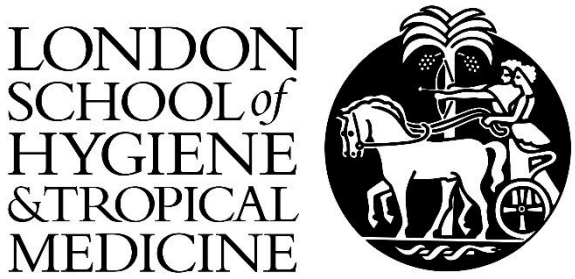

School-based malaria control in Kenya: Evaluating heterogeneity in risk, impact and process

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Declaration by candidate

I, Katherine Elizabeth Halliday, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Signed……..... Date……05/10/15……

KATHERINE ELIZABETH HALLIDAY

Abstract

School children are increasingly recognised as an important target for malaria control. However, limited evidence exists on the relative benefits of alternative school-based malaria control interventions or how impact may vary according to transmission intensity. Nested within the first evaluation of school-based intermittent screening and treatment (IST), in a region of low-to-moderate transmission, this thesis aimed to investigate the influence of heterogeneity in underlying transmission risk and variation in the fidelity of the intervention on the impact and possible operational applications of IST. The thesis utilized data from a cluster-randomised trial, over two years, evaluating the impact of school-based IST on health and education of children in 101 schools in south coast, Kenya. In the intervention children were screened for *Plasmodium falciparum* infection using rapid diagnostic tests (RDTs) once a term, with those found RDT-positive treated with artemether-lumefantrine.

Cross-sectional analysis of baseline data, indicated marked heterogeneity in *P. falciparum* infection and anaemia at school-level. *P. falciparum* infection was strongly associated with anaemia, but health status was not associated with educational performance. Subsequent analysis of the impact of IST showed no benefit on the primary health or education outcomes even when stratified by baseline *Plasmodium* prevalence, or number of treatments received. Latent class analysis suggested reasonable diagnostic performance of RDTs, with seasonal variability observed. Bayesian geostatistical analysis of the spatial and temporal heterogeneity of school-level *P. falciparum* infection highlighted the relative stability in observed heterogeneity, despite periodic treatment of infection. Analysis at the individual-level suggested overdispersion of *P. falciparum* infection with prevalence-based models showing a greater proportion of individuals repeatedly infected than expected even after accounting for exposure.

Collectively, these findings indicate that, in such a locally heterogeneous transmission setting, school-based IST provided no health or education benefits, with a number of factors identified for lack of impact. The persistence of high infection levels in certain schools despite periodic treatment highlights the importance of fine-scale targeting with a need for community-wide coverage in high-risk clusters. Moreover, the findings suggest a potential role for school-level screenings in both the identification of communities for targeted control, and periodic monitoring of impact.

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Abbreviations

ACT	Artemisinin Combination Therapy
Adj.MD	Adjusted mean difference
Ajd.OR	Adjusted odds ratio
Adj.RR	Adjusted risk ratio
AE	Adverse event
AL	Artemether Lumefantrine
API	Annual parasite incidence
AQ	Amodiaquine
AS	Artesunate
BMIZ	Body Mass Index for age
CHW	Community health worker
CV	Coefficient of variation
DHS	Demographic and Health Survey
DP	dihydroartemisinin-piperaquine
DOMC	Division of Malaria Control
EGMA	Early Grade Maths Assessment
EGRA	Early Grade Reading Assessment
EIR	Entomological Inoculation Rate
FRESH	Focusing Resources on Effective School Health
FU1	Follow-up 1
FU2	Follow-up 2
GEE	Generalized estimating equations
GMAP	Global Malaria Action Plan
GPS	Global Positioning System
HALI	Health and Literacy Intervention
HAZ	Height For Age
Hb	Haemoglobin
HRP-2	Histadine Rich Protein - 2
KEMRI	Kenya Medical Research Institute
ICC	Intraclass correlation coefficient
IEC	Information Education Communication
IPT	Intermittent Preventive Treatment
IQR	Inter-quartile range
IRS	Indoor Residual Spraying
IST	Intermittent Screening and Treatment

ITNs	Insecticide-treated nets
LCA	Latent class analysis
LLIN	Long-lasting insecticidal net
LST	Land surface temperature
MAP	Malaria Atlas Project
MIS	Malaria Indicator Survey
MOE	Ministry of Education
MoPHS	Ministry of Public Health and Sanitation
MSaT	Mass screen and treat
NMCP	National Malaria Control Programme
NMS	National Malaria Strategy
NPV	Negative predictive value
P. falciparum	*Plasmodium falciparum*
*Pf*PR	Parasite rate
PCA	Principal Components Analysis
PCR	Polymerase chain reaction
PPV	Positive predictive value
PTA	Parent Teacher Association
PTK	Pupil Treatment Kit
ROR	Relative odds ratio
RBM	Roll Back Malaria
RDT	Rapid diagnostic test
SAP	Statistical analysis plan
SD	Standard deviation
SES	Socioeconomic status
SHN	School Health and Nutrition
SP	Sulphadoxine-pyrimethamine
SSA	sub-Saharan Africa
STROBE	Strengthening the reporting of Observational Studies in Epidemiology
TAC	Teacher Advisory Centre
TEA-Ch	Tests of everyday attention for children
WAZ	Weight for Age
WBC	White blood cells
WHO	World Health Organisation

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

Chapter 1. Introduction

1.1 BACKGROUND AND CONTEXT

The last decade has seen a global decline in malaria transmission [1,2], with substantial reductions in malaria-related mortality, morbidity and transmission observed in multiple countries across sub Saharan Africa (SSA) including The Gambia [3,4], Kenya [5,6], Zanzibar [7] and Zambia [8]. The World Health Organisation (WHO) estimates overall reductions in malaria incidence rates of 29% globally and 31% in Africa between 2000 and 2012 [9]. However, despite these declines, predominantly the result of the increased investment and the resulting scale-up of prevention and control interventions [10], combined with social and economic development [11,12], nearly 800 million people in Africa were estimated to be at risk of *Plasmodium falciparum* transmission in 2010 [13]. Hence, continued and strengthened efforts are required to address the burden of malaria worldwide.

These calls have been met by renewed commitment to the goal of elimination proposed by the WHO's Global Malaria Programme in 2006 [14] and consolidated in 2008 by the Roll Back Malaria initiative in the form of a Global Malaria Action Plan (GMAP), with a set of targets including elimination of malaria in ten new countries by 2015 [15]. The pursuit of these targets has led to a shift in focus from the case management of clinical disease and targeting of interventions at traditionally high risk groups (children under 5 years old and pregnant women), to a more inclusive approach aimed at interruption of community-wide transmission [14]. This is to be achieved through universal coverage of interventions [16], increased surveillance [17] and treatment of asymptomatic *Plasmodium* infection [18].

This paradigm shift has highlighted additional vulnerable groups such as school-age children, who experience some of the highest age-specific parasite rates [19,20] regardless of transmission intensity, and as such are important contributors to reservoirs of transmission [21-24]. Moreover,

due to a delay in acquired immunity as a result of decreasing *Plasmodium* infection exposure, this age-group is expected to experience increasing episodes of clinical malaria [25,26]. Despite this, school children are often overlooked by control efforts and continue to demonstrate the lowest coverage of malaria prevention interventions, including long lasting insecticide-treated nets [27-29].

Health and education sectors are increasingly recognising the importance of malaria control in school children for reducing the burden of both acute clinical and asymptomatic *P. falciparum* infections [30,31], with a growing body of evidence indicating the potentially beneficial impact school-based malaria control can have on children's overall health and educational development [32-35]. Furthermore, with schools providing a logistically accessible group of potentially infected individuals, it may be that school-based interventions can offer wider-scale benefits to the communities they serve, both directly and indirectly. As a result, there is ever-increasing interest in the incorporation of school-based malaria control into wider community control measures and the ways in which this can be achieved in SSA.

Historically, studies of school-based malaria control have focused on interventions such as presumptive case management [36], chemoprophylaxis [37-39] and intermittent preventive treatment (IPT) [33,40]. However, due to expanding drug resistance, consequent changes in drug policies across much of SSA, and the requirement of clinical confirmation of infection prior to artemisinin combination therapy (ACT) treatment, many of these approaches are not practically scalable without modifications.

Furthermore, a significant consequence of reduction in transmission is the development of increasingly fractal heterogeneity, defined as irregular patterns of transmission across varying geographical scales, with the variation in *P. falciparum* infection risk becoming more pronounced across multiple scales. While the relative proportion of the population living in regions of hyper- and holoendemic transmission (greater than 50% parasite prevalence) has decreased between 2000 and 2010, the proportion living in mesoendemic and low transmission

regions (less than 50% parasite prevalence) has risen [41]. The highly unequal distribution of *Plasmodium* risk in space, across fine geographical scales, in regions of moderate and low transmission, is likely to influence the efficacy and resultant impact of interventions, and is a significant consideration when planning effective control programmes. However, evaluations of the effects of school-based malaria control have thus far been undertaken in intense and/or seasonal transmission settings only [33,35,38,40,42], with no apparent studies to date conducted in moderate and low transmission settings.

For these reasons, there is a need first, for rigorous evaluation of the impact of alternative strategies of school-based malaria control; secondly, for such evaluations to be conducted in varying transmission settings, with a specific emphasis on low-to-moderate transmission, as many countries and regions transition into these endemicity classes; and finally, for consideration of the influence of heterogeneity at geographic and individual levels, on the impact of interventions implemented in low-to-moderate transmission settings where extensive localised variation in underlying risk is likely.

This thesis aims to explore the extent of underlying heterogeneities in a region of low-to-moderate transmission intensity and to examine the influence of this on the impact, process and potential applications of a new school-based malaria control intervention implemented. This is investigated using data from a cluster randomised controlled trial evaluating a programme of school-based intermittent screening and treatment (IST) for malaria, conducted in region of low-to-moderate transmission in south coast, Kenya. In this, the first study to evaluate the screen and treat approach delivered through schools, school children were screened using rapid diagnostic tests (RDTs) once a term, and those (with or without symptoms) found RDT-positive were treated with artemether-lumefantrine (AL).

This opening chapter provides an overview of the current status of malaria epidemiology and control with a particular focus on the burden in school children and control initiatives delivered through schools. Additionally, the evidence for heterogeneity in transmission at varying scales

is reviewed and the potential for such heterogeneity to influence the impact of control initiatives is discussed.

1.2 BIOLOGY AND PATHOLOGY OF MALARIA

1.2.1 Malaria parasite and vector

The causative agent of malaria is a protozoan parasite, of the genus *Plasmodium*, with five species known to infect humans: *P. falciparum, P. vivax, P. ovale* and *P. malariae* and more recently *P. knowlesi*, recognised as responsible for a zoonotic form of human malaria infection in Southeast Asia [43]. Of these species, *P. falciparum* is the most virulent, in part due to the ability of parasitized erythrocytes to bind strongly to the endothelium of blood vessels, and each other, causing sequestration and clumping within the blood vessels of organs including the brain [44,45], leading to potentially fatal complications. *P. falciparum* is the predominant infecting species in most regions of Africa and this thesis shall focus on infections caused by this organism.

The *Plasmodium* parasite is transmitted by a female mosquito vector of the *Anopheles* genus. Of the more than 400 species of *Anopheles* known, between 40 and 70 species have been associated with the transmission of malaria, with *Anopheles gambiae* sensu lato, considered the most important in Africa followed by *Anopheles funestus*, both of which are anthrophilic species complexes [46]. *Anopheles arabiensis*, a species within the *Anopheles gambiae* s.l. complex, is notable for distinct characteristics such as a lower sporozoite rate [47].

1.2.2 *Plasmodium falciparum* lifecycle

P. falciparum infection occurs when an infected female *Anopheles* mosquito inoculates an individual with sporozoites during a blood meal. The infective, motile parasite forms invade hepatocytes in the liver (Figure 1.1). During this asymptomatic liver stage infection (exo-erythrocytic schizogony), lasting approximately 6 days, the sporozoites mature into schizonts. These rupture releasing merozoites into the blood stream, which infect erythrocytes [48]. During

this blood stage of infection (erythrocytic schizogony) merozoites undergo asexual multiplication, progressing through trophozoite stages, developing into schizonts in the erythrocytes, which then rupture releasing further merozoites. In the absence of immunity, this eyrthocytic cycle will lead to clinical manifestations with each merozoite producing up to 20 replications every 48 hours; often presenting as cyclical fever. At this stage merozoite surface antigens are susceptible to opsonising antibodies and macrophage, lymphocyte and cytokine responses [49].

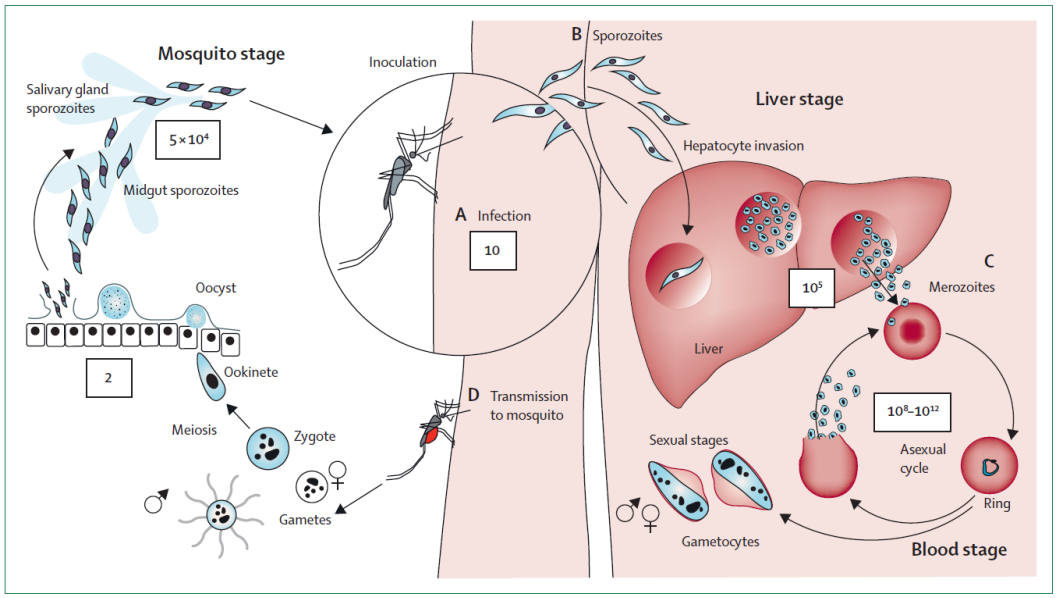


Figure 1.1: *Plasmodium falciparum* lifecycle. Taken from White 2014 [50]. The numbers in boxes represent the estimated number of parasites at each stage, with 10^{12} the equivalent of approximately 2% parasitaemia in an adult.

A proportion of merozoites differentiate into sexual forms (gametocytes) in the erythrocytic cycle [48]. Gametocytes, while non-pathogenic to humans, are ingested by *Anopheles* whilst feeding and are the forms responsible for transmission. In the stomach of the *Anopheles*, male and female gametocytes generate zygotes (sporogonic cycle), which mature into motile ookinetes. These develop in the midgut wall into oocysts, rupturing to release sporozoites. The sporozoites travel to the salivary glands and can inoculate a human during feeding. This sexual stage, sporogony, in the *Anopheles* takes approximately 14 days (range 10-21 days) and is a function of vector species and extrinsic conditions such as ambient temperature [51].

1.2.3 Pathology of malaria

Infection with *P. falciparum* can manifest in several forms (Figure 1.2), including clinical (uncomplicated) malaria, severe malaria (with complications) which can lead to death, and asymptomatic infection, with the manifestation largely a function of the degree of acquired immunity of the individual [25,52]. Clinical or uncomplicated malaria typically occurs in a non-immune individual, with symptoms becoming apparent at the asexual stage of erythrocytic schizogony some six to ten days after inoculation [50]. Clinical manifestations characteristically present as shaking chills (fever with rigors) with a variety of non-specific symptoms such as headache, generalised arthralgia and myalgia, malaise and sweats [50,53]. Haemolytic anaemia commonly occurs in individuals during and in the period following a clinical attack of *P. falciparum* infection, due to the destruction of erythrocytes by the parasite and by the pro-inflammatory mediated response [54]. A differential diagnosis between malaria and other common childhood infections can be difficult, and in endemic areas, malaria has typically been treated on the basis of a presumptive diagnosis on presentation with a fever or history of fever [55,56]. In the case of prompt and effective treatment an infected individual can recover without sequelae, although neurological and cognitive impairments have been documented in children suffering repeated clinical attacks, especially in the younger age-groups [57,58].

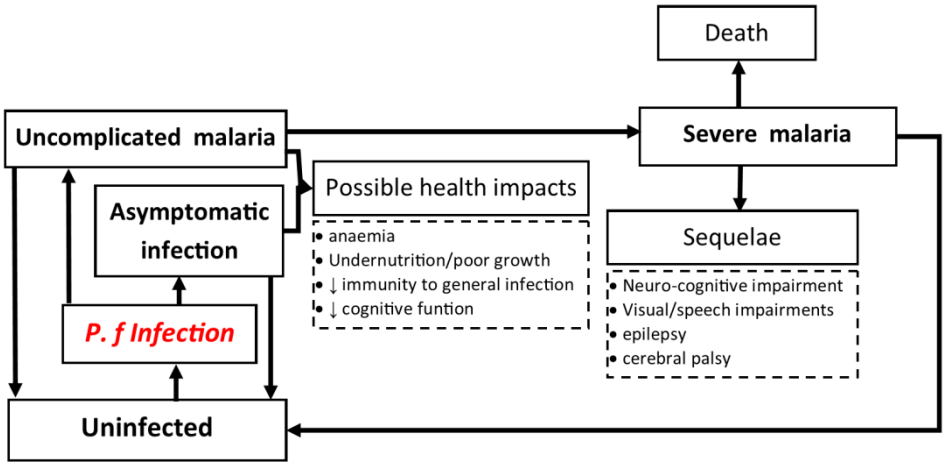


Figure 1.2: Diagrammatic representation of the pathology states of *P. falciparum* infection including possible pathways and negative impacts associated with each stage. Adapted from Snow *et al.* [59]

In some cases serious clinical complications develop, resulting in severe malaria. In adults such complications often lead to multiorgan damage and renal failure [49]. In children, severe malaria is more likely to be characterised by acute respiratory distress, severe anaemia (Hb<50g/L), prostration and cerebral malaria, the last of which is associated with central nervous system complications, including convulsions and coma [60]. In all severe cases complications such as acidosis, haemoglobinuria, pulmonary oedema and hypoglycaemia can occur, further worsening the prognosis [60]. Sequelae following severe malaria, particularly cerebral malaria, are highly variable and can include neurological and developmental impairments [57,61,62] as well as the possibility of blindness, epilepsy and cerebral palsy [63].

In the presence of exposure-related functional immunity, individuals are more likely to experience low intensity infections as the immune system is better able to regulate parasite growth and replication, meaning infected individuals may not experience clinical signs and symptoms [64-66]. Although there are no strict diagnostic criteria for defining asymptomatic infection, the absence of fever or any other clinical symptom for at least a week, in the presence of a positive blood smear and/or PCR result is often used to define infections as asymptomatic [67,68]. It is notable that subpatent (submicroscopic) infections where the parasite density is below the threshold detectable by microscopy- thus requiring molecular techniques for detection- are also asymptomatic. Theoretical and empirical evidence indicates that such subpatent infections also contribute to the pool of infectious individuals sustaining transmission [69-71].

Despite the lack of acute symptoms, it has been suggested that chronic asymptomatic infection can have long-term morbidity consequences, including haemolytic anaemia [54]. Although the documented evidence of anaemia is higher in those experiencing a clinical attack, with anaemia positively correlated with parasite density [72], studies have shown that chronic low intensity infections are also associated with anaemia [73-75], with re-analysis of published data depicting a negative relationship between *P. falciparum* infection and Hb in preschool children and pregnant women [76]. The exact mechanism of anaemia in the case of asymptomatic infection

has been debated. While many studies refer to the direct destruction of the erythrocytes by the parasite and the pro-imflammatory mediator response during the blood stage of infection [54], the role of erythropoiesis (erythrocyte production in the bone marrow) inhibition in the development of anaemia in low intensity infections is more contentious. Several studies have found suppression of erythropoiesis associated with increased nitric oxide levels [77] and bone marrow suppression [78] in chronic infections, while others have suggested that in the case of low intensity chronic infections there is an adequate increase in erythropoiesis to respond to the degree of anaemia [79,80]. There has also been a suggestion of asymptomatic infection leading to a deficiency in iron absorption exacerbating microcytic and hypochromic anaemia [81] although this finding has not been replicated [82].

1.3 MEASUREMENT OF *P. FALCIPARUM* TRANSMISSION

Central to an understanding of the epidemiology of malaria and subsequent impact of interventions is intensity of malaria transmission, which determines exposure to inoculation, in turn contributing to the acquisition of immunity, subsequently influencing the age profiles of morbidity and mortality. Transmission of infectious diseases such as *P. falciparum* is measured by the basic reproduction number R_0, the number of new cases an infection generates upon introduction to a naive population [83]. Traditionally, vector based metrics such as the EIR (entomological inoculation rate, number of infective bites from infected mosquitoes per person per unit time) have been used to determine transmission in relation to malaria [84,85], whereby transmission is considered stable when the EIR is high and the vector population is largely irresponsive to minor environmental changes. As the EIR decreases, transmission becomes unstable and the vector population becomes highly susceptible to extrinsic conditions.

Logistical and ethical problems of measuring EIR through methods such as human landing catches have led to the adoption of parasite prevalence indices such as parasite rate (*Pf*PR), commonly measured in surveys globally, and annual parasite incidence (API) per 1000

population, obtained from routine health facility passive surveillance [13,86]. Smith et al [87] have developed a model to approximate the relationship between EIR and *Pf*PR, demonstrating that dramatic reductions in EIR are required to produce slight reductions in *Pf*PR.

*Pf*PR has traditionally been measured by microscopy, however, in the last two decades the use of RDTs to detect circulating *Plasmodium* antigen has offered a more operationally attractive method of measuring *Pf*PR in the field and health facilities [88]. However, with declining transmission, and increasingly low density, there is a need for the use of even more sensitive molecular methods of detection of infections, such as loop-mediated-isothermal amplification (LAMP) and nested polymerase chain reaction (PCR) [89]. However, these methods currently have operational and cost restrictions. A further consideration of *Pf*PR is that it is subject to problems such as the strong influence of seasonality on parasite density. More recently, the use of antibody immune responses, determined through enzyme-linked immunoabsorbant assays (ELISA), has been suggested as a more robust measure of transmission, with the construction of age-specific seroconversion rates proving a useful tool for determining historic transmission exposure and changes in transmission intensity over time [90]. The persistence of antibodies makes these serological measures stable despite seasonal influences that affect EIR and *Pf*PR, but constrain their use as a measure of current transmission [91].

While the original stable/unstable classification, based on EIR, has been important in the past, a more relevant scale for categorising transmission and classifying endemicity is one based on the more operationally feasible *Pf*PR (measured in 2-10 years as standard, often used in reference to a broader population group). Holoendemic (>75% $Pf\text{PR}_{2\text{-}10}$), hyperendemic (50-75% $Pf\text{PR}_{2\text{-}10}$), mesoendemic (10-50% $Pf\text{PR}_{2\text{-}10}$), hypoendemic (0-10% $Pf\text{PR}_{2\text{-}10}$) or low (unstable) transmission (<5% $Pf\text{PR}_{2\text{-}10}$), have been used to define priorities and action phases by the Global Malaria Eradication programme [92], and recently used by Noor et al [41] to document the change in risk of *P. falciparum* infection in the last decade, in relation to proportions of population in various endemicity classes.

1.4 *P. FALCIPARUM* INFECTION EPIDEMIOLOGY

The latest estimates from model-based geostatistics suggest that close to 800 million people were at risk of *P. falciparum* infection in 2010 [13]. However, the spatial distribution of this infection exhibits marked heterogeneity, where incidence and prevalence of infection are unevenly distributed across populations [93,94]. This non-random distribution of infection can be manifested at various scales, exhibiting global, regional, community, household and even individual-level heterogeneity [95,96]. *P. falciparum* transmission is subject to variation from numerous sources including ecological factors affecting vector density, dispersal, biting rate, lifespan and sporogonic rate; and human behaviour and personal protection factors influencing parasite virulence, host attractiveness, duration of infection and gametocyte carriage [94,95,97,98]. In the current environment of declining transmission, regions previously exposed to intense, seemingly homogeneous transmission, where the majority of individuals were exposed and infected at some point, are now experiencing moderate or low transmission whereby the variability in exposure and infection is more pronounced, with clusters and foci of transmission becoming apparent [99-102]. Such spatial heterogeneity in transmission is an important consideration when planning operationally effective control strategies, as the localised patterns of the human infectious reservoir have important implications for the potential impact of control measures, especially those aimed at transmission reduction [96,103]. These considerations will be covered later in the chapter.

1.4.1 Age profiles of disease burden

A key biological determinant of the epidemiology and burden of clinical disease is the development of exposure-related immunity, determined by prior exposure and is consequently strongly influenced by transmission intensity in the environment. Each infection episode survived is thought to confer additional antibody-mediated immunity and cross protection can be

gained from *Plasmodium* clones with similar variant surface antigens [52]. Therefore, with increasing age, exposure, and survived infection episodes, the immune system is better able to constrain parasite replication and parasite density, reducing the severity of symptoms. Thus, in regions of high transmission, a strong age profile is usually observed, with the majority of malaria-attributed deaths focussed in the first few years of life, with a rapid decline in cases of clinical and severe malaria over that period, as functional immunity is acquired and the future infective bites result in asymptomatic infection.

However, in regions of low transmission (where the exposure to infective inoculation is infrequent) the acquisition of immunity is slower and the morbidity age profile becomes less defined, leaving all ages at risk of uncomplicated or severe malaria [25,104,105]. Empirical evidence from studies of hospitalisations with severe malaria have shown support for the age profiles with increasing age of hospitalisation accompanying decreased transmission intensity [106] and theoretical and empirical evidence is gradually building of the changing age profiles as transmission declines [5,26]. On the other hand, a consistent pattern, regardless of transmission intensity [20] is that of *Plasmodium* infection prevalence, (*Pf*PR) which typically increases in the first two years of life, at which point it plateaus and slowly declines into adolescence and adulthood [19]. As a result school-aged children experience some of the highest age-specific rates of *Plasmodium* infection [19,20,28,107], regardless of transmission intensity (Figure 1.3), with an estimated 212 million children aged 5 to 14 years classified as at risk of *P. falciparum* infection in 2010 [13]. Such infection carries with it not only a direct risk of morbidity and mortality, but may also have secondary health, developmental and educational consequences for the children, in addition to sustaining transmission in the wider community.

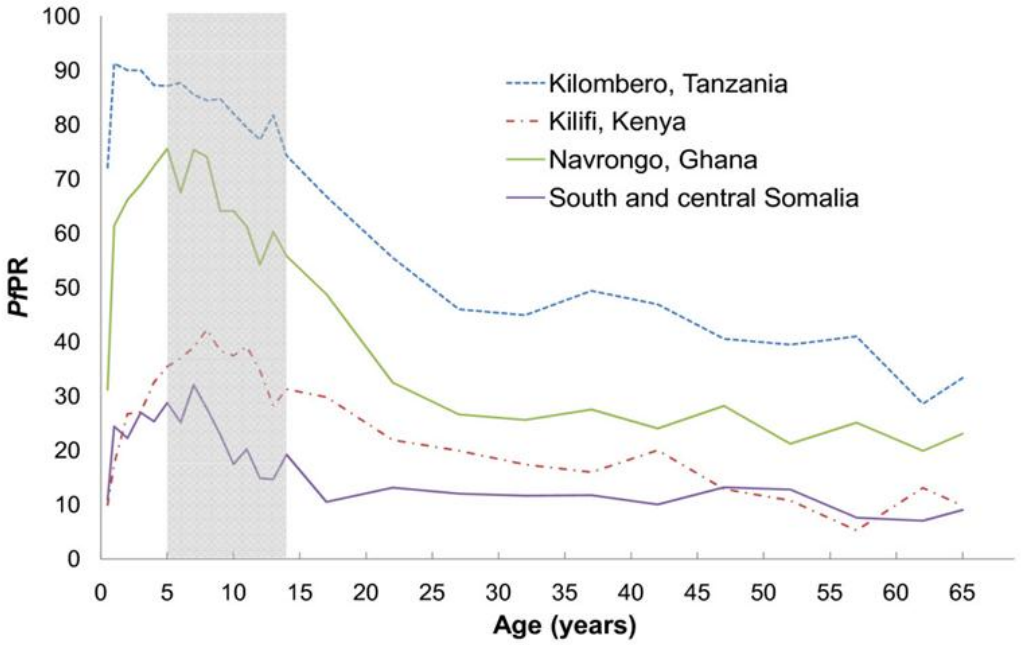


Figure 1.3: The relationship between age and *Plasmodium falciparum* parasite rate (*Pf*PR). Taken from Brooker et al (2009) [20]. Each line represents the age profile for the populations living in varying transmission settings, data from Smith et al 2007 [19] used. South and Central Somalia *Pf*PR is 24.6%, Kilifi *Pf*PR is 37.5%, Navarongo *Pf*PR is 66.9% and Kilombero *Pf*PR is 83.7%. The grey box represents the typical age of primary school children.

1.5 *P. FALCIPARUM* INFECTION IN SCHOOL CHILDREN

1.5.1 Health burden of *P. falciparum* infection in school children

In spite of the degree of immunity often acquired by the time a child reaches school age, malaria remains an important source of morbidity and mortality in school-aged children in malaria endemic regions [108], with the risks even greater in unstable transmission regions [109]. Clarke *et al*. observed a six-fold greater incidence of clinical attacks in school children in a region experiencing unstable versus stable transmission in Kenya. After accounting for the differing lengths of transmission period in the two regions this equated to an estimated incidence of 51.2 per 100 children per year and 25.6 attacks per 100 children per year, in unstable and stable regions respectively [110]. While mortality due to malaria in this age-group remains low in relative terms, it is estimated that up to 16.5% of adolescent deaths in Africa are related to malaria [111], and based on population projections for 1995, model-based estimates gave a median mortality

rate of 2.17 (1.64-2.86) per 1000 population for children aged 5-9 years living in stable endemic transmission regions in comparison to 9.4 (7.1-12.4) in the 0-4 years age range [63].

Anaemia is a significant public health problem in school-age children throughout Africa and Asia [112] but the complex multifactorial aetiology of anaemia makes establishing the causal chain in different settings complicated. However empirical research has found associations between malaria parasitaemia and anaemia in school children infected with low density asymptomatic infection [75,113-115], with population-based modelling supporting the relationship, demonstrating prevalence of anaemia strongly related to parasite prevalence [116]. The presence of co-infection with soil transmitted helminths (STH) or schistosomes has been documented to further increase the odds of anaemia [117]. Despite accounting for potential confounders where possible, the cross sectional nature of this evidence limits the conclusions to be drawn on the relative contribution of malaria parasitaemia in regions and age-groups where polyparasitism [117-119] and undernutrition [120] are particularly prevalent [115,121,122]. However, a cohort study of 65 children in Ghana found school children with asymptomatic *P. falciparum* infection had significantly lower haemoglobin (Hb) concentrations compared to uninfected counterparts, and among this group there was a significant reduction in Hb over the 4 month duration of infection [74], although the small numbers followed must be considered.

Additionally, an increasing body of evidence documents improvements in haemoglobin concentration as a result of effective malaria control interventions [33,40,123,124] with distribution of insecticide-treated nets (ITNs) to schoolgirls aged 12-13 years in western Kenya, associated with a 0.34g/dL (95% confidence interval [CI] 0.02-0.66) increase in mean Hb [125,126]. A review by Korenromp *et al*. quantified an average increase of 0.76g/dL (95%CI: 0.64-0.81) across 29 studies conducted in endemic malaria settings following 1-2 years of malaria control interventions [127], although these studies were mainly conducted in children under 5 years rather than school-aged children. The relationship between parasitaemia and nutritional status has also been debated with some evidence of a relationship between the two [128]

especially in relation to acute malnutrition in the form of wasting [129]. Cohort study findings indicated a negative change in body mass index (BMI) with increasing parasite density over the transmission season in Kenyan adolescent and adult males [130] and trial evidence from Nigeria in 1954 indicated suppression of parasitaemia was related to an increased growth rate in school-children [131].

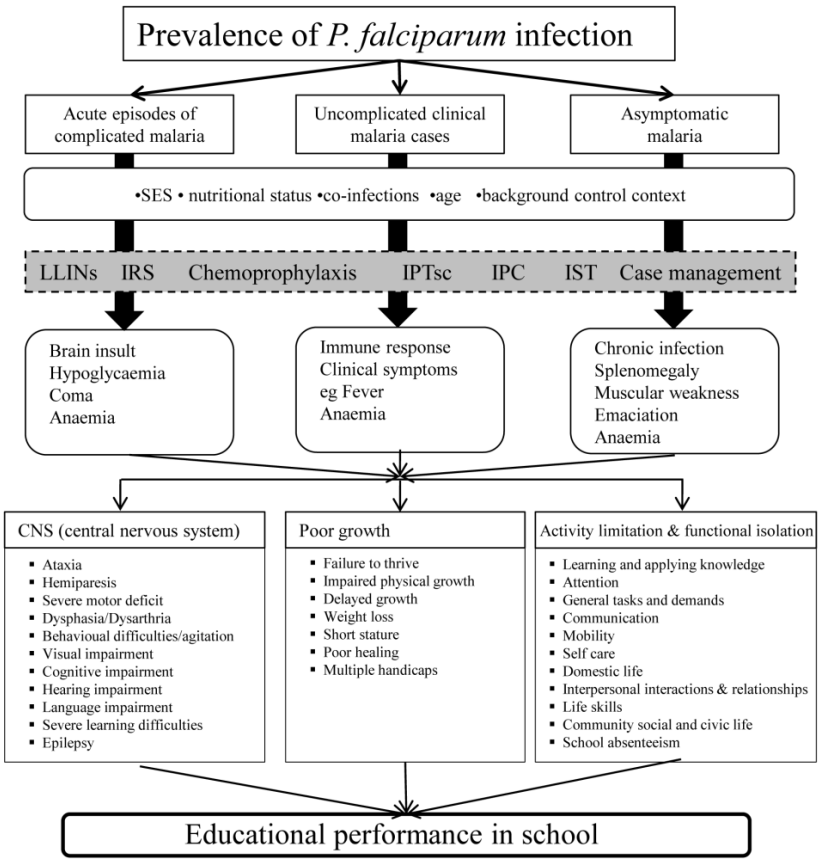
1.5.2 Cognitive and educational burden

The negative impacts of severe, particularly cerebral, malaria on cognitive constructs such as attention, working memory, learning and sensory motor impairments are well established. Pooled evidence of six studies found that of 1552 survivors of cerebral malaria, 16% suffered cognitive deficits on discharge [57], and 24% of Kenyan children followed nine years after an episode of severe malaria had one or more cognitive impairment against 10% of control children [61,132]. Boivin *et al*. observed a 3.7 fold (95%CI: 1.3-10.7 fold) increase in risk of cognitive deficits in children (aged 5 to 12 years) six months after hospitalisation for cerebral malaria, when compared to community controls; with number of seizures and duration of coma significantly associated with cognitive deficits in the group [62]. Many of these studies documenting the relationships between severe malaria and both cognition and longer term academic performance suffer from small sample sizes. However, the consistent findings of strong relationships and large effect sizes provide robust evidence for such associations.

The evidence concerning cognitive and learning deficits following uncomplicated clinical attacks in school children is less well defined. The association between malaria, and other infections with immune system activation, may be suggestive of short term effects on cognitive and learning abilities, but the direct evidence of this is limited [133]. A matched case-control study in Sri Lanka demonstrated children aged 6 to 14 years, presenting with malarial fever, performed significantly worse on tests of maths and language than comparison groups with non-malarial fevers, both at time of presentation and two weeks later, although scores had improved after two

weeks [134], suggestive of short-term negative effects. The view that repeated clinical attacks may cause longer term problems for learning in school has been postulated, and prospective study evidence also from Sri Lanka indicates repeated clinical malaria episodes as a predictor of poorer education performance, with children suffering five or more attacks over a six year period to score significantly (15%) lower than children experiencing less than three attacks [135]. It must be mentioned that these infections included both *P. falciparum* and *P. vivax* infections. However, the findings of Boivin *et al.* in Uganda indicated no increased risk of cognitive impairments in 54 children aged 5-12 years who had experienced an episode of uncomplicated malaria when compared to 89 community controls when assessed six months after discharge [62]. Figure 1.3 demonstrates the hypothesised causal pathway of *P. falciparum* infection on overall school performance.

Figure 1.4: Hypothesised pathways of *P. falciparum* infection on school performance (adapted from Thuilliez et al [136]). The pathways of severe (complicated), uncomplicated and asymptomatic malaria are related to their potential health consequences and subsequent possible developmental, behavioural and cognitive impacts.



LLINs –Long-lasting insecticidal nets, IRS – indoor residual spraying, IPTsc – intermittent preventive treatment in school children IPC – intermittent parasite clearance, IST – intermittent screening and treatment

Relatively few studies have documented a direct association between asymptomatic *P. falciparum* infection in school children and cognitive impairments [137]. However the evidence base is growing, with initial evidence provided by a matched study of parasitaemic (those with between 750-5000 parasites/µl) and control schoolboys in the Yemen [114] whereby performance on fine motor function tests was slower in the parasitaemic group. Moreover, a recent study in a high transmission setting in Uganda, where the overall prevalence of *P. falciparum* was 30.1%, and where 61% of parasitaemic children had parasite densities <1000 parasites/µl found evidence of impaired cognitive performance in those infected. Lower scores were observed in tests of sustained attention (adjusted mean difference (AMD): -1.6 (95%CI: -2.40 to -0.81)) and abstract reasoning (AMD: -0.6 (95%CI: -1.01 to -0.21)) in those children with parasitaemia, with evidence of a negative dose response relationship between parasite density and sustained attention scores [138]. Evidence from a longitudinal study in a high transmission setting of Mali also documented a dose response between parasite density and cognitive performance [136] with both clinical and asymptomatic malaria associated with significantly lower cognitive scores. Children who experienced a clinical attack scored between 0.37 and 0.54 less than those who did not, and children with asymptomatic infection scored between 0.108 and 0.186 lower than uninfected children [136], although these differences were relatively small in relation to the standard deviation. Despite this evidence coming from cross-sectional studies where a causal link cannot be established, increasingly evidence is indicating that malaria control in high transmission settings can have beneficial effects on sustained attention [33,139]. *Plasmodium* infection has been linked to grade repetition in Mali [140] and a relationship between malaria and absenteeism has been documented in a number of randomised trials, whereby malaria control has led to reduced absenteeism over control groups [42,124,141]. While overall, evidence suggests between 3 and 8% of all-cause school absenteeism can be attributed to malaria in endemic areas [108,109,142], this leaves a large proportion to be explained by alternative causes.

Whether observed associations between parasitaemia and cognition are mediated by anaemia and a reduced oxygen carrying capacity is unclear. An important consideration of the associations between *P. falciparum* infection with both anaemia and with cognition and educational performance is the potential confounding of other factors, particularly helminth infections and undernutrition. All of these factors commonly coexist and are all inextricably linked to socioeconomic status (SES), making careful consideration of confounding essential when investigating the impact of a malaria control intervention on these constructs.

1.5.3 The significance of school children as contributors to the parasite reservoir

Asymptomatic infections typically go undetected and untreated, maintaining a crucial reservoir of infection for onward transmission [24]. Model-based estimates have suggested substantial infection periods of up to six months [87] and modelling derived from multiple observations of infection in Ghanaian children estimated a duration of 194 days (95% CI: 191-196), longest in children in the age group 5-9 years when compared with infants and older children [143]. Chronic low density infections have been found to persist through the dry season in highly seasonal transmission settings [144], acting as a source of infection to mosquito vectors in the following rainy season [102]. Bousema *et al*. demonstrated that a large proportion of asymptomatic children develop gametocytaemia, and although the duration declines with increasing age [22], school-age children still constitute a significant reservoir of infection given the high prevalence of low intensity parasitaemia in this age group [23,24]. As a result, it has been argued that interruption of transmission in communities and hence progress towards malaria elimination, will only be achieved if infection rates can be substantially reduced among this age group.

1.6 MALARIA PREVENTION AND CONTROL STRATEGIES

A number of prevention and control strategies exist to tackle the burden of malaria. While traditionally the approach has been focussed on case management and targeting of interventions at groups at higher risk of mortality and morbidity, namely those under 5 years old and pregnant

women [18], in 2008 there was a call for universal coverage of interventions in a bid to move towards transmission reduction. Following on from this, WHO in 2012 launched the *T3: Test. Treat. Track Initiative*, in which the provision of diagnostic testing and treatment, and the role of surveillance and targeting clusters of infection was emphasised [17]. The mainstays of malaria control can be broadly characterised as vector control: through use of long-lasting insecticidal nets (LLINs) and Indoor Residual Spraying (IRS), and treatment based approaches including case management and intermittent preventive treatment (IPT). In recent years, strategies based on screening and treatment of infection have emerged and are under investigation in endemic as well as elimination-focussed settings. Community-wide control strategies covering school children as members of the wider community are summarised before examining more closely prevention and control interventions delivered through schools.

1.6.1 Vector control strategies

The protective efficacy of ITNs or LLINs has been demonstrated repeatedly across all transmission settings since the 1980s [145-147]. The majority of studies looking at protective efficacy against mortality and clinical malaria episodes have focussed on children under five years. Evidence from an intense transmission setting in western Kenya demonstrated a 16% protective efficacy of ITNs for mortality among children aged 1-59 months [148] , with meta-analyses supporting these findings, demonstrating all-cause mortality protective efficacy in this age group of 17% and 18% [147,149]. However, a cohort study conducted in Kenya during a two-year period of rapid scale up in net use demonstrated a protective efficacy of 44% (95% CI: 4-67%) in relation to all-cause mortality in children aged 1-59 months [150]. Pooled analyses of four trials also associated sleeping under an ITN with a 51% (95% CI: 0.46-0.54%) reduction in uncomplicated malaria in children [149]. A further pooled analysis using data from multiple malaria indicator surveys demonstrated a relative reduction of 24% (95% CI: 1-42%) of parasitaemia in children under five years sleeping under an ITN, with extensive heterogeneity of protective efficacy observed between surveys [151].

There is, however limited evidence from randomised trials of the efficacy of ITNs specifically in older children, with one trial showing significant reduction in anaemia in 12-13 year old girls in western Kenya, but not older girls, possibly due to increased functional immunity in the older girls, however, no impact on clinical malaria, parasite prevalence or all-cause morbidity, was detected [125]. A 25% reduction in all-cause mortality was found in children aged 1-9 years in treated villages, one year after initial introduction of ITNs in 1992 as well as decreased parasitaemia observed [146]. Furthermore cross sectional evidence has commonly demonstrated decreased odds of *P. falciparum* infection in school-aged children sleeping under nets compared with those not [28], however variation in protective efficacy observed varies by transmission intensity [152,153] and age [154].

In relation to coverage of ITNs, Kenya, similarly to other countries, has seen a substantial increase in distribution of ITNs through mass campaigns in the last decade, with overall coverage increasing from 7.1 to 67.3% between 2004 and 2006 [155]. Despite this, extensive variations in coverage and use have been documented within and between countries [151], and equity of coverage by age remains low. School-age children consistently demonstrate systematically lower rates of coverage and usage across all settings [27,28] indicating that even within households there is variation in coverage and use of personal protection. Furthermore, observational evidence has suggested that of all age groups school children are most likely to sleep under poorer quality nets [156].

Historically, indoor residual spraying (IRS) with dichlorodiphenyltrichloroethane (DDT) played an important role in eradication and elimination programmes in the 1940s and 50s [157], and although use of DDT has begun to decline, IRS with alternative insecticides such as pyrethroids has remained in use in a number of malaria control programmes [158-160]. Although shown to be effective as a standalone tool, the contribution of IRS to the patterns of reducing transmission must be considered within the context of ITN/LLIN use, whereby evidence has demonstrated

that a combination of IRS with ITNs is associated with a reduced prevalence of infection over and above each strategy alone [161]. In relation to coverage of IRS, as well as providing direct benefits at the household level, indirect benefits have been shown in communities where coverage was high despite the house not being sprayed directly [162]. However a recent study has shown that in a region of high LLIN coverage, IRS does not appear to provide additional benefits in relation to either clinical malaria or vector density [163]. Furthermore, the increasing deployment of pyrethroid insecticides for IRS, as well as in treating LLINs, has resulted in the development of pockets of resistance in key malaria vectors such as *Anopheles gambiae* [164]. The increasing reports of emerging physiological resistance in *An. gambiae* from experimental trials have significant implications for the future impact of these two important malaria control methods [165,166]. Additionally, the development of behavioural resistance in response to wide-scale exposure to insecticide is likely to further compromise the effectiveness of IRS and LLIN strategies [167].

1.6.2 Antimalarial drug regimens

A number of chemotherapeutic compounds for the treatment of malaria have been utilised over the last century. Following the long-standing use of the natural compound, quinine [168], the first drug to be widely used was the synthetic compound chloroquine (CQ), a 4-aminoquinoline which acts by interfering with parasite haem detoxification [169]. However, due to large-scale use and the slow elimination of the compound, by the 1990s, *P. falciparum* resistance to CQ had spread and sulfadoxine pyrimethamine (SP) was recommended for the treatment of malaria in CQ-resistant regions. Sulfadoxine is a slowly eliminated sulfonamide (elimination half-life of four to nine days), which acts as a competitive inhibitor of plasmodial dihydropteroate synthase, a key enzyme in the synthesis of folic acid in the parasite [170]. It is administered in a fixed dose formulation with pyrimethamine, which acts on schizonts and pre-erythrocytic forms of the parasite by inhibiting dihydrofolate reductase, blocking synthesis of the nucleic acids in the parasite [171,172].

Substantial advantages of SP include high tolerability and the low-cost, single-dose treatment regimen of the drug. Furthermore, the relatively long-acting nature of SP means that in addition to treatment, it can also provide post-treatment prophylaxis for approximately one month [173]. However, again, intensive use and relatively slow elimination of SP resulted in the spread of plasmodial resistance through the acquisition of mutations in *dhps* and *dhfr* for sulfadoxine and pyrimethamine respectively [172,174]. This resistance rendered both SP monotherapy and SP in combination with other antimalarials such as amodiaquine (AQ), a 4 amino-quinoline with similar properties to chloroquine [175], relatively ineffective across much of Southeast Asia, eastern and southern Africa by 2000.

However, the emergence of artemisinin drugs, first extracted from the Chinese *Artemisia* plant in the early 1990s, provided an extremely effective alternative [176,177]. By 2006, WHO guidelines stated that ACTs should be the first line treatment for uncomplicated malaria [170] and by 2009, many malaria control programmes in eastern and southern Africa were withdrawing non-ACTs such as SP-AQ from use for mass treatment or prophylaxis, although it remained in use for IPTp, as discussed in section 1.6.3.

Artemisinins are fast-acting, broad-activity schizonticides, active against all stages of the asexual parasites, with the additional benefit of being gametocidal, enabling effective reduction of transmission [178]. Although they induce rapid parasite clearance with an estimated 100-1000 fold parasite reduction per asexual cycle [170], the artemisinin compounds have a short elimination half-life of approximately one hour. Thus to extend the antimalarial action and reduce the potential for the development of resistance it is necessary to administer in combination with an antimalarial with a different mechanism of action and a longer elimination half-life. The result of such a combination is that any parasites not rapidly cleared or resistant to the artemisinin can be targeted by the slowly eliminated compounds [179]. Artemether-lumefantrine (AL) is the combination currently recommended as first-line therapy in many countries, administered as a six-dose regimen over three-days. Artemether is biotransformed to the active dihydroartemisinin

in vivo where it has a peak plasma concentration three hours after administration and produces a rapid reduction in parasite numbers through damage to the parasite via enhanced free radical mechanisms [177]. Artemether (20mg) is co-formulated with 120mg lumefantrine, a compound similar in structure and action to the quinine and mefloquine group of antimalarials. It is absorbed more slowly than artemisinin and has a peak plasma concentration approximately 10 hours after a single dose and a longer elimination half life of three to six days [177]. An alternative combination of dihydroartemisinin piperaquine (DP) has more recently been introduced and recommended by WHO as of 2011. Advantages of DP over AL include the once-daily dose of DP for three days and the longer elimination half-life of piperaquine (compared to lumefantrine) of 48 days [180,181].

Despite high treatment efficacy and tolerability, the rapid elimination of artemisinin derivatives means the post-treatment prophylaxis conferred by ACTs is usually dependent on the partner drug [182]. A review of studies comparing AL and DP in relation to post-treatment prophylaxis found DP to reduce risk of re-infection by 79% and 44% at days 28 and 42 respectively in comparison to AL. No difference was observed in treatment failure rates [181]. ACTs are not currently recommended for mass presumptive treatment due to resistance concerns, especially with issues of compliance to a multiple-day regimen, unlike the single dose-regimen of SP. Moreover, a recent trial into comparing a two-day (condensed) regimen of DP with the standard three day was stopped due to concerns over delayed repolarisation in the heart [183].

1.6.3 Treatment based strategies

Prompt and efficient case management is recognised as an essential aspect of any control programme. Increasing access to effective diagnosis and treatment is of prime importance with expanded use of RDTs in health facilities and wider availability of ACTs (first-line case management drug across SSA) cited as the Test and Treat components of the *T3 Initiative*. To expand coverage, there is increasing deployment of diagnosis and treatment using RDTs across the spectrum of health providers covering the public sector and the formal and informal private

sector [184] as well as successful programmes of community health workers (CHWs) trained to implement case management in communities [185,186].

An additional treatment approach is intermittent preventive treatment (IPT) involving periodic administration of a full therapeutic/prophylactic dose of antimalarials, usually delivered to certain high risk groups [187]. The most widespread use of IPT has been in pregnant women, IPTp, delivered at ante-natal clinic visits as policy in many African countries, aimed at clearing placental malaria and therefore reducing maternal anaemia, low birthweight and neonatal mortality. Pooled analyses from 32 countries associated IPTp with sulfadoxine-pyrimethamine (SP) with a 25% (20-29) reduction in the odds of low birthweight and decreased risk (protective efficacy: 20% [10-30]) of neonatal mortality [188]. With a considerable proportion of first pregnancies occurring in adolescent girls across SSA [111], consideration of IPTp in relation to school-age girls is not unwarranted. IPT for infants (IPTi) delivered to children aged between 3 and 24 months in high perennial settings alongside the expanded programme of immunization (EPI) has been trialled in various countries including Ghana and Tanzania, with findings from six studies all showing significant protective efficacy against uncomplicated malaria, with a range of 20.8% to 59.4% [189].

The strong empirical and theoretical evidence of the beneficial impact of IPT delivered to children under five years during the short intense transmission season in West Africa [190,191], has led to seasonal malaria chemoprevention (SMC) being recommended by WHO for regions of highly seasonal malaria transmission. Here a complete dose of amodiaquine plus sulfadoxine-pyrimethamine (AQ-SP) is provided for children aged 3-59 months during the high transmission season to clear parasitaemia and prevent clinical malaria [192]. A maximum of four doses is provided across the malaria season to retain therapeutic concentrations of the drugs in the system during the period of greatest risk, with delivery of IPT integrated into existing community-based programmes. There is now increasing interest in extending this malaria control strategy to include

older children (up to ten years) using early childhood development (ECD) centres in schools as a delivery route.

1.6.4 Interventions under investigation

With declining transmission and increasing awareness of resistance and the need to preserve the efficacy of ACTs, there are moves away from mass presumptive treatment strategies and towards methods of active case detection of asymptomatic as well as symptomatic infections. Additionally, in settings of low and moderate transmission, exhibiting pronounced heterogeneity in risk of *Plasmodium* infection with focal transmission, targeted interventions are increasingly preferred. Reactive case detection is one such strategy, whereby the individuals living within a specified radius of detected index cases are reactively screened for parasitaemia. In both Swaziland and Zambia additional cases above and beyond passive detection at health facilities were detected using this method [193,194], but as yet there is no evidence on the potential impact this strategy could have on transmission. In the case of reactive screen and treat, index cases have been clinical cases passively detected at health facilities. However in approaching elimination, it has been argued that this will be inadequate for detecting all low density infections in the community and proactive case detection is currently re-emerging as a favoured strategy [195]. Proactive case detection, the screening of high risk populations and treatment of those positive, was used for the eradication campaigns of the 1960s [196] and more recently in Brazil [197] and the screening of communities identified as experiencing high transmission will likely become important again. Interventions such as community mass Screen and Treat (MSaT) campaigns using RDTs and ACTs [107,198] will be discussed in more detail in Section 1.7.5. Both MSaT and reactive case detection fall in line with the T3 (Test Treat Track) initiative of WHO, with additional benefits of providing data for surveillance. Considerations such as screening tool and coverage are central to the success of these strategies.

1.7 SCHOOL-BASED MALARIA CONTROL

Given the recognised importance of *P. falciparum* infection to school populations in relation to morbidity, education and onward transmission, global efforts are underway to provide malaria prevention and control services through schools, in addition to the coverage provided by the community-wide prevention and control initiatives discussed above [34], from which school children also benefit. With ministries of health and education increasingly looking to incorporate malaria control into their school health and nutrition programmes [199], a number of school-based approaches have been investigated.

1.7.1 Delivery of prevention interventions and knowledge through schools

The ever increasing primary school enrolment across Africa [200] makes schools logistically effective channels for delivery of interventions to school children and surrounding community members. The potential importance of schools for delivery of preventative methods is recognised by WHO's latest recommendations for achieving universal coverage, which specifically cite schools as important channels for distribution of ITNs [201]. A study in 1988 of the distribution of bed nets to boarding school students in an intense transmission region of Kenya led to a 97.3% reduction in attack rates, over children not provided with nets [202]. More recently, the delivery of LLINs through schools in a low unstable transmission setting in Tana River, in support of the national distribution campaigns, found that despite significantly higher net-use reported in the intervention group following school-based distribution, this did not translate to reductions in *P. falciparum* infection or anaemia (Gitonga et al. unpublished). These contrasting impacts observed are likely primarily due to the differential transmission intensities, but also mention must be made of the fact that early studies of bed-net distribution through schools were conducted within in a context of absent or highly limited net-use, whereas current studies are conducted in a context of variable net coverage from health facility and community distribution campaigns. Despite this, school-based distribution has benefits for increased coverage and use.

Evidence indicates school-based malaria education can be associated with positive changes in children's knowledge and practices [203] and that children can act as agents of health education in their communities. For example, programmes of school child-conveyed malaria education in Lao PDR and Ghana demonstrated increased knowledge among community members [204,205]. Admittedly, the before-after intervention design used by these studies, assessing knowledge and behaviour change through self-reported questionnaires, often with small study numbers and no control groups, does leave them subject to bias. However, preliminary evidence from a randomised controlled trial in a high seasonal transmission setting in Mali more recently demonstrated that a school-based net education programme in support of a universal LLIN distribution campaign led to significantly higher net use in the intervention group than the control group, with the difference sustained throughout the dry season [139], although again, no consequent impact on *P. falciparum* or anaemia prevalence was seen.

1.7.2 School-based case management

A number of studies have investigated the prompt case management of clinical malaria in schools through presumptive treatment of uncomplicated malaria by school teachers. The use of a diagnostic algorithm followed by chloroquine treatment, in Ghana [206] and Tanzania [207], demonstrated teachers were capable providers of treatment. However, the absence of a comparison group, or measurement of outcomes, in either study, limited the evaluation of effects of this approach on health or schooling. The introduction of pupil treatment kits (first aid kits including SP for presumptive treatment of malaria by teachers) in Malawi was associated with an apparent drop in malaria-specific mortality rates from 1.28 to 0.44 deaths per 1000 student years when compared with three years before the intervention [36]. Although subsequent retrospective cohort analyses showed no evidence of a reduction of all-cause mortality in schools with PTKs over matched controls, significant reductions in absenteeism, grade repetition and drop-out were found [208]. Changes in malaria treatment drug policy and the requirement of clinical diagnosis prior to treatment with ACT in children over five years, has necessitated a new approach to school-based case management, with a current study evaluating the impact of a

school-based programme of malaria diagnosis and treatment by teachers using RDTs and ACTs, on school attendance in Southern Malawi [209]. These case management strategies operating in schools, although useful for tackling malaria-related morbidity in school children, have little or no impact on reduction of transmission as the asymptomatic reservoir of infection is not addressed.

1.7.3 School-based chemoprophylaxis

In order to reduce transmission and move towards elimination, infection, as opposed to simply cases of malaria, must also be treated. This has traditionally been done through mass drug administration approaches in schools. Evidence from randomised controlled trials of chemoprophylaxis (weekly or daily doses of antimalarial treatment), in school children dates back to the 1950s. Chemoprophylaxis has been demonstrated to significantly reduce parasitaemia [37,39,42,202,210,211] in intervention versus control groups, although protective efficacy was as low as 50-70% in some studies, despite reportedly consistent antimalarial prophylaxis [37,210]. Studies have also reported significantly lower attack rates of clinical episodes [38,124], beneficial impacts on nutritional status [131], reduction in absenteeism and increased educational achievement [42,124] in intervention groups receiving chemoprophylaxis when compared to control groups. The majority of these studies did not assess Hb concentration as an outcome, but in Sri Lanka, chemoprophylaxis targeting both *P. falciparum* and *P. vivax*, was associated with improved Hb [124]. With the exception of the recent study in Sri Lanka, all of these chemoprophylaxis studies were conducted in high transmission settings in Africa over twenty years ago, and only in a few schools (between one and four), with small study populations, leading to concerns of sufficient power. Despite the beneficial impacts found, chemoprophylaxis has never been widely implemented due to unsustainable costs and concerns over encouraging drug resistance [210,212].

1.7.4 Intermittent preventive treatment of school children

In recent years, intermittent preventive treatment among schoolchildren (IPTsc), based on the approach of IPTp and IPTi interventions described in Section 1.6.2, has been evaluated. The first cluster randomised trial conducted in a high transmission setting in Kenya demonstrated that providing SP in combination with amodiaquine (AQ), once a school term, to all school children regardless of (unknown) infection status, reduced the risk of anaemia by 48% and resulted in significant increases in sustained attention scores; mean difference 7.74 (95% CI: 2.83-10.65 P=0.005) [33]. A subsequent trial in a high seasonal transmission setting in Mali found a benefit of IPTsc using SP/AS (artesunate) or AQ/AS, administered during the rainy season, in reducing anaemia and asymptomatic parasitaemia, as well as incidence of clinical malaria [40].

Due to the continued susceptibility of the *Plasmodium* parasites to SP therapeutic compounds in West and Central Africa, such mass drug administration (MDA) approaches remain viable. Recent empirical research observed that clearance of parasites with one full dose of SP/AS at the end of the transmission season in Mali, led to a significant reduction in parasitaemia throughout the dry season, with a highly beneficial impact on haemoglobin concentration and on sustained attention scores in the intervention over control group [139]. Additionally, a trial is underway in the Democratic Republic of Congo investigating IPT using SP compared to SP-piperaquine and to an untreated control group [213]. However, the extensive resistance to SP in eastern and southern Africa, the subsequent withdrawal of the primary drugs for IPT in 2009, and the prohibited use of ACTs such as AL for mass presumptive treatment, has meant that the use of IPTsc as originally evaluated is not currently appropriate. The use of dihydroartemisinin-piperaquine (DP) for IPTsc in Uganda has been found to be an efficacious, safe option for reduction in risk of parasitaemia 42 days following treatment, presenting a potential option for IPTsc in the future [214]. Recent empirical evidence showed a substantial impact of IPT with DP given every term and given every month on asymptomatic parasitaemia, with monthly treatment also reducing the incidence of clinical malaria by 96% and the prevalence of anaemia by 40% [35]. Currently the status of DP as the second-line treatment in many east African countries has

precluded its up-scaled use for mass presumptive treatment, but this may change in the near future. However, these challenges have necessitated research into alternative strategies for malaria control in schools both in terms of beneficial impacts on health and education and reducing ongoing transmission in these malaria endemic regions of SSA.

1.7.5 Intermittent screening and treatment of school children

Intermittent screening and treatment (IST) for malaria has been proposed as a possible alternative strategy to IPT for tackling asymptomatic infections. IST involves periodic screening for *P. falciparum* infection, using a rapid diagnostic test (RDT) by a public health worker, with those found to be positive for *P. falciparum* parasitaemia (with or without symptoms) treated with a full regimen of antimalarial treatment. This strategy is in line with the requirement for parasitological confirmation of infection prior to treatment with ACTs, hence reducing the over-use of antimalarial drugs and subsequent threat of resistance [17]. There is an expanding body of theoretical and empirical research into appropriate uses and settings for screening and treatment. Research evidence from a moderate stable transmission region in Ghana found IST during antenatal clinic visits for pregnant women to be equally efficacious to the counterfactual SP-IPTp whether using SP or AQ-AS for treatment [215]. Qualitative evidence suggested high user and provider acceptability of such a strategy [216,217].

Recent modelling work evaluating the impact of population-based screening and treatment (mass screening and treatment, MSaT) as a component of a suite of malaria control interventions indicated high coverage of twice yearly rounds of MSaT plus indoor residual spraying in addition to intense scale-up of LLINs could significantly reduce *P. falciparum* prevalence in high transmission settings and reduce prevalence to below 1% in moderate to low transmission settings [107]. Further modelling work suggested that while such IST campaigns would have the greatest impact in high transmission settings, high rates of reinfection would require continued regular high coverage campaigns to sustain the gains, whereas in low transmission settings the

impact gained from a single round of IST could be sustained for up to three years [198]. Disappointingly however, a recent evaluation of community-wide IST performed in Burkina Faso, where transmission is intense and highly seasonal, found three successive screen and treat campaigns prior to the rainy season resulted in no significant reduction in clinical attacks in the following rainy season [218] and no decreased prevalence of *P. falciparum* infection the following dry season, despite substantially lower *P. falciparum* prevalence in the intervention group at screening rounds two and three at monthly intervals [219].

1.8 EVALUATION OF MALARIA CONTROL INITIATIVES

The prevailing context in which a new intervention is implemented is of crucial importance to the subsequent impact. Rigorous evaluation of impact of any intervention involves examination of the difference in outcome in the groups with and without the intervention [220] with six key principles of successful evaluation of impact outlined as: (i) considering the causal chain, (ii) the use of a credible control, (iii) understanding the context, (iv) anticipating heterogeneity (e.g. in sample populations, implementation, impact), (v) rigorous analysis, and (vi) employing mixed methods [221]. In particular, the concept of heterogeneity is an important consideration when evaluating the impact of any intervention.

1.8.1 The concept of heterogeneity in impact evaluations

The simplest definition of heterogeneity is the presence of variation [222], however there are multiple perspectives from which to view the concept of heterogeneity. For the purposes of this thesis, heterogeneity will be considered in several contexts. Firstly, heterogeneity in risk whereby distribution of infection is aggregated within the host population, where a minority are frequently and heavily infected, while the majority remain free of infection [93,223]; this can take a spatial dimension (spatial heterogeneity) relating to the variation of values in space [222]. Finally

statistical heterogeneity is defined as variation in intervention effects between studies or subgroups within studies beyond that which would be expected by chance [224,225].

The key sources of statistical heterogeneity in impact can be classified into non-random variability in: baseline characteristics of populations, underlying context (for instance concurrent programmes), coverage of and compliance to the intervention, and fidelity of the intervention process or tool. All these factors can vary at different scales, ranging from the individual through the household to the community and from the small spatial scale to large spatial scales. A challenge in the current climate of declining transmission is the rapidly changing malaria epidemiology, with increasingly marked heterogeneity in risk of *Plasmodium* infection apparent across various scales [41,226,227]. With this comes the need to assess critically the impact of an intervention while also considering the potential influence of underlying variation in transmission, even at local scales [228]. The remainder of this chapter will address the importance of identifying sources of heterogeneity at various scales and their possible influence when considering the impact, process and applications of interventions.

1.8.2 Heterogeneity in transmission

Heterogeneity is frequently found in the investigation of parasitic diseases, where incidence and prevalence of infection and clinical disease are not evenly distributed across populations [93,94]. In relation to malaria, this non-random spatial distribution of infection can be manifested at various scales, exhibiting global, regional, community, household and even individual-level heterogeneity [95,96]. Large-scale school surveys have been used to provide an operationally efficient method of depicting such variation [229,230]. Figure 1.5 uses school surveys to depict the extent of variation in *Pf*PR between and within countries in East and West Africa.

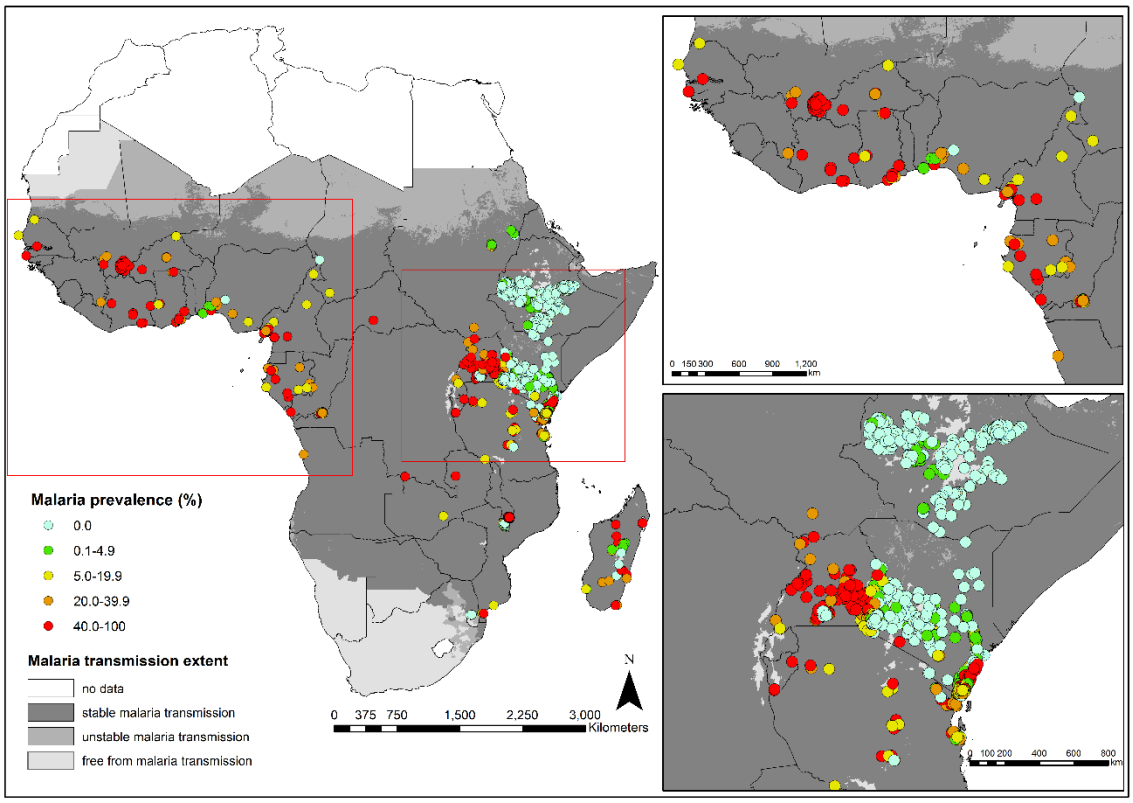


Figure 1.5 Heterogeneity in prevalence of *P. falciparum* infection across East and West Africa, as depicted by 1280 school surveys. Points mapped using data accessed from the Malaria Atlas Project (www.map.ox.ac.uk) with additional data from Kenya [229,231] , Mali [139], Malawi [232] and Ethiopia [230].

P. falciparum transmission is subject to variation from numerous sources that exert influence over vector density, dispersal, biting rate, lifespan and sporogonic rate as well as parasite virulence, host attractiveness, duration of infection and gametocyte carriage [233]. The elements contributing to heterogeneity in transmission can be largely grouped into ecological and environmental factors, human and behavioural factors and individual and intrinsic host factors [94].

1.9 SOURCES OF HETEROGENEITY IN TRANSMISSION

1.9.1 Environmental determinants of heterogeneity in transmission

Spatial heterogeneity in risk of *P. falciparum* infection is largely a function of ecological heterogeneity (variation of environmental factors in space and time). Large-scale trends in ecological factors such as temperature and altitude create global and regional variations in patterns of endemicity, while simultaneously slight modifications of environmental correlates can generate microgeographical heterogeneity in *Plasmodium* transmission.

Of critical importance to mosquito lifespan and sporogonic duration, is ambient temperature, with mosquito death defined as occurring below 5 and above 40°C and survival rate declining rapidly at the margins [234]. Altitude and temperature are closely related, and in practical terms, increasing altitude is often used as a metric for defining limits of transmission and providing an operationally useful regional classification of transmission intensity [235]. Recent modelling work suggests the most efficient transmission occurs between 25 and 27°C [236], however even slight alterations in temperature have been found to affect vector distribution and abundance and development [237-239]. Geographical and temporal variability in precipitation determines the provision of temporary breeding sites, as female *Anopheles gambiae s.l.* breed abundantly in temporary turbid freshwater bodies created in rainy seasons [234] in addition to permanent and semi-permanent sites [240]. A wealth of evidence from geostatistical modelling demonstrates associations between clustering of *P.falciparum* infection and reduced distance to waterbodies [95,241-243], and in regions where transmission is very low, waterbodies often create defined foci of transmission.

The intra-annual fluctuation of temperature and rainfall creates seasonality in transmission, causing oscillations in vector density across the year in large parts of SSA [85,244]. Human-influenced environmental change such as differences in agricultural practices have also been linked to variation in vectorial species and density [245], and the creation of man-made

development projects such as large-scale irrigation systems and dams have been observed to be associated with increased incidence of malaria, especially in areas of unstable transmission [246]. However, in stable transmission regions changes in malaria vector species associated with such irrigation schemes and the socioeconomic benefits they bring appear to outweigh the costs in terms of increased risk of malaria [247]. Extensive evidence exists on the impact of global and local level urbanisation and social development on transmission of malaria [11,248].

1.9.2 Household level factors affecting transmission

As well as heterogeneity in transmission between communities largely on the basis of environmental factors, clustering of both clinical malaria and asymptomatic infection within households is also recognised [193,249,250], with distance of household to breeding site a crucial determinant [95,243]. However, household construction, density and presence of animals and household use of protective measures are important in addition to the geographical location of the compound as they determine the degree of contact between mosquitoes and humans. Cross sectional research has demonstrated greater odds of infection in households of lower SES [12,243,251-253], which has been directly related to household construction [254]. The presence of full window screening and closed eaves have been independently associated with significantly lower prevalence of *P. falciparum* infection [255], corroborated by entomological evidence, with associations between the presence of mosquitoes in houses with mud walls and open eaves [256]. Household crowding (both in terms of limited sleeping rooms and number of residents) has also been associated with increased transmission in various settings [252,256-258] with a capture recapture study suggesting memorized site fidelity in *An. arabiensis*, whereby the vector returns to the location of its last blood meal [259], However further corroborative evidence is required to validate this assertion. Additionally, use of personal protection (as discussed in Section 1.6) within a community and within households, can also affect risk of *P. falciparum*.

1.9.3 Individual level heterogeneity

Intrinsic host factors such as genetic polymorphisms are, on the whole, involved more in modifying the individual's response to *P. falciparum* infection and its manifestation rather than preventing infection itself. For instance, polymorphisms such as sickle cell trait and the thalassaemias have been associated with decreased clinical infection and lower parasite densities, but not reduced prevalence of infection [260]. Although limited research has been conducted on the effect such intrinsic polymorphisms have on *P. falciparum* transmission potential directly, it would be logical to suspect that a mutation that protects from clinical manifestations and constrains the parasitaemia to low intensities, may increase the duration of untreated asymptomatic infection with gamocytaemia, in turn increasing transmission. Various studies have investigated the relative contribution of genetics to *Plasmodium* infection [261-263], with genetic effects found as a significant determinant of parasite density in Ugandan children during cross sectional surveys [263].

1.10 HETEROGENEITY IN IMPACT: MALARIA CONTROL

1.10.1 Heterogeneity in impact between studies

When planning a new intervention strategy it is important to consider such factors discussed above as well as the prevailing prevention and control context, as this will likely modify the impact of the new strategy. In the context of malaria control, meta-analyses have frequently been used to explore the impact of interventions implemented in differing contexts and countries. One such example is IPTi, where the delivery of IPTi using SP alongside the expanded programme of immunization (EPI) was analysed across three sites in Ghana and one each in Tanzania, Mozambique and Gabon. Although the trials were very similar, heterogeneity in protective efficacy against clinical malaria was observed [189] with the protective efficacy in the high perennial transmission setting in Tanzania twice that of the five alternative settings, where high seasonality or seasonal peaks were present. In addition to the influence of varying transmission

intensity, the contextual difference of high net coverage in this region of Tanzania (68%), in comparison to the range of 0-20% coverage seen in the other five sites is likely to have been important in explaining the heterogeneity in impact seen [264]. Such high levels of net use would substantially reduce re-infection between rounds of IPTi. Results of pooled analyses of the protective efficacy of IRS and ITNs against parasitaemia in children under five years, from national Demographic and Health Survey (DHS) and Malaria Indicator Survey (MIS) data, found that transmission setting modified the protective efficacy of the interventions in addition to the prevailing malaria control context (in the form of nets) [265]. In moderate transmission settings, a combination of IRS and ITNs provided increased protection against parasitaemia compared with either intervention alone. But in low transmission settings, only IRS alone had a significant protective efficacy, and in high transmission settings, both interventions were singularly effective, but combination of the two did not increase protection [265]. However, these findings were from observational survey data where it is often not possible to control for crucial confounders [266]. Recent randomised and non-randomised evaluations of the combination of IRS and LLINs, conducted in differing transmission settings with varying IRS insecticides, LLIN and IRS coverage rates and vector susceptibility, have demonstrated mixed results [267]. Whereas a trial in Tanzania demonstrated additional protection of IRS over and above LLINs alone, a similar trial in the Gambia observed no added protection of the combination of IRS and LLINs in comparison with LLINs only [163,267,268]. Nevertheless the findings demonstrate that baseline heterogeneity in control programme context must be considered in the process of evaluation of an additional intervention, due to the impact this can exert on the success of a new intervention as well as the effect on baseline transmission.

The potential differential impact of malaria control interventions in regions of varying transmission intensity has also been explored in theoretical studies, with modelling used to predict the potential impact of malaria control interventions when varying parameters of underlying transmission intensity. Okell *et al*. used this approach to predict the potential impact on transmission of case management with ACTs when the baseline transmission parameter (using

altitude as a proxy) was varied across six settings, predicting that percentage reduction in prevalence of infection and incidence of clinical cases would be greatest in settings with lowest baseline parasite prevalence [269]. Again, modelling work estimating the potential impact of population-based IST implemented as part of a suite of interventions, would potentially have differential impact across various transmission settings as discussed in Section 1.7.5 [107].

To date, evaluations of school-based malaria interventions (Section 1.7), although demonstrating a largely beneficial impact, have been evaluated in high perennial or high seasonal transmission settings. In an environment of declining transmission, and subsequently increasing baseline heterogeneity, there is real value in evaluating these approaches in regions of low to moderate transmission intensity. This would allow the comparison of school-based approaches across varying endemicities.

1.10.2 Heterogeneity in impact between subgroups within a trial

As discussed in section 1.9, heterogeneity occurs on multiple levels and, as such, local variations must additionally be considered, especially in a region of low-to-moderate transmission where heterogeneity in infection often occurs. To investigate this, heterogeneity in impact within the trial can be quantified through stratified analyses of potentially important subgroups. Heterogeneity of impact (intervention effect on study outcomes) can occur in the presence of non-random variability in the direction or magnitude of the impact [270], and, as such, reports of average impact may be less informative than reports of differential impacts across subgroups or contexts [271]. While it is possible to account for between-group heterogeneity through effective randomisation, the same is not true of within-group heterogeneity. If this is not considered, and extensive variation is present within study groups, the application of outcomes to individual participants is difficult. The presence of such heterogeneity can lead to a type II error, whereby a statistical difference between groups is obscured, even when present [270,272]

Heterogeneity of impact has long been of interest to economists and those evaluating social welfare and education interventions where important potential determinants of heterogeneity in impact include background characteristics of the participant populations, such as age, sex, SES and baseline education levels and compliance to the intervention. For example, an evaluation of an education intervention providing textbooks to schools with sparse resources demonstrated non-uniformity of impact, with only the children in the top two quintiles of pre-intervention test performance demonstrating an improvement [273]. School feeding programmes have also been seen to have greater impacts on the enrolment and attendance at school for girls, compared to boys, in several low income countries [274]. In other examples, a conditional cash transfer programme in Mexico had a greater impact on school enrolment in girls [275] whilst a family health programme in Brazil had the greatest impact in the poorest regions [276].

In the context of malaria control programmes, the differential impact of ITN distribution and utilisation has been routinely investigated, often stratifying by age to quantify any heterogeneity in protective efficacy. During a community programme of ITN distribution in a high transmission setting in western Kenya, ITNs were associated with a 0.34g/dL (95%CI 0.02-0.66) increase in mean Hb and a reduction in all cause anaemia in school girls age 12-13 years, whereas no effect was seen on either of these outcomes for girls aged 14-18 years [125]. Protective efficacy of ITNs on mortality in Kenya was found to be significant, 23% (11-34%) in 1-11 month infants, but not so in 12-59 month children, 7% (-6-19%), although no formal statistical interaction was found between groups [148]. Guyatt *et al*. investigated the impact of two separate campaigns of ITNs and IRS in the highlands of western Kenya, and found a strong protective effect of both interventions, when stratified by age-group, with the exception of ITNs in children aged 5-15 years where there was no significant protective efficacy against parasitaemia. However, those reported not sleeping under the distributed nets were excluded, and as there is a differential usage of nets in age groups with school children having low usage [27], this resulted in very small numbers in the analysis of ITNs [277]. Finally, Bejon *et al*. found a protective effect of ITNs against febrile malaria among younger (12-42 months) but not older children (42-80 months)

[278]. Despite fairly consistent findings of greater protective effects in the younger age-groups, the studies were not necessarily powered to detect differential effects between age-groups and thus sample sizes may have been insufficient to formally assess interactions between age groups and protective efficacy of ITNs.

The investigation of heterogeneity in impact of malaria control programmes according to localised spatial variation in transmission is less common, but has been addressed in Bioko, Equatorial Guinea. The use of IRS and ITNs conferred substantial reductions in under-5 mortality (42-18%) across five years of intervention, but with spatial variation in impact exhibited [279]. Serological, parasitological, child mortality and entomology indicators demonstrated heterogeneity in impact on transmission by region, with only three of the four regions exhibiting evidence of decreased transmission from the time the programme started [280]. Suggestions of different behaviour or insufficient IRS coverage have been offered to explain this variation in impact [279]. Such spatial heterogeneity in impact leaves the door open for targeting interventions. Overall investigation of the micro-epidemiology of the region and baseline heterogeneity in distribution of *P. falciparum* is crucial, due to the potential modification of effect this can exert on the intervention [281].

1.10.3 Fidelity of the intervention: variability in performance of tool

Another potential source of heterogeneity is variability in the performance of the intervention tools, for example efficacy of drugs used or performance of the diagnostic screening tool. Examination of absolute performance measures, as well as the variability in these is critical when evaluating overall impact as well as internal and external validity of the intervention. This is of particular importance for a screening and treatment intervention, whereby the treatment of infected individuals is conditional on the accurate diagnosis of *P. falciparum* infection during screening, as poor sensitivity will result in attenuation of the success and impact of the intervention. Underlying heterogeneity in the population may exert influence on the performance

of diagnostic screening tools [282]. Such variation can include intrinsic factors such as age, concomitant health states or infections and their effect on parasite density, and extrinsic factors such as the influence of climate on the test cassette itself [283].

1.10.4 Influence of seasonality on the impact of a malaria control intervention

The importance of seasonality in the effect of an intervention must not be overlooked. Whether comparing a region of perennial transmission with a region of seasonal transmission, or whether comparing the effect of an intervention delivered in one site where there is seasonal variation, this is likely to modify any effect. For instance, the delivery of a screen and treat intervention must consider the optimal time for screening rounds in order to achieve the maximal impact through treatment of infections. Tiono *et al*. conducted three community screen and treat campaigns in the dry season to clear baseline parasitaemia before the rains in a highly seasonal transmission setting. However, no effect was found on the clinical episodes experienced in rainy season or on parasitaemia prevalence in the following dry season [218]. Recent evidence from Tanzania has also demonstrated that a combination of ITNs with IRS conferred significantly greater benefit than ITNs alone, and this effect was strongest in the peak transmission season [268].

1.10.5 A framework for investigating heterogeneities in the impact of school-based intermittent screening and treatment

This thesis will consider some of the above issues in the context of an evaluation of school-based intermittent screening and treatment. Figure 1.6 depicts a conceptual framework for the evaluation of a programme of school-based IST, incorporating potential influences on the impact of the intervention. As shown, the context in which the intervention is delivered may exert multiple influences on the impact observed. A combination of environmental and socio-demographic factors at various scales will determine the underlying *P. falciparum* transmission intensity, which in turn may affect the performance of the screening tool in relation to the

detection of infected individuals. Once infected individuals are identified, compliance to the full regimen of treatment (also influenced by individual characteristics) and efficacy of the treatment will affect successful clearance of the parasites. Additionally, the duration of screening intervals will likely influence the rate of re-infection between rounds of IST, in conjunction with the localised transmission intensity. Finally, all of these factors must be considered within the context of the coverage achieved by the intervention. The thesis uses this framework of conceptual mechanisms and influences to explore the impact of IST on anaemia, *P. falciparum* infection, and cognitive and educational performance in this setting, addressing specific objectives as outlined below.

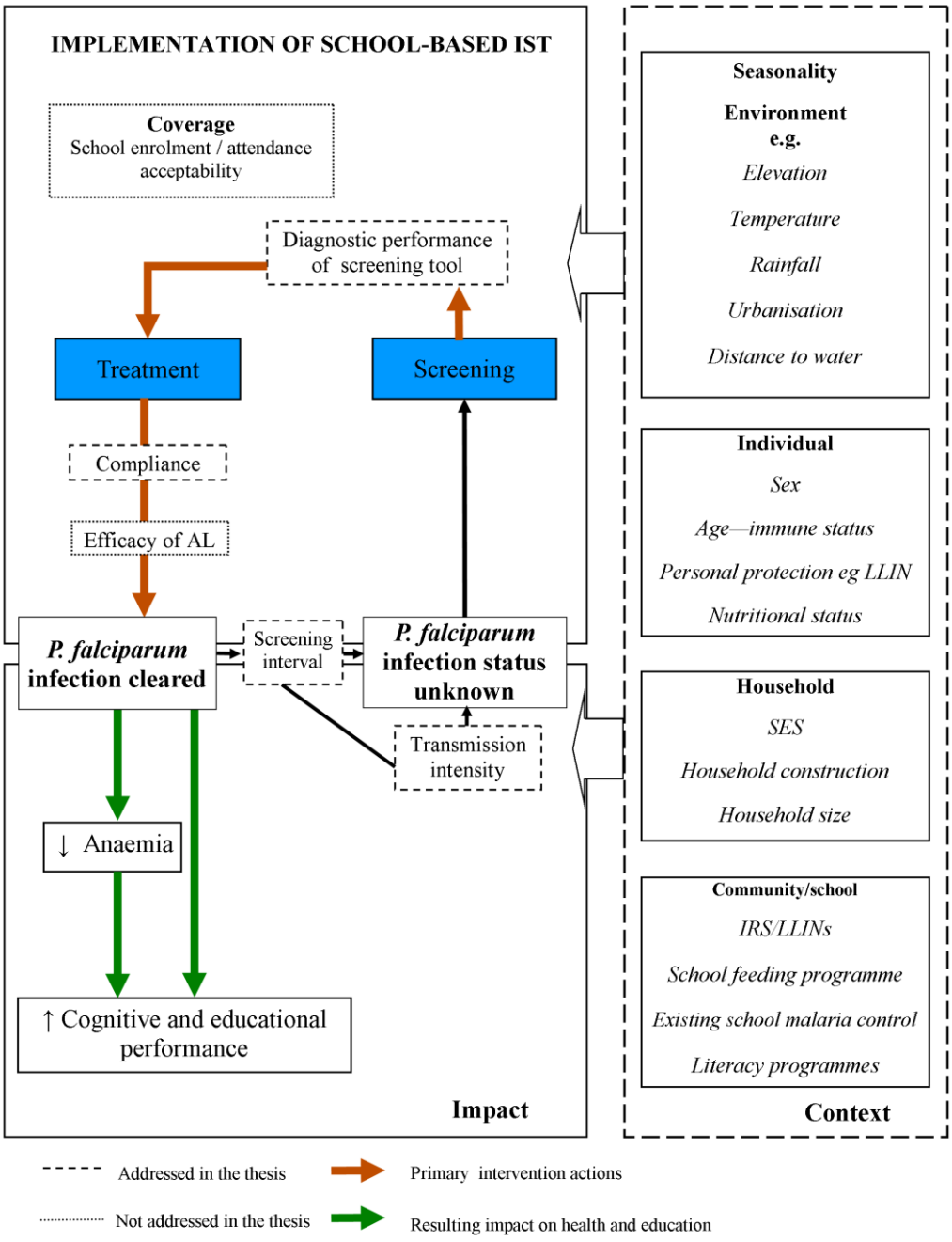


Figure 1.6. A conceptual framework for the evaluation of impact of a programme of intermittent screening and treatment, delivered through schools, intended to reduce parasitaemia, in turn decreasing the prevalence of anaemia and increasing cognitive and educational performance (IST – Intermittent screening and treatment, AL – artemether lumefantrine, SES – socioeconomic status, LLINs – long-lasting insecticidal nets, IRS – indoor residual spraying).

1.11 AIMS AND OBJECTIVES OF THE THESIS

1.11.1 Overall Aim

To investigate heterogeneity in (i) spatial and temporal patterns of *Plasmodium falciparum* infection, (ii) the process of the intervention and (iii) the impact, of a programme of school-based malaria control using intermittent screening and treatment (IST), in a low-to-moderate transmission setting in south coast, Kenya.

1.11.2 Specific objectives

1. To describe the epidemiology and underlying heterogeneity in patterns of *P. falciparum* infection and anaemia in school children and their association with measures of cognition, attention and educational achievement in a low-to-moderate transmission setting in south coast, Kenya (Chapter 3).

2. To quantify the overall impact and any heterogeneities in impact of a programme of school-based malaria control using IST for malaria among school children in a low-to-moderate transmission setting in south coast, Kenya (Chapter 4).

3. To investigate the diagnostic performance of rapid diagnostic tests for screening *P. falciparum* infection, and explore the influences of individual, local transmission and seasonal factors in a low-to-moderate transmission setting (Chapter 5).

4. To quantify the spatial and temporal heterogeneity of school-level *P. falciparum* infection and associated ecological and socioeconomic covariates in a low-to-moderate transmission setting (Chapter 6).

5. To investigate the heterogeneity of *P. falciparum* infection at the individual level in relation to the extent of repeated infections in the presence of treatment in a region of low-to-moderate transmission in south coast, Kenya (Chapter 7).

1.11.3 Thesis Summary

Using data from a large scale cluster randomised controlled trial of school-based IST this thesis aims to address the above objectives in order to further understand the role of heterogeneity in the implementation of malaria control in a low-to-moderate transmission setting and the implications of this for the impact and further applications of delivering IST through schools.

Chapter 1 provides an overview of the context and justification for this thesis. Chapter 2 introduces the study design and methods of the Health and Literacy Intervention (HALI) Project, the randomised controlled trial on which this thesis is based. Chapter 3 presents data from the baseline cross-sectional surveys in which the initial epidemiology of *Plasmodium falciparum* infection and anaemia are described, with an investigation of the risk factors for both, and their association with correlates of cognition, attention and educational achievement. Chapter 4 evaluates both the overall impact of the IST intervention on the health and education outcomes among school children as well as the presence of heterogeneity in impact according to pre-specified subgroups such as baseline *Plasmodium* prevalence. Chapter 5 presents repeated measures data of diagnostic pairs (microscopy and RDT) from four screening surveys. Diagnostic accuracy is estimated in the absence of a reference standard, investigating the influence of factors such as seasonality on performance. Chapter 6 uses spatially explicit models within a Bayesian framework to investigate the extent of spatial and temporal heterogeneity in *Plasmodium* infection at the school level across the two year study period as well as identifying drivers of such variation. Chapter 7 investigates the dispersion of *Plasmodium* infection at the individual-level in the cohort of school children in the IST intervention group and examines the extent of repeated infections within children at five discrete time-points using a prevalence-based analysis. Chapter 8 provides a discussion of the overall findings of the analyses presented and the implications for the use of IST in low-moderate heterogeneous transmission settings in the move towards the goals of elimination. Recommendations for future research arising from this thesis will be discussed.

Chapter 2. The Health and Literacy Intervention Project

2.1 OVERVIEW

As illustrated in Chapter 1, school children experience some of the highest age-specific *Plasmodium falciparum* parasite rates [19,20]. In addition to contributing to significant morbidity, infection with *Plasmodium* can impair cognitive performance and education and such a burden in this group provides a source of ongoing transmission to other community members. As such this group is of critical relevance when planning malaria control and transmission reduction strategies. However, as elucidated in Chapter 1, there is limited experimental evidence of the benefits of alternative school-based malaria interventions, with a specific gap in the knowledge regarding low-to-moderate transmission settings.

This chapter describes the Health and Literacy Intervention (HALI) Project, a cluster randomised controlled trial conducted between 2010 and 2012, with the principal aim of evaluating the impact of school-based intermittent screening and treatment (IST) for malaria on the health and education of school children, in an area of low to moderate malaria transmission on the Kenyan coast [284]. Alongside the IST intervention an enhanced literacy instruction intervention was implemented with early grade primary teachers (in class 1), to evaluate the potential synergy of health and education interventions on educational outcomes. The research presented in this thesis is set within the overarching framework of this larger trial but focuses only on the IST intervention, with a particular emphasis on the impact and the influence of heterogeneity in risk and intervention process, on both the overall and any differential impact of the intervention. The study setting, design, randomisation, intervention and assessment procedures of the HALI project are discussed in this chapter to provide the context in which the data presented in subsequent chapters were collected.

The information in this chapter is primarily taken from the peer reviewed publications:

Halliday KE, Okello G, Turner EL, Njagi K, Mcharo C, Kengo J, Allen E, Dubeck MM, Jukes MCH & Brooker SJ. (2014) Impact of intermittent screening and treatment for malaria among school children in Kenya: A cluster randomised trial. PLoS Medicine 11:1.

Brooker S, Okello G, Njagi K, Dubeck M, Halliday K, Inyega H & Jukes MC (2010). Improving educational achievement and anaemia of school children: design of a cluster randomised trial of school-based malaria prevention and enhanced literacy instruction in Kenya. Trials, 11:93

The study was conceived and designed by Professor Brooker and Dr Jukes. My role was as research coordinator for the duration of the study. This role involved sensitisation at the district and community levels, designing, piloting and implementation of survey tools, management of the informed consent process, coordination of data collection, data management, cleaning and analysis. My role included coordination of data collection, with specific emphasis on the health intervention and health assessment training and supervision. I was responsible for the data management and cleaning of all databases.

Following data collection, I conducted the baseline analysis presented in Chapter 3 and although the main trial analysis was conducted by the trial statistician, I was responsible for the secondary analyses of differential impact and compliance to the intervention presented in Chapter 4. As a result of the rich dataset collected during the trial I was able to further explore the various sources of heterogeneity present and their potential impact on the outcomes of the trial. I was responsible for conceiving and conducting the various latent class, spatial, and prevalence-based analyses presented in Chapters 5 to 7 with high level statistical support.

2.2 INTRODUCTION TO THE HALI PROJECT

Building on the success of studies of school-based intermittent preventive treatment (IPT) in improving health and sustained attention outcomes of school children in a high transmission setting in western Kenya [33] and in reducing the incidence of clinical malaria in a high seasonal transmission setting in Mali [40], there was strong Kenyan governmental interest in investigating the reproducibility of this approach in alternative transmission settings, such as moderate and low perennial transmission regions. Site selection was made in close consultation with the Ministries of Education and Health in Kenya. Kwale County (specifically Kwale and Msambweni districts) was suggested as a suitable site to replicate the IPT study conducted in Bondo, western Kenya [33], at the direct request of the Permanent Secretary for Education for several reasons: firstly, in terms of educational achievement, the districts are among the poorest performing in Kenya [285], thus as this intervention was designed to improve educational achievement of school children through both health and literacy interventions, Kwale county was proposed as a suitable site; secondly, the districts experience low-to-moderate stable malaria transmission [229]. Finally, the districts have not benefitted from extensive disease control research as have other parts of the country and the province, such as the north Coast.

However, changes to the drug policy in Kenya and other countries in Eastern and Southern Africa in late 2009 led to the withdrawal of the principal drugs used for IPT at that time - sulfadoxine-pyrimethamine (SP) and amodiaquine (AQ) thereby limiting its potential implementation. As discussed in section 1.6.2, SP was withdrawn due to concerns of extensive resistance in the region and AQ monotherapy was also withdrawn due to plans to combine it with artesunate in the future in a combination therapy. No alternative candidate drugs were identified as suitable for mass administration. As such, following extensive consultations with the Ministry of Health (MOH) at the National level, an alternative school-based malaria control strategy, intermittent screening and treatment (IST), using rapid diagnostic tests (RDTs) to screen, and artemether lumefantrine (AL) to treat, children with *P. falciparum* parasitaemia, was outlined as a possible strategy, having been proposed in the recent "*Malaria-Free Schools Initiative*", as part of the Kenya National Malaria

Strategy 2009-2017 [31]. AL was selected for treatment due to its status as the first-line antimalarial drug in Kenya. At this time dihydroartemisinin-piperaquine (DP) had not yet been firmly established as a possible first-line treatment by WHO. A once-a- term screening interval was selected on logistical grounds as the most feasible schedule that the ministries of health and education could adopt as part of a large-scale school health programme.

Despite the substantially beneficial impact of IPT on anaemia, *P. falciparum* infection and sustained attention, observed in the previous randomised evaluation conducted in Western Kenya, these benefits did not translate into improved educational achievement [33]. Reasons suggested for the lack of impact on educational achievement included an insufficient follow-up period of one year and an educational environment (in relation to resources such as textbooks or quality instruction) too poor to facilitate an impact on learning in school. It was postulated that health-evoked improvements in educational performance may be more readily detected when the teaching and learning environment is richer and the time period investigated is extended [284]. Thus it was hypothesised that simultaneous implementation of two interventions (both health and education) was necessary to optimise school instruction in order to maximise the impact of malaria control on educational performance (Figure 2.1).

Consequently, two interventions were delivered through the selected schools: (i) a malaria control intervention based on intermittent screening and treatment; and (ii) a literacy intervention based on a programme of training and support for class 1 teachers in teaching Swahili and English. Both interventions were developed within the context of current government strategies and guidelines, and were designed to be replicable on a large scale, within existing school programmes. The Health and Literacy Intervention (HALI) project was formulated as a factorial cluster randomised controlled trial to evaluate the impact of the interventions on health and educational outcomes in school children [284].

This thesis shall focus only on the IST intervention, and does not address the impact of the literacy intervention. However, in brief, the literacy intervention was implemented with class 1 teachers, who attended an initial three day residential training workshop in February 2010 with day-long follow-up workshops in July 2010 and in February 2011 when they were teaching class 2 (Figure 2.5). A teacher manual was provided with 140 partially scripted lessons, bonus lessons and games [286]. The training workshops sought to provide teachers with background information about literacy acquisition to improve their instruction, to guide them in the use of the manual for promoting efficient reading acquisition, and to give them the opportunity to customize materials for their classrooms. Teachers implemented the literacy intervention within their routine teaching activities [284]. During the two year intervention the study team provided ongoing weekly text message support to the teachers providing instructional tips and motivation to implement lesson plans.

In addition to the overall impact of improved quality of literacy instruction on educational outcomes, the factorial design of the trial allowed investigation of the differential effects of the IST intervention on education performance against backgrounds of contrasting quality instruction. However, the lack of interaction detected (interaction effect *p*-values of 0.45, 0.26, and 0.60 for the three key literacy outcomes) between the two interventions in class 1 where both were implemented, means the results of the two interventions can be reported independently. The results of the literacy intervention will be reported separately, targeting an education research audience, as the literacy intervention was focused purely on enhanced English and Swahili literacy instruction and was not intended to have an impact on health.

From this point onwards the focus shall be specifically on the IST intervention, whereby the hypothesised causal chain of action is diagnosis and treatment of *P.falciparum* infection, leading to haematological recovery, an increase in sustained attention and an eventual impact on education achievement (Figure 2.1). Furthermore, as previously displayed in Figure 1.6, the thesis shall further explore the influence of various sources of heterogeneity along this causal

pathway, on the impact of the intervention, for example in the underlying transmission intensity, and in process, in relation to the diagnostic performance of the screening tool.

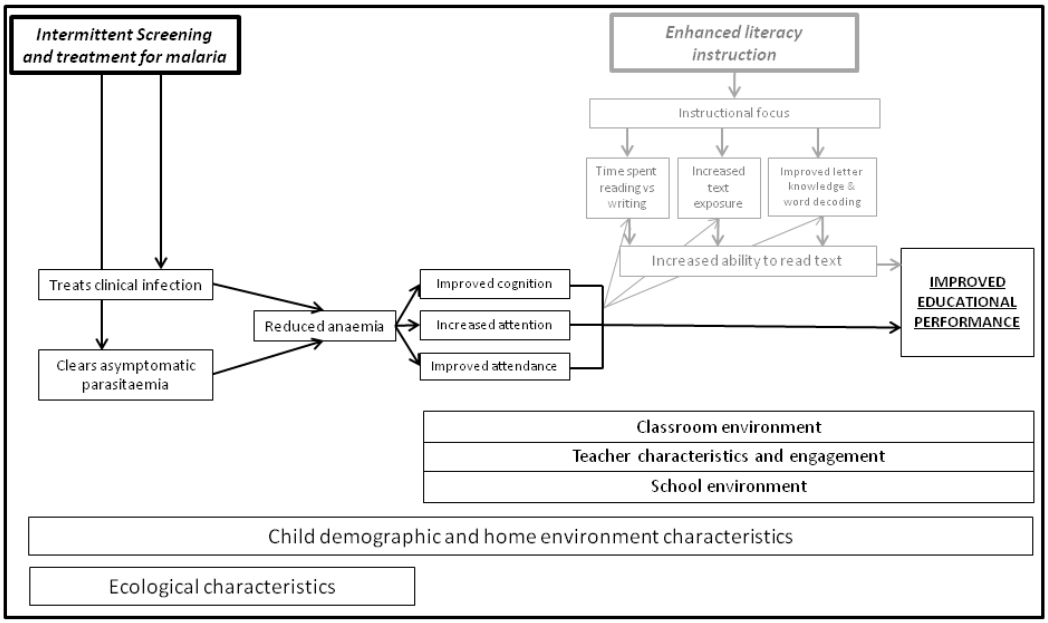


Figure 2.1. Hypothesised causal pathway of the intermittent screening and treatment intervention alongside related contextual factors (literacy intervention presented in grey)

2.3 METHODS

2.3.1 Study setting and population

The trial was conducted from January 2010 to March 2012 in Kwale and Msambweni districts, both now part of Kwale County, on the south Kenyan coast (Figure 2.2).

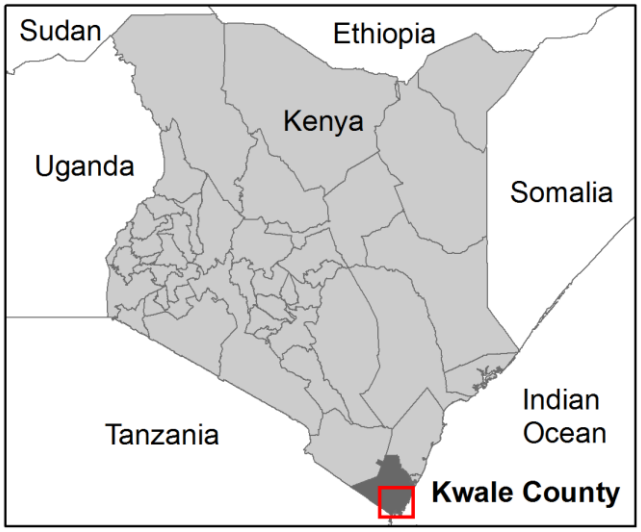


Figure 2.2. The location of the study site in Kenya. Kwale County, covering both Kwale and Msambweni districts, is highlighted

The study region covers an area of approximately 1600km^2 with an altitude range of 0-200m above sea level. The region is hot and humid with average temperatures between 22-33°C. Two rainy seasons dominate: April-June and October-November, with a mean annual precipitation of 1200mm along the coastline [287,288]. The Ramisi and Umba rivers are the predominant water bodies in the region, with additional smaller permanent and seasonally transient streams and marshes [289]. Malaria transmission in the area is moderate and perennial, with seasonal peaks following the two rainy seasons (April-July and September-November) [290]. The primary malaria vectors are *Anopheles gambiae s.l.* and *Anopheles funestus* [287,291]. Intensity of malaria transmission has been declining in recent years: school surveys conducted in 2010 reported prevalences of *P. falciparum* of 9-24% [231,292], compared to 64% in 1998 [287].

Overall reported mosquito net use in the region is relatively high, at 63% net use reported by school children in coastal Kenya in 2009 [153], with the communities having benefited from universal coverage campaigns. Prior to the study, mass albendazole treatment was provided to all schools in 2009 as part of the national school deworming programme. During the two year trial period, albendazole was delivered through households as part of the National lymphatic filariasis campaign in 2011, although coverage was not extensive and praziquantel was delivered to schools in the area in June 2011. The vast majority of the population in these districts belong to the Mijikenda ethnic group, with Digo and Duruma the predominant subgroups [293]. The region is primarily rural, with subsistence farming of maize and cassava practiced by many of the communities, although titanium mining has recently become an important source of employment. In economic and educational terms, the districts are ranked the seventh poorest in Kenya and consistently have some of the worst performing schools in the national school examinations [285].

Kwale District has 85 schools across four zones, and in two of these an alternative literacy intervention study was underway. Therefore only 20 schools from Mkongani and Shimba Hills zones were included in our study, allowing the two interventions to proceed without leakage. In

Msambweni District, 81 of the 112 schools were selected, with schools in Lunga Lunga and Mwereni zones greater than 70 km away from the project office excluded because of logistical considerations in visiting them.

2.3.2 Study Design

The study was designed as a factorial, cluster randomized trial to investigate the impact of two interventions: (i) the impact of school-based IST of malaria on the health, sustained attention and education of school children, and (ii) the impact of a literacy intervention on education [284]. In order to evaluate the potential interaction between the two interventions, schools were randomised to one of four groups, receiving either: (i) IST alone; (ii) the literacy intervention alone; (iii) both interventions combined; or (iv) control group where neither intervention was implemented (Figure 2.3).

Recruitment and baseline sample collection were conducted in January-March 2010 using children randomly selected from classes 1 (age range: 5-15 years) and 5 (age range: 8-20 years). The wide age ranges in each class reflect the variability in children's age at enrolment and their variable progress, related to grade repetition. Prior to randomisation and consenting a school census of all 101 schools included in the trial was conducted by trained personnel and used as a sampling frame from which 25 children from class 1 and 30 children from class 5 were randomly selected using random number tables. Fewer children were selected from class 1 because of the extra educational assessments undertaken with these children and the low feasibility of conducting the tests in a single day. School children eligible for inclusion into the study were those enrolled in participating schools in either classes 1 and 5 in January 2010, who also had informed parental consent and a willingness to participate. Exclusion criteria included a lack of parental informed consent, unwillingness of the child to participate, a known allergy or history of adverse reaction to study medications, and known or suspected sickle-cell trait.

Both classes received the IST intervention, but the literacy intervention was delivered only to children in class 1 and as they advanced to class 2, as it focused on the initial stages of literacy acquisition. Education outcome measures were assessed in the same children at 9 and 24 months and health outcome measures at 12 and 24 months.

2.3.3 Sensitisation recruitment and consent

Sensitization took place at national, provincial and district levels prior to visiting the schools. At the national level, the study was approved by the Division of Malaria Control at the Ministry of Public Health and Sanitation and the Director of Basic Education, and School Health and Nutrition unit at the Ministry of Education [294]. Meetings were held with the Provincial Medical Officer and the Provincial Director of Education in Mombasa, as well as district health and education management teams in both Kwale and Msambweni. Finally, school head teachers and Teachers' Advisory Centres (TAC) tutors were informed of the study [284].

In January and February 2010, following both enumeration and the random selection of children for study enrolment, meetings were held at each of the 101 schools, to which all parents and guardians were invited, with a particular emphasis on those with children in classes 1 and 5. All aspects of the study were explained, with emphasis placed on the fact that participation of their children in the study was voluntary and they had the opportunity to opt out at any time. Written informed consent was sought from parents or guardians. As coastal Kenya is predominantly inhabited by the Mijikenda ethnic group, incorporating multiple subgroups with diverse mother-tongue languages, there was a potential challenge of language barriers in areas where parents/guardians could not understand Kiswahili well. This was a particular problem in explaining some technical and scientific aspects of the study. To address this, only fieldworkers local to the region who were proficient in the specific local languages were used to explain the study in local mother tongue to ensure parents/guardians were making fully informed decisions.

Initially parent/guardian attendance at the school based consent meetings was low in a number of schools, introducing potential selection bias, as those parents who did not attend may have been systematically different from those who did, for example they might have been of lower socioeconomic status or living in more remote areas and therefore were less able to afford the time and economic costs of attending. To minimise this bias, if parents failed to attend the meeting a follow-up meeting was arranged, and in the event of low turn-out at the follow-up meeting, home visits were undertaken to obtain consent. Although multiple school meetings and household visits incurred additional time and expense and could have lead to an increased feeling of pressure to participate, the one-to-one discussions between parents and field officers is likely to have created a more in-depth understanding of the nature of the study [294]. Furthermore, the majority of parents found at home cited routine economic and household activities as reasons for non-attendance rather than due to refusal or lack of interest.

During the initial stages of the study, some parents had concerns over the finger prick required for the RDT and teachers reported rumours of blood stealing, covert HIV testing, and the safety of the study drugs, leading to withdrawals. This was a particular problem in four schools, located close to one another. The rumours were closely linked to previous interactions and experiences with school-based development programmes and health care interventions. As the study was unblinded and the fears were predominantly related to the RDT conducted during the intervention, this was a potential source of attrition bias. To address these concerns, additional school and community meetings were held, with the involvement of community elders, whether they were parents at the school or not. Attendees were shown the malaria RDTs which had been and study staff and teachers volunteered to have a finger prick blood sample taken as one way of allaying parents' fears. A local community liaison team was established to act as a link between communities and the research team and were present in the schools on assessment days. Finally, parents were encouraged to some to the school and witness these activities. These supplemental meetings, supervised by senior field staff to ensure no coercion, were popular, with the majority of parents re-consenting and additional parents requesting to consent.

As the study enrolled school children both parental/guardian consent and child assent was required for the child's participation. Verbal assent was collected by both the health and education assessment teams prior to any activities. Some children dissented, either verbally or by leaving the classroom. This was largely attributed their fears about the finger prick. This dissent was not always accepted by parents, who on discovering, occasionally tried to demand the health team test the child, despite the health workers insisting that both child assent and parental consent were important. The discord between parental consent and child assent proved a challenge, as supporting dissenting behaviour based on unnecessary fear could have undermined the success of the study as well as allowed concerns to persist [294]. In contrast, ignoring children's dissent could have undermined their ability to make autonomous decisions to participate in the study or not. To allay the children's fears, teachers and health workers were often tested first in front of the children.

2.3.4 Sample size

The sample size was based on methods designed for cluster-randomized trials [295] and assumed that 101 eligible schools would be randomized to the four intervention groups, with an average of 50 children per school. Based on data collected previously in the study area, the baseline prevalence of anaemia was assumed to be 20% and the coefficient of variation (CV) 0.2. In order to detect a 25% reduction in the prevalence of anaemia between the two groups, based on previous work in Kenya [33], the sample size required to give a study with a power of 80% at a two-sided significance level of 5%, was a total of 27 schools in each arm with 50 children per school. A sample size of 101 schools with 25 children per class (i.e. analysing classes 1 and 5 separately), will enable us to detect, with 80% power and 5% significance, an approximate difference of 0.2 standard deviations (SDs) between arms of the trial in educational achievement (assuming an intraclass correlation coefficient (ICC) of 0.2 and a pre-post correlation of 0.7), and a difference of approximately 0.15 SD in tests of sustained attention (assuming an ICC of 0.1 and a pre-post correlation of 0.7) [33]. The increased number of schools required for the sustained attention and

educational achievement outcomes provided greater power (97%) to detect a 25% reduction in the prevalence of anaemia, or alternatively 85% power to detect a 20% reduction.

2.3.5 Randomisation

The 101 schools were randomised in two stages (Figure 2.3). In Kenya, schools are aggregated into sets of between three and six closely located schools, which regularly meet and share information, supported by a Ministry of Education Teacher Advisory Centre tutor. Our 101 study schools formed 24 of these sets of schools, which were randomised either to receive the literacy intervention or to serve as the literacy control. Randomisation of these sets of schools was stratified by (i) set size, to ensure equal numbers of schools in the experimental groups; and (ii) average primary school leaving exam scores of the school sets, to balance the two study groups for school achievement. This randomisation procedure was designed to minimize contamination of the literacy intervention methods across the study groups. In stage two, the IST intervention was randomly allocated at the level of the school, with the 101 schools re-stratified by (i) literacy intervention group assignment and (ii) quintiles of average school exam scores, producing 10 strata overall.

This two-stage randomisation procedure was conducted during two separate public randomisation ceremonies, the first held with the district health and education officials, where the sets of schools were randomly assigned to either literacy intervention or control groups and the second with head teachers and parent teacher association representatives where the schools were randomly assigned to the IST intervention and control groups [284]. These public ceremonies played an important role in ensuring transparency in the process of study group allocation and fully explaining to communities the necessity for random selection as well as strengthening the trust between the research team and communities. They were carried out with active involvement from the meeting participants who selected the sealed envelopes containing the names of the school sets or schools and placed them into one of two boxes representing the control and intervention groups.

However, as the consent process was incomplete at the time of randomisation and the study was unblinded, allocation was concealed from the schools, communities and study team conducting the randomisation, sensitisation and consenting. During the ceremonies the literacy groups were known as "A" and "B" and the IST groups as "1" and "2". A sealed envelope containing the group information was provided by an individual external to the study who had performed the random assignment of "A", "B", "1" and "2" to intervention and control and this envelope was opened by the research team only after the finalisation of sensitisation and consenting so as to minimise selection bias.

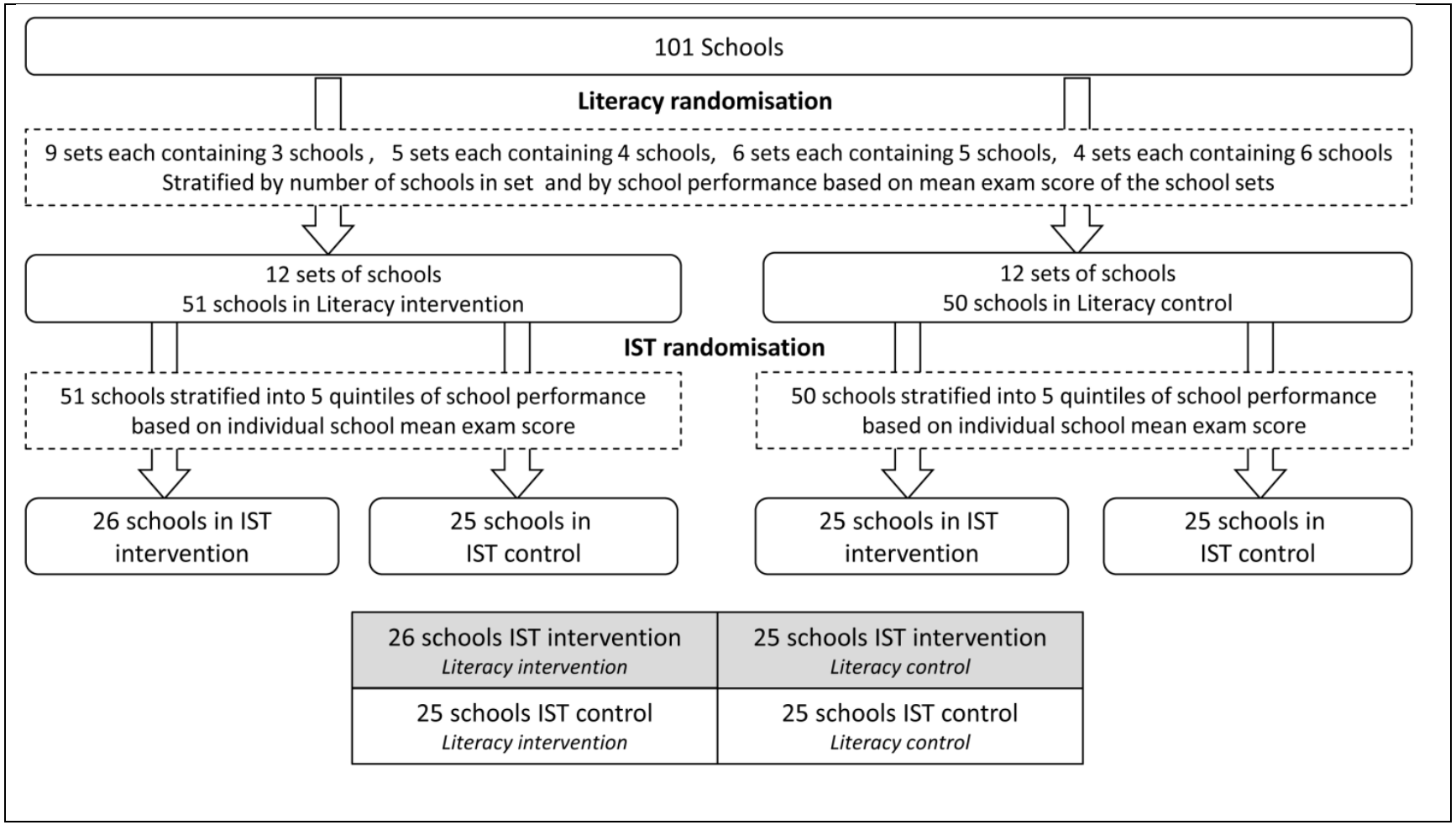


Figure 2.3. Diagram depicting the two-stage randomisation procedure.

For the purposes of this thesis, the schools are evaluated as two study groups, IST intervention and control. As such, Figure 2.4 depicts the study group allocation of the IST intervention across the 101 schools in the HALI project.

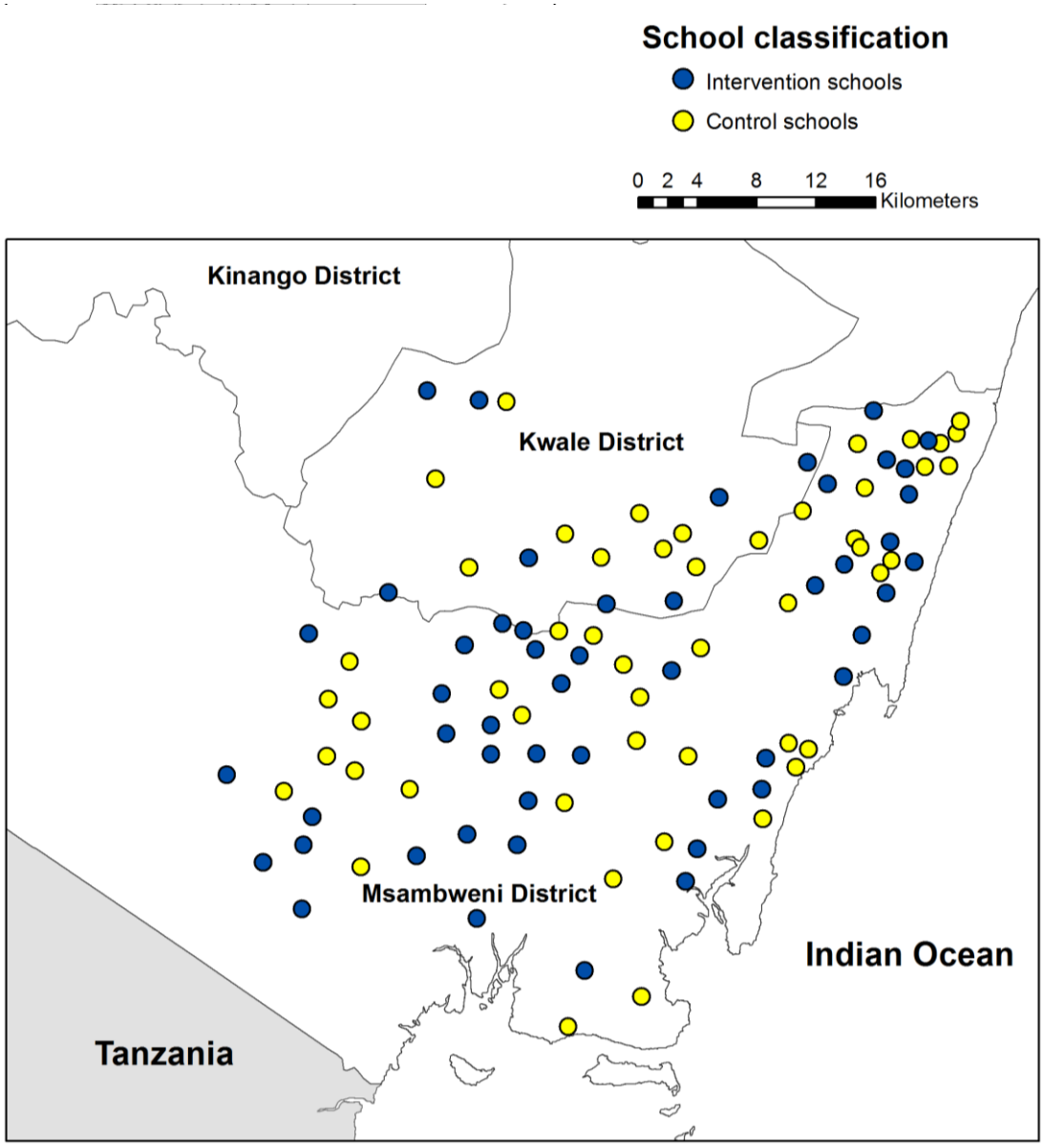


Figure 2.4. Map of the study area and 101 study schools. Schools assigned to the IST intervention are shown in blue and schools assigned to the control group are shown in yellow.

2.3.6 Intermittent Screening and Treatment (IST) intervention

During IST, children were screened once a school term for malaria parasitaemia using an RDT (ParaCheck-*Pf* device, Orchid Biomedical Systems), which is able to detect *P. falciparum*

antigen. Screening was conducted by laboratory technicians. Repeat visits were made to follow-up children absent on the day of screening. Children (with or without malaria symptoms) found to be RDT-positive were treated with a six dose regimen of AL (artemether 20 mg/lumefantrine 120 mg, Coartem, Novartis) over three days. Doses of AL were based on weight, with children stratified into one of the four categories (<15 kg, 15–24.9 kg, 25–34.9 kg, and ≥35 kg). AL was given at a dose of 20/120 mg to children <15 kg, 40/240 mg to children 15–24.9 kg, 60/360 mg to children 25–34.9 kg, and 80/480 mg to those who weighed ≥35 kg. Parents or older siblings of children were called and a nurse explained that their child was infected with malaria parasites and required treatment. Doses 1, 3, and 5 were given under direct observation at the school by the study nurses.

Children were given milk and biscuits with the AL and observed for 30 minutes after drug administration. If vomiting occurred during this period, drugs were re-administered. If vomiting occurred on a second occasion, this was noted but the drugs were not given again. Such children were not excluded from the trial and they were eligible to receive drugs on the subsequent two days. The parents/older siblings, or study children themselves if in the older classes, were given doses 2, 4, and 6 each day for evening administration and provided with instructions on treatment. Children absent from school on days two or three of treatment were followed up at their home by the nurse, and provided with the doses. Supervised treatment was defined as nurses administering and directly observing doses 1, 3, and 5 taken on three consecutive mornings in the school and recording doses 2 and 4 reported by the child as having been taken the previous evenings. No direct confirmation of whether dose 6 was taken was recorded by the nurse. The record of supervised treatment was used as a proxy for compliance. Adverse events were monitored by the study team for 24 hours after each treatment, and a further 28 days thereafter using a passive surveillance system in schools. Travel costs were reimbursed and treatment charges waived. Adverse experiences were monitored until the event was cured or had stabilised. Agranulocytosis and hepatotoxicity were not assessed because of logistical constraints.

2.3.7 Timeline of interventions and assessments

Following sensitisation and recruitment, baseline education surveys were conducted in February 2010. Health surveys in were conducted in March 2010 alongside the first round of screening and treatment in the IST intervention schools. Figure 2.5 displays the timing of rounds of screening and treatment in relation to baseline and follow-up surveys.

Five rounds of screening and treatment were implemented. The first round was conducted alongside baseline health assessments in March 2010, the second round in July 2010, the third in September 2010, the fourth in March 2011, and the final round in October 2011 (Figure 2.5). As the study region experiences moderate seasonal peaks in transmission following the two rainy seasons, April-July and September-November, the screenings were timed to cover both the dry and wet seasons.

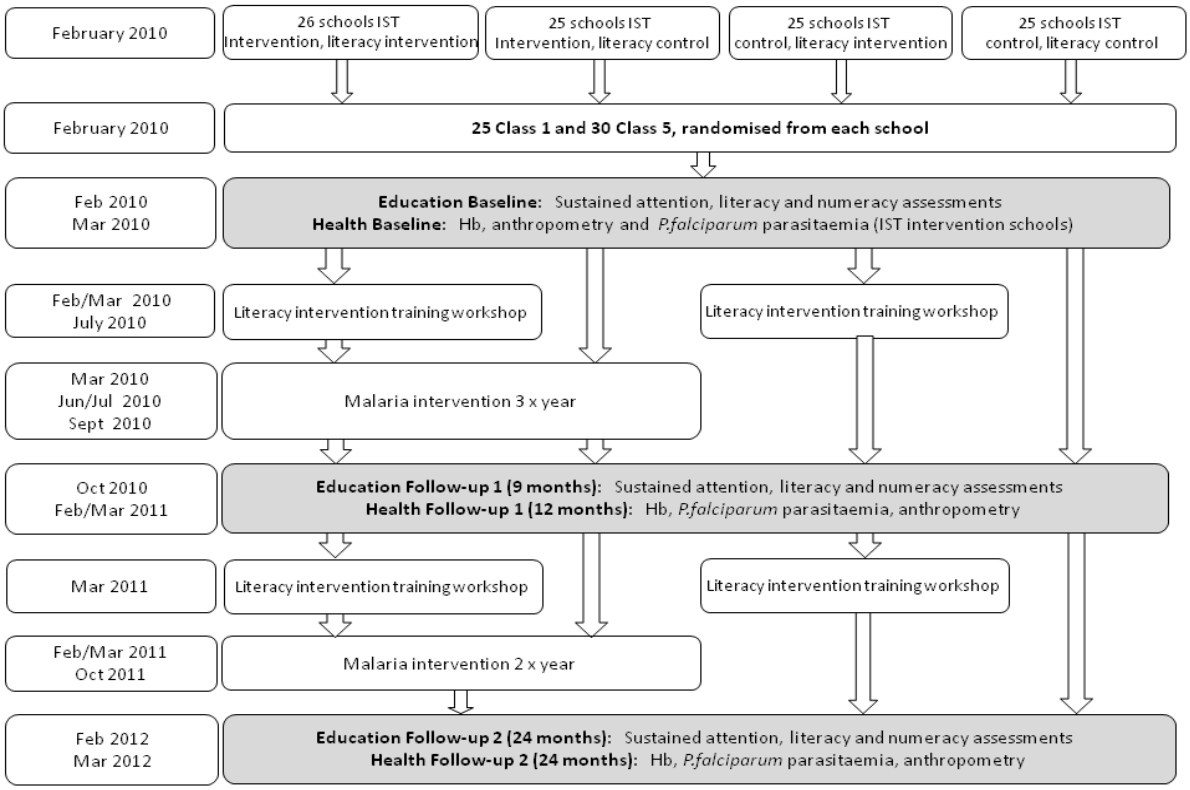


Figure 2.5. Timeline of study activities conducted in the 101 schools by study group.

2.3.8 Health assessments

At enrolment, all children's height and weight were measured, axillary temperature was digitally recorded, and finger-prick blood samples were obtained to determine haemoglobin concentration (Hb). Children known or suspected (as a result of reports from parents, teachers or children themselves) to be homozygous for sickle cell trait, or pregnant were excluded. Any child found with Hb <80 g/L was referred by the nurse to the nearest health facility for iron therapy, and any child found with Hb <50 g/L was taken to the hospital for transfusion. In the intervention group, baseline parasitaemia was measured through preparation of thick and thin blood films during the first round of screening, but baseline parasitaemia was not measured in the control group owing to the ethical constraints of testing for malaria but not treating children found to be infected in the control schools, which was of particular importance at baseline as the intervention involved screening for *Plasmodium falciparum* infection.

Cross-sectional health surveys were carried out in the intervention and control groups at 12 and 24 months (Figure 2.5). During these surveys, temperature, weight, and height were measured and a finger-prick blood sample collected for determination of malaria parasitaemia and Hb. Children with an axillary temperature ≥37.5 °C were tested using an RDT, providing an on-the-spot diagnosis for malaria and treatment administered as per national guidelines. Hb was measured using a portable haemoglobinometer (Hemocue, Ängelholm, Sweden). Thick and thin blood films were stained with Giemsa, asexual parasites were counted against 200 white blood cells (WBCs), and parasite density was estimated assuming an average WBC count of 8,000 cells/µl. A smear was considered negative after reviewing 100 high-powered fields. Thin blood smears were reviewed for species identification. All blood slides were read independently by two microscopists who were blinded to group allocation. Discrepant results were resolved by a third microscopist. In the IST intervention group at the 12-month follow-up, a round of IST was provided alongside the health surveys. Thus in addition to the blood-slide collected and Hb measured, RDTs were conducted on children in this group and AL treatment provided on the basis of the results.

During recruitment a questionnaire was administered to parents/guardians to record information on residence, family size, ownership of possessions, mosquito net use by them and their children, recent deworming of the child, house construction, and parental education.

2.3.9 Attention and education assessments

Tests of sustained attention and educational achievement were administered at baseline, 9 months, and 24 months. Table 2.1 outlines the education assessments undertaken, but in brief, sustained attention was a primary outcome assessed through the code transmission test, adapted from the TEA-Ch (Tests of everyday attention for children) battery [296]. To avoid floor effects, (in which the assessment is too challenging to establish the range of abilities in the target population), a simpler measure of sustained attention, the pencil tap test [297], was used at baseline for the younger cohort. The secondary outcome of educational achievement was measured through tests of literacy and numeracy. At baseline, a test of cognitive non-verbal reasoning, was assessed in Class 1 by the Raven's Progressive Matrices task [298]. All educational assessments were piloted prior to use in the baseline and follow-up evaluations. During piloting, the assessments were conducted under the same assessment conditions on two occasions a week apart, with the correlation between the scores at the two time points providing a reliability score. The inclusion criteria for the tests used in this trial was a Cronbach's alpha correlation of 0.7 or above, indicating a well-constructed test with consistent administration.

The educational assessments were conducted separately to the health assessment both for logistical reasons and to avoid bias during the educational assessments due to apprehension of the finger-prick. The education assessments preceded health assessments by an average of a week, at baseline and 24-month follow-up. However, during the first follow-up, the education assessments were conducted at the end of the school year (9 months) and the health assessments were conducted at end of a full year (12 months).

Table 2.1: Education assessments used in the HALI Project for children in classes 1 and 5

Construct assessed	Class	Assessment	Administration	Source	Description	Baseline	9 month follow-up	24 month follow-up	Chapters
Sustained attention									
	1	Pencil tap	Individual	Adapted from Luria's measures 1966 [297]	Assesses sustained attention and executive control. Children were required to tap a pencil on the desk a predetermined number of times in response to the assessor's taps, while completing a shading (distraction task) simultaneously.	✓			3 & 4
	1	1 digit code transmission	Group	TEA-Ch (Tests of everyday attention for children) battery [296]	A recorded list of digits is read aloud and children are required to listen for a code – two consecutive occurrences of the number 5 - and then record the number that preceded the code.		✓	✓	3 & 4
	5	2 digit code transmission	Group	TEA-Ch (Tests of everyday attention for children) battery [296]	A recorded list of digits is read aloud and children are required to listen for a code – two consecutive occurrences of the number 5 - and then record the two numbers that preceded the code.	✓	✓	✓	3 & 4
Literacy									
	1	Spelling	Group	Adapted from PALS (Phonological Awareness Literacy Screening) [299]	Children are required to spell five 3-letter words with credit given for phonetically acceptable choices for each letter as well as for the correct overall spelling.	✓	✓	✓	3 & 4
	5	Spelling	Group	Adapted from PALS (Phonological Awareness Literacy Screening) [299]	Children are required to spell 25 words with credit given for correctly spelling the features and sound combinations of the word as well as for the correct overall spelling.	✓	✓	✓	3 & 4
	5	Silly sentences	Group	Developed by Baddeley, et al (1995). Speed and Capacity of Language Processing Test (SCOLP)[300]	Designed to evaluate verbal intelligence and language comprehension. Children are required to read a set of 40 sentences in English and tick whether they are true or false based on what they understood from reading the sentence.	✓	✓	✓	3
Numeracy									
	1	Early Grade Maths Assessment	Individual	Developed by RTI under the ED data II project USAID [301]	A suite of tasks designed to orally assess foundational mathematic skills, including number identification, quantity discrimination, addition and subtraction. Addition is the task analysed in this thesis	✓	✓		3 & 4
	1	Written numeracy	Group		A written arithmetic test consisting of a combination of addition, subtraction, multiplication and division. 38 sums in total. Time limited			✓	4
	5	Written numeracy	Group		A written arithmetic test consisting of a combination of addition, subtraction, multiplication and division. 38 sums in total. Time limited	✓	✓	✓	3 & 4
Cognitive function									
	1	Ravens colour progressive matrices	Individual	Raven [298]	Provide a measure of general intelligence in children 5-11 years. 24 non-verbal reasoning tasks, in order of difficulty, focused on pattern recognition.	✓	✓	✓	3

2.3.10 Quality control and data capture

All members of the study team were trained in the study objectives, methods of effective communication with study participants, and collection of high quality data. Study members received additional training specific to the tasks they performed within the study, including the process of information dissemination and informed consent, interviewing techniques and administration of education assessments. Paper-based data collection was employed for the cognitive and education assessments, the health assessments and the socio-demographic questionnaires. The completed data collection forms were submitted daily to the study coordination team on return to the office and these were stored in a locked data office until shipped to the data entry companies in Nairobi and Busia for double entry. Cleaning and consistency checks of all data were performed by the author and discrepancies were resolved using the raw data paper forms.

Task specific standard operating procedures were developed and followed during training, piloting and survey operations. The community liaison officers were trained in the process of disseminating information and collecting written informed consent and completion of questionnaires. All questionnaires were administered in Kiswahili. The education teams undertook a ten-day training workshop on administering and scoring of assessments and a further ten-day pilot training in five schools outside of the study region. Test-retest reliability data was collected for all assessments to ensure sufficient reliability. Inter-rater reliability was assessed for the Spelling Test because of its relatively complex scoring procedures and was found to be high (Fleiss' kappa 0.87 – 0.93) (Jukes *et al*. in preparation). During the education assessments each school was visited by a team of six education assessors, with individual-level tasks administered by four assessors in class 1 and group-level tasks administered by two assessors in class 5.

All technicians (performing the rapid diagnostic tests) and nurses (taking anthropometric measurements and treating with AL) involved in the health screening and assessment surveys received additional training, by an experienced trainer from the Kenya Medical Research Laboratories, Nairobi, and the district pharmacist, on the clinical and laboratory measurements involved prior to each round of screening or health assessments. The Hemocue machines were sent for calibration and cleaning before each round of health assessments. The blood slides collected for assessment of parasitaemia were sent to laboratory technicians at KEMRI Nairobi where they were double-read. No central lot-testing testing of RDTs was conducted prior to the screening rounds [302]. Rotating spotcheck supervision visits were made to all the teams in the field by the author and her coordination team.

Results of study group allocation were only unveiled to both the study team and the community members after completion of the informed consent process in order to eliminate selection bias, and during the trial an effort was made to maintain the field assessors blinded to the group to which schools were assigned and the microscopists conducting the blood slide readings in Nairobi were blind to group allocation.

2.3.11 Ethics Statement

The study was approved by the Kenya Medical Research Institute and National Ethics Review Committee (SSC No. 1543), the London School of Hygiene & Tropical Medicine Ethics Committee (5503), and the Harvard University Committee on the Use of Human Subjects in Research (F17578-101). Prior to the randomization, meetings were held with community and school leaders and parents/guardians in each school to explain the study objectives and procedures. Parents/guardians of all children in classes 1 and 5 were requested to provide individual written informed consent and they were given the option to withdraw their child from the study at any time. Prior to every IST round or assessment, the procedures were explained to

the children and they were required to provide verbal assent. An independent data monitoring committee reviewed the trial protocol, data analysis plan and preliminary results. The study is registered with ClinicalTrials.gov, NCT00878007.

2.4 CONCLUSIONS

This chapter has described the design and operation of the trial, the data from which forms the basis for analysis in the future chapters. The following chapter uses the baseline health, demographic, socioeconomic and education data collected in the IST intervention study group to investigate the epidemiology of *P. falciparum* infection and anaemia in school children in this low-to-moderate transmission setting in Kenya, with an examination of risk factors for both. Additionally the contribution of *P. falciparum* infection, anaemia and other factors to sustained attention and educational performance shall be investigated. Future chapters will present the overall impact of the intervention described above and any differential impact and assess the heterogeneity in underlying transmission risk and implementation of the intervention on the impact and operational applications of IST. The subsequent chapters use the repeated measures data from the health assessment surveys and the IST intervention screening rounds. However, as not all surveys were included in each chapter, the numbering of surveys was redefined in the methodology for each chapter.

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…The study was conceived and designed by Professor Simon Brooker and Dr Matthew Jukes. My role was as research coordinator for the duration of the study. This involved designing, piloting and implementation of survey tools, coordination of data collection, data management, cleaning and analysis. I conceived of the paper with Professor Simon Brooker. I conducted the analyses and drafted the manuscript with high level statistical guidance from Dr Elizabeth Turner and Dr Elizabeth Allen and additional input on the draft from all authors

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SUPERVISOR/SENIOR AUTHOR'S SIGNATURE (3 above) ……..…… ……

Chapter 3. *Plasmodium falciparum*, anaemia and cognitive and educational performance in an area of low to moderate malaria transmission: Baseline results of the cluster randomised trial

3.1 OVERVIEW

As discussed in Chapter 1, the context in which an intervention is introduced is an important consideration, as a variety of extrinsic and intrinsic correlates can modify the effect of the intervention, giving rise, potentially, to heterogeneity in impact of the intervention. Malaria control interventions such as IST are intended to repeatedly clear *Plasmodium falciparum* infection, resulting in a reduction in risk of anaemia. As such, knowledge of the baseline epidemiological variation in risk of these pathologies and associated factors is crucial to developing more effective ways to implement such malaria control strategies. Evidence from modelling work has demonstrated that altering parameters of transmission intensity has a strong effect on the resulting impact of interventions such as IPTi and treatment with ACTs whether through case management, mass treatment or screen and treat campaigns [198,269,303,304]. Examination of the heterogeneity in risk, and of the individual, household and school-level risk factors associated with such variation, is crucial for the subsequent evaluation of the overall impact, and for understanding the potential differential impact of any malaria control intervention.

This chapter uses the data collected from 2400 school children in the IST intervention group during the baseline surveys of the HALI Project to describe the epidemiology of both *P. falciparum* infection (determined by microscopy) and anaemia in the region, and the extent of heterogeneity in both at the school-level. Possible factors contributing to any observed

heterogeneity in risk of these health states at baseline are explored. In addition their association with correlates of cognition, attention and educational achievement is assessed as these are outcomes on which the IST intervention is hypothesised to impact through the mediating pathway of health.

This chapter has been peer reviewed and published in Tropical Medicine and International Health: *Halliday KE, Karanja P, Turner EL, Okello G, Njagi K, Dubeck MM, Allen E, Jukes MCH & Brooker SJ. (2012)* Plasmodium falciparum, *anaemia and cognitive and educational performance among school children in an area of moderate malaria transmission: baseline results of a cluster randomized trial on the coast of Kenya. Trop Med Int Health 17: 532-549.* I coordinated the data collection, performed data cleaning and analyses and drafted the manuscript presented in this chapter, with high level statistical guidance from Dr Elizabeth Turner and Dr Elizabeth Allen.

3.2 INTRODUCTION

The health of school children has received increasing attention over the last two decades, and there are increased efforts to implement school health programmes, delivering anthelmintics and micronutrients [199,305]. Less emphasis has been given to malaria as a health problem facing school children despite them experiencing some of the highest age-specific rates of *Plasmodium* infection [19,20,306], which can have a number of direct and indirect consequences, including anaemia [307]. The control of malaria is associated with significant improvements in haemoglobin levels among both young children [127] and children of school-age [33,40,123,307]. Malaria may have additional consequences for children's cognitive performance and ultimately educational achievement [57,58,114,137,308]. For instance, malaria has been related to increased absenteeism [108,141], grade repetition [140], and poorer educational achievement [134,309]. Studies in Kenya and Sri Lanka suggest that malaria

prevention can improve school attendance, sustained attention, and educational achievement [33,42,124,310].

The consequences of malaria for school children and the benefits of school-based malaria control are likely to vary in different settings, particularly according to intensity of malaria transmission and the relative contribution of other causes of anaemia and poor education outcomes. A previous study in an area of perennial high malaria transmission in western Kenya [33] investigated the impact of intermittent preventive treatment for malaria in schools, and found a large impact on children's concentration in class and a 48% reduction in the rates of anaemia. However, no effect on educational achievement was observed. To investigate this result further and find whether the benefits of malaria control are observed in settings with different intensities of malaria transmission, and different educational standards, a cluster randomised trial was conducted to investigate the impact of an alternative school-based malaria intervention, intermittent screening and treatment (IST), in an area of low-to-moderate malaria transmission on the coast of Kenya [284]. The current chapter presents data from the baseline cross-sectional survey of this trial and explores variation in risk of *Plasmodium falciparum* infection and anaemia, and the individual, household and school-level risk factors associated with such variation, as well as correlates of cognition, attention and educational achievement.

3.3 METHODS

3.3.1 Design

The study design and methods of the intervention trial have been previously detailed in chapter 2 and are briefly summarized below in regards to the baseline data collection. The current investigation uses baseline cross-sectional data collected between February and March 2010 in the 51 intervention schools which were allocated to receive the intermittent screening and

treatment for malaria. No baseline data were collected on *P. falciparum* infection for the 50 control schools (not receiving the IST intervention) due to ethical considerations about screening for malaria and not providing treatment. Results reported here on *Plasmodium* infection are based on expert microscopy. Reporting of the current study has been verified in accordance with the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) checklist [311].

3.3.2 Recruitment

A school census of all schools included in the trial was conducted by trained personnel and was used as a sampling frame from which 25 children from class 1 and 30 children from class 5 were randomly selected using random number tables. Fewer children were selected from class 1 because of the extra educational assessments undertaken with these children and the feasibility of conducting the tests in a single day.

3.3.3 Health and education surveys

As described in chapter two, finger prick blood samples were obtained from all children to assess haemoglobin concentration (Hb) using a portable haemoglobinometer (Hemocue, Ängelholm, Sweden) and to prepare thick and thin blood smears for malaria microscopy. Children with severe anaemia (Hb< 80g/L) were referred to the nearest health facility for iron therapy as per the national guidelines. Height and weight were measured to the nearest 0.1cm and 0.1kg using Leicester portable fixed base stadiometers and electronic balances, respectively and axillary temperature was digitally recorded.

Educational assessments are detailed in Table 2.1. In brief, age-appropriate tests of sustained attention were conducted in each class: the pencil tap test and the code transmission test adapted from the TEA-Ch (Tests of everyday attention for children) group [296] for class 1 and class 5 children, respectively. Non-verbal reasoning was assessed in Class 1 by the Raven's Progressive

Matrices task [298]. A range of class-specific literacy and numeracy tests were conducted in individualized and small-group settings. The tests were extensively piloted and adapted to the context.

3.3.4 Risk factors

During the informed consent process, a questionnaire was administered to parents/guardians to record household information on residence, family size, ownership of possessions, mosquito net use by them and their children, recent deworming of the child, household construction (eg roof and wall materials) and education level of the parent. For children in class 1, additional information on household literacy, the language spoken in the household and reading practices was recorded. At each school, a questionnaire was administered to the head-teacher to collect information on school demography, sanitation facilities, presence of school feeding and other health programmes. School locations were mapped using a Global Positioning System (GPS) receiver (eTrex Garmin Ltd., Olathe, KS). Elevation of schools was recorded and used as a geographical marker of distance from the coast.

3.3.5 Statistical analysis

Data were double-entered using customised data entry screens in Microsoft Access (Microsoft Corporation, Seattle, USA). Consistency checks were performed and all discrepancies and queries verified against the original paper forms. Health data were linked by school location and mapped using ArcGIS 9.3.1 (Environmental Systems Research Institute Inc. Redlands, CA, US).

P. falciparum infection was defined on the basis of duplicate slide readings. Anaemia was defined using age and sex corrected WHO thresholds [312], with no correction made for altitude. The anthropometric indices, z-scores of height-for-age (HAZ), weight-for-age (WAZ) and body mass index-for-age (BMIZ), were calculated using the AnthroPlus software for children aged 5-19 years [313], assuming a mid-year age for each child because of doubts over the correct date of birth. Weight-for-age z-score was only calculated for children aged 5-10 years. Children were

classified as stunted, underweight or thin if HAZ, WAZ and BMIZ respectively were less than 2 standard deviations below the reference median. Age of the children was provided by themselves and by their parents. Ages provided by the children were used to calculate anaemia and anthropometric indices as they were considered more reliable. A sensitivity analysis using parent-reported ages for all multivariable models indicated minimal sensitivity. Age was modelled as a categorical variable for the *P. falciparum* and anaemia risk factor analyses and as a continuous variable for the attention and education analyses, due to the smaller age ranges observed once stratified by class.

Household asset data (Appendix 3.1) were used to derive an index of socio-economic status (SES), based on the entire trial population. The principal component analysis (PCA) approach proposed by Filmer and Pritchett [314] was used. Variables included into the PCA included, ownership of a bicycle, motorcycle, mobile phone, radio, television, as well as presence of electricity, pit latrine, and brick and cement construction materials. The first principal component explained 30.6% of the overall variability and gave greatest weight to the household construction materials followed by ownership of a television (Appendix 3.1). The resultant scores were divided into quintiles so that households could be classified according to relative SES. No internal validation of the index was undertaken. Finally, elevation (a proxy for distance from the coastline) was divided into tertiles.

Analyses were performed using STATA version 11.0 (STATA Corporation, College Station, TX, USA). The outcomes of interest examined were prevalence of *P. falciparum* and of anaemia (binary outcomes) and scores for spelling, number identification, numeracy, comprehension, code transmission and pencil tapping tasks (continuous outcomes). Univariable associations between the health related outcomes and risk factors were assessed using multilevel logistic regression, accounting for school-level clustering [315]. Variables demonstrating an association at the 10% significance level were subsequently included into a multivariable, multilevel logistic regression model, accounting for school-level clustering. Stepwise elimination was used to

create the final model using a 5% significance level for retention in the model. Age and sex were treated as *a priori* risk factors and retained in multivariable models. *A priori* interactions between net use with age and sex and between school-feeding and elevation (distance from the coast) were investigated.

Analysis of the cognitive and education outcomes was stratified by class, and focused on associations with *P. falciparum* infection and with anaemia, additionally accounting for age and sex as *a priori* risk factors. For the pencil tap assessment of sustained attention in class 1 children, the analysis was split into two due to the significant proportion of children who were disengaged and scored zero. The proportion of children engaged in the task was examined by different variables using multilevel logistic regression accounting for school-level clustering. For each of the spelling assessments in classes 1 (score 0-20) and 5 (score 0-43); the numeracy in classes 1 (score 0-20) and 5 (score 0-38); the Ravens assessment in class 1 (score 0-20); the sentence comprehension in class 5 (score 0-40); the code transmission assessment of sustained attention in class 5 children (score 0-20) and the analysis of children who were engaged in the pencil tap task (score 1-20), the effect of explanatory variables was quantified by mean differences in test performance using linear regression. Bootstrapping was used to account for non-normality of the scores, whereby schools were resampled to account for school-level clustering [316]. Bias-corrected confidence intervals based on the bootstrap resamples were obtained. Significant ($p<0.1$) variables identified in univariable analysis were considered for the multivariable model which employed stepwise elimination.

3.4 RESULTS

3.4.1 Study population description

Of the 3,850 children randomly identified as eligible for inclusion in the study, further processes of selection (Figure 3.1) resulted in a total of 2,400 children (1160 in class 1 and 1240 in class 5) who were included in the analysis, with a mean of 48 children per school (range 26-60). No

systematic differences in individual and household characteristics were observed between included children and those children excluded due to missing health data Table 3.1. The mean age of children in the present analysis was 10.3 years (range 5-18 years) and the male/female ratio was 0.95 (Table 3.2).

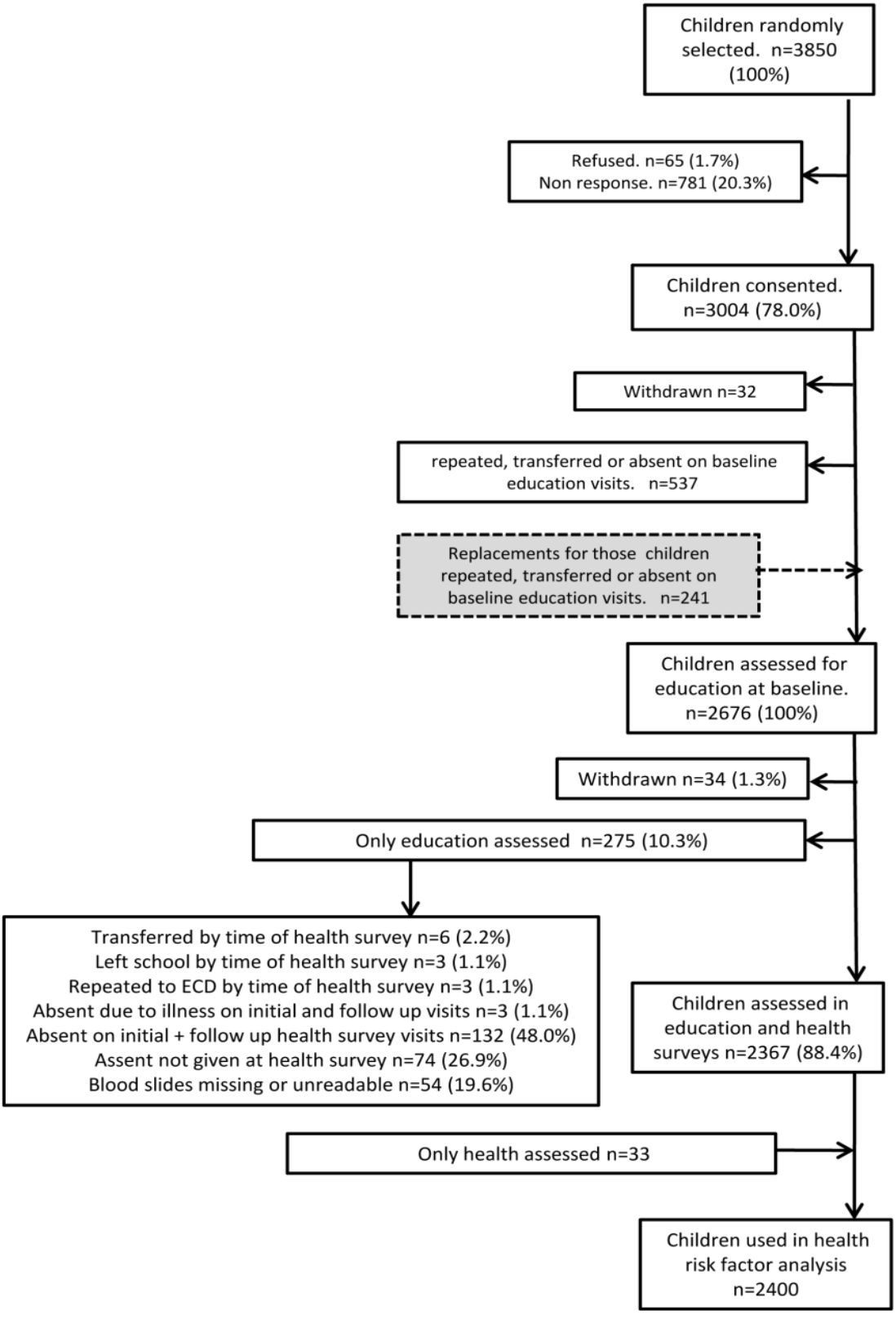

Figure 3.1. Data flow diagram for the education and health surveys conducted in school children in 51 schools on the South Coast of Kenya, 2010.

Table 3.1: Characteristics of study children with health data only or health and education data (included in analysis) and study children with education data only (excluded from analysis).

Variable	Children with health data (included in this analysis) N (%)[a], n=2400	Children with only education data (excluded from analysis) N (%)[a] n=275
Division[b]		
Diani	636 (26.5)	51 (18.6)
Lunga Lunga	960 (40.0)	128 (46.5)
Msambweni	312 (13.0)	60 (21.8)
Kubo	492 (20.5)	36 (13.1)
Sex		
Male	1167 (48.6)	132 (48.0)
Female	1233 (51.4)	143 (52.0)
Age (yrs)		
	10.34 (2.81)	10.31 (2.97)
Age groups (yrs)		
5-10	940 (39.2)	111 (40.4)
11-12	860 (34.6)	87 (31.6)
13-18	630 (26.2)	77 (28.0)
Education level of household head		
No schooling	814 (34.3)	95 (35.9)
Primary	1228 (51.8)	141 (53.2)
Secondary	255 (10.8)	22 (8.3)
College/degree	74 (3.1)	7 (2.6)
Number of people in household		
	7.06 (2.52)	7.11 (2.34)
Number of children in household		
	4.82 (2.18)	4.76 (2.11)
SES quintile		
Poorest	577 (24.2)	74 (27.9)
Poor	504 (21.1)	54 (20.4)
Median	423 (17.7)	63 (23.8)
Less poor	459 (19.3)	41 (15.5)
Least poor	422 (17.7)	33 (12.4)
Child sleeps under a net		
No	880 (37.2)	87 (33.0)
Yes	1489 (62.8)	177 (67.0)
Child been dewormed in last year		
No	442 (18.6)	59 (23.4)
Yes	1824 (77.0)	193 (76.6)
Malaria control activities in school [b]		
No	1814 (74.1)	224 (81.5)
Yes	586 (25.9)	51 (18.5)
School feeding programme in school [b]		
No	1115 (46.5)	143 (52.0)
Yes	1285 (53.5)	132 (48.0)

[a] Displayed as number and percentage except for continuous variables, displayed as Mean and Standard Deviation (SD),

[b] Measured at the school level.

3.4.2 *Plasmodium falciparum* and anaemia

The overall prevalence of *P. falciparum* was 13.0% (95% confidence interval [CI]: 8.9-17.0%); only 11 infected children had documented fever. Infection prevalence varied markedly by school, ranging from 0 to 75.0% (Figure 3.2a.), with no infected children found in seven schools and a prevalence exceeding 40% in three schools. Overall, 45.5% (95% CI: 42.0-48.9%) of children were anaemic and 1.1% (95% CI: 0.7-1.5) were severely anaemic. The mean haemoglobin concentration was 117.5g/L (95% CI: 116.4-118.6). Marked heterogeneity was also observed in the school-level prevalence of anaemia (range: 26.3-80.0%) (Figure 3.2b.).

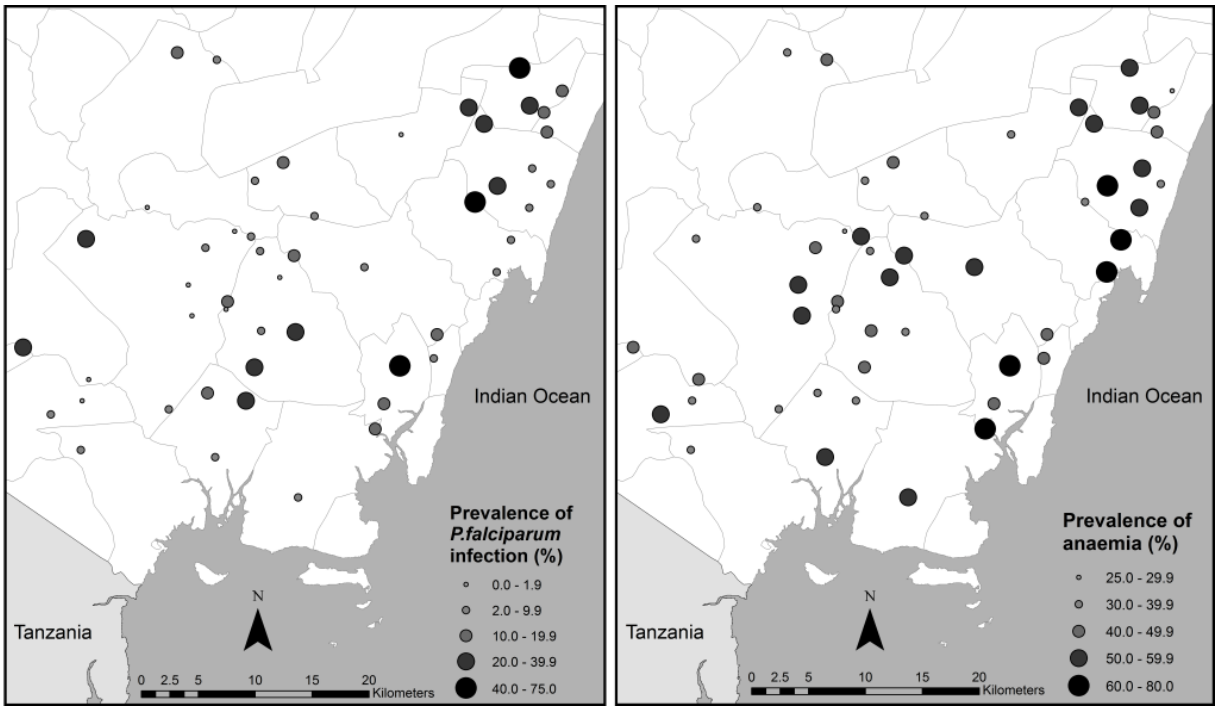

Figure 3.2. a) The geographical distribution of *Plasmodium falciparum* infection in 51 schools on the south coast of Kenya, 2010. b) The geographical distribution of anaemia (adjusted for age and sex) in 51 schools on the South Coast of Kenya, 2010.

3.4.3 Risk factors for *P. falciparum* infection and anaemia

The relative frequencies of individual, household and school-level risk factors for *P. falciparum* infection and anaemia are shown in Table 3.2. Overall, 62.8% (95% CI: 58.7-67.0) of children were reported as sleeping under a mosquito net, but usage varied markedly by school (range: 26.4-93.3%). In univariable analysis, *P. falciparum* infection was significantly associated with being male, younger age, stunting, absence of deworming, education level of household head, increased number of people in the household, fewer mosquito nets in the household, and not sleeping under a mosquito net. In the multivariable model, higher odds of *P. falciparum* infection were significantly associated with being male, younger age groups, increasing number of people living in the child's household and the child not sleeping under a net (Table 3.3), with children sleeping under s net approximately 30% less likely to be infected.

In univariable analysis, anaemia was significantly associated, with male sex, younger age, *P. falciparum* infection, being stunted, education level of household head, not attending a school with an active school feeding programme and attending school at lower elevation, closer to the coast (Table 3.2). In multivariable analysis, increased odds of anaemia were significantly associated with *P. falciparum* infection, with the odds increasing with increasing parasite density, (AOR [adjusted odds ratio]: 3.68, 95%CI: 2.12-6.38 p<0.001) for children with high intensity infection versus those with no infection, and for children who were stunted (Table 3.3) where stunted children were almost 10% more likely to be anaemic. Significantly lower odds of anaemia were associated with children who were female, being aged 10-12 years old versus 5-9 years old. The effect of a school feeding programme on anaemia was modified by elevation of school (distance from coast); and thus is presented by stratum specific odds ratios. School feeding was associated with lower odds of anaemia in schools closest to the coast (AOR: 0.46, 95%CI: 0.28-0.76 p=0.003) with no evidence of an association for schools positioned further from the coast

Table 3.2.: Univariable analysis for associations between *P. falciparum* infection and anaemia and potential risk factors for both health outcomes among school children in 51 schools on the south coast of Kenya, 2010.

Variable	Number of children (%)[a] **n=2400**	Number of children (%) with *P. falciparum* **n=311**	Odds Ratio (95% CI)	P-value[f]	Number of children (%) with anaemia **n=1091**	Odds Ratio (95% CI)	P-value[f]
CHILD-LEVEL							
Sex							
Male	1167 (48.6)	173 (14.8)	1		566 (48.5)	1	
Female	1233 (51.4)	138 (11.2)	0.67 (0.51-0.87)	0.003	525 (42.6)	0.76 (0.65-0.90)	0.001
Age (per additional year)[b]							
	10.34 (2.81)		0.92 (0.87-0.96)	<0.001		1.00 (0.97-1.03)	0.863
Age groups (years)							
5-9	940 (39.2)	139 (14.8)	1		458 (48.7)	1	
10-12	830 (34.6)	120 (14.5)	0.90 (0.67-1.21)	<0.0001	337 (40.6)	0.71 (0.59-0.87)	0.002
13-18	630 (26.2)	52 (8.3)	0.42 (0.29-0.61)		296 (47.0)	0.96 (0.78-1.18)	
***P. falciparum* infection status**							
Not infected	2089 (87.0)				914 (43.8)	1	
Infected	311 (13.0)	-	-		177 (56.9)	1.66 (1.28-2.15)	<0.001
***P. falciparum* density (p/µl)**							
No infection (0)	2089 (87.0)				914 (43.8)	1	
Low (1-999)	237 (9.9)	-	-		124 (52.3)	1.37 (1.03-1.82)	<0.0001
Medium/high (1000>)	74 (3.1)				53 (71.6)	3.28 (1.93-5.57)	
WAZ (z scores)[c,d,e]							
Not underweight	709 (75.8)	102 (14.4)	1		343 (48.4)	1	
Underweight	227 (24.2)	37 (16.3)	1.37 (0.86-2.17)	0.188	113 (49.8)	1.06 (0.77-1.46)	0.726
HAZ (z scores)[c]							
Not stunted	1790 (74.8)	223 (12.5)	1		794 (44.4)	1	
Stunted	603 (25.2)	88 (14.6)	1.31 (0.97-1.76)	0.083	294 (48.8)	1.27 (1.04-1.54)	0.016
BMIZ (z scores)[c]							
Not thin	1950 (81.5)	262 (13.4)	1		895 (45.9)	1	
Thin	442 (18.5)	49 (11.1)	0.85 (0.60-1.22)	0.377	193 (43.7)	0.91 (0.73-1.12)	0.363
Child been Dewormed in last year[d]							
No	442 (19.5)	72 (16.3)	1		181 (41.0)	1	
Yes	1824 (80.5)	220 (12.1)	0.70 (0.50-0.97)	0.033	843 (46.2)	1.19 (0.95-1.48)	0.130
HOUSEHOLD-LEVEL							
Education level of household head							
No schooling	814 (34.3)	131 (16.1)	1		392 (48.2)	1	
Primary	1228 (51.8)	153 (12.5)	0.83 (0.63-1.11)		523 (42.6)	0.79 (0.66-0.95)	
Secondary	255 (10.8)	17 (6.7)	0.50 (0.28-0.89)	0.056	126 (49.4)	1.05 (0.78-1.41)	0.047
College/degree	74 (3.1)	6 (8.1)	0.54 (0.22-1.34)		34 (46.0)	0.91 (0.56-1.50)	

Table 3.2 continued

Variable	Number of children (%)[1] **n=2400**	Number of children (%) with *P. falciparum* **n=311**	Odds Ratio (95% CI)	P-value[5]	Number of children (%) with anaemia **n=1091**	Odds Ratio (95% CI)	P-value[5]
Water source							
Uncovered (stream/river/dam)	318 (13.4)	63 (19.8)	1		135 (42.5)	1	
Covered (well/borehole/piped)	2066 (86.7)	246 (11.9)	0.76 (0.49-1.20)	0.240	948 (45.9)	1.05 (0.80-1.38)	0.742
SES							
Poorest	577 (24.2)	89 (15.4)	1		270 (46.8)	1	
Poor	504 (21.1)	67 (13.3)	0.94 (0.64-1.38)		240 (47.6)	1.01 (0.79-1.29)	
Median	423 (17.7)	61 (14.4)	1.00 (0.67-1.48)	0.206	171 (40.4)	0.75 (0.58-0.98)	0.207
Less poor	459 (19.3)	48 (10.5)	0.83 (0.55-1.27)		206 (44.9)	0.89 (0.69-1.15)	
Least poor	422 (17.7)	44 (10.4)	0.66 (0.42-1.03)		197 (46.7)	0.93 (0.72-1.22)	
Number of people in the house[b]							
	7.06 (2.52)	-	1.06 (1.00-1.12)	0.036	-	1.01 (0.98-1.05)	0.390
Number of children in the house[b]							
	4.82 (2.18)	-	1.04 (0.98-1.11)	0.183	-	1.03 (0.99-1.07)	0.196
Child sleeps under a net							
No	880 (37.2)	140 (15.9)	1		406 (46.1)	1	
Yes	1489 (62.8)	166 (11.2)	0.62 (0.47-0.82)	<0.001	666 (44.7)	0.95 (0.80-1.13)	0.554
If yes, is the net treated?							
No	278 (8.7)	34 (12.2)	1		129 (46.4)	1	
Yes	1161 (78.2)	127 (11.0)	1.06 (0.67-1.68)	0.879	518 (44.6)	0.93 (0.71-1.22)	0.643
Don't Know	46 (3.1)	5 (10.9)	0.83 (0.27-2.58)		18 (39.1)	0.74 (0.39-1.41)	
Number of nets in the house[d]							
No nets	360 (17.0)	55 (15.3)	1		170 (47.2)	1	
1-2 nets	655 (30.9)	98 (15.0)	1.01 (0.67-1.50)		286 (43.7)	0.84 (0.64-1.10)	
3-4 nets	810 (38.2)	85 (10.5)	0.65 (0.43-0.99)	0.003	362 (44.7)	0.90 (0.69-1.17)	0.423
>=5 nets	295 (13.9)	26 (8.8)	0.46 (0.27-0.80)		136 (46.1)	0.96 (0.70-1.33)	
SCHOOL-LEVEL							
School Malaria control activities							
No	1814 (75.6)	238 (13.1)	1		835 (46.0)	1	
Yes	586 (24.4)	73 (12.5)	1.20 (0.48-3.00)	0.697	256 (43.7)	0.90 (0.66-1.24)	0.531
School feeding programme							
No	1115 (46.5)	139 (12.5)	1		555 (49.8)	1	
Yes	1285 (53.5)	172 (13.4)	0.89 (0.40-1.97)	0.775	536 (41.7)	0.73 (0.57-0.94)	0.017
Elevation (meters)							
0-50	708 (29.5)	120 (17.0)	1		370 (52.3)	1	
51-100	919 (38.3)	119 (13.0)	0.59 (0.24-1.50)	0.176	410 (44.6)	0.73 (0.54-0.99)	0.013
101-200	773 (32.2)	72 (9.3)	0.39 (0.15-1.05)		311 (40.2)	0.61 (0.45-0.84)	

[a]Displayed as number and percentage except for continuous variables, displayed as mean and standard deviation (SD). [b]Modelled as continuous variable. [c]WAZ – (weight-for-age) HAZ- (height-for-age) BMI – (body-mass-index-for-age). Underweight, stunted and thin defined as WAZ, HAZ and BMIZ z-scores <2SD. [d]Characteristics with missing data vary (all below 2% missing except deworming 5.6% missing, number of nets owned 11.7% missing, and WAZ 61% missing). [e]WAZ only calculated for children aged 5 years to 10 years. [f]P-value is from likelihood ratio test comparing multilevel logistic regression models (adjusting for school level clustering), with and without character of interest.

Table 3.3. Multivariable risk factor analysis for *P. falciparum* infection and anaemia among school children in 51 schools on the south coast of Kenya, 2010.

Variable	***P. falciparum* Infection**[d]			**Anaemia**[e]		
	Adjusted[f] Odds Ratio	95% confidence interval	P-value[g]	Adjusted[f] Odds Ratio	95% confidence interval	P-value[g]
Sex						
Male	1			1		
Female	0.68	0.51-0.89	0.005	0.80	0.67-0.95	0.009
Age (years)						
5-9	1			1		
10-12	0.87	0.64-1.18		0.71	0.58-0.87	
13-18	0.37	0.25-0.54	<0.001	0.97	0.78-1.20	0.002
Child sleeps under a net						
No	1					
Yes	0.60	0.45-0.79	<0.001	-	-	-
Number of people in the house[a]						
	1.07	1.01-1.14	0.014	-	-	-
***P. falciparum* density (p/µl)**						
No infection				1		
Low (1-999)				1.41	1.05-1.89	
Medium/high (>=1000)	-	-	-	3.68	2.12-6.38	<0.001
HAZ (z scores)						
Not stunted				1		
Stunted	-	-	-	1.26	1.03-1.54	0.022
Education level of household head						
No schooling				1		
Primary	-	-	-	0.78	0.64-0.94	
Secondary				1.12	0.83-1.50	0.014
College/degree				0.89	0.53-1.48	
Effect of elevation (m) by absence/presence of school feeding[b]						
No school feeding 0-50				1		
51-100				0.58	0.40-0.83	
101-200				0.58	0.34-1.00	
School feeding 0-50				1		0.003[h]
51-100				1.30	0.79-2.15	
101-200				1.32	0.63-2.76	
Effect of school feeding programme by elevation (m)[b,c]						
0-50 No school feeding				1		
School feeding				0.46	0.28-0.76	
51-100 No school feeding				1		
School feeding				1.05	0.72-1.51	0.003[h]
101-200 No school feeding				1		
School feeding				0.82	0.48-1.39	

[a] modelled as continuous variable,
[b] There was statistical evidence of an interaction between elevation of schools and presence of a school feeding programme on anaemia, therefore the stratum specific results are reported both for school feeding and elevation (P-value derived from Likelihood Ratio Test comparing the model with school feeding and elevation variables separately with the model also including the interaction between the two variables is p=0.042)
[c] At elevation group 50-100m 184 children have school feeding and 524 do not. At group 51-100m 468 children have school feeding and 451 do not. At elevation group 101-200m 633 children have school feeding and 140 do not,
[d] n=2369 observations included for children with complete data for all variables
[e] n=2364 observations included for children with complete data for all variables
[f] Adjusted for variables included in final multivariable regression model as shown,
[g] P-value derived from Likelihood Ratio Test in multivariable multilevel, logistic regression model, adjusted for school-level clustering,
[h] P-value is derived from Likelihood Ratio Test comparing the model with both the school feeding and elevation variables and their interaction term with the model without either of the variables.

3.4.4 Associations with cognition and educational achievement

Results from the univariable analysis of the associations between cognition and educational achievement and health and other factors are presented in Appendices 3.2, 3.3 and 3.4. Results from multivariable analysis are presented in Tables 3.4 and 3.5, which report significant associations between scores and several child-level variables. In all tasks, increasing age was associated with higher scores among children in class 1, but with lower scores among children in class 5. For several tasks, girls were found to have lower scores than boys, such as in spelling and comprehension in class 5 where girls scored on average more than a mark lower than the boys. Neither *P. falciparum* infection, irrespective of parasite density, or anaemia were found to be associated with any cognitive or educational outcome. Interestingly, poor engagement in the attention task for class 1 was associated with eating breakfast and attending a school with school feeding, and better spelling performance in class 5 was found among children who were classified as thin on the basis of BMI.

A number of household factors were also associated with the cognition and educational achievement scores. Higher household socio-economic status was associated with higher scores in the comprehension task in class 5 and the spelling in both classes. Lower scores were associated with living in a house with a high number of children for the class 5 comprehension task. Higher parental education levels were associated with higher scores in the class 5 comprehension and class 1 numeracy tasks. School environment and educational administrative zones were found to be associated with several of the tasks, with lower literacy and numeracy scores associated with children learning in classrooms without desks, and significantly higher spelling and numeracy scores in class 5 as well as higher attention and cognitive scores in class 1 found in children schooling in coastal Diani zone.

Table 3.4: Multivariable risk factor analysis - associations of *P. falciparum* infection and anaemia with a test of sustained attention and a test of literacy in children in classes 1 and 5

Variable	Attention Assessments						Literacy assessments			
	Pencil Tap – Class 1				**Code transmission – Class 5**		**Spelling - Class 1**		**Spelling – Class 5**	
	Adjusted OR for engagement (95% CI) n=1122	P-value[b]	Mean adjusted difference in performance children who were engaged[c,d] (95% CI) n=998	P-value[e]	Mean adjusted difference in performance[c] (95% CI) n=1227	P-value[e]	Mean adjusted difference in performance[c] (95% CI) n=1127	P-value[e]	Mean adjusted difference in performance[c] (95% CI) n=1216	P – value[e]
CHILD LEVEL										
***P.falciparum* density (p/µl)**										
No infection (0)	1									
Low (1-999)	1.00 (0.53-1.80)		-0.05 (-1.01, 1.12)		0.06 (-1.42, 1.52)		0.63 (-0.56, 2.12)		-1.00 (-2.90, 0.89)	
Medium/High (≥1000)	6.38 (0.85-47.87)	0.053	-0.00 (-1.28, 1.23)	1.000	-0.97 (-3.40, 1.03)	0.638	1.14 (0.08, 2.28)	0.102	-2.49 (-6.80, 1.31)	0.205
Anaemia status										
Not anaemic	1									
Anaemic	1.20 (0.82-1.77)	0.353	0.17 (-0.41, 0.77)	0.169	0.34 (-0.25, 0.95)	0.250	0.35 (-0.18, 0.98)	0.343	0.69 (-0.28, 1.68)	0.170
Sex										
Male	1									
Female	0.80 (0.55-1.17)	0.249	**-0.62 (-1.16, 0.00)**	**0.037**	-0.61 (-1.37, 0.07)	0.102	0.43 (-0.13, 1.02)	0.268	**-1.32 (-2.14, -0.46)**	**0.003**
Age (years)[a]										
	1.16 (1.03-1.31)	**0.014**	**0.44 (0.22, 0.62)**	**<0.001**	**-0.30 (-0.47, -0.10)**	**0.003**	**0.27 (0.06, 0.47)**	**0.025**	**-1.32 (-1.65, -1.03)**	**<0.001**
BMIZ (z score)										
Not thin										
Thin	-	-	-	-	-	-	-	-	**1.12 (0.13, 2.11)**	**0.026**
Eat breakfast before school										
No	**1**		-	-	-	-	-	-	-	-
Yes	**0.46 (0.28-0.74)**	**0.001**								
HOUSEHOLD-LEVEL										
SES quintile										
Poorest										
Poor			**-0.17 (-1.17, 0.72)**				**0.09 (-0.59, 0.91)**		**-0.14 (-1.32, 1.34)**	
Median	-	-	**-0.85 (-1.80, 0.04)**		-	-	**0.49 (-0.33, 1.38)**		**2.30 (1.24, 3.52)**	**<0.001**
Less poor			**-0.92 (-2.00, 0.17)**	**0.026**			**1.20 (0.43, 2.17)**	**0.006**	**1.42 (0.30, 2.60)**	
Least poor			**-1.27 (-2.33, -0.16)**				**1.48 (0.59, 2.46)**		**3.32 (1.95, 4.80)**	
SCHOOL-LEVEL										
School Feeding Programme										
No	**1**									
Yes	**0.62 (0.39-0.98)**	**0.039**	-	-	-	-	-	-	-	-
Seating in classroom										
Desks or tables and chairs										
Floor	-	-	-	-	-	-	**-1.76 (-3.55, -0.48)**	**0.026**	-	-
Division										
Diani										
Lunga Lunga			**-0.18 (-1.16, 0.74)**						**-3.53 (-5.79, -1.49)**	
Msambweni	-	-	**-1.20 (-2.40, -0.08)**	**0.016**	-	-	-	-	**-3.95 (-6.17, -2.13)**	**<0.001**
Kubo			**-1.58 (--2.78, -0.39)**						**-3.79 (-6.19, -1.33)**	

[a] Modelled as a continuous variable, [b] P-value derived from Likelihood Ratio test of model with and without variable of interest in multivariable multilevel logistic regression analysis (adjusting for school level clustering).
[c] Positive values indicate an increased score over reference group and negative values indicate a decreased score over reference group (95% CI is the bias corrected confidence interval),
[d] Only children found to be engaged in task are included. [e] P-value is from multivariable Wald test derived from multivariable linear regression, bootstrapped and adjusted for school level clustering

Table 3.5: Multivariable risk factor analysis - associations of *P. falciparum* infection and anaemia with a test of cognition and numeracy in children in classes 1 and 5

Variable	Cognitive non verbal reasoning Assessment		Comprehension Assessment		Numeracy Assessments			
	Ravens test – Class 1		**Silly sentences – Class 5**		**Number identification -Class 1**		**Written numeracy – Class 5**	
	Mean difference in performance in children who were engaged[b] (95% CI) n=1118	P-value[c]	Mean difference in performance[b,] (95% CI) n=1211	P-value[c]	Mean difference in performance[b] (95% CI) n=1119	P-value[c]	Mean difference in performance[b] (95% CI) n=1219	P –value[c]
CHILD-LEVEL								
***P.falciparum* density (p/µl)**								
No infection (0)								
Low (1-999)	-0.48 (-0.97, 0.02)		-0.03 (-2.07, 0.88)		-0.10 (-0.60, 0.37)		0.07 (-1.28, 1.16)	
Medium/High (≥1000)	0.23 (-0.51, 1.23)	0.151	-1.09 (-3.78, 2.95)	0.799	-0.15 (-0.84, 0.57)	0.876	0.51 (-1.18, 2.57)	0.866
Anaemia status								
Not anaemic								
Anaemic	-0.01 (-0.27, 0.25)	0.936	0.14 (-0.65, 0.86)	0.960	0.34 (0.00, 0.70)	0.053	-0.21 (-0.89, 0.47)	0.540
Sex								
Male								
Female	-0.08 (-0.33, 0.14)	0.524	**-1.08 (-1.84, -0.18)**	**0.005**	0.03 (-0.28, 0.32)	0.841	0.09 (-0.64, 0.74)	0.800
Age (years) [a]								
	0.13 (0.02, 0.25)	**0.029**	**-0.56 (-0.88, -0.27)**	**<0.001**	**0.26 (0.13, 0.38)**	**<0.001**	-0.02 (-0.26, 0.19)	0.870
HOUSEHOLD-LEVEL								
SES quintile								
Poorest								
Poor			**-0.19 (-1.24, 0.92)**					
Median	-	-	**1.54 (0.37, 2.74)**		-	-	-	-
Less poor			**1.07 (0.01, 2.15)**	**<0.001**				
Least poor			**2.43 (1.18, 3.68)**					
No. of children in household								
			-0.24 (-0.40, -0.08)	**0.026**	-	-	-	-
Education of household head								
No schooling								
Primary	-	-	**-0.06 (-0.81, 0.64)**		-	-	-	-
Secondary			**1.11 (-0.35, 1.93)**	**0.012**				
College/degree			**3.33 (1.10, 4.88)**					
Parent is literate								
No								
Yes	-	-	-	-	**0.49 (0.18, 0.82)**	**0.003**	-	-
SCHOOL-LEVEL								
Seating arrangement in classroom								
Desks or tables and chairs								
Floor	-	-	-	-	**-0.78 (-1.34, -0.30)**	**0.005**	-	-
Division								
Diani								
Lunga Lunga	**-0.87 (-1.34, -0.45)**						**-2.48 (-3.85, -0.88)**	
Msambweni	**0.51 (-0.31, 1.43)**	**<0.001**	-	-	-	-	**-1.69 (-3.53, -0.00)**	**<0.001**
Kubo	**-0.47 (-1.08, 0.22)**						**-3.76 (-6.38, -1.94)**	

[a] Modelled as a continuous variable, [b] Positive values indicate an increased score over reference group and negative values indicate a decreased score over reference group (95% CI is the bias corrected confidence interval),
[c] P value is from multivariable Wald test derived from multivariable linear regression, bootstrapped and adjusted for school level clustering

3.5 DISCUSSION

The evidence presented here shows that in this moderate malaria transmission setting there is marked variation in the prevalence of *P. falciparum*, with some schools having no microscopy-detected *Plasmodium* infections and prevalence reaching 75% in other schools. Such heterogeneity is likely to influence the impact of a malaria control initiative, such as IST, implemented in this region. There was also evidence that infection is strongly associated with anaemia, with the odds higher with increasing density of infection. The results also show potentially important variation in the malaria burden between the sexes and age groups, and by school. The scale of observed health problems strongly supports the need for school health programmes aimed at reducing the health burden of malaria in school children. Despite this health burden, the analysis of educational data suggested no association between current health status and measurements of sustained attention and educational achievement.

The geographical heterogeneity observed in the prevalence of *Plasmodium* infection is likely to reflect a complexity of factors that influence vector distribution and density as well as vector-human contact and human infection[97]. The principal malaria vectors in the study are *Anopheles gambiae s.l.* and *An. funestus*, which in our study area, have been shown to exhibit strong spatial and temporal heterogeneity related, in part, to variation in rainfall [287] and more recently, variation in mosquito net use and type of household construction [288]. Human-vector contact and human infection may also be influenced by proximity to vector breeding sites [28,317] and variation in personal protection measures [318] and net use [28]. Geostatistical analysis presented in Chapter 6 will investigate the environmental correlates of the observed variation in infection patterns. Such geographical heterogeneity in infection risk has particular implications for the targeting of malaria interventions as well as for the possible impact of intervention [319]. School-level variation in the prevalence of anaemia may reflect the observed geographical variation in the prevalence of *Plasmodium* infection, but is also likely to be due to differences in food availability, the prevalence of helminth infection, and other important aetiological factors for anaemia.

The protective effect of sleeping under a mosquito net is consistent with previous cross-sectional findings [28,154], whilst the strong association between *P. falciparum* infection and anaemia has been observed in other school-aged populations in East Africa [73,75,113,126]. The impact of chronic *P. falciparum* infection on haemoglobin levels is attributed to increased red blood cell destruction and decreased red blood cell production [54,320,321], with high density infections intensifying these processes. However, anaemia is mutifactorial and the findings of this study highlight additional contributory factors: stunting, indicative of poor nutritional intake for a sustained period during the childhood growth phase, was associated with increased odds of anaemia. This nutritional relationship is supported by the finding that at sea level, in the schools nearest the coast where the soil is infertile and the crop growing potential is poor, the presence of a school feeding programme at the child's school appears to be associated with a 50% decrease in odds of anaemia. Few studies to date have measured the effect of school feeding on anaemia [322], although provision of iron fortified porridge and biscuits and cakes as part of school feeding programmes have been shown to be associated with a reduction in anaemia in Kenya, South Africa and Peru [323-325]. Micronutrient deficiency is commonly found among school-aged children in malaria endemic areas [120], and infection with *P. falciparum* is bound to further increase the stress on the haemoglobin status in individuals who are already anaemic [326,327].

The lack of observed association between health status and sustained attention and education may not necessarily reflect an absence of effect of malaria on education. First, asymptomatic *P. falciparum* can persist for over three months, and as children may be constantly re-infected it is probable that infection has a cumulative effect on cognitive function over an extended period of time. Thus, the single time point of our cross-sectional design may not sufficiently capture the effects of recurrent, chronic infection over an extended period [136,328]. Second, the cross-sectional design meant that we were unable to capture information on past clinical attacks, which have previously been shown to be related to poor educational achievement [309]. Third, malaria

is just one of many contributing factors to poorer cognitive and educational performance, with socio-economic status and the educational environment of children's homes playing an important role, as highlighted in the present study.

The association found in both classes between higher literacy and attention scores and indicators of SES is supported by previous findings where SES has been found to be strongly related to psychometric and education test scores in school children [328]. Increased SES is likely to be associated with increased stimulation, increased access to reading material, and ownership of school related materials, factors previously shown to be associated with increased academic achievement [329]. This is supported by the fact that increased education of household heads and increased literacy was associated with improved performance in comprehension in class 5 and numeracy in class 1. As expected, there was a positive relationship between age and assessment scores for children in class 1. By contrast, increasing age was associated with lower scores in assessments in class 5. This seemingly contradictory observation could be attributed to the older children in class 5 having repeated earlier years due to poor educational performance, as is frequently seen in low income countries [330,331]. Also poor children enrol in school later [332]. The poorer scores in attention (class 1) and literacy (class 5) assessments observed in females are consistent with the recognised disparity between sexes in access to education and support in many low income settings [32]. The strong variation in educational performance by administrative division is an indicator that there are aspects of the school divisional organisation and management, such as the availability of books, the teacher-child contact time and the quality of teaching, that may influence educational outcomes [330,333,334]. The importance of the school environment is further demonstrated by the lower literacy and numeracy scores observed in class 1 children who learn in classrooms with no desks.

3.6 CONCLUSIONS

In conclusion, we found a strong geographical variation in the prevalence of *P. falciparum* infection, underscoring the need for geographical targeting of malaria interventions. The observed strong association between infection and anaemia provides evidence of the, presumably cumulative, negative effects of asymptomatic *P. falciparum* infection on the haemoglobin status of school children. The aim of the trial of IST was to provide an indication of how much of this effect could be reversed in the presence of a school-based control initiative and whether malaria control could also improve the cognitive and educational performance of children in this low to moderate transmission setting. The presence of such variation in underlying risk of both *P. falciparum* and anaemia as well as in current intervention and socioeconomic context within the region of implementation is suggestive of the potential for variability in impact, even across this relatively localised geographical region. Examination of the presence of differential impact provides important information in relation to the external validity of any intervention, particularly in the current climate of decreasing transmission and increasingly fractal heterogeneity of transmission. The following chapter evaluates both the overall impact of school-based IST on health and education outcomes as well as investigating the presence of any heterogeneity in impact on the basis of the context of the implementation in this setting.

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…The study was conceived and designed by Professor Brooker and Dr Matthew Jukes. My role was as research coordinator for the duration of the study. This involved designing, piloting and implementation of survey tools, coordination of data collection, data management, cleaning and analysis. For this paper, Dr Elizabeth Turner conducted the main trial analysis and missingness analyses. I conducted the additional secondary analysis of differential impact by subgroups, analysis of compliance and was responsible for the figures as well as presentation and interpretation of results. I drafted the manuscript and additional input was provided from Dr Elizabeth Turner and Professor Simon Brooker and other authors.

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Chapter 4. The impact of intermittent screening and treatment for malaria among school children in Kenya: a cluster randomised trial.

4.1 OVERVIEW

The results presented in Chapter 3 indicated extensive variation in both the health and education status of school children in this region. Given the striking heterogeneity observed at school-level for both anaemia and *P. falciparum* infection during the baseline surveys, it is important to consider the potential influence this could have on variation in effectiveness of school-based intermittent screening and treatment for malaria in addition to evaluating the overall impact. In the current chapter, the data collected in the trial described in Chapter 2 is used to present an evaluation of the impact of school-based IST on the health and education of school children in a low-to-moderate transmission setting. The overall impact is evaluated as is any variation in impact on health outcomes in relation to baseline heterogeneity of factors such as *P. falciparum* transmission.

This chapter has been published in PLoS Medicine: *Halliday KE, Okello G, Turner EL, Njagi K, Mcharo C, Kengo J, Allen E, Dubeck MM, Jukes MCH & Brooker SJ. (2014) Impact of intermittent screening and treatment for malaria among school children in Kenya: A cluster randomised trial. PLoS Med.11:1* Slight modifications to the publication content have been made: the trial design and methods have been summarised for this chapter as they were presented in detail in Chapter 2, and additional pre-specified subgroup analyses are included in this chapter.

I oversaw the data collection, data management and data cleaning. The main trial impact analyses and missing data analyses were conducted by the trial statistician, Dr Elizabeth Turner. I was responsible for the additional secondary analysis of differential impact by subgroups, analysis of

compliance and was responsible for the figures as well as presentation and interpretation of results. I drafted the manuscript and additional input was provided from Dr Elizabeth Turner and Professor Simon Brooker and other authors.

4.2 INTRODUCTION

In many malaria endemic countries, successful control programmes have recently contributed to reductions in the level of malaria transmission [2,4,6] alongside other factors such as socioeconomic progress [12], and as a consequence, immunity to malaria is acquired more slowly and the burden of clinical malaria is shifting from the very young to older children [3,5]. Recent success in malaria control has also prompted a renewed emphasis on malaria elimination, leading to a shift in focus from targeting only clinical malaria to also identifying and treating asymptomatic malaria parasitaemia [2,51]. Infection rates are typically highest among school-aged children [19,20], who, due to recent improvements in primary school access, are increasingly enrolled in school [108,335]. Tackling such parasitaemia, whether or not it results in clinical disease, is important for two reasons. First, an increasing body of evidence is showing that chronic untreated *Plasmodium* infections can negatively affect children's health [54,74] and cognitive function [58,137,309], including sustained attention [138], and ultimately, their educational achievement [114,124]. Second, with the move towards elimination in low-moderate transmission settings [102,336], there is a need to tackle untreated reservoirs of infection, to which school children are important contributors [22,337]. Yet, surprisingly, there remains a lack of consistent policy and technical guidance [34] on which interventions can reduce the burden of malaria among school children and which can cost-effectively be delivered through existing school systems.

Previous studies have highlighted the beneficial impact of school-based intermittent preventive treatment (IPT) on health and cognitive function in high [33] and high, seasonal [40] malaria transmission settings. However, the recent withdrawal of the primary drugs for IPT, sulfadoxine-

pyrimethamine (SP) and amodiaquine (AQ), in many east African countries, precluded further investigation of IPT using SP+AQ. A possible alternative to IPT is intermittent screening and treatment (IST), whereby individuals are periodically screened for *Plasmodium* infection using a rapid diagnostic test (RDT) and those infected (whether symptomatic or not) are treated with a full course of first-line drug treatment, artemether-lumefantrine (AL). The potential of IST was first highlighted by modelling work [107,198], and its comparable efficacy to IPT in antenatal care has been evaluated [215], although a recent trial in Burkina Faso indicated no impact of IST on community-wide malaria transmission [218]. This chapter reports the results of a cluster randomised trial investigating the impact of IST in schools on health and education outcomes in school children in a low-to-moderate transmission setting on the south coast of Kenya [284]. A particular focus is placed on the potential differential impact of IST on anaemia and *P. falciparum* infection, explored using pre-specified subgroups to identify heterogeneity in impact.

4.3 METHODS

4.3.1 Study design

The study design and methods of the IST intervention trial adhered to, and are reported, according to the CONSORT guidelines [338] and have been previously detailed in Chapter 2 but are briefly summarised here. The trial was conducted from January 2010 to March 2012 in Kwale and Msambweni districts on the south Kenyan coast. Recruitment and baseline sample collection were conducted in January–March 2010 using children randomly selected from classes 1 (age range: 5-15 years) and 5 (age range: 8-20 years). Education outcome measures were assessed in the same children at 9 and 24 months and health outcome measures at 12 and 24 months.

The sample size was based on methods designed for cluster randomised trials and assumed that 101 eligible schools would be randomised to the four intervention groups, with an average of 50 children per school. The 101 schools were randomised in two stages (Chapter 2, Figure 2.1), with sets (aggregations of between three to six closely located schools) of schools randomised to

literacy intervention or control and then individual schools within these two groups randomised to IST intervention or control.

4.3.2 Intermittent screening and treatment (IST) intervention

During IST, children were screened once a school term for malaria parasitaemia using a RDT (ParaCheck-*Pf* device, Orchid Biomedical Systems), which is able to detect *P. falciparum.* Screening was conducted by laboratory technicians. Children (with or without malaria symptoms) found to be RDT-positive were treated with a six dose regimen of AL. Doses 1, 3, and 5 were given under direct observation at the school by the study nurses. Five rounds of screening and treatment were implemented. The first round was conducted alongside baseline health assessments in March 2010, the second round in July 2010, the third in September 2010, the fourth in March 2011, and the final round in October 2011.

4.3.3 Health and education assessments

At enrolment, children's height and weight were measured to the nearest 0.1cm and 0.1kg using Leicester portable fixed base stadiometers and electronic balances, respectively, axillary temperature was digitally recorded, and finger-prick blood samples were obtained to determine haemoglobin concentration (Hb). Baseline parasitaemia, determined by microscopy, was measured in the intervention group during the first round of screening but was not measured in the control group. During follow-up surveys, temperature, weight, and height were measured and a finger-prick blood sample collected for determination of malaria parasitaemia via thick and thin blood films duplicate read by expert microscopists and Hb was measured. Children with an axillary temperature ≥37.5 °C were tested using a RDT, providing an on-the-spot diagnosis for malaria and treatment was administered as per national guidelines. Tests of sustained attention and educational achievement were administered at baseline, 9 months, and 24 months. Sustained attention was a primary outcome assessed through the code transmission test [296]. To avoid floor effects, a simpler measure of sustained attention, the pencil tap test [297], was used at baseline

for the younger cohort. Educational achievement was measured through tests of literacy and numeracy (Chapter 2 Table 2.1).

4.3.4 Data analysis

Data from the paper-based forms were double-entered, consistency checks were performed, and all analysis was conducted using Stata software version 12.1. The pre-specified primary outcome measures were the prevalence of anaemia, defined according to age and sex corrected World Health Organization (WHO) thresholds: Hb < 110 g/l in children under 5 years; < 115 g/l in children 5 to 11 years; < 120 g/l in females 12 years and over and males 12 to 15 years old; and < 130 g/l in males over 15 years, with no adjustment made for altitude [312] and sustained attention. The pre-specified secondary outcomes were the prevalence of *P. falciparum* and scores for spelling and arithmetic. Reported information on ownership of household assets and household construction was used to construct wealth indices using principal component analysis [314] and resulting scores were divided into quintiles. Anthropometric measurements were processed using the WHO Anthroplus Stata macro [313] to derive indicators of stunting, thinness, and underweight. The analyses described here correspond to a pre-specified statistical analysis plan, approved by both the data monitoring committee and trial steering committee before any data were examined.

Baseline school and child characteristics, together with baseline measurements of the study outcomes, were summarized by study groups separately, with class-specific study outcomes reported separately by class. Counts and percentages were used for categorical variables. Means and standard deviations, or medians and the limits of the inter-quartile range (IQR), were reported for continuous variables. Coefficients of variation (CVs) for the binary (health) outcomes and intraclass correlation coefficients (ICCs) for the continuous (cognitive and education) outcomes were calculated from the baseline measures using appropriate formulae [295].

The effectiveness of the IST intervention was assessed using generalized estimating equations (GEE) with robust standard errors and an exchangeable correlation matrix to allow for clustering within schools. All main analyses used the intention-to-treat principle whereby children were analysed in the intervention group that they were assigned to, even if the child moved schools or did not fully comply. The primary pre-specified analysis adjusted for age (as a continuous variable), sex, and the baseline measure of the outcome, except for baseline *P. falciparum*, which was not measured in the control schools. As randomisation of schools to the IST intervention was stratified on the basis of both literacy intervention assignment and school mean exam score, all adjusted analyses presented account for these two stratification factors. Data for classes 1 and 5 combined were used for the health outcome analyses. However, as different assessments were administered for classes 1 and 5 for the evaluation of attention (e.g., pencil tap for class 1 and code transmission for class 5), literacy, and numeracy outcomes, analyses were conducted for each class separately. Separate GEE analyses were conducted for the first and second follow-ups. No formal adjustment was made for multiple testing, therefore *p*-values should be interpreted with due caution. However, as specified in the statistical analysis plan, formal testing was restricted to two primary and three secondary pre-specified outcomes.

For comparison purposes, we also obtained estimates from an unadjusted model that did not adjust for baseline outcome measures, child characteristics, or study design (literacy group and mean school-exam score) and hence retained all study children assessed at follow-up regardless of whether they had baseline measures. Secondary analyses were conducted additionally adjusting for stunting, school-feeding programme, and socioeconomic status (SES) on top of the pre-specified variables. These additional adjustments had no notable impact on the effect estimates and are not presented.

In order to gain power and account for missing data, random effects models, using a likelihood-based approach, were fitted to the one-year and two-year follow-up data simultaneously (Appendices 4.1-4.7). Additional sensitivity analyses were conducted to examine intervention

effects when children who had transferred from their original school were excluded from the analyses (Appendix 4.8).

Despite the study not being powered on the basis of detecting heterogeneity in impact, with previous evidence indicating extensive heterogeneity of both anaemia and *P. falciparum* infection in the study region, analyses were conducted to examine the effect of IST on the primary health outcomes of anaemia and *P. falciparum* infection by pre-specified subgroups. Baseline risk of infection was considered as an important subgroup, as a proxy for transmission intensity, and as such schools were classified into low (<5%), medium (5-19%) and high (≥20%) prevalence groups. Baseline infection data were used for the IST intervention schools and control school prevalences were estimated using 12 month follow-up data, on the assumption that *P. falciparum* infection would remain fairly stable in the absence of a malaria control intervention. Additionally, variation in impact on malaria outcomes was assessed by existing malaria control interventions, namely tertiles of school-level reported net use. The use of a child-level GEE model allowed the stratification of analyses by child characteristics and the child-level subgroups considered potentially important in terms of modification of the IST effect on anaemia were the child's baseline anaemia status and nutritional status (with stunting used as a proxy).

GEE models for a single follow-up time point (12 or 24 months) were specified as below, with stratification by stunting used as an example:

$$log\left(ANAEMIA_{ij}\right) = \beta_0 + \beta_1 IST_{ij} + \beta_2 STUNT_{ij} x IST_{ij} + \beta_3 STUNT_{ij} + \beta_4 X_{ij}$$

For student *i* in school *j*, $ANAEMIA_{ij}$ denotes the presence (with a value of 1) or absence (with a value of 0) of anaemia at the 12 or 24 month follow-up; IST_{ij} is a binary indicator variable for the *i*th child in the *j*th school with value 1 if the child is in the intervention group and 0 if not (in practice, intention-to-treat analyses were conducted so that all children from the same school had the same value of IST_{ij}); similarly $STUNT_{ij}$ is a binary indicator variable, with a value of 1 if stunted, 0 if not; and X_{ij} is a vector of exogenous individual and school-level covariates for student

i including gender, age, baseline anaemia status in addition to school-mean exam score and literacy group assignment to account for the stratification. An exchangeable working correlation matrix was specified to account for clustering by school. β_0 is the intercept and represents the log-probability of anaemia in the absence of intervention, for a non-stunted child, with all covariates set to 0. The parameter β_1 represents the effect of the IST intervention on anaemia for children who are not stunted (*STUNT* = 0), which is quantified as the difference in log-probability of anaemia for IST vs. no IST for non-stunted children, with all exogenous variables held constant. The parameter β_2 represents the difference in effect of IST on anaemia for stunted children vs. non-stunted children, so that $\beta_1 + \beta_2$ is the difference in log-probability of anaemia for IST vs. no IST for stunted-children, with all exogenous variables held constant. A significant p-value for β_2 indicates the presence of an interaction. β_3 represents the effect of stunting on anaemia for children in the absence of intervention (*IST* = 0), which is quantified as the difference in log-probability of anaemia for stunting vs. no stunting for children, with all exogenous variables held constant.

Finally, analyses of the individual-level effects of IST on *P. falciparum* infection and anaemia were conducted within the intervention group according to the frequency of AL treatments the child required across the study duration on the basis of the RDT screening rounds. All subgroup analyses were conducted for the one-year and two-year follow-up outcomes separately.

4.4 RESULTS

4.4.1 Trial profile and baseline data

One hundred and one schools were randomised to one of the two study groups (Chapter 2, Figure 2.3). In total, 7,337 children aged between 5 and 20 years (median: 10 years and IQR: 8-13 years) were randomly selected in January 2010 of which 5,772 (78.7%) parents consented, with no real differences found between groups in terms of percentage of parents refusing and not attending the meetings. Overall, 5,233 children were initially enrolled, of which 5,176 (98.9%) children were eligible for follow-up after the baseline assessments. Characteristics of the children included in each of the study groups are shown in Table 4.1. The numbers of children per school ranged from 18 to 58 but overall were well balanced between groups (control: median, 52; IQR, 50–54 and intervention: median, 53; IQR, 50–55). A difference in percentage of children unavailable for the baseline health surveys was observed between the groups with 5.1% and 10.1% unavailable in the control and intervention groups, respectively (Figure 4.1.).

Children in the two study groups were broadly similar in regard to age, sex, anthropometric indices, bednet use, and household characteristics, with some slight apparent differences in school size and SES (Table 4.1). The primary outcomes, anaemia and educational measures, were also similar between groups at baseline; anaemia prevalence was 45.2% and 45.5% in control and intervention groups, respectively. The prevalence of *P. falciparum*, assessed only in the intervention group at baseline, was 12.9%.

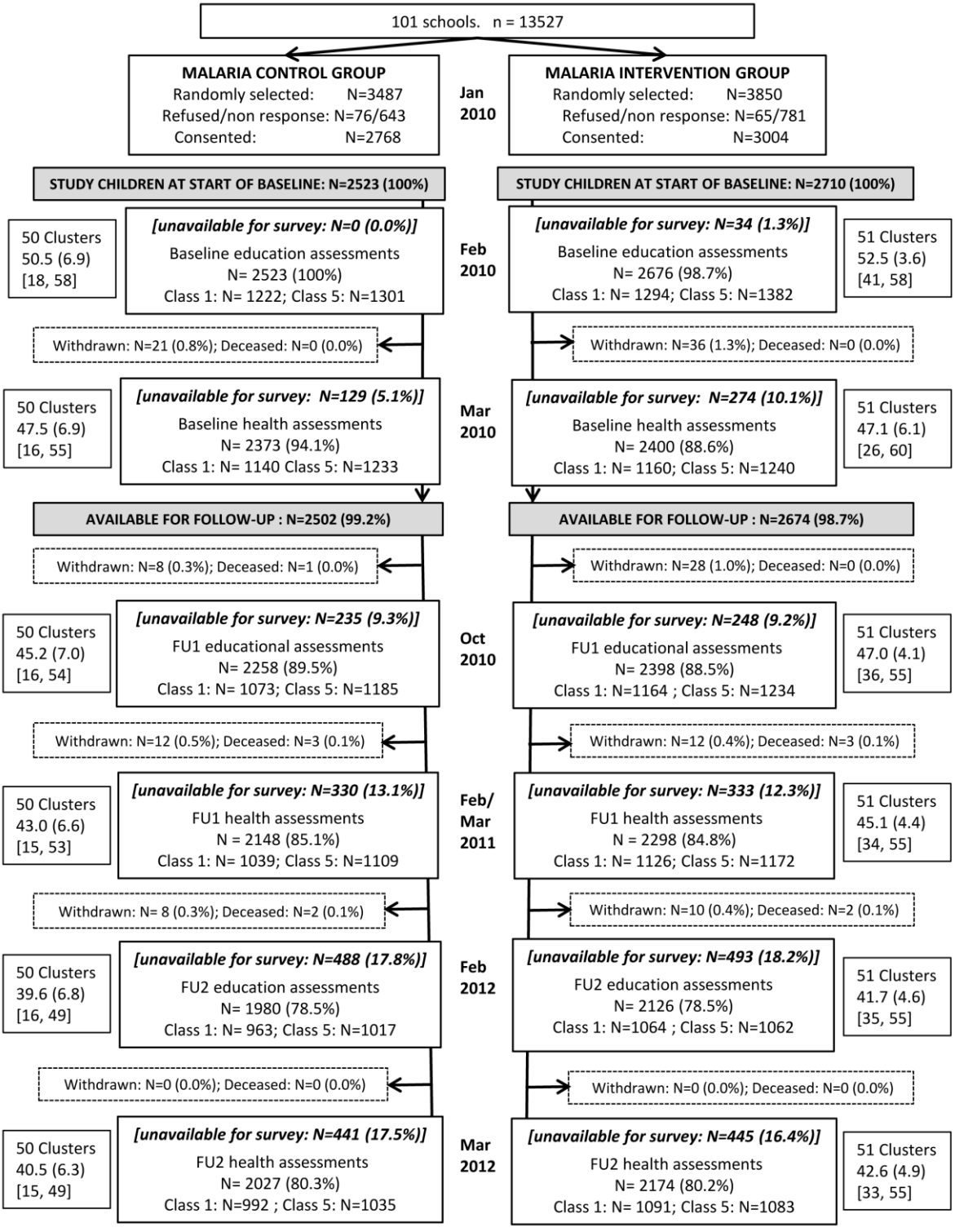


Figure 4.1. Trial profile. The flow of children and clusters in the 50 control 51 IST intervention groups at all assessment points throughout the two-year study period. FU1 indicates follow-up 1 and FU2 indicates follow-up 2. Cluster size is presented as mean (SD) [min, max]

Table 4.1. Baseline characteristics of 5,233 study children in the control and IST intervention schools

Characteristics; *n* (%)[a]		**Control**	**Intervention**
School characteristics[b]		**50 schools**	**51 schools**
Exam score	Mean (SD)	223.4 (27.7)	225.8 (29.0)
School size	Median (IQR) [min, max]	505 (308, 961) [85, 4,891]	568 (389, 692) [225, 1,344]
Enrolled class 1	Mean (SD) [min, max]	24.4 (3.3) [10, 30]	25.8 (1.5) [23, 30]
Enrolled class 5	Mean (SD) [min, max]	26.0 (4.6) [8, 30]	27.3 (3.3) [16, 32]
School programmes	Feeding	22 (44.0)	27 (52.9)
	De-worming	50 (100.0)	49 (96.1)
	Malaria control	9 (18.4)	12 (23.5)
Child characteristics[b]		**2,523 children**	**2,710 children**
Age[c]	Mean (SD)	10.1 (2.8)	10.3 (2.8)
	5–9	1,041 (41.2)	1,069 (39.5)
	10–12	877 (34.8)	925 (34.1)
	13–20	605 (24.0)	716 (26.4)
Sex	Male	1,257 (49.8)	1,319 (48.7)
Child sleeps under net	Usually	1,668 (67.3)	1,682 (63.1)
	Treated net [d]	1,357 (83.3)	1,308 (80.5)
	Last night [d]	1,606 (96.3)	1,609 (95.7)
Nutritional status	Underweight	266 (27.0)	231 (23.9)
	Stunted	600 (25.2)	612 (24.9)
	Thin	482 (20.2)	450 (18.3)
Household characteristics[b]			
Parental education	No schooling	726 (29.4)	925 (34.7)
	Primary schooling	1,292 (52.2)	1,381 (51.8)
	Secondary schooling	353 (14.3)	278 (10.4)
	Higher education	102 (4.1)	83 (3.1)
SES	Poorest	440 (17.7)	655 (24.4)
	Poor	483 (19.5)	564 (21.0)
	Median	465 (18.7)	495 (18.5)
	Less poor	524 (21.1)	509 (19.0)
	Least poor	572 (23.0)	458 (17.1)
Household size	1–5	697 (28.1)	703 (26.4)
	6–9	1,444 (58.3)	1,580 (59.3)
	10–31	338 (13.6)	382 (14.3)
Study endpoints-baseline[e]		**2,523 children**	**2,710 children**
Anaemia prevalence[f] (k = 0.21)	Age-sex specific	1,073 (45.2)	1,114 (45.5)
	Severe (<70 g/l)	14 (0.6)	14 (0.6)
	Moderate (70–89 g/l)	43 (1.8)	55 (2.2)
	Mild (90–109 g/l)	530 (22.3)	518 (21.1)
	None (≥110 g/l)	1,786 (75.3)	1,864 (76.1)
Haemoglobin (g/l)	Mean (SD)	117.3 (13.0)	117.5 (13.7)
***P. falciparum* prevalence**[f,g] (k = 1.03)		—	311 (12.9)
Class 1[f,h]		**1,222 children**	**1,317 children**
Score: 0–20 (ICC = 0.07)	Sustained attention[i]	11.9 (6.7) [0, 20]	12.1 (6.6) [0, 20]
Score: 0–20 (ICC = 0.29)	Spelling	8.6 (4.5) [0, 19]	7.7 (4.4) [0, 20]
Score: 0–30 (ICC = 0.11)	Arithmetic	2.6 (2.4) [0, 17]	2.6 (2.5) [0, 15]
Class 5[f,h]		**1,301 children**	**1,393 children**
Score: 0–20 (ICC = 0.23)	Sustained attention[i]	9.9 (6.0) [0, 20]	10.4 (5.7) [0, 20]
Score: 0–78 (ICC = 0.09)	Spelling	27.9 (11.8) [0, 63]	25.8 (11.2) [1, 59]
Score: 0–38 (ICC = 0.22)	Arithmetic	29.4 (5.6) [0, 38]	28.5 (5.8) [0, 38]

[a]Percent non-missing children in each study group presented for categorised data. For continuous data mean (SD) [min,max] is presented; [b]All characteristics have less than 2% missing data with the exception of following indicators (reported as control/intervention): stunted and thin both (138/248 [5.5/9.2%] missing), underweight (1,538/1,744 [61.0/64.4%] missing), net use last night (661/840 [26.2/31.0%] missing). [c]In Class 1, mean (SD) for age is: 7.8 (1.7) and in Class 5, mean (SD) for age is:12.5 (1.6) [d]Percentages of treated nets and children sleeping under a net last night are presented only for those children who were reported as usually sleeping under a net; [e]Study endpoints have less than 5% missing data at baseline with the exception of the following (reported as control/intervention): Hb (147/255 [5.8/9.4%] missing), *P. falciparum* infection (274 [10.1%] missing in intervention group), class 5 attention (79/72 [6.1/5.2%] missing). [f]Coefficient of variation (k) estimated for binary outcomes using available baseline (i.e., only using data from IST schools for *P. falciparum*) and interclass correlation coefficient (ICC) estimated for continuous outcomes using baseline measures. [g]Not measured at baseline in the control group; [h]Presented as mean (SD) [min,max]. [i]In class 1 sustained attention was measured by the "pencil tap test" and in class 5 sustained attention was measured by the "two digit code transmission test."

4.4.2 Compliance with screening and treatment

During the 24 months of intervention, an average of 2,340 children (88.4% of eligible study children) in the 51 intervention schools were screened at each visit, of whom, on average 17.5% were RDT-positive (Table 4.2). Of the study children, 84.0% were screened at four or more IST rounds and 66.8% were screened at all five rounds. By the fifth screening round, 3.3% children were lost due to withdrawal or death and a further 17.7% of children were lost due to out-migration. The percentage of children RDT-positive at each screening ranged from 14.9% to 19.2%, with no distinct trend over time. Overall, 99.1% of RDT-positive results led to treatment across the five screening rounds and 92.6% of these were recorded as receiving the fully supervised six-dose treatment regime (Table 4.2). There was an apparent decline in full supervision (a proxy for compliance) with time, falling from 96.9% at the first round to 81.7% at the fifth round. RDT performance, examined against a "gold standard" of expert microscopy, revealed consistently high specificity, greater than 90% at all rounds, whereas sensitivity was more variable ranging from 68.7% to 94.6% across surveys, with higher sensitivity observed during the wet season compared to the dry season (Table 4.2.).

Table 4.2. Summary information for 2,710 study children in the IST intervention group by screening round

IST Round	Season	Study Children[a]	*n* (%) Screened	*n* (%) RDT Positive	*n* (%) Treated	*n* (%) Supervised Treatment[b]	RDT Sensitivity /Specificity[c]
Feb–Mar 2010	Dry	2,674 (98.7)	2,454 (91.8)	453 (18.5)	449 (99.1)	435 (96.9)	78.5/90.6
Jun–Jul 2010	Wet	2,654 (97.9)	2,430 (91.6)	466 (19.2)	465 (99.8)	440 (94.6)	89.2/90.4
Sept 2010	Wet	2,651 (97.8)	2,368 (89.3)	444 (18.8)	443 (99.8)	422 (95.3)	94.6/90.3
Feb–Mar 2011	Dry	2,631 (97.1)	2,291 (87.1)	340 (14.8)	335 (98.5)	306 (91.3)	68.7/91.9
Oct 2011	Wet	2,621 (96.7)	2,157 (82.3)	345 (16.0)	338 (98.0)	276 (81.7)	NA
TOTALS		**13,231**	**11,700 (88.4)**	**2,048 (17.5)**	**2,030 (99.1)**	**1,879 (92.6)**	**82.7/90.8**

Sensitivity and specificity of RDTs compared to expert microscopy is displayed.
[a]Study children are shown as a percentage of the 2,710 initially eligible for the intervention and loss at each stage represents withdrawals and/or deaths. Child transfer events are not included.
[b]Children treated who were directly observed taking doses 1, 3, and 5 in school at the correct time and who reported taking the evening doses. [c]Microscopy results not available for visit 5

4.4.3 Follow up

Of the 5,233 children enrolled initially, 4,446 (85.0%) were included in the 12-month follow-up health survey and 4,201 (80.3%) were included in the 24-month health survey (Figure 4.1.). At 12 and 24 months, children lost to follow-up across both study arms were largely similar to children followed up (Appendices 4.1 and 4.2) with slightly lower spelling scores in those children lost to follow-up across both groups and a higher proportion of children whose parents had no schooling in those lost to follow-up in the intervention schools. The prevalence of *P. falciparum*, in the intervention group, was lower in children lost to follow-up (8.6%) compared to those followed-up (13.6%) at both 12 and 24 months.

Overall, 4,656 (89.0%) of children were included in the 9-month follow-up education survey and 4,106 (78.5%) in the 24-month follow-up survey. Children unavailable for the follow-up educational surveys at 9 and 24 months were similar across the two study groups (Appendices 4.4 and 4.5), with a slight imbalance in SES and parental education categories seen between children available and unavailable for the survey in the intervention group. Additionally baseline prevalence of *P. falciparum* was lower in children lost to follow-up (9.1%) compared to those followed-up (13.3%) in the intervention arm.

As intention-to-treat analysis was performed, no adjustment was made for children transferring between schools and study groups at the follow-ups. Overall, 308 children were recorded as transferred by the end of the study. Of those, 46 (0.9%), 71 (1.8%), and 308 (5.9%) children were assessed in a different school from their initial enrolment school, at 9-month, 12-month, and 24-month follow-ups, respectively. Sensitivity analysis excluding these transfers resulted in no change in direction or magnitude of results (Appendix 4.8).

4.4.4 Effect of IST on anaemia and *P.falciparum* infection

At 12-months follow-up, 2,148 children in the control schools and 2,298 in the intervention schools provided a finger-prick blood sample for Hb assessment, and at 24 months 2,027 and 2,174 children provided finger-prick samples in the control and intervention groups, respectively. There was no significant difference in the prevalence of anaemia between children in the two groups at 12- or 24-month follow-ups (adjusted risk ratio [Adj.RR]: 1.03, 95% CI 0.93–1.13, p = 0.621 and Adj.RR: 1.00, 95% CI 0.90–1.11, p = 0.953), respectively (Table 3); the same was observed in relation to mean Hb. There was also no significant difference in the prevalence of *P. falciparum* between study groups at 12 or 24 months (Adj.RR: 0.71, 95% CI 0.46–1.11, p = 0.131 and Adj.RR: 1.53, 95% CI 0.89–2.62, p = 0.124).

Table 4.3. Effect of the IST intervention at 12- and 24-months follow-up on health outcomes anaemia and *P. falciparum* prevalence for study children.

Outcome	**Control (50 Schools)**		**Intervention (51 Schools)**		**Risk Ratio[a] (95% CI)**	***p*-Value**	**Cluster-Size; Range (Average)**
	N	***n* (%)[b]**	*N*	***n* (%)[b]**			
12-month follow-up	2,478		2,631				
Prevalence of anaemia[c]							
Unadjusted	2,146	837 (39.0%)	2,297	920 (40.1%)	1.03 (0.91,1.16)	0.646	15–55 (44.0)
Adjusted	2,048	788 (38.5%)	2,142	858 (40.1%)	1.03 (0.93,1.13)	0.621	15–55 (41.5)
Prevalence of *P. falciparum*							
Unadjusted	2,106	302 (14.3%)	2,276	243 (10.7%)	0.76 (0.49,1.18)	0.221	11–55 (43.4)
Adjusted[d]	2,106	302 (14.3%)	2,276	243 (10.7%)	0.71 (0.46,1.11)	0.131	11–55 (43.4)
24-months follow-up	2,468		2,619				
Prevalence of anaemia[c]							
Unadjusted	2,027	809 (39.9%)	2,173	910 (41.9%)	1.05 (0.91,1.21)	0.514	15–55 (41.6)
Adjusted	1,935	765 (39.5%)	2,027	842 (41.5%)	1.00 (0.90,1.11)	0.953	14–55 (39.5)
Prevalence of *P. falciparum*							
Unadjusted	2,001	169 (8.5%)	2,139	253 (11.8%)	1.42 (0.84,2.42)	0.192	15–55 (41.0)
Adjusted[d]	2,001	169 (8.5%)	2,139	253 (11.8%)	1.53 (0.89,2.62)	0.124	15–55 (41.0)

Results presented (i) for all children with outcome data (unadjusted) and (ii) for those with baseline measurements of each outcome and accounting for age, sex, and stratification effects as the primary pre-specified analysis. *N*, number of children eligible for follow-up (not withdrawn or deceased). Adjusted, for baseline age, sex, school mean exam score and literacy group (to account for stratification), and baseline measure of the outcome, where available; unadjusted, all children with outcome measures, not adjusted for any baseline or study design characteristics.

[a]Risk ratios (intervention/control) presented for binary outcomes (anaemia and *P. falciparum* prevalence) and are obtained from GEE analysis accounting for school-level clustering.

[b]Number and percentage with outcome.

[c]Age-sex specific anaemia was defined using age and sex corrected WHO thresholds of Hb: <110 g/l in children under 5 years; <115 g/l in children 5 to 11 years; <120 g/l in females 12 years and over and males 12 to 14.99 years old; and <130 g/l in males ≥15 years. All female adolescents are assumed to not be pregnant.

[d]Not including baseline *P. falciparum* infection

4.4.5 Heterogeneity of effect of IST on health outcomes

Subgroup analysis of the impact of the IST intervention on anaemia according to *Plasmodium* prevalence at baseline (using 12-month estimates for the control group as a proxy for baseline), demonstrated no differential impact by prevalence category (<5%, 5%–19.9%, and ≥20%) at either follow-up (p = 0.578 and p=0.840, for interaction test at 12- and 24-month follow-up, respectively, Table 4.4). However, the corresponding analysis conducted in relation to *P. falciparum* infection demonstrated apparent variation in effect of IST in the different *Plasmodium* prevalence subgroups (p < 0.001 at both 12- and 24-month follow-up, Table 4.4). At the 12-month follow-up in the schools with the lowest baseline prevalence (<5%) those in the intervention group appeared to be at over four and a half times the risk of *P. falciparum* infection than the control group, however in the moderate prevalence subgroup (5%–19.9%) those in the intervention group appeared less than half as likely to be infected. Whereas at 24 months the only group in which a significant impact was observed, was in the high baseline prevalence group (≥20%), in which the intervention appeared to increase the risk of *P. falciparum* infection. Overall therefore, there was no consistent trend observed in the differential impact across the two follow-ups in the three subgroups.

Table 4.4. Effect of the IST intervention at 12 and 24 months follow-up on the prevalence of anaemia and *P.falciparum* infection, by baseline prevalence category of *P.falciparum* (control school prevalence estimated using 12 month follow-up data) with adjustment for age, sex and stratification effects.

Baseline prevalence of *P. falciparum* infection	**Control (50 schools)**		**Intervention (51 schools)**		**Risk ratio[c] (95% CI)**	**p-value**
	N	**n(%)**	***N***	**n(%)**		
Follow-up 12 months						
Prevalence of Anaemia	2478		2631			
<5%	787	265 (33.7%)	751	270 (36.0%)	1.01 (0.84,1.23)	
5-19.9%	606	220 (36.3%)	858	358 (41.7%)	1.09 (0.95,1.26)	0.578
≥20%	655	303 (46.3%)	533	230 (43.2%)	0.99 (0.87,1.13)	
Prevalence of *P. falciparum*	2478		2631			
<5%	813	13 (1.6%)	781	56 (7.2%)	4.69 (2.18,10.08)	
5-19.9%	629	75 (11.9%)	946	52 (5.5%)	0.41 (0.28,0.75)	<0.001
≥20%	664	214 (32.2%)	549	135 (24.6%)	0.83 (0.55,1.24)	
Follow-up 24 months						
Prevalence of Anaemia	2468		2619			
<5%	740	264 (35.7%)	710	243 (34.2%)	0.95 (0.78,1.16)	
5-19.9%	572	226 (39.5%)	803	364 (45.3%)	0.99 (0.86,1.14)	0.840
≥20%	623	275 (44.1%)	514	235 (45.7%)	1.03 (0.86,1.24)	
Prevalence of *P. falciparum*	2468		2619			
<5%	774	7 (0.9%)	735	17 (2.3%)	2.53 (0.90,7.13)	
5-19.9%	595	55 (9.2%)	876	90 (10.3%)	0.92 (0.47,1.80)	<0.001
≥20%	632	107 (16.9%)	528	146 (27.7%)	1.89 (1.06, 3.36)	

N=numbers not withdrawn or died by the time of follow-up.
[a] Control school *P.falciparum* prevalence was estimated using 12 month follow-up data. [b] Number and (% with outcome
[c] Risk ratios presented are obtained from GEE analysis accounting for school-level clustering, age, sex, school mean exam score and literacy group (to account for stratification), and baseline outcome (in the case of anaemia).

No differential impact of IST on anaemia was observed when the analysis was stratified by individual-level baseline anaemia status (Table 4.5) or by baseline nutritional status (Table 4.6). Similarly no variation in impact of IST on *P. falciparum* infection was shown when analyses were stratified by these individual-level subgroups (results not shown).

Table 4.5. Effect of the IST intervention at 12 and 24 months follow-up on the prevalence of anaemia, by presence or absence of anaemia at baseline at the child-level, with adjustment for age, sex and stratification effects.

Prevalence of anaemia	**Control (50 schools)**		**Intervention (51 schools)**		**Risk ratio[b] (95% CI)**	**p-value**
		n (%)[a]		**n (%)[a]**		
Follow-up 12 months	N=2478		N=2631			
Baseline anaemia status						
Not anaemic	1119	254 (22.7%)	1156	279 (24.1%)	1.04 (0.85,1.28)	
Anaemic	929	534 (57.5%)	986	579 (58.7%)	1.02 (0.93,1.13)	0.848
Follow-up 24 months	N=2468		N=2619			
Baseline anaemia status						
Not anaemic	1068	258 (24.2%)	1108	304 (27.4%)	1.14 (0.93,1.41)	
Anaemic	867	507 (58.5%)	919	538 (58.5%)	1.00 (0.90,1.11)	0.113

N=numbers not withdrawn or died by the time of follow-up.
[a] Number and percentage with outcome
[b] Risk ratios presented are obtained from GEE analysis accounting for school-level clustering, age, sex, school mean exam score and literacy group (to account for stratification), and baseline anaemia.

Table 4.6. Effect of the IST intervention at 12 and 24 months follow-up on the prevalence of anaemia, by presence or absence of stunting at baseline at the child-level, with adjustment for age, sex and stratification effects.

Prevalence of anaemia	**Control (50 schools)**		**Intervention (51 schools)**		**Risk ratio[b] (95% CI)**	**p-value**
		n (%)[a]		**n (%)[a]**		
Follow-up 12 months	N=2478		N=2631			
Anthropometric status						
Not stunted	1528	574 (37.6%)	1594	615 (38.6%)	1.02 (0.92,1.13)	
Stunted	520	214 (41.2%)	540	241 (44.6%)	1.05 (0.91,1.22)	0.605
Follow-up 24 months	N=2468		N=2619			
Anthropometric status						
Not stunted	1437	539 (37.5%)	1513	604 (39.9%)	1.01 (0.90,1.14)	
Stunted	498	226 (45.4%)	506	236 (46.6%)	0.98 (0.85,1.12)	0.656

N=numbers not withdrawn or died by the time of follow-up.
[a] Number and percentage with outcome
[b] Risk ratios presented are obtained from GEE analysis accounting for school-level clustering, age, sex, school mean exam score and literacy group (to account for stratification), and baseline anaemia.

As shown in Table 4.7 no heterogeneity of impact of IST on *P. falciparum* infection was found according to school-level prevalence categories of child-reported net use (<60%, 60%–74.9%, and ≥75%) and a similar lack of differential impact was observed when stratification was performed on the basis of individual-level net use (results not shown).

Table 4.7. Effect of the IST intervention at 12 and 24 months follow-up on the prevalence of *P. falciparum* infection by school-level prevalence of reported net use with adjustment for age, sex and stratification effects.

Prevalence of *P. falciparum* infection	**Control (50 schools)**		**Intervention (51 schools)**		**Risk ratio[c] (95% CI)**	**p-value**
		n (%)[b]		**n (%)[b]**		
Follow-up 12 months	N=2478		N=2631			
Prevalence of baseline net use						
<60.0%	730	106 (14.5%)	939	124 (13.2%)	0.74 (0.44,1.25)	
60.0-74.9%	665	133 (20.0%)	757	92 (12.2%)	0.76 (0.35,1.64)	0.713
≥75%	711	63 (8.9%)	580	27 (4.7%)	0.41 (0.10,1.62)	
Follow-up 24 months	N=2468		N=2619			
Prevalence of baseline net use						
<60.0%	692	79 (11.4%)	884	122 (13.8%)	1.36 (0.63,2.97)	
60.0-74.9%	627	70 (11.2%)	724	101 (14.0%)	1.39 (0.62,3.09)	0.975
≥75%	682	20 (2.9%)	531	30 (5.7%)	1.66 (0.34,8.07)	

N=numbers not withdrawn or died by the time of follow-up.
[a] Control school *P. falciparum* prevalence was estimated using 12 month follow-up data.
[b] Number and percentage with outcome
[c] Risk ratios presented are obtained from GEE analysis accounting for school-level clustering, age, sex, school mean exam score and literacy group (to account for stratification), and baseline anaemia.

Stratification of the analysis, within the intervention group only, by frequency of positive results and hence AL treatments received during the study duration, exhibited no differential impact of the intervention in relation to anaemia (Table 4.8). However, in relation to *P. falciparum* infection, children who had been found RDT -positive and subsequently treated on an increased number of screening rounds exhibited increased risk of *P. falciparum* infection at both the 12- and 24-month follow-ups, with a strong dose response relationship observed across the subgroups.

Table 4.8. Effect of the IST intervention on anaemia at 12 and 24 months follow-up within the IST intervention group by number of positive results and subsequent AL treatments received at the individual-level.

Number of positive RDT results and hence AL treatments received[a]	Intervention (51 schools)		Risk ratio[d] (95% CI)	p-value
	N[b]	n[c](%)		
Follow-up 12 months				
Prevalence of Anaemia	2293			
0	1417	545 (38.5%)	0	
1	588	241 (41.0%)	0.99 (0.90, 1.09)	0.839
2-3	288	131 (45.5%)	1.04 (0.91, 1.19)	
Prevalence of *P. falciparum*	2266			
0	1401	89 (6.4%)	0	
1	583	74 (12.7%)	1.41 (1.01, 1.95)	<0.001
2-3	282	80 (28.4%)	2.38 (1.50, 3.77)	
Follow-up 24 months				
Prevalence of Anaemia	2169			
0	1336	546 (40.9%)	0	
1-2	563	233 (41.4%)	0.96 (0.88, 1.05)	0.470
3-5	270	129 (47.8%)	1.03 (0.88, 1.21)	
Prevalence of *P. falciparum*	2139			
0	1150	53 (4.6%)	0	
1-2	774	116 (15.0%)	1.95 (1.45, 2.63)	<0.001
3-5	215	84 (39.1%)	3.37 (2.17, 5.24)	

[a] This represents the number of treatments received by each follow-up (12 and 24 months). The maximum number of IST rounds and thus AL treatments by the 12 month follow-up was three and the maximum number of IST rounds and thus AL treatments by the 24 month follow-up was five

[b] Number of children in each subgroup at the time of follow-up

[c] Number and percentage of children with anaemia or *Plasmodium* infection at follow-up

[d] Risk ratios presented are obtained from GEE analysis accounting for school-level clustering, age, sex, school mean exam score and literacy group (to account for stratification), and baseline outcome (in the case of anaemia).

4.4.6 Effect of IST on attention and educational achievement

At both 9- and 24-months follow-up, there was no statistical difference in mean scores for sustained attention between study groups in either class with adjusted mean difference (Adj.MD): −0.44, 95% CI −1.09 to 0.21, p = 0.180 and Adj.MD: 0.28, 95% CI −0.23 to 0.79, p = 0.283 for classes 1 and 5, respectively at the 24-month follow-up (Table 4.9). Similarly there was no significant difference between groups on scores for spelling in the older class at 9- and 24-month follow-ups (Adj.MD: −0.31, 95% CI −1.26 to 0.63, p = 0.515 and Adj.MD: 0.71, 95% CI −0.34 to 1.76, p = 0.183) nor for arithmetic at either follow-up (Table 4.10).

However, at 9-months follow-up, children in the younger class in the intervention group had lower mean adjusted scores for the spelling task and the same trend was observed at 24 months (Adj.MD: −0.65, 95% CI −1.11 to −0.20, p = 0.005) (Table 4.10). Similarly at 24 months, in the younger class, children in the intervention group scored on average 0.60 points lower in the arithmetic assessments than children in the control group (Adj.MD: −0.60, 95% CI: −1.02 to −0.19, p = 0.005).

4.4.7 Surveillance for adverse events

Active surveillance found that 4.5% (92/2,030) children reported one or more adverse effects within 2 days of receiving treatment, including headache (68; 3.3%), stomach ache (38; 1.9%), dizziness (17; 0.8%), vomiting (7; 0.3%), and pruritis (10; 0.5%). During the 24 months of follow-up, 11 children died: five in the intervention group and six in the control group. Cause of death was investigated and included yellow fever, heart defect, leukaemia, drowning, trauma, pneumonia, and paediatric HIV. In the intervention group, none of these deaths occurred within 30 days of the screening and treatment and therefore were not attributed to the intervention.

Table 4.9. Effect of the IST intervention at 9- and 24-months follow-up on sustained attention outcomes for younger (class 1) and older (class 5) children.

Outcome	Control (50 Schools)		Intervention (51 Schools)		Mean Difference[a] (95% CI)	*p*-Value	Cluster-Size; Range (Mean)
	N	Mean (SD)[b]	*N*	Mean (SD)[b]			
9-months follow-up							
Class 1 (median age:8, range: 5-15)	1210		1281				
Sustained attention[c] (score: 0–20)							
Unadjusted	1,070	8.48 (3.63)	1,162	8.43 (3.76)	−0.04 (−0.58 to 0.51)	0.895	8–27 (22.1)
Adjusted	1,030	8.52 (3.65)	1,144	8.43 (3.77)	−0.13 (−0.66 to 0.39)	0.623	5–27 (21.7)
Class 5 (median age:12, range: 8-18)	1283		1,365				
Sustained attention[d] (score: 0–20)							
Unadjusted	1,180	13.38 (5.45)	1,231	13.35 (5.13)	−0.09 (−0.77 to 0.56)	0.799	8–30 (23.9)
Adjusted	1,178	13.38 (5.45)	1,221	13.40 (5.10)	−0.21 (−0.81 to 0.39)	0.490	8–30 (23.8)
24-months follow-up							
Class 1 (median age:8, range: 5-15)	1201		1,269				
Sustained attention[c] (score: 0–20)							
Unadjusted	960	13.45 (5.15)	1,059	13.20 (4.96)	−0.26 (−0.95 to 0.43)	0.456	8–26 (20.0)
Adjusted	923	13.49 (5.15)	1,041	13.18 (4.96)	−0.44 (−1.09 to 0.21)	0.180	4–25 (19.6)
Class 5 (median age:12, range: 9-18)	1267		1,350				
Sustained attention[d] (score: 0–20)							
Unadjusted	1,007	14.22 (4.90)	1,052	14.66 (4.60)	0.40 (−0.14 to 0.94)	0.144	6–31 (20.4)
Adjusted	1,006	14.21 (4.90)	1,044	14.70 (4.58)	0.28 (−0.23 to 0.79)	0.283	6–29 (20.3)

Results presented (i) for all children with outcome data (unadjusted) and (ii) for those with baseline measurements of each outcome and accounting for age, sex, and stratification effects as the primary pre-specified analysis. *N*, number of children eligible for follow-up (not withdrawn or deceased). Adjusted, for baseline age, sex, school mean exam score and literacy group (to account for stratification), and baseline measure of the outcome, where available; unadjusted, all children with outcome measures, not adjusted for any baseline or study design characteristics.

[a]Mean difference (intervention-control) are obtained from GEE analysis accounting for school-level clustering.

[b]Mean score and SD at follow-up.

[c]Pencil tap test was conducted at baseline and single digit code transmission task was conducted at 9- and 24-months follow-ups.

[d]Double digit code transmission was conducted at baseline and both follow-ups.

Table 4.10. Effect of the IST intervention at 9- and 24-months follow-up on educational achievement outcomes for younger (class 1) and older (class 5) children.

Outcome; *N* (%)	Control (50 Schools)		Intervention (51 Schools)		Mean Difference[a] (95% CI)	*p*-Value	Cluster-Size; Range (Mean)
	N	Mean (SD)[b]	*N*	Mean (SD)[b]			
9-months follow-up							
Class 1 (median age:8, range: 5-15)	1,210		1,281				
Spelling (score: 0–20)[c]							
Unadjusted	1,068	11.70 (4.59)	1,162	10.47 (4.57)	−1.23 (−2.21 to −0.24)	0.015	8–27 (22.1)
Adjusted	1,060	11.69 (4.59)	1,133	10.49 (4.58)	−0.67 (−1.26 to −0.08)	0.026	8–27 (21.7)
Arithmetic (score: 0–20)[d]							
Unadjusted	1,071	4.21 (3.13)	1,162	4.04 (3.26)	−0.17 (−0.60 to 0.26)	0.433	8–27 (22.1)
Adjusted	1,069	4.21 (3.12)	1,143	4.07 (3.28)	−0.21 (−0.54 to 0.12)	0.214	8–27 (21.9)
Class 5 (median age:12, range: 8-18)	1,283		1,365				
Spelling (score: 0–75)[e]							
Unadjusted	1,169	31.34 (12.61)	1,223	28.73 (12.36)	−2.73 (−5.26 to −0.19)	0.035	8–30 (23.7)
Adjusted	1,154	31.37 (12.60)	1,214	28.76 (12.34)	−0.31 (−1.26 to 0.63)	0.515	8–30 (23.4)
Arithmetic (score: 0–30)[f]							
Unadjusted	1,180	31.15 (5.49)	1,229	30.72 (5.17)	−0.49 (−1.40 to 0.42)	0.294	8–30 (23.9)
Adjusted	1,173	31.14 (5.50)	1,210	30.73 (5.17)	0.13 (−0.41 to 0.68)	0.629	8–30 (23.6)
24-months follow-up							
Class 1 (median age:8, range: 5-15)	1,201		1,269				
Spelling (score: 0–20)[c]							
Unadjusted	961	12.03 (3.05)	1,062	11.04 (3.49)	−0.97 (−1.54 to −0.40)	0.001	8–26 (20.0)
Adjusted	954	12.02 (3.05)	1,036	11.04 (3.50)	−0.65 (−1.11 to −0.20)	0.005	8–25 (19.7)
Arithmetic (score: 0–30)[g]							
Unadjusted	962	5.97 (3.05)	1,061	5.38 (2.97)	−0.59 (−1.08 to −0.10)	0.018	8–26 (20.0)
Adjusted	960	5.97 (3.04)	1,042	5.40 (2.97)	−0.60 (−1.02 to −0.19)	0.005	8–25 (19.9)
Class 5(median age:12, range: 9-18)	1,267		1,350				
Spelling (score: 0–78)[e]							
Unadjusted	1,010	35.28 (12.91)	1,060	33.97 (12.79)	−1.58 (−4.01 to 0.85)	0.202	6–31 (20.5)
Adjusted	996	35.33 (12.85)	1,052	34.04 (12.75)	0.71 (−0.34 to 1.76)	0.183	6–29 (20.3)
Arithmetic (score: 0–30)[f]							
Unadjusted	1,016	21.20 (5.47)	1,062	20.15 (5.68)	−1.07 (−2.15 to 0.00)	0.050	6–31 (20.6)
Adjusted	1,009	21.20 (5.48)	1,045	20.18 (5.69)	−0.49 (−1.32 to 0.34)	0.243	6–29 (20.3)

Results presented (i) for all children with outcome data (unadjusted) and (ii) for those with baseline measurements of each outcome and accounting for age, sex, and stratification effects as the primary pre-specified analysis. *N*, number of children eligible for follow-up (not withdrawn or deceased). Adjusted, for baseline age, sex, school mean exam score and literacy group (to account for stratification) and baseline measure of the outcome, where available; unadjusted, all children with outcome measures, not adjusted for any baseline or study design characteristics. [a]Mean difference (intervention-control) for scores on spelling and arithmetic are obtained from GEE analysis accounting for school-level clustering. [b]Mean score and SD at follow-up. [c]The same class 1 spelling task was given at baseline, 9- and 24-months follow-ups, with different words used follow-up and at baseline, hence baseline adjustment is for the same task. [e]The same class 5 spelling task was given at baseline, 9- and 24-months follow-ups, with different words used for the 24-month follow-up. [f]Same arithmetic task conducted at baseline, 9- and 24-months follow-ups, with different sums used for the 24-month follow-up. [g]Addition task conducted at baseline and arithmetic task containing addition, subtraction, multiplication, and division conducted at 24-months follow-up, hence baseline adjustment for different task.for the 24-month follow-up. [d]The same addition task conducted at 9-months

4.5 DISCUSSION

School-based malaria control is increasingly recognised as an important potential component for integrated school health packages [199]. However, as yet there is no consensus about the most effective malaria interventions for the alternative transmission settings. To our knowledge, we conducted the first cluster randomised trial of the impact of school-based IST of malaria. We failed to detect any overall benefit of IST using AL on the health, attention, or educational achievement of school children in this low-moderate malaria transmission setting. No evidence was found of heterogeneity in impact of IST on the primary outcome of anaemia according to a variety of subgroups such as baseline *Plasmodium* prevalence. Nor was there any convincing evidence of consistent variation in impact of IST on *P. falciparum* infection over the two years.

The reasonably high follow-up rates of, on average, 87.0% and 79.4% at the first and second follow-ups, respectively, equal between groups at each follow-up, suggest sample bias was not responsible for the lack of impact observed. The higher proportion of children unavailable for baseline health assessments was driven by a few initially apprehensive schools [294], which were subsequently assessed throughout the study and included in the unadjusted analyses. The differential baseline prevalence of *P. falciparum* in those children available and unavailable for follow-up in the intervention group may reflect a higher proportion of withdrawal and absenteeism on screening and assessment days in schools in low transmission regions, where there was no treatment benefit. However, such a situation is unlikely to have masked any impact of IST as historical exposure and current parasite prevalence is highly predictive of subsequent malaria risk [66,223], and as such these children were less likely to have been infected and thus gain any potential benefit from treatment over the study period.

The absence of apparent differences between control and intervention groups in relation to either *Plasmodium* infection or anaemia at 12 or 24 months are contradictory to predictions from simulation analyses of mass screening and treatment in a moderate transmission setting [107,198].

One reason for these contrasting results may be the different coverage rates, where the simulations assumed 80% intervention coverage of the whole community in contrast to this study where the IST intervention covered two classes of the school populations only. In this low-moderate transmission setting less than 20% of children screened were eligible for treatment at each round.

The lack of differential impact on anaemia observed when schools were stratified by baseline school-level prevalence of *Plasmodium* (a proxy for transmission intensity) and by number of treatments received at the individual level, suggests there was no impact on long-term health even amongst the children receiving AL treatment. Moreover the lack of variation in impact of IST on anaemia in relation to baseline anaemia or stunting at the child-level, indicates that the underlying health status of the individual had no influence on the effectiveness of the intervention. Although there was evidence of differential impact of IST on *P. falciparum* infection according to baseline school-level prevalence of *Plasmodium*, there was no consistent pattern to the variation either across the subgroups or across the two follow ups. The apparent significantly increased risk of *P. falciparum* infection in the low prevalence groups may to be due to the small numbers involved. While the IST intervention was associated with a reduction in risk of *P. falciparum* infection in the medium prevalence subgroup at 12 months, an increased risk was present in the high prevalence subgroup at 24 months. Given these findings there would appear to be no meaningful heterogeneity in impact. Consideration should be given to the possibility of misclassification of schools into these prevalence categories as baseline prevalence in the control schools was inferred using data collected at 12 months.

A possible explanation for the lack of impact of IST on anaemia at the group or individual level is high, localised, rates of re-infection and acquisition of new infections between screening rounds allowing no time for haematological recovery, indicated by the remarkably similar percentage of children RDT positive at each screening round. Further support for the importance of re-infection is provided by the increased risk of *P. falciparum* infection observed at both the 12- and 24-month follow-ups in children who were RDT-positive and treated with AL at multiple screening rounds. This propensity for aggregation of *Plasmodium* infections in certain individuals despite periodic

treatment will be further explored in Chapter 7. The use of AL may have contributed to rapid re-infection rates as it affords short (14–28 days) post-treatment protection [339,340]. Such a protection period would have provided extensive time at risk of acquiring new infections before the next round of IST at least three months later. A potential alternative would be dihydroartemisinin-piperaquine (DP) [341], which would afford a longer post-treatment prophylaxis period than AL between screening rounds and has recently been successfully evaluated as part of IPT in Uganda [214]. Additionally, increased frequency of screening, six times a year as opposed to three, could reduce the time at risk for parasite carriage and allow for haematological recovery, but would be logistically and financially prohibitive. The marked, but stable heterogeneity of *Plasmodium* infection observed over the two years (school-level prevalence range: 0%–75%) resulted in several schools experiencing no infection throughout all screening rounds, and a small sample of schools exhibiting repeatedly high proportions of RDT positive study children at each round. This heterogeneity, compounded by the large proportion of untested and therefore untreated asymptomatic carriers remaining in the communities likely led to study children in localised hotspots being exposed to high risk of infection immediately after treatment [102]. Analyses of the stability infection at the school-level, and the environmental correlates of such patterns, will be presented in Chapter 6.

The evaluation identified two further limitations of the IST approach. First, there was variability in RDT performance between screening rounds, with lowest RDT sensitivity during the dry season. However, diagnostic performance in this analysis, was estimated assuming microscopy as a "gold standard," and in light of concerns of the diagnostic accuracy of such reference tests, alternative methods of estimation for two or more malaria diagnostic tools in the absence of a "gold standard" have been suggested [342-344]. Chapter 5 shall explore the diagnostic performance of RDTs and expert microscopy as well as the influence of individual, local transmission and seasonal factors during the two-year study period. The recent study conducted in Burkina Faso failed to show a significant reduction in parasitaemia in the dry season following community-wide screening and treatment campaigns in the previous dry season [218], suggesting

that screening and treatment with RDTs is not sensitive enough to reduce transmission even when delivered in a mass campaign. The use of PCR would constitute a more sensitive tool, additionally detecting subpatent infections that contribute to transmission [71,345,346], but would be operationally challenging. Second, there was a decline in supervised treatment over time, as it became logistically difficult for children who were absent on screening day and subsequently treated on a repeat visit to be followed up on treatment day two and three by the nurse. Such children and/or their guardians and older siblings were given the full regimen with instructions on how to take the doses at home over the three days [347]. Altering the treatment supervision by the nurse from three days to the first day only would greatly reduce the cost of the IST intervention [348]. Although evidence indicates that unsupervised treatment is as effective at clearing parasitaemia as fully supervised treatment in clinical cases [349], unsupervised compliance may be lower when treating asymptomatic infection. Low efficacy of AL in the study is possible. No specific treatment efficacy evaluation was performed during this trial; however, although there is mixed evidence as to whether there is a slight decline in efficacy of AL in Kenya [350,351], overall treatment success is thought to remain reasonably high.

In a region such as coastal Kenya, where food security is particularly low [352,353] and malaria transmission is low-to-moderate, it is probable that factors such as long term nutritional status, short term access to food, and helminth infections are stronger contributors to the aetiology of anaemia in this setting than parasitaemia [121]. These factors would result in a limited impact on anaemia though a programme targeting malaria only, rather than a package containing a combination of school-feeding, deworming, and malaria control. This study thus contrasts with the previous IPT study conducted in Nyanza province, Kenya [33], where malaria is predicted to be the greatest contributor to anaemia [121], enabling a malaria control programme to have a large impact on anaemia directly.

Our finding of no significant differences between groups for sustained attention in either the younger or older classes at either follow-up is consistent with expectations, based on the lack of

effect of IST on the assumed mediator, health. Likewise with the adjusted literacy and numeracy scores in the older class at both follow-ups, no significant differences between groups were found. However, in the younger class at both 9 and 24 months, there was an apparent negative effect of the IST intervention on literacy scores and on arithmetic scores at 24 months. This seemingly negative impact of IST was found only in the younger class, where the literacy intervention was implemented. As no statistical interaction between the two interventions was detected in the younger class, the differences between study groups cannot be attributed to an effect of the literacy intervention. Because of the multiple tests conducted, this finding could be due to chance. If we were to use a highly conservative Bonferroni correction for the 16 tests (two health and six education outcomes, all at two follow-ups) from adjusted models, the apparent negative effects on spelling and arithmetic would lie close to the updated significance level.

Alternatively, these findings could demonstrate a negative effect of the by-term screening, involving an uncomfortable finger prick [216], with the intervention group experiencing increased apprehension of the finger prick during the education assessments as they associated the presence of our research team with the IST process [294], or reduced classroom attendance throughout the year in this group to avoid the IST intervention, or a combination. However, attendance measured at health and education assessment visits indicated no significant differences in attendance between the groups. Findings of negative educational or cognitive effects of health interventions are rare but not unprecedented [354] and suggest the need for experimental evaluations to test assumptions about the educational benefits of health programs. The finding of low overall achievement levels and minimal learning is consistent with the international literature and findings from Kenya [293]. The causes are well documented and include a lack of a culture of literacy, lack of effective teaching methods, poorly resourced teachers with large classes, poor health of children, and competition for children's time at home [330,334].

Our study has a number of limitations. First, given the nature of the intervention, it was not possible to blind the parents, participants, or field officers delivering the IST intervention to

experimental assignment, which could have led to a possible "John Henry" effect whereby children in the control group adjust their behaviour as they know they are not receiving the intervention, for example in risk aversion and treatment seeking behaviour. Biomedical and educational assessors were blinded where feasible. Second, study children's access to alternative malaria treatments outside of the school-based IST rounds was not monitored during the two years of the trial. However, due to the randomised design of the trial and the fact that the majority of infections in this age group and population were asymptomatic at assessment and screening points, we have no reason to suspect that study children's access to treatment outside of this trial differed greatly across study groups. Finally, the lack of multiple testing adjustments may have increased the possibility of type 1 error, and results should be interpreted in light of this possible error.

4.6 CONCLUSION

In summary, the findings in this chapter show there are no health or education benefits of implementing school-based IST with AL in a low to moderate transmission setting such as this study site. Possible reasons for the absence of an impact are the marked geographical heterogeneity in transmission whereby a high proportion of children screened do not require treatment and those who do largely live in focal high transmission regions; a rapid rate of re-infection following AL treatment between screening rounds; the variable reliability of RDTs as the implementation tool, and the relative contribution of malaria to the aetiology of anaemia in this setting. Following chapters will explore these reasons in greater detail, with an analysis of the diagnostic accuracy of RDTs for screening and treatment presented in Chapter 5, the spatial and temporal heterogeneity of transmission at the school level investigated in Chapter 6 and individual-level re-infection addressed in Chapter 7.

Nevertheless, despite the lack of impact of school-based IST in this setting, our results do highlight a potential role for schools as screening platforms. School screenings using RDTs could

provide an operationally efficient method to initially identify transmission hotspots for targeted community control [355]. School surveys have proved a useful platform for defining heterogeneities in *Plasmodium* transmission over large geographical areas in a more rapid and low cost manner than community surveys [229,230]. The results from this study's screening rounds present a case for the use of schools in also depicting local transmission heterogeneities, which can be extrapolated to the local community [356] and aid in developing targeted community-wide comprehensive interventions, with biennial school screenings used to monitor the success of these interventions. The use of schools in this way is a focus of current research. Chapter 6 will further explore the spatial and temporal heterogeneity observed across all 101 schools, whilst also investigating the environmental and socio-economic factors related to such variation, and the effect of screening interval duration on school-level prevalence.

Chapter 5. The accuracy of rapid diagnostic tests and expert microscopy in screening for *Plasmodium falciparum* infection among school children in south coast, Kenya

5.1 OVERVIEW

Results presented in Chapter 4 demonstrated that in the low-to-moderate transmission setting of coastal Kenya, school-based IST, as implemented in this study, was not effective in improving the health, sustained attention or educational achievement of children. The absence of a differential impact on the primary outcome, anaemia, observed when analyses were stratified by school or child-level baseline characteristics, or when stratified by the frequency of treatments received in the intervention group, suggests there was no impact on long-term health even amongst those children at greatest risk of infection.

A possible factor contributing to the lack of impact, highlighted in Chapter 4, was variable sensitivity of the diagnostic tool, rapid diagnostic test (RDT), used to detect *Plasmodium falciparum* infections during screening rounds. The fidelity of IST is particularly dependent on the accuracy of the screening tool, as it relies on treatment of infected individuals, conditional on a true positive diagnosis during screening, unlike an intervention such as IPT where treatment is administered presumptively. Implementation of IST in a low-to-moderate transmission setting involves regular screenings of largely negative and asymptomatic individuals, and so RDTs need to reliably detect low intensity infections, in order to promote clearance of the parasite reservoir in the population and reduce transmission. Poor RDT sensitivity would result in attenuation of the success and impact of IST.

This chapter uses paired diagnostic data collected from the cohort of study children in the IST intervention group at four screening rounds to estimate the diagnostic accuracy of RDTs in the absence of a reference standard. These estimates will be compared to those from a model assuming expert microscopy as a "gold" or reference standard, as presented in Chapter 4. Additionally, potential influences of individual, school-level and seasonal factors on the variability of diagnostic performance will be explored.

This chapter has been prepared for submission in a modified form. *Halliday KE, Turner EL, Okello G, Njagi K, Pullan RL, Brooker SJ. (2014) The diagnostic accuracy of rapid diagnostic tests and expert microscopy for screening Plasmodium falciparum infection among school children in the South coast, Kenya.* I coordinated the data collection and data entry, conducted the data cleaning and analysis and drafted the manuscript with high level statistical guidance from Dr Rachel Pullan and Dr Elizabeth Turner.

5.2 INTRODUCTION

The increasingly recognised heterogeneous patterns of underlying malaria parasitaemia [94], resulting from widespread reductions in transmission intensity, and the renewed focus on malaria elimination, have led to a growing emphasis on accurate identification and treatment of reservoirs of asymptomatic *Plasmodium falciparum* infection. Identification has been achieved primarily through active surveillance approaches such as large-scale population-based surveys [159,229]. In addition to allowing more efficient targeting of vector control methods such as LLINs, IRS and larval source management [102,103], effective detection of localised regions of high transmission has enabled the increasing deployment of treatment-based interventions, including local reactive screening [193,194], and mass screen and treat campaigns [218,357]. Such strategies are only feasible with a practical and sensitive field diagnostic tool that can provide reliable, real-time information.

Microscopy and RDTs are currently the most widely used diagnostic tools for malaria [17,358]. While there is a large body of evidence on the diagnostic performance of these tools for case management of uncomplicated and severe malaria (usually with high levels of parasitaemia) in clinic settings [359-361], their diagnostic performance during surveys of largely asymptomatic populations is less firmly established. There are relatively limited data on the diagnostic accuracy of RDTs in non-clinic settings, with those conducted so far demonstrating variable diagnostic performance [362-365]. Despite variation, these studies have generally observed comparatively lower estimates of RDT specificity and higher estimates of sensitivity when compared to a reference standard of microscopy. Evidence from large-scale community-wide household surveys in Ethiopia [366], and countrywide school surveys in Kenya, [367] estimated the diagnostic performance of RDTs against a reference of microscopy, with estimates of sensitivity substantially higher and specificity substantially lower in Kenya (96.1% and 70.8%) than those observed in Ethiopia (47.5% and 98.5%) respectively.

Such variation in diagnostic performance may be due to population characteristics, for example age-related immunity. External conditions affecting the operation of the test may also play a role, with all factors liable to vary across time [283,368,369]. Additionally, quality control of RDT devices is an important consideration for diagnostic test performance (as discussed in Section 2.3.4), with quality assurance testing advised at various points during supply chain management, transport and storage and field deployment [370]. Diagnostic performance of RDTs has been associated with subject age [364,365], with a study in Tanzania observing higher sensitivity in children than adults and highly variable specificity across age-groups [365]; transmission intensity [359,371]; density of infection, with higher sensitivity observed at higher parasite density [372]; and recent treatment history [364]. These studies were based on results from single time-point survey data, with seasonal influences not explicitly assessed. Several large-scale studies conducted in patients presenting to clinics, found an association between seasonality and diagnostic accuracy, with relative sensitivity higher in the rainy seasons (high transmission) and specificity higher in the dry season (low transmission) [359,373,374]. However, there is sparse evidence from evaluations of diagnostic accuracy using repeated measures data, allowing the analysis of multiple factors over time, whilst incorporating individual-level and school-level variance.

In the studies referenced above, evaluation of diagnostic accuracy was performed through examination of the results of the index test (RDT) in comparison with results of an assumed reference or "gold" standard, usually expert microscopy or PCR, believed to provide the best approximation of true infection status [375]. However, the use of an imperfect reference standard leads to misclassification of infection status and biased estimates of the index test performance [376]. Given the recognised limitations of techniques such as microscopy to act as a perfect reference test [377,378], there has been a move towards approaches evaluating diagnostic performance in the absence of a reference standard. Latent class analysis (LCA), often performed within a Bayesian framework, assumes a single unobserved (latent) true disease prevalence for each population, and is used to model dependencies between the observed results of two or more

diagnostic tests, without assuming a reference standard [377,379,380]. Such analyses also allow the incorporation of dependence between tests, conditional on disease status, when appropriate [381,382]. Bayesian LCA has been used in a variety of veterinary and medical fields including cancer screening [383] and diagnosis of infectious diseases such as leptospirosis [384] and dengue [385] and the methods are increasingly being adopted for evaluation of malaria diagnostics [342-344,368]. Recent reviews of this approach for evaluating diagnostics [376,386] have suggested that improved estimates of sensitivity and specificity can be obtained, providing the key assumptions of model identifiability are met. This chapter evaluates the performance of RDTs and expert microscopy for screening *P. falciparum* infection using LCA within a Bayesian framework.

5.3 METHODS

These analyses use data collected as part of a longitudinal, cluster randomised trial investigating the impact of a school-based malaria control intervention, intermittent screening and treatment (IST), in 101 primary schools in coastal Kenya [284,357]. Full details of this trial are presented in Chapter 2. In brief, school-based IST involves periodic screening of children using an RDT to detect *P. falciparum* parasites, with RDT-positive children (with or without malaria symptoms) treated with artemether-lumefantrine (AL). Fifty one schools were randomly allocated to receive the IST intervention and the data presented are from a cohort of 2,674 children in these schools, originally in classes 1 and 5, assessed at four screening rounds across one year, Survey 1: February/March 2010; Survey 2: June/July 2010; Survey 3: September 2010 and Survey 4: February/March 2011 (Figure 5.1). Surveys one and four correspond to the dry season in Kenya and surveys two and three cover the period during and following the rains, when transmission peaks [287,290,291]. Reporting of the study has been verified in accordance with the STARD (Standards for the reporting of diagnostic accuracy studies) checklist [387].

5.3.1 Screening survey procedures and diagnostic tools

At each school survey finger-prick blood samples were obtained from the randomly selected 25 class 1 and 30 class 5 children on which a ParaCheck-*Pf* malaria RDT (Orchid Biomedical Systems, Goa, India) was performed. The finger-prick blood sample was additionally used to prepare a blood slide with a thin and thick smear. During the first survey, haemoglobin concentration (Hb) was also assessed using a portable haemoglobinometer (Hemocue, Ängelholm, Sweden). The surveys were conducted by local facility and hospital-based laboratory technologists with experience in the two diagnostic methods, who received additional training from an expert microscopist from Kenya Medical Research Institute (KEMRI) Laboratories, Nairobi.

Paracheck-*Pf* RDT is an immunoassay for the detection of *P. falciparum* specific histidine rich protein-2 (HRP-2) antigen circulating in whole blood [388]. The RDTs were performed as per the manufacturer's instructions and were opened immediately prior to use. When the internal control line was absent, indicating an invalid result, the test was repeated. The devices were procured through the Kenya Medical Supplies Agency (KEMSA) and were used within the shelf life of 24 months. They were stored at room temperature within the recommended temperature range and transported to schools in cooler boxes.

Blood slides were labelled and air-dried horizontally in a covered slide tray in the school. Thin smears were fixed in methanol and the slides stained with 2% Giemsa for 30 minutes at the end of each day. Blood slides were transported to Nairobi and read at KEMRI Laboratories by expert microscopists. The microsopists were blinded to the field-based RDT result of the individual. Parasite densities were determined from thick smears by counting the number of asexual parasites per 200 white blood cells, assuming a white blood cell count of 8,000/µl. A smear was considered negative after reviewing 100 high-powered fields. Thin blood smears were reviewed for species identification. Two independent microscopists read the slides, with a third microscopist resolving

discrepant results. *P. falciparum* infection was defined on the basis of duplicate slide readings and parasite density taken as the mean parasites/µl of two positive slide readings.

5.3.2 Possible correlates of diagnostic accuracy

During the first survey (baseline), individual demographic and health information was collected, including age as reported by the child, sex, and anaemia, here defined using the threshold of 110g/L haemoglobin with no correction made for age, sex or altitude. School-level prevalence of *P. falciparum* infection at baseline was categorised into four subgroups <10%, 10-19.9% and 20-39.9% and ≥40%. Combined prevalence (RDT positive and/or microscopy positive) was used for this categorisation. The presence of fever was classified as axillary temperature ≥37.5°C, measured using a digital thermometer at each survey.

5.3.3 Data analyses

Data were double-entered, consistency checks performed and all descriptive analyses conducted using Stata software version 13.1 (Stata Corporation, College Station TX). Bayesian analyses were conducted using Winbugs 1.4 (Medical Research Council, Cambridge, UK and Imperial College London, UK) [389]. Overall and survey specific estimates of *P. falciparum* infection prevalence were determined independently by expert microscopy and Paracheck RDT, and using a combined measure (microscopy and/or RDT positive) with confidence intervals adjusted for correlation within schools. The Kappa κ-statistic was used to determine the level of agreement between the diagnostic tests [390] with values of 0.21-0.4 indicative of fair agreement, 0.41-0.6, moderate agreement; 0.61-0.8, substantial agreement; and 0.81-1.0, almost perfect agreement. Multinomial hierarchical modelling was used to investigate correlates of test discordance at the level of the child, school and survey. Diagnostic performance was estimated, using the traditional approach assuming expert microscopy as the gold standard and through latent class analyses, comparing both RDT and expert microscopy assuming a reference standard. Finally, based on the

results of the multinomial model, variability in the estimated diagnostic performance is investigated through stratification of the LCA.

5.3.3.1 Bayesian multinomial hierarchical model

A multinomial hierarchical modelling approach was used to initially explore potential correlates of test discordance at the individual (age, sex, anaemia at baseline, and microscopist discrepancy), school (prevalence category – proxy for transmission intensity) and survey (screening round) level. This approach allowed additional quantification of the variability observed in Chapter 4, whilst simultaneously accounting for additional covariates and dependence within schools and within children, across repeated measures [391]. For the given scenario, four mutually exclusive diagnostic outcome combinations are possible ((i) RDT negative and microscopy negative, (ii) RDT positive and microscopy positive, (iii) RDT positive and microscopy negative, (iv) RDT negative and microscopy positive). Covariates associated with these outcomes were first examined at the univariable level using multilevel multinomial logistic models in STATA, accounting for dependence within schools and within children, with the random effects at each level assumed as equal across the outcomes. Covariates retained from univariable analyses were included in a multinomial hierarchical model within a Bayesian framework, whereby random effects at the level of the child and the school could vary across the multiple outcomes.

The four mutually exclusive diagnostic outcome combinations were used to construct a product multinomial outcome at the level of the individual, with the concordant negative combination taken as the base comparison group. The effect of the covariates on the probability of the outcome falling in a particular category was assessed through logistic regression. The model was specified as:

$$\boldsymbol{Y}_{ijk} \sim MN\left(1, \boldsymbol{p}_{ijk}\right)$$

$$\text{where } \boldsymbol{Y}_{ijk} = \left(Y_{ijk1,}\ Y_{ijk2,}\ Y_{ijk3,}\ Y_{ijk4,}\right)$$

where vector $\boldsymbol{Y}_{ijk}$ is a multinomial, within one trial Y_{ijkl} is equal to either 1 or 0, with the probability of being in any one of four classes represented by $\boldsymbol{p}_{ijk}$. Therefore:

$$\boldsymbol{Y}_{ijkl} \sim Binomial(1, p_{ijkl})$$

$$\text{where } \boldsymbol{p}_{ijk} = (p_{ijk1,}\ p_{ijk2,}\ p_{ijk3,}\ p_{ijk4,}\ )$$

l indexes the four diagnostic outcome combinations at survey *i*, for individual *j*, in school *k*. Thus $\boldsymbol{p}_{ijkl}$ is the probability of: concordant RDT and microscopy negative (*l=1*); concordant RDT and microscopy positive (*l=2*); discordant RDT positive and microscopy negative (*l=3*); and discordant RDT negative and microscopy positive (*l=4*) at survey *i*, for individual *j*, in school *k*. The model for the reference group p_{ijk1} is specified as:

$$log(p_{ijk1}) = \alpha_1 + \sum \beta_{1n} X_{ijk} + u_k + v_j$$

And the models for *l* =2,3,4 are specified as:

$$log\left(\frac{p_{ijkl}}{p_{ijk1}}\right) = \alpha_1 + \alpha_l + \sum \beta_{ln} X_{ijk} + u_k + v_j \ \ for\ l = 2,3,4$$

The intercept for the reference group is α_1 and the intercept for infection status (*l*) is denoted by α_l where α_l is the difference in log probabilities from the reference group (concordant negative) for groups *l* =2,3,4. $\Sigma\beta_{ln}$ is a vector of regression coefficient parameters related to a matrix of *n* covariates (X_{ijk}) for which covariate values can vary across schools, individuals and surveys. A non-informative flat beta prior (a flexible distribution implying no prior knowledge) was assigned to the intercept and the β coefficients were assigned a normal prior with a mean of 0 and a precision of 1×10^{-6}.

The unstructured school-level correlation (random intercepts) for the diagnostic outcome combinations are denoted by u_{kl} and assume a multivariate normal distribution to account for potential dependency between outcomes at the school-level, assuming an unstructured covariance matrix:

$$(u_{k2,}u_{k3,}u_{k4}) \sim MVN(0, \Omega^{-1})$$

Ω^{-1} denotes the covariance matrix of the multivariate normal prior. As RDT and microscopy concordant negative was taken as the reference category $u_1 = 0$.

$$\begin{bmatrix} u_2 \\ u_3 \\ u_4 \end{bmatrix} \sim N_3 \left(\begin{bmatrix} 0 \\ 0 \\ 0 \end{bmatrix}, \begin{bmatrix} \tau_{22} & \tau_{23} & \tau_{24} \\ \tau_{23} & \tau_{33} & \tau_{34} \\ \tau_{24} & \tau_{34} & \tau_{44} \end{bmatrix} \right)$$

The variance arises from the diagonal element of the matrix and the off-diagonals are the correlation components between diagnostic outcome combinations. The variance components of the covariance matrix were assigned a Wishart distribution (a multivariate scaled χ^2 distribution): $\Sigma^{-1} \sim Wishart\ (\Omega, p)$ where Ω = scale matrix for the covariance matrix and p = degrees of freedom (3) of multinomial outcome. The diffuse inverse Wishart prior takes the form $p(\Omega^{-1}) \sim$ Wishart(p, Q).

The unstructured child-level correlation (random intercepts) for the diagnostic outcome combinations are denoted by v_{jl} and also assume a multivariate normal distribution assuming an unstructured covariance matrix to account for potential dependency between infection status outcomes at the child level.

For both the multinomial hierarchical models and the latent class models described below, a burn-in of 20,000 iterations was run with two chains using a Markov Chain Monte Carlo (MCMC) algorithm. Model convergence and autocorrelation were assessed through inspection of the trace plots and Gelman Reubin statistic. Convergence was achieved after 20,000 iterations and a further 10,000 iterations were run, thinning every 10 to obtain stored samples from the marginal posterior distribution of each parameter. Summary statistics from the stored values of the posterior distributions of the model parameters were calculated.

5.3.3.2 Estimation of diagnostic accuracy assuming a reference standard

As a basis for comparison, the standard approach was used to estimate the diagnostic accuracy of the RDT, whereby the RDT (considered the index test), was evaluated against expert microscopy, assumed to be the reference standard comparator [375]. Overall summaries of sensitivity (proportion of true positives identified as such), specificity (proportion of true negatives identified as such), positive predictive value (PPV, proportion of those with positive test results correctly diagnosed) and negative predictive value (NPV, proportion of those with negative test results correctly diagnosed) with 95% confidence intervals were estimated using a chi squared distribution based on standard procedures for contingency diagnostic tables (using the diagt command in STATA) [392]. No adjustments were made for correlations within students or schools, as is standard for this type of analysis.

5.3.3.3 Estimation of diagnostic accuracy in the absence of a reference standard

The use of microscopy as a reference standard has several constraints, such as the limits of sensitivity, where it has been estimated that *P. falciparum* infection prevalence measured by microscopy is only 50.8% of that measured by PCR in surveys of endemic populations, with poorer sensitivity in low transmission settings [71]. Given the recognised imperfections of microscopy as a reference standard, sensitivity and specificity of both diagnostic methods were estimated assuming the absence of a reference standard, through Bayesian LCA. Here an unobserved (latent or "true") infection status was assumed, with the tests acting as imperfect classifiers of this "true" infection status, and a probabilistic model was used to model the relationship between the two imperfect tests [393], with the dependency between tests (either independent or dependent of infection status) used to obtain estimates of the diagnostic accuracy of each without using either one as the reference.

A product multinomial outcome was assumed at the school level (with numbers of (i) RDT negative and microscopy negative, (ii) RDT positive and microscopy positive, (iii) RDT positive

and microscopy negative, (iv) RDT negative and microscopy positive). The models were initially constructed on the basis of comparing two or more tests in two or more populations, assuming independence between tests conditional on infection status, an approach developed by Hui and Walter [377]. Each of the 51 schools at every survey was considered a separate population *k* (thus *k*=1,…204), with an associated latent true *P. falciparum* prevalence, denoted by π_k. The two diagnostic tests are represented as T_j (j = 1, 2), with both tests conducted in a sample of *N* subjects from *k* populations. A positive test result is designated by T_j^+ and a negative result by T_j^-, while the true result or underlying infection status is represented by *D*, with D^+ in truly infected subjects and D^- in truly non-infected subjects. The sensitivity *S* of test *j* is the conditional probability of a truly infected subject correctly identified as such by the test where $S_j = \mathrm{P}(T_j^+|D^+)$ and the specificity *C* of test *j* is the conditional probability of a truly non-infected subject being identified as negative by the test, where $C_j = \mathrm{P}(T_j^-|D^-)$. Diagnostic performance was estimated for each test assuming sensitivity and specificity were unknown but remained constant across populations, but that prevalence of infection varied across populations.

As children in each school were screened for *P. falciparum* infection independently by microscopy and RDT, the data formed 2 x 2 contingency tables for each school population (*k*) Where X denotes the counts of data in each of the response categories:

		T_1	
		+	-
T_2	+	X_{k++}	X_{k-+}
	-	X_{k+-}	X_{k--}

Independent multinomial distributions were modelled where:

$$\boldsymbol{y}_k \sim Multi[N_k(p_{k++}, p_{k+-}, p_{k-+}, p_{k--})]$$

And, assuming conditional test independence, the multinomial probabilities take the form:

$$p_{k++} = P\ (T_1^+, T_2^+ | k\text{th population}) = \pi_k[S_1 S_2] + (1-\pi_k)[(1-C_1)(1-C_2)]$$

$$p_{k+-} = P\ (T_1^+, T_2^- | k\text{th population}) = \pi_k[S_1(1-S_2)] + (1-\pi_k)[(1-C_1)C_2]$$

$$p_{k-+} = P\ (T_1^-, T_2^+ | k\text{th population}) = \pi_k[(1-S_1)S_2] + (1-\pi_k)[C_1(1-C_2)]$$

$$p_{k--} = P\ (T_1^-, T_2^- | k\text{th population}) = \pi_k[(1-S_1)(1-S_2)] + (1-\pi_k)[C_1 C_2]$$

k=1,2,3...204

The population-specific estimates were then pooled to provide overall estimates of sensitivity, specificity and true *P. falciparum* prevalence, and these estimates obtained from the LCA were used to calculate the PPV (proportion of positive results that are truly positive) and NPV (proportion of negative results that are truly negative).

$$PPV_j = \mathrm{P}(T_j^+ | D^+) = \frac{S_j \pi}{S_j \pi + (1-C_j)(1-\pi)}$$

$$NPV_j = \mathrm{P}(T_j^- | D^-) = \frac{C_j \pi}{C_j(1-\pi) + (1-S_j)\pi}$$

Here π is the true, but unknown, prevalence. Non-informative beta (1, 1) prior distributions were assigned to the sensitivity and specificity of the diagnostic tests and to the true prevalence. Sensitivity analyses were conducted to assess the influence of the prior distributions on the resulting parameter estimates. Use of alternative non-informative (uniform) prior distributions or more restrictive beta distributions had no meaningful effect on the estimates of sensitivity and specificity or on the true prevalence obtained.

Crucially, however, the model above assumes conditional independence between tests, whereby the result of one test is independent and unrelated to the result of another, whether the unknown infection status is positive or negative. As the tests both rely on blood products for diagnosis, however, it may be that they are correlated on the basis of infection status. To assess the validity of this assumption, the model was extended to include a measure of covariance between the tests within each of the two infection classes (infected and uninfected), thereby assuming the tests are conditional both on the result of the alternative test and on infection status, as described by Gardner *et al.* and Dendukuri and Joseph [381,382].

Covariance between tests for infected individuals is denoted by $covD^+ = \mathrm{P}(T_1^+ T_2^+ | D^+) - S_1 S_2$ and for uninfected individuals is denoted by $covD^- = \mathrm{P}(T_1^- T_2^- | D^-) - C_1 C_2$.

The multinomial probabilities including the additional covariance parameter take the form:

$$p_{k++} = P\,(T_1^+, T_2^+ | k\text{th population}) = \pi_k[S_1 S_2 + covD^+] + (1-\pi_k)[(1-C_1)(1-C_2) + covD^-]$$

$$p_{k+-} = P\,(T_1^+, T_2^- | k\text{th population}) = \pi_k[S_1(1-S_2) - covD^+] + (1-\pi_k)[(1-C_1)C_2 - covD^-]$$

$$p_{k-+} = P\,(T_1^-, T_2^+ | k\text{th population}) = \pi_k[(1-S_1)S_2 - covD^+] + (1-\pi_k)[C_1(1-C_2) - covD^-]$$

$$p_{k--} = P\,(T_1^-, T_2^- | k\text{th population}) = \pi_k[(1-S_1)(1-S_2) + covD^+] + (1-\pi_k)[C_1 C_2 + covD^-]$$

The two covariance parameters were assumed to take generalised beta distributions using a uniform prior with the covariance specified as below, and the upper and lower bounds of the covariance parameters were derived using the method described by Branscum et al [380]:

$$(S_1 - 1)(1 - S_2) \leq covD^+ \leq min(S_1, S_2) - S_1 S_2$$

$$(C_1 - 1)(1 - C_2) \leq covD^- \leq min(C_1, C_2) - C_1 C_2$$

The covariance was used to calculate the conditional correlation between tests using:

$$\rho D^+ = \frac{covD^+}{\sqrt{S_1(1-S_1)\,S_2(1-S_2)}} \qquad \text{and} \qquad \rho D^- = \frac{covD^-}{\sqrt{C_1(1-C_1)\,C_2(1-C_2)}}$$

Where ρD^+ is the correlation between test outcomes in infected individuals, and ρD^- is the correlation between test outcomes in uninfected individuals.

The inclusion of the covariance terms results in additional parameters to be estimated and the associated increase of two degrees of freedom, necessitating constraints on the model to maintain identifiability. Probabilistic constraints were incorporated whereby expert opinion was used to inform the prior beta distributions for test specificities [343,344]. The beta distribution for the specificity of microscopy was assumed to have a mode of 0.9 and 5th percentile of 0.7

$(\alpha,\beta)=(15.03,2.56)$ and for the sensitivity was assumed to have a mode of 0.7 and 5th percentile of 0.5 $(\alpha,\beta)=(13.32,6.28)$ based on expert opinion of the performance of microscopy [343]. The RDT sensitivity and specificity and prevalence parameters were given non-informative beta priors. Sensitivity analyses were conducted, varying the given beta distributions both in relation to (a) restrictiveness and (b) the parameters on which the informative distributions were placed, to assess the influence of the prior information on resulting parameter estimates. These variations in prior distributions did not significantly influence the parameter estimates. Model fit was examined to assess the appropriateness of the assumption of conditional independence against conditional dependence through comparison of the deviance information criterion (DIC), with a smaller DIC indicative of a better fitting model while also considering parsimony.

5.3.3.4 Stratified analysis of diagnostic accuracy

Correlates found to be associated with test discordance in the hierarchical multinomial model were further explored and used as a basis for stratification of latent class models to assess differential diagnostic performance. Sensitivity and specificity of the two diagnostic tools were estimated for the subgroups of children.

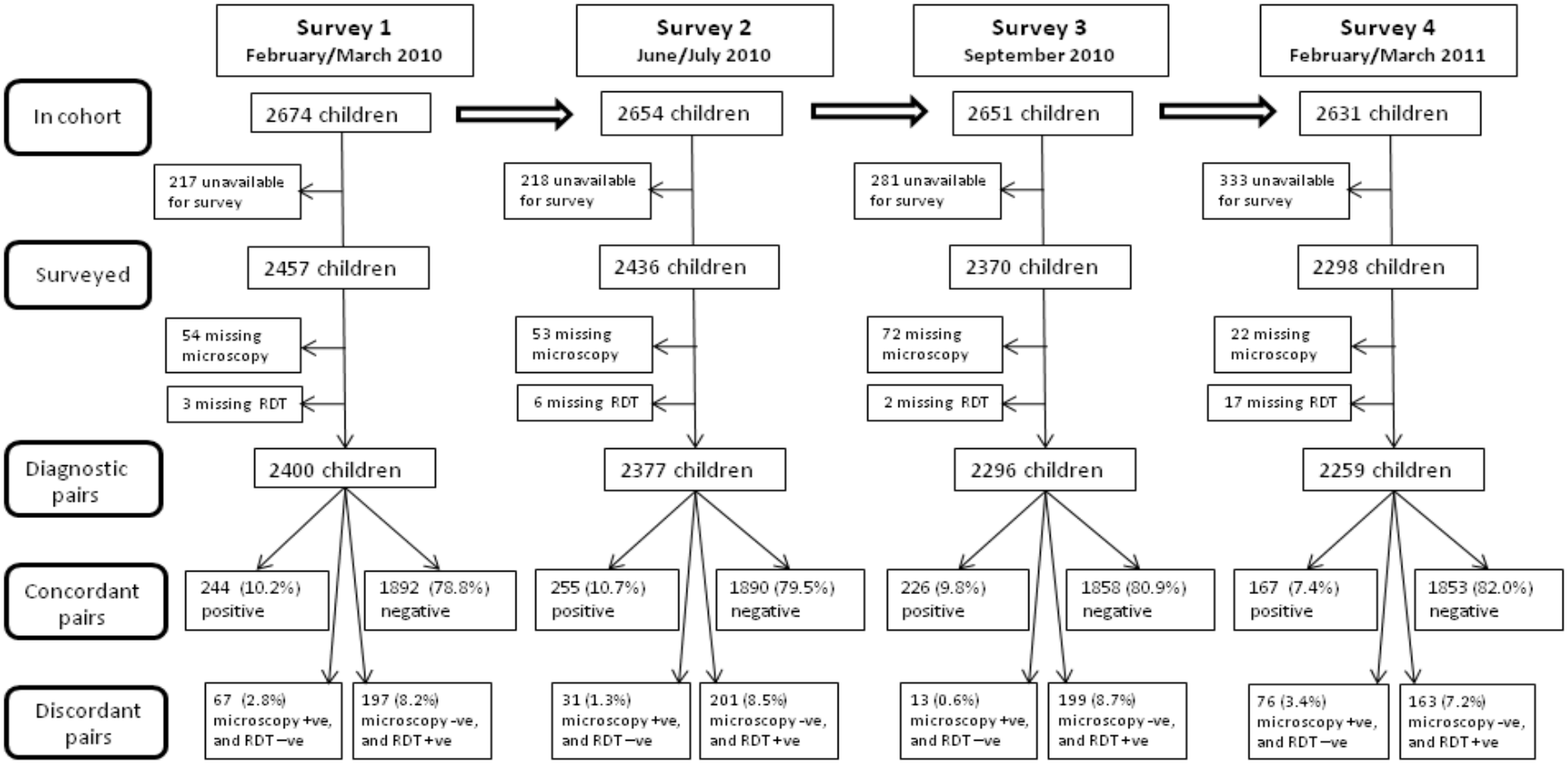


Figure 5.1 Data flow diagram for four screening surveys conducted in school children in 51 schools across a 12 month period.

5.4 RESULTS

5.4.1 Data Summary

A total of 2,674 children were initially enrolled from the 51 schools, with valid RDT and microscopy diagnostic pairs obtained from 2,400 children in Survey 1 (baseline); 2,377 in Survey 2; 2,296 in Survey 3 and 2,258 in Survey 4 (Figure 5.1), totalling 9,331 diagnostic pairwise comparisons overall. A mean of 48 children per school (range: 26-60) were assessed during the first survey, and by Survey 4 a mean of 45 (range: 33-55) children per school were assessed. Figure 5.1 summarises the flow of data. The mean age of children at baseline was 10.3 years (range 5-18 years) and the male/female ratio was 0.95 (Table 5.1).

Table 5.1 Baseline characteristics of the 2674 children in the initial cohort as identified in Figure 5.1

Characteristics at baseline	**Number (%)**
CHILD	
Sex	
Male	1299 (48.6)
Female	1375 (51.4)
Age (years)	
5-9	1051 (39.3)
10-12	917 (34.3)
13-20	706 (26.4)
Anaemic (<110 g/L)	
No	1864 (76.0)
Yes	587 (24.0)
SCHOOL	
Apparent School prevalence (%)[a]	
<10	674 (25.2)
10-20	988 (36.9)
20-40	590 (22.1)
>40	422 (15.8)

[a] calculated using a combined reference (positive in microscopy, RDT or both)

Overall 1,839 cases of *P. falciparum* infection were detected by either or both diagnostic tests across all surveys (i.e. combined prevalence was 19.7%, [95% confidence interval [CI]: 15.3-24.2%]), with 760 (8.1%) children positive by RDT only and 187 (2.0%) positive by microscopy only. Apparent *P. falciparum* infection prevalence, as determined by microscopy alone, was

11.6% (95% CI: 8.3-14.8%), in comparison with 17.7% (95% CI: 13.5-21.9%) by RDT. The κ-statistic of 0.60 (95% CI: 0.57-0.62) indicates moderate to substantial overall agreement between RDT and microscopy. Of the 9,331 slides examined, one observation of *P. ovale* and thirty observations of *P. malariae* were reported, of which fourteen were mixed infections with *P. falciparum*. Thirteen of the mixed infections were detected by RDT and a further five of the single *P. malariae* infections were also detected by RDT. From this point the analyses will only consider *P. falciparum* infection.

The geometric mean parasite density of apparent infections determined through microscopy was 474.2 parasites/μl (95%CI: 435.6-516.1 parasites/μl) with densities below 500 parasites/μl in 60% of apparent infections, and below 160 parasites/μl in 25% of apparent infections. Of the 9,331 slides read, 8% were discrepant and required a third reading for confirmation of infection status, constituting a kappa κ-statistic of 0.64 (95%CI: 0.62-0.67) between first and second microscopists in relation to presence/absence of infection.

5.4.2 Factors associated with test discordance

The prevalence of fever (axillary temperature ≥37.5°C) over the study period was minimal, 1.3%, with a range of 0.7-2.7% across surveys, and no statistical difference observed between those with positive and negative RDT results. Therefore, the presence of fever was not assessed as a risk factor for discordance in the multinomial hierarchical model. In the univariable analysis, anaemia at baseline, discrepancies between microscopists and increased combined school-level *P. falciparum* prevalence were significantly associated with increased relative odds of discordant (RDT positive and microscopy negative) results in comparison with the base group of concordant negative results, whereas being female and the survey conducted in February 2011 (Survey 4) were associated with decreased relative odds of this outcome (Appendix 5.1). Surveys 2 and 3, conducted during the rainy season, were associated with decreased relative odds of discordant (RDT negative and microscopy positive) results whereas discrepancies between microscopists, and combined school-level *P. falciparum* prevalence were associated with increased relative odds

of RDT negative and microscopy positive results in comparison with the base group of concordant negative results.

As shown in Table 5.2, the results of the Bayesian multivariable hierarchical model demonstrate that survey period remained associated with test discordance, with slightly higher relative odds of discordant (RDT positive and microscopy negative) results in Surveys 2 and 3, and substantially lower odds of discordant (RDT negative and microscopy positive) results in Surveys 2 and 3 (Relative odds ratio [ROR] 0.56 [95% Bayesian credible interval [BCI]: 0.34-0.91] and ROR 0.25 [95% BCI: 0.13-0.47]) respectively. As expected, with increasing combined school-level prevalence, the relative odds increase substantially for all outcomes in comparison with the base outcome of concordant negative with very similar relative odds ratios observed across both the discordant outcome combinations. The relative odds of discordance were greater than twice, fivefold and fifteen fold higher than that of the concordant base group for the three apparent school prevalence categories (10-19.9, 20-39.9 and ≥40.0%) when compared to the base category of <10%. Anaemia at baseline and female gender were associated with increased and decreased relative odds respectively of both concordant positive and discordant (RDT positive and microscopy negative) results.

Interestingly, cases in which the two microscopists' readings were discrepant were associated with approximately three times higher relative odds of a concordant positive result or a discordant (RDT positive/microscopy negative) result in comparison to the base outcome. However, discrepancies between microscopists were associated with twenty-five times greater odds of a discordant (RDT negative and microscopy positive) result (ROR 25.41 [95% BCI: 17.99-36.34]).

The addition of the covariates covered in Table 5.2 to a null model (not shown), led to no significant change in the child-level variance for any of the outcomes, which was reasonably low in all cases except for the concordant positive outcome (Table 5.2). However, school-level variance for each outcome decreased significantly after accounting for the covariates. In the case

of the discordant (RDT positive microscopy negative) outcome, the variance decreased from 0.84 (BCI: 0.54-1.30) to 0.11 (BCI: 0.06-0.19) and for the discordant (RDT negative microscopy positive) outcome, the variance decreased from 1.53 (BCI: 0.84-2.67) to 0.40 (BCI: 0.15-0.80).

Table 5.2: Bayesian multivariable multinomial hierarchical model of correlates of discordance.

	Concordant Outcome		**Discordant Outcomes**			
Characteristic	RDT positive, Microscopy positive		RDT positive Microscopy negative		RDT negative, Microscopy positive	
	Relative odds ratio (ROR[a])	95% Bayesian credible interval (BCI)	ROR[a]	95% BCI	ROR[a]	95% BCI
Feb 2010						
Jul 2010	**1.41**	**1.12, 1.78**	**1.27**	**1.00, 1.60**	**0.56**	**0.34, 0.91**
Sept 2010	1.18	0.93, 1.48	**1.26**	**1.00, 1.57**	**0.25**	**0.13, 0.47**
Feb 2011	0.80	0.63, 1.03	1.02	0.81, 1.30	0.98	0.66, 1.48
No discrepancy between microscopists						
Discrepancy between microscopists	**2.96**	**2.28, 3.38**	**3.30**	**2.57, 4.22**	**25.41**	**17.99, 36.34**
Male						
Female	**0.81**	**0.66, 0.99**	**0.81**	**0.68, 0.96**	1.05	0.75, 1.47
Not anaemic						
Anaemic	**1.62**	**1.29, 2.03**	**1.29**	**1.05, 1.58**	1.01	0.67, 1.51
Age (years) 5-9						
10-12	0.83	0.66, 1.05	0.94	0.77, 1.16	1.33	0.89, 2.00
13-20	**0.60**	**0.46, 0.79**	0.92	0.74, 1.15	1.09	0.71, 1.68
Prevalence (%) <10						
10.0-19.9	**2.83**	**2.02, 3.98**	**2.76**	**2.10, 3.64**	**2.91**	**1.51, 5.74**
20.0-39.9	**8.24**	**5.89, 11.51**	**5.32**	**3.99, 7.21**	**7.64**	**4.13, 14.57**
≥40.0	**44.21**	**28.96, 66.09**	**15.46**	**10.71, 22.51**	**15.86**	**7.46, 34.54**
Child-level RE variance	1.32	0.94, 1.72	0.54	0.28, 0.79	0.40	0.19, 0.75
School-level RE variance	0.13	0.07, 1.25	0.11	0.06, 0.19	0.40	0.15, 0.80

[a]ROR denotes the relative odds ratio, of the relative odds compared with the base outcome (RDT negative, Microscopy negative) for those exposed vs unexposed for each characteristic.
RORs in bold indicate those significant at the 5% significance level, as determined by the 95% BCI

5.4.3 Diagnostic performance

The overall estimate of RDT sensitivity, using the traditional approach of assuming expert microscopy as the reference standard, was 82.7% (95% CI: 80.3-84.9%), which when combined with the apparent microscopy prevalence of 11.6% gave rise to a low positive predictive value of 54.0% (95% CI: 51.6-56.4%). Specificity was higher, estimated at 90.8% (95% CI: 90.1-91.4%).

Given the recognised limitations of the reference standard approach, the subsequent analyses of diagnostic performance uses LCA in the absence of a reference standard. The assumption of conditional independence was acceptable based on comparison with a model allowing dependence between tests conditional on disease status. The inclusion of the covariance terms, increasing model complexity, did not improve the model fit according to the DIC, which was 2,464 compared to 2,431 for the conditional dependent and conditional independent models respectively (Appendix 5.2). Correlation between tests in uninfected individuals was non-significant (ρ=0.12 [95% BCI: -0.02-0.32] and within infected individuals correlation between tests was moderate (ρ=0.48 [95% Bayesian Credible Interval (BCI): 0.43-0.52]). Models presented assume conditional independence.

The use of LCA allowed the estimation of performance for both RDTs and expert microscopy simultaneously, with the results demonstrating superior performance of RDTs overall. As displayed in Table 5.3, estimated overall sensitivity of RDTs from the LCA, at 81.6% (95% BCI: 79.0-84.1%), was comparable to that from the reference standard model. However, a considerable difference was observed in the estimated sensitivity of microscopy, with sensitivity estimated to be only 58.7% (95% BCI: 55.1-62.3%), substantially lower than the 100% assumed in the reference standard model. This sensitivity estimate for microscopy resulted in a significantly higher overall estimate of true *P. falciparum* prevalence of 20.9%. Both tests exhibited a high degree of specificity with the mean estimates and their associated Bayesian credible intervals greater than 95% for each (Table 5.3).

Table 5.3: Overall and survey-specific estimates of sensitivity and specificity of RDTs and expert microscopy as evaluated using latent class analysis, assuming the absence of a reference standard.

	Overall N=9331	Survey 1 N=2400 (Feb-Mar 2010)	Survey 2 N=2377 (Jun-Jul 2010)	Survey 3 N=2296 (Sept 2010)	Survey 4 N=2259 (Feb-Mar 2011)
True prevalence	***20.9 (19.7-22.1)***	***18.9 (16.6-21.4)***	***20.6 (18.6-22.7)***	***18.4 (16.4-20.5)***	***22.5 (20.1-25.0)***
	RDT diagnostic performance				
Sensitivity (95% BCI)	**81.6 (79.0-84.1)**	**77.6 (72.4-82.4)**	**91.8 (86.6-96.1)**	**97.5 (93.6-99.8)**	**65.5 (58.8-72.0)**
Specificity (95% BCI)	**97.9 (97.0-98.7)**	**94.2 (92.4-96.2)**	**98.2 (96.5-99.7)**	**97.7 (95.9-99.4)**	**99.1 (97.8-99.9)**
Positive predictive value (95% BCI)	91.0 (87.2-94.7)	75.8 (68.0-84.2)	93.0 (86.3-98.8)	90.6 (83.0-97.6)	95.3 (89.0-99.6)
Negative predictive value (95% BCI)	95.3 (94.5-96.0)	94.7 (93.2-96.1)	97.9 (96.4-99.1)	99.4 (98.5-100)	90.8 (88.3-93.0)
	Microscopy diagnostic performance				
Sensitivity (95% BCI)	**58.7 (55.1-62.3)**	**73.4 (64.1-82.5)**	**59.7 (53.7-66.1)**	**58.9 (52.0-66.2)**	**50.4 (44.1-57.1)**
Specificity (95% BCI)	**99.9 (99.7-100)**	**99.9 (99.4-100)**	**99.4 (98.7-100)**	**99.5 (99.0-99.9)**	**99.9 (99.5-100)**
Positive predictive value (95% BCI)	99.4 (98.1-100)	99.1 (96.8-100)	96.1 (91.8-99.4)	96.2 (92.8-99.0)	99.0 (96.3-100)
Negative predictive value (95% BCI)	90.2 (88.9-91.4)	94.1 (91.3-96.5)	90.5 (88.4-92.5)	91.4 (89.3-93.5)	87.4 (84.7-90.0)

N refers to number of test pairs in each group

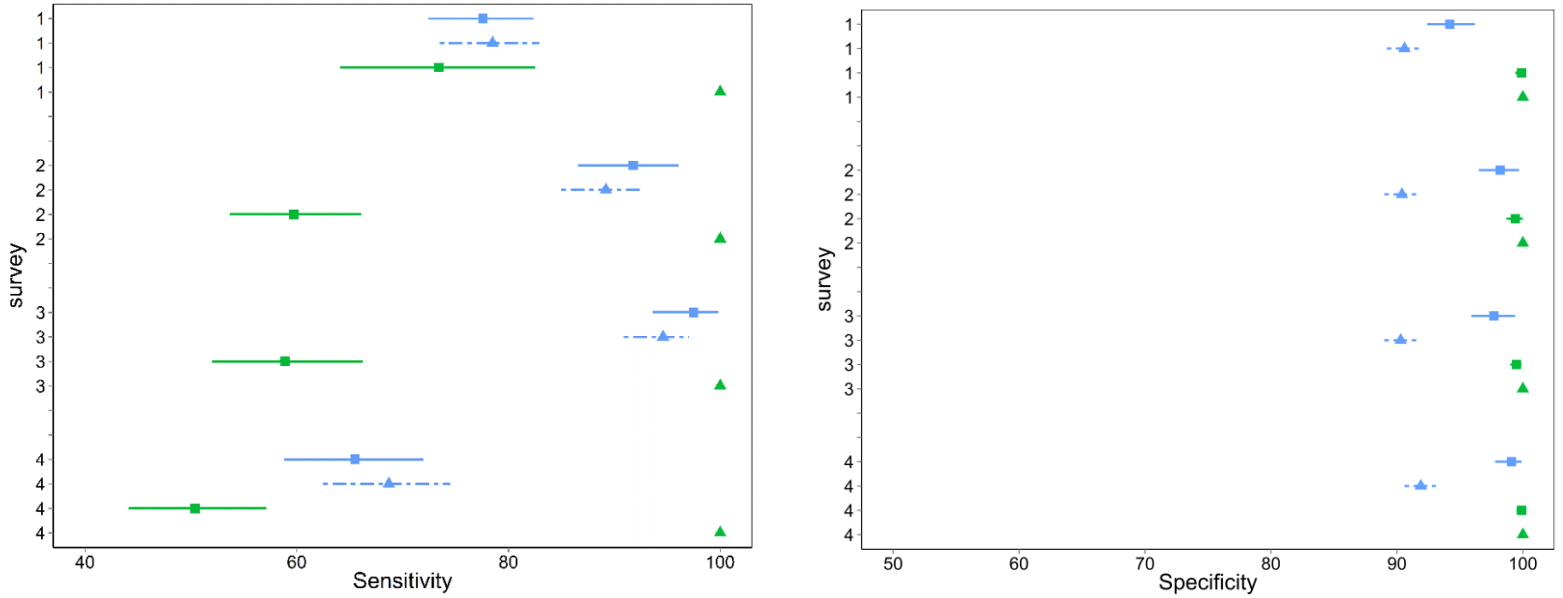
5.4.4 Survey-specific diagnostic performance

As already observed from the results presented in Chapter 4, the sensitivity of RDTs was highly variable across the surveys, when compared to expert microscopy as the reference standard. The multinomial hierarchical model also highlighted the differential odds of discordance between test results across the four surveys. Table 5.3 displays a similar pattern of RDT diagnostic variability across surveys using Bayesian LCA. RDT sensitivity varied substantially across the surveys, with sensitivities over 90% observed during Surveys 2 and 3 conducted during the rainy season. In contrast, estimated sensitivity was considerably lower during the surveys conducted in the dry season (Surveys 1 and 4), where the estimated sensitivity of RDTs fell as low as 65.5% (95% BCI: 58.8-72.0%).

Regardless of the variability in sensitivity observed, RDTs performed better than microscopy at all surveys, with the latter also exhibiting variability over a lower range, with greatest sensitivity during Survey 1 at 73.4% (95% BCI: 64.1-82.5%) and poorest sensitivity at Survey 4 at 50.4% (95% BCI: 44.1-57.1%), as shown in Table 5.3. This is also demonstrated in Figure 5.2 where the sensitivity of microscopy as estimated by LCA is significantly lower than that of the RDTs (estimated by either approach), and of the 100% assumed by the reference standard approach. In comparison, consistently high specificity estimates were generated by the LCA modelling at all surveys for both tests, ranging from 94.2% to 99.1% (Table 5.3) with regard to RDT specificity, and even higher estimates observed for microscopy, exceeding 99% for all surveys. As shown in Figure 5.2, the specificity of RDTs estimated by LCA is slightly lower than that estimated by the reference standard model, but remains consistently above 90% across all surveys.

Estimates of sensitivity and specificity stratified by sex and baseline anaemia did not differ significantly across the subgroups and are not presented. In relation to school-level prevalence increased odds of discordance were seen across both discordant outcome groups as well as the concordant positive group, in accordance with the increased infections detected. School-level prevalence groups were not addressed further in relation to differential diagnostic performance.

Figure 5.2: Survey specific (a) sensitivity (b) specificity of RDTs and expert microscopy as estimated by latent class analysis and by the reference standard approach. Square points represent the mean posterior estimate and associated 95% Bayesian credible interval from the latent class analysis and triangular points represent the mean estimate and 95% confidence interval for RDT from the reference standard approach and the assumed 100% sensitivity and specificity of microscopy.



For the reference standard model, the sensitivity and specificity of expert microscopy are assumed to be 100%

5.4.5 Survey specific prevalence estimates

Figure 5.3 demonstrates a consistent pattern in the prevalence estimates across the four surveys, with the microscopy data providing the lowest prevalence in all cases, and the RDT, combined and true (unobserved) estimates all relatively close, not differing significantly in Surveys 1 to 3. However, in Survey 4, where the sensitivities of both tests were estimated to be lowest, the true prevalence was significantly higher than the apparent prevalence as determined by either microscopy or RDT.

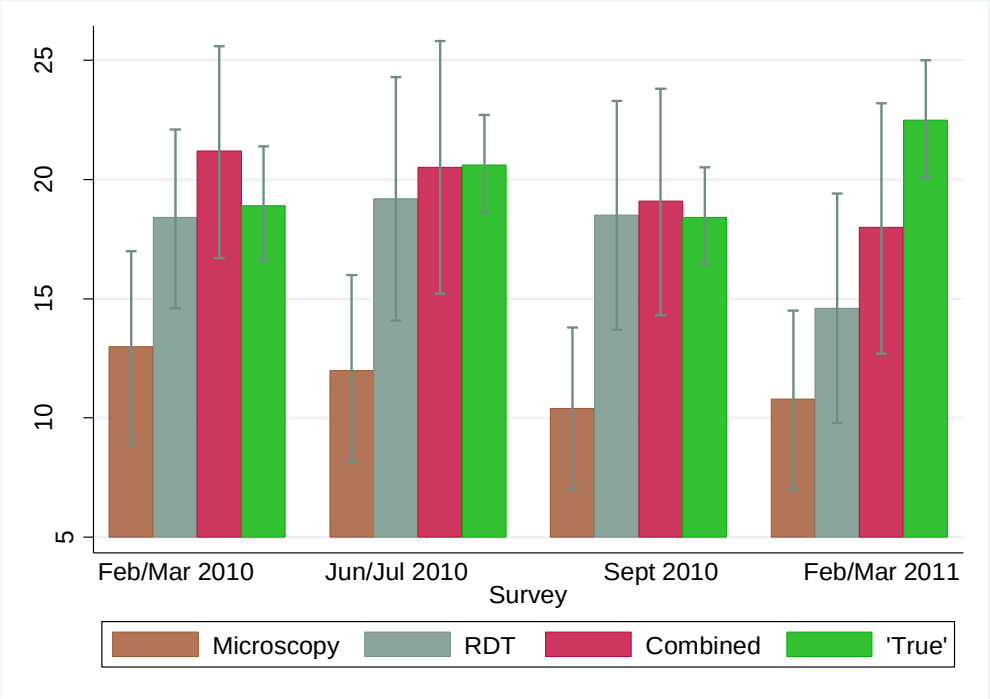


Figure 5.3: Apparent mean *P. falciparum* prevalence at the four surveys as determined by expert microscopy, RDT and a combined reference (RDT positive and/or microscopy positive), alongside the estimates of assumed "true" prevalence. 95% confidence interval presented for all but "true" prevalence where the Bayesian credible interval is presented.

5.4.6 Relationship between parasite density and diagnostic performance

Variation in mean parasite density (although only available for infections detected by microscopy) was examined as a possible explanation for the differential diagnostic performance observed across surveys. As shown in Figure 5.4a, geometric mean parasite density (among those determined as infected by microscopy), although variable, was observed to be higher in the two surveys conducted in the rainy season, where the sensitivity of RDT was highest.

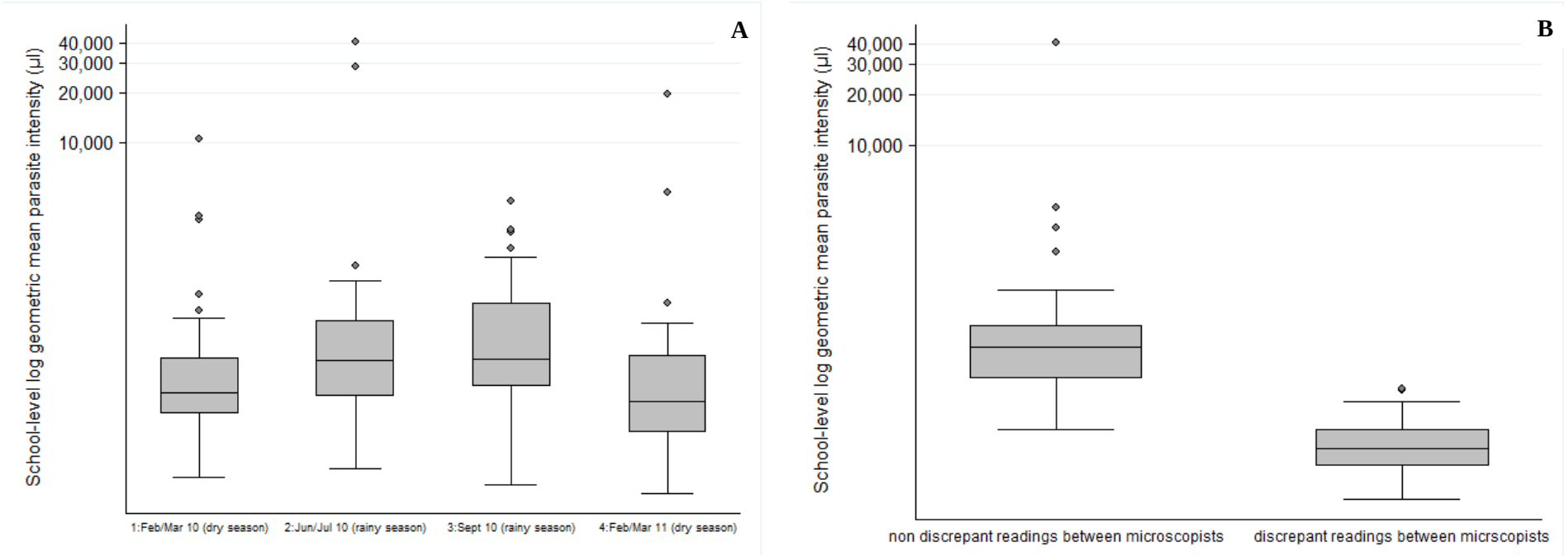


Figure 5.4: Box plots of school-level log geometric mean parasite intensity in those children determined as infected with *P. falciparum* on the basis of a positive microscopy slide by (A) survey and by (B) discrepant vs non discrepant first and second microscopy readings.

To further assess the relationship between estimates of diagnostic performance and density of infections, analysis was stratified on the basis of whether the two microscopists agreed on the infection status or gave discrepant results. Discrepancies between microscopists generally occur at low parasite density thresholds, and this factor was found significantly associated with discordance in the multinomial hierarchical model. Figure 5.4b indicates a substantially reduced geometric mean parasite density (among those determined as infected by microscopy) for those slides where the microscopists were not in initial agreement. As shown in Table 5.4 stratification into two subpopulations on the basis of discrepancies between microscopists indicates significantly reduced estimates of RDT sensitivity, from 93.1(95% BCI: 90.6-95.3) to 52.5 (95% BCI: 46.5-58.7) in cases where microscopists' readings were in agreement, and discrepant, respectively. A parallel but less marked decline was observed in specificity. In both subpopulations the estimated sensitivity of microscopy was low, with specificity remaining high.

Table 5.4 Estimates of sensitivity and specificity of RDTs and expert microscopy as evaluated using latent class analysis, assuming the absence of a reference standard, when stratified by cases of non-discrepant microscopy slide readings and discrepant microscopy slide readings.

	Non-discrepant (N=8581)	Discrepant (N=750)
True prevalence	***15.3 (14.3-16.3)***	***52.6 (46.4-58.8)***
	RDT diagnostic performance	
Sensitivity (95% BCI)	**93.1(90.6-95.3)**	**52.5 (46.5-58.7)**
Specificity (95% BCI)	**97.1 (96.3-97.9)**	**87.0 (80.9-93.1)**
Positive predictive value (95% BCI)	85.2 (81.1-89.1)	81.7 (71.7-91.1)
Negative predictive value (95% BCI)	98.7 (98.2-99.2)	62.2 (55.2-69.0)
	Microscopy diagnostic performance	
Sensitivity (95% BCI)	**64.1 (60.1-67.9)**	**61.7 (53.7-70.3)**
Specificity (95% BCI)	**99.8 (99.6-100)**	**98.2 (94.0-100)**
Positive predictive value (95% BCI)	98.1 (96.2-99.7)	97.5 (91.1-99.9)
Negative predictive value (95% BCI)	93.9 (93.0-94.8)	69.7 (60.8-78.5)

N refers to number of test pairs in each group

5.5 DISCUSSION

As the need for routine surveillance and screening of *Plasmodium* infection intensifies, alongside the expanded adoption of control methods requiring parasitological confirmation prior to treatment, the need for accurate screening tools also increases [17]. Poor sensitivity in the screening tool will attenuate the impact of any intervention reliant on detecting low density asymptomatic *Plasmodium* infections. The results presented here constitute the first evaluation of diagnostic performance of RDTs and microscopy using Bayesian LCA for a longitudinal study where influences such as seasonality are investigated. Overall the use of LCA suggested superior diagnostic performance of RDTs over expert microscopy, with estimates of sensitivity in both diagnostic tools observed to vary considerable across surveys, assumed to reflect seasonality affecting transmission and parasite density. The findings are discussed in relation to the IST intervention under evaluation, and in relation to the broader implications for the use of RDTs for surveillance and monitoring in low-to-moderate transmission settings.

The traditional "gold standard" for malaria diagnostics is expert microscopy, with WHO specifying that diagnostic tools must have greater than 95% sensitivity and specificity, in comparison to expert microscopy, in order to be deemed suitable for use in the field [394]. However, the current findings suggest that the HRP-2 based RDT constitutes a more sensitive tool for screening primarily asymptomatic populations in a region of low-moderate transmission than expert microscopy, which in turn has previously been shown to demonstrate higher sensitivity in comparison to field microscopy [395-397]. The LCM estimates of RDT sensitivity are comparable with those from the traditional reference-standard approach, and while specificity estimates are lower than estimated by the traditional approach they remain above 90%.

In the same manner, the poor sensitivity of microscopy relative to RDTs, using the LCA approach, suggests it is inappropriate to use expert microscopy as a reference standard. Both overall, and when stratified by survey, microscopy demonstrated significantly lower sensitivity in comparison

with RDTs, with posterior estimates ranging from 50.4 to 73.4%. Interestingly, these current findings are more consistent with analyses evaluating the diagnostic performance of both RDTs and microscopy against a third reference standard such as PCR. For example, results from a *Plasmodium* prevalence survey in Angola indicated superior performance of RDTs over microscopy when compared to a reference standard of PCR, 73% versus 60% sensitivity [398].

Additionally, these results are corroborated by previous studies employing similar methods, in which diagnostic performance was evaluated assuming no reference standard. A review of diagnostic accuracy across nine field studies documented a pooled HRP-2 RDT sensitivity greater than that of microscopy using latent class analysis [342]. Similarly Gonçalves *et al.* [344] found low sensitivity of microscopy in comparison to RDTs and PCR using this approach in a low transmission setting in São Tomé and Príncipe, with the difference most pronounced in afebrile children under five years. Speybroek *et al*. [343] observed a median sensitivity of 53% for microscopy in comparison to ELISA and PCR during a survey conducted in Vietnam, a similar transmission setting to this Kenyan setting.

A possible explanation for the inferior sensitivity of microscopy in comparison to RDTs in this setting relates to the predominance of low density parasitaemic infections. Typically microscopy is argued to have a lower density detection threshold than RDTs, with microscopy able to detect 40 parasites/μl and RDTs closer to 140-1400 parasites/μl [399]. However, it has been suggested that in the case of low density infections, HRP-2 antigen is released and potentially detectable over the duration of the infection, despite the erythrocytic parasite levels fluctuating above and below microscopy detection limits [400]. The agreement between first and second microscopists in our study, although substantial, was slightly lower than has been found in other studies [401,402]. Densities of 160 parasites/μl or less in 25% of the slides confirmed as positive, indicates a reasonably high proportion of low density infections in this setting, possibly contributing to misclassification of infection status due to inter-observer error. This source of error has been shown to increase sharply with decreasing parasite densities, with very low

agreement typically found in slides with less than 100 parasites/μl [401,402]. The significantly lower parasite densities exhibited in positive slides where microscopy readings were discrepant is indicative of parasite density-dependence in the differential diagnostic accuracy observed. Loop-mediated isothermal amplification (LAMP) may present a more sensitive alternative to RDTs and microscopy in such a low-to-moderate transmission setting, with sensitivity estimates similar to those of PCR in clinic settings [89]. However, as yet, this option is still not cost or operationally effective for field based surveys or screening and treatment based programmes.

The variation in RDT sensitivity estimates that we observed across surveys is consistent with a pattern of seasonal variation, with sensitivity substantially higher in surveys conducted in the higher transmission rainy season than in the surveys conducted in the dry season. Although this is a region of perennial transmission with seasonal peaks as opposed to marked seasonal transmission, the higher geometric mean parasite densities observed in the surveys conducted during and following the rains, suggest seasonal fluctuations in parasite densities, with increased likelihood of high density infections in the period of relatively higher transmission. This finding of seasonal variation in RDT performance is supported by evidence from seasonal transmission settings, such as that from Burkina Faso, where in both symptomatic and asymptomatic children, significantly higher sensitivity and lower specificity of the RDT device was observed in the high transmission season, compared to the low transmission season, possibly related to the threefold higher parasite density [373,374]. The increased geometric mean parasite density observed in the surveys conducted in the wet season would appear to support this. However, as such density measures are not available from RDTs, infections missed by microscopy are not included in this consideration.

The seasonal variation in RDT performance could additionally in part be attributed to the extrinsic environmental conditions and their effect on the operation of the tool. In the hot dry season, the temperatures in the vehicles and the schools in which the RDTs were transported and used increased above 36°C, exceeding the recommended heat stability limits. However evidence

suggests that HRP-2 RDTs, in particular Paracheck RDTs, are extremely heat stable and can function after storage at 60°C [360,403] and as such, external conditions are less likely to have affected diagnostic performance than parasite density.

Seasonality has also been related to specificity of HRP-2 RDTs [359], with lower specificity following the high transmission season attributed to a greater number of cleared infections with circulating antigens still present for up to two weeks [404]. Although the LCA findings indicate a lower RDT specificity than that estimated for microscopy, the specificity is still reasonable. High specificity has commonly been found in evaluations of malaria diagnostics using Bayesian LCA [342-344]. It may be that in this setting, low density chronic infections are more liable to go untreated, resulting in a lower proportion of individuals with cleared parasites but remaining circulating antigens. However, study children's access to treatment outside of the screening and treatment visits was not monitored.

Latent class analysis is recognised as a more robust method than the reference standard approach, when due to the lack of a reliable reference standard, the true infection status of the population is unknown, leading to bias in the estimates of diagnostic accuracy of the index test [386]. LCA, within a Bayesian framework, enables estimation of sensitivity and specificity in the absence of a reference standard, and thus this source of bias is reduced. However, LCA is not without its potential limitations. A key assumption of this approach is that the diagnostic accuracy of both tests is constant across compared populations, and where this is not the case, bias will be introduced into the estimates [378]. Additionally, the extent of variation in prevalence between populations influences the estimates obtained from LCA, biasing sensitivity towards populations with the highest infection prevalence, and thus the most data [405].

Furthermore if the test outcomes in true positive (and true negative) individuals are highly correlated the assumption of conditional independence is not viable. The adequacy of the assumption of conditional independence between tests has been a subject of extensive debate in

relation to various tests including malaria diagnostics, with Speybroek *et al*. [343] proposing the incorporation of conditional dependence as appropriate. This assumption requires increased probabilistic and deterministic constraints, due to the increase in estimable parameters over and above the degrees of freedom, resulting in a greater dependence on prior knowledge in the specification of prior distributions. The analyses presented here suggested that the assumption of conditional independence was reasonable, consistent with previous analyses using Bayesian LCA to evaluate the accuracy of malaria diagnostic tools [342,344]. Sensitivity analyses using more restrictive (informative) priors had no significant influence on the posterior estimates in these analyses. However, the moderate correlation observed between positive tests in the conditional dependence model, disregarded due to worse model fit, may suggest a slight overestimation of diagnostic performance for both tests in this setting [405].

On the basis of biological plausibility, an assumption of conditional independence is reasonable, as although the methods of each test are based on blood products, they identify distinct biological phenomena, with the RDT detecting circulating antigen and microscopy detecting the whole parasite. Nevertheless the inclusion of a third diagnostic test, such as PCR or LAMP, in the analysis, would strengthen future analysis by increasing the degrees of freedom and subsequent identifiability of the model. However it should be appreciated that tools such as RDTs and PCR may be more likely to be conditionally dependent on disease status because individuals who have recently cleared infection and have truly negative blood samples may test positive by PCR and by RDT for a period of up to two weeks in the case of RDT and shorter for PCR [368].

Although controlled for in models investigating factors associated with discordant results, multiple covariate influences were not accounted for simultaneously when estimating the diagnostic accuracy in the various population subgroups. The stratification of populations into subgroups based on certain determinants, and their subsequent separate analysis has been argued to be a practical means of understanding how the performance of diagnostic tests may vary across smaller biologically determined groups or groups subject to the same local conditions. This

approach has been applied in previous latent class analyses of malaria diagnostics [342,344]. The subgroups presented here were analysed separately, with the populations included in each subgroup analysis assumed to have the same characteristics, resulting in unbiased overall estimates. However, it should be noted that the subsequent pooling of such performance estimates is not appropriate, as this violates the assumption that sensitivity and specificity are constant across populations. For instance, pooled estimates will be more strongly influenced by the estimates with the most data (the greatest prevalence), leading to bias if performance varies systematically on the basis of prevalence [405].

While the incorporation of multiple covariates into these LCA models may improve the inferences on diagnostic performance [405], extending the model in this way adds further complexity, risking over-fitting and non-identifiabilty. Vectors of covariates have been incorporated into Bayesian LCAs assessing accuracy of diagnostic tools for cervical cancer screening and Chagas disease [383,406]. In veterinary epidemiology, diagnostic accuracy for Johnes disease in cows was considered a function of covariates across repeated measures in herds within a Bayesian framework [407]. While this approach might provide additional future insight for the analysis of malaria diagnostics across repeated measures, a necessary constraint on this analysis was the assumption that the infection status remained constant across the observed timepoints, a non-viable assumption for repeated measures of *P. falciparum.*

5.6 CONCLUSIONS

In conclusion, these findings suggest that HRP-2 RDTs are a more sensitive screening tool than expert microscopy for surveillance or screen and treat campaigns, despite not matching the WHO guideline threshold of greater than 95% sensitivity in all screening surveys. In addition, RDTs constitute a more operationally attractive method for large-scale field-based activities than field or expert microscopy, or costly alternatives such as PCR, where a recent study investigating a mobile laboratory in Cambodia provided estimates of $2.75 per sample screened with real-time PCR including extraction and $3.75 for species identification [408]. Nevertheless, the

significance of the variable RDT sensitivity by survey (65.5-97.5%) cannot be overlooked, and may have contributed to the lack of impact of the IST intervention if many of the low density infections were missed during the screening process. The findings indicate that the timing of screening surveys must be carefully considered, with screening perhaps more reliable in the rainy season when the transmission intensity is likely to be higher, resulting in greater mean parasite densities which are more easily detectable. However, as transmission declines and the emphasis on interrupting transmission becomes greater, a move towards more sensitive tools such as LAMP for IST interventions will be required.

While variability in the process of IST may constitute one reason for the lack of impact of this intervention in this low-to-moderate transmission setting, additional factors, external to the process of the intervention, such as the heterogeneity in transmission intensity may have played an influential role in the lack of impact of the IST intervention. The following chapter explores school-level heterogeneity in transmission and the effect of this on re-infection between screening rounds.

Chapter 6. Spatial and temporal heterogeneity of asymptomatic *Plasmodium falciparum* parasitaemia among Kenyan school children

6.1 OVERVIEW

Heterogeneity in risk of *P. falciparum* infection at a localised scale may have contributed to the lack of impact of IST on *P. falciparum* infection and anaemia observed in Chapter 4 as a large proportion of children screened did not require treatment and those children infected and treated were likely rapidly exposed to re-infection from surrounding community members. Chapter 3 highlighted marked school-level heterogeneity in *P. falciparum* infection at baseline. This was, however, established from examination of one cross sectional time point prior to IST implementation. In order to fully understand the effect of such heterogeneity on the success of a control strategy it is important to establish the extent to which the micro geographical variation in transmission is temporally stable in the face of periodic intervention, as this may explain the persistence of transmission during the trial. Additionally, the degree of spatial and temporal stability in transmission also has substantial implications for the targeting of future sustained control strategies in regions of low to moderate transmission. This chapter uses microscopy data collected at six surveys across the study period in the cohorts of children in both the intervention and control groups to further investigate the spatio-temporal patterns of *Plasmodium* infection in this low to moderate transmission setting using geostatistical modelling within a Bayesian framework. While recognising that the results presented in Chapter 5 suggested imperfect diagnostic performance of both microscopy and RDTs, both techniques are used widely in the field and to allow for the inclusion of the data from control schools in the spatio-temporal analyses presented, microscopy data is used in this chapter.

This chapter has been prepared for submission in a modified form: *Halliday KE, Pullan R, Okello G, Njagi K, Kinyua K, Cano-Ortega J, Turner EL, & Brooker SJ. (2014) Spatial and temporal heterogeneity of asymptomatic Plasmodium falciparum parasitaemia among Kenyan school children.* I coordinated the data collection, entry and cleaning, as well as conceiving and conducting the data analysis with high level technical support provided by Dr Rachel Pullan.

6.2 INTRODUCTION

The spatio-temporal dynamics of malaria have been of longstanding interest [290,409-411], with the majority of studies characterising clinical malaria episodes in time and space [100,412-414], especially in relation to vector dynamics [250,415] and epidemic detection [416-419]. However, in an environment of declining transmission [2] where clinical malaria is less common, coupled with a recent focus on malaria elimination and targeted interventions, there is an increasing need to explore spatio-temporal patterns of asymptomatic *Plasmodium* infection across fine geographical scales [102]. Such knowledge is critical for identifying the drivers of heterogeneity underlying transmission dynamics, both in terms of developing an understanding of the lack of impact of school-based IST in this low-moderate transmission setting and for the planning of locally targeted control initiatives, in turn enhancing elimination efforts [103,420].

In one of the few longitudinal studies investigating spatio-temporal dynamics of malaria, Bejon *et al*. (2010) followed a community cohort over twelve years on the coast of Kenya and showed that whilst spatial clusters of clinical malaria were transitory, clusters of asymptomatic *Plasmodium* infection were stable over time [99]. Temporally stable clusters of parasitaemia have also been demonstrated in Mali [249], Peru [421] and Sudan [422,423]. The principal method used by these studies to detect clustering has been the spatial scan statistic [424], the results from which are critically dependent on a number of assumptions, such as the size and shape of the scanning window and upper cluster size threshold [425-427]. Furthermore, the difficulty of adequately controlling for possible environmental confounders can lead to a high

possibility of false positive clusters detected [427,428]. An alternative approach used to quantify and understand spatio-temporal patterns is geostatistical modelling within a Bayesian framework [427,429], which has previously been used to investigate spatial relationships between *Plasmodium* infection and environmental covariates, using single time point and assembled survey data [241,243,248,430-433] and is increasingly employed to explore spatio-temporal patterns of infectious diseases [242,434,435].

This chapter investigates the spatial and temporal heterogeneity of asymptomatic *Plasmodium* infection over 24 months, using a cohort of school children on the south coast of Kenya. The environmental, seasonal and socioeconomic factors associated with the distribution of school-level infection in space and time are explored using Bayesian geostatistical modelling [429]. Implications for the impact of a school-based programme of screening and treatment, and for informing future targeted malaria control and monitoring decisions, are also examined.

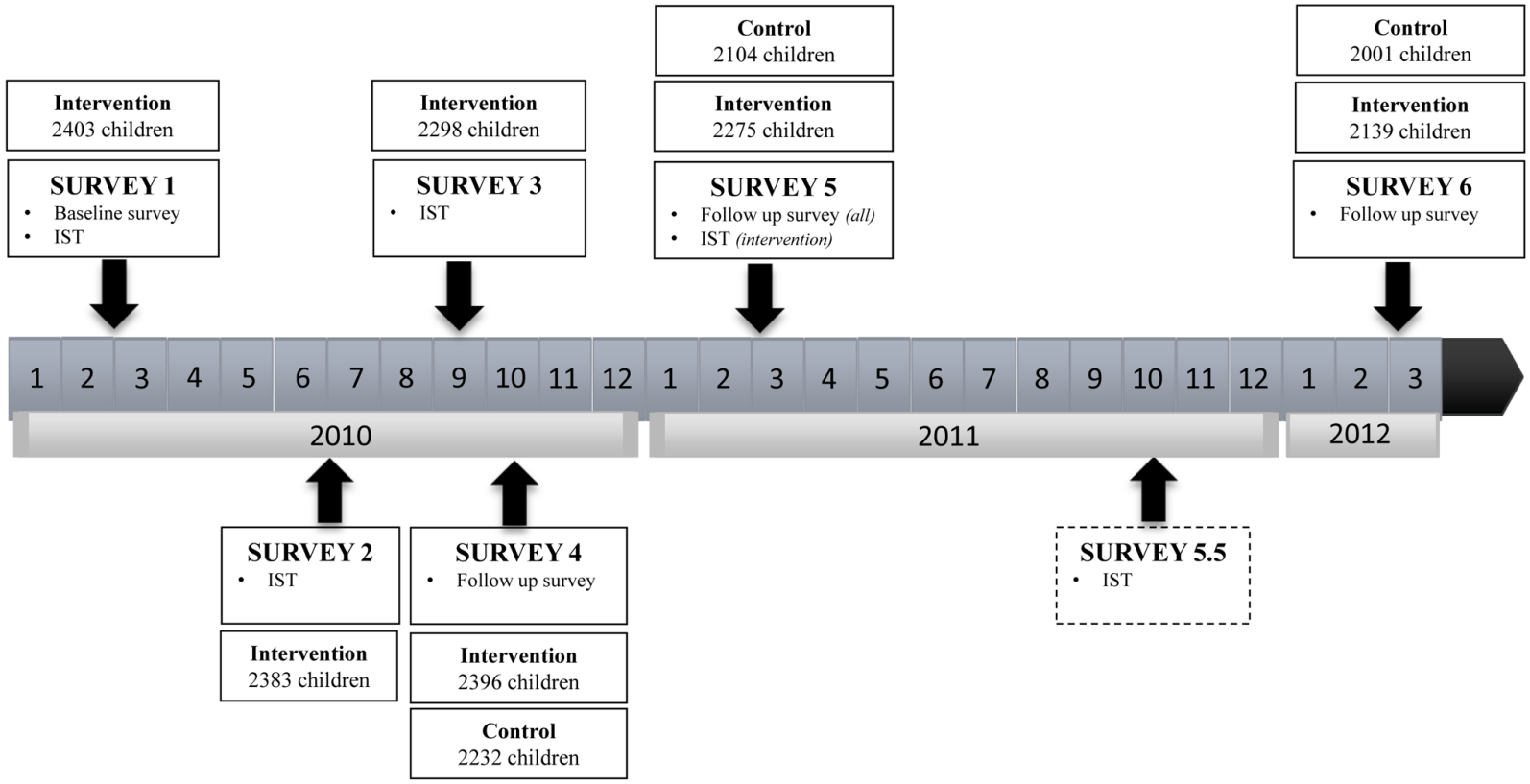
6.3 METHODS

6.3.1 Design

The analysis uses data from blood slides collected as part of a longitudinal, cluster randomised trial investigating the impact of a school-based malaria control intervention, Intermittent Screening and Treatment (IST), in 101 primary schools in Coastal Kenya [284,357]. The study is described in detail in Chapter 2, but, in brief 51 schools were randomly allocated to the IST intervention group and 50 schools to the control group (Figure 6.1). These data represent a cohort of children followed between February 2010 and March 2012. In order to maximise data and to ensure a sufficiently robust semivariance by increasing the spatial locations, the models utilise data from the 51 IST intervention schools assessed at six survey time points (Surveys 1 to 6) and includes data collected from the additional 50 control schools assessed at three survey time points (Surveys 4 to 6) (Figure 6.1). Surveys 1 to 3 correspond to the IST rounds conducted in the intervention schools where blood slides were collected in addition to the RDTs performed.

The IST round conducted in October 2011 is not included, as for logistical considerations, blood slides were not collected. Surveys 4 to 6 refer to the health assessment surveys conducted at 9, 12 and 24 months in all 101 schools (Figure 6.1).

Figure 6.1 Timeline of surveys and activities conducted in all 101 study schools across the 24 month study period. Number of children assessed at each survey in both the intervention and control groups is depicted.



Survey 5.5. (dotted box) is not included in the analyses as blood slides were not collected at this survey due to logistical constraints. However RDT-detected cases were treated with AL.

6.3.2 Parasitological surveys

As described in Chapter 2, 25 and 30 children were randomly selected from classes 1 and 5 respectively and enrolled into the study in January 2010. At each of the six surveys, children were asked to provide finger-prick blood samples, and thick-and-thin films were made, Giemsa-stained, and examined by two independent microscopists, with a third microscopist resolving discrepancies. At the IST intervention screening surveys, the same blood sample was used to perform an RDT and treat on the basis of the result.

6.3.3 Field collected data

During recruitment, a questionnaire was administered to children's parents/guardians to record household information, including ownership of possessions, household construction and mosquito net use. School-level proportions of net use were calculated and quintiles of SES were derived from individual scores assigned to each child during a principal components analysis described in Chapter 3 and Appendix 3.1 [231]. The proportions of children falling in the lowest and highest wealth quintiles respectively were calculated for each school. School coordinates were recorded using a handheld Global Positioning System (GPS) receiver (eTrex Garmin Ltd., Olathe, KS).

6.3.4 Environmental and remote sensing data

For each school, a range of environmental data were extracted using ArcMAP 10 (Environmental Systems Research Institute Inc. Redlands, CA, US), including time constant and time varying covariates. For 27 of the 51 IST intervention schools, locations of children's households were also mapped from which a median school catchment zone was defined and applied as a uniform buffer (1.13km radius) to all 101 schools. Use of a buffer facilitated the extraction of environmental data from the area in which the school children lived and slept and where they were likely to become infected. Furthermore, although mosquito flight distance may

be highly variable and dependent on local ecology of a region, this radius is consistent with the average 0.3-1.5 km mosquito dispersion range [436,437]. The mean and standard deviation of continuous environmental variables was extracted for each school buffer and for categorical variables, the category covering the majority of the buffer zone was extracted. A detailed description of the environmental covariates extracted is provided in Appendix 6.1, however, a brief description is provided here.

The time constant covariates included elevation, at 90 metre resolution, obtained from a Shuttle Radar Topography Mission (SRTM) [438]; Euclidean distance to permanent and temporary waterbodies using a digital water-body map devised from digital elevation models [439]; land cover as determined through the Global Land Cover Classification [440]; estimates of mean annual temperature and precipitation obtained from the WorldClim data source [439] and estimates of potential evapotranspiration (PET) and aridity data modelled from this data were obtained from CGIARCSI [441]. Peri-urban and rural areas were defined on the basis of a combination of spatial gridded (2010) population density data settlement points and night-time lights obtained from AfriPop [442,443].

The time varying covariates included Normalized Difference Vegetation Index (NDVI) estimates obtained from the VEGETATION 2 sensor onboard the SPOT 5 satellite system, which provides 10 day composites at 1km resolution [444]. For each survey, the mean, maximum and standard deviation NDVI values were calculated using a lag time of one month prior to the survey, in addition to the survey period [244,431], and standardised. Long term average monthly precipitation data, calculated for the period 1950–2000 were obtained [439] at 1 km resolution and these averages were used to create mean precipitation values for each survey averaged over a lag period of two months to the end of each survey round. Land surface temperature (LST) was obtained from data measured by the Moderate Resolution Imaging Spectroradiometer sensor [445] and mean LST values were calculated, averaged over a lag

period of six weeks to the end of each survey [431]. Figure 6.2 depicts a conceptual framework of environmental and socioeconomic factors often linked to *Plasmodium* infection risk, as well their hypothesised causal pathways, all of which were investigated in these analyses.

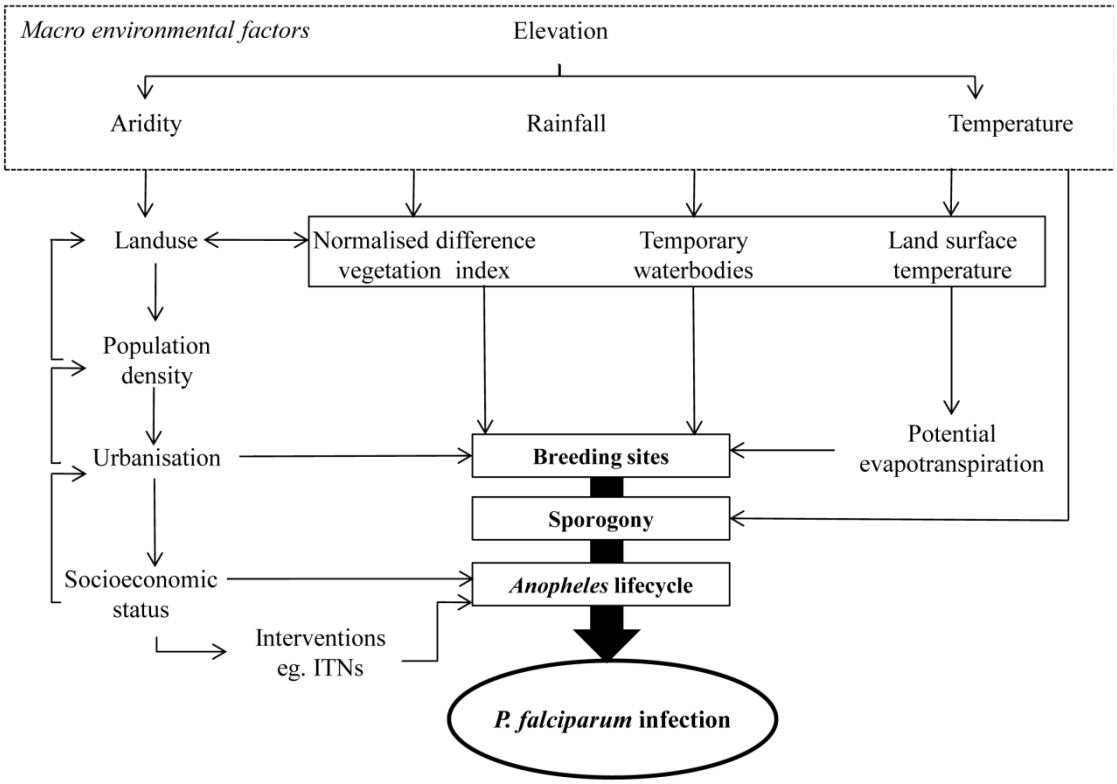


Figure 6.2 Conceptual framework of environmental and socioeconomic factors and their hypothesised associations with *P. falciparum* prevalence.

6.3.5 Data analysis

The raw data were mapped to visualise the geographical variation in school-level *P. falciparum* prevalence and the extent of spatial dependence was determined using semivariograms. Binomial logistic regression using a frequentist approach was used to identify environmental and socioeconomic factors, at the school-level, associated with *P. falciparum* infection. A series of models were constructed within a Bayesian framework to (i) sequentially account for aspatial, and spatial variation and risk factors, and (ii) explore the temporal nature of the data.

6.3.5.1 Spatial exploration of data

Semivariograms were computed to visually examine the spatial structure (autocorrelation) of the raw school-level *P. falciparum* infection prevalence. Semivariograms define the range, if any, over which spatial dependence exists, through determination of the semivariance, the measure of dissimilarity of two observations as a function of the distance between them [426,427]. This is calculated as half the squared difference between observation pairs. Semivariance values are grouped and averaged across lag distances, with the maximum lag distance approximately half the distance between the two furthest observations.

Figure 6.3 A diagrammatic representation of a semivariogram

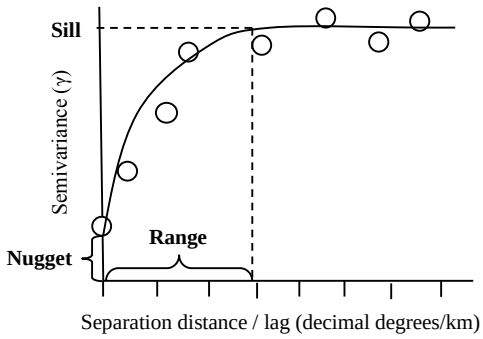


Figure 6.3 shows a diagram of a semivariogram and its key features. The nugget is the semivariance at a distance of zero, representing the spatially uncorrelated (stochastic) variation, with larger nugget values indicating a lower degree of spatial dependence. The sill is the asymptotic value of the semivariogram and represents the point of maximum semivariance. Finally the range represents the distance at which the spatial autocorrelation between observations falls below 5% and pairs of observations separated by a distance greater than the range are equally dissimilar to each other [427].

Log transformed observed prevalence was used, due to a skewed distribution of school-level infection, and a weighted least squares model variogram was fitted. The spatial structure was examined first by using the mean prevalence across all survey timepoints, for all 101 schools,

and then using the survey-specific prevalence to explore the temporal stationarity in the spatial dependence observed.

6.3.5.2 Frequentist analysis

Initial examination of univariable associations of school-level *P. falciparum* prevalence with environmental and socioeconomic covariates was conducted through a frequentist approach, using binomial grouped logistic regression models in Stata Version 12.0 (Stata Corporation, College Station TX) [446]. Candidate variables were selected for multivariable analysis using the criteria of Wald test $p<0.2$. Robust standard errors were used to adjust for dependence between children within schools. As a result of the treatment provided at the surveys in the intervention schools, it was necessary to account for varying treatment intervals (one month at Survey 4; three months at Survey 3; and greater than four months at Surveys 2, 5 and 6 [Figure 6.1]) in the 51 intervention schools. Initial associations were assessed whilst controlling for treatment interval so as to account for the important effect of the intervention. Non-linearity was assessed through scatter plots (Appendix 6.2) and addressed through categorisation based on clear natural breaks in the variable associations. Assessment of collinearity was made between pairs of eligible covariates, and if exhibiting a correlation coefficient greater than 0.8, the variable lying closer to infection on the assumed causal pathway (Figure 6.2), and with a lower Akaike Information Criterion (AIC), was retained [447]. Backwards-stepwise elimination (criteria: Wald test $p<0.1$) was employed for the multivariable binomial logistic regression to generate a minimum adequate model.

6.3.5.3 Bayesian analysis overview

Covariates retained in the frequentist multivariable model were incorporated into a series of Bayesian binomial logistic regression models, sequentially including non-spatial, spatial and spatio-temporal random effects using Winbugs 1.4 (Medical Research Council, Cambridge, UK and Imperial College London, UK) [389]. Null models were fitted to view the extent of spatial

and aspatial variation in the data; subsequent models included covariates (from the minimum adequate frequentist multivariable model) for comparison of fit on the basis of deviance information criterion (DIC). Smaller DICs demonstrating a difference of between five and 10 or more units, are indicative of better fitting models while also considering model parameterisation and parsimony. First, an unstructured, non-spatial random effect was included to account for within-school correlation and the repeated measures nature of the data. Second, an additional structured spatial random effect was estimated using an isotropic exponential spatial decay function and a mean of zero, based on Euclidean distance between school locations [429]. Finally, an explicit spatio-temporal (space-time interaction) model was fitted with separate spatial random effects placed on each survey, making the assumption that spatial correlation was present at all surveys, but the extent of correlation could vary between surveys. Figure 6.4 provides a schematic representation of the analysis strategy conducted within a Bayesian framework.

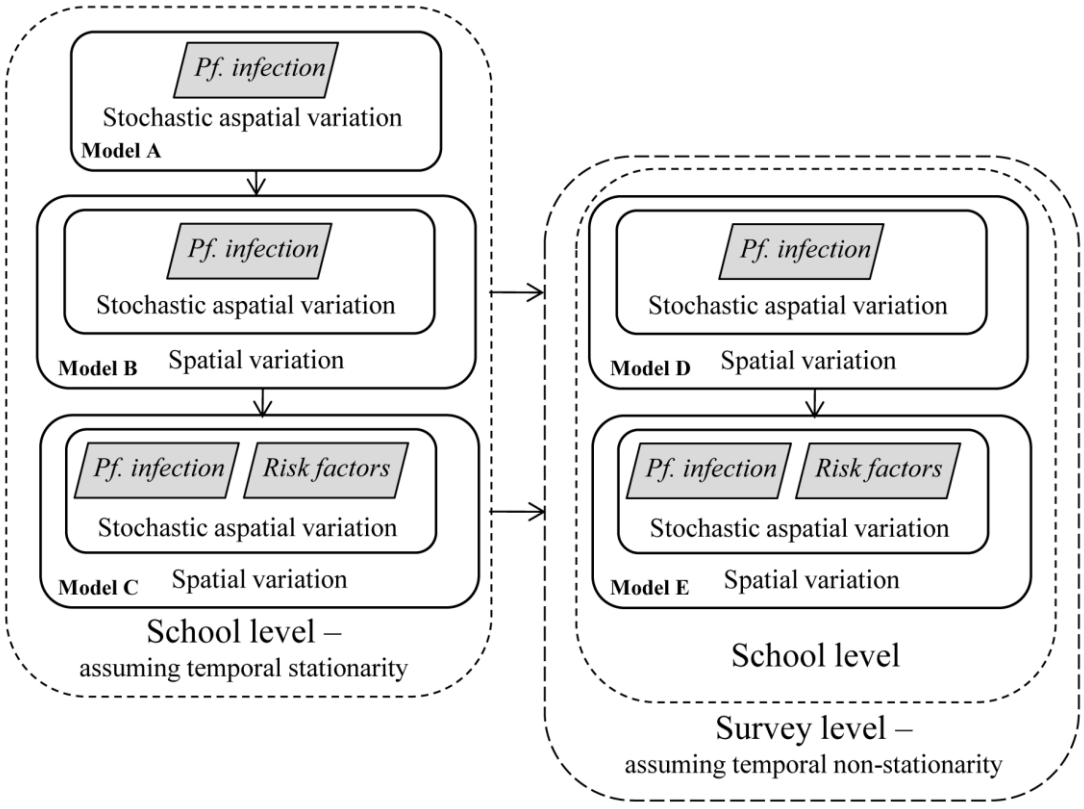


Figure 6.4: Schematic of analysis strategy adopted for the Bayesian risk analyses. The various models presented and discussed below are labelled (Models A-E)

6.3.5.4 Bayesian model specification

In all models, Y_{ij} denotes the number of children positive, of n_{ij} children tested in each school i (i=1,...,51) at each survey j (j=1,...,6). The model assumes a Binomial distribution with the probability of infection represented by $p_{ij.}$

$$Y_{ij} \sim Binomial(p_{ij}, n_{ij})$$

A logit transformed probability $logit(p_{ij})$ was used as a base on which to expand the models including covariate matrices, unstructured non-spatial random effects and structured spatial random effects.

$$logit(p_{ij}) = \alpha + \sum_{k=1}^{K} \beta_k x_{ijk} + \sum_{l=1}^{L} \beta_l z_{il} + u_i + v_i$$

The intercept is denoted by α to which a non-informative uniform flat prior was assigned. $\Sigma\beta_k$ is a vector of k regression coefficient parameters related to a matrix of time varying covariates (x_{ijk}) for which the values change across the surveys (j). $\Sigma\beta_l$ is a vector of l regression coefficient parameters related to a matrix of time constant factors (z_{il}), which are stable across all surveys. The β coefficients were assigned an uninformative normal prior with a mean of 0 and a precision of 1×10^{-6}. The unstructured school-level heterogeneity (random effect) is denoted by u_i which takes account of non-spatial within-school correlation and the variation from the repeated measures for each school. This was given a normal distribution with a mean of 0 and a gamma distribution on the precision. The models incorporating spatial structure included a structured random effect (v_i) accounting for correlation in space, with a mean of 0 and gamma distribution assigned to the precision. It is defined by an isotropic exponential spatial correlation function:

$$f(d_{ab}; \phi) = exp[-(\phi d_{ab})^k]$$

Where d is the distance between pairs of school locations (ab). ϕ denotes the rate of spatial decay (decline in spatial correlation). A uniform prior was given to this spatial decay with parameters (5 and 200) creating a lower bound of 0.005 decimal degrees (the minimum distance

between observed data points) and an upper bound of 0.2 decimal degrees (the maximum distance between observed points). This equates to a range of 1.7-66.8km. The degree of spatial smoothing is shown by *k* and was given a value of 1.

In accounting for the temporal aspect of the data, inclusion of a first order autoregressive function placed on the survey time point was considered, to explicitly model the temporal correlation. However, this overrode important survey level covariates such as treatment interval (in intervention schools) and seasonality, without improving model fit and as such was not used for further modelling. Instead, time was accounted for through the incorporation of these survey-specific fixed effects. Finally the model was expanded to account for temporally non-stationary spatial clustering by including a separate isotropic exponential spatial correlation function (spatial random effect) for each survey *j*.

For all models, Bayesian inference was implemented using a Markov Chain Monte Carlo (MCMC) algorithm. Two chains were run consecutively, and a burn-in of 10,000 iterations was performed. Following this, the convergence was examined using the MCMC dynamic traces of the model parameters using the dynamic trace plot history, kernel density plots and autocorrelation plots. A further 10,000 iterations were run with every tenth observation of the posterior distribution of the parameters stored, from which the posterior estimates were calculated. Range of spatial autocorrelation was calculated using the formula $(1 / \phi)*3$ and was multiplied by 111.3, to convert from decimal degrees to meaningful km. Residuals were examined before and after addition of random effects and covariates in the models.

6.3.5.5 Model validation

Although not designed to produce a risk map, model validation was performed using training and hold-out (validation) datasets to assess the predictive performance and classification accuracy of the best fitting model on the basis of risk thresholds. The data, consisting of 456

school-level observations across the 101 schools and six surveys, were divided so as to create ten alternative training datasets, with ten corresponding validation datasets each consisting of ten randomly selected schools. The models were fit to each of the training datasets in turn predicting the prevalence of the schools in the corresponding validation datasets. An assumption of 100 children tested at each location was made, to give the posterior distribution mean *P. falciparum* infection as percentage of infection at each survey at each school. Incorporation of spatial variation was performed through inclusion of spatial random effects, computed through kriging [448] (interpolation based on the distance between prediction locations and observed data locations). These were added to the sum of the coefficients for the covariates included in the Bayesian model and the values of the parameters at each of the prediction locations.

Point estimates of infection prevalence from the sampled mean posterior distribution at each of the six surveys were compared with the observed infection prevalence at each school and survey point. The correlation between observed and predicted school-level prevalence at each visit was calculated, as well as the mean error (ME; a measure of bias of the predictions), and the mean absolute error (MAE; providing a measure of accuracy of the predictions versus observed data). Additionally, the classification accuracy of the models was assessed through examination of the area under the curve (AUC) of the receiver operating characteristic (ROC). This is a measure of the ability of model predictions to correctly discriminate true prevalence thresholds, and in this case the predictive points were assessed in response to prevalence categories using the cut-offs 5%, 10% and 20%.

6.4 RESULTS

6.4.1 Survey data description

A total of 456 surveys conducted over two years are presented: six surveys in all 51 IST intervention schools and three surveys in the 50 control schools. Overall a mean of 45.3 children

were surveyed per school (range 26-60), and the number of children screened at each survey round is presented in Figure 6.1. The vast majority of *Plasmodium* infections found were asymptomatic, with clinical cases (blood slide positive plus temperature ≥37.5°C) found on less than 2% of the 20,231 individual assessments conducted across the six surveys.

Figure 6.5 presents the mean and median infection prevalence by survey in the intervention and control schools. In the intervention schools, the highest mean *Plasmodium* infection prevalence was exhibited at the baseline survey (Survey 1), 12.94% (95% confidence interval [CI]: 8.86 – 17.03%). Also in the intervention schools, despite lower mean prevalence found at all subsequent surveys, only that in Survey 4 (where treatment was given only one month previously) was significantly lower than the baseline prevalence with a mean prevalence four-fold lower than at baseline. While a declining mean prevalence of infection was observed across the three surveys in which the control cohort was assessed, only at Survey 4 was a significant difference exhibited between the intervention and control schools 3.46 (95%CI: 2.25-4.68) and 15.91 (95%CI: 11.36-20.45) respectively. The right skewed nature of the school-level prevalence of *P. falciparum* infection resulted in a lower median than mean prevalence at all surveys, with wide interquartile ranges displayed.

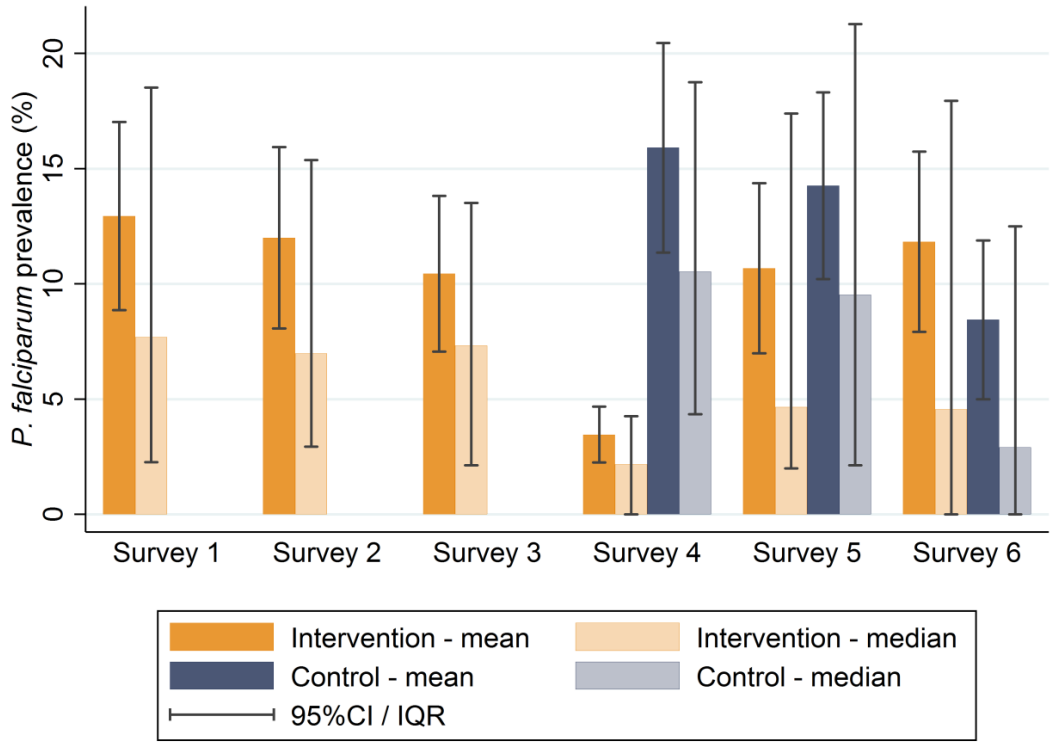


Figure 6.5: *P. falciparum* infection prevalence for the intervention and control groups at the multiple surveys. Means with 95% CIs are presented, as well as medians with IQRs. *P. falciparum* infection was only measured in control schools at Surveys 4 to 6 and thus is only represented at these three survey timepoints.

6.4.2 Heterogeneity in space and time

Marked heterogeneity in school-level *Plasmodium* infection was observed, with *Plasmodium* prevalence ranging from 0-75%. As shown in Figure 6.6, consistently high infection prevalence appears relatively restricted to a few (approximately 10%) of the schools throughout the study period, with this minority of schools displaying prevalences greater than 20% at the repeated surveys. However, two control schools appear to show anomalously low *Plasmodium* prevalence on at least one of the surveys.

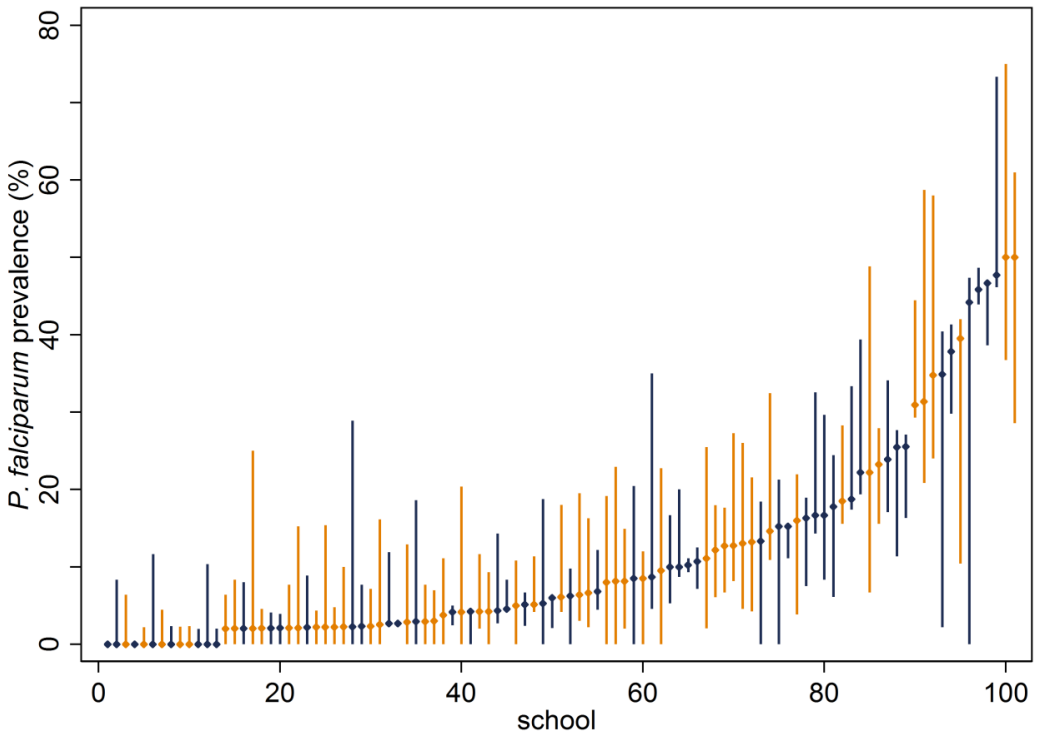


Figure 6.6: School-level prevalence of *Plasmodium falciparum* infection in ascending order. The lines depict the minimum and maximum observed prevalence of five surveys in the intervention schools (excluding Survey 4 due to the treatment given only one month prior to this survey) and of the three surveys in control schools. The circles depict the median prevalence observed across the surveys. Intervention schools are shown in orange and control schools in navy

The spatial distribution of school-level infection across the study site at all surveys is presented in Figure 6.7A-F. Substantial geographical variation in prevalence was observed, with this variation appearing spatially structured. A subset of schools in the northeast of the site exhibited high infection prevalence, and low *Plasmodium* prevalences were observed in the central and south western regions of the site. This spatial distribution appears, from mapping the raw data, to be remarkably stable over time. Similar patterns are observed when considering the 51 intervention schools only and all 101 schools together.

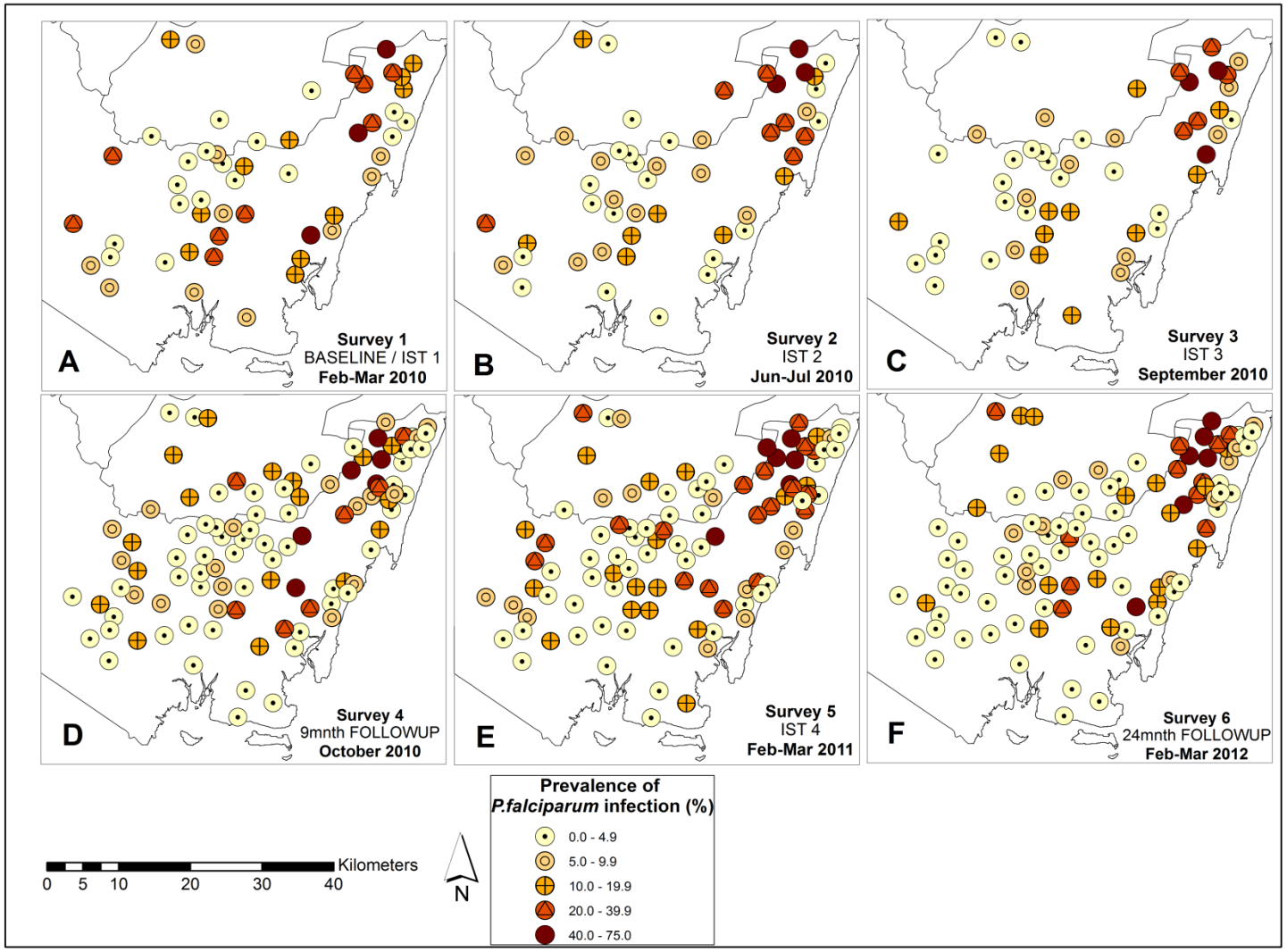


Figure 6.7: The geographical distribution of *Plasmodium falciparum* infection (as determined by microscopy) in the 51 intervention schools at: **(A)** Survey 1 **(B)** Survey 2 **(C)** Survey 3 and in the 51 intervention schools plus the 50 control schools at **(D)** Survey 4 **(E)** Survey 5 **(F)** Survey 6. The fifth IST round was conducted in October 2011 but is not included in the analysis as blood slides were not collected for logistical reasons.

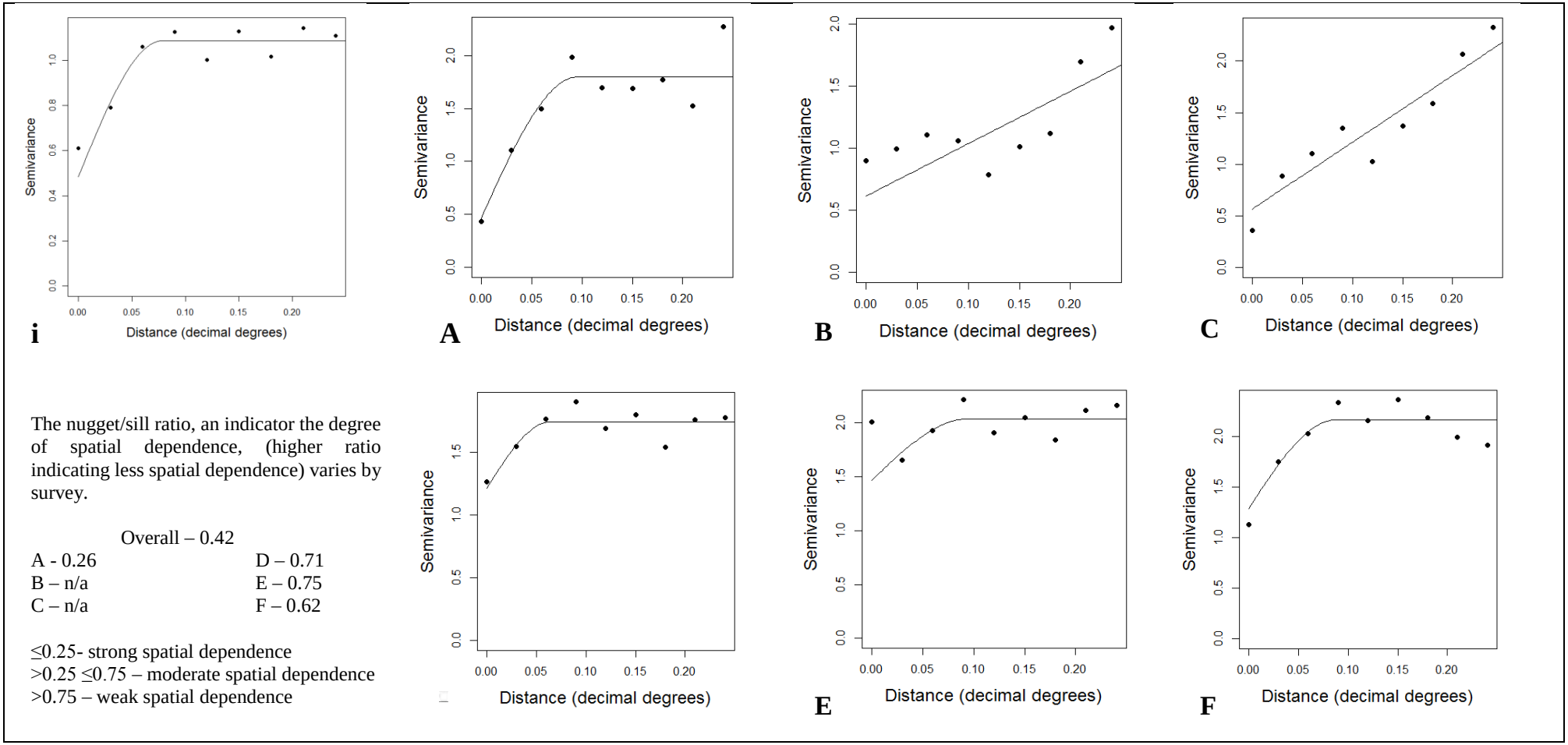


Figure 6.8: Semivariograms of log transformed raw *P. falciparum* infection prevalence data **(i)** overall mean prevalence across all surveys in the 101 schools. Also by survey: **(A)** Survey1: February/March 2010, **(B)** Survey 2: June/July 2010, **(C)** Survey 3: September 2010, **(D)** Survey 4: October 2010, **(E)** Survey 5: February/March 2011, **(F)** Survey 6: February/March 2012. Models for Surveys 1-3 **(A-C)** use 51 intervention schools, models for Surveys 4-6 **(D-F)** use 101 schools.

As displayed in Figure 6.8i, the combined school-level semivariogram indicated moderate spatial dependence across the site. Survey-specific semivariograms of the raw school-level *P. falciparum* prevalence data exhibited a degree of spatial structure at all surveys, although the extent and trends in spatial dependence observed varied by survey. Semivariograms for Surveys 2 and 3 (Figure 6.8B and C), which were conducted in the rainy season, suggest apparent large-scale spatial trends across the region, as indicated by the lack of an asymptote. The greatest degree of spatial autocorrelation was observed at Survey 1 (Figure 6.8A), with substantially less spatial structure at Surveys 4 to 6, indicated by a large nugget (Figure 6.8D-F). In particular, only minimal spatial autocorrelation was displayed at Survey 5 (Figure 6.8E), exhibited by the highest nugget/sill ratio.

6.4.3 Initial associations of covariates with *P. falciparum* prevalence

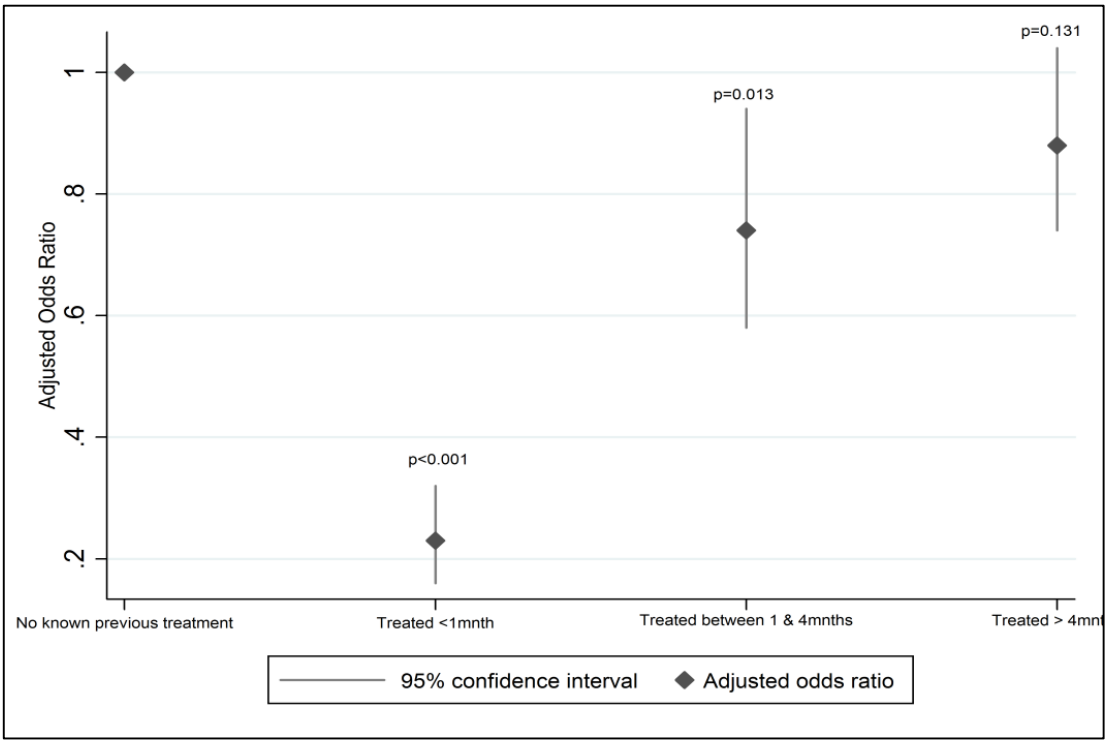
Associations between *P. falciparum* infection and both environmental and socioeconomic covariates from the frequentist analyses are presented in Table 6.1. Univariable analyses indicated associations between school-level odds of infection and: treatment interval; season of survey; NDVI; landuse; rural/periurban distinction; distance from waterbodies; annual precipitation; PET, aridity and distance from road. In multivariable analyses, a number of these associations were not retained in the minimum adequate model, including: aridity; distance from road; distance from permanent waterbodies; landuse and NDVI (Table 6.1). Of the sociodemographic variables, higher school-level SES (on the basis of proportion of students in the richest wealth quintile) and greater percentage net use were significantly associated with decreased prevalence of *P. falciparum* infection at univariable analysis stage and were retained in the final multivariable model. A strong treatment interval response relationship was exhibited, with shorter intervals between treatments associated with significantly reduced infection prevalence, with greater than three times the odds of infection as the treatment interval increased from one month to up to four months, and four-fold higher odds when the treatment interval exceeded four months. When treatment intervals exceeded four months the odds of infection did not differ significantly from the base category of no previous treatment (Figure6.9).

Table 6.1: Univariable and multivariable analysis of school-level environmental and sociodemographic and seasonal covariates with *P. falciparum* prevalence.

	Univariable Frequentist analyses		**Multivariable Frequentist analyses**	
Variable [a]	Odds ratio [b] (95% CI)	P-value[c]	Odds ratio [b] (95% CI)	P-value[c]
Time Varying Covariates				
Time since treatment				
No known previous treatment [d]	1		1	
Treated ≤ 1 month ago [e]	0.25 (0.17-0.36)		0.23 (0.16-0.32)	
Treated > 1 month & <4 months [f]	0.79 (0.56-1.11)	<0.001	0.74 (0.58-0.94)	<0.001
Treated ≥ 4 months ago [g]	0.88 (0.65-1.20)		0.88 (0.74-1.04)	
Season				
Dry	1		1	
Rainy	1.15 (0.97-1.36)	0.110	1.16 (1.00-1.34)	0.052
Normalised Difference Vegetation Index [h]	1.11 (1.00-1.24)	0.054		
Precipitation (mm) [i]	1.00 (1.00-1.00)	0.220		
Land Surface Temperature (°C) [i]	0.99 (0.97-1.01)	0.266		
Time Constant Covariates				
Landuse				
Forest	1			
Croplands	0.65 (0.42-0.99)	0.046		
Rural/periurban				
Rural	1		1	
Periurban	0.36 (0.24-0.55)	<0.001	0.52 (0.33-0.83)	0.006
Population density (ppl per km^2)	1.00 (1.00-1.00)	0.967		
Distance of school from road (km)				
<0.8	1			
≥0.8	0.71 (0.48-1.06)	0.092		
Distance school - permanent waterbody (km)				
<10	1			
≥10	2.20 (1.54-3.15)	<0.001		
Distance school - temporary waterbody (km)	0.94 (0.88-0.99)	0.026	0.93 (0.90-0.96)	<0.001
Mean elevation (m above sea level)				
<100	1			
≥100	0.85 (0.55-1.31)	0.463		
Mean annual precipitation (mm)				
<1200	1		1	
≥1200	2.69 (1.96-3.69)	<0.001	2.03 (1.40-2.94)	<0.001
Mean annual temperature (ºC)				
< 25.5	1		1	
≥25.5 & < 26.1	1.35 (0.86-2.11)	0.079	2.32 (1.50-3.58)	<0.001
≥ 26.1	1.59 (1.06-2.39)		2.50 (1.55-4.04)	
Potential evapotranspiration (PET) (mm)	0.98 (0.97-0.99)	<0.001	0.98 (0.97-0.98)	<0.001
Aridity	2.69 (1.95-3.72)	<0.001		
Sociodemographic Covariates				
Mean age at survey (years)	0.98 (0.84-0.15)	0.838		
Proportion females at survey	1.21 (0.26-5.71)	0.805		
Proportion in highest wealth quintile	0.41 (0.14-1.20)	0.104	0.40 (0.32-0.51)	<0.001
School-level net coverage (%)	0.99 (0.97-1.00)	0.025	0.99 (0.98-1.00)	0.026

[a] All variables were assessed in the univariable model while controlling for treatment interval, thus the odds ratio presented is adjusted for treatment interval.
[b] Obtained from Binomial regression analysis accounting for school-level clustering
[c] Obtained through multivariate Wald test,
[d] Survey one in intervention schools an Surveys 4 to 6 in control schools
[e] Survey 4 in intervention schools
[f] Survey 3 in intervention schools
[g] Surveys 2, 5 and 6 in intervention schools.
[h] NDVI averaged over a month, with a lag time of a month for each survey and then standardised.
[i] Precipitation and LST averaged over the current and previous six weeks of each survey

Figure 6.9: The adjusted association between treatment interval (time since last treatment) and the odds of *P. falciparum* infection. Odds ratios shown are adjusted for seasonality, rural/periurban, distance from temporary waterbody, mean annual precipitation, mean annual temperature, PET, SES and mean net use.



6.4.4 Bayesian risk analysis

Table 6.2 displays the results of the Bayesian non-spatial, spatial and spatio-temporal models constructed. Despite the similarity between the non-spatial and spatial null models (Models A and B) in relation to the model fit and DIC, the results indicate that the unstructured between-school variation detected in Model A was largely spatial rather than aspatial, as the non-spatial variance parameter decreased from 1.37 to 0.09 between Models A and B and the spatial variance parameter was 1.63 in Model B. The distance at which spatial correlation falls below 5% was 19.19km (Bayesian Credible Interval [BCI]: 9.99 - 53.58), one third of the maximum distance of the site, indicating a significant degree of spatial dependence exists between schools.

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Table 6.2: Estimates from Bayesian hierarchical logistic regression models of asymptomatic *P.falciparum* infection. (A) non-spatial null model (B) spatial null model (C) Spatial model with covariates (D) spatial null model with separate spatial random effect for each survey, (E) spatial covariate model with separate spatial random effect for each survey

Odds ratio (95% Bayesian CI)	**Model A**	**Model B**	**Model C**	**Model D**	**Model E**
Treatment No known previous			1		1
≤1 month	-		0.17 (0.12, 0.22)		0.17 (0.10, 0.27)
>1 month & ≤3 months	-		0.61 (0.49, 0.75)		0.55 (0.27, 0.94)
>3 month	-		0.79 (0.68, 0.91)		0.68 (0.45, 1.01)
Season Dry	-		1		1
Rainy			1.31 (1.17, 1.47)		1.47 (1.11, 1.93)
Urbanisation Rural			1		1
Peri urban†			0.32 (0.14, 0.65)		0.37 (0.14, 0.81)
Distance to temp waterbodies (km)	-		0.94 (0.86, 1.02)		0.90 (0.86, 0.95)
Annual precipitation (mm) < 1200	-		1		1
≥ 1200			1.88 (0.73, 3.60)		4.36 (2.79, 6.46)
Relative SES	-		0.13 (0.02, 0.52)		0.09 (0.01, 0.37)
Net coverage (%)	-		0.98 (0.97, 0.99)		0.98 (0.97, 0.99)
α Intercept	-2.52 (-2.76, -2.28)	-2.63 (-3.49, -1.81)	-0.81 (-1.92, 0.36)	-2.65 (-3.00, -2.30)	-0.67 (-1.61, 0.25)
σ^2 Non-spatial variation	1.37 (1.00, 1.92)	0.09 (0.01, 0.33)	0.11 (0.12, 0.38)	1.31 (0.90, 1.85)	0.56 (0.35, 0.84)
σ^2 Spatial variation		1.63 (0.78, 3.53)	0.97 (0.31, 2.26)		
φ Rate of decay in spatial correlation		17.40 (6.23, 33.41)	14.41 (5.34, 36.66)		
† range (km)		19.19 (9.99, 53.58)	23.17 (9.11, 62.53)		
σ^2 spatial variation survey 1				0.78 (0.34, 1.56)	0.65 (0.26, 1.36)
σ^2 spatial variation survey 2				0.34 (0.10, 0.80)	0.31 (0.08, 0.75)
σ^2 spatial variation survey 3				0.28 (0.05, 0.83)	0.36 (0.07, 1.06)
σ^2 spatial variation survey 4				0.63 (0.25, 1.34)	0.20 (0.05, 0.46)
σ^2 spatial variation survey 5				0.66 (0.33, 1.34)	0.73 (0.39, 1.23)
σ^2 spatial variation survey 6				0.94 (0.32, 2.19)	0.87 (0.32, 2.01)
φ Spatial decay [Range (km)] survey 1				97.11 (19.32, 194.00) [3.44 (1.72, 17.28)]	105.5 (22.20, 194.90) [3.16 (1.71, 15.04)]
φ Spatial decay [Range (km)] survey 2				96.11 (11.84, 194.3) [3.47 (1.72, 28.20)]	90.65 (9.42, 193.20) [3.68 (1.73, 35.45)]
φ Spatial decay [Range (km)] survey 3				74.62 (7.05, 192.2) [4.47 (1.74, 47.36)]	48.39 (5.72, 183.40) [6.90 (1.82, 58.37)]
φ Spatial decay [Range (km)] survey 4				6.48 (5.04, 10.06) [51.53 (33.19, 66.12)]	118.0 (33.00, 195.80) [2.83 (1.71, 10.12)]
φ Spatial decay [Range (km)] survey 5				149.30 (71.74, 197.9) [2.24 (1.69, 4.47)]	150.9 (75.22, 198.11) [2.21 (1.69, 4.44)]
φ Spatial decay [Range (km)] survey 6				14.35 (5.71, 31.88) [23.27 (10.47, 58.48)]	13.61 (5.61, 29.93) [24.53 (11.16, 59.52)]
DIC	**2390**	**2387**	**2173**	**1863**	**1857**

The addition of a survey-level variable to account for treatment interval, whilst simultaneously modelling the temporal aspect of the data as a fixed effect, improved the model fit and parsimony (DIC: 2192 vs. 2387) especially through removal of the variation caused by the anomalous pattern of the infection data in Survey 4 in the intervention schools, but the school-level aspatial or spatial variation was not reduced (model not shown).

Incorporation of the environmental and socioeconomic covariates into a spatial model within a Bayesian framework on top of the treatment variables (Model C), resulted in several changes in the environmental covariate relationships with infection. The odds of infection remained significantly lower in periurban regions (Adj.OR [adjusted odds ratio]: 0.32, 95% BCI 0.14 – 0.65). However, interestingly where the two environmental variables commonly related to vector breeding sites had demonstrated associations with *P. falciparum* infection in non-spatial models, with odds of infection decreasing as distance from temporary waterbodies increased and locations with mean annual precipitation greater than 1200mm exhibiting two and a half greater odds of infection than locations with less than 1200mm precipitation annually, both these covariates were no longer significantly associated with *P. falciparum* prevalence (Model C). As in the non-spatial model, the odds of *P. falciparum* infection were still reduced in schools with higher net coverage and where there was a higher level of wealth. Annual temperature (fitted as a categorical variable) and PET (fitted as continuous) were excluded from the final minimum adequate model as they did not reach the point of convergence.

Although none of the time-variable environmental covariates were found to be significantly associated with infection in the multivariable models, seasonality (categorised into a binary variable based on whether the survey was conducted in a wet or dry season) was significantly associated with *Plasmodium* prevalence, with higher odds of infection associated with surveys conducted in the rainy season (Adj.OR 1.31, 95% BCI 1.17 – 1.47). The other temporal covariate included, and already mentioned, was time since last treatment where a negative dose-response relationship was observed between infection and treatment interval, with odds of infection

decreasing as the time since last treatment decreased. Despite Model C, including both spatial random effects and covariates, demonstrating an improvement in the DIC, implying an improved model fit, the addition of these covariates did not appear to significantly alter the spatial or aspatial variation or the range over which the spatial dependence between schools declines.

Apparent non-stationarity between surveys, observed in the semivariograms (Figure 6.8), was addressed through fitting a separate school-level spatial random effect to each survey timepoint, i.e. a space-time interaction (Models D and E, Table 6.2). These models greatly improved the model fit, as evidenced by the DIC, suggesting the spatial structure varied by survey. At surveys 1, 2, 3 and 5 there was evidence of small-scale second order spatial effects, with correlation between a few nearby schools detected (as the minimum distance between schools is 1.3km), while Surveys 4 and 6 exhibited spatial structure over a greater range, as shown in Model D. At Survey 4 the range at which the correlation falls below 5% is 51km, three quarters of the maximum distance between schools and at Survey 6 this spatial decay was approximately one third of the maximum distance. The addition of the covariates to this model (Model E) provided an increased fit, with all environmental and socioeconomic covariates significantly associated with *P. falciparum* infection prevalence. These covariates explained a substantial degree of the spatial structure observed at Survey 4, reducing the range of spatial decay down to 2.83km, in line with the range of spatial correlation exhibited in the other surveys. However, interestingly there was no effect on the spatial structure of Survey 6. The inclusion of the covariates also explained a significant degree of residual non-spatially structured variation between schools, with the overall non-spatial variance reduced from 1.31 (0.90, 1.85) to 0.56 (0.35, 0.84) between Models D and E. This is also demonstrated in Figure 6.10, in which these residuals are drawn towards the posterior mean, following the inclusion of the covariates in the model.

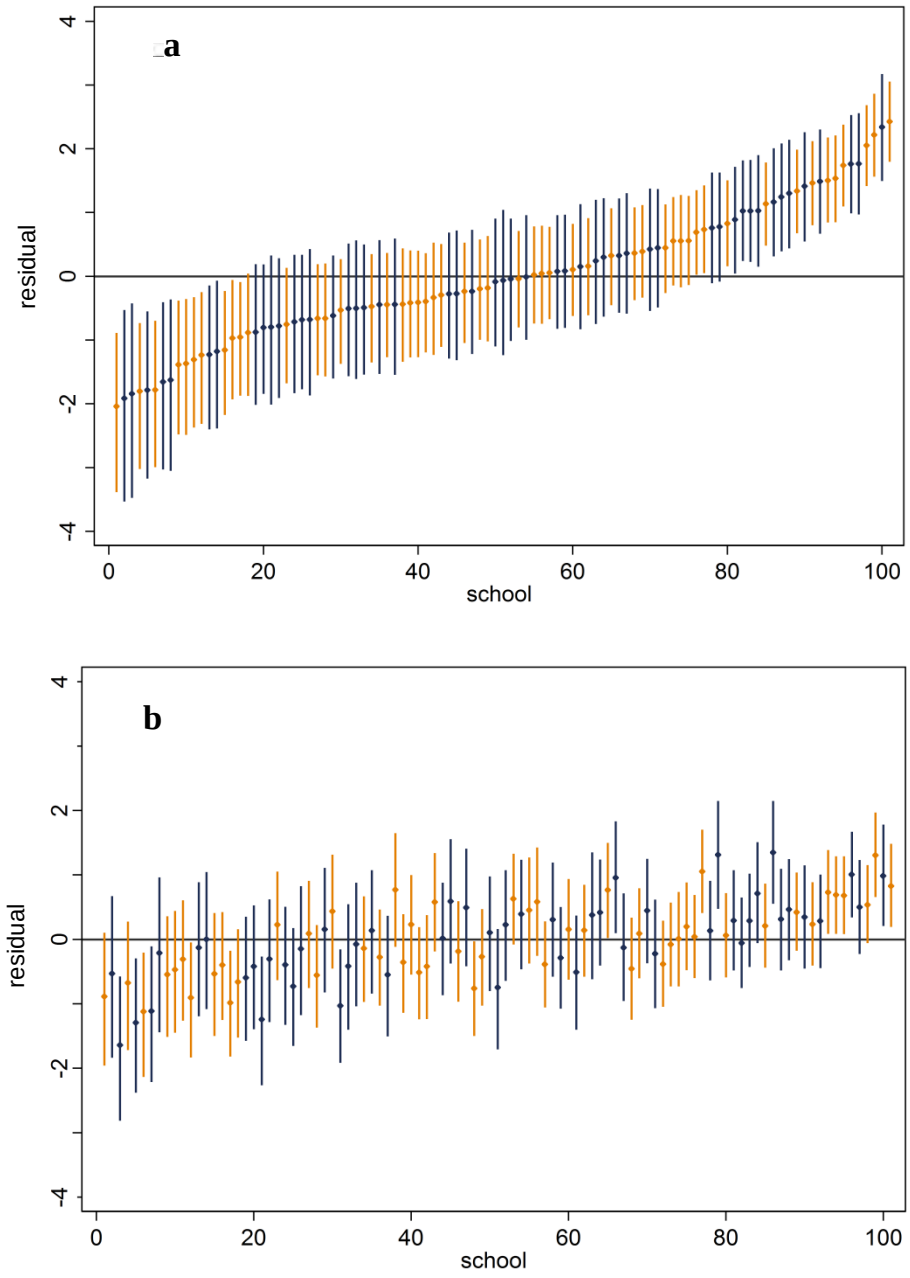

Figure 6.10: School-level residuals (a) from the null model with separate spatial random effects for each survey (Model D) plotted against the posterior mean (for comparison purposes, the residuals are ordered in ascending order) (b) the same school-level residuals following the inclusion of the covariates in the first spatio-temporal model (Model E) again plotted against the posterior mean, and it can be seen that the residuals have been pulled in towards the zero-centred mean and the Bayesian credible intervals cross the mean in the majority of cases, demonstrating that a large proportion of the residual variation is random noise that does not differ from expected, having accounted for the covariates. Intervention schools are shown in orange and control schools in navy.

In summary, the inclusion of school-level spatial random effects indicates a large proportion of the variation is spatially structured (Model B), however, the inclusion of covariates in this spatial model could not explain this spatial dependence (Model C). The space-time interaction model, allowing for temporal non-stationarity across survey timepoints is the best fit for the observed data (Model D), with the covariates explaining a large proportion of the stochastic variation and the spatial dependence in Survey 4 (Model E). The differing range of spatial dependence observed in Survey 6, and the lack of impact of the covariates on this dependence suggests an anomaly.

Figure 6.11 displays the survey specific residuals from Model E, indicating that at Surveys 1 to 4, the model, through the inclusion of covariates and both the structured and unstructured random effects, has sufficiently explained the spatial dependence observed in the raw data. However, at Surveys 5 and 6 a degree of variation remains unaccounted for, as seen by the schools exhibiting both higher and lower than expected variation from the posterior mean in several intervention and control schools (Figure 6.11D and E). Despite a lot of the variation having been explained by the space-time interaction and covariates, the non-spatial residuals displayed in Figure 6.11i indicate persistently high infection risk in a selection of schools in the northeast of the site where the high transmission was initially detected.

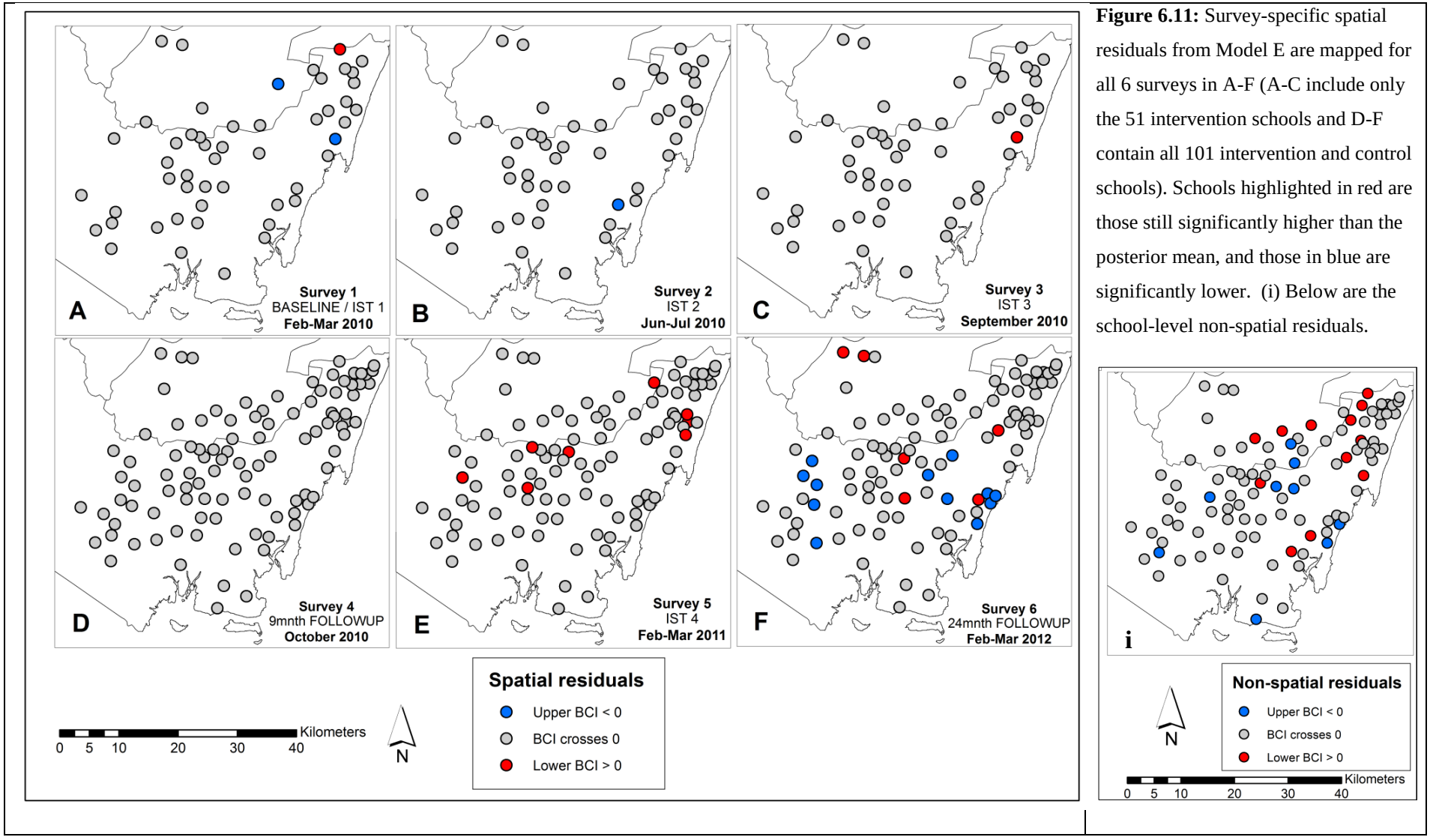

Figure 6.11: Survey-specific spatial residuals from Model E are mapped for all 6 surveys in A-F (A-C include only the 51 intervention schools and D-F contain all 101 intervention and control schools). Schools highlighted in red are those still significantly higher than the posterior mean, and those in blue are significantly lower. (i) Below are the school-level non-spatial residuals.

6.4.5 Validation and prediction

Validation performed using Model E, including covariates and accounting for temporal non-stationarity in spatial correlation across surveys, showed a good predictive performance, with a correlation of 0.61 exhibited between observed and predicted prevalence (Table 6.3). The models were found to underpredict school-level prevalence on average, but not significantly so. However a mean absolute error of 7.50 suggests an overall more substantial difference in observed and predicted prevalence, arising from the schools at the high end of the distribution, where observed prevalences were as high as 75%. The classification accuracy, shown by the area under the receiver operating characteristic, was high for all thresholds, improving as the prevalence threshold increased. The AUC at the prevalence threshold of 20% was excellent at 0.86 (95%CI: 0.80 - 0.90). Examination of the validation statistics by study group indicated a better predictive performance for the intervention schools at all thresholds and a lower classification accuracy at Survey 5 when compared to Surveys 4 and 6.

Table 6.3: Validation statistics showing the threshold discriminatory ability and correlation of predictions made from the Bayesian logistic regression model including covariates and assuming separate school-level spatial random effects for each survey.

Validation Statistics	All 101 schools	Intervention schools	Control schools
Area under the ROC curve (95% CI)*			
Exceeds 5% threshold	**0.78 (0.74-0.82)**	**0.80 (0.76-0.85)**	**0.73 (0.65-0.80)**
Survey 4	0.85 (0.79-0.92)		
Survey 5	0.72 (0.60-0.81)		
Survey 6	0.76 (0.65-0.85)		
Exceeds 10% threshold	**0.82 (0.78-0.86)**	**0.87 (0.82-0.91)**	**0.73 (0.65-0.81)**
Survey 4	0.85 (0.78-0.92)		
Survey 5	0.72 (0.60-0.81)		
Survey 6	0.86 (0.77-0.92)		
Exceeds 20% threshold	**0.86 (0.80-0.90)**	**0.90 (0.84-0.94)**	**0.79 (0.69-0.87)**
Survey 4	0.86 (0.73-0.94)		
Survey 5	0.77 (0.63-0.87)		
Survey 6	0.86 (0.78-0.93)		
Correlation coefficient	0.61	0.66	0.54
Mean error	-0.39	-0.24	-0.71
Mean absolute error	7.50	6.65	9.25

*Validation statistics in bold shown for surveys 1-6 in intervention schools and 4-6 in control schools combined. However, stratified AUCs only shown for surveys 4-6 individually so as to include predictions for all 101 schools

6.5 DISCUSSION

Developing an understanding of the microepidemiological dynamics of malaria transmission in a region over time can form a basis for efficient resource allocation, through guiding the implementation of targeted control programmes and monitoring the subsequent initiatives [103]. This repeated measures dataset has afforded the opportunity of investigating the utility of school-based *P. falciparum* screening for: 1) examining patterns of *Plasmodium* transmission at the local level; 2) identifying drivers of heterogeneity at the school level; 3) exploring possible reasons for the lack of impact of IST on health and educational outcomes observed in the previous chapter; 4) predicting regions of high transmission and consequently planning appropriate interventions and, 5) monitoring transmission over time in the presence of such control initiatives.

The mapped raw data provide clear evidence of marked spatial heterogeneity in asymptomatic *P.falciparum* infection over a relatively small geographical area in this region of low-to-moderate transmission. While school-level prevalences ranged from 0 to 75%, there was localised clustering of high transmission in the north east of the site. Such fine-scale variation of *Plasmodium* infection has been previously demonstrated in household-level surveys within districts [249,317,449].

A major strength of this study is the temporal, as well as spatial dimension to the data, resulting from examination of a cohort over two years. The finding of a lack of significant change in overall mean *Plasmodium* prevalence between surveys across both the control schools (undergoing no treatment) and the intervention schools in spite of periodic treatment, is suggestive of the intensity of localised transmission from the wider reservoir of infected individuals in the community. This, alongside the observation that schools initially exhibiting high infection prevalence continued to do so at subsequent surveys, and schools with low prevalence, on the whole, remained low at all surveys, is indicative of stability of transmission over time. In this respect these findings are consistent with previous studies also documenting temporally stable clusters of asymptomatic infection over time in settings of low to moderate

transmission [99,422]. However, whereas in these studies, conducted over more than a decade, parasitaemia surveys were conducted annually, the current study has further expanded on this to explore the stability of infection within years, enabling the influence of seasonality to be assessed [249]. Bayesian geostatistical analysis was used to quantify the role of seasonal, environmental and socioeconomic covariates in determining *P. falciparum* infection within a spatio-temporal context.

The findings of the Bayesian spatio-temporal model suggest environmental and socioeconomic factors are strong drivers of local variation in *P. falciparum* transmission, with significant associations maintained after accounting for both spatial and aspatial variation. These factors found to be related to *Plasmodium* infection, are consistent with much previous evidence and knowledge of vector distribution [250]. The dichotomy in transmission risk above and below the mean annual precipitation in Kwale of 1200mm [287], is consistent with the understanding that increased precipitation leads to an increased likelihood of water pooling, creating mosquito breeding sites [432,450] although it must be noted that cases have been observed whereby intense rains in regions have been associated in reduced transmission that season [451]. The higher odds of infection in schools closer to temporary waterbodies may be attributed to the breeding preferences of the key vectors in the region *(Anopheles gambiae s.l.* and *An. funestus s.l)* [291,452] for fresh, clear water sources with low vegetation [243,450,453-455]. Reduced risk of *Plasmodium* transmission in urban areas is commonly attributed to the reduction in clean freshwater breeding sites and increase in pollution, limiting vector reproduction and dispersion as well as the often increased socioeconomic status and improved household construction in these areas [11,12,242,248,256,456,457]. Similarly the reduction in odds of *Plasmodium* infection in wealthier communities is frequently recognised [12] and ample evidence exists on the impact of global and local development on transmission of malaria [11,248]. The finding of a protective effect of increased net coverage on *Plasmodium* infection is also well documented [154,231,416,432,456].

Despite the heterogeneity in *Plasmodium* infection across the region and the temporal stability observed from the mapped raw data, there was a degree of variation in the spatial dependence across the surveys, highlighted by the survey-specific semivariograms. The incorporation of a space-time interaction in the Bayesian geostatistical model resulting in a better model fit, supports the finding of non-stationarity over time, as does the finding of significant associations between the environmental covariates and infection risk in this model (Model E).

The persistently elevated range of spatial correlation in Survey 6 in this model would seem to be anomalous. A possible reason for this could be the modification of the covariate associations with *Plasmodium* infection by time. Just as there appears to be an interaction between survey timepoint and spatial structure there could be an influence of survey timepoint on covariate associations, with alternative factors responsible for the variation seen in this final survey. The addition of covariate-time or covariate-season interaction terms could be used to further explore the relationships at Survey 6.

The observed association between seasonality and risk of *P. falciparum* infection in these analyses, whereby increased odds of infection were related to surveys conducted in the wet season, is consistent with previous research where seasonality has been shown to modify the incidence of malaria but not affect the spatial clusters over time [249,414]. Although visually the school-level prevalences appear to exhibit no obvious change in spatial distribution of infection across seasons, the semivariograms indicate the presence of deterministic spatial trends across the site in the wet seasons, most probably from the increased influence of environmental factors such as precipitation at these timepoints. The lack of extreme values in the mapped spatial residuals from the space-time covariate model for Surveys 2, 3 and 4, conducted in the wet season, indicate that the modelling was able to adequately address these deterministic trends. The model residuals also suggest that explaining the variation was more problematic in the dry season (Surveys 1, 5 and 6), adding weight to the possibility of seasonal modification of the covariate relationships with infection.

The apparent temporal stability of infection despite treatment during the IST rounds in intervention schools indicates the importance of re-infection and new infections acquired between screening rounds, likely due to transmission from untreated community members. Furthermore the significant dose response relationship between treatment interval and risk of *Plasmodium* infection is further evidence of the influence of re-infection on the lack of impact of the IST intervention. Children attending schools with low prevalence of infection are likely to have a low risk of exposure outside of school in their communities, whereas those attending schools with high prevalence are likely to come from regions where a large proportion of the community is infected and transmission is high. A three month interval is in excess of the recognised pharmacological protection period of a course of AL treatment [339,340], leaving individuals at risk of re-infection through transmission from family and surrounding community members between surveys.

The infection patterns observed in space and time in this cohort, despite treatment, must be considered in the context of the intervention delivery in the 51 intervention schools. Firstly, it is assumed that all infections were detected during screening and subsequently treated in the intervention schools. However, as infection was screened using RDTs, and these analyses utilised microscopy results, the comparative diagnostic performance of the two methods will affect this assumption. As was demonstrated in Chapter 5, a fair proportion of discordance between the two diagnostic methods was observed. Furthermore, no confirmation of parasite clearance was made following treatment with AL. Although the markedly lower prevalences at Survey 4 (a month following treatment) in the intervention schools, are indicative of treatment success, parasitaemia levels may have been temporarily reduced to subpatent levels, with subsequent recrudescence leading to microscopically detectable levels [339,346] at the following survey. The use of serology to measure antibody responses, and polymerase chain reaction (PCR) measuring subpatent infections could resolve some of these concerns regarding transmission exposure and low detection thresholds, known to be important for transmission [71,345].

Despite the robust geostatistical analyses used, methodological and data limitations must be considered. The persistence of residual risk in schools in the north east of the region following incorporation of covariates and spatial correlation, as well as indicating a hotspot of infection, suggests the presence of a geographical non-stationarity. The addition of location-specific geostatistical random effects for the north east and south west of the study site to account for this were not incorporated due to a lack of known biological relevance and the small sample size. Instead the inclusion of environmental covariates to investigate the spatial dependence was preferred. In order to more fully explore this cluster of high infection in the north east, geographically weighted regression could have been employed to account for potential differences in covariate relationships with infection across the region [458]. However, this was not appropriate due to the increased power required and the limited spatial locations sampled in this site.

The localised area over which the heterogeneity was examined may have led to difficulties in resolving reasonably large-scale spatial processes with relatively small-scale distances between schools. Although the majority of satellite and remote sensed data used was at a resolution of $1km^2$ or less (minimum distance between schools was 1.3km), the use of a buffer for extraction of the environmental data may have diluted the variation between schools making it difficult to adequately capture the fine scale heterogeneity in transmission. The use of high resolution satellite imagery such as Quickbird satellite imagery at 61-82m may improve the ability of the models to detect additional important environmental relationships with infection prevalence at the school level [459].

The enduring heterogeneity in school-level infection with a reasonably stable tail at the high end of the distribution has important implications for targeted control, suggesting screening of schools can assist in guiding county level control programmes with regards to fine-scale targeting to specific schools and their surrounding villages. These modelling results, though not

appropriate for widespread predictive risk mapping due to the restricted geographical range, indicated strong classification accuracy at various prevalence thresholds, especially the highest threshold, enabling reliable identification of the schools at highest risk of transmission. The model (Model E) used, relied on covariate data for predictions using a small degree of residual spatial and aspatial variation. However in a real unknown predictive exercise, socioeconomic data would not be available, reducing the practicality of using modelling to identify high-risk schools on such a scale. With school surveys providing an operationally efficient and cost effective method of rapidly screening for infection [229], this would still seem the preferred strategy for identification of schools at high risk over predictive modelling at such small geographical scales.

The operational implications of these findings suggest that due to the geographical stability of *P. falciparum* infection observed across a localised region, sustained targeting of screen and treat, or mass treatment campaigns, to the minority of schools exhibiting high transmission, could substantially reduce the extent of spatial heterogeneity and overall transmission in the region, in support of elimination goals. However, as shown in Chapter 4, transmission was not reduced over the two year period through screening and treatment of school children once a term. A key indication of these findings is that treatment intervals must be carefully considered due to the substantial dose response relationship between time since last treatment and risk of infection. Recent evidence from Uganda has demonstrated that mass treatment of school children with dihydroartemisinin-piperaquine each term can significantly reduce parasitaemia, with monthly treatment leading to an even greater impact [35], which is consistent with the findings of this study where a one month treatment interval led to substantial reductions in parasitaemia in comparison with the three and four month intervals. The extension of such intermittent mass treatment to the wider catchment areas of these targeted schools would further reduce the extent of re-infections and incident infections between treatment rounds. The strong associations between infection prevalence and environmental factors such as precipitation and proximity to

waterbodies point to a possible supplementary role for targeted ecological vector control initiatives such as larviciding.

6.6 CONCLUSIONS

In conclusion, the distinct spatial heterogeneity, alongside the rapid bounce back in school-level infection in spite of treatment, suggests that there is a need for sustained high net coverage as well as focal coverage of interventions, potentially through community screening and treatment or presumptive treatment in communities in addition to school-level coverage in the high-risk schools and their catchment areas. Periodic screening of school children for infection can prove an efficient strategy providing real-time, cost effective, prevalence data, providing an insight into the transmission dynamics and heterogeneity in communities on which to base resource targeting decisions as well as for use in monitoring and evaluating interventions, as shown by the relative stability of infection in this study. The significant relationship of environmental covariates with school-level prevalence also indicates a role for integrated vector management in providing a comprehensive suite of malaria control interventions in high-risk schools and their surrounding communities [460].

However, it is important to note that despite using a cohort of school children, these analyses presented investigate infection at the unit of the school, meaning individual child-level correlation is not considered. This restricts the ability to determine whether the temporal stability of infection observed was due to clustering of infections within individual children across surveys, or to infections in different children at each survey. The following chapter will explore the individual-level heterogeneity in infection and determine the extent of overdispersion of infection events using the cohort of children in the IST intervention group.

Chapter 7. Evidence for individual-level heterogeneity in *P. falciparum* infection: Repeat infections following treatment in a cohort of school children in south coast, Kenya.

7.1 OVERVIEW

Chapter 6 demonstrated extensive geographical heterogeneity in school-level *Plasmodium falciparum* infection prevalence, with this variation proving stable over time. Schools exhibiting high prevalence of infection at baseline were generally found to maintain high prevalence at the following surveys, in spite of treatment of infected individuals at each survey. Meanwhile, schools with low initial prevalence, for the most part, repeatedly demonstrated low prevalence at the following surveys. Given the analysis was conducted at the school level it was not possible to establish whether such stability was primarily due to re-infections in the same individuals over time or to new infections in different children. However, stratified analysis within the intervention group in Chapter 4 indicated increased risk of *P. falciparum* infection in children who had more AL treatments during the IST rounds. Further knowledge regarding aggregation of repeat infections in specific children would assist in informing strategies for targeting individuals who help sustain transmission. This chapter uses longitudinal, repeated measures RDT-detected infection data collected among the intermittent screening and treatment (IST) intervention cohort of school children. Heterogeneity of *P. falciparum* infection at the individual-level is investigated allowing for the influence of exposure at the school and individual-level to be factored into analyses. While recognising that the results presented in Chapter 5 suggested imperfect diagnostic performance of both microscopy and RDTs, both techniques are widely used in the field and due to the key assumption in the models used, of clearance of all infections at each survey, RDT-detected infection data are used for these analyses.

The analyses in this chapter were instigated to address an interesting issue arising from the analyses conducted in the previous chapters. I coordinated the data collection, entry and cleaning, as well as conducting the data analysis. Dr Rachel Pullan and Dr Elizabeth Turner provided high level technical support.

7.2 INTRODUCTION

The overdispersion of parasitic infections such as soil-transmitted helminths and *Plasmodium* is well recognised, with a small proportion of individuals within endemic communities experiencing a disproportionately high percentage of infections [93]. There is a significant body of evidence on the overdispersion of clinical malaria episodes in various transmission settings [142,461,462], but the dispersion of *P. falciparum* infections is less well studied. With evidence of temporal stability in clusters of *Plasmodium* infection observed across a fine spatial scale [99,249], it is reasonable to assume that repeated infections occur in individuals living in focal areas of intense transmission, who are consistently highly exposed. However, it remains less clear whether the clusters of infection found over time are experienced by the same individuals, who have a higher intrinsic susceptibility to infection once exposure is accounted for, or by different individuals living in close proximity to each other and subject to the same environmental risk factors, i.e. primarily a localised high exposure environment [97]. The answer to this question has implications for targeting control programmes at those most important for sustaining transmission, especially as prevalence drops as a result of large-scale control. For example, should programmes be targeted at all children in high-risk schools (and their surrounding communities), as opposed to an approach of targeting particular individuals and their households who contribute substantially to transmission in a region.

This chapter examines the extent of repeated *P. falciparum* infections in the presence of periodic treatment of asymptomatic infections detected by RDT screening in a cohort of children in a low to moderate transmission region of coastal Kenya. The dispersion of repeated RDT-detected infections over five discrete time observation processes is first examined using Poisson and negative binomial regression models. Subsequently, the extent to which the proportion of children experiencing repeated infections exceed that predicted by a prevalence-based model before and after accounting for covariates is investigated.

7.3 METHODS

7.3.1 Design and data

The data used come from the 51 schools assigned to receive the IST intervention, whereby children were screened for *P. falciparum* infection using RDTs and treated with AL if found to be parasitaemic. Five rounds of IST were implemented across the two year study period, in February/March 2010, June/July 2010, September 2010, February/March 2011, with the final round in October 2011. The RDT results at each round form the basis for these analyses [284,357].

The prevalence-based models comparing observed and predicted proportion of individuals with repeated infections incorporating school-level and individual-level covariate information. Transmission intensity (exposure) was estimated using the school-level baseline prevalence of *P. falciparum* infection at the first round of screening (February/March 2010). Individual-level sociodemographic factors were established through a questionnaire, administered to parents/guardians on enrolment of their children into the study. The variables included in the models were: age, sex, nutritional status (stunting) and socioeconomic status (SES quintiles), number of people in the household and reported bednet use. The inclusion of these factors was based on the findings of risk analyses presented in Chapters 3 and 4 and thus were assumed *a-priori* to be risk factors for *Plasmodium* infection.

7.3.2 Data analysis

Data were first summarised for the children screened once or more during the intervention period. Subsequent analysis was then conducted to (i) determine the dispersion of the observed infection data, and (ii) examine the role of both school-level transmission exposure and individual-level sociodemographic factors in accounting for repeat infections within children through the use of prevalence-based models. These analyses included only the 1785 children who were screened by an RDT at all five rounds of IST. Only those children with complete RDT screening data were included for several reasons: firstly, children assessed at every screening round could be assumed to have had equal exposure and secondly, it could be assured that if the child was RDT positive during the previous screening round, they would have received treatment to clear the infection, resulting in the detection of incident infections at following screening surveys.

7.3.2.1 Analysis of the pattern of dispersion of the observed infection data

The initial dispersion of the observed *Plasmodium* infection data was examined by fitting both a Poisson model (assuming no over-dispersion and that the variance is equal to the mean) and a negative binomial model which assumes over-dispersion of the outcome whereby the variance exceeds the mean, in Stata Version 12.0 (Stata Corporation, College Station TX) [446]. As all children included were present at all surveys, they were assumed to have the same person-time at risk. The estimated number of infections per child was predicted using both models, and a comparison made between the fitted probability distribution of multiple *Plasmodium* infections, with the observed frequency of multiple infections. Model fit was further compared through the Akaike information criterion (AIC), with a lower AIC indicative of a better model fit. For the negative binomial model the dispersion parameter (κ) was assessed to determine whether the null hypothesis of the Poisson distribution could be rejected on the basis of a value of κ significantly greater than zero.

7.3.2.2 Analysis of repeated infections using a prevalence-based model

Subsequent analyses were performed using an approach adopted by Carlton et al [463] in the investigation of repeated schistosome infections. These analyses were conducted within a Bayesian framework using Winbugs 1.4 (Medical Research Council, Cambridge, UK and Imperial College London, UK) [389]. The first screening and treatment round was defined as time zero (T_0) and was used to establish baseline prevalence (as a measure of local transmission intensity) at the school-level. Screening rounds two to five were defined as: T_1, T_2, T_3, T_4 and were viewed as follow-up surveys at which repeat infections were analysed in 1785 study children.

Initially a simple, prevalence-based model was used to examine the ratio of observed (O_{RI}) to predicted (P_{RI}) proportion of the population with repeat infections, whereby the individual-level probability of infection at each of the follow-up screening rounds was estimated using a null logistic model. In the model, Y_{ij} denotes the outcome as infected Y_{ij} =1 or uninfected Y_{ij} =0 of the *ith* individual (*i*=1…1785) at the *jth* follow-up screening (*j*=1…4). A Bernoulli distribution was assumed for each individual at each screening round, with the probability of infection represented by $p_{ij.}$ Assuming $Y_{ij} \sim Bern(p_{ij})$, a logit transformed probability *logit*(p_{ij}) was used for the null model. The product of these individual-level probabilities was calculated to estimate the probability of being infected at multiple rounds, assuming independence between rounds (i.e. being RDT-positive on the previous follow-up had no effect on infection status at the next follow-up as it was assumed all infections were treated and cleared). The mean of these probabilities across the population was taken as an estimate of the predicted proportion of the population experiencing infection at multiple follow-up screening rounds.

Due to the treatment of infections detected at each screening round, with the assumption that all infections are cleared, it was assumed that all subsequent infections at screening round T_x were incident. Using the standard formula for the relationship between prevalence, incidence and duration, it was assumed that prevalence at T_x represents incident infections post T_{x-1}. Hence

the probability of infection at T_x was assumed to be the equivalent of the incidence of infection between the two screening rounds (T_x and T_{x-1}) multiplied by the time between the two rounds, which can be equated to the prevalence at T_x [463]. This assumes that incident infections are cleared before the next screening round. Therefore, for example, the probability of infection at all four rounds was $P_{RI} = P(Y_{T1} = 1) * P(Y_{T2} = 1) * P(Y_{T3} = 1) * P(Y_{T4} = 1)$. This was compared to the observed proportion of children infected at all four follow-ups (screening round) to provide a ratio of observed to predicted (O_{RI}/P_{RI}). A ratio greater than 1 indicated a certain proportion of individuals were susceptible to repeated infection, over and above that predicted by a basic model, based on the product of the probability of infection at each follow-up. Repeat infections were characterised in terms of both (a) consecutive infections and (b) total number of times infected (not necessarily consecutively) at the four screening rounds following baseline screening (T_0).

The basic prevalence model was then expanded to account for, in the first instance, transmission intensity at the school-level, and secondly to account for additional individual-level socio-demographic exposures, in order to estimate the probability conditional on these covariates. For example, the probability of infection at all four rounds, conditional on exposure, was $P_{RI} = P(Y_{T1} = 1 \mid \boldsymbol{X}) * P(Y_{T2} = 1 \mid \boldsymbol{X}) * P(Y_{T3} = 1 \mid \boldsymbol{X}) * P(Y_{T4} = 1 \mid \boldsymbol{X})$ where Y is infection status for a given child at a given time point (where for ease index *i* is not included to denote the child), $\boldsymbol{X}$ is a matrix of *K* covariates for all 1785 children at all of the 4 follow-up time points to indicate that the probability for a given child depends on covariates of other children. In practice, only baseline covariates were used to yield a 1785 x *K* matrix of covariates for all children included in the analysis. This model still assumes the probability of infection is independent across follow-ups and can only be related through the use of the same covariates at each round (**X**). The logit model above was used as a base on which to incorporate the matrix of exposure covariates.

$$logit(p_{ij}) = \alpha + \sum_{k=1}^{K} \beta_k x_{ijk}$$

This model assumes that the effect of covariates is the same at each time-point (since β_k does not change with time). In practice x_{ijk} is the same for each child at each time point as only baseline covariates are included in the model, and therefore $x_{ijk} = x_{ij}$. However, sensitivity analyses were conducted to assess the effect of allowing both the baseline risk and/or the influence of covariates to vary across the screening rounds (follow-up surveys). Such changes did not result in an improved model fit or meaningfully different parameter estimates and thus were not implemented in the final model. A non-informative uniform flat prior was assigned to the intercept (α). β is a vector of *k* regression coefficient parameters related to a matrix of covariates ($\boldsymbol{X}$). The β coefficients were assigned an uninformative normal prior with a mean of 0 and a precision of 1×10^{-6}.

Bayesian inference was implemented using a Markov Chain Monte Carlo (MCMC) algorithm. Two chains were run consecutively, and a burn-in of 10,000 iterations was performed. Following this, the convergence was examined using the MCMC dynamic traces of the model parameters using the dynamic trace plot history, kernel density plots and autocorrelation plots. A further 10,000 iterations were run with every tenth observation of the posterior distribution of the parameters stored, from which the posterior estimates were calculated.

As previously noted, the school-level prevalence at baseline (T_0) was included to account for local exposure to transmission in both the transmission exposure model and the transmission and sociodemographic exposure model. Covariates included in the transmission and sociodemographic exposure model at the individual-level were age, sex, reported bed-net use, stunting (a proxy for nutritional status), SES and number of people in the household. The product of these adjusted individual-level probabilities was calculated to estimate the expected proportion of the population with repeated infection and the ratio of observed to the adjusted expected estimates was calculated.

7.4 RESULTS

7.4.1 Survey data description

Of the initial 2710 study children at baseline, 2602 were screened at least once during the IST rounds, with 66 children only screened on one occasion and 1785 (68.6%) children screened at all five screening rounds (Table 7.1). A certain proportion of children were found RDT positive at every visit at which they were screened, for example, of the children screened at all five occasions, 29 (1.6%) were RDT positive at each round.

Table 7.1: Frequency of screening rounds attended against RDT positive results. A total of five screening rounds were conducted

		Frequency of RDT positive results n (%)						
		0	1	2	3	4	5	Total
Frequency of RDT screenings	1	52 (78.8)	14 (21.2)	0	0	0	0	66
	2	66 (71.7)	23 (25.0)	3 (3.3)	0	0	0	92
	3	129 (62.6)	47 (22.8)	22 (10.7)	8 (3.9)	0	0	206
	4	251 (55.4)	119 (26.3)	55 (12.1)	17 (3.8)	11 (2.4)	0	453
	5	940 (52.7)	445 (24.9)	199 (11.1)	121 (6.8)	51 (2.9)	29 (1.6)	1785
Total		1438	648	279	146	62	29	2602

A comparison between the 1785 children screened at all five rounds who were included in the repeated infection analyses and children excluded due to incomplete screening data, showed that the children excluded did not differ significantly in respect of demographic, socioeconomic or health characteristics (Table 7.2). Importantly, baseline *P. falciparum* infection was similar across the two groups, 18.9% versus 17.6% in those included and excluded respectively.

Table 7.2: Characteristics of children present at all five screening rounds of the IST intervention with a complete set of RDT results from all time points (included in analysis), and children who missed one or more screening round of the IST intervention and hence have incomplete data (excluded from analysis).

Variable	Children with full screening results(included in analysis) N (%)[1], n=1785	Children with incomplete screening results (excluded from analysis) N (%)[1] n=817
Baseline *P. falciparum* prevalence (%)		
	338 (18.9)	111 (17.6)
Sex		
Male	859 (48.1)	402 (49.2)
Female	926 (51.9)	415 (50.8)
Age (yrs)		
	10.2 (2.7)	10.6 (3.0)
Age groups (yrs)		
5-10	723 (40.5)	303 (37.1)
11-12	637 (35.7)	257 (31.5)
13-18	425 (23.8)	257 (31.5)
Education level of household head		
No schooling	569 (32.2)	314 (39.1)
Primary	936 (53.0)	394 (49.1)
Secondary	207 (11.7)	68 (8.5)
College/degree	53 (3.0)	27 (3.4)
SES quintile		
Poorest	411 (23.1)	219 (27.2)
Poor	372 (21.0)	176 (21.8)
Median	309 (17.4)	163 (20.2)
Less poor	352 (19.8)	134 (16.6)
Least poor	332 (18.7)	114 (14.2)
Child sleeps under a net		
No	663 (35.9)	313 (39.1)
Yes	1131 (64.1)	488 (60.9)
Child stunted at baseline		
No	1336 (75.1)	473 (75.1)
Yes	443 (24.9)	157 (24.9)
Child anaemic at baseline		
No	972 (54.5)	344 (54.8)
Yes	812 (45.5)	284 (45.2)

[1]Displayed as number and percentage except for continuous variables, displayed as Mean and Standard Deviation (SD).
[2]Measured at the school level.

7.4.2 Evidence of overdispersion of infection

A visual examination of the dispersion of observed RDT-detected *Plasmodium* infections plotted against the predicted probability distribution, as shown in Figure 7.1, indicated a significantly better fit for the negative binomial distribution than for the Poisson distribution, as shown by the results of a likelihood ratio chi squared test that the dispersion parameter (k) is zero, (chi squared test statistic 239.2 $p<0.001$). The Poisson distribution assumes that the number of infections has a variance equal to the mean, whereas the negative binomial model indicates that the variance exceeds the mean. The dispersion parameter obtained from fitting the negative binomial model

was 0.84 (95%CI: 0.69-1.02), significantly greater than 0, demonstrating overdispersion in the infection data. The AIC of the negative binomial model was also lower than that of the Poisson model (4615 compared to 4852) providing evidence of a better fit to the observed data. Overall the evidence suggests individual-level heterogeneity in infection events, indicating that all children do not experience the same rates of infection. In total 71.4% of infection events were experienced by only 22.4% of the children. Notably, over 50% of children were RDT negative every time they were screened. The Poisson distribution significantly underestimated this percentage of consistently RDT-negative children, as well as overestimating the percentage of children experiencing *P. falciparum* infections on one or two screenings. Again the Poisson distribution predicted an underestimated proportion of children infected three to five times, whereas the negative binomial model distribution was similar to the observed distribution of infection events (Figure 7.1).

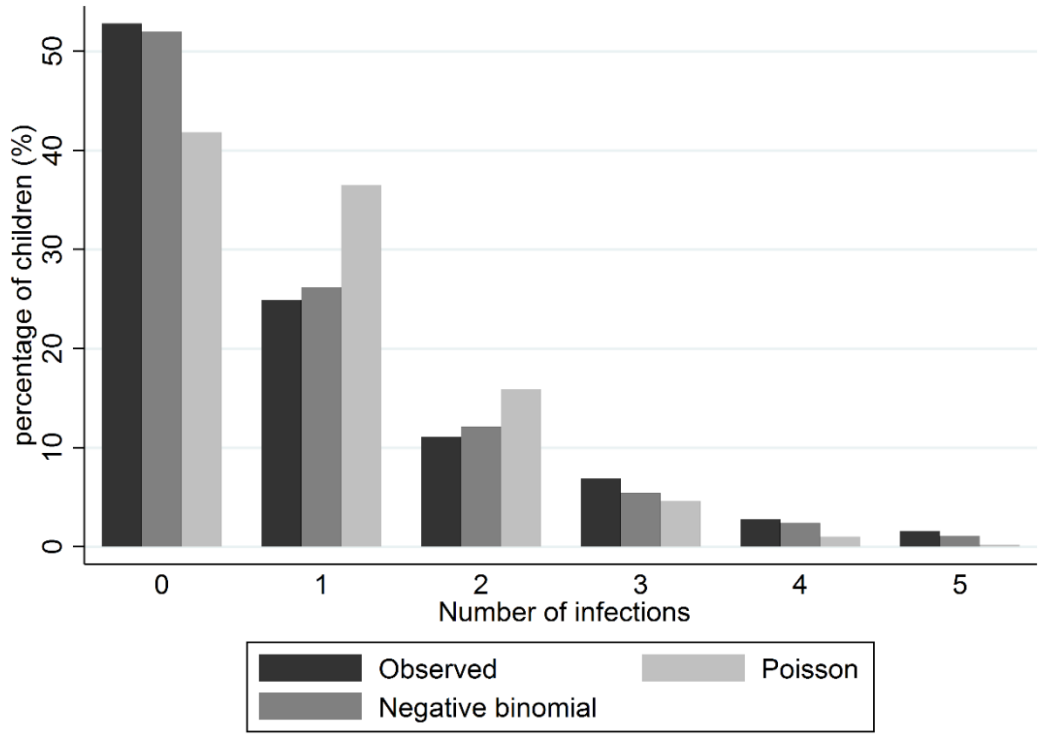

Figure 7.1: Observed *P. falciparum* infections across the cohort of individuals measured at five discrete observations. The probability distribution of infection events estimated from the Poisson and negative binomial models is shown compared to the observed prevalence (using data from 1785 children observed at 5 time points).

7.4.3 Prevalence-based models of repeat infections

Table 7.3 shows the prevalence of infection at each of the four follow-up surveys, each representing the assumed incidence in the time since the previous survey. Overall prevalence of infection at the first follow-up (T_1) was 18.9%, however when stratified by RDT result in the first screening survey, those who were RDT-positive (and subsequently treated) at baseline, were more than twice as likely to be infected at T_1 than those who were RDT-negative at baseline. The same pattern was observed at all subsequent surveys. In relation to cumulative infections, 7.51% of children were RDT-positive at T_1 and T_2, 3.53% were positive at T_1, T_2, and T_3 and 2.46% of children were RDT-positive on all four follow-up surveys. When stratified by baseline RDT result, the relative percentages of children with cumulative infections were between three- and ten-fold higher amongst the children found infected at the first screening survey as opposed to those who were uninfected.

Table 7.3: The distribution of *P. falciparum* infections at individual screening rounds and cumulatively across screening rounds displayed overall and stratified by baseline infection status.

			Infected at baseline (T_0)			
	All N=1785		**No** N=1447		**Yes** N=338	
	Single infection n (%)	Cumulative infections n (%)	Single infection n (%)	Cumulative infections n (%)[a]	Single infection n (%)	Cumulative infections n (%)[a]
Infected at T_1	338 (18.94)		226 (15.62)		112 (33.14)	
Infected at T_2	334 (18.71)	134 (7.51)	226 (15.62)	77 (5.32)	108 (31.95)	57 (16.86)
Infected at T_3	263 (14.73)	63 (3.53)	148 (10.23)	26 (1.80)	115 (34.02)	37 (10.95)
Infected at T_4	282 (15.80)	44 (2.46)	172 (11.89)	15 (1.04)	110 (32.54)	29 (8.58)

[a] Percentages are of the N populations ("not infected at baseline" and "infected at baseline")

The observed proportions of children experiencing at least two consecutive infections and at least two infections overall were 1.70 and 1.30 times greater, respectively, than predicted by the basic prevalence model (Table 7.4). In relation to at least three infections, the observed proportion of children was more than six and four fold higher than predicted, considering consecutive infections and cumulative infections respectively. The ratio of observed to predicted proportion of children infected with *P. falciparum* at all four follow-up screening rounds was 29.40. Thus the ratio of observed to predicted demonstrated a strong dose response relationship when considering increasing number of infections experienced.

Table 7.4: The observed and predicted proportions with consecutive infections and combinations of multiple infection events over the four follow-up screening rounds. Results are shown for (i) the basic prevalence-based model, (ii) models accounting for local transmission intensity and (iii) models accounting for local transmission intensity and individual-level socio-demographic factors.

		Observed % population with repeated infections 95% BCI	Predicted % population with repeated infections 95% BCI	Ratio (observed /predicted) 95% BCI	Model type
Consecutive surveys	Infected on at least two consecutive surveys T_1 T_2 or T_2 T_3 or T_3 T_4	13.11 (11.58, 14.76)	7.73 (7.01, 8.48)	1.70 (1.55, 1.87)	Basic prevalence model[a]
			10.24 (9.44, 11.10)	1.28 (1.18, 1.39)	Transmission exposure model[b]
			10.36 (9.49, 11.22)	1.27 (1.17, 1.38)	Transmission & socio-demographic exposure model[c]
	Infected on at least three consecutive surveys T_1 T_2 T_3 or T_2 T_3 T_4	6.10 (5.04, 7.32)	0.91 (0.78, 1.05)	6.78 (5.84, 7.82)	Basic prevalence model[a]
			2.51 (2.16, 2.92)	2.45 (2.10, 2.82)	Transmission exposure model[b]
			2.59 (2.22, 2.97)	2.38 (2.06, 2.75)	Transmission & socio-demographic exposure model[c]
Multiple surveys	Infected on any 2 or more surveys	17.76 (16.01, 19.61)	13.73 (12.53, 14.97)	1.30 (1.19, 1.42)	Basic prevalence model[a]
			16.14 (15.02, 17.29)	1.10 (1.03, 1.18)	Transmission exposure model[b]
			16.25 (15.08, 17.41)	1.09 (1.02, 1.18)	Transmission & socio-demographic exposure model[c]
	Infected on any 3 or more surveys	7.39 (6.22, 8.70)	1.73 (1.49, 1.99)	4.30 (3.71, 4.96)	Basic prevalence model[a]
			4.34 (3.78, 4.95)	1.71 (1.49, 1.96)	Transmission exposure model[b]
			4.45 (3.88, 5.07)	1.67 (1.46, 1.91)	Transmission & socio-demographic exposure model[c]
All surveys	Infected at all follow-up surveys T_1 T_2 T_3 T_4	2.46 (1.80, 3.30)	0.08 (0.07, 0.10)	29.40 (23.9, 35.9)	Basic prevalence model[a]
			0.67 (0.53, 0.84)	3.73 (2.95, 4.69)	Transmission exposure model[b]
			0.71 (0.56, 0.89)	3.51 (2.78, 4.40)	Transmission & socio-demographic exposure model[c]

[a]Basic prevalence model – null model based only on prevalence of infection at each screening round
[b]Transmission exposure model – model accounting for school-level prevalence of infection at baseline
[c]Transmission & socio-demographic exposure model – model accounting for school-level prevalence of infection at baseline and individual-level age, sex, stunting, SES, net use, people per household

Once accounting for school-level baseline (T_0) prevalence in these models, the proportion of children predicted to experience repeated infections increased significantly, with a substantial decrease in the ratio of observed to expected in all cases (Table 7.4). The decrease in ratio of 29.40 to 3.73 on addition of school-level prevalence when considering children RDT-positive at all four follow-up surveys provides strong evidence for the importance of local transmission intensity on the risk of repeated infections. After additional inclusion of individual-level socio-demographic covariates, there was a further decline in each of the ratios, however these were not large reductions. A comparison of repeated consecutive infections and cumulative infections indicates a greater difference between observed and predicted percentage, unexplained by the models, when considering consecutive infections. In all combinations of repeated infections examined, having accounted for transmission exposure at the school-level and additional exposure at the individual-level, there remained a significant excess in the proportion of children observed to have repeated infections than expected.

Table 7.5: Associations between the individual-level exposure covariates included in the transmission and socio-demographic exposure models.

Characteristic	Adjusted OR (95% BCI)[b]
Age (years)[a]	
	0.96 (0.94-0.99)
Sex	
Male	1
Female	0.98 (0.86-1.11)
Nutritional status	
Not stunted	1
Stunted	1.18 (1.01-1.37)
Net use	
Does not sleep under net	1
Sleeps under net	0.81 (0.70-0.93)
SES	
Poorest	1
Poor	1.28 (1.06-1.55)
Median	1.21 (0.97-1.49)
Less poor	1.08 (0.88-1.35)
Least poor	1.07 (0.87-1.33)
People in household[a]	
	1.03 (1.00-1.05)

[a]per 1 unit increase
[b]adjusted odds ratio and 95% Bayesian credible interval from

Examination of associations between repeated infections and the socio-demographic covariates included in the full exposure models showed decreased odds of repeated infection with increasing age (Table 7.5). No significant association was observed between sex and odds of repeated infection and in relation to SES, despite a slight increase in odds of repeated infection in children in the second wealth quintile, no association was seen for the other relative wealth categories. Stunting was associated with increased odds of repeated infection (Adj.OR 1.18, 95%CI: 1.01-1.37) as was increased numbers of people living in the household (Adj.OR 1.03, 95%CI: 1.00-1.05). Use of a bednet was associated with a significant protective effect (Adj.OR 0.81, 95%CI: 0.70-0.93), as expected.

7.5 DISCUSSION

These findings indicate that despite treatment, heterogeneity in individual risk of *Plasmodium* infection was present in this setting, with certain children particularly vulnerable to *P. falciparum* infection. This finding is maintained to a degree, even once factors such as age, sex, nutritional status, SES, household crowding, net use and local transmission intensity are accounted for. The results may provide an explanation for the lack of impact on long term health (anaemia) observed in the children who received multiple AL treatments during the IST visits in the intervention, as repeated re-infection in these children would not have allowed the process of haematological recovery.

The overdispersion of infections observed in this cohort of children is consistent with findings of other studies of heterogeneity in parasitic infections [93] and clinical episodes of malaria [461,462]. It can also be related to overdispersed vector distribution and transmission, with a study conducted in the Gambia demonstrating that when *An. gambiae s.l* are abundant in the wet season, vector numbers collected under bednets best fit a negative binomial distribution,

whereby over a third of bednets had no mosquitoes and others had up to seventeen [464]. Possible reasons for the overdispersed frequency distribution were suggested to be related to distance from breeding sites, quality of nets and household construction [464]. In addition to increased variability in infection frequency than would be expected from the Poisson distribution, a high percentage of children (over half) did not experience any RDT-detected *Plasmodium* infections across the five screening rounds, suggesting the use of a zero inflated negative-binomial model may have resulted in an improved fit. This was found by Cairns *et al.* when examining cohort data of clinical malaria episodes in Ghana [462]. Although this high proportion of children experiencing no infection could represent a reduced susceptibility to infection, the more likely explanation is decreased exposure to infection [66].

A similar pattern of heterogeneity of infection between individuals is also exhibited through the use of prevalence-based models, with a substantially greater proportion of children observed to experience repeated infections than would be expected in a situation where children are assumed equally exposed, and a strong dose response observed across the ratios with increasing numbers of infections. The finding that the majority of this difference can be explained by local transmission intensity is concordant with evidence both from previous research and the previous chapter demonstrating extensive, temporally stable, local variation in risk of *P. falciparum* infection [99,249,422], whereby local regions of high transmission are likely related to the number and extent of breeding sites in close proximity [317,452,465]. The additional reduction in the excess risk of repeated infection on accounting for individual-level socio-demographic factors, albeit small, is also consistent with the associations observed in previous chapters. SES, although significantly associated with risk at the school level, as found in Chapter 6, appears not to exert much influence on infection risk at the individual-level, as was also found in Chapter 3.

Despite the importance of these factors in explaining the rate of infection among the children, the residual increased risk of repeat infections suggests some children were more liable to infection even after accounting for these covariates. This residual excess risk could be due to

unobserved heterogeneity in exposure, with the most likely form of this being highly localised environmental exposures within the school catchment areas. The association of such ecological covariates is an important consideration when making inferences regarding the risk of repeated infections, as factors such as distance of household to a waterbody may have a substantial impact on individual-level infection [28,100]. The future inclusion of household location, spatial dependence and associated covariates at high resolution would allow a more thorough examination of risk due to individual-level susceptibility over and above environmental risk factors.

However, the increased repeat infections observed could also represent a degree of increased innate susceptibility in certain individuals. Although there is limited evidence for the influence of genetic polymorphisms in modifying infection risk [94], various studies have investigated the relative contribution of genetics in relation to other factors in the variance in *Plasmodium* infection observed between individuals [261-263] with genetic effects found to be a significant determinant of parasite density in Ugandan children at a single timepoint [263]. The frequently-observed overdispersion of clinical malaria attacks has been largely attributed to the acquisition of functional immunity with repeated exposure to inoculation. However, evidence for acquisition of immunity to infection in individuals following continued exposure and repeated infection is mixed [97], with some evidence to suggest uninfected children are unexposed rather than "immune" to infection [66]. Studies have examined time to infection following treatment, stratified by age, in order to explore this, and whilst some have found evidence for a relationship between age and length of time to infection or re-infection [466,467], others have observed no variation by age [468,469]. Furthermore, variation in susceptibility has been found in West Africa between sympatric groups exhibiting similar behaviours in terms of preventive measures. The Fulani population in West Africa and West Sudan have persistently shown decreased susceptibility to asymptomatic infection, asexual parasite density and gametocyte carriage [470-472].

Both approaches were subject to a number of important assumptions and the results must be interpreted in light of these assumptions. Firstly, both set of analyses examine the assumed incident infections. The use of all five screening visits for the initial Poisson and negative binomial models means that not all individuals were infection free at enrolment, however they were all were screened with an RDT and those found positive were administered a full dose of AL, so providing the screening picked up all infections and the treatment was effective, infections detected at subsequent screenings should be incident infections. The prevalence-based models did not include the first round of IST, because the assumptions applying to the standard formula used (prevalence = incidence x duration) could not be satisfied as incidence and duration were not known at T_0. Hence in this approach the prevalence at baseline was used as a proxy for transmission.

Secondly, the supposition that all infections were incident and independent highlights two further critical assumptions of both approaches: (i) the screening tool (an RDT) was 100% sensitive, and as such all infections were detected at every screening round, and (ii) that all infections detected were successfully cleared by the dose of AL given by the nurse, relying on full compliance. These two assumptions are unlikely to be fully satisfied, as is evidenced from the comparison of RDT and microscopy performance presented in Chapter 5. Given the observed sensitivity of the RDTs, some low density infections are likely to have been missed. This would have led to missed treatment, resulting in infections detected at the following round incorrectly classified as incident. Furthermore, subpatent and recrudescent infections would also violate this assumption of independence [71].

Thirdly, the prevalence-based models rely on the typical asymptomatic infection having a longer duration than the intervals between follow-ups (screening rounds). Empirical and theoretical evidence supports this, with untreated asymptomatic infections found to last up to 18 months [87,144]. However it is possible that between screening rounds children may have become infected with *P. falciparum*, which cleared before the following screening round. This could be

due to either self-limitation of the infection, or treatment sought at a health facility in the event of clinical symptoms. These potentially undetected infections were not accounted for in either analysis and could have led to an underestimation of the number of infections per child. Treatment for malaria external to that received in the study was not monitored, although the predominantly asymptomatic nature of infection observed during the surveys may suggest that the proportion of children seeking treatment between the surveys would have been reasonably low.

Additionally, on account of the discrete observation time-points of the data as opposed to the typical approach of passive surveillance for incidence of clinical disease or intensive active surveillance for incident infections, there is a strong possibility that the results are an underestimation of the number of overall and repeated infection events. With only five screening rounds conducted, the maximum number of infections that could be detected were constrained to five, whereas for instance, Poisson and negative binomial distributions would usually allow values to be larger.

Finally, it was assumed that all children had the same time at risk, however, with AL treatment providing a post-exposure prophylaxis period of up to four weeks [340], the number of treatments a child received across the screening surveys is likely to have modified the time at risk slightly, thus it may be appropriate to take this into account in future analyses. The Poisson and negative binomial models assume that the rate of infection is constant over time, an assumption that would be violated by the seasonal nature of transmission. However, as all children are subject to the same seasonal effects and were screened at the same points during the year, this is unlikely to be a critical limitation when assessing the variation of infection rate between children. In a future study of this sort, it would also be useful to collect covariate information for each screening interval as this would likely provide a more reliable method for accounting for exposure over time in these models.

7.6 CONCLUSIONS

In summary, the individual-level heterogeneity in *Plasmodium* infection indicates that certain children were more vulnerable to repeat infections than others, likely acting as a substantial reservoir of transmission. Once detected however, such children could provide useful sentinels for the focal treatment of other individuals in the household. Whether the increased risk of repeat infections is the result of increased exposure or susceptibility with a genetic component, other family members are likely to also experience increased risk, especially as protective behaviours and other exposure-related factors will also be very similar in these households. This supports a role for reactive screen and treat, whereby children are examined for infection at school and tracked to the household where screening and treatment of additional members of the compound is conducted [195]. However, the relative importance of local transmission (at the school-level) over the individual-level characteristics, suggests that from an operational standpoint, interventions targeted at certain schools and their catchment areas would be sufficiently effective in reaching individuals at high risk of repeat *P. falciparum* infections.

Chapter 8. Summary and discussion of findings

8.1 OVERVIEW

The renewed emphasis on eliminating malaria and the introduction of a more inclusive approach aimed at interrupting community-wide transmission has highlighted school-aged children as an important target population [108]. School health programmes provide a logical and affordable platform for tackling the malaria burden among school-age children, although limited evidence exists on the best approach to controlling malaria in this group [34]. Moreover, with increasing declines in transmission, regions of low-to-moderate transmission are expanding [41] and heterogeneity in risk of *Plasmodium* infection is becoming increasingly pronounced across multiple scales [94,102]. Identifying, understanding and accounting for such increased heterogeneity in transmission is emerging as an important feature of designing appropriate malaria control initiatives and evaluating their impact.

This thesis sought to investigate the extent of heterogeneity in *Plasmodium* infection in a region of low-to-moderate transmission intensity, as well as the influence of heterogeneity on the impact, process and potential implications for school-based malaria control. These aims were met through detailed analysis of data from the evaluation of a programme of intermittent screening and treatment (IST) delivered through schools in coastal Kenya, with a particular focus on heterogeneity in risk at the school- and individual-level, as well as on seasonality. This chapter provides a summary of findings, and discussion, with a view to providing policy-relevant guidance on the potential of school-based IST, and the wider implications of the findings for malaria-control strategies within the context of increasingly fractal heterogeneity. Avenues for future research are also identified.

8.2 SUMMARY AND DISCUSSION OF FINDINGS

A review of evidence for the burden of malaria among school-aged children presented in Chapter 1 highlighted the negative health and cognitive impacts which *P. falciparum* infection can potentially have on school children, whilst also illustrating (i) the inadequate coverage of school-age children by community-wide interventions, (ii) a lack of consensus about the optimal strategies to be delivered through schools, and (iii) the absence of evidence on school-based malaria control in moderate and low transmission settings. With this in mind, Chapter 2 described a cluster randomised trial conducted to evaluate the impact of school-based IST on the health and education of school children in south coast, Kenya. This trial was notable in two respects: it was the first trial to provide evidence regarding school-based malaria control in a low-to-moderate perennial transmission setting and it was the first to evaluate a programme of IST delivered through schools.

An initial description of the underlying epidemiology of *P. falciparum* infection and anaemia, using the baseline surveys conducted across the 51 intervention schools, was presented in Chapter 3. The results demonstrated that in this transmission setting, despite the overall moderate *P. falciparum* prevalence of 13%, substantial heterogeneity was observed across this relatively small geographical area, with school-level prevalence ranging from 0% to 75%. The burden of *P. falciparum* infections consisted, almost exclusively, of low density asymptomatic infections. Increasing age was associated with a reduced risk of *P. falciparum* infection, especially in the older age-group (13-18 years) where the odds of infection were less than half of those in the youngest age-group (5-9 years). Net use was also associated with a reduced risk of infection. While neither malaria nor anaemia were found related to cognitive or educational performance, the high prevalence of anaemia and strong, positive dose-dependent relationship exhibited between *Plasmodium* infection intensity and anaemia, adds to the body of evidence regarding the burden of asymptomatic infection on the health of school children. This supported the assertion that sustained clearance of *P. falciparum* parasitaemia through a successful school-

based malaria control programme could contribute significantly to a reduction in anaemia. However, the finding of such extensive variation in underlying transmission intensity could have implications for the impact of such a programme and are discussed below.

Studies conducted in intense, and intense-seasonal transmission settings have shown school-based malaria control through intermittent preventive treatment (IPT) to have substantial beneficial impacts on both health and education of school children [33,35,40,139]. By contrast, evaluation of the impact of school-based IST in this low-to-moderate transmission setting, presented in Chapter 4, demonstrated no long-term benefits on either the health or education of school children. Supplemental analysis of impact according to baseline *Plasmodium* prevalence categories, a proxy for transmission intensity, also indicated no meaningful heterogeneity of impact, with no beneficial effect found, even in those schools in regions with the highest transmission intensities, as might have been expected on the basis of evidence from the studies referenced above. Stratification on the basis of number of rapid diagnostic test (RDT)-positive results, and subsequent AL treatments received, also revealed no impact on long-term health (anaemia) even amongst the children receiving more than one AL treatment.

Evaluation of cost of the IST intervention revealed the estimated economic cost per child screened to be $6.24 (US$ 2010), and per year (three rounds) to be $18.72 [348]. Although sensitivity analyses indicated costs could be reduced by as much as 40% through various modifications, including choice of RDT and nurse supervision of only the first ACT dose at the point of screening, this intervention would still be relatively expensive in comparison with alternative school-based malaria control interventions. School-based IPT using SP-AQ was estimated to cost $3.17 per child per year for three IPT rounds (adjusted to US$ 2010) [348,473] although using ACT in place of SP-AQ for IPT, as is now required in east Africa, would increase this cost. Further comparisons with vector control methods include that with LLIN distribution, where estimates range from $1.38 to $1.90 per treated net year of protection (US$ 2005), IRS, where estimates ranged from US$ 3.27 to US$3.90 per person-year of protection (US$ 2005)

[474], and larval source management, where estimates ranged from US$0.94 to US$2.50 (US$ 2006) per person-year of protection [240,475].

Whereas interventions such as IPT and seasonal malaria chemoprevention (SMC) involve administration of treatment presumptively, in the case of IST where treatment is only provided for individuals detected as infected by screening, the intervention is highly dependent on the accuracy of the screening tool for detecting all infections. Thus performance of the diagnostic screening tool (RDT) was explored as a possible explanation for the lack of impact observed. Findings presented in Chapter 5 indicated support for the use of RDTs over expert microscopy for such a large-scale screening intervention in this population of predominantly asymptomatic school children. The use of latent class analysis (LCA) allowed a robust comparison of the two methods, without requiring the often flawed assumption of a perfect reference standard. Although LCA resulted in superior sensitivity estimates of RDTs over microscopy at all screening rounds (ranging between 6% and 60% greater sensitivity at rounds one and three respectively), the extensive variation in the estimates of RDT sensitivity by screening round – assumed to reflect seasonality - emphasised the need to consider the influence of such variation on the impact of an IST intervention.

Additionally, the increased performance of the screening tool in the wet season emphasised the relative importance of seasonality on the scheduling of screening rounds, whether for control or surveillance programmes, even in a perennial transmission settings such as this. The apparent increased sensitivity of both RDTs and microscopy in the rainy season, was likely to be related to the higher parasite density found in the rainy seasons when transmission is more intense [374]. Overall the predominance of low-intensity infections in school children in low-moderate transmission settings, such as found in this study area, is consistent with evidence from various countries where infections are more frequently low density in low transmission regions [71], possibly related to fewer multiple infections which often occur in higher transmission settings. Abundance of low parasite density infections speak to the need for more sensitive molecular

diagnostic tools such as LAMP or PCR for screening and although this need will only increase in importance in moving towards elimination [71,195], these methods are not currently feasible for field-based screening due to high cost and operational restrictions. A recent study in which a mobile laboratory for conducting real time PCR in the field was piloted in Cambodia, demonstrated that despite proving operationally possible, such a facility was associated with high costs, of US$200,000 to set up the mobile laboratory and US$2.75 per sample screened (real time PCR and DNA extraction) and US$3.75 for species identification [408].

The finding, in Chapter 4, that individuals who received multiple AL treatments during IST rounds were at significantly higher risk of *P. falciparum* infection when followed up at 12 and 24 months, highlighted the role of re-infection between screening rounds in contributing to the lack of beneficial impact observed. Analyses presented in Chapters 6 and 7 further explored the extent of heterogeneity and re-infection at both the level of the school and individual. The results from Chapter 6 showed remarkable temporal stability in the spatial heterogeneity observed in Chapter 3, in spite of periodic treatment of infection. The strong positive dose-response relationship observed between *Plasmodium* prevalence and time since last screening and treatment round, provided important evidence for the influence of duration of treatment interval in relation to the potential for re-infection and the need for high local coverage of the intervention to reduce re-infection.

The use of geostatistical modelling to explore the spatio-temporal patterns observed, provided further insight into both the spatial structure of *P. falciaprum* infection risk and the associated environmental and socioecomomic correlates. A range of factors including high rainfall and decreased distance to temporary water, as well as increased school-level net use and SES were significantly associated with school-level infection. From these school-level analyses presented in Chapter 6 it was not possible to establish whether the stability in transmission was primarily due to re-infections in certain children over time or new infections in alternative children within the same school. However, the findings from Chapter 7 demonstrated overdispersion of repeated

Plasmodium infections among children, indicating the presence of heterogeneity in individual risk in this setting. This aggregation of infections within certain children, despite repeated AL treatments, adds weight to the role of re-infection at the individual-level in contributing to the lack of differential beneficial impact of the intervention in those children at highest risk.

However the findings of these analyses must be considered in the context of methodological limitations discussed in detail in each chapter. In brief, the use of a parent questionnaire, with no confirmation made of responses through household visits may have led to information bias when collecting risk factor information, however, the collection of this data prior to group allocation should have eliminated the issue of systematically different collection across groups. When considering impact of the IST intervention, the unblinded nature of the intervention had the potential to cause selection and attrition bias due to some parents' and children's fears of the finger prick and RDT. All possible steps to minimise these biases were taken during the sensitisation, recruitment and consent process, including public randomisation and allocation concealment until after the consent process, and an emphasis on community participation and strong community engagement throughout [294]. Furthermore as treatment efficacy was not evaluated within this study and with research in coastal Kenya indicating apparent recrudescence following AL treatment doubled from 6% to 15% between 2005 and 2008 [350], it is possible that a proportion of the low density infections in children treated with ACT during IST could reflect treatment failure and very low-level recrudescent infections rather than the assumed re-infections. The potential presence of lower-density recrudescent infections in the IST intervention group, as opposed to the control group may, by virtue of the limited sensitivity of microscopy, have led to differential misclassification of the *P. falciparum* infection outcome between control and intervention groups. Moreover, recrudescent infections would have affected the findings in Chapters 6 and 7 of the aggregation of infections in a proportion of schools and individuals. Furthermore the examination of risk at the school-level, when infection occurs at the household level, compounded by the use of reasonably large-scale spatial processes and environmental data with relatively small-scale distances between schools may have obscured

important relationships between environmental exposures and *P. falciparum* infection. Finally the inclusion of results of a third diagnostic test for the estimation of diagnostic performance in Chapter 5 would have strengthened the analyses through the addition of degrees of freedom, increasing model identifiability.

Whilst overall the findings of this thesis have indicated that school-based IST for malaria is an inappropriate strategy for low-to-moderate transmission settings, this does not mean that either school-based approaches to malaria control, or screening and treatment, should be altogether disregarded in such transmission settings. In working towards an elimination agenda, the focus of malaria control has moved from targeting interventions to groups at highest risk of morbidity and mortality, to targeting all individuals within specific geographical locations of high transmission intensity. The collective evidence from Chapters 4, 6 and 7 of the aggregation of repeated infections in certain schools and even in certain individuals, in spite of repeated treatment, has promising implications for the future application of school-level screening as a component of a wider strategy of targeted malaria control.

In particular, the substantial geographical heterogeneity observed alongside the rapid rebound of school-level infection in spite of treatment, suggests a role for sustained focal coverage of malaria interventions. With consistently high infection prevalence observed to be relatively restricted to a few schools, targeting of interventions to high-risk schools could be a more effective and cost effective strategy than providing interventions to all schools in a given district. The use of rapid school screening surveys carried out to initially identify these high-risk schools would seem to be a practical strategy. Furthermore, expanding interventions such as focal community screen and treat [223], or mass drug administration to the communities served by the schools experiencing high transmission, would considerably increase the effectiveness of focal control, limiting the rate of re-infection. The significant associations of environmental factors such as distance to temporary waterbodies observed in Chapter 6, suggest that the

incorporation of environmental vector control methods, such as community-based environmental management and larval source management, could further enhance a treatment-based strategy [476,477]. In turn, school-level screenings conducted at annual intervals could prove useful for monitoring the impact of such interventions.

The residual individual-level risk observed in Chapter 7, after accounting for transmission exposure and individual-level exposures, such as age and net use, pointed to an increased susceptibility to re-infection. Whether this risk resulted from genetic susceptibility or unobserved heterogeneity in the form of localised household-level exposure, the findings suggest a programme of reactive screen and treat could be successful in this setting. The reactive screening of households of clinical malaria cases identified in clinics has proven a useful strategy in low transmission settings [193,194]. Furthermore, a precedent for the use of school screenings in actively detecting index cases has been set in other parasitic infections such as schistosomiasis and lymphatic filariasis, where reactive screening of households has been conducted on the basis of infected children identified in school surveys [478,479].

8.3 FUTURE DIRECTIONS

Whilst school screenings might prove useful for identifying and targeting interventions towards high transmission schools and their surrounding communities, and for identifying and targeting interventions at infected children and their surrounding family members, future work, as discussed below, is required in order to refine both strategies, as well as the process of school-level screening as a whole.

The use of schools as a platform from which to screen children for informing decisions on targeted community control depends on whether the school *Plasmodium* infection prevalence is representative of that in the surrounding community. Previous studies have shown school

prevalence of infection to be broadly representative of the communities they serve in the case of schistosomiasis, soil transmitted helminths and crucially *P. falciparum* infection [356,480,481]. However, further research is necessary to elucidate whether such a targeted approach can simultaneously tackle clinical cases. For instance, a matched comparison between clinical cases from health facilities records corresponding to school catchment areas in which school surveys are conducted, would increase understanding of the relationship between clinical episodes in the wider community and school-level asymptomatic infection.

While the findings of Chapter 7 indicate a possible role for reactive screening and treatment of households, additional analyses of the spatial heterogeneity of infection within school catchment areas would add additional weight to this suggestion. Recent evidence from Bejon *et al.* [227] depicted the presence of hotspots within community hotspots in relation to febrile malaria, detecting clusters at a resolution as high as the household. The investigation of the heterogeneity in risk of *P. falciparum* infection between households within school catchment areas would provide important additional information on whether asymptomatically-infected children would reliably lead to other infected individuals in and around their compound for reactive control measures. At the same time, inclusion of high resolution satellite data on factors such local waterbodies would allow distance of household to local waterbody to be estimated and this could help tease out whether the excess risk of repeated infection observed in Chapter 7 was due to genetic susceptibility or unobserved heterogeneity in the form of fine- scale environmental exposure. Furthermore, ground surveys conducted in the study area in which both temporary and permanent waterbodies are mapped would enable more accurate estimation of distance of schools and households from potential breeding sites. The addition of entomological data collection to establish the effectiveness of the breeding sites through larval surveys would further strengthen conclusions of the over-dispersion of transmission within small geographical areas [464].

In relation to the use of screening and treatment delivered through communities and schools, a number of modifications could be considered, which might improve the impact. In order to reduce the rate of re-infection and new infections between screening rounds, a closer match between the period of post-treatment prophylaxis and screening interval should be explored. This could be achieved by shortening of the screening interval. However, this would have prohibitive operational and cost implications on a large scale as indicated in section 8.2, where decreasing the treatment interval to two months would result in a cost of US$37.44 per child screened per year. An alternative would be the use of antimalarials with an extended post-treatment prophylactic period. Dihydroartemisinin Piperaquine (DP), currently serving as the second line therapy in Kenya, would be a potentially viable option having now been prequalified and recommended by WHO since 2011. DP was recently found successful in reducing parasitaemia in the context of IPT with a three month treatment interval in a high transmission setting in Uganda [35]. No examination of treatment efficacy was performed in this current study. However, given the use of directly observed treatment for three of the six doses of AL in this trial, it would be expected efficacy would be higher than if all six doses were unsupervised. Before wide-scale use of screening and treatment in schools and communities, it may be appropriate to conduct research into treatment efficacy of AL or DP as provided through this approach, in an operational setting.

Given the predominance of low density *Plasmodium* infections detected in this setting, there may be benefit to incorporating the use of a molecular detection method in a future school survey in the region, in order to establish the level of subpatent infections present and the influence such findings would have on the impact of future control strategies in the area. However, this would provide useful contextual information and would not currently be advised for routine screenings.

IST as implemented in this study required the teachers to perform an essential facilitation role through organising the children for IST by the health team on the screening days. However,

qualitative research indicated that on balance community members would be happy to see teachers taking on a greater role, for example in supervising the subsequent doses of AL on days two and three [347]. Moreover, an ongoing study in southern Malawi is evaluating the use of teachers in school-based case management for malaria, in which between two and four teachers per school have been trained to diagnose uncomplicated malaria using RDTs and treat with ACTs [209]. An evaluation of the seven day training workshop indicated that teachers could provide a safe and accurate service. However, were teachers to conduct school-based screening, careful consideration of role conflicts and increased burden on already stretched teacher workloads would be required as unlike case management, where only children reporting with symptoms are tested, all children in school would be tested and treated if parasitaemic at the expense of teaching time.

Overall, there remains a requirement for additional research into alternative strategies for school-based malaria control and tackling the burden of anaemia, especially in such a locally heterogeneous setting. The high prevalence of anaemia (45.3%) with a multi-factorial aetiology in school children in this coastal region calls for increased investigation into integrated school-health programmes tackling geohelminth infections, urinary schistosomiasis, school feeding and nutrient supplementation in addition to malaria control. Such integrated school-health programmes could build on the success of the integrated child health programme delivering health messages, vitamin A supplementation and immunizations through biannual child health days [482]. The government approval of the use of DP for IPT in specific schools targeted for treatment on the basis of school level screenings could allow this as a feasible alternative to screening and treatment as part of a comprehensive suite of interventions on a focal scale. The staging of teacher-led school-health days in which albendazole praziquantel and DP are administered to all children, in addition to workshops run on topics such as long-lasting insecticidal net care, and messages of good water sanitation and hygiene practices, would benefit from economies of scope. The implementation of an effective home-grown school feeding programme throughout the year would further strengthen this package of services [483].

As is the case with any method of control or surveillance delivered through schools, success is reliant on high levels of enrolment and attendance. Despite substantial improvements, school enrolment remains highly variable at multiple scales [484]. Further analyses into the influence of such variation in levels of enrolment and attendance on the impact of school-based malaria control and surveillance are warranted. This is especially true for screening and treatment interventions, reliant on identifying and treating infected individuals [485].

8.4 CONCLUSIONS

In analysing the heterogeneities in the impact and process of school-based IST for malaria control in a low-to-moderate transmission setting, this thesis has highlighted several important issues. Marked heterogeneity in transmission was exhibited in this region. However findings indicated that in such a setting, IST as implemented in the study, provided no health or education benefits to school children, even to those in schools with high *Plasmodium* prevalence, where children benefited from AL treatment. The lack of both overall and differential impact suggests school-based IST is not an appropriate strategy for low-to-moderate transmission settings. The school-level spatial heterogeneity, stable over time, and the aggregation of repeated infections in certain children indicated high re-infection as critical contributor to lack of impact. Encouragingly, however, these findings also have important implications for future malaria control strategies in low-to-moderate transmission settings. The potential for school-level screening to (a) identify high risk schools and their surrounding communities for the implementation of a targeted suite of interventions and (b) identify infected children in order to conduct reactive screening and treatment of additional members of the household, should be explored.

References

1. Feachem RG, Phillips AA, Hwang J, Cotter C, Wielgosz B, Greenwood BM, Sabot O, Rodriguez MH, Abeyasinghe RR, Ghebreyesus TA, Snow RW (2010) Shrinking the malaria map: progress and prospects. Lancet 376: 1566-1578.
2. O'Meara WP, Mangeni JN, Steketee R, Greenwood B (2010) Changes in the burden of malaria in sub-Saharan Africa. Lancet Infect Dis 10: 545-555.
3. Ceesay SJ, Casals-Pascual C, Erskine J, Anya SE, Duah NO, Fulford AJC, Sesay SSS, Abubakar I, Dunyo S, Sey O, Palmer A, Fofana M, Corrah T, Bojang KA, Whittle HC, Greenwood BM, Conway DJ (2008) Changes in malaria indices between 1999 and 2007 in The Gambia: a retrospective analysis. Lancet 372: 1545-1554.
4. Ceesay SJ, Casals-Pascual C, Nwakanma DC, Walther M, Gomez-Escobar N, Fulford AJC, Takem EN, Nogaro S, Bojang KA, Corrah T, Jaye MC, Taal MA, Sonko AAJ, Conway DJ (2010) Continued decline of malaria in The Gambia with implications for elimination. PloS one 5: e12242.
5. O'Meara WP, Bejon P, Mwangi TW, Okiro EA, Peshu N, Snow RW, Newton CRJC, Marsh K (2008) Effect of a fall in malaria transmission on morbidity and mortality in Kilifi, Kenya. Lancet 372: 1555-1562.
6. Kalayjian BC, Malhotra I, Mungai PL, Holding PA, King CL (2013) Marked decline in malaria prevalence among pregnant women and their offspring from 1996 to 2010 on the south kenyan coast. Am J Trop Med Hyg 89: 1129-1134.
7. Aregawi MW, Ali AS, Al-mafazy A-w, Molteni F, Katikiti S, Warsame M, Njau RJA, Komatsu R, Korenromp E, Hosseini M, Low-Beer D, Bjorkman A, D'Alessandro U, Coosemans M, Otten M (2011) Reductions in malaria and anaemia case and death burden at hospitals following scale-up of malaria control in Zanzibar, 1999-2008. Malar J 10: 46.
8. Chizema-Kawesha E, Miller JM, Steketee R, Mukonka V, Mukuka C, Mohamed AD, Miti SK, Campbell CC (2010) Scaling Up Malaria Control in Zambia: Progress and Impact 2005–2008. Am J Trop Med Hyg 83: 480-488.
9. World Health Organization (2013) World malaria report: 2013. Geneva: World Health Organization.
10. Steketee RW, Campbell CC (2010) Impact of national malaria control scale-up programmes in Africa: magnitude and attribution of effects. Malar J 9: 299.
11. Hay SI, Guerra CA, Tatem AJ, Atkinson PM, Snow RW (2005) Urbanization, malaria transmission and disease burden in Africa. Nat Rev Microbiol 3: 81-90.

12. Tusting LS, Willey B, Lucas H, Thompson J, Kafy HT, Smith R, Lindsay SW (2013) Socioeconomic development as an intervention against malaria: a systematic review and meta-analysis. Lancet 382: 963-972.

13. Gething PW, Patil AP, Smith DL, Guerra CA, Elyazar IRF, Johnston GL, Tatem AJ, Hay SI (2011) A new world malaria map: *Plasmodium falciparum* endemicity in 2010. Malar J 10: 378.

14. Delacollette C, Rietveld A (2006) WHO GMP-informal consultation on malaria elimination: setting up the WHO agenda. World Health Organization, Tunis.

15. Roll Back Malaria Partnership (2008) A Global Malaria Action Plan Geneva: World Health Organisation,.

16. Roll Back Malaria (2005) Global Strategic Plan 2005-2015. Geneva: WHO.

17. World Health Organisation (2012) Test. Treat. Track. Scaling up diagnostic testing, treatment and surveillance. Geneva: WHO.

18. Aregawi M, Cibulskis R, Otten M, Williams R, Dye C (2008) World Malaria Report. Geneva: World Health Organization.

19. Smith DL, Guerra CA, Snow RW, Hay SI (2007) Standardizing estimates of the *Plasmodium falciparum* parasite rate. Malar J 6: 131.

20. Brooker S, Kolaczinski JH, Gitonga CW, Noor AM, Snow RW (2009) The use of schools for malaria surveillance and programme evaluation in Africa. Malar J 8: 231.

21. Drakeley C, Akim N, Sauerwein R, Greenwood B, Targett G (2000) Estimates of the infectious reservoir of *Plasmodium falciparum* malaria in The Gambia and in Tanzania. Trans R Soc Trop Med Hyg 94: 472-476.

22. Bousema JT, Gouagna LC, Drakeley CJ, Meutstege AM, Okech BA, Akim IN, Beier JC, Githure JI, Sauerwein RW (2004) *Plasmodium falciparum* gametocyte carriage in asymptomatic children in western Kenya. Malar J 3: 18.

23. Baliraine FN, Afrane YA, Amenya DA, Bonizzoni M, Menge DM, Zhou G, Zhong D, Vardo-Zalik AM, Githeko AK, Yan G (2009) High prevalence of asymptomatic *Plasmodium falciparum* infections in a highland area of western Kenya: a cohort study. J Infect Dis 200: 66-74.

24. Ogutu B, Tiono AB, Makanga M, Premji Z, Gbadoe AD, Ubben D, Marrast AC, Gaye O (2010) Treatment of asymptomatic carriers with artemether-lumefantrine: an opportunity to reduce the burden of malaria? Malar J 9: 30.

25. Snow RW, Omumbo JA, Lowe B, Molyneux CS, Obiero JO, Palmer A, Weber MW, Pinder M, Nahlen B, Obonyo C, Newbold C, Gupta S, Marsh K (1997) Relation between severe malaria morbidity in children and level of *Plasmodium falciparum* transmission in Africa. Lancet 349: 1650-1654.

26. Griffin JT, Ferguson NM, Ghani AC (2014) Estimates of the changing age-burden of *Plasmodium falciparum* malaria disease in sub-Saharan Africa. Nat Commun 5.
27. Noor A, Kirui V, Brooker S, Snow R (2009) The use of insecticide treated nets by age: implications for universal coverage in Africa. BMC Public Health 9: 369.
28. Pullan RL, Bukirwa H, Staedke SG, Snow RW, Brooker S (2010) *Plasmodium* infection and its risk factors in eastern Uganda. Malar J 9: 2.
29. Kilian A, Koenker H, Baba E, Onyefunafoa EO, Selby RA, Lokko K, Lynch M (2013) Universal coverage with insecticide-treated nets - applying the revised indicators for ownership and use to the Nigeria 2010 malaria indicator survey data. Malar J 12: 314.
30. UNESCO, UNICEF, World Bank, World Health Organisation, Education International (2000) Focussing Resources on Effective School Health: a FRESH start to enhancing the quality and equity of education. World Education Forum Final Report. . World Education Forum - Dakar, Senegal. .
31. Division of Malaria Control Ministry of Public Health and Sanitation (2009) National Malaria Strategy 2009-2017. Kenya NMCP.
32. Jukes MC, Drake LJ, Bundy DA (2008) School health, nutrition and education for all: levelling the playing field. Wallingford, UK: CABI.
33. Clarke SE, Jukes MCH, Njagi JK, Khasakhala L, Cundill B, Otido J, Crudder C, Estambale BBA, Brooker S (2008) Effect of intermittent preventive treatment of malaria on health and education in schoolchildren: a cluster-randomised, double-blind, placebo-controlled trial. Lancet 372: 127-138.
34. Brooker S (2009) Malaria Control in Schools: A toolkit on effective education sector responses to malaria in Africa. UK.
35. Nankabirwa JI, Wandera B, Amuge P, Kiwanuka N, Dorsey G, Rosenthal PJ, Brooker SJ, Staedke SG, Kamya MR (2014) Impact of Intermittent Preventive Treatment With Dihydroartemisinin-Piperaquine on Malaria in Ugandan Schoolchildren: A Randomized, Placebo-Controlled Trial. Clin Inf Dis 58: 1404-1412.
36. Pasha O, Del Rosso J, Mukaka M, Marsh D (2003) The effect of providing fansidar (sulfadoxine-pyrimethamine) in schools on mortality in school-age children in Malawi. Lancet 361: 577-578.
37. Nevill C, Lury J, Mosobo M, Watkins H, Watkins W (1994) Daily chlorproguanil is an effective alternative to daily proguanil in the prevention of *Plasmodium falciparum* malaria in Kenya. Trans R Soc Trop Med Hyg 88: 319-320.
38. Hogh B, Thompson R, Lobo V, Dgedge M, Dziegiel M, Borre M, Gottschau A, Streat E, Schapira A, Barreto J (1994) The influence of Maloprim chemoprophylaxis on cellular and humoral immune responses to *Plasmodium falciparum* asexual blood stage antigens

in schoolchildren living in a malaria endemic area of Mozambique. Acta Trop 57: 265-277.

39. Weiss WR, Oloo AJ, Johnson A, Koech D, Hoffman SL (1995) Daily primaquine is effective for prophylaxis against falciparum malaria in Kenya: comparison with mefloquine, doxycycline, and chloroquine plus proguanil. J Infect Dis 171: 1569-1575.
40. Barger B, Maiga H, Traore OB, Tekete M, Tembine I, Dara A, Traore ZI, Gantt S, Doumbo OK, Djimde AA (2009) Intermittent preventive treatment using artemisinin-based combination therapy reduces malaria morbidity among school-aged children in Mali. Trop Med Int Health 14: 784-791.
41. Noor AM, Kinyoki DK, Mundia CW, Kabaria CW, Mutua JW, Alegana VA, Fall IS, Snow RW (2014) The changing risk of *Plasmodium falciparum* malaria infection in Africa: 2000–10: a spatial and temporal analysis of transmission intensity. Lancet 383: 1739-1747.
42. Colbourne MJ (1955) The effect of malaria supression in a group of Accra school children Trans R Soc Trop Med Hyg 49: 356-369.
43. Cox-Singh J, Davis TM, Lee KS, Shamsul SS, Matusop A, Ratnam S, Rahman HA, Conway DJ, Singh B (2008) *Plasmodium knowlesi* malaria in humans is widely distributed and potentially life threatening. Clin Infect Dis 46: 165-171.
44. Trager W, Rudzinska MA, Bradbury PC (1966) The fine structure of *Plasmodium falciparum* and its host erythrocytes in natural malarial infections in man. Bull World Health Organ 35: 883.
45. Ponsford MJ, Medana IM, Prapansilp P, Hien TT, Lee SJ, Dondorp AM, Esiri MM, Day NP, White NJ, Turner GD (2012) Sequestration and microvascular congestion are associated with coma in human cerebral malaria. J Infect Dis 205: 663-671.
46. Depinay J-MO, Mbogo CM, Killeen G, Knols B, Beier J, Carlson J, Dushoff J, Billingsley P, Mwambi H, Githure J (2004) A simulation model of African *Anopheles* ecology and population dynamics for the analysis of malaria transmission. Malar J 3: 29.
47. Highton R, Bryan JH, Boreham P, Chandler J (1979) Studies on the sibling species *Anopheles gambiae* Giles and *Anopheles arabiensis* Patton (Diptera: *Culicidae*) in the Kisumu area, Kenya. Bull Entomol Res 69: 43-53.
48. Centers for Disease Control and Prevention (2010) Malaria: Biology. In: CDC, editor. Global Health - Division of Parasitic Diseases and Malaria,. Atlanta, GA, USA: Centers for Disease Control and Prevention,.
49. Greenwood BM, Fidock DA, Kyle DE, Kappe SH, Alonso PL, Collins FH, Duffy PE (2008) Malaria: progress, perils, and prospects for eradication. J Clin Invest 118: 1266-1276.
50. White NJ, Pukrittayakamee S, Hien TT, Faiz MA, Mokuolu OA, Dondorp AM (2014) Malaria. Lancet 383: 723-735.

51. Greenwood BM (2008) Control to elimination: implications for malaria research. Trends Parasitol 24: 449-454.

52. Smith T, Felger I, Tanner M, Beck HP (1999) Premunition in *Plasmodium falciparum* infection: insights from the epidemiology of multiple infections. Trans R Soc Trop Med Hyg 93 Suppl 1: 59-64.

53. Molyneux M (1989) Malaria--clinical features in children. J R Soc Med 82: 35.

54. Menendez C, Fleming AF, Alonso PL (2000) Malaria-related anaemia. Parasitol Today 16: 469-476.

55. Pagnoni F, Convelbo N, Tiendrebeogo J, Cousens S, Esposito F (1997) A community-based programme to provide prompt and adequate treatment of presumptive malaria in children. Trans R Soc Trop Med Hyg 91: 512-517.

56. Gosling RD, Drakeley CJ, Mwita A, Chandramohan D (2008) Presumptive treatment of fever cases as malaria: help or hindrance for malaria control? Malar J 7: 132.

57. Holding PA, Snow RW (2001) Impact of *Plasmodium falciparum* malaria on performance and learning: review of the evidence. Am J Trop Med Hyg 64: 68-75.

58. Fernando SD, Rodrigo C, Rajapakse S (2010) The 'hidden' burden of malaria: cognitive impairment following infection. Malar J 9: 366.

59. Snow RW (2000) The burden of malaria: understanding the balance between immunity, public health and control. J Med Microbiol 49: 1053-1055.

60. World Health Organization (2000) Severe falciparum malaria. Trans R Soc Trop Med Hyg 94: 1-90.

61. Carter JA, Ross AJ, Neville BG, Obiero E, Katana K, Mung'ala-Odera V, Lees JA, Newton CR (2005) Developmental impairments following severe falciparum malaria in children. Trop Med Int Health 10: 3-10.

62. Boivin MJ, Bangirana P, Byarugaba J, Opoka RO, Idro R, Jurek AM, John CC (2007) Cognitive impairment after cerebral malaria in children: a prospective study. Pediatrics 119: e360-366.

63. Snow RW, Craig M, Deichmann U, Marsh K (1999) Estimating mortality, morbidity and disability due to malaria among Africa's non-pregnant population. Bull World Health Organ 77: 624-640.

64. Gupta S, Snow RW, Donnelly CA, Marsh K, Newbold C (1999) Immunity to non-cerebral severe malaria is acquired after one or two infections. Nat Med 5: 340-343.

65. Stevenson MM, Riley EM (2004) Innate immunity to malaria. Nat Rev Immunol 4: 169-180.

66. Bejon P, Warimwe G, Mackintosh CL, Mackinnon MJ, Kinyanjui SM, Musyoki JN, Bull PC, Marsh K (2009) Analysis of immunity to febrile malaria in children that distinguishes immunity from lack of exposure. Infect Immun 77: 1917-1923.

67. Alves FP, Durlacher RR, Menezes MJ, Krieger H, Silva LHP, Camargo EP (2002) High prevalence of asymptomatic *Plasmodium vivax* and *Plasmodium falciparum* infections in native Amazonian populations. Am J Trop Med Hyg 66: 641-648.

68. Laishram DD, Sutton PL, Nanda N, Sharma VL, Sobti RC, Carlton JM, Joshi H (2012) The complexities of malaria disease manifestations with a focus on asymptomatic malaria. Malar J 11: 29.

69. Schneider P, Bousema JT, Gouagna LC, Otieno S, Van de Vegte-Bolmer M, Omar SA, Sauerwein RW (2007) Submicroscopic *Plasmodium falciparum* gametocyte densities frequently result in mosquito infection. Am J Trop Med Hyg 76: 470-474.

70. Ouédraogo AL, Bousema T, Schneider P, de Vlas SJ, Ilboudo-Sanogo E, Cuzin-Ouattara N, Nébié I, Roeffen W, Verhave JP, Luty AJ (2009) Substantial contribution of submicroscopical *Plasmodium falciparum* gametocyte carriage to the infectious reservoir in an area of seasonal transmission. PloS one 4: e8410.

71. Okell LC, Ghani AC, Lyons E, Drakeley CJ (2009) Submicroscopic infection in *Plasmodium falciparum*-endemic populations: a systematic review and meta-analysis. J Infect Dis 200: 1509-1517.

72. Nkuo-Akenji TK, Chi PC, Cho JF, Ndamukong KKJ, Sumbele I (2006) Malaria and helminth co-infection in children living in a malaria endemic setting of mount Cameroon and predictors of anemia. J Parasitol 92: 1191-1195.

73. Olsen A, Magnussen P, Ouma J, Andreassen J, Friis H (1998) The contribution of hookworm and other parasitic infections to haemoglobin and iron status among children and adults in western Kenya. Trans R Soc Trop Med Hyg 92: 643-649.

74. Kurtzhals JA, Addae MM, Akanmori BD, Dunyo S, Koram KA, Appawu MA, Nkrumah FK, Hviid L (1999) Anaemia caused by asymptomatic *Plasmodium falciparum* infection in semi-immune African schoolchildren. Trans R Soc Trop Med Hyg 93: 623-627.

75. Koukounari A, Estambale B, Kiambo Njagi J, Cundill B, Ajanga A, Crudder C, Otido J, Jukes MC, Clarke SE, Brooker S (2008) Relationships between anaemia and parasitic infections in Kenyan schoolchildren: a Bayesian hierarchical modelling approach. Int J Parasitol 38: 1663-1671.

76. Brooker S, Akhwale W, Pullan R, Estambale B, Clarke SE, Snow RW, Hotez PJ (2007) Epidemiology of *Plasmodium*-helminth co-infection in Africa: populations at risk, potential impact on anemia and prospects for combining control. Am J Trop Med Hyg 77: 88.

77. Anstey NM, Granger DL, Hassanali MY, Mwaikambo ED, Duffy PE, Weinberg JB (1999) Nitric oxide, malaria, and anemia: inverse relationship between nitric oxide production and hemoglobin concentration in asymptomatic, malaria-exposed children. Am J Trop Med Hyg 61: 249-252.

78. Helleberg M, Goka BQ, Akanmori BD, Obeng-Adjei G, Rodriques O, Kurtzhals JAL (2005) Bone marrow suppression and severe anaemia associated with persistent *Plasmodium falciparum* infection in African children with microscopically undetectable parasitaemia. Malar J 4: 56.

79. Verhoef H, West CE, Ndeto P, Burema J, Beguin Y, Kok FJ (2001) Serum transferrin receptor concentration indicates increased erythropoiesis in Kenyan children with asymptomatic malaria. The American journal of clinical nutrition 74: 767-775.

80. Verhoef H, West CE, Kraaijenhagen R, Nzyuko SM, King R, Mbandi MM, van Laatum S, Hogervorst R, Schep C, Kok FJ (2002) Malarial anemia leads to adequately increased erythropoiesis in asymptomatic Kenyan children. Blood 100: 3489-3494.

81. Cercamondi CI, Egli IM, Ahouandjinou E, Dossa R, Zeder C, Salami L, Tjalsma H, Wiegerinck E, Tanno T, Hurrell RF, Hounhouigan J, Zimmermann MB (2010) Afebrile *Plasmodium falciparum* parasitemia decreases absorption of fortification iron but does not affect systemic iron utilization: a double stable-isotope study in young Beninese women. Am J Clin Nutr 92: 1385-1392.

82. Verhoef H (2010) Asymptomatic malaria in the etiology of iron deficiency anemia: a malariologist's viewpoint. Am J Clin Nutr 92: 1285-1286.

83. Anderson R, May R (1992) Infectious Disease of Humans: Dynamics and Control; OUP, editor. Oxford.

84. Macdonald G (1956) Epidemiological basis of malaria control. Bull World Health Organ 15: 613-626.

85. Hay SI, Rogers DJ, Toomer JF, Snow RW (2000) Annual *Plasmodium falciparum* entomological inoculation rates (EIR) across Africa: literature survey, Internet access and review. Trans R Soc Trop Med Hyg 94: 113-127.

86. Hay SI, Guerra CA, Gething PW, Patil AP, Tatem AJ, Noor AM, Kabaria CW, Manh BH, Elyazar IRF, Brooker S, Smith DL, Moyeed RA, Snow RW (2009) A world malaria map: *Plasmodium falciparum* endemicity in 2007. PLoS Med 6: e100048.

87. Smith DL, Dushoff J, Snow RW, Hay SI (2005) The entomological inoculation rate and *Plasmodium falciparum* infection in African children. Nature 438: 492-495.

88. Moody A (2002) Rapid diagnostic tests for malaria parasites. Clin Microbiol Rev 15: 66-78.

89. Hopkins H, González IJ, Polley SD, Angutoko P, Ategeka J, Asiimwe C, Agaba B, Kyabayinze DJ, Sutherland CJ, Perkins MD (2013) Highly sensitive detection of malaria parasitemia in a malaria-endemic setting: performance of a new loop-mediated isothermal amplification kit in a remote clinic in Uganda. J Infect Dis 208: 645-652.

90. Drakeley C, Cook J (2009) Chapter 5. Potential contribution of sero-epidemiological analysis for monitoring malaria control and elimination: historical and current perspectives. Adv Parasitol 69: 299-352.

91. Corran P, Coleman P, Riley E, Drakeley C (2007) Serology: a robust indicator of malaria transmission intensity? Trends Parasitol 23: 575-582.

92. Hay SI, Smith DL, Snow RW (2008) Measuring malaria endemicity from intense to interrupted transmission. Lancet Infect Dis 8: 369-378.

93. Woolhouse ME, Dye C, Etard J-F, Smith T, Charlwood J, Garnett G, Hagan P, Hii J, Ndhlovu P, Quinnell R (1997) Heterogeneities in the transmission of infectious agents: implications for the design of control programs. Proc Natl Acad Sci 94: 338-342.

94. Bousema T, Baidjoe A (2013) Heterogeneity in malaria transmission: underlying factors and implications for disease control. In: Takken W, Koenraadt CJM, editors. Ecology of parasite-vector interactions. The Netherlands: Wageningen Academic Publishers. pp. 197-220.

95. Clark TD, Greenhouse B, Njama-Meya D, Nzarubara B, Maiteki-Sebuguzi C, Staedke SG, Seto E, Kamya MR, Rosenthal PJ, Dorsey G (2008) Factors determining the heterogeneity of malaria incidence in children in Kampala, Uganda. J Infect Dis 198: 393-400.

96. Hansen E, Buckee CO (2013) Modeling the human infectious reservoir for malaria control: does heterogeneity matter? Trends Parasitol 29: 270-275.

97. Greenwood BM (1989) The microepidemiology of malaria and its importance to malaria control. Trans R Soc Trop Med Hyg 83: 25-29.

98. Breman JG (2001) Ears of the hippopotamus: manifestations, determinants, and estimates of the malaria burden. Am J Trop Med Hyg 64 1-11.

99. Bejon P, Williams TN, Liljander A, Noor AM, Wambua J, Ogada E, Olotu A, Osier FHA, Hay SI, Farnert A, Marsh K (2010) Stable and unstable malaria hotspots in longitudinal cohort studies in Kenya. PLoS Med 7: e1000304.

100. Bousema T, Drakeley C, Gesase S, Hashim R, Magesa S, Mosha F, Otieno S, Carneiro I, Cox J, Msuya E, Kleinschmidt I, Maxwell C, Greenwood B, Riley E, Sauerwein R, Chandramohan D, Gosling R (2010) Identification of hot spots of malaria transmission for targeted malaria control. J Infect Dis 201: 1764-1774.

101. Bejon P, Turner L, Lavstsen T, Cham G, Olotu A, Drakeley CJ, Lievens M, Vekemans J, Savarese B, Lusingu J, von Seidlein L, Bull PC, Marsh K, Theander TG (2011) Serological evidence of discrete spatial clusters of *Plasmodium falciparum* parasites. PloS one 6: e21711.

102. Bousema T, Griffin JT, Sauerwein RW, Smith DL, Churcher TS, Takken W, Ghani A, Drakeley C, Gosling R (2012) Hitting hotspots: spatial targeting of malaria for control and elimination. PLoS Med 9: e1001165.

103. Carter R, Mendis KN, Roberts D (2000) Spatial targeting of interventions against malaria. Bull World Health Organ 78: 1401-1411.

104. Carneiro I, Roca-Feltrer A, Griffin JT, Smith L, Tanner M, Schellenberg JA, Greenwood B, Schellenberg D (2010) Age-patterns of malaria vary with severity, transmission intensity and seasonality in sub-Saharan Africa: a systematic review and pooled analysis. PloS one 5: e8988.

105. Brasseur P, Badiane M, Cisse M, Agnamey P, Vaillant MT, Olliaro PL (2011) Changing patterns of malaria during 1996–2010 in an area of moderate transmission in southern Senegal. Malar J 10: 203.

106. Reyburn H, Mbatia R, Drakeley C, Bruce J, Carneiro I, Olomi R, Cox J, Nkya WM, Lemnge M, Greenwood BM, Riley EM (2005) Association of transmission intensity and age with clinical manifestations and case fatality of severe *Plasmodium falciparum* malaria. J Amer Med Assoc 293: 1461-1470.

107. Griffin JT, Hollingsworth TD, Okell LC, Churcher TS, White M, Hinsley W, Bousema T, Drakeley CJ, Ferguson NM, Basanez MG, Ghani AC (2010) Reducing *Plasmodium falciparum* malaria transmission in Africa: a model-based evaluation of intervention strategies. PLoS Med 7: e1000324.

108. Brooker S, Guyatt H, Omumbo J, Shretta R, Drake L, Ouma J (2000) Situation analysis of malaria in school-aged children in Kenya - what can be done? Parasitol Today 16: 183-186.

109. Bundy D, Lwin S, Osika J, McLaughlin J, Pannenborg C (2000) What should schools do about malaria? Parasitol Today 16: 181-182.

110. Clarke SE, Brooker S, Njagi JK, Njau E, Estambale B, Muchiri E, Magnussen P (2004) Malaria morbidity among school children living in two areas of contrasting transmission in western Kenya. Am J Trop Med Hyg 71: 732-738.

111. Lalloo DG, Olukoya P, Olliaro P (2006) Malaria in adolescence: burden of disease, consequences, and opportunities for intervention. Lancet Infect Dis 6: 780-793.

112. Hall A, Bobrow E, Brooker S, Jukes MC, Nokes K, Lambo JK, Guyatt HL, Bundy DA, Adjei S, Wen S-T (2001) Anaemia in schoolchildren in eight countries in Africa and Asia. Public Health Nutr 4: 749-756.

113. Stoltzfus RJ, Chwaya HM, Tielsch JM, Schulze KJ, Albonico M, Savioli L (1997) Epidemiology of iron deficiency anemia in Zanzibari schoolchildren: the importance of hookworms. Am J Clin Nutr 65: 153-159.

114. Al Serouri AW, Grantham-McGregor SM, Greenwood B, Costello A (2000) Impact of asymptomatic malaria parasitaemia on cognitive function and school achievement of schoolchildren in the Yemen Republic. Parasitol 121: 337-345.

115. Bustinduy AL, Parraga IM, Thomas CL, Mungai PL, Mutuku F, Muchiri EM, Kitron U, King CH (2013) Impact of polyparasitic infections on anemia and undernutrition among

Kenyan children living in a *Schistosoma haematobium*-endemic area. Am J Trop Med Hyg 88: 433-440.

116. Carneiro IA, Smith T, Lusingu JP, Malima R, Utzinger J, Drakeley CJ (2006) Modeling the relationship between the population prevalence of *Plasmodium falciparum* malaria and anemia. Am J Trop Med Hyg 75: 82-89.
117. Midzi N, Mtapuri-Zinyowera S, Mapingure MP, Sangweme D, Chirehwa MT, Brouwer KC, Mudzori J, Hlerema G, Mutapi F, Kumar N, Mduluza T (2010) Consequences of polyparasitism on anaemia among primary school children in Zimbabwe. Acta Trop 115: 103-111.
118. Brooker S, Miguel E, Moulin S, Louba A, Bundy D, Kremer M (2000) Epidemiology of single and multiple species of helminth infections among school children in Busia District, Kenya. East Afr Med J 77: 157-161.
119. Pullan R, Brooker S (2008) The health impact of polyparasitism in humans: are we under-estimating the burden of parasitic diseases? Parasitol 135: 783.
120. Best C, Neufingerl N, van Geel L, van den Briel T, Osendarp S (2010) The nutritional status of school-aged children: why should we care? Food Nutr Bull 31: 400-417.
121. Pullan RL, Gitonga C, Mwandawiro C, Snow RW, Brooker SJ (2013) Estimating the relative contribution of parasitic infections and nutrition for anaemia among school-aged children in Kenya: a subnational geostatistical analysis. BMJ Open 3.
122. Magalhães RJS, Langa A, Pedro JM, Sousa-Figueiredo JC, Clements AC, Nery SV (2013) Role of malnutrition and parasite infections in the spatial variation in children's anaemia risk in Northern Angola. Geospat Health 7: 341-354.
123. Geerligs PDP, Brabin BJ, Eggelte TA (2003) Analysis of the effects of malaria chemoprophylaxis in children on haematological responses, morbidity and mortality. Bull World Health Organ 81: 205-216.
124. Fernando D, de Silva D, Carter R, Mendis KN, Wickremasinghe R (2006) A randomized, double-blind, placebo-controlled, clinical trial of the impact of malaria prevention on the educational attainment of school children. Am J Trop Med Hyg 74: 386-393.
125. Leenstra T, Phillips-Howard PA, Kariuki SK, Hawley WA, Alaii JA, Rosen DH, Oloo AJ, Nahlen BL, Kager PA, ter Kuile FO (2003) Permethrin-treated bed nets in the prevention of malaria and anemia in adolescent schoolgirls in western Kenya. Am J Trop Med Hyg 68: 86-93.
126. Leenstra T, Kariuki SK, Kurtis JD, Oloo AJ, Kager PA, Ter Kuile FO (2004) Prevalence and severity of anemia and iron deficiency: cross-sectional studies in adolescent schoolgirls in western Kenya. Eur J Clin Nutr 58: 681-691.

127. Korenromp EL, Armstrong-Schellenberg JRM, Williams BG, Nahlen BL, Snow RW (2004) Impact of malaria control on childhood anaemia in Africa -- a quantitative review. Trop Med Int Health 9: 1050-1065.

128. Friedman JF, Kwena AM, Mirel LB, Kariuki SK, Terlouw DJ, Phillips-Howard PA, Hawley WA, Nahlen BL, Shi YP, Ter Kuile FO (2005) Malaria and nutritional status among pre-school children: results from cross-sectional surveys in western Kenya. Am J Trop Med Hyg 73: 698-704.

129. Takakura M, Uza M, Sasaki Y, Nagahama N, Phommpida S, Bounyadeth S, Kobayashi J, Toma T, Miyagi I (2001) The relationship between anthropometric indicators of nutritional status and malaria infection among youths in Khammouane Province, Lao PDR. Southeast Asian J Trop Med Public Health 32: 262-267.

130. Friedman JF, Kurtis JD, Ramadhan M, Opollo M, Lanar DE, Duffy PE (2003) Malaria is related to decreased nutritional status among male adolescents and adults in the setting of intense perennial transmission. J Infect Dis 188: 449-457.

131. Archibald HM, Bruce-Chwatt LJ (1956) Suppression of malaria with pyrimethamine in Nigerian school-children. Bull World Health Organ 15: 775.

132. Carter JA, Mung'ala-Odera V, Neville BG, Murira G, Mturi N, Musumba C, Newton CR (2005) Persistent neurocognitive impairments associated with severe falciparum malaria in Kenyan children. J Neurol Neurosurg Psychiatry 76: 476-481.

133. Holding PA, Kitsao-Wekulo PK (2004) Describing the burden of malaria on child development: what should we be measuring and how should we be measuring it? Am J Trop Med Hyg 71: 71-79.

134. Fernando D, de Silva D, Wickremasinghe R (2003) Short-term impact of an acute attack of malaria on the cognitive performance of schoolchildren living in a malaria-endemic area of Sri Lanka. Trans R Soc Trop Med Hyg 97: 633-639.

135. Fernando SD, Gunawardena DM, Bandara MR, De Silva D, Carter R, Mendis KN, Wickremasinghe AR (2003) The impact of repeated malaria attacks on the school performance of children. Am J Trop Med Hyg 69: 582-588.

136. Thuilliez J, Sissoko MS, Toure OB, Kamate P, Berthelemy JC, Doumbo OK (2010) Malaria and primary education in Mali: a longitudinal study in the village of Doneguebougou. Soc Sci Med 71: 324-334.

137. Kihara M, Carter JA, Newton CRJC (2006) The effect of *Plasmodium falciparum* on cognition: a systematic review. Trop Med Int Health 11: 386-397.

138. Nankabirwa J, Wandera B, Kiwanuka N, Staedke SG, Kamya MR, Brooker SJ (2013) Asymptomatic *Plasmodium* infection and cognition among primary schoolchildren in a high malaria transmission setting in Uganda. Am J Trop Med Hyg 88: 1102-1108.

139. Clarke SE, Rouhani S, Diarra S, Bamadio M, Jones R, Traore D, Jukes MCH, Thuillez J, Sacko M, Brooker S, Lee S, Roschnik N. Intermittent parasite clearance in schoolchildren: Impact on cognition in an area of highly seasonal transmission; 2013; Washington, DC. ASTMH 62nd Annual Meeting.

140. Thuilliez J (2010) Fever, malaria and primary repetition rates amongst school children in Mali: combining demographic and health surveys (DHS) with spatial malariological measures. Soc Sci Med 71: 314-323.

141. Kimbi HK, Awah NW, Ndamukong KJ, Mbuh JV (2005) Malaria infection and its consequences in school children. East Afr Med J: 92-97.

142. Trape J-F, Zoulani A, Quinet M (1987) Assessment of the incidence and prevalence of clinical malaria in semi-immune children exposed to intense and perennial transmission. Am J Epidemiol 126: 193-201.

143. Felger I, Maire M, Bretscher MT, Falk N, Tiaden A, Sama W, Beck HP, Owusu-Agyei S, Smith TA (2012) The dynamics of natural *Plasmodium falciparum* infections. PloS one 7: e45542.

144. Babiker HA, Gadalla AA, Ranford-Cartwright LC (2013) The role of asymptomatic *P. falciparum* parasitaemia in the evolution of antimalarial drug resistance in areas of seasonal transmission. Drug resistance updates : reviews and commentaries in antimicrobial and anticancer chemotherapy 16: 1-9.

145. Snow RW, Rowan KM, Lindsay SW, Greenwood BM (1988) A trial of bed nets (mosquito nets) as a malaria control strategy in a rural area of The Gambia, West Africa Trans R Soc Trop Med Hyg 82: 212-215.

146. D'Alessandro U, Olaleye B, Langerock P, Aikins M, Thomson M, Cham M, Greenwood B, McGuire W, Bennett S (1995) Mortality and morbidity from malaria in Gambian children after introduction of an impregnated bednet programme. Lancet 345: 479-483.

147. Lengeler C (2004) Insecticide-treated bed nets and curtains for preventing malaria (review). Cochrane Database Syst Rev: John Wiley & Sons, Ltd.

148. Phillips-Howard PA, Nahlen BL, Kolczak MS, Hightower AW, ter Kuile FO, Alaii JA, Gimnig JE, Arudo J, Vulule JM, Odhacha A, Kachur SP, Schoute E, Rosen DH, Sexton JD, Oloo AJ, Hawley WA (2003) Efficacy of permethrin-treated bed nets in the prevention of mortality in young children in an area of high perennial malaria transmission in western Kenya. Am J Trop Med Hyg 68: 23-29.

149. Eisele TP, Larsen D, Steketee RW (2010) Protective efficacy of interventions for preventing malaria mortality in children in *Plasmodium falciparum* endemic areas. Int J Epidemiol 39: i88-i101.

150. Fegan GW, Noor AM, Akhwale WS, Cousens S, Snow RW (2007) Effect of expanded insecticide-treated bednet coverage on child survival in rural Kenya: a longitudinal study. Lancet 370: 1035-1039.

151. Lim SS, Fullman N, Stokes A, Ravishankar N, Masiye F, Murray CJ, Gakidou E (2011) Net benefits: a multicountry analysis of observational data examining associations between insecticide-treated mosquito nets and health outcomes. PLoS Med 8: e1001091.

152. Mboera L, Kamugisha ML, Rumisha SF, Kisinza WN, Senkoro KP, Kitua AY (2008) Malaria and mosquito net utilisation among schoolchildren in villages with or without healthcare facilities at different altitudes in Iringa District, Tanzania. Afr Health Sci 8: 114-119.

153. Gitonga CW, Edwards T, Karanja PN, Noor AM, Snow RW, Brooker SJ (2012) *Plasmodium infection*, anaemia and mosquito net use among school children across different settings in Kenya. Trop Med Int Health 17: 858-870.

154. Noor AM, Moloney G, Borle M, Fegan GW, Shewchuk T, Snow RW (2008) The use of mosquito nets and the prevalence of *Plasmodium falciparum* infection in rural South Central Somalia. PloS one 3: e2081.

155. Noor AM, Amin AA, Akhwale WS, Snow RW (2007) Increasing coverage and decreasing inequity in insecticide-treated bed net use among rural Kenyan children. PLoS Med 4: e255.

156. Githinji S, Herbst S, Kistemann T, Noor AM (2010) Mosquito nets in a rural area of Western Kenya: ownership, use and quality. Malar J 9: 250.

157. Mabaso ML, Sharp B, Lengeler C (2004) Historical review of malarial control in southern African with emphasis on the use of indoor residual house-spraying. Trop Med Int Health 9: 846-856.

158. Sharp BL, Kleinschmidt I, Streat E, Maharaj R, Barnes KI, Durrheim DN, Ridl FC, Morris N, Seocharan I, Kunene S (2007) Seven years of regional malaria control collaboration—Mozambique, South Africa, and Swaziland. Am J Trop Med Hyg 76: 42.

159. Jima D, Getachew A, Bilak H, Steketee RW, Emerson PM, Graves PM, Gebre T, Reithinger R, Hwang J (2010) Malaria indicator survey 2007, Ethiopia: coverage and use of major malaria prevention and control interventions. Malar J 9: 10.1186.

160. Ratovonjato J, Randrianarivelojosia M, Rakotondrainibe ME, Raharimanga V, Andrianaivolambo L, Le Goff G, Rogier C, Ariey F, Boyer S, Robert V (2014) Entomological and parasitological impacts of indoor residual spraying with DDT, alphacypermethrin and deltamethrin in the western foothill area of Madagascar. Malar J 13: 21.

161. Kleinschmidt I, Schwabe C, Shiva M, Segura JL, Sima V, Mabunda SJA, Coleman M (2009) Combining indoor residual spraying and insecticide-treated net interventions. Am J Trop Med Hyg 81: 519-524.

162. Skarbinski J, Mwandama D, Wolkon A, Luka M, Jafali J, Smith A, Mzilahowa T, Gimnig J, Campbell C, Chiphwanya J (2012) Impact of indoor residual spraying with lambda-cyhalothrin on malaria parasitemia and anemia prevalence among children less than five years of age in an area of intense, year-round transmission in Malawi. Am J Trop Med Hyg 86: 997.

163. Pinder M, Jawara M, Jarju LBS, Salami K, Jeffries D, Adiamoh M, Bojang KA, Correa S, Kandeh B, Kaur H, Conway DJ, D'alessandro U, Lindsay SW (2014) Efficacy of indoor residual spraying with dichlorodiphenyltrichloroethane against malaria in Gambian communities with high usage of long-lasting insecticidal mosquito nets: a cluster-randomised controlled trial. Lancet pii: S0140-6736: 61007-61002.

164. Ranson H, N'Guessan R, Lines J, Moiroux N, Nkuni Z, Corbel V (2010) Pyrethroid resistance in African anopheline mosquitoes: what are the implications for malaria control? Trends Parasitol 27: 91-98.

165. N'Guessan R, Corbel V, Akogbeto M, Rowland M (2007) Reduced efficacy of insecticide treated nets and indoor residual spraying for malaria control in pyrethroid resistance area. Emerg Infect Dis 13: 199-206.

166. Asidi A, N'Guessan R, Akogbeto M, Curtis C, Rowland M (2012) Loss of household protection from use of insecticide-treated nets against pyrethroid-resistant mosquitoes, Benin. Emerg Infect Dis 18: 1101-1106.

167. Gatton ML, Chitnis N, Churcher TS, Donnelly MJ, Ghani AC, Godfray CJ, Gould F, Hastings I, Marshall J, Ranson H, Rowland M, Shaman J, Lindsay SW (2013) The importance of mosquito behavioural adaptations to malaria control in Africa. Evolution 67: 1218-1230.

168. Achan J, Talisuna A, Erhart A, Yeka A, Tibenderana JK, Baliraine FN, Rosenthal PJ, D'Alessandro U (2011) Quinine, an old anti-malarial drug in a modern world: role in the treatment of malaria. Malar J: 144.

169. Wellems TE, Plowe CV (2001) Chloroquine-resistant malaria. JID 184: 770-776.

170. World Health Organisation (2010) Guidelines for the treatment of malaria - 2nd Edition. Geneva: WHO.

171. Sirawaraporn W, Yuthavong Y (1984) Kinetic and molecular properties of dihydrofolate reductase from pyrimethamine-sensitive and pyrimethamine-resistant *Plasmodium chabaudi*. Mol Biochem Parasit 355-367.

172. Hopkins Sibley C, Hyde JE, Sims PFG, Plowe CV, Kublin JG, Mberu EK, Cowman AF, Winstanley PA, Watkins WM, Nzila AM (2001) Pyrimethamine-sulfadoxine resistance in *Plasmodium falciparum*: what next? Trends Parasitol 17: 582-588.

173. Cairns M, Gosling R, Carneiro I, Gesase S, Mosha JF, Hashim R, Kaur H, Lemnge M, Mosha FW, Greenwood B, Chandramohan D (2010) Duration of protection against clinical malaria provided by three regimens of intermittent preventive treatment in Tanzanian infants. PloS one 5: e9467.

174. White NJ (2004) Antimalarial drug resistance. The Journal of clinical investigation 113: 1084-1092.

175. Hwang J, Bitarakwate E, Pai M, Reingold A, Rosenthal PJ, Dorsey G (2006) Chloroquine or amodiaquine combined with sulfadoxine-pyrimethamine for uncomplicated malaria: a systematic review. Trop Med Int Health 11: 789-799.

176. Hatz C, Abdulla S, Mull R, Schellenberg D, Gathmann I, Kibatala P, Beck H-P, Tanner M, Royce C (1998) Efficacy and safety of CGP 56697 (artemether and benflumetol) compared with chloroquine to treat acute falciparum malaria in Tanzanian children aged 1-5 years. Trop Med Int Health 3: 498-504.

177. White NJ, van Vugt M, Ezzet F (1999) Clinical pharmacokinetics and pharmacodynamics of artemether-lumefantrine. Clin Pharmacokinet 37: 105-125.

178. Makanga M (2014) A review of the effects of artemether-lumefantrine on gametocyte carriage and disease transmission. Malar J 13.

179. Djimde AA, Lefevre G (2009) Understanding the pharmacokinetics of Coartem. Malar J 8(Suppl 1).

180. Myint HY, Ashley E, Day NPJ, Nosten F, White NJ (2007) Efficacy and safety of dihydroartemisinin-piperaquine. Trans R Soc Trop Med Hyg 101: 858-866.

181. Akpaloo W, Pursell E (2014) Does the use of dihydroartemisinin-piperaquine in treating patients with uncomplicated falciparum malaria reduce the risk for recurrent new falciparum infection more than artemether-lumefantrine? Malar Res Treat 263674.

182. White NJ (2008) How antimalarial drug resistance affects post-treatment prophylaxis. Malar J 7.

183. Manning J, Vanachayangkula P, Lon C, Spring M, So M, Sea D, Se Y, Somethy S, Phann S-T, Chann S, Sriwichai S, Buathong N, Kuntawunginn W, Mitprasat M, Siripokasupkul R, Teja-Isavadharm P, Soh E, Timmermans A, Lanteri C, Kaewkungwal J, Auayporn M, Tang D, Meng Chour C, Prom S, Haigney M, Cantilena L, Saunders D (2014) Randomized, double-blind, placebo-controlled clinical trial of a two-day regimen of dihydroartemisinin-piperaquine for malaria prevention halted for concern over prolonged corrected QT interval. Antimicrob Agents Chemother 58: 6056-6067.

184. Kamal-Yanni MM, Potet J, Saunders PM (2012) Scaling-up malaria treatment: a review of the performance of different providers. Malar J 11: 1-10.

185. Mubi M, Janson A, Warsame M, Mårtensson A, Källander K, Petzold MG, Ngasala B, Maganga G, Gustafsson LL, Massele A (2011) Malaria rapid testing by community health workers is effective and safe for targeting malaria treatment: randomised cross-over trial in Tanzania. PloS one 6: e19753.

186. Chanda P, Hamainza B, Moonga HB, Chalwe V, Pagnoni F (2011) Community case management of malaria using ACT and RDT in two districts in Zambia: achieving high adherence to test results using community health workers. Malar J 10: 158.

187. White NJ (2005) Intermittent presumptive treatment for malaria. PLoS Med 2: e3.

188. Eisele TP, Larsen DA, Anglewicz PA, Keating J, Yukich J, Bennett A, Hutchinson P, Steketee RW (2012) Malaria prevention in pregnancy, birthweight, and neonatal mortality: a meta-analysis of 32 national cross-sectional datasets in Africa. Lancet Infect Dis 12: 942-949.

189. Aponte JJ, Schellenberg D, Egan A, Breckenridge A, Carneiro I, Critchley J, Danquah I, Dodoo A, Kobbe R, Lell B, May J, Premji Z, Sanz S, Sevene E, Soulaymani-Becheikh R, Winstanley P, Adjei S, Anemana S, Chandramohan D, Issifou S, Mockenhaupt F, Owusu-Agyei S, Greenwood B, Grobusch MP, Kremsner PG, Macete E, Mshinda H, Newman RD, Slutsker L, Tanner M, Alonso P, Menendez C (2009) Efficacy and safety of intermittent preventive treatment with sulfadoxine-pyrimethamine for malaria in African infants: a pooled analysis of six randomised, placebo-controlled trials. Lancet 374: 1533-1542.

190. Cissé B, Sokhna C, Boulanger D, Milet J, Bâ EH, Richardson K, Hallett R, Sutherland C, Simondon K, Simondon F (2006) Seasonal intermittent preventive treatment with artesunate and sulfadoxine-pyrimethamine for prevention of malaria in Senegalese children: a randomised, placebo-controlled, double-blind trial. Lancet 367: 659-667.

191. Cairns M, Roca-Feltrer A, Garske T, Wilson AL, Diallo D, Milligan PJ, Ghani AC, Greenwood BM (2012) Estimating the potential public health impact of seasonal malaria chemoprevention in African children. Nat Commun 3: 881.

192. World Health Organization (2013) WHO Policy Recommendation: Seasonal Malaria Chemoprevention (SMC) for *Plasmodium falciparum* malaria control in highly seasonal transmission areas of the Sahel sub-region in Africa. Geneva.

193. Stresman GH, Kamanga A, Moono P, Hamapumbu H, Mharakurwa S, Kobayashi T, Moss WJ, Shiff C (2010) A method of active case detection to target reservoirs of asymptomatic malaria and gametocyte carriers in a rural area in Southern Province, Zambia. Malar J 9.

194. Sturrock HJ, Novotny JM, Kunene S, Dlamini S, Zulu Z, Cohen JM, Hsiang MS, Greenhouse B, Gosling RD (2013) Reactive case detection for malaria elimination: real-life experience from an ongoing program in Swaziland. PloS one 8: e63830.

195. Sturrock HJW, Hsiang MS, Cohen JM, Smith DL, Greenhouse B, Bousema T, Gosling RD (2013) Targeting asymptomatic malaria infections: active surveillance in control and elimination. PLoS Med 10: e1001467.

196. Ch'en C, Liang K (1956) Malaria surveillance programme in Taiwan. Bull World Health Organ 15: 805.

197. Macauley C (2005) Aggressive active case detection: a malaria control strategy based on the Brazilian model. Social science & medicine 60: 563-573.

198. Kern SE, Tiono AB, Makanga M, Gbadoe AD, Premji Z, Gaye O, Sagara I, Ubben D, Cousin M, Oladiran F, Sander O, Ogutu B (2011) Community screening and treatment of asymptomatic carriers of *Plasmodium falciparum* with artemether-lumefantrine to reduce malaria disease burden: a modelling and simulation analysis. Malar J 10: 210.

199. Bundy D (2011) Rethinking School Health: A key component of education for all. Washington: World Bank.

200. World Bank (2014) World Bank Development Indicators In: International Bank for Reconstruction and Development / The World Bank, editor. Washington D.C. USA.

201. World Health Organisation (2013) WHO Recommendations for Achieving Universal Coverage with Long-Lasting Insecticidal Nets in Malaria Control. Geneva: WHO.

202. Nevill CG, Watkins WM, Carter JY, Munafu CG (1988) Comparison of mosquito nets, proguanil hydrochloride, and placebo to prevent malaria. BMJ, 297: 401-403.

203. Okabayashi H, Thongthien P, Singhasvanon P, Waikagul J, Looareesuwan S, Jimba M, Kano S, Kojima S, Takeuchi T, Kobayashi J, Tateno S (2006) Keys to success for a school-based malaria control program in primary schools in Thailand. Parasitol Int 55: 121-126.

204. Nonaka D, Kobayashi J, Jimba M, Vilaysouk B, Tsukamoto K, Kano S, Phommasack B, Singhasivanon P, Waikagul J, Tateno S, Takeuchi T (2008) Malaria education from school to community in Oudomxay province, Lao PDR. Parasitol Int 57: 76-82.

205. Ayi I, Nonaka D, Adjovu JK, Hanafusa S, Jimba M, Bosompem KM, Mizoue T, Takeuchi T, Boakye DA, Kobayashi J (2010) School-based participatory health education for malaria control in Ghana: engaging children as health messengers. Malar J 9: 98.

206. Afenyadu GY, Agyepong IA, Barnish G, Adjei S (2005) Improving access to early treatment of malaria: a trial with primary school teachers as care providers. Trop Med Int Health 10: 1065-1072.

207. Magnussen P, Ndawi B, Sheshe AK, Byskov J, Mbwana K (2001) Malaria diagnosis and treatment administered by teachers in primary schools in Tanzania. Trop Med Int Health 6: 273-279.

208. Simwaka BN, Simwaka K, Bello G (2009) Retrospective analysis of a school-based malaria treatment programme demonstrates a positive impact on health and education outcomes in Mangochi district, Malawi. J Dev Effect 1: 492-506.

209. Witek-McManus S, Mtali A, Ali D, Sande J, Mathanga D, Mwenda R, Mazinga C, Chimuna T, George J, Roschnik N, Halliday KE, Brooker S (2013) Evaluation of the effectiveness of a training programme for school teachers in performing and interpreting malaria rapid diagnostic tests safely and accurately in Zomba, Malawi. Submitted.

210. Watkins W, Oloo A, Gilles H, Brandling-Bennett A, Howells R, Koech D (1987) Inadequacy of chlorproguanil 20 mg per week as chemoprophylaxis for falciparum malaria in Kenya. Lancet 329: 125-128.

211. Pividal J, Viktinski V, Streat E, Schapira A (1992) Efficacy of dapsone with pyrimethamine (Maloprim) for malaria prophylaxis in Maputo, Mozambique. East Afr Med J 69: 303.

212. Laing AB (1984) The impact of malaria chemoprophylaxis in Africa with special reference to Madagascar, Cameroon, and Senegal. Bull World Health Organ 62 Suppl: 41-48.

213. Doua JY, Matangila J, Lutumba P, Van Geertruyden JP (2013) Intermittent preventive treatment: efficacy and safety of sulfadoxine-pyrimethamine and sulfadoxine-pyrimethamine plus piperaquine regimens in schoolchildren of the Democratic Republic of Congo: a study protocol for a randomized controlled trial. Trials 14: 311.

214. Nankabirwa J, Cundill B, Clarke S, Kabatereine N, Rosenthal PJ, Dorsey G, Brooker S, Staedke SG (2010) Efficacy, safety, and tolerability of three regimens for prevention of malaria: a randomized, placebo-controlled trial in Ugandan schoolchildren. PloS one 5: e13438.

215. Tagbor H, Bruce J, Agbo M, Greenwood B, Chandramohan D (2010) Intermittent screening and treatment versus intermittent preventive treatment of malaria in pregnancy: a randomised controlled non-inferiority trial. PloS one 5: e14425.

216. Smith LA, Jones C, Adjei RO, Antwi GD, Afrah NA, Greenwood B, Chandramohan D, Tagbor H, Webster J (2010) Intermittent screening and treatment versus intermittent preventive treatment of malaria in pregnancy: user acceptability. Malar J 9: 18.

217. Paintain LS, Antwi GD, Jones C, Amoako E, Adjei RO, Afrah NA, Greenwood B, Chandramohan D, Tagbor H, Webster J (2011) Intermittent screening and treatment versus intermittent preventive treatment of malaria in pregnancy: provider knowledge and acceptability. PloS one 6: e24035.

218. Tiono AB, Ouedraogo A, Ogutu B, Diarra A, Coulibaly S, Gansane A, Sirima SB, O'Neil G, Mukhopadhyay A, Hamed K (2013) A controlled, parallel, cluster-randomized trial

of community-wide screening and treatment of asymptomatic carriers of *Plasmodium falciparum* in Burkina Faso. Malar J 12: 79.

219. Tiono AB, Guelbeogo MW, Sagnon NF, Nébié I, Sirima SB, Mukhopadhyay A, Hamed K (2013) Dynamics of malaria transmission and susceptibility to clinical malaria episodes following treatment of *Plasmodium falciparum* asymptomatic carriers: results of a cluster-randomized study of community-wide screening and treatment. BMC Infect Dis 13: 535.
220. White H (2009) Some reflections on current debates in impact evaluation. New Delhi: International Initiative for Impact Evaluation (3ie)
221. White H (2009) Theory-based impact evaluation: principles and practice. J Dev Effect 1: 271-284.
222. Kolasa J, Rollo CD (1991) Introduction: the heterogeneity of heterogeneity: a glossary. Ecological heterogeneity. New York: Springer. pp. 1-23.
223. Bousema T, Kreuels B, Gosling R (2011) Adjusting for heterogeneity of malaria transmission in longitudinal studies. J Infect Dis 204: 1-3.
224. Higgins J, Thompson S, Deeks J, Altman D (2002) Statistical heterogeneity in systematic reviews of clinical trials: a critical appraisal of guidelines and practice. J Health Serv Res Policy 7: 51-61.
225. Higgins J, Green S (2011) Cochrane handbook for systematic reviews of interventions. The Cochrane Collaboration Version 5.1.0
226. Kreuels B, Kobbe R, Adjei S, Kreuzberg C, von Reden C, Bäter K, Klug S, Busch W, Adjei O, May J (2008) Spatial variation of malaria incidence in young children from a geographically homogeneous area with high endemicity. J Infect Dis 197: 85-93.
227. Bejon P, Williams TN, Nyundo C, Hay SI, Benz D, Gething PW, Otiende M, Peshu J, Bashraheil M, Greenhouse B, Bousema JT, Bauni E, Marsh K, Smith DL, Borrmann S (2014) A micro-epidemiological analysis of febrile malaria in Coastal Kenya showing hotspots within hotspots. Elife 3: e02130.
228. Alonso PL, Brown G, Arevalo-Herrera M, Binka F, Chitnis C, Collins F, Doumbo OK, Greenwood B, Hall BF, Levine MM (2011) A research agenda to underpin malaria eradication. PLoS Med 8: e1000406.
229. Gitonga CW, Karanja PN, Kihara J, Mwanje M, Juma E, Snow RW, Noor AM, Brooker S (2010) Implementing school malaria surveys in Kenya: towards a national surveillance system. Malar J 9: 306.
230. Ashton RA, Kefyalew T, Tesfaye G, Pullan RL, Yadeta D, Reithinger R, Kolaczinski JH, Brooker S (2011) School-based surveys of malaria in Oromia Regional State, Ethiopia: a rapid survey method for malaria in low transmission settings. Malar J 10: 25.

231. Halliday KE, Karanja P, Turner EL, Okello G, Njagi K, Dubeck MM, Allen E, Jukes MCH, Brooker SJ (2012) *Plasmodium falciparum*, anaemia and cognitive and educational performance among school children in an area of moderate malaria transmission: baseline results of a cluster randomized trial on the coast of Kenya. Trop Med Int Health 17: 532-549.

232. Mathanga D, Halliday KE, Ali D, Sande J, Mazinga C, Verney A, Mtali A, Jones R, Witek-McManus S, Roschnik N, Brooker S (2014) The high burden of malaria among school children in southern Malawi. Am J Trop Med Hyg In Press.

233. Takken W, Lindsay SW (2003) Factors affecting the vectorial competence of *Anopheles gambiae*: a question of scale In: Takken W, Scott TW, editors. Ecological Aspects for Application of Genetically Modified Mosquitoes. The Netherlands: Kluwer Academic Publishers. pp. 75.

234. Hay SI, Omumbo J, Craig M, Snow RW (2000) Earth observation, Geographic Information Systems and *Plasmodium falciparum* malaria in Sub-Saharan Africa. Remote sensing and geographical information systems in epidemiology. London: Academic Press. pp. 174-206.

235. Drakeley CJ, Carneiro I, Reyburn H, Malima R, Lusingu JPA, Cox J, Theander TG, Nkya WMMM, Lemnge MM, Riley EM (2005) Altitude-dependent and -independent variations in *Plasmodium falciparum* prevalence in northeastern Tanzania. J Infect Dis 191: 1589-1598.

236. Lunde TM, Bayoh MN, Lindtjørn B (2013) How malaria models relate temperature to malaria transmission. Parasit Vectors 6: 20.

237. Kelly-Hope LA, Hemingway J, McKenzie FE (2009) Environmental factors associated with the malaria vectors *Anopheles gambiae* and *Anopheles funestus* in Kenya. Malar J 8: 268.

238. De Souza D, Kelly-Hope L, Lawson B, Wilson M, Boakye D (2010) Environmental factors associated with the distribution of *Anopheles gambiae ss* in Ghana; an important vector of lymphatic filariasis and malaria. PloS one 5: e9927.

239. Walker M, Winskill P, Basáñez M-G, Mwangangi JM, Mbogo C, Beier JC, Midega JT (2013) Temporal and micro-spatial heterogeneity in the distribution of *Anopheles* vectors of malaria along the Kenyan coast. Parasit Vectors 6: 311.

240. Fillinger U, Lindsay SW (2011) Larval source management for malaria control in Africa: myths and reality. Malar J 10.

241. Gosoniu L, Vounatsou P, Sogoba N, Smith T (2006) Bayesian modelling of geostatistical malaria risk data. Geospat Health 1: 127-139.

242. Noor AM, Gething PW, Alegana VA, Patil AP, Hay SI, Muchiri E, Juma E, Snow RW (2009) The risks of malaria infection in Kenya in 2009. BMC Infect Dis 9: 180.

243. Raso G, Silue KD, Vounatsou P, Singer BH, Yapi A, Tanner M, Utzinger J, N'Goran EK (2009) Spatial risk profiling of *Plasmodium falciparum* parasitaemia in a high endemicity area in Cote d'Ivoire. Malar J 8: 252.

244. Hay SI, Snow RW, Rogers DJ (1998) Predicting malaria seasons in Kenya using multitemporal meteorological satellite sensor data. Trans R Soc Trop Med Hyg 92: 12-20.

245. Ijumba JN, Mosha FW, Lindsay SW (2002) Malaria transmission risk variations derived from different agricultural practices in an irrigated area of northern Tanzania. Medical and veterinary entomology 16: 28-38.

246. Keiser J, De Castro MC, Maltese MF, Bos R, Tanner M, Singer BH, Utzinger J (2005) Effect of irrigation and large dams on the burden of malaria on a global and regional scale. Am J Trop Med Hyg 72: 392-406.

247. Ijumba JN, Lindsay SW (2001) Impact of irrigantion on malaria in Africa: paddies paradox. Med Vet Entomol 15: 1-11.

248. Gosoniu L, Msengwa A, Lengeler C, Vounatsou P (2012) Spatially explicit burden estimates of malaria in Tanzania: bayesian geostatistical modeling of the malaria indicator survey data. PloS one 7: e23966.

249. Gaudart J, Poudiougou B, Dicko A, Ranque S, Toure O, Sagara I, Diallo M, Diawara S, Ouattara A, Diakite M, Doumbo OK (2006) Space-time clustering of childhood malaria at the household level: a dynamic cohort in a Mali village. BMC Public Health 6: 286.

250. Oesterholt MJAM, Bousema JT, Mwerinde OK, Harris C, Lushino P, Masokoto A, Mwerinde H, Mosha FW, Drakeley CJ (2006) Spatial and temporal variation in malaria transmission in a low endemicity area in northern Tanzania. Malar J 5: 98.

251. Banguero H (1984) Socioeconomic factors associated with malaria in Colombia. Soc Sci Med 19: 1099-1104.

252. Koram KA, Bennett S, Adiamah JH, Greenwood BM (1995) Socio-economic risk factors for malaria in a peri-urban area of The Gambia. Trans R Soc Trop Med Hyg 89: 146-150.

253. Matthys B, Vounatsou P, Raso G, Tschannen AB, Becket EG, Gosoniu L, Cisse G, Tanner M, N'Goran EK, Utzinger J (2006) Urban farming and malaria risk factors in a medium-sized town in Cote d'Ivoire. Am J Trop Med Hyg 75: 1223-1231.

254. Ayele DG, Zewotir TT, Mwambi HG (2012) Prevalence and risk factors of malaria in Ethiopia. Malar J 11: 195.

255. Bradley J, Rehman AM, Schwabe C, Vargas D, Monti F, Ela C, Riloha M, Kleinschmidt I (2013) Reduced prevalence of malaria infection in children living in houses with window screening or closed eaves on bioko island, equatorial Guinea. PloS one 8: e80626.

256. Kirby MJ, Green C, Milligan PM, Sismanidis C, Jasseh M, Conway DJ, Lindsay SW (2008) Risk factors for house-entry by malaria vectors in a rural town and satellite villages in The Gambia. Malar J 7: 2.

257. Ghebreyesus TA, Haile M, Witten KH, Getachew A, Yohannes M, Lindsay SW, Byass P (2000) Household risk factors for malaria among children in the Ethiopian highlands. Trans R Soc Trop Med Hyg 94: 17-21.

258. Temu EA, Coleman M, Abilio AP, Kleinschmidt I (2012) High prevalence of malaria in Zambezia, Mozambique: the protective effect of IRS versus increased risks due to pig-keeping and house construction. PloS one 7: e31409.

259. McCall PJ, Mosha FW, Njunwa KJ, Sherlock K (2001) Evidence for memorized site-fidelity in Anopheles arabiensis. Trans R Soc Trop Med Hyg 95: 587-590.

260. Willcox M, Björkman A, Brohult J (1983) Falciparum malaria and beta-thalassaemia trait in northern Liberia. Ann Trop Med Parasitol 77: 335-347.

261. Mackinnon MJ, Gunawardena D, Rajakaruna J, Weerasingha S, Mendis KN, Carter R (2000) Quantifying genetic and nongenetic contributions to malarial infection in a Sri Lankan population. Proc Natl Acad Sci 97: 12661-12666.

262. Mackinnon MJ, Mwangi TW, Snow RW, Marsh K, Williams TN (2005) Heritability of malaria in Africa. PLoS Med 2: e340.

263. Pullan RL, Bukirwa H, Snow RW, Brooker S (2010) Heritability of *Plasmodium* Parasite Density in a Rural Ugandan Community. Am J Trop Med Hyg 83: 990.

264. Menendez C, Schellenberg D, Macete E, Aide P, Kahigwa E, Sanz S, Aponte JJ, Sacarlal J, Mshinda H, Tanner M, Alonso PL (2007) Varying efficacy of intermittent preventive treatment for malaria in infants in two similar trials: public health implications. Malar J 6: 132.

265. Fullman N, Burstein R, Lim SS, Medlin C, Gakidou E (2013) Nets, spray or both? The effectiveness of insecticide-treated nets and indoor residual spraying in reducing malaria morbidity and child mortality in sub-Saharan Africa. Malar J 12: 62.

266. Lines J, Kleinschmidt I (2013) Combining malaria vector control interventions: some trial design issues. Pathog Glob Health 107: 1-4.

267. Lines J, Kleinschmidt I (2014) Is malaria control better with both treated nets and spraying. Lancet pii: S0140-6736: 61306-61304.

268. West PA, Protopopoff N, Wright A, Kivaju Z, Tigererwa R, Mosha FW, Kisinza W, Rowland M, Kleinschmidt I (2014) Indoor Residual Spraying in Combination with Insecticide-Treated Nets Compared to Insecticide-Treated Nets Alone for Protection against Malaria: A Cluster Randomised Trial in Tanzania. PLoS Med 11: e1001630.

269. Okell LC, Drakeley CJ, Bousema T, Whitty CJM, Ghani AC (2008) Modelling the impact of artemisinin combination therapy and long-acting treatments on malaria transmission intensity. PLoS Med 5: e226.

270. Segal JB, Weiss C, Varadhan R (2011) Understanding Heterogeneity of Treatment Effects in Pragmatic Trials with an Example of a Large, Simple Trial of a Drug Treatment for Osteoporosis (White Paper). Center for Medical Technology Policy, MD, USA.

271. Plewis I (2002) Modelling impact heterogeneity. J R Stat Soc: Series A (Statistics in Society) 165: 31-38.

272. Varadhan R, Segal JB, Boyd CM, Wu AW, Weiss CO (2013) A framework for the analysis of heterogeneity of treatment effect in patient-centered outcomes research. J Clin Epidemiol 66: 818-825.

273. Glewwe P, Kremer M, Moulin S (1998) Textbooks and test scores: evidence from prospective evaluation in Kenya. Cambridge, Massachusettes, USA: Harvard University.

274. Gelli A, Meir U, Espejo F (2007) Does provision of food in school increase girls enrollment? Evidence from schools in sub-Saharan Africa. Food Nutr Bull 28: 149-155.

275. Shultz TP (2004) School subsidies for the poor: Evaluating the Mexican Progresa Poverty Program. J Dev Econ 74: 199-250.

276. Rocha R, Soares RR (2010) Evaluating the impact of community-based health interventions: evidence from Brazil's Family Health Program. Health Econ 19: 126-158.

277. Guyatt HL, Corlett SK, Robinson TP, Ochola SA, Snow RW (2002) Malaria prevention in highland Kenya: indoor residual house-spraying vs. insecticide-treated bednets. Trop Med Int Health 7: 298-303.

278. Bejon P, Ogada E, Peshu N, Marsh K (2009) Interactions between age and ITN use determine the risk of febrile malaria in children. PLoS One 4: e8321.

279. Overgaard HJ, Reddy VP, Abaga S, Matias A, Reddy MR, Kulkarni V, Schwabe C, Segura L, Kleinschmidt I, Slotman MA (2012) Malaria transmission after five years of vector control on Bioko Island, Equatorial Guinea. Parasit Vectors 5: 253.

280. Cook J, Kleinschmidt I, Schwabe C, Nseng G, Bousema T, Corran PH, Riley EM, Drakeley CJ (2011) Serological markers suggest heterogeneity of effectiveness of malaria control interventions on Bioko Island, equatorial Guinea. PloS one 6: e25137.

281. Ioannidis J, Lau J (1997) The impact of high-risk patients on the results of clinical trials. J Clin Epidemiol 50: 1089-1098.

282. Begg CB (1987) Biases in the assessment of diagnostic tests. Stat Med 6: 411-423.

283. Greiner M, Gardner I (2000) Epidemiologic issues in the validation of veterinary diagnostic tests. Prev Vet Med 45: 3-22.

284. Brooker S, Okello G, Njagi K, Dubeck MM, Halliday KE, Inyega H, Jukes MCH (2010) Improving educational achievement and anaemia of school children: design of a cluster randomised trial of school-based malaria prevention and enhanced literacy instruction in Kenya. Trials 11: 93.

285. Oketch M, Mutisya M (2013) Evolutional of Educational Outcomes in Kenya Nairobi: Paper commissioned for the EFA Global Monitoring Report 2013/4, Teaching and learning: Achieving quality for all.

286. Dubeck MM, Jukes M, Brooker S, Drake TL, Inyega H (2015) Designing a program of teacher professional development to improve children's achievement in coastal Kenya. Int J Educ Dev Submitted

287. Mbogo CM, Mwangangi JM, Nzovu J, Gu W, Yan G, Gunter JT, Swalm C, Keating J, Regens JL, Shililu JI, Githure JI, Beier JC (2003) Spatial and temporal heterogeneity of *Anopheles* mosquitoes and *Plasmodium falciparum* transmission along the Kenyan coast. Am J Trop Med Hyg 68: 734-742.

288. Mutuku FM, King CH, Mungai PL, Mbogo CM, Mwangangi JM, Muchiri EM, Walker ED, Kitron U (2011) Impact of insecticide-treated bed nets on malaria transmission indices on the south coast of Kenya. Malar J 10: 356.

289. Mwangangi JM, Mbogo CM, Muturi EJ, Nzovu J, Githure JI, Yan G, Minakawa N, Novak R, Beir JC (2007) Spatial distribution and habitat characterisation of *Anopheles* larvae along the Kenyan coast J Vector Borne Dis 44: 44-51.

290. Snow RW, Schellenberg JR, Peshu N, Forster D, Newton CR, Winstanley PA, Mwangi I, Waruiru C, Warn PA, Newbold C, et al. (1993) Periodicity and space-time clustering of severe childhood malaria on the coast of Kenya. Trans R Soc Trop Med Hyg 87: 386-390.

291. Mwangangi JM, Mbogo CM, Orindi BO, Muturi EJ, Midega JT, Nzovu J, Gatakaa H, Githure J, Borgemeister C, Keating J, Beier JC (2013) Shifts in malaria vector species composition and transmission dynamics along the Kenyan coast over the past 20 years. Malar J 12: 13.

292. Bustinduy AL, Thomas CL, Fiutem JJ, Parraga IM, Mungai PL, Muchiri EM, Mutuku F, Kitron U, King CH (2011) Measuring fitness of Kenyan children with polyparasitic infections using the 20-meter shuttle run test as a morbidity metric. PLoS Negl Trop Dis 5: e1213.

293. Dubeck MM, Jukes MCH, Okello G (2012) Early primary literacy instruction in Kenya. Comp Educ Rev 56: 48-68.

294. Okello G, Jones C, Bonareri M, Ndegwa SN, McHaro C, Kengo J, Kinyua K, Dubeck MM, Halliday KE, Jukes MC, Molyneux S, Brooker SJ (2013) Challenges for consent and

community engagement in the conduct of cluster randomized trial among school children in low income settings: experiences from Kenya. Trials 14: 142.

295. Hayes R, J., Moulton L, H. (2009) Cluster Randomised Trials Hall C, editor. London, UK: CRC Press.
296. Manly T, Robertson IH, Anderson V, Nimmo-Smith I (1999) Test of everyday attention for children : TEA-Ch. Bury St Edmunds, UK: Company TVT.
297. Luria AR (1966) Higher Cortical Functions in Man. New York: Basic Books.
298. Raven JC, Styles I, Raven MA (1998) Raven's progressive Matrices: CPM parallel test booklet. UK: Oxford Psychologists Press.
299. Invernizzi M, Sullivan A, Meier J, Swank L (2004) PALS: Phonological awareness literacy screening. Charlottesville, VA: University of Virginia.
300. Baddeley A, Gardner JM, Grantham-McGregor S (1995) Cross-cultural cognition: Developing tests for developing countries. Appl Cognitive Psych 9: S173-S195.
301. Research Triangle Institute (RTI) International (2009) Early Grade Mathematics Assessment Toolkit. Washington: USAID, EDDATA II.
302. WHO-TDR-FIND (2010) Methods manual for laboratory quality control testing of malaria rapid diagnostic tests, Version Six. Geneva.
303. Ross A, Penny M, Maire N, Studer A, Carneiro I, Schellenberg D, Greenwood B, Tanner M, Smith T (2008) Modelling the epidemiological impact of intermittent preventive treatment against malaria in infants. PloS one 3: e2661.
304. Okell LC, Griffin JT, Kleinschmidt I, Hollingsworth TD, Churcher TS, White MJ, Bousema T, Drakeley CJ, Ghani AC (2011) The potential contribution of mass treatment to the control of *Plasmodium falciparum* malaria. PloS one 6: e20179.
305. Bundy DA, Shaeffer S, Jukes M, Beegle K, Gillespie A, Drake L, Lee SF, Hoffman AM, Jones J, Mitchell A, Barcelona D, Camara B, Golmar C, Savioli L, Sembene M, Takeuchi T, Wright C (2006) School-based Health and Nutrition Programs. In: Jamison DT, Breman JG, Measham AR, Alleyne G, Claeson M et al., editors. Disease Control Priorities in Developing Countries Second Edition. Washington: Oxford University Press. pp. 1091-1108.
306. Guerra CA, Gikandi PW, Tatem AJ, Noor AM, Smith DL, Hay SI, Snow RW (2008) The limits and intensity of *Plasmodium falciparum* transmission: implications for malaria control and elimination worldwide. PLoS Med 5: e38.
307. Draper CC (1960) Effect of malaria control on haemoglobin levels. Bmj 1: 1480-1483.
308. Vitor-Silva S, Reyes-Lecca RC, Pinheiro TR, Lacerda MV (2009) Malaria is associated with poor school performance in an endemic area of the Brazilian Amazon. Malar J 8: 230.

309. Fernando D, Wickremasinghe R, Mendis KN, Wickremasinghe AR (2003) Cognitive performance at school entry of children living in malaria-endemic areas of Sri Lanka. Trans R Soc Trop Med Hyg 97: 161-165.

310. Jukes MCH, Pinder M, Grigorenko EL, Smith HB, G. W, Bariau EM, Sternberg RJ, Drake LJ, Milligan P, Cheung YB, Greenwood BM, Bundy DA (2006) Long-term impact of malaria chemoprophylaxis on cognitive abilities and educational attainment: follow-up of a controlled trial. PLos Clin Trials 1: e19.

311. Von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP (2007) The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. Prev Med 45: 247-251.

312. McLean E, Cogswell M, Egli I, Wojdyla D, de Benoist B (2009) Worldwide prevalence of anaemia, WHO Vitamin and Mineral Nutrition Information System, 1993-2005. Public Health Nutr 12: 444-454.

313. World Health Organization (2009) WHO AnthroPlus for personal computers manual: software for assessing growth of the world's children and adolescents. Geneva: WHO.

314. Filmer D, Pritchett LH (2001) Estimating wealth effects without expenditure data--or tears: an application to educational enrollments in states of India. Demography 38: 115-132.

315. Rabe-Hesketh S, Skrondal A (2008) Multilevel and Longitudinal Modeling Using Stata. College Station, Texas: STATA Press.

316. Efron B, Tibshirani RJ (1994) Introduction to the Bootstrap (Monographs on Statistics and Applied Probability): Chapman & Hall CRC.

317. Clarke SE, Bogh C, Brown RC, Walraven GEL, Thomas CJ, Lindsay SW (2002) Risk of malaria attacks in Gambian children is greater away from malaria vector breeding sites. Trans R Soc Trop Med Hyg 96: 499-506.

318. Snow RW, Peshu J, Forster D, Bomu G, Mitsanze E, Ngumbao E, Chisengwa R, Schellenberg JR, Hayes RJ, Newbold CI, Marsh K (1998) Environmental and entomological risk factors for the development of clinical malaria among children on the Kenyan coast. Trans R Soc Trop Med Hyg: 381-385.

319. Kleinschmidt I, Torrez M, Schwabe C, Benavente L, Seocharan I, Jituboh D, Nseng G, Sharp B (2007) Factors influencing the effectiveness of malaria control in Bioko Island, equatorial Guinea. Am J Trop Med Hyg 76: 1027-1032.

320. Abdalla S, Weatherall DJ, Wickramasinghe SN, Hughes M (1980) The anaemia of *P. falciparum* malaria Br J Haematol 46: 171-183.

321. Phillips RE, Pasvol G (1992) Anaemia of *Plasmodium falciparum* malaria. Baillieres Clin Haematol 5: 315-330.

322. Jomaa LH, McDonnell E, Probart C (2011) School feeding programs in developing countries: impacts on children's health and educational outcomes. Nutr Rev: 83-98.

323. Van Stuijvenberg ME, Kvalsvig JD, Faber M, Kruger M, Kenoyer DG, Benade AJ (1999) Effect of iron-, iodine-, and beta-carotene-fortified biscuits on the micronutrient status of primary school children: a randomized controlled trial. Am J Clin Nutr: 497-503.

324. Jacoby ER, Cueto S, Pollitt E (1998) When science and politics listen to each other: good prospects from a new school breakfast program in Peru. Am J Clin Nutr 67: 795s-797s.

325. Andang'o PE, Osendarp SJ, Ayah R, West CE, Mwaniki DL, De Wolf CAD, Kraaijenhagen R, Kok FJ, Verhoef H (2007) Efficacy of iron-fortified whole maize flour on iron status of schoolchildren in Kenya: a randomised controlled trial. Lancet 369: 1799-1806.

326. Topley E (1968) Common anaemia in rural Gambia. 3. A spontaneously remitting anaemia possibly precipitated by malarial parasitaemia. Trans R Soc Trop Med Hyg: 602-606.

327. Nussenblatt V, Semba RD (2002) Micronutrient malnutrition and the pathogenesis of malarial anemia. Acta Trop: 321-337.

328. Jukes M, Nokes C, Alcock KJ, Lambo JK, Kihamia C, Ngorosho N, Mbise A, Lorri W, Yona E, Mwanri L, Baddeley A, Hall A, Bundy DA (2002) Heavy schistosomiasis associated with poor short-term memory and slower reaction times in Tanzanian schoolchildren. Trop Med Int Health 7: 104-117.

329. Walker SP, Grantham-McGregor SM, Himes JH, Williams S, Duff EM (1998) School performance in adolescent Jamaican girls: associations with health, social and behavioural characteristics, and risk factors for dropout. J Adolescence 21: 109-122.

330. Heyneman SP, Loxley WA (1983) The effect of primary-school quality on academic achievement across twenty-nine high- and low-income countries. Am J Sociol 88: 1162-1194.

331. Fehrler S, Michaelowa K, Wechtler A (2009) The effectiveness of inputs in primary education: Insights from recent student surveys for sub-Saharan Africa. J Dev Stud 45: 1545-1578.

332. Fentiman A, Hall A, Bundy DA (1999) School enrolment patterns in rural Ghana: A comparative study of the impact of location, gender, age and health on children's access to basic schooling. Comp Educ 35: 331-349.

333. Boissiere M (2004) Determinants of primary education outcomes in developing countries.; Department. WBOE, editor. Washington D.C.: World Bank.

334. Bhargava A, Jukes M, Ngorosho D, Khilma C, Bundy DA (2005) Modeling the effects of health status and the educational infrastructure on the cognitive development of Tanzanian schoolchildren. Am J Hum Biol 17: 280-292.

335. Japan International Cooperation Agency (JICA) (2012) Basic education sector analysis report: Kenya. Japan.

336. Moonen B, Cohen JM, Snow RW, Slutsker L, Drakeley C, Smith DL, Abeyasinghe RR, Rodriguez MH, Maharaj R, Tanner M, Targett G (2010) Operational strategies to achieve and maintain malaria elimination. Lancet 376: 1592-1603.

337. Githeko AK, Brandling-Bennett AD, Beier M, Atieli F, Owaga M, Collins FH (1992) The reservoir of *Plasmodium falciparum* malaria in a holoendemic area of western Kenya. Trans R Soc Trop Med Hyg 86: 355-358.

338. Campbell MK, Elbourne DR, Altman DG (2004) CONSORT statement: extension to cluster randomised trials. BMJ (Clinical research ed) 328: 702-708.

339. Sowunmi A, Gbotosho GO, Happi CT, Adedeji AA, Fehintola FA, Folarin OA, Tambo E, Fateye BA (2007) Therapeutic efficacy and effects of artemether-lumefantrine and amodiaquine-sulfalene-pyrimethamine on gametocyte carriage in children with uncomplicated *Plasmodium falciparum* malaria in southwestern Nigeria. Am J Trop Med Hyg 77: 235-241.

340. Woodring JV, Ogutu B, Schnabel D, Waitumbi JN, Olsen CH, Walsh DS, Heppner DG, Jr., Polhemus ME (2010) Evaluation of recurrent parasitemia after artemether-lumefantrine treatment for uncomplicated malaria in children in western Kenya. Am J Trop Med Hyg 83: 458-464.

341. Nambozi M, Van Geertruyden J-P, Hachizovu S, Chaponda M, Mukwamataba D, Mulenga M, Ubben D, D'Alessandro U (2011) Safety and efficacy of dihydroartemisinin-piperaquine versus artemether-lumefantrine in the treatment of uncomplicated *Plasmodium falciparum* malaria in Zambian children. Malar J 10: 50.

342. Ochola LB, Vounatsou P, Smith T, Mabaso MLH, Newton CRJC (2006) The reliability of diagnostic techniques in the diagnosis and management of malaria in the absence of a gold standard. Lancet Infect Dis 6: 582-588.

343. Speybroeck N, Praet N, Claes F, Van Hong N, Torres K, Mao S, Van den Eede P, Thi Thinh T, Gamboa D, Sochantha T, Thang ND, Coosemans M, Buscher P, D'Alessandro U, Berkvens D, Erhart A (2011) True versus apparent malaria infection prevalence: the contribution of a Bayesian approach. PloS one 6: e16705.

344. Gonçalves L, Subtil A, de Oliveira MR, do Rosário V, Lee P-W, Shaio M-F (2012) Bayesian latent class models in malaria diagnosis. PloS one 7: e40633.

345. Okell LC, Bousema T, Griffin JT, Ouédraogo AL, Ghani AC, Drakeley CJ (2012) Factors determining the occurrence of submicroscopic malaria infections and their relevance for control. Nat Commun 3: 1237.

346. Dinko B, Oguike MC, Larbi JA, Bousema JT, Sutherland CJ (2013) Persistent detection of *Plasmodium falciparum, P. malariae, P. ovale curtisi* and *P. ovale wallikeri* after ACT treatment of asymptomatic Ghanaian school-children. Int J Parasitol Drugs Drug Resist 3: 45-50.

347. Okello G, Ndegwa SN, Halliday KE, Hanson K, Brooker SJ, Jones C (2012) Local perceptions of intermittent screening and treatment for malaria in school children on the south coast of Kenya. Malar J 11: 185.

348. Drake TL, Okello G, Njagi K, Halliday KE, Jukes MC, Mangham L, Brooker S (2011) Cost analysis of school-based intermittent screening and treatment of malaria in Kenya. Malar J 10: 273.

349. Piola P, Fogg C, Bajunirwe F, Biraro S, Grandesso F, Ruzagira E, Babigumira J, Kigozi I, Kiguli J, Kyomuhendo J, Ferradini L, Taylor W, Checchi F, Guthmann J-P (2005) Supervised versus unsupervised intake of six-dose artemether-lumefantrine for treatment of acute, uncomplicated *Plasmodium falciparum* malaria in Mbarara, Uganda: a randomised trial. Lancet 365: 1467-1473.

350. Borrmann S, Sasi P, Mwai L, Bashraheil M, Abdallah A, Muriithi S, Frühauf H, Schaub B, Pfeil J, Peshu J (2011) Declining responsiveness of *Plasmodium falciparum* infections to artemisinin-based combination treatments on the Kenyan coast. PloS one 6: e26005.

351. Agarwal A, McMorrow M, Onyango P, Otieno K, Odero C, Williamson J, Kariuki S, Kachur SP, Slutsker L, Desai M (2013) A randomized trial of artemether-lumefantrine and dihydroartemisinin-piperaquine in the treatment of uncomplicated malaria among children in western Kenya. Malar J 12: 254.

352. National Coordination Agency for Population and Development (2005) Kwale District Strategic Plan 2005-2010 for implementation of the National Population Poilcy for Sustainable Development. Kenya. 1-55 p.

353. Kenya Food Security Steering Group (2012) The 2011/12 Short Rains Season Assessment Report. Kenya 1-42 p.

354. Hamadani JD, Fuchs GJ, Osendarp SJ, Khatun F, Huda SN, Grantham-McGregor SM (2001) Randomized controlled trial of the effect of zinc supplementation on the mental development of Bangladeshi infants. Am J Clin Nutr 74: 381-386.

355. Takem EN, Affara M, Amambua-Ngwa A, Okebe J, Ceesay SJ, Jawara M, Oriero E, Nwakanma DC, Pinder M, Clifford C, Taal MA, Sowe M, Suso P, Mbaye A, Drakeley CJ, D'Alessandro U (2013) Detecting Foci of Malaria Transmission with School Surveys: A Pilot Study in the Gambia. PloS one 8: e67108.

356. Stevenson JC, Stresman GH, Gitonga CW, Gillig J, Owaga C, Marube E, Odongo W, Okoth A, China P, Oriango R (2013) Reliability of school surveys in estimating geographic variation in malaria transmission in the Western Kenyan highlands. PloS one 8: e77641.

357. Halliday KE, Okello G, Turner EL, Njagi K, Mcharo C, Kengo J, Allen E, Dubeck MM, Jukes MC, Brooker SJ (2014) Impact of intermittent screening and treatment for malaria among school children in Kenya: a cluster randomised trial. PLoS Med 11: e1001594.

358. World Health Organisation (2012) World Malaria Report. Geneva: WHO.

359. Abeku TA, Kristan M, Jones C, Beard J, Mueller DH, Okia M, Rapuoda B, Greenwood B, Cox J (2008) Determinants of the accuracy of rapid diagnostic tests in malaria case management: evidence from low and moderate transmission settings in the East African highlands. Malar J 7: 202.

360. Ashton RA, Kefyalew T, Tesfaye G, Counihan H, Yadeta D, Cundill B, Reithinger R, Kolaczinski JH (2010) Performance of three multi-species rapid diagnostic tests for diagnosis of *Plasmodium falciparum* and *Plasmodium vivax* malaria in Oromia Regional State, Ethiopia. Malar J 9: 297.

361. Endeshaw T, Graves PM, Ayele B, Mosher AW, Gebre T, Ayalew F, Genet A, Mesfin A, Shargie EB, Tadesse Z, Teferi T, Melak B, Richards FO, Emerson PM (2012) Performance of local light microscopy and the ParaScreen Pan/Pf rapid diagnostic test to detect malaria in health centers in Northwest Ethiopia. PloS one 7: e33014.

362. Singh N, Saxena A, Sharma VP (2002) Usefulness of an inexpensive, Paracheck test in detecting asymptomatic infectious reservoir of *Plasmodium falciparum* during dry season in an inaccessible terrain in central India. J Infect 45: 165-168.

363. Neumann CG, Bwibo NO, Siekmann JH, McLean ED, Browdy B, Drorbaugh N (2008) Comparison of blood smear microscopy to a rapid diagnostic test for in-vitro testing for *P. falciparum* malaria in Kenyan school children. East Afr Med J 85: 544-549.

364. Keating J, Miller JM, Bennett A, Moonga HB, Eisele TP (2009) *Plasmodium falciparum* parasite infection prevalence from a household survey in Zambia using microscopy and a rapid diagnostic test: implications for monitoring and evaluation. Acta Trop 112: 277-282.

365. Laurent A, Schellenberg J, Shirima K, Ketende SC, Alonso PL, Mshinda H, Tanner M, Schellenberg D (2010) Performance of HRP-2 based rapid diagnostic test for malaria and its variation with age in an area of intense malaria transmission in southern Tanzania. Malar J 9: 294.

366. Endeshaw T, Gebre T, Ngondi J, Graves PM, Shargie EB, Ejigsemahu Y, Ayele B, Yohannes G, Teferi T, Messele A, Zerihun M, Genet A, Mosher AW, Emerson PM, Richards FO (2008) Evaluation of light microscopy and rapid diagnostic test for the detection of malaria under operational field conditions: a household survey in Ethiopia. Malar J 7: 118.

367. Gitonga CW, Kihara JH, Njenga SM, Awuondo K, Noor AM, Snow RW, Brooker SJ (2012) Use of rapid diagnostic tests in malaria school surveys in Kenya: does their under-performance matter for planning malaria control? Am J Trop Med Hyg 87: 1004-1011.

368. Schachterle SE, Mtove G, Levens JP, Clemens EG, Shi L, Raj A, Munoz B, Reller ME, West S, Dumler JS, Sullivan D, Team PP (2011) Prevalence and density-related

concordance of three diagnostic tests for malaria in a region of Tanzania with hypoendemic malaria. J Clin Microbiol 49: 3885-3891.

369. Mouatcho JC, Goldring JD (2013) Malaria rapid diagnostic tests: challenges and prospects. J Med Microbiol 62: 1491-1505.
370. World Health Organisation - Regional Office for the Western Pacific, UNICEF / UNDP / World Bank / WHO Special Programme for Research and Training in Tropical Disease, Foundation for New Innovative Diagnostics (FIND) (2008) Initiative for Quality Assurance of Malaria Rapid Diagnostic Tests - Outline of product testing and associated protocols 1-43 p.
371. Mboera LEG, Fanello CI, Malima RC, Talbert A, Fogliati P, Bobbio F, Molteni F (2006) Comparison of the Paracheck-Pf test with microscopy, for the confirmation of *Plasmodium falciparum* malaria in Tanzania. Ann Trop Med Parasitol 100: 115-122.
372. Forney JR, Magill AJ, Wongsrichanalai C, Sirichaisinthop J, Bautista CT, Heppner DG, Miller RS, Ockenhouse CF, Gubanov A, Shafer R, DeWitt CC, Quino-Ascurra HA, Kester KE, Kain KC, Walsh DS, Ballou WR, Gasser RA, Jr. (2001) Malaria rapid diagnostic devices: performance characteristics of the ParaSight F device determined in a multisite field study. J Clin Microbiol 39: 2884-2890.
373. Bisoffi Z, Sirima SB, Menten J, Pattaro C, Angheben A, Gobbi F, Tinto H, Lodesani C, Neya B, Gobbo M, Van den Ende J (2010) Accuracy of a rapid diagnostic test on the diagnosis of malaria infection and of malaria-attributable fever during low and high transmission season in Burkina Faso. Malar J 9: 192.
374. Diarra A, Nebie I, Tiono A, Sanon S, Soulama I, Ouedraogo A, Gansane A, Yaro JB, Ouedraogo E, Traore AS, Sirima SB (2012) Seasonal performance of a malaria rapid diagnosis test at community health clinics in a malaria-hyperendemic region of Burkina Faso. Parasit Vectors 5: 103.
375. Banoo S, Bell D, Bossuyt P, Herring A, Mabey D, Poole F, Smith PG, Sriram N, Wongsrichanalai C, Linke R (2008) Evaluation of diagnostic tests for infectious diseases: general principles. Nat Rev Microbiol 8: S16-S28.
376. Menten J, Boelaert M, Lesaffre E (2013) Bayesian meta-analysis of diagnostic tests allowing for imperfect reference standards. Stat Med 32: 5398-5413.
377. Hui SL, Walter SD (1980) Estimating the error rates of diagnostic tests. Biometrics 36: 167-171.
378. Enøe C, Georgiadis MP, Johnson WO (2000) Estimation of sensitivity and specificity of diagnostic tests and disease prevalence when the true disease state is unknown. Prev Vet Med 45: 61-81.
379. Rindskopf D, Rindskopf W (1986) The value of latent class analysis in medical diagnosis. Stat Med 5: 21-27.

380. Branscum AJ, Gardner IA, Johnson WO (2005) Estimation of diagnostic-test sensitivity and specificity through Bayesian modeling. Prev Vet Med 68: 145-163.

381. Gardner IA, Stryhn H, Lind P, Collins MT (2000) Conditional dependence between tests affects the diagnosis and surveillance of animal diseases. Prev Vet Med 45: 107-122.

382. Dendukuri N, Joseph L (2001) Bayesian approaches to modeling the conditional dependence between multiple diagnostic tests. Biometrics 57: 158-167.

383. Martinez EZ, Louzada-Neto F, Derchain SFM, Achcar JA, Gontijo RC, Sarian LOZ, Syrjanen KJ (2008) Bayesian estimation of performance measures of cervical cancer screening tests in the presence of covariates and absence of a gold standard. Cancer Inform 6: 33-46.

384. Limmathurotsakul D, Turner EL, Wuthiekanun V, Thaipadungpanit J, Suputtamongkol Y, Chierakul W, Smythe LD, Day NPJ, Cooper B, Peacock SJ (2012) Fool's gold: Why imperfect reference tests are undermining the evaluation of novel diagnostics: a reevaluation of 5 diagnostic tests for leptospirosis. Clin Infect Dis 55: 322-331.

385. Pan-ngum W, Blacksell SD, Lubell Y, Pukrittayakamee S, Bailey MS, de Silva HJ, Lalloo DG, Day NPJ, White LJ, Limmathurotsakul D (2013) Estimating the true accuracy of diagnostic tests for dengue infection using bayesian latent class models. PloS one 8: e50765.

386. van Smeden M, Naaktgeboren CA, Reitsma JB, Moons KG, de Groot JA (2013) Latent Class Models in Diagnostic Studies When There is No Reference Standard—A Systematic Review. Am J Epidemiol 179: 423-431.

387. Bossuyt PM, Reitsma JB, Bruns DE, Gatsonis CA, Glasziou PP, Irwig LM, Lijmer JG, Moher D, Rennie D, de Vet HCW, Standards for Reporting of Diagnostic Accuracy (2003) Towards complete and accurate reporting of studies of diagnostic accuracy: the STARD initiative. BMJ 326: 41-44.

388. Proux S, Hkirijareon L, Ngamngonkiri C, McConnell S, Nosten F (2001) Short communication: Paracheck-Pf®: a new, inexpensive and reliable rapid test for *P. falciparum* malaria. Trop Med Int Health 6: 99-101.

389. Lunn DJ, Thomas A, Best N, Spiegelhalter D (2000) WinBUGS - a Bayesian modelling framework: concepts, structure and extensibility. Stat Comput 10: 325-337.

390. Landis JR, Koch GG (1977) The measurement of observer agreement for categorical data. Biometrics: 159-174.

391. Pullan R, Bethony JM, Geiger SM, Cundill B, Correa-Oliveira R, Quinnell R, Brooker S (2008) Human Helminth Co-infection: analysis of spatial patterns and risk factors in a Brazilian community. PLoS Negl Trop Dis 2: e352.

392. Seed PT, Tobias A (2001) sbe36_1: Summary statistics for diagnostic tests. . Stata Technical Bulletin: College Station, TX: Stata Press. pp. 90-93.

393. Pepe MS, James H (2006) Insights into latent class analysis of diagnostic test performance. Philos Math 8: 474-484.

394. World Health Organisation (1999) New Perpectives: Malaria Diagnosis, Report of a joint WHO/USAID informal consultation Geneva. 1-29 p.

395. Coleman RE, Maneechai N, Rachaphaew N, Kumpitak C, Miller RS, Soyseng V, Thimasarn K, Sattabongkot J (2002) Comparison of field and expert laboratory microscopy for active surveillance for asymptomatic *Plasmodium falciparum* and *Plasmodium vivax* in western Thailand. Am J Trop Med Hyg 67: 141-144.

396. McKenzie FE, Sirichainthop J, Miller RS, Gasser Jr RA, Wongsrichanalai C (2003) Dependence of malaria detection and species diagnosis by microscopy on parasite density. Am J Trop Med Hyg 69: 372.

397. de Oliveira AM, Skarbinski J, Ouma PO, Kariuki S, Barnwell JW, Otieno K, Onyona P, Causer LM, Laserson KF, Akhwale WS (2009) Performance of malaria rapid diagnostic tests as part of routine malaria case management in Kenya. Am J Trop Med Hyg 80: 470-474.

398. Fançony C, Sebastião YV, Pires JE, Gamboa D, Nery SV (2013) Performance of microscopy and RDTs in the context of a malaria prevalence survey in Angola: a comparison using PCR as the gold standard. Malar J 12: 284.

399. Satoguina J, Walther B, Drakeley C, Nwakanma D, Oriero EC, Correa S, Corran P, Conway DJ, Walther M (2009) Comparison of surveillance methods applied to a situation of low malaria prevalence at rural sites in The Gambia and Guinea Bissau. Malar J 8: 274.

400. Bell DR, Wilson DW, Martin LB (2005) False-positive results of a *Plasmodium falciparum* histidine-rich protein 2-detecting malaria rapid diagnostic test due to high sensitivity in a community with fluctuating low parasite density. Am J Trop Med Hyg 73: 199-203.

401. Kilian A, Metzger W, Mutschelknauss E, Kabagambe G, Langi P, Korte R, Sonnenburg Fv (2000) Reliability of malaria microscopy in epidemiological studies: results of quality control. Trop Med Int Health 5: 3-8.

402. Billo MA, Diakité M, Dolo A, Diallo M, Poudiougou B, Diawara SI, Johnson ES, Rice JC, Krogstad DJ, Doumbo OK (2013) Inter-observer agreement according to malaria parasite density. Malar J 12: 335.

403. Chiodini PL, Bowers K, Jorgensen P, Barnwell JW, Grady KK, Luchavez J, Moody AH, Cenizal A, Bell D (2007) The heat stability of *Plasmodium* lactate dehydrogenase-based and histidine-rich protein 2-based malaria rapid diagnostic tests. Trans R Soc Trop Med Hyg 101: 331-337.

404. Iqbal J, Siddique A, Jameel M, Hira PR (2004) Persistent histidine-rich protein 2, parasite lactate dehydrogenase, and panmalarial antigen reactivity after clearance of *Plasmodium falciparum* monoinfection. J Clin Microbiol 42: 4237-4241.

405. Toft N, Jørgensen E, Højsgaard S (2005) Diagnosing diagnostic tests: evaluating the assumptions underlying the estimation of sensitivity and specificity in the absence of a gold standard. Prev Vet Med 68: 19-33.

406. Pereira GdA, Louzada F, Barbosa VdF, Ferreira-Silva MM, Moraes-Souza H (2012) A General Latent Class Model for Performance Evaluation of Diagnostic Tests in the Absence of a Gold Standard: An Application to Chagas Disease. Comput Math Methods Med 2012.

407. Norton S, Johnson WO, Jones G, Heuer C (2010) Evaluation of diagnostic tests for Johne's disease (*Mycobacterium avium* subspecies paratuberculosis) in New Zealand dairy cows. J Vet Diagn Invest 22: 341-351.

408. Canier L, Khim N, Kim S, Sluydts V, Heng S, Dourng D, Eam R, Chy S, Khean C, Loch K, Ken M, Lim H, Siv S, Tho S, Masse-Navette P, Gryseels C, Uk S, Van Roey K, Grietens KP, Sokny M, Thavrin B, Chuor CM, Durnez L, Coosemans M, Menard D (2013) An innovative tool for moving malaria PCR detection of parasite reservoir into the field. Malar J 12.

409. Lindsay SW, Campbell H, Adiamah JH, Greenwood AM, Bangali JE, Greenwood BM (1990) Malaria in a peri-urban area of The Gambia. Ann Trop Med Parasitol 84: 553-562.

410. Schellenberg JA, Newell JN, Snow RW, Mung'ala V, Marsh K, Smith PG, Hayes RJ (1998) An analysis of the geographical distribution of severe malaria in children in Kilifi District, Kenya. Int J Epidemiol 27: 323-329.

411. Chadee DD, Kitron U (1999) Spatial and temporal patterns of imported malaria cases and local transmission in Trinidad. Am J Trop Med Hyg 61: 513-517.

412. Mabaso MLH, Vounatsou P, Midzi S, Da Silva J, Smith T (2006) Spatio-temporal analysis of the role of climate in inter-annual variation of malaria incidence in Zimbabwe. Int J Health Geogr 5: 20.

413. Gething PW, Atkinson PM, Noor AM, Gikandi PW, Hay SI, Nixon MS (2007) A local space-time kriging approach applied to a national outpatient malaria data set. Comput Geosci 33: 1337-1350.

414. Abellana R, Ascaso C, Aponte J, Saute F, Nhalungo D, Nhacolo A, Alonso P (2008) Spatio-seasonal modeling of the incidence rate of malaria in Mozambique. Malar J 7: 228.

415. Ribeiro JM, Seulu F, Abose T, Kidane G, Teklehaimanot A (1996) Temporal and spatial distribution of anopheline mosquitos in an Ethiopian village: implications for malaria control strategies. Bull World Health Organ 74: 299-305.

416. Brooker S, Clarke S, Njagi JK, Polack S, Mugo B, Estambale B, Muchiri E, Magnussen P, Cox J (2004) Spatial clustering of malaria and associated risk factors during an epidemic in a highland area of western Kenya. Trop Med Int Health 9: 757-766.

417. Ernst KC, Adoka SO, Kowuor DO, Wilson ML, John CC (2006) Malaria hotspot areas in a highland Kenya site are consistent in epidemic and non-epidemic years and are associated with ecological factors. Malar J 5: 78.

418. Coleman M, Coleman M, Mabuza AM, Kok G, Coetzee M, Durrheim DN (2009) Using the SaTScan method to detect local malaria clusters for guiding malaria control programmes. Malar J 8: 68.

419. Wimberly MC, Midekisa A, Semungiguse P, Teka H, Henebry GM, Chuang T-W, Senay GB (2012) Spatial synchrony of malaria outbreaks in a highland region of Ethiopia. Trop Med Int Health 17: 1192-1201.

420. Dolgin E (2010) Targeting hotspots of transmission promises to reduce malaria. Nat Med 16: 1055.

421. Bautista CT, Chan AST, Ryan JR, Calampa C, Roper MH, Hightower AW, Magill AJ (2006) Epidemiology and spatial analysis of malaria in the Northern Peruvian Amazon. Am J Trop Med Hyg 75: 1216-1222.

422. Mirghani SE, Nour BYM, Bushra SM, Elhassan IM, Snow RW, Noor AM (2010) The spatial-temporal clustering of *Plasmodium falciparum* infection over eleven years in Gezira State, The Sudan. Malar J 9: 172.

423. Nourein AB, Abass MA, Nugud AHD, El Hassan I, Snow RW, Noor AM (2011) Identifying residual foci of *Plasmodium falciparum* infections for malaria elimination: the urban context of Khartoum, Sudan. PloS one 6: e16948.

424. Kulldorf M, Nagarwalla N (1995) Spatial disease clusters: detection and inference. Stat Med 14: 799-810.

425. Patil GP, Taillie C (2004) Upper level set scan statistic for detecting arbitrarily shaped hotspots. Environ Ecol Stat 11: 183-197.

426. Pfeiffer D, Robinson T, Stevenson M, Stevens K, Rogers D, Clements ACA (2008) Spatial Analysis in Epidemiology. Oxford, UK: Oxford University Press.

427. Pullan RL, Sturrock HJW, Soares Magalhaes RJ, Clements ACA, Brooker SJ (2012) Spatial parasite ecology and epidemiology: a review of methods and applications. Parasitology 139: 1870-1887.

428. Read S, Bath PA, Willett P, Maheswaran R (2013) New developments in the spatial scan statistic. J Inform Sci 39: 36-47.

429. Diggle PJ, Tawn JA, Moyeed RA (1998) Model-based geostatistics. Appl Statist 47: 299-350.

430. Silue KD, Raso G, Yapi A, Vounatsou P, Tanner M, N'Goran EK, Utzinger J (2008) Spatially-explicit risk profiling of *Plasmodium falciparum* infections at a small scale: a geostatistical modelling approach. Malar J 7: 111.

431. Riedel N, Vounatsou P, Miller JM, Gosoniu L, Chizema-Kawesha E, Mukonka V, Steketee RW (2010) Geographical patterns and predictors of malaria risk in Zambia: Bayesian geostatistical modelling of the 2006 Zambia national malaria indicator survey (ZMIS). Malar J 9: 37.

432. Gosoniu L, Veta AM, Vounatsou P (2010) Bayesian geostatistical modeling of Malaria Indicator Survey data in Angola. PloS one 5: e9322.

433. Raso G, Schur N, Utzinger J, Koudou BG, Tchicaya ES, Rohner F, N'Goran EK, Silue KD, Matthys B, Assi S, Tanner M, Vounatsou P (2012) Mapping malaria risk among children in Cote d'Ivoire using Bayesian geo-statistical models. Malar J 11: 160.

434. Wang XH, Zhou XN, Vounatsou P, Chen Z, Utzinger J, Yang K, Steinmann P, Wu XH (2008) Bayesian spatio-temporal modeling of *Schistosoma japonicum* prevalence data in the absence of a diagnostic 'gold' standard. PLoS Negl Trop Dis 2: e250.

435. Pullan RL, Gething PW, Smith JL, Mwandawiro CS, Sturrock HJ, Gitonga CW, Hay SI, Brooker S (2011) Spatial modelling of soil-transmitted helminth infections in Kenya: a disease control planning tool. PLoS Negl Trop Dis 5: e958.

436. Midega JT, Mbogo CM, Mwambi H, Wilson MD, Ojwang G, Mwangangi JM, Nzovu J, Githure JI, Yan G, Beier JC (2007) Estimating dispersal and survival of *Anopheles gambiae* and *Anopheles funestus* along the Kenyan coast by using mark–release–recapture methods. J Med Entomol 44: 923-929.

437. Thomas CJ, Cross DE, Bogh C (2013) Landscape movements of *Anopheles gambiae* malaria vector mosquitoes in rural Gambia. PloS one 8: e68679.

438. US Geological Survey Global 3 ARC second elevation data.

439. Hijmans RJ, Cameron SE, Parra JL, Jones PG, Jarvis A (2005) Very high resolution interpolated climate surfaces for global land areas. Int J Climatol 25: 1965-1978.

440. FAO Africover Project (Food and Agriculture Organization of the United Nations, FAO).

441. Zomer RJ, Trabucco A, Bossio DA, Verchot LV (2008) Climate change mitigation: A spatial analysis of global land suitability for clean development mechanism afforestation and reforestation. Agric Ecosyst Environ 126: 67-80.

442. Tatem AJ, Noor AM, von Hagen C, Di Gregorio A, Hay SI (2007) High resolution population maps for low income nations: combining land cover and census in East Africa. PloS one 2: e1298.

443. Tatem AJ (2009) Kenya AfriPop Data 2009 (alpha version). Gainesville, United States: Emerging Pathogens Institute, University of Florida.

444. Spotimage SPOT Vegetation Programme (SPOT5).

445. US Geological Survey Early Warning Explorer. In: Famine Early Warning System Network, editor.

446. StataCorp (2011) Stata Statistical Software: Release 12. College Station, TX: StataCorp LP.

447. Richards SA (2005) Testing ecological theory using the information-theoretic approach: examples and cautionary results. Ecology 86: 2805-2814.

448. Krige D (1966) Two-dimensional weighted moving average trend surfaces for ore-evaluation. J South Afr Inst Min Metall 66: 13-38.

449. Haque U, Magalhães RJS, Reid HL, Clements AC, Ahmed SM, Islam A, Yamamoto T, Haque R, Glass GE (2010) Spatial prediction of malaria prevalence in an endemic area of Bangladesh. Malar J 9: 120.

450. Stresman GH (2010) Beyond temperature and precipitation: ecological risk factors that modify malaria transmission. Acta Trop 116: 167-172.

451. Lindsay SW, Bodker R, Malima R, Msangeni HA, Kisinza W (2000) Effect of 1997-98 El Nino on highland malaria in Tanzania Lancet 355: 989-990.

452. Zhou G, Munga S, Minakawa N, Githeko AK, Yan G (2007) Spatial relationship between adult malaria vector abundance and environmental factors in western Kenya higlands. Am J Trop Med Hyg 77: 29-35.

453. Sumba LA, Okoth K, Deng AL, Githure J, Knols BGJ, Beier JC, Hassanali A (2004) Daily oviposition patterns of the African malaria mosquito *Anopheles gambiae Giles* (Diptera: *Culicidae*) on different types of aqueous substrates. J Circadian Rhythms 2: 1-7.

454. Fillinger U, Sonye G, Killeen G, Knols BGJ, Becker N (2004) The practical importance of permanent and semipermanent habitats for controlling aquatic stages of Anopheles gambiae sensu lato mosquitoes: operational observations from a rural town in western Kenya. Trop Med Int Health 9: 1274-1289.

455. Sattler MA, Mtasiwa D, Kiama M, Premji Z, Tanner M, Killeen G, Lengeler C (2005) Habitat characterization and spatial distribution of Anopheles sp. mosquito larvae in Dar es Salaam (Tanzania) during an extended dry period. Malar J 4.

456. Giardina F, Gosoniu L, Konate L, Diouf MB, Perry R, Gaye O, Faye O, Vounatsou P (2012) Estimating the burden of malaria in Senegal: Bayesian zero-inflated binomial geostatistical modeling of the MIS 2008 data. PloS one 7: e32625.

457. Govoetchan R, Gnanguenon V, Azondekon R, Agossa RF, Sovi A, Oke-Agbo F, Osse R, Akogbeto M (2014) Evidence for perennial malaria in rural and urban areas under the Sudanian climate of Kandi, Northeastern Benin. Parasit Vectors 24.

458. Brunsdon C, Fotheringham AS, Charlton ME (1996) Geographically weighted regression: a method for exploring spatial nonstationarity. Geogr Anal 28: 281-298.

459. Sawaya KE, Olmanson LG, Heinert NJ, Brezonik PL, Bauer ME (2003) Extending satellite remote sensing to local scales: land and water resource monitoring using high-resolution imagery. Remote Sens Environ 88: 144-156.

460. World Health Organisation (2012) Handbook for integrated vector management. Geneva: WHO. 1-67 p.

461. Mwangi TW, Fegan G, Williams TN, Kinyanjui SM, Snow RW, Marsh K (2008) Evidence for over-dispersion in the distribution of clinical malaria episodes in children. PLoS One 3: e2196.

462. Cairns ME, Asante KP, Owusu-Agyei S, Chandramohan D, Greenwood BM, Milligan PJ (2013) Analysis of partial and complete protection in malaria cohort studies. Malar J 12: 355.

463. Carlton EJ, Hubbard A, Wang S, Spear RC (2013) Repeated *Schistosoma japonicum* Infection Following Treatment in Two Cohorts: Evidence for Host Susceptibility to Helminthiasis? PLoS Negl Trop Dis 7: e2098.

464. Lindsay SW, Armstrong-Schellenberg JRM, Zeiler HA, Daly JF, Salum FM, Wilkins HA (1995) Exposure of Gambian children to *Anopheles gambiae* malaria vectors in an irrigated rice production area. Medical and veterinary entomology: 50-58.

465. Thomas C, Lindsay S (2000) Local-scale variation in malaria infection amongst rural Gambian children estimated by satellite remote sensing. Trans R Soc Trop Med Hyg 94: 159-163.

466. Sokhna C-S, Rogier C, Dieye A, Trape J-F (2000) Host factors affecting the delay of reappearance of *Plasmodium falciparum* after radical treatment among a semi-immune population exposed to intense perennial transmission. Am J Trop Med Hyg 62: 266-270.

467. Dent AE, Bergmann-Leitner ES, Wilson DW, Tisch DJ, Kimmel R, Vulule J, Sumba PO, Beeson JG, Angov E, Moormann AM (2008) Antibody-mediated growth inhibition of *Plasmodium falciparum*: relationship to age and protection from parasitemia in Kenyan children and adults. PLoS One 3: e3557.

468. Michon P, Cole-Tobian JL, Dabod E, Schoepflin S, Igu J, Susapu M, Tarongka N, Zimmerman PA, Reeder JC, Beeson JG (2007) The risk of malarial infections and disease in Papua New Guinean children. Am J Trop Med Hyg 76: 997.

469. Tran TM, Li S, Doumbo S, Doumtabe D, Huang C-Y, Dia S, Bathily A, Sangala J, Kone Y, Traore A (2013) An intensive longitudinal cohort study of Malian children and adults reveals no evidence of acquired immunity to *Plasmodium falciparum* infection. Clin Infect Dis 57: 40-47.

470. Modiano D, Petrarca V, Sirima B, Nebie I, Diallo D, Esposito F, Coluzzi M (1996) Different response to *Plasmodium falciparum* malaria in west African sympatric ethnic groups. Proc Natl Acad Sci 93: 13206-13211.

471. Torcia MG, Santarlasci V, Cosmi L, Clemente A, Maggi L, Mangano VD, Verra F, Bancone G, Nebie I, Sirima BS (2008) Functional deficit of T regulatory cells in Fulani,

an ethnic group with low susceptibility to *Plasmodium falciparum* malaria. Proc Natl Acad Sci 105: 646-651.

472. Bostrom S, Giusti P, Arama C, Persson J-O, Dara V, Traore B, Dolo A, Doumbo O, Troye-Blomberg M (2012) Changes in the levels of cytokines, chemokines and malaria-specific antibodies in response to *Plasmodium falciparum* infection in children living in sympatry in Mali. Malar J 11: 109.
473. Temperley M, Mueller DH, Njagi JK, Akhwale W, Clarke SE, Jukes MCH, Estambale B, Brooker S (2008) Costs and cost-effectiveness of delivering intermittent preventive treatment through schools in western Kenya. Malar J 7.
474. Yukich J, Lengeler C, Tediosi F, Brown N, Mulligan J-A, Chavasse D, Stevens W, Justino J, Conteh L, Maharaj R, Erskine M, Mueller DH, Wiseman V, Ghebremeskel T, Zerom M, Goodman C, McGuire D, Urrutia JM, Sakho F, Hanson K, Sharp B (2008) Costs and consequences of large-scale vector control for malaria. Malar J 7.
475. Worrall E, Fillinger U (2011) Large-scale use of mosquito larval source management for malaria control in Africa: a cost analysis. Malar J 10.
476. Lindsay SW, Kirby MJ, Baris E, Bos R (2004) Environmental management for malaria control in the East Asia and Pacific (EAP) region. Washington D.C. USA: 4 The International Bank for Reconstruction and Development / The World Bank.
477. Tusting LS, Thwing J, Sinclair D, Fillinger U, Gimnig J, Bonner KE, Bottomley C, Lindsay SW (2013) Mosquito larval source management for controlling malaria (Review). The Cochrane Collaboration. Oxford, UK: John Wiley & Sons Ltd. pp. 1-92.
478. Massara CL, Peixoto SV, Enk MJ, Barros HdS, Carvalho OdS, Sakurai E, Schall V (2006) Evaluation of an improved approach using residences of schistosomiasis-positive school children to identify carriers in an area of low endemicity. Am J Trop Med Hyg 74: 495-499.
479. Drexler N, Washington CH, Lovegrove M, Grady C, Milord MD, Streit T, Lammie P (2012) Secondary mapping of lymphatic filariasis in Haiti-definition of transmission foci in low-prevalence settings. PLoS Negl Trop Dis 6: e1807.
480. Guyatt H, Brooker S, Donnelly C (1999) Can prevalence of infection in school-aged children be used as an index for assessing community prevalence? Parasitology 118: 257-268.
481. Rodrigues L, Wheeler J, Shier R, Guerra H, Pimenta F, Lima e Costa M (2000) Predicting the community prevalence of schistosomiasis mansoni from the prevalence among 7-to 14-year-olds. Parasitology 121: 507-512.
482. Doherty T, Chopra M, Tomlinson M, Oliphant N, Nsibande D, Mason J (2010) Moving from vertical to integrated child health programmes:experiences from a multi-country

assessment of the Child Health Days approach in Africa. Trop Med Int Health 15: 196-305.

483. Bundy DA, Burbano C, Grosh M, Gelli A, Jukes M, Drake L (2009) Rethinking school feeding: Social safety nets, child development, and the education sector; Bank. TW, editor. Washington, D.C. USA: The World Bank.

484. UNESCO Institute for Statistics (2011) Global Education Digest. Regional Profile: Sub-Saharan Africa.

485. Nokes C, Bundy DA (1993) Compliance and absenteeism in school children: implications for helminth control. Trans R Soc Trop Med Hyg 87: 148-152.

486. Kolenikov S, Angeles G (2009) Socioeconomic status measurement with discrete proxy variables: Is principal component analysis a reliable answer? Rev Income Wealth 55: 128-165.

487. Vyas S, Kumaranayake L (2006) Constructing socio-economic status indices: how to use principal components analysis. Health Policy Plan 21: 459-468.

488. Thomson MC, Obsomer V, Kamgno J, Gardon J, Wanji S, Takougang I, Enyong P, Remme JH, Molyneux DH, Boussinesq M (2004) Mapping the distribution of Loa loa in Cameroon in support of the African Programme for Onchocerciasis Control. Filaria J 3: 7.

Appendices

APPENDIX 3.1

The principal component analysis (PCA) approach proposed by Filmer and Pritchett [314] was used to create composite wealth scores for the study population at baseline across both study groups. These wealth scores were used in all analyses including those restricted to sub-sets of the study population e.g. the risk analysis presented in Chapters 1 and 7 where only the children in the IST intervention group are included. Household asset factors were not available for 59 of the 5177 children at baseline and these children were not included in the PCA calculations. The variables included into the PCA included, ownership of a bicycle, motorcycle, mobile phone, radio, television, as well as presence of electricity, pit latrine, and brick and cement construction materials for walls and floors respectively. Roofing material was not included in the PCA as the nature of the roof material and design, such as the presence of open eaves may be directly related to mosquito access and thus was examined individually. All variables were coded as discrete binary categories. Although this method was originally designed for use with continuous data, the PCA results were compared with results from a polychoric PCA [486], more suitable for discrete data and there was no difference in how children were classified on the basis of wealth quintiles.

PCA produces principal components (sets of linear combinations of assets) in a way so that the first principal component captures the largest variance in the assets, and subsequent components are orthogonal to the previous component, each with maximum variance [487].

The following formula depicts the index derived for each household asset using PCA.

$$A_j = \sum_{i=1}^{n} f_i(a_{ji} - a_i)/s_i$$

Where A_j is the asset index for each child/household *j=1,...5118*. f_i is the scoring factor for each asset of the household. a_{ji} denotes the i^{th} asset (*i=1,...9)* of the j^{th} household, a_i is the mean of the i^{th} asset across households and s_i is the standard deviation of the i^{th} asset. In these analyses the household is equivalent to the child.

Table A3.1: Scoring factors for the principal component and summary statistics for the assets calculated from the PCA analysis for assets reported by the parents of 5118 children in south coast, Kenya in 2010

							Means		
Asset/Variable	Scoring factor	Mean	SD	Scoring factor /SD	Poorest	Poor	Median	Less poor	Least poor
Owns bicycle	0.220	0.530	0.499	0.441	0.148	0.361	0.687	0.731	0.762
Owns motorcycle	0.232	0.049	0.216	1.074	0.000	0.001	0.112	0.003	0.200
Owns radio	0.311	0.624	0.485	0.641	0.137	0.488	0.780	0.814	0.944
Owns television	0.376	0.095	0.293	1.283	0.000	0.000	0.003	0.061	0.413
Owns mobilephone	0.335	0.592	0.491	0.682	0.000	0.512	0.708	0.848	0.940
Has electricity	0.309	0.037	0.189	1.635	0.000	0.000	0.000	0.006	0.179
Has latrine in compound	0.298	0.583	0.493	0.604	0.228	0.412	0.569	0.819	0.912
House has brick walls	0.406	0.243	0.429	0.946	0.000	0.035	0.109	0.330	0.751
House has cement floor	0.445	0.225	0.418	1.065	0.000	0.001	0.033	0.255	0.839

The mean value of the index is 0 and so a moving from 0 (not having the asset) to 1 (possessing the asset) changes the index by f_i/s_i (scoring factor/SD) [314]. Thus a household with a bicycle has an asset indicator higher by 0.441 than a household that does not. The first principal component explained 30.6% of the overall variability and gave greatest weight to household construction materials and ownership of a television and the least weight to ownership of a bicycle. The first Eigenvalue (variance for the first principal component) was 2.76 and the second was 1.32. The resultant scores form the first principal component were divided into quintiles so that households could be classified according to relative SES.

APPENDIX 3.2

Table A3.2: Univariable analyses for associations of *P. falciparum* infection and anaemia and additional potential risk factors with a test of cognition (Ravens test), numeracy (Number Identification test) and a test of literacy (Spelling test) in class 1 children on the south coast of Kenya, 2010.

Risk factor	Number of children n (%)[1,2] 1135	RAVENS COGNITIVE TEST class 1			NUMBER IDENTIFICATION TEST class 1			SPELLING TEST class 1		
		Mean score[3] (0-20) (SD)	Mean difference between test performance (95% CI)	P value[4]	Mean score[3] (0-20) (SD)	Mean difference between test performance (95% CI)	P value[4]	Mean score[3] (0-20) (SD)	Mean difference between test performance (95% CI)	P value[4]
CHILD LEVEL										
Sex										
Male	572 (50.4)	7.45 (2.66)			3.24 (2.90)			7.51 (4.27)		
Female	563 (49.6)	7.39 (2.50)	-0.06 (-0.33, 0.19)	0.679	3.14 (2.60)	-0.10 (-0.45, 0.19)	0.542	7.68 (4.57)	0.17 (-0.41, 0.80)	0.578
Age (years)[5]										
	12.56 (1.55)	7.42 (2.58)	0.09 (-0.01, -0.20)	0.106	3.19 (2.75)	0.25 (0.12, 0.37)	<0.001	7.59 (4.42)	0.23 (0.02, 0.44)	0.030
***P.falciparum* density (p/µl)**										
No infection (0)	949 (83.6)	7.43 (2.57)			3.18 (2.78)			7.45 (4.42)		
Low (1-999)	136 (12.0)	7.17 (2.50)	-0.26 (-0.70, 0.18)	0.365	3.29 (2.77)	0.12 (-0.46, 0.67)	0.901	8.22 (4.64)	0.77 (-0.54, 2.20)	0.179
Medium/High (≥1000)	50 (4.4)	7.86 (3.03)	0.43 (-0.42, 1.43)		3.14 (2.29)	-0.04 (-0.72, 0.67)		8.52 (3.52)	1.07 (-0.12, 2.34)	
Anaemia status										
Not anaemic	596 (52.5)	7.38 (2.57)			3.06 (2.61)			7.44 (4.45)		
Anaemic	539 (47.5)	7.46 (2.59)	0.08 (-0.21, 0.36)	0.564	3.33 (2.90)	0.27 (-0.02, 0.64)	0.105	7.76 (4.39)	0.32 (-0.28, 0.97)	0.324
WAZ (z scores) [2]										
Not wasted	683 (75.6)	7.35 (2.47)			3.00 (2.62)			7.48 (4.48)		
Wasted	221 (24.4)	7.63 (2.94)	0.28 (-0.15, 0.75)	0.221	3.20 (2.72)	0.20 (-0.26, 0.59)	0.357	7.63 (4.58)	0.15 (-0.73, 0.95)	0.733
HAZ (z scores)										
Not stunted	850 (75.0)	7.39 (2.52)			3.19 (2.75)			7.62 (4.51)		
Stunted	283 (25.0)	7.53 (2.75)	0.15 (-0.27, 0.59)	0.494	3.22 (2.77)	0.03 (-0.39, 0.42)	0.884	7.51 (4.15)	-0.12 (-0.74, 0.47)	0.723
BMIZ (z scores)										
Not thin	923 (81.5)	7.47 (2.57)			3.20 (2.78)			7.67 (4.40)		
Thin	209 (18.5)	7.22 (2.62)	-0.25 (-0.64, 0.10)	0.164	3.17 (2.64)	-0.03 (-0.38, 0.38)	0.880	7.20 (4.53)	-0.48 (-1.14, 0.29)	0.203
Child been dewormed in last year										
No	277 (25.8)	7.49 (2.60)			3.24 (2.87)			7.62 (4.14)		
Yes	796 (74.2)	7.41 (2.53)	-0.08 (-0.49, 0.38)	0.716	3.23 (2.76)	-0.01 (-0.47, 0.44)	0.964	7.66 (4.52)	0.05 (-0.76, 0.78)	0.905
Child missed school previous week[2]										
No	651 (62.4)	7.35 (2.60)			3.07 (2.71)			7.40 (4.37)		
Yes	393 (37.6)	7.51 (2.50)	0.16 (-0.12, 0.46)	0.296	3.42 (2.84)	0.36 (-0.02, 0.74)	0.063	7.89 (4.53)	0.49 (-0.01, 1.04)	0.070
Child ate breakfast before assessed										
No	331 (39.5)	7.12 (2.26)			3.01 (2.67)			7.33 (4.23)		
Yes	791 (70.5)	7.53 (2.68)	0.41 (0.07, 0.73)	0.015	3.24 (2.78)	0.23 (-0.19, 0.63)	0.256	7.69 (4.51)	0.35 (-0.31, 1.00)	0.282
Child failed a grade[2]										
No	719 (66.3)	7.40 (2.54)			3.09 (2.66)			7.54 (4.52)		
Yes	365 (33.7)	7.32 (2.52)	-0.08 (-0.37, 0.27)	0.642	2.50 (2.97)	0.40 (0.05, 0.80)	0.038	7.82 (4.25)	0.27 (-0.36, 0.85)	0.367
Family has books at home										
No	788 (71.4)	7.39 (2.58)			3.11 (2.69)			7.44 (4.41)		
Yes	315 (28.6)	7.30 (2.59)	-0.18 (-0.60, 0.17)	0.373	3.39 (2.98)	0.28 (-0.08, 0.67)	0.149	7.95 (4.51)	0.51 (-0.19, 1.20)	0.145

Risk factor	Number of children n (%)[1,2] 1135	Mean score[3] (0-20) (SD)	Mean difference between test performance (95% CI)	P value[4]	Mean score[3] (0-20) (SD)	Mean difference between test performance (95% CI)	P value[4]	Mean score[3] (0-20) (SD)	Mean difference between test performance (95% CI)	P value[4]
HOUSEHOLD LEVEL Education Level of household head										
No schooling	388 (34.5)	7.23 (2.41)			2.98 (2.62)			7.29 (4.18)		
Primary	593 (52.8)	7.45 (2.65)	0.22 (-0.08, 0.57)		3.21 (2.63)	0.23 (-0.14, 0.55)		7.66 (4.41)	0.37 (-0.26, 1.00)	
Secondary	103 (9.2)	7.92 (2.83)	0.69 (0.05, 1.37)	0.130	3.74 (3.11)	0.76 (-0.02, 1.46)	0.167	8.06 (5.04)	0.77 (-0.46, 1.97)	
College/degree	39 (3.5)	7.64 (2.35)	0.41 (-0.25, 1.16)		3.77 (4.40)	0.79 (-0.60, 2.39)		7.95 (5.35)	0.65 (-1.08, 2.55)	0.611
Child sleeps under a net										
No	373 (33.4)	7.48 (2.75)			3.17 (2.69)			7.51 (4.17)		
Yes	745 (66.6)	7.35 (2.45)	-0.13 (-0.45, 0.23)	0.432	3.21 (2.81)	0.04 (-0.27, 0.37)	0.794	7.65 (4.54)	0.13 (-0.49, 0.77)	0.684
SES quintiles										
Poorest	287 (25.4)	7.36 (2.46)			3.03 (2.76)			7.04 (4.34)		
Poor	256 (22.6)	7.38 (2.52)	0.02 (-0.36, 0.35)		3.16 (2.66)	0.13 (-0.39, 0.59)		7.24 (4.28)	0.21 (-0.48, 1.01)	
Median	196 (17.3)	7.37 (2.73)	0.00 (-0.53, 0.58)	0.860	3.06 (2.73)	0.02 (-0.52, 0.57)	0.202	7.50 (4.07)	0.46 (-0.39, 1.34)	
Less poor	203 (18.0)	7.45 (2.46)	0.09 (-0.32, 0.52)		3.17 (2.79)	0.14 (-0.48, 0.81)		8.11 (4.34)	1.07 (0.22, 2.06)	0.052
Least poor	189 (16.7)	7.61 (2.83)	0.25 (-0.28, 0.71)		3.74 (2.86)	0.61 (-0.01, 1.22)		8.43 (4.97)	1.39 (0.44, 2.44)	
Household size[5]	7.20 (2.60)	7.40 (2.56)	-0.07 (-0.14, -0.01)	0.041	3.20 (2.77)	0.01 (-0.06, 0.08)	0.859	7.60 (4.42)	0.04 (-0.07, 0.16)	0.515
Number of children in house[5]	5.06 (2.25)	7.40 (2.56)	-0.07 (-0.14, -0.01)	0.045	3.20 (2.77)	0.00 (-0.09, 0.10)	0.932	7.60 (4.42)	0.09 (-0.05, 0.22)	0.193
Parent is literate										
No	398 (35.5)	7.27 (2.38)			2.92 (2.52)			7.15 (4.15)		
Yes	722 (64.5)	7.51 (2.69)	0.24 (-0.08, 0.56)	0.154	3.34 (2.87)	0.41 (0.087, 0.76)	0.021	7.83 (4.56)	0.68 (-0.01, 1.33)	0.050
Language parents speak with child										
Mothertongue	925 (82.8)	7.47 (2.61)			3.16 (2.77)			7.68 (4.38)		
English/Swahili	192 (17.2)	7.21 (2.56)	-0.26 (-0.72, 0.23)	0.265	3.33 (2.69)	0.17 (-0.41, 0.87)	0.594	7.07 (4.68)	-0.61 (-1.90, 0.99)	0.434
SCHOOL LEVEL Child teacher ratio										
15-34	187 (16.5)	7.66 (2.54)			3.34 (2.61)			7.61 (4.58)		
35-44	299 (26.3)	7.40 (2.40)	-0.26 (-0.80, 0.31)		2.95 (2.33)	-0.38 (-1.42, 0.66)		7.43 (4.58)	-0.18 (-2.33, 2.42)	
45-54	350 (30.8)	7.72 (3.05)	0.07 (-0.58, 0.68)	0.003	3.82 (3.16)	0.48 (-0.61, 1.54)	0.063	9.01 (4.52)	1.40 (-0.53, 3.84)	<0.001
55-64	120 (10.6)	6.78 (2.23)	-0.88 (-1.35, -0.38)		2.5 0 (2.70)	-0.84 (-2.08, 0.30)		5.98 (3.29)	-1.64 (-3.69, 0.75)	
≥65	179 (15.8)	7.03 (1.96)	-0.62 (-1.32, -0.06)		2.65 (2.49)	-0.69 (-1.76, 0.22)		6.15 (3.50)	-1.46 (-3.19, 0.69)	
Seating arrangement in class										
Desks or tables and chairs	967 (85.2)	7.36 (2.57)			3.32 (2.87)			7.86 (4.46)		
Floor	168 (14.8)	7.76 (2.65)	0.40 (-0.41, 1.21)	0.338	2.46 (1.84)	-0.85 (-1.40, -0.25)	0.003	6.00 (3.80)	-1.87 (-3.70, -0.57)	0.022
School malaria control activities										
No	867 (76.4)	7.39 (2.55)			3.18 (2.72)			7.49 (4.48)		
Yes	268 (23.6)	7.52 (2.69)	0.14 (-0.38, 0.64)	0.519	3.22 (2.86)	0.04 (-0.67, 0.74)	0.915	7.92 (4.22)	0.42 (-1.05, 1.83)	0.576
School feeding programme										
No	525 (46.3)	7.56 (2.72)			3.27 (2.56)			7.61 (4.25)		
Yes	610 (53.7)	7.30 (2.46)	-0.27 (-0.70, 0.16)	0.240	2.12 (2.91)	-0.14 (-0.74, 0.53)	0.661	7.57 (4.56)	-0.04 (-1.41, 1.33)	0.952
Administrative Division										
Diani	303 (26.7)	7.79 (2.72)			3.63 (2.58)			8.99 (4.38)		
Lunga Lunga	457 (40.3)	6.98 (2.47)	-0.81 (-1.28, -0.40)		3.06 (3.00)	-0.57 (-1.21, 0.11)		7.20 (4.03)	-1.79 (-3.28, -0.23)	
Msambweni	139 (12.2)	8.22 (2.79)	-0.43 (-0.34, 1.33)	>0.001	2.87 (2.31)	-0.76 (-1.55, -0.03)	0.141	6.79 (4.89)	-2.19 (-4.82, 0.50)	0.080
Kubo	236 (20.8)	7.32 (2.32)	-0.47 (-1.08, 0.13)		3.08 (2.66)	-0.55 (-1.56, 0.79)		7.03 (4.54)	-1.95 (-3.93, 0.42)	

[1] 1135 observations included for Ravens test. 1134 observations included for number identification. 1131 observations included for spelling test. Percentage children per characteristic shown for 1135 children.

[2]All missing <3% with the exception of WAZ-20.3%, children missed school previous week-8.0%, child failed a grade-4.5% [3]Positive values indicate an increased score over reference group and negative values indicate a decreased score over reference group (95% CI is the bias corrected confidence interval) [4] P value is from multivariable Wald test derived from multivariable linear regression, bootstrapped and adjusted for school level clustering

APPENDIX 3.3

Table A3.3: Univariable analyses for associations of *P. falciparum* infection and anaemia and additional potential risk factors with a test of sustained attention (pencil tapping) in class 1 children on the south coast of Kenya, 2010.

	PENCIL TAP ATTENTION TEST class 1						
	Probability of children engaging in the task				Scores if children are engaged		
Risk factor	Number of children (%)[1,2] n=1135	Number of children engaged in task (%)[3] n= 998	OR of engagement; (95% CI)	P- value[4]	Mean score if engaged (1-20) (SD)	Mean difference between test performance[5] (95% CI)	P value[6]
CHILD LEVEL							
Sex							
Male	572 (50.4)	513 (89.7)	1		14.17 (4.99)		
Female	563 (49.6)	485 (86.2)	0.71 (0.49, 1.02)	0.064	13.36 (5.11)	-0.80 (-1.35, 0.21)	0.006
Age (years)	12.56 (1.55)	12.56 (1.55)	1.20 (1.07, 1.35)	0.002	13.78 (5.07)	0.57 (0.36, 0.74)	<0.001
***P.falciparum* density (p/µl)**							
No infection (0)	949 (83.6)	929 (87.4)	1		13.71 (5.07)		
Low (1-999)	136 (12.0)	121 (89.0)	1.12 (0.61, 2.04)	0.131	14.13 (4.91)	0.42 (-0.53, 1.53)	0.564
Medium/High (≥1000)	50 (4.4)	48 (96.0)	3.48 (0.82, 14.82)		14.13 (5.31)	0.42 (-0.82, 1.58)	
Anaemia status							
Not anaemic	596 (52.5)	515 (86.4)	1		13.63 (5.04)		
Anaemic	539 (47.5)	483 (89.6)	1.28 (0.88, 1.87)	0.198	13.94 (5.08)	0.31 (-0.28, 0.90)	0.290
WAZ (z scores)							
Not wasted	683 (75.6)	597 (87.4)	1		13.55 (5.08)		
Wasted	221 (24.4)	188 (85.1)	0.80 (0.51, 1.26)	0.341	13.62 (5.06)	-0.09 (-0.85, 0.65)	0.891
HAZ (z scores)							
Not stunted	850 (75.0)	742 (87.3)	1		13.68 (5.01)		
Stunted	283 (25.0)	254 (89.8)	1.29 (0.83-2.02)	0.249	14.07 (5.22)	0.39 (-0.37, 1.06)	0.278
BMIZ (z scores)							
Not thin	923 (81.5)	816 (88.4)	1		13.79 (5.00)		
Thin	209 (18.5)	179 (85.7)	0.82 (0.52, 1.29)	0.394	13.70 (5.40)	-0.09 (-0.85, 0.65)	0.820
Child been dewormed in last year							
No	277 (25.8)	241 (87.0)	1		14.27 (4.86)		
Yes	796 (74.2)	704 (88.4)	1.19 (0.77, 1.83)	0.430	13.75 (5.04)	-0.52 (-1.29, 0.29)	0.205
Child missed schl in previous week							
No	651 (62.4)	564 (86.6)	1		13.84 (5.00)		
Yes	393 (37.6)	351 (89.3)	1.28 (0.86, 1.92)	0.220	13.85 (5.13)	0.00 (-0.57, 0.61)	0.990
Child ate breakfast on day of test							
No	331 (29.5)	307 (92.8)	1	<0.001	13.63 (5.00)		
Yes	791 (70.5)	680 (89.0)	0.46 (0.29, 0.75)		13.81 (5.13)	0.17 (-0.52, 0.86)	0.627
Child failed a grade							
No	719 (66.3)	635 (88.3)	1		13.55 (5.11)		
Yes	365 (33.7)	320 (87.7)	0.92 (0.62, 1.37)	0.688	14.25 (4.96)	0.71 (0.00, 1.45)	0.062
HOUSEHOLD LEVEL							
Education Level of household head							
No schooling	388 (34.5)	338 (87.1)	1		14.25 (4.96)		
Primary	593 (52.8)	523 (88.2)	1.18 (0.78, 1.77)		13.70 (5.06)	-0.55 (-0.27, 1.46)	
Secondary	103 (9.2)	93 (90.3)	1.65 (0.78, 3.51)	0.405	12.69 (5.09)	-1.57 (-2.90, -0.18)	0.110
College/degree	39 (3.5)	36 (92.3)	2.08 (0.60, 7.28)		13.33 (5.84)	-0.92 (-2.93, 0.87)	

	Probability of children engaging in the task				Scores if children are engaged		
Risk factor	Number of children (%)[1,2] n=1135	Number of children engaged in task (%)[3]n= 998	OR of engagement; (95% CI)	P- value[4]	Mean score if engaged (1-20) (SD)	Mean difference between test performance[5] (95% CI)	P value[6]
Child sleeps under a net							
No	373 (33.4)	332 (89.0)	1		14.42 (5.00)		
Yes	745 (66.6)	654 (87.8)	0.95 (0.63, 1.43)	0.796	13.47 (5.08)	-0.96 (-1.73, -0.23)	0.013
SES quintiles							
Poorest	287 (25.4)	257 (89.6)	1		14.48 (5.04)		
Poor	256 (22.6)	227 (88.7)	0.94 (0.54, 1.65)		14.27 (4.99)	-0.21 (-1.23, 0.69)	
Median	196 (17.3)	174 (88.8)	1.01 (0.55, 1.86)	0.830	13.43 (4.97)	-1.05 (-2.04, -0.17)	0.002
Less poor	203 (18.0)	177 (87.2)	0.86 (0.48, 1.54)		13.16 (5.20)	-1.31 (-2.42, -0.38)	
Least poor	189 (16.7)	162 (85.7)	0.73 (0.41, 1.31)		13.02 (5.01)	-1.46 (-2.53, -0.40)	
Household size							
	7.20 (2.60)	7.20 (2.60)	1.06 (0.98, 1.16)	0.134	13.78 (5.07)	0.18 (0.06, 0.32)	0.007
Number of children in house							
	5.06 (2.25)	5.06 (2.25)	1.09 (0.99, 1.21)	0.068	13.78 (5.07)	0.06 (0.08, 0.19)	0.428
Parent is literate							
No	398 (35.5)	348 (87.4)	1		14.11 (5.02)		
Yes	722 (64.5)	639 (88.5)	1.18 (0.79, 1.74)	0.421	13.61 (5.08)	-0.50 (-1.12, 0.13)	0.122
Language parents speak with child							
Mother tongue	925 (82.8)	818 (88.4)	1		13.96 (4.94)		
English/Swahili	192 (17.2)	167 (87.0)	0.88 (0.53, 1.44)	0.605	12.79 (5.56)	-1.17 (-2.09, -0.18)	0.017
Family has books at home							
No	788 (71.4)	692 (87.8)	1		13.98 (4.95)		
Yes	315 (28.6)	279 (88.6)	1.16 (0.76, 1.76)	0.481	13.25 (5.29)	-0.72 (-1.43, -0.09)	0.042
SCHOOL LEVEL Child teacher ratio							
15-34	187 (16.5)	167 (89.3)	1		13.87 (5.04)		
35-44	299 (26.3)	257 (86.0)	0.73 (0.35, 0.51)		13.45 (5.02)	-0.42 (-1.82, 1.05)	
45-54	350 (30.8)	308 (88.0)	0.86 (0.42, 1.75)	0.821	13.80 (5.12)	-0.08 (-1.48, 1.50)	0.862
55-64	120 (10.6)	109 (90.8)	1.18 (0.45, 3.08)		13.80 (5.26)	-0.08 (-1.47, 1.77)	
≥65	179 (15.8)	157 (87.7)	0.83 (0.36, 1.88)		14.16 (4.94)	-0.28 (-1.19, 1.94)	
Seating arrangement in classroom							
Desks or tables and chairs	967 (85.2)	848 (87.7)	1		13.77 (5.09)		
Floor	168 (14.8)	150 (89.3)	1.13 (0.58, 2.20)	0.709	13.82 (4.92)	0.05 (-0.70, 1.12)	0.914
School malaria control activities							
No	867 (76.4)	757 (87.3)	1		13.57 (5.07)		
Yes	268 (23.6)	241 (89.9)	1.30 (0.74, 2.29)	0.351	14.43 (5.00)	0.86 (-0.21, 1.73)	0.089
School feeding programme							
No	525 (46.3)	475 (90.5)	1		14.00 (5.03)		
Yes	610 (53.7)	523 (85.7)	0.63 (0.40, 0.99)	0.049	13.58 (5.09)	-0.42 (-1.21, 0.36)	0.321
Administrative Division							
Diani	303 (26.7)	271 (89.4)	1		14.16 (5.06)		
Lunga Lunga	457 (40.3)	415 (90.8)	1.17 (0.67, 2.03)		14.35 (4.95)	0.19 (-0.70, 1.15)	
Msambweni	139 (12.2)	120 (86.3)	0.74 (0.37, 1.49)	0.032	12.90 (5.19)	-1.26 (-2.44, -0.19)	<0.001
Kubo	236 (20.8)	192 (81.4)	0.51 (0.29, 0.92)		12.56 (4.98)	-0.60 (-2.87, -0.39)	

[1] 1135 observations included for Pencil-tap attention test. Displayed as number and percentage except for continuous variables, displayed as mean and standard deviation (SD). [2]All missing <3% with the exception of WAZ- 20.3%, children missed school previous week-8.0%, child failed a grade-4.5% [3]Only children found to be engaged in task are included. [4]P value is from likelihood ratio test comparing multilevel logistic regression models (adjusting for school level clustering), with and without character of interest. [5]Positive values indicate an increased score over reference group and negative values indicate a decreased score over reference group (95% CI is the bias corrected confidence interval) [6] P-value is from multivariable Wald test derived from multivariable linear regression, bootstrapped and adjusted for school level clustering

APPENDIX 3.4

Table A3.4: Univariable analyses for associations of *P. falciparum* infection and anaemia and additional potential risk factors with a test of cognition (Silly Sentences), numeracy (Written Numeracy test), literacy (Spelling test) and sustained attention (Code Transmission test) in class 5 children on the south coast of Kenya, 2010.

		SILLY SENTENCES COMPREHENSION TEST class 5			WRITTEN NUMERACY TEST class 5			SPELLING TEST class 5			CODE TRANSMISSION TEST class 5		
Risk factor	Number of children N (%)[1,2] 1229	Mean score[3] (0-40) (SD)	Mean difference between test performance (95% CI)	P value[4]	Mean score[3] (0-38) (SD)	Mean difference between test performance (95% CI)	P value[4]	Mean score[3] (0-20) (SD)	Mean difference between test performance (95% CI)	P value[4]	Mean score[3] (0-20) (SD)	Mean difference between test performance (95% CI)	P value[4]
CHILD LEVEL													
Sex													
Male	578 (47.0)	29.33 (6.30)			28.56 (5.81)			22.54 (8.48)			10.69 (5.52)		
Female	651 (53.0)	28.56 (6.48)	-0.77 (-1.51, 0.03)	0.052	28.59 (5.74)	0.04 (-0.66, 0.73)	0.917	21.69 (7.93)	-0.86 (-1.67, 0.07)	0.051	10.19 (5.73)	-0.50 (-1.20, 0.15)	0.166
Age (years)[5]													
	12.58 (1.52)	28.93 (6.40)	-0.70 (-1.03,-0.44)	0.039	28.58 (5.77)	-0.04 (-0.27, 0.17)	0.732	22.09 (8.20)	-1.37 (-1.72,-1.04)	<0.001	10.43 (5.64)	-0.26 (-0.44,-0.07)	0.008
***P.falciparum* density (p/µl)**													
No infection (0)	1106 (90.0)	28.94 (6.44)			28.51 (5.86)			22.12 (8.27)			10.43 (5.63)		
Low (1-999)	101 (8.2)	28.94 (5.97)	0.00 (-1.63, 1.42		29.25 (5.14)	0.74 (-0.47, 2.06)		22.16 (7.44)	0.04 (-1.97, 2.28)		10.57 (5.84)	0.15 (-1.32, 1.68)	
High (1000>)	22 (1.8)	28.18 (6.75)	-0.76 (-3.60, 2.77)	0.890	29.00 (3.82)	0.49 (-1.21, 2.61)	0.474	20.36 (8.17)	-1.75 (-6.11, 2.46)	0.725	9.59 (5.15)	-0.84 (-3.16, 1.27)	0.829
Anaemia status													
Not anaemic	696 (56.6)	28.97 (6.50)			28.57 (5.92)			21.94 (8.54)			10.31 (5.66)		
Anaemic	533 (43.4)	28.86 (6.28)	-0.11 (-0.92, 0.71)	0.793	28.59 (4.80)	0.02 (-0.74, 0.77)	0.949	22.28 (7.75)	0.34 (-0.76, 1.45)	0.528	10.58 (5.61)	0.27 (-0.33, 0.84)	0.360
HAZ (z scores)													
Not stunted	916 (74.7)	29.09 (6.55)			28.80 (5.66)			22.28 (8.20)			10.35 (5.71)		
Stunted	311 (25.3)	28.40 (5.94)	-0.69 (-1.42, 0.06)	0.070	27.92 (6.06)	-0.88 (-1.76,-0.03)	0.042	21.51 (8.21)	-0.77 (-2.00, 0.33)	0.202	10.64 (5.43)	0.29 (-0.38, 0.96)	0.410
BMIZ (z scores)													
Not thin	1000 (81.5)	28.80 (6.47)			28.51 (5.83)			21.83 (8.26)			10.35 (5.63)		
Thin	227 (18.5)	29.44 (6.11)	0.65 (-0.22, 1.60)	0.164	28.85 (5.55)	0.34 (-0.39, 1.14)	0.400	23.21 (7.90)	1.38 (0.26, 2.49)	0.016	10.77 (5.69)	0.43 (-0.41, 1.24)	0.302
HOUSEHOLD LEVEL Education of household head													
No schooling	415 (34.2)	28.20 (6.10)			28.23 (5.84)			21.18 (7.80)			9.91 (4.48)		
Primary	614 (50.6)	28.74 (6.47)	0.55 (-0.19, 1.28)		28.57 (5.89)	0.34 (-0.25, 0.99)		21.95 (8.24)	0.77 (-0.33, 1.65)		10.64 (5.75)	0.74 (-0.27, 1.46)	
Secondary	150 (12.4)	30.45 (6.64)	2.25 (1.03, 3.46)	<0.001	29.36 (5.13)	1.13 (0.31, 2.19)	0.207	23.84 (8.39)	2.66 (1.38, 4.00)	<0.001	10.75 (5.61)	0.83 (-0.38, 2.13)	0.232
College/degree	34 (2.8)	33.24 (5.33)	5.04 (3.06, 6.74)		29.12 (5.69)	0.88 (-1.03, 3.10)		25.76 (9.39)	4.58 (0.93, 8.49)		11.06 (5.43)	1.15 (-0.90, 2.96)	
Child sleeps under a net													
No	494 (40.6)	28.39 (6.51)			28.83 (5.65)			21.41 (8.27)			10.19 (5.83)		
Yes	722 (59.4)	29.28 (6.33)	0.88 (0.02, 1.80)	0.059	28.39 (5.85)	-0.44 (-1.14, 0.30)	0.214	22.52 (8.16)	1.12 (0.17, 2.13)	0.027	10. 57 (5.51)	0.37 (-0.31, 0.97)	0.253
Child been dewormed in last year													
No	155 (13.4)	28.01 (6.75)			27.66 (6.43)			21.26 (8.63)			9.81 (5.64)		
Yes	1003 (86.6)	29.12 (6.33)	1.11 (-0.46, 2.59)	0.166	28.72 (5.68)	1.06 (-0.59, 2.89)	0.231	22.21 (8.13)	0.95 (-1.23, 3.10)	0.397	10.52 (5.61)	0.72 (-0.39, 1.78)	0.186
SES quintile													
Poorest	283 (23.2)	27.64 (6.38)			27.99 (5.86)			20.04 (8.28)			9.90 (5.50)		
Poor	240 (19.7)	27.67 (6.58)	0.03 (-0.98, 1.19)		28.30 (5.88)	0.31 (-0.59, 1.57)		20.40 (8.33)	0.36 (-1.03, 1.70)		10.44 (5.69)	0.53 (-0.25, 1.49)	
Median	222 (18.2)	29.30 (6.29)	1.66 (0.49, 2.83)	<0.001	28.70 (6.06)	0.71 (-0.17, 1.66)	0.057	22.68 (7.45)	2.64 (1.48, 4.08)		9.98 (5.60)	0.07 (-0.96, 1.20)	0.064
Less poor	246 (20.2)	29.24 (6.18)	1.61 (0.60, 2.71)		28.77 (6.11)	0.78 (-0.32, 1.93)		22.38 (8.23)	2.34 (1.08, 3.65)	<0.001	11.06 (5.77)	1.16 (0.38, 2.15)	
Least poor	228 (18.7)	31.10 (5.99)	3.46 (2.30, 4.70)`		29.28 (4.75)	1.29 (0.34, 2.40)`		25.40 (7.53)	5.36 (3.70, 7.06)		10.80 (5.61)	0.89 (0.13, 1.84)	
Household size[5]													
	7.21 (2.61)	28.92 (6.42)	-0.22 (-0.39,-0.06)	0.008	28.58 (5.77)	-0.06 (-0.20,-0.06)	0.388	22.07 (8.22)	-0.26 (-0.50 -0.06)	0.014	10.42 (5.65)	-0.05 (-0.18, 0.08)	0.435
Number of children in house[5]													
	5.06 (2.25)	28.92 (6.42)	-0.30 (-0.48, 0.12)	0.001	28.58 (5.77)	-0.06 (-0.22, 0.07)	0.393	22.07 (8.22)	-0.38 (-0.61,-0.17)	0.001	10.42 (5.65)	-0.07 (-0.21, 0.06)	0.290

		SILLY SENTENCES COMPREHENSION TEST class 5			WRITTEN NUMERACY TEST class 5			SPELLING TEST class 5			CODE TRANSMISSION TEST class 5		
Risk factor	Number of children N (%)[1,2] 1229	Mean score[3] (0-40) (SD)	Mean difference between test performance (95% CI)	P value[4]	Mean score[3] (0-38) (SD)	Mean difference between test performance (95% CI)	P value[4]	Mean score[3] (0-20) (SD)	Mean difference between test performance (95% CI)	P value[4]	Mean score[3] (0-20) (SD)	Mean difference between test performance (95% CI)	P value[4]
SCHOOL LEVEL													
Child teacher ratio													
15-34	187 (15.2)	30.82 (5.27)			29.04 (4.17)			23.76 (6.65)			9.89 (5.58)		
35-44	325 (26.4)	29.24 (5.98)	-1.58 (-3.76, 1.09)		29.12 (5.38)	0.09 (-1.82, 1.94)		22.42 (8.16)	-1.34 (-3.82, 1.75)		11.19 (5.78)	1.30 (-0.26, 3.03)	
45-54	382 (31.1)	29.14 (7.12)	-1.68 (-4.51, 1.37)	0.132	29.50 (5.88)	0.45 (-1.38, 2.13)	0.125	22.94 (8.42)	-0.83 (-3.60, 2.32)	0.046	9.89 (5.92)	-0.00 (-1.39, 1.30)	0.186
55-64	140 (11.4)	27.94 (5.65)	-2.89 (-5.03, 0.23)		28.09 (4.80)	-0.95 (-2.52, 0.96)		20.84 (7.81)	-2.93 (-5.29,-0.22)		11.08 (4.51)	1.19 (-0.27, 2.43)	
≥65	195 (15.9)	26.87 (6.47)	-3.96 (-7.21, 0.06)		25.82 (7.14)	-3.22 (-6.04,-0.25)		19.16 (8.68)	-4.61 (-7.80,-0.87)		10.24 (5.47)	0.34 (-1.26, 1.74)	
School malaria control activities	915 (74.4)	28.77 (6.39)			28.26 (5.81)			22.06 (8.15)			10.34 (5.62)		
No	314 (25.6)	29.38 (6.44)	0.61 (-2.17, 2.99)	0.642	29.51 (5.56)	1.25 (-0.46, 2.93)	0.168	22.17 (8.38)	0.11 (-2.78, 3.01)	0.940	10.68 (5.70)	0.34 (-0.76, 1.80)	0.600
Yes													
School feeding programme													
No	567 (46.1)	29.68 (6.71)			29.39 (5.44)			23.46 (8.05)			10.63 (5.72)		
Yes	662 (53.9)	28.28 (6.05)	-1.40 (-3.41, 0.51)	0.145	27.88 (5.95)	-1.51 (-3.06, 0.01)	0.056	20.92 (8.15)	-2.55 (-4.78 -0.51)	0.017	10.25 (5.57)	-0.38 (-1.39, 0.53)	0.436
Administrative Division													
Diani	327 (26.6)	30.93 (6.34)			30.54 (4.10)			25.15 (7.16)			11.12 (5.99)		
Lunga Lunga	494 (40.2)	28.17 (6.24)	-2.76 (-5.11,-0.13)		28.08 (6.26)	-2.46 (-3.95,-0.87)		20.71 (8.36)	-4.32 (-6.67,-2.15)		10.01 (5.32)	-1.11 (-2.42, 0.14)	
Msambweni	161 (13.1)	28.11 (6.51)	-2.82 (-5.22, 0.12)	0.083	28.86 (5.06)	-1.68 (-3.55,-0.12)	<0.001	21.67 (7.78)	-3.48 (-6.10,-1.23)	<0.001	11.28 (5.59)	0.16 (-1.75, 1.90)	0.105
Kubo	247 (20.1)	28.31 (6.20)	-2.62 (-5.31,-0.01)		26.80 (6.31)	-3.74 (-6.38,-1.90)		21.07 (8.44)	-4.07 (-7.02,-1.22)		9.80 (5.67)	-1.32 (-2.60, 0.03)	

[1] 1229 observations included for Silly Sentences test. 1219 observations included for Written Numeracy test. 1228 observations included for Spelling test. 1227 observations included for Code Transmission test. Percentage children per characteristic shown for 1229 children.

[2]All variables missing <3%

[3]Positive values indicate an increased score over reference group and negative values indicate a decreased score over reference group (95% CI is the bias corrected confidence interval)

[4]P value is from multivariable Wald test derived from multivariable linear regression, bootstrapped and adjusted for school level clustering

APPENDIX 4.1

Table A4.1 Baseline measures for 5233 study children with missing 12 months follow-up health data vs. those not missing 12 months follow-up health data across both the control and IST intervention groups.

Characteristic; n (%) [a]		CONTROL GROUP		INTERVENTION GROUP	
		Missing outcome data	Outcome data available	Missing outcome data	Outcome data available
Child characteristics		N=375	N=2148	N=412	N=2298
Age	Mean (sd)	10.4 (3.1)	10.1 (2.8)	10.6 (3.1)	10.3 (2.8)
	5-9	155 (41.3)	886 (41.2)	155 (37.6)	914 (39.8)
	10-12	107 (28.5)	770 (35.9)	120 (29.1)	805 (35.0)
	13-20	113 (30.1)	492 (22.9)	137 (33.3)	579 (25.2)
Sex	Male	193 (51.5)	1064 (49.5)	208 (50.5)	1111 (48.3)
Child sleeps under net	Usually	229 (63.6)	1439 (67.9)	238 (60.1)	1444 (63.7)
	Last night	223 (97.4)	1383 (96.1)	225 (94.5)	1384 (95.8)
Nutritional Status	Underweight	42 (30.7)	224 (26.4)	26 (22.6)	205 (24.1)
	Stunted	80 (24.1)	520 (25.3)	72 (22.4)	540 (25.2)
	Thin	64 (19.3)	418 (20.4)	47 (14.6)	403 (18.8)
Household characteristics					
Parental Education	No schooling	101 (28.2)	625 (29.6)	158 (39.6)	767 (33.8)
	Primary schooling	180 (50.3)	1112 (52.6)	196 (49.1)	1185 (52.2)
	Secondary schooling	59 (16.5)	294 (13.9)	30 (7.5)	248 (10.9)
	Higher education	18 (5.0)	84 (4.0)	15 (3.8)	68 (3.0)
Socioeconomic status	Poorest	67 (18.6)	373 (17.6)	98 (24.5)	557 (24.4)
	Poor	84 (23.3)	399 (18.8)	88 (22.0)	476 (20.9)
	Median	63 (17.5)	402 (18.9)	84 (21.0)	411 (18.0)
	Less poor	60 (16.7)	464 (21.8)	72 (18.0)	437 (19.2)
	Least poor	86 (23.9)	486 (22.9)	58 (14.5)	400 (17.5)
Household size	1-5	122 (33.9)	575 (27.1)	117 (29.5)	586 (25.8)
	6-9	193 (53.6)	1251 (59.0)	211 (53.3)	1369 (60.3)
	10-31	45 (12.5)	293 (13.8)	68 (17.2)	314 (13.8)
Study endpoints-baseline		Class 1 N=183 Class 5 N=192	Class 1 N=1039 Class 5 N=1109	Class 1 N=191 Class 5 N=221	Class 1 N=1126 Class 5 N=1172
Anaemia prevalence	Age-sex specific	144 (44.4)	929 (45.3)	128 (41.6)	986 (46.0)
	Severe (<70g/L)	2 (0.6)	12 (0.6)	0 (0.0)	14 (0.7)
	Moderate (70-89 g/L)	10 (3.1)	33 (1.6)	7 (2.3)	48 (2.2)
	Mild (90-109 g/L)	66 (20.4)	464 (22.6)	55 (17.9)	463 (21.6)
	None (≥110 g/L)	246 (75.9)	1540 (75.2)	246 (79.9)	1618 (75.5)
Haemoglobin (g/L)	Mean (sd)	117.7 (13.6)	117.3 (12.9)	118.9 (13.3)	117.3 (13.7)
***P.falciparum* prevalence** [b]		- -	- -	26 (8.6)	285 (13.6)
Class 1 [c]					
Score: 0-20	Sustained attention [d]	11.9 (6.7) [0, 20]	11.9 (6.7) [0, 20]	11.8 (6.6) [0, 20]	12.2 (6.6) [0, 20]
Score: 0-20	Spelling	8.0 (4.2) [0, 19]	8.7 (4.5) [0, 19]	7.4 (4.5) [0, 19]	7.7 (4.4) [0, 20]
Score: 0-30	Arithmetic	2.4 (2.3) [0, 12]	2.6 (2.4) [0, 17]	2.3 (2.6) [0, 13]	2.6 (2.5) [0, 15]
Class 5 [c]					
Score: 0-20	Sustained attention [d]	9.9 (6.1) [0, 20]	9.9 (6.0) [0, 20]	9.6 (5.7) [0, 20]	10.6 (5.7) [0, 20]
Score: 0-78	Spelling	24.0 (11.6) [0, 51]	28.6 (11.7) [0, 63]	24.2 (11.1) [0, 56]	26.1 (11.2) [0, 59]
Score: 0-38	Arithmetic	28.6 (6.1) [5, 38]	29.5 (5.5) [0, 38]	27.2 (7.0) [1, 38]	28.8 (5.5) [0, 38]

[a] % of non-missing children in each study group presented for categorised data. For continuous data mean(sd) [min,max] is presented;
[b] Not measured at baseline in the control group;
[c] Presented as mean(sd) [min,max]
[d] In class 1 sustained attention was measured by the "pencil tap test" and in class 5 sustained attention was measured by the "two digit code transmission test".

APPENDIX 4.2

Table A4.2 Baseline measures for 5233 study children with missing 24 months follow-up health data vs. those not missing 24 months follow-up health data across both the control and IST intervention groups.

Characteristic; n (%) [a]		**CONTROL GROUP**		**INTERVENTION GROUP**	
		Missing outcome data	Outcome data available	Missing outcome data	Outcome data available
Child characteristics		N=496	N=2027	N=536	N=2174
Age	Mean (sd)	10.5 (3.1)	10.0 (2.8)	10.9 (3.1)	10.2 (2.7)
	5-9	196 (39.5)	845 (41.7)	184 (34.3)	885 (40.7)
	10-12	140 (28.2)	737 (36.4)	149 (27.8)	776 (35.7)
	13-20	160 (32.3)	445 (22.0)	203 (37.9)	513 (23.6)
Sex	Male	240 (48.4)	1017 (50.2)	248 (46.3)	1071 (49.3)
Child sleeps under net	Usually	308 (64.4)	1360 (68.0)	324 (62.4)	1358 (63.3)
	Last night	298 (96.8)	1308 (96.2)	310 (95.7)	1299 (95.7)
Nutritional Status	Underweight	50 (28.6)	216 (26.7)	27 (18.7)	204 (24.8)
	Stunted	102 (23.0)	498 (25.7)	106 (24.3)	506 (25.0)
	Thin	76 (17.1)	406 (20.9)	66 (15.1)	384 (19.0)
Household characteristics					
Parental Education	No schooling	147 (30.8)	579 (29.0)	203 (39.0)	722 (33.6)
	Primary schooling	237 (49.7)	1055 (52.9)	257 (49.4)	1124 (52.4)
	Secondary schooling	75 (15.7)	278 (13.9)	42 (8.1)	236 (11.0)
	Higher education	18 (3.8)	84 (4.2)	18 (3.5)	65 (3.0)
Socioeconomic status	Poorest	95 (19.8)	345 (17.2)	124 (23.8)	531 (24.6)
	Poor	105 (21.9)	378 (18.9)	115 (22.0)	449 (20.8)
	Median	87 (18.2)	378 (18.9)	99 (19.0)	396 (18.3)
	Less poor	73 (15.2)	451 (22.5)	105 (20.1)	404 (18.7)
	Least poor	119 (24.8)	453 (22.6)	79 (15.1)	379 (17.6)
Household size	1-5	158 (33.1)	539 (26.9)	144 (27.7)	559 (26.0)
	6-9	262 (54.8)	1182 (59.1)	298 (57.4)	1282 (59.7)
	10-31	58 (12.1)	280 (14.0)	77 (14.8)	305 (14.2)
Study endpoints-baseline		Class 1 N=230 Class 5 N=266	Class 1 N=992 Class 5 N=1035	Class 1 N=226 Class 5 N=310	Class 1 N=1091 Class 5 N=1083
Anaemia prevalence	Age-sex specific	206 (47.0)	867 (44.8)	194 (45.9)	920 (45.4)
	Severe (<70g/L)	2 (0.5)	12 (0.6)	1 (0.2)	13 (0.6)
	Moderate (70-89 g/L)	8 (1.8)	35 (1.8)	9 (2.1)	46 (2.3)
	Mild (90-109 g/L)	98 (22.4)	432 (22.3)	83 (19.6)	435 (21.4)
	None (≥110 g/L)	330 (75.3)	1456 (75.2)	330 (78.0)	1534 (75.6)
Haemoglobin (g/L)	Mean (sd)	117.3 (13.3)	117.3 (12.9)	118.5 (13.6)	117.3 (13.7)
***P.falciparum* prevalence** [b]		- -	-	37 (8.9)	274 (13.8)
Class 1 [c]					
Score: 0-20	Sustained attention [d]	11.6 (6.7) [0, 20]	11.9 (6.7) [0, 20]	11.6 (6.8) [0, 20]	12.3 (6.5) [0, 20]
Score: 0-20	Spelling	8.5 (4.1) [0, 19]	8.6 (4.6) [0, 19]	7.7 (4.7) [0, 19]	7.6 (4.4) [0, 20]
Score: 0-30	Arithmetic	2.6 (2.3) [0, 12]	2.6 (2.4) [0, 17]	2.6 (2.8) [0, 15]	2.6 (2.4) [0, 12]
Class 5 [c]					
Score: 0-20	Sustained attention [d]	9.8 (6.1) [0, 20]	9.9 (6.0) [0, 20]	9.4 (5.5) [0, 20]	10.7 (5.7) [0, 20]
Score: 0-78	Spelling	24.2 (11.4) [0, 52]	28.9 (11.7) [0, 63]	22.5 (10.7) [1, 51]	26.7 (11.1) [1, 59]
Score: 0-38	Arithmetic	28.6 (6.2) [4, 38]	29.6 (5.4) [0, 38]	27.3 (6.4) [3, 38]	28.8 (5.6) [0, 38]

[a] % of non-missing children in each study group presented for categorised data, where data is continuous mean(sd) is presented.
[b] Not measured at baseline in the control group;
[c] Presented as mean(sd) [min,max]
[d] In class 1 sustained attention was measured by the "pencil tap test" and in class 5 sustained attention was measured by the "two digit code transmission test"

APPENDIX 4.3

Table A4.3 Results from missing data analysis for anaemia. Effect of the IST intervention at 12 and 24 months follow-up on the primary health outcome of anaemia for study children combined using a longitudinal, random effects regression modelling approach. Results presented (i) for all children with either 12 or 24 months follow-up measurements of the outcome (unadjusted), (ii) for those with baseline measurements of the outcome and accounting for age, sex and stratification effects as the primary pre-specified analysis, and (iii) for those additionally with baseline measures of parental education, SES and baseline educational level (measured by baseline spelling) as further predictors of missingness.

Prevalence of anaemia[a]		**Control (50 schools) n (%)[b]**		**Intervention (51 schools) n (%)[b]**	**Odds ratio[c] (95% CI)**	**p-value [d]**	**ICC (95% CI)**	
							School	Child
Unadjusted								
12-month	2146	837 (39.0%)	2297	920 (40.1%)	1.08 (0.74,1.43)			
24-month	2027	809 (39.9%)	2173	910 (41.9%)	1.12 (0.77,1.48)	0.758	0.07 (0.05,0.10)	0.50 (0.45,0.54)
Adjusted								
12-month	2048	788 (38.5%)	2142	858 (40.1%)	1.09 (0.79,1.40)			
24-month	1935	765 (39.5%)	2027	842 (41.5%)	1.11 (0.80,1.42)	0.890	0.06 (0.04,0.08)	0.38 (0.33,0.43)
Adjusted for predictors of missingness								
12-month	1998	768 (38.4%)	2083	832 (39.9%)	1.05 (0.77,1.34)	0.789		
24-month	1889	747 (39.5%)	1969	820 (41.7%)	1.09 (0.79,1.38)		0.05 (0.03,0.07)	0.34 (0.32,0.42)

[a] Age-sex specific anaemia was defined using age and sex corrected WHO thresholds of haemoglobin concentration: <110g/l in children under 5 years; <115g/l in children 5 to 11 years; <120g/l in females 12 years and over and males 12 to 14.99 years old; and <130g/l in males ≥ 15 years. All female adolescents are assumed to not be pregnant

[b] Number and percentage with outcome

[c] Odds ratios (intervention/control) presented for anaemia are obtained from random effects logistic regression analysis accounting for school-level clustering and repeated measures of children for the comparison of the intervention effect at 12 months to 24 months

[d] p-value for the comparison of the intervention effect at 12 months to 24 months

Unadjusted: All children with outcome measures, not adjusted for any baseline or study design characteristics.

Adjusted: for baseline age, sex, school mean exam score and literacy group (to account for stratification) and baseline measure of the outcome, where available.

Adjusted for predictors of missingness: for baseline age, sex, school mean exam score and literacy group (to account for stratification) and baseline measure of the outcome, where available. Additionally adjusted for parental education, SES and baseline educational level as measured by baseline spelling score (standardized by subtracting year-group baseline mean and scaled by year-group sd).

APPENDIX 4.4

Table A4.4 Baseline measures for study children with missing 9 months follow-up education data vs. those not missing 9 months follow-up education data across both the control and intervention groups.

Characteristic; n (%) [a]		**CONTROL GROUP**		**INTERVENTION GROUP**	
		Missing outcome data	Outcome data available	Missing outcome data	Outcome data available
Child characteristics		N=265	N=2258	N=312	N=2398
Age	Mean (sd)	10.0 (3.2)	10.1 (2.8)	10.5 (3.1)	10.3 (2.8)
	5-9	125 (47.2)	916 (40.6)	121 (38.8)	948 (39.5)
	10-12	71 (26.8)	806 (35.7)	91 (29.2)	834 (34.8)
	13-20	68 (26.0)	536 (23.7)	100 (32.1)	616 (25.7)
Sex	Male	134 (50.6)	1123 (49.7)	157 (50.3)	1162 (48.5)
Child sleeps under net	Usually	167 (66.8)	1501 (67.3)	174 (58.6)	1508 (63.7)
	Last night	164 (98.2)	1442 (96.1)	169 (97.1)	1440 (95.5)
Nutritional Status	Underweight	38 (34.9)	228 (26.0)	17 (19.5)	214 (24.3)
	Stunted	55 (24.6)	545 (25.2)	44 (19.2)	568 (25.4)
	Thin	48 (21.4)	434 (20.1)	39 (17.0)	411 (18.4)
Household characteristics					
Parental Education	No schooling	81 (32.5)	645 (29.0)	127 (42.8)	798 (33.7)
	Primary schooling	128 (51.4)	1164 (52.3)	141 (47.5)	1240 (52.3)
	Secondary schooling	30 (12.0)	323 (14.5)	17 (5.7)	261 (11.0)
	Higher education	10 (4.0)	92 (4.1)	12 (4.0)	71 (3.0)
Socioeconomic status	Poorest	55 (22.0)	385 (17.2)	84 (28.1)	571 (24.0)
	Poor	54 (21.6)	429 (19.2)	66 (22.1)	498 (20.9)
	Median	42 (16.8)	423 (18.9)	53 (17.7)	442 (18.6)
	Less poor	46 (18.4)	478 (21.4)	62 (20.7)	447 (18.8)
	Least poor	53 (21.2)	519 (23.2)	34 (11.4)	424 (17.8)
Household size	1-5	90 (36.0)	607 (27.2)	88 (29.6)	615 (26.0)
	6-9	118 (47.2)	1326 (59.5)	171 (57.6)	1409 (59.5)
	10-31	42 (16.8)	296 (13.3)	38 (12.8)	344 (14.5)
Study endpoints-baseline		Class 1 N=149 Class 5 N=116	Class 1 N=1073 Class 5 N=1185	Class 1 N=153 Class 5 N=159	Class 1 N=1164 Class 5 N=1234
Anaemia prevalence	Age-sex specific	93 (42.9)	980 (45.5)	98 (45.2)	1016 (45.5)
	Severe (<70g/L)	1 (0.5)	13 (0.6)	1 (0.5)	13 (0.6)
	Moderate (70-89 g/L)	8 (3.7)	35 (1.6)	9 (4.1)	46 (2.1)
	Mild (90-109 g/L)	43 (19.8)	487 (22.6)	44 (20.3)	474 (21.2)
	None (≥110 g/L)	165 (76.0)	1621 (75.2)	163 (75.1)	1701 (76.1)
Haemoglobin (g/L)	Mean (sd)	116.6 (14.1)	117.4 (12.9)	117.5 (15.0)	117.5 (13.6)
***P.falciparum* prevalence** [b]		- -	- -	19 (9.1)	292 (13.3)
Class 1 [c]					
Score: 0-20	Sustained attention [d]	11.0 (6.8) [0, 20]	12.0 (6.6) [0, 20]	12.3 (6.7) [0, 20]	12.1 (6.6) [0, 20]
Score: 0-20	Spelling	8.2 (4.3) [0, 19]	8.6 (4.5) [0, 20]	7.1 (4.2) [0, 18]	7.7 (4.4) [0, 20]
Score: 0-30	Arithmetic	2.8 (2.8) [0, 13]	2.5 (2.3) [0, 17]	2.8 (2.9) [0, 13]	2.5 (2.4) [0, 15]
Class 5 [c]					
Score: 0-20	Sustained attention [d]	9.8 (5.8) [0, 20]	9.9 (6.0) [0, 20]	9.5 (5.8) [0, 20]	10.6 (5.6) [0, 20]
Score: 0-78	Spelling	24.6 (11.1) [2, 52]	28.2 (11.8) [0, 63]	25.1 (11.2) [1, 51]	25.9 (11.2) [1, 59]
Score: 0-38	Arithmetic	28.3 (6.6) [5, 38]	29.5 (5.5) [0, 38]	27.8 (7.2) [3, 38]	28.6 (5.6) [0, 38]

[a] % of non-missing children in each study group presented for categorised data, where data is continuous mean(sd) is presented.
[b] Not measured at baseline in the control group;
[c] Presented as mean(sd) [min,max]
[d] In class 1 sustained attention was measured by the "pencil tap test" and in class 5 sustained attention was measured by the "two digit code transmission test".

APPENDIX 4.5

Table A4.5 Baseline measures for study children with missing 24 months follow-up education data vs. those not missing 24 months follow-up education data across both the control and intervention groups.

Characteristic; n (%) [a]		**CONTROL GROUP**		**INTERVENTION GROUP**	
		Missing outcome data	Outcome data available	Missing outcome data	Outcome data available
Child characteristics		N=543	N=1980	N=584	N=2126
Age	Mean (sd)	10.5 (3.1)	10.0 (2.8)	10.9 (3.1)	10.2 (2.7)
	5-9	213 (39.2)	828 (41.8)	202 (34.6)	867 (40.8)
	10-12	161 (29.7)	716 (36.2)	167 (28.6)	758 (35.7)
	13-20	169 (31.1)	436 (22.0)	215 (36.8)	501 (23.6)
Sex	Male	271 (49.9)	986 (49.8)	270 (46.2)	1049 (49.3)
Child sleeps under net	Usually	343 (65.2)	1325 (67.8)	345 (61.0)	1337 (63.7)
	Last night	334 (97.4)	1272 (96.0)	328 (95.1)	1281 (95.8)
Nutritional Status	Underweight	49 (26.1)	217 (27.2)	37 (22.8)	194 (24.1)
	Stunted	114 (23.7)	486 (25.5)	121 (25.0)	491 (24.8)
	Thin	90 (18.7)	392 (20.6)	74 (15.3)	376 (19.0)
Household characteristics					
Parental Education	No schooling	167 (31.8)	559 (28.7)	229 (40.4)	696 (33.1)
	Primary schooling	258 (49.1)	1034 (53.1)	271 (47.8)	1110 (52.9)
	Secondary schooling	82 (15.6)	271 (13.9)	46 (8.1)	232 (11.0)
	Higher education	18 (3.4)	84 (4.3)	21 (3.7)	62 (3.0)
Socioeconomic status	Poorest	102 (19.4)	338 (17.3)	138 (24.3)	517 (24.5)
	Poor	119 (22.6)	364 (18.6)	125 (22.0)	439 (20.8)
	Median	92 (17.5)	373 (19.1)	110 (19.3)	385 (18.2)
	Less poor	86 (16.3)	438 (22.4)	109 (19.2)	400 (18.9)
	Least poor	128 (24.3)	444 (22.7)	87 (15.3)	371 (17.6)
Household size	1-5	163 (31.0)	534 (27.3)	152 (26.9)	551 (26.3)
	6-9	293 (55.7)	1151 (58.9)	335 (59.2)	1245 (59.3)
	10-31	70 (13.3)	268 (13.7)	79 (14.0)	303 (14.4)
Study endpoints-baseline		Class 1 N=259 Class 5 N=284	Class 1 N=963 Class 5 N=1017	Class 1 N=253 Class 5 N=331	Class 1 N=1064 Class 5 N=1062
Anaemia prevalence	Age-sex specific	213 (44.9)	860 (45.3)	211 (44.8)	903 (45.6)
	Severe (<70g/L)	2 (0.4)	12 (0.6)	1 (0.2)	13 (0.7)
	Moderate (70-89 g/L)	10 (2.1)	33 (1.7)	9 (1.9)	46 (2.3)
	Mild (90-109 g/L)	104 (21.9)	426 (22.4)	91 (19.3)	427 (21.6)
	None (≥110 g/L)	358 (75.5)	1428 (75.2)	370 (78.6)	1494 (75.5)
Haemoglobin (g/L)	Mean (sd)	117.4 (13.4)	117.3 (12.9)	118.7 (13.6)	117.2 (13.7)
***P.falciparum* prevalence** [b]		- -	-	47 (10.2)	264 (13.6)
Class 1 [c]					
Score: 0-20	Sustained attention [d]	11.8 (6.6) [0, 20]	11.9 (6.7) [0, 20]	11.9 (6.6) [0, 20]	12.2 (6.6) [0, 20]
Score: 0-20	Spelling	8.5 (4.2) [0, 19]	8.6 (4.6) [0, 19]	7.6 (4.6) [0, 19]	7.7 (4.4) [0, 20]
Score: 0-30	Arithmetic	2.5 (2.3) [0, 12]	2.6 (2.4) [0, 17]	2.6 (2.7) [0, 13]	2.6 (2.4) [0, 15]
Class 5 [c]					
Score: 0-20	Sustained attention [d]	9.9 (6.1) [0, 20]	9.9 (6.0) [0, 20]	9.6 (5.6) [0, 20]	10.7 (5.7) [0, 20]
Score: 0-78	Spelling	25.4 (11.6) [0, 53]	28.6 (11.7) [0, 63]	23.1 (11.1) [1, 59]	26.6 (11.1) [1, 59]
Score: 0-38	Arithmetic	28.7 (6.3) [4, 38]	29.5 (5.3) [0, 38]	27.7 (6.3) [3, 38]	28.8 (5.6) [0, 38]

[a] % of non-missing children in each study group presented for categorised data, where data is continuous mean(sd) is presented.
[b] Not measured at baseline in the control group;
[c] Presented as mean(sd) [min,max]
[d] In class 1 sustained attention was measured by the "pencil tap test" and in class 5 sustained attention was measured by the "two digit code transmission test".

APPENDIX 4.6

Table A4.6 Results from missing data analysis for sustained attention. Effect of the IST intervention at 9 and 24 months follow-up on sustained attention outcomes for younger (class 1) and older (class 5) children combined using a longitudinal, random effects regression modeling approach. Results presented (i) for all children with either 9 or 24 months follow-up measurements of the outcome (unadjusted), (ii) for those with baseline measurements of the outcome and accounting for age, sex and stratification effects as the primary pre-specified analysis, and (iii) for those additionally with baseline measures of parental education, SES and baseline educational level (measured by baseline spelling) as further predictors of missingness

Sustained attention (score: 0-20)	**Control (50 schools)**		**Intervention (51 schools)**		**Mean difference [d] (95% CI)**	**p-value [e]**	**ICC (95% CI)**	
							School	Child
CLASS 1 [b]		**Mean (SD) [a]**		**Mean (SD) [a]**				
Unadjusted								
9-months	1070	8.48 (3.63)	1162	8.43 (3.76)	-0.05 (-0.53,0.44)			
24-months	960	13.45 (5.15)	1059	13.20 (4.96)	-0.25 (-0.75,0.24)	0.409	0.04 (0.02,0.06)	0.17 (0.13,0.22)
Adjusted								
9-months	1030	8.52 (3.65)	1144	8.43 (3.77)	-0.16 (-0.61,0.30)			
24-months	923	13.49 (5.15)	1041	13.18 (4.96)	-0.41 (-0.88,0.06)	0.311	0.03 (0.02,0.05)	0.13 (0.09,0.18)
Adjusted for predictors of missingness								
9-months	1013	8.54 (3.67)	1118	8.43 (3.77)	-0.02 (-0.43,0.46)			
24-months	908	13.49 (5.16)	1017	13.19 (5.00)	-0.21 (-0.67,0.26)	0.385	0.02 (0.01,0.04)	0.12 (0.08,0.17)
CLASS 5 [c]		**Mean (SD) [a]**		**Mean (SD) [a]**				
Unadjusted								
9-months	1180	13.38 (5.45)	1231	13.35 (5.13)	-0.07 (-0.65,0.51)			
24-months	1007	14.22 (4.90)	1052	14.66 (5.13)	0.31 (-0.29,0.91)	0.083	0.04 (0.03,0.07)	0.52 (0.49,0.55)
Adjusted								
9-months	1178	13.38 (5.45)	1221	13.40 (5.10)	-0.14 (-0.65,0.37)			
24-months	1006	14.21 (4.90)	1044	14.70 (5.10)	0.19 (-0.33,0.72)	0.122	0.04 (0.03,0.07)	0.40 (0.36,0.44)
Adjusted for predictors of missingness								
9-months	1141	13.39 (5.42)	1203	13.40 (5.10)	-0.02 (-0.54,0.51)			
24-months	971	14.24 (4.85)	1028	14.69 (4.58)	0.29 (-0.25,0.84)	0.160	0.05 (0.03,0.07)	0.37 (0.34,0.42)

[a] Mean score and sd at follow-up [b] Pencil tap test was conducted at baseline and single digit code transmission task was conducted at 9 and 24 months follow-ups.

[c] Double digit code transmission was conducted at baseline and both follow up visits.

[d] Mean difference (intervention-control) presented for continuous outcomes (scores on attention task) and are obtained from random effects regression analysis accounting for school-level clustering and repeated measures on children. [e] p-value for the comparison of the intervention effect at 12 months to 24 months **Unadjusted**: All children with outcome measures, not adjusted for any baseline or study design characteristics.

Adjusted: for baseline age, sex, school mean exam score and literacy group (to account for stratification) and baseline measure of the outcome, where available.

Adjusted for predictors of missingness: for baseline age, sex, school mean exam score and literacy group (to account for stratification) and baseline measure of the outcome, where available. Additionally adjusted for parental education, SES and baseline educational level as measured by baseline spelling score (standardized by subtracting year-group baseline mean and scaled by year-group sd).

APPENDIX 4.7

Table A4.7: Results from missing data analysis for spelling. Effect of the IST intervention at 9 and 24 months follow-up on spelling outcomes for younger (class 1) and older (class 5) children combined using a longitudinal, random effects regression modeling approach. Results presented (i) for all children with either 9 or 24 months follow-up measurements of the outcome (unadjusted), (ii) for those with baseline measurements of the outcome and accounting for age, sex and stratification effects as the primary pre-specified analysis, and (iii) for those additionally with baseline measures of parental education, SES and baseline educational level (measured by baseline spelling) as further predictors of missingness.

Spelling score	**Control (50 schools)**		**Intervention (51 schools)**		**Mean difference [d] (95% CI)**	**p-value [e]**	**ICC (95% CI)**	
							School	Child
CLASS 1 [b]		**Mean (SD) [a]**		**Mean (SD) [a]**				
Unadjusted								
9-months	1068	11.70 (4.59)	1162	10.47 (4.57)	-1.24 (-2.00,-0.48)			
24-months	961	12.03 (3.05)	1062	11.04 (3.49)	-0.98 (-1.74,-0.21)	0.094	0.20 (0.15,0.25)	0.61 (0.58,0.65)
Adjusted								
9-months	1060	11.69 (4.59)	1133	10.49 (4.58)	-0.79 (-1.28,-0.30)			
24-months	954	12.02 (3.05)	1036	11.04 (3.50)	-0.54 (-1.04,-0.05)	0.116	0.10 (0.07,0.14)	0.43 (0.39,0.47)
Adjusted for predictors of missingness								
9-months	1049	11.70 (4.59)	1121	10.49 (4.58)	-0.75 (-1.23,-0.27)			
24-months	944	12.05 (3.03)	1025	11.03 (3.50)	-0.54 (-1.02,-0.05)	0.178	0.09 (0.07,0.14)	0.42 (0.38,0.46)
CLASS 5 [c]		**Mean (SD) [a]**		**Mean (SD) [a]**				
Unadjusted								
9-months	1169	31.34 (12.61)	1223	28.73 (12.36)	-2.69 (-5.10,-0.27)			
24-months	1010	35.28 (12.91)	1060	33.97 (12.79)	-1.70 (-4.13,0.73)	0.001	0.21 (0.16,0.26)	0.85 (0.84,0.87)
Adjusted								
9-months	1154	31.37 (12.60)	1214	28.76 (12.34)	-0.28 (-1.16,0.60)			
24-months	996	35.33 (12.85)	1052	34.04 (12.75)	0.68 (0.22,1.58)	0.001	0.08 (0.06,0.12)	0.43 (0.40,0.47)
Adjusted for predictors of missingness								
9-months	1131	31.49 (12.69)	1198	28.69 (12.36)	-0.18 (-1.07,0.70)			
24-months	974	35.57 (12.81)	1037	33.98 (12.77)	0.73 (-0.18,1.63)	0.003	0.08 (0.06,0.12)	0.43 (0.40,0.47)

[a] Mean score and sd at follow-up based on the data [b] The same class 1 spelling task was given at baseline, 9 and 24 months follow-ups, with different words used for the 24 month follow-up and was scored 0-20.
[c] The same class 5 spelling task was given at baseline, 9 and 24 months follow-ups, with different words used for the 24 month follow-up and was scored 0-78.
[d] Mean difference (intervention-control) presented for continuous outcomes (scores on spelling task) and are obtained from random effects regression analysis accounting for school-level clustering and repeated measures on children. [e] p-value for the comparison of the intervention effect at 12 months to 24 months **Unadjusted**: All children with outcome measures, not adjusted for any baseline or study design characteristics.
Adjusted: for baseline age, sex, school mean exam score and literacy group (to account for stratification) and baseline measure of the outcome, where available.

Adjusted for predictors of missingness: for baseline age, sex, school mean exam score and literacy group (to account for stratification) and baseline measure of the outcome, where available. Additionally adjusted for parental education, SES and baseline educational level as measured by baseline spelling score (standardized by subtracting year-group baseline mean and scaled by year-group sd).

APPENDIX 4.8

Table A4.8 Sensitivity analyses considering transfers across the study period. Effect of the IST intervention at 12 and 24 months follow-up on health outcomes for study children. Results presented (i) for all children with either 12 or 24 months follow-up measurements of the outcome (unadjusted) with children who transferred schools excluded and (ii) for those with baseline measurements of each outcome and accounting for age, sex and stratification effects as the primary pre-specified analysis with children who transferred schools excluded.

Outcome	Control (50 schools)		Intervention (51 schools)		Risk ratio[b] (95% CI)	p-value	Cluster-size; range (average)
		n (%)[a]		n (%)[a]			
12 month follow-up	N=2439		N=2574				
Prevalence of anaemia[c]							
Unadjusted	2117	827 (39.1%)	2255	906 (40.2%)	1.03 (0.91,1.16)	0.640	15-54 (43.3)
Adjusted	2023	780 (38.6%)	2106	847 (40.2%)	1.02 (0.93,1.13)	0.670	15-54 (40.9)
Prevalence of *P.falciparum*							
Unadjusted	2078	300 (14.4%)	2235	243 (10.9%)	0.76 (0.49,1.19)	0.234	11-54 (42.7)
Adjusted [d]	2078	300 (14.4%)	2235	243 (10.9%)	0.72 (0.46,1.11)	0.139	11-54 (42.7)
24 months follow-up	N=2362		N=2417				
Prevalence of anaemia[c]							
Unadjusted	1929	770 (39.9%)	1999	843 (42.2%)	1.06 (0.91,1.22)	0.463	15-52 (39.3)
Adjusted	1845	728 (39.5%)	1862	780 (41.9%)	1.01 (0.90,1.12)	0.920	14-52 (37.1)
Prevalence of *P.falciparum*							
Unadjusted	1908	162 (8.5%)	1972	239 (12.2%)	1.42 (0.83,2.43)	0.206	15-52 (38.8)
Adjusted [d]	1908	162 (8.5%)	1972	239 (12.2%)	1.49 (0.86,2.57)	0.154	15-52 (38.8)

N=number of children eligible for follow up (not withdrawn or deceased)

[a] Number and percentage with outcome

[b] Risk ratios presented for binary outcomes (anaemia & *P. falciparum* prevalence) and are obtained from GEE analysis accounting for school-level clustering.

[c] Age-sex specific anaemia was defined using age and sex corrected WHO thresholds of haemoglobin concentration: <110g/l in children under 5 years; <115g/l in children 5 to 11 years; <120g/l in females 12 years and over and males 12 to 14.99 years old; and <130g/l in males ≥ 15 years. All female adolescents are assumed to not be pregnant

[d] Not including baseline *P.falciparum* prevalence

Unadjusted: All children with outcome measures, not adjusted for any baseline or study design characteristics.

Adjusted: for baseline age, sex, school mean exam score and literacy group (to account for stratification) and baseline measure of the outcome, where available

APPENDIX 5.1

Table A5.1: Univariable results of correlates of test discordance from multinomial multilevel analyses

Characteristic	RDT positive, Microscopy positive			RDT positive Microscopy negative			RDT negative, Microscopy positive			School and child level variance
	ROR[a]	95% CI	p-value	ROR[a]	95% BCI	p-value	ROR[a]	95% BCI	p-value	
Feb 2010										
Jul 2010	1.05	0.86, 1.29	0.625	1.03	0.82, 1.28	0.814	**0.47**	**0.30, 0.72**	**0.001**	*1.16 (0.76-1.77)*
Sept 2010	0.94	0.76, 1.15	0.558	1.02	0.82, 1.28	0.829	**0.20**	**0.12, 0.36**	**<0.001**	0.69 (0.51-0.92)
Feb 2011	**0.66**	**0.53, 0.83**	<0.001	**0.80**	**0.64, 1.01**	**0.062**	1.10	0.78, 1.55	0.591	
Non-discrepant slides										*1.09 (0.71-1.65)*
Discrepant slides	**3.16**	**2.49, 4.02**	**<0.001**	**3.58**	**2.80, 5.58**	**<0.001**	**31.70**	**22.70, 44.26**	**<0.001**	0.66 (0.49-0.90)
Male										*1.15 (0.76-1.76)*
Female	**0.82**	**0.69, 0.97**	**0.018**	**0.80**	**0.67, 0.95**	**0.012**	1.14	0.84, 1.56	0.395	0.67 (0.50-0.90)
Not anaemic										*1.14 (0.75-1.73)*
Anaemic	**1.63**	**1.35, 1.96**	**<0.001**	**1.39**	**1.14, 1.68**	**0.001**	0.86	0.61, 1.23	0.423	0.67 (0.49-0.90)
Age (years) 5-9										
10-12	0.85	0.70, 1.03	0.107	0.95	0.77, 1.17	0.627	**1.42**	**0.99, 2.05**	**0.058**	*1.16 (0.76-1.76)*
13-20	**0.66**	**0.53, 0.82**	<0.001	0.95	0.76, 1.19	0.658	1.38	0.93, 2.05	0.105	0.68 (0.50-0.91)
Prevalence (%) <10										
10.0-19.9	**4.54**	**2.28, 9.06**	**<0.001**	**3.80**	**2.20, 6.57**	**<0.001**	**5.77**	**1.31, 25.48**	**0.001**	*0.20 (0.11-0.33)*
20.0-39.9	**9.91**	**5.06, 9.42**	**<0.001**	**7.84**	**4.58, 13.40**	**<0.001**	**12.87**	**3.06, 54.07**	**<0.001**	0.79 (0.60-1.04)
≥40.0	**39.87**	**20.36, 78.08**	**<0.001**	**15.85**	**9.19, 27.31**	**<0.001**	**38.74**	**9.38, 160.02**	**<0.001**	

[a]ROR denotes the relative odds ratio, of the relative odds compared with the base outcome (RDT negative, Microscopy negative) for those exposed vs unexposed for each characteristic.
RORs in bold indicate those significant at the 10% significance level.

APPENDIX 5.2

Table A5.2: Bayesian latent class analyses of diagnostic accuracy of Paracheck RDT and expert microscopy in the absence of a reference standard, assuming conditional dependence

	RDT diagnostic performance	Microscopy diagnostic performance
True prevalence	***30.9 (28.31-33.6)***	
Sensitivity (95% BCI)	**52.6 (48.3-57.2)**	**38.0 (34.4-41.9)**
Specificity (95% BCI)	**97.2 (96.3-98.1)**	**99.7 (99.3-99.9)**
PPV (95% BCI)	89.3 (85.6-92.7)	98.3 (96.1-99.7)
NPV (95% BCI)	82.1 (79.1-85.1)	78.2 (75.1-81.2)
Accuracy (95% BCI)	83.4 (80.9-85.9)	80.6 (77.9-83.3)
Correlation positives	0.48 (0.43-0.52)	
Correlation Negatives	0.12 (-0.02-0.32)	
Covariance (sensitivity)	0.12 (0.10-0.13)	
Covariance (specificity)	0.1 (-0.0- 0.4)	
DIC	2463.760	

APPENDIX 6.1

Table A6.1: Environmental, climatic, topographic and demographic factors analysed: sources of data and geoprocessing.

Type	Variable	Unit	Spatial resolution	Source		Processing
Environmental	Normalised difference vegetation index (NDVI)		1km^2	SPOT 5 VEGETATION project http://www.spot-vegetation.com/	variable across surveys	NDVI allows monitoring of seasonal variation in vegetation status. SPOT-VEGETATION provides 10 daily synthesized products. NDVI data are provided in digital values (8-bytes) and the true NDVI value (-1 to 1) was calculated using the following formula ((digital number*0.004)-0.1) [488]. Mean, maximum and standard deviation were calculated by survey averaged using a lag time of one month. Values were standardised.
	Landcover	Categorical	300 x 300 m	Global Land Cover Network (FAO) http://www.glcn.org/index_en.jsp	constant across surveys	Global land cover data from 2005 for the region. Processed from vectorial data to raster data which include information on density and type of vegetation such as shrubland and mosaic vegetation/croplands.
	Potential evapo-transpiration (PET)		900 x 900 m	**Global Aridity Index (Global-PET) The CGIAR Consortium for Spatial Information** http://csi.cgiar.org/Aridity/Global_Aridity_PET_Methodolgy.asp	constant across surveys	PET-a measure of the ability of the atmosphere to remove water through evapo-transpiration. Modelled using the data available from WorldClim Global climate data.
	Aridity Index	Aridity units	900 x 900 m	**Global Aridity Index (Global-Aridity) The CGIAR Consortium for Spatial Information** http://csi.cgiar.org/Aridity/Global_Aridity_PET_Methodolgy.asp	constant across surveys	Mean aridity Index from 1950-2000 is calculated as: *Aridity Index (AI) = Mean Annual Precipitation / Mean Annual Potential Evapo-Transpiration.* Aridity Index values decrease with more arid conditions.

Topographical	Euclidean distance to any / permanent / temporary waterbodies	km	90 x 90 m	Digital Chart of the World http://www.diva-gis.org/gdata	constant across surveys	Euclidean (straight line)distance from each cell in the raster dataset to any water body or the nearest permanent or temporary waterbody
	Altitude	Meters	90 x 90 m	USGS-SRTM http://srtm.csi.cgiar.org/	constant across surveys	Digital Elevation Model (DEM) was obtained from the Shuttle Radar Topography Mission, US Geological Survey (SRTM) to a 250 m-spatial resolution. A higher spatial resolution was processed as well as processing to correct blanks or *NoData* values.
Climate	Temperature Precipitation	°C mm	1 x 1 km	WorldClim (based on 1950-2000 interpolation) http://www.worldclim.org/	constant across surveys	Long term average monthly precipitation data for the period 1950–2000 was interpolated using a thin-plate smoothing spline algorithm at 1 km resolution. Annual mean, maximum and SD values were created for each and on the basis of non-linearity, mean annual precipitation was categorised. Monthly average precipitation values were also extracted and calculated using an average over a lag period of two months to the end of each survey
	Land surface temperature (LST)	°C	5 x 5 km	USGS-Early Warning Explorer Famine Early Warning System Network http://earlywarning.usgs.gov:8080/EWX/index.html	variable across surveys	LST was obtained from data measured by the Moderate Resolution Imaging Spectroradiometer sensor in dekads (10 day averages). Mean LST values were calculated averaged over a lag period of six weeks to the end of each survey
Population	Rural/urban classification	Categorical	30 arc seconds 1km^2	Global Rural-Urban Mapping project (GRUMPv1) http://sedac.ciesin.columbia.edu/data/set/grump-v1-urban-extents	constant across surveys	Distinction of rural, periurban and urban areas with the mask based on population counts, settlement points and night-time lights
	Euclidean distance from main road	km			constant across surveys	Euclidean (straight line)distance from each cell in the raster dataset to a vector dataset of roads
	Population density	Pop/km^2	3 arc seconds 100 x 100 m	AfriPop project http://www.worldpop.org.uk/data/data_sources/	constant across surveys	Combines census data with remote sensed data to provide gridded predictions of population.

APPENDIX 6.2

Figure A6.2: Scatter plots of school level *P. falciparum* prevalence against environmental covariates at all surveys in 101 schools. The red line indicates the line of best fit and the blue line displays the lowess fit.