and just over 20% in 2009 (104). In 2012, the Ministry of Health, with technical support from Evidence Action's Deworm the World Initiative and the Partnership for Child Development, and five years of funding from the Child Investment Fund Foundation (CIFF), launched a National SBD Programme which targeted, among others, several districts in the Western Province, including Bungoma (105). A baseline study carried out right before the launch of the programme surveyed over 6000 children from 60 primary schools in the Western Province and found an overall hookworm, *A. lumbricoides* and *T. trichiura* infection prevalence of 25.1%, 24.2% and 5.8%, respectively (106). However, the range of school prevalence varied considerably, with some schools having more than 50% prevalence of hookworm or *A. lumbricoides*

The baseline prevalence of STH infection in schools in Bungoma District (previous to the launch of the national SBD programme) was 49.4%, with 28.0% prevalence of *A. lumbricoides* and 44.3% prevalence of hookworm in SAC (107). According to the reports issued in 2013 and 2014 (108,109), over 5 million SAC in Kenya received albendazole treatment in 2013 and over 6 million received it in 2014. Moreover, over 80,000 SAC are being treated annually in Bungoma South, where the present study took place.

Besides the national SBD programme, other local organisations also distribute deworming treatment in the area, most notably the Primary School Deworming Project initiated in 1998 by the non-governmental organisation (NGO) International Child Support in the neighbouring Busia district (18). Albendazole is also administered to infected children and adults at the hospitals and locally available in pharmacies, so there is thought to be considerable poorly reported and unprogrammed deworming in the area (110).

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1.5. Thesis outline

Chapter 2 explains the details of the fieldwork and the findings of the baseline cross-sectional survey. These include the individual and household characteristics of the study population, the age-distribution of infection, spatial distribution and aggregation of infection by village and household, risk factors associated with individual STH infection and age distribution of morbidity, specifically anaemia.

Chapter 3 describes the findings of the study's community-wide MDA and the follow-up cross-sectional survey. These include the treatment coverage and treatment efficacy, characteristics of the

followed-up population vs. those who were lost to follow-up (compliance), the effect of the community MDA on the age-distribution of STH infections, and an analysis of the reinfections observed three months post-treatment.

Chapter 4 focuses on the immuno-epidemiology of STH infections, comparing the age-distribution of infection with the age-distribution of antibody titres specific to defined parasite antigens. It looks at differences in antibody titres between infected and uninfected individuals, correlations between antibody titres and intensity of infection, and compares individuals and age group antibody titres before and three months after treatment. It also analyses the potential of using immunoassays as diagnostic tools for STH control programmes.

Chapter 5 is the discussion, which provides an overview of findings, the study limitations, the implications of the findings for STH control policy, and future work required to better understand the epidemiology of STH infections in populations under MDA.

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CHAPTER 2. COMMUNITY DISTRIBUTION OF SOIL-TRANSMITTED HELMINTH INFECTIONS IN FOUR VILLAGES IN BUNGOMA, KENYA IN THE CONTEXT OF THE NATIONAL SCHOOL-BASED DEWORMING PROGRAMME

2.1. Summary

Background

A school-based deworming programme to control soil-transmitted helminth (STH) infections has been ongoing in western Kenya since 2012, as well as in many other endemic countries. These programmes deliver repeated anthelmintic treatment to school age children (SAC), which is necessary due to frequent reinfections. Identifying the factors associated with maintenance of infection can improve the effectiveness of control programmes.

The aim of this study was to investigate the distribution of STH infection and its associated morbidity across all age classes, in a setting with an ongoing school-based preventive chemotherapy programme, as well as the risk factors associated with continued infection in individuals, in order to better understand the effect of school-based preventive chemotherapy on STH infections across the whole community.

Methods

A cross-sectional survey was carried out in four rural villages near Bungoma, western Kenya, between March and April 2014. The study population (n=1,464) had an age range of 2 to 93 years, with 40.2% male participants. Between one and four stool samples were collected from each participant, two slides from each sample were analysed using the Kato-Katz thick smear method, and intensity of STH infections was recorded. Finger prick blood samples were collected to test for anaemia by measuring haemoglobin (Hb) concentration. Aggregation and clustering of infection were investigated, and logistic and negative binomial regression were used to assess correlations between prevalence and intensity of STH infections and possible risk factors.

Results

A low prevalence of *Ascaris lumbricoides* (7.4%) and *Necator americanus* (6.4%) infections, and zero prevalence of *Trichuris trichiura*, were found in the study area, with heterogeneous distributions between villages, schools and households. The age profiles of the study population observed were similar to those of previous studies, but *A. lumbricoides* infection levels peaked in pre-school age children (pre-SAC) rather than SAC. Both STH infections were highly aggregated in the population, and

most infections were low intensity (60.4% for *A. lumbricoides* and 97.9% for *N. americanus*). Young age, residing in one of the villages and poor sanitation were the most significant risk factors for *A. lumbricoides* infection, while old age, farming, and poverty-related factors were identified as significant risk factors for *N. americanus* infection. Anaemia was not significantly associated with either *A. lumbricoides* or *N. americanus* infection, but 26.9% of the study participants were anaemic, and SAC were found to have a lower proportion of anaemia than other age groups.

Conclusions

There is a need to expand the national deworming programme to pre-SAC and adults, as they currently carry the heaviest burdens of *A. lumbricoides* and *N. americanus* infections, respectively. There is also a need to identify the households where infection is clustered, as these individuals might be predisposed to heavier burdens of infection or be systematic non-compliers, and thus might be driving reinfection in treated individuals. Finally, public health measures need to be taken to improve sanitation and the socioeconomic conditions of the population, as well as farming practices, as these factors were found to be associated with a significant risk of infection.

2.2. Introduction

STH infections remain a public health problem in many poor communities living in rural or deprived urban areas of developing countries all over the tropical and subtropical world (1–3). While these infections are not associated with considerable mortality, they pose a high risk of morbidity for specific groups of the population. These high-risk groups include pre-school age children (pre-SAC, 2-4 years old), school age children (SAC, 5-14 years old) and women of reproductive age, in particular, pregnant women (4).

Control of STH infections through preventive chemotherapy - the administration of single-dose anthelmintic treatments to high-risk groups without prior diagnosis - is the most common approach in STH control programmes (5). The large-scale donation of anthelminthic drugs by GlaxoSmithKline (albendazole) and Johnson & Johnson (mebendazole) has enabled the expansion and significant success of school-based deworming (SBD) programmes in many endemic countries (4). The fundamental aim of these programmes is to control and prevent morbidity caused by STH infections, which is defined as the elimination of moderate and high intensity infections (4,6). This is achieved by reducing individual worm loads and maintaining low prevalence and intensity of infection, currently through repeated annual or biannual preventive chemotherapy. Repeated treatments are required because reinfection occurs due to limited effective immunity. Ideally, the goal would be to reduce

infection rates below a threshold upon which transmission is no longer sustainable, which can be generally defined as a basic reproductive number lower than one ($R_0 < 1$) – the average number of secondary cases generated by one infected individual in an otherwise uninfected population is lower than one, thus the infection dies out (7). In high endemicity areas, however, reinfection occurs rapidly, and infections tend to return to pre-treatment levels within 12 months of mass drug administration (8).

Difficulties in reaching the transmission breakpoint are partly due to heterogeneity in the distribution of STH infections. The distribution of worms among hosts in an endemic community usually follows the Pareto Principle, commonly known as the 80/20 Rule: most infected individuals (~80%) harbour only a few worms (~20%), while a few "wormy" people (~20%) harbour heavy infections, carrying ~80% of the worms (9). The probability distribution of worms per host or egg output per host are typically well described by the negative binomial distribution, as described in many published studies of STH epidemiology (7,9). This aggregation of parasites in a small number of hosts can translate into clustering of infection by household and other social groups, such as schools (10), within which exposure to infection is increased. Over the years, numerous studies have investigated the risk factors associated with heterogeneity in STH infection and reinfection (11–13). The effect of each risk factor is dictated by the worm species involved and its mode of infection. A*scaris lumbricoides, T. trichiura* and *A. duodenale* infections are acquired via the faecal-oral route. Transmission may occur through ingestion of infective eggs (larvae in the case of *A. duodenale*) when touching the mouth with soiled hands, eating or drinking contaminated foodstuffs and water, or through direct ingestion of soil, a practice known in Kenya as *pica* (14). Infection by *N. americanus* requires penetration of the skin by infective larvae found in the soil, which can be associated with farming and walking barefoot. Poor hygiene and sanitation, as well as low socioeconomic status, are also associated with higher risk of any STH infection (15,16).

A national SBD programme to control STH infections has been ongoing in Kenya since 2012 (17,18). It is based on targeted preventive chemotherapy, distributing albendazole tablets through schools to SAC and pre-SAC across 143 endemic sub-counties. The programme has been successful in achieving consistently high levels of treatment coverage and reducing the prevalence of combined STH infection in schools, from 34.8% (confidence interval [CI]: 29.9%-40.4%) in 2012, pre-treatment, to 19.7% (CI: 15.3%-25.3%) in 2014, after two rounds of SBD, although success has varied between counties (19). However, the impact of the national SBD programme on the untreated population and individuals not in the SAC category remains unknown. While the intensity of STH infections in school children was expected to be reduced following the repeated annual treatments, no information was available on

the distribution of STH infection in the whole population or the levels of morbidity associated with infection.

The aim of this study was therefore to assess the distribution of STH infections across all age classes, their heterogeneity and clustering, as well as the human risk factors associated with infections, in a setting with an ongoing preventive chemotherapy programme, in order to better understand the effect of school-based preventive chemotherapy on STH infections across the whole community. The study focused on four communities in Bungoma County, western Kenya, where the national SBD programme was first rolled out in 2012 (17). In a cross-sectional study targeting the entire age range of the population, demographic, socioeconomic and parasitological data, as well as a survey on water, sanitation and hygiene (WASH), were collected to assess 1) the heterogeneity of the distribution of STH infections and associated morbidity in the community, and 2) the potential risk factors associated with maintenance of infection in this setting after two years of a large-scale preventive chemotherapy programme. At present the vast majority of STH endemic countries use school-based preventive chemotherapy as their main form of STH control, thus findings from this study will be of interest beyond Kenya.

2.3. Materials and Methods

2.3.1. Ethical considerations and treatment

Ethical approval was obtained from the Kenya Medical Research Institute (KEMRI Scientific Steering Committee [SSC] no. 2687), the Kenyan National Ethics Review Committee, and the Imperial College London Research Ethics Committee (ICREC 13_3_10). Before visiting the schools in this study, the district public health authorities, district education authorities and the school administrators were informed about the aims and procedures of the study. Written informed consent was obtained from participants aged 18 and over and parents/guardians provided written informed consent for their child's participation (17 years old and younger). Children younger than 12 years consented verbally and children between 12-17 years of age were informed about the aims of the study and also signed their own assent forms. Participation was voluntary and withdrawal was possible at any time without further obligation or exclusion from anthelminthic treatment. All parasitological and survey data were coded and treated confidentially. Additionally, all community members registered in the study were treated free of charge with a single oral dose of albendazole (400mg) soon after sample collection, regardless of sample provision or the results of the diagnostic tests.

2.3.2. Study area and population

All residents of four rural villages in Bungoma District, in the Western Province of Kenya, were invited to participate in this study. The villages - Siangwe, Siaka, Sangalo and Nasimbo - are all within a 5 km area of each other and 10 km away from Bungoma, the nearest town (Figure 2.1). In terms of administrative divisions, these villages were all located within the West Sangalo sub-location (Figure 2.2), which was part of the Sangalo location of Bungoma South sub-district, in Bungoma District.

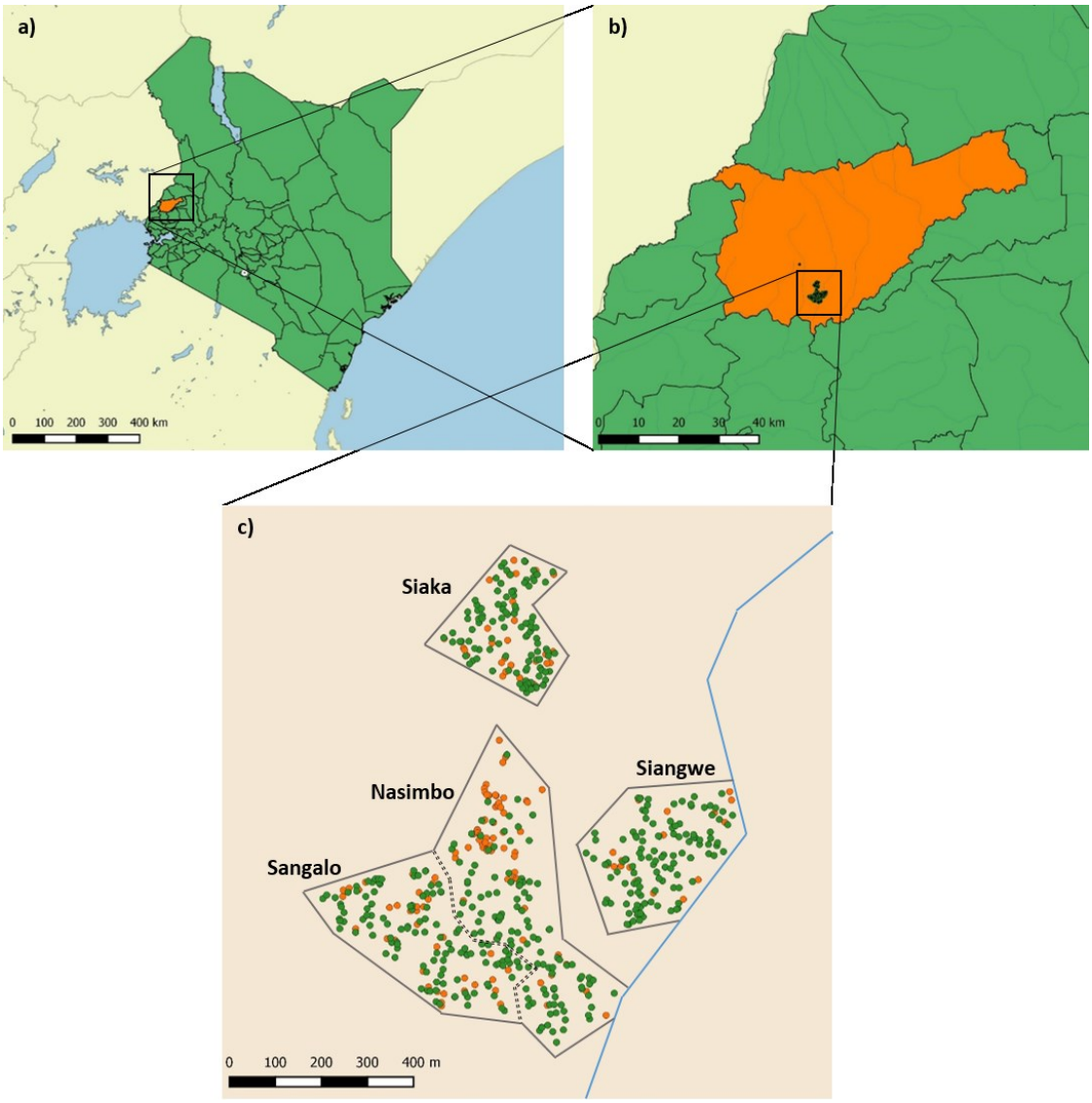


Figure 2.1. Study location: district, villages and households. a) Kenya in green, with Bungoma County highlighted in orange in the western side of the country. **b)** Bungoma County (excluding Mt Elgon sub-county) with Bungoma Town and the study villages represented in black. **c)** Individual households within the study villages. Siaka and Siangwe are relatively isolated villages, while Sangalo and Nasimbo are intertwined and separated by a dirt road (represented by a dashed line). Households which provided stool samples – participant households – are represented by the green circles, whilst those enrolled but which did not provide stool samples are shown in orange. These maps were drawn using Quantum Geographic Information Systems (QGIS™) and Global Positioning System (GPS) coordinates gathered during study recruitment in Nov-Dec 2013. The blue line represents a small river, which marks the border between sub-locations.

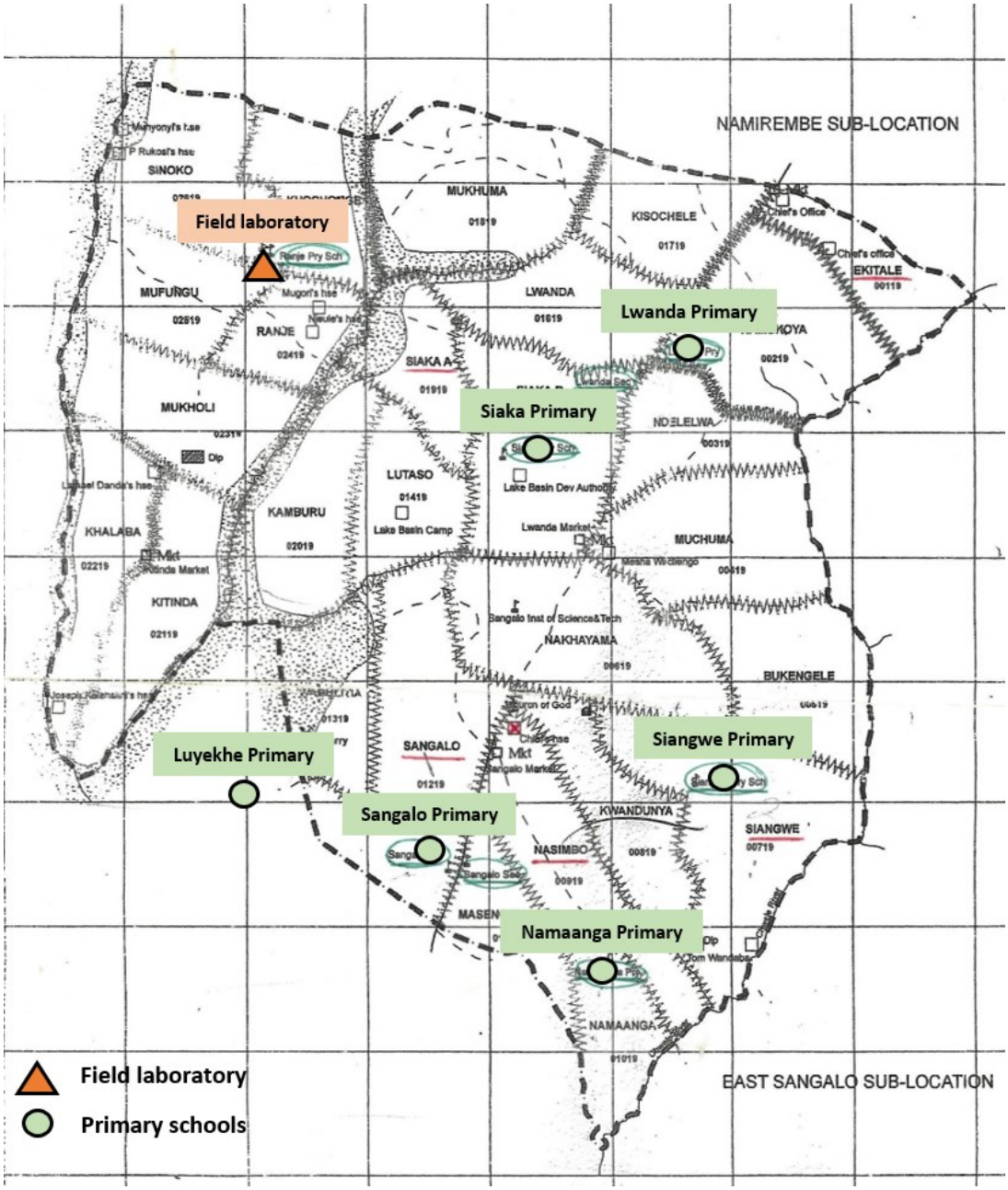


Figure 2.2. Map of West Sangalo sub-location. Map prepared by the Kenya National Bureau of Statistics: Carthography/GIS Unit in August 2009, and provided for study purposes by the sub-chief Robert Barasa. Map boundaries are not exact. The orange triangle depicts the location of the field laboratory. The green circles pinpoint the location of the primary schools visited during the study. Luyekhe Primary School is located in a neighbouring sub-location, not represented.

2.3.2.1. Selection of study site

The original selection criteria set to define two groups of two villages, where one group had approximately 50% prevalence of school-based combined STH infection before the launch of the national SBD programme (high risk, according to the World Health Organization [WHO] (4)) and the other group had school-based combined STH prevalence between 20-50%. (moderate risk). This was not possible due to 1) lack of timely access to official epidemiological data collected previous to the national SBD programme, and 2) the fact that parasitological data was collected from a few random schools in each district, and thus no data was available for a group of schools (and thus villages) close to each other. Due to time and logistical constraints, it was not possible to run a pilot study in the area to assess prevalence of infection in suitable schools and villages. Instead, the study site was selected based on available parasitological data collected in January 2013, approximately six months after the first round of the national SBD programme, for a different KEMRI research project (SSC project no. 2242). This included five primary schools in five sub-locations of Bungoma District, which presented varying levels of STH prevalence (0.08-34% - personal communication).

Permission and assistance to conduct the study in the district were first sought from the Bungoma District Public Health Officer, the Education Deputy Officer and the Bungoma District Hospital. Following a meeting with the chief of the Sangalo location at the weekly *baraza*, two sub-locations were selected as possible study sites, East Sangalo and West Sangalo, with the latter eventually being chosen as the most suitable (Table 2.1). The only epidemiological information available for the West Sangalo sub-location was the prevalence of STH infection based on a sample of 50 children from Siangwe Primary School collected in January 2013; infection levels were unknown elsewhere in this sub-location. Siangwe was thus selected as one of the study villages. The three other villages were selected according to advice from the local sub-chief, village managers and community health workers (CHWs), as the most similar to (Nasimbo) and the most distinct from (Siaka and Sangalo) Siangwe, regarding perceived socioeconomic characteristics of residents and thus, possibly, infection levels.

2.3.2.2. Study enrolment

Study participants were enrolled through household visits carried out between November and December 2013. Training was provided to village managers and CHWs on the details of the study and how to create awareness in the communities prior to the beginning of the activities. They were also trained on how to approach possible study participants, obtaining informed consent, conducting a socio-demographic and WASH survey and recording global positioning system (GPS) coordinates for each household. A schedule was agreed upon for awareness and enrolment activities in the four

villages, and teams composed of one study manager, one village manager or CHW and one study assistant were formed. Recruitment was initiated in Siangwe village; the village was geographically divided into three main areas and a team allocated to each sector, where all households were then visited. Siaka, Sang'alo and Nasimbo followed, with recruitment lasting an average of five days per village.

Criteria	East Sangalo	*Pos (+)/ Neg (-)*	West Sangalo	*Pos (+)/ Neg (-)*
*Known STH prevalence**	32% in Mufule Primary School	(+)	24% in Siangwe Primary School	(+/-)
Proximity to Bungoma Town	Further (20 km+)	(-)	Closer (up to 10 km)	(+)
Field laboratory facilities (nearest option available)	Crowded health centre, 15km+ from study villages, in a different sub-location	(-)	Newly built health centre within the same sub-location	(+)
Road conditions	Bad, mud-covered	(-)	Relatively good	(+)
Sub-chief cooperation	Reluctant/suspicious	(-)	Enthusiastic	(+)
Village participation	Disadvantaged due to poorly dedicated staff	(+/-)	Dedicated and receptive village managers and CHWs, previous work with NGOs	(+)

Table 2.1. Study site selection criteria. Two sub-locations within Bungoma District were proposed as possible study sites, East and West Sangalo. Following meetings with the local sub-chiefs, village managers and community health workers, as well as visits to possible study villages and field laboratory facilities, this table was built containing the available information, which eventually led to the selection of West Sangalo sub-location as the study site.

* parasitological data collected in January 2013 for another KEMRI study conducted in Bungoma District (KEMRI SSC no. 2242)

In total, 4,773 individuals from 843 households were enrolled (Figure 2.3). The geographical coordinates of households were recorded using a GPS receiver (eTrex Garmin Ltd., Olathe, KS, US). A socio-demographic survey on individual information (age, sex and profession/school attended) and household characteristics (e.g. education level of head of household, and materials of the roof, walls and floor) was performed. An additional household WASH survey about water sources, hand washing habits, and ownership and conditions of the latrine(s) used by the household was also performed.

The final number of study participants selected for sample collection was reduced to 3,530 due to logistical and financial constraints, with selections made at a household level. In Siaka, the households located further away from the meeting point were excluded due to uncertainties regarding village boundaries; in the other three villages, households were selected by dividing them into groups of walking proximity (based on the GPS coordinates previously gathered) and then applying a random

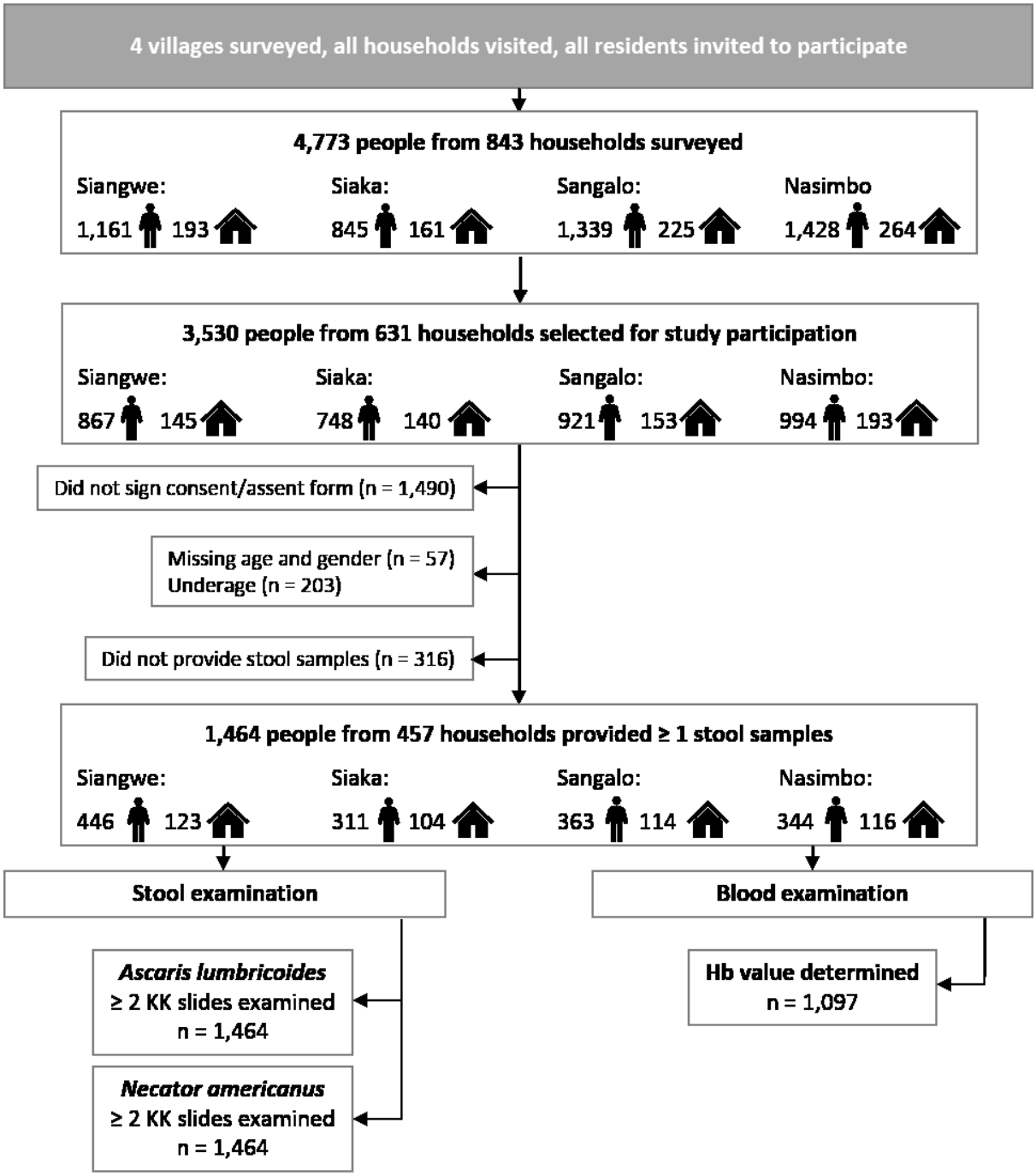


Figure 2.3. Flow chart describing study participation. The final study population included all individuals who provided one or more stool samples and had complete records, i.e. signed consent/assent form and demographic (age and gender) data. Some of these individuals (n=1,907) also provided one blood sample for haemoglobin (Hb) measurement. Symbols shown represent the number of individuals and the number of households in each village.

number generator. While samples were not collected from individuals in the households not selected for the study, they were still provided with albendazole treatment shortly after the cross-sectional survey to simulate a community-wide mass deworming campaign, as well as to comply with the ethical regulations.

2.3.3. Field and laboratory procedures

Sample collection was conducted between March and April 2014, during the "long rains" season. It took place at previously designated community meeting points in each village, generally Christian churches centrally located, and at the six primary schools with the largest number of children selected for participation in the study: Siangwe Primary, Siaka Primary, Lwanda Primary, Sangalo Primary, Luyekhe Primary and Namaanga Primary (Figure 2.2). Sample collection at the primary schools was carried out due to the study taking place over the school term, to ensure the majority of the enrolled school-age children would provide samples.

When collection was conducted at the community meeting points, CHWs from each village distributed labelled containers, pieces of newspaper and small sticks to each selected household, at least one day in advance, with instructions on how to collect stool samples from each enrolled household member and when to take them to the meeting point. On collection day, a second labelled pot was given out for each sample delivered, with instructions to return it the following day – this aimed to improve the goal of receiving two stool samples from each individual. Sample collection at the community meeting points was staggered in a way that only up to 200 individuals were scheduled to deliver their samples on a given day. This was achieved by dividing the villages into walking proximity groups, according to the CHWs and village managers' knowledge, and only residents enrolled in each household group would be required to provide samples over the following two days. This staggering enabled the maintenance of a manageable number of samples to be analysed in the laboratory each day.

When collection was conducted in primary schools, children enrolled in the study were called out from each class (if present). Informed verbal consent was then obtained and individuals were asked to sign assent forms if older than 12 years old. Each children was then provided with a small plastic container, a piece of newspaper, a small stick and toilet paper, and they were explained in the local language how to collect their own small stool sample in the school latrine, which they quickly delivered. Since none of the schools had more than 200 children enrolled in the study, there was no need to stagger sample collection; each school was visited twice, on two consecutive days, in order to collect two samples from each child. On occasion, some of these children provided extra samples at the community meeting points, on their household's collection days.

2.3.3.1. Collection points

Two teams were deployed each day to two villages or schools. Each team consisted of three to five technicians, the villages' CHWs and village manager and one study manager. The technicians and study manager would set up the collection point while the village manager and CHWs would gather the study participants, either from the community or at the schools. When a study participant (or a family) arrived at the sample collection point, a team member would keep a record for each participant with 1) name, 2) individual code, 3) whether a stool sample was provided on that date and 4) the result of the haemoglobin (Hb) measurement carried out on-site. The individual code written on each stool container brought by the study participant would be confirmed and the sample stored in a cold box. Sample collection points usually functioned between 8:00 and 12:00, after which the samples would be taken to the field laboratory for processing and examination.

2.3.3.2. Laboratory procedures

Stool samples were analysed in the field laboratory within 5 hours of collection from the participants. A double Kato-Katz thick smear (20) was prepared from each sample, as recommended by the WHO (6). Two microscope slides per stool were prepared using the standard template. All slides were examined under optical microscopes within 60 min of preparation for the presence of hookworm eggs, and later for the remaining STH eggs. Two different technicians examined slides A and B to account for a reading bias, and a random sample of 5% of each day's slides was kept and re-examined for quality control. There was no upper limit to egg counts in order to record all intensities of infection.

2.3.4. Data management and statistical analysis

All data were entered in Microsoft Excel 2010 (version 14.0) and later updated to Microsoft Excel 2013 (version 15.0, Microsoft Corporation). Any discrepancies found during data cleaning were checked against the original paper records and resolved accordingly, where possible. Survey and parasitological data were linked to household locations, and maps were created using QGIS™ 2.12 'Lyon' (21).

All participants who had at least one stool sample examined were included in the final analyses. The total number of eggs counted for each STH species in the Kato-Katz analysis was multiplied by 24 to obtain the faecal egg count (FEC) in units of eggs per gram (EPG) – a standard measure of infection intensity. The arithmetic mean EPG for each individual was calculated from the total number of Kato-Katz slides per person (which ranged between 2-8 Kato-Katz slides). The ranges of light, moderate and heavy infections used were 1-4,999, 5,000-49,999 and 50,000+ EPG, respectively, for *A. lumbricoides*

and 1-1,999, 2,000-3,999 and 4,000+ EPG, respectively, for *N. americanus*, following WHO references (22). Individuals were classified into “non-anaemic”, “light”, “moderate” and “severe” anaemia using WHO thresholds, corrected for age and sex, with an adjustment made for altitude since the study site was located over 1000m above sea level (23,24). For the purposes of this study age was a categorical variable divided into eight age groups: 2-4, 5-9, 10-14, 15-19, 20-29, 30-39, 40-49 and 50+ years. The first age group, 2-4, represents the pre-SAC as defined by the WHO (0-4 years); however, children younger than 24 months were excluded from the study due to lack of international consensus on the safety of albendazole treatment in this age group (25). Children aged 5-14 are the WHO definition of SAC, which in his study were divided into two 5-year groups. Adults (WHO definition: older than 15 years) were divided into five groups to allow the visualization of changes in infection prevalence and intensity with age.

The prevalence of each STH species was calculated for each age group, and their 95% binomial confidence intervals (CI) were obtained using the Wilson method (26). The Williams mean intensity of STH infection was calculated for each age group including all individuals, both infected and uninfected (27). This variation of the geometric mean, obtained by adding 1 to all logarithmic transformed data values, calculating the mean and then subtracting 1 from the result, was used to accommodate the zero values of uninfected individuals. Asymmetric 95% CIs on the mean intensity of infection were calculated using the logarithmic transformed values and subtracting 1 to obtain the final confidence limits. The aggregation parameter of the negative binomial distribution *k* was calculated using the corrected moment estimate (28),

$$k = \frac{\bar{x}^2 - \frac{v}{n}}{(v + \bar{x})}$$

where n is the number of individuals in the population, $\bar{x}$ is the arithmetic mean intensity of infection, and v is the variance around the mean.

Statistical analyses were carried out using Stata version 13.1 (29). The outcome variables of interest examined were the prevalence of *A. lumbricoides* and *N. americanus* (binary variables) and the mean intensity of *A. lumbricoides* and *N. americanus* infections (continuous variables). Univariable associations between the binary variables of interest and risk factors were assessed using logistic regression, while negative binomial regression was used to assess correlations between the continuous variables of interest and risk factors. Significant categorical variables (p<0.05) were used to obtain adjusted odds ratios in multivariable analysis. Logistic regression was also applied to search for associations between *A. lumbricoides* infection, *N. americanus* infection and categories of anaemia.

2.3.4.1. Principal component analysis (PCA)

Data collected through the household socio-demographic and WASH surveys were used to derive an index of socioeconomic status (SES) using the principal component analysis (PCA), a statistical method of multivariate analysis (30,31). Fifteen variables, including education level of the head of household, household characteristics and sanitary conditions were used in this analysis (Table 1 of Annex III). Variables used in PCA must be binary or continuous, therefore categorical variables had to be recoded into binary variables (e.g. education level was recoded into four new variables: edlevel_a – no education; edlevel_b – any primary education; edlevel_c – any secondary education; edlevel_d – secondary education complete or higher). A total of 48 binary and continuous variables were used in Stata's *pca* command (32).

The output of the *pca* command showed the first principal component (PC) had an eigenvalue of 7.2, which explained 15.0% of the total variation in the dataset. The first 10 PCs had a cumulative eigenvalue of 25.8, which explained 53.8% of the variance. For simplification, only the first PC was used to calculate the households' socioeconomic score. The eigenvectors' table of the first PC had thirteen variables with scores above 0.1 (Table 2 of Annex III), with the household floor material (cement), wall material (cement) and type of latrine (pit latrine with cement floor) having the highest scores. A new SES score variable was then created in Stata which used the eigenvectors from all of the selected variables as weights - except the continuous variable, which caused the distribution to be non-normal. This new SES score variable outputted the sum of the twelve weighted variables for each household and varied discretely between 0.00 and 2.14, with $\bar{x} = 0.79$. The *xtile* command was then used to create a new categorical variable which divided households into five quintiles, or SES groups, from "poorest" to "least poor" in socio economic terms.

2.4. Results

2.4.1. Study population

Of the 3,530 individuals from 710 households randomly selected to participate in the study, 1,464 (41.5%) provided at least one stool sample for analysis, and 1,097 of these also provided a blood sample to measure Hb concentration (31.1%) (Figure 2.3). Study participation was significantly different between villages ($\chi^2 = 56.4$, $p<0.001$); Siangwe was the village with the highest study participation (51.4%) while Nasimbo had the lowest (34.6%). When compared to the national Kenyan population pyramid, the randomly selected study population had a relatively similar age and sex distribution (Figures 2.4a and 2.4b). However, in the final study population, who actually provided

samples for the study, SAC were oversampled (52.0% of the study population) and adults, in particular male adults aged 20 to 59 years, were under sampled (only 5.4% of the study population) (Figures 2.4c and 2.4d). The final study population consisted of 875 female participants (59.8%) and 589 male participants (40.2%), with a median age of 11 and an age range of 2 to 93 years.

The population of the four villages was significantly different in terms of age distribution (χ^2 = 34.7, p=0.031) and several household characteristics (Table 2.2). In summary, the village of Siaka was the most well developed and educated village, with the lowest percentage of uneducated head of households (2.6%), the highest percentage of participants in the least poor SES quintile (22.4%) and the highest percentage of participants living in households with cement floors (26.0%), iron sheet roofing (99.3%), access to improved water sources (57.0%) and ventilated improved pit (VIP) latrines (16.8%). On the other hand, Sangalo had the highest proportion of uneducated participants (8.3%), the highest proportion of participants in the poorest SES quintile (38.6%) and the highest percentage of participants living in households with mud floor (92.5%), thatch roof (7.0%) and pit latrines with mud floor (70.6%). Siangwe had the highest proportion of participants without access to improved water sources (77.4%) and Nasimbo had the highest proportion of participants living in households without a pit latrine (16.1%).

2.4.2. Village and school distribution of STH infection

The prevalence of STH infection was lower than 20% in all four villages (Table 2.3). The overall prevalence of any STH infection was 13.3% and significantly different between the four villages (χ^2=29.6, p<0.001). The prevalence of *A. lumbricoides* infection ranged between 14.3% in Siangwe and 2.6% in Siaka, with significant differences between the villages (χ^2=49.4, p<0.001). Hookworm infection, on the other hand, ranged between 7.6% prevalence in Nasimbo and 3.5% in Siaka, with no significant differences between villages. The hookworm species detected was later identified as *N. americanus* following an expulsion study and PCR analysis of stool samples (33), which did not detect the presence of *A. duodenale*. Only eight individuals (0.5%) were found to be co-infected with *A. lumbricoides* and *N. americanus*, and no *T. trichiura* eggs were observed in the samples provided by the study participants.

The prevalence of STH infection was also lower than 20% in the six primary schools visited (Table 2.4). Siangwe Primary had the highest prevalence of *A. lumbricoides* (16.0%) and any STH infection (17.8%), which were both significantly different between schools (χ^2=18.1, p=0.003 and χ^2=11.7, p=0.039, respectively). Lwanda Primary had the lowest prevalence of *A. lumbricoides* (1.8%) and any STH infection (5.3%). Hookworm prevalence was fairly low and similar among the schools, between 4.8%

and 2.1%. A total of 720 children between 5 and 14 years old participated in the study during these school visits, 94.5% of the total participants of this age group.

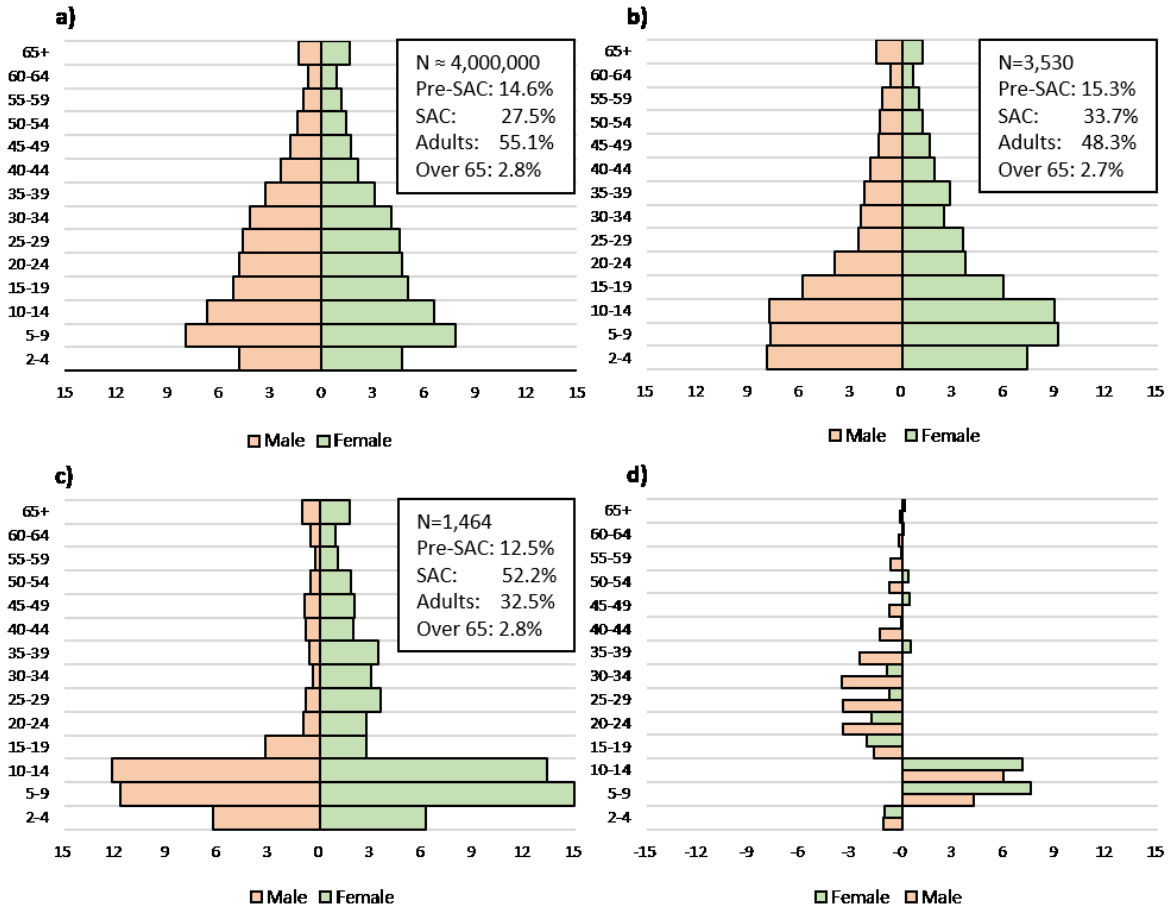


Figure 2.4. Kenyan and study population pyramids. a) Kenyan national population pyramid for the year 2014, adapted from data obtained from the US Census Bureau International Database; **b)** Population randomly selected for participation in the study (n=3,530); **c)** Population of study participants (n=1,464); **d)** Over-sampling (positive values, right) and under-sampling (negative values, left) of groups of participating individuals in relation to the national population pyramid. The *x* axis denotes percentage of the total population, while the *y* axis indicates the age groups. The first age group (2-4) excludes children younger than two years of age, who were not included in the study.

	Siangwe		Siaka		Sangalo		Nasimbo		Overall		Difference		
Individual characteristics	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	χ2	P-value	
Sex													
Male	39.0	(174/446)	37.0	(115/311)	39.7	(144/363)	45.3	(156/344)	40.2	(589/1464)			
Female	61.0	(272/446)	63.0	(196/311)	60.3	(219/363)	54.7	(188/344)	59.8	(875/1464)	5.4	0.142	ns
Age groups													
2-4	11.7	(52/446)	9.6	(30/311)	13.2	(48/363)	13.7	(47/344)	12.1	(177/1464)			
5-9	26.9	(120/446)	23.2	(72/311)	30.0	(109/363)	26.5	(91/344)	26.8	(392/1464)			
10-14	23.3	(104/446)	29.3	(91/311)	22.3	(81/363)	27.3	(94/344)	25.3	(370/1464)			
15-19	7.0	(31/446)	8.0	(25/311)	5.0	(18/363)	4.9	(17/344)	6.2	(91/1464)			
20-29	11.2	(50/446)	7.1	(22/311)	5.8	(21/363)	8.1	(28/344)	8.3	(121/1464)			
30-39	5.6	(25/446)	8.4	(26/311)	10.2	(37/363)	7.0	(24/344)	7.7	(112/1464)			
40-49	6.7	(30/446)	4.5	(14/311)	7.2	(26/363)	4.4	(15/344)	5.8	(85/1464)			
50+	7.6	(34/446)	10.0	(31/311)	6.3	(23/363)	8.1	(28/344)	7.9	(116/1464)	**34.7**	**0.031**	*
Occupation													
Student	54.3	(242/446)	56.9	(177/311)	55.4	(201/363)	55.2	(190/344)	55.3	(810/1464)			
Farmer	17.0	(76/446)	14.1	(44/311)	17.9	(65/363)	14.8	(51/344)	16.1	(236/1464)			
Trader	4.3	(19/446)	5.5	(17/311)	4.7	(17/363)	3.8	(13/344)	4.5	(66/1464)			
Teacher/Civil servant	1.6	(7/446)	1.3	(4/311)	1.4	(5/363)	1.7	(6/344)	1.5	(22/1464)			
Specialist labour	0.2	(1/446)	1.6	(5/311)	1.4	(5/363)	0.6	(2/344)	0.9	(13/1464)			
Other	4.0	(18/446)	4.8	(15/311)	2.7	(10/363)	4.1	(14/344)	3.9	(57/1464)			
Unknown	18.6	(83/446)	15.8	(49/331)	16.5	(60/363)	19.8	(68/344)	17.8	(260/1464)	13.0	0.792	ns
Anaemia													
Non-anaemic	72.9	(250/343)	71.9	(120/167)	77.6	(205/264)	69.8	(206/295)	73.1	(781/1069)			
Mild	21.3	(73/343)	16.8	(28/167)	14.0	(37/264)	19.0	(56/295)	18.1	(194/1069)			
Moderate	5.2	(18/343)	9.6	(16/167)	7.2	(19/264)	10.2	(30/295)	7.8	(83/1069)			
Severe	0.6	(2/343)	1.8	(3/167)	1.1	(3/264)	1.0	(3/295	1.0	(11/1069)	13.3	0.149	ns
Household characteristics													
Education level of head of household													
None	6.8	(30/443)	**2.6**	**(8/305)**	8.3	(30/361)	5.4	(18/331)	6.0	(86/1440)			
Primary incomplete	24.4	(108/443)	24.6	(75/305)	16.1	(58/361)	27.2	(90/331)	23.0	(331/1440)			
Primary complete	24.6	(109/443)	31.1	(95/305)	29.9	(108/361)	23.9	(79/331)	27.1	(391/1440)			
Secondary incomplete	12.2	(54/443)	14.1	(43/305)	14.4	(52/361)	9.4	(31/331)	12.5	(180/1440)			
Secondary/Higher education	32.0	(142/443)	27.5	(84/305)	31.3	(113/361)	34.1	(113/331)	31.4	(452/1440)	**32.2**	**0.001**	**
Socioeconomic status													
Poorest	25.2	(91/361)	10.8	(27/250)	38.6	(114/295)	19.2	(50/261)	24.2	(282/1167)			
Very poor	21.3	(77/361)	20.4	(51/250)	23.7	(70/295)	21.1	(55/261)	21.7	(253/1167)			
Poor	21.9	(79/361)	19.2	(48/250)	15.6	(46/295)	32.2	(84/261)	22.0	(257/1167)			
Less poor	19.4	(70/361)	**27.2**	**(68/250)**	12.5	(37/295)	15.7	(41/261)	18.5	(216/1167)			
Least poor	12.2	(44/361)	**22.4**	**(56/250)**	9.5	(28/295)	11.9	(31/261)	13.6	(159/1167)	**102.5**	**<0.001**	***
Floor													
Mud	85.3	(378/443)	74.0	(216/292)	92.5	(332/359)	91.2	(302/331)	86.2	(1228/1425)			
Cement	14.7	(65/443)	**26.0**	**(76/292)**	7.5	(27/359)	8.8	(29/331)	13.9	(197/1425)	**55.9**	**<0.001**	***
Roof													
Thatch	5.9	(26/443)	**0.7**	**(2/295)**	7.0	(25/357)	5.5	(18/330)	5.0	(71/1425)			
Iron sheets	94.1	(417/443)	99.3	(293/295)	93.0	(332/357)	94.5	(312/330)	95.0	(1354/1425)	**15.5**	**0.001**	**
Water source													
Non-improved	77.4	(343/443)	43.0	(132/307)	64.8	(234/361)	70.9	(243/343)	65.5	(952/1454)			
Improved	22.6	(100/443)	**57.0**	**(175/307)**	35.2	(127/361)	29.1	(100/343)	34.5	(502/1454)	**101.1**	**<0.001**	***
Latrine													
Absent	11.3	(42/371)	**7.1**	**(21/297)**	13.2	(47/357)	16.1	(54/336)	12.0	(164/1361)			
Pit latrine with mud floor	61.2	(227/371)	43.1	(128/297)	70.6	(252/357)	69.9	(235/336)	61.9	(842/1361)			
Pit latrine with cement floor	25.1	(93/371)	33.0	(98/297)	13.2	(47/357)	13.1	(44/336)	20.7	(282/1361)			
Ventilated improved pit latrine	2.4	(9/371)	**16.8**	**(50/297)**	3.1	(11/357)	0.9	(3/336)	5.4	(73/1361)	**175.1**	**<0.001**	***

Table 2.2. Individual and household characteristics of the study population, stratified by village. Differences between villages were examined using the Kruskall-Wallis rank test. * $p<0.05$, statistically significant; ** $p<0.01$, very significant; *** $p<0.001$, extremely significant; ns $p>0.05$, not significant.

Village	Siangwe		Siaka		Sangalo		Nasimbo		Overall		Difference		
	%	n	%	n	%	n	%	n	%	n	χ2	P-value	
A. lumbricoides	14.3	(64/446)	3.9	(12/311)	6.3	(23/363)	2.6	(9/344)	7.4	(108/1464)	**49.4**	**<0.001**	***
N. americanus	6.7	(30/446)	3.5	(11/311)	7.4	(27/363)	7.6	(26/344)	6.4	(94/1464)	5.7	0.125	ns
Co-infection	1.1	(5/446)	0.0	(0/311)	0.8	(3/363)	0.0	(0/344)	0.5	(8/1464)	**7.6**	**0.050**	*
Any STH infection	20.0	(89/446)	7.4	(23/311)	12.9	(47/363)	10.2	(35/344)	13.3	(194/1464)	**29.6**	**<0.001**	***

Table 2.3. Prevalence of STH infections in the study villages, as determined by Kato-Katz. Differences between villages measured using the Kruskall-Wallis rank test. * $p<0.05$, statistically significant; ** $p<0.01$, very significant; *** $p<0.001$, extremely significant; ns $p>0.05$, not significant.

Primary School	Luyekhe		Lwanda		Namaanga		Sangalo		Siaka		Siangwe		Difference		
	%	n	%	n	%	n	%	n	%	n	%	n	χ2	P-value	
A. lumbricoides	4.2	(3/71)	1.8	(1/57)	9.6	(12/125)	7.5	(11/146)	7.3	(7/96)	16.0	(36/225)	**18.1**	**0.003**	**
N. americanus	2.8	(2/71)	3.5	(2/57)	4.0	(5/125)	4.8	(7/146)	2.1	(2/96)	2.7	(6/225)	2.0	0.852	ns
Any STH infection	7.0	(5/71)	5.3	(3/57)	13.6	(17/125)	11.0	(16/146)	9.4	(9/96)	17.8	(40/225)	**11.7**	**0.039**	*

Table 2.4. Prevalence of STH infections in primary schools visited during the study. Differences between schools were measured using the Kruskall-Wallis rank test. * $p<0.05$, statistically significant; ** $p<0.01$, very significant; *** $p<0.001$, extremely significant; ns $p>0.05$, not significant.

In terms of the geographical distribution of STH infection (Figures 2.5 and 2.6), Siangwe had the largest number of households where participants were found to be infected with *A. lumbricoides* (n=41) or any STH infection (n=56). The aggregation of *A. lumbricoides* infection per household was also evident, with several houses in all villages having more than one person infected (Figure 2.5a), while most of the households with heavier combined intensity of *A. lumbricoides* infection were aggregated in Siangwe (Figure 2.6a). Infection with *N. americanus* was more geographically widespread (Figure 2.5b), with little household aggregation (only 1 or 2 participants infected per house) and generally light intensity of infection in the majority of households (Figure 2.6b). Siangwe also had the largest proportion of households where both infections were identified (n=13, 10.6%) while Siaka had the household with the highest number of infected household members (six diagnosed with *A. lumbricoides* and one with *N. americanus* infection). Overall, there was a significant difference in the household distribution of *A. lumbricoides*, *N. americanus* and double STH infections between the four villages (χ^2=51.3, p<0.001).

2.4.3. Age and sex distribution of STH infection

The prevalence of *A. lumbricoides* infection generally decreased with age (Figure 2.6a), with the peak prevalence in the group 2-4 years old (12.4%) and significant differences between age groups (χ^2=30.1, p<0.001). Females had overall higher prevalence of infection than males (χ^2=2.31, p=0.129, not significant), with infected participants in all age groups and peak prevalence of *A. lumbricoides* infection (19.3%) in girls 2-4 years old (χ^2=7.63, p=0.006 – *A. lumbricoides* infection significantly more prevalent in young girls vs. boys). On the other hand, no males older than 30 years were observed to be infected with *A. lumbricoides* and the peak prevalence of infection (8.3%) was in the age group 15-19 years. *Necator americanus* infection prevalence, on the contrary, generally increased with age (Figure 2.6b), peaking in the group 50 years and older (27.6%), again with significant differences between age groups (χ^2=115.9, p<0.001). Prevalence in male participants increased with age, peaking at 20.8% in adult men aged 40-49 years, but females aged 50 years and older had the highest prevalence of *N. americanus* infection at 33.8% (χ^2=4.90, p=0.027 – *N. americanus* infection significantly more prevalent in older women vs. men).

The distribution of mean intensity of *A. lumbricoides* was very similar to the prevalence distribution, peaking in the age group 2-4 years old and decreasing with age (Figure 2.6c). There was a marked gender difference in mean intensity of infection in the age group 2-4 years age, where girls generally harboured heavier *A. lumbricoides* infections (H=7.40, p=0.006 – *A. lumbricoides* intensity of infection significantly higher in young girls vs. boys), and there were significant differences in mean intensity of

A. lumbricoides infection between the age groups (H=30.5, *p*<0.001). The mean intensity distribution of *N. americanus* was also very similar to the prevalence distribution, increasing with age and peaking in the oldest group, with significant differences between age groups (H=119.0, p<0.001) (Figure 2.6d). The mean intensity peaks of *N. americanus* infection were again observed in males aged 40-49 years (H=3.79, p=0.052) and females aged 50 years and above (H=4.08, p=0.043).

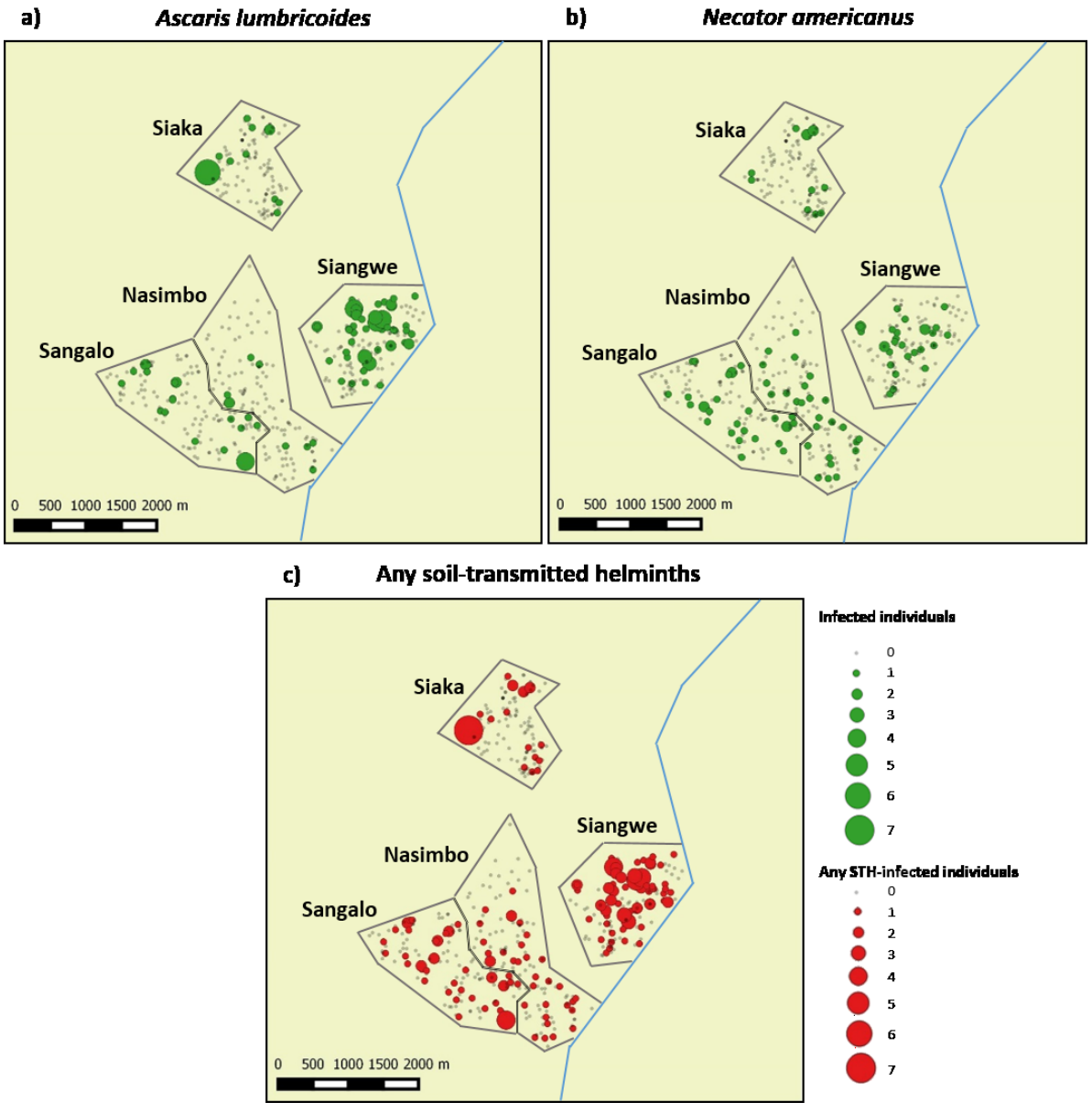


Figure 2.5. Geographical distribution of STH infection in participant households. Households where at least one individual provided at least one stool sample but where infection was not detected are represented by small grey circles (corresponding to size 0 in the legend). The green circles in **a)** highlight households where individuals were found to be infected with *A. lumbricoides* and in **b)** households where individuals were found to be infected with *N. americanus*. The red circles in **c)** identify households where any STH species was detected. The diameter of the green and red circles represents the number of individuals found to be infected in each household as per the legend (range: 0 to 7 infected individuals). A maximum of two individuals per household were found to be infected with *N. americanus*.

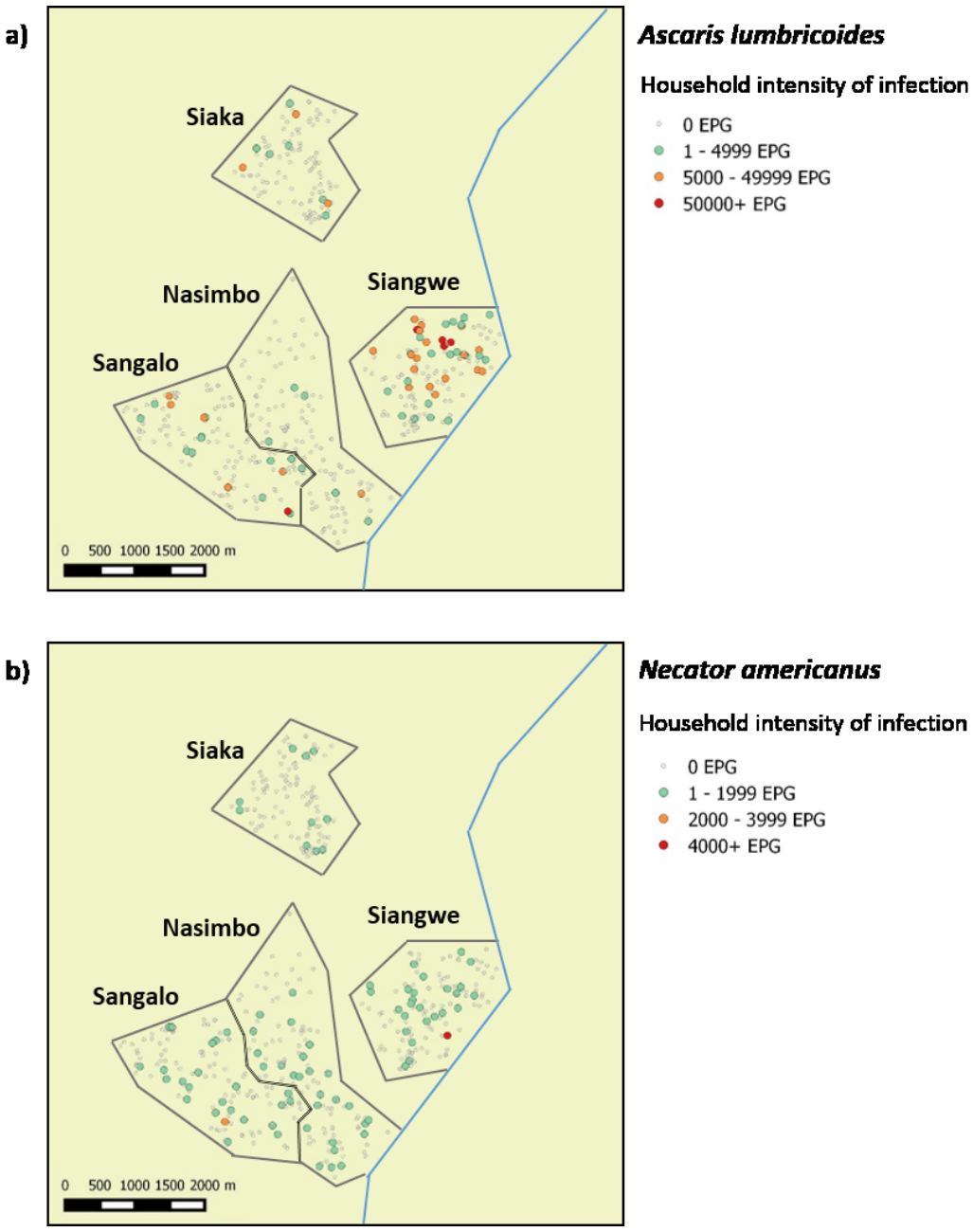


Figure 2.6. Household distribution of intensity of *A. lumbricoides* and *N. americanus* infection. Households where at least one individual provided at least one stool sample but infection was not detected are represented by small grey circles. Coloured circles represent households where at least one individual was found to be infected with either **a)** *A. lumbricoides* infection or **b)** *N. americanus* infection. Green circles represent households where, according to WHO guidelines, the total intensity of infection (all individuals' EPG added together) was found to be light (1–4999 EPG for *A. lumbricoides* and 1–1999 EPG for *N. americanus*). Orange circles represent households where the total intensity of infection was found to be moderate (5000–49999 EPG for *A. lumbricoides* and 2000–3999 EPG for *N. americanus*). Red circles represent households where the total intensity of infection was found to be heavy (50000 EPG and above for *A. lumbricoides* and 4000 EPG and above for *N. americanus*).

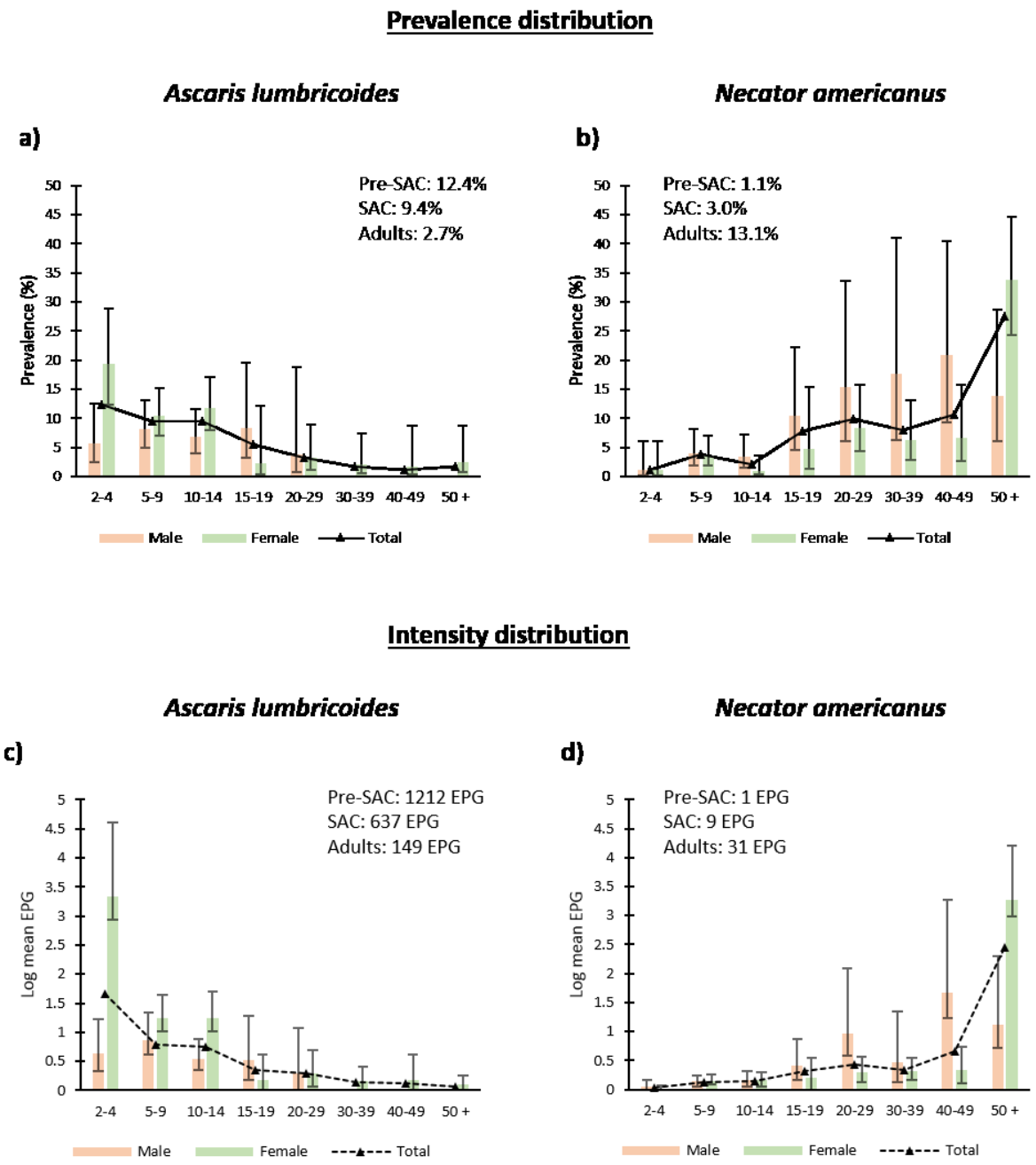


Figure 2.7. Prevalence and intensity distribution of STH infections. Distribution of infection prevalence and mean intensity according to age and gender. Mean intensity of infection was calculated using Williams mean, a variation of geometric mean where 1 is added to all values to allow the logarithmic transformation of zeros (uninfected individuals). Vertical lines represent binomial 95% confidence intervals (CIs) in **a)** and **b)** and normal 95% CIs (applied to the logarithmic transformed values) in **c)** and **d)**.

2.4.4. Frequency distribution of infection

The majority of the study participants (n=1,270, 86.7%) were found to be uninfected (EPG=0), and the frequency of EPG loads decreased rapidly with increasing intensity of infection (Figure 2.8). The mean intensity of *A. lumbricoides* infection in the population was 545 EPG, ranging between zero and 46,806 EPG, while the mean intensity of *N. americanus* infection was 14 EPG, ranging between zero and 5,604 EPG.

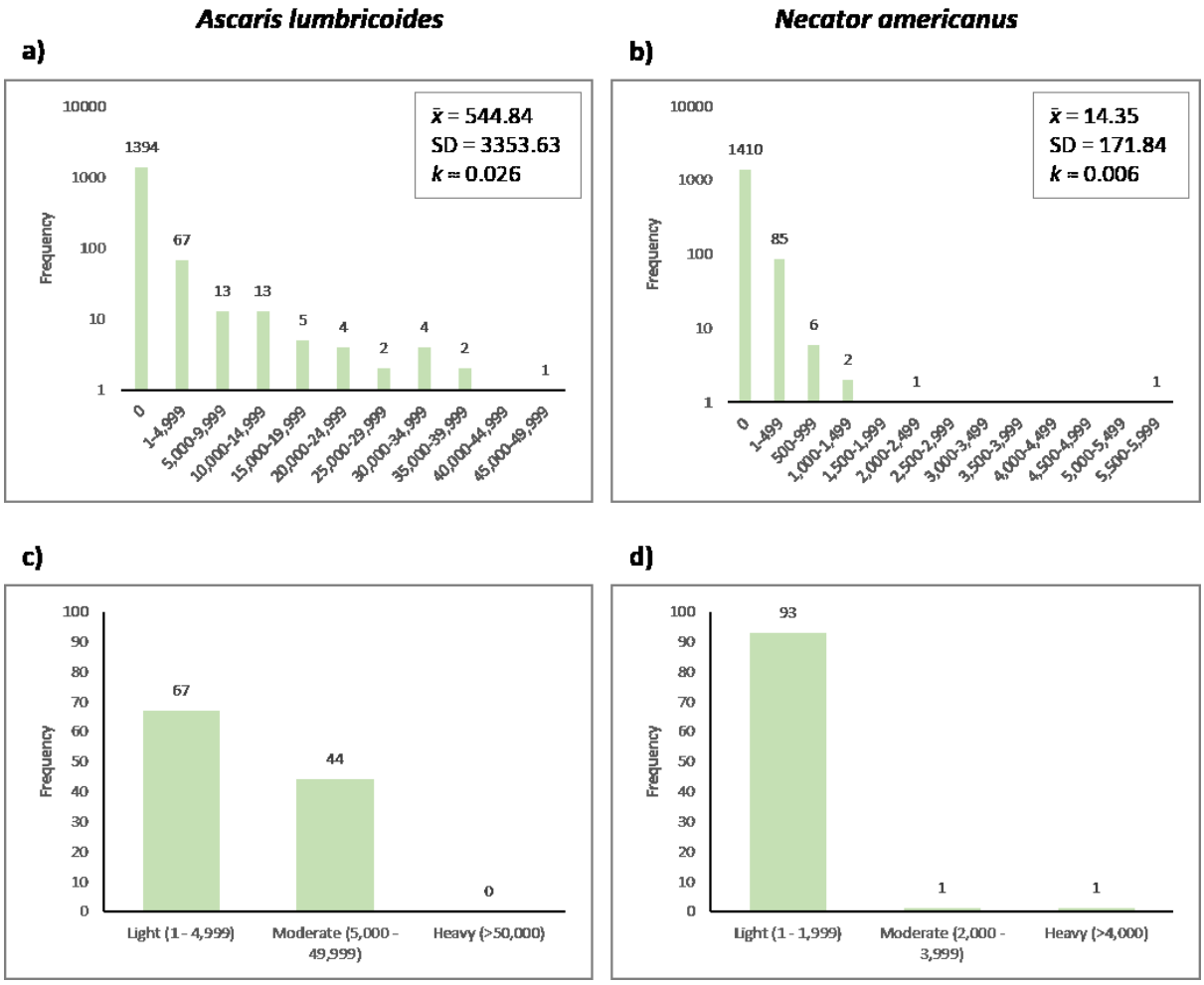


Figure 2.8. Frequency distribution of EPG. The distribution of individual intensity levels of *A. lumbricoides* infection, as measured by EPG, is represented in **a)** and that of *N. americanus* infections in **b)**. Both graphs are presented with a logarithmic *y* axis to enable visualisation. The frequency distribution of individual intensity levels of *A. lumbricoides* in terms of WHO intensity categories is presented in **c)** and that of *N. americanus* in **d)**. The arithmetic mean ($\bar{x}$), standard deviation (SD) and aggregation parameter *k* of the frequency histograms are provided. *k* was calculated using the corrected moment estimate. Numbers above columns shown the individuals falling in to each EPG category. EPG loads are used as proxy for the number of worms harboured by individuals.

The small measurements of the negative binomial aggregation parameter *k* for both frequency distributions were suggestive of strong aggregation of the parasite populations, as well as the high variance-to-mean ratios (index of dispersion) of D = 20,642 for *A. lumbricoides* and D = 2,058 for *N. americanus*. Using the WHO categories of infection intensity, most infected individuals harboured light *A. lumbricoides* infections (n=67/111, 60.4%) or light *N. americanus* infections (n=93/95, 97.9%) (Figures 2.8c and 2.8d). There were positive non-linear correlations between the prevalence and mean intensity of *A. lumbricoides* (R^2=0.73) and *N. americanus* (R^2=0.80) infection (Figure 2.9), showing the data is well described by the negative binomial distribution.

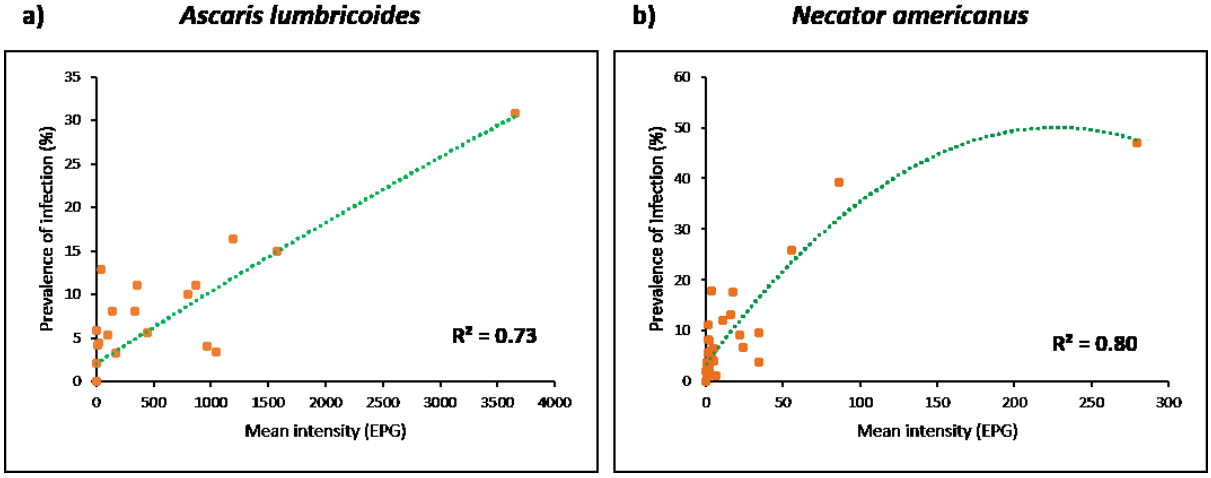


Figure 2.9. Relationship between prevalence and mean intensity of a) *A. lumbricoides* and b) *N. americanus* infection. Each point corresponds to one age group in one of the four villages, e.g. children between the age of 2-4 in Siangwe (total=32 points). The trendline coefficients of determination (R^2) provide good indications that the data coincide with a negative binomial distribution (R^2 close to 1). The mean intensity of infection increases with increasing prevalence of infection in the population, and the density-dependence effect is visible in the case of *N. americanus* infection.

2.4.5. Risk factors for STH infections

Children up to 14 years old, particularly pre-SAC, were at higher risk of *A. lumbricoides* infection than other age groups (OR=8.09, p=0.005), as well as residents of Siangwe village (Table 2.5). Improved pit latrines were found to be a protective factor against *A. lumbricoides* infection after adjusting for age, but the infection risk was increased if the latrine had no roof (OR=3.51, p<0.001) or if it was dirty, with visible urine or faeces in the floor (OR=1.68, p=0.024). Age and village were the most important predictors of *A. lumbricoides* infection in this population, with ownership, type and conditions of latrine also significant. On the other hand, younger age was a protective factor against *N. americanus* infection, with pre-SAC having the smallest odds of infection (OR=0.03, p<0.001) (Table 2.6). Having an improved pit latrine in the household was also a significant protective factor, as well as spending a higher weekly amount on toilet paper and soap. Being a farmer increased the odds of having *N. americanus* infection, even after adjusting for age (adj. OR=2.12, p=0.029). Finally, living in a household whose head had a low education level or a household that did not own a mobile phone were both risk factors for *N. americanus* infection.

2.4.6. Age and sex distribution of anaemia

The majority of the population was non-anaemic (73.1%, n=781), but still more than one in four individuals (26.9%, n=288) had some level of anaemia. Overall, women had a significantly higher prevalence of any anaemia than men (27.6% vs. 25.9%, χ^2=9.9, p=0.020), as well as a higher prevalence of moderate and severe anaemia (9.6% and 1.2%, respectively, Table 2.7). Significant differences were also found in the distribution of anaemia between age groups (χ^2=79.4, p<0.001), with women between 20 to 49 years old having much higher prevalence of mild, moderate and severe anaemia than their male counterparts (Figure 2.10). The only males found to have severe anaemia were boys between the ages of 2-4 years old.

2.4.7. Association between STH infections and anaemia

In terms of individual co-infection, no significant associations were found between the two STH infections (*A. lumbricoides* and *N. americanus)*, as only eight co-infections were observed (0.5% prevalence, OR=1.18, p=0.664). There was a significant association between *A. lumbricoides* infection and mild anaemia (OR=9.43, p=0.030), but not after adjusting for age and village (adj. OR=6.73, p=0.069). No significant association was found between *N. americanus* infection and anaemia.

Variables	Proportion infected			Intensity of infection		
Individual characteristics	OR (95% CI)	*p*-value	Adj. OR (95% CI)	*p*-value	Epg ratio (95% CI)	*p*-value
Age groups						
2-4	8.09 (1.86-35.10)	0.005 **	8.42 (1.92-36.96)	0.005 **	8.30 (1.95-35.34)	0.004 **
5-9	5.94 (1.41-25.04)	0.015 *	5.90 (1.39-25.07)	0.016 *	5.64 (1.36-23.40)	0.017 *
10-14	5.96 (1.41-25.15)	0.015 *	6.33 (1.49-27.00)	0.013 *	6.02 (1.45-25.02)	0.014 *
15-19	3.31 (0.63-17.49)	0.158 ns	3.10 (0.58-16.58)	0.186 ns	3.01 (0.58-15.54)	0.187 ns
20-29	1.95 (0.35-10.85)	0.446 ns	1.64 (0.29-9.25)	0.573 ns	1.67 (0.31-9.11)	0.555 ns
30-39	1.04 (0.14-7.49)	0.972 ns	1.11 (0.15-8.11)	0.917 ns	1.12 (0.16-7.98)	0.908 ns
40-49	0.68 (0.06-7.61)	0.753 ns	0.59 (0.05-6.67)	0.669 ns	0.60 (0.05-6.64)	0.678 ns
50+	1		1		1	
Household characteristics						
Village						
Siangwe	1		1		1	
Siaka	0.24 (0.13-0.45)	<0.001 ***	0.23 (0.12-0.44)	<0.001 ***	0.24 (0.13-0.45)	<0.001 ***
Sangalo	0.40 (0.25-0.66)	<0.001 ***	0.38 (0.23-0.63)	<0.001 ***	0.39 (0.24-0.63)	<0.001 ***
Nasimbo	0.16 (0.08-0.32)	<0.001 ***	0.14 (0.07-0.30)	<0.001 ***	0.15 (0.08-0.31)	<0.001 ***
Latrine type						
Absent	1		1		1	
Pit latrine, mud floor	0.57 (0.32-1.01)	0.055 ns	0.49 (0.26-0.90)	0.021 *	0.54 (0.31-0.94)	0.028 *
Pit latrine, cement floor	0.55 (0.27-1.12)	0.100 ns	0.41 (0.19-0.87)	0.020 *	0.49 (0.25-0.96)	0.039 *
Ventilated improved latrine	0.24 (0.05-1.08)	0.064 ns	0.22 (0.05-1.05)	0.058 ns	0.27 (0.06-1.20)	0.086 ns
Latrine roof						
Completely waterproof	1		1		1	
With holes	0.86 (0.52-1.44)	0.576 ns	0.78 (0.46-1.34)	0.372 ns	0.80 (0.49-1.33)	0.396 ns
No roof	3.51 (1.76-7.00)	<0.001 ***	2.60 (1.24-5.48)	0.012 *	2.68 (1.41-5.08)	0.003 **
Latrine floor						
Good condition	1		1		1	
Cracked	0.57 (0.34-0.96)	0.035 *	0.57 (0.33-0.98)	0.042 *	0.57 (0.34-0.96)	0.035 *
Broken	0.31 (0.04-2.29)	0.250 ns	0.43 (0.06-3.29)	0.416 ns	0.43 (0.06-3.14)	0.407 Ns
Latrine cleanliness						
Clean	1		1		1	
Unclean	1.68 (1.07-2.64)	0.024 *	1.62 (1.00-2.63)	0.049 *	1.62 (1.03-2.55)	0.036 *
Very unclean	1.51 (0.52-4.44)	0.451 ns	1.50 (.049-4.61)	0.479 ns	1.51 (0.53-4.29)	0.435 ns

Table 2.5. Risk factors associated with *A. lumbricoides* infection, determined using multivariate logistic regression modelling. The original models included 27 explanatory variables. Individual variables: age, sex, village, occupation, farmer (Y/N), infection with *N. americanus* and soil eating. Household variables: socioeconomic status, education level of head of household, floor, walls and roof materials of the household, type of latrine, handwashing products used, conditions of latrine floor, walls, roof and hole covering, cleanliness of latrine, cleansing materials visible in the latrine, weekly expenditure on soap and toilet paper and ownership of mobile phone, television, radio and electricity. Stepwise backwards logistic regression was performed, keeping only explanatory variables with *p*-values <0.05. EPG ratio (IRR) was calculated using dispersion(constant). Adjusted OR and EPG ratio were adjusted for age and village, except IRR of latrine type, adjusted for village only. * $p<0.05$ significant; ** $p<0.01$ very significant; *** $p<0.001$ extremely significant; ns $p>0.05$, not significant.

Variables	Proportion infected				Intensity of infection	
Individual characteristics	OR (95% CI)	*p*-value	Adj. OR (95% CI)	*p*-value	Epg ratio (95% CI)	*p*-value
Age groups						
2-4	0.03 (0.01-0.13)	<0.001 ***	0.03 (0.01-0.11)	<0.001 ***	0.03 (0.01-0.12)	<0.001 ***
5-9	0.10 (0.05-0.20)	<0.001 ***	0.09 (0.05-0.18)	<0.001 ***	0.10 (0.05-0.18)	<0.001 ***
10-14	0.06 (0.03-0.13)	<0.001 ***	0.06 (0.03-0.13)	<0.001 ***	0.06 (0.03-0.14)	<0.001 ***
15-19	0.22 (0.09-0.52)	0.001 **	0.21 (0.09-0.52)	0.001 **	0.23 (0.10-0.52)	<0.001 ***
20-29	0.29 (0.14-0.59)	0.001 **	0.24 (0.11-0.52)	<0.001 ***	0.26 (0.13-0.51)	<0.001 ***
30-39	0.23 (0.10-0.51)	<0.001 ***	0.19 (0.08-0.44)	<0.001 ***	0.20 (0.09-0.44)	<0.001 ***
40-49	0.31 (0.14-0.69)	0.004 **	0.28 (0.13-0.64)	0.002 **	0.30 (0.15-0.64)	0.002 **
50+	1		1		1	
Farmer						
No	1		1		1	
Yes	2.25 (1.17-4.31)	0.015 *	2.12 (1.08-4.15)	0.029 *	1.99 (1.07-3.70)	0.030 *
Household characteristics						
Education level of head of household						
None	3.17 (1.50-6.68)	0.002 **	1.80 (0.79-4.08)	0.161 ns	1.89 (0.91-3.92)	0.088 ns
Primary incomplete	1.88 (1.06-3.33)	0.031 *	1.55 (0.84-2.84)	0.158 ns	1.54 (0.87-2.71)	0.136 ns
Primary complete	0.73 (0.37-1.44)	0.359 ns	0.76 (0.38-1.54)	0.452 ns	0.76 (0.39-1.48)	0.416 ns
Secondary incomplete	1.52 (0.75-3.09)	0.246 ns	1.77 (0.85-3.71)	0.129 ns	1.66 (0.83-3.32)	0.149 ns
Secondary/higher education	1		1		1	
Owns mobile phone						
No	1		1		1	
Yes	0.37 (0.22-0.64)	<0.001 ***	0.54 (0.30-0.99)	0.046 *	0.57 (0.34-0.98)	0.041 *
Soap (weekly expenditure)	0.995 (0.99-0.999)	0.016 *	0.997 (0.99-1.00)	0.077 ns	0.996 (0.99-1.00)	0.061 ns
Toilet paper (weekly expenditure)	0.984 (0.97-0.995)	0.005 **	0.985 (0.97-0.997)	0.013 *	0.986 (0.97-0.997)	0.010 *
Latrine type						
Absent	1		1		1	
Pit latrine, mud floor	0.58 (0.33-1.01)	0.055 ns	0.57 (0.31-1.03)	0.062 ns	0.55 (0.32-0.93)	0.027 *
Pit latrine, cement floor	0.49 (0.24-0.99)	0.045 *	0.46 (0.22-0.97)	0.042 *	0.44 (0.22-0.86)	0.017 *
Ventilated improved latrine	0.11 (0.01-0.86)	0.035 *	0.10 (0.01-0.80)	0.030 *	0.11 (0.01-0.80)	0.029 *

Table 2.6. Risk factors associated with *N. americanus* infection, determined using multivariate logistic regression modelling. The original models included 27 explanatory variables. Individual variables: age, sex, village, occupation, farmer (Y/N), infection with *A. lumbricoides* and soil eating. Household variables: socioeconomic status, education level of head of household, floor, walls and roof materials of the household, type of latrine, handwashing products used, conditions of latrine floor, walls, roof and hole covering, cleanliness of latrine, cleansing materials visible in the latrine, weekly expenditure on soap and toilet paper and ownership of mobile phone, television, radio and electricity. Stepwise backwards logistic regression was performed, keeping only explanatory variables with *p*-values <0.05. EPG ratio (IRR) was calculated using dispersion(constant). Adjusted OR and EPG ratio were adjusted for age, except age groups which were adjusted for latrine type.
* $p<0.05$ significant; ** $p<0.01$ very significant; *** $p<0.001$ extremely significant; ns $p>0.05$, not significant.

	Male		Female		Total	
Status	%	n	%	n	%	n
Non-anaemic	74.1	306	72.4	475	73.1	781
Mild	20.3	84	16.8	110	18.1	194
Moderate	4.8	20	9.6	63	7.8	83
Severe	0.7	3	1.2	8	1.0	11
Total	100	413	100	656	100	1069

Table 2.7. Prevalence of male and female anaemia, stratified by WHO categories. Individuals were classified into the four categories of anaemia using WHO thresholds of haemoglobin concentration (*26*), corrected for age and sex and adjusted for altitude (-2 g/L in all categories to adjust for residence between 1000 and 1500m above sea level). All females age 15+ included in this analysis were assumed to be non-pregnant, as per study exclusion criteria. Significant differences were found between the distribution of male and female individuals into anaemia categories (Pearson's χ^2=9.9, *p*=0.020).

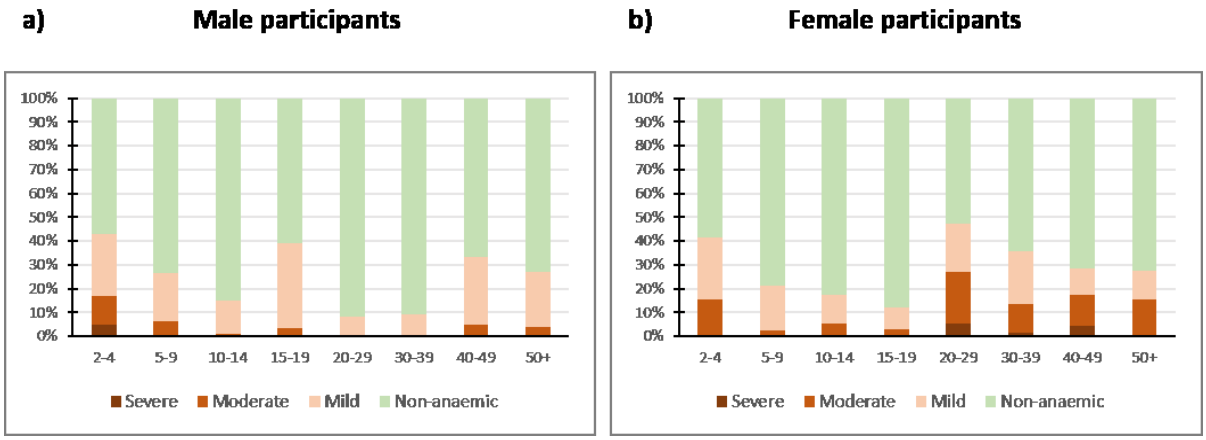


Figure 2.10. Age and sex distribution of anaemia. Cumulative distribution of four categories of anaemia in the male **a)** and female **b)** study population. The maximum *y* axis (100%) corresponds to the total number of individuals in the age group, e.g. 65 males in group 2-4 years old, of which 57% are non-anaemic (n=37), 26% have mild anaemia (n=17), 12% have moderate anaemia (n=8) and 5% have severe anaemia (n=3). Individuals were classified into the four categories of anaemia using WHO thresholds of haemoglobin concentration (*26*), corrected for age and sex and adjusted for altitude (-2 g/L in all categories to adjust for residence between 1000 and 1500m above sea level). All females age 15+ included in this analysis were assumed to be non-pregnant, as per study exclusion criteria.

2.5. Discussion

Overall, the prevalence and intensity of STH infection in the study area was relatively low.

Less than 20% of the study participants were diagnosed with any STH infection and 83% of these harboured only light intensity infections. Low prevalence and intensity of infection were expected in the study area, which had benefited from a high coverage of deworming treatment provided by the national school-based deworming (SBD) programme since 2012 (34). Several modelling studies have previously shown that STH infection levels should be reduced over time by repeated annual treatment (35,36), which these findings confirm, since the prevalence of *A. lumbricoides* and hookworm infections reported before the launch of the national SBD programme were 30.7% and 44.0%, respectively (37). However, the infection levels on which the national SBD programme was justified were based on SAC sampling in primary schools, which does not always represent infection levels across the whole community. Prevalence of STH infections in the community might have been lower or higher than those detected in primary-school children alone, depending on the proportion of STH infection in the community which were harboured by SAC (38). Regardless, the repeated annual treatment provided to SAC does reduce overall levels of infection in the community due to reducing the overall release of infective stages into the environment (39). The reduced contamination risk will lead to a lower reinfection rate and lower infection prevalence and intensity, at least for as long as annual deworming treatment is provided, and potentially longer. The reduction in transmission depends on the baseline endemicity, thus achieving infection levels which are low enough to enable a discontinuation of annual deworming programmes will vary between areas which had historically high or low infection intensities and endemicity.

The large number of light intensity infections found in this study may also be partially explained by the recent treatment provided by the national SBD programme and the extra deworming campaign reported by some participants, which took place eight and three months before this study, respectively. Light intensity infections are defined by a reduced number of EPG, which is used as proxy for a small number of adult worms harboured by an individual. Newly reinfected individuals are expected to have light intensity infections, as *A. lumbricoides* and *N. americanus* require two to three months from ingestion or skin penetration to reach full maturation, the capacity to produce eggs and, thus, be detected by Kato-Katz.

It is likely that the prevalence and intensity of STH infection were actually higher than the figures observed in this study. The Kato-Katz diagnostic technique was used in this study due to its low cost, fast and easy use in the field, access to highly trained parasitologists to perform it and because most STH control programmes still use this technique as their main parasitological diagnostic procedure

(40,41). However, it lacks sensitivity at low infection intensities, including post-treatment (42). Newer diagnostic tools such as qPCR can detect low intensity infections in Kato-Katz negative individuals, as reported in Easton *et al.* 2016 from a study in Bungoma (33).

The age-prevalence and age-intensity profiles observed for *A. lumbricoides* and *N. americanus* infections were consistent with the ones found in previous literature.

The full age profile of STH infections in the study area pre-national SBD programme are unknown. A study carried out in western Kenya in 1994 found 27% prevalence of *A. lumbricoides* infection in pre-SAC and SAC, which in adults decreased to 7%, as well as much higher intensity of infection in SAC than in the two other age groups (*40*). It also found that prevalence and intensity of hookworm infection increased with age. These profiles can be found in most moderately endemic untreated populations (7,45,46), and thus the pre-treatment age distribution in the study area is assumed to have been similar.

Since the launch of the national SBD programme in 2012, SAC have been targeted with annual preventive chemotherapy, which is expected to have reduced prevalence and, particularly, intensity of STH infection in this age group. In fact, the peak prevalence and peak intensity of *A. lumbricoides* infection were found in the pre-SAC and not the SAC group. This might be an indication that, despite the short interval since the last treatment round, the national SBD programme is having a positive impact in this section of the population. The treatment coverage of the national SBD programme in the previous year was reportedly high (18,37) and is assumed to have been similar in all schools in the area. The observed STH infections in SAC are, thus, assumed to be mostly due to reinfection since last treatment (and presumably some due to non-clearance), which is consistent with these findings.

On the other hand, the age profiles of hookworm infection are very similar to those expected of an untreated population. While the national SBD programme might be accountable for the small reduction in prevalence between the 5-9 (3.8%) and the 10-14 year olds (2.2%), adults still have much higher prevalence and intensity of infection, particularly the oldest age group (27.6%). This is consistent with the predictions of various modelling studies, which stressed the fact that most of the hookworm infection in endemic populations would not be controlled by treating SAC alone (38,43,47). The hookworm age profiles observed were also very similar to those of a study conducted in a community in rural Uganda with previous periodic SBD treatment (48).

Infection was heterogeneously distributed in the population, with village and household clusters, as well as individual aggregation.

The village of Siangwe, as well as its primary school, had a significantly higher prevalence of STH infection, in particular of *A. lumbricoides* infection, than the other villages and schools. This geographic clustering was not observed for hookworm infection, which had a more homogeneous distribution, not only among villages and schools but also between households. While there were only one or two individuals diagnosed with hookworm per household, some households had three, four and up to six participants diagnosed with *A. lumbricoides* infection. Overall, 23.8% of all participant households contained 43.5% of all individuals diagnosed with any STH infection. This spatial clustering of infection by village and by household has previously been reported in other studies (12,48), and it may be problematic for STH control programmes. The inclusion of these clusters of infection in monitoring and evaluation (M&E) studies is essential to correctly determine the prevalence and intensity of remaining STH infection. Individuals living in these villages or households may be either predisposed to infection due to genetic, environmental or behavioural factors, or be persistent non-compliers, being thus responsible for maintaining transmission in the population. The correct identification of clusters of infection will improve the success of control programmes.

In terms of individuals, 60% of *A. lumbricoides* and 98% of hookworm-diagnosed participants were found to harbour light intensity infections. The frequency distribution of intensity of infection (Figures 2.7a and b) showed that while the mean intensity of infection was relatively low due to the high number of light infections, the variance was much higher due to the small number of moderately and heavily infected individuals. The strong positive linear correlation between the variance and the mean intensity of infection was indicative of a negative binomial distribution, and the low values of the aggregation parameter *k* obtained for both *A. lumbricoides* and *N. americanus* infections provided further evidence of the overdispersion of worms in the host population (9,49). The individual aggregation of *A. lumbricoides* worms in the study population was indeed observed in a study carried out in Bungoma, where, in a worm burden range of 1 to 64 *A. lumbricoides* worms expelled following treatment with albendazole, 92.5% of the participants expelled only up to four worms (33).

School-based deworming, as a deworming strategy targeted at the group with heaviest worm burdens, should have a major impact on the distribution of worms in the population, at least in the short-term. In fact, while SAC as a group might have harboured the heaviest burdens of *A. lumbricoides* infection pre-national SBD programme, they are now on a par with pre-SAC, who were found to carry a slightly higher proportion of moderate intensity *A. lumbricoides* infections (5.1% vs. 4.1%). The national SBD programme is, however, unlikely to have had a similar effect on hookworm infection, and other treatment strategies are required to impact hookworm infections and transmission.

Associations between STH infections and demographic, socio-economic and WASH risk factors.

Contamination with *A. lumbricoides* requires egg ingestion, either through consumption of contaminated water or foodstuffs or due to poor hygiene, particularly poor hand hygiene. Children are likely to accidentally ingest eggs by playing in the soil and putting their hands in their mouths without washing them, particularly toddlers, but since no individual data on handwashing practices was collected in this study no associations can be made between hand hygiene and *A. lumbricoides* infection. While no significant association was found between water and infection, Siangwe had both the highest proportion of households using non-improved water sources (77.4%) and the highest proportion of households with *A. lumbricoides* infection (34.2%), while Siaka had the lowest proportion of households using non-improved water sources (43.0%) and the second lowest prevalence of *A. lumbricoides* infection (3.9%). Significant associations were found, however, between *A. lumbricoides* infection and latrine type and conditions. The risk of infection significantly decreased with improving latrine type, as expected. The association with ventilated improved latrines was not significant, though, possibly because only 5.4% of the study population (73 individuals in 26 households, 18 of those in Siaka) had access to this type of latrine, but also because a ventilated latrine does not reduce the risk of exposure in comparison with a non-ventilated pit latrine with cement floor. Having a latrine without a roof (allowing rain to fall inside) or with an unclean floor were both associated with increased risk of *A. lumbricoides*. The associations between *A. lumbricoides* infection and latrine use, type and conditions have been thoroughly described in previous literature (50). The findings of this study further corroborate the view that improvements in sanitation and hygiene are associated with decreased risk of *A. lumbricoides* infection.

Hookworm infection is acquired through skin penetration of larvae hatched in contaminated soil, and is primarily associated with farming, walking barefoot and, more generally, poverty (50,51). Young age was found to be the strongest protective factor against hookworm, which is a clear difference between the two parasites. A significant association was found between hookworm infection and being a farmer (Table 2.6), which remained significant after adjusting for age. Two thirds of adults in most villages work in the fields, even when farming is not their main occupation. The majority of them are women, with the highest proportion in the oldest age group, which also had the highest prevalence and intensity of hookworm infection. Living in a household whose head had a low education level or did not own a mobile phone were found to be risks for hookworm infection, possibly because of their association with poverty. Spending more money weekly on soap and toilet paper, which is negatively associated with poverty, were found to be protective factors against hookworm infection, as well as improved latrines. Transmission might occur via skin contact with contaminated muddy soil in unimproved latrines, and households which don't own a latrine might make a larger contribution to

transmission of infection. In fact, Siaka had the highest proportion of 1) ventilated improved latrines, 2) households in the least poor socioeconomic status, and 3) traders and specialist labourers, as well as the lowest proportion of farmers, and it also had the lowest prevalence of hookworm infection. Unfortunately, no data was collected on shoe-wearing, so no associations can be made between hookworm infection and walking barefoot.

While genetic factors were not investigated in this study, the presence of multiple *A. lumbricoides* infections per household could indicate a potential genetic predisposition associated with familial relationships, as well as shared behaviours or similar lack of treatment uptake (non-compliance). Previous studies have shown genetic similarities between *A. lumbricoides* worms collected from individuals living in the same and nearby households (52), and the evidence points towards a stronger contribution of household-associated exposure risk factors to clustering, rather than predisposition (12,48). Interestingly, the one household in Siaka where most infected individuals were found was in the second-lowest socio-economic status quintile and all six children were found to be infected. This observation highlights the role of poverty in the maintenance of STH infection.

No significant associations were found between anaemia and STH infection.

In contrast to previous studies, no significant associations were found between anaemia and STH infections (after adjusting for age) (53,54). However, anaemia due to helminth infections is generally associated with moderate to heavy worm burdens, and the great majority of STH infections harboured by this study's population were light intensity.

Women of reproductive age had the highest proportion of moderate and severe anaemia in the population, supporting previous work (55). Interestingly, all the moderately anaemic individuals who had concomitant hookworm infection were women above the age of 30 years. This association was not significant, which might be explained by the small sample size (n=5). Interestingly, SAC had a lower proportion of anaemia than the other age groups. Assuming all other factors not accounted for are equally distributed in the community, this might be seen as a positive impact of the national SBD programme. Since the main goal of the programme is to eliminate morbidity in SAC due to STH infections, this may be evidence that the goal is, at least in part, being achieved.

Due to the small number of individuals tested for anaemia who were found to be co-infected (n=7), no association was found between co-infection and anaemia. However, 43% (n=4) of the co-infected individuals had mild anaemia, a proportion much higher than the 23.7% (n=18) and 26.1% (n=18) of any anaemia in individuals with *A. lumbricoides* and *N. americanus* infections, respectively, and the 27.2% (n=249) of any anaemia in uninfected individuals. It is important to note that western Kenya is

a high malaria prevalence area, and since malaria is known to be associated with anaemia (54,56), this could partly explain the 27.2% prevalence of any anaemia in non-helminth infected individuals.

Study limitations

Enrolment in the study was randomized by household instead of individual participant in order to increase compliance, assuming that if all household occupants are recruited, instead of only one or a few family members, they are more likely to participate. Household-based enrolment also eased the work of the CWHs, who would have to visit a higher number of households daily to reach the same sample size if only some people from each house had been enrolled. This household randomization may have biased the children:adult or gender ratio of the population structure, but as the initial recruitment population structure mirrored to a degree the national population structure, this bias should have been minimal. Besides, the rural population structure should have a slightly larger proportion of children than the national population due to high reproduction rates in these communities and migration of working age individuals to urban centres.

However, the structure of the final (as opposed to the initially enrolled) study population did differ from the national population. This resulted from oversampling of SAC and undersampling of adults and pre-SAC. The study took place during the school term and, in order to ensure the participation of enrolled SAC, six schools were visited to collect samples from these children. Compliance is typically very high when sampling takes place in schools because children are readily available (57). Sampling adults, on the other hand, depends on their willingness to spend part of the day away from work and/or regular daily activities to bring the samples to a meeting point. It is a time-consuming task, and it might not be possible to visit the meeting point due to incompatibility with work hours or unanticipated events (e.g. sickness or funeral). Men in particular often have their place of occupation away from the family compound or even away from the village, making it particularly challenging to obtain their participation in the study. This difference in population structure may have biased our overall infection findings, but age and gender were included as variables in the analyses.

In terms of morbidity, no other data besides Hb measurements were collected. It is not possible to attribute to STH infections all the anaemia found, as no data on nutritional factors or other illnesses which might cause anaemia (e.g. sickle cell, malaria) were gathered. Still, nutritional factors are expected to be fairly similar between and within villages, and vary mostly between households with very distinct socio-economic status. Prevalence and intensity of malaria was not measured in this survey, but a follow-up survey conducted in the same villages in August and September found a community prevalence level (i.e. in all age groups) of 23.8%. As this survey took place in the rainy

season, the prevalence of malaria can be expected to have been slightly higher, and possibly close to the 27.2% prevalence of anaemia found through Hb measurements. Sickle cell is also known to be more prevalent in Africa in regions with historically high prevalence of malaria, such as western Kenya (58,59), and it may also be related to higher anaemia levels.

The use of duplicate Kato-Katz slides obtained from at least one sample per person (but up to four samples to a total of eight slides) might have biased the results. The Kato-Katz technique is known to have poor sensitivity but high specificity, making it more likely to obtain false negative than false positive results (60). Because repeated sampling compliance was low, particularly in the adult male groups, using only data from individuals who provided at least two stool samples would result in very small sample sizes in some age groups. While using data from individuals who provided only one sample might have erroneously increased the proportion of individuals recorded as uninfected (due to the low sensitivity of the technique), this is counter-balanced by the increased power provided by a larger sample size. Previous studies have shown that analysing only one sample with duplicate Kato-Katz slides (as opposed to two samples) does not significantly alter the prevalence observed for *A. lumbricoides* infection, but it might underestimate the prevalence of hookworm infection (57,61,62). Indeed, a study carried out in Bungoma analysing stool samples from the same individuals using both Kato-Katz and qPCR found a 4% higher prevalence of *A. lumbricoides* (17% vs. 13%) and 11% higher prevalence of *N. americanus* (18% vs 7%) detected by qPCR in relation to Kato-Katz (33). In future studies, the exact number of samples and Kato-Katz slides tested per individual should be included in the analyses in order to correct the estimated levels of infection.

The generally low prevalence and intensity of STH infection in the study population might also be associated with general improvements in socio-economic and WASH conditions in the area, as well as the national SBD programme. It is not possible, with the data gathered in this study, to accurately estimate the contribution of the national SBD programme to the general decrease in STH infection in the region, due to the absence of good individual-based compliance data within the national SBD programme. An important lesson for future work is to improve compliance information recording in national or regional deworming programmes.

Conclusions

This study demonstrates that despite progress made by the national deworming programme, STH infections, particularly hookworm infection, are not likely not be completely controlled through chemotherapy alone if only children are treated. The age patterns and aggregation of infection observed, together with the risk factors identified, emphasize the fact that transmission of STH

infection in the area is persistent, and additional intervention measures need to be taken into consideration for implementation. Adults should be included in the treatment schedule, particularly those whose main occupation might increase their exposure to soil contaminated with hookworm and, to a lesser extent, *A. lumbricoides* (e.g. farmers, brick makers, builders). Efforts to reach pre-SAC should be increased, as they were found to be 1.3 times more likely to carry *A. lumbricoides* worms than SAC (12.4% vs. 9.4% prevalence). Efforts should also be made to identify and treat households where infection is clustered, as these individuals might be predisposed to heavier burdens of infection or be systematic non-compliers to treatment, and are likely to play a major role in sustaining infection in the village communities. However, previous studies have highlighted the heavier costs of selective versus targeted (SBD) or community-wide deworming (63), and a cost-effective solution to identifying infection clusters is still required. The sensitivity of diagnostic techniques also needs to be improved in order to detect reducing prevalence in areas with ongoing deworming programmes (64). This will be increasingly important in monitoring and evaluation surveys of intensified control programmes, in areas where mass drug administration (MDA) has reduced STH prevalence to low levels. Finally, with infection levels decreasing, the focus needs to change from morbidity control to transmission control, going beyond preventive chemotherapy (65,66). Public health measures need to be implemented to increase health education and improve sanitation, including access to clean water and soap. These measures will, in turn, improve the socio-economic conditions of the population, alleviating poverty, which this study suggests is the key factor in the control and eventual elimination of STH infections.

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CHAPTER 3. REINFECTION WITH SOIL-TRANSMITTED HELMINTHS: IMPACT OF ONE ROUND OF COMMUNITY-WIDE MASS DRUG ADMINISTRATION

3.1. Summary

Background

School-based deworming has been implemented in many countries to control soil-transmitted helminth (STH) infections. However, the different worm species are overdispersed in the community and often infection may not be most prevalent in school aged children (SAC), and hence the successful control of STH-derived morbidity in this age group may not lead to transmission elimination or interruption within the total population. This is particularly the case for hookworm infections, where the highest intensities of infection are typically found in adult age groups (1). The research described in this chapter investigates the short-term impact of one round of community mass drug administration (MDA) on *A. lumbricoides* and *N. americanus* infection within four rural Kenyan villages.

Methods

Stool samples from 763 individuals (age range: 2-87) from Siangwe, Siaka, Sangalo and Nasimbo villages in Bungoma District, western Kenya, were analysed by duplicate Kato-Katz during two cross-sectional surveys in 2014. The first survey (baseline) in this longitudinal study was carried out in Mar-Apr 2014, followed by community MDA of albendazole in May 2014, and the second survey (follow-up) took place three months post-treatment in Aug-Sep 2014, when samples were collected from the same individuals. Parasitological data collected during the two surveys, as well as treatment coverage and efficacy data, collected during and up to four weeks post-treatment from all infected participants, were used to evaluate the impact of one round of community MDA on this population exposed to school-based deworming (SBD) since 2012.

Results

A total of 1,464 study participants (30.7% of the total registered population of 4,773) participated in the study baseline and 1,170 (24.5%) participated the study follow-up, with only 763 individuals (16.0%) being effectively followed-up. Treatment coverage was high, with 93.9% of followed-up study participants reportedly receiving 400mg albendazole tablets. The treatment was deemed effective for both *A. lumbricoides* and *N. americanus*, with cure rates (CR) of 98.9% and 91.5% and egg reduction rates (ERR) of 99.7% and 95.8%, respectively, as measured three weeks post-treatment. Though the community MDA failed to clear STH infection in the four villages, the proportion infected with *A. lumbricoides* decreased by 81.6% between study baseline and follow-up, and infection with *N. americanus* was reduced by 79.1%. Only eight individuals (6.7%) were reinfected at follow-up, thus

predisposition to heavy or light infection and risk factors associated with reinfection could not be assessed.

Conclusions

Despite the short evaluation period, a high-coverage community-based MDA of albendazole was not able to clear STH infections in this setting. However, it had a marked impact on the levels of infection in the community, in particular *N. americanus*, showing that expanding deworming to include adults will increase treatment effectiveness in areas where hookworm is endemic.

3.2. Introduction

Soil-transmitted helminth (STH) infections are still common in sub-Saharan Africa and other areas in developing countries in Asia and South America (2,3). They typically affect the rural and deprived sectors of the population, whose poverty is maintained by the harmful effects of chronic infection on health, nutrition, education and productivity (4). The most common strategy to control STH is via preventive chemotherapy, which consists of the large-scale distribution of anthelminthic drugs (usually albendazole or mebendazole) to an endemic population without previous diagnosis (2,3,5). This is typically targeted at SAC due to the high levels of infection usually observed in this age group (5-14 years) and because schools offer a cost-effective platform for mass drug administration (6,7).

STH infections have long been recognised in Kenya as a cause of morbidity, with published research studies and medical reports recording the presence of both species of hookworm and *A. lumbricoides* infections throughout the country, going back to surveys in the early 1910s (8). Both *A. lumbricoides* and *N. americanus* have long been endemic in the western region, with studies reporting up to 72.6% prevalence of hookworm in adults in 1958 (9) and up to 64% prevalence of *A. lumbricoides* in SAC in 1981 (10). Several campaigns against both STH infections have taken place in various parts of the country since the 1920s (8). However, the first nationwide programme, which included the Western Province, was initiated in 2009, with annual school-based administration of albendazole to SAC living in areas with estimated prevalence of any STH between 20% and 50% and bi-annual if above 50% (11–13). The national school-based deworming (SBD) programme was restarted in 2012 (11) and albendazole has since been administered to SAC and pre-SAC in the Western Province (including primary schools attended by children enrolled in this study) on a fairly regular basis, although this varies by region (11,14) (see Figures 3.1 and 3.2). Besides the national SBD programme, some local organisations carry out periodic deworming campaigns in the area, and both children and adults can have access to deworming drugs either at health facilities or drug dispensaries. The latest official

government figures on treatment coverage in Kenya, as reported to the World Health Organization (WHO), show that 42% of pre-SAC and 59% of SAC in need of preventive chemotherapy received albendazole tablets in 2014 (15).

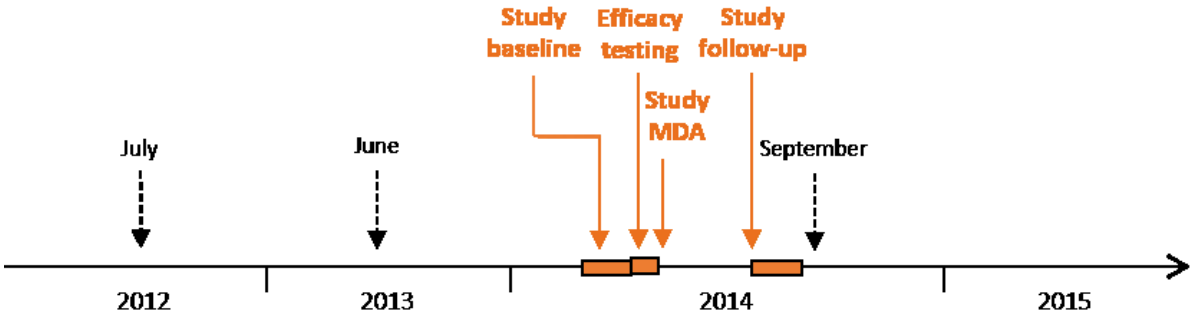


Figure 3.1. Timeline of study stages and deworming campaigns in Bungoma since the launch of the National School-Based Deworming Programme. A school-based deworming programme first started distributing 400mg single oral doses of albendazole to SAC in Bungoma in July 2012 and for the second time in June 2013, as well as. The study baseline (first cross-sectional survey) took place in Mar-Apr 2014, followed by the efficacy testing in mid to late April and the study's community-based MDA in mid-May 2014, which provided 400mg single oral doses of albendazole to all participating residents of four rural villages in Bungoma District. The study follow-up took place three months later in Aug-Sep 2014 and was immediately followed by the third round of the national SBD programme in late September 2014.



Figure 3.2. Advertising poster for the Kenya National School-Based Deworming Programme of 2014. kiSwahili advertisement of the school deworming day in Bungoma District. The poster states that treatment is free (no payment required), available for children aged 2-14 regardless of school registration, and parents are informed to take their children aged 2-14 to school on the 25th September 2014 from 9am.

The Kenyan national SBD programme aims to achieve the target of treating at least 75% of children living in endemic areas and eliminate morbidity caused by STH infections by 2020 (16), in line with the current WHO treatment policy for STH.

With the success of the national SBD programme (despite being short of the 75% target), and with generally increasing improvements in sanitation, access to clean water and health education, STH infection levels have been slowly decreasing over the country (8,17,18), and many areas now have very low STH infection prevalence. In these settings, it may be possible to attempt to eliminate transmission of infection through MDA alone. This might be possible by expanding preventive chemotherapy to other age groups of the population in an MDA programme similar to the one for lymphatic filariasis, which targets the whole community above five years of age (19) often using the same drug, albendazole. This approach might be particularly effective in areas affected by hookworm infection, since adults (15+ years), who do not directly benefit from school-based programmes, typically harbour most of the hookworms in the community (20–22), as supported by the findings presented in Chapter 2. Still, in any given area endemic for STH infections, while children might harbour most of the worms, in the case of *Ascaris* and *Trichuris*, infected adults may still maintain transmission of infection in the community if left untreated (23).

One of the most characteristic features of the epidemiology of STH infections is the highly-aggregated distribution of worms per person in the community, so that most individuals harbour light intensity infections while a few harbour heavy worm burdens. This aggregation or overdispersion of worms in the population can partly be explained by an individual's predisposition to heavy or light infection, which has previously been demonstrated for both *A. lumbricoides* and hookworm (24,25). The processes responsible for predisposition might be related to differences in behavioural or environmental exposure to infection, genetic susceptibility, ability to generate an effective immune response or a combination of these factors (26,27). In addition, individuals of all age groups can become reinfected after treatment, and there is limited evidence of effective immune responses to prevent re-infection. The reinfection rate depends on a series of factors, namely the helminth species' life expectancy, the intensity of transmission in the community, the treatment efficacy and the treatment coverage (28).

The aims of this study were to 1) quantify the impact of a community-wide treatment intervention on *A. lumbricoides* and *N. americanus* infections, 2) investigate evidence for predisposition using data from successfully followed-up individuals, and 3) identify risk factors associated with residual infection and reinfection. These findings from rural Kenya are of interest to public health specialists and programme managers when considering treatment strategies and deworming goals, particularly in

areas where deworming campaigns are currently ongoing and the prevalence of infection has reached low levels.

3.3. Materials and Methods

3.3.1. Ethical considerations and treatment

As referred in Chapter 2, ethical approval was obtained from the Kenya Medical Research Institute (KEMRI Scientific Steering Committee [SSC] no. 2687), the Kenyan National Ethics Review Committee, and the Imperial College London Research Ethics Committee (ICREC 13_3_10). Written informed consent was obtained from participants aged 18 and over and parents/guardians provided written informed consent for their child's participation (17 years old and younger). Children younger than 12 years consented verbally and children between 12-17 years of age were informed about the aims of the study and also signed their own assent forms. Participation was voluntary and withdrawal was possible at any time without further obligation or exclusion from anthelminthic treatment. All parasitological and survey data were coded and treated confidentially. Additionally, all community members registered in the study were treated free of charge with a single oral dose of albendazole (400mg) soon after the study baseline, regardless of sample provision or the results of the diagnostic tests.

3.3.2. Study area and population

The study was carried out in four rural villages near Bungoma, western Kenya, where 3,530 individuals from 631 households were randomly selected to participate (see Chapter 2). The four villages (Siangwe, Siaka, Sangalo and Nasimbo) lay in an area endemic for STH, where primary schools had been involved in the national SBD programme since its launch in 2012. At the beginning of this study's first cross-sectional survey in March 2014, most pre-SAC and SAC living in these villages had been dewormed by the national SBD programme in June 2013 and more recently in November 2013, nearly four months earlier, in a deworming campaign ran by an unknown organization.

Exclusion criteria were based on international safety regulations for albendazole (29,30), the deworming drug administered during the study, which stipulate that the 400mg tablets should not be provided to children under 24 months of age and women in the first trimester of pregnancy. Thus, children younger than two years old were excluded and, due to uncertainty regarding pregnancy stages, all pregnant women were also excluded.

3.3.3. Field and laboratory procedures

This study involved two cross-sectional surveys, the first in March and April 2014 (i.e. study baseline, see Chapter 2) and the second four months later in August-September 2014 (i.e. study follow-up) (Figure 3.1). Immediately after the first survey, all study participants found to be infected were treated with 400mg albendazole in order to test the efficacy of the drug (see 3.3.3.2. below), and in mid-May 2014 the same deworming drug was distributed by community health workers (CHWs) to all other eligible individuals registered in the study to simulate a community-wide MDA campaign. All study participants found to be infected at study follow-up were also treated immediately after the survey in mid-September. The 2014's round of the national SBD programme took place on the 25th September, soon after the conclusion of the study, and therefore does not affect the results presented here.

3.3.3.1. Sample collection

At study baseline (Chapter 2), which took place during the school term in March and April 2014, six primary schools were visited to collect stool samples from enrolled children, while samples from all other participants were collected at village meeting points. At study follow-up, which took place over the school holidays in August and September 2014, sample collection took place only at meeting points. During both surveys, an attempt was made to collect two stool samples over two consecutive days from each study participant. Attempts were also made at study follow-up to resample all individuals who participated in the study baseline. Each sample provided was analysed within 8h from collection using the duplicate Kato-Katz method to obtain a measure of intensity of STH infection (see Chapter 2).

3.3.3.2. Drug efficacy

Following baseline sample collection (described in Chapter 2), all individuals found to be infected with any STH were treated with 400mg albendazole tablets and followed-up over four weeks to measure treatment efficacy. All previously infected participants from two villages – Siangwe and Sangalo – were assigned for weekly efficacy analysis (weeks one to four post-treatment), and previously infected participants from the two other villages were assessed only once, three weeks post-treatment. Small stool samples were obtained from each previously infected participant, as well as from one or two non-infected family members, to avoid stigmatising infected individuals, and in an attempt to increase compliance. Participants from each village were divided into daily groups to ensure that all participants from a single village could be tested in the same week. CHWs and village managers started distributing small plastic containers on Sunday to the first group of participants to be collected and

analysed on Monday morning. On Monday afternoon, they would distribute containers to the following group to be analysed on Tuesday and so on until all previously infected participants from each village were tested. In the villages assessed weekly, this process was repeated at weeks 1, 2, 3 and 4 post-treatment. In the two other villages this process took place only three weeks post-treatment. Stool samples collected during treatment efficacy analysis were analysed using the duplicate Kato-Katz method as previously mentioned in Chapter 2, and faecal egg counts were recorded to allow calculation of both cure rates (CRs) and egg reduction rates (ERRs).

3.3.3.3. Community-wide drug distribution

Following the study baseline and treatment efficacy testing, 400mg albendazole tablets were delivered to the CHWs and village managers involved in the study, to be distributed among all 4,773 individuals who had been visited and had verbally agreed to participate in the study during the recruitment phase (Nov-Dec 2013 – see Chapter 2). The CHWs and village managers were provided with a list of the individuals to be treated and asked to record for each person whether treatment was provided, the treatment date and whether ingestion of the tablet was directly observed. Besides the community-wide MDA, deworming tablets were also provided to the six primary schools where sample collection took place during the study baseline. All six schools received enough tablets to treat all registered students, regardless of their participation in the study or residency in the study villages.

3.3.4. Statistical analysis

The study population was divided into eight age groups to allow a series of analyses on the effect of age on STH infection and treatment impact. In addition, results are presented for the three age groups defined by the World Health Organization (WHO): the pre-SAC (ages 2-4, taking into account the exclusion of children below the age of two), SAC (5-9 and 10-14), and adults (15-19, 20-29, 30-39, 40-49 and 50+).

When comparing characteristics of the study population against the participants who were lost to follow-up, Kruskall-Wallis rank tests were used to measure differences in categorical variables between the two populations. Logistic regression was used to measure differences in means (prevalence of infection and treatment coverage), and negative binomial regression was used to measure differences in intensity of infection between the two populations (continuous non-random variable).

Treatment coverage is usually reported as *n/N*, where *n* is the number of people treated and *N* can either be the total number of people in the population or the number of eligible people. When measuring compliance instead of coverage, *N* can also be the number of people who are offered treatment and *n* the number of people who actually take/ingest the treatment (31).

In this study, *n* refers to the number of people recorded as having been given deworming tablets (who might or might not have ingested them), and *N* refers to the total eligible population surveyed at the beginning of the study (Nov-Dec 2013 – see Chapter 2). From this population, which represents a study census of the four villages involved, children younger than two years of age and pregnant women were excluded, since they could not receive deworming treatment due to safety regulations. Individuals who died or migrated during the period between the study recruitment and MDA were also excluded.

The **treatment efficacy** of 400mg albendazole tablets for both *A. lumbricoides* and *N. americanus* infection was calculated based on the reduction in infected individuals, CR, and the reduction in faecal egg counts, ERR. The ERR was calculated using the group-based arithmetic mean formula recommended by Vercruysse *et al.* and the WHO (32,33):

$$ERR = 100 \times \frac{arithmetic\ mean\ [pre\ treatment\ EPG] - arithmetic\ mean\ [post\ treatment\ EPG]}{arithmetic\ mean\ [pre\ treatment\ EPG]}$$

This formula ignores individual variability, so the effect of confounding factors cannot be easily measured using statistical analysis. However, it is considered to be the most sensitive formula to measure population level changes in drug efficacy and to capture variation more effectively than geometric mean formulas.

The prevalence of each STH species was calculated, and their 95% confidence intervals (CI) were obtained using either the positive binomial (normal) distribution for discrete variables (sample mean and standard error) or the Student's *t* distribution when *n* was lower than 60 individuals. The Williams mean intensity of STH infection for each age group was also calculated for both infected and uninfected individuals, and asymmetric 95% CIs were calculated using the logarithmically transformed values. This procedure was adopted because the intensity measures had an aggregated distribution, with variance greater than the mean (a negative binomial distribution for discrete counts).

Predisposition to STH infection was assessed using the Spearman's rho, Kendall's tau and pairwise correlation non-parametric methods applied to the non-normal distribution of EPG (variance greater

than the mean in value) (34). These methods are used to measure the strength of association between two variables, in this case between infection at baseline (pre-treatment) and follow-up (post-treatment). The continuous variable of eggs-per-gram (EPG) was used instead of the binary positive/negative to investigate whether heavier infection pre-treatment (i.e. higher EPG) was associated with heavier infection post-treatment. Due to the very small number of reinfections (n=8), this analysis was not viable for *N. americanus* infection, but could be performed for *A. lumbricoides*. An attempt was made at identifying factors associated with both residual infection (infection detected during treatment efficacy testing) and reinfection, using models of logistic regression and negative binomial regression. However, due to the small numbers in both groups of individuals, these analyses could not be carried out.

3.4. Results

3.4.1. Characteristics of study population

The population surveyed in the four villages involved in this study totalled 4,773 individuals (Figure 3.3). This population was reduced to 3,530 individuals randomly selected for participation, due to logistical and financial constraints (see Chapter 2). During the first cross-sectional survey (study baseline, Chapter 2), a total of 1,464 eligible individuals (41.5% of the randomly selected population) provided at least one stool sample for analysis, while 1,170 eligible individuals (33.1%) provided samples during this follow-up study. However, only 763 individuals (21.6%) participated in both surveys and were thus successfully followed-up and considered as the study population in this chapter. 701 individuals only delivered samples during the study baseline and 407 others only during the follow-up.

When comparing the individual and household characteristics of the participants who were successfully followed-up (n=763) and the ones who were lost to follow-up (n=701), the two groups are similar, although there was a significant difference between their age distribution – a larger proportion of adults in comparison to children was lost to follow-up (Table 3.1). The population lost to follow-up also had a significantly lower uptake of deworming treatment during the study's MDA and a significantly lower prevalence of *A. lumbricoides* infection, even after adjusting for age (OR=1.99, CI:1.30-3.06, p=0.002 - see Table 3.2). The mean intensity of *A. lumbricoides* infection was also significantly lower in the population lost to follow-up, but this difference became non-significant once adjusted for age (IRR=3.45, CI: 0.81-14.68, p=0.094).

The followed-up population had only 30.5% adult participants (aged above 15 years) and only 37.6% male participants overall (Table 3.3). As previously seen in Chapter 2, the population pyramid of the study baseline revealed oversampling of pre-SAC and SAC, as well as undersampling of both male and female adults in comparison with the national demographic profile (Figures 3.4a and 3.4b). This contrast was further amplified in the followed-up population, which had a particularly small proportion of male adults of working age (15-59) (Figure 3.4c).

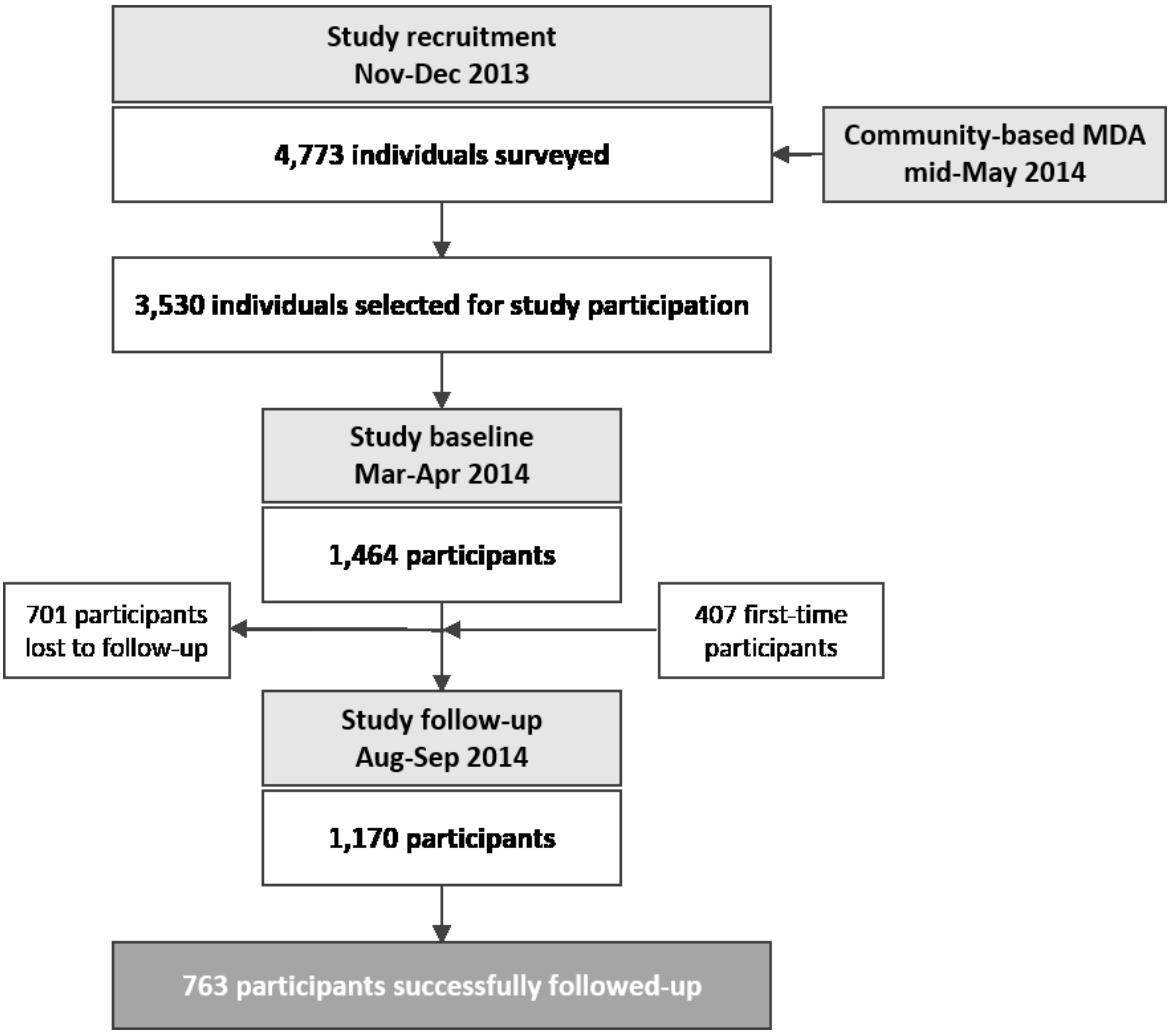


Figure 3.3. Diagram of the study population. In the initial study recruitment carried out in Nov-Dec 2013, a total of 4,773 people were registered in the study. Due to logistical reasons, this number was reduced to 3,530 individuals, based on a randomized household selection (see Chapter 2). Out of these 3,530 individuals, 1,464 participated in the study baseline and 1,170 participated in the study follow-up, with only 763 participating in both surveys. The community-based MDA, which took place between the study baseline and follow-up in mid-May 2013, was targeted at all the individuals surveyed in the initial study recruitment (excluding ineligible people and migrants).

	Followed-up		Lost to follow-up		Difference		
Individual characteristics	%	n	%	n	χ^2	P-value	
Sex							
Male	37.6	(287/763)	43.1	(302/701)			
Female	62.4	(476/763)	56.9	(399/701)	3.3	0.070	ns
Age groups							
2-4	14.3	(109/763)	9.7	(68/701)			
5-9	30.9	(236/763)	22.2	(156/701)			
10-14	24.2	(185/763)	26.4	(185/701)			
15-19	5.0	(38/763)	7.6	(53/701)			
20-29	7.2	(55/763)	9.4	(66/701)			
30-39	5.1	(39/763)	10.4	(73/701)			
40-49	5.1	(39/763)	6.6	(46/701)			
50+	8.1	(62/763)	7.7	(54/701)	23.1	<0.001	***
Occupation							
At school (primary or secondary)	72.4	(477/659)	65.1	(396/608)			
Farmer	17.6	(116/659)	19.7	(120/608)			
Trader	3.9	(26/659)	6.6	(40/608)			
Teacher/Civil servant	1.7	(11/659)	1.8	(11/608)			
Specialist labour	0.8	(5/659)	1.3	(8/608)			
Other	3.6	(24/659)	5.4	(33/608)	5.9	0.015	*
Household characteristics							
Education level of head of household							
None	5.2	(39/744)	6.7	(47/696)			
Primary incomplete	24.9	(185/744)	21.0	(146/696)			
Primary complete	26.5	(197/744)	27.9	(194/696)			
Secondary incomplete	13.4	(100/744)	11.5	(80/696)			
Secondary/Higher education	31.3	(223/744)	32.9	(229/696)	0.6	0.425	ns
Socioeconomic status							
Poorest	26.2	(158/602)	21.9	(124/565)			
Very poor	19.3	(116/602)	24.2	(137/565)			
Poor	21.6	(130/602)	22.5	(127/565)			
Less poor	20.6	(124/602)	16.3	(92/565)			
Least poor	12.3	(74/602)	15.0	(85/565)	0.3	0.569	ns
Floor							
Mud	86.5	(639/739)	85.7	(589/686)			
Cement	13.5	(100/739)	14.1	(97/686)	0.04	0.842	ns
Roof							
Thatch	4.6	(34/741)	5.4	(37/684)			
Iron sheets	95.4	(707/741)	94.6	(647/684)	0.07	0.788	ns
Water source							
Non-improved	62.5	(471/754)	68.7	(481/700)			
Improved	37.5	(283/754)	31.3	(219/700)	4.2	0.039	*
Latrine							
Absent	12.7	(89/698)	11.3	(75/663)			
Pit latrine with mud floor	59.7	(417/698)	64.1	(425/663)			
Pit latrine with cement floor	23.3	(163/698)	17.9	(119/663)			
Ventilated improved pit latrine	4.1	(29/698)	6.6	(44/663)	0.05	0.822	ns

Table 3.1. Characteristics of study population and population lost to follow-up. Comparing characteristics between the study population (n=763) and the population lost to follow-up (n=701). χ^2 was calculated using the Kruskall-Wallis rank test. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; *ns* $p \geq 0.05$, not significant.

	Followed-up		Lost to follow-up		Difference			
Treatment coverage	***%***	***n***	***%***	***n***	***OR***	***CI***	***p-value***	
	93.8	(702/748)	90.3	(626/693)	1.63	1.11-2.41	0.014	*
Infection prevalence	***%***	***n***	***%***	***n***	***OR***	***CI***	***p-value***	
A. lumbricoides	9.8	(75/763)	4.7	(33/701)	2.21	1.45-3.37	<0.001	***
N. americanus	6.7	(51/763)	6.1	(43/701)	1.10	0.72-1.67	0.668	ns
Mean intensity of infection	***Mean***	***SD***	***Mean***	***SD***	***IRR***	***CI***	***p-value***	
A. lumbricoides	849	4218	214	1983	3.97	1.17-13.48	0.027	*
N. americanus	13.2	113.1	15.6	218.6	0.85	0.29-2.49	0.767	ns

Table 3.2. (cont.) Characteristics of study population and population lost to follow-up. Followed-up individuals were more likely to have been infected with *A. lumbricoides* at baseline than individuals who were lost to follow-up. This difference remained significant after adjusting for age: OR=1.99 (CI:1.30-3.06, p=0.002). There was no significant difference in baseline prevalence of *N. americanus* between individuals who were followed-up and lost to follow-up. The OR (odds ratio) was obtained using logistic regression, while the IRR (incidence-rate ratio) was obtained using negative binomial regression. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; *ns* $p \geq 0.05$, not significant.

Age group	National population (%)	% male	Study baseline population (%)	% male	Followed-up population (%)	% male
Pre-SAC (2-4 years)	9.5	50.2	12.1	50.3	14.3	48.6
SAC (5-14 years)	29.1	50.1	52.0	45.8	55.2	40.9
Adults (15+ years)	61.4	49.7	35.9	28.8	30.5	26.6
Total	100	**49.9**	100	**40.2**	100	**37.6**

Table 3.3. Comparing national, study baseline and followed-up population. Proportion of population (percentage) in each major age group as defined by the WHO. The national population of Kenya in 2014 (data from US Census Bureau International Database (52)) was adapted to exclude children younger than two years old. The study baseline population refers to individuals which participated in the first cross-sectional survey (study baseline) carried out in Mar-Apr 2014 (n=1,464). The followed-up population refers to the individuals who participated in both cross-sectional surveys and were thus successfully followed-up (n=763).

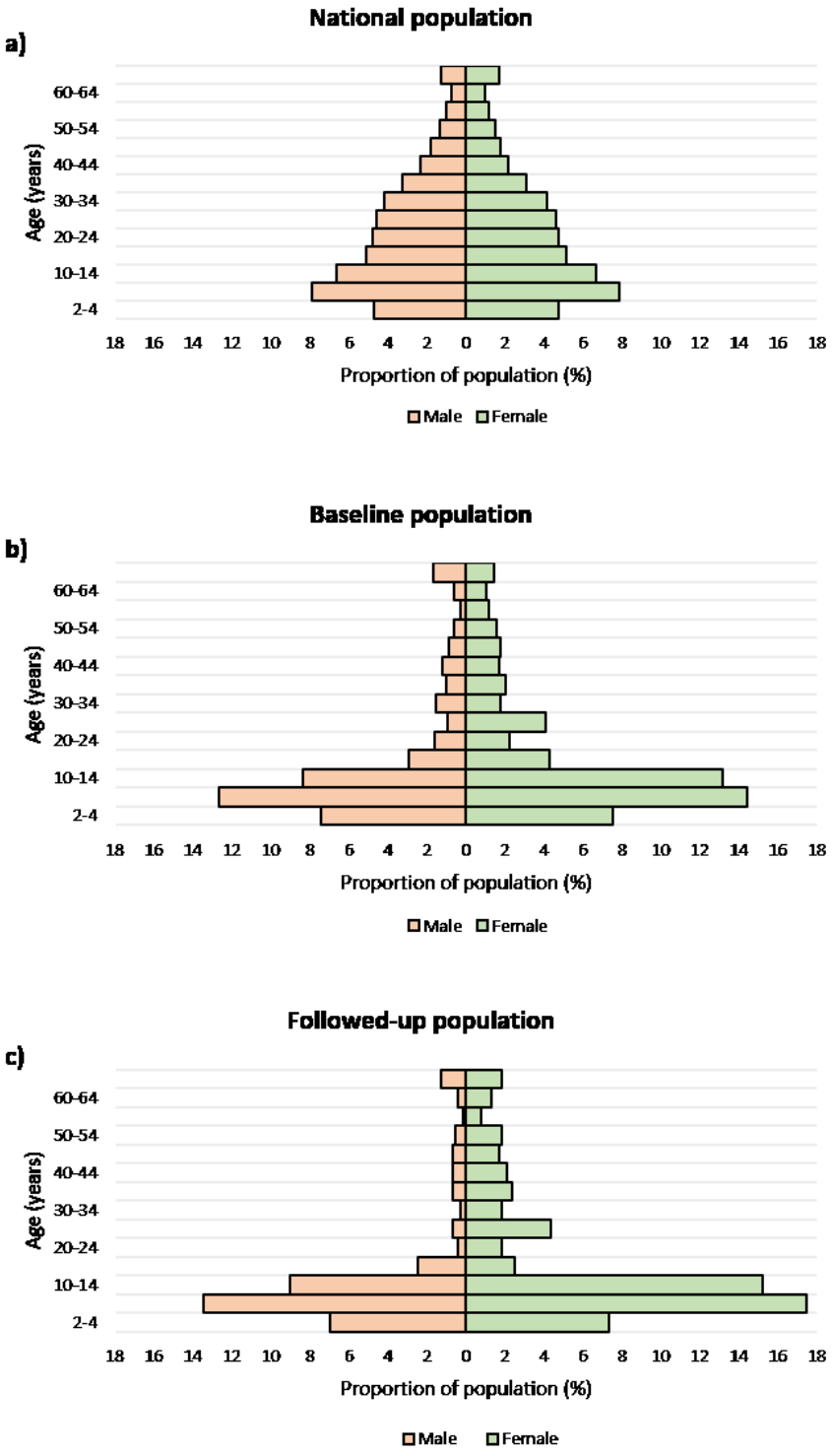


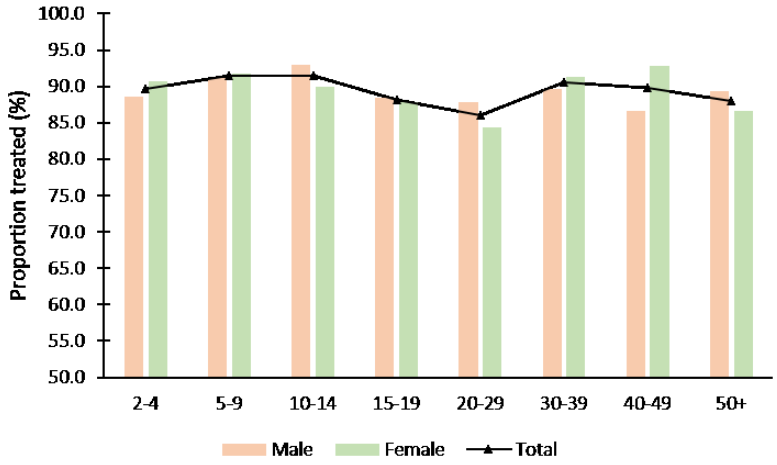
Figure 3.4. Pyramid diagram of Kenyan and study population. a) Kenyan national population pyramid for the year 2014, adapted to exclude children younger than two years old from data obtained from the US Census Bureau International Database (52); **b)** Population pyramid of study baseline participants (n=1,464); **c)** Population pyramid of individuals who participated in both cross-sectional surveys and was thus successfully followed-up – study population (n=763).

3.4.2. Treatment coverage

A total of 4,773 residents – the initially surveyed population – were targeted for MDA, but full treatment records could only be obtained for 3,687 eligible individuals. A total of 36 female residents could not be treated due to pregnancy, and 34 participants (of various age groups) had migrated out of the study population area. The overall treatment coverage of the total population eligible for treatment achieved during the study's community-based MDA was 89.5%, with no significant differences between the 89.6%, 91.4% and 88.3% coverage in pre-SAC, SAC and adults, respectively (Figure 3.5). The difference in treatment coverage between men and women was also non-significant, with 89.7% of male and 89.4% of females receiving treatment, but there was a significant difference between villages (χ^2=19.6, p<0.001). Siaka had the highest treatment coverage at 97.7%, and Nasimbo had the lowest at 86.3% (Table 3.4).

The treatment coverage in the subset of the study population (those successfully followed-up, n=763) was overall higher than that of the whole MDA. Of the followed-up population, 93.9% received treatment, including 96.3% of the pre-SAC, 94.0% of the SAC and 92.4% of the adults (Figure 3.6). The younger adults (15-19 years) had a slightly lower treatment uptake than other age groups, but this difference was not significant. The lower treatment uptake in young women when compared to their male counterparts was partly due to pregnancy, but the majority of female non-compliers (4 of 5) was not available during treatment distribution. There were still differences in treatment coverage in this subset of the population between the villages (Table 3.5), with Siaka achieving 100% of coverage and Sangalo only 87.3%, but overall these differences were not significant (χ^2=3.8, p=0.289).

Some study participants were treated more than once during the course of the study. This includes individuals who were found to be infected with any STH at both study baseline and study follow-up (n=10, excluding one pregnant female who was not treated), which were treated immediately after each survey. It also includes individuals who did not participate in the study baseline but were treated during the community MDA and later found to be infected at study follow-up (n=14). Some individuals who were found to be infected at study baseline might have also received treatment during the MDA, despite the fact they were dewormed immediately upon diagnostic (numbers not recorded – possibly all infected individuals). Also not recorded but possibly treated twice were SAC attending any of the six schools visited by the study – they might have been dewormed both at school and at home during the community MDA.



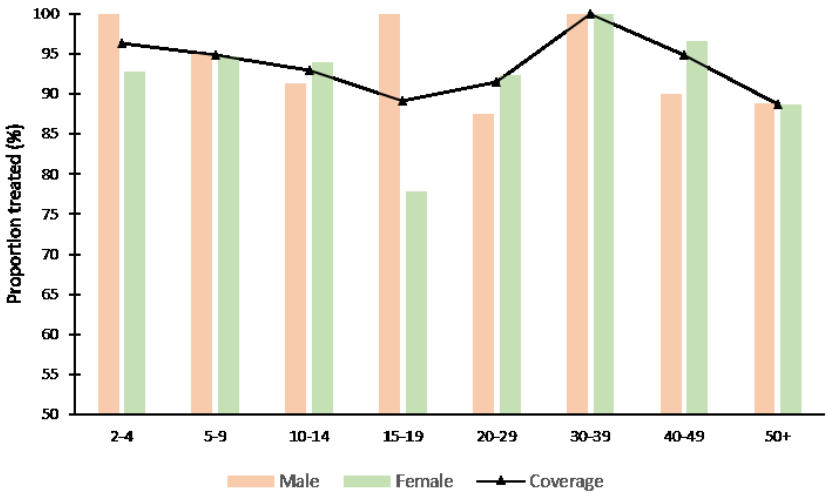
Pre-SAC: 89.6%; SAC: 91.4%; Adults (15+): 88.3%
Overall: 89.5%

Figure 3.5. Treatment coverage stratified by age group (n=3,687). Proportion of people in each age group who were provided with 400 mg albendazole tablets. Total number (3,687) distinct from initial census population (4,773) due to exclusion of ineligible and migrant individuals. Minimum *y* axis set to 50%.

Village	Treated	Not treated	Total	% treated
Siangwe	729	84	813	89.7
Siaka	708	17	725	**97.7**
Sang'alo	738	106	844	87.4
Nasimbo	1126	179	1305	**86.3**
Total	3301	386	3687	89.5

Kruskall-Wallis rank test
$\chi^2(3) = 19.590$; p-value = 0.0002

Table 3.4. Treatment coverage stratified by village (n=3,687). Proportion of people in each village who were provided with 400 mg albendazole tablets. The Kruskall-Wallis rank test used treated vs. non-treated individuals (including drug refused, not at home and blanks/unreachable). The 3 degrees of difference refer to the 4 groups analysed (villages). Differences in coverage between age groups are not statistically significant (p>0.05, not represented). Green and orange highlight the highest and lowest % treated, respectively.



Pre-SAC: 96.3%; SAC: 94.0%; Adults (15+): 92.4%
Overall: 93.9%

Figure 3.6. Treatment coverage stratified by age group (n=763). Proportion of people in each age group who participated in both cross-sectional surveys and were provided with 400 mg albendazole tablets. Minimum *y* axis is set to 50%.

Village	Treated	Not treated	Total	% treated
Siangwe	288	16	304	94.7
Siaka	149	0	149	**100**
Sang'alo	131	19	150	**87.3**
Nasimbo	134	11	145	92.4
Total	702	46	748	93.9

Kruskall-Wallis rank test
$\chi^2(3) = 3.758$; p-value = 0.2888

Table 3.5. Treatment coverage stratified by village (n=763). Proportion of people in each village who participated in both cross-sectional surveys and were provided with 400mg albendazole tablets. Kruskall-Wallis rank test used treated vs. non-treated individuals (including drug refused, not home and blanks/unreachable). The 3 degrees of difference refer to the 4 groups analysed (villages). Differences in coverage between age groups are not statistically significant (p>0.05). Green and orange highlight the highest and lowest % treated, respectively.

3.4.3. Treatment efficacy

The overall CR for *A. lumbricoides* and *N. americanus*, defined as the proportion of infected individuals who were cleared of infection three weeks after albendazole treatment, was 98.9% and 91.5%, respectively (Table 3.6). The overall ERR for *A. lumbricoides* and *N. americanus*, defined as the group-based arithmetic mean difference in EPG between study baseline and three weeks post-albendazole treatment, was 99.7% and 95.8%, respectively. There were complete baseline and treatment efficacy records for 90 individuals infected with *A. lumbricoides* and 59 with *N. americanus*. Out of these, one person was still infected with *A. lumbricoides* three weeks post-treatment (1.1%), while five failed to clear *N. americanus* infection (8.5%). CR and ERR differed by age group - *N. americanus* infection in adults was found to have both the lowest CR (90.5%) and lowest ERR (95.2%).

	Pre-treatment infections (n)	*Post-treatment infections (n)*	***CR (%)***	*Pre-treatment mean EPG*	*Post-treatment mean EPG*	***ERR (%)***
A. lumbricoides						
Pre-SAC (2-4)	16	0	100	11647.9	0	100
SAC (5-14)	63	1	98.4	7433.4	31.8	99.6
Adults (15+)	11	0	100	4506.0	0	100
Total	90	1	**98.9**	7824.8	22.3	**99.7**
N. americanus						
Pre-SAC (2-4)	1	0	100	96.0	0	100
SAC (5-14)	16	1	93.7	216.5	6.8	96.9
Adults (15+)	42	4	90.5	178.9	8.6	95.2
Total	59	5	**91.5**	187.7	7.9	**95.8**

Table 3.6. Treatment efficacy: cure rates and egg reduction rates. The total *n* used in these calculations corresponds to the subset of all individuals found to be infected with either *A. lumbricoides* or *N. americanus* at study baseline, whose data matched that of individuals who participated in efficacy testing. Post-treatment infections are defined as any positive egg counts found during testing conducted three weeks post-treatment, if available – data from weeks two and four post-treatment were used only when data from week three were not available. Arithmetic mean EPG were used to calculate ERR using Vercruysse's formula (see 3.3.3). CR: Cure Rate; ERR: Egg Reduction Rate

3.4.4. Distribution of STH infection at study baseline and follow-up

At study baseline, the overall prevalence of *A. lumbricoides* infection in the followed-up population (n=763) was 9.8%, with marked differences between age groups (pre-SAC:20.2%, SAC:11.2%, adults:2.6%, Figure 3.7a). The mean intensity of infection followed the same pattern, with pre-SAC having the highest burden of infection (peak arithmetic mean EPG: 2021.1), which overall decreased with age (Figure 3.7b). Meanwhile, the overall prevalence of *N. americanus* infection in this population was 6.7%, with prevalence generally increasing with age to 30.6% in the eldest age group (pre-

SAC:1.8%, SAC:3.6%, adults:14.6%, Figure 3.8a), as is the typical pattern for this STH infection (1). The mean intensity of *N. americanus* infection also increased with age, with the eldest age group having the highest burden of infection (peak arithmetic mean EPG: 76.0, Figure 3.8b).

Three months post-anthelminthic treatment, the overall prevalence of *A. lumbricoides* infection was reduced to 1.8%. Pre-SAC remained the age group with the highest prevalence of infection (pre-SAC=2.8%, SAC=1.9%, adults=1.3%), as well as mean intensity (arithmetic mean EPG: 314.2), but both measures of infection were very low in all age groups (Figure 3.9). The overall prevalence of *N. americanus* infection at study follow-up was 1.4%, and the eldest age group remained the one with the highest prevalence at 3.2% (pre-SAC=1.8%, SAC=1.2%, adults=1.7%, Figure 3.10). The mean intensity of *N. americanus* infection was below 2 EPG in all age groups, and the peak intensity shifted to the age group of 5-9 years (arithmetic mean EPG: 1.9).

In summary, at study baseline, 68 individuals were diagnosed with *A. lumbricoides*, 44 with *N. americanus* and 7 with double infection (Figure 3.11). At follow-up, 13 individuals were diagnosed with *A. lumbricoides*, 10 with *N. americanus* and 1 with double infection. There was a total of seven *A. lumbricoides* and one *N. americanus* reinfections. Prevalence and mean intensity of *A. lumbricoides* were reduced by 81.6% and 83.7%, respectively, while the prevalence and mean intensity of *N. americanus* infection were reduced by 79.1% and 93.9%, respectively, in the followed-up population (Table 3.7). While the overall reduction post-MDA treatment was very significant, it varied with age. The age groups with the highest prevalence and intensity of *A. lumbricoides* infection at baseline (pre-SAC and SAC) had significant reductions in both measures of infection (Table 3.8), while the age groups with lowest levels of infection either had non-significant reductions or maintained the infection levels. Similarly, the age groups with highest prevalence and intensity of *N. americanus* infection at baseline (20-29 and 50+) had significant reductions in both measures of infection, particularly the eldest age group.

3.4.5. Predisposition and factors associated with residual infection/reinfection

Comparing individual measurements of the intensity of infection between study baseline and follow-up, no significant values of Spearman's rho, Kendall's tau and pairwise correlation were obtained for *A. lumbricoides* infection (Table 3.9). The lack of significant associations may be due to the low number of reinfections recorded. The reduced number of individuals reinfected with *N. americanus* prevented this analysis of predisposition. Due to the limited number of individuals with residual infection (see 3.4.3) or reinfection (see Figure 3.11), it was also not possible to investigate individual or household factors that might have been correlated with non-clearance or resurgence of STH infection.

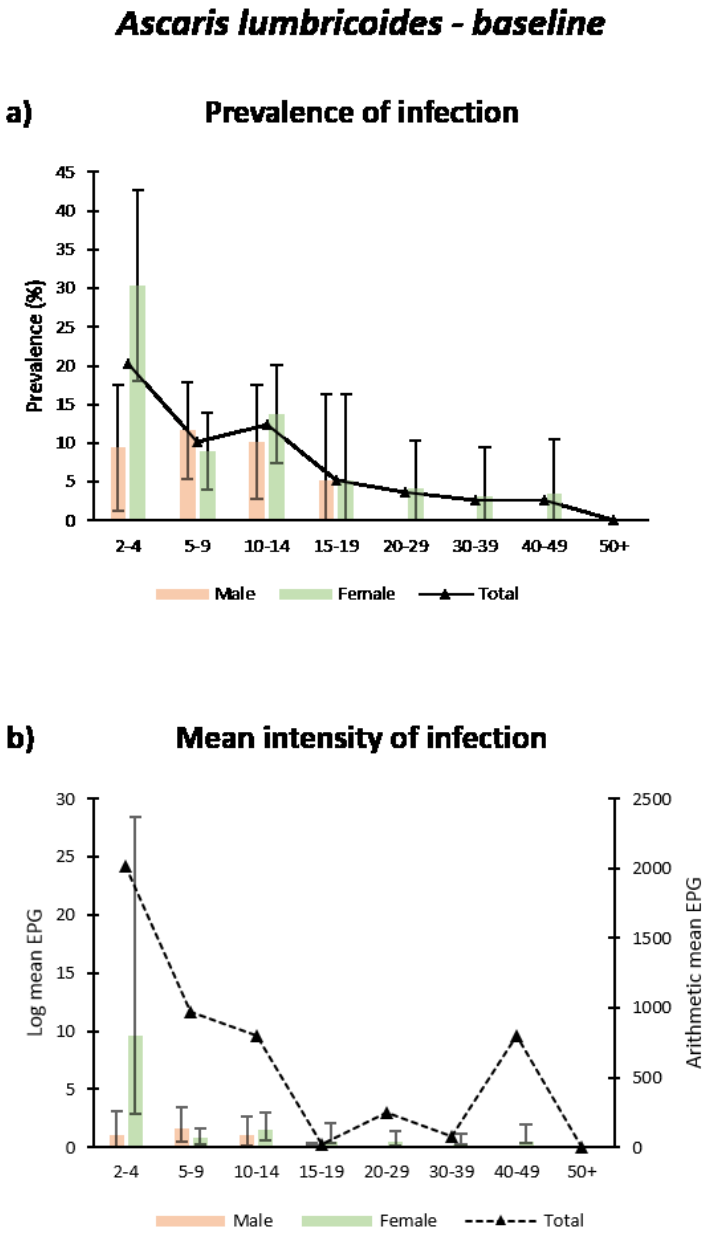


Figure 3.7. Age-related prevalence and intensity of *A. lumbricoides* infection at study baseline. a) Prevalence of *A. lumbricoides* infection distributed by gender (columns) and age group (line)*;* **b)** Mean intensity of *A. lumbricoides* infection distributed by gender (columns) and age group. Logarithmically transformed EPG (left *y* axis) refer to data in columns, while arithmetic mean EPG (right *y* axis) refer to data represented by the dotted line. One single case of moderate-to-heavy *A. lumbricoides* infection (mean EPG: 31,344) in the group 40-49 years of age, which included a total of 39 individuals (38 non-infected), caused the spike observed in **b)**. Vertical lines represent 95% normal confidence intervals (**a**) and 95% binomial confidence intervals (**b**). Both graphs use only data from followed-up individuals (n=763).

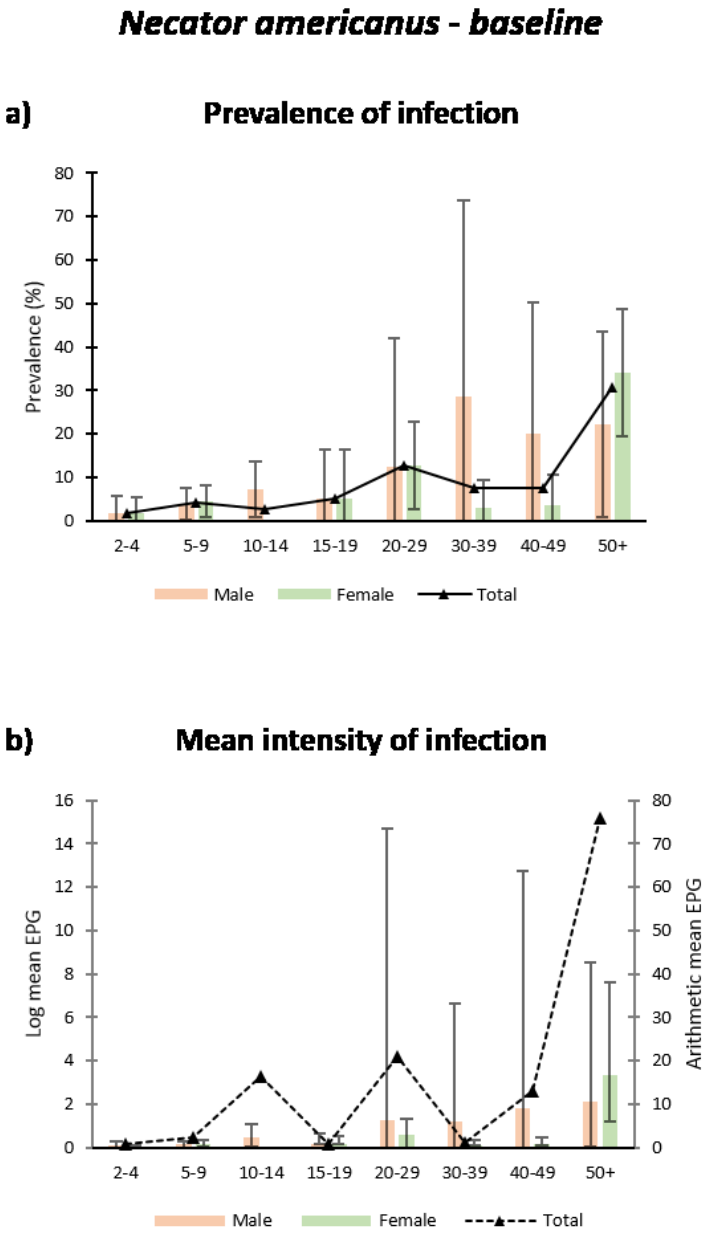


Figure 3.8. Age-related prevalence and intensity of *N. americanus* infection at study baseline. a) Prevalence of *N. americanus* infection distributed by gender (columns) and age group (line)*;* **b)** Mean intensity of *N. americanus* infection distributed by gender (columns) and age group. Logarithmically transformed EPG (left *y* axis) refer to data in columns, while arithmetic mean EPG (right *y* axis) refer to data represented by the dotted line. Vertical lines represent 95% normal confidence intervals (**a**) and 95% binomial confidence intervals (**b**). Both graphs use only data from followed-up individuals (n=763).

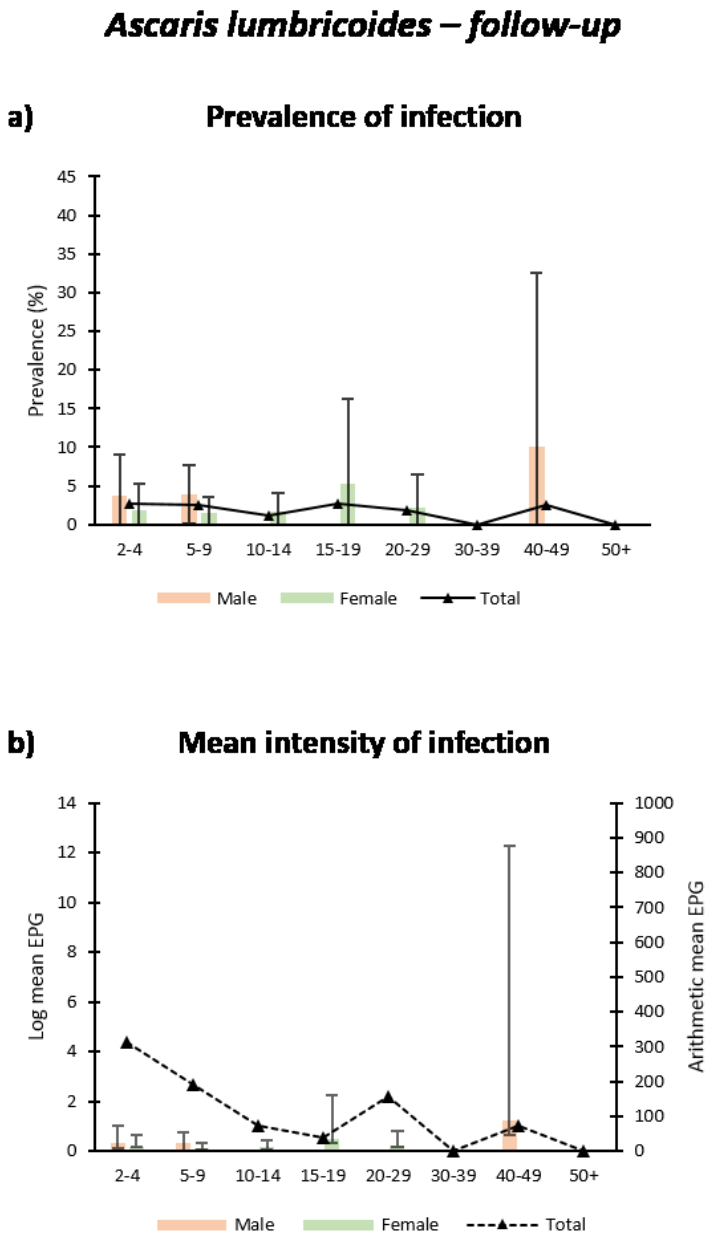


Figure 3.9. Age-related prevalence and intensity of *A. lumbricoides* infection at study follow-up. **a)** Prevalence of *A. lumbricoides* infection distributed by gender (columns) and age group (line)*;* **b)** Mean intensity of *A. lumbricoides* infection distributed by gender (columns) and age group. Logarithmically transformed EPG (left *y* axis) refer to data in columns, while arithmetic mean EPG (right *y* axis) refer to data represented by the dotted line. Vertical lines represent 95% normal confidence intervals (**a**) and 95% binomial confidence intervals (**b**). Both graphs use only data from followed-up individuals (n=763).

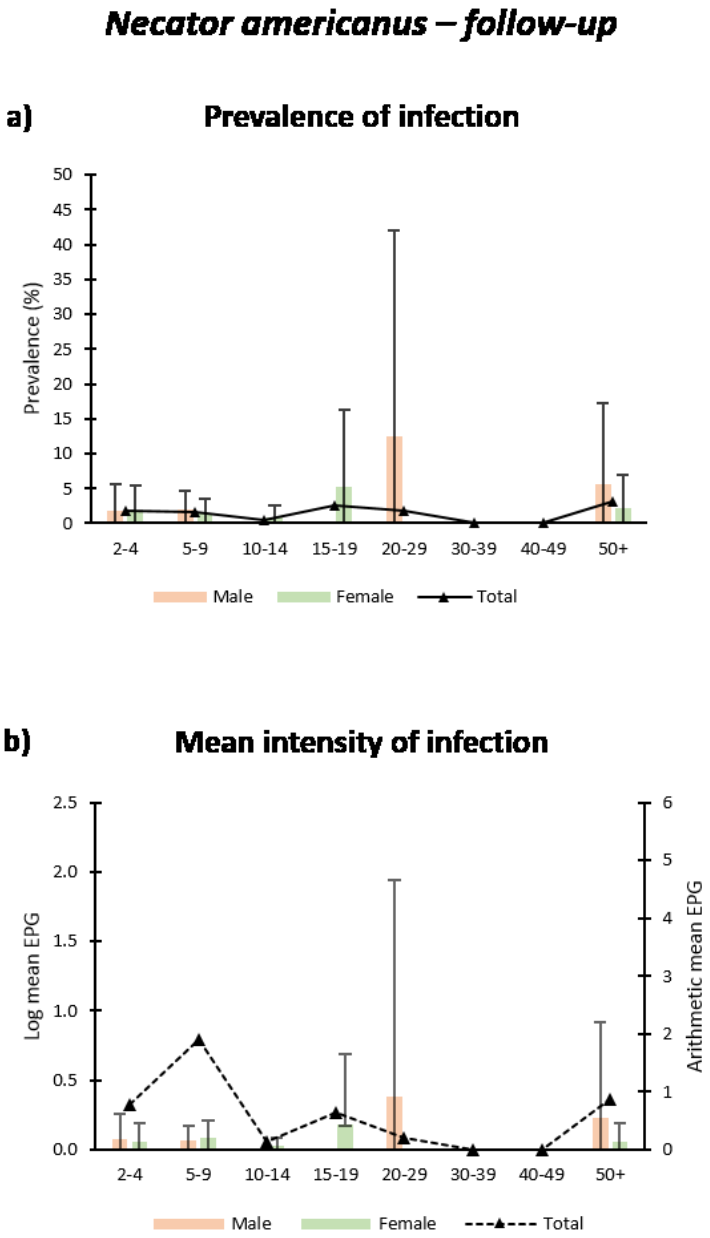


Figure 3.10. Age-related prevalence and intensity of *N. americanus* infection at study follow-up. **a)** Prevalence of *N. americanus* infection distributed by gender (columns) and age group (line)*;* **b)** Mean intensity of *N. americanus* infection distributed by gender (columns) and age group. Logarithmically transformed EPG (left *y* axis) refer to data in columns, while arithmetic mean EPG (right *y* axis) refer to data represented by the dotted line Vertical lines represent 95% normal confidence intervals (**a**) and 95% binomial confidence intervals (**b**). Both graphs use only data from followed-up individuals (n=763).

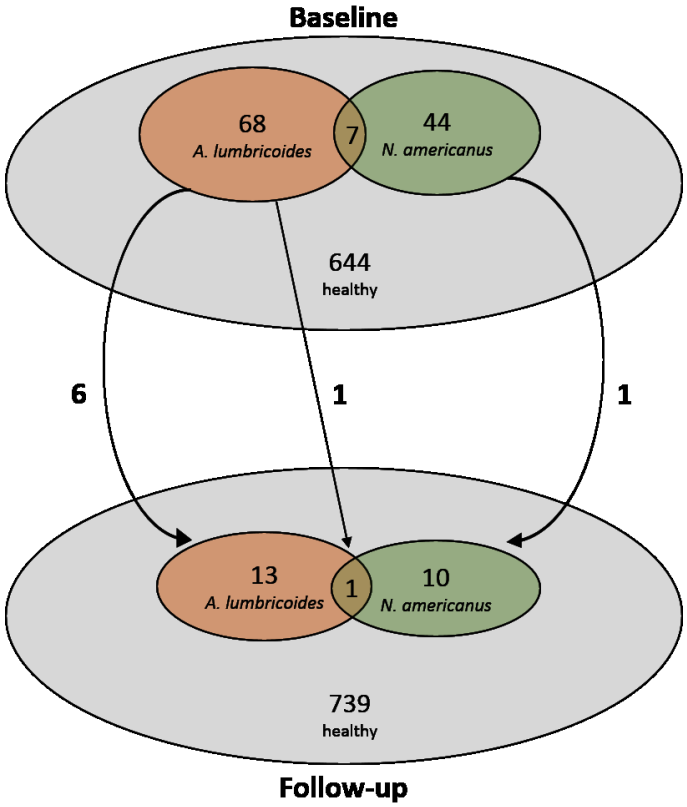


Figure 3.11. Venn diagram of infections at study baseline and follow-up. There were 644 STH-negative (healthy) individuals at study baseline, 68 single *A. lumbricoides* infections, 44 single *N. americanus* infections and 7 co-infections. Arrows indicate reinfections: 6 reinfections with *A. lumbricoides*, 1 reinfection with *N. americanus* and 1 reinfection with *A. lumbricoides* which became a co-infection with *N. americanus* at follow-up. *n*=763 at both baseline and follow-up.

	Baseline	***Follow-up***	***Absolute reduction (% / epg)***	***Relative reduction (%)***
	Prevalence (%)	Prevalence (%)		
A. lumbricoides	9.8	1.8	8.0	81.6
N. americanus	6.7	1.4	5.3	79.1
	Intensity (EPG*)	Intensity (EPG*)		
A. lumbricoides	848.9	138.5	711	83.7
N. americanus	13.2	0.8	12	93.9

Table 3.7. Overall reduction in prevalence and intensity of STH infection between baseline and follow-up. Absolute reduction calculated as *1)* the difference between prevalence at baseline and follow-up and *2)* difference between arithmetic mean EPG at baseline and follow-up. Relative reduction calculated as 100 – [(follow-up x 100)/baseline]. Only data from followed-up population were used (n=763). * arithmetic mean EPG

	Baseline	Follow-up		t-test			
A. lumbricoides							
Prevalence	**%**	**%**	***n***	***t***	**CI**	**p-value of Ha≠0**	
2-4	20.2	2.8	109	4.77	10.2-24.7	<0.001	***
5-9	10.2	2.5	236	3.78	3.6-11.6	<0.001	***
10-14	12.4	1.1	185	4.61	6.5-16.2	<0.001	***
15-19	5.3	2.6	38	0.57	-6.7-11.9	0.571	ns
20-29	3.6	1.8	55	0.57	-4.5-8.2	0.568	ns
30-39	2.6	0.0	39	1.00	-2.6-7.8	0.324	ns
40-49	2.6	2.6	39	-	-	-	-
50+	0.0	0.0	62	-	-	-	-
Total	***9.8***	***1.8***	***763***	***7.28***	***5.8-10.2***	***<0.001***	***
Log-transformed mean intensity	$\bar{x}$	$\bar{x}$	***n***	***t***	**CI**	**p-value of Ha≠0**	
2-4	1.58	0.23	109	4.35	0.73-1.97	<0.001	***
5-9	0.75	0.18	236	3.78	0.27-0.87	<0.001	***
10-14	0.85	0.09	185	4.21	0.40-1.11	<0.001	***
15-19	0.23	0.19	38	0.15	-0.51-0.59	0.885	ns
20-29	0.32	0.16	55	0.54	-0.41-0.71	0.590	ns
30-39	0.21	0.00	39	1.00	-0.21-0.62	0.324	ns
40-49	0.27	0.20	39	0.18	-0.62-0.75	0.856	ns
50+	0.00	0.00	62	-	-	-	-
Total	***0.72***	***0.14***	***763***	***6.82***	***0.41-0.75***	***<0.001***	***
N. americanus							
Prevalence	**%**	**%**	***n***	***t***	**CI**	**p-value of Ha≠0**	
2-4	1.8	1.8	109	-	-	-	-
5-9	4.2	1.7	236	1.74	-0.3-5.4	0.083	ns
10-14	2.7	0.5	185	1.64	-0.4-4.8	0.103	ns
15-19	5.3	2.6	38	0.57	-6.7-11.9	0.571	ns
20-29	12.7	1.8	55	2.19	0.9-21.8	0.033	*
30-39	7.7	0.0	39	1.78	-1.1-16.4	0.083	ns
40-49	7.7	0.0	39	1.78	-1.1-16.4	0.083	ns
50+	30.6	3.2	62	4.17	14.3-40.6	<0.001	***
Total	***6.7***	***1.4***	***763***	***5.25***	***3.3-7.2***	***<0.001***	***
Log-transformed mean intensity	$\bar{x}$	$\bar{x}$	***n***	***t***	**CI**	**p-value of Ha≠0**	
2-4	0.06	0.07	109	-0.13	-0.14-0.12	0.894	ns
5-9	0.14	0.07	236	1.38	-0.03-0.17	0.168	ns
10-14	0.14	0.02	185	1.80	-0.01-0.26	0.074	ns
15-19	0.14	0.08	38	0.45	-0.21-0.33	0.657	ns
20-29	0.51	0.05	55	2.30	0.06-0.87	0.026	*
30-39	0.22	0.00	39	1.78	-0.03-0.46	0.084	ns
40-49	0.35	0.00	39	1.73	-0.06-0.77	0.092	ns
50+	1.37	0.10	62	4.21	0.67-1.87	<0.001	***
Total	***0.27***	***0.05***	***763***	***5.24***	***0.14-0.30***	***<0.001***	***

Table 3.8. Reduction in prevalence and intensity of STH infection between baseline and follow-up. T-tests were used to compare prevalence and mean intensity of *A. lumbricoides* and *N. americanus* infection at baseline and follow-up for each age group. Logarithmically transformed mean intensity of infection was used to allow comparisons to be made. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; *ns* $p \geq 0.05$, not significant; - no change observed between baseline and follow-up.

	Spearman's rho	p-value	Kendall's tau	p-value	Pairwise correlation	p-value
A. lumbricoides	-0.5000	0.2532	-0.3333	0.3675	-0.3072	0.5028
N. americanus	NA	NA	NA	NA	NA	NA

Table 3.9. Predisposition to infection. Three non-parametric methods were used to assess predisposition using the EPG values of individuals infected at both baseline and follow-up (reinfections). There were only seven *A. lumbricoides* reinfections (n=7) and one *N. americanus* reinfection (n=1), thus it was not possible to carry out the tests for *N. americanus*.

3.5. Discussion

As seen in the previous chapter, at the time of this study's baseline survey, *A. lumbricoides* and *N. americanus* infections were still present in western Kenya, where a national SBD programme has been ongoing since 2012. The two infections presented distinct age-distributions in the community but were similarly substantially reduced by the study's community-based MDA, which covered over 75% of the population in all age groups. Deworming treatment with 400 mg albendazole tablets displayed satisfactory efficacy against both worm species, and reinfection was very low three months post-treatment.

Compliance with the study was relatively low

The two cross-sectional surveys carried out by this study aimed to assess infection levels in the same individuals at two points in time, and 763 individuals of all age groups were succesfully sampled at both time points. However, study participation and adherence by adults, in particular male adults, was poor. The factors behind this pattern of behaviour have previously been discussed in Chapter 2. Still, study fatigue, absence due to unrelated factors or the perception of not benefiting from the study might have contributed to the decision not to participate in the second survey. Prevalence and intensity of *A. lumbricoides* infection, of which people can more easily be aware of than hookworm infection due to visual expulsion of adult worms periodically and following treatment, was significantly lower in the individuals lost to follow-up than in the ones who adhered to the study. The prevalence of *N. americanus* infection was very similar in both groups. The perception of their children or themselves not being infected might thus have reduced willingness to participate. Similarly, receiving

deworming treatment after the first survey might have been seen as a good enough benefit for non-adherents, and possibly served as a pretext against further participation. Still, the treatment coverage in the followed-up population was significantly higher than in those lost to follow-up, thus it is possible that some improvements felt or observed following treatment might have increased willingness to participate in the study follow-up.

Treatment coverage achieved during the study's community-based MDA was higher than that achieved by standard control programmes.

The coverage of albendazole treatment distributed during the study community MDA was high, with over 75% of all age groups receiving treatment. However, the definition of coverage used in this study is one of "medication received" rather than "medication ingested", and as such coverage rates might not reflect true compliance in this setting. This is due to a lack of consistent drug delivery records, where some CHWs and village managers recorded drugs "ingested" and drugs "left in the house" but others made no such distinction, either recording everyone as having swallowed the tablet or not taking notes regarding the drug delivery method. Moreover, while not everyone listed in the treatment records provided to CHWs and village managers were treated, some people who were not listed were offered treatment. These were presumably previous village residents who could not be reached during the initial study census, new village members who had recently moved in, or human errors with names and records during either recruitment or treatment. Any new additions were not considered when estimating treatment coverage.

In general, treatment coverage tends to be overestimated - the numerator *n* is usually higher than the reality, and the denominator *N* is usually smaller than it should (31). While the numerator in this study excludes ineligible individuals (i.e. younger children and pregnant women), which brings it closer to reality, it does include individuals who were treated more than once and the number of deworming tablets distributed but not ingested. This study would have benefited from improved recording methods of compliance to treatment, including specifying ingestion/directly observed treatment (DOT) versus reception of drug (e.g. to be ingested later by absent household members), as well as recording treatment coverage following the second cross-sectional survey. This is a common failure in detail for most STH MDA programmes (31). In the future, studies of compliance or adherence to treatment should aim to collect accurate, detailed and individual (longitudinal patterns) data on treatment coverage and compliance to several rounds of MDA, as it would allow the identification of individuals/families who are persistent non-compliers. These non-compliers might carry heavier STH infections and consequently might be responsible for continued transmission of infection in the

community. A study by Edwards *et al.* in 2014 examined non-participation in an annual azithromycin MDA programme and identified persistent non-participation (i.e. non-compliance) at both household and individual level. Geographical clustering of non-participation was also identified and linked with hotspots of infection, showing the importance of identifying these individuals or groups to control transmission of infection (35). Previous studies have identified strategies to increase compliance with preventive treatment and MDA programmes, including improving demand by increasing trust in drug distributors and implementing clear and timely pre-MDA education campaigns (36,37).

A single dose of 400 mg albendazole was highly efficacious against both STH infections

Besides coverage data, reliable estimates of treatment efficacy are also necessary for an improved analysis of reinfection rates and, consequently, the impact of a deworming programme. Only one individual (1.1%) failed to clear *A. lumbricoides* infection, and five (8.5%) failed to clear *N. americanus* infection up to three weeks post-treatment. This period of evaluation should allow for infection clearance to be observed, as it allows time for the drug to act and worms to be expelled (38). On the other hand, it does not permit enough time for newly acquired worms (reinfections) to fully mature and start producing eggs, as ingested *Ascaris* eggs require 10-12 weeks and hookworm L3 larvae 6-8 weeks to mature into egg-producing adults worms (39,40). Since the diagnostics method used in both the surveys and the treatment efficacy testing was the same (duplicate Kato-Katz slides), differences in sensitivity and specificity can be disregarded. Measurements of CRs have previously been shown to vary with the intensity of infection pre-treatment (32), so ERRs based on arithmetic means, which are recommended by the WHO (*32*, *35*), were additionally used. The CRs obtained, though overall high, tended to increase with increasing pre-treatment mean EPG (Table 3.6), confirming the findings by Vercruysse *et al.* (32). The ERRs obtained for *A. lumbricoides* and *N. americanus* were both above the WHO recommended thresholds (95% for *A. lumbricoides* and 90% for hookworms), thus the efficacy of the deworming treatment with 400mg albendazole tablets was considered satisfactory in this setting (33), raising no concerns of drug resistance. Still, it was more effective against *A. lumbricoides* than *N. americanus* infection, which is in agreement with previous literature (5,42,43). The most recent review of albendazole efficacy, measured in SAC from six countries with and without ongoing MDA programmes, also found albendazole to have an overall higher efficacy against *Ascaris* than hookworm (*A. lumbricoides*: CR=98.2%, ERR=99.5%; hookworm: CR=87.8%, ERR=94.8%) (32). This difference in treatment efficacy highlights the need to adapt approaches in deworming programmes depending on the STH species endemic in each community.

Community-based MDA had a very significant impact in reducing levels of *Ascaris* infection in pre-SAC and hookworm infection in adults

The strongest evidence for implementing distinct deworming strategies depending on the endemic STH species comes from the pool of literature documenting the differences in the age distribution of *Ascaris* and hookworm infections in the population. *Ascaris* infection customarily presents its peak prevalence and intensity in children (SAC and pre-SAC) and hookworm in older age groups, particularly in settings with low to moderate prevalence of infection (see Chapter 1, Figure 1.5) (27,44–46). These patterns were again observed in this study during the first cross-sectional survey, with higher prevalence and intensity of *A. lumbricoides* infection in pre-SAC and higher prevalence and intensity of *N. americanus* infection in older adults. The community-based MDA showed clear benefits against both helminth infections, with very significant reductions in prevalence and intensity of both helminth infections three months after the MDA (Tables 3.7 and 3.8). The particularly high relative reduction in mean intensity of hookworm infection, which was mostly observed in the oldest age group, and the significant decline in the prevalence and intensity levels of *A. lumbricoides* infection in pre-SAC, show the importance of extending the deworming programme to age groups besides SAC. This study clearly showed that SAC significantly benefited from a community-based MDA, as the prevalence and intensity levels of *A. lumbricoides* infection in this age group were significantly reduced. However, prevalence and intensity of *N. americanus* infection in this community would remain largely unaffected by a school-based deworming programme, as SAC harboured very low levels of this infection. Previous literature, including a study in western Kenya by Olsen in 1998 (47), determined that only 14% of the hookworm infected individuals were reached through SBD programmes, while nearly 50% of the *Ascaris*-infected individuals were reached through SBD. More recent studies have also highlighted this issue in relation to hookworm-endemic communities, particularly as SBD programmes bring down the overall levels of STH infection (23,48,49). Preventive chemotherapy strategies used against another neglected tropical disease, lymphatic filariasis, have included successful MDA campaigns distributing albendazole in combination with ivermectin or diethylcarbamazine citrate (DEC) to entire at-risk populations, treating STH infections as a by-product (50). Most of Kenya is non-endemic for lymphatic filariasis, and thus adults in this setting are not targeted by MDA campaigns providing albendazole treatment. This study adds to the evidence that it would be beneficial for a community-based programme, similar to the ones implemented for lymphatic filariasis, to be extended to areas where hookworm is endemic.

The small number of reinfections prevented the analysis of predisposition and risk factors associated with maintenance of infection

The small number of individuals who were reinfected post-treatment prevented the assessment of predisposition to heavy or light infection in the community. The key causes of reinfection are 1) the fact that only a fraction of the community is treated - thus the need for good coverage data - and 2) the fact that the environment can remain contaminated for an extended period. In fact, *Ascaris* eggs have been shown to remain infective in soil, under some conditions, for up to three months (27). Reinfection with STH is known to happen quickly, in some cases bouncing back to pre-treatment levels six months to one year post-treatment (51) in areas of high transmission intensity. However, this study had only a period of three months between the MDA and the second cross-sectional survey. This is a very short period, taking into consideration that new infections require up to 12 weeks from ingestion or skin penetration to worm maturity and egg detection in stool by Kato-Katz (39). Besides, the previous national SBD campaign had taken place nearly seven months earlier and a smaller deworming campaign only four months earlier and, thus, prevalence and intensity of STH infection were already low-to-moderate at study baseline. Had the study been designed to allow for a longer interval between treatment and the post-treatment survey, reinfection figures would likely have been higher, allowing analyses of predisposition and risk factors associated with reinfection.

Conclusion

This study complements the evidence arguing for a change in current deworming strategies, which are targeted at pre-SAC and SAC, by showing that community-based approaches have a positive impact on *A. lumbricoides* infection, which was here shown to be more prevalent in pre-SAC, and also more obviously on hookworm infection, which was once again shown to be more prevalent in the adult population. Neither of these age groups is regularly targeted by deworming campaigns (though parents are encouraged to include their pre-SAC in the Kenyan national SBD programme since 2014), but this study has shown they are the ones carrying most of the burden of infection in the community. In a setting where SBD is being provided annually and STH infection levels are slowly decreasing, there is an increased need to reach the last few infected individuals. This should be achieved by improving treatment delivery strategies, treatment coverage and compliance records and diagnostic methods (to reduce false negative results, particularly of hookworm). Resistance to albendazole (and other anthelminthic drugs) has not yet presented cause for concern, but its relatively reduced efficacy against hookworm and in particular *T. trichiura*, the third STH species targeted by SBD programmes,

highlights the need to evaluate the situation in each setting and adapt deworming policies appropriately.

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CHAPTER 4. IMMUNO-EPIDEMIOLOGY OF ASCARIS LUMBRICOIDES AND NECATOR AMERICANUS IN A COMMUNITY TARGETED BY THE NATIONAL SCHOOL-BASED DEWORMING PROGRAMME

4.1. Summary

Background

Reinfection with soil-transmitted helminths (STH) post-treatment, where parasites survive to reproductive maturity in the human host, reflect an, at least partially, ineffective immune response against the parasites. This lack of protective immunity enables repeated and often rapid reinfection, creating the need for repeated treatment through long-term control programmes. Immunoassays measuring individual antibody titres against specific STH antigens can provide valuable insights into the immuno-epidemiology of STH infections, and possibly a new way of measuring programme impact and recording rates of reinfection. This study describes the seroprevalence of STH antibodies in four communities in Bungoma, western Kenya, where *Ascaris lumbricoides* and *Necator americanus* are endemic. It also measures correlations between antibody titres and intensity of infection, whilst examining the impact of community-wide anthelminthic treatment on seroprevalence of STH antibodies.

Methods

Blood and stool samples were collected from 2273 individuals from four rural villages in Bungoma District, western Kenya, during two cross-sectional surveys carried out in Mar-Apr and Aug-Sep 2014. Stool samples were analysed using duplicate Kato-Katz slides. Plasma was extracted from the blood samples and analysed using indirect ELISA to measure antibody titres against two *N. americanus* recombinant proteins (Na-ASP-2 and Na-SAA-2) and two *Ascaris* antigens, *A. suum* haemoglobin (AsHb) and *A. lumbricoides* extract (AlExt). Prevalence and intensity of infection were analysed together with antibody seroprevalence against the four antigens, searching for correlations between EPG and antibody titres at individual and community level, as well as differences in seroprevalence between age groups and changes post-community-wide treatment.

Results

The prevalence and intensity age-profiles of *A. lumbricoides* infection at study baseline were not correlated with *Ascaris* seroprevalence profiles, but there was a significant correlation between the age-prevalence profile of *N. americanus* and the seroprevalence profile of Na-ASP-2 antibodies. There

was also a pattern of increasing anti-*Ascaris* antibodies and decreasing *A. lumbricoides* EPG with age, contrasting with a trend towards increasing *N. americanus* seropositivity and EPG with age. However, there was some evidence of cross-reactivity between the four antigens tested, with particularly strong correlations between anti-AlExt and both the anti-*Necator* antibodies. No correlations were found between infection status or EPG levels and quantitative antibody response, and no significant changes in seroprevalence were found three months after community-wide deworming treatment. Finally, IgG4 antibody titres were generally low in in the whole population, including the majority of individuals found to be infected and co-infected.

Conclusions

Immunoassays might be used as alternative diagnostic methods in populations targeted by deworming programmes, but cross-reactivity between species and difficulties in distinguishing between past, prepatent and current infections remain. Their use might thus be limited to an analysis of community-wide exposure rather than accurate individual diagnosis of present or past infection. Following repeated rounds of mass drug administration, seroconversion in young children could be a good marker of continued exposure to infection.

4.2. Introduction

The patterns in the age-distribution of intensity of infection of soil-transmitted helminths (STHs) seen in endemic regions can be explained by age-related changes in exposure, as well as changes in acquired immunity to infection (1). Age-related exposures have previously been briefly discussed in Chapter 2, with farming (carried out by adults) standing out as one of the most significant risks of hookworm infection in the study population. Acquired immunity to STH infection, however, has a more complex association with age and other risk factors. While most hosts are generally able to acquire a strong immune response against bacterial and viral infections, being able to clear the pathogen and mount efficient immune responses at second exposure, STHs are able to establish chronic infections and reinfections post-treatment. Depending on the STH species and the prevailing local transmission intensity, the prevalence and intensity of STH infection might increase with age in the case of hookworms but generally show a convex pattern for *Ascaris* and *Trichuris* in the human population (2,3). To date, no effective STH vaccines have been developed for human infection, thus deworming treatment needs to be provided regularly to control infection (4).

The response of the human immune system to STH infection can be measured by the presence and concentration of parasite-specific antibodies. Over the years, several studies have measured

circulating blood levels of various human antibody isotypes produced specifically against STH and similar parasites. Evidence indicates that antibody seroprevalence tends to reflects the current intensity of infection (5–8), but antibody responses are not completely protective against reinfection (9,10). In a study in India in 1987, specific IgE against excretory/secretory (ES) antigens of *A. duodenale* L3 larvae were found to have both diagnostic and prognostic value (11). Patients infected with *A. duodenale* had elevated specific IgE antibodies, which decreased to levels seen in uninfected people soon after treatment, suggesting that IgE antibodies do not reflect past infections. In another study, the titre of *Ascaris*-specific IgG4 was shown to decay relatively quickly (becoming negative within six months) following successful monthly treatment with pyrantel pamoate (12). On the other hand, a study from Papua New Guinea found significant negative correlations between hookworm burden and anti-adult ES IgE, IgM and IgG, which might point towards either a degree of protective immunity or parasite-induced immunosuppression (13).

The majority of field studies have been carried out in communities with high STH prevalence and high transmission intensity, which had not previously been influenced by mass drug administration (MDA) programmes. However, many countries endemic for STH (including Kenya) have now launched school-based deworming (SBD) programmes, as recommended by the WHO, distributing regular preventive chemotherapy of albendazole or mebendazole to school age and, to a lesser extent, pre-school age children (14–16). In areas endemic for lymphatic filariasis, MDA campaigns have also been distributing albendazole (in conjunction with ivermectin) to whole communities (4,17). These campaigns have been shown to alter the dynamics of STH infection in the population, reducing prevalence and transmission of infection (18), and may have an effect on individual immunity to infection and population seroprevalence profiles. A study comparing humoral responses in three settings with different STH endemicity levels found evidence for a peak shift in total IgE, where the population with the highest total IgE levels had a steeper increase in IgE at an earlier age than the other two populations with lower total IgE levels (7 years vs. 10 years and 17 years). The population with highest total IgE levels also had higher prevalence of STH infection (45% hookworm and 20% *A. lumbricoides*), and the peak in IgE was associated with the peak in *A. lumbricoides* prevalence (19). A more recent study comparing anti-*Ascaris suum* haemoglobin (AsHb) IgG4 seroprevalence in Indonesia pre- and post-6 rounds of lymphatic filariasis MDA with albendazole found that IgG4 seroprevalence decreased over the years along with *A. lumbricoides* prevalence, and antibody levels seemed to reflect recent exposure to *Ascaris* infection (6). In a recent study of *Schistosoma mansoni* and *N. americanus* co-infection in Ugandan school children, anti-somatic adult hookworm IgG4 levels were found to increase with age up to the late teens (maximum age: 16 years) and to decrease significantly two months post-albendazole treatment, with greater reductions in heavily infected children (20). The same study

found no significant changes between anti-larval Na-ASP-2 IgG4 and increasing age or post-treatment. Previous studies, however, have found significant positive associations between *N. americanus* egg and worm counts and IgG4, as well as elevated IgG4 in infected and reinfected individuals six-months post-treatment in comparison with non-infected and cured individuals (21–23). These findings have led to the suggestion that specific IgG4 antibodies might be used for immunodiagnostic of hookworm infection.

The present study took place in an area of western Kenya endemic for both *A. lumbricoides* and *N. americanus*, where transmission intensity was historically high but has recently been reduced due to a successful annual SBD campaign, ongoing since 2012. Few studies on the immuno-epidemiology of STH infections have been carried out in a setting such as this. There is limited knowledge at present about the seroprevalence profiles encountered in a population receiving targeted MDA (SBD) treatment.

The aims of this study were to elucidate:

(1) how anti-STH antibody seroprevalence varies with age in an endemic, MDA-treated population;
(2) possible correlations between current STH infection, its intensity, and anti-STH antibody seroprevalence;
(3) the impact of anthelminthic treatment and reinfection on anti-STH antibody seroprevalence, and whether seroprevalence profiles can reflect epidemiological events.

4.3. Materials and Methods

4.3.1. Ethical considerations and treatment

As mentioned in the previous chapters, ethical approval was obtained from the Kenya Medical Research Institute (KEMRI), the Kenyan National Ethics Review Committee (SSC No. 2687), and the Imperial College London Research Ethics Committee (13_3_10). Written informed consent was obtained from all study participants before data collection. Participation was voluntary, and withdrawal had no further obligations or loss of benefits. All survey, parasitological and serological data were coded and treated confidentially. No HIV, human DNA or other non-specified tests were carried out using the blood samples provided, as stated in the information sheets provided to study participants (see Appendix I).

4.3.2. Study area and population

The study was carried out in four rural villages in Bungoma County, in the Western Province of Kenya. A total of 3,530 individuals above two years of age were randomly selected to participate (see Chapter 2). Exclusion criteria for this study were based on international safety regulations for albendazole (24,25), which stipulate that the 400mg tablets used in the study should not be provided to children under 24 months of age and women in the first trimester of pregnancy. Thus, children younger than two years old were excluded and, due to uncertainty regarding pregnancy stages, all known pregnant women were also excluded.

As mentioned in the previous chapters, the four villages (Siangwe, Siaka, Sangalo and Nasimbo) lay in an area endemic for STH, where primary schools had been targeted by the National SBD Programme since its launch in 2012. At the start of this study in March 2014, most pre-SAC and SAC living in these villages had last been dewormed by the National SBD Programme in June 2013. The area is not endemic for lymphatic filariasis, so no other MDA of albendazole is thought to have occurred in these villages. Individuals might have self-medicated or been prescribed deworming drugs when attending health facilities, however, through personal communications in the community, this is reported to be low.

4.3.3. Field procedures

4.3.3.1. Collection of blood samples

Where possible, blood sample collection was performed on the day that each participant provided his/her first stool sample at the sample collection points in both primary schools and community meeting points (see Chapter 2). Sample collection took place between 08:00-12:00. Following registration and delivery of the stool sample, a small (up to 300 µl) capillary blood sample was collected from each participant into an EDTA Microvette® CB 300 tube, using a lancet to pierce the middle finger. Each tube was labelled with the participant's unique identification code and stored at 0-4°C before being processed in the field laboratory.

4.3.3.2. Whole blood fractionation and long-term storage

On each collection day, blood samples were centrifuged in the field laboratory at 10,000 rpm for 5 minutes (Heraeus® Biofuge Pico) to separate into 3 phases: clear supernatant solution of blood plasma, buffy coat (thin layer of leukocytes and platelets), and erythrocytes sediment. Up to 0.5 ml of plasma per person was stored in FluidX® tubes at 0-4°C until transferred to a -20°C freezer at the end

of the day. At the end of each study trip they were transported at 0-4°C into a -80°C freezer located in the KEMRI-CDC facilities in Kisumu, Western Kenya. They were then shipped to the United Kingdom on dry ice and stored at -80°C at Imperial College London before being moved in dry ice to a -20°C freezer at the London School of Hygiene & Tropical Medicine for the final processing and analyses.

4.3.4. Laboratory procedures

4.3.4.1. Enzyme-linked immunosorbent assay (ELISA)

The plasma samples were analysed using ELISA to quantify human IgG subclass 4 (IgG4) antibody titres specific against two *Ascaris* and two *N. americanus* antigens (see below). The ELISA protocol generally consisted of the following steps (see Appendix III for solutions used in this protocol):

Day 1: 96-well flat-bottom plates were coated with 50 µl of the antigen solution (diluted in coating buffer – PBS to reach the test concentration) and incubated overnight at 4°C.

Day 2: Plates were washed three times with PBS-Tween solution, coated with 150 µl of blocking buffer, and incubated for three hours at room temperature. Following this period, plates were again washed three times with PBS-Tween solution and coated with samples (columns 1 to 10) and controls (columns 11 and 12), with each sample transferred into duplicated wells in the ELISA plate (e.g. sample A1 in sample plate transferred into A1 and A2 in the ELISA plate). ELISA plates were then incubated overnight at 4°C.

Day 3: Plates were washed five times with PBS-Tween solution and coated with 50 µl of IgG4 conjugate solution diluted at 1:2,000 in PBS-Tween. Plates were incubated for three hours at room temperature and then washed five times with PBS-Tween solution. Finally, 150 µl of TMB substrate solution were added to each well, incubated at room temperature in the dark for 15 minutes for the assay to develop, and the reaction was stopped by adding 50 µl of 0.2M sulphuric acid (H_2SO_4). Plates were then read at 450 nm using a microplate reader.

The optimal concentration of each antigen and of the plasma samples to be used in the ELISA were tested using samples collected from individuals in a pilot study village, Ranje. This was the village where the field laboratory was located, within 5 km of the study villages, so the samples collected from residents of this village are expected to be very similar to those collected from study participants. The test concentrations of both samples and antigens were obtained from standard curves drawn using serial dilutions of either samples or antigens (Figure 4.1). Following these titrations, it was determined that plasma samples would be used in a 1:200 dilution and the antigens would be used in the concentrations shown in Table 4.1.

Test and control samples were diluted with recon buffer into deep wells before transferring in duplicate into the test plates. For the majority of the antigens, only two negative controls were used in quadruplicate, in wells 11 and 12 A and B (negative control 1) and C and D (negative control 2) and recon buffer only in wells E to H. For the plates using antigen AsHb, however, serial dilutions (1:2) of a positive control were used in wells 11 and 12 A to F and recon buffer only in wells G and H. The positive control was provided by Dr Johnny Vlaminck and consisted of a pool of AsHb-positive plasma samples, previously confirmed positive by Western blot (personal communication).

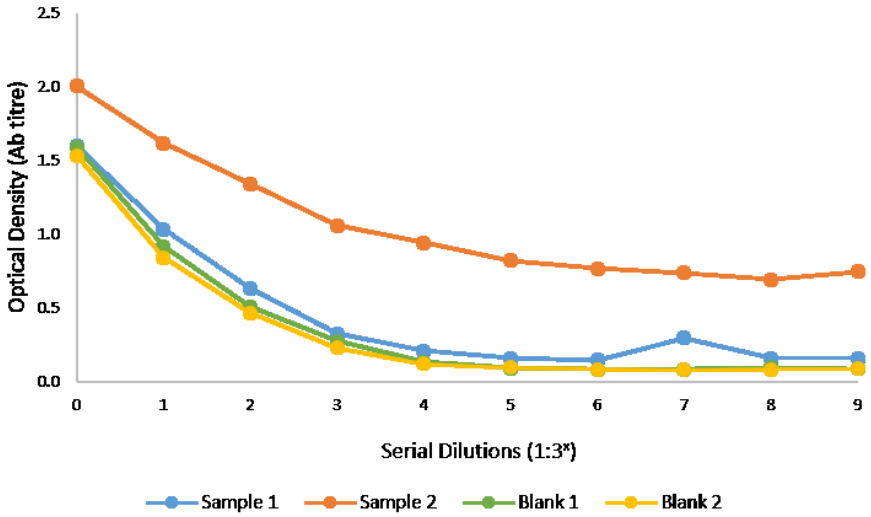


Figure 4.1. Standard curves obtained during test titration for AlExt antigen. This test used two plasma samples from two residents of the pilot village (Samples 1 and 2) and two samples from two volunteers from the LSHTM (Blanks 1 and 2), who were seronegative for malaria antigens and assumed to be seronegative for helminth antigens, acting as negative controls 1 and 2. Ten serial dilutions of 1:3 were used, starting with the neat protein (Point 0 = 787µg/ml). Dilution 5 (1:3^5 or 1:243) was chosen as the test concentration, the one where the negative controls became linear, which corresponded to a concentration of 3.2µg/ml AlExt.

Antigen	Initial concentration	Test concentration	Cut-off value (OD)
AlExt	787 µg/ml	3.2 µg/ml	0.2317
AsHb	534 µg/ml	1.0 µg/ml	0.3000
Na-ASP-2	2600 µg/ml	0.5 µg/ml	0.1670
Na-SAA-2	140 µg/ml	0.05 µg/ml	0.1797

Table 4.1. Concentrations and cut-off values of each antigen used in ELISA. The test concentrations were determined by drawing standard curves such as the one in Figure 4.1 and selecting the antigen dilution at which the difference between the control and positive samples was greater and/or the optical density became linear. The cut-off value (minimum positive OD value) for each antigen was determined in Stata using the *fmm* package (see section 4.3.5).

4.3.4.2. Antigens

Four different antigens were used in this study:

i) *Ascaris suum* haemoglobin (**AsHb**) protein,

ii) *Necator americanus Ancylostoma* secreted protein (ASP) 2 (**Na-ASP-2**),

iii) *Necator americanus* surface-associated antigen (SAA) 2 (**Na-SAA-2**), and

iv) *Ascaris lumbricoides* PBS extract (**AlExt**).

The AsHb antigen was provided by the University of Ghent, Belgium, where it was obtained from adult *Ascaris suum* worms collected from pigs in a local abattoir and purified following previously described protocols (26,27). The *Necator* recombinant proteins were both provided by the Baylor College of Medicine in Houston, TX, where they were cloned and purified following previously described protocols (28,29). The *A. lumbricoides* antigen was produced specifically for this study using worms collected from infected pilot study participants. Summarily, two 5 cm (2 in) sections of adult *A. lumbricoides* worms (one female and one male) were frozen in liquid nitrogen, ground into a fine powder with mortar and pestle and added to centrifuge tubes containing 1x PBS. This mixture was then repeatedly sonicated, centrifuged for 30 min at 12,000 rpm and 4 ^{o}C, until finally the supernatant was collected and stored at -20 ^{o}C. The protein content of the supernatant was quantified using the Bradford protein quantification method (30). From the standard curve obtained, a mean concentration of **787ug/ml** of AlExt protein was estimated. No further analyses of the AlExt protein content were carried out.

These antigens were selected based on 1) availability of the materials, including the *A. lumbricoides* worms used to produce AlExt, and 2) a concern with using refined, specific proteins, due to previously reported cross-reactivity of worm extract-type products and expected improved success of single-protein antigens (31).

4.3.4.3. Sampling replication error

One ELISA plate containing repeated sample/antigen combinations was read repeatedly to obtain a measure of the error (i.e. variance) between readings of the same sample/antigen combination in a plate, as well as between readings over time. Summarily, three antigens were used (AsHb, Na-ASP-2 and Na-SAA-2), in combination with two samples previously found to be positive for each antigen, making a total of three antigens and six positive samples. The general ELISA protocol was followed, except that no controls were used and each sample/antigen combination was pipetted ten times into the plate, using only the central 60 wells of the plate (Figure 4.2). This allowed ten repeated

measurements of each sample/antigen combination in the same plate. Following the completion of the assay, the plate was read ten times in the microplate reader, a procedure that lasted approximately ten minutes between the first and last reading, and simulated the reading of ten plates. This allowed ten repeated measurements over time of the sample/antigen combinations repeated ten times in the plate, making a total of 100 readings for each sample/antigen combination.

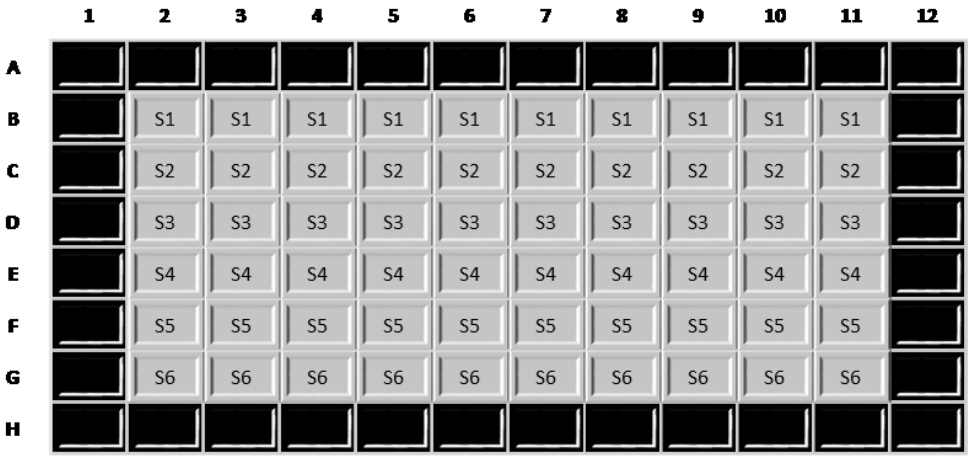


Figure 4.2. Schematic diagram of the replication error test plate. This test used six samples collected from six residents of the pilot village (samples S1 through S6). Summarily, three antigens were used (AsHb: rows B and C, Na-ASP-2: rows D and E and Na-SAA-2: rows F and G), in combination with two samples previously found to be positive for each antigen, making a total of three antigens and six positive samples. Each of the six sample/antigen combination was pipetted ten times into the plate using the same concentration, covering the central 60 wells of the plate. This provided a measure of error on readings of samples in the same plate. The plate was then read ten times in the microplate reader to simulate the reading of ten plates. This provided a measure of error in readings between plates.

4.3.5. Data management and statistical analysis

All data were entered in Microsoft Excel 2010 (version 14.0) and later updated to Microsoft Excel 2013 (version 15.0, Microsoft Corporation). Any discrepancies found during data cleaning were checked against the original paper records and resolved accordingly or excluded from the analysis. Statistical analyses were carried out using Stata version 13.1 (32), unless otherwise specified.

Due to the small number of followed-up individuals in some age categories, most analyses were performed using the baseline study population, while the followed-up population was used to investigate the effect of treatment on individual and group prevalence and seroprevalence of STH infections. All participants who had at least one stool sample and one blood sample collected and

examined during the baseline study were included in the first set of analyses. This set includes Tables 4.2 and 4.3 and Figures 4.5 to 4.14. Only participants who had at least one stool sample and one blood sample collected and examined both at baseline and follow-up were included in the second set of analyses, which includes Tables 4.4 and 4.5 and Figures 4.15 to 4.18.

As mentioned in previous chapters, EPG for each STH species were obtained by multiplying by 24 the arithmetic mean faecal egg counts of KK slides for each individual, and age was considered as a categorical variable divided into eight age groups: 2-4 (pre-SAC), 5-9 and 10-14 (SAC), 15-19, 20-29, 30-39, 40-49 and 50+ years (adults). The prevalence of each STH species was calculated for each age group, and their 95% binomial confidence intervals (CI) were obtained using either the normal distribution (positive binomial distribution for discrete variables, with sample mean and standard error) or the Student's *t* distribution when *n* was lower than 60 individuals. The geometric mean intensity of STH infection for each age group was also calculated for both infected and uninfected individuals, and asymmetric 95% CIs were calculated using the log-transformed values (33).

The IgG4 seroprevalence for each age group was calculated as the proportion above the cut-off of optical density (OD) defined for each antigen (see section below), and 95% CIs were calculated as per prevalence. The median and interquartile range (IQR) of OD per age group were used as the equivalent to the mean intensity of infection and CI. The median was used instead of the mean due to the reduced effect of the few very large values on the median, and OD values were multiplied by 100 to facilitate presentation.

The aggregation parameter of the negative binomial distribution (*k*) was calculated using the corrected moment estimate (34),

$$k = \frac{\bar{x}^2 - \frac{v}{n}}{(v + \bar{x})}$$

where n is the number of individuals in the study population, $\bar{x}$ is the arithmetic mean intensity of STH infection in the population (or arithmetic mean OD in the population), and v is its variance.

A non-parametric ranking correlation test (Kendall's tau) was used to measure the strength of association between variables (e.g. age-prevalence and age-seroprevalence in Figures 4.5 and 4.6). Logistic regression was used to obtain p-values for binary variables (prevalence and seroprevalence), while negative binomial regression was used for continuous variables (mean EPG). Pearson's correlation coefficients were used to measure correlation between antigens using individual values in Table 4.3. Chi-squared tests were used to compare proportions and means of each age group at baseline (pre-treatment) and follow-up (post-treatment) in Figures 4.15 and 4.16. Finally, Wilcoxon

signed-rank tests were used to examine differences between paired individual seroprevalence levels pre-treatment and post-treatment in Figures 4.17 and 4.18.

4.3.5.1. Cut-off values of OD

To generate an OD cut-off value above which samples were considered antibody positive, a finite mixture model was applied in Stata (*fmm* package) to the distribution of OD values for each separate antigen. This model uses maximum likelihood methods to fit the distribution of OD values as the sum of two Gaussian distributions: a distribution of seronegatives and a distribution of seropositives. The cut-off for seropositivity of each antigen (Table 4.1) was defined as the mean OD of the Gaussian corresponding to the seronegative population plus three standard deviations (35).

4.4. Results

4.4.1. Characteristics of study population

A total of 3,530 individuals living in four rural villages in western Kenya were randomly selected for participation in this study, as previously described in Chapter 2. At least one stool and one blood sample were successfully collected from 2,273 individuals at two cross-sectional surveys carried out in Mar-Apr and Aug-Sep 2014 (study participation was 64%). During the first cross-sectional survey, which took place in March and April 2014, a total of 1,268 eligible individuals provided at least one stool sample and one blood sample for analysis (35.9% of the randomly selected population), while 1,005 eligible individuals (28.5%) provided samples during the follow-up study in August and September 2014. A total of 607 individuals were successfully followed-up, providing samples at both cross-sectional surveys (47.9% of the 1,268 participants) (Figure 4.3).

The population pyramids of both the study baseline and the followed-up participants revealed oversampling of pre-SAC and SAC, as well as undersampling of working-age adults (15-59) in comparison with the national demographic profile (Figure 4.4). Adult male participants were particularly under-represented, making up only 22.0% and 17.0% of all working age adults in the baseline and followed-up populations, respectively.

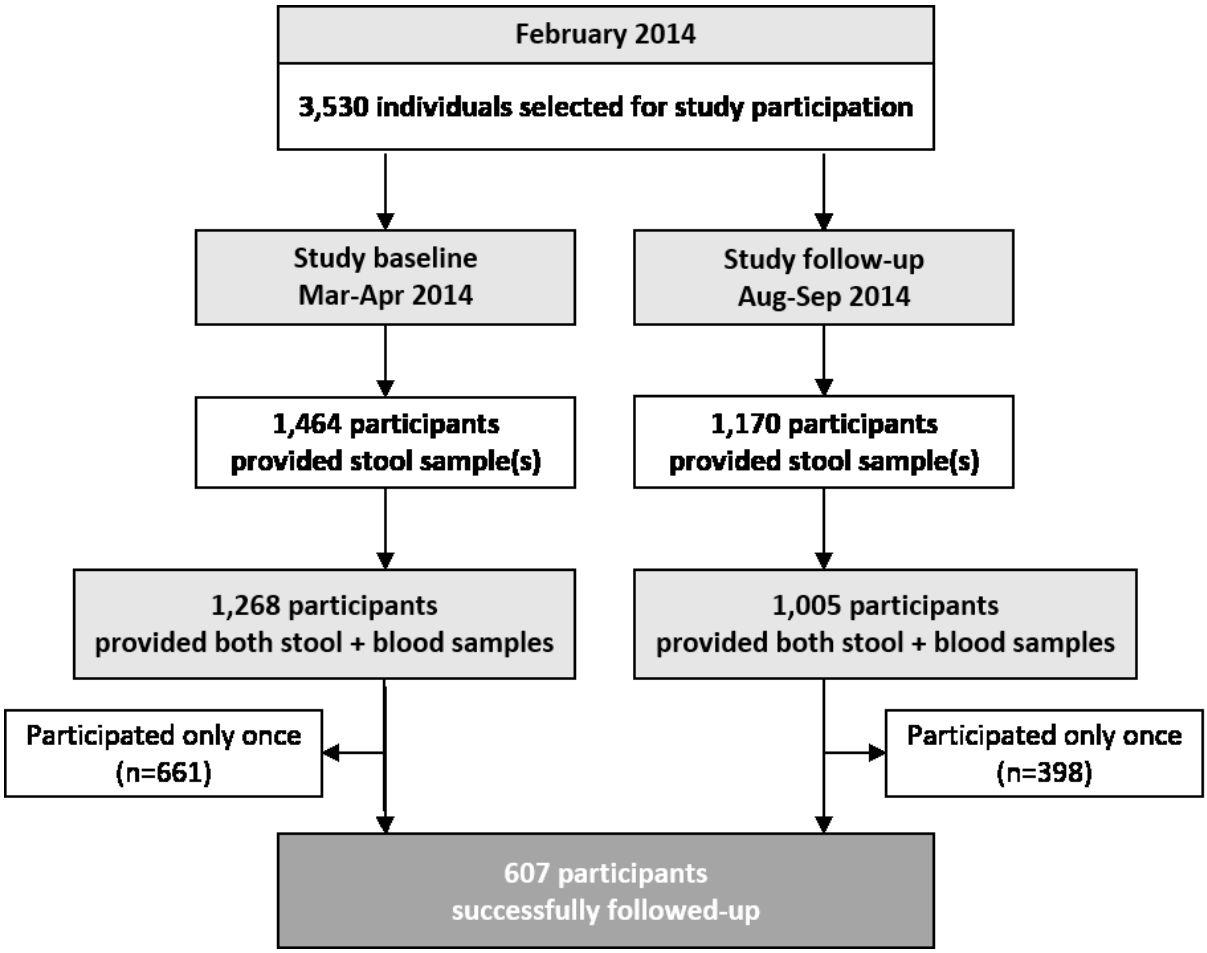


Figure 4.3. Flowchart of study population. A total of 3,530 adults and children with signed informed consent forms and complete socio-demographic information were selected for study participation in February 2014 (see previous chapters). During the study baseline in March and April 2014 1,464 individuals provided at least one stool sample for analysis, but only 1,268 provided both stool and blood samples. At follow-up in August and September 2014, 1,005 participants provided stool and blood samples for analysis, but 661 of the previous participants were lost to follow-up and 398 individuals participated in the study for the first time at follow-up. In the end, 607 participants provided stool and blood samples at both time points and were thus successfully followed-up.

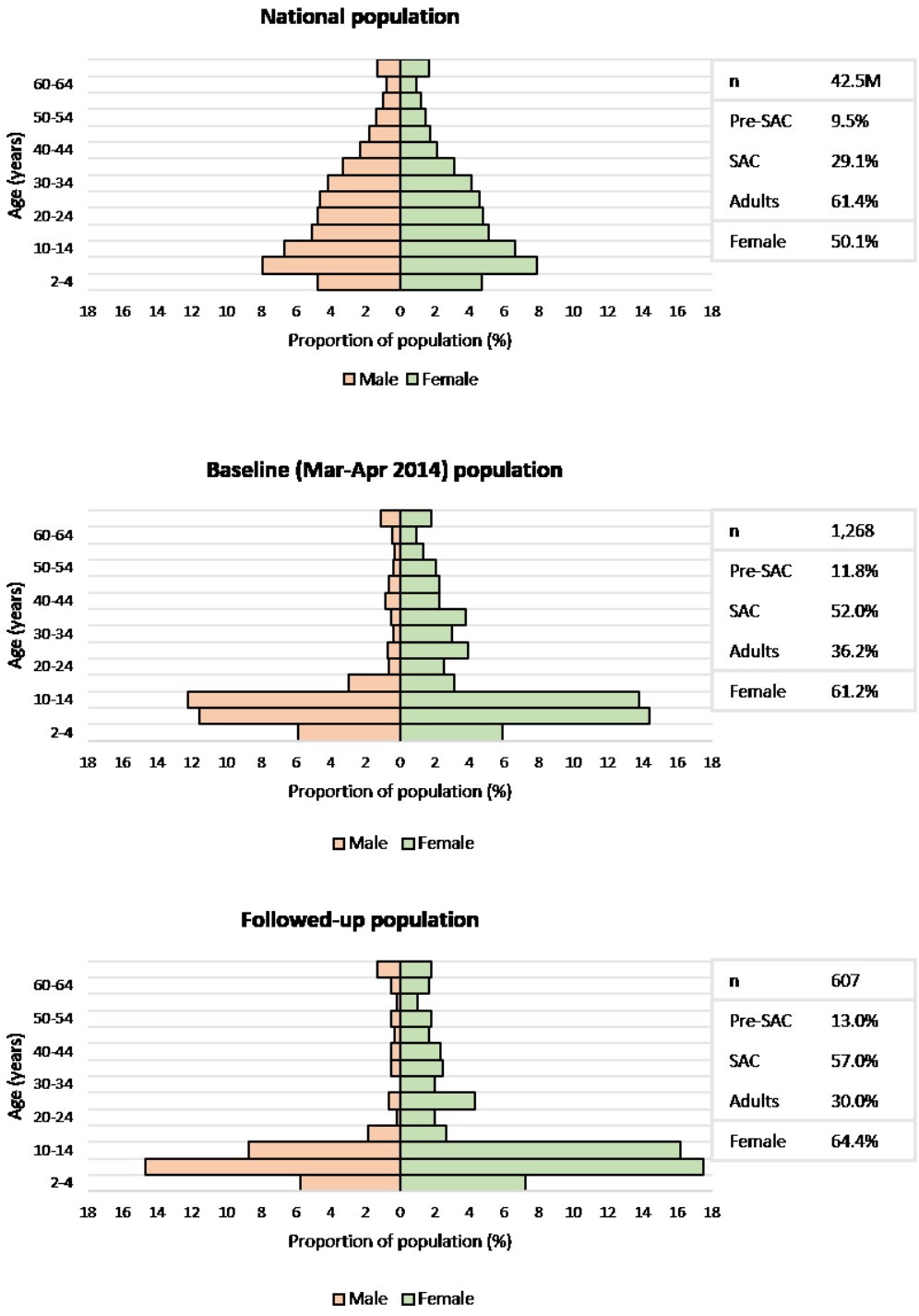


Figure 4.4. Pyramid diagrams of study population. The top diagram represents the national Kenyan population, which totalled 42.5 million people in 2014 (51). The middle diagram shows the population which participated (provided stool and blood samples) in the first cross-sectional survey (study baseline) carried out in Mar-Apr 2014. The bottom diagram represents the population that participated in both cross-sectional surveys and was thus successfully followed-up. Age groups in all diagrams are 5-year groups, except the bottom and top groups, which are, respectively, 2 to 4 years and 65 years and above. The tables next to the diagrams show the total number of individuals represented, the proportion in the three major age groups and the proportion female in that population.

4.4.2. Replication error

The sampling replication assay revealed a discrepancy in the variation of mean OD and variance of sample measurements over time. While samples with mean OD values up to 1.5 had very little variation in mean and variance between readings of the test plate (Figure 4.5), the sample with a mean OD value above 2.0 varied greatly between the first and last readings. Interestingly, the mean OD of this sample decreased and the variance increased with time, i.e. the ten repeated measurements of this sample in the plate were more similar and of higher OD when read shortly after finishing the ELISA. While there was barely a difference in the mean and variance OD of samples with lower antibody titres, the sample with a high antibody titre had a reduction of 1.2 in mean OD (3.5 to 2.3) and a fivefold increase in variance between the first and the last readings (0.004 to 0.019). Another result to note is the fact that the maximum variance between the ten repeated measurements of each sample on a test plate reading was 0.019 (0.005 when the high OD sample is excluded). Due to the small error values observed in this test, results were taken as consistent between plates.

4.4.3. Age-distribution of prevalence and intensity of STH infections and serological responses

As previously shown in Chapters 2 and 3, *A. lumbricoides* and *N. americanus* infections were both present in this population, with low prevalence (7.3% and 6.6%, respectively) and low to moderate intensity of infection, as measured by Kato-Katz (Table 4.2). *A. lumbricoides* infection had significantly higher prevalence and arithmetic mean intensity in the younger age groups of the baseline study population (pre-SAC and SAC), while *N. americanus* infection had significantly higher prevalence and arithmetic mean intensity in the older age groups, particularly in the group 50 years and older.

In terms of antibody titres, 16.0% and 21.3% of the population were found to be seropositive for the two *Ascaris* antigens, AlExt and AsHb, respectively, with median ODs of 7.5 and 8.0. The seroprevalence of anti-AsHb and anti-AlExt antibodies had very similar age-distributions, with a small peak in children aged 5 to 9 years, followed by a low seroprevalence in individuals aged 20 to 29 and a plateau in adults above the age of 30 (Figure 4.6). The age-distributions of seroprevalence of the two *Ascaris* antibodies were found to be significantly correlated, with a Kendall's tau of 0.868 (p=0.005). The age-distribution of median OD values of both anti-*Ascaris* antibodies was also very similar. There was only a small variation in median OD between the age groups, but the age groups of 2 to 4 years and 20 to 29 years had the lowest median OD values, as well as lowest seroprevalence levels.

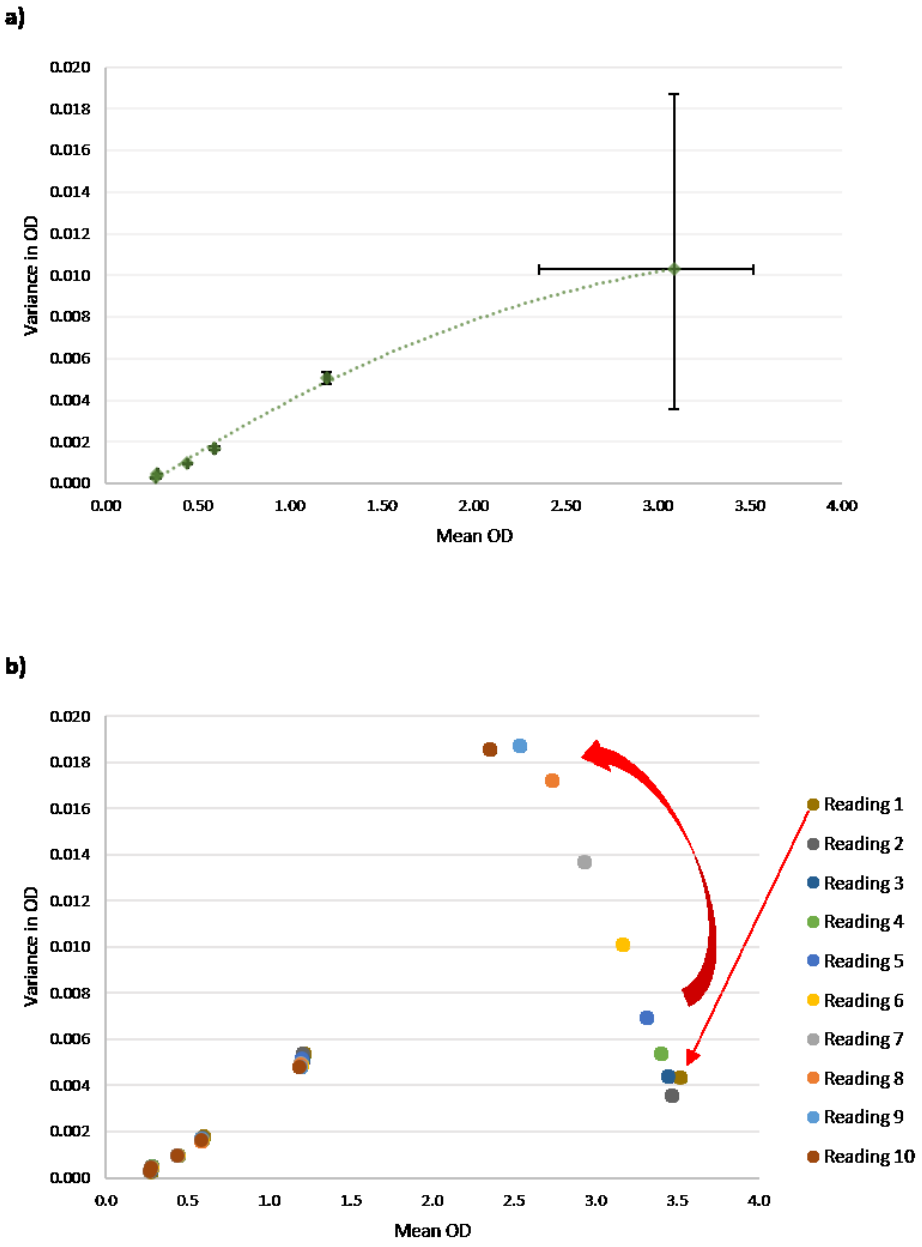


Figure 4.5. Replication error test. The arithmetic mean OD and variance was calculated for each of the six samples and each of the ten plate readings. The points in **a)** represent the average mean OD and variance for each sample, with vertical bars showing the maximum and minimum variance in OD of the sample between readings and horizontal bars showing the maximum and minimum arithmetic mean OD between the ten readings of the plate. The coloured points in **b)** show the mean and variance of each sample in each of the readings, with arrows showing the direction of change over time (approximately 30 to 60 seconds between plate readings).

		A. lumbricoides infection					*N. americanus* infection				
		Kato-Katz		ELISA			Kato-Katz		ELISA		
Age Group	N (%)	Prevalence (n)	Mean EPG (SD)		AlExt	AsHb	Prevalence (n)	Mean EPG (SD)		Na-ASP-2	Na-SAA-2
2-4	150 (11.8)	14.0% (21)	1407 (5997)	Prevalence (n/N)	12.0% (18/150)	18.0% (27/150)	1.3% (2)	1 (8)	Prevalence (n/N)	16.0% (24/150)	19.3% (29/150)
				OD (IQR)	5.8 (3.7-12.2)	5.7 (0.8-21.4)			OD (IQR)	4.4 (2.4-10.0)	5.5 (1.7-11.7)
5-9	329 (25.9)	9.7% (32)	784 (3682)	Prevalence (n/N)	18.3% (60/328)	23.1% (76/329)	4.6% (15)	2 (15)	Prevalence (n/N)	22.8% (75/329)	26.3% (86/327)
				OD (IQR)	8.6 (5.0-18.1)	9.7 (3.0-27.0)			OD (IQR)	6.1 (3.1-15.7)	7.1 (2.9-18.4)
10-14	330 (26.0)	8.8% (29)	422 (2568)	Prevalence (n/N)	13.7% (45/328)	19.2% (63/328)	2.1% (7)	9 (123)	Prevalence (n/N)	20.6% (68/330)	20.0% (66/330)
				OD (IQR)	8.3 (4.3-15.3)	8.4 (2.6-24.5)			OD (IQR)	5.5 (3.1-13.7)	7.0 (2.7-14.4)
15-19	78 (6.1)	5.1% (4)	350 (2953)	Prevalence (n/N)	12.8% (10/78)	14.1% (11/78)	6.4% (5)	5 (31)	Prevalence (n/N)	24.4% (19/78)	28.6% (22/77)
				OD (IQR)	6.9 (4.8-16.5)	8.2 (1.8-18.7)			OD (IQR)	6.5 (3.1-16.1)	7.2 (3.1-24.8)
20-29	99 (7.8)	2.0% (2)	138 (1068)	Prevalence (n/N)	8.1% (8/99)	14.1% (14/99)	9.1% (9)	12 (77)	Prevalence (n/N)	20.2% (20/99)	17.3% (17/98)
				OD (IQR)	6.2 (3.7-10.4)	3.7 (0.3-14.1)			OD (IQR)	4.5 (2.6-13.3)	3.6 (1.7-10.3)
30-39	98 (7.7)	2.0% (2)	35 (304)	Prevalence (n/N)	23.5% (23/98)	30.6% (30/98)	8.2% (8)	4 (24)	Prevalence (n/N)	29.6% (29/98)	29.9% (29/97)
				OD (IQR)	8.0 (4.0-21.6)	11.2 (2.7-40.5)			OD (IQR)	6.2 (2.3-24.7)	6.4 (2.3-22.6)
40-49	77 (6.1)	1.3% (1)	407 (3572)	Prevalence (n/N)	18.2% (14/77)	23.7% (18/76)	10.4% (8)	23 (102)	Prevalence (n/N)	24.7% (19/77)	33.8% (26/77)
				OD (IQR)	6.4 (3.7-17.5)	6.2 (0.6-28.4)			OD (IQR)	7.1 (2.2-15.9)	6.0 (1.8-32.6)
50+	107 (8.4)	1.9% (2)	1 (8)	Prevalence (n/N)	23.4% (25/107)	29.0% (31/107)	28.0% (30)	113 (575)	Prevalence (n/N)	31.8% (34/107)	29.0% (31/107)
				OD (IQR)	7.2 (4.1-21.9)	9.0 (1.1-44.1)			OD (IQR)	7.5 (3.2-22.2)	6.7 (2.2-20.5)
Total	**1,268**	**7.3% (93)**	**540 (3318)**	**Prevalence (n/N)**	**16.0% (203/1265)**	**21.3% (270/1265)**	**6.6% (84)**	**16 (184)**	**Prevalence (n/N)**	**22.7% (288/1268)**	**24.2% (306/1263)**
				OD (IQR)	**7.5 (4.2-16.3)**	**8.0 (1.9-25.8)**			**OD (IQR)**	**5.6 (2.3-15.0)**	**6.4 (2.4-17.5)**

nsmitted helminth (STH) infections

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e OD (IQR) is the median OD value ar

pelow the cut-off (the whole range).

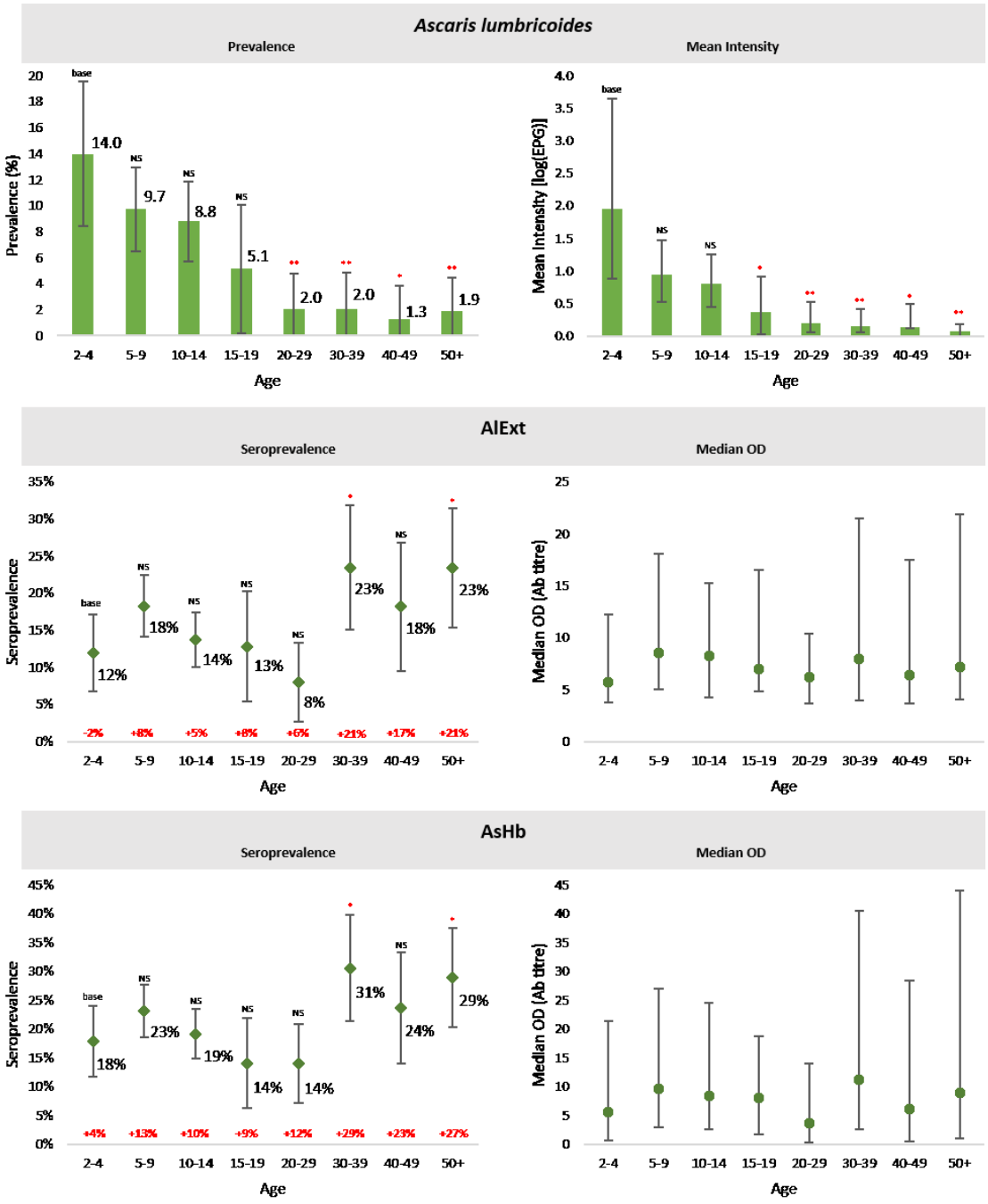


Figure 4.6. Age distribution of *A. lumbricoides* infection, anti-AlExt and anti-AsHb antibody titres. These graphs refer to the baseline survey population (n=1,268). The top graphs represent the age-distribution of prevalence and geometrical mean intensity of *A. lumbricoides* infection. The middle and bottom graphs show the age distribution of seroprevalence of anti-AlExt and anti-AsHb antibodies on the left and the age distribution of median antibody titre (OD) on the right. The seroprevalence represents the proportion of individuals with OD above the cut-off value for each antigen, while the median OD represents the median value in the whole range of OD (both above and below cut-off values). Vertical lines represent normal 95% confidence intervals, except for median OD graphs, where they represent the interquartile range (25-75% around the mean OD values). Percentages in red in seroprevalence graphs show the difference between seroprevalence and prevalence of *A. lumbricoides* for each age group. Statistically significant differences are signalled in red: *: p<0.05; **: p<0.01; ***: p<0.001; NS: not significant (p>0.05); base: base test

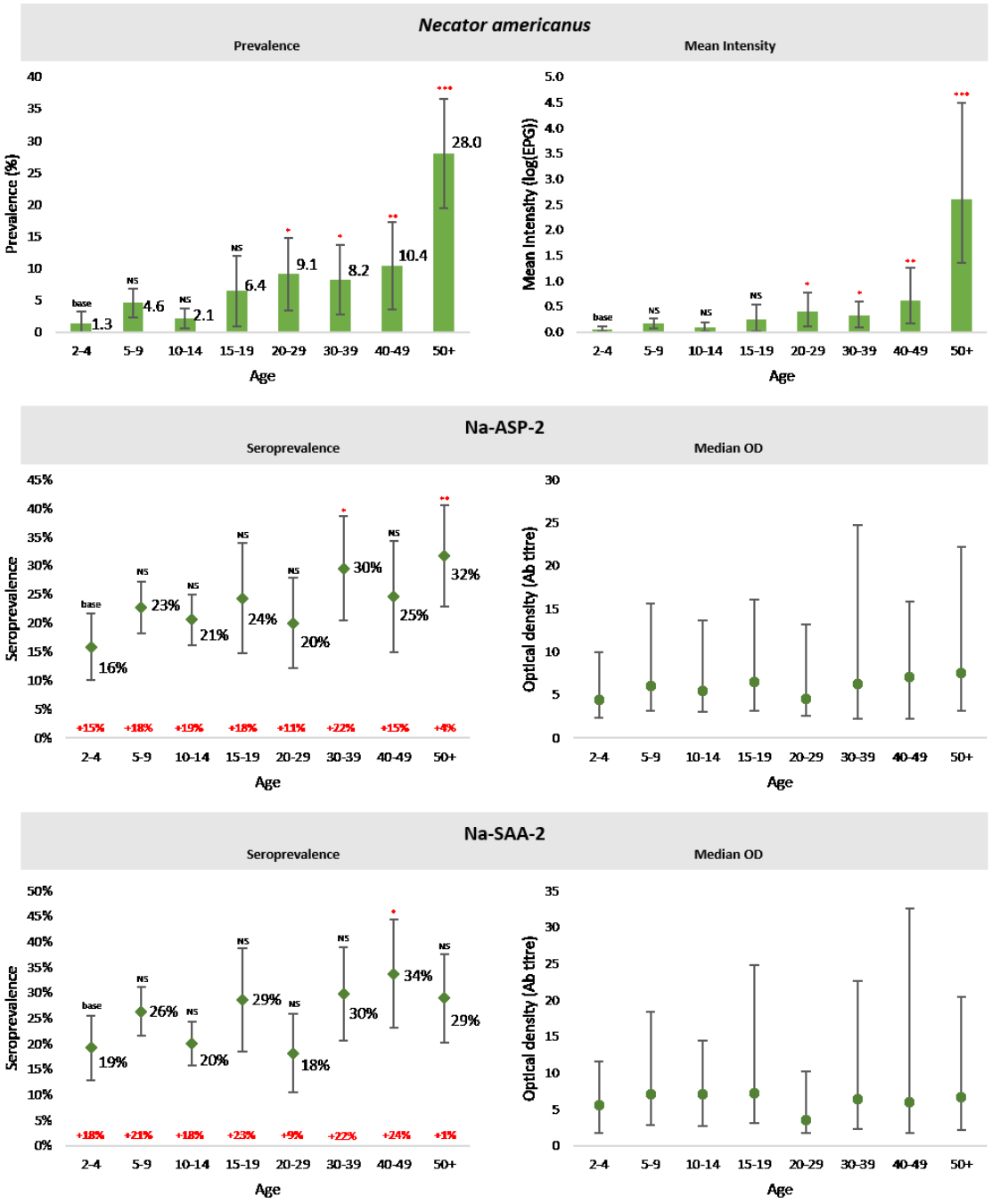


Figure 4.7. Age distribution of *N. americanus* infection, anti-Na-ASP-2 and anti-Na-SAA-2 antibody titres. These graphs refer to the baseline survey population (n=1,268). The top graphs represent the age distribution of prevalence and geometrical mean intensity of *N. americanus* infection. The middle and bottom graphs show the age distribution of seroprevalence of anti-Na-ASP-2 and anti-Na-SAA-2 antibodies on the left and the age distribution of median antibody titre (OD) on the right. The seroprevalence represents the proportion of individuals with OD above the cut-off value for each antigen, while the median OD represents the median value in the whole range of OD (both above and below cut-off values). Vertical lines represent normal 95% confidence intervals, except for the median OD graphs, where they represent the interquartile range (25-75% around the mean OD values). Percentages in red in seroprevalence graphs show the difference between seroprevalence and prevalence of *N. americanus* for each age group. Statistically significant differences are signalled in red: *: p<0.05; **: p<0.01; ***: p<0.001; NS: not significant (p>0.05); base: base test group

A total of 22.7% and 24.2% of the population were seropositive for the two hookworm antigens, Na-ASP-2 and Na-SAA-2, respectively, with median ODs of 5.6 and 6.4. The age-distribution of seroprevalence of the anti-*Necator* antibodies generally increased with age (in line with the prevalence and intensity of infection), while the pre-SAC and the group aged 20 to 29 remained the groups with lowest seroprevalence of both anti-NA-ASP-2 and anti-Na-SAA-2 antibodies (Figure 4.7). There was again a positive correlation between the two seroprevalence profiles, with Kendall's tau of 0.691 (p=0.025). The age-distributions of median OD of the two *Necator* antibodies was also very similar, with very small variation between all age groups.

When measuring correlation between the age profiles of prevalence of infection and seroprevalence of anti-STH antibodies, the patterns for both *Ascaris* antibodies showed no close correlation with the observed prevalence profile. Only the age-distribution of anti-Na-ASP-2 antibody seroprevalence was significantly correlated with the age-distribution of *N. americanus* prevalence (Kendall's τ=0.643, p=0.035). None of the age-distributions of anti-STH antibodies median OD were significantly correlated with the profile of mean intensity of infection. When looking at correlations between age distribution of any STH infection (Figure 4.8) and antibody seroprevalence, none of the antibodies was significantly correlated with prevalence of any STH (AlExt: Kendall's τ= - 0.074, p=0.900; AsHb: τ=- 0.109, p=0.803; Na-ASP-2: τ=- 0.143, p=0.711; Na-SAA-2: τ=- 0.182, p=0.618).

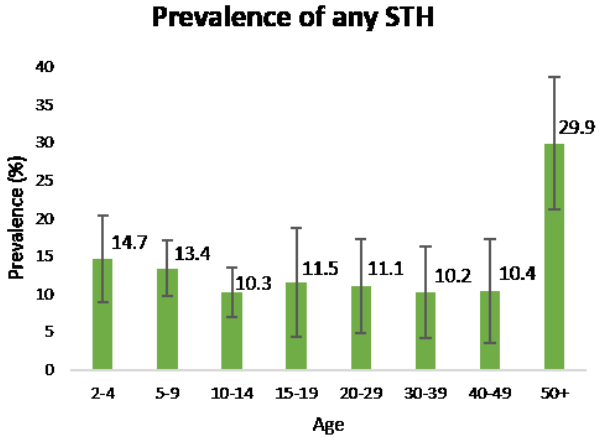


Figure 4.8. Age distribution of any STH infection in the baseline survey population (n=1,268). This figure represents the proportion found to be infected with either *A. lumbricoides* or *N. americanus* in each age group. Vertical lines represent normal 95% confidence intervals.

In terms of individual infection, the frequency histograms of individual intensity of *A. lumbricoides* and *N. americanus* infections both followed a negative binomial distribution, with small values of the aggregation parameter *k* reflecting a high degree of aggregation (high values=low aggregation; low values=high aggregation), as well as high variance-to-mean ratios (index of dispersion) (Figure 4.9). All antibody titre histograms followed the negative binomial distribution, with variance much greater than the mean and seronegative individuals outnumbering the seropositive individuals.

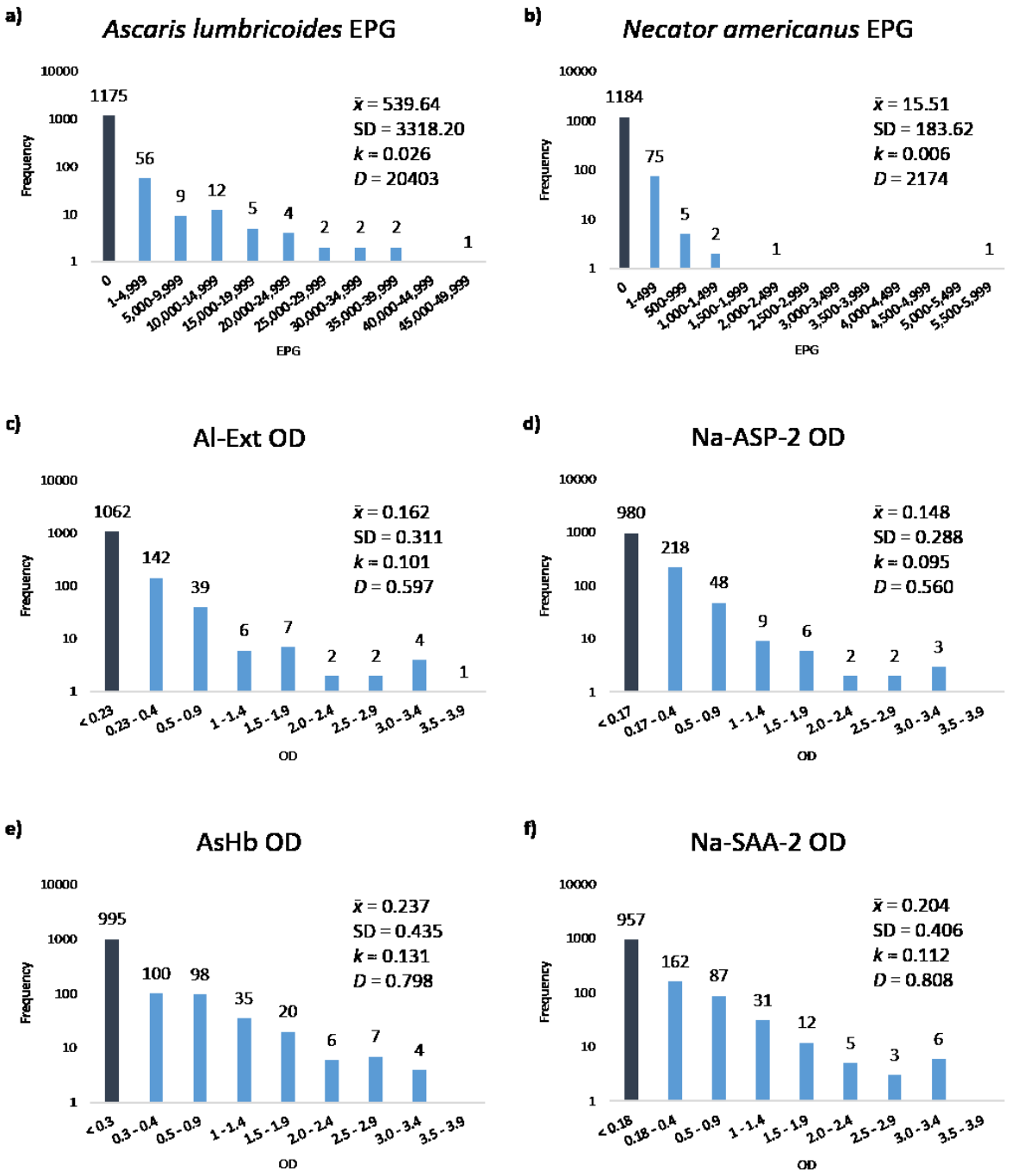


Figure 4.9. Frequency distribution of EPG and OD. The distribution of individual intensity levels of *A. lumbricoides* infection, as measured by EPG, is represented in **a)** and that of *N. americanus* infections in **b)**. The distribution of individual antibody titres against AlExt, Na-ASP-2, AsHb and Na-SAA-2 are represented in **c)**, **d)**, **e)** and **f)**, respectively. All graphs are presented with a logarithmic *y*-axis to enable visualisation. The arithmetic mean ($\bar{x}$), standard deviation (SD) aggregation parameter (k) and index of dispersion (D) of the frequency histograms are provided. k was calculated using the corrected moment estimate (see section 4.3.5) and D is the variance-to-mean ratio. Numbers above columns show the number of individuals in each EPG and OD category. EPG loads are used as proxy for the number of worms harboured by individuals, while OD values correspond to the antibody titre against each specific antigen. Dark columns show frequency of individuals with zero EPG (a, b) or OD below cut-off levels (c -e).

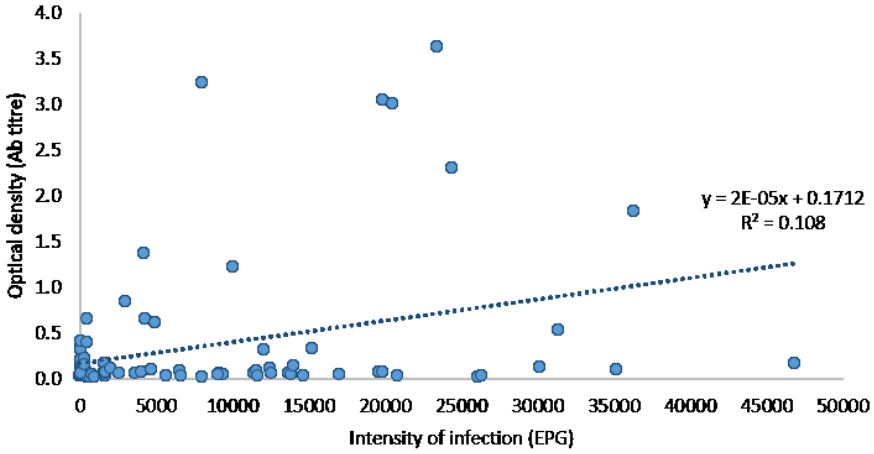
4.4.4. Anti-helminth antibodies and infection status

When analysing only the study participants who were found to be infected at baseline, no strong correlations were found between individual intensity measurements of *A. lumbricoides* (EPG) or *N. americanus* infections (EPG) and anti-STH antibody titres (OD), even when stratifying individuals by major age groups (Figures 4.10 to 4.13). The only significant correlation was found between anti-Na-SAA-2 antibody titres and light *N. americanus* infections (EPG < 2,000) (Kendall's tau=0.151, p=0.048, Figure 4.12). Interestingly, individuals found to be co-infected (n=7, not shown) did not present particularly high anti-STH antibody titres, and were seronegative for the four antigens - except for one individual carrying light *N. americanus* and moderate to high *A. lumbricoides* infection, who was found to be seropositive for all antigens.

When the mean EPG and mean antibody titres for each of the eight age groups were plotted, two almost opposite patterns emerged for each helminth species (Figure 4.14). While the mean *A. lumbricoides* EPG decreases with age, the average anti-*Ascaris* antibody titres generally increase, so that the points generally move left and up in the chart with increasing age. The charts for *N. americanus* are very different: the mean EPG increases with age and so do the mean anti-*Necator* antibody titres, thus the points in the chart move to the right and up with increasing age. Though there are differences between the two *Ascaris* graphs and the two *Necator* graphs, the overall patterns are very similar within species.

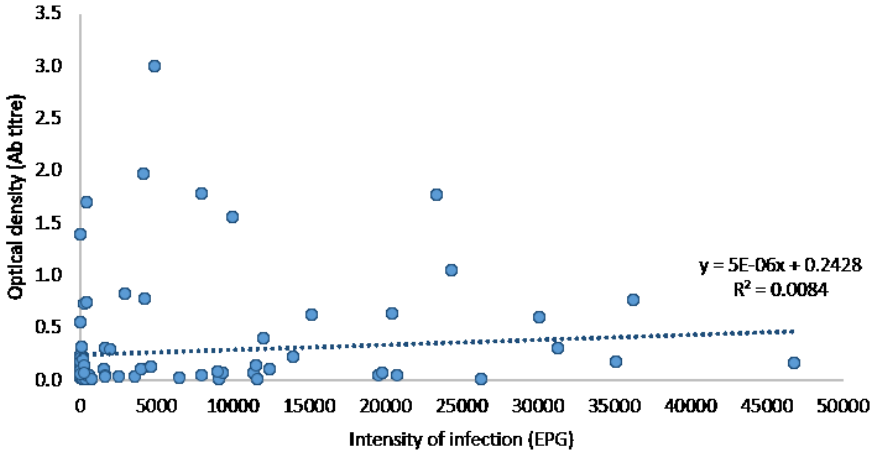
A comparison was made between the data obtained in this study and that obtained in an already published longitudinal study of anti-AsHb IgG4 in an MDA-targeted community in Indonesia (6). The proportion of individuals in the present study's baseline survey falling into the EPG-positive or negative and antigen-positive or negative populations was found to be very similar to the proportions of EPG-positive or negative and antigen-positive or negative populations in Indonesia two years into a community-wide albendazole MDA programme (Figure 4.15). This analysis also enabled the observation that, at study baseline, the proportion of infected individiduals who were seronegative was approximately four times larger than the proportion of infected seropositive individuals.The seronegative proportion of uninfected individuals was also close to four times larger than the seropositive proportion.

a)



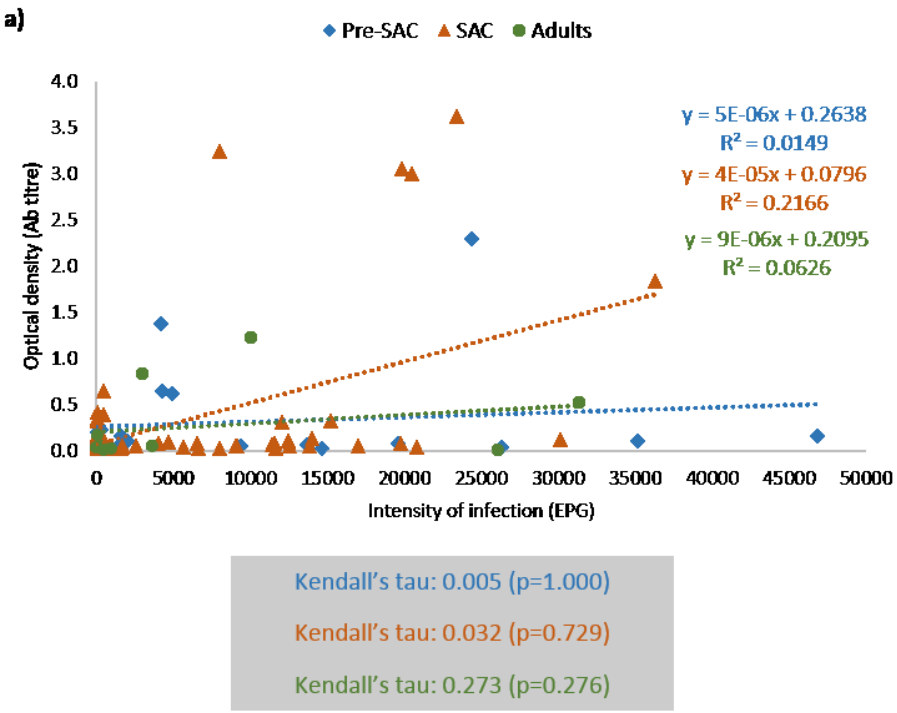
Kendall's tau: 0.060 (p=0.399)

b)



Kendall's tau: 0.022 (p=0.755)

Figure 4.10. Scatterplots of individual *A. lumbricoides* EPG loads vs. antibody titres. Each individual's *A. lumbricoides* EPG load is plotted against his anti-AlExt antibody titre in **a)** and his anti-AsHb antibody titre in **b)**. Both graphs refer to the baseline survey population (n=1,268), but only infected individuals (*A. lumbricoides* EPG>0) are represented. No upper or lower limit was set to OD values. The dashed trendline's equation and R^2 are shown, while the Kendall's tau and p-value below each graph show the correlation strength between EPG and Ab titre.



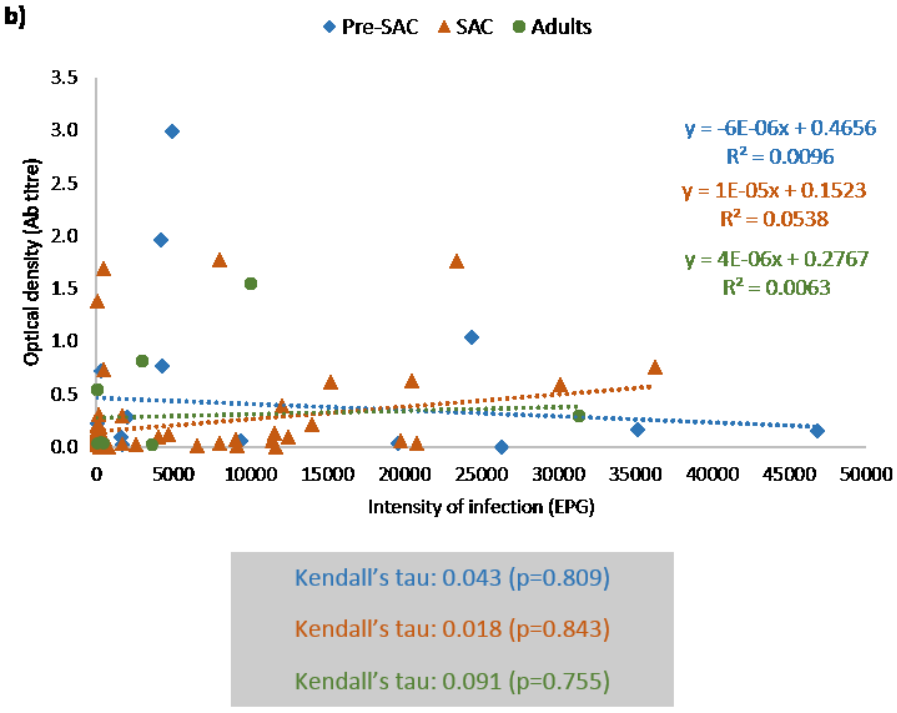


Figure 4.11. Age-based scatterplots of individual *A. lumbricoides* EPG loads vs. antibody titres. Each individual's *A. lumbricoides* EPG load is plotted against his anti-AlExt antibody titre in **a)** and his anti-AsHb antibody titre in **b)**. The different colours of the data points, trendlines and text correspond to the three major age groups. Both graphs refer to the baseline survey population (n=1,268), but only infected individuals (*A. lumbricoides* EPG>0) are represented. No upper or lower limit was set to OD values. The dashed trendline's equation and R^2 are shown, while the Kendall's tau and p-value below each graph show the correlation strength between EPG and Ab titre.

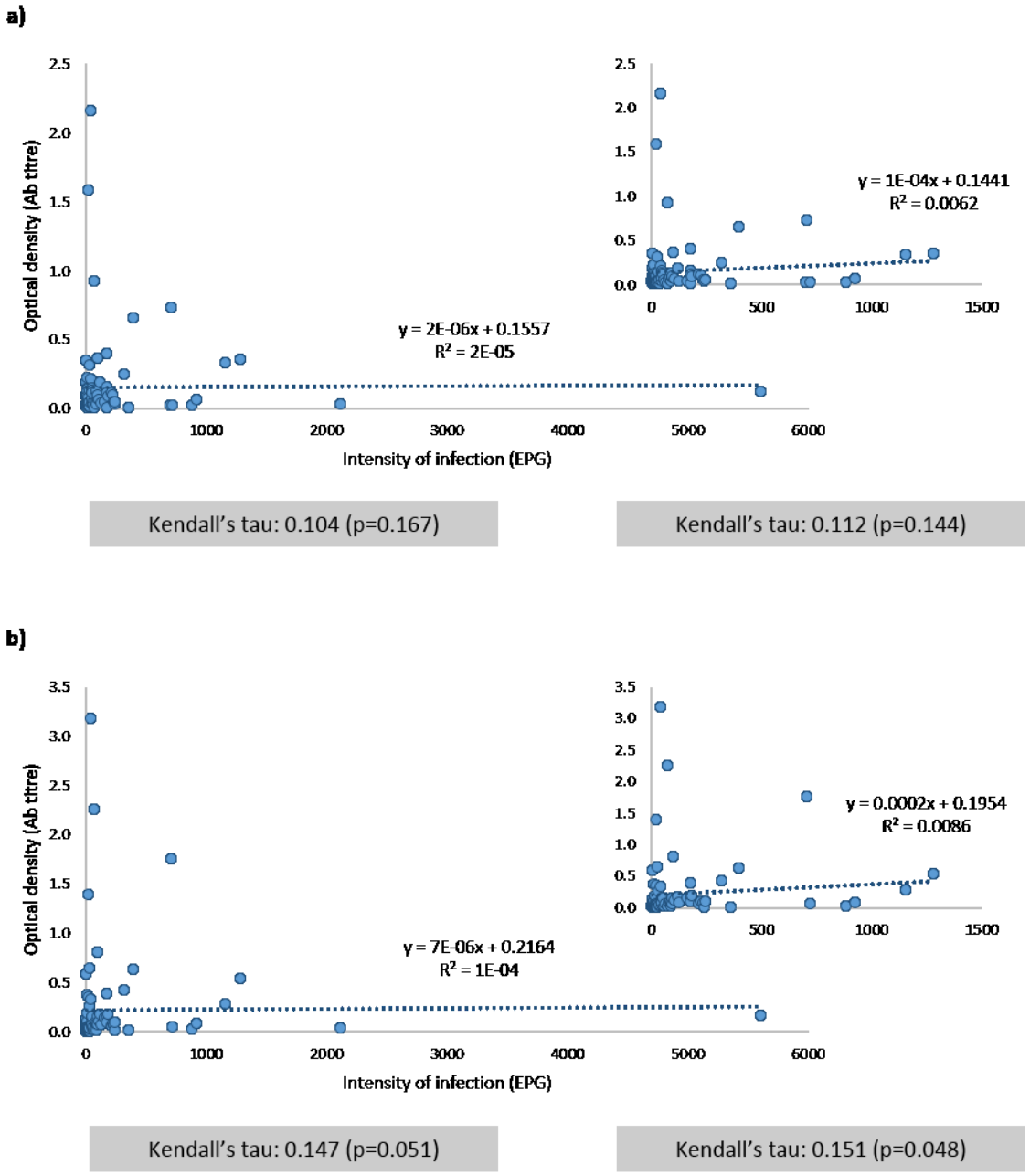


Figure 4.12. Scatterplots of individual *N. americanus* EPG loads vs. antibody titres. Each individual's *Necator americanus* EPG load is plotted against his anti-Na-ASP-2 antibody titre in **a)** and his anti-Na-SAA-2 antibody titre in **b)**. All four graphs refer to the baseline survey population (n=1,268), but only infected individuals (*N. americanus* EPG>0) are presented in the larger graphs, and only individuals with light infections (*N. americanus* EPG<2,000) are presented in the smaller, inset graphs. No upper or lower limit was set to OD values. The dashed trendline's equation and R^2 are shown, while the Kendall's tau and p-value below each graph show the correlation strength between EPG and Ab titre.

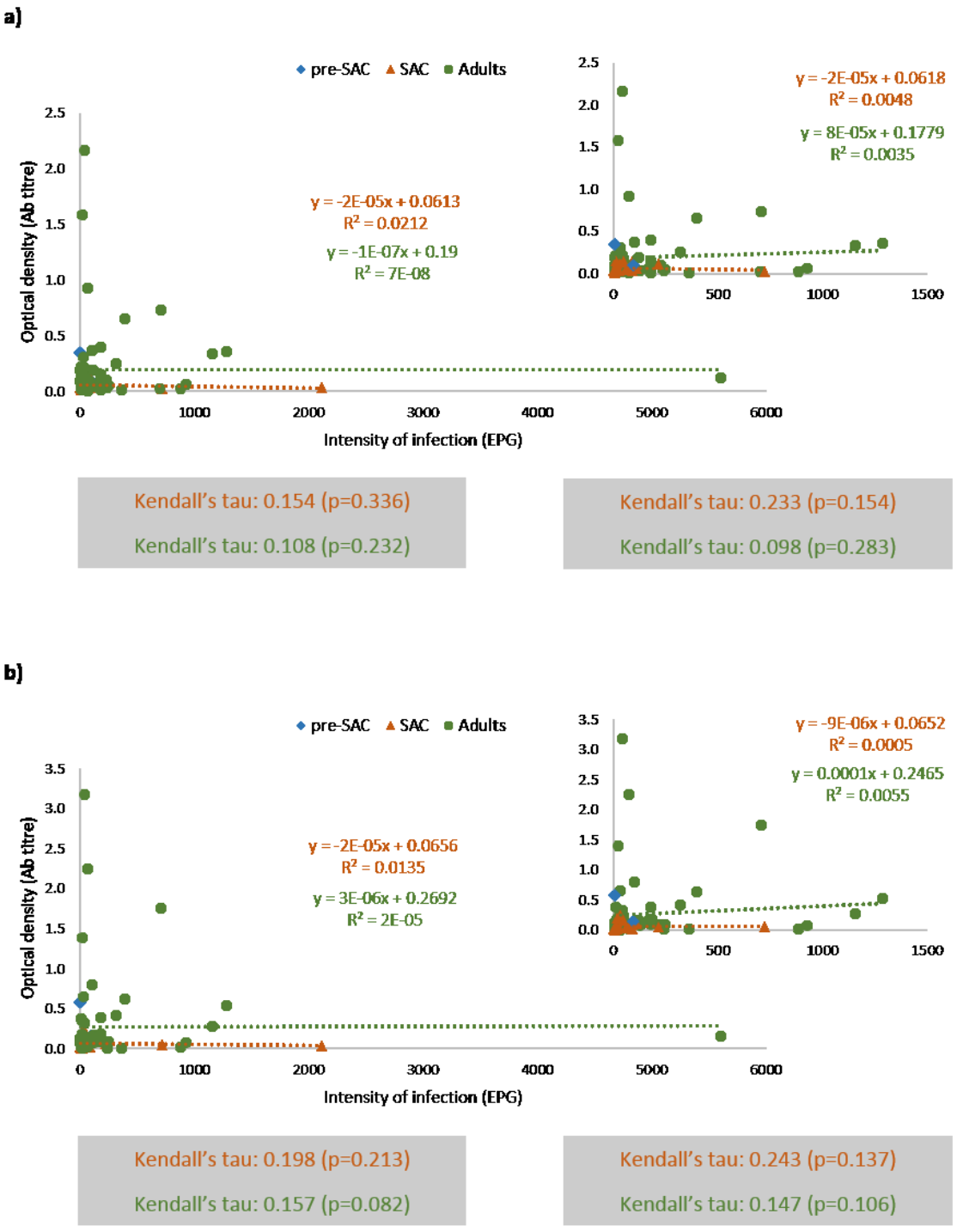


Figure 4.13. Age-based scatterplots of individual *N. americanus* EPG loads vs. antibody titres. Each individual's *N. americanus* EPG load is plotted against his anti-Na-ASP-2 antibody titre in **a)** and his anti-Na-SAA-2 antibody titre in **b)**. The different colours of the data points, trendlines and text correspond to the three major age groups (only two pre-SAC individuals are presented). All four graphs refer to the baseline survey population (n=1,268), but only infected individuals (*N. americanus* EPG>0) are presented in the larger graphs, and only individuals with light infections (*N. americanus* EPG<2,000) are presented in the smaller, inset graphs. No upper or lower limit was set to OD values. The dashed trendline's equation and R^2 are shown, while the Kendall's tau and p-value below each graph show the correlation strength between EPG and Ab titre.

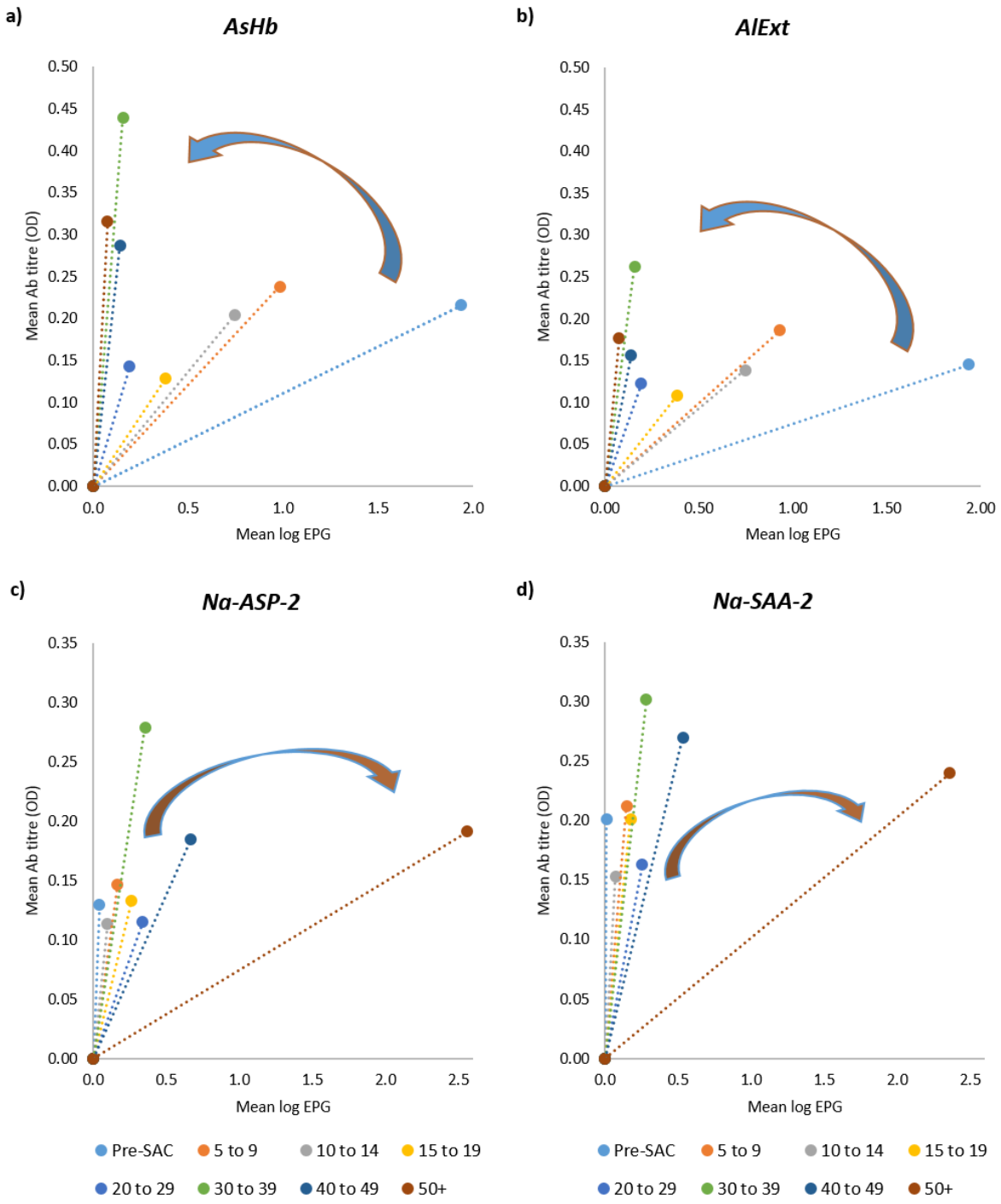


Figure 4.14. Patterns of average EPG loads and average antibody titres with age. These graphs show the distinct trends for each species in average EPG vs. antibody titre with age. Each point represents one age group, with colour identification in the legend, with the geometric mean of *A. lumbricoides* EPG vs. arithmetic mean anti-AsHb antibody titre in **a)** and anti-AlExt antibody titre in **b)**, and the geometric mean of *N. americanus* EPG vs. anti-Na-ASP-2 antibody titre in **c)** and anti-Na-SAA-2 antibody titre in **d)**. These graphs refer to the baseline survey population (n=1,268), including both infected and uninfected individuals, as well as OD values both above and below cut-off. The curved arrows indicate the general direction of change with increasing age.

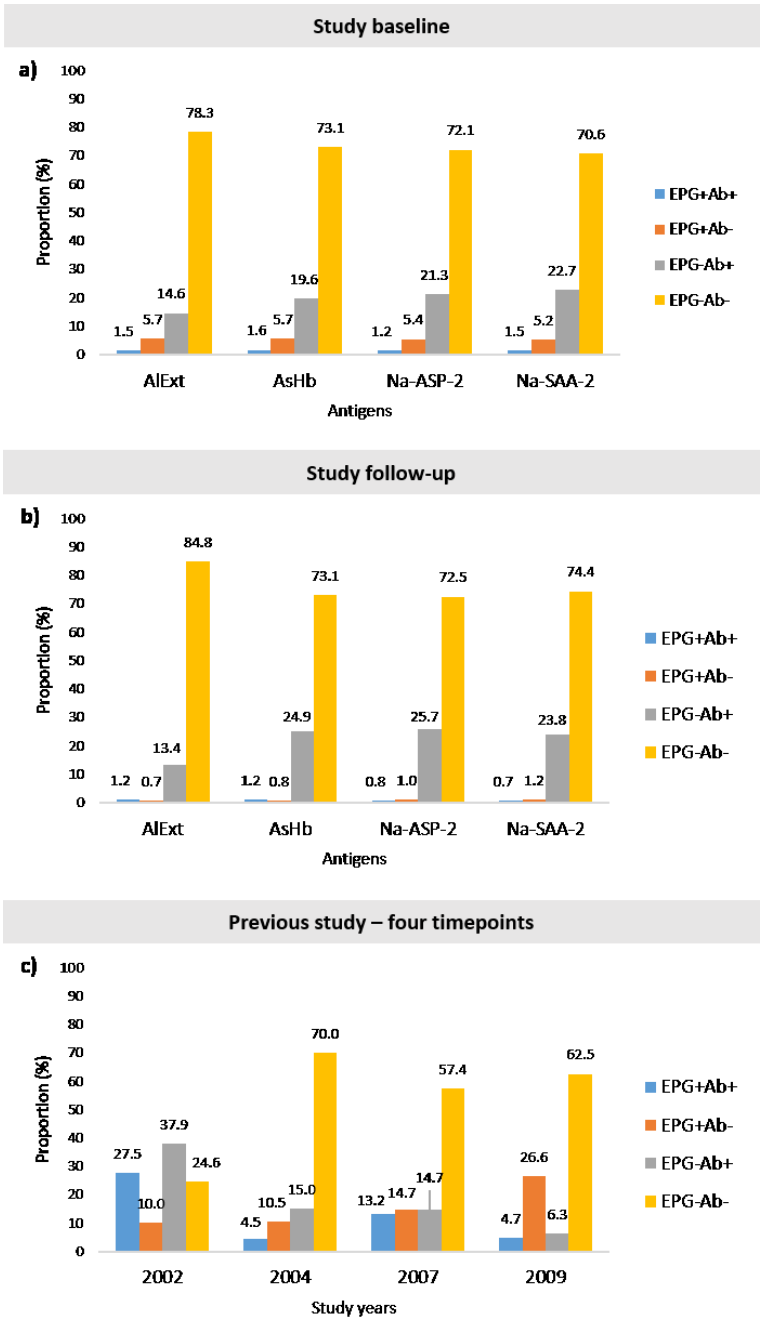


Figure 4.15. Comparison of proportions infected/uninfected/seropositive/seronegative between a previous longitudinal study and this study. Graphs **a)** and **b)** show the proportions of individuals in the baseline survey population (n=1,268) and the follow-up survey population (n=607), respectively, who were infected and seropositive (EPG+Ab+), infected and seronegative (EPG+Ab-), uninfected and seropositive (EPG-Ab+) or uninfected and seronegative (EPG-Ab-) for each of the four antigens tested. The graph shown in **c)** displays the findings of a previous study analysing *A. lumbricoides* infection and anti-AsHb IgG4 antibody levels in a population pre-community-wide MDA (2002), after two rounds of MDA (2004), prior to the final (sixth) round of MDA (2007) and two years after the last round of MDA (2009) (data adapted from [4]). EPG+ for AlExt and AsHb refers to *A. lumbricoides* EPG>0, while EPG+ for Na-ASP-2 and Na-SAA-2 refers to *N. americanus* EPG>0. Seropositivity is defined as OD values above cut-off for each specific antibody. **NB.** All data presented in graph **c)** relates to anti-AsHb IgG4 antibodies, while only AsHb data in graphs **a)** and **b)** refer to the same antigen/antibody combination.

Finally, correlations between the four anti-STH antibodies were analysed. Overall, the four anti-STH antibodies showed similar age distributions in terms of seroprevalence and median OD values (see Figures 4.6 and 4.7). Strong correlations were found, as expected, between the two *Ascaris* antibody titres and between the two *N. americanus* antibody titres, using both the age-group values and individual values (Table 4.3). Strong correlations were also found between anti-AlExt antibodies and the two *Necator* antibodies, but not between anti-AsHb antibodies and Na-ASP-2 or Na-SAA-2.

a

Age-group values	AlExt	AsHb (τ, p-value)	Na-ASP-2 (τ, p-value)	Na-SAA-2 (τ, p-value)
AlExt	**1**	0.868**	0.741*	0.642*
AsHb	-	1	0.618*	**0.593^{NS}**
Na-ASP-2	-	-	**1**	0.691*
Na-SAA-2	-	-	-	1

b

Individual values	AlExt	AsHb (R^2, p-value)	Na-ASP-2 (R^2, p-value)	Na-SAA-2 (R^2, p-value)
AlExt	1	0.281***	0.405***	0.085***
AsHb	-	**1**	0.041*	**0.018^{NS}**
Na-ASP-2	-	-	**1**	0.382***
Na-SAA-2	-	-	-	**1**

Table 4.3. Correlation between the four antigens – a measurement of cross-reactivity. Table **a)** shows the Kendall's tau rank correlation (τ) measurements between mean age-group values of IgG4 seroprevalence for each antigen. The values used are those presented in the age profiles of Figures 4.6 and 4.7 (n=8). Each age group's IgG4 seroprevalence against one antigen was compared against the same age group's seroprevalence against another antigen. All age groups were analysed simultaneously. Table **b)** shows the Pearson's correlation coefficients (R^2) between individual IgG4 antibody titres against each antigen. Each individual's IgG4 titre against one antigen was compared against his own IgG4 titre against another antigen. These comparisons were made at population level, and only values between the cut-off and a maximum OD = 2.0 for each antigen were used in this analysis (n range=127 to 212). The maximum OD value (2.0) is derived from observations made in the replication error test (see Figure 4.5). Statistically significant differences are signalled with different shades of grey and asterisks: *: p<0.05; **: p<0.01; ***: p<0.001; NS: not significant (p>0.05).

4.4.5. Impact of anthelminthic treatment on anti-STH seroprevalence

Three months after a community-wide deworming round, significant reductions were observed in prevalence and mean intensity of *A. lumbricoides* infections in pre-SAC and SAC, as well as in prevalence and mean intensity of *N. americanus* infection in the eldest age group (50 years and older) (Tables 4.4 and 4.5 and Figure 4.16). However, none of these reductions were accompanied by significant changes in anti-STH antibody titres, and in fact no consistent trends of rise or decline of antibody titres were observed (Figure 4.17). The only significant difference observed was an increase in seroprevalence of anti-AsHb antibodies in children 5 to 9 years old (p=0.011) post-treatment.

When examining individual changes in seroprevalence after community-wide treatment, there were no significant differences between baseline and follow-up levels of AlExt or AsHb seroprevalence, but there were significant reductions in the seroprevalence levels of Na-ASP-2 (z-score: 8.277, p<0.001) and Na-SAA-2 (z-score: 11.889, p<0.001). When focusing only on the individuals who were found to be carrying *A. lumbricoides* infections at baseline, there was still no significant difference in anti-*Ascaris* antibody titres three months post-treatment, even when comparing only individuals who were also seropositive at baseline (Figure 4.18). However, there was a significant decrease in seroprevalence levels of Na-SAA-2 in individuals who were infected with *N. americanus* at study baseline (z-score: 2.484, p=0.013, Figure 4.19). Importantly, seven of the individuals in Figures 4.18a and 4.18b (12.1%) were reinfected/still infected at follow-up, while only one hookworm reinfection was found in the individuals in Figures 4.19 (2.6%). Removing these individuals from the analyses does not alter the values for *Ascaris* antigens but the decrease in Na-SAA-2 seroprevalence levels post-treatment becomes more significant (z-score: 2.738, p=0.006). When comparing the antibody titres of all individuals, both those who were found to be infected and those who were not (i.e. population-level analysis), only the average anti-Na-SAA-2 antibody titre was significantly reduced three months post-treatment (from OD=0.22 to OD=0.12, *t*: -5.16, p<0.001). The average anti-Na-ASP-2 and anti-AsHb antibody titres were also reduced, though not significantly (*t*: -1.82, p=0.069 and *t*: -0.72, p=0.470, respectively), while the average anti-AlExt antibody titre remained mostly unchanged (*t*: 0.08, p=0.932).

Age Group	N (%)	Baseline					Follow-up				
		Prevalence (n)	Intensity (SD)		AlExt	AsHb	Prevalence (n)	Intensity (SD)		AlExt	AsHb
2-4	**79 (13.0)**	**21.5% (17)**	**2029 (6337)**	**Prevalence (n/N)**	**13.9 (11/79)**	**21.5 (17/79)**	**3.8% (3)**	**428 (2870)**	**Prevalence (n/N)**	**16.5 (13/79)**	**21.5 (17/79)**
				OD (IQR)	6.4 (4.3-16.2)	5.5 (3.6-24.9)			OD (IQR)	5.9 (3.9-12.2)	7.5 (3.0-21.1)
5-9	**195 (32.1)**	**9.2% (18)**	**905 (4060)**	**Prevalence (n/N)**	**17.5 (34/194)**	**20.6 (39/189)**	**2.6% (5)**	**235 (2105)**	**Prevalence (n/N)**	**14.9 (29/195)**	**31.3 (61/195)**
				OD (IQR)	8.6 (5.2-16.5)	9.4 (2.8-24.1)			OD (IQR)	8.4 (4.4-19.5)	10.6 (4.2-27.1)
10-14	**151 (24.9)**	**11.9% (18)**	**692 (3536)**	**Prevalence (n/N)**	**10.7 (16/149)**	**17.4 (26/149)**	**1.3% (2)**	**88 (837)**	**Prevalence (n/N)**	**7.3 (11/151)**	**24.5 (37/151)**
				OD (IQR)	7.6 (4.3-15.2)	7.3 (2.3-19.7)			OD (IQR)	7.5 (4.0-16.1)	8.0 (4.2-20.9)
15-19	**27 (4.4)**	**7.4% (2)**	**34 (173)**	**Prevalence (n/N)**	**7.4 (2/27)**	**11.1 (3/27)**	**3.7% (1)**	**52 (273)**	**Prevalence (n/N)**	**11.1 (3/27)**	**25.9 (7/27)**
				OD (IQR)	5.9 (3.1-15.2)	4.3 (1.4-45.2)			OD (IQR)	6.8 (4.4-15.3)	7.6 (4.1-24.5)
20-29	**43 (7.1)**	**4.7% (2)**	**317 (1614)**	**Prevalence (n/N)**	**9.3 (4/43)**	**16.3 (7/43)**	**2.3% (1)**	**194 (1285)**	**Prevalence (n/N)**	**13.9 (6/43)**	**11.6 (5/43)**
				OD (IQR)	7.1 (4.1-10.3)	1.9 (0.2-15.6)			OD (IQR)	6.9 (3.7-18.2)	6.9 (4.1-14.0)
30-39	**30 (4.9)**	**3.3% (1)**	**99 (543)**	**Prevalence (n/N)**	**36.7 (11/30)**	**40.0 (12/30)**	**0.0% (0)**	**0 (0)**	**Prevalence (n/N)**	**30.0 (9/30)**	**33.3 (10/30)**
				OD (IQR)	8.7 (4.3-34.7)	7.7 (1.9-98.1)			OD (IQR)	9.8 (3.7-45.7)	10.1 (1.7-53.7)
40-49	**29 (4.8)**	**3.4% (1)**	**1081 (5820)**	**Prevalence (n/N)**	**24.1 (7/29)**	**27.6 (8/29)**	**0.0% (0)**	**0 (0)**	**Prevalence (n/N)**	**20.7 (6/29)**	**31.0 (9/29)**
				OD (IQR)	9.5 (3.9-15.2)	10.8 (2.2-30.6)			OD (IQR)	12.0 (4.2-22.1)	10.9 (7.4-24.2)
50+	**53 (8.7)**	**0.0% (0)**	**0 (0)**	**Prevalence (n/N)**	**18.9 (10/53)**	**20.7 (11/53)**	**0.0% (0)**	**0 (0)**	**Prevalence (n/N)**	**20.7 (11/53)**	**24.5 (13/53)**
				OD (IQR)	6.3 (4.4-20.2)	6.6 (0.6-35.3)			OD (IQR)	7.7 (4.2-17.8)	7.2 (1.9-16.7)
Total	**607**	**9.7% (59)**	**807 (3954)**				**2.0% (12)**	**170 (1672)**			

Table 4.4. Age distribution, prevalence and intensity of *Ascaris lumbricoides* infection and serological population at baseline and follow-up. This table refers to the successfully followed-up population who timepoints (N=607). Intensity (SD) is the arithmetic mean intensity of *A. lumbricoides* infection and the s percentage of people with OD values above the cut-off for each antigen, while OD (IQR) is the median O (25% to 75% of values around the median), including values both above AND below the cut-off (the who 100 to facilitate data presentation.

Age Group	N (%)	*Baseline*					*Follow-up*				
		Prevalence (n)	Intensity (SD)		Na-ASP-2	Na-SAA-2	Prevalence (n)	Intensity (SD)		Na-ASP-2	Na-SAA-2
2-4	**79 (13.0)**	**0.0% (0)**	**0 (0)**	**Prevalence (n/N)**	**19.0 (15/79)**	**22.8 (18/79)**	**2.5% (2)**	**1 (7)**	**Prevalence (n/N)**	**29.1 (23/79)**	**26.6 (21/79)**
				OD (IQR)	5.2 (3.4-14.0)	5.7 (2.8-12.4)			OD (IQR)	4.5 (2.3-13.5)	3.4 (2.0-11.0)
5-9	**195 (32.1)**	**4.1% (8)**	**3 (19)**	**Prevalence (n/N)**	**23.6 (46/195)**	**27.3 (53/194)**	**2.6% (5)**	**3 (20)**	**Prevalence (n/N)**	**24.6 (48/195)**	**23.1 (45/195)**
				OD (IQR)	5.9 (3.0-10.0)	7.0 (3.2-20.1)			OD (IQR)	3.7 (1.8-10.5)	3.6 (1.5-8.8)
10-14	**151 (24.9)**	**3.3% (5)**	**20 (182)**	**Prevalence (n/N)**	**23.2 (35/151)**	**25.8 (38/147)**	**0.7% (1)**	**0.2 (2)**	**Prevalence (n/N)**	**18.5 (28/151)**	**19.9 (30/151)**
				OD (IQR)	5.3 (2.3-15.4)	6.4 (2.2-16.3)			OD (IQR)	3.3 (1.8-7.7)	3.0 (1.5-7.5)
15-19	**27 (4.4)**	**3.7% (1)**	**0.4 (2)**	**Prevalence (n/N)**	**18.5 (5/27)**	**25.9 (7/27)**	**3.7% (1)**	**0.9 (5)**	**Prevalence (n/N)**	**29.6 (8/27)**	**25.9 (7/27)**
				OD (IQR)	7.3 (1.4-14.4)	7.1 (3.0-24.3)			OD (IQR)	4.4 (2.5-11.3)	3.3 (1.9-10.7)
20-29	**43 (7.1)**	**14.0% (6)**	**26 (116)**	**Prevalence (n/N)**	**23.3 (10/43)**	**18.6 (8/43)**	**0.0% (0)**	**0 (0)**	**Prevalence (n/N)**	**23.3 (10/43)**	**25.6 (11/43)**
				OD (IQR)	5.4 (2.5-12.2)	5.2 (2.1-13.8)			OD (IQR)	3.7 (2.1-8.9)	3.4 (0.9-10.6)
30-39	**30 (4.9)**	**3.3% (1)**	**0.6 (3)**	**Prevalence (n/N)**	**46.7 (14/30)**	**36.7 (11/30)**	**0.0% (0)**	**0 (0)**	**Prevalence (n/N)**	**50.0 (15/30)**	**43.3 (13/30)**
				OD (IQR)	11.8 (4.3-38.8)	8.5 (2.5-39.7)			OD (IQR)	8.6 (1.6-58.6)	5.4 (1.7-28.1)
40-49	**29 (4.8)**	**6.9% (2)**	**15 (74)**	**Prevalence (n/N)**	**34.5 (10/29)**	**37.9 (11/29)**	**0.0% (0)**	**0 (0)**	**Prevalence (n/N)**	**44.8 (13/29)**	**37.9 (11/29)**
				OD (IQR)	10.1 (4.1-22.2)	8.8 (2.6-62.2)			OD (IQR)	6.5 (2.8-16.9)	7.2 (2.2-14.5)
50+	**53 (8.7)**	**30.2% (16)**	**81 (263)**	**Prevalence (n/N)**	**28.3 (15/53)**	**30.2 (16/53)**	**3.8% (2)**	**1 (6)**	**Prevalence (n/N)**	**30.2 (16/53)**	**22.6 (12/53)**
				OD (IQR)	5.6 (3.3-12.2)	5.8 (1.9-17.5)			OD (IQR)	5.5 (2.2-18.2)	3.3 (1.8-8.1)
Total	**607**	**6.4% (39)**	**15 (126)**				**1.8% (11)**	**1 (12)**			

Table 4.5. Age distribution, prevalence and intensity of *Necator americanus* infection and serological same population at baseline and follow-up. This table refers to the successfully followed-up populatio two timepoints (n=607). Intensity (SD) is the arithmetic mean intensity of *N. americanus* infection and th the percentage of people with OD values above the cut-off for each antigen, while OD (IQR) is the me values (25% to 75% of values around the median), including values both above AND below the cut- multiplied by 100 to facilitate data presentation.

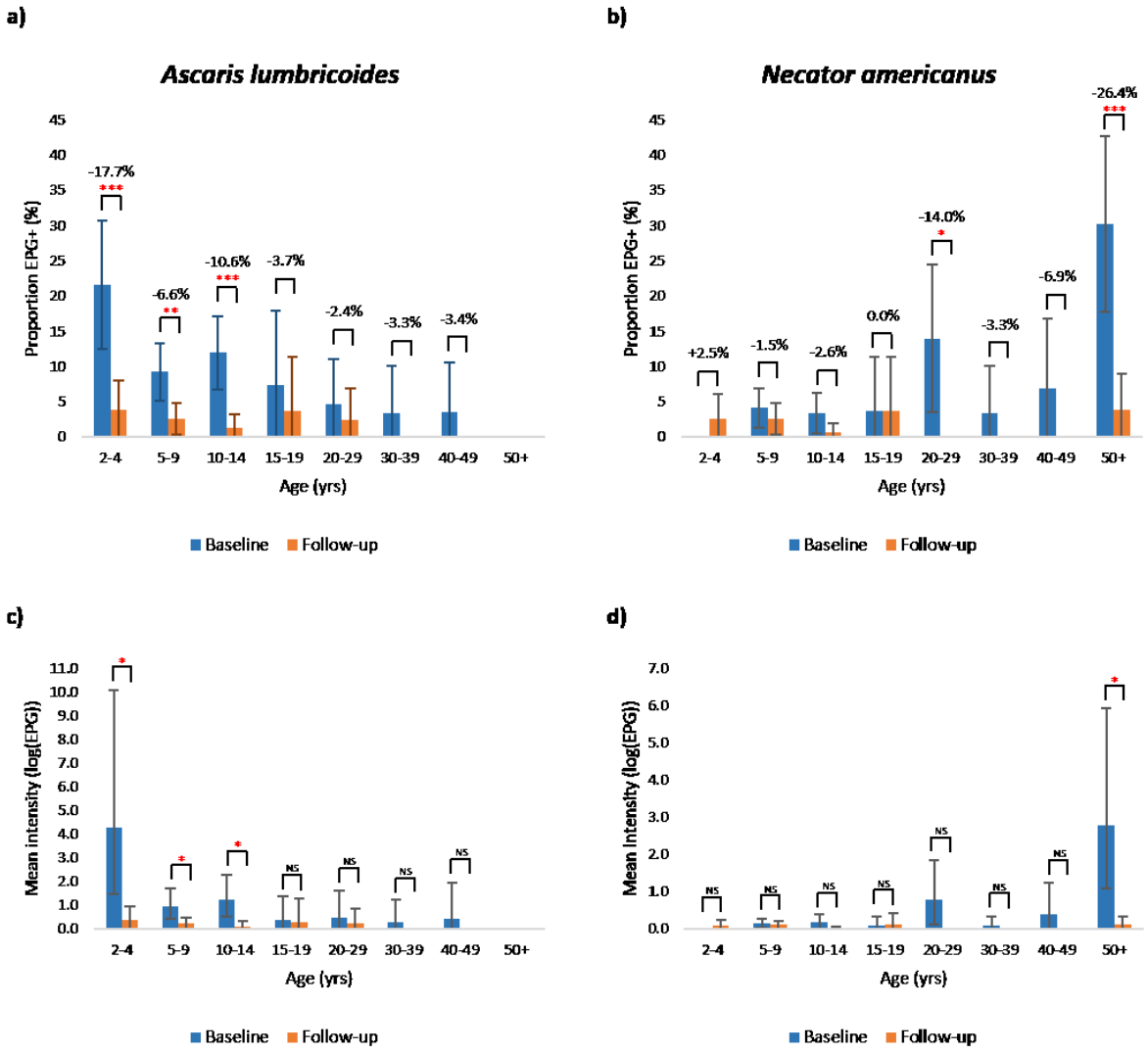


Figure 4.16. Age distribution of prevalence and intensity of *A. lumbricoides* and *N. americanus* infections at baseline and follow-up. These graphs refer to the study population successfully followed-up (n=607). Graphs **a)** and **b)** show the age distribution of prevalence of *A. lumbricoides* and *N. americanus* infections, respectively, at study baseline and at study follow-up (three months post-MDA). Graphs **c)** and **d)** show the age distribution of the geometrical mean intensity of *A. lumbricoides* and *N. americanus* infections, respectively, at study baseline and study follow-up. Vertical lines represent normal 95% confidence intervals and percentages above them show the change in prevalence between the two timepoints for each age group. Statistically significant changes in prevalence are signalled with red asterisks: *: p<0.05; **: p<0.01; ***: p<0.001; NS: not significant (p>0.05)

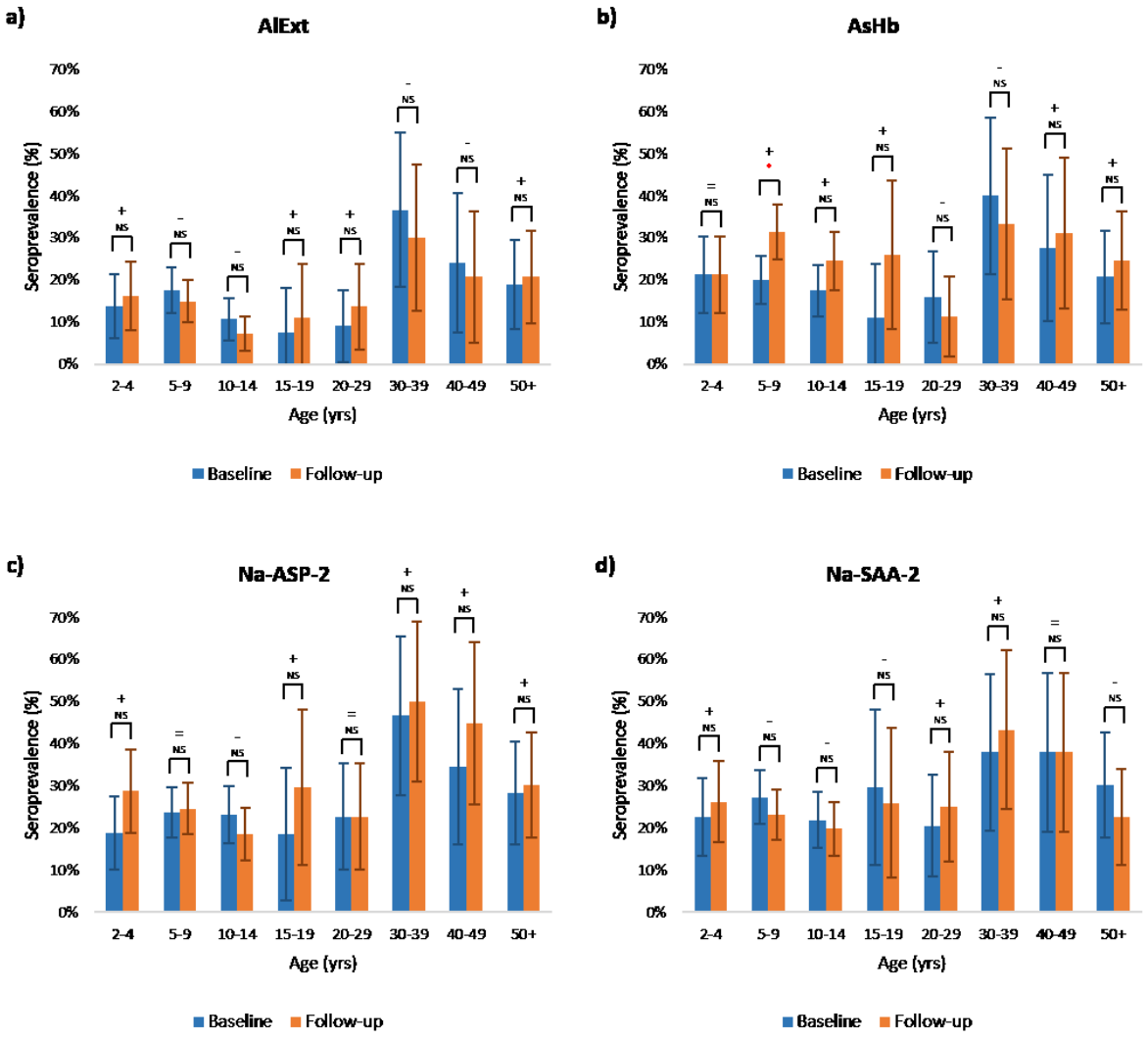


Figure 4.17. Age distribution of seroprevalence of anti-STH antibodies at baseline and follow-up. Graphs **a)**, **b)**, **c)** and **d)** show the age distribution of seroprevalence of IgG4 antibodies against AlExt, AsHb, Na-ASP-2 and Na-SAA-2, respectively, at study baseline and at study follow-up (three months post-MDA). Seroprevalence is defined as the proportion (%) with OD values above cut-off for each antigen. All four graphs refer to the study population successfully followed-up (n=607). Vertical lines represent normal 95% confidence intervals and signs above them show the direction of change in seroprevalence between the two timepoints for each age group: + increase; - decrease; = no change. Statistically significant changes are signalled with red asterisks: *: p<0.05; **: p<0.01; ***: p<0.001; NS: not significant (p>0.05)

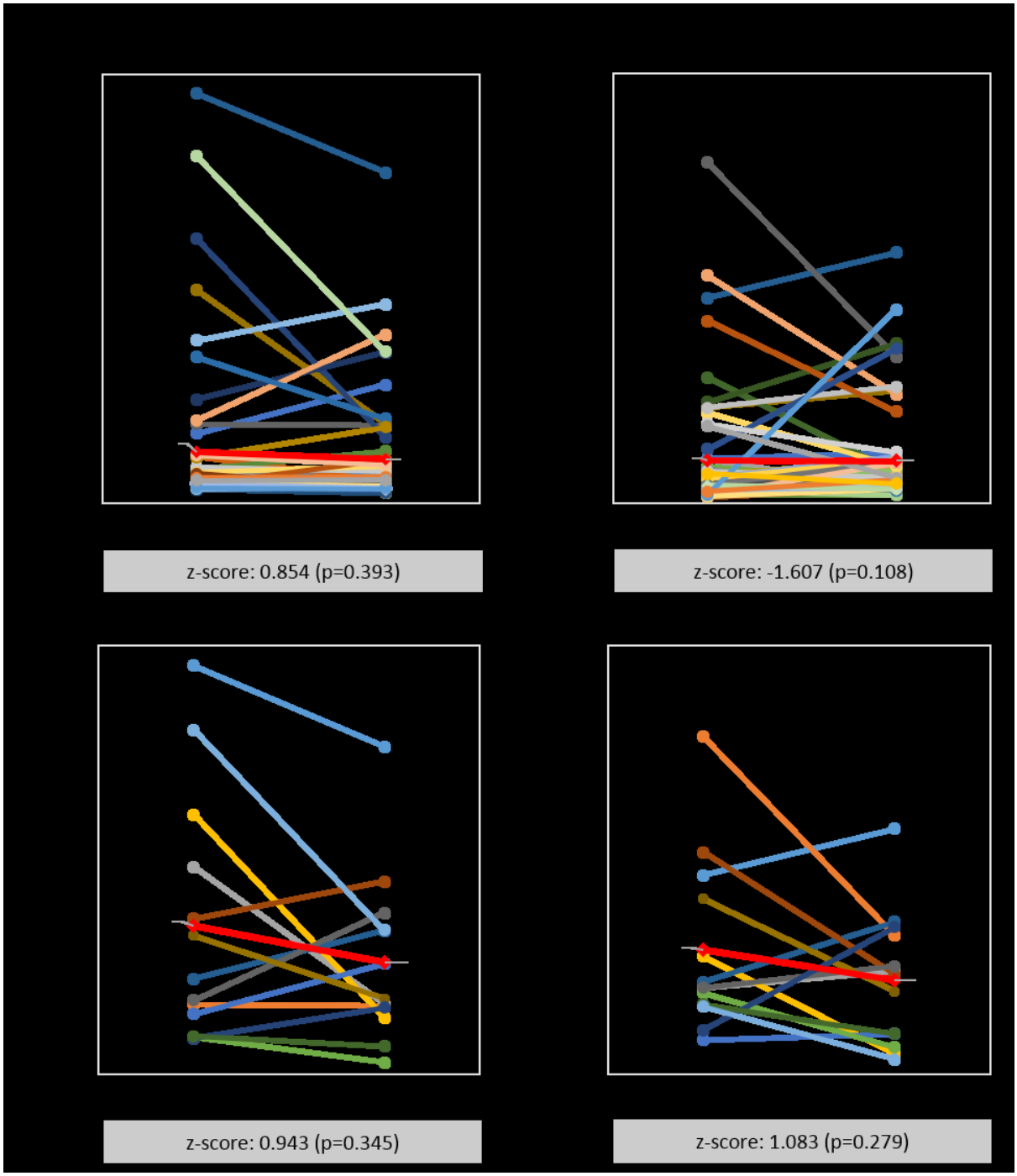


Figure 4.18. Individual change in anti-*Ascaris* antibody titres with deworming treatment. Graphs **a)** and **b)** show the individual change in anti-AlExt and anti-AsHb IgG4 antibody, respectively, for all individuals in the successfully followed-up population who were found to be infected with *A. lumbricoides* (EPG>0) at baseline (n=58). Graphs **c)** and **d)** show the individual change in anti-AlExt and anti-AsHb IgG4 antibody, respectively, for all individuals in the successfully followed-up population who were found to be infected with *A. lumbricoides* (EPG>0) and seropositive (OD above cut-off) at baseline (n=13). Each colour represents one individual, except the red dots and lines, which represent the average (arithmetic mean) change in OD between baseline and follow-up. The z-scores and p-values below the graphs were obtained using the Wilcoxon signed-rank test.

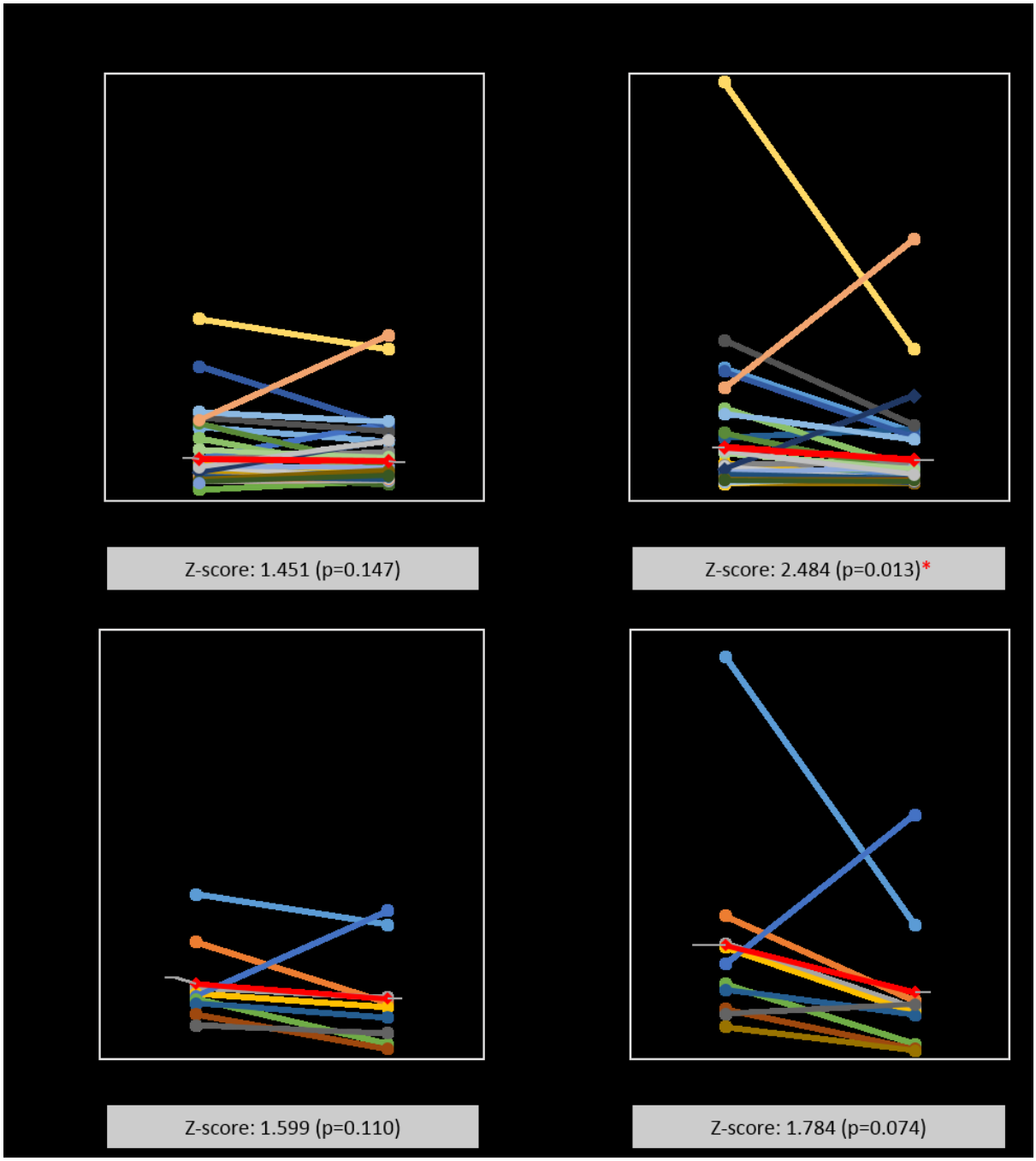


Figure 4.19. Individual change in anti-*Necator* antibody titres with deworming treatment. Graphs **a)** and **b)** show the individual change in anti-Na-ASP-2 and anti-Na-SAA-2 IgG4 antibody, respectively, for all individuals in the successfully followed-up population who were found to be infected with *N. americanus* (EPG>0) at baseline (n=39). Graphs **c)** and **d)** show the individual change in anti-Na-ASP-2 and anti-Na-SAA-2 IgG4 antibody, respectively, for all individuals in the successfully followed-up population who were found to be infected with *N. americanus* (EPG>0) and seropositive (OD above cut-off) at baseline (n=9 for Na-ASP-2 and 10 for Na-SAA-2). Each colour represents one individual, except the red dots and lines, which represent the average (arithmetic mean) change in OD between baseline and follow-up. The z-scores and p-values below the graphs were obtained using the Wilcoxon signed-rank test.

4.5. Discussion

This study took place in an area of western Kenya endemic for both *A. lumbricoides* and *N. americanus*, where prevalence of STH infections was historically high but has been reduced in recent years likely due to a successful annual SBD programme, ongoing since 2012 (see Chapter 3). A limited number of immuno-epidemiology studies of STH infections have been carried out in populations receiving periodic anthelminthic treatment, thus this study aimed to investigate the seroprevalence profiles encountered in such a population, as well as the correlations between intensity of STH infections and antibody seroprevalence and changes in antibody titres following one round of MDA. The main findings of this study are as follows.

The age-profiles of *A. lumbricoides* prevalence and intensity of infection were not correlated with *Ascaris* seroprevalence profiles, but there was a correlation between the age-prevalence profile of *N. americanus* and the Na-ASP-2 seroprevalence profile.

AS previously seen in Chapters 2 and 3, there was a very strong distinction between the prevalence and intensity profiles of *A. lumbricoides* and *N. americanus*, with the younger age groups having a significantly heavier burden of *A. lumbricoides* infection and the older age groups having a significantly heavier burden of *N. americanus* infection. The seroprevalence profiles, however, did not reflect these differences. While the seroprevalence profiles obtained for antibodies against the two *Ascaris* antigens were fairly distinct from the prevalence and intensity profiles for *A. lumbricoides* infection, the profiles obtained for *N. americanus* were more similar. In fact, the seroprevalence profile of Na-ASP-2 antibodies was significantly positively correlated with the *N. americanus* prevalence profile.

One possible explanation for these differences would be that seropositivity increases with recent exposure to STH infection, being associated with intensity of infection. In other words, it may be more reflective of recent infection (e.g. over the last six months) than all past exposure over longer periods. Increased exposure to hookworm infection in adulthood – which has been shown as a strong correlation between *N. americanus* infection and adult farmers in Chapter 1 – may lead to the observed increase in *Necator* antibody titres with age. The small peak observed in the group 5 to 9 years old in the *Ascaris* seroprevalence profiles can also be explained by an increased exposure to *A. lumbricoides* infection in this age group, while the low seroprevalence levels of pre-SAC can be explained by their young age and the time required to mount specific antibody responses, particularly IgG4 (36).

Another possible explanation would be that seropositivity, at least partially, protects from reinfection. In fact, adults, who have presumably been repeatedly exposed to *A. lumbricoides* during their lifetime, were found to have much higher titres of both anti-AsHb and anti-AlExt antibodies than children and young adults, and this might provide a level of protection against the establishment of new infections – thus explaining their low prevalence and intensity levels. It would also alternatively explain why pre-SAC, who had the lowest seroprevalence of *Ascaris* antibodies, have the heaviest burden of *A. lumbricoides*. While the antibody titres against *N. americanus* in adults are also high, they might be counterbalanced by an increased exposure to infection, which allows more larvae to escape the immune response and establish as adults, overcoming the protective effect of antibodies.

No correlations were found between infection status and antibody response.

Unlike what was found in a study in India in 1989 (37), no strong positive correlations were observed between individual burden of *A. lumbricoides* infection and antibody titres. However, the observed prevalence of *A. lumbricoides* and *N. americanus* infections was much lower in the current study than in India, which might have reduced the power of these analyses. Still, a positive trendline was observed between *A. lumbricoides* EPG and anti-AlExt antibodies, which was mostly associated with SAC and male participants (NB infected male participants were all younger than 20 years old), but this correlation was not significant (Kendall's tau: 0.06, p=0.399). Moreover, while previous studies have argued that specific IgG4 could be used for immunodiagnostic of hookworm infection due to a positive correlation with intensity of infection (22,23), the present study found only a small significant positive correlation between individual *N. americanus* EPG and anti-Na-SAA-2 antibody titres (Kendall's tau: 0.151, p=0.048), but no other significant correlations in the study community.

One hypothesis for the absence of correlations is that not all current infections were detected (i.e. missing EPGs). This might have been due to IgG4 being detected during prepatent infections, when antibodies were being produced only in response to larval migration, and/or because the Kato-Katz smear used in parasitological diagnosis did not detect all current infections due to low sensitivity. In fact, both *Necator* antigens used were purified L3 larvae surface antigens (28,29), anti-AsHb antibodies have previously been detected in pigs during lung passage of larvae (Prof. Peter Geldhof, personal communication) and some of the proteins present in the adult worm PBS extract must surely be present in *A. lumbricoides* larval stages too. The low sensitivity of Kato-Katz has been discussed in the previous chapters, and in a previous publication (38). There is also no individual data available on past infections, so there is a possibility that antibodies present are a remnant of cured infections.

One other possible explanation for the absence of correlations is immunomodulation, either by the parasite to subdue the production of antibodies and, thus, increase the number of egg-producing adult worms, or by self-regulation of the immune system, which limits the production of antibodies (and other immune responses) in order to avoid overactivation, especially when large numbers of parasites are established. Several studies have revealed the existence of these immunomodulatory and immunoregulatory events (39,40).

There was a trend towards increasing *Ascaris* seropositivity and decreasing *A. lumbricoides* intensity of infection with increasing age, and increasing *N. americanus* seropositivity and intensity of infection with age.

Antibody responses to both infections generally increased with age, regardless of which age groups carried heavier burdens of infection. In hookworm infection, both mean EPG and mean antibody titres increased with age, while mean *A. lumbricoides* EPG showed a negative association with age and mean antibody titres, especially in the case of anti-AsHb antibodies. Three hypotheses might explain these observations.

A first hypothesis is that there are differences in exposure to each parasite species, which affect the development of equally effective antibodies. An increased exposure to *Ascaris* antigens from early childhood might lead to the development of a strong, protective antibody response in adulthood, shown by the high antibody titres and very low intensity of infection in the eldest age groups. An increased exposure to *Necator* antigens starting only in adulthood might lead to a delay in the development of protective antibody responses, which are not as effective in the older age groups – thus the high prevalence and intensity levels of infection in adults.

A second hypothesis is that exposure to both parasites is equal for individuals of all age groups, but antibody responses mounted against *A. lumbricoides* are only effective in protecting from reinfection in older age, while antibodies developed against *N. americanus* are overall less protective than those against *Ascaris*. While it is unlikely that high-affinity IgG4 antibodies, which vary only on the epitope they recognise, have such different effectiveness, it is possible that *Ascaris* and *Necator* possess different mechanisms of immunomodulation and evasion, which render IgG4 antibodies less effective against hookworm infection.

A final hypothesis is that seropositivity is in fact correlated with infection status, and the high anti-AsHb antibody titres observed in the adult age groups are really an effect of cross-reactivity with hookworm antigens. This hypothesis is supported by the next finding of the study.

There is evidence of cross-reactivity between the four antigens tested.

Despite having very low intensity of infection, 5 to 9 year old children were found to have relatively high antibody titres against *N. americanus* antigens, while adults had the highest seroprevalence of *Ascaris* antibodies despite having the lowest intensity. These disparities might reflect past infection, but also raise the question of cross-reactivity due to exposure to similar antigens of other parasites. Both hypotheses are probably correct, and an accurate distinction cannot be made using the data available.

Cross-reactivity was previously observed in a study carried out in a co-endemic area, where hookworm co-infected individuals with a combination of *A. lumbricoides*, *S. mansoni* or both had higher seroprevalence levels of anti-hookworm IgG4 than hookworm mono-infected individuals (41). It was also identified in an analysis of IgG4 as a possible marker for serodiagnosis of *A. lumbricoides*, where excretory/secretory antigens cross-reacted with hookworm and other helminth infections (42). The existence of cross-reactivity was further confirmed in a study where orthologue genes for AsHb were found in *N. americanus*, *Ancylostoma ceylanicum* and *Toxocara canis* (6). This led to samples from hookworm mono-infected individuals showing the presence of anti-AsHb antibodies, which might have happened to some extent in this study. A previous analysis of cross-reactivity between *A. lumbricoides* and *N. americanus* had also obtained results similar to be the ones obtained in the current study (43).

The fact that practically all the antigens were found to be significantly correlated with each other provides a hint that there might be significant cross-reactivity between them. AsHb is expected to be a major constituent of AlExt, so a strong correlation between these antigens was to be expected (27). The same could be expected of the two *Necator* antigens, since they are both derived from *N. americanus* L3-stage surface antigens (28,29). Besides, a blood sample containing antibodies against one antigen derived from one worm species should in principle contain antibodies against more antigens of that same species. However, the strong correlations observed between AlExt and the *Necator* antigens suggest the existence of antigens similar to Na-ASP-2 and Na-SAA-2 in AlExt, which is a broader, unspecific antigen mix. The weaker but still significant correlation between AsHb and Na-ASP-2 might also be an indication of the existence of similar antigens between the two species, though it shows that these antigens elicit more specific antibody responses than AlExt.

It is also possible that these correlations reflect instead a substantial level of past or prepatent co-infections. All individuals in this study are in principle exposed to infection with both worm species, and in fact the seropositive results found in the study are likely to reflect a mixture of prepatent infections, current patent infections and recently cleared infections. The data available is not sufficient

to distinguish between these stages, and since IgG4 antibodies have a relatively short half-life (36), seronegativity also cannot exclude the possibility of past infections.

No significant changes in seroprevalence were found three months after community-wide deworming treatment.

The community-wide administration of albendazole following the study baseline had a major impact in reducing the prevalence and intensity of both infections in the study population, but no significant changes were observed in the seroprevalence profiles. Individual antibody titres generally decreased between the two timepoints, but only the average anti-Na-SAA-2 antibody titre at population-level was significant reduced. Several studies have mentioned a decrease in IgG4 seroprevalence levels following anthelminthic treatment, as well as strong correlations between seroprevalence and infection status (6,20–23). It was thus expected that IgG4 antibody levels would decrease together with prevalence and intensity of STH infection post-treatment, but our results did not fully support this.

There are two possible explanations for this lack of significant change in seroprevalence between the two study timepoints. The first is because, even in the absence of reinfection, IgG4 antibodies can remain in circulation for up to six months, according to a study in West Bengal, India (12). It is possible that the period between the two study timepoints was not sufficient for a significant reduction in circulating antibody levels to have occurred. However, antibodies in general have very short half-lives, and IgG4 is thought to have the longest at 21 days, which still falls short of three months (36). As cells were removed during the separation of plasma samples, life-long immunity cannot account for the seroprevalence measured by ELISA, which only takes into account circulating antibodies.

A more likely explanation would be that exposure to infection remains constant after treatment, and thus antibody stimulation due to reinfection will likely continue during this period. The IgG4 antibody isotype is only produced after chronic and repeated exposure to an antigen (44), so it is likely that individuals who had previously produced this antibody isotype at study baseline would produce it again following re-exposure. The seropositivity detected three months after deworming might be due to 1) larval passage or prepatent infection, as well as male-only infections, and thus not detectable by KK, or 2) it might be due to very light intensity infections, which could possibly be misdiagnosed as false-negative by KK. The follow-up study was carried out three months post-treatment, a period during which reinfection could have taken place at any time, and which would allow the maturation of these newly acquired infections, since both *A. lumbricoides* and *N. americanus* worms mature over a period of two months (45,46). It is also possible that not all infecting larvae present at time of

treatment were eliminated by the albendazole treatment, and may have completed maturation soon after. Albendazole has been shown to be active against immature stages of helminths in sheep and other animals (47), but no information is available on albendazole activity against larvae of *A. lumbricoides* or *N. americanus* in human infection.

There was similarly low seropositivity in infected individuals for all antigens tested.

The proportions of the study population who were found to be infected or uninfected, and seropositive or seronegative, were very similar to the proportions found in a study of *A. lumbricoides* infection in Indonesia, two years into a community-based MDA of albendazole (6). It is important to note that the proportion of infected individiduals who were seronegative was larger than the proportion of infected and seropositive individuals, a tendency which, in the study in Indonesia, increased greatly after the end of the MDA programme. Interestingly, the proportions found were similar for all antigens tested, which might again point towards cross-reactivity between the two species or, otherwise, to a commonality in the dynamics of different STH infections. Furthermore, in a separate analysis, almost all individuals found to be co-infected with *A. lumbricoides* and *N. americanus* were found to be seronegative for the four antigens.

Repeated deworming treatment reduces the time of exposure to adult worms by eliminating long-term chronic infections, as well as indirectly reducing the exposure to larvae by eliminating egg-producing adults and, thus, environmental contamination and transmission of infection. Since IgG4 class-switching occurs only following prolonged exposure to a specific antigen, it is possible that MDA programmes increase IgG4 seronegativity in the whole population, through a reduced exposure to the pathogens. In terms of co-infections, it is possible that co-infecting helminth species have a synergistic effect on immunomodulation, in such a way that IgG4 antibody titres become negative. In a study in Brazil, hookworm and *A. lumbricoides* infections were found to act synergistically, and individuals with heavy hookworm infection were more likely to have heavy *Ascaris* infection (48). Another study found lower cell proliferation in individuals co-infected with hookworm and *Schistosoma mansoni*, compared with those with hookworm mono-infection (49).

Study limitations and further work

Taking into consideration the great complexity of the immune system, the major limitation of this study is the fact that only one antibody isotype was analysed. The analysis of other antibody isotypes, namely IgE and other gamma immunoglobulins, as well as the analysis of cytokines and cells of the

immune system, would enable a wider range of observations. This would, however, require larger blood sample volumes to be collected, which would require venepuncture and the recruitment of nurses to collect these samples, a protocol not covered by available funds or ethical clearance in this study. Venous blood collection would also likely reduce the size of the study population, by reducing the number of both children and adults willing to cooperate. Finally, the analysis of cytokines and blood cells would require more complex storage and processing methods, as well as access to a well-equipped laboratory in the field.

The three-month period between the two timepoints of this study was determined by the practical aspects of fieldwork, but different timepoints would enable more interesting evaluations. Serological data from the endemic equilibrium state, before the launch of the National SBD Programme, would have provided "true" baseline seroprevalence data for the study population. Collection of serological samples one month following anthelmintic treatment would have reduced the reinfection period, enabling a stronger analysis of the deworming treatment on seroprevalence, as IgG4 antibody titres in cured and non-reinfected individuals would be expected to decrease approximately by half (36). Conversely, samples collected six months post-treatment (instead of three) could have provided a stronger measure of reinfection, since during this period a larger proportion of the study population would likely become reinfected, increasing the power of the analyses.

A final limitation was the lack of control samples for the ELISAs. No positive controls were used to test three of the four antigens in the ELISAs, and thus it was not possible to obtain standard curves and determine the actual concentration of antibody in each sample, limiting the ability to compare results with other studies. This did not, however, limit the within-study, between-individuals and between-timepoints comparisons reported here. The negative controls used were obtained from European volunteers, who were seronegative for malaria antigens but not previously tested for STH antigens, thought they were found to be seronegative in preliminary ELISA tests. Ideally, samples from seronegative and seropositive individuals of different ages and residing in the study area should have been used as negative and positive controls, respectively. The seronegativity and seropositivity of each control sample could be validated using the western blot technique, which would have confirmed the presence or absence of antibodies against the specific antigens tested in the control plasma samples (50). Using control samples obtained from study participants would also have provided stronger, more accurate cut-offs for each antigen.

Conclusions

This study was conducted to investigate the seroprevalence profiles of *Ascaris* and *Necator* IgG4 antibodies in a population where children had been repeatedly dewormed since 2012 through the Kenyan National SBD Programme. In communities targeted by deworming campaigns, it is vital to understand the dynamics of immune responses to STH, particularly those associated with deworming and reinfection. A significant positive correlation was found between the hookworm infection and seroprevalence age-profiles at study baseline, but no correlations were found between *Ascaris* infection and antibody titres. Contrary to findings from previous studies, no correlations were found between individual antibody titres and intensity of infection. However, significant correlations were found between individual titres of almost all antigens, which, taking into account the findings of previous immuno-epidemiological studies, is a strong indication of considerable cross-reactivity between the two species. IgG4 antibody titres against all four antigens tested were generally low in in the whole population, including the majority of individuals found to be infected. Finally, despite very significant reductions in prevalence and intensity of STH infection, and a general decrease in population seropositivity, no significant changes in seroprevalence were observed three months post-MDA.

As the prevalence of STH infection goes down with time, it becomes crucial to accurately measure the impact of control measures. Kato-Katz is known for its suboptimal sensitivity, especially when measuring hookworm infection, thus other methods are required. Immunoassays, such as indirect ELISA measuring anti-helminth antibodies might be an alternative, if the antibodies measured are short-lived and the antigens tested are species-specific. In low-prevalence communities, antibody titres could provide a measure of recent infection and thus help to identify continued transmission. Cross-reactivity remains an issue, and distinguishing between past, prepatent and current infections will likely remain difficult. However, at population-level, immunoassays may provide a much-needed overview of the levels of exposure, and thus transmission of infection in the population, with age seroprevalence profiles reflecting the true impact of deworming control programmes.

4.6. References

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CHAPTER 5. DISCUSSION

This thesis presents an epidemiological study of soil-transmitted helminths (STH) in a community in Bungoma, Kenya, which had been targeted by the national school-based deworming (SBD) programme for two years prior to the start of this project. A particular focus of the project was immuno-epidemiological studies, in an attempt to detect markers of recent and past infection within individuals in a low transmission setting. This research provides insights into the impact of a transition from SBD into community-wide deworming strategies and the use of enzyme-linked immunosorbent assays (ELISA) as diagnostic tools for the so-called 'endgame' of trying to eliminate STH transmission. The thesis reports the results of fieldwork, laboratory studies and statistical analyses. This discussion provides an overview of the findings and their limitations, and outlines possible directions for future research.

5.1. Summary of the research findings

Chapter 2 reports the basic epidemiological findings on the distribution of infection in the communities studied. The prevalence and intensity of STH infections in the study area was quite low, with only 7.4% and 6.4% prevalence of *A. lumbricoides* and *N. americanus* infections, respectively, found in the 1,464 individuals tested at study baseline. The age-prevalence and age-intensity profiles observed for *A. lumbricoides* and *N. americanus* infections were very distinct and consistent with the ones reported in many previous studies. Both prevalence and intensity of *Ascaris* infection peaked in preschool age children (pre-SAC) and decreased as individuals aged, while prevalence and intensity of hookworm infection increased with age to a peak in the older age groups. Besides age classes, infection was heterogeneously distributed in the population, with village and household clusters, as well as individual aggregation. Significant associations were found between STH infections and demographic, socioeconomic and WASH risk factors. *Ascaris* infection was significantly associated with young age (pre-SAC and SAC), residing in one of the villages (Siangwe) and poor sanitation (not owning a pit latrine or bad latrine conditions). Hookworm infection was significantly associated with old age (50+ years), farming and poverty-associated factors, as well as the type of latrine owned. A quarter of the study population was found to be anaemic (26.9%), with higher prevalence of moderate and severe anaemia in pre-SAC and adult women. However, no significant associations were found between anaemia and STH infection.

Chapter 3 documents the impact of chemotherapy on an individual basis, and describes coverage and compliance to treatment, as well as drug efficacy. Only 763 individuals who participated in the study baseline (52.1%) participated for a second time in the study follow-up. Adults of working age were

particularly non-compliant with the study (34.0% of the population lost-to-follow-up), and non-complying individuals had significantly lower prevalence and intensity of *Ascaris* infection (4.7% vs. 9.8% prevalence in followed-up population, $p<0.001$). Treatment coverage was above 75% in all age groups, but there were difficulties defining treatment coverage and recording compliance to treatment. The egg reduction rates (ERRs) obtained for *A. lumbricoides* and *N. americanus* were both above the World Health Organization (WHO) recommended thresholds (95% for *A. lumbricoides* and 90% for hookworms), thus the efficacy of single dose 400mg albendazole tablets was considered satisfactory. Significant post-treatment reductions in prevalence and intensity of infection in pre-SAC (*Ascaris*) and adults (hookworm) strongly supported the need to expand deworming strategies to age classes beyond SAC.

Chapter 4 reports the results of the immuno-epidemiological studies. The age-profiles of seroprevalence obtained for antibodies against the two *Ascaris* antigens were fairly distinct from the infection prevalence and intensity profiles for *A. lumbricoides* infection. The profiles obtained for *N. americanus* prevalence and seroprevalence were more similar, and there was a significant positive correlation between the age-prevalence of *N. americanus* eggs per gram (EPG) of stool and the age-seroprevalence of anti-Na-ASP-2 antibodies ($p=0.035$). No significant correlations were found between infection status or EPG and antibody titres. The majority of the study participants, both infected and uninfected, were found to be seronegative for all antibodies – there was a negative binomial distribution of antibody titres, as well as of intensity of infection. Most co-infected individuals were also found to be seronegative for all antibodies. The mean intensity of *N. americanus* infection and the mean antibody titres of *Necator* antigens were found to both increase with age, while the mean antibody titres of *Ascaris* antigens increased and the mean intensity of *A. lumbricoides* infection decreased with age. No significant changes in age-seroprofiles were observed three months post-treatment, but cured individuals generally had a decrease in antibody titres. Positive correlations between different antibodies indicate cross-reactivity between species. This was particularly apparent between the *Ascaris* extract antigen and the *Necator* antigens.

5.2. Limitations

This study had some limitations common to the results reported in all chapters. The first was the time constraints imposed by logistical issues and the constraints of a PhD study time frame. The first cross-sectional study was originally intended to be carried out approximately six months after the national SBD programme round of June 2013. However, delays in obtaining ethical approval and an unexpected deworming campaign carried out in Bungoma by an unidentified organization in November 2013

resulted in the first survey being delayed until March 2014 and taking place only four months after an uncertain proportion of the study population had been dewormed. This delay in turn resulted in the need to expedite the second cross-sectional survey, so that it would take place immediately before the national SBD programme in September 2014. This resulted in a reinfection interval of only three to four months after the study's community-wide MDA round, instead of the planned six months. The shorter periods between deworming rounds resulted in infection prevalence and intensity levels below those expected, which made some analyses impossible, including those of predisposition to light versus heavy infection. Longer reinfection periods could also possibly have enabled the detection of significant changes in seroprevalence of STH-specific antibodies following the study's MDA. This study would have been improved by access to up-to-date information about the public health services being provided in the study site (especially past compliance to drug treatment for STH infection), and by better communication between the various organizations providing public health services in the field. A detailed individual survey of previous treatments could have been carried out to identify the persons treated in November 2013, though these surveys can be problematic due to recollection bias, including a possible misidentification of tablets taken.

The second limitation was the structure of the final study population. While the study enrolment made at household level represented an accurate ratio of children to adults and male to female participants, the low level of participation in the study (only 41.5% of those recruited), particularly of adult male individuals, led to a distinct final population structure where adults were undersampled. This pattern is not atypical in past reported studies of STH infection in many regions of the world (1,2). The high proportion of participants lost to follow-up (47.9%) further reduced the power of analyses of reinfection and predisposition, as well as those of immunological responses to past and present infection. The fact that the households located at the most northern areas of Nasimbo village were the furthest away from the meeting points in the first cross-sectional survey might have led to the low participation levels observed in this village, as well as to an oversampling of children in this same village, who were still reached through the school visits. Efforts were made to increase study participation and compliance, including increasing the number of meeting points from the first to the second survey, but adults, particularly males, could not always be reached using our sampling strategies due to working away from the family home for most of the day.

A third limitation was the use of Kato-Katz as the parasitological diagnostic method for the presence or absence of STH infection. This method was used due to its simplicity and speed of use, the fact that technicians were already experienced in this technique (no training required), and because this method is still the most widely used to assess the prevalence and infection intensity of STH, as currently recommended by the WHO. It is, however, known to have low sensitivity, particularly at low

infection intensities (3,4). We aimed to increase the sensitivity by collecting two stool samples from each individual, and preparing two Kato-Katz slides from each sample, so that most study participants had four readings. In addition, the Kato-Katz technique presents further limitations for quantifying hookworm infections, as the eggs degrade rapidly after staining, with the slide needing to be read within one hour. Attempts were made to process samples rapidly to minimise this, but hookworm infection intensity and prevalence may have been higher than reported. The same timelines and protocols were used at all stages of the study, minimising this limitations effect on ERR measurements. Some children provided samples both at school and at the village meeting points (totalling up to four samples for some individuals), and some study participants only provided one stool sample. Due to these discrepancies, individual EPG counts were calculated from an average of two to an average of eight Kato-Katz slides, which might have biased the results since worm eggs were less likely to be missed in individuals who provided more samples, affecting both prevalence and geometric mean infection intensity measures (5). EPG counts are characterised by a high variance (negative binomial distributed), so logarithmic counts were used in parametric statistical procedures and arithmetic plus geometric means were used where appropriate.

In terms of the findings of Chapter 2, the analysis of risk factors could have been enhanced if the WASH survey had been conducted individually instead of at household level. Otherwise, more thorough individual and household WASH surveys could have been conducted, which would enable stronger analyses of risk factors at both individual and household level, as these practices might vary greatly both within and between households, potentially differing between ages, sexes, occupations and other variables. The analysis of STH-associated morbidity was quite limited, as no other data besides haemoglobin measurements were used. The division of the population into five quintiles of socio-economic status (SES) might have reduced the power of the analysis of SES as a risk factor, as the population was quite homogeneous in socio-economics terms. While the “poorest” and “least poor” SES groups were fairly different in terms of the variables used in the PC analysis (see Appendix III), the three middle groups were quite similar. The fact that the division into SES groups was made at household level but then adapted to individuals might have further diluted any significant observations that could be made regarding SES as a risk factor for STH infection.

The main limitations in Chapter 3 were those associated with recording treatment coverage and compliance, a problem common to many epidemiological studies of STH infections (6). While the treatment record for all individuals found to be infected in the first cross-sectional survey was accurate (as they still participated in the worm expulsion and treatment efficacy studies immediately afterwards), the records obtained for the non-infected study participants and non-participant recruited individuals were problematic. Despite the fact that all Community Health Workers (CHWs)

were trained to observe and record only the ingestion of the deworming tablets, the treatment records included a large number of households where the drugs were delivered to one family member, to be ingested later by the entire family. It is uncertain whether those houses were dewormed, and if so whether all the individuals received a tablet. In addition, individuals who were infected might have received two doses of treatment, as most CHWs recorded also treating them during the MDA. Improved training and supervision, including demonstrations of the correct procedures during the first day of treatment, could possibly have improved the quality of MDA treatment records. No treatment records were made following the second cross-sectional survey, and thus there is no longitudinal data of compliance to treatment.

A limitation of the findings reported in Chapter 4 was the small number of blood samples obtained, particularly from adult men, which prevented some analyses of seroprevalence between sexes from being carried out. More importantly, only one antibody isotype was tested, which limits the full understanding of immunological responses to STH infections. The original concept was to quantify a range of antibody isotypes, which included IgE, pan-IgG and IgM, against specific larval and adult worm antigens from both *A. lumbricoides* and *N. americanus*. Difficulties in obtaining antigens, which resulted in further delays, and the small amount of blood collected from each study participant dictated the need to reduce the number of analyses performed. The ELISA technique had a recurrent procedural error, in which the border wells of the test plates were not suitably washed. This led to some results having to be excluded from the analyses and some blood samples being reanalysed, but it should not have an impact on the results obtained. The lack of confirmed negative controls, and the use of only one positive control (for the AsHb antigen), prevented the use of standard curves and determination of antibody concentration in each sample.

5.3. Implications of findings in the current context of STH control

This study identified a series of factors which may be sustaining STH infections in the study area, as well as in other endemic areas and countries where SBD is implemented as an STH infection control measure. The analysis of risk factors identified strong correlations between young age and *Ascaris* infection, as well as old age and hookworm infection. This difference in the age-distribution of the two STH infections has been consistently reported in past studies (7,8), and seems to become even more divergent in populations subjected to SBD programmes. In countries where SBD control programmes are implemented to control STH infections, monitoring and evaluation (M&E) surveys, particularly those carried out towards the end of the programmes, will need to include pre-SAC and adults in order to correctly identify the most prevalent STH species and possible remaining clusters of infection. This

study found clusters of *Ascaris,* but not of hookworm infection, at school, village and household level. Depending on the most prevalent species, it may be beneficial to adapt treatment strategies to target the individuals more likely to be sustaining transmission of infection in defined locations (selective chemotherapy). Should hookworm be found to the most prevalent species, community-wide MDA, such as the round provided during this study, should have a more significant impact in reducing prevalence and intensity of infection than SBD, as it will also reach the older age classes carrying the heaviest burden of hookworm infection. On the other hand, should *A. lumbricoides* be the most prevalent species, not only should SBD be expanded to include pre-SAC (if not already included), but identifying the individuals with heavier burden of infection and providing treatment to all of their household members, might have a very significant impact in reducing transmission of infection (9). While it was not possible to identify individual predisposition to infection in this study, the household clusters of *Ascaris* infection observed seem to indicate that some families might be predisposed to infection, and thus providing treatment to all these household residents might have a significant impact in reducing transmission of *Ascaris* infection in the long-term. Expanding treatment to adults and/or pre-SAC can be achieved in a cost-effective way by integrating albendazole MDA for STH with control programmes for other NTDs (such as LF), vaccination or distribution of vitamin A. The Tanzanian government has successfully delivered an integrated lymphatic filariasis MDA programme with the measles rubella immunization campaign in 2014, in a country endemic for five NTDs (10), and the WHO recommends distributing deworming together with vitamin A supplementation (11). Reaching adults, particularly men of working age who might work away from the homestead during the day, may possibly be accomplished by having more than one day of MDA in each village, with drug delivery being made outside of working hours and/or door-to-door or at social gathering and work sites. Advertising MDA campaigns through the radio and text messages would work particularly well in Bungoma, since the large majority of the households owned a radio and a mobile phone.

In this study, as well as in previously reported epidemiological studies of STH infection, poor sanitation was found to be positively associated with both *Ascaris* and hookworm infections. Good quality sanitation facilities, which include the construction of pit latrines in houses which don't own one, and upgrading unimproved to improved pit latrines (following WHO/UNICEF Joint Monitoring Programme for Water Supply and Sanitation (JMP) sanitation ladder standards), which in turn need to be maintained clean and in good condition, should help reduce the transmission of both *Ascaris* and hookworm infections (12). However, building a new latrine is an expensive endeavour for a family in rural Bungoma, as well as in most areas worldwide where STH infections are endemic. Water, sanitation and hygiene (WASH) projects should be a priority in areas where STH infections are endemic, which the local government should invest in for the long-term benefit of the community, as

it would also reduce prevalence of other infectious diseases such as schistosomiasis and diarrhoea. A number of NGOs are currently involved in collaborations with endemic countries, sponsoring and researching new ways of improving WASH conditions in deprived areas (13). These projects need to be affordable, popular and sustainable, made in consultation with the communities to assess their needs, maximise their utility, and ensure the maintenance of all infrastructure built. Maintaining clean facilities has been shown to be an important factor in sustaining the impact of WASH (14). Moreover, socio-economic conditions have long been known to indirectly increase the risk of STH infections (15,16), and this study has also found significant associations between hookworm infection and poverty-related factors. Microfinancing enterprises such as One Acre Fund (headquartered in Bungoma) can help alleviate poverty for families living in rural areas, where a small loan of cash or seeds can help improve their income. Farming in this study was also found to be significantly associated with hookworm infection, and it is the main occupation of most adults living in the area. Hookworm larvae present in contaminated soil can easily infect farmers working with bare hands and bare feet or wearing sandals. Health education campaigns targeted at adults, explaining not only the benefits of wearing gloves and boots when farming but also the importance of sanitation, hand-washing, and proper cleaning and cooking of vegetables, could have a significant impact in reducing transmission of hookworm infection. This behaviour change would be more successful if gloves and boots were provided or loaned separately or in conjunction with other agricultural microfinance loans.

The very low prevalence and intensity of STH infections found in this study, as well as those found in other areas where MDA programmes have been sustained for a number of years, showcase the success of preventive chemotherapy programmes but also the importance of M&E of these programmes. While albendazole was found to be satisfactorily effective against both *Ascaris* and hookworm infections in this setting, the worldwide use of this drug requires monitoring of its efficacy to ensure drug resistance does not become a problem (17–19). Accurate records of treatment coverage and, more importantly, of adherence and compliance to treatment are vital when many years of coverage are planned. Drug resistance may develop in a population when the drug pressure is high but long-term compliance is low. However, at present little is understood about the factors that might induce resistance over and above natural genetic variability in parasites that recombine via sexual reproduction and the strong selective pressure provided by treatment. Longitudinal records of individual adherence and compliance to treatment, where these are defined as directly observed ingestion of the tablets, should be preferred by M&E teams to accurately represent the drug pressure and quickly identify spots of resistance. On the same subject, communication between agents providing related public health services in-country needs to be improved. In order for M&E surveys to accurately report an STH control programme's direct impact, other sources of deworming need to be

accounted for, whether it is provision of deworming in health centres, self-treatment from drug dispensaries and particularly organizations providing deworming campaigns outside of government-based treatment programmes. Better communication to avoid providing overlapping services would prevent the waste of time and resources, as well as improve the accuracy of the programme outcomes measured.

Better, more sensitive diagnostic techniques which can be used quickly and cheaply in the field are also required to improve detection, not only of low intensity infections but also of hookworm, which is currently problematic using Kato-Katz. Very low prevalence and intensity infections were recorded in this study using Kato-Katz, but another study in the same population using both qPCR and Kato-Katz found a much larger prevalence of hookworm infection using qPCR than Kato-Katz (18.3% vs. 7.2%) (20). However, qPCR cannot provide quick results in the field, mainly due to the equipment required, and is more expensive than Kato-Katz (3,21). A rapid diagnostic test such as the point-of-care circulating cathodic-antigen test (POC-CCA) for *Schistosoma mansoni* (22) would be a great improvement, given all the three major STH species have close contact with blood in their life cycles within the human host (though only during larval stages in the case of *A. lumbricoides*), but research is still needed to produce a similar test for STH.

Immunoassays such as ELISA can be very useful when measuring exposure to infection or to detect transmission of infection, as they can not only detect the presence of egg-producing adult worms but also pre-patent larvae. However, with the existing knowledge of helminth immunology, no distinction can be made between current infection and past infection/exposure, and thus ELISA cannot be accurately used as a diagnostic method in endemic populations. No STH antigens are currently commercially available, which increases the difficulty of expanding the use of ELISA for STH research, as antigens will need to be produced for each individual study and may not be comparable between studies. Research into more specific antigens is also necessary. The lack of correlation found in this study between IgG4 antibodies and egg counts, and the cross-reactivity found between antigens highlight the need for more research to identify and produce specific recombinant antigens, which can be used to distinguish between prepatent and current infections, as well as between helminth species in co-infected populations and individuals (23–25).

The proportion of study participants in Bungoma who were found to be infected by Kato-Katz assessments, but seronegative by ELISA, was larger than the proportion of infected and seropositive individuals. Similar findings were reported in a study carried out in Indonesia, where the proportion of the population infected but seronegative was even greater after the end of the MDA programme (26). Should this tendency also take place following the end of the national SBD programme in Kenya

and elsewhere, it is possible that the communities targeted by SBD programmes in fact become more vulnerable to reinfection due to loss of antibodies after the end of a deworming programme. In this scenario, deworming will provide an immediate benefit to the children dewormed during the length of the control programme, but without concomitant improvements in socio-economic and WASH conditions the population might not reap long-term benefits, as without protective antibodies infection might still occur later in life. More research is necessary to understand helminth immunity, but it seems clear that in the absence of improvements to reduce exposure to infection (i.e. behaviour change, improving WASH and socio-economic conditions), a control measure which might render individuals less immune to reinfection (and thus more prone to heavy infection) without eliminating transmission might make matters worse. In this sense, deworming to control morbidity due to heavy infection in some individuals might not be as successful in the long term as aiming for elimination of transmission in the entire population (27–29).

5.4. Future Work and Concluding Remarks

Despite the long history of epidemiological study of STH infections, much remains to be understood . While early studies showed the many benefits of deworming children, more recent studies and reviews looking at the impact of SBD programmes on children's height, weight, educational improvements and other benefits have proven inconclusive (30–32). In a time when policies need more and more to be evidence-based and resources are limited, studies showing the clear benefits of deworming and the cost-effectiveness of deworming programmes are in demand (33). These studies need to be longitudinal in nature to be able to analyse and quantify the long-term effects of deworming. In other words, following treated children in their communities over a period of possibly ten or more years is required – from the start of an STH control programme, through to its end, and at least as many more years after its conclusion. While most academic research studies are not able to cover such a long period, data sharing platforms such as the Global Atlas of Helminth Infections (GAHI, 34) might provide future researchers with enough detail to follow-up on studies carried out before or during the initial years of deworming programmes. The Kenyan national SBD programme has now completed its 5-year agenda and is expected to continue (Children's Investment Fund Foundation, personal communication). Future studies carried out in Kenya should make use of data collected during this arm of the programme to analyse changes over time.

The analysis of risk factors for each STH infection will remain an important aspect of baseline surveys carried out prior to the launch of STH treatment programmes to identify issues that need to be included in the control programme, as well as of M&E surveys carried out during the programme to

identify risks associated with remaining infection and the determinants of reinfection. These surveys should collect both individual and household-level data with clearly defined variables which are appropriate to each setting and adapted as necessary to local conditions, while remaining standardized enough to enable comparisons between studies.

Novel methodological approaches are available for diagnostics of STH infections. These include the loop-mediated isothermal amplification (LAMP), which is a simpler and faster PCR analysis still being tested to compare sensitivity and specificity against current methods (35,36), and the FECPAK software, which uses a cell counter to count eggs automatically, and which is being adapted from veterinary use to application in human populations (37). The genomes of the three STH species (*A. lumbricoides*, *N. americanus* and *T. trichiura*) have been sequenced and are available online (38). The sequenced genome can be used for epidemiological studies of parasite population genetics, and may enable a direct analysis of transmission of infection by identifying "who-infects-whom". It may also provide possible targets for the discovery of new anthelminthic treatments, which will prove vital should the parasites develop resistance to the very few drugs currently available. This may prove particularly important for *Trichuris*, for which no single currently available anthelminthic drug is satisfactorily effective (39).

Recent advances in immunology, including flow cytometry and mass cytometry techniques, can be used to perform deep-immunophenotyping (extensive profiles of receptors and other markers present on the cell surface) of the immune cells intervening in responding to STH infection when comparing to uninfected individuals, the cells involved in responses to heavy or light STH infection, those active in children in comparison with adults, or in individuals infected with different STH species. Cytokine assay kits are available commercially, which can be used to quantify several cytokines and metabolites associated with inflammatory and regulatory processes. In particular, the Luminex technology can also be used to quantify a range of antibodies simultaneously, using specific antigens from different species or from different stages of the parasites (40). STH-specific antigens are not standardized or commercially available, and the identification of very specific antigens, as well as the production of recombinant proteins is a vast area of future work. These antigens will also enable the production of point-of-care (POC) tests such as the POC-CCA for *S. mansoni*, which can potentially greatly improve the STH diagnostics done in the field at baseline and during STH control programmes.

The past decade has seen enormous progress in the gearing up of MDA programmes worldwide to control STH infection. Much less attention has been given to detailed epidemiological studies of impact and what factors determine the persistence of infection in long-treated communities. Detailed longitudinal compliance studies should complement drug efficacy monitoring studies, and

implementation science and anthropological studies should help understanding the factors associated with the slow uptake of water, hygiene and sanitation messages. This study has attempted to advance understanding of the factors that sustain transmission post wide-scale treatment and has reported the advantages of community-wide MDA over that induced by school-based treatment.

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APPENDIX I: PROJECT DOCUMENTATION

Patient Information Sheet

Kenya Medical Research Institute / Imperial College London

Study on the distribution of parasitic infections among communities in Kenya, to improve deworming programs and their evaluation

Principal investigators:
Dr Charles Mwandawiro[1], Prof. Roy Anderson[2], Ms. Alice Easton[2], Ms Rita Oliveira[2]
Co-investigators:
Prof. Simon Brooker[3,4], Prof. Sammy Njenga[1], Dr. Jimmy Kihara[1], Mr. Cassian Mwatele[1], Ms. Faith Mwende[1]

1) Eastern and Southern Africa Centre of International Parasite Control, KEMRI, Nairobi, Kenya; 2) Imperial College London, United Kingdom; 3) KEMRI-Wellcome Trust Research Programme, Nairobi, Kenya; 4) London School of Hygiene and Tropical Medicine, United Kingdom

Introduction: What is KEMRI?
We are staff from the Kenya Medical Research Institute (KEMRI). KEMRI is a government organization that carries out medical research. Research is different from normal treatment because research aims to find better ways of preventing and treating illness in the future for everybody's benefit. You and other people living in this village are being invited to take part in a research study.

Before you decide whether to participate, it is important for you to understand why the research is being done and what it will involve. Please listen to or read the following information and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information.

Purpose of the study: What is this research about?
Worm infections are found in much of rural Kenya, and children are most likely to have worm infections. We are conducting research on the occurrence of worms in order to generate information important for running deworming programs. There is a national program to distribute deworming medication, and efforts are on-going to evaluate the impact of this program. By testing members of this community for infection with worms, we hope to learn how to improve the ways in which we measure impact of such programs.

Why have I been chosen?
We are visiting four villages, and we hope to test up to 2500 willing people. National survey data suggest that many people have worm infections in Bungoma South, but this is a very large area, so we are focusing on just four villages in West Sangalo.

Do I have to take part?
It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect your or your children's access to any public health service.

What will happen to me if I [and/or my children] take part?
At all points in time, we will come to you in order to give the treatment and collect stool samples. In some cases, we may come by your house to request to you walk to a nearby sample collection point. You will not be charged anything for treatment for worms, and we cannot pay you to participate in this study. You should try to eat something in the morning before being treated. If you take part, we would like to:

- Take stool samples on two consecutive days to test for worm infection;
- Take a blood sample from your finger, which we will use to measure how your body has responded to any past worm infections you may have had; we will also test your blood for malaria parasites and anaemia;

● Provide you with oral treatment for worms and ask you to collect 48 hours of stool output in a plastic container, which we will collect to test for worms, as worms are pushed out of the body following treatment;

● Take stool samples a couple more times to test for worm reinfection: once three weeks after treatment from a smaller group of randomly selected people to check how effective the treatment was, again six to ten months later;

● Take fingerprick blood samples one more time from everyone at six months to ten months after the first treatment to measure anaemia and to test for malaria; and if you're 25 years old or younger to measure immunity as well;

● Provide you with oral treatment for worms after the follow-up timepoint and again collect 48 hours of stool output in a container.

What drug will be used?
The drug we will use to treat worms is albendazole; it has been around for many years and it is safe for use in most people. It is the same drug the government uses to treat schoolchildren in Kenya as part of the national deworming program.

What are the potential side effects of this treatment?
If you have many worms living in your intestines, you may experience discomfort following treatment. This discomfort is due to the worms being pushed out of your body, which in the long-term is a good thing. If you do experience strong pain following treatment, please contact Charles Mwandawiro, Alice Easton or Rita Oliveira (see below) and we will investigate quickly.

What are the possible disadvantages and risks of taking part?
The risks associated with participating in this study are minimal, as the drugs used are known to be very safe. This drug is not approved for use in children under two and pregnant women. If you become pregnant during the study, please tell the researchers the next time they visit, and you will be excused from participation from then on. The blood samples will be collected by experienced researchers by pricking your finger with a needle, so you will only feel discomfort for a moment. The needles used are sterile and will only be used once, so there is no risk of contamination.

What are the possible benefits of taking part?
You and members of your community will receive treatment for worms that live in the gut of many people. You will also receive free malaria treatment if you have signs of malaria infection, and anaemia measurements. We cannot promise the study will help you, but the information we get might help improve the treatment of people with worm infections.

What will happen after the study stops?
School children in this community will still receive treatment for worms through the national deworming program following the end of this study.

Who will have access to the information I give?
All information which is collected about you during the course of the research will be kept strictly confidential. Any information about you which leaves the KEMRI offices will have your name and address removed so that you cannot be recognised from it.

What will happen to the samples and results?
Stool samples will be studied in Kenya, and portions of these samples will be taken to the United States, where we will use machines available there to analyse the DNA of worms collected. In addition to looking for genetic material from worms, we will test for other infections like Giardia. The material exported will not be attached to your name, so you do not risk personal embarrassment from the export of stool. By allowing us to transport your stool to the US, this will enable us to use sophisticated technology to analyse it. We will work with researchers and technicians in Kenya to determine how further integration of these techniques into the Kenyan health and research infrastructure could be beneficial.

Blood samples will be taken to England, where we will use machines available there to measure your immunity to worms. You will be told of your (or your children's) worm, anaemia and malaria status. If you (or your children) have a positive malaria test, you will receive treatment. If you have anaemia, we will refer you to the nearest health centre.
Your genetic material will not be analysed—just that of the worms. We will not test any of your samples for HIV. Summaries of the results will be published in academic journals, and be used by governments to improve their deworming programs.

Who is organizing and funding the research?
KEMRI, the London School of Hygiene and Tropical Medicine and Imperial College London are organizing the research. The work is funded by the Bill and Melinda Gates Foundation and GlaxoSmithKline, which also donates the drugs.

Who has reviewed/approved the study?
All research at KEMRI is approved by national independent expert committees in Nairobi to make sure the research is conducted properly and that participants' safety and rights are respected. This research has also been approved by a review board at Imperial College London.

What if I have any questions?
For information about this study, you can contact the researcher who is responsible for this study:

Dr. Charles Mwandawiro
Kenya Institute for Medical Research
P.O Box 54840-00200, Nairobi
Telephone: 020 272 2541
Mobile: 0722 339242

Ms Rita Oliveira
Mobile: **0717177066**

Ms Alice Easton
Mobile: **0788271734**

If you have questions about your rights as a study participant, concerns about the research or if you want to ask someone independent anything about this research please contact:

The Secretary, KEMRI Ethics Review Committee
P. O. BOX 54840-00200, Nairobi
Telephone: 020 2722541, 0722205901, 0733400003
E-mail address: erc@kemri.org.

This research is supported by the KEMRI and Imperial College London, who will pay for any treatment or compensation in the unlikely event of any injury resulting from this study.

Subject/Parent/Guardian Consent Form

Kenya Medical Research Institute / Imperial College London

Survey of the distribution of parasitic infections among communities in Kenya, in order to improve deworming programs and their evaluation

I have had the research explained to me. I have understood all that has been read and had my questions answered satisfactorily. I understand that participation is voluntary and I [and my child] are free to withdraw at any time, without giving any reason, without medical care or legal rights being affected. I understand that there is no monetary compensation for participation.

Yes/No *please circle* **I agree to participate in this research**
Yes/No *please circle* **I agree to my stool samples being collected and stored**
Yes/No *please circle* **I agree to my blood samples being collected and stored**
Yes/No *please circle* **I agree to my samples being taken outside of Kenya**

If also consenting for children to participate:
Yes/No *please circle* **I agree to allow my child[ren] listed below (unless specified below) to take part in this research**
Yes/No *please circle* **I agree to their stool samples being collected and stored**
Yes/No *please circle* **I agree to their blood samples being collected and stored**
Yes/No *please circle* **I agree to their samples being taken outside of Kenya**

Subject/parent/guardian's signature: _____ **Date**_____

Subject/Parent/guardian's name: _____ **Time**_____
(Please print name)

I certify that I have followed all the study specific procedures described in the SOP for obtaining informed consent.
Designee/investigator's signature: _____ **Date**_____

Designee/investigator's name: _____ **Time**_____
(Please print name)

Only necessary if the subject/parent/guardian cannot read:
I *attest that the information concerning this research was accurately explained to and apparently understood by the subject/parent/guardian and that informed consent was freely given by the subject/parent/guardian.

Witness' signature: _____ **Date**_____

Witness' name: _____ **Time**_____
(Please print name)

Child (ages 12-17) Consent Form

Kenya Medical Research Institute / Imperial College London

Survey of the distribution of parasitic infections among communities in Kenya, in order to improve deworming programs and their evaluation

Child (or if unable, parent on their behalf) /young person to circle all they agree with:

Yes/No Has somebody explained this project to you?
Yes/No Have you asked all the questions you want?
Yes/No Have you had your questions answered in a way you understand?
Yes/No Do you understand it's OK to stop taking part at any time?
Yes/No Do you know that stool and blood samples will be collected a few times over a year?
Yes/No Are you happy to take part?

If any answers are 'no' or you **don't** want to take part, **don't** sign your name!

I DO CONSENT:		**I hereby agree to take part in this study**

I DO NOT CONSENT:		**I do not wish to take part in this study**

Child's name		
Child's Signature or Mark		Date:

I certify that I have followed all the study specific procedures described in the SOP for obtaining informed consent.

Designee/investigator's signature: _____ **Date** _____

Designee/investigator's name: _____ **Time** _____
(Please print name)

Only necessary if the child cannot read:

I *attest that the information concerning this research was accurately explained to and apparently understood by the child and that assent was freely given.

Witness' signature: _____ **Date** _____

Witness' name: _____ **Time** _____
(Please print name)

*A witness is a person who is independent from the trial or a member of staff who was not involved in gaining the consent

APPENDIX II: ALBENDAZOLE INFO SHEET

1 NAME OF THE MEDICINAL PRODUCT

Albendazole.
Zentel/Albenza manufactured by GlaxoSmithKline

2 QUALITATIVE AND QUANTITATIVE COMPOSITION

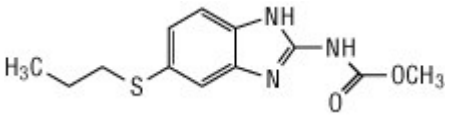
Tablet containing either 200 mg or 400 mg albendazole.
4 % w/v suspension to be taken orally; 4 g albendazole per 100 ml.
2 % w/v suspension to be taken orally; 2 g albendazole per 100 ml.

3 PHARMACEUTICAL FORM

Tablet
Suspension

4 CLINICAL PARTICULARS

Albendazole is an orally administered broad-spectrum anthelmintic. Chemically, it is methyl 5-(propylthio)-2-benzimidazolecarbamate. Its molecular formula is C12H15N3O2S. Its molecular weight is 265.34. It has the following chemical structure:



Albendazole is a white to off-white powder. It is soluble in dimethylsulfoxide, strong acids, and strong bases. It is slightly soluble in methanol, chloroform, ethyl acetate, and acetonitrile. Albendazole is practically insoluble in water. Each white to off-white, film-coated tablet contains 200 mg of albendazole.
Inactive ingredients consist of: carnauba wax, hypromellose, lactose monohydrate, magnesium stearate, microcrystalline cellulose, povidone, sodium lauryl sulfate, sodium saccharin, sodium starch glycolate, and starch.

4.1 Therapeutic indications

Albendazole is a benzimidazole carbamate with antihelmintic and antiprotozoal activity against the following intestinal and tissue parasites: Round-worm (*Ascaris lumbricoides*), pin-worm (*Enterobius vermicularis*), hook-worm (*Necator americanus, Ancylostoma duodenale*), whip-worm (*Trichuris trichiura*), thread-worm (*Strongyloides stercoralis*), tape-worm (*Taenia* spp and Hymenolepis nana only in the case of associated parasitism), Chlonorchiasis (*Chlonorchis sinensis*), Opisthorchiasis (*Opisthorchis viverrini*) and cutaneous larva migrans; Giardiasis (*G.lamblia, G.duodenalis, G.intestinalis, Lamblia intestinalis*) in children.

4.2 Posology and method of administration

Dosage

Indications	Age	Dose	Period
- Round-worm - Pin-worm* - Hook-worms - Whip-worm	adults and children over 2 years of age	400 mg [two 200 mg or one 400 mg tablet(s) or 10 ml 4% or 20 ml 2% suspension]#	single dose
	children 1-2 years of age	200 mg (one 200 mg tablet or 5 ml 4% or 10 ml 2% suspension)	single dose
- Strongyloidiasis - Taeniasis - Hymenolepiasis=	adults and children over 2 years of age	400 mg (#see above)	one dose per day for 3 days
- Chlonorchiasis - Opisthorchiasis	adults and children over 2 years of age	400 mg (#see above)	two doses per day for 3 days
- Giardiasis	children 2 - 12 years of age only	400 mg (#see above)	one dose per day for 5 days

*In order to obtain a complete cure in the case of pin-worm infestation, prescribe strict measures of hygiene, also treat the relatives and individuals sharing the same housing.

=In cases of proven Hymenolepiasis, retreatment in 10-21 days is recommended.

Method of Administration

If the patient is not cured after three weeks, a second course of treatment is indicated.

No special procedures, such as fasting or purging, are required.

The tablets can be chewed or taken with water. Some people, particularly young children, may experience difficulties swallowing the tablets whole and should be encouraged to chew the tablets with a little water, alternatively the tablets may be crushed.

4.3 Contraindications

Albendazole should not be administered during pregnancy, or in women thought to be pregnant.

Albendazole is contra-indicated in patients with a known history of hypersensitivity to the drug (albendazole or constituents).

4.4 Special warnings and precautions for use

In order to avoid administering Albendazole during early pregnancy, women of childbearing age should initiate treatment during the first week of menstruation or after a negative pregnancy test.

Treatment with Albendazole may uncover pre-existing neurocysticercosis, particularly in areas with high taenosis infection. Patients may experience neurological symptoms e.g. seizures, increased intracranial pressure and focal signs as a result of an inflammatory reaction caused by death of the parasite within the brain. Symptoms may occur soon after treatment, appropriate steroid and anticonvulsant therapy should be started immediately.

Albendazole suspension contains benzoic acid which is a mild irritant to the skin, eyes and mucous membrane. It may increase the risk of jaundice in newborn babies.

Elderly

Experience in patients 65 years of age or older is limited. Reports indicate that no dosage adjustment is required, however, albendazole should be used with caution in elderly patients with evidence of hepatic dysfunction (see Hepatic Impairment and Pharmacokinetics).

Renal impairment

Since renal elimination of albendazole and its primary metabolite, albendazole sulfoxide, is negligible, it is unlikely that clearance of these compounds would be altered in these patients. No dosage adjustment is required, however, patients with evidence of renal impairment should be carefully monitored.

Hepatic impairment

Since albendazole is rapidly metabolized by the liver to the primary pharmacologically active metabolite, albendazole sulfoxide, hepatic impairment would be expected to have significant effects on the pharmacokinetics of albendazole sulfoxide. Patients with abnormal liver function test results (transaminases) prior to commencing albendazole therapy should be carefully monitored.

4.5 Interaction with other medicinal products and other forms of interaction

Praziquantel has been reported to increase the plasma levels of the albendazole active metabolite.

Ritonavir, phenytoin, carbamazepine and phenobarbital may have the potential to reduce plasma concentrations of the active metabolite of albendazole; albendazole sulfoxide. The clinical relevance of this is unknown, but may result in decreased efficacy, especially in the treatment of systemic helminth infections. Patients should be monitored for efficacy and may require alternative dose regimens or therapies.

4.6 Fertility, pregnancy and lactation

Albendazole should not be administered during pregnancy or in women thought to be pregnant (see Contraindications).

It is not known whether albendazole or its metabolites are secreted in human breast milk. Thus Albendazole should not be used during lactation unless the potential benefits are considered to outweigh the potential risks associated with treatment.

4.7 Effects on ability to drive and use machines

Adverse effects on the ability to drive or operate machinery have not been observed.

4.8 Undesirable effects

Data from large clinical studies were used to determine the frequency of very common to rare undesirable reactions. The frequencies assigned to all other undesirable reactions (i.e. those occurring at < 1/1000) were mainly determined using post-marketing data and refer to a reporting rate rather than a true frequency.

The following convention has been used for the classification of frequency:

Very common	≥1/10		
Common	≥1/100	and	<1/10
Uncommon	≥1/1000	and	<1/100
Rare	≥1/10,000	and	<1/1000
Very rare	< 1/10,000		

Immune system disorders

Rare: Hypersensitivity reactions including rash, pruritis and urticaria.

Nervous system disorders

Uncommon: Headache and dizziness.

Gastrointestinal disorders
Uncommon: Upper gastrointestinal symptoms (e.g. epigastric or abdominal pain, nausea, vomiting) and diarrhoea.

Hepatobiliary disorders
Rare: Elevations of hepatic enzymes

Skin and subcutaneous tissue disorders
Very rare: Erythema multiforme, Stevens-Johnson syndrome

4.9 Overdose
Significant toxicity and mortality were shown in male and female mice at doses exceeding 5,000 mg/kg; in rats, at estimated doses between 1,300 and 2,400 mg/kg; in hamsters, at doses exceeding 10,000 mg/kg; and in rabbits, at estimated doses between 500 and 1,250 mg/kg. In the animals, symptoms were demonstrated in a dose-response relationship and included diarrhea, vomiting, tachycardia, and respiratory distress.
One overdosage has been reported with ALBENZA in a patient who took at least 16 grams over 12 hours. No untoward effects were reported. In case of overdosage, symptomatic therapy and general supportive measures are recommended.

5 PHARMACOLOGICAL PROPERTIES
5.1 Pharmacodynamic properties
Albendazole belongs to the benzimidazole class of anthelmintics. Benzimidazoles bind to nematode tubulin, a protein necessary for the formation and viability of microtubules. This occurs primarily in absorptive intestinal cells resulting in the absence of microtubules in the intestinal cells of the nematode, with the result that these cells cannot absorb nutrients, thus causing a consequent reduction in glycogen and effective starvation of the parasites. Structural differences have been shown to exist between tubulin from mammalian and helminth sources, resulting in the preferential toxicity of albendazole to the helminth and not to the host. Benzimidazoles have also been shown to inhibit the fumarate reductase system of helminths and impair energy production.

5.2 Pharmacokinetic properties
Albendazole is poorly absorbed from the gastrointestinal tract due to its low aqueous solubility. Albendazole concentrations are negligible or undetectable in plasma as it is rapidly converted to the sulfoxide metabolite prior to reaching the systemic circulation. The systemic anthelmintic activity has been attributed to the primary metabolite, albendazole sulfoxide. Oral bioavailability appears to be enhanced when albendazole is coadministered with a fatty meal (estimated fat content 40 g) as evidenced by higher (up to 5-fold on average) plasma concentrations of albendazole sulfoxide as compared to the fasted state.

Maximal plasma concentrations of albendazole sulfoxide are typically achieved 2 to 5 hours after dosing and are on average 1.31 mcg/mL (range 0.46 to 1.58 mcg/mL) following oral doses of albendazole (400 mg) in 6 hydatid disease patients, when administered with a fatty meal. Plasma concentrations of albendazole sulfoxide increase in a dose-proportional manner over the therapeutic dose range following ingestion of a fatty meal (fat content 43.1 g). The mean apparent terminal elimination half-life of albendazole sulfoxide typically ranges from 8 to 12 hours in 25 normal subjects, as well as in 14 hydatid and 8 neurocysticercosis patients.

Following 4 weeks of treatment with albendazole (200 mg three times daily), 12 patients' plasma concentrations of albendazole sulfoxide were approximately 20% lower than those observed during the first half of the treatment period, suggesting that albendazole may induce its own metabolism.

Special Patient Populations

- Elderly

Although no studies have investigated the effect of age on albendazole sulfoxide pharmacokinetics, data in twenty-six hydatid cyst patients (up to 79 years) suggest pharmacokinetics similar to those in young healthy subjects. The number of elderly patients treated for either hydatid disease or neurocysticercosis is limited, but no problems associated with an older population have been observed.

- Renal Impairment

The pharmacokinetics of albendazole in patients with impaired renal function have not been studied.

- Hepatic Impairment

The pharmacokinetics of albendazole in patients with impaired hepatic function have not been studied.

5.3 Preclinical safety data

DRUG	FDA PREG-NANCY CATEGORY	PLACENTAL PASSAGE (NEWBORN/MATERNAL RATIO)	ANIMAL REPRODUCTION STUDIES	CONCERNS IN HUMAN PREGNANCY	RECOMMENDED USE IN PREGNANCY
Albendazole	C	Unknown	Teratogenic (skeletal malformations) in rats and rabbits, but not in mice.	No experience, animal data concerning.	Consider in second, third trimester for severe diarrhoea with documented *Microsporidia* infection.

6 PHARMACEUTICAL PARTICULARS

6.1 List of excipients

Tablets 200 mg	Tablets 400 mg	Suspension (2%, 4%)
Lactose	Lactose	Aluminium magnesium silicate
Maize starch	Microcrystalline cellulose	Carboxymethylcellulose sodium
Polyvidone	Maize starch	Glycerin
Sodium lauryl sulphate	Croscarmellose sodium	Polysorbate 80
Sodium starch glycollate	Povidone K30	Sorbitan monolaureate
Microcrystalline cellulose	Sodium lauryl sulphate	Potassium sorbate
Sodium saccharin	Sunset yellow lake	Benzoic acid (see Warnings and Precautions)
Magnesium stearate*	Sodium saccharin	Sorbic acid
	Magnesium stearate*	Silicone antifoam 1510
	Flavourings	Saccharin sodium
Film coating		Flavourings
Methylhydroxypropylcellulose 15		
Methylhydroxypropylcellulose 5		
Propylene glycol.		

6.2 Incompatibilities

Not to be mixed or diluted
Grapefruit juice should not be consumed around the time of administration
Avoid the introduction of contamination during use
Efficacy is lowered by concurrent use of antiepileptics

6.3 Shelf life

The expiry date is indicated on the packaging.
Standard is three years.

6.4 Special precautions for storage

Tablets: Store below 25°C.
Suspensions: Store below 30°C and protect from direct sunlight.

6.5 Nature and contents of container

Tablets: ***Blister packs, polypropylene containers and cap.***
Suspensions: ***Glass/plastic bottle with aluminium cap.***

6.6 Special precautions for disposal

Do not contaminate ponds, waterways or ditches with the product or used containers. Unused product and waste material should be disposed of in accordance with current practice for pharmaceutical waste under national waste disposal regulations.

7 DATE OF FIRST AUTHORISATION/RENEWAL OF THE AUTHORISATION

Date of issue: 23 Jul 2010 - GlaxoSmithKline

Sources:
GlaxoSmithKline: personal correspondence.

Stanford: http://www.stanford.edu/group/parasites/ParaSites2005/Albendazole/index.htm

Irish Medicines Board (veterinary):
http://www.imb.ie/images/uploaded/swedocuments/LicenseSPC_10019-050-001_29112010104905.pdf

Mayo Clinic: http://www.mayoclinic.com/health/drug-information/DR600027
http://dailymed.nlm.nih.gov/dailymed/archives/fdaDrugInfo.cfm?archiveid=12271

WHO. 2010. 2nd WHO model list of essential medicines for children (March 2010 update)
www.chartcaribbean.org

APPENDIX III: PCA ANALYSIS – FURTHER DETAILS

Variable	Type	List of categories
Education level	Categorical	No education, Any primary, Secondary incomplete, Secondary complete or above
Household characteristics		
Wall material	Categorical	Cement/Bricks, Clay/Mud
Floor material	Categorical	Cement/Tiles/Linoleum, Mud/Earth
Roof material	Categorical	Tiles, Iron sheets, Grass/Thatch
Drinking water source	Categorical	Stream/River, Spring, Borehole, Well, Piped/Tap
Asset ownership	Binary categories	Mobile, TV, Radio, Electricity, Solar power, Other power sources (e.g. generator)
Hand wash products	Categorical	Only water, Water and soap, Water and ash
Weekly household expenses	Continuous	Soap, Toilet Paper
Sanitary conditions		
Toilet/latrine ownership	Binary	Y / N
Type of latrine	Categorical	Pit latrine w/ mud floor, Pit latrine w/ cement floor, Ventilated pit latrine, Composting toilet, Flush toilet
Latrine floor	Categorical	Good, Cracked, Broken
Latrine walls	Categorical	Fully private, With holes, No walls
Latrine roof	Categorical	Fully waterproof, With holes, No roof
Latrine hole	Categorical	Fully covered, Uncovered, No cover at all
Cleansing materials	Categorical	Toilet paper/tissues, Leaves/newspaper, No cleansing materials

Table I.1. Variables used in PCA analysis to derive index of SES. (Chapter 2, PCA sub-section)

Variables	Eigenvector (weight)
Household floor material: Cement/Tiles/Linoleum	0.2856
Household wall material: Cement/Bricks	0.2757
Latrine type: Pit latrine w/ cement floor	0.2329
Latrine walls: Fully private	0.2283
Latrine roof: Fully waterproof	0.2060
Latrine floor: Good	0.2053
Electricity	0.1924
TV ownership	0.1889
Weekly expenses: Toilet paper **(continuous variable)**	0.1864
Education level: Secondary complete and above	0.1852
Latrine cleansing materials: Toilet paper/tissues	0.1507
Latrine type: Ventilated (VIP)	0.1252
Radio ownership	0.1087

Table I.2. PCA output – eigenvectors for variables with score above 0.1 in the first principal component. (Chapter 2, PCA sub-section)

APPENDIX IV: SOLUTIONS USED IN ELISA PROTOCOL

Solution	**Reagents**
Coating buffer (1 L) pH 9.4-9.6	Na_2CO_3 – 1.59 g $NaHCO_3$ – 2.93 g Distilled water – up to 1 L Adjust pH, if necessary, using 5% HCl
20x PBS (phosphate buffered saline) (10 L) pH 7.2	Na_2HPO_4 – 167 g NaH_2PO_4 – 57 g NaCl – 850 g Distilled water – up to 10L
PBS-Tween wash solution (5 L)	20x PBS – 250 mL Tween 20 – 2.5 mL Distilled water – up to 5 L
Blocking buffer (1 L)	Skimmed milk powder – 10g PBS-Tween – up to 1 L
Reconstitution **buffer** (1 L) (used to dilute samples)	20x PBS – 50 mL Sodium azide – 1 g Distilled water – up to 1 L
IgG4 conjugate solution 1:2000 (10 mL)	PBS-Tween – 10 mL Mouse anti-human IgG4 pFc'-HRP – 5 µl (Southern biotech® 9190-05)
Substrate solution (TMB)	3,3',5,5'-Tetramethylbenzidine (TMB) Liquid Substrate System for ELISA (Sigma-Aldrich)
Stop solution (0.2 M H_2SO_4) (1 L)	H_2SO_4 concentrate – 107 mL Distilled water – add H_2SO_4 to ~800 mL of water, then top up to 1 L