SEASONAL PATTERNS OF MALARIA AND ITS HEALTH RELATED CONSEQUENCES AMONG ADOLESCENT FEMALES IN RURAL MALAWI

Thesis submitted in accordance with the requirements of the
University of Liverpool
for the degree of Doctor of Philosophy

by

Gertrude Takamani Chapotera Kalanda

Liverpool School of Tropical Medicine
December 2008

DEDICATION

Dedicated to the memory of my father,

Zakeyo Takamani Gwazanyoni Chapotera

A man of vision and tremendous courage

&

to my loving son,

Lonjezo Timothy Kalanda,

a hero and more....., who has a prayer in his heart.

"Pray, Hope and Don't Worry" --- St Pio of Pietrelcina

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

DECLARATION OF WORK DONE

This research study was established as a research project based at Nchalo, Malawi for the duration of the study. My (GK) contribution to the study was as follows:

Activity	Responsible
(1) Cross-sectional surveys	
Project management	GK
Questionnaire administration	GK & research team
Specimen collection and processing	GK & research nurse
Reading malaria slides	Laboratory technician
Sub-sample	GK
Quality control	Others
Urine ELISA tests	Others
Data entry	GK and others
Data cleaning and analysis	GK
(2) AGLIT data	
Project management	Others
Data collection and entry	Others
Data cleaning and analysis	GK

ACKNOWLEDGEMENTS

I would like to thank my primary supervisor Professor Bernard Brabin for his support, advice, patience and hard work from conception of the PhD research project and seeing it through to completion. To my secondary supervisor, Dr Luis Cuevas, thanks for his support and advice always. They always urged me on especially during the many times I stumbled. Special thanks to my advisory panel Drs Feiko ter Kuile and Tim O'Dempsey for their encouragement and interest in this work. Many thanks to Dr Grace Malenga and Dr Peter Kazembe for their inspiration and encouragement in my previous malaria work and consequently the pursuit of these studies. I wish to also express my gratitude to the Gates Malaria Partnership for sponsoring this research work.

This study would not have been possible without the support of the adolescent girls who participated and their communities for their willingness. My special thanks to them. I would like to thank all who worked on the project and became good friends, especially Fidelia Noah, Regina Machila, Sr Orleen and her team of laboratory technicians, Mr Isaac and all health surveillance assistants from the participating villages. Thanks to staff at St. Montfort Hospital, AGLIT and Malaria Alert Centre for their support and assistance. I also thank Edward Senga, Dr Kelias Msyamboza, Dr Maliwichi and Dr Albert Mkumbwa who helped me set up in Nchalo.

My gratitude goes to Dr Teunnis Eggelte for doing the antimalarial metabolites ELISA tests on the urine samples. Thanks to Greg Harper for logistics, Maria Midgley for the quality control work, Drs Brian Faragher, Imelda Bates and Martin Donnelly for their technical expertise, Dr Anja Terlow, Jenny Hill, Jean Taylor, Sue Cain and Pauline Anderson for their untiring support and putting up with me. Thanks to fellow PhD students for encouragement and sharing experiences.

To my family, thanks for their love and support throughout the years, more especially my parents for the opportunities and encouragement to pursue my dreams. To Fr Micheal Williams and the extended family in Liverpool, thank you for your love and support. To Lonje my son, thanks for keeping me going through challenging moments and keeping me physically and mentally fit. Your enthusiasm for life is contagious.

GLOSSARY OF TERMS

Adolescence	- Period of growth between 10 to 19 years of age.
Adrenarche	- Onset of dehydroepiandrosterone (DHEA) and DHEA-sulphate (DHEA-S) production from the adrenal zona reticularis.
Anaemia	- Haemoglobin of less than 12 gm per decilitre (Hb <12 g/dl).
Anthropometry	- Technique of expressing quantitatively the form of the body through a reliable scientific system of measuring and taking observations on the musculoskeletal system, brain and other organs.
Arm circumference	- Measurement done on the mid-upper arm, used to assess total body muscle mass and in some circumstances protein-energy malnutrition.
Body Mass Index	- Index that uses weight and height to measure body fat store. It is calculated as weight in kilogrammes divided by the square of height in metres.
Gynaecological age	- Period between current and age at onset of menses.
ITN coverage	- Proportion of the target population possessing an ITN.
ITN adherence	- Proper deployment of ITNs.
Percentiles	- Number that corresponds to one of 100 equal divisions in a range of values; a measure of relative location. The 5^{th} percentile means that 5 % of values in the data set are less than or equal to it and 95 % greater than or equal to it.

Puberty - Period or process of morphological and physiological growth changes as the gonads change from infantile to adult stages of development.

Stable malaria transmission – Intense malaria transmission which can be seasonal or perennial, and under which conditions host acquired immunity develops with increasing age.

Stunting - Slowing of skeletal growth that results in reduced stature or length. It is measured using minus two standard deviations from median height-for-age of the reference population.

Underweight - Condition measured by weight-for-age which acts as a composite measure of stunting and wasting. It is measured as weight below minus two standard deviations of the median weight-for-age of the reference population.

Undernutrition - Body mass index less than the 5^{th} percentile of the values for the reference population.

Unstable malaria transmission – low or moderate malaria transmission, which renders individuals highly susceptible to malaria due to no or low acquired immunity, leading to a risk of epidemic malaria.

Wasting - Condition that result from the loss of body tissue and fat. It is measured as weight-for-length/height or BMI-for-age below the minus 2 z-score line.

Z-score - A standard deviation score that indicates how far a measurement is from the median.

ABBREVIATIONS

ACT	Artemisinin based combination therapy
ACC/SCN	United Nations Administration Committee on Coordination/ Sub-committee on Nutrition
AFHS	Adolescent Friendly Health Services
AGLIT	Adolescent Girls Literacy programme
AOR	Adjusted odds ratio
BMI	Body mass index
CDC	Centres for Disease Control
CHW	Community health workers
CI	Confidence interval
DHEA	Dehydroepiandrosterone
DHEA- S	Dehydroepiandrosterone sulphate
DHEA(S)	Dehydroepiandrosterone and dehydroepiandrosterone sulphate
EIR	Entomological inoculation rate
ELISA	Enzyme linked immunosorbent assay
FSH	Follicle stimulating hormone
GEE	Generalizing estimating equations
HAZ	Height-for-age z-score
HIV/ AIDS	Human immunodeficiency virus/ acquired immunodeficiency syndrome
HSV	Herpes simple virus
IL – 6	Interleukin 6
IPT	Intermittent preventive therapy
IPTp	Intermittent preventive therapy in pregnancy

IRS	In – door residual spraying
ITNs	Insecticide treated bed nets
LH	Leuteinizing hormone
MDGs	Millennium development goals
MUAC	Mid-upper arm circumference
NCHS	National Center for Health Statistics (USA)
OR	Odds ratio
P. f	*Plasmodium falciparum*
PCR	Polymerase chain reaction
PRR	Prevalence risk ratio
RBM	Roll Back Malaria
RDT	Rapid diagnostic test
ROC	Receiver operating curves
RR	Relative risk
SP	Sulphadoxine-pyrimethamine
SUCOMA	Sugar Corporation of Malawi Limited
TNF - α	Tumour necrotic factor alpha
UTI	Urinary tract infections
WAZ	Weight-for-age z-score
WHO	World Health Organisation
UNESCO	United Nations Educational Scientific and Cultural Organisation
UNICEF	United Nations Children Fund

ABSTRACT

Seasonal patterns of malaria and its health related consequences among adolescent girls in rural Malawi

Gertrude T.C. Kalanda

Introduction and objectives: The prevention and control of malaria in young women is a key strategy in reproductive health. This study presents the results of cross-sectional surveys amongst female adolescents living in the Shire valley, rural Malawi, an area holoendemic to malaria. The patterns of malaria and magnitude of associated factors were determined, and considered in relation to an adolescent girls literacy programme (AGLIT) aimed at improving health seeking behaviour and malaria knowledge.

Methodology: Two community-based cross-sectional malaria, nutritional and health surveys of non-pregnant adolescent girls were conducted using single-stage cluster sampling during the dry (Sept- Dec 2005; 15 villages, n=477), and wet seasons (Jan- June 2006; 19 villages, n=786), to determine magnitude and factors associated with malaria parasitaemia. Parasites on microscopy defined parasitaemia prevalence, and parasitaemia with fever (≥37.5°C) defined symptomatic malaria. AGLIT programme cross-sectional surveys were completed prior to (n=933) and following a 9 month curriculum (n=860) and malaria knowledge change was assessed.

Results: Mean ± one standard deviation values for chronological age, gynaecological age and age at menarche were 13.2±2.9, 2.4±1.6, and 14.3±1.2 years, respectively. Stunting prevalence was 8.4% and 13.6 % in the dry and wet seasons, respectively (p=0.009); 19.2% had low weight-for-age, 23.4% low body mass index (BMI) for age and 69.6% low mid-upper-arm-circumference (MUAC) for age with no seasonal variation. When adjusted for season, height for age z-scores and low MUAC for age showed no significant associations with co-variates; BMI z-score was associated with maternal education [PRR 1.23, 95% CI 1.02-1.48, p=0.03]. Anaemia prevalence (Hb<12 g/dl) was 57.0% in the wet and 60.7% in the dry season (p=0.20) and severe anaemia (Hb<8 g/dl) prevalence 3.6% in the wet and 5.3% in the dry seasons (p=0.18). Chronic malnutrition was prevalent and potential exposure to *Schistosomiasis* was not infrequent, especially in the wet season.

P. falciparum malaria prevalence was 5.7% in the wet and 3.4% in the dry season (p=0.07). Significant seasonal prevalence was apparent in younger adolescents <14 years [PRR 2.28, 95% CI 1.08-4.82, p=0.03]. Factors associated with malaria parasitaemia were chronological age [PRR 0.92, 95 % CI 0.87-0.97, p = 0.02], gynaecogical age [PRR 0.63, 95 % CI 0.40-0.98, p=0.04] and paternal education [PRR 0.88, 95 % CI 0.80-0.97, p=0.009], adjusted for season of survey. Symptomatic malaria prevalence was 2.8% in the dry and 3.6% in the wet season (p=0.35), and was associated with chronological age [PRR 0.93,95 % CI 0.86-0.98; p=0.02] and paternal education [PRR 0.83, 95 % CI: 0.74-0.94; p=0.002], with borderline significance for gynaecological age [PRR 0.65, 95 % CI 0.40-1.01, p=0.05]. Puberty corresponded with a reversal of the age dependent decline in malaria prevalence.

ITNs were readily available in households 77.1 %, (dry) and 82.9 % (wet), (p=0.010). Mean±sd ITN number per household was 1.6±1.3 (dry) and 1.7±1.2 (wet), (p=0.02). ITN access was 46.6%. Factors associated with ITN access were unmarried [PRR 0.27, 95% CI 0.10-0.73, p=0.01], postmenarche [PRR 2.01, 95% CI 1.28-3.17, p=0.002], maternal education [PRR 1.06, 95% CI 1.01-1.12, p=0.02], and participant literacy [PRR 1.65, 95% CI 1.12-2.43, p=0.01]. ITN use was 63.8% (dry) and 88.2% (wet), p<0.001. This was associated with season [PRR 2.04, 95% CI 1.58-2.63, p<0.001], gynaecogical age [PRR 1.29, 95 % CI 1.19-1.41, p<0.001] and participant education [PRR 1.12, 95 % CI 1.06-1.18, p<0.001]. Malaria prevalence was not associated with ITN access [PRR 0.93, 95% CI 0.55-1.57, p=0.79] or ITN use [PRR 2.09, 95% CI 0.62-7.06, p=0.24].These results indicate good ITN access, low usage and no significant improvements in malaria prevalence suggesting infrequent adherence.

Self-treatment with an antimalarial drug was 12.9 % in pregnant and 2.4 % in non-pregnant adolescents, (p=0.008). The rate of antimalarial drug self-treatment in the first trimester was 3.2 %, which was comparable to that for non-pregnant adolescents (p=0.51). Factors associated with self-treatment were gynaecological age [PRR 1.51, 95% CI 1.18-1.93; p<0.001], literacy [PRR 1.10, 95% CI: 1.02-1.20; p =0.02) and symptomatic malaria [PRR 6.57, 95% CI: 1.85-23.37; p=0.004]. Inadvertent antimalarial drug exposure in the first trimester should be recognised and evaluated.

Malaria knowledge (MK) in out-of-school adolescent girls (mean±sd age 14±2.5yrs) attending AGLIT, was assessed at enrolment and evaluation. 87.1% had poor MK. Associated factors were young age [AOR 1.49, 95% CI 1.05-2.13, p=0.03] and maternal illiteracy [AOR 6.44, 95% CI 1.25-33.12, p=0.03] in unmarried girls; and having no previous child [AOR 3.59, 95% CI 1.21-10.67, p=0.02] in married. MK improved post-AGLIT class attendance and regression analysis showed that 18% of improved MK was attributable to acquired literacy skills, assuming illiteracy at enrolment.

Conclusion: These female adolescents experienced some malaria risk with no significant seasonal difference. Malaria prevalence was influenced by puberty. Lack of access to preventive measures, low literacy, anaemia and undernutrition were prevalent. Improved strategies for malaria control in pre-pregnant adolescent girls are required and should possibly be integrated as part of Adolescent Friendly Health Services provision.

CHAPTER ONE

INTRODUCTION AND AIMS

1.1 Malaria in adolescent girls as a public health problem

The prevention and control of malaria is a key strategy in reproductive health in malaria endemic areas. At high risk of malaria infection are adolescent girls who prematurely become pregnant (WHO 2007). However, information on malaria risk before pregnancy is limited because malaria infection among adolescents in these endemic areas is thought to be infrequent and asymptomatic and therefore to remain undetected and untreated. The evidence for this is limited (Lalloo *et al.* 2006). Therefore, understanding the problem of malaria infection in the pre-pregnant state and its prevention and control strategies is of importance and could contribute to reducing malaria risk in pregnancy.

The approach of this research study utilizes the public health problem-solving paradigm (Guyer 1998). As such, this work addresses the epidemiology of malaria as it applies to the adolescent as well as issues related to adolescent help-seeking as well as health literacy. The paradigm includes six steps, with modifications to fit particular circumstances and depending on the expanse of knowledge already, with the 3 latter stages being applicable to policy development. The steps are: (a) defining the problem; (b) measuring the magnitude of the problem; (c) developing a conceptual framework for key determinants of the problem, including biological, epidemiological, socio-cultural, economic and political determinants; (d) identifying and developing interventions and prevention strategies; (e) setting priorities among

strategies and recommending policies; and (f) implementing programmes and their evaluation. This research does not go as as far as steps (d) to (f) because these specifically involve policy, however, its findings could contribute towards these.

1.2 Adolescence

Adolescence is defined as the process of growing up which covers the period between childhood and maturity (Brook 1985). It encompasses the physical changes of puberty as well as the psychosocial developmental process. These changes vary widely in both amount and duration, between individuals, between males or females, as well as among populations (Neinstein 1996).

Puberty is a collective term embracing all those morphological and physiological changes present in the growing boy or girl as the gonads change from the infantile to the adult stages of development (Marshall 1978). It is a result of complex developmental processes which occur in the central nervous system and endocrine system (Marshall 1978). These changes involve nearly all organs and structures of the body. The principal manifestations include the adolescent growth spurt, the development of gonads in relation to secondary reproductive organs and secondary sex characteristics, changes in body composition and the development of the circulatory and respiratory systems. Puberty is not an isolated event but a continuous process, usually beginning and ending within the second decade of life. This transition does not occur by a uniform synchronous process (Neinstein 1996) and may be interrupted by environmental as well as host factors influencing the adolescent's health.

The adolescence period can be conceptualized into three psychosocial developmental phases: early adolescence (approximate ages 10 – 13 years); middle

adolescence (approximate ages 14 – 17 years); and late adolescence (approximate ages 17 – 21 years) (Neinstein 1996). These phases, which overlap, are determined by the physiological stage of maturation, how the individual responds to all the changes of puberty and the life tasks of the individual (Neinstein 1996).

For public health assessments, adolescence is defined as the period of growth between 10 to 19 years of age (World Health Organization 2001). Information on the timing and occurrence of the pubertal phases is not routinely collected and therefore age is sometimes used to arbitrarily demarcate this process into early adolescence from 10 to 14 years and late adolescence from 15 to 19 years (United Nations Population Fund 2007). Medically, adolescent problems can be encompassed within the specialties of paediatrics and adult medicine (Brook 1985). In this thesis, the World Health Organisation (WHO) definition of adolescence is adopted, unless stated otherwise.

1.3 Adolescent health

In 2005, the estimated 1.2 billion adolescents constituted nearly one fifth of the world population (The World Bank Group 2007). These are the world's political and economic future and understanding their health situation is of great importance. Adolescents world-wide are prematurely taking up challenging adult roles including parenthood and labour activities which may make them vulnerable to interruption of education, early and even single parenthood, exposure to injury and infectious diseases. This affects their general health, physiological and psychological well being. Because of relatively low mortality rates during adolescence, this period had long been regarded as one of the healthiest periods of life. As a result, little attention had been given to adolescent health and nutrition.

Access to good health services for adolescents is often limited and even the uptake may be poor, thereby increasing their risk of morbidity and mortality (World Health Organization 2001). These adolescents have a right to access adolescent friendly health services that can protect them from HIV/AIDS and other threats to their health and well-being such as malaria (World Health Organization 2001). The period of adolescence offers a last window of opportunity to correct problems inflicted in childhood and to prepare for the demands of adult life, and this opportunity should not be missed.

Despite a shift toward later marriage in many parts of the world, 82 million girls in developing countries who are now aged 10 to 17 years will be married before their 18th birthday. This makes adolescent females more vulnerable to undesirable health outcomes than adolescent males. Gender considerations are therefore fundamental in health programming (United Nations Population Fund 2007).

1.4 Epidemiology of malaria infection

Malaria is a parasitic disease which can be transmitted to people of all ages. It is caused by parasites of the species *Plasmodium* that are spread from person to person through the bites of infected mosquitoes. Malaria infection is mostly found in the tropical and sub-tropical regions of the world and approximately 40 % of the world's population is at risk of acquiring this infection (WHO 2000). It causes more than 300 million acute illnesses and at least one million deaths annually (Roll Back Malaria 2004). The Roll Back Malaria (RBM) Partnership estimates that ninety percent of these deaths occur in Africa south of the Sahara, mostly in young children (Roll Back Malaria 2004).

The risk and epidemiology of malaria for different geographical areas and age groups is determined by malaria transmission intensity (MacDonald 1957). At one extreme is unstable transmission that occurs under conditions of low transmission with risk of epidemics. This is due to no or a low level of acquired malarial immunity, and leads to a risk of clinical disease occurring in children as well as adults with an increased risk of severe forms of the disease. In contrast, stable transmission results from a high transmission intensity which exposes children to multiple infections from an early age, with a peak incidence in the second and third year of life (MacDonald 1957). Under these conditions, immunity develops from early infancy and malaria incidence declines with increasing age. The frequency of clinical malaria is therefore correspondingly reduced in adults and is usually in mild form (Bruce-Chwatt 1963). Under stable conditions, when severe malaria occurs, clinical manifestations differ by age group. In children, cerebral malaria, anaemia, respiratory distress and metabolic acidosis are common manifestations of severe malaria, whereas in adults cerebral malaria, renal failure and hepatic impairment are the most common features (Marsh *et al.* 1995).

The endemicity pattern and level of transmission intensity are determined by the number of infective bites per person per year, often expressed as the entomological inoculation rate (EIR). However, a wide spectrum of variation within the classifications of unstable and stable malaria transmission exists as the precise relationship between frequency of parasite exposure, functional immunity and disease risk still remains ill defined (Snow *et al.* 1999). For example, different patterns of transmission were noted within short distances of the same area in Brazzaville, Congo (Trape 1987) and summation of various studies showed that the spectrum of stable

endemicity may be maintained over a wide range of transmission pressures from approximately one to one thousand infective bites per year (Marsh & Snow 1999).

There are five *Plasmodium* species of public health importance. Four species of *Plasmodium* (*Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae* and *Plasmodium ovale*) have been known to cause malaria in humans and their severity of illness varies. The fifth parasite *Plasmodium knowlesi* (White 2008), which is a fatal simian malaria parasite, was reported in the human population of Malaysian Borneo using molecular tools (Cox-Singh *et al.* 2008; Singh *et al.* 2004) and has been confirmed in others parts of South-east Asia (Cox-Singh *et al.* 2008; Kamtele *et al.* 2008; Luchavez *et al.* 2008; Ng *et al.* 2008). *P. falciparum* malaria is the commonest malaria species throughout the sub-tropics and tropics (Snow & Gilles 2002). *P. vivax* has the widest geographical range, being prevalent in many temperate zones as well as the sub-tropics and tropics. *P. malariae* is patchily present over the same range as *P. falciparum* but much less common. *P. ovale* is chiefly found in tropical Africa and occasionally in the West Pacific (Snow & Gilles 2002).

P. falciparum malaria infection is the primary focus of this thesis because it is the species of major public health importance in Malawi. Over 85 % of the malaria infections are due to *P. falciparum* alone, with a small number of mixed infections with *P. ovale* and *P. malariae*, variable according to location (Ministry of Health and Population 2001). In the lower Shire valley where this research study was conducted, *P. falciparum* is predominant in 99.5 % with a small proportion of *P. malariae* (0.5 %), (Mzilahowa 2005).

1.5 Strategies for malaria prevention and control

The key to controlling malaria is a combination of both preventive and curative approaches. Those at risk must have access to the most effective preventive measures, and those suffering from malaria must have access to prompt and effective treatment (Roll Back Malaria partnership 2004). The RBM Partnership recommends that people suffering from malaria, especially children and pregnant women, need to be diagnosed and given effective and affordable drug treatment within 24 hours of the first symptoms appearing (Roll Back Malaria partnership 2004). In the wake of rapidly developing resistance to many currently used antimalarial drugs, combination treatment preferably with an artemisinin-based combination therapy (ACT) is recommended.

Preventive measures in those at risk of malaria infection include personal protection and mosquito control. Chemoprophylaxis is recommended for non-immune populations and for some vulnerable groups such as in patients post-splenectomy. In pregnant women intermittent preventive treatment (IPTp) is recommended, which involves providing pregnant women with antimalarial drugs at defined intervals during their antenatal care visits. IPTp has been shown to substantially reduce the risk of life-threatening complications which occur in mothers and babies as a consequence of malaria in pregnancy. Insecticide-treated mosquito bed nets, if used properly, are one of the best ways to prevent mosquitoes from biting people and infecting them with malaria (Hill *et al.* 2006). They are especially recommended for use among young children, pregnant women, and those living with HIV/AIDS. Other insecticide-treated materials such as curtains and clothes (Kimani *et al.* 2006) have been shown to be effective in community studies but their usage is not emphasized in current policy. Spraying the inside of people's houses with insecticide to kill malaria mosquitoes, known as indoor residual spraying (IRS), is also used to control mosquito populations.

IRS is especially recommended in epidemic-prone areas or dense urban settings, and in countries where there is a strong, well-organized public sector delivery mechanism. Other interventions at individual level include use of body and space repellents.

All the means to control malaria would be useless if they do not reach and are not used by those who need them. Adolescents living in malarious areas need to have access to all these life-saving interventions, although this particular risk group has received little priority in the context of malaria control interventions. This may be partly because they are often considered to be covered under the umbrella of antenatal care, but this excludes the specific needs of the non-pregnant adolescent girl.

1.6 Study aims and objectives

The aim of this research was to describe the magnitude and factors associated with *P. falciparum* malaria among adolescent girls (10- 19 years old) in southern Malawi.

Specific objectives:

1. To describe nutritional and health characteristics of non-pregnant adolescent females
2. To describe the point prevalence of *Plasmodium falciparum* parasitaemia and symptomatic malaria infection in non-pregnant adolescent girls during the dry (low transmission) and wet (high transmission) seasons at the community level.
3. To describe the factors associated with *P. falciparum* malaria infection in non-pregnant adolescent girls.

4. To determine the access and utilisation of ITNs for malaria prevention in non-pregnant adolescent girls and the effects of reported ITN availability, access and use on malaria, anaemia and nutritional status.
5. To assess antimalarial and other drug self-treatment rates for presumed malaria infections in non-pregnant and pregnant adolescent females.
6. To assess malaria knowledge, factors associated with poor malaria knowledge and the impact of acquired literacy on malaria knowledge among the adolescent girls attending an adolescent girl's literacy programme (AGLIT).

1.7 Thesis structure

This thesis is presented in nine chapters. Chapter one summarises the background information to the studies presented in this thesis. Chapter two is a review of relevant literature. Chapter 3 describes the methodology used in the study. Chapters four to eight present the results. All result chapters have sections that give a brief introduction, the objective, any additions to the methodology that relate to specific chapter objectives, results with the related discussion and conclusions. Chapter four describes the study population, with emphasis on nutritional and health characteristics (objective 1). Chapter five describes seasonal malaria prevalence and its associated factors (specific objectives 2 and 3). Chapters six and seven address objectives 4 and 5, respectively. Chapter eight addresses objective 6. Research findings are summarized in Chapter nine with a general discussion of the conclusions emphasising their relevance for public health.

CHAPTER TWO

LITERATURE REVIEW

The objectives of this review were to summarise available data on host susceptibility to *Plasmodium falciparum* malaria in female adolescents, the prevalence and burden of malaria- related disease, the estimates of efficacy of malaria-related interventions and their access in this specific age group.

2.1 Host susceptibility to *P. falciparum* infection

Host susceptibility to infectious diseases such as *P. falciparum* infection is age and sex dependent among adolescents. Intrinsic features of the immune system that change with age may determine key characteristics of the immune response, depending upon the frequency, conditions of exposure and whether the infection is acute or chronic (Baird 1998). Patterns of infection between males and females are likely to be different due to inter-related differences in exposure, immunity acquisition, immune response, hormonal differences or they might reflect differences in access to health services or compliance with treatment (Brabin 2004). Exposure to infective vectors is related to behaviour and work patterns in males and females, which are frequently distinct (Brabin & Brabin 1992a). This differential exposure influences the evolution of the age-specific disease profiles according to the specific conditions and environments under which the individual lives (Abebe *et al.* 2002).

Evidence for the effects of host genetic factors on the risk of acquiring *P. falciparum* infection and for developing severe malaria complications is substantial (Hill 2006). Erythrocyte variants that are protective for malaria include sickle cell trait (HbAS), Haemoglobin C, Haemoglobin E, the alpha- and beta-thalassaemias, glucose-

6-phosphate dehydrogenase deficiency and pyruvate kinase deficiency (Aidoo *et al.* 2002; Brabin & Perrin 1985; Brabin & Brabin 1990; Hill 2006; Min-Oo *et al.* 2007; Min-Oo & Gros 2005). Genetic factors can influence different immune responses to malaria infection. These include adhesion molecules, interferon receptors, CD36, CD40L and various cytokines, e.g. tumour necrosis factor (TNF) 2 and interleukins (IL) 4 and IL-12p40 (Stevenson & Riley 2004).

Also of importance is the differential way individuals and communities access health care. Access to health care for malaria susceptible individuals may be variable within families, between rural and urban settings or in relation to distance and geographical location. All these biological and social factors can influence host immunity and sub-clinical or clinical disease manifestations. Host susceptibility to malaria infection is therefore complex and multi-factorial at the individual, community as well as population level.

2.1.1 Host susceptibility in pregnant women

Pregnant women are more susceptible to malaria infection than non-pregnant women and this susceptibility is greatest in the first and second pregnancy (Brabin 1983). This is due to alterations in host responses influenced by changes in metabolic, haematological and immunological parameters during pregnancy (Brabin 1985). Young maternal age also increases susceptibility, which may relate to influences on these same parameters coupled with nutritional and developmental changes. The prevalence of malaria varies with gestational age, with a peak in early pregnancy between 13 and 16 weeks gestation (Brabin *et al.* 1990; Diagne *et al.* 1997; Rogerson *et al.* 2000; Steketee *et al.* 1996). Comprehensive reviews on malaria in pregnancy emphasise the importance of the altered general immune response to

infection during pregnancy. These include changes in T-lymphocyte sub-populations, polymorphonuclear leukocyte function, peripheral blood lymphocyte responses, serum immunoglobulin concentrations, immunosuppressive serum factors, circulating immune complexes, and maternal immunologic recognition mechanisms (Brabin 1985; Rogerson *et al.* 2007).

Antibody responses in pregnancy are complex and their concentrations in the blood are influenced by gestational changes in plasma volume. The level of serum IgG decreases significantly with advancing gestation, falling to values below those in non-pregnant women towards term (Brabin 1985). These quantitative values cannot be interpreted in relation to protection in pregnancy (Brabin 1985). Changes in total serum IgA and IgM are less well-defined during pregnancy (Brabin 1985; Okoko *et al.* 2003). There is some evidence for an association between the production of antibodies recognizing specific antigens on infected placental erythrocytes and protection against *P. falciparum* malaria (Ricke *et al.* 2000). The placenta is well described as showing high parasite densities due to sequestration of infected red blood cells when the peripheral circulation is free of parasites. Pregnancy-associated malaria susceptibility in part results from a sub-set of infected erythrocytes sequestering in the placenta. During the first pregnancy, infected red cells expressing receptors for condroitin sulphate A (CSA) preferentially sequester in the placenta. Specific antibodies develop against a surface protein on these infected erythrocytes in the placenta which bind to CSA on syncytioblast. These antibodies are usually absent in the first pregnancy but increase in prevalence in subsequent pregnancies and they are partly the basis for explaining parity-specific immunity in pregnancy (Fried *et al.* 1998).

In pregnancy there is a transient alteration of cell-mediated immunity (CMI). The mechanism behind the depressed CMI is not well understood; and pregnancy hormones are implicated as partly responsible (Brabin 1997). This transient alteration of immunity influences immunologic tolerance allowing acceptance of the implantation of the fetal allograph in her uterus (Wegmann 1993). The lack of a strong maternal cell-mediated anti-fetal immune response, combined with the hormonally influenced immune response suggests that the cellular immune system in normal pregnancy is biased towards a type II helper cell (Th2)-like rather than the type I helper cell (Th1)-like response (Wegmann *et al.* 1993). Although this immunological change in pregnancy is biologically advantageous to the fetus, the consequences of this altered immunity may lead to increased susceptibility to infectious diseases caused by intracellular pathogens; including viruses, malaria and some parasitic infections, together with the potential for interaction between these infections in the mother (Auchus & Rainey 2004). For example, interaction between HIV and *P. falciparum* in pregnancy has been well described.

Despite considerable evidence for immunological changes related to malaria in pregnancy, studies into other factors such as hormones, which could influence this susceptibility, are few. Malaria has been associated with reduced oestradiol production in late pregnancy (Watkinson *et al.* 1985) and raised serum cortisol levels in primigravidae have been reported in placental malaria (Bouyou-Akotet *et al.* 2005). However, evidence for prolactin effects has been inconclusive (Rogerson *et al.* 2007). A recent study from the Gambia explored the associations of blood group phenotypes in relation to placental malaria pathology (Loscertales & Brabin 2006). Blood group O was significantly associated with increased placental malaria infection in primiparae and reduced the risk of infection in multiparae; and this

parity-related susceptibility was not present with other ABO phenotypes. Data from a study of pregnant women in Malawi has also shown this same association of the blood group O phenotype with decreased malaria prevalence in multigravidae (Senga *et al.* 2007). From a review of the role of the ABO blood group system, it has been suggested that altered cytoadherence, rather than red blood cell invasion, is the mechanism in action (Cserti & Dzik 2007).

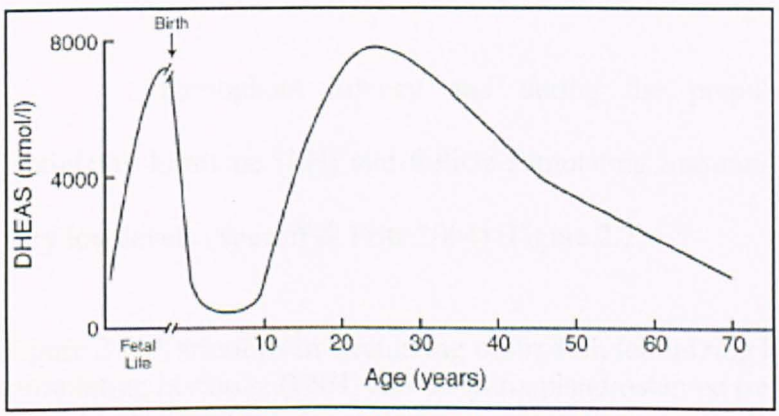
2.1.2 Hormonal changes in adolescents and susceptibility to infection

Complex hormonal changes are associated with growth and puberty. Hormonal changes in adolescent females and males differ and these may modify malaria risk between the sexes. As puberty approaches, changes of adrenarche, decreasing repression of the gonadostat (i.e. the hypothalamic–pituitary system controlling gonadotrophins) and gradual amplification of the gonadotrophin-releasing hormone (GnRH) and its interactions with gonadotrophin and ovarian steroid hormones occur (Speroff & Fritz 2004).

Adrenarche, i.e. the onset of dehydroepiandrosterone (DHEA) and DHEA-sulphate (DHEA-S) production from the adrenal zona reticularis, can be detected at around 6 years of age, which results in the development of axillary and pubic hair occurring in both girls and boys at about age 8 years (Auchus & Rainey 2004). These are also produced in smaller quantities in the testes in males and the ovaries in the female (Marshall 1978). DHEA-S concentrations are high in the newborn, as this hormone is produced by the fetal adrenal glands during pregnancy. By one year of age, the fetal adrenal is replaced by the definitive adrenal cortex, which initially makes little DHEA-S. Hence, DHEA-S production declines precipitously during the first months of life and remains low until adrenarche (Figure 2.1), (Auchus & Rainey 2004). The

rise in the circulating concentrations of DHEA and DHEA-S is the biochemical hallmark of adrenarche. Importantly, the rise in DHEA-S occurs prior to the increase of either oestrogens or other androgens associated with puberty. Circulating DHEA-S concentrations continue to rise and peak during the second decade of life and levels are higher in males than females (Auchus & Rainey 2004). Adrenarche does not appear to be under the direct control of gonadotrophins or adrenocorticotropic hormone (ACTH) during puberty as the circulating levels of these androgens change without corresponding changes in cortisol and ACTH (Speroff & Fritz 2004).

Fig. 2.1: Variation in circulating dehydroepiandrosterone sulphate (DHEA-S) concentrations throughout human life.



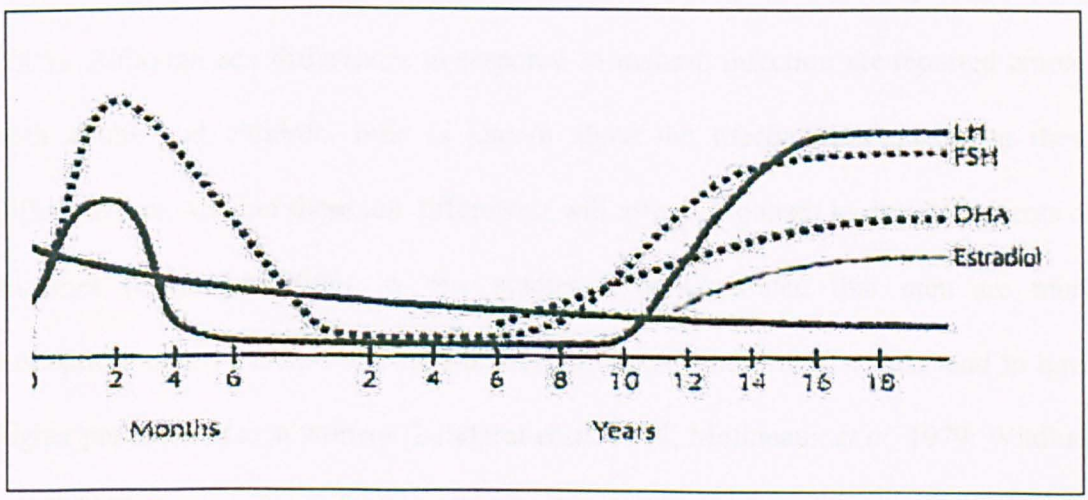
(Source: Auchus & Rainey 2004. With permission from Willey-Blackwell publishers)

The role of DHEA and its sulphate DHEA-S (described as DHEA(S) when referring to both) has been evaluated recently in relation to *P. falciparum* malaria in adolescents. In both experimental animals and human in vitro studies DHEA(S) showed anti-inflammatory properties and down-regulated IL-6 and TNF-α (Coutinho *et al.* 2007). Among non-pregnant adolescent girls in western Kenya, DHEA(S) was positively associated with acquisition of malaria immunity in adolescent girls (Leenstra *et al.* 2003b). In adolescent boys from the same population, DHEA(S) showed positive correlation with malaria immunity acquired during puberty (Kurtis *et*

al. 2001). In a Philippines study, DHEA(S) showed a dose-dependent association with several inflammatory markers that relate to malaria immunity (Coutinho *et al.* 2007). Furthermore, raised DHEA(S) levels were associated with both reduced *Schistosoma japonicum* infection (Coutinho *et al.* 2007) and parasite re-infection (Kurtis *et al.* 2006). This evidence suggests that endocrine status and development during puberty modifies host susceptibility to parasitic infections. There is variable secretion affecting the dynamics of the interconversion between DHEA and DHEA-S. Unlike DHEA-S, DHEA levels slightly differ by age and sex. DHEA is generally higher among women than men and significantly different during puberty (around 11 to 15 years) and in the pre-menopausal period (36 to 45 years) (Sulcova *et al.* 1997). It is not known how this affects sex-dependent immunity.

Throughout infancy and during the prepubertal period, oestradiol, leutinizing hormone (LH) and follicle-stimulating hormone (FSH) are suppressed to very low levels (Speroff & Fritz 2004) (Figure 2.2).

Figure 2.2: Variations in circulating oestradiol, leutinizing hormone (LH), follicle-stimulating hormone (FSH) and dehydroepiandrosterone (referred to as DHA in this figure) in human life



Source: Speroff & Fritz 2004

Although low, FSH and LH display evidence of pulsatile secretion during this period and increase during the progress through the stages of puberty (Dunkel *et al.* 1992). Changes in the pattern of gonadotrophin responses to the hypothalamic-releasing hormone GnRH accompany normal pubertal maturation in girls (Sizonenko 1989). FSH responses to GnRH are initially pronounced but decrease steadily throughout the onset of puberty, while LH responses are low in prepubertal girls and increase strikingly during puberty (Job *et al.* 1972). So FSH appears to rise initially and plateau in midpuberty while LH tends to rise more slowly and reaches adult levels in late puberty. Increased amplitude and frequency of pulsatile GnRH are believed to provoke progressively enhanced responses of FSH and LH secretion (Bridges *et al.* 1994). By mid- to late puberty, maturation of the positive feedback relationship between oestradiol and LH is established, leading to ovulatory cycles.

Circulating steroid hormones have been linked to increased susceptibility to infection in field and laboratory studies; and males are more susceptible to many protozoan infections than females (Klein 2000; Klein 2004; Roberts 2001). Most studies of malaria do not distinguish between the responses of males and females, and thus, the prevalence of sex differences may be under-reported (Allotey & Gyapong 2005). Although sex differences in response to malaria infection are reported among both adults and children, little is known about the mechanisms mediating these differences or whether these sex differences will affect responses to drug treatments or vaccines (Cernetich 2006). A few studies have illustrated that men are more susceptible than women. For example, several studies indicate that men tend to have higher parasitemia than women (Landgraf *et al.* 1994; Molineaux *et al.* 1979; Wildling *et al.* 1995) and more severe symptoms of malaria infection (Casalino *et al.* 2002). Among Ghanaian school children, parasite density was significantly higher for boys

than girls around puberty (8 to 16 years), suggesting that circulating sex steroids may influence this outcome, although the prevalence of *P. falciparum* infection does not significantly differ between the sexes (Landgraf *et al.* 1994). Recent studies with a rodent malaria model indicated that males and females respond differently to malaria infection and suggested that the underlying immunological mechanisms were likely to involve heightened IFN-γ and regulatory T-cell responses observed in the female mice (Cernetich 2006). These data make a compelling case for evaluating how males and females differ in their responses to malaria infection in epidemiological studies.

2.2 *P. falciparum* malaria in adolescent females in stable transmission areas

Adolescent malaria infection in endemic areas is often asymptomatic and therefore can remain undetected and untreated. This is due to the acquisition of partial immunity from childhood, which translates into relatively lower incidence rates of symptomatic disease compared to children less than 5 years of age. Malaria reporting has traditionally grouped adolescents with children older than 5 years of age and adults. At high risk of malaria infection are adolescent girls who prematurely become pregnant, and since as many as one in every six births in developing countries is to an adolescent female aged between 15 and 19 years, this is a significant burden of disease (World Health Organization & UNICEF 2002). These adolescent mothers will have an increased risk of severe anaemia, preterm delivery, stillbirth and neonatal death (Brabin *et al.* 1998a; Brabin & Brabin 1992b). For this reason, understanding how malaria behaves in the adolescent female is of major public health importance.

2.2.1 Malaria prevalence

The problem of malaria in adolescents has been overshadowed by the huge burden of disease in young children and pregnant women. Although much has been

learnt about the epidemiology and clinical effects of malaria infection, its understanding and relevance in adolescence has been neglected (Lalloo *et al.* 2006). Lalloo *et al.* (2006) were the first to present a comprehensive analysis of the problem of malaria in the adolescent. These authors present current evidence that this is a substantial and preventable problem. From seven available studies from six countries (four African countries, one from Papua New Guinea and one from Burma), (Bloland *et al.* 1999; Colbourne 1955; Rogier & Trape 1993; Rogier *et al.* 1999; Smith *et al.* 1994; Soe *et al.* 2001; Trape 1987), which provided an overall sample size of 1,068 adolescents, they estimated that the incidence of clinical malaria in adolescents in areas of stable transmission varied from 0.13 to 1.18 attacks per year (Table 2.1). They present further African estimates of clinical malaria, reported by Brooker *et al.* (2000) among schoolchildren 10 - 20 years old, to be 0.252 attacks per year.

Table 2.1: Incidence of clinical malaria in adolescents (both sexes) from stable transmission areas

Location	Year of study	N	Age	Incidence – episodes per year (95% CI)	Reference
Kenya	1992 -95	262	10-14	0.22 (0.15 – 0.31)	Bloland *et al.* 1999
Republic of Congo					
Daily survey	1982 - 84	48	11-13	0.76 (0.01 – 4.04)	Trape *et al.* 1987
Weekly survey		53	11-13	1.18 (0.73 – 1.83)	
Senegal	1990	14	11-14	0.22 (0.15 – 0.31)	Rogier *et al.* 1993
Senegal	1990 -93	41	10-19	0.13 (0.08 – 0.19)	Rogier *et al.* 1999
Ghana	1952	248	12-20	0.22 (0.15 – 0.31)	Coulbourne 1955
Papua New Guinea	1991	355	10-19	0.28	Smith *et al.* 1994
Burma	1999	47	10-14	0.22 (0.15 – 0.31)	Soe *et al.* 2001

Source: Lalloo *et al.* 2006

Incidence measurements while valuable, are more difficult to obtain, are more costly and require longitudinal sampling. Although incidence rates are reported

in some studies, prevalence estimates are mostly available as malariometric studies are usually cross-sectional. Prevalence will under-estimate risk for malaria infection with short clearance times and super-infection. The prevalence will give a point estimate for a particular time and location. Many studies do not distinguish the adolescent sex, preventing estimation of malaria burden in adolescent females. A further limitation of cross-sectional adolescent data is the lack of age-specific prevalence for the different adolescent age groups between the intervals 10 to 19 years. This limits comparability of data between pre-pubertal and pubertal adolescents. It is important to measure age-specific prevalence in order to establish comparative estimates between studies with different age ranges. In some cross-sectional studies, prevalence estimates of malaria have included adolescents. These are limited to a few reports: five studies in the non-pregnant (Table 2.2) and three studies in pregnant adolescents (Table 2.3). The incidence rates and prevalence estimates of *P. falciparum* malaria infection among adolescents as outlined above indicates this is a significant public health problem an understanding of which is essential in order to control malaria infection in this specific risk group.

Table 2.2: Prevalence of parasitaemia in non-pregnant female adolescents from stable transmission areas

Location	Sample size	Age (years)	Prevalence (%)	Reference
Tanzania ¶	§ (M, F)		[dry, rainy, post-rainy]	Lusingu *et al.* 2004
Hypoendemic (Magamba)	255 (132, 123)	10 – 14 15 – 19	8, 15, 14 0, 7, 6	
Mesoendemic (Ubiri)	250 (139, 111)	10 – 14 15 – 19	13, 39, 20 33, 55, 24	
Holoendemic (Mgombe)	254 (115, 139)	10 – 14 15 - 19	57, 77, 88 44, 58, 63	
Kenya, western *	575	12 -18	[overall, dry , post-rainy] 27.7, 20.4, 33.7	Leenstra *et al.* 2004
Papua New Guinea (Wosera region) ¶				
2001 – 2003	§2119	10 – 19	31.0	Kasehagen *et al.* 2006
1990 - 1992	-	10 – 19	52.0	
Malawi, Chikwawa	52	10 - 14 15 - 19	25 17.5	Ngwira 2005
Democratic Republic of Congo				
(1) Rural Brazaville *	§616	10 -14	82.0	Trape *et al.* 1987
Low transmission (Linzolo)	137	15 -19	70.1	
(2) Urban Brazaville *				Trape *et al.* 1987
High transmission (Massina)	50	10 -14	84	
Very low transmission (Moungali)	103	10 -14	12.6	
Moderate transmission (Talangai)	60	10 -14	71.7	
Low transmission (Bacongo)	64	10 -14	46.9	
Very low transmission (Poto-poto)	58	14 -15	7.0	

* Schoolchildren; ¶ both sexes; M, F = males, females; [ ] = season-specific prevalence; transmission levels are reportedly defined according to entomological inoculation rates and/ or spleen rates; § N represents 0 to 19 yrs as adolescent-specific N was not reported but their malaria prevalence reported.

Table 2.3: Prevalence of clinical malaria in pregnant female adolescents from stable transmission areas

Location	Sample size	Age (years)	Prevalence (%)	Reference
Malawi, Chikwawa	615	13 - 19	34.4	Brabin *et al.* 1998
Mozambique (Matola & Boane)	600 G1 & G2	< 21	33.3	Challis *et al.* 2004
Tanzania, Morogoro region	528	Pregnant adolescents	G1 – 43.3 G2 – 28.1	Uddenfeldt- Wort *et al.* 2006

G1 = primigravidae; G2 = secundigravidae

2.2.2 Severe disease estimates

In high transmission areas in Africa, age-specific patterns of severe disease show a rapid decrease over the first few years of life such that significant severe disease does not appear to occur much after 5 years of age in these areas. This decline in the incidence of severe disease occurs at an even earlier age than declines in mild malaria. One would expect this to lead to a very low incidence of severe malaria in adolescents. Cerebral malaria, anaemia, respiratory distress and acidosis are the commonest manifestations of severe malaria in children; whereas in adults, cerebral malaria, renal failure, and hepatic impairment are more common (Marsh *et al.*1995). The pattern of severe malaria may however vary within the same age group between different geographic regions (Greenwood *et al.* 1991).

Lalloo et al. (2006) reported three studies that estimated the incidence of severe disease and these are summarised in Table 2.4 (Carme *et al.* 1992; Greenberg *et al.* 1989; Trape *et al.* 1987).

Table 2.4: Incidence of severe malaria in adolescents (both sexes) from stable transmission areas

Location	Age	Incidence (per 1000 population/ year)	Reference
Republic of Congo, Kinshasa	10 - 13	0.35	Greenberg *et al.* 1989
Republic of Congo, Brazaville	10 - 14	0.05	Trape *et al.* 1987
Republic of Congo, Brazaville	10 - 14	0.13	Carme *et al.* 1987

Source: Lalloo *et al.* 2006

These studies were hospital-based, conducted in paediatric wards and included both male and female adolescents between 10 – 14 years of age. The incidence ranged from 0.05 to 0.35 per 1000 adolescents per year. Different paediatric admission criteria exist between and within geographical locations and often older adolescents with malaria infection are admitted into adult wards, including gynaecological wards. This poses a problem in estimating incidence of disease in older adolescents. Severe malaria studies are rarely conducted in non-pregnant adult populations. Community-based morbidity studies that stratify adolescents are also rare. All these limitations may contribute to an under-estimation of the severe disease burden in adolescent females.

2.2.3 Sub-clinical disease estimates

Not all malaria infection results in acute morbidity. Semi-immune adolescents may have persistent malaria infection that is asymptomatic. This may remain undetectable depending on the physiological effect it has on the individual as well as the sensitivity of malaria diagnostic tests. The sensitivity for detecting malaria infection by standard light microscopy at low parasitaemia density is variable. Adolescents may be harbouring such low levels of infection. For example, studies from Senegal showed that parasite density decreased with age. No significant seasonal variation of either parasite prevalence or density was observed among children

between 1- 14 years in Dakar, Senegal (Trape *et al.* 1985). The authors concluded that *P. falciparum* clinical malaria was unlikely to occur in children under 15 years if the parasite/ leucocyte ratio was less than 1.5, and that a clinical malaria diagnosis was very probable if the parasite/ leucocyte ratio was higher than 2.

While it has been shown in endemic areas that *P. falciparum* sub-microscopic infections contribute to malaria-associated anemia and inflammation (McElroy *et al.* 2000), the clinical and epidemiological relevance of such infections remains unclear. It also remains unclear if there would be similar haematological consequences for the adolescent. Current evidence also shows that cerebral malaria is frequently associated with sub-microscopic infections in semi-immune individuals (Giha *et al.* 2005).

Hypothetically, prolonged carriage of *P. falciparum* may suppress growth through the production of growth-depressing cytokines (Ntab *et al.* 2007). Tumor necrosis factor-α (TNF) is produced in response to malaria (Friedman *et al.* 2003) and is known to inhibit the production of insulin-like growth factor (IGF), which mediates the effect of growth hormone. However, although biologically plausible, little evidence is available to support this hypothesis at the present time. In male African adolescents, malaria-induced TNF production was associated with significantly lower body mass index (Friedman *et al.* 2003).

2.2.4. Mortality

Data on malaria-related mortality in adolescence is limited and anecdotal evidence from paediatric reports suggests its occurrence is rare (Lalloo *et al.* 2006). Using data derived from children under 5 years old, Snow and others (2000) have estimated median mortality rates for adolescents at about 0.80 malaria deaths per 1000

per year. Table 2.5 summarizes the estimated population mortality rates in areas of stable transmission as reported by Lalloo *et al.* (2006).

Table 2.5: Mortality estimates in adolescents in stable transmission areas

Location	Age	Mortality (per 1000 admissions/ year)	Reference
Republic of Congo	10 -13	0.1	Greenberg *et al.* 1989
Republic of Congo	10 – 14	0.0	Trape *et al.* 1987
Republic of Congo (celebral malaria)	10 – 14	0.01	Carme *et al.* 1987
Nigeria	11-15	0.3	Bruce-Chwatt 1952
Papua New Guinea	10-19	0.4	Genton *et al.* 1995

Source: Lalloo *et al.* 2006

These ranged between 0.0 to 0.4 malaria deaths per 1000 adolescents per year. The authors further discussed the proportion of adolescent deaths attributed to malaria as compared to other diseases and age groups cited from several published and unpublished sources. These proportions ranged from around 6.3 % of deaths attributed to malaria in adolescents less than 16 years in Malawi, to around 9.1 % among 11-15 year old adolescents in Nigeria. These estimates present compelling evidence that adolescents who have been considered to be at a comparatively low risk from malaria infection are dying from this preventable disease.

2.3 *P. falciparum* malaria in adolescents in unstable transmission areas

Adolescent malaria infection in areas of unstable transmission commonly presents as clinical malaria at all ages, including the adolescent. Lalloo *et al.* (2006) showed a wide variation in incidence from studies conducted in parts of South America and South-east Asia (Belizario *et al.* 1997; Camargo *et al.* 1994; Camargo *et*

al. 1996; Camargo *et al.* 1999; Konradsen *et al.* 1997; Luxemburger *et al.* 1996). The results are summarized in Table 2.6.

Table 2.6: Incidence of clinical malaria in adolescents (both sexes) from unstable transmission areas

Location	Year	N	Age	Incidence – episodes per year (95% CI)	Reference
Western Thailand	1992 - 93	86	12 -15	1.00 (0.95 – 1.05)	Luxemburger *et al.* 1996
Philippines	1991 - 93	4585	11 – 19	0.026 (0.022 – 0.031)	Belizario *et al.* 1997
Brazil	1989 - 91	875	11 - 15	0.25 (0.22 – 0.28)	Camargo *et al.* 1996
Brazil	1991 - 92	40	11 – 15	1.08 (1.04 – 1.12)	Camargo *et al.* 1994
Brazil	1994 - 95	39	11 - 15	0.35 (0.22 – 0.52)	Camargo *et al.* 1999
Sri Lanka	1995	72	5 - 13	0.99 (0.96 – 1.02)	Konradsen *et al.* 1997

Source: Lalloo et.al. 2006

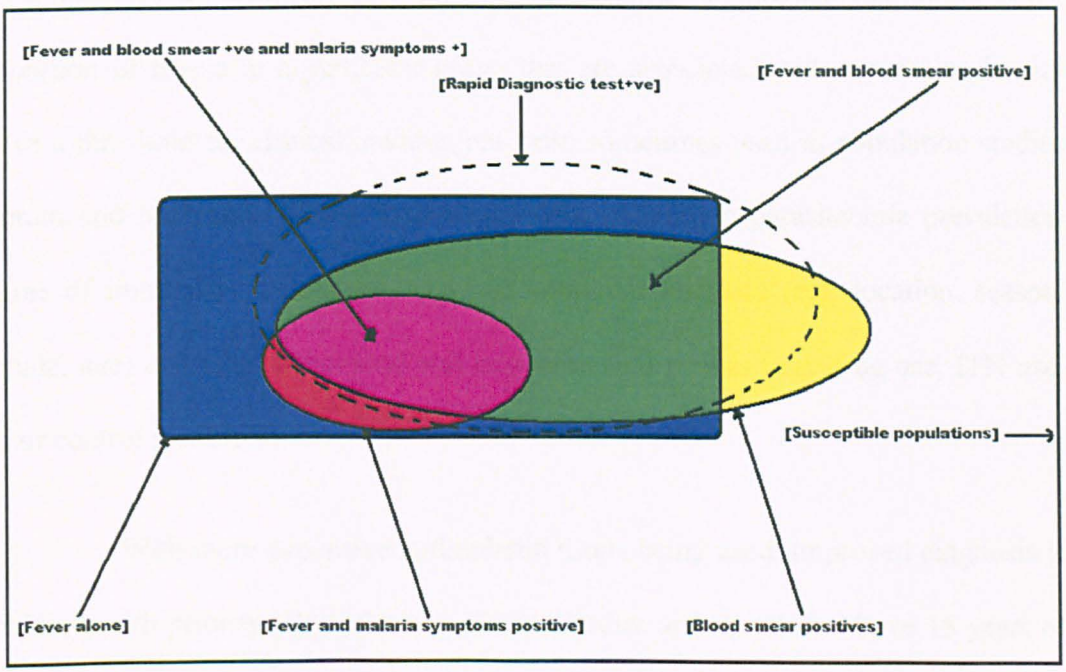
The incidence in adolescents was similar to that in younger age groups, and sometimes even higher. Incidence varied from as low as 0.026 (95 % confidence interval: 0.022 – 0.031) episodes per year among 11 to 19 years old adolescents in the Phillipines to as high as 1.08 (95 % confidence interval: 1.04 – 1.12) episodes per year among 11 to 15 year old adolescents in Brazil. In contrast to stable transmission areas where *Plasmodium falciparum* predominates, *Plasmodium vivax* was the more important cause of clinical illness.

2.4 Malaria diagnosis

Clinical diagnosis of malaria remains imprecise leading to occurrences of both under-diagnosis and over-diagnosis (Bell *et al.* 2005). The relationship between the clinical severity of malaria and the density of malaria parasitaemia is also complex. Scientific quantification of mis-diagnosis or its effects have not been adequately investigated; and this has an impact on malaria treatment decisions, classification of

cases in studies of pathogenesis or therapy, in intervention trials and monitoring trends in epidemiologic records (Snow *et al.* 2005). Figure 2.3 illustrates a schematic presentation of malaria infection in a malaria endemic population and how the detection of parasites relates to clinical diagnosis (Koram & Molyneux 2007).

Figure 2.3: Venn diagram of malaria infection categories in a susceptible population from a malaria endemic area



Adapted from Koram and Molyneux 2007.

Malaria parasites (i.e. blood smear positive) in the presence of related signs and symptoms such as fever often results into a correct diagnosis of clinical malaria (purple). However, a small proportion could be missed (red) depending on diagnostic procedures. The presence of malaria parasites (large oval with a continuous line) either with fever (green) or in the absence of fever (yellow) does not necessarily equate to a clinical diagnosis of malaria as a febrile illness in a malaria-susceptible population could be due to malaria or other infectious diseases. Fever is usually present

in malaria infection; consequently, presumptive treatment is widely applied for febrile illnesses among high risk groups in malaria endemic areas with limited laboratory support (green+ purple). This leads to an over-diagnosis of malaria. However fever as well as most malaria-related symptoms and signs have poor specificity (Chandramohan *et al.* 2002; Mwangi *et al.* 2005). For example, up to 30 % of febrile children fulfil diagnostic criteria for both malaria and respiratory infections (O'Dempsey *et al.* 1993; Redd *et al.* 1996). Malaria attributable fraction, i.e. the proportion of fevers in a particular group that are associated with a parasite density above a threshold for clinical malaria, has been sometimes used in population studies (Koram and Molyneux 2007). This varies with population parasitaemia prevalence, degree of immunity, factors related to transmission intensity (e.g. location, season, altitude, age) and other behavioral and environmental factors (e.g. drug use, ITN use, vector control measures).

With more expensive antimalarial drugs being used, improved diagnosis is a public health priority (Barnish *et al.* 2004). Studies among adults above 15 years of age have demonstrated the persistence of sub-microscopic infections, which proportionally increased with age, suggesting continued development of immunity that is able to restrict parasites to low densities with a sub-clinical but not sterilizing infection (Mayor *et al.* 2007).

Laboratory methods for detecting malaria parasites employ light microscopy (LM), polymerase chain reaction (PCR) techniques and malaria rapid diagnostic tests (RDT). Light microscopy for malaria diagnosis is widely accepted due to its simplicity, relative low cost, and ability to identify infecting species and to quantify parasitaemia (Bharti *et al.* 2007). Though LM is time-consuming, labor-

intensive and requires considerable expertise, it remains the gold standard for detecting parasitaemia in suspected malaria infection (Jonkman *et al.* 1995). The most important short-comings of LM are the relatively low sensitivity at low parasite levels and reliance on the microscopists' expertise. Although experienced microscopists can detect up to 20 parasites/μL, routine diagnostic laboratories have a much lower sensitivity of detection (Roshanravan *et al.* 2003). This has probably resulted in underestimation of malaria infection prevalence, especially for those with low parasitemia and asymptomatic malaria.

PCR- based methods, which are highly sensitive and specific techniques for the detection of all species of malaria parasite in whole blood, have been shown consistently to be highly sensitive for malaria diagnosis (Coleman *et al.* 2006;Cox-Singh *et al.* 1997). RDTs, based on immunochromatographic assays that detect malaria antigen, have also been developed to offer accurate, reliable, rapid, cheap and easily available alternatives to LM. These can detect all plasmodia species or can be made species-specific and have shown high sensitivity and specificity when compared to LM (Murray *et al.* 2003). They include an antigen capture assay for detection of water soluble antigen – histidine rich protein 2 (HRP2) which is specific for *P.falciparum* , and an antigen capture test for the detection of parasite lactate dehydrogenase (pLDH) for detection of *P.falciparum* and human infective plasmodium species (Murray *et al.* 2003; Wongsrichanalai *et al.* 2007). Possible reasons for a false-negative RDT result include low parasite density, different malaria species for a specific test, or interpreting the RDT before the test line has fully developed (Marx *et al.* 2005). Limitations of pLDH and HRP2 include false negative reactions at low parasite densities as sensitivity decreases markedly at < 100parasites/ul blood, which may follow recent self-treatment. Possible reasons for a false-positive RDT result include persistent

antigenemia after antimalarial treatment, detection of gametocytes when asexual forms are not present, RDT detection of low-density microscopy-negative infections, or presence of antigenemia early in infection before parasites are detected by microscopy (Hopkins *et al.* 2007). Some recommend the use of both LM and PCR for the characterization of any diagnostic test for testing validity of RDTs, especially with species-specific diagnosis when evaluating malaria efficacy and treatment as recrudescent infection can be distinguished from re-infection (Rodulfo *et al.* 2007). Debate on the relative merits of microscopy and PCR methods for the detection and speciation of *Plasmodium* spp. infections is not straightforward since each method has particular advantages which prescribe a specific utility. LM is dependent upon the rapid cost effective diagnosis in a field setting, while the highly specific and sensitive PCR is standard for use in malaria research and reference laboratories (Boonma *et al.* 2007).

2.5 Malaria co-morbidity

Malaria and other infections frequently coincide, and their interaction is of considerable public health importance. These include interaction with HIV and other parasitic infections. Animal models suggest that helminth infections may alter susceptibility to malaria, although the results are conflicting (Bejon *et al.* 2008). It is probable that the host response to pathogens might be determined by complex interactions among host and concomitant parasites.

Some animal studies demonstrate that concurrent nematode infection strongly modulates multiple aspects of immunity to blood-stage malaria and consequently impairs the development of protective antimalarial immunity (Su *et al.* 2005). No evidence of any relationship was observed in another study which reported the same degree of protective antimalarial immunity (Spiegel *et al.* 2003). In human studies conducted among malaria endemic populations in Africa, helminths have been reported to increase susceptibility to clinical malaria in an age-dependent manner (Lyke *et al.* 2005), reduce the risk or made no difference (Shapiro *et al.* 2005) while *S. mansoni* was shown to cancel malaria susceptibility (Yoshida *et al.* 2000). Lyke et al. (2005) noted that the protective effect disappeared after the first infection of the malaria season although the course of these infections over multiple transmission seasons is unclear. On the contrary, another study showed that the protective effect was related to the intensity of parasite load (Sokhna *et al.* 2004). These malaria interactions with other parasitic infections could be related to how environmental factors affect the disease epidemiology of the various parasites. Studies into geographical mapping of the parasite clusters within community environments in relation to immunity acquisition are pivotal.

2.6 Malaria prevention in adolescents

Personal protection through use of insecticide-treated bed nets (ITNs), repellents and environmental control of mosquitoes are relevant for adolescents just like in the general population. Chemoprophylaxis or intermittent preventive therapy is not usually recommended in adolescents since they are not a priority high risk group for malaria prevention in endemic countries.

2.6.1 Insecticide-treated bed nets

Insecticide-treated bed nets (ITNs), if used properly, are one of the best ways to prevent mosquitoes from biting and infecting people with malaria (Hill, Lines, & Rowland 2006). A systematic review of twenty-two randomized controlled trials on the impact of ITNs has shown that insecticide-treated bed nets are highly effective in reducing malaria morbidity and mortality in areas of unstable and stable malaria transmission (Lengeler 2000). Lengeler reported that in areas of unstable malaria transmission, ITNs reduced episodes of uncomplicated malaria among users by 62 % compared to non-users, and by 43 % when compared to users of untreated bed nets. In areas of stable malaria transmission, ITNs reduced episodes of uncomplicated malaria by 50 % compared to non-users, and by 39 % compared to users of untreated bed nets. In stable transmission areas, ITNs also had a beneficial impact on severe malaria (protective efficacy 45 %, 95 % confidence interval 20 % to 63 %), parasite prevalence (protective efficacy 13 %), and high parasitaemia (protective efficacy 29 %).

Five of the studies in the Lengeler review reported child mortality. In children, ITNs provided 17 % protective efficacy (PE) compared to no nets (relative risk 0.83, 0.76 to 0.90), and 23 % PE compared to untreated nets (relative risk 0.77,

0.63 to 0.95). Child growth, for both height and weight also improved with ITN use (Lengeler 2000; ter Kuile *et al.* 2003).

ITNs can also substantially reduce the number of malaria transmitting mosquitoes. For example, in a randomized trial in Kenya, ITNs reduced the number of malaria transmitting mosquitoes by approximately 71.5 %. Some of this effect was compromised if one or more household residents did not sleep under a net (23.8 %, p=0.498) and if no resident slept under a net (5.2 %, p = 0.907), or if bed nets had not been re-treated within six months (16.9 %, p = 0.694), (Gimnig *et al.* 2003b).

2.6.2 Impact of ITNs in adolescents

There are few studies on the impact of ITNs in adolescents. In a large trial in Kenya the impact of ITNs in adolescents was assessed in schoolchildren of 4 to 13 years old. ITNs had no association with linear growth or indicators of protein-energy malnutrition (Friedman *et al.* 2003), but they were associated with improved body composition showing an increase in percent lean body mass of 1.2 % (p = 0.04). In the same trial, amongst non-pregnant adolescent girls 12 to 18 years of age, ITN use approximately halved prevalence of mild anaemia (<12 g/dL) in younger girls aged less than 12 years (OR = 0.38, 0.21 to 0.69), but had no association with anaemia in the older girls (OR = 0.79, 0.43 to 1.45) (Leenstra *et al.* 2003). Similarly, mean haemoglobin improved in the younger girls by 0.34 g/ dL (0.02 to 0.66), but not in the older girls (0.14 g/ dL, -0.24 to 0.53). There was no impact on other malaria-related morbidity.

2.6.3 Impact of ITNs in pregnant women

In areas of low and unstable malaria, women do not acquire substantial antimalarial immunity and are susceptible to acute and sometimes severe malaria. In

areas with stable malaria transmission, infection with *P. falciparum* malaria during pregnancy is characterized by low-grade, and often persistent or recurrent parasitaemias (Brabin 1983). These women may experience placental parasitaemia in the absence of microscopically detectable peripheral parasitaemia and may be asymptomatic (Brabin *et al.* 2004a). Prevention of malaria through ITN use in pregnant women living in these endemic conditions is beneficial.

A systematic review of randomised controlled trials of ITNs for the prevention of malaria in pregnancy showed that use of ITNs significantly improved pregnancy outcome at the individual as well as the community level in sub-Saharan Africa (Gamble *et al.* 2006). ITNs reduced placental malaria in all parities (RR 0.79, 0.63 to 0.98), low birthweight (RR 0.77, 0.61 to 0.98), fetal loss (RR 0.67, 0.47 to 0.97), anaemia and clinical malaria although the results for the latter were not significant. In areas of low transmission, only one study which met review criteria showed a significant reduction for all pregnancies in anaemia prevalence (RR 0.63, 0.42 to 0.93) and fetal loss (RR 0.21, 0.05 to 0.92) with ITN use. There was no effect on clinical malaria or low birth weight prevalence.

2.6.4 Uptake of ITNs

ITN coverage is influenced by the processes and the economics which effect ITN distribution. Underlying social and political factors as well as the access to follow up re-treatment will also influence coverage. For example, in Tanzania, payment of fees for re-treatment, inadequate logistical arrangements, access (e.g. limited number of days for community re-treatment) were all identified as important factors affecting re-treatment rates (Winch *et al.* 1997).

ITN adherence is influenced by a range of environmental factors (e.g. temperature, mosquito presence), household social factors (such as sleeping arrangements) and technical factors of the dwelling unit associated with how the ITN is hang (Alaii *et al.* 2003). The changing patterns of seasonal ITN use and difficulties in encouraging community-sustainable approaches to ITN use have been emphasised (Winch *et al.* 1997). In a large scale study in Kenya where participant adherence was directly observed, low temperatures (RR = 1.31, p = 0.039) and child age less than five years (RR = 0.86, p = 0.001) were significantly associated with lower ITN adherence (Allai *et al.* 2003). Mosquito numbers, relative wealth, number of household occupants and educational level of the household head had no association with adherence in the same study.

2.7 Adolescent growth and nutrition in developing countries

Nutrition influences growth and development throughout infancy, childhood and adolescence, but it is in adolescence that nutrient needs are the greatest (World Health Organization 2005). Individuals can gain up to 15 % of their ultimate height and 50 % of their adult weight during adolescence, and this rapid growth is accompanied by an increase in demand for nutrients (World Health Organization 2005). Changes occurring in body composition during the puberty-related growth spurt vary in timing and with maturation rate, making assessment of nutritional status complex. Adolescent boys build more muscle mass, gain weight at a faster rate, have a larger skeleton and deposit less fat than girls; and boys tend to grow for a longer period of time (Heald & Gong 1999). For adolescent girls, the greatest gain in weight and height normally occurs in the year preceding menarche, although the growth spurt continues for two years after menarche (Heald & Gong 1999).

Food insecurity, inadequate maternal and child care, poor health services and environmental exposures lead to malnutrition through the combined influences of poor diet and disease (ACC/ SCN 1997). Infectious diseases influence nutritional status because they decrease food intake, impair nutrient absorption, increase nutrient losses and metabolic requirements through catabolism and impair transport of nutrients to target tissues (Stephensen 1999). In addition, the acute phase response to infection and the production of pro-inflammatory cytokines may directly affect the process of bone remodeling required for long bone growth (World Health Organization 2005). Such infections may be acute, chronic or sub-clinical and thus asymptomatic. Malnutrition in adolescents can result in the well described anthropometric deficits of stunting and wasting.

2.7.1 Anthropometric nutritional assessment

Nutritional status is commonly described using anthropometry, which assesses body composition, size and proportion. These measurements are compared to a reference standard for a healthy population of the same ages. In adolescents, this reference should preferably be derived from local data if available, be sex and age-specific and indicate the associated changes of puberty (ACC/ SCN 1997). Local reference data is not usually available in developing countries, which limits interpretation of anthropometric data in relation to health determinants and outcomes among adolescents. Commonly used anthropometric indices for assessing nutrition in adolescence include height-for-age, weight-for-age, body mass index (BMI) and mid-upper arm circumference (Marshall 1978). A consensus into developing age-specific international growth standards for school-aged children and adolescents for clinical and public health applications was recently reached following an evaluation into current standards (Butte *et al.* 2007).

The World Health Organisation recommends the reference values of the National Center for Health Statistics (NCHS) from the United States of America (USA) (NCHS 1987). Height, weight and body mass index are plotted on NCHS growth charts using these standards and anthropometric values below the 5th percentile are considered to indicate undernutrition (Woodruff & Duffield 2000). Use of reference data from the USA has limitations. Cordeiro et al. (2006) considered that due to increasing obesity in the USA, particularly among adolescents, the age distributions of weight and BMI in the NCHS growth charts are less reliable as healthy reference values. The average age at menarche is also different ranging from 10 to 12 years in the United States compared to between 13 to 16 years of age in many developing countries (Cordeiro *et al.* 2006; NCHS 1974). NCHS growth charts used as reference

values for developing countries may therefore overestimate undernutrition. Physiological age as opposed to chronological age has been recommended in comparing adolescents from developing countries with the reference population. Standards for assessing physiological age using breast and genitalia developmental stages have been developed, and these are widely used in clinical settings (Tanner 1962). However, assessing Tanner breast and genitalia stages in the field outside the clinical setting is logistically problematic. In such cases, a validated self-assessment for Tanner staging can be used, as well as age at menarche for girls as a proxy for physiological age (Brooks-Gunn *et al.* 1982; Chan *et al.* 2008; Taylor *et al.* 2001).

Height-for-age reflects linear growth. Linear growth retardation, known as stunting, results from a slowing of skeletal growth and is an important marker reflecting cumulative inadequacies in nutrition and health (Norgan 2000). Stunting is defined as height below minus two standard deviations of that expected with reference to an international growth standard (ACC/ SCN 1997). In populations with a high rate of childhood malnutrition, stunting is commonly observed among adolescents (Kurz & Johnson-Welch 1994). Stunting impairs body size and causes growth faltering which may lead to reduced physical work capacity, poor intellectual or behavioral development (ACC/ SCN 1997). In girls, this causes cephalopelvic disproportion which can lead to obstructed labour in pregnancy (Rush 2000).

Weight-for-age is commonly used as an indicator for undernutrition in children because it is easier to measure than height. It reflects linear growth and weight accumulation achieved pre- and post-natally over the long-term, as well as weight accumulation in the short-term (ACC/ SCN 1997). Underweight is usually defined as weight below minus two standard deviations of that expected on the basis of the

international growth reference. Children who are stunted in early life may attain normal weight for height later on but can remain short. Weight alone is therefore not a good measure for assessing malnutrition in adolescence (ACC/ SCN 1997).

The body mass index (BMI) is used to assess thinness or obesity. BMI-for-age can be used to assess undernutrition by comparison with age and sex-matched values in the NCHS data and is useful for measuring acute undernutrition in a population (de Onis & Habicht 1996). For assessment of chronic undernutrition, height-for-age comparisons with reference data are recommended. Mid-upper arm circumference (MUAC) is another useful measure for undernutrition. The rapid addition of soft tissue during puberty results in a rapid increase in arm circumference, hence MUAC changes with age and pubertal development during adolescence. For this reason, age-specific reference comparisons are required.

2.7.2 Adolescent under-nutrition in sub-Saharan Africa

Anthropometric measurements do not distinguish specific causes of undernutrition and therefore are a general measure of nutritional outcome. There is little information on adolescent undernutrition available from African studies. Table 2.7 summarizes estimates of malnutrition in adolescents from nine studies.

Table 2.7: Magnitude of malnutrition in adolescents from sub-Saharan Africa

Location	Sample size	Age (years)	Mean HAZ ± SD (95% CI); {Stunting %}	Mean WAZ ± SD (95% CI); {Underweight %}	Mean BMIZ ± SD (95% CI) {Wasted %}	Mean MUACZ ±SD (95% CI) { Wasted %}	Other measures (%)	Reference
Studies in adolescent males (M) and females (F)								
Ghana	475 F 469 M	12 - 13 12 - 13	- 2.3 ± 1.0; {57.5} - 2.2 ± 0.9; {55.7}	- 1.7 ± 0.8; {39.8} - 2.0 ± 0.7; {53.3}	-	20.4 ± 2.0 19.1 ± 1.4	36.8 -anaemia 38.8 -anaemia	(PCD) 1998
Western Kenya §	272 (M & F); Schoolchildren	10 - 13	-1.6 (-1.7,-1.4); {30.0}	-1.6 (-1.7,-1.4); {26.8}	-1.5 (-1.8,-1.3)	-1.2 (-1.3,-1.0); {3.9}	-	Friedman *et al.* 2005
Tanzania	465 F 430 M	12 - 13 12 - 13	- 3.1 ± 1.0; {84.1} - 2.7 ± 0.8; {80.9}	- 2.2 ± 0.6; {64.9} - 2.3 ± 0.6; {70.7}	- -	18.7 ± 1.7 17.7 ± 1.5	75.1 -anaemia 75.4 -anaemia	PCD 1998
Benin ¶	F (NA) M (NA)	10 -13 10 -13	{27} {55}	- -	- -	{14} {32}	-	Kurz & Galloway 2000
Cameron¶	F (NA) M (NA)	10 -13 10 -13	{8} {19}	- -	- -	{2} {8}	32.0 – IDA (both sexes)	Kurz & Galloway 2000
Studies in adolescent females only								
Western Kenya	938 Schoolchildren	12 - 18	-0.77 (-0.86, 0.68) {12.1}	-	{15.6}	-	-	Leenstra *et al.* 2005
Malawi β	Rural (118)	10 - 19	{40.0}	-	40	-	27 -low retinol	Fazzio-Tirrozzo *et al.* 1998
Malawi	Pregnant (190)	12 - 19	-	-	20.4±1.8	24.1±2.0; {17.9}¥	-	Kalanda *et al.* 2006
Nigeria β	386 rural ‡ 845 urban ‡	14 - 19 14 - 19	{10.0} {5.0}	- -	- -	- -	-	Brabin *et al.* 1997

Adapted from WHO 2005

HAZ - Height-for-age Z-score, WAZ - Weight-for-age Z-score, BMIZ - BMI Z-score, MUACZ - Mid-upper arm circumference for age Z-score, CI – confidence interval, IDA – iron deficiency anaemia, PCD - Partnership for Child Development; NA –sample size not available, M- males, F - females

§: Stunting, wasting and underweight were defined as HAZ, BMIZ, and WAZ<-2 SD from the reference median, respectively.

¶: Stunting was HAZ < 5th percentile, wasting was BMI < 5th percentile.

¥: Under-nutrition was defined (at first attendance) as either maternal height < 155 cm, weight less < 50 kg, MUAC < 23 cm, or BMI < 18.5 kg/m^2.

β: stunting and wasting defined as < 2nd percentile.

‡ Postmenarche adolescents only

The NCHS was used as the reference data set in all these studies with the definition of stunting or undernutrition based on a cut-off at the 5th percentile or a z-score less than two standard deviations. These results vary between the sexes, among different age groups and can not be generalized across different locations. However they highlight the scarcity of data on adolescent nutritional status and demonstrate that many adolescents are deprived of adequate nutrition. Several of the studies also reported estimates of anaemia, iron and vitamin A deficiency.

Five studies reported estimates for both males and females, but only for younger adolescents (≤ 13 years) (Friedman *et al.* 2005; Kurz & Galloway 2000; The Partnership for Child Development 1998). Maturation levels of females were not reported. Stunting was estimated for three studies from Ghana, Kenya and Tanzania and prevalence was high ranging from 30.0 % to 84.1 % and comparable between males and females. Undernutrition prevalence was also high ranging from 26.8 % to 70.7 % for the same locations. Two studies, from Benin and Cameron reported lower estimates, but values were higher in males.

One study in non-pregnant female adolescents showed that stunting and thinness were common and decreased markedly with age and sexual maturation (Leenstra *et al.* 2005). These were school-going adolescents from 12 to 18 years from western Kenya and a third of the females were post-menarcheal. Median age at the start of puberty was 12.1 (11.7, 12.3) years, measured using Tanner breast development > B2. Median age at menarche was 15.0 (14.9, 15.1) years. Both measures were delayed by approximately 2 years compared to the NCHS/ WHO reference population. Stunting was prevalent in young girls (12-13 years) (20.0%) but relatively uncommon in older adolescents 14 -18 years (11.6 %). Similarly, thinness

was less frequent in young girls 12-13 years (20.1%) and uncommon in older adolescents (14 -18 years) (4.3 %). Girls with late menarche were more likely to be stunted (adjusted HAZ mean difference -0.47 [95 % CI -0.86 to -0.08]) and thin (adjusted BMIZ mean difference -0.85 [95 % CI -1.12 to -0.58]) compared to those with early menarche, even after adjusting for age.

Three further studies were in pregnant adolescents: two from Malawi which showed up to 40 % prevalence of stunting (Fazio-Tirrozzo *et al.* 1998; Kalanda *et al.* 2006) and one study from Nigeria (Brabin *et al.* 1997). In the latter study, stunting in menarcheal adolescent females was relatively low at 5 % among rural and 10 % among urban participants. Undernutrition in childhood and adolescence will lead to poor physical and mental development, poor school performance, reduced adult size and capacity for physical work. Undernutrition influences the host's immune response against infection and potentiates the effects of infection through decreased food intake and impaired nutrient absorption, transport and utilization in target tissues. This creates a maladaptive cycle between immunity and nutritional morbidity (Stephensen 1999). Improving nutritional status in girls and young women, including when they they become pregnant would be expected to prevent low birth weight in malarious areas (Allen *et al.* 1998).

2.7.3 Anaemia in adolescence

Anaemia prevalence is highest in the developing world where approximately 42 % of children less than five years of age and 53 % of children 5 to 14 years of age are anaemic (ACC/ SCN 2000). The aetiology of anaemia is usually multi-factorial. These factors include micronutrient deficiency, haemoglobinopathies, and infections with malaria, schistosomiasis, worm infestations (e.g. hookworm, trichuris, and ascaris), tuberculosis and other chronic diseases.

Most studies of anaemia in sub-Saharan Africa have focused on children less than 12 years of age (DeMaeyer & els-Tegman 1985). In areas with stable malaria transmission, malaria is the likely predominant cause of anaemia in young children under five years of age, and the prevalence of anaemia in this age group has been shown to be a sensitive indicator to changes in malaria exposure (Desai *et al.* 2005). By modeling the relationship between malaria prevalence and anaemia, it has been estimated that only 3.3 % of anaemia in women of child-bearing age is attributable to malaria (Caneiro *et al.* 2006). Whether malaria is a major contributor to anaemia in adolescents as it is in young children is unknown as there is scarcity of data regarding the adverse consequences of malaria in adolescence.

Among adolescents, anaemia aetiology is probably more complex and multi-factorial than in the younger children. While nutritional deficiencies, most importantly iron deficiency, are regarded as the most important cause of anaemia in adolescents, multiple other causes co-exist with this deficiency. These include helminth infections, most importantly hookworm and schistosomiasis (ACC/ SCN 2000). Many girls are often already anaemic by the time they become pregnant and the pregnancy period is too short to reduce pre-existing anaemia, particularly when many women do

not seek pre-natal care until their second or third trimester (DeMaeyer & els-Tegman 1985; Kurz 1996; Nelson 1996).

Maternal anaemia is associated with reduced physical capacity (Haas & Brownlie 2001; Kurz & Galloway 2000), even in mild forms (Nelson *et al.* 1994), reduced cognitive function and school performance (Nelson 1996; Stoltzfus 2001), poor pregnancy outcomes such as preterm birth, low birth weight (Allen 2000) and reduced growth in early infancy (Perez *et al.* 2005).

2.7.4 Iron and other nutritional deficiency anaemias

The prevalence of anaemia remains high in less developed countries (Tolentino & Friedman 2007). Tolentino and Friedman reviewed the current knowledge on anaemia and underscore the importance of micronutrient deficiencies of iron and vitamins. The co-existence of high levels of parasitic diseases are also contributory factors for the slow progress in reducing anaemia prevalence in these countries.

Iron deficiency is estimated to affect the health of more than one billion people world-wide (ACC/ SCN 2000). Some mild to moderate forms of iron deficiency may exist in which the individual is not yet anaemic but tissue functionality may be affected. Young children are most affected due to increased requirements during periods of rapid growth and imbalanced diets that are poor in bio-available iron during the critical post-weaning period, especially in areas with inadequate or no iron fortification in food preparations (Stoltzfus 2001). During adolescence, iron requirements increase dramatically in both males and females as a result of the expansion of the total blood volume and the increase in lean body mass. The onset of menses in young females further increases these requirements. The overall iron

requirements increase from a pre-adolescent level of approximately 0.7- 0.9 mg Fe per day to as much as 2.2 mg Fe/d or perhaps even more in heavily menstruating young women of which less than 10 % is absorbed (Beard 2000). These increased requirements are associated with the timing of the growth spurt as well as sexual maturation and the onset of menses. Adolescent girls are unlikely to acquire substantial iron stores during this time period because intakes may average as little as 10-11 mg Fe/d (Beard 2000). In developing countries, iron requirements are even higher in the presence of infectious diseases (Brabin & Brabin 1992a). The low iron stores in these young women of reproductive age will make them susceptible to iron deficiency anaemia during pregnancy because dietary intakes alone are insufficient, in most cases, to meet the requirements of pregnancy (Beard 2000).

Deficiencies of various vitamins are important contributors to anaemia. Vitamin A deficiency has long been recognized to cause anaemia, especially in close relation to iron deficiency (Semba & Bloem 2002). Vitamin A appears to influence anaemia via modulation of haematopoiesis, by enhancement of immunity to infectious diseases and through the modulation of iron metabolism (Bloem 1995). In adolescent girls, sex hormone patterns correlate significantly with serum retinol which could be important for reproductive function, and severe deficiency has been associated with sterility and amenorrhea (Brabin & Brabin 1992b). Studies suggest that vitamin A could improve haematologic indicators and enhance efficacy of iron supplementation in adolescents (Brabin *et al.* 2004a).

Folic acid, which is of special importance in pregnancy, is necessary for cell growth and repair; and essential for the formation and maturation of red blood cells. Demands for folate increase because of the rapid growth of the fetus and utero-

placental organs (Tamura & Picciano 2006). In the presence of malaria infection, assessment of folate status may pose challenges as *Plasmodium spp* synthesize folate and cause red cell haemolysis to which the body responds by stimulating red cell precursors. This leads to folate depletion and megaloblastic anaemia (Fleming 1989). On the other hand, reticulocytes also have high folate content and a high number in the blood may lead to over-estimation of folate status.

Vitamin B12 is also necessary for red cell synthesis and its deficiency is associated with megaloblastic anaemia. The contribution of B12 deficiency to the world-wide prevalence of anaemia has not been well established. Tolentino and Friedman (2007) reported the prevalence of Vitamin B12 deficiency as ranging between 18 % to 41 % in pre-school children in Kenya and Mexico, respectively; 16 % among pregnant women in Malawi and 40 % among individuals in all age groups in Latin America.

Anaemia prevalence should be estimated routinely in adolescents, and there is a scarcity of information on this morbidity in this age group. Nutritional anaemia assessments are more complicated, but it is only through improved understanding of this pathology that appropriate interventions can be considered, and in particular the role of multi-micronutrient supplementation prior to or during pregnancy.

2.8 Adolescent friendly health services

Adolescent friendly health services (AFHS) represent an approach which brings together high standards and qualities that young people seek in health care. Services need to be accessible, equitable, acceptable, appropriate, comprehensive, effective and efficient (McIntyre 2002). They can be stand alone, linked to health facilities, school health programmes or community outreach services. Barriers in adolescents' access to AFHS are influenced by socioeconomic as well as political factors; hence responding to health and development needs of adolescents requires broader participation. Creating the right political and community support and the engagement of the adolescents themselves is crucial (World Health Organization & UNICEF 2002).

2.8.1 Delivery of AFHS and associated challenges

Implementation of AFHS in developing countries has mostly been championed in relation to sexual and reproductive health issues; hence this discussion often uses examples from this field. This highlights the lack of comprehensive knowledge of adolescent health issues across different disciplines and gives an example of differential vulnerability which may be influenced by age, maturation and related demands for specialized services. Younger adolescents or those with delayed sexual debut may be under-represented in this case.

Providing quality AFHS is challenging. To ensure that the services reach people who are vulnerable, that services are equitable, inclusive and do not discriminate, AFHS could be delivered as an essential comprehensive package of services. Multi-component interventions are necessary and these require multi-sectoral involvement from health, education, social and community sectors. An adolescent

sexual health programme in rural Tanzania assessed the feasibility and sustainability of such an approach (Obasi *et al.* 2006). The intervention comprised community mobilization, participatory reproductive health education in primary schools (teacher-led and peer-assisted), youth-friendly reproductive health services through training and supervision of health workers and community-based condom provision for youth. Within three years, substantial improvements in knowledge, reported attitudes and some reported sexual behaviours, were noted; but it had no consistent impact on biological outcomes (HIV and HSV2 prevalence) within a three-year trial period (Ross *et al.* 2007). The school-based interventions posed some challenges related to low enrolment and attendance rates, limited teacher training and little access to teaching resources (Plummer *et al.* 2007). The need for complementary strategies to access out-of-school youth and the wider community was identified (Obasi *et al.* 2006).

Delivery of AFHS should be by competent, motivated providers who are good at communicating so that the adolescents and their communities are involved and find the services acceptable. For example, in Uganda, both male and female adolescents articulated the need for a "face of welcome" and the possibility of having some of their peers trained as community based distributors for their family planning needs (Flaherty *et al.* 2005). Adolescents can be empowered to be health change agents for their own health and in the community, for example, through school-based health programmes (Magnussen *et al.* 2001; Onyango-Ouma *et al.* 2005). Innovative strategies for maintaining a system of quality improvement in clinic procedures which includes adequate staff support and motivation need to be identified and properly implemented accordingly. For example, in South Africa, an accreditation system for clinics providing AFHS showed improvements in how providers determined

adolescents health needs in the community, their knowledge of adolescent rights, availability of adolescent-specific information and better attitudes of staff towards adolescent sexual and reproductive health needs (Dickson *et al.* 2007).

Adolescents complain that they have limited access to information which impacts on lack of knowledge of their health needs and could lead to a delay in recognizing the need to seek care (World Health Organization 2007). This could be related to lack of education, limited exposure to media messages and inability to comprehend printed communication materials. Associated costs of seeking and obtaining care are also important obstacles to the use of health services. These costs include user fees, travel and opportunity costs of the adolescent and carer. This may delay the decision to seek care or limit access to care. Economic-related decisions may be worsened when the condition is perceived as non-threatening or self-limiting (World Health Organization 2007). Institutional strategies e.g. lack of targeted subsidies for adolescents for ITNs may propagate such perceptions within communities.

2.9 Health seeking behaviour for malaria in adolescents

Health seeking behavior is the seeking of help for specific health needs, including health services (in the formal health care system or from traditional healers and pharmacists) as well as seeking health-related information (World Health Organisation 1998). Barker et al. (2007) proposed the use of "help-seeking" when addressing the needs of adolescents. This is defined as any action or activity carried out by an adolescent who perceives themselves as needing personal, psychological, affective assistance, health or social services, with the purpose of meeting this need in a positive way. They propose three categories of adolescent help-seeking behaviour.

The first category is health-seeking behaviour as already known. The second category is help-seeking for normative developmental needs, including help in completing school, or help related to vocational training, or employment-seeking; relationship formation and concerns; understanding the changes associated with sexuality or puberty; or other concerns that are frequently associated with adolescence. The third category is help-seeking behaviour related to personal stress or problems, as in the case of family crises; family violence or victimization by abuse; relationship stresses; acute financial needs; homelessness; and needs or problems related to chronic or acute ill-health. A decision-making model for understanding adolescent help-seeking within a given social context has been proposed, encompassing both the individual and exogenous factors related to the nature and use of available social supports (Barker 2007). This discussion focuses on health seeking behaviour for malaria illnesses.

Health seeking behaviour studies have mostly focused on treatment seeking and much less on information seeking. In the case of adolescents, information seeking is of high importance also. Adolescent sources of malaria-related information include peers, school, parents, clinics and public media. However, barriers to access the right information exist.

2.9.1 Determinants of malaria treatment practices

Prompt treatment with an effective antimalarial drug is important for preventing malaria complications and deaths (WHO 2007). Treatment practices within the family and community are inter-related with malaria epidemiology patterns, associated malaria treatment policy as well as illness belief systems (McCombie 2002). Reviews into literature on treatment seeking and self-treatment for malaria reveal the complexity of the issues (McCombie 1996; McCombie 2002; Williams & Jones 2004).

Factors that influence treatment choices include cultural beliefs about cause and cure of illness which in turn relate to the illness definitions and perceptions, type of drugs in use, issues of access, cost and provider attitudes.

McCombie (1996) reviewed care seeking patterns and their determinants among studies published between 1985 and 1993. Studies were mostly from Africa and Asia, with a small number from South America. Treatment seeking patterns were very variable. Use of the formal health sector ranged from 10 to 99 %, self-treatment from 4 to 87 % and exclusive reliance on traditional remedies was rare. The first response to most episodes began with self-treatment with modern and traditional medicines. Exclusive reliance on self-treatment was evident in almost half of the cases, usually with an antimalarial drug, and under-dosing was very common. McCombie (2002) added to the available knowledge up to 2002 with special focus on self-treatment.

Overall, the treatment choices may be determined by location, age, educational level, severity perception and prior experience to the disease. Treatment patterns between urban and rural locations and all these are inter-related to access. Urban populations tend to have better access to health facilities and pharmacies. Younger age, e.g. children less than five years old, are more likely to be taken to health facilities (Kaseje *et al.* 1987; Molyneux *et al.* 1999; Slutsker *et al.* 1994). Higher educational level of the mother has been associated with promptness to seek care from a health provider (Tarimo *et al.* 1998) and better knowledge of antimalarials (Tarimo *et al.* 2001). Higher educational level of the head of household has also been associated with higher likelihood of attending a clinic (Slutsker *et al.* 1994), and this could be related to socio-economic status.

In locations where formal community-based access is available, self-treatment may be less preferred. For example, participants in a community-based cross-sectional study from an epidemic prone area in Ethiopia preferred visiting community-based health workers (33 %), followed by use of public health facility (23 %) or private clinics (17 %). Home treatment was chosen as the last resort (3%) and this was in spite of high self-diagnosis (77 %) (Deressa 2007). In Vietnam, provision of a good comprehensive package for access to early microscopic diagnosis and free treatment based on community involvement reduced self-treatment. Self-treatment decreased from 74 % to 8 % in seven years although self-diagnosis increased from 68 % to 100 % (Giao *et al.* 2005). A prospective study from a rural malaria holo-endemic community in Nigeria showed that community health workers were preferred to self-treatment or other treatment choices (Onwujekwe *et al.* 2006). The use of community health workers (CHWs) increased from 0 % to 26.1 % ($p < 0.05$), while self-treatment in the homes decreased from 9.4 % to 0 % ($p < 0.05$) after the implementation of the CHW strategy. Use of patent medicine dealers also decreased from 44.8 % to 17.9 % ($p < 0.05$).

Experience of the disease could also play a role in treatment choices. For example, a prospective study in rural Gambia assessed the treatment choices and uptake for *P falciparum* malaria infection, with monthly examination of malaria blood slides (Von *et al.* 2002). Although access to treatment was good, only 20 % of infected individuals sought medical treatment within a period of 14 days before or after a malaria positive blood film. Within 2 months of a positive film, only 27 % requested treatment. Likelihood to seek treatment was associated with higher parasite count of >500 per microlitre (odds ratio 5.2, 95 % CI 3.6, 7.4) and higher malaria prevalence at village level. Only 42 % of the malaria positive slides had high parasitaemia counts.

Factors related to reporting of prior antimalarial use and its ascertainment are important. For example, participants may under-report prior use in order to access antimalarial drugs provided by the study, hence the use of tests to detect antimalarial levels in blood or urine is useful (Nsimba *et al.* 2002; Nsimba & Rimoy 2005; Nwanyanwu *et al.* 1996).

Study design may also influence estimates of treatment rates. McCombie (2002) argued that mild illnesses were more likely to be forgotten especially if they were not treated. As a result, prospective studies and those that use shorter recall periods may underestimate estimates. Variation in the choice of terms used to designate fever or malaria could result in different episodes of illness being recalled. Clinical presentation and associated differential diagnoses for malaria also need consideration in study design (Tanner & Vlassoff 1998). For example in endemic areas, clinical malaria commonly presents as a febrile illness with parasitaemia. The infection can however be asymptomatic with non-specific signs hence resulting in mis-diagnosis and poor treatment.

2.9.2 Self-treatment for malaria infection

Self-treatment is any treatment that does not involve consulting a health care provider or traditional healers. Thus an individual or carer who visits a shop or pharmacy to purchase medication is included in this definition. However, the definition has limitations because in some cases visits to shops or pharmacies may involve advice seeking and consultation (McCombie 2002). Self-treatment entails self-diagnosis in most cases; hence definitions of malaria may be variable according to the cultural understanding of malaria. This leads to diversity in the self-treatment practices for malaria (McCombie 2002). Some studies combined home treatment with traditional

remedies; others noted proportions that accessed treatment outside formal health providers but did not discuss types and sources of treatment. When described, self-treatment may involve a number of behaviours with very different potential impacts on an episode of disease. These could include use of cool baths, use of herbal remedies and purchase of drugs from a shop.

Adolescents experience illness and actively participate in their own health care as part of the essential adaptive skills of life. Little is known on how adolescents respond to perceived malaria illness. Studies in school-going adolescents have shown that knowledge and use of both herbal and pharmaceutical medicines is substantial (Geissler *et al.* 2001). In schoolchildren age 11 to 17 years in rural western Kenya, participants experienced on average 25 illness episodes each during 30 weeks of follow-up (Geissler *et al.* 2000). Illnesses included colds, headache, abdominal complaints and injuries; fever was a common symptom. One fifth (21%) of the illness episodes were associated with absence from school. In 72 % of the episodes, an adult caretaker was not consulted for treatment. Of these, 81 % remained untreated, while 19 % were treated by the children themselves with either herbal or western medicines. Of all episodes involving treatment with pharmaceutical drugs, two thirds were provided or facilitated by adults and one third taken by the children themselves without adult involvement. Among older adolescents more than 14 years old, boys had better access (34 %) to self-treatment with drugs than girls (9%). Similar trends were observed among younger adolescents with a narrower gap. These differences in self-treatment practices and choice of medicines between girls and boys may have reflected the higher income potential of boys, who could earn money by fishing. The most commonly used pharmaceuticals were antimalarials (mainly chloroquine), pain-killers

and anti-pyretics (mainly aspirin and paracetamol), which were stocked in most small shops in the village at low prices and readily sold to children.

In pregnant adolescents, health seeking behavior is influenced by a large number of factors operating at the individual, family, community and societal levels (World Health Organization 2007). The decision making process surrounding adolescent's ability to seek care is complex, showing variations among countries and cultures, and could be severely limiting in some cases. The pregnant adolescent may have less autonomy, being dependent on her partner, mother-in-law or parents for approval and access to services (World Health Organization 2007). Addressing the adolescent's educational, social, economic, nutritional, psychological, as well as medical needs is more likely to result in better pregnancy outcomes for the mother and child, and also broadens the adolescent's life options (World Health Organization 2007).

Self-treatment also raises issues of drug regulation and rational drug use, under-dosing or over-dosing, drug pilferage from public health institutions into the private sector. Self-treatment based on insufficient knowledge and limited funds could lead to misuse of pharmaceuticals drugs. With increasing resistance to chloroquine and SP, most African countries are moving to antimalarial combination therapy. Self-treatment may be used by women following self-diagnosis of malaria (Espino & Manderson 2000; Nyamongo 2002) or due to lack of access to health facilities (Hotchkiss & Gordillo 1999; Mugisha *et al.* 2002) and this could lead to inadvertent exposure to antimalarial drugs. In a Ugandan study on perceptions on malaria in pregnancy, most women could not differentiate symptoms of malaria from those of early pregnancy (Mbonye *et al.* 2006). It was considered that fever or febrile illness

was a normal sign of pregnancy and this was related to the use of herbs and clay as a first resort to treat pregnancy ailments. Safety information of antimalarial drugs during pregnancy is limited (Nosten *et al.* 2006) and uncertainty still remains over the neurotoxicity of artemisinins, currently recommended as part of antimalarial combination therapy (ACTs) (Toovey 2006). The risks of inadvertent and unnecessary exposure to ACTs in early human pregnancy are unknown. Exploration of more gender-sensitive approaches that take into account endemicity levels and cultural settings would be beneficial for better malaria control (Tanner & Vlassoff 1998).

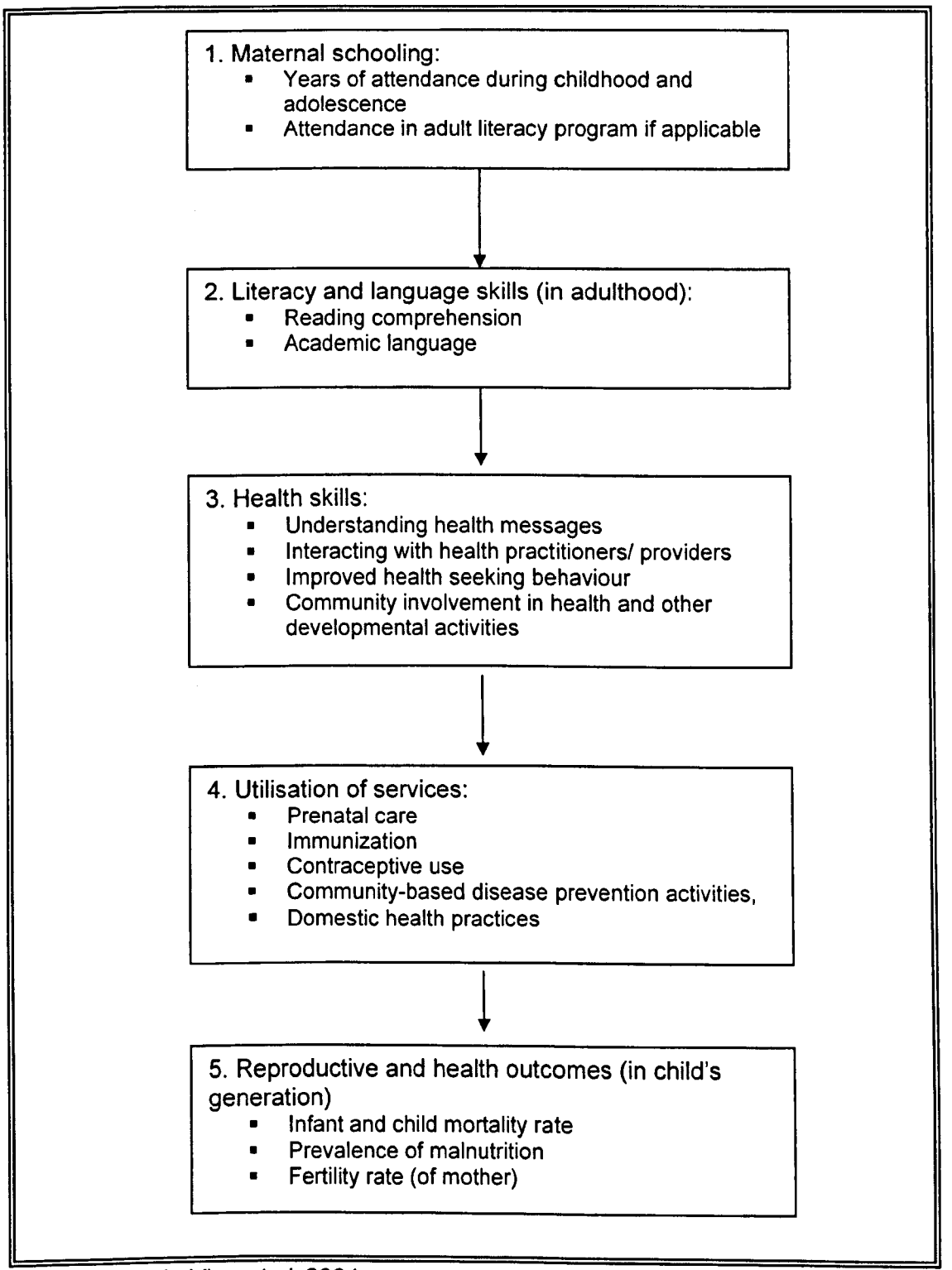
2.10 Adolescent health literacy

Literacy is fundamental to any nation's development. A literate person is defined as one who engages in all activities in which literacy is required for effective functioning of his or her reference group and community and also for enabling him or her to use reading, writing and calculation for their own and the community's development (UNESCO 1995). Health literacy is defined as representing the cognitive and social skills which determine the motivation and ability of individuals to gain access to, understand and use information in ways which promote and maintain good health (World Health Organisation 1998). The health literacy concept provides an opportunity to shift our thinking from a simple transfer of knowledge to that which enables people to take an active role in bringing about change in environments that influence their health (Nutbeam & Kickbusch 2000). Health literacy is especially seen as a more active process empowering women (Renkert & Nutbeam 2001). This conceptual understanding of health literacy, which is a continuum of health literacy that includes basic or functional health literacy to interactive and critical health literacy, is not easy to measure objectively (Nutbeam 2000). In developed countries, various objective tests for health literacy exist while in developing countries, the basic

definition of literacy as ability to read and write is mostly applied. Nutbeam & Kickbusch (2000) highlighted the global recognition of health literacy towards the end of the twentieth century, yet still lacking a measurable programme definition. They emphasised the fundamental and long-established relationship between access to education, population literacy levels and health status.

Fig 2.4 illustrates a hypothetical model of the maternal health-literacy pathway (LeVine *et al.* 2004).

Figure 2.4: Hypothetical influences of maternal literacy on health and child development



Adapted from LeVine *et al.* 2004

Maternal literacy and language skills gained from schooling influence health literacy which in turn impact on the health knowledge and behaviour for health skills and utilisation of health services. In a systematic review of published observational studies covering a 23 year period (1980-2003) in the United States of America (USA), the aetiological association of health literacy was assessed against a wide range of health outcomes (Dewalt *et al.* 2004). The studies were mostly evaluated based on tests using word recognition and pronunciation or prose writing to define literacy levels as low or high. Although the findings may be limited due to the larger number of cross-sectional study designs compared to cohort studies and other reporting issues; a high correlation between the various literacy testing instruments was noted and it was concluded that they do essentially measure the same underlying construct. From the analysis, the authors found compelling evidence that people with low literacy were generally 1.5 to 3 to times more likely to have an adverse health outcome than those with higher literacy.

Access to education, especially for girls, is probably one of the fundamental actions which can improve public health in the world's poorest countries (Nutbeam & Kickbusch 2000b). Adolescent girls' education and literacy coverage among malaria endemic countries are variable, and so is their malaria knowledge. In some adolescent studies, education has been shown to have an association with malaria knowledge (Rodriguez *et al.* 2003) and has been identified as a significant predictor of knowledge on HIV/ AIDS (Khan 2002). Access to relevant forms of information for adolescent girls before pregnancy may be centrally important for preventing pregnancy morbidity and even mortality. It is therefore crucial to understand how such information is acquired and how best to empower adolescent girls for their own and future families' health.

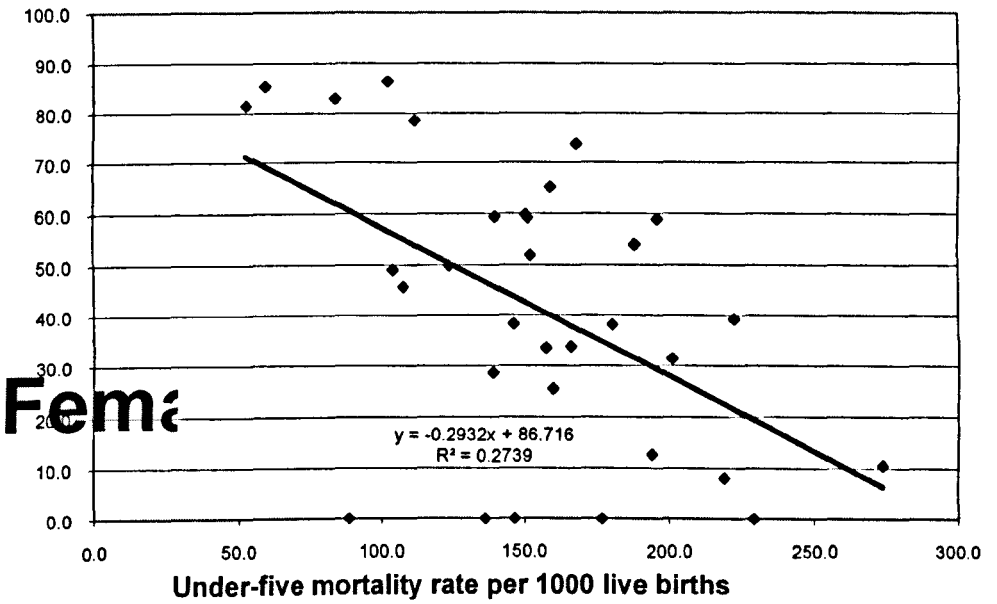
Using data from World Fertility Surveys (WFS) and re-analysed by Cochrane, Cleland *et al.* (1988) the authors showed that the education-mortality association is appreciably stronger in childhood than in infancy. Maternal schooling of 4 to 6 years duration was associated with a fall in infant mortality of about 20 %. The author showed that the inverse education-mortality relationship was found in all major regions of the developing world. The association was very pronounced, but appreciably closer in childhood than in infancy, and even a modest exposure of the mother to formal schooling was associated with reduced risks of death in most contexts (Cleland & Van Ginneken 1988). The authors explored associations between education and each of the following: reproductive health, socioeconomic status and use of health facilities.

Between education and reproductive health, relatively high risks of infant death were associated with younger mothers (less than 20 years old), older mothers (35 and over) and short birth intervals. There was also the tendency for educated women to reproduce at low risk ages because of postponement of marriage and earlier cessation of childbearing. In relation to socioeconomic status, a few years of primary schooling was associated with a 20 % drop in the probability of dying between age 1 and 5 years, a few more years of primary schooling brought about another 10 % fall, and a further 20 % drop with secondary schooling. The tendency was universal for better educated women to marry similarly advantaged men and hence enjoy relatively high standards of living. As such, interaction between education with income and household facilities might be expected. On the other hand, it was equally plausible to argue that availability of good water and sanitation might erode the educational advantage. Cleland *et al.* (1998) summised that the economic advantage associated with education (income, water and latrine facilities, clothing, housing quality etc) probably accounted for about

one-half of the overall education-mortality association. On use of health facilities, inter-relationships between maternal education, health service provision and childhood mortality was complex and variable. There were countries whose primary health services were so weak that they had no effect on the health of mothers or children. There were also countries whose services tended to accentuate educational disparities because of differential access. Finally, there were countries perhaps only a few in numbers, with services sufficient to offset the advantage of education and bring about greater equality in health and survivorship.

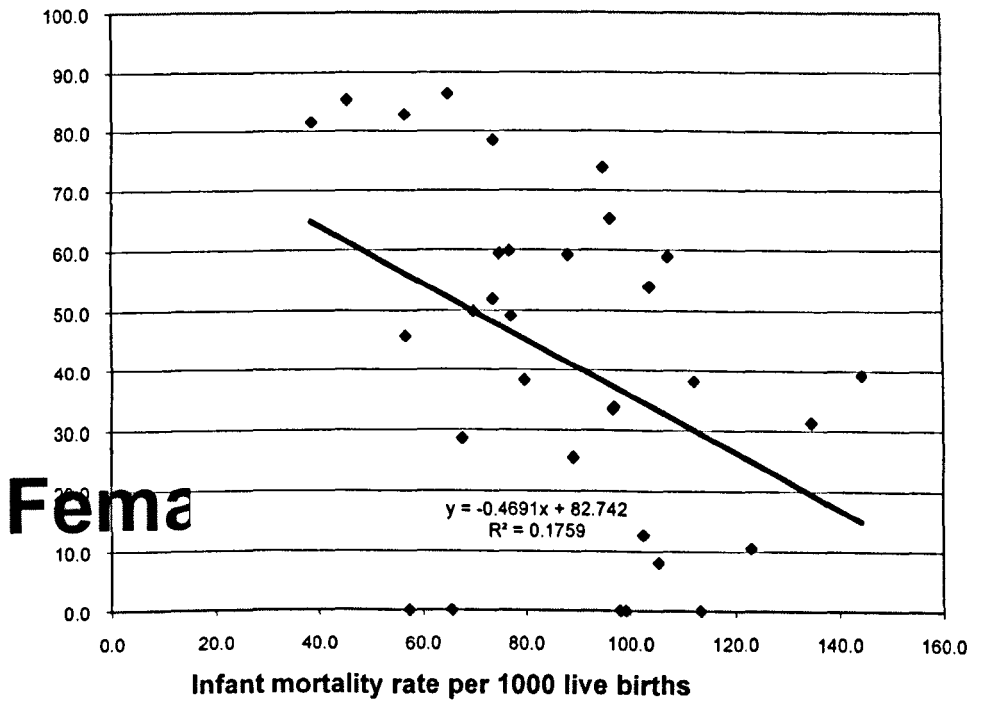
Figures 2.5 and 2.6 show the crude relationship of female literacy and childhood mortality estimates using data from UNESCO (UNESCO Institute for Statistics 2004) and demographic health surveys, respectively. The inverse relationship is apparent between female literacy with under-five mortality (Figure 2.5) and infant mortality (Figure 2.6).

Figure 2.5: Under 5 mortality and female literacy.



Source: UNESCO and own analysis

Figure 2.6: Infant mortality and female literacy



Source: UNESCO and own analysis

CHAPTER THREE

STUDY METHODOLOGY

This chapter outlines the study methodology for the main study except for data related to Chapter 8, which is presented in the relevant chapter.

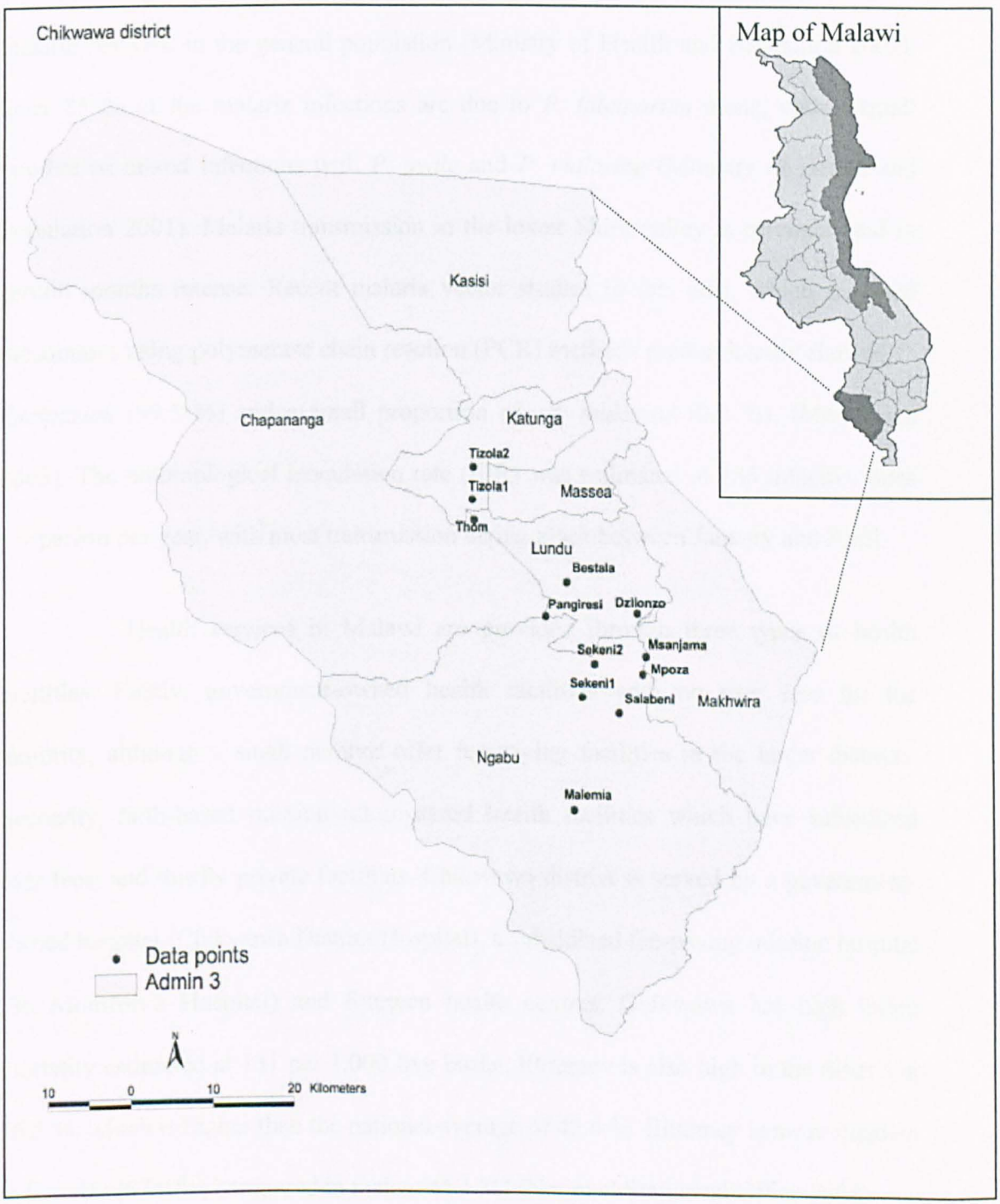
3.1 Study location

The study was conducted in Chikwawa district, located in the lower Shire valley, southern Malawi (Figure 3.1). Malawi had an estimated population of 9.9 million people during its previous census in 1998, with an estimated inter-censal growth rate of 2.0 % per annum (Malawi National Statistical Office 2004). A landlocked country in south-eastern Africa, it spans an area of about 118,500 square kilometers with altitude ranging from 1,000 to 2,000 metres above sea level. The country is organized into 29 Administrative Districts, which are further divided into traditional authorities (TAs) comprising specific village groups. It is mainly an agricultural country with a gross domestic product of 156 USD per capita (Malawi national Statistical Office 2004).

Malawi has a sub-tropical climate, which is relatively dry and strongly seasonal. The hot dry season lasts from September to October with average temperatures varying between 25 and 37 degrees Celsius and humidity around 50 %. The warm wet season occurs from November to April, during which 95% of the annual precipitation occurs. Annual average rainfall varies from 725mm to 2,500mm with humidity reaching up to 87 % and mean temperatures of 17 to 27 degrees Celsius. The low-lying areas of the lower Shire Valley are vulnerable to floods during heavy

rains. A cool dry winter season occurs from May to August with temperatures ranging between 4 and 10 degrees Celsius, (Malawi Meteorological Services 2007).

Figure 3.1: Map of Chikwawa district showing location of study villages



20 villages not plotted are proximate to the named villages in the map. For full list of villages, see Appendix 1.

Malaria transmission in Malawi is stable and year round, with topographical and seasonal variations. Transmission is highest along the lower-lying areas and during the rainy season. Malawians experience an estimated 30 to 50 infective bites per person per year, translating into about eight million episodes of malaria per year in the general population (Ministry of Health and Population 2001). Over 85 % of the malaria infections are due to *P. falciparum* alone, with a small number of mixed infections with *P. ovale* and *P. malariae* (Ministry of Health and Population 2001). Malaria transmission in the lower Shire valley is perennial and in certain months intense. Recent malaria vector studies in this area, which screened mosquitoes using polymerase chain reaction (PCR) methods predominantly showed *P. falciparum* (99.5 %) and a small proportion of *P. malariae* (0.5 %), (Mzilahowa 2005). The entomological inoculation rate (EIR) was estimated at 183 infective bites per person per year, with most transmission taking place between January and April.

Health services in Malawi are provided through three types of health facilities. Firstly, government-owned health facilities with no user fees for the majority, although a small number offer fee-paying facilities in the larger districts. Secondly, faith-based mission-administered health facilities which have subsidized user fees, and thirdly private facilities. Chikwawa district is served by a government-owned hospital (Chikwawa District Hospital), a subsidized fee-paying mission hospital (St. Montfort's Hospital) and fourteen health centres. Chikwawa has high infant mortality estimated at 131 per 1,000 live births. Illiteracy is also high in the district at 56.5 %, which is higher than the national average of 42.4 %. Illiteracy is more frequent in females (67.9 %) compared to males (45.1 %) (National Statistical Office 2004).

This study was concentrated within villages in the St. Montfort's Hospital catchment population. The largest sugar estate in the country, SUCOMA, is located in this area and provides seasonal employment for surrounding villages. Some of the selected villages had been involved in an Adolescent Girls Literacy (AGLIT) village-based project targeting illiterate girls out of school. This project aimed to improve health-seeking behaviour and prevent early pregnancy through health literacy interventions and has developed appropriate teaching curricula, including a malaria curriculum. An evaluation specific to the malaria literacy curriculum was done prior to commencement of this study and is presented in Chapter eight.

3.2 Study population

Chikwawa district had a projected population of 437,678 for 2005 (National Statistical Office 2004). Farming is the main occupation. Subsistence farming is mainly of maize, rice, sorghum, seasonal vegetables, bananas, sweet potatoes and poultry which are the main food sources. Small-scale farming of vegetables, cotton, goat and cattle herding provide additional income for some families. The target population was adolescent girls aged 10 to 19 years who comprised 11.1 % of the total district population (National Statistical Office 2004). Previous studies from 1993 in pregnant women from this same area reported a high malaria prevalence of 34.4 % among pregnant adolescent girls during the wet season, who also had high prevalence of anaemia (92.6 % had Hb <11g/dl) (Brabin *et al.* 1998b; Kalanda *et al.* 2006).

3.3 Study design

Two community-based cross-sectional surveys of adolescent girls were completed during the dry season between September and the first week of December

2005 and during the wet season and post-rainy period from January to June 2006. An observational study design was selected as no interventions were introduced. Although prevalence bias can affect the risk estimates obtained from prevalence risk ratios, the cross-sectional design was selected because malaria infection is relatively an acute infectious disease. Therefore, this bias would not be as substantial. In addition, the cross-sectional design could minimize survey bias which can occur when the same participants are surveyed sequentially as some behavioral patterns can alter simply because the population is under study. On the other hand, a prospective cohort study, which has the advantage to the cross-sectional design because it eliminates prevalence bias and provides time-to-event data that better estimates risk, would have been ideal. However, this would have required more substantial financial resources and additional logistics as well as a longer period of follow-up which was beyond the scope of this project.

3.4 Field procedures

The research team comprised the research student, a research nurse and a research assistant. The health surveillance assistant (HSA) responsible for the particular village cluster also assisted and this person resided in the village community. An additional student completing a Master's project joined the team for part of the second survey. Training in the field procedures was provided for all research team members. Administrative procedures prior to survey included a meeting that was held at district level and permission was requested from village chiefs for the survey to take place in the selected villages. Village meetings were pre-arranged before the dates of the survey. On the day of survey, an open meeting was held to further brief village leaders, parents and participants of survey procedures before requesting participants to be interviewed. Demographic and health information was collected by team members

using a pre-tested interviewer-administered structured questionnaire (Appendix 2). Laboratory materials were stored in an air-conditioned room at a local field office, which was rented for the period of the study and these were transported as required to the field on the day of the survey. Participants were requested to provide blood and urine samples.

3.4.1 Questionnaire interviews

Participants were privately interviewed using the questionnaire by a member of the research team. Written consent was requested using a standard information sheet in the Chichewa language (Appendix 3). Signed consent was requested or a thumb print if they were not able to sign (Appendix 4). The questionnaire contained information on participant identification, age, occupation, marital status, household characteristics, a brief medical history, history of onset of menarche, history of fever in the three days prior to the survey, history of antimalarial and other drug use, availability and use of insecticide treated bednets and a basic literacy assessment. Medical history was elicited for (a) an ever history of tuberculosis, diabetes, sickle cell disease, hypertension and asthma, and (b) history of urinary tract infections and sexually transmitted infections during adolescence. A series of prompts for symptoms were used for the latter group. A history of fever was assessed through a three-day history prior to study date and by measurement as part of the physical examination. Participants were requested to read letters and words as well as copying a word in order to assess literacy. Total literacy scores depicted tri-modal distribution and were thus categorized into illiterate, semi-literate and literate. Health cards were sought when available for further validation of some participant information. Mission hospitals had introduced use of these cards some years before the government introduced them in about 1996. Individuals would usually acquire the health cards at

birth or when seeking help for an illness at both mission and government health facilities. A small fee of about 15 Malawi Kwacha was usually charged for these health cards.

3.4.2 Physical examination

Physical measurements were completed by the research student and a Master's student assisted in obtaining some of the anthropometric measurements under supervision. The general health status of the girls was recorded. Temperature, height, weight and mid-upper arm circumference (MUAC) measurements were taken. Temperature was measured using a Braun Thermoscan ear thermometer in °C. Fever was defined as tympanic temperature equal to and above 37.5 °C.

Height, weight and MUAC measurements were taken with the participants wearing light clothing and no shoes; no repeated measures were done and this could cause observational error as test-retest reliability could not be checked. Height was measured using a portable minimiter aligned to a locally made wooden stand and standardized using a fixed wall chart at the hospital. This was recorded to the nearest millimetre and later converted to centimetres during data cleaning. Weight was measured to 0.1 kilogrammes on a digital Seca model 770 scale (Seca Inc., Columbia, MD, USA). MUAC was measured to the nearest 0.1 cm at the mid-point of the left upper arm between the acromion process and the tip of the olecranon using a non-stretch insertion tape. The weighing scale was only standardized at the beginning of the surveys, which could affect the validity of the measurements and could lead to systematic error in one direction by either overestimating or underestimating the true measures. Height-for-age and weight-for-age z-scores were obtained using the 1978 CDC/ WHO normalized versions of the 1977 NCHS reference curves. The CDC 2000

reference population was used to obtain BMI for age z-scores because the 1977 NCHS does not have complete reference values for older adolescents.

Self-assessment of breast development was done using a pictorial chart for breast development stages designed by Tanner (Appendix 4). This type of assessment has been validated in adolescents in industrialized settings (Brooks-Gunn *et al.* 1982; Chan et *al.* 2008; Taylor *et al.* 2001). Information on its validation in sub-Saharan Africa of developed country settings is limited but its use among adolescents has been reported in western Kenya (Leenstra *et al.* 2005). Identification of pregnant participants was through questionnaire interview (directly asking if the participant was pregnant or from the history of the last normal menstrual period if it was indicated this was delayed) and confirmed by urine pregnancy tests. Pregnant participants were referred for antenatal care if they had not already started attending.

3.4.3 Blood collection

Blood was collected by finger prick for checking malaria and haemoglobin. Paracheck Pf rapid test (Orchid Biomedical Systems, Goa, India) was used for screening for malaria infection exposure. This test detects *P. falciparum*-specific histidine rich protein II (HRP II) in whole blood samples and excludes *Pf* infection with high accuracy. According to the manufacturer's instructions, a finger-prick blood sample was placed in the relevant well, six drops of the buffer solution were applied to another well and results read within three minutes. A thick blood smear for malaria microscopy was obtained, allowed to dry in the open air for up to 20 minutes and transported to the laboratory in the evening. Slides were stained within 48 hours using Field stain and read under x 100 light microscopy by the laboratory technician at St. Montfort's Hospital, who was an experienced malaria microscopist

(referred to as Reader 1). In order to achieve 10 % precision assuming 90 % sensitivity and specificity, the investigator (referred to as Reader 2) completed separate quality control checks on the 280 randomly selected slides using random numbers independently generated by a statistician in Liverpool. Slides were considered negative when no parasites were seen per 200 white blood cells counted. There was good inter-rater agreement of microscopy results: 93.3 % agreement, kappa statistic 0.684 (standard error 0.058). Parasite density ranged from 40 to 8000 parasites per μl, mean 682.5 (±1086). A futher 20 slides were cross-checked by a malaria microscopist expert (referred to as Expert reader) at the Liverpool School of Tropical Medicine in order to validate the quality control screening of the investigator. Though limited in sample number due to the microscopist's time limitations, the results showed good agreement 89.4 % agreement, kappa statistic 0.641 (standard error 0.214).

A portable battery operated photometer (HemoCue, Angelholm, Sweden) was used for haemoglobin estimation. A HemoCue cuvette was filled and read according to manufacturer's instructions. On each morning of the field work, the Hemocue was calibrated using the control cuvette provided by the manufacturer. Anaemia was defined as haemoglobin less than 12 grammes per decilitre. Severe anaemia was defined as haemoglobin less than 8 grammes per decilitre. Participants with a haemoglobin level of less than 10 g per deciliter were given a one-month course of ferrous sulphate tablets (200 mg iron) according to prevailing clinical guidelines. Those with a haemoglobin level of less than 8 g per deciliter were referred to the hospital for further evaluation.

3.4.4 Urine collection

Urine was collected in 200 ml containers in the field. Urine was tested for pregnancy using an hCG One-step Pregnancy Test Strip (ACON Biotech. Co., Ltd.). Samples were transported in a cooler box for storage at the hospital laboratory, then alliquoted into 5 ml containers and frozen awaiting further transportation to Liverpool for further analysis on antimalarial drug metabolites. Due to an observation that some of the urine samples were bloody, a record of whether urine appeared bloody or not was added while the study had already commenced.

Detection of chloroquine and sulphadoxine-pyrimethamine in urine

The drug metabolite assays were undertaken by Dr. T.A. Eggelte at the Amsterdam Medical Centre, the Netherlands using an ELISA procedure. Two different ELISA tests were used for the detection of chloroquine in urine. The first ELISA was based on the use of a monoclonal antibody F73-8 which recognizes the quinoline part of the chloroquine molecule and therefore not only detects chloroquine but also a number of chloroquine metabolites. The second ELISA used a monoclonal antibody F149-12 which is much more specific and shows only low cross reactivity with chloroquine metabolites and amodiaquine. In the ELISA test, 96–well microtitre plates were coated with a solution of the monoclonal antibodies for 3 hours. Plates were washed with phosphate-buffered saline (PBS) / 0.05% Tween-20 and stored. Urine samples were tested in a 1:10 dilution in PBS. To the wells of the microtitre plate 50 µl of urine dilution was added together with 50 µl of a chloroquine calibration series. After addition of 50 µl of a solution of a chloroquine–peroxidase conjugate in PBS/0.05% Tween-20/1% bovine serum albumin (BSA) to all wells, the plate was incubated for one hour at room temperature. Different chloroquine horseradish peroxidase (CQ-HRP) conjugates were used in combination with the two different antibodies. After washing of the plate with PBS/0.5% Tween-20 100 µl of a

tetramethyle benzidine (TMB)/H2O2 substrate solution in Citrate/Acetate buffer pH = 4 was added to all wells of the plate. After 20 minutes the reaction was stopped by addition of 50 µl per 1 ml of H2SO4. Some samples were also tested using a dipstick assay which was based on the use of chloroquine monoclonal antibody F73-8. The procedure for the detection of pyrimethamine and sulfadoxine was similar to that of chloroquine. For pyrimethamine a monoclonal antibody F131-23 was used and for sulfadoxine antibody F115-5 which detects also the main metabolite of sulfadoxine e.g. N4 Acetylsulfadoxine.

3.5 Statistical methods and data analysis

3.5.1 Sample size

Since the aim of this research is merely to measure the point prevalence of malaria, no hypothesis is being tested. Win Episcope 2.0 software (de Blas *et al.* 2000) was used for sample size calculation. In order to detect malaria parasitaemia prevalence of 35 % with accuracy (i.e. 95 % confidence intervals) of ± 5 %, we calculated that this required a total of 350 adolescent girls. Due to expected clustering of individuals and villages in these community-based studies, we used a conservative estimate of 2.0 for the cluster design effect, meaning that the planned sample size was doubled to 700 participants per survey. An estimated malaria prevalence of 35 % was selected based on previous studies conducted in 1994. If prevalence is actually lower than 35 % due to improved malaria control strategies over time, thereby requiring a reduced sample size, effect would be to increase accuracy of the estimate. Single-stage random cluster sampling was used, with clusters selected by probability proportional to size (Bennett *et al.* 1991), with villages being identified as clusters. Montfort catchment population villages and their estimated populations for 2005 were computed in a spreadsheet. An independent statistician selected the villages based on cumulative

population density sampling. The basic sampling unit was adolescent girls 10 – 19 years old. A rapid census of adolescent girls was carried out through enumeration at household visits in the selected villages. Adolescent girls were then randomly selected from the census lists prior to each survey by an independent statistician with no intention of repeated participant selection.

The St. Monfort catchment population had ninety villages. These villages are all located on the western side of the Shire River and served by the one hospital and therefore assumed to harbor similar populations. Thirteen villages were surveyed during the dry season survey and nineteen villages during the wet season survey, three of which had been surveyed during the dry season. All selected villages agreed to take part in the study. Due to delays in obtaining ethical clearance, the dry season survey was delayed leading to a reduced sample size than planned.

3.5.2 Data analysis

Data was entered in EpiInfo version 3 (Centres for Disease Control (CDC) 2002) and analyzed in Stata 8.0 (Stata Corporation 2003) and SPSS version 14 and 15 (LEAD Technologies Inc. 2006). Data were explored for distribution, data type, checking the use of correct coding, missing variables, typos and abnormal values and relevant data cleaning performed. No statistical manipulations were done for missing data and these were analysed as system missing. Comparisons were made between dry and wet seasons. Differences between proportions were compared by the chi-square test. Normally distributed continuous data were compared by the Student's t-test. Generalised estimating equations (GEE) algorithm by multiple regression was used in order to adjust for clustering effects. This analysis was done as it allows for within-cluster and between-cluster correlations inherent in community-based cross-sectional

data and thereby making the estimates of prevalence risk ratios (PRR) and p-values more precise. Univariate and multivariate analyses were completed to identify and quantify associated factors. Potential variables for consideration in a logistic regression model were identified using a p-value cut-off of 0.2. Estimates of PRR and their 95 % confidence intervals were calculated. Statistical significance was p-value < 0.05.

3.5.3 Definitions

Mararia parasites

Malaria parasitaemia was defined as presence of *P. falciparum* parasites by light microscopy. Symptomatic malaria was defined as the presence of *P. falciparum* parasites on light microscopy in the presence of fever. Paracheck rapid test positive result is reported as rapid test positivity. Fever was defined as tympanic temperature equal to and above 37.5 °C.

Age and maturation

Chronological age was calculated from the reported date of birth. If exact age was unknown, the mid-point of the month and / or year was used to estimate age if an appropriate age was available from the participant, parent or guardian. Several prompts for memorable national or local events were used to assist parents and guardians in remembering the month and year of birth if they had forgotten. Gynaecological age is the period between current age and age at onset of menses. This was calculated from the reported date of onset of menses, or as the number of years since the onset of menses reported by participant if the date was unknown. If gynaecological age was less than a year, it was recorded as 0.5 years.

Anaemia

Anaemia was defined as haemoglobin less than 12 grammes per decilitre. Severe anaemia was defined as haemoglobin less than 8 grammes per decilitre.

Anthropometric

Stunting was defined as height-for-age less than minus 2 standard deviations. Underweight was defined as weight-for-age less than minus 2 standard deviations. Body mass index (BMI) was calculated as weight in kilogrammes divided by the square of height in metres. Undernutrition was defined as BMI less than the 5th percentile of the values for the reference population.

ITNs

Ownership of an insecticide treated bed net (ITN) and its associated access and use by the participant was elicited from the interview using standard household questions for WHO Roll Back Malaria indicators (WHO 2006). ITN availability was defined as the reported presence of at least one ITN in the household. ITN access was defined as access by the participant to an ITN within the household. ITN use was measured using the standard indicator of use of an ITN during the night prior to the survey date, although the validity varies and could be over-estimated in ITN intervention studies. Additionally a non-standard measure of ITN use was measured as the reported number of nights per week that the participant slept under an ITN and this is elaborated as such when used in the results and discussion. This has been previously used during rapid vector control assessments together with the standard measure in Malawi because of concerns of over-estimation of the RBM question in assessing ITN use (personal communication D. Chiphwanya, National Malaria Control programme).

Assessment on use of treatment in response to illness

Use of antipyretic and antimalarial drugs was elicited from the interview using open ended questions and the interviewer recorded the relevant drugs mentioned. The list of drugs included Aspirin, Paracetamol/ Panadol, Chloroquine, SP/ Fansidar/ Novidar, Quinine, Halfan/ Halofantrine, Co-trimoxazole/ Bactrim and Artemisinin derivatives. For those who sought treatment from a health facility, the health passport was inspected when available. Ascertainment of drugs from other sources could not be made.

3.6 Ethical approval

Ethical approval was given by the Malawi Health Sciences Research committee and Liverpool School of Tropical Medicine Ethics Committee. Information sheets and consent forms used in the study are included as appendices 3 and 4, respectively.

3.7 Sample population description

A total of 1315 adolescent females were recruited, which represented a 95.8 % acceptance rate as 57 refused to participate in the study. Eighteen (1.4%) participants were re-sampled during the wet season and these have been excluded from analyses leaving 1297 participants: 496 in dry and 801 in wet season. High proportions of participants consented to the various tests being done. The rapid malaria tests were available for 99.5 %, blood slides for 97.1 % and pregnancy tests for 87.4 % of the participants. Urine was collected for 88.1 % of the participants and bloody urine colour was checked for 78.8 % of participants (44.7 % in the dry season and 99.1 % in wet season samples).

CHAPTER FOUR

NUTRITIONAL AND HEALTH CHARACTERISTICS OF NON-PREGNANT ADOLESCENT FEMALES

4.1 Introduction

The health of adolescents has been neglected for many years because of their lower morbidity and mortality than younger children. Nevertheless, adolescents are vulnerable to many health-related life risks through specific lifestyles factors, food habits, physical activity patterns and health behaviours established during childhood and during adolescence (World Health Organization & UNICEF). Adolescents are predisposed to significant health problems ranging from sexually transmitted diseases, including HIV/ AIDS, physical injury, mental health problems and several nutritional deficiencies.

Undernutrition is highly prevalent in childhood and adolescence in many developing countries (World Health Organization 1995). The cumulative effect of poor nutrition and health during childhood is manifest in linear growth retardation which is an important indicator reflecting chronic undernutrition (ACC/ SCN 1997). Despite the importance of linear growth retardation, anthropometric studies among adolescents in developing countries are very limited. For African studies, stunting and thinness have been reported as common among school-going non-pregnant adolescent females from western Kenya although the prevalence of these decreased markedly with increasing age and sexual maturation (Leenstra *et al.* 2005). Previous studies of pregnant adolescents from Malawi also have reported a 40 % prevalence of stunting (Fazio-Tirrozzo *et al.* 1998; Kalanda *et al.* 2006; Brabin *et al.* 1997).

The aim of this analysis was to describe the health and nutritional characteristics of the non-pregnant adolescent females who participated in the cross-sectional surveys of this study.

4.2 Statistical analysis

Data was analyzed in SPSS 14.0 and 15.0 and in Stata 8.0 using the GEE (generalized estimating equations) algorithm by multiple regression adjusted for clustering effects. The EpiNut programme in Epi-info 2000 was used to calculate z-scores obtained using the 1978 CDC/ WHO normalized versions of the 1977 NCHS reference curves. The CDC 2000 reference population was used to obtain BMI for age z-scores because the NCHS did not have reference values for older adolescents. Nutritional variables were described according to both chronological age and gynaecological age. Statistical significance was considered at a p-value < 0.05. Three measures of adolescent nutritional status i.e. height for-age z-score (HAZ), body mass index for-age z-scores (BMI) and low MUAC for age were explored for associated factors. Univariate and multivariate analyses were completed to identify and quantify factors associated with the measures of nutrition and prevalence risk ratios (PRR) were estimated. Potential variables for consideration for a multiple regression analysis were identified using a p-value of < 0.2. Univariate analyses for combined data are presented both adjusted and unadjusted for season. As season is one of the co-variates, this limits identification of seasonal effects on the risk factor analysis for the outcomes being measured.

4.3 Results

4.3.1 Sample population description

A total of 1297 participants were surveyed: 496 in dry and 801 in wet season. Table 4.1 summarises the socio- demographic characteristics of the participants. The majority of the population relied on subsistence farming and some sources of unskilled work depending on seasonal availability. Water for household use was mostly provided through communal pipes and taps usually in the villages proximal to the sugar estate, and boreholes or other natural sources in those villages farther away from the estate. However, utilisation of these water sources changed by season due to logistical factors. Most of the participants were school-going, although about one third were illiterate.

Age, gynaecological age, participant occupation, maternal education, paternal education, marital status and type of dwelling house were comparable for the study samples in the two seasons. Those seen in the dry season had a higher number of years of schooling ($p < 0.001$) and a higher literacy rate than those adolescents seen during the wet season survey. This could almost be entirely explained by the fact that the question sought 'completed years of schooling'; the dry season survey occurred during holidays after the academic year had ended while the wet season survey occurred during the academic calendar before year completion. Fewer girls who were surveyed in the wet season had attended AGLIT literacy classes and the overall attendance was low and this corresponded to lower literacy levels. Farming was also more frequently undertaken by parents of wet season participants ($p < 0.001$). Thirty-four of the participants (2.6 %) were pregnant, leaving 477 and 786 non-pregnant participants in the dry and wet seasons, respectively.

Table 4.1: Socio- demographic characteristics of non-pregnant and pregnant adolescent girls by season of survey

Characteristic		Dry season (n = 496)	Wet season (n = 801)	p-value‡
Mean (sd)				
Age in years		13.4 (2.8)	13.2 (2.8)	0.184
Gynaecological age in years		2.4 (1.6)	2.4 (1.7)	0.677
Education - years of schooling		3.9 (2.4)	3.4 (2.0)	<0.001
Maternal years of schooling		5.1 (3.4)	4.7 (3.3)	0.162
Paternal years of schooling		7.6 (3.3)	7.4 (3.3)	0.362
Number (Percentage)				
Married		66 (13.3)	78 (9.7)	0.208
Pregnant		19 (3.8)	15 (1.9)	0.027
Attended AGLIT		29 (5.8)	21 (2.6)	0.003
Literacy level:	Illiterate	143 (28.8)	309 (38.6)	
	Semi-literate	39 (7.9)	87 (10.9)	<0.001
	Literate	314 (63.3)	405 (50.6)	
Earned/ earning cash		21 (4.5)	38 (4.7)	0.935
Occupation:	student (in-school)	354 (71.4)	595 (74.3)	
	farming	59 (11.9)	100 (12.5)	0.298
	other unskilled work	69 (13.9)	88 (11.0)	
	SUCOMA/ Trade/ Business	14 (2.8)	18 (2.2)	
Maternal occupation:	Farming	276 (55.6)	629 (78.5)	
	Civil service	7 (1.4)	12 (1.5)	
	Ganyu	20 (4.0)	26 (3.2)	<0.001
	other unskilled work	156 (31.5)	87 (10.9)	
	SUCOMA/ Trade/ Business	25 (5.0)	33 (4.1)	
	Deceased	12 (2.4)	14 (1.7)	
Paternal occupation:	Farming	191 (38.5)	372 (46.4)	
	Civil service	26 (5.2)	22 (2.7)	
	Ganyu	19 (3.8)	28 (3.5)	0.004
	other unskilled work	88 (17.7)	98 (12.2)	
	SUCOMA/ Trade/ Business	149 (30.1)	242 (30.2)	
	Deceased	23 (4.6)	39 (4.9)	
Type of house:	Grass thatched	319 (64.3)	551 (68.8)	
	Corrugated iron roof	174 (35.1)	247 (30.8)	0.180
	Missing	3 (0.6)	3 (0.4)	
Water source:	Borehole	127 (25.6)	136 (17.0)	
	Tap/ piped	268 (54.0)	527 (65.8)	<0.001
	River	11 (2.4)	97 (12.1)	
	Well	86 (17.3)	37 (4.6)	
	Missing	4 (0.7)	4 (0.5)	

‡ Adjusted for clustering effect

4.3.2 Health characteristics of non-pregnant adolescent females

Table 4.2 summarises the health characteristics of the non-pregnant participants.

Table 4.2: Health characteristics of non-pregnant adolescents by season of survey

Characteristic	N	Dry season (n =477)	N	Wet season (n =786)	p-value‡
Mean (sd)					
Chronological age in years	477	13.2 (2.8)	786	13.1(2.7)	0.622
Gynaecological age in years	477	2.2 (1.5)	786	2.4 (1.7)	0.419
Age at menarche in years	477	14.4 (1.2)	786	14.3 (1.2)	0.640
Body temperature in °C	460	37.4 (0.4)	757	37.2 (0.4)	< 0.01
Education - years of schooling	476	3.9 (2.1)	784	3.4 (2.0)	< 0.001
Number (percentage)					
Attended AGLIT	476	29 (6.1)	784	21 (2.7)	0.003
Literacy level: Illiterate Semi-literate Literate	464	134 (28.9) 38 (8.2) 292 (62.9)	775	299 (38.6) 85 (11.0) 391 (50.8)	0.001
Pubertal (Tanner B >=2)	471	283 (60.1)	773	428 (55.4)	0.178
Post-menarche	477	152 (31.9)	786	212 (27.0)	0.058
Tanner breast staging: B1 B2 B3 B4 B5	474	109 (39.9) 48 (17.6) 30 (11.0) 14 (5.1) 72 (26.4)	773	345 (44.6) 146 (18.9) 110 (14.2) 64 (8.3) 108 (14.0)	0.083
Own a health book	467	185 (39.6)	785	347 (44.2)	0.085
Smoke	475	1 (0.2)	785	3 (0.4)	0.188
Alcohol	452	2 (0.4)	784	2 (0.2)	0.576
Fever history– in past 3 days	472	85 (18.0)	785	153 (19.5)	0.497
Body temperature ≥ 37.5 °C	460	87 (18.2)	757	189 (23.5)	0.027
Tuberculosis	475	1 (0.2)	785	6 (0.7)	0.256
Asthma		4 (0.8)		20 (2.5)	0.032
Sexually transmitted diseases		0 (0.0)		3 (0.2)	0.271
Urinary tract infections		48 (9.8)		406 (49.9)	< 0.001
Diabetes		0 (0.0)		0 (0.0)	1.000
Hypertension		0 (0.0)		2 (0.3)	0.271
Sickle cell disease		0 (0.0)		0 (0.0)	1.000
Bloody urine	213	8 (3.8)	779	221 (28.4)	< 0.001
Malaria rapid test positive	477	56 (11.7)	780	147 (18.8)	0.001
Malaria microscopy positive	475	16 (3.4)	779	44 (5.7)	0.070
Symptomatic malaria	460	13 (2.8)	757	27 (3.6)	0.347

‡ Adjusted for clustering effect

Mean age was 13.1 (± 2.7) years and mean gynaecological age 2.3 (± 1.6) years. Age, gynaecological age, age at menarche, maternal education, paternal education, marital status and type of dwelling house were comparable in the two seasonal surveys. The proportion of post-menarcheal girls was higher during the wet season with boarderline significance and no seasonal difference was observed by Tanner stage or in the mean age at menarche. Significant differences between the dry and wet seasons were observed for mean body temperature, proportion of febrile participants, and the mean number of years of schooling, AGLIT attendance and literacy level. Body temperature ranged between 35.5 °C to 39.2 °C, mean 37.3 °C and 21.5 %, with a higher proportion of febrile girls (≥ 37.5 °C) in the wet season than in the dry season (23.5 % versus 18.2 %). Although mean temperature is lower for the wet season by 0.2 °C, the proportion that was febrile in the wet season was more than in the dry season. The mean difference in years of schooling was 0.5 years, higher in the dry than wet season, which could almost be entirely explained by the fact that the question sought 'completed years of schooling'; the dry season survey occurred during holidays after the academic year had ended while the wet season survey occurred during the academic calendar before year completion. This could also explain the significant differences in literacy level. Fewer girls who were surveyed in the wet season had attended AGLIT literacy classes and the overall attendance was low and this corresponded to lower literacy levels. Smoking and alcohol intake were very rare.

The commonest history of morbidity was symptoms associated with a urinary tract infection which were more frequent in participants in the wet than dry season surveys (49.9 % versus 9.8 %, $p < 0.001$). Urine samples collected during the wet season were significantly bloodier in appearance (28.4 %) than in the dry season

survey (3.8 %). The correlation between reported urinary symptoms and bloody urine was 0.233, p< 0.001 (Spearman's rho). Rapid test positivity had significantly higher prevalence in the wet season while malaria microscopy positivity was of borderline statistical significance between the two surveys (p = 0.07). Symptomatic malaria was more common in the wet than dry season (p = 0.347).

4.3.3 Nutritional assessment

Prevalence of malnutrition and seasonal variation

Table 4.3 summarises the nutritional characteristics of the participants. Mean haemoglobin was significantly higher by 0.3g/dl in the wet compared to the dry season (p = 0.022). Mean z-scores for height (p < 0.001) and weight (p = 0.024) were lower in the wet than dry season. The proportion classified as stunted was higher in the wet (13.6 %) than the dry season (8.4 %), (p = 0.009).

Table 4.3: Nutritional assessment of non-pregnant adolescents, by season of survey

Characteristic	Dry season	Wet season	p-value‡
Mean (sd)			
Haemoglobin in g/dl	11.2 (1.9)	11.5 (1.8)	0.022
Height-for-Age Z-score	-0.61 (1.21)	-0.88 (1.12)	<0.001
Weight-for-Age Z-score	-1.25 (0.77)	-1.37 (0.73)	0.024
BMI-for-Age Z-score	-1.40 (1.49)	-1.27 (1.28)	0.086
Number (percentage)			
Stunting (HAZ < -2SD)	39/ 467 (8.4)	99/ 729 (13.6)	0.009
Underweight (WAZ < -2SD)	78/ 467 (16.7)	127/ 729 (20.3)	0.179
BMI-for-Age <5th percentile	164/ 467 (35.1)	254/ 729 (34.8)	0.922
BMI-for-Age < -2SD	120/ 467 (25.7)	158/ 729 (21.7)	0.146
Low MUAC-for-Age	339/ 467 (72.9)	520/ 729 (68.1)	0.073
Anaemia (Hb < 12 g/Dl)	182/ 300 (60.7)	437/ 775 (56.4)	0.204
Severe anaemia (Hb < 8 g/Dl)	16/ 300 (5.3)	28/ 775 (3.6)	0.203

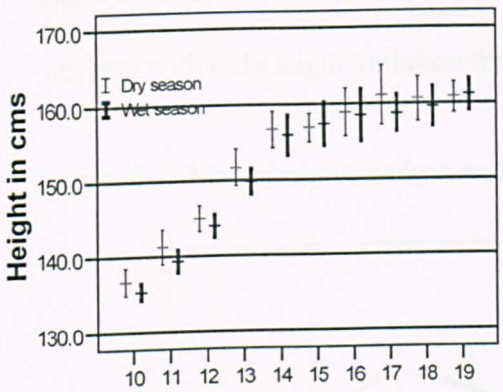
‡ Adjusted for clustering; HAZ - Height-for-Age Z-score; WAZ - Weight-for-Age Z-score; BMIZ - Body mass index-for-Age Z-score.

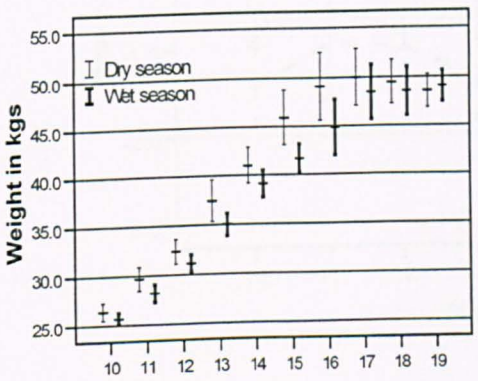
Age and pubertal maturation

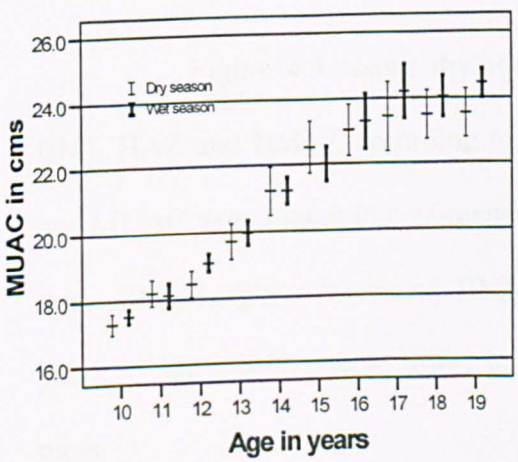
Anthropometric parameters by chronological and gynaecological age for dry and wet season participants are shown in Figure 4.1. Mean height, weight, BMI, and MUAC increased sequentially with age plateauing at approximately 17 years of age. Mean height and weight were lower in wet season compared to dry season participants in those younger than 16 years of age and this difference became less marked in older girls. These seasonal differences were smaller for MUAC measurements. When plotted by gynaecological age these differences were much smaller, except for weight, which in the first two years after menarche was consistently lower in the wet season.

Figure 4.1: Mean height, weight and MUAC, by chronological or gynaecological age (error bars represent 95 % CI)

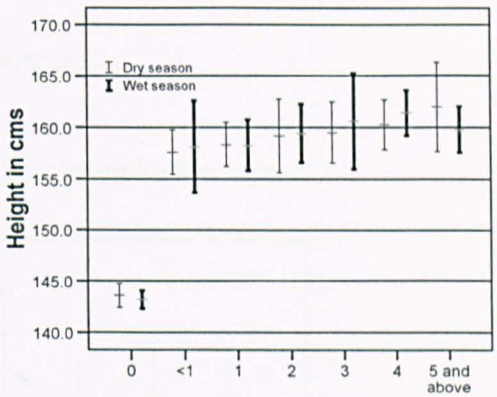
Chronological age

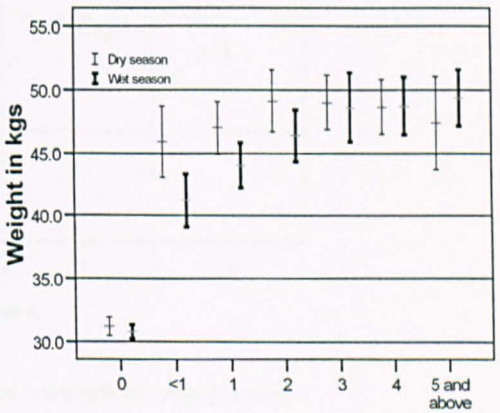


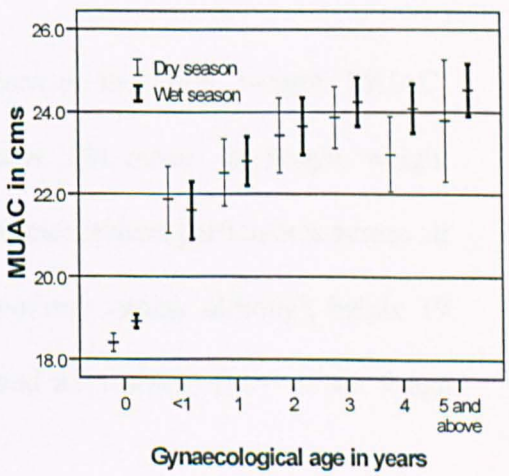




Gynaecological age



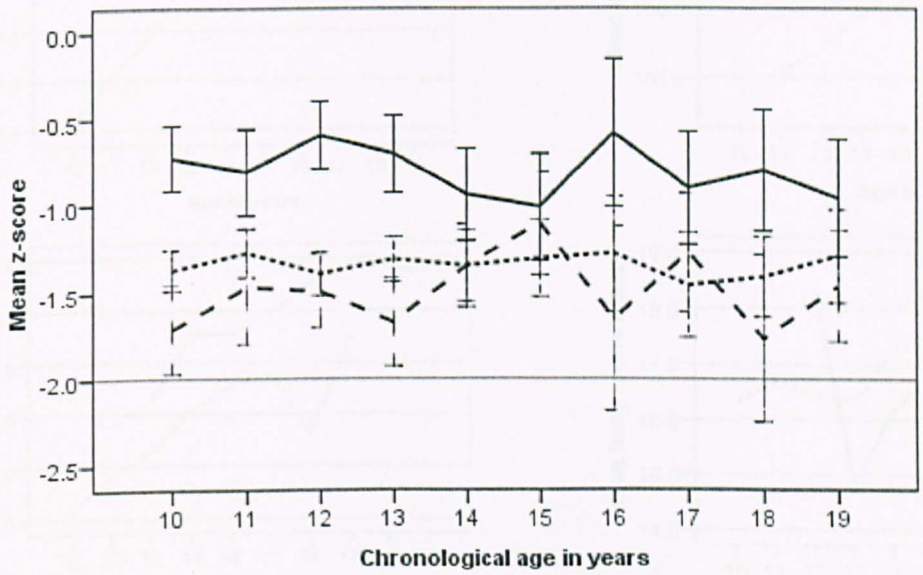




Onset of menarche and z-scores

Figure 4.2 shows trends in z-scores for height, weight and body mass index by age, without stratification by menarche status. Deficits in z-scores remained uniform with only slight variation throughout adolescence.

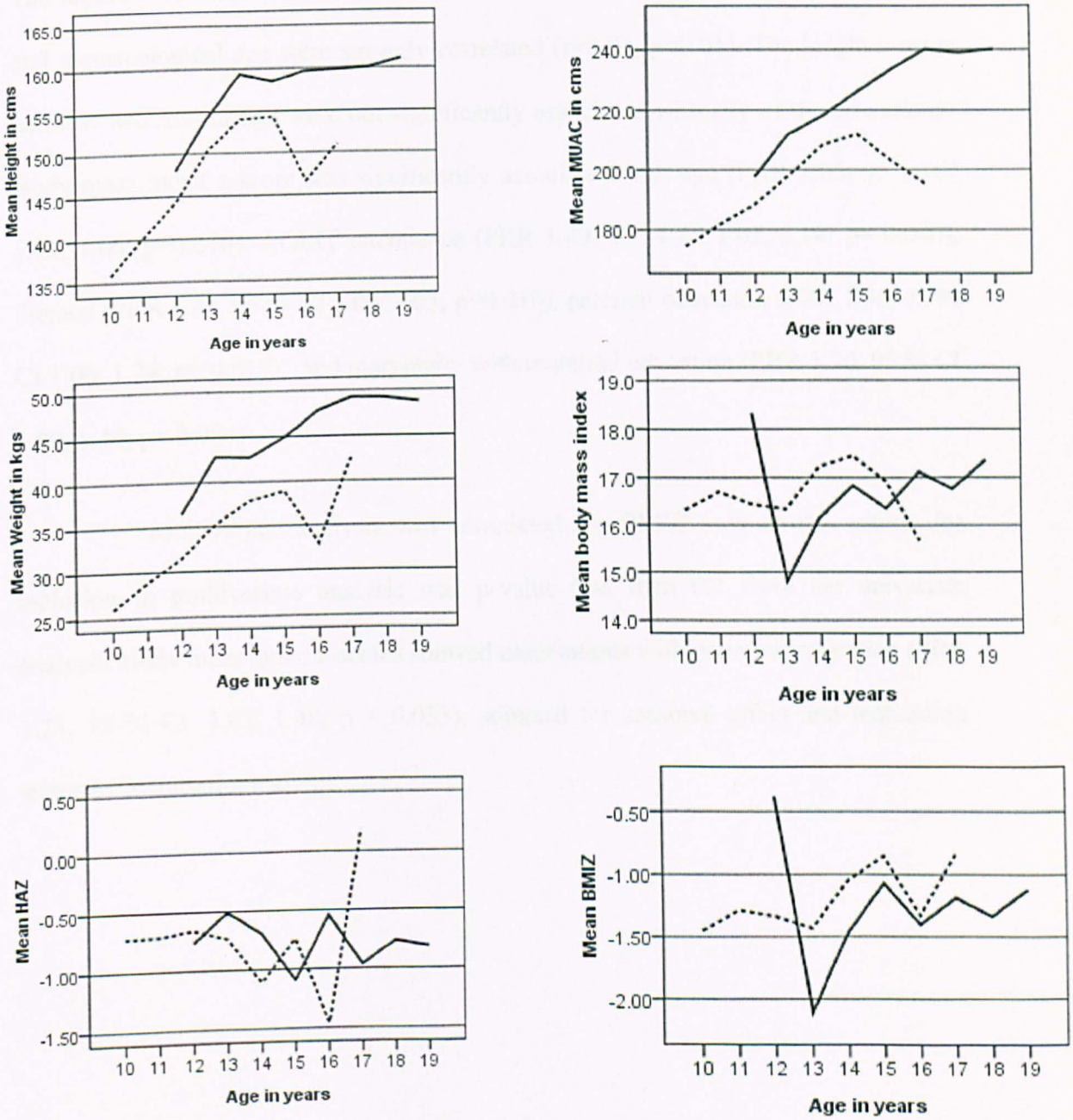
Figure 4.2: Mean height, weight and BMI z-scores by chronological age



Continuous line = height-for-age z-score, stippled line = weight-for-age z-score; broken line = BMI-scores; error bars = 95 % CI

Figure 4.3 shows the age-related differences in height, weight, MUAC, BMI, HAZ and BMI-Z according to menarche status. The means for height, weight and MUAC were higher in post-menarcheal than pre-menarcheal participants across all ages. BMI, height z-score and BMI z-score estimations varied, although below 19 years of age z-scores were lower in post-menarcheal adolescents for 6 of the 7 age plots.

Figure 4.3: Mean height, weight, mid-upper arm circumference (MUAC) body mass index (BMI), height-for-age z-score (HAZ) and BMI z-score (BMIZ) according to menarche status



Key: Continuous line – postmenarche, dotted line - premenarche

Table 4.4 presents the univariate regression analyses for height z-scores, BMI z-scores or low MUAC–for-age, adjusted and unadjusted for season. Adjusted and unadjusted estimates were reasonably comparable except for AGLIT attendance. The adjusted estimates are therefore reported for better precision. Chronological age and gynaecological age were strongly correlated (r=0.83, p=0.01). The height z-score and low MUAC-for-age were not significantly associated with any of the co-variates. Body mass index z-score was significantly associated with age (PRR 1.03, 95 % CI 1.01, 1.06; p=0.016), AGLIT attendance (PRR 1.49, 95 % CI 1.02, 2.18; p= 0.040), literacy (PRR 1.02, 95 % CI 1.00, 1.03; p=0.010), paternal education (PRR 1.36, 95 % CI 1.06, 1.74; p= 0.017), and marginally with maternal education (PRR 1.20, 95 % CI 1.00, 1.44; p= 0.054).

Multivariate analysis was completed for BMIZ only as the criteria for inclusion in multivariate analysis was p-value less than 0.2 from the univariate analysis. Body mass index z-scores showed associations with maternal education (PRR 1.23; 95 % CI: 1.02, 1.48; p = 0.033), adjusted for seasonal effect and maturation status by gynaecological age.

Table 4.4: Univariate analysis of factors associated with height for age z-score (HAZ), body mass index for age z-score (BMIZ) and low mid-upper arm circumference (MUAC)-for-age

Co-variate	HAZ				BMI z-score				Low MUAC for Age			
	¶		†		¶		†		¶		†	
	PRR (95 % CI)	p	PRR (95 % CI)	p	PRR (95 % CI)	p	PRR (95 % CI)	p	PRR (95 % CI)	p	PRR (95 % CI)	p
Age, yrs	0.98 (0.95, 1.01)	0.128	0.98 (0.95, 1.01)	0.156	1.03 (1.01,1.06)	0.016	1.03 (1.01,1.06)	0.006	1.01 (0.97,1.06)	0.502	1.02 (0.97,1.06)	0.494
Age, yrs (premenarcheal only)	0.97 (0.92, 1.03)	0.388	0.97 (0.91, 1.03)	0.322	1.06 (1.01,1.12)	0.050	1.06 (1.00,1.13)	0.058	1.02 (0.93,1.13)	0.659	1.02 (0.92,1.23)	0.699
Age, yrs (postmemacheal only)	0.99 (0.93, 1.06)	0.819	0.99 (0.93, 1.06)	0.814	1.04 (0.99,1.11)	0.130	1.05 (0.99,1.11)	0.130	1.08 (0.97,1.21)	0.179	1.08 (0.96,1.21)	0.190
Gynaecological age yrs	0.98 (0.92, 1.03)	0.427	0.98 (0.92, 1.04)	0.514	1.04 (0.99, 1.10)	0.139	1.05 (0.99, 1.10)	0.079	1.07 (0.97, 1.18)	0.164	1.07 (0.97, 1.19)	0.152
Attended AGLIT: No	reference		reference		reference		reference		reference		reference	
Yes	0.84 (0.57, 1.26)	0.409	0.93 (0.63, 1.39)	0.728	1.49 (1.02, 2.18)	0.040	1.39 (0.97, 2.00)	0.075	1.44 (0.73, 2.87)	0.293	1.52 (0.77, 3.00)	0.232
Literacy score	1.00 (0.99, 1.01)	0.867	1.00 (0.99, 1.01)	0.568	1.02 (1.00,1.03)	0.010	1.02 (1.00,1.03)	0.012	0.99 (0.96, 1.01)	0.204	0.99 (0.97, 1.01)	0.294
Education, years ≥ 4	reference		reference		reference		reference		reference		reference	
< 4	1.00 (0.87, 1.18)	0.988	1.04 (0.90, 1.20)	0.617	0.95 (0.82, 1.11)	0.539	0.94 (0.81, 1.09)	0.440	1.13 (0.88, 1.45)	0.334	1.15 (0.90, 1.48)	0.253
Paternal education, ≥4 (years)	reference		reference		reference		reference		reference		reference	
< 4	0.95 (0.72, 1.24)	0.690	0.95 (0.72, 1.24)	0.709	1.36 (1.06, 1.74)	0.017	1.37 (1.06, 1.76)	0.015	0.79 (0.50, 1.25)	0.317	0.79 (0.50, 1.26)	0.320
Maternal education ≥ 4 (years)	reference		reference		reference		reference		reference		reference	
< 4	0.98 (0.80, 1.20)	0.842	0.98 (0.80, 1.20)	0.969	1.20 (1.00, 1.44)	0.054	1.20 (1.00, 1.45)	0.050	1.22 (0.88, 1.21)	0.238	1.22 (0.86, 1.67)	0.274

¶Adjusted for season survey group; †Unadjusted for season survey group; HAZ – height for-age z-score, BMIZ – body mass index for-age z-score, mid-upper arm circumference (MUAC)-for-age; PRR – prevalence risk ratio; AGLIT - Adolescent girls literacy programme.

4.4 Discussion

In this sample of 10 to 19 year old females, low z-scores for height, weight, BMI as well as low MUAC were prevalent in comparison to the reference population. This indicates that chronic undernutrition was common in this population. Although seasonal differences were observed, the improved anthropometric status of dry season participants compared to those in the wet season could relate to the higher proportion of post-menopausal girls participating in the dry season surveys, as following menarche anthropometric measures improved for all ages. The significant difference in AGLIT attendance between the two surveys could be due to chance or reflect the non-random village coverage of the AGLIT project whose recruitment selection related to the vulnerabilibity factors for access to education and illiteracy.

Childhood malnutrition is highly prevalent in rural Malawi (Maleta *et al.* 2003) and the stunting and undernutrition in these adolescents therefore reflects a continuum of chronic nutritional deficiency from childhood through to adolescence. Previous studies in rural adolescents aged 12 to 19 years from the Shire Valley, who were in early pregnancy (under 18 weeks gestation), have shown high levels of chronic undernutrition with evidence of catch up in linear growth in late adolescence (Kalanda *et al.* 2006). Prolonged catch-up growth of children stunted during pre-school ages has been reported in other African studies with catch-up being attained to the WHO/ NCHS reference values by the age of 20 to 22 years (Coly *et al.* 2006).

The age at menarche in this population was 2 to 4 years later than the reference NCHS population. Similar delays in onset of menarche among undernourished adolescents have been described in other developing country populations (Chowdhury *et al.* 2000; Dreizen *et al.* 1967; Simondon *et al.* 1997). Mean

z-score estimates for the Malawian adolescents were derived using NCHS reference data and are therefore confounded as the onset of menarche in the NCHS reference dataset is approximately 2 years earlier than that for this Malawian population. This mismatch could lead to an overestimation of stunting and undernutrition in pre-menopausal adolescent (Butte *et al.* 2007). This discrepancy could explain, at least in part the poorer anthropometric status in pre-menopausal compared to post-menopausal adolescents. It also could explain why seasonal differences in anthropometric values were reduced when gynaecological rather than chronological age was used for classification, as the former adjusts for age of onset of menarche.

The high proportion of participants with low MUAC measurements when compared to the USA reference population should be interpreted in the context of more rapid fat deposition in healthy American adolescents during puberty (Codeiro *et al.* 2006; NCHS 1974). The overall pattern of adolescent nutrition and growth in this Malawian population supports the conclusion of prolonged linear growth with restricted catch-up. However, because of some misclassification, the analysis emphasizes the importance of developing an appropriate reference standard showing growth deviations in adolescents from countries with later onset of menarche. This would allow for nutritional differences related to age of menarche between Western and African populations to be better assessed.

The results show that participant age, paternal illiteracy, maternal illiteracy (though borderline) as well as AGLIT attendance were associated with increased undernutrition as measured by BMI in the univariate analysis. By menarche status, the significant univariate association with age is however only significant in premenarcheal participants, reflecting the relevance of puberty in assessing growth.

The criteria for selection into the literacy programme were illiteracy and not attending school. This prior selection was reflected in these nutrition characteristics indicating an already vulnerable population of adolescents. Initial attendance at AGLIT classes equates to a proxy measure for this vulnerability. Around a third of participants were illiterate.In multivariate analysis, only maternal illiteracy significantly predicted an increased risk of undernutrition. Maternal illiteracy has been associated with poor health and nutrition during childhood (Caldwell 1979; Caldwell 1986).

Most participants were in good health although symptoms associated with urinary tract infections were common and there was significant correlation of the UTI symptoms with haematuria. This area is endemic for infection with schistosomiasis (Bowie *et al.* 2004) and the high haematuria prevalence indicates the magnitude of this problem in these communities. Episodes of haematuria were probably confused with urinary tract infection. Environmental parasitic infestations are almost universal in people living in poverty and population-based studies into their relationship to growth faltering in children are advocated (Salazar-Lindo *et al.* 2004). A small seasonal increase in mean haemoglobin was observed during the wet season, which was significant. This may be due to differences in survey populations or could indicate differences in food availability or infection exposure from other pathogens. Malaria was more frequent in the wet season when diagnosis was based on the rapid diagnostic test (RDT), which assesses current and recent exposure in the previous 2 to 4 weeks, although not more frequent when based on malaria microscopy. The higher RDT positivity compared to the malaria microscopy prevalence estimates reflect the fact that the RDT used would assess period prevalence in terms of exposure to malaria infection. Malaria microscopy measures only current malaria parasitaemia with

parasites densities above 50/ μl blood (Cruciani *et al.* 2004). The rapid diagnostic test used in this study measures histidine rich protein II.

Undernutrition in childhood and adolescence leads to poor physical and mental development, effects school performance and reduces adult size and capacity for physical work (ACC/ SCN 1997). Undernutrition also influences the host's immune response against infection and potentiates the effects of infection through decreased food intake and impaired nutrient absorption, transport and utilization in target tissues. In adolescent girls from rural African communities, this is at a critical stage prior to their first pregnancy. The potential role of nutritional interventions during this period should be considered and few studies have addressed this possibility in this target age group (ACC/ SCN 2000).

4.5 Conclusions

Stunting and undernutrition were common amongst non-pregnant female adolescents in these poor rural communities of the Shire valley and exposure to malaria infection and infection with schistosomiasis was not infrequent. Nutritional interventions should be considered in this population as a strategy to improve their health and in order to improve nutritional status prior to pregnancy. Such interventions could include fortified foods or multi-micronutrient supplements. There is a need for randomized controlled trials to assess their benefits in these nutritionally vulnerable populations. Increased public health control measures are required to address the high prevalence of schistosomiasis and substantial exposure to malaria.

CHAPTER FIVE

SEASONAL MALARIA PREVALENCE IN NON-PREGNANT ADOLESCENT FEMALES AND ASSOCIATED FACTORS

5.1 Introduction

Susceptibility to *Plasmodium falciparum* malaria is age dependent such that adolescents from malaria endemic countries acquire partial immunity with increasing age due to frequent childhood exposure to malaria parasites (MacDonald 1957). As such, malaria infection among adolescents in these endemic areas is thought to be infrequent and asymptomatic and therefore to remain undetected and untreated. The evidence for this is limited and there is little data on malaria prevalence in adolescents (Lalloo *et al.* 2006). A review of malaria burden in adolescents from seven countries with a cumulative sample size of 1,068 estimated the incidence of clinical malaria in adolescents at 0.13 to 1.18 attacks per year in stable transmission areas (Lalloo *et al.* 2006).

Gender also influences malaria susceptibility due to differential exposure to infective vectors, which relates to differences in work, behavioral patterns and possibly host susceptibility between males and females, translating into differing infection risk (Brabin & Brabin 1992a). This differential exposure influences the intrinsic features of the immune response (Abebe *et al.* 2002). Endocrine factors may also be important, but this aspect has been little studied. In animal studies, raised estradiol has been associated with decreased parasitaemia and increased cerebral malaria incidence, while the adrenal hormone, dehydroepiandrosterone (DHEA) had no effect (Libonati *et al.* 2006). Testosterone in experimental animals was reported to

impair efficacy of protective malaria vaccination (Wunderlich *et al.* 1993), and suppressed the protective responses to both liver and blood stage malaria (Krucken *et al.* 2005). Few human studies suggest that host susceptibility to malaria infections is modified by endocrine status and development during puberty (Kurtis *et al.* 2001; Leenstra *et al.* 2003). Kurtis *et al.* (2001) showed that DHEAS levels positively correlated with malaria immunity acquired in puberty and Leenstra *et al.* (2003) showed that increasing DHEAS levels were positively associated with acquisition of malaria immunity during puberty. It is not known if gynaecological age is associated with malaria prevalence.

5.2 Objective

The objective of this study was to describe the point prevalence and associated factors of malaria parasitaemia and symptomatic malaria in non-pregnant adolescent females during the dry and wet seasons.

5.3 Statistical analysis

Data was analysed in Stata 8.0 and SPSS 15.0. Comparisons were made between dry and wet seasons, adjusted for clustering effects using the generalized estimating equations analysis algorithm with independent correlation matrix. Malaria parasite prevalence is assessed on the basis of microscopy results. Symptomatic malaria was defined as malaria parasitaemia with recorded fever (tympanic temperature ≥ 37.5 ° C). A rapid malaria diagnostic test was also used as an indicator of current or recent malaria exposure, which represents exposure within the 2 to 4 weeks prior to the time of the test. Statistical significance was considered at a p-value < 0.05. Potential variables for consideration for a logistic regression analysis were identified using a cut-off p-value of < 0.2. Single variables were entered for univariate

analysis, with and without adjusting for the effects of survey season. Univariate and multivariate analyses were done to identify and quantify factors associated with malaria parasitaemia and symptomatic malaria.

5.4 Results

5.4.1 Reliability of microscopy results

Comparison of microscopy results are presented in Table 5.1. There was good agreement between readers 1 and 2; agreement was 93.3 %, kappa statistic 0.684 (standard error 0.058). In comparison to expert microscopy, agreement was 89.4 %, kappa statistic 0.641 (standard error 0.214).

Table 5.1: Comparison of light microscopy results in non-pregnant adolescents

Reader 1 and Reader 2			
		Reader 1	
		Positive	Negative
Reader 2	Positive	252	8
	Negative	5	2
Agreement		93.3 %	
Kappa statistic		0.684	
Standard error		0.058	

Reader 2 and Expert reader			
		Expert reader	
		Positive	Negative
Reader 2	Positive	0	1
	Negative	2	17
Agreement		89.4 %	
Kappa statistic		0.641	
Standard error		0.214	

5.4.2 Prevalence of malaria parasites and related malariometric measures

Malaria prevalence and related malariometric indices are presented in Table 5.2.

Table 5.2: Malaria prevalence and malariometric variables by season of survey

Variable	N	Dry season n =477	N	Wet season n =786	p-value‡
Malaria microscopy positive	475	16 (3.4)	779	44 (5.7)	0.070
Malaria rapid test positive†	477	56 (11.7)	780	147 (18.8)	0.001
Symptomatic malaria	460	13 (2.8)	757	27 (3.6)	0.347
Fever – within prior 3 days	472	85 (18.0)	785	153 (19.6)	0.497
Body temperature ≥ 37.5 °C	460	87 (18.2)	757	189 (23.5)	0.027
Mean body temperature in °C §	460	37.4 (0.4)	757	37.2 (0.4)	< 0.01
Mean haemoglobin in g/dl §	300	11.2 (1.9)	775	11.5 (1.8)	0.022
Anaemia (Hb < 12 g/dl)	300	182 (60.7)	775	437 (56.4)	0.204
Severe anaemia (Hb < 8 g/dl)	300	16 (5.3)	775	28 (3.5)	0.203
ITN owned in household	458	353 (77.1)	782	648 (82.9)	0.010
Mean no. of ITNs in household §	458	1.6 (1.3)	782	1.7 (1.2)	0.020
Mean no. of ITNs in household* §	207	2.4 (1.1)	372	2.5 (1.0)	0.281
Participant owned/ accessed ITN	458	207 (45.2)	782	372 (47.6)	0.439
Used ITN previous night *	207	132 (63.7)	372	328 (88.2)	<0.001
No. of nights/week using ITN§ *	207	2.6 (1.9)	372	4.9 (2.2)	<0.0001

Values are number (percentage), except § mean (standard deviation); ‡ Adjusted for clustering
* Among participants who own an ITN; †Paracheck Pf test

Malaria parasites prevalence was higher in the wet season by a difference of 2.3 %, although this difference was not significant (p = 0.070). The rapid test results showed that the proportion with a positive result was significantly higher in the wet than the dry season (p = 0.001). Although the mean temperature was lower for the wet season than in the dry season, the proportion who were febrile in the wet season was higher than that in the dry season (p = 0.027). The prevalence of symptomatic malaria was 2.8 % in the dry and 3.6 % in the wet season (p = 0.347). Anaemia prevalence showed no seasonal variation and did not differ according to menarche status (p = 0.147). Severe anaemia prevalence showed no seasonal variation. Most households owned at least one ITN and ownership increased in the wet season (p = 0.010). Less than half of the participants owned or had access to an ITN and no seasonal differences in ownership were observed. Among the participants with access to an ITN, utilization

was significantly lower in the dry than the wet season and hot weather was most frequently cited for non-use.

The prevalence of participant parasitaemia and fever categories based on alternative parasite density cut-offs is summarized in Table 5.3. Prevalence estimates for parasitaemia with or without fever were lower for higher parasite density categories.

Table 5.3: Prevalence of parasitaemia and fever categories based on alternative parasite density cut-offs

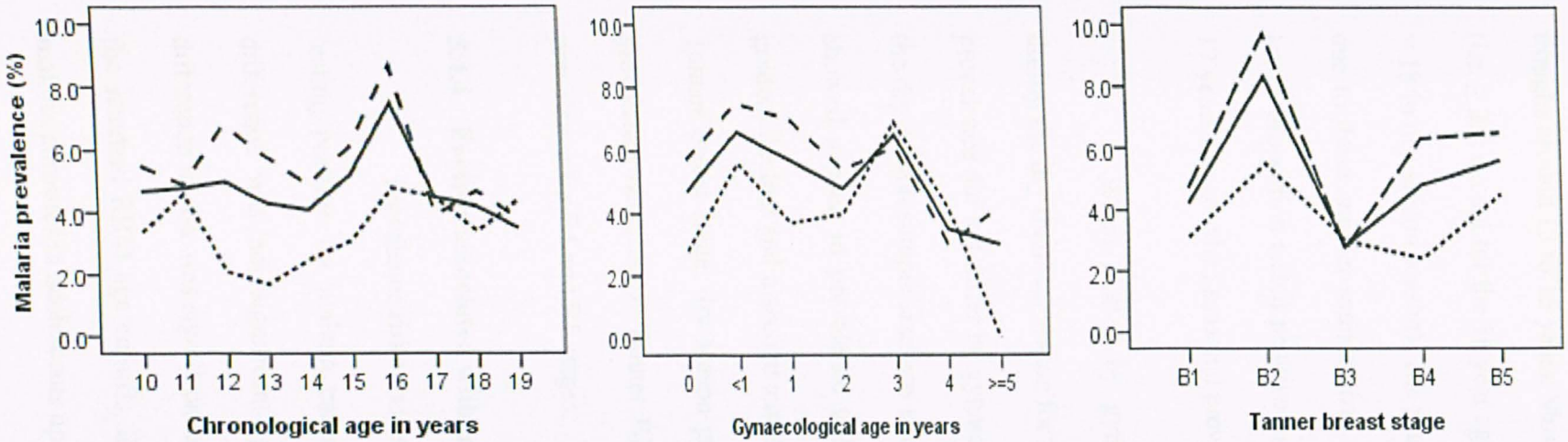
Participant category†	Any parasites	≥ 100/ µl	≥ 500/ µl	≥ 800/ µl	≥ 1000/ µl
Parasitaemia with fever	40 (3.3)	27 (2.2)	18 (1.5)	12 (1.0)	11 (0.9)
Parasitaemia with no fever	20 (1.6)	14 (1.2)	8 (0.7)	5 (0.4)	3 (0.4)

Data is for combined seasons (N=1217); brackets = percentage;
†definition of parasitaemia according to cut-off level shown; fever is tympanic temperature ≥ 37.5 °C.

5.4.3 Age-specific malaria parasite prevalence

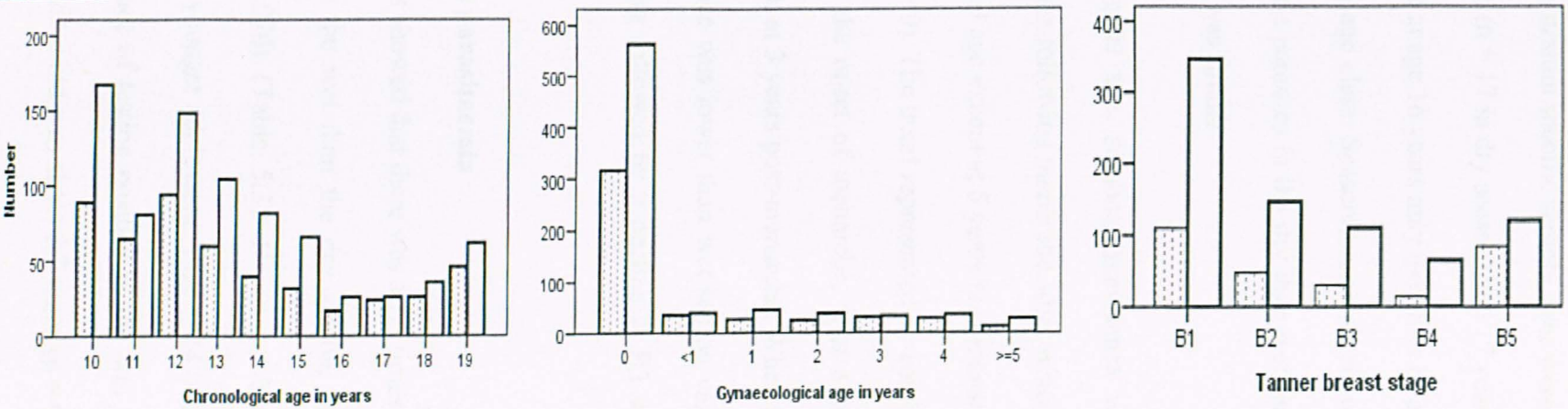
Trends in age-specific malaria prevalence for smear positive parasitaemia are presented in Figure 5.1.

Figure 5.1: Prevalence of malaria parasitaemia in non-pregnant adolescents by chronological age, gynaecological age and Tanner breast stage



Stippled line = dry season; broken line = wet season; continuous = combined data

Sample sizes



Stippled bars = dry season; clear bars = wet season

Using the trendline for combined data by chronological age, an age-dependent decline in malaria prevalence was observed with chronological age with troughs around 13 to 15 years. Most of the stratum specific sample sizes were adequate (i.e. ≥ 25) except for the 16 year age group (n = 17 in dry season) and 17 year group (n = 18 in dry season season). The peak value at age 16 years may therefore be artefactual due to these smaller sample sizes for the age class. Seasonal analysis showed that a lower proportion tested positive for malaria parasites in the dry than wet season until 17 years of age when seasonal prevalence was similar.

When plotted by gynaecological age, seasonal prevalence was lower during the dry than wet season for two years following menarche, after which seasonal prevalence did not differ by gynaecological age except at 5 years post-menarche when the dry season sample size was small (n = 9). The trend represented by combined data showed a peak in prevalence following the onset of menarche, with a subsequent gradual decline and a second smaller peak at 3 years post-menarche. When plotted by Tanner breast stage, dry season prevalence was lower than wet season values. Peak prevalence occurred at Tanner B2 staging followed by a decline at B3 with rising prevalence for B4 and B5 stages.

5.4.4 Factors associated with malaria parasitaemia

Prevalence risk ratio estimates showed that there was a 1.7 times chance of testing positive for malaria parasites in the wet than the dry season, though this difference was not significant (p = 0.070) (Table 5.3). However, this seasonal difference in risk was significant among younger adolescents (age $\leq$ 14 years). Using the standard RBM age cut-offs, the chance of testing positive in the wet season for malaria parasites in adolescents up to 14 years old was about 2.3 times (p = 0.031) that

in the dry season. Using a physiological age cut-off, the chance of testing positive for malaria parasites in the wet season in early adolescence (i.e. up to 13 years old) was 2.7 times (p = 0.036) that in the dry season. Risk estimates did not differ for older physiological age groups (Table 5.4).

Table 5.4: Prevalence risk ratios (PRR) of malaria parasitaemia in wet compared to dry season by adolescent age groups

Adolescent group	PRR (95 % CI)	p-value‡
All	1.71 (0.96, 3.08)	0.070
10 - 14 years	2.28 (1.08, 4.82)	0.031
15 - 19 years	0.99 (0.37, 2.63)	0.988
Early adolescence (10 – 13 years)	2.73 (1.06, 5.74)	0.036
Middle adolescence (14 – 17 years)	1.82 (0.65, 5.12)	0.254
Late adolescence (18 – 19 years)	0.16 (0.02, 1.49)	0.109

‡ Adjusted for clustering

Univariate regression analyses for factors associated with malaria parasitaemia are presented in Table 5.5. Results are presented both unadjusted and adjusted for season of survey.

Table 5.5: Univariate analysis of factors associated with malaria parasite prevalence adjusted and unadjusted for season

	Malaria parasites					
Co-variate	Yes	No	Season Unadjusted		Season adjusted	
	Mean (sd)	Mean (sd)	PRR (95% CI)	p-value‡	PRR (95% CI)	p-value‡
Chron. Age, years	13.3 (2.6)	13.1 (2.7)	0.96 (0.92,0.98)	0.031	0.96 (0.93,0.98)	0.030
Gynae. Age, years	0.5 (1.0)	0.7 (1.3)	0.65 (0.45, 0.95)	0.027	0.66 (0.46, 0.96)	0.031
Education, years	3.6 (2.0)	3.6 (2.2)	1.00 (0.99,1.01)	0.988	1.00 (0.99,1.01)	0.853
Maternal education	5.0 (3.4)	4.8 (3.5)	1.00 (0.99,1.01)	0.539	1.00 (0.99,1.01)	0.513
Paternal education	5.9 (3.2)	7.4 (3.3)	0.99 (0.98,0.99)	0.005	0.99 (0.98,0.99)	0.006
Tanner breast stage	2.6 (1.6)	2.3 (1.5)	1.02 (1.00,1.03)	0.010	1.02 (1.00,1.03)	0.009
ITNs in household	1.7 (1.3)	1.7 (1.2)	1.00 (0.99,1.01)	0.854	1.00 (0.99,1.01)	0.780
Nights/wk under ITN	4.8 (1.9)	4.2 (2.4)	1.00 (0.99,1.02)	0.327	1.00 (0.99,1.02)	0.551
Literacy score	7.0 (5.3)	7.8 (5.4)	1.00 (0.99,1.00)	0.470	1.00 (0.99,1.00)	0.597
HAZ	- 0.9(1.2)	- 0.7 (1.2)	0.99 (0.98,1.00)	0.087	0.99 (0.98,1.00)	0.140
WAZ	- 1.2 (0.8)	- 1.3 (0.7)	1.01 (0.99,1.03)	0.321	1.01 (0.99,1.03)	0.235
BMIZ	- 1.5 (1.3)	- 1.3 (1.3)	0.99 (0.98,1.00)	0.228	0.99 (0.98,1.00)	0.195
	Number	%				
Attended AGLIT: No	57 / 1203	4.7	1.00		1.00	
Yes	3 / 50	6.0	1.40 (0.41, 4.65)	0.594	1.57 (0.46, 5.30)	0.471
Illiterate	22 / 430	5.1	1.00		1.00	
Semi-literate	9 / 123	7.3	1.42 (0.64, 3.17)	0.394	1.42 (0.64, 3.18)	0.391
Literate	29 / 681	4.3	0.80 (0.45, 1.42)	0.450	0.85 (0.48, 1.51)	0.571
ITN in household: No	14 / 235	6.0	1.00		1.00	
Yes	46 / 997	4.6	0.74 (0.40, 1.37)	0.342	0.71 (0.38, 1.33)	0.288
Accessed ITN: No	33 / 658	5.0	1.00		1.00	
Yes	27 / 575	4.7	1.02 (0.60, 1.73)	0.952	1.01 (0.59, 1.72)	0.971
ITN prev night:No	36 / 769	4.7	1.00		1.00	
Yes	24 / 462	5.2	1.22 (0.71, 2.09)	0.475	1.15 (0.67, 1.99)	0.608
Low MUAC-FA: No	23 / 366	6.3	1.00		1.00	
Yes	37 / 854	4.3	0.66 (0.39, 1.14)	0.137	0.69 (0.39, 1.16)	0.158
Hb < 12 g/Dl: No	20 / 454	4.4	1.00		1.00	
Yes	34 / 619	5.5	1.21 (0.69, 2.15)	0.504	1.24 (0.70, 2.19)	0.464

PRR – prevalence risk ratio; ‡ adjusted for clustering; sd – standard deviation; Chron. Age - chronological age; Gynae. Age - gynaecological age; HAZ – height for age z-score; WAZ – weight for age z-score; MUAC – mid-upper arm circumference; AGLIT – adolescent girls literacy programme; ITN – insecticide treated bed net.

Chronological age, gynaecological age, paternal education and pubertal stage by Tanner breast staging were associated with malaria parasitaemia in univariate analysis. There was no significant association with anthropometric indices, maternal or

participant literacy, availability and access to an ITN in households, or the number of nights of ITN use per week.

In multivariate analysis, seven co-variates were entered (chronological age, gynaecological age, paternal education, Tanner breast stage, HAZ, BMIZ and low MUAC-FA). The final model showed that malaria prevalence was significantly associated with chronological age (PRR 0.92, 95 % CI 0.87, 0.97, p = 0.025), gynaecogical age (PRR 0.63, 95 % CI 0.40, 0.98, p = 0.045) and paternal education (PRR 0.88, 95 % CI 0.80, 0.97, p = 0.009), adjusted for season of survey.

5.4.5 Prevalence of symptomatic malaria and its associated factors

Symptomatic malaria was present in 13 (2.8 %) participants during the dry and in 27 (3.6 %) during the wet season and the seasonal difference was not significant (p = 0.347). The increased risk for symptomatic malaria in the wet than the dry season was 1.5 times (PRR 1.50, 95 % CI 0.64, 3.50, p = 0.347). Chronological age, paternal education, Tanner breast stage and height-for-age z-score were significantly associated with symptomatic malaria in univariate regression analysis, adjusted for season (Table 5.6). Chronological age, gynaecological age, paternal education, Tanner breast stage, and HAZ were entered for multivariate analysis. In multivariate analysis adjusted for season, significant associations were observed with chronological age (PRR 0.93, 95 % CI: 0.86, 0.98; p = 0.023) and paternal education (PRR 0.83, 95 % CI: 0.74, 0.94; p = 0.002) and borderline significance with gynaecological age (PRR 0.65, 95 % CI 0.40, 1.01, p = 0.053).

Table 5.6: Univariate analysis of factors associated with symptomatic malaria prevalence adjusted and unadjusted for season

	Symptomatic malaria					
Co-variate	Yes	No	Season Unadjusted		Season adjusted	
	Mean (sd)	Mean(sd)	PRR (95% CI)	p-value‡	PRR (95% CI)	p-value‡
Chron. Age, years	13.7 (2.9)	13.2 (2.8)	1.23 (1.02,1.50)	0.033	1.23 (1.02,1.49)	0.033
Gynae. Age, years	0.7 (1.2)	0.7 (1.4)	0.65 (0.45, 0.98)	0.047	0.66 (0.46, 0.96)	0.041
Education, years	3.7 (1.9)	3.6 (2.2)	1.01 (0.87,1.16)	0.905	1.02 (0.88,1.17)	0.834
Maternal education	4.5 (3.2)	6.1 (4.3)	0.97 (0.88,1.07)	0.521	1.00 (0.99,1.01)	0.513
Paternal education	5.4 (3.4)	8.7 (3.7)	0.83 (0.74,0.93)	0.002	0.99 (0.98,0.99)	0.006
Tanner breast stage	2.9 (1.7)	2.4 (1.5)	1.52 (1.15,2.03)	0.003	1.53 (1.15,2.03)	0.003
ITNs in household	1.7 (1.3)	1.7 (1.2)	0.99 (0.77,1.28)	0.956	0.99 (0.76,1.28)	0.915
Nights/wk under ITN	4.4 (2.0)	4.1 (2.4)	1.06 (0.77,1.46)	0.722	1.04 (0.72,1.49)	0.839
Literacy score	7.0 (6.8)	7.6 (5.6)	0.99 (0.94,1.05)	0.829	1.00 (0.94,1.06)	0.896
HAZ	- 1.3 (1.2)	- 0.8(1.2)	0.69 (0.50,0.93)	0.017	0.70 (0.51,0.95)	0.022
WAZ	- 1.1 (0.8)	-1.3 (0.7)	1.43 (0.87,2.35)	0.153	1.49 (0.90,2.46)	0.235
BMIZ	- 1.4 (1.1)	-1.3 (1.3)	0.92 (0.73,1.17)	0.501	0.92 (0.72,1.16)	0.474
	Number	%				
Attended AGLIT: No	37 / 1203	3.1	1.00		1.00	
Yes	3 / 50	6.0	1.89 (0.56, 6.34)	0.302	2.02 (0.60, 6.83)	0.258
Illiterate	14 / 430	3.3	1.00		1.00	
Semi-literate	5 / 123	4.1	1.26 (0.44, 3.57)	0.665	1.26 (0.44, 3.57)	0.665
Literate	21 / 681	3.1	0.95 (0.48, 1.88)	0.876	0.97 (0.49, 1.94)	0.932
ITN in household: No	8 / 235	3.4	1.00		1.00	
Yes	32 / 997	3.2	0.94 (0.43, 2.07)	0.880	0.92(0.42, 2.01)	0.844
Accessed ITN: No	19 / 658	2.9	1.00		1.00	
Yes	21 / 575	3.7	1.24 (0.66, 2.33)	0.508	1.23 (0.67, 2.31)	0.515
ITN prev night:No	22 / 769	2.9	1.00		1.00	
Yes	18 / 462	3.9	1.36 (0.72, 2.56)	0.346	1.32 (0.70, 2.51)	0.393
Low MUAC-FA: No	15 / 366	6.3	1.00		1.00	
Yes	25 / 854	2.9	0.71 (0.39, 1.36)	0.296	0.71 (0.37, 1.37)	0.313
Hb < 12 g/Dl: No	11 / 454	2.4	1.00		1.00	
Yes	25 / 619	4.0	1.69 (0.83, 3.48)	0.151	1.71 (0.83, 3.50)	0.146

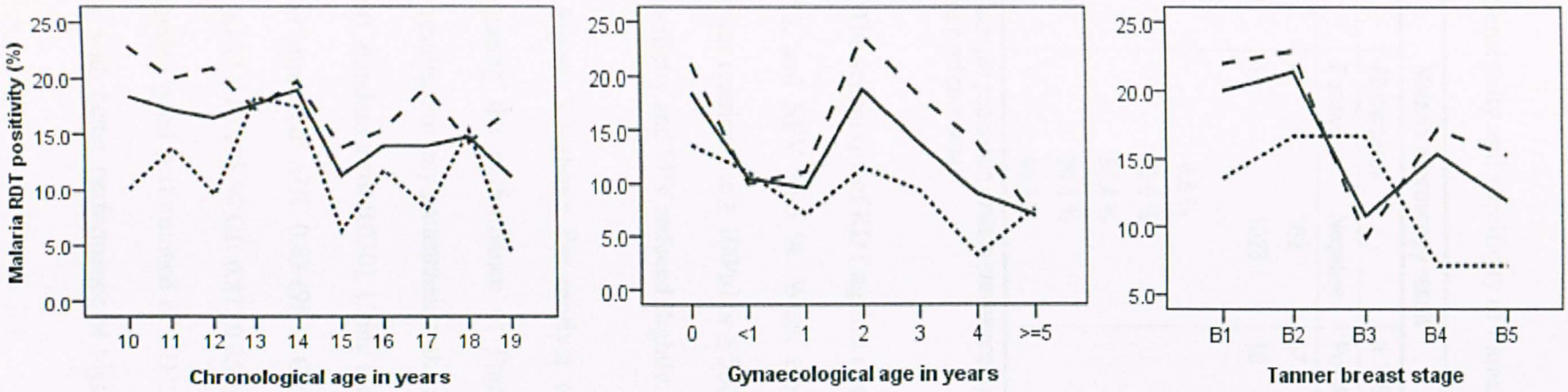
PRR – prevalence risk ratio; ‡ adjusted for clustering; sd – standard deviation; Chron. Age - chronological age; Gynae. Age - gynaecological age; HAZ – height for age z-score; WAZ – weight for age z-score; MUAC – mid-upper arm circumference; AGLIT – adolescent girls literacy programme; ITN – insecticide treated bed net.

5.4.6 Validity of Paracheck-Pf rapid diagnostic test

Using the malaria rapid diagnostic test (RDT) as a proxy measure of malaria infection exposure, 56 (11.7 %) of the participants tested positive in the dry

and 147 (18.8 %) in the wet seasons ($p = 0.001$). The seasonal trends by chronological age, gynaecological age and Tanner breast stage showed a similar pattern to those observed based on microscopy with an age-dependent decline by chronological age and pubertal reversal in the first 2 years post menarche, and at B2/B3 Tanner breast stages (Figure 5.2).

Figure 5.2: Trends in malaria Paracheck Pf rapid diagnostic test (RDT) positivity by chronological age, gynaecological age and Tanner breast stage



Stippled line = dry season; broken line = wet season; continuous = combined data

Results of RDT findings compared to microscopy are shown in Table 5.7.

Table 5.7: Sensitivity and specificity of Paracheck Pf RDT against microscopy

	Malaria microscopy result					
	All parasites		≥ 100 parasites/ µl		≥ 500 parasites/ µl	
	Positive	Negative	Positive	Negative	Positive	Negative
RDT positive	42	162	37	167	21	183
RDT negative	18	1028	10	1036	5	1041
Prevalence	4.8 %		3.8 %		2.1 %	
Sensitivity	70.0 %		78.7 %		80.8 %	
Specificity	86.4 %		86.1 %		85.0 %	
PPV	20.6 %		18.1 %		10.3 %	
NPV	98.3 %		99.0 %		99.5 %	

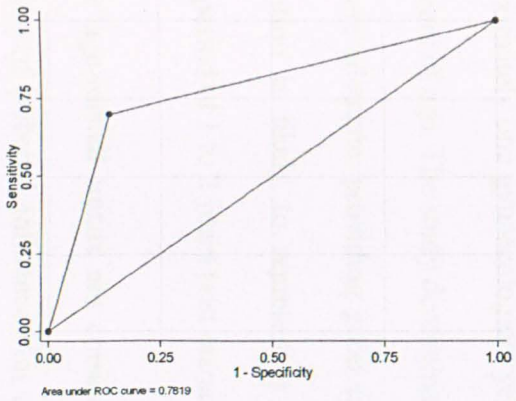
Prevalence is sample population malaria prevalence by microscopy, PPV – positive predictive value, NPV – negative predictive value

The sensitivity of RDT against microscopy was 70.0 %, specificity 86.4 %, PPV 20.6 %, and NPV 98.3 %. When the criterion for presence of parasites by microscopy was restricted to ≥ 100/µl or ≥ 500/µl, RDT sensitivity and NPV improved although specificity and PPV reduced slightly.

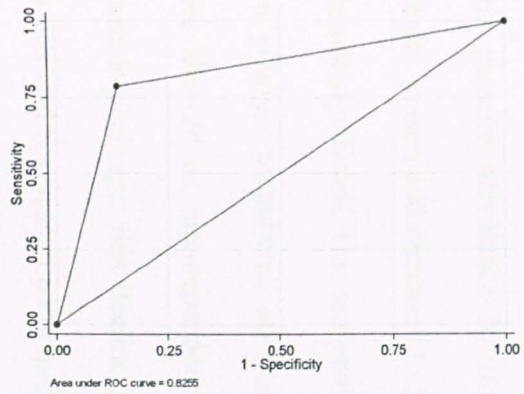
Figure 5.3 shows the receiver operating characteristic (ROC) analysis curves comparing the performance of Paracheck RDT test results against light microscopy results. For any parasitaemia, the area under curve (AUC) was 0.78 (95 % CI: 0.76, 0.80, standard error 0.030). Using the 100 and 500/µl parasite density cut-off, the AUC was improved: AUC 0.83 (95 % CI: 0.78, 0.89, standard error 0.031) for the former and AUC 0.83 (95 % CI: 0.81, 0.85, standard error 0.040) for the latter. This shows reasonably good performance of RDT in identifying participants with malaria parasitaemia, with better performance at higher parasite density levels of more than 500/µl.

Figure 5.3: Receiver operating curves of Paracheck Pf rapid malaria diagnostic test compared to malaria light microscopy

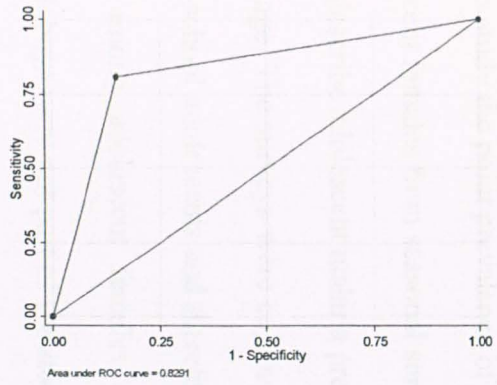
Any parasitaemia



Using 100 parasites / µl as cut-off



Using 500 parasites / µl as cut-off



5.5 Discussion

In this study the point prevalence of malaria parasitaemia and symptomatic malaria in adolescent females from seasonal surveys are described and this is possibly the first study to describe adolescent malaria prevalence by gynaecological as opposed to chronological age. The surveys were community-based and captured both school-going and out-of-school adolescents and therefore should be representative of malaria prevalence risk among adolescent females in these rural village communities. Comparisons with boys were not part of the study design and therefore the results can not be generalized to adolescent males.

Malaria parasite prevalence by microscopy was 3.4 % in adolescent females during the dry season and increased to 5.7 % during the wet season, although this difference was not significant. This seasonal difference was more pronounced and was significant in younger prepubertal adolescents. By chronological age, malaria parasite prevalence showed an age-dependent decline with increasing age. When analysed by gynaecological age, this decline showed a reversal at one year post-menarche in both wet and dry season participants suggesting a pubertal influence on acquisition of age dependent malaria immunity post-menarche. This reversal spanned a period of approximately one gynaecological year and occurred between one and two gynaecological years of age. The study denominators for each gynaecological age class before 5 years were adequate, providing good sample sizes, justifying the conclusion that this observation is likely to represent a pubertal reversal of age-dependent immunity over a period of 1 to 2 years post menarche.

These age-related trends are consistent with existing observational and biological evidence of pubertal influences on malaria immunity. Increased malaria

transmission in the wet season was apparent in the younger participants as their risk of malaria parasitaemia was significantly 2.2 times increased compared to the dry season, yet this seasonal effect was not significant in the older adolescents. This indicates that immunity was better developed in the older adolescents. The multivariate regression analyses, which adjusted for seasonal effects, confirm that this observation was not due to solely to chronological age but also to the effects of gynaecological age. Changes in the levels of the DHEA- sulphate (DHEAS), at the onset of puberty onset and the velocity of these changes are highest as puberty progresses and have been positively associated with the pubertal levels in acquisition of malaria immunity in African non-pregnant adolescent girls (Leenstra *et al.* 2003). A positive correlation with malaria immunity acquired in puberty among African adolescent boys has also been shown in the same Kenyan adolescent population (Kurtis *et al.* 2001). A dose-dependent association of DHEAS with inflammatory markers related to malaria immunity has also been demonstrated with increased levels of DHEAS correlating with increased levels of the inflammatory markers IL-6 and TNF-α (Coutinho *et al.* 2007). The endocrinological correlates of the initial pubertal reversal of immunity are unclear. Higher DHEAS levels later in puberty would be temporarily associated with declining malaria prevalence in older adolescents with later acquisition of age-dependent immunity. An increased risk of malaria parasitaemia has been associated with higher blood cortisol concentrations in pregnant women (Vleugels *et al.* 1987; Vleugels *et al.* 1989), but whether this correlation holds in non-pregnant individuals is unclear.

Better understanding of the endocrinological mechanisms involved would be beneficial in modeling of malaria risk in non-pregnant female populations. This has relevance to the potential use of malaria vaccines which may be targeted at young non-pregnant adolescent girls prior to their first pregnancy, in order to protect them from

malaria risk during their subsequent initial pregnancy. It is unclear whether gynaecological age would modify malaria infection risk in adolescents when pregnant, or whether intermittent preventative therapy for pre-pregnant adolescents would reduce malaria-related complications in subsequent adolescent pregnancy. In the present survey parasite prevalence was lower than might be expected in an area considered to have high transmission. This may be due to more widespread use of ITNs in these communities as well as possible recent climatic changes. The level of the malaria prevalence in this study would not justify routine use of an intermittent preventive antimalarial treatment strategy, especially as undetected pregnancies may lead to inadvertent antimalarial drug exposures to the fetus in the first trimester. Much greater emphasis is required on improving access to and utilization of ITNs in this age group as in principle 100 % utilization is required in order to reduce current and early pregnancy parasitaemic risk.

This cross-sectional data indicates that among adolescent females under conditions of high transmission, malaria infection was not infrequent during the dry as well as the wet season. Seasonal patterns of malaria have previously been reported in Kenyan school-going non-pregnant female adolescents (12 – 18 years) which ranged from 20.4 % during the dry season to 33.7 % during the post-rainy season (Leenstra *et al.* 2004). Other African cross-sectional studies have reported prevalence estimates ranging from as 8 % (Lusingu *et al.* 2004) during the low transmission season to as high as 84 % among younger adolescents of 10 to 14 years of age (Trape *et al.* 1987).

In univariate analysis, symptomatic malaria was significantly higher among those with lower z-scores for height, which did not remain significant in multivariate analysis. This association with stunting indicates the importance of

chronic undernutrition and its link with poverty as well as poor nutrition. Poorer households are less likely to access health services which may be compounded by illiteracy, which was also associated with stunting in the multivariate analysis presented in Chapter four. Stunting was not associated with a positive RDT result which may indicate that the association with symptomatic malaria was related more to decreased use of health services, rather than reflecting an association with malaria exposure risk.

Symptomatic malaria was present in only 3.2 % of the participants and no seasonal differences were observed. Symptomatic malaria was defined on the basis of measured fever and the presence of parasites on light microscopy using a standard methodology (WHO 2000) and these results reflect malaria diagnosis under field conditions. Light microscopy has limitations at low parasite density (< 20 parasites/ μl) and reduced sensitivity leading to an under-estimation of malaria infection (Roshanravan *et al.* 2003). Exposure to malaria infection as measured by RDT positivity was significantly higher in the wet than dry season. The higher RDT result reflects its assessment of period prevalence in terms of exposure to both current malaria and previous malaria infection in the last 2 to 4 weeks. RDT sensitivity was only 70 % indicating a proportion of false negative results, which is more likely to occur with low parasite densities in blood, especially below 100 parasites per μl blood. The present results showed reasonably good agreement of the RDT compared to light microscopy, especially at higher parasite density. Reduced RDT sensitivity may occur in areas of high transmission as many individuals with a good level of acquired immunity will have low parasite densities (Cruciani *et al.* 2004). Most RDT validation studies have been conducted in areas of low to moderate transmission where high sensitivies were observed, and validation studies in areas of intense transmission are

limited or have reported lower sensitivities (Marx 2005). Persistence of HRP-2, on which Paracheck is based, has been reported after day 14 in up to 10 % of patients after malaria therapy and this would limit distinctions between clinically important parasitaemia from otherwise successfully treated infections (Moody 2002; Murray 2003). The seasonal RDT positivity pattern indicates a seasonal exposure to malaria infection. The RDT prevalence estimates parallel changes with microscopy prevalence, providing supporting evidence for the observation of an early pubertal reversal of age dependent malaria immunity based on parasitaemia prevalence.

Increasing chronological age and better participant education were both associated with significantly reduced malaria prevalence after adjusting for the confounding effects of season and adolescent maturation. The age-related changes are best explained as due to the acquisition of age dependent immunity, which should be apparent in the older adolescent age group. The significant association with paternal education is of interest, especially as an association with maternal education or AGLIT attendance was not identified. One explanation is that this reflects household income levels related to more frequent employment of better educated fathers. Income levels were not assessed in the study as it was felt they would be unreliable. The high level of significance of the association makes it unlikely that this finding was due to chance. The lack of an association of malaria risk with attendance at the rural AGLIT programme designed to improve adolescent health literacy could be interpreted as an indication that this knowledge based programme failed to improve malaria preventive practices and reducing malaria risk. The selection of girls for the AGLIT programme was based on those with no previous education or literacy skills and this group might be considered to be at increased not decreased risk for malaria. Therefore the absence of increased malaria risk might be interpreted to be indicative of a beneficial effect of

the AGLIT programme. Further issues related to AGLIT attendance are discussed in Chapter eight.

5.6 Conclusion

The results provide some evidence for a pubertal reversal of malaria prevalence which could delay the onset of adult age-dependent immunity. Biomedical studies to explore the cause-effect relationship of pubertal changes and malaria are required in order to test this hypothesis of a pubertal influence on malarial immunity. These non-pregnant adolescent females were at not infrequent risk of malaria. Parasitaemia was associated with anaemia and lower paternal education, which could result from poor access to health services. Compromise of these girls health status during adolescence increases their risk of morbidity during their subsequent first pregnancy. The continuity of these effects could be cumulative as during the first pregnancy there is an additional markedly increased risk for *P. falciparum* parasitaemia and anaemia.

CHAPTER SIX

ACCESS AND UTILISATION OF INSECTICIDE TREATED BEDNETS AMONG NON-PREGNANT ADOLESCENT FEMALES

6.1 Introduction

Insecticide-treated bed nets (ITNs) are highly effective in reducing malaria morbidity and mortality (Hill *et al.* 2006). A review of ITN trials among malaria endemic populations of all ages showed that this intervention can reduce episodes of uncomplicated malaria by as much as 50 % when compared to no use of nets (Lengeler 2000; Lengeler 2004). From fourteen randomized trials which included all ages, they estimated 45 % (95 % CI: 20 to 63 %) protective efficacy for the occurrence of severe malaria, 13 % protective efficacy for parasite prevalence, and 29 % efficacy for high density parasitaemia. Confidence intervals for the latter estimates were not calculated because results included cluster and individual randomized trials. In children (from five randomized trials), they reported 17 % (95 % CI: 10 to 24 %) protective efficacy for reduction in parasite prevalence, and significantly improved weight-for-age z-scores in Gambian and Kenyan children (Lengeler 2004; ter Kuile *et al.* 2003). ITNs also substantially reduce the number of malaria transmitting mosquitoes (Gimnig *et al.* 2003). Despite their efficacy, adherence is mostly low, which relates to a number of environmental factors such as night temperature and mosquito presence, household factors such as sleeping arrangements and physical factors in the household associated with the hanging of ITN, for example, absence of beds and mud walls (Alaii *et al.* 2003).

Information on the use and impact of ITNs in adolescence is limited. Studies from western Kenya among school-going adolescents showed benefits only among younger adolescents less than 15 years of age but not in older adolescents. ITN use improved body fat composition as measured by arm circumference but not linear growth (Friedman *et al.* 2003), reduced prevalence of all cause anaemia i.e. haemoglobin level <12 g/dL (adjusted OR 0.38, 95 % CI: 0.21, 0.69) and improved mean haemoglobin concentrations by 0.34 g/dL (95 % CI: 0.02, 0.66), (Leenstra *et al.* 2003). There was no associated impact on other malaria-related morbidity.

In pregnancy, several African trials have shown that ITNs significantly improved pregnancy outcome: with reduced placental malaria across all parities, and reduced low birth weight, fetal loss, maternal anaemia and clinical malaria prevalence (Gamble *et al.* 2006). One in six of these pregnant women are adolescent. In Malawi, although pregnant adolescents have a higher risk of malaria infection and poorer pregnancy outcomes (Brabin 1983; Brabin *et al.* 1998), their access to and use of ITNs in the Shire valley has been shown to be limited compared to pregnant adults (Msyamboza *et al.* 2008).

6.2 Objective

To measure the determinants of access and use of ITNs for malaria prevention in non-pregnant adolescents during the dry and wet seasons and the effects of reported ITN availability, access and use on malaria, anaemia and nutritional status.

6.3 Statistical analysis

Data was analysed in Stata 8.0 (Stata Corporation 2003) and SPSS 15.0 (LEAD Technologies Inc. 2006). Univariate and multivariate analyses were completed

to identify and quantify factors associated with ITN availability, access and use, modeled under the binomial distribution. Results reported were not adjusted for season because season is one of the known associated factors influencing ITN use in other African studies and therefore required exploration in these analyses. However, season adjusted results are presented alongside in univariate analyses for information in order to show that indeed most of the co-variates were not influenced by season, except in the case of AGLIT attendance where season adjusted results may be applicable. Factors for inclusion in the multivariate regression analysis were selected if achieving a p-value of 0.2 in the univariate analysis. Statistical significance was considered at a p-value < 0.05.

6.4 Results

6.4.1 Seasonal patterns of ITN availability, access and use.

Most participants came from households that owned at least one ITN: 77.1 % and 82.9 % in dry and wet seasons respectively, p = 0.010 (Table 6.1). Correspondingly, the mean number of ITNs per household was lower in the dry than wet season in these households among all participants (p = 0.020). This seasonal improvement did not translate into better access of ITNs in the adolescents as the proportion of participants with access to an ITN was not high and was comparable in the two seasons (45.2 % in dry and 47.6 % in the wet season). Equally, the mean number of ITNs in households where the participant reported having access to an ITN did not show any seasonal variation (p= 0.281).

Among the participants with access to an ITN, the use of ITNs in the previous night to survey day or by the estimated number of nights of use per week was significantly lower in the dry than the wet season. These two measures of ITN use are

estimating different aspects of ITN utilization: the former reflects community coverage while the latter reflects community as well as time coverage. Participants attributed hot weather and reduced mosquito nuisance as the reason for their reduced ITN use during the dry season.

Table 6.1: Seasonal patterns of reported ITN availability, access and use in non-pregnant adolescents by season of survey

Variable	N	Dry season n =477	N	Wet season n =786	p-value‡
ITN availability					
No.(%) households with ITN(s)	458	353 (77.1)	782	648 (82.9)	0.010
Mean (sd) ITNs in household	458	1.6 (1.3)	782	1.7 (1.2)	0.020
Mean (sd) ITNs in household*	207	2.4 (1.1)	372	2.5 (1.0)	0.281
ITN access					
No. (%) participants accessed ITN	458	207 (45.2)	782	372 (47.6)	0.439
*ITN use**					
No. (%) used ITN previous night	207	132 (63.8)	372	328 (88.2)	<0.001
Mean (sd) nights/week using ITN	207	2.6 (1.9)	372	4.9 (2.2)	<0.0001

‡ Adjusted for clustering; * among participants who had access to an ITN.

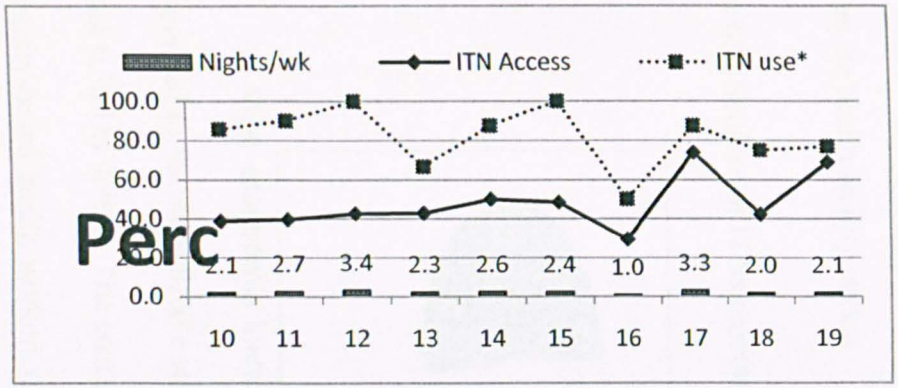
Patterns of ITN access and use by age are shown in Figure 6.1. ITN access increased with increasing chronological and gynaecological age in both seasons. Group specific sample sizes were adequate (i.e. $\geq$ 25) except for the 16 year chronological age group in dry season (n = 17) and gynaecological age 5 in dry season (n = 9). The reported ITN use during the previous night varied randomly both by chronological and gynaecological age in the dry season, with less variation and better use in the wet season. Seasonal variability in the estimated number of nights per week using an ITN was apparent by chronological and gynaecological ages.

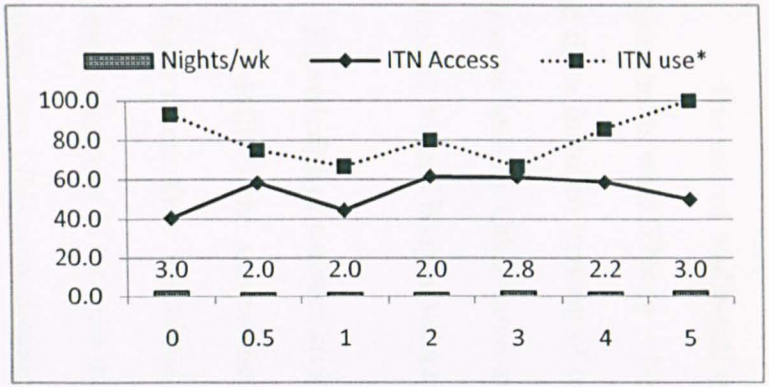
Figure: 6.1: ITN access and use in non-pregnant adolescent girls by chronological and gynaecological age, by season of survey

Chronological

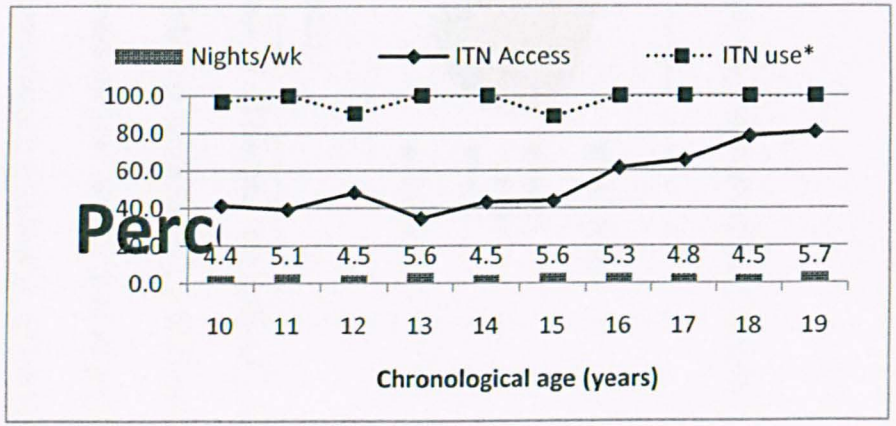
Gynaecological age

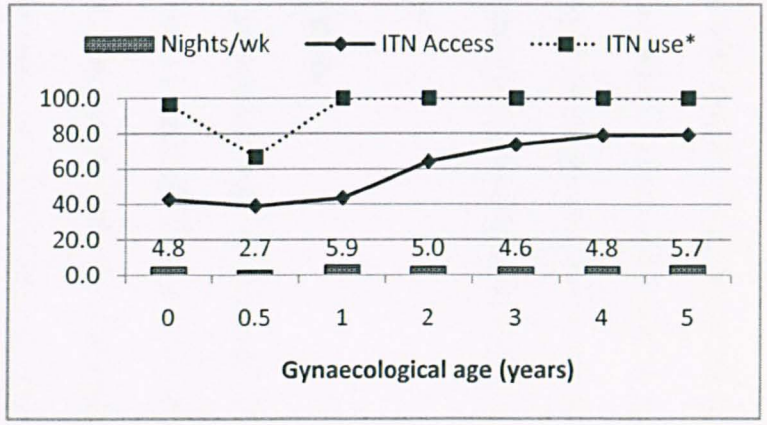
Dry season





Wet season





* Reported ITN use during the night prior to survey date.
Denominator for ITN access is all responding participants (n = 1240) and participants with ITN access is denominator for ITN use and nights/ week (n = 579)

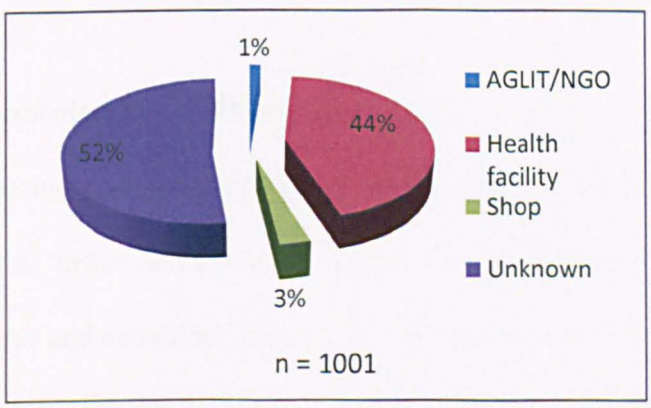
Use of other mosquito control measures

The use of traditional methods was common among participants coming from households with ITNs (n = 1001) as an additional mosquito control measure (52 %) and these included burning of cow dung or branches and leaves of the Neem tree (*Azadirachta indica*). Other common preventive measures included the use of coils (15 %), sprays (2 %) and body repellents (<1 %).

6.4.2 Knowledge of sources and cost of ITNs

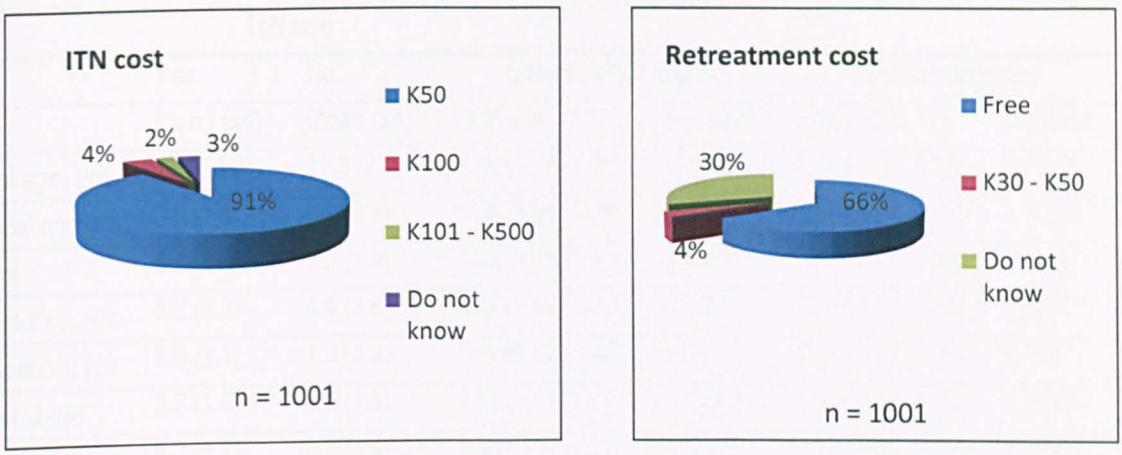
Half of the adolescents from households with at least one ITN did not know from where their household ITNs were procured (Figure 6.2). Of those that knew, the majority of ITNs were purchased from health facilities with a small number from shops; few ITNs were donations from either AGLIT or other non-governmental organizations (NGOs). Health surveillance assistants, antenatal and under-five clinics acted as the health facility outlets.

Figure 6.2: Sources of ITNs reported by non-pregnant adolescent females



Most adolescents knew the cost of ITNs and the majority of purchases corresponded to the subsidized cost of 50 Malawi Kwacha (K), which was equivalent to about 0.30 US dollars. The cost of re-treatment was usually free as provided by the community-based health workers (health surveillance assistants), or alternatively at a cost of 30 to 50 Kwachas (Figure 6.3).

Figure 6.3: Knowledge of ITN and re-treatment costs in non-pregnant adolescents



6.4.3 Factors associated with ITN availability

In univariate logistic regression analysis, household availability of ITNs was significantly associated with season (PRR 1.47, 95 % CI: 1.11, 1.96, p = 0.008) and paternal education (PRR 1.06, 95 % CI: 1.00, 1.12, p = 0.049). These factors remained significantly associated in multivariate analysis: season (PRR 1.55; 95 % CI: 1.04, 2.31, p = 0.032) and paternal education (PRR 1.06; 95 % CI: 1.00, 1.12, p = 0.041).

6.4.4 Factors associated with ITN access

Increasing chronological age or gynaecological age, better participant or maternal education, better literacy level, higher Tanner breast stage, higher BMI z-score, marital status and menarche status were significantly associated with ITN access in univariate analysis (Table 6.2). These variables together with paternal education and AGLIT attendance were entered for multivariate analysis as they satisfied the criteria of $p < 0.2$.

Table 6.2: Univariate analysis of factors associated with ITN access in non-pregnant adolescents

	ITN use					
Co-variate	Yes	No	Season Unadjusted		Season adjusted	
	Mean (sd)	Mean (sd)	PRR (95% CI)	p-value‡	PRR (95% CI)	p-value‡
Chronological age, yrs	13.6 (3.0)	12.3 (2.2)	1.20 (1.14, 1.27)	< 0.001	1.21 (1.15, 1.27)	< 0.001
Gynaecological age,yrs	0.9 (1.6)	0.3 (0.9)	1.53 (1.35, 1.74)	< 0.001	1.53 (1.35, 1.74)	< 0.001
Education, yrs	4.0 (2.3)	3.2 (1.9)	1.20 (1.13, 1.28)	< 0.001	1.20 (1.13, 1.28)	< 0.001
Maternal education, yrs	5.2 (3.3)	4.4 (3.6)	1.07 (1.02, 1.12)	0.008	1.07 (1.02, 1.12)	0.009
Paternal education, yrs	7.6 (3.1)	7.3 (3.3)	1.04 (0.99, 1.09)	0.131	1.04 (0.99, 1.09)	0.150
Tanner breast stage	2.7 (1.6)	1.9 (1.3)	1.41 (1.27, 1.55)	< 0.001	1.41 (1.28, 1.56)	< 0.001
Literacy score	8.2 (5.2)	6.7 (5.4)	1.05 (1.03, 1.08)	< 0.001	1.06 (1.03, 1.08)	< 0.001
HAZ	0.8 (1.1)	-0.8 (1.3)	0.99 (0.88, 1.11)	0.847	0.97 (0.86, 1.10)	0.681
WAZ	-1.3 (0.7)	-1.3 (0.8)	1.07 (0.88, 1.30)	0.502	1.07 (0.88, 1.30)	0.503
BMIZ	-1.2 (1.1)	-1.4 (1.3)	1.16 (1.04, 1.29)	0.007	1.16 (1.04, 1.29)	0.006
	Number (%)					
Season: Dry	207 / 458 (45.2)		1.00	0.588	n/a	
Wet	372 / 782 (47.6)		0.93 (0.71, 1.21)			
Attended AGLIT: No	547 / 957 (57.2)		1.00	0.131	1.00	0.140
Yes	29 / 42 (69.1)		1.67 (0.86, 3.26)		1.66 (0.85, 3.23)	
Unmarried: No	92 / 107 (86.0)		1.00	< 0.001	1.00	< 0.001
Yes	484 / 892 (54.3)		0.19 (0.11, 0.34)		0.19 (0.11, 0.34)	
Premenarche	361 / 864 (41.8)		1.00	< 0.001	1.00	< 0.001
Postmenarche	232 / 391 (59.3)		2.63 (1.94, 3.55)		2.62 (1.94, 3.54)	
Illiterate	166 / 334 (49.7)		1.00		1.00	
Semi-literate	43 / 98 (43.9)		0.79 (0.50, 1.24)	0.311	0.79 (0.50, 1.24)	0.311
Literate	360 / 553 (65.1)		1.89 (1.43, 1.24)	< 0.001	1.89 (1.43, 2.49)	< 0.001
Low MUAC-F-Age: No	166 / 291 (57.0)		1.00	0.824	1.00	0.845
Yes	392 / 678 (57.8)		1.03 (0.78 , 1.36)		1.03 (0.7, 1.36)	

‡ Adjusted for clustering; HAZ – height-for-age z-score; WAZ - weight for-age z-score- ; BMIZ - body mass index for-age z-score; AGLIT – adolescent girls literacy programme; MUAC – mid-upper arm circumference

Marital status, menarche status, maternal education and literacy level were significantly associated with ITN access in multivariate analysis (Table 6.3). These results show that being unmarried compared to being married was associated with a 73 % reduced chance of accessing an ITN, adjusted for the other three co-variates in the model (i.e. menarche status, maternal education and literacy level). Post-menarcheal participants were twice as likely to access an ITN compared to premenarcheal participants when adjusted for marital status, maternal education and literacy level. For

each year improvement in maternal education, there was a 6 % increased chance of ITN access when adjusted for marital status, menarche status and literacy level. Being literate compared to illiterate or semi-literate improved ITN access by 65 %, adjusted for marital status, maternal education and menarche status.

Table 6.3: Multivariate analysis of factors associated with ITN access in non-pregnant adolescents

Characteristic	PRR (95% CI)	p-value‡
Married	1.00	
Unmarried	0.27 (0.10, 0.73)	0.012
Premenarche	1.00	
Postmenarche	2.01 (1.28, 3.17)	0.002
Maternal education, years	1.06 (1.01, 1.12)	0.016
Illiterate	1.00	
Semi-literate	1.05 (0.57, 1.93)	0.882
Literate	1.65 (1.12, 2.43)	0.011

‡ Adjusted for clustering effects

6.4.5 Factors associated with ITN use

ITN use was based on use during the night prior to survey. Increasing chronological age or gynaecological age, better participant or maternal education, better literacy level, higher Tanner breast stage, higher BMI z-score, season, marital status, AGLIT attendance and menarche status were significantly associated with ITN access in univariate analysis (Table 6.4). These variables together with paternal education were entered for multivariate analysis as they satisfied the criteria of $p < 0.2$.

Table 6.4: Univariate analysis of factors associated with ITN use in non-pregnant adolescents

	ITN use *					
Co-variate	Yes	No	Season Unadjusted		Season adjusted	
	Mean (sd)	Mean (sd)	PRR (95% CI)	p-value‡	PRR (95% CI)	p-value‡
Chronological age, yrs	13.8 (3.0)	12.5 (2.3)	1.20 (1.14, 1.26)	<0.001	1.20 (1.15, 1.26)	<0.001
Gynaecological age,yrs	1.0 (1.6)	0.4 (1.0)	1.46 (1.30, 1.62)	<0.001	1.47 (1.32, 1.64)	<0.001
Education, yrs	4.0 (2.3)	3.4 (2.0)	1.13 (1.06, 1.20)	<0.001	1.15 (1.08, 1.21)	<0.001
Maternal education, yrs	5.4 (3.2)	4.4 (3.5)	1.09 (1.04 ,1.14)	0.001	1.09 (1.04 ,1.15)	0.001
Paternal education, yrs	7.7 (3.1)	7.4 (3.3)	1.04 (0.99, 1.09)	0.141	1.04 (0.99, 1.09)	0.133
Tanner breast stage	2.7 (1.6)	2.0 (1.4)	1.31 (1.20, 1.44)	<0.001	1.34 (1.22, 1.47)	<0.001
Literacy score	8.1 (5.3)	7.1 (5.4)	1.04 (1.01, 1.06)	0.002	1.04 (1.02, 1.07)	<0.001
HAZ	-0.8 (1.1)	-0.7 (1.3)	0.95 (0.84, 1.07)	0.391	0.96 (0.85, 1.08)	0.468
WAZ	-1.3 (0.7)	-1.3 (0.8)	0.95 (0.79, 1.26)	0.629	0.98 (0.81, 1.20)	0.629
BMIZ	-1.1 (1.1)	-1.4 (1.3)	1.20 (1.07, 1.34)	0.002	1.19 (1.07, 1.34)	0.002
	Number (%)					
Season: Dry	132 / 207 (63.8)		1.00	<0.001	n/a	
Wet	328 / 372 (88.2)		1.66 (1.28, 2.17)			
Attended AGLIT: No	439 / 951 (46.2)		1.00	0.089	1.00	0.050
Yes	24 / 40 (60.0)		1.75 (0.92, 3.33)		1.92 (1.00, 3.69)	
Unmarried: No	381 / 884 (43.1)		1.00	<0.001	1.00	<0.001
Yes	82 / 107 (76.6)		0.23 (0.14, 0.37)		0.22 (0.14, 0.35)	
Premenarche	109 / 523 (20.8)		1.00	<0.001	1.00	<0.001
Postmenarche	173 / 456 (37.9)		2.32 (1.75, 3.08)		2.40 (1.81, 3.21)	
Illiterate	137 / 332 (41.3)		1.00		1.00	
Semi-literate	37 / 98 (37.8)		0.86 (0.54, 1.37)	0.534	0.85 (0.54, 1.36)	0.510
Literate	285 / 548 (52.0)		1.54 (1.17, 2.03)	0.002	1.65 (1.25, 2.18)	<0.001
Low MUAC-F-Age: No	134 / 287 (46.7)		1.00	0.892	1.00	0.988
Yes	311 / 674 (46.1)		0.98 (0.74, 1.29)		1.00 (0.75, 1.32)	

* Among those who had access to an ITN; ‡ adjusted for clustering; HAZ – height-for-age z-score; WAZ - weight for-age z-score- ; BMIZ - body mass index for-age z-score; MUAC – mid-upper arm circumference; AGLIT – adolescent girls literacy programme

In multivariate analysis, season, gynaecological age and participant education were the most significant predictors of ITN use, each adjusted for the other co-variates in the model (Table 6.5).

Table 6.5: Multivariate analysis of factors associated with ITN use in non-pregnant adolescents

Characteristic	PRR (95% CI)	p-value‡
Dry season Wet season	1.00 2.04 (1.58, 2.63)	< 0.001
Gynaecological age, yrs	1.29 (1.19, 1.41)	< 0.001
Participant education, years	1.12 (1.06, 1.18)	< 0.001

6.4.6 Relationship of malaria with ITN availability, access and use

The presence of parasites showed some reduction with ITN availability, but these results were not significant (PRR 0.76, 95 % CI 0.41, 1.41) (Table 6.6). No significant associations were observed with the other malariometric variables either. Modeling ITN availability as number of ITNs in the household did not change the results. ITN access showed no significant associations with malaria parasitaemia or any of the other malariometric variables. ITN use compared to non-use, as measured by use during the previous night, was associated with reduced tympanic temperature ($p < 0.003$), higher haemoglobin ($p=0.046$) and reduced anaemia status (0.012) (Table 6.6).

Table 6.6: Univariate analysis of adolescent ITN availability, access and use with malaria parasites and other malariometric measures

Outcome variable	ITN availability		No of ITNs in household		ITN access		ITN use	
	PRR (95% CI)	p-value	PRR (95% CI)	p-value	PRR (95% CI)	p-value	PRR (95% CI)	p-value
No malaria parasites*	1.00	0.391	1.00	0.886	1.00	0.795	1.00	0.236
Malaria parasitaemia	0.76 (0.41, 1.41)		0.98 (0.71, 1.34)		0.93 (0.55, 1.57)		2.09 (0.62, 7.06)	
Parasite count per µl†	0.85 (0.57, 1.27)	0.427	1.01 (0.82, 1.24)	0.932	1.06 (0.75, 1.49)	0.741	1.29 (0.66, 2.55)	0.457
Paracheck negative	1.00	0.234	1.00	0.084	1.00	0.183	1.00	0.656
Paracheck positive	0.80 (0.55, 1.15)		0.85 (0.71, 1.02)		0.81 (0.60, 1.10)		0.79 (0.58, 1.08)	
Symp malaria:No	1.00	0.880	1.00	0.956	1.00	0.508	1.00	0.466
Yes	0.94 (0.43, 2.07)		0.99 (0.67, 1.46)		1.24 (0.66, 2.33)		1.36 (0.72, 2.56)	
Body temperature °C†	0.83 (0.56, 1.23)	0.356	0.92 (0.76, 1.10)	0.354	0.89 (0.66, 1.22)	0.478	0.68 (0.49, 0.93)	0.003
Temperature < 37.5°C	1.00	0.922	1.00	0.445	1.00	0.840	1.00	0.101
Temperature ≥ 37.5°C	1.02 (0.73, 1.42)		1.06 (0.91, 1.25)		1.03 (0.79, 1.33)		0.88 (0.67, 1.15)	
Haemoglobin g/dl	1.01 (0.93, 1.10)	0.805	1.01 (0.97, 1.05)	0.533	1.00 (0.93, 1.07)	0.936	1.54 (1.01, 2.34)	0.046
Hb < 12 g/dl: No	1.00	0.692	1.00	0.983	1.00	0.922	1.00	0.012
Yes	1.06 (0.78, 1.45)		1.00 (0.86, 1.16)		0.99 (0.77, 1.26)		0.53 (0.32, 0.87)	

p- values are adjusted for clustering, * by malaria light microscopy; symp malaria – malaria parasites with tympanic temperature ≥ 37.5°C;
†An ITN being available in the household compared to no ITN in household was associated with a ([1-PRR]*100) % reduction in the outcome variable measure as a continuous variable.

6.5 Discussion

The purpose of this analysis was to measure access and use of ITNs by non-pregnant adolescent females and their determinants as well as their effects on malariometric measures. The administered questions on ITNs were based on reported measures. Their reliability was not validated and mis-classification errors could occur and no direct observations of ITN use were made. Evidence from these surveys indicates that efforts to make ITNs more widely available in households are working; household ITN availability was more than 75 % in each season of survey. This is also supported by the fact that many households had several bed nets. Availability of at least one ITN in households was high at the times of both surveys and increased significantly during the wet season. Season remained significant in multivariate analysis of ITN availability. Paternal education was also a significant determinant of ITN availability, which could reflect better family wealth and awareness. The majority of ITNs were acquired from health facilities at a subsidized cost, through antenatal clinics, under five children's clinics, or from health surveillance assistants (HSAs). These routes of access reflect existing ITN implementation strategies for the district. The HSAs also provided a community re-treatment service at no cost, or individuals could purchase re-treatment packs from retailers.

ITN access was comparable in the two seasons (46 % overall) and this varied with chronological age and maturity; ITN access was lower in early to mid-adolescence. Being married, post-menarcheal, higher maternal education and better literacy were significantly associated with ITN access in multivariate analysis. Being unmarried was associated with a 73 % reduced chance of accessing an ITN and being post-menarcheal was associated with two times increased likelihood of ITN access.

ITNs may be prioritized for specific age groups, for example, in younger adolescents because of their higher malaria risk associated with developing malarial immunity and the increased pubertal reversal of immunity, and in older married adolescents because of their higher risk of malaria during pregnancy. The association with body mass index z-scores shown in univariate analysis probably relates to younger age rather than better nutrition, as discussed in Chapter 4. Access was better in households with at least two ITNs probably reflecting simple numerical availability. Previous studies from male and female adolescents in peri-urban Malawi reported 36 % access and showed an inverse relationship with age, with decreasing use in older adolescents (Muula & Misiri 2004). Low ITN access among rural school-going children who included adolescents has been reported from Tanzania (Edson & Kayombo 2007). Socio-economic factors such as wealth, access to health care and education also have been shown to be important predictors of ITN possession (Binka & Akweongo 2006; Winch *et al.* 1997).

Reported ITN use during the previous night is a population coverage measure while the estimated nights of ITN use per week reflects a measure of community coverage with a time dimension. The former is dependent on the previous night's temperatures while the later tries to estimate average use with consideration that temperatures are likely to fluctuate during the week. Of course, week by week temperatures are likely to vary, but the weekly estimate was meant to minimize biases towards most recent behavior. The general promotion message for ITN use advocates use every night. With an estimate of only 64 % use in the previous night in the dry season, for example, it is of interest why the remaining 36 % did not use their ITN. It is not known if those who used their ITNs the previous night are the same as those who use them every night or whether the 36 % non-users are persistent non-users. The

proportion using ITNs in any one night relates to community coverage and not just individual compliance, whereas estimating the number of nights of use per week provides a better assessment of variance in nightly use. This reflects not just community coverage but also personal protection for the individual concerned and hence is relevant for improving programming from the perspective of strategy development. Nonetheless, both measures are prone to report bias and are likely to be correlated to recent night-time temperatures.

Season, gynaecological age and participant education were the major determinants of ITN use in multivariate analysis. Participants were twice as likely to sleep under an ITN in the wet as dry season. Changing patterns of seasonal ITN use have been observed and difficulties in encouraging community-sustainable approaches to improve ITN use and coverage throughout the year remain a challenge (Winch *et al.* 1997). A review of several National Surveys has shown disparity between availability and consistent use of ITNs, and seasonal variation was partially attributed to this observation (Korenromp *et al.* 2003). While challenges to availability could be eventually overcome by strategies based on large-scale distribution efforts, issues related to use may still remain depending on household and individual factors affecting adherence. Increasing maturity by gynaecological age influenced behavior for ITN use, possibly due to better awareness because of life experiences. The significant association with paternal education could be a reflection of better wealth and awareness. Additional uses of traditional methods of mosquito bite prevention were also common. This indicates that a comprehensive approach to prevention should be emphasized and that communities should be receptive to broad based strategies without sole emphasis on ITNs as the effective intervention.

The influence of ITNs on reducing malaria parasite prevalence was not significantly associated with either availability of ITNs in the household or their access. ITN use during the previous night was significantly associated with reduced tympanic temperature and improved haemoglobin concentration. This could reflect the spatial influence of the increased number of ITNs on mosquito survival and the sum repellent effect of a greater density of ITNs leading to reduced overall risk of infection over several months. Other studies have reported no significant association between presence or use of bed nets and malaria parasitaemia prevalence (Appleyard *et al.* 2008; Leenstra *et al.* 2003a). The cross-sectional nature of the data in the present study limits its interpretation in comparison to results from randomized trials of ITN use which have reported more significant beneficial associations. Nevertheless, the cross-sectional survey identified benefits which could reflect chronic exposure to ITNs even though differences in current parasitaemia were not related to use.

A scale-up of ITN use is taking place across African countries (Roll Back Malaria Partnership 2005) . Scaling up coverage to at least 80 % ITN use by young children and pregnant women by 2010 is the consensus target of the Millennium Development Goals and RBM Partnership and 100 % use is the optimal target (Millennium Project 2005). It has been suggested that communal benefits can make large impacts on malaria disease burden only if appreciable levels of coverage are achieved in the population as a whole, but precise coverage targets for achieving this remain to be determined (Hawley *et al.* 2003; Killeen & Smith 2006; Le Menach *et al.* 2007). Using field-parameterized kinetic models of mosquito host availability, it has been shown that if the majority of people living in malaria-endemic Africa regularly used existing ITN technologies, non-users would receive communal protection (Killeen *et al.* 2007). This would mean that ITN users (e.g. the targeted vulnerable

groups of children and pregnant women) would gain a multiplicative combined effect on exposure of both personal and communal benefits. The improvements in haemoglobin and reduction in anaemia in these adolescents in Malawi could be biomarkers of these effects.

Should malaria prevention and intervention programs target adolescents or advocate ITNs as part of adolescent health friendly services? ITNs can protect not only the individuals and households that use them, but also members of the surrounding community (Binka *et al.* 1998; Gimnig *et al.* 2003a; Hii *et al.* 2001; Howard *et al.* 2000; Maxwell *et al.* 1999). Adolescent involvement in health-related issues in the household may not be uniform and could vary with cultural perceptions of maturity. Nonetheless, availability of more ITNs in households corresponded with better access for adolescents. The increased malaria prevalence with increased risk following menarche identified in this study, and the beneficial effects shown for ITN use support the promotion of increased coverage for this risk group. Improved access to preventive measures among adolescents could therefore potentially reduce the prevalence of parasitaemia, with consequent reduction of the risk for developing malaria-related anaemia in pregnancy as adolescents enter their first pregnancy.

The correlation with education and literacy also highlight the importance of improved health education and awareness. This aspect of health care delivery is considered further in Chapter eight.

6.6 Conclusion

ITNs were accessible to at least half of the adolescent females in this study, although this group was not prioritized for distribution within their

communities, which had high ITN coverage. Their utilization was low and highly dependent on seasonal factors. Access and use of ITNs did not translate into significant improvements in current malaria prevalence indicating more frequent adherence and coverage was required, although anaemia was reduced suggesting the influence of a chronic effect. While prioritizing personal protection for the most vulnerable groups, targeting other vulnerable groups such as adolescents before they become pregnant would enhance equitable malaria prevention as well as improving community-wide protection. This could be of particular importance as these young women enter their first pregnancy.

CHAPTER SEVEN

MALARIA SELF-TREATMENT AMONG ADOLESCENT FEMALES

7.1 Introduction

Self-treatment, that is, any treatment that does not involve consulting a health care provider or traditional healers may be used by girls and women following self-diagnosis for malaria (Espino *et al.* 2000; Nyamongo 2002) or due to lack of access to health facilities (Hotchkiss & Gordillo 1999; Mugisha *et al.* 2002). Adolescent girls may seek malaria treatment on the basis of symptoms they experience in early pregnancy, which may or may not relate to malaria infection (Mbonye 2003). This inadvertent and unnecessary exposure to antimalarial drugs is a potential hazard in the first trimester of pregnancy. For example, uncertainty still remains over the neurotoxicity of artemisinin, currently recommended for antimalarial combination therapy (ACTs) (Toovey 2006b).

The risks of inadvertent and unnecessary exposures to antimalarial drugs in early human pregnancy are little studied because pregnant women are systematically excluded from clinical trials. However, there are concerns that unnecessary fetal exposures can occur with a drug known to be teratogenic in animal experiments (Nosten *et al.* 2006). Antimalarial drugs that are currently used in pregnancy include chloroquine, amodiaquine, quinine, azithromycin, sulphadoxine-pyrimethamine, mefloquine, artemisinin derivatives, atovaquone-proguanil and lumefantrine (Nosten *et al.* 2006). Halofantrine, tetracycline, doxycycline and primaquine should not be used in pregnancy due to their known fetal toxicity (Nosten *et al.* 2006) and there are concerns

about chlorproguanil dapsone because of the safety of dapsone in women with glucose-6-phosphate dehydrogenase deficiency (Brabin *et al.* 2004).

In a recent review on the safety of antimalarial drugs in pregnancy, Nosten and others (2006) underscore the absence of adequate safety data, especially in the first trimester. For example, although chloroquine has been a drug of choice for treating malaria in pregnancy for decades, studies on its efficacy and tolerability have been limited. Animal studies have shown evidence of *in utero* effects on rat lungs and neurons (Okanlawon *et al.* 1993); as well as a risk of accumulation in eye and ear tissues at high doses. It has been implicated in fetal toxicity in some earlier studies. Safety evidence for sulphadoxine-pyrimethamine, which is widely used in pregnancy, is limited. Animal studies in rats have shown a causal association with cleft palate at high doses and increased embryotoxicity when used with folic acid (Chung *et al.* 1993). Conversely, in humans, it has not been associated with apparent toxicity except in one study which reported an association with cleft palate. It has been used in the first trimester with no reported associated toxicity. Quinine, the oldest drug used in malaria treatment, remains widely used. It is considered safe during pregnancy although evidence is limited. From animal studies, its teratogenic effects on the nervous system have been reported although some studies have not observed this finding. In humans, it is considered safe at treatment doses, with hypoglycaemia, auditory nerve damage and possibly optic nerve damage as side effects. It is a known arbotifacient at higher doses.

Safety data for ACT use in pregnancy is limited and these drugs are not recommended in the first trimester of pregnancy unless no other drugs are available (e.g. for rescue therapy with quinine failure) in multi-drug resistant malaria infections.

They can be used during the second and third trimesters of pregnancy (World Health Organization 2003). Animal studies have shown that artemisinins are toxic (Boareto *et al.* 2008; Clark *et al.* 2004; Toovey 2006a; Toovey 2006b). Extrapolating results from animal studies to pregnant women remains difficult due to an inadequate understanding of the mechanisms of toxicity in animal studies (Gordi & Lepist 2004; Longo *et al.* 2006). Animal studies into less toxic artemisinins for use during pregnancy, such as artemisone could be promising (D'Alessandro *et al.* 2007). A recent review on the relationship between artemisinin compounds and adverse pregnancy outcomes suggested that artemisinins were effective and unlikely to be the cause of fetal loss or abnormalities when used in late pregnancy (Dellicour *et al.* 2007). However, due to limited data, none of the studies had adequate power to rule out rare serious adverse events, even in second and third trimesters. Larger studies and post-marketing pharmacovigilance studies would provide better information. A recent retrospective analysis of 50 Karen pregnant women with recurrent *P. falciparum* infections (62 malaria episodes) who were treated with dihydroartemisinin piperaquine between June 2006 and January 2007, showed the drug was well tolerated and no adverse events were recorded (Rijken *et al.* 2008). The gestational ages of these women ranged from 9.2 to 39.1 weeks, mean 23.7 ± 8.2 weeks and 26.0 % were primigravidae.

Little is known on how adolescents respond to perceived malaria illness. Knowledge on their use of antimalarial drugs, in particular self-treatment, during the first trimester of pregnancy is required in order to develop a clearer malaria treatment strategy for non-pregnant adolescents, especially for those potentially at risk of inadvertent and possibly unnecessary drug exposures early in pregnancy, in particular to artemisinin drugs.

7.2 Objective

To assess antimalarial and other drug self-treatment rates for presumed malaria infections in non-pregnant and pregnant adolescent females.

7.3 Statistical analysis

Data was analyzed in Stata 8.0 and SPSS 15.0. Malaria self-treatment was defined as administration of drugs at home without seeking formal health care. Factors associated with self-treatment were assessed. Univariate and multivariate analyses were completed to identify and quantify factors associated with self-treatment. Variables for inclusion in the multivariate regression analysis were identified using $p < 0.2$. Statistical significance was considered at $p< 0.05$.

7.4 Results

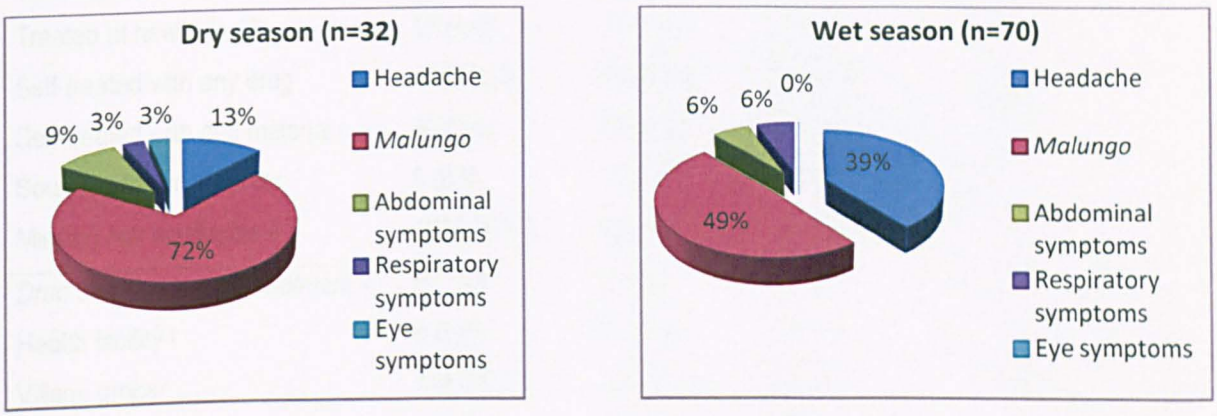
Results are presented for non-pregnant adolescents in section 7.4.1, pregnant adolescents in section 7.4.2 and comparisons made between these two groups in section 7.4.3.

7.4.1 Seasonal patterns of treatment choices in non-pregnant adolescent females

A total of 276 participants had a temperature above 37.5 °C (21.5 %) and this percentage was greater in the wet season (23.5 %) than in the dry season (18.2 %) ($p = 0.027$). Of these, 238 also reported a history of fever within 3 days prior to the survey. In addition, many participants for both seasons commonly complained of headaches (31%), *malungo* (56%) and less frequently, abdominal pains with or without diarrhea (7%), respiratory symptoms (5%) or eye symptoms (1%). *Malungo* means unspecified symptoms of rigors, loss of appetite, tiredness or general weakness which

could be synonymous with a malaria illness and is usually attributed to malaria by villagers. Fever alone or in the presence of its related symptoms was also perceived to be a malaria related illness. Season-specific frequencies of symptoms in febrile participants are presented in Figure 7.1.

Figure 7.1: Fever-related symptoms in non-pregnant adolescent girls



Treatment choices for perceived malaria illnesses in these non-pregnant adolescents are summarized in Table 7.1. Approximately 25 % were verified by health passports.

Table 7.1: Treatment choices and drugs used in non-pregnant adolescent females, by season of survey

Variable	All (n = 1263)	Dry season (n = 477)	Wet season (n = 786)	p-value
Response to illness:				
Any form of treatment	171 (13.5)	61(12.8)	110 (14.0)	0.769
No treatment	67 (5.3)	24 (5.0)	43 (5.5)	
Not applicable$^{\Delta}$	1025 (81.2)	392 (82.2)	633 (80.5)	
Treatment choices:				
Treated at health facility	17 (1.4)	7 (1.5)	10 (1.3)	
Self-treated with any drug	143 (11.3)	50 (10.5)	93 (11.8)	0.418
Self-treated with anti-malarial	30 (2.4)	16 (3.4)	14 (1.8)	
Sought traditional healer	3 (0.2)	1 (0.2)	2 (0.3)	
Missing Not applicable $^{\Delta}$	1070 (84.7)	403 (84.5)	667 (84.9)	
Drug sources for self-treatment †	*n= 143*	*n= 50*	*n = 93*	
Health facility ‡	8 (5.6)	5 (10.0)	3 (3.2)	
Village grocer	124 (86.7)	43 (86.0)	81 (87.1)	0.263
Vendor	9 (6.3)	2 (4.0)	7 (7.4)	
Family/ friends	2 (1.4)	0 (0.0)	2 (2.1)	
Drugs used $^{\beta}$	*n = 201*	*n = 66*	*n = 135*	
Aspirin	80 (39.8)	22 (33.3)	58 (43.0)	
Paracetamol	83 (41.3)	26 (39.4)	57 (42.2)	
SP	28 (13.9)	15 (22.7)	13 (9.6)	0.146
Quinine	2 (1.0)	1 (1.5)	1 (0.7)	
Halofantrine	1 (0.5)	0 (0.0)	1 (0.7)	
ACT	0 (0.0)	0 (0.0)	0 (0.0)	
Co-trimoxazole	7 (3.5)	2 (3.0)	5 (3.7)	

Values are number (percentage); SP - Sulphadoxine-pyrimethamine; ACT – artemisinin based compound; SP - Sulphadoxine-pyrimethamine.
Δ Not applicable because they did not have a reason for seeking care
† Sample size includes only those who self-treated with any drug
‡ Drugs prescribed and acquired from a health facility previously but not for index illness
$^{\beta}$ Sample n = total number of drug uses reported

Up to 13.5 % had taken some form of treatment, 11.3 % self-treated with drugs while at home, 1.4 % consulted a health facility and 0.2 % consulted a traditional healer; these patterns showed no seasonal variation. Self-treatment with an

antimalarial drug was 3.4 % in the dry and 1.8 % in the wet season. Drugs for self-treatment were mostly from local village grocers (86.7 %) and smaller numbers from vendors (mobile sellers of various items) (6.3 %), health facilities (5.6 %) or friends or family (1.4%).

Aspirin and paracetamol were the commonly used anti-pyretics. Sulphadoxine-pyrimethamine, which was the recommended first line antimalarial drug during the study period, was the predominant antimalarial drug used (13.9 %) with use higher in dry than the wet season. Artemisinin-based compounds were not used by any participants for self-treatment.

7.4.2 Seasonal patterns of treatment choices in pregnant adolescent females

Information on malaria treatment was available for 31 out of the 34 pregnant adolescents who attended the surveys and their characteristics are summarized in Table 7.2. Mean age was 18.1 (±1.3) years, gynaecological age 3.5 (±1.2) years, number of education years 3.7 (±1.2) and 80.7 % of participants were married. These parameters were comparable between the pregnant participants enrolled in both seasonal surveys. Fever prevalence was 25.8 % with no seasonal difference. Fourteen were primigravida and seventeen were secundigravida and only 3 (9.7 %) had attended antenatal care. Nine were surveyed while in their first trimester.

Table 7.2: Summary characteristics of pregnant adolescents

Variable	All n = 31	Dry season n = 17	Wet season n = 14	p-value*
Age, years [a]	18.1 (1.3)	18.2 (1.0)	17.9 (1.7)	0.598
Gynaecological age, years [a]	3.5 (1.4)	3.7 (1.2)	3.4 (1.6)	0.502
Education - years of schooling [a]	3.7 (2.9)	3.9 (3.3)	3.4 (2.4)	0.661
Literacy score [a]	8.0 (5.5)	9.5 (4.8)	6.5 (5.9)	0.154
Attended AGLIT	4 (12.9)	3 (17.7)	1 (7.1)	0.400
Body temperature [a]	37.2 (0.3)	37.3 (0.3)	37.1 (0.3)	0.101
Fever (body temp ≥ 37.5 °C)	8 (25.8)	4 (23.5)	4 (28.6)	0.750
Married	25 (80.7)	16 (94.1)	9 (64.3)	0.062
Attended ANC [b]	3 (9.7)	2 (11.8)	1 (7.1)	0.232
Gravida 1	14 (45.2)	7 (41.2)	7 (50.0)	0.624
Gravida 2	17 (54.8)	10 (58.8	7 (50.0)	
Gestation:				
First trimester	9 (29.0)	5 (29.4)	4 (28.6)	
Second trimester	8 (25.8)	5 (29.4)	3 (21.4)	0.958
Third trimester	5 (16.1)	2 (11.8)	3 (21.4)	
Missing	9 (29.0)	5 (29.4)	4 (28.6)	

Values are number (%) except [a] where values are mean (standard deviation); * Adjusted for clustering. AGLIT – adolescent girls literacy programme; ANC – antenatal clinic, [b] had attended ANC for pregnancy booking or illness.

Treatment choices and sources of drugs

Table 7.3 shows the treatment choices and drugs used among the 31 pregnant adolescents. Eight (25.8 %) had fever (temperature ≥ 37.5 °C). Other fever-related symptoms mentioned were headache in one participant and *malungo* in another participant. Five participants (16.1 %) had self-treated with some drug and four (12.9 %) with an antimalarial drug. Sources of drugs were either the health facility or the village grocer. The only drugs used were Aspirin and SP. The gestational ages of these four participants were 10, 16, 23 weeks and unknown for one participant.

Table 7.3: Treatment choices and drugs used for febrile episodes in pregnant adolescents

Variable	All (n=31)	Dry season (n=17)	Wet season (n=14)	p-value
Treatment choices:				
No treatment *	2 (6.5)	1 (5.9)	1 (7.1)	
Treated at health facility	1 (3.2)	0 (0.0)	1 (7.1)	
Self-treated with any drug	5 (16.1)	3 (17.6)	2 (14.3)	0.890
Self-treated with anti-malarial	4 (12.9)	3 (17.6)	1 (7.1)	
Sought traditional healer	0 (0.0)	0 (0.0)	0 (0.0)	
Not applicable **	19 (61.3)	10 (58.8)	9 (64.3)	
Drug sources for self-treatment †	*n = 5*	*n = 3*	*n = 2*	
Health facility ‡	2 (40.0)	2 (66.7)	0 (0.0)	0.400
Village grocer	3 (60.0)	1 (33.3)	2 (100)	
Drugs used β				
Aspirin	4 (50.0)	3 (75.0)	1 (25.0)	1.000
SP	4 (50.0)	3 (75.0)	1 (25.0)	

* Had an illness but did not seek care
** Did not have an illness to warrant seeking care
† Sample size includes only those who self-treated with any drug
‡ Drugs prescribed and acquired from a health facility which could or could not for index illness
β Sample n = total number of drug uses reported
SP - Sulphadoxine-pyrimethamine

Laboratory confirmation of antimalarial drug use

SP use was detected in urine in 7 samples of those reporting antimalarial use (20.0 %) and chloroquine in 3 (8.7 %). The ELISA tests also showed some borderline sensitivity in some samples (results not presented) which could relate to SP use in the previous 2 to 3 months. Chloroquine cross-reacts with amodiaquine and there is a possibility that some of those with chloroquine detected may have taken amodiaquine.

7.4.3 Comparison of treatment rates in non-pregnant and pregnant adolescents

Self-treatment with an antimalarial drug was 12.9 % in pregnant and 2.4 % in non-pregnant adolescents, (p=0.008). The rate of antimalarial drug self-treatment in the first trimester was 3.2 %, which was comparable to that for non-pregnant adolescents (p = 0.509), Table 7.4.

Table 7.4: Comparison of antimalarial self-treatment rates in pregnant and non-pregnant adolescents

	Pregnant (n = 31)	Non-pregnant (n = 1263)	p-value
No. (%) self-treated with antimalarial drug	4 (12.9)	31 (2.4)	0.008†
No. (%) self-treated with antimalarial drug during 1st trimester	1 (3.2)	-	0.509†

† Pregnant compared to non-pregnant using Fisher exact test

7.4.4 Factors associated with antimalarial drug self-treatment

Univariate analysis showed that chronological or gynaecological age, Tanner breast stage, the presence of malaria aparasites on microscopy and a diagnosis of symptomatic malaria were all positively associated with anti-malarial self-treatment, whereas the wet season survey was inversely associated (Table 7.5). In multi-variate analysis, predictors of anti-malarial self-treatment were gynaecological age (PRR 1.51, 95 % CI: 1.18, 1.93; p <0.001), high literacy score (PRR 1.10, 95 % CI: 1.02, 1.20; p =0.020) and a symptomatic malaria diagnosis (PRR 6.57, 95 % CI: 1.85, 23.37; p =0.004).

Table 7.5: Univariate analysis of factors associated with anti-malarial self-treatment

Characteristic		PRR (95% CI)	p-value‡
Pregnant		4.08 (0.93, 17.94)	0.062
Chronological age, years		1.27 (1.13, 1.43)	<0.001
Gynaecological age years		1.53 (1.26, 1.86)	<0.001
Education, years		1.00 (0.85, 1.18)	0.971
Mother's education, years		1.02 (0.96, 1.05)	0.357
Father's education, years		1.02 (0.98, 1.05)	0.305
Tanner breast stage		1.42 (1.08, 1.86)	0.013
Literacy score†		1.07 (0.99, 1.15)	0.081
Season:	Dry	1.00	
	Wet	0.44 (0.21, 0.90)	0.026
Malaria parasitaemia		1.00 4.25 (1.57, 11.53)	0.004
Rapid test positive		1.00 0.93 (0.38, 2.28)	0.876
Symptomatic malaria		1.00 4.09 (1.28, 13.07)	0.018

‡ Adjusted for clustering; † Literacy assessment score as a continuous measure

7.5 Discussion

These community-based surveys aimed to assess self-treatment rates for malaria infection in adolescent females. The results are based on reported use and verification was done only for health facility treatment from available health books. Sulphadoxine-pyrimethamine (SP) was the recommended first line antimalarial drug for treating uncomplicated malaria and for the presumptive treatment of malaria within these communities (Ministry of Health 2003). According to prevailing malaria policy and rational prescribing standards, SP and Quinine were the only available and accessible antimalarial drugs through the primary level public health facilities. Artemisinin-based compounds (ACTs) and other antimalarial drugs were also available through private clinics, traders and private pharmacies. For example, the

sugar factory-owned private clinic (SUCOMA) stocked a wide range of antimalarial drugs including ACTs.

Self-treatment with an antimalarial drug was 2.4 % in non-pregnant and 12.9 % in pregnant adolescents. 3.2 % pregnant adolescents were estimated toself-treat with an antimalarial drug during the first trimester, although this represented only one of the 31 women. This first trimester treatment rate was comparable to that determined for non-pregnant women. Despite the small sample size of pregnant compared to non-pregnant adolescents, the difference in treatment rates between these two groups was significant indicating that pregnant women were more likely to self-treat. Significant predictors for anti-malarial self-treatment were increasing maturity (gynaecological age), improved literacy and symptomatic malaria. Maternal or paternal literacy were not associated with treatment behaviour.

Previous studies of treatment behaviour from Malawi have been among care-givers of children under five years of age and were conducted when chloroquine was the first line antimalarial drug. These showed high rates of antimalarial drug treatment at home with estimates as high as 56 % (Nwanyanwu *et al.* 1996; Slutsker *et al.* 1994). In an urban setting following the introduction of SP, self-treatment in these young children was reported as 34 %, and associated with easy access to shops (Holtz *et al.* 2003). Re-analyzed data from a nationwide Demographic Health Survey conducted in 2000 in Malawi showed that higher socio-economic status, maternal literacy, paternal education and media access positively influenced self-treatment for malaria (Kazembe *et al.* 2007).

In the combined adolescent seasonal surveys, drugs were mostly sourced from the village grocers. Other African studies have shown the popularity of private

medicine sellers (Abuya *et al.* 2007). Strategies to improve rational use are challenging due to the wide coverage and variation between local communities. Sulphadoxine-pyrimethamine was the commonest antimalarial drug in the present study while ACTs were not used by any of the participants. Use of anti-pyretic drugs (Aspirin and Paracetamol) was common. Of concern were the small numbers of drugs used for self-treatment which had originated from the health facility but which was still kept in the home. One participant indicated self-treatment with Quinine which is only indicated for the treatment of complicated malaria. Self-treatment when based on insufficient knowledge in the individual or drug provider is likely to lead to misuse of pharmaceutical drugs (Ruebush *et al.* 1995). In addition, the visual appearances for tablets of available malarial and non-malarial drugs in the country, such as SP and Paracetamol, were similar such that reliance on tablet recognition by appearance would be unreliable if drugs were not in their original packaging (Launiala & Honkasalo 2007).

The accessibility of antimalarial drugs within communities ensures prompt treatment of suspected malaria infection, sometimes at the expense of misdiagnosis and inadequate treatment (Wirima 1994). Community-based interventions to improve presumptive diagnosis and provide antimalarial drugs have been shown to be feasible and effective (Kidane & Morrow 2000; Pagnoni *et al.* 1997; Winch *et al.* 2003). Under-dosing in young children has been previously reported and is a potential problem (Terlouw *et al.* 2003), although information on dosing based on weight related to treatments in adolescents is limited. A study among Kenyan and Ugandan school-children of both sexes, 10 to 18 years old showed that although self-treatment was common, knowledge about indications and dosages were lacking (Geissler *et al.* 2001).

Malawi changed to Coartem (Artemether-Lumenfantrine) as first-line antimalarial drug towards the end of 2007. Its availability and access is likely to be different from SP because presumptive treatment with combination therapies is not highly advocated as was the case with SP. With ACTs, drug dosing issues are crucial. In a study of dosing regimens, for example, two age categories were identified for infants because of high growth rate (Taylor *et al.* 2006). It is unclear if the adolescent growth spurt introduces a similar consideration, and the effectiveness of dosing regimens in this group remains unclear.

One of the thirty-one pregnant participants (3.2 %) had SP self-treatment during the first trimester, and although this sample size was small, inadvertent exposure to antimalarials in the first trimester of pregnancy is probably not uncommon; and a wider discussion and increased awareness is required to reduce the potential adverse consequences of these exposures, especially to ACT therapy which is now much more widely available.

7.6 Conclusion

Adolescent females frequently self-treated for perceived malaria infection and this could contribute to inadvertent drug exposures in early pregnancy. Use of self-treatment could relate to several factors influencing access as these were not a popular treatment choice. Adolescent-friendly strategies to improve malaria seeking behaviour in malaria endemic areas during the pre-pregnancy period are required to facilitate improved understanding and use of antimalarials and potentially to reduce inadvertent exposures in the first trimester.

CHAPTER EIGHT

IMPROVING HEALTH LITERACY AND MALARIA KNOWLEDGE IN OUT OF SCHOOL ADOLESCENT GIRLS

8.1 Introduction

Access to education, especially for girls, is probably one of the fundamental actions which could improve public health in the world's poorest countries (Nutbeam & Kickbusch 2000). Acquisition of literacy, numeracy and language skills through formal education are contributory to health literacy (LeVine *et al.* 2004); hence health literacy is seen as an active process empowering women (Renkert & Nutbeam 2001). However, girls continue to drop-out of school world-wide. For instance, in spite of universal free primary school education since 1995 in Malawi, an estimated 10.5 % of girls drop-out annually and 60 % of those enrolled do not attend school regularly. Eventually, about half of these girls do not complete school beyond four years and this could lead to generations of illiterate mothers if this trend is not reverted (Government of Malawi & UNICEF 2005). Non-formal education for such adolescents presents a last chance of attaining literacy before they become mothers.

Previous studies in the Shire valley of southern Malawi highlighted the link between high illiteracy levels and pregnancy complications, especially among young primigravidae, and improved literacy was one of the interventions recommended (Brabin *et al.* 1998). In response to this, an Adolescent Girls Literacy project (AGLIT) was established in Chikwawa district, an area with an estimated population of 45,418 adolescent females. This was a village-based combined literacy and health education programme targeting illiterate girls 10 to 19 years of age who

were out of school. The project aimed to improve health-seeking behaviour and prevent early pregnancy through health literacy interventions. The basic hypothesis was that health promotion was likely to be most effective if the girls were literate and that literacy attainment would also make them more self-confident in acting on their new knowledge and raise their social standing within the community (Hogg *et al.* 2005). After a one year pilot in 1997, a 36-week course was developed and a teaching curriculum covering general life skills and health was developed. This curriculum included malaria as one of the ten main health topics. In each village, lessons were delivered for two hours, five afternoons per week, generally under a tree or a shelter. Lessons were conducted in Chichewa, the main local language common to all ethnic groups. AGLIT coverage expanded slowly in the first two years while the curriculum continued to be structured and was extended widely from 2000 when it was reaching 40 villages annually.

8.2 Objective

This analysis aimed to assess malaria knowledge, factors associated with poor malaria knowledge and the impact of the acquired literacy on malaria knowledge among the adolescent girls attending the AGLIT programme.

8.3 Methods

Study participants: Illiterate out of school adolescent girls aged 10 to 19 years from Chikwawa district.

Study design: cross-sectional survey at enrolment and an evaluation on completion of the AGLIT programme.

8.3.1 Selection of villages and participants

Villages were selected based on the extent of village commitment to the programme. This was based on villagers' willingness to (a) collect names of girls fitting the selection criteria; (b) to attend a meeting to discuss with AGLIT what the programme participation involved and (c) to form a Village Literacy Committee. Girls were selected if they were illiterate and out of school. Age eligibility was always difficult to ascertain and it was agreed to advertise the age range as 10 to 19 years for guidance to give the committees. Age was based on self-reporting or as provided by parents and guardians. Prompts using local events were made to estimate ages for those whose ages were not known. Lists of participants were checked by comparing names with local primary school enrolment registers in order to avoid "poaching" girls from formal schooling. Participants were also informally tested by asking them to read a standard sentence to find out whether they were really illiterate. Once AGLIT was well established and became known in the area, the main spread of information to other villages was by word -of-mouth. Village chiefs used to approach AGLIT to ask if their girls could participate. AGLIT staff would then visit villages and assess their eligibility.

8.3.2 Malaria curriculum

The curriculum on malaria (Appendix 8) was delivered using various ways of engaging participants. For example, participants would explore the effects of malaria in their lives and their communities through discussion of daily activities within their families and conducting simple surveys. They would discuss and copy out survey questions. They were encouraged to ask and find out about recent malaria episodes and to share the results within their families. Subsequently they would also engage their families in discussing its effects on family earnings and costs of

prevention strategies such as bed nets or applying their numerical skills. They would report back by writing sentences about the survey results as part of assigned homework.

8.3.3 Field procedures

Participants were enrolled in March 2003. Demographic and health information was collected from participants using a questionnaire (Appendix 6). Health knowledge of diarrhoeal disease, baby feeding practices, malaria, sexually transmitted infections and HIV/ AIDS was assessed. Girls attended health literacy classes using a curriculum that covered varied health topics with the main themes on malaria, reproductive health, diarrhoeal diseases, primary health care and HIV/AIDS. Use of strip cartoons, drama, dance and song and organization into youth clubs were among the strategies used in curriculum delivery. At the end of the curriculum in November 2003, health information was collected and health-related knowledge was assessed using a questionnaire similar to the one at enrolment (Appendix 7). Participant identity was recorded using 'name' and 'village' but not using an identity number. In addition literacy and numeracy skills were assessed.

8.3.4 Assessment of literacy and malaria knowledge

At enrolment, participants could not read or write as according to the informal assessment done when ascertaining eligibility to participate in the programme and literacy was therefore not part of the assessments recorded on the enrolment questionnaire. Therefore the reliability of classifying all participants as illiterate at baseline is not known. This limits the quantification and interpretation of literacy and numeracy skills acquired as assessed at evaluation because an assumption of illiteracy is therefore uniformly applied. At evaluation, literacy assessment included tests on the

ability to read letters, words, phrases, write and copy words. Numeracy tests included identification of commonly used numbers, a simple mental arithmetic sum, and interpretation of dates, a weight measure and monetary value. Total scores were calculated and literacy and numeracy categories assessed using a binary variable (low or adequate score) based on whether they could or could not complete the prescribed assessment test.

Malaria knowledge was assessed at enrolment and evaluation. A malaria knowledge score was estimated based on the total number of correct answers to five questions on malaria knowledge (Table 8.1). Each correct answer was one mark and an incorrect answer was zero marks. Two categories of 'poor malaria knowledge' and 'adequate malaria knowledge' were created based on the malaria knowledge scores, using three as the cut-off point. This approach was used to indicate that scoring less than 60 % of the total score (i.e. < 3 out of 5 malaria questions correctly answered) reflected poor knowledge and scoring more than 60 % of the total score (i.e. ≥ 3 out of 5 questions correctly answered) reflected adequate malaria knowledge.

Table 8.1: Malaria knowledge questions for AGLIT surveys

Question	Right answer(s)	Wrong answer(s)
Drinking river water can cause malaria	No	Yes
What is the best treatment to cure malaria?	Fansidar or Quinine	Buy Aspirin from store Buy other drugs from store Other or no answer
Number of days for treatment on SP/ Fansidar	One day	All other answers
Do mosquitoes give you malaria?	Yes	No Don't know
Most important for malaria prevention	Use bednet Measures to avoid mosquito bites	Burning cow dung Other or Don't know

8.3.5 Data management and limitations

Data was entered in Epi Info version 3.3 at the AGLIT field offices. Data at enrolment and evaluation was unlinked and attempts to link these two data sets using name and village residence were problematic due to changes in these two variables. Within the duration of the surveys, there was a re-demarcation of enumeration areas in relation to the nation-wide 2004 general elections which resulted in some villages being split, new ones created and a small number of boundaries moved. In addition, some participants' names changed due to marriage or other reasons. Paired analysis of enrolment and evaluation data was therefore not undertaken and results are presented based on the two cross-sectional surveys pre- and post AGLIT attendance. This limits the assessment of temporal trends in malaria knowledge scores and co-variates within individuals; hence the analysis is completed at population level and therefore prone to cross-sectional bias. Sample sizes for some co-variates showed substantial missing values. No imputation methods for missing values were applied. This means that regression analysis results could be biased towards participants who responded but the direction of bias is unknown.

8.3.6 Data analysis

Analysis was completed in SPSS 15.0. Malaria knowledge scores were normally distributed both at enrolment and evaluation. A descriptive analysis of enrolment data was completed. Differences between 'poor' and 'adequate' malaria knowledge groups were tested using students t-test for normally distributed data, chi-square for categorical data and Fisher's exact test for categorical data with expected cells less than 5. A p-value of less than 0.2 was considered for inclusion of variables for logistic regression. Univariate and multivariate logistic regression analyses for significant associations between malaria knowledge and explanatory variables were completed. P-value < 0.05 was considered significant. A post AGLIT attendance

evaluation of literacy attainment was completed. Data analysis to estimate measured change in malaria knowledge scores was completed for villages as a unit, and comparisons made between malaria knowledge at enrolment and on post AGLIT evaluation based on the questionnaire interview responses.

8.3.7 Definitions

Age: reported age by participant or parent or guardian, or estimated from probing questions related to local calendar events.

Malaria knowledge: a composite score from questions that were answered correctly as indicated in Table 8.1.

Self-reported malaria: diagnosed or presumed malaria illness reported by participant in the previous month.

ITN use: corresponded to 'yes' reponses to a question asking if the participant used an ITN on most nights.

Level of class attendance: semi-quantitative based on four options assessed by the AGLIT teacher: stopped attending, missed at least a whole month, missed at least a whole week or had regular attendance.

8.4 Enrolment survey results

Nine hundred and thirty-three participants (933) were enrolled. One hundred and ninety-one participants did not know their exact age although they were clearly adolescents by observation. Age was normally distributed, mean 14 ± 2.5 years; the reliability of these age estimates is unknown. Table 8.2 summarises the descriptive characteristics at enrolment. The majority of the girls (65.5 %) had never been to

school and none had attended beyond 4 years of primary school. Some adolescents were married (13.4 %) and 2.7 % were pregnant. One in eleven (9.1 %) participants was already mothers. One hundred and eight participants (11.6 %) had lost their mother and 229 (24.7 %) their father. About half (53.3 %) reported experiencing malaria in the previous month with no difference according to marital status. Access to any type of bed net was 20 %, to an ITN 16.2 % and ITN use was 54.2%.

Table 8.2: Characteristics of AGLIT adolescent girls at enrolment

Characteristic	***Value***
Mean (sd) age in years [a b]	14.0 (2.5)
Participant level of education:	
None	611 / 933 (65.5)
Standard 1	127 / 933 (13.6)
Standard 2	128 / 933 (13.7)
Standard 3	57 / 933 (6.1)
Standard 4	10 / 933 (1.1)
Married	125 / 933 (13.4)
Pregnant, from history	25 / 933 (2.7)
Low MUAC-for-age †	316 / 720 (43.9)
Has child/ children	76 / 838 (9.1)
Father deceased	229 / 928 (24.7)
Mother deceased	108 / 932 (11.6)
Self-reported malaria ‡	496 / 931 (53.3)
Bed net ownership	187 / 933 (20.0)
Bed nets treated with insecticides	151 / 933 (16.2)
ITN use [c]	136 / 251 (54.2)

Values are number (percentage) except [a] where values are mean (sd); [b] n = 742;
[c] based on the question if they used an ITN on most nights (yes/no) and missing values could be explained by those who had no access to an ITN
† Below – 2SD; ‡ within the previous month

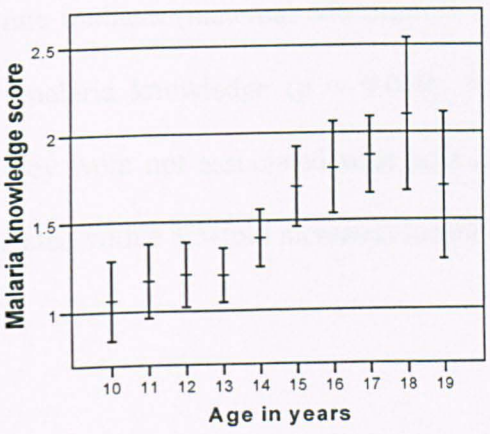
8.4.1 Malaria knowledge and associated factors at enrolment

Mean malaria scores by age are shown in Figure 8.1. Knowledge scores were lower among younger adolescents, increasing with age except for a decline at 19

years; and these did not differ with years of schooling (Figure 8.2). At enrolment 813 (87.1 %) were classified as having poor malaria knowledge and 120 (12.9 %) with adequate malaria knowledge.

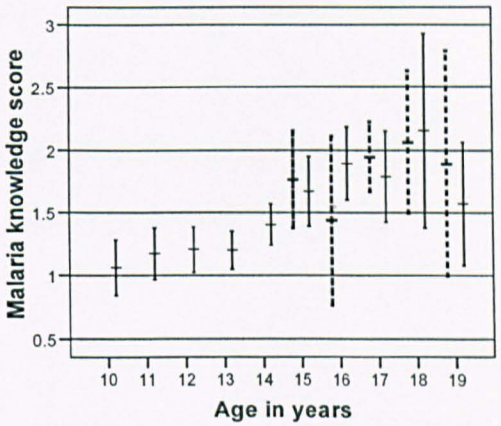
Figure 8.1: Trends in mean malaria knowledge scores by age and marital status

By age



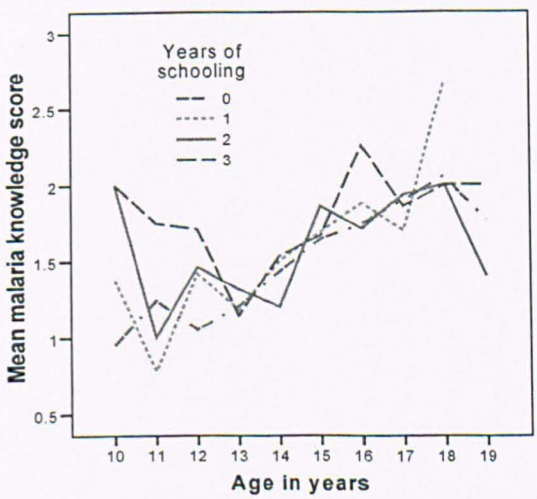
(Error bars, 95 % confidence interval)

By age and marital status



(Error bars, 95 % confidence interval; continuous – unmarried, broken – married)

Figure 8.2: Trends in malaria knowledge scores by age and educational level



The univariate analysis of factors associated with poor malaria knowledge is shown in Table 8.3. Marital status confounded the association of malaria knowledge with age as it was associated with both malaria knowledge and age (OR 2.10, 95 % CI 1.82, 2.43 $p<0.001$). The analysis is therefore stratified by marital status. In both

unmarried and married participants, there was no significant association between poor malaria knowledge with participant or paternal educational, loss of any parent or being an adolescent mother.

In unmarried participants, for each yearly decrement, there was a 1.3 times increased likelihood of having poor malaria knowledge ($p < 0.001$). Adolescents with illiterate mothers (maternal education $\leq$ 4 years) were 2.7 times more likely to have poor malaria knowledge ($p = 0.049$). For married participants, age and maternal illiteracy were not associated with poor malaria knowledge and not being a mother associated with a 3.5-fold increased likelihood of poor malaria knowledge ($p= 0.017$).

Table 8.3: Univariate analysis of factors associated with poor malaria knowledge in AGLIT girls at enrolment, stratified by marital status

	Married (n = 125)				Unmarried (n = 798)			
Co-variate	***Malaria knowledge***				***Malaria knowledge***			
	Poor	**Adequate**	**Crude OR (95 % CI)**	**p-value**	**Poor**	**Adequate**	**Crude OR (95 % CI)**	**p-value**
Age in years [a]	16.4 (1.6)	16.4 (1.8)	0.75 (0.50, 1.13)	0.166	13.4 (2.1)	14.5 (2.1)	0.79 (0.71, 0.89)	<0.001
Age in descending order			1.53 (0.92, 2.55)	0.098			1.32 (1.16, 1.49)	<0.001
Participant education (yrs)								
None	42 / 72 (58.3)	17 / 24 (70.8)	ref		483 / 724 (66.7)	51 / 84 (60.7)	ref	
Standard 1 to 4	30 / 72 (41.7)	7 / 24 (29.2)	1.73 (0.64, 4.70)	0.276	241 / 724 (33.3)	33 / 84 (39.3)	0.77 (0.48, 1.23)	0.272
Mother alive	64 / 72 (88.9)	20 / 24 (83.3)	ref		643 / 723 (88.9)	72 / 84 (85.7)	ref	
Mother deceased	8 / 72 (11.1)	4 / 24 (16.7)	0.63 (0.17, 2.30)	0.474	80 / 723 (11.1)	12 / 84 (14.3)	0.75 (0.39, 1.43)	0.379
Father alive	61 / 72 (84.7)	18 / 24 (75.0)	ref		543 / 721 (75.3)	56 / 84 (66.7)	ref	
Father deceased	11 / 72 (15.3)	6 / 24 (25.0)	0.54(0.18, 1.67)	0.280	178 / 721 (24.7)	28 / 84 (33.3)	0.66 (0.40, 1.11)	0.086
Maternal education > 4 yrs	3 / 7 (42.9)	1 / 2 (50.0)	ref		54 / 115 (47.0)	12 / 17 (70.6)	ref	
Maternal education ≤ 4 yrs	4 / 7 (57.1)	1 / 2 (50.0)	1.33 (0.06, 13.12)	0.858	61 / 115 (53.0)	5 / 17 (29.4)	2.71 (1.01, 8.19)	0.049
Paternal education > 4 yrs	19 / 27 (70.4)	6 / 7 (85.7)	ref		160 / 217 (73.7)	26 / 32 (81.3)	ref	
Paternal education ≤ 4 yrs	8 / 27 (29.6)	1 / 7 (14.3)	2.52 (0.26, 14.51)	0.412	57 / 217 (26.3)	6 / 32 (18.7)	1.54 (0.60, 3.94)	0.361
Has at least 1 child	35 / 72 (48.6)	5 / 24 (20.8)	ref		631 / 641 (98.4)	70 / 73 (95.9)	ref	
Has no children	37 / 72 (51.4)	19 / 24 (79.2)	3.59 (1.21, 10.62)	0.021	10 / 641 (1.6)	3 / 73 (4.1)	0.37 (0.10, 1.37)	0.123
Normal MUAC-for-age	55 / 66 (83.3)	18 / 22 (81.8)	ref		280 / 549 (51.0)	43 / 69 (62.3)	ref	
Low MUAC-for-age †	11 / 66 (16.7)	4 / 22 (18.2)	0.90 (0.25, 3.18)	0.870	169 / 549 (49.0)	26 / 69 (37.7)	1.59 (0.95, 2.66)	0.076

Values under *'Malaria knowledge'* columns are number (percentage) except [a] where values are mean (sd); the values mean prevalence of poor or adequate malaria knowledge (according to column) within that co-variate classification; values are reported excluding missing values; † < - 2SD of NCHS reference.

In multivariate analysis, age and maternal illiteracy were significant predictors of poor malaria knowledge in unmarried participants. Not being a mother was the only significant predictor among married participants (Table 8.4).

Table 8.4: Multivariate logistic regression analysis of factors associated with poor malaria knowledge

	β (SE)	OR (95 % CI)	p-value
Unmarried participants			
Age*	0.399 (0.181)	1.49 (1.05, 2.13)	0.026
Maternal education ≤ 4 yrs (ref > 4 yrs)	1.863 (0.835)	6.44 (1.25, 33.12)	0.027
Married participants			
Has no children (ref has children)	1.279 (0.555)	3.59 (1.21, 10.67)	0.021

* Referent age is 19 years old, comparison in descending order.

8.4.2 Malaria knowledge and ITNs

The association of malaria knowledge with reported malaria illness and ITN access and use was evaluated (Table 8.5). As marital status was associated with both malaria knowledge and ITN variables, results were stratified by marital status. In logistic regression analysis there was no association between malaria knowledge and ITN access or use in the unmarried group. In married participants, access to an ITN was associated with a 50 % reduced odds of poor compared to good malaria knowledge (p = 004). Access to an untreated bednet was not related to malaria knowledge.

Table 8.5: Malaria knowledge, self-reported malaria and ITN characteristics, stratified by marital status

	Married				Unmarried			
Variable	**Poor (n= 96)**	**Adequate (n=39)**	**Crude OR (95%CI)**	**p-value**	**Poor (n= 717)**	**Adequate (n=81)**	**Crude OR (95 %CI)**	**p-value**
No malaria	14 / 19 (73.7)	4 / 16 (25.0)	ref		85 /177 (48.0)	14 / 29 (48.3)	ref	
Self-reported malaria†	5 / 19 (26.3)	12 / 16 (75.0)	0.71 (0.10, 5.18)	0.739	92 / 177 (52.0)	15 / 29 (51.7)	1.01 (0.46, 2.22)	0.980
ITN access								
No bed net	54 / 70 (77.1)	11 / 25 (44.0)	ref		572/697 (82.0)	64/ 84 (76.2)	ref	
Untreated bed net	3 / 70 (4.3)	2 / 25 (8.0)	0.31 (0.05, 2.05)	0.201	20 /69 7 (2.9)	2 / 84 (2.4)	1.12 (0.26, 4.90)	0.881
Treated bed net (ITN)	13 / 70 (18.6)	12 / 25 (48.0)	0.51 (0.32, 0.83)	0.003	103/697 (15.1)	18 / 84 (21.4)	0.65 (0.37, 1.15)	0.135
No ITN§	57 / 70 (85.2)	13 / 25 (52.0)	ref		592/697 (84.9)	66 /84 (78.6)	Ref	
ITN	13 / 70 (18.6)	12 / 25 (48.0)	0.50 (0.32, 0.78)	0.004	105 / 70 (15.1)	18 / 25 (21.4)	0.65 (0.37, 0.1.14)	0.130
ITN use ‡								
No or low ITN use	2 / 16 (12.5)	2 / 14 (14.3)	ref		33 /125 (26.4)	5 / 20 (25.0)	Ref	
Frequent ITN use	14/ 16 (87.5)	12 / 14 (85.7)	0.50 (0.19, 1.29)	0.886	92/ 125 (73.6)	15 / 20 (75.0)	0.93 (0.31, 2.76)	0.895

§ Has either untreated bed net or no bed net; † previous month; ‡ in response to the question of ITN use on most nights (yes or no)

8.5 Evaluation survey results

A total of 860 girls were evaluated following their completion of the AGLIT programme and 73 (7.8 %) were lost to follow up. Class attendance varied; 3.6 % were reported to have stopped attending, 9.3 % missed at least a whole month and 21.2 % missed at least a whole week. Overall, 48.6 % of the girls were classified as regular attenders with the remainder irregular attendees. Self-reported malaria prevalence was 51.6 % (437/847) and did not differ according to poor or adequate malaria knowledge at the time of the survey (p-value 0.98). This prevalence was comparable to that observed at enrolment (53.2 %) (p = 0.54).

8.5.1 Acquisition of reading, writing and numeracy skills on completion

Results of literacy, writing skills and numeracy are summarised in Table 8.6. Basic literacy, defined as being able to read and write, was attained in at least half of the participants (reading 55 %, writing 56.8 %). Numeracy was attained by 46.5 % and for numeracy applications (e.g. in dates and measures), 35.1 %. Total scores for literacy (i.e. for reading, writing skills) and numeracy was categorised into a binary variable of low or adequate which showed that 49.7 % had adequate literacy (402/800).

Table 8.6: Assessment of literacy and numeracy among AGLIT girls at evaluation survey

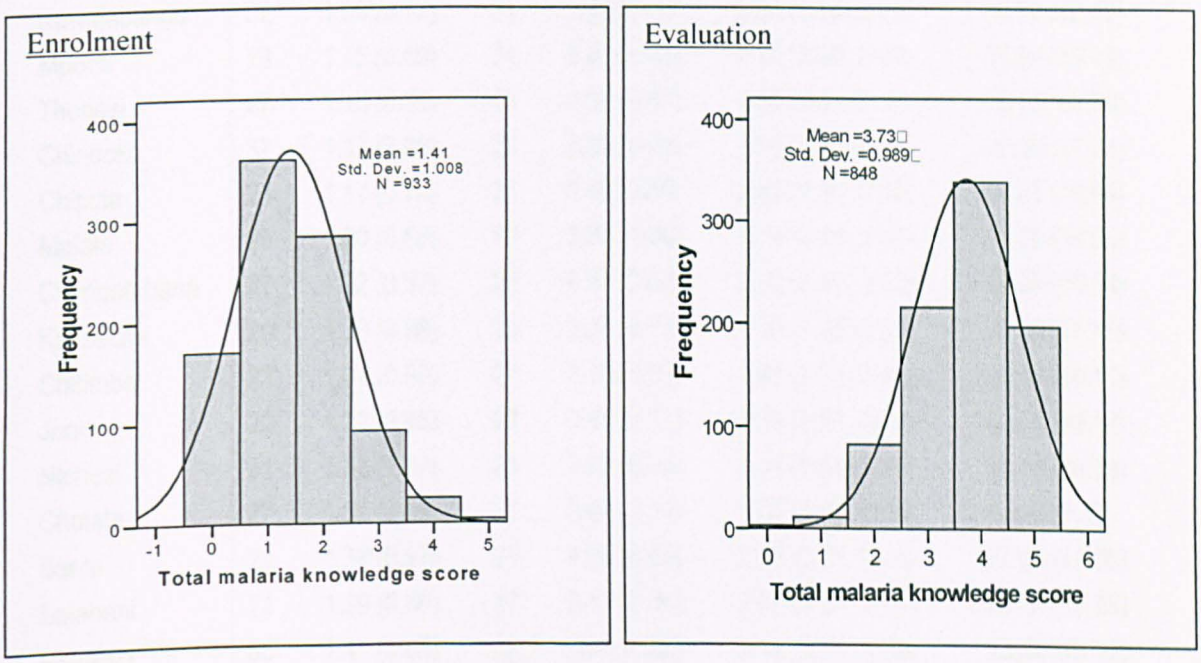
Literacy		***Number (%)***
1. Reading alphabetical letters	None: All 6 letters:	42 / 860 (4.9) 521 / 860 (60.6)
2. Reading words	None: All 5 words:	185 / 860 (21.5) 473 / 860 (55.0)
3. Reading health-related phrases:		
AIDS kills (*edzi imapha*)	None: All words in the phrase:	342 / 848 (40.3) 394 / 848 (46.5)
Avoid promiscuity (*tipewe chiwerewere*)	None: All words in the phrase:	397 / 845 (47.0) 412 / 845 (48.8)
Eat mangoes (*idyani mango*)	None: All words in the phrase:	340 / 846 (40.2) 385 / 846 (45.5)
Eggs are a body building food (*mazira amamanga thupi*)	None: All words in the phrase:	345 / 848 (40.7) 409 / 848 (48.2)
We sleep under a net to avoid mosquito bites (*tigone muneti kopewa udzudzu*)	None: All words in the phrase:	321 / 848(37.9) 406 / 848(47.9)
When going to Lilongwe Malita took a minibus (*malita popita ku Lilongwe anakwera basi*)	None: All words in the phrase:	359 / 848(42.3) 377/ 848 (44.5)
4. Writing skills		
Can hold pencil correctly	-	824 / 848 (97.2)
Can copy word (*kalata*)	-	804 / 848 (94.8)
Can write word on her own	-	482 / 848 (56.8)
Numeracy		
Can recognise 12 numbers	None: All 12 numbers:	9 / 860 (1.0) 400 / 860 (46.5)
Can do a simple mental sum	-	530 / 860 (63.1)
Can understand numbers (*15 November 2000, 16.8.2002, 20 kg and K30*)	None All 4 numbers	259 / 860 (30.1) 302 / 860 (35.1)

(*italics*) – phrases as administered in Chichewa language.

8.5.2 Changes in malaria knowledge and behaviour scores following AGLIT attendance

Mean scores for malaria knowledge at evaluation improved when compared to enrolment (Figure 8.3).

Figure 8.3: Distribution of total malaria knowledge scores at enrolment and evaluation



Due to difficulties in linking participant information individually at enrolment and evaluation, a paired analysis of malaria knowledge was not possible. This was completed using village as the analysis unit and results are summarized in Table 8.7. Seventy-three (8.5 %) participants had some missing values. The change in malaria knowledge score between enrolment and evaluation was calculated and compared with mean attained literacy scores for villages participating in the post-AGLIT surveys and following girls' completion of the AGLIT curriculum.

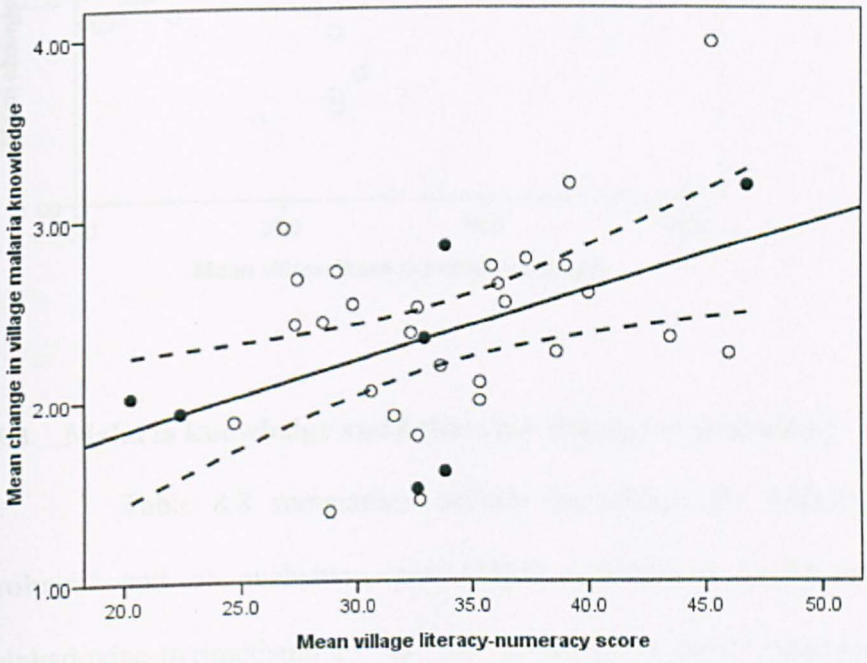
Table 8.7: Change in malaria knowledge and evaluation literacy scores by village

Village	Malaria knowledge score					Literacy at evaluation
	Enrolment †		Evaluation			
	n	Mean (sd)	n	Mean (sd)	Change (95 % CI)	Mean score (sd)
Mafunga	26	0.65 (0.80)	26	4.62 (0.64)	3.97 (3.58, 4.36)	45.27 (12.09)
Dwaliti	16	0.81 (0.91)	14	4.00 (0.64)	3.19 (2.61, 3.76)	46.85 (8.67)
Chituwi	33	0.88 (1.29)	32	3.23 (0.94)	2.35 (1.80, 2.89)	43.54 (9.91)
Nsingano	32	0.94 (0.95)	33	3.48 (1.06)	2.54 (2.05, 3.03)	36.41 (15.18)
Chamboko	20	1.05 (0.94)	24	4.00 (0.72)	2.95 (2.45, 3.45)	26.83 (15.61)
Kuwalabango	22	1.05 (0.72)	31	3.58 (1.09)	2.53 (2.04, 3.02)	29.77 (18.78)
Mpoza	13	1.15 (0.69)	24	3.25 (1.16)	2.10 (1.50, 2.70)	35.27 (15.12)
Thenesi	27	1.15 (0.99)	19	4.00 (0.67)	2.85 (2.37, 3.33)	33.76 (16.73)
Chindoko	37	1.17 (0.96)	25	3.88 (0.93)	2.71 (2.23, 3.19)	29.08 (17.21)
Chipula	24	1.17 (0.70)	23	3.59 (0.80)	2.42 (1.98, 2.92)	27.25 (16.69)
Mafale	20	1.20 (0.89)	18	3.94 (1.00)	2.74 (2.14, 3.34)	35.78 (16.65)
Chimpambana	27	1.22 (0.97)	23	4.00 (0.82)	2.78 (2.28, 3.28)	37.29 (16.34)
Kanseche	20	1.30 (0.98)	13	3.23 (0.73)	1.93 (1.35, 2.51)	22.38 (11.15)
Chitimbe	23	1.31 (0.99)	21	3.19 (0.93)	1.88 (1.31, 2.45)	24.70 (16.11)
Joni	20	1.32 (0.85)	22	3.91 (0.75)	2.59 (2.11, 3.07)	40.01 (16.17)
Ntchesi	31	1.32 (1.27)	23	3.96 (0.98)	2.64 (2.04, 3.24)	36.10 (16.68)
Chatala	27	1.37 (0.69)	27	3.63 (1.11)	2.26 (1.77, 2.75)	45.96 (9.39)
Santu	24	1.38 (0.97)	21	4.05 (0.59)	2.67 (2.21, 3.13)	27.35 (11.60)
Salabeni	24	1.39 (0.66)	17	3.41 (0.94)	2.02 (1.50, 2.54)	20.29 (17.23)
Nkhwazi	36	1.41 (0.86)	32	3.84 (0.85)	2.43 (2.02, 2.84)	28.50 (16.12)
Nyasa	32	1.44 (1.19)	26	2.88 (1.07)	1.44 (0.86, 2.02)	32.67 (16.18)
Machokola	29	1.45(1.05)	23	3.64 (0.90)	2.19 (1.66, 2.72)	33.55 (16.17)
Maluwati	24	1.46 (0.90)	22	3.73 (0.98)	2.27 (1.72, 2.82)	38.58 (14.90)
Mwalija	22	1.50 (0.91)	21	4.24 (0.54)	2.74 (2.80, 3.18)	39.05 (13.64)
Jombo	23	1.52 (0.85)	20	3.89 (1.15)	2.37 (1.75,2.98)	32.27 (16.01)
Phingo	26	1.58 (0.58)	25	3.38 (0.92)	1.80 (1.38, 2.22)	32.57 (17.37)
Maya	17	1.60 (0.74)	16	3.94 (1.18)	2.34 (1.66, 3.02)	32.93 (20.08)
Njereza	32	1.61 (0.95)	26	4.81 (0.40)	3.20 (2.84, 3.56)	39.15 (14.84)
Dzongwe	27	1.63 (0.84)	28	4.14 (1.08)	2.51 (2.00, 3.02)	32.58 (14.46)
Kalima	20	1.79 (1.13)	20	3.70 (0.80)	1.91 (1.30,2.52)	31.58 (16.40)
Mikanzo	25	1.84 (1.28)	18	3.22 (0.94)	1.38 (0.72, 2.04)	28.83 (15.63)
Kulima	17	1.90 (1.15)	6	3.50 (0.84)	1.60 (0.73, 2.47)	33.75 (16.92)
Matsukambiya	28	1.93 (1.24)	28	3.93 (1.09)	2.00 (1.38, 2.61)	35.26 (16.04)
Moznyeti	21	2.05 (0.80)	20	4.10 (0.97)	2.05 (1.50, 2.60)	30.61 (16.33)
Namatchuwa	24	2.29 (1.20)	19	3.79 (0.86)	1.50 (0.88, 2.12)	32.58 (17.88)
All	869	1.39 (0.35)	787	3.77 (0.40)	2.38 (2.34, 2.42)	34.00 (6.26)

†Listed in ascending order of knowledge scores

Mean malaria knowledge scores significantly improved for all villages with means ranging from 1.38 to 3.20 units. The proportion with poor malaria knowledge in each village declined. In four villages all girls assessed had adequate malaria knowledge at the final evaluation. There was a positive linear correlation between mean literacy-numeracy scores at village level on completion of the AGLIT curriculum and mean change in village malaria knowledge scores (Figure 8.4). The R^2 value indicates the proportion of improvement that can be explained by the model.

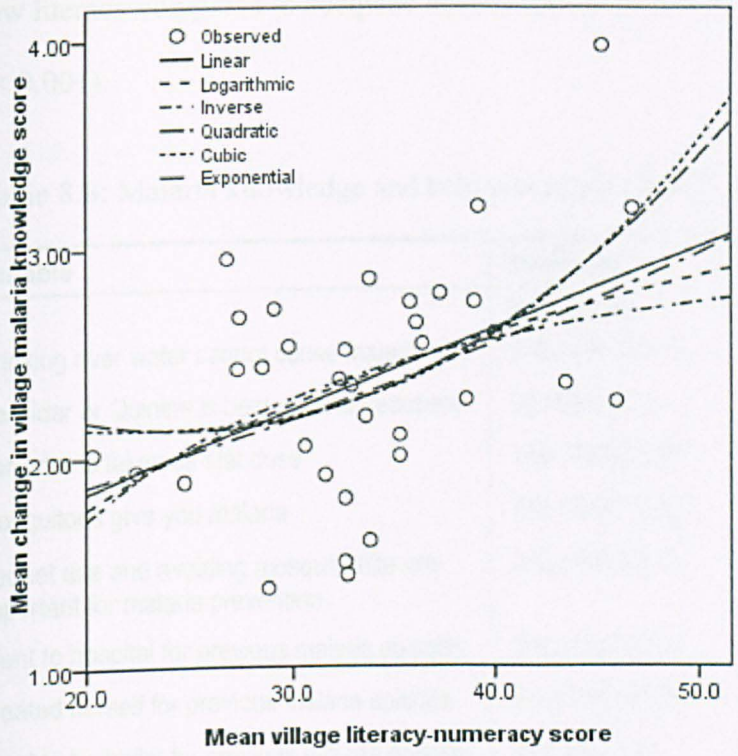
Figure 8.4: Scatter-plot of literacy scores and change in malaria knowledge scores



Each point represents a village, R = 0.45; mean change in village malaria knowledge (MK) = 0.98+0.04(Mean village literacy-numeracy score), R^2 = 0.18, p-value 0.012. Stippled lines = 95 % CI.
Closed circles indicate villages with sample size < 20 participants

Other models were explored and compared to the linear model including quadratic, cubic, logarithmic, inverse and exponential (Figure 8.5). The linear model was the most parsimonious at explaining the data.

Figure 8.5: Comparison of linear model with alternative model fits for association of literacy and malaria knowledge scores.



8.5.3 Malaria knowledge and behaviour changes at evaluation

Table 8.8 summarises malaria knowledge and behaviour variables at enrolment and at evaluation post-AGLIT attendance. Knowledge of Fansidar (sulphadoxine-pyrimethamine) as the preferred malaria treatment, drug dose, knowledge that mosquitoes transmit malaria and the perception of malaria preventive measures (bednet use and avoidance of mosquito bites) all showed improvements following completion of the AGLIT attendance. Reported malaria behaviour for seeking care from a health facility also improved ($p < 0.001$). There was no change in reported behaviour of care seeking from the herbalist ($p = 0.62$), or home self-treatment ($p = 0.64$). There were improvements of pregnant adolescent's knowledge that malaria can harm the fetus ($p < 0.001$), as well as for behaviour for taking

intermittent presumptive antimalarial treatment during pregnancy (p = 0.03). Poor malaria knowledge at evaluation showed a significant association with classification of low literacy compared to adequate literacy (crude odds ratio 2.83; 95 % CI 1.74, 4.59; p< 0.001).

Table 8.8: Malaria knowledge and behaviour post-AGLIT attendance

Variable	Enrolment	Evaluation	
	Number (%)	Number (%)	p-value
Drinking river water cannot cause malaria	849 / 936 (90. 7)	725 / 860 (84.3)	0.00004
Fansidar or Quinine is best malaria treatment	90 / 931 (9.7)	557 / 860 (64.8)	<0.00001
Fansidar is taken as stat dose	158 / 759 (20.8)	345 / 852 (40.5)	<0.00001
Mosquitoes give you malaria	715 / 933 (76.6)	836 / 853 (98.0)	<0.00001
Bednet use and avoiding mosquito bite are important for malaria prevention	242 / 936 (28.7)	751 / 860 (83.1)	<0.00001
Went to hospital for previous malaria episode	246 / 584 (37.0)	240 / 396 (60.6)	<0.00001
Treated herself for previous malaria episode	239 / 584 (40.9)	168 / 396 (42.4)	0.64
Went to herbalist for previous malaria episode	38 / 583 (6.5)	29 / 396 (7.3)	0.62
In pregnancy			
Malaria can harm your unborn child	9 / 25 (36.0)	28 / 31 (90.3)	0.00002
Took IPT in index pregnancy	8 / 25 (32.0)	19 / 31 (61.3)	0.03

IPT – intermittent preventive treatment

8.6 Discussion

In this study, knowledge on malaria prevention and control among illiterate adolescents was assessed prior to and following attendance at a combined literacy and health education programme in rural southern Malawi. Malawi is a country where an estimated 65.3 % of the population lives in poverty (Government of Malawi 1998), and these adolescent participants represent a highly vulnerable group of girls most of whom (65 %) had never accessed formal education. One in four had lost a father and one in nine their mother. By middle adolescence, some of the girls had already started entering into marriage.

Following attendance at AGLIT classes, basic literacy was achieved in 56% of participants. Although the complete drop-out rate was low at 3.6 %, attendance rates were problematic as 47.8 % had irregular attendance. Commonly cited reasons for absence were illness, bereavement and income-generating commitments (Hogg *et al.* 2005). Out of school adolescent girls are difficult to reach and they are not an automatic target group for most health interventions. They have also not benefited from school-based interventions. The decision on whether adolescents attend school or literacy programs lies with parents and guardians. From inception of the AGLIT project, the role the community could play in encouraging girls to enroll was recognized and support through community participation was a pre-requisite for sustaining attendance (Hogg *et al.* 2005). Another dimension, which is applicable to adolescents, was that with the opportunity of free time and energy to spare away from domestic responsibilities, girls used it their own way for entertainment such as playing cards when their parents and carers had released them from domestic chores (Hogg *et al.* 2005). These out of curriculum activities acted to re-enforce knowledge gained by AGLIT attendance.

In this group of AGLIT participants, those who were younger than 15 years should have been accessible to free universal primary education at the time they attained school-going age of 5 to 6 years in line with prevailing policy on education. Those factors which affect girls' school enrolment and attendance may also affect their access to literacy programmes, health facilities or other public health services (Fuller *et al.* 1995). In one of its Millennium Development Goals, Malawi plans to make primary school education compulsory by 2012 as one of the measures for achieving universal access to primary education by 2015 (Government of Malawi, 2005). The Malawi government however recognises that poverty remains a challenge to achieving this

target due to its influence on domestic demands related to food availability and poor health status for the most impoverished and vulnerable families. If enrolment were to improve, the challenge of irregular attendance would still need to be addressed. Though effective in improving knowledge and behaviour in adolescents, even school – based health programs are dependent on reducing rates of absenteeism (Plummer *et al.* 2007).

Before commencement of classes, most participants had poor malaria-related knowledge and this differed by marital status. In the unmarried group, poor malaria knowledge was best predicted by young age and having an illiterate mother. Having an illiterate mother was associated with a 2.7 times chance of poor malaria knowledge, and each yearly decrement increased by 30 % the chance of having poor malaria knowledge compared to those a year older. In the married group, having had a child was associated with improved malaria knowledge. This could relate to improved knowledge due to lived experiences and possibly to exposure to antenatal clinics or under-five clinics. Improved maternal education has been consistently linked to better health outcomes in developing countries, and a central component of the inverse maternal education – child mortality relationship is health literacy (Caldwell 1979; Caldwell 1986). Low maternal literacy is associated with increased infectious disease morbidity (Dewalt *et al.* 2004; Wolf *et al.* 2004), while improvement of literacy in illiterate mothers has been shown to improve child survival in a study from Central America (Sandiford *et al.* 1995).

The regression line in Figure 8.4 indicates that about 18 % of the improvement in malaria knowledge could be explained by literacy attainment post-AGLIT attendance. Its interpretation is limited by the assumption of universal illiteracy

at baseline. Lower improvement scores in malaria knowledge were significantly associated with poorer literacy attainment. It is uncertain the extent to which poor literacy attainment was related to irregular AGLIT attendance. Some village clusters in this analysis also had small sample sizes (n <20) (Figure 8.4), limiting the validity of this comparison for these smaller village groups.

Empowering adolescents by improving health literacy should improve their health-related behaviour through their adolescent and adult years. In unmarried adolescents, knowledge on malaria was not related to reported access to an ITN, whereas for married adolescents, better malaria knowledge was associated with improved ITN access. Unmarried adolescents may be more restricted in making decisions, and married adolescents may have been more empowered through their pregnancy or marriage, or have better access through antenatal distribution of ITNs.

Adolescents can be empowered to influence health changes for themselves and in their community (Magnussen *et al.* 2001; Onyang-Ouma *et al.* 2005). The results of this study provide some evidence that improving adolescent literacy leads to better health-related knowledge and behavioral changes related to malaria control. Since 2001, AGLIT classes have also developed youth clubs for post-literacy activities such as cultivating gardens. This study offers evidence of an out-of-school strategy which was successful in providing health literacy to adolescent girls, thereby empowering them for motherhood and improving health seeking behaviours related to malaria control.

8.7 Conclusion

These findings indicate that improving adolescent health literacy in vulnerable out of school girls is an effective strategy for improving knowledge and behaviour related to malaria prevention and control prior to pregnancy. Innovative approaches to attract and sustain girls' attendance at the AGLIT classes require further research. This would enable those girls who are irregular attendees to gain greater benefits from this health literacy strategy.

CHAPTER NINE

GENERAL OVERVIEW

The findings presented in this thesis underscore the importance of pre-pregnancy interventions related to malaria prevention and control in adolescent girls. The objectives of the research study were to determine the magnitude and factors associated with malaria infection in adolescent females in rural Malawi, and to relate this information to strategies for preventive interventions. Efforts to improve malaria control are continuously improving and emphasis is shifting from malaria control towards eradication due to experience with effective interventions which include ACTs and anti-vector measures (World Health Organization 2007).

The ability to generalize these approaches to female adolescent populations depends on understanding prevailing demographic, health and environmental conditions. Observational studies in public health are useful in measuring critical health parameters, but these always reflect specific population groups and often relate to groups with high health risk or exposures. This is why much emphasis in malaria research is given to children and pregnant women; hence the intermediate group of adolescents is often neglected. The adolescent population in this study was from a stable malaria transmission area in one of the poorest sub-Saharan countries with a predominantly agro-based economy. Low literacy and early motherhood were common in these adolescents from the lower Shire river valley, a situation which was not unusual for other parts of Malawi or sub-Saharan Africa. This is a highly vulnerable age group that is least understood due to paucity of health information on their burden of disease or health seeking behaviour. The setting in the

Shire valley could be considered comparable to many other parts of sub-Saharan Africa where malaria is holoendemic.

In this study sample, two independent samples were planned and socio-demographic characteristics were mostly comparable in the two seasons although those seen in the dry season had a higher number of years of schooling which could be explained by the timing difference of the academic calendar. Although the cluster sampling design tries to minimize sampling error by using probability density sampling and ensuring a large sample size in this community-based study, sampling error could still have occurred as evidenced by the higher proportion of postmenopausal participants in the dry than the wet season. Some sources of error could be due to non-random variability of some characteristics. This was observed with significant differences in AGLIT attendance between the two surveys reflecting the non-random village coverage of the AGLIT project and hence related to the vulnerability factors for access to education and illiteracy.

This chapter aims to summarize the main findings for public health practice and prioritize areas for future work. The findings presented in this thesis may assist in policy formulation for Adolescent Friendly Health Services (AFHS) and for interventions directly or indirectly related to malaria prevention and control before pregnancy. This discussion is presented in five sections corresponding to each of the study objectives. Each section considers (a) lessons learnt and conclusions, (b) implications for public health and (c) areas for future research.

9.1 Health and nutritional status of adolescent females

9.1.1 Lessons learnt and conclusions

Stunting and undernutrition were common amongst non-pregnant female adolescents in these poor rural communities of the Shire valley. Although comparison of seasonal variation in nutritional levels were limited by potential sampling error, seasonal variation in food availability and infection exposure could increase the risk of micro-nutrient deficiencies and anaemia as shown by the significant decrement in haemoglobin concentration during the wet season. Moderate or severe anaemia was not frequent, which could promote a perception of reasonable health in these adolescents, although the majority was anaemic prior to their first pregnancy. Participants who were illiterate and those who had an illiterate mother were more likely to be undernourished, and stunting was associated with increased risk for symptomatic malaria. This raises the issue of illiteracy as a proxy for poor health outcomes, or poor health seeking behaviour. The use of the NCHS reference data for assessing anthropometric measures in African populations could lead to misclassification of adolescent nutritional status due to their delayed onset of puberty and differences in body fat composition and prolonged catch-up growth.

9.1.2 Implications for public health

Clinicians and other health workers attending adolescents need to be aware of the public health importance of chronic undernutrition throughout childhood. This encourages a more holistic approach to managing adolescent illness in order to lessen missed opportunities for alleviating adolescent-related health problems. The process of poor growth from the first three years of life is not easily reversed and generally persists unless nutritional status improves in early life (Martorell *et al.* 1994). Hence recognition of malnutrition as an underlying risk factor for many illnesses in children in developing countries is emphasized by paediatricians (Allen & Lagunju 2007). Although maturation delay may be corrected by later catch-up growth, this period may

not be sufficient for it to be completed prior to the onset of the first pregnancy. For this reason these undernourished adolescents continue to experience slowed growth which can persist into early adulthood (Kalanda *et al.* 2006).

9.1.3 Future research

Anthropometric research is needed in order to improve descriptions of patterns of growth and nutrition in adolescent African populations (Butte *et al.* 2007). This would provide reference data for developing adolescent growth curves. Age-based guides for height or MUAC, which separate pre-menarcheal from post-menarcheal adolescents, would be important for standardization, as well as for identifying potential problems of undernutrition which require nutritional interventions. Height measurements below 145 to 150 centimetres can be used to measure of stunting and this approach has been used to screen for cephalo-pelvic disproportion in pregnancy. Improved adolescent screening methodologies related to puberty are required in order to identify particular risk groups to enable earlier interventions.

The presence of nutritional deficiencies may be reflected not only in growth deficits but also in various manifestations of micronutrient deficiency. Randomized controlled trials are required to assess the benefits of pre-natal nutritional interventions such as fortified foods or multi-micronutrient supplements, in these nutritionally vulnerable adolescent populations. It is uncertain whether iron supplementation or food fortification in this pre-pregnancy age group would increase malaria risk when these young women experience their first preganancy, and specific randomized trials are required to address this. This is an important area for further

research as it is uncertain whether early gestational susceptibility to *P. falciparum* malaria in primigravidae is altered by the mother's nutritional status.

9.2 Prevalence of malaria infection in female adolescents

9.2.1 Lessons learnt and conclusions

Study participants were at risk of malaria, which was associated with anaemia. Estimates of malaria parasitaemia prevalence showed an age-dependent decline with increasing chronological age. There was pubertal reversal of the trend of decreasing age-related malaria prevalence which suggests a reversal in the development of adult age-dependent immunity. This finding is consistent with the existing observational and biological evidence for the endocrine influence of puberty on malarial immunity. This is a subject that has been little studied and there is a scarcity of information on the influence of endocrine factors on malaria, and puberty changes are a good example of their potential importance.

Malaria parasiteamia prevalence was associated with a lack of access to adequate preventive measures and low participant education. Symptomatic malaria, defined on the basis of measured fever and the presence of parasites on light microscopy was uncommon. Although the burden of malaria exposure reflected in rapid diagnostic positivity, which measured current and past parasitaemia (previous 2 to 4 weeks) was much higher. Low paternal education and stunting were associated with increased prevalence of symptomatic malaria. This observation is a reflection of poverty, poor nutrition and poorer access to public health interventions or health services in this population group.

9.2.2 Implications for public health

Pubertal influences on the susceptibility of malaria infection and related acquisition of immunity underscore the importance of hormonal influences on malaria infection. Improved understanding of this phenomenon would help determine how malaria immunity continues to develop beyond childhood. Knowledge of pre-pregnancy malaria in adolescents is important for improved targeting and timing of interventions in order to reduce malaria-related complications of pregnancy. This is relevant for the evaluation of malaria vaccines targeted at reducing malaria risk in pregnancy, which are under development and are based on immunization against VAR2 CSA antigens associated with placental malaria parasite sequestration. Non-pregnant adolescents are likely to be the target group for these immunization programmes should an efficacious vaccine be developed. The adolescent age for timing such vaccination and the influence of puberty on vaccine responses is animprotant consideration.

The presence of parasites at sub-clinical levels is important immunologically, but is not without complications such as anaemia. With scaling up of interventions for malaria prevention such as the use of ITNs (Killeen *et al.* 2008) and promising use of potential vaccines (Greenwood *et al.* 2008), the epidemiology of malaria infection in adolescents will change, highlighting the importance of some form of monitoring and surveillance in this age group. The consistent use of insecticide treated bed nets in younger children may lead to lower levels of age dependent malaria immunity in older children and adolescents leading to increased risk patterns for infection and disease in adolescence.

9.2.3 Future research

The value of documenting gynaecological age in relation to altered malaria infection risk in adolescents or pregnancy should be confirmed. This could influence parity-specific risk in primigravidae as early adolescent pregnancies may present with the combined risk of puberty and pregnancy. With application of gynaecological age, pre-menarcheal adolescents all fall into one sub-group. Conversely, Tanner breast staging separates out pre-menarcheal adolescents, but tends to group post-menarcheal adolescents into two groups as onset of menses corresponds to both B4 and B5 Tanner stages. A method to combine these categories to allow an extension of the existing demarcations of prepubertal, pubertal and post-pubertal categories would be preferable. Identification and validation of such a combined measure would be required and this could provide a non-invasive clinical indicator, which could be used where physiological measurement of hormones such as DHEAS was not possible.

9.3 ITN access and utilisation

9.3.1 Lessons learnt and conclusions

ITN scale up interventions in the malaria programme in this study area have successfully led to high household ITN coverage which has ensured ITN access to at least half of the adolescent females surveyed even though they are not a current priority group according to prevailing policy. ITN access was significantly associated with maternal literacy, gynaecological age and marital status. However, access was lower during mid-adolescence, which corresponded to the period of pubertal influence on malarial immunity, a time when ITNs could provide the necessary prevention from malaria infection. Use of the ITNs was low and varied by season, which is an important challenge for many ITN implementation programmes. This was evident possibly because ITN access and use in these adolescents was not translated into

significant improvements in malaria prevalence which would promote better coverage and more frequent adherence. Nevertheless, availability of an ITN in the household was associated with reduced malaria parasite prevalence, which reflected the importance of the communal benefits of ITNs.

9.3.2 Implications for public health

These findings support the need for enhanced promotion in order to increase coverage and use in this risk group with potential personal and communal gains. This should complement the current policy of prioritizing young children and pregnant women. Change of current policy to one that is equitable to adolescents is required, which raises the issues of distribution mechanisms, cost and the issue of subsidies for implementation outside of antenatal clinics. Various innovations in delivering ITNs through the public or private or hybrid-combined approaches co-exist in varied settings. Improving correct utilization of ITNs that are already available in communities requires a multi-pronged approach. This is linked to poverty reduction, for example, through education and economic empowerment. Adolescents are an ideal target group for such interventions especially by provision of Adolescent Friendly Health Services (AFHS).

9.3.3 Future research

Trials of AFHS service packages focusing on preventing malaria and related infectious diseases are needed to provide better understanding and in order to develop strategies for ITN delivery to this group. These might be combined with sexual health strategies, targeted immunization (e.g. with tetanus or human papilloma virus vaccines) and food fortification or supplementation.

9.4 Malaria self-treatment practices

9.4.1 Lessons learnt and conclusions

These adolescents frequently self-treated for presumed malaria infection, which could potentially contribute to substantial inadvertent antimalarial drug exposures in early pregnancy, an issue of vital importance especially with more widespread access of ACTs. Anti-malarial self-treatment was significantly associated with increasing maturity, improved literacy and symptomatic malaria. Although the estimates are limited by the small sample size, they nevertheless indicate that the evidence for exposure to anti-malarials during early pregnancy is a cause for concern and they provide the first estimates for first trimester exposure from a community as opposed to health facility based surveys. Modelling suggests that the probability an embryo will encounter artemisinins during the critical six-week period (at week four to week ten of gestation) through accidental exposure is 12% for areas where adults receive on average one treatment with three days of artemisinin-based combination therapy per year(Dellicour *et al.* 2008). This exposure estimate includes treatment of confirmed and presumed malaria.

Adolescent-friendly strategies to improve malaria seeking behaviour in malaria endemic areas during the pre-pregnancy period are required to facilitate improved understanding and use of anti-malarials. The issue also arises whether young adolescents with delayed menstrual periods should not be treated with ACTs, or even whether pregnancy testing should be more widely available prior to treatments. The adolescents readily accepted pregnancy testing in these surveys and compliance was not an issue so long as confidentiality was assured. Greater use of pregnancy testing may be feasible in adolescent girls although currently it is infrequently done. There use should be considered before treating uncomplicated malaria in girls who may be in

early pregnancy. Quinine may be the preferred choice in these circumstances until further trial data is available on the safety of artemisinin combination therapy in pregnancy.

9.4.2 Implications for public health

In the face of limited data on drug safety of ACTs in pregnancy especially in the first trimester, antimalarial self-treatment is potentially dangerous. Although accessibility of anti-malarial drugs within communities ensures prompt treatment of suspected malaria infections, mis-diagnosis and inadequate treatment are inevitable. This has an impact on the development of drug resistance, treatment costs and possible influences in the clinical presentation of malarial illness when partially treated. Improving access to health services and more positive health seeking behaviour should reduce incorrect treatments.

9.4.3 Future research

Further research into patterns of treatment behaviour by adolescents is needed particularly where policy has changed to first-line treatment with ACTs. Risk assessment tools for potential pregnancy in adolescent girls seeking treatment for malaria need to be developed. Trials of interventions aimed at reducing self-treatment and other inappropriate treatment seeking behaviour as well as enhancing good treatment seeking practices would provide valuable information.

9.5 Adolescent health literacy

9.5.1 Lessons learnt and conclusions

Improving adolescent health literacy in vulnerable out of school girls was effective for improving knowledge and behaviour related to malaria prevention and control prior to pregnancy. This shows the importance of health literacy and not just

education in bringing about public health gains. Absenteeism from classes posed a challenge. While regular attendees achieved better basic literacy, conversely, poor improvement in malaria knowledge was significantly associated with poor literacy attainment. Before commencement of classes, poor knowledge of malaria-related issues was best predicted by young age and having an illiterate mother. Maternal experience was also a good 'teacher' as evidenced by the improved malaria knowledge of married adolescents. Experience of malaria or related issues can be obtained at individual, peer or community level. Acquisition of malaria knowledge through health literacy combines all these approaches and re-enforces the competence of these young people.

9.5.2 Implications for public health

Success of such a programme relies on community willingness to participate and not the girls alone. This study offers evidence of an out-of-school strategy which was successful in providing health literacy to adolescent girls, thereby empowering them for motherhood and improving health seeking behaviours related to malaria control. Improving access to formal education for girls still remains a priority. However, the parallel development of functional health literacy for out-of-school girls is important for improving health outcomes in a higher risk group.

9.5.3 Future research

Innovative approaches to attract and sustain girls' attendance in literacy programmes such as the AGLIT require further research. This would enable girls who are irregular attendees to benefit more from this health literacy strategy. Replication of adolescent health literacy programmes in other settings would provide a broader

understanding of the role of this approach, which has its greatest relevance in regions with high illiteracy rates and poor school attendance.

9.6 Summary of outcome measures and associated factors

The summary of the multi-variate factor analyses shows ten participant characteristics that were associated with one or more of the outcomes measured (Table 9.1). This highlights the inter-connection of these factors and their varied associations with the prevalence of malaria, or knowledge related to it, in this population. For example, season of survey was significantly associated with low height-for-age z-scores, low MUAC-for age, the use of ITNs, and the prevalence of malaria parasitaemia. Stunting was also associated with symptomatic malaria. The association of these factors to the individual as well as in the community is complex and inter-related. The number of significant associations provides a cogent argument for promoting improved strategies for malaria control in adolescent girls. Greater attention to this risk group should have long-term consequences not only for the individual, but for the future mother and her children.

Table 9.1: Summary of multivariate factor analyses in the rural adolescent female surveys

	HAZ	BMI-Z	Low MUAC-FA	Malaria knowledge	ITN access	ITN use	Malaria parasitaemia	Symptomatic malaria	Anti-malarial self-treatment
Co-variate									
Season (ref Dry)	n/a	n/a	n/a	n/a	n/a	2.04 *** (1.58, 2.63)	n/a	n/a	n/a
Age (yrs), ascending	-	-	-	1.49 * ‡ (1.05, 2.13)	-	-	0.92 * (0.87, 0.97)	0.93 * (0.86, 0.98)	-
Gynaecol. age	-	-	-	-	2.03 ** ¥ (1.28, 3.17)	1.29 *** (1.19, 1.41)	0.63 * (0.40, 0.98)	0.65 β (0.40, 1.01)	1.51 *** (1.18, 1.93)
Participant education	-	-	-	-	1.65 ** (1.12, 2.43)	1.12 *** (1.06, 1.18)	-	-	1.10 * † (1.02, 1.20)
Maternal education ¶	-	1.19 * (0.99,1.43)	-	6.44 * ‡ (1.25, 33.12)	1.06 * (1.01,1.12)	-	-	-	-
Paternal education	-	-	-	-	-	-	0.88 ** (0.80, 0.9)	0.83 ** (0.74, 0.94)	-
Unmarried (ref married)	-	-	-	{3.59 * § (1.21,10.67)}	0.27 ** (0.10, 0.73)	-	-	-	-
Symptomatic malaria	-	-	-	-	-	-	-	-	6.57 ** (1.85, 23.37)

Values are adjusted PRR except AOR for malaria knowledge column, brackets are 95 % CI; p-values are * <0.05, ** <0.01 and *** <0.001 except β which means borderline statistical significance; n/a in season row means not applicable because PRRs were adjusted for seasonal effect except under ITN use; ¶ maternal education is years of schooling except under malaria knowledge column it is maternal illiteracy (<4 years schooling) compared to literacy (≥ 4 years schooling); ‡ unmarried participants only; § married participants only; { } is has children; ¥ postmenarche compared to premenarche; † literacy score as continuous variable.

BIBLIOGRAPHY

1. Abebe F, Gaarder PI, Petros B and Gundersen SG. Differences in prevalence, intensity of infection and parasite-specific antibody levels do not predict different age-infection profiles. A study in two communities endemic for *Schistosoma mansoni* infections. APMIS 2002;110:535-44.
2. Abuya TO, Mutemi W and Karisa B. Use of over-the-counter malaria medicines in children and adults in three districts in Kenya: Implications for private medicine retailer interventions. *Malaria Journal* 2007;6:57.
3. ACC/ SCN. Third Report on the World Nutrition situation. UN Adminstration Committee on Coordination/ Sub-committee on Nutrition. 1997. Geneva.
4. ACC/ SCN. Fourth Report on the World Nutrition situation. UN Adminstration Committee on Coordination/ Sub-committee on Nutrition. 2000. Geneva.
5. Aidoo M, Terlouw DJ, Kolczak MS, McElroy PD, ter Kuile FO, Kariuki S, Nahlen BL, Lal AA and Udhayakumar V. Protective effects of the sickle cell gene against malaria morbidity and mortality. *Lancet* 2002;359:1311-2.
6. Alaii JA, Hawley WA, Kolczak MS, ter Kuile FO, Gimnig JE, Odhacha A, Oloo AJ, Nahlen BL and Philips-Howard PA. Factors affecting use of permethrin-treated bed nets during a randomized controlled trial in western Kenya. *Am J Trop Med Hyg* 2003;68:137-41.
7. Allen LH. Anaemia and iron deficiency: effects on pregnancy outcome. *Am J Clin Nutr* 2000;71:1280S-1284.
8. Allen SJ, Raiko A, O'Donnel A, Alexander NDE and Clegg JB. Causes of preterm delivery and intrauterine growth retardation in a malaria endemic region of Papua New Guinea. *Arch Dis Child Fetal Neonatal Ed* 1998;79:F135-F140.
9. Allotey P and M. Gyapong. The gender agenda in the control of tropical diseases: a review of current evidence. 2005. World Health Organization Special Programme for Research, Geneva, Switzerland.
10. Appleyard, B Tuni M, Cheng Q, Chen N, Bryan J and McCarthy JS. Malaria in Pregnancy in the Solomon Islands: Barriers to Prevention and Control. *Am J Trop Med Hyg* 2008;78:449-54.
11. Asobayire FS, Adou P, Davidsson L, Cook JD and Hurrell RF. Prevalence of iron deficiency with and without concurrent anaemia in population groups with high

prevalences of malaria and other infections: a study in Cote d'Ivoire. *Am J of Clin Nutr* 2001; 74:776-782.

12. Atzori C, Bruno A, Chichino G, Cevini C, Bernuzzi AM, Gatti S, Comolli G and Scaglia M. HIV-1 and parasitic infections in rural Tanzania. *Ann Trop Med Parasitol.* 1993;87:585-93.
13. Auchus RJ and Rainey WE. Adrenarche - physiology, biochemistry and human disease. *Clinical Endocrinology* 2004;60:288-96.
14. Baird JK. Age dependent characteristics of protection v. susceptibility to *Plasmodium falciparum. Ann Trop Med Parasitol* 1998;92:367-90.
15. Barker G. Adolescents, social support and help-seeking behaviour: an international literature review and programme consultation with recommendations for action. http://www.who.int/child-adolescent-health/publications/ [WHO discussion papers on adolescence]. 2007. Accessed 10-10-2007.
16. Barnish G, Bates I and Iboro J. Newer drug combinations for malaria. *BMJ* 2004;328:1511-2.
17. Beard JL. Iron Requirements in Adolescent Females. *Journal of Nutrition* 2000;130:440.
18. Bejon P, Mwangi TW, Lowe B, Peshu N, Hill AV and Marsh K. Helminth infection and eosinophilia and the risk of *Plasmodium falciparum* malaria in 1- to 6-year-old children in a malaria endemic area. *PLoS Negl Trop Dis* 2008;2:e164.
19. Belizario VY, Saul A, Bustos MD, Lansang MA, Pasay CJ, Gatton M and Salazar NP. Field epidemiological studies on malaria in a low endemic area in the Philippines. *Acta Trop* 1997;63:241-56.
20. Bell DR, Jorgensen P, Christophel EM and Palmer KL. Malaria risk: estimation of the malaria burden. *Nature* 2005;437:E3-E4.
21. Bennett S, Woods T, Liyanage WM and Smith DL. A simplified general method for cluster-sample surveys of health in developing countries. *World Health Stat Q* 1991;44:98-106.
22. Bharti AR, Patra KP, Chuquiyauri R, Kosek M, Gilman RH, Llanos-Cuentas A and Vinetz JM. Polymerase Chain Reaction detection of *Plasmodium vivax* and *Plasmodium falciparum* DNA from stored serum samples: Implications for retrospective diagnosis of malaria. *Am J Trop Med Hyg* 2007;77:444-6.

23. Binka FN, Indome F and Smith T. Impact of spatial distribution of permethrin-impregnated bed nets on child mortality in rural Northern Ghana. *Am J Trop Med Hyg* 1998;59:80-5.

24. Bloem MW. Interdependence of vitamin A and iron: an important association for programmes of anaemia control. *Proc Nutr Soc.* 1995;54:501-8.

25. Ayisi J, Boriga DA, Oloo AJ, Beach R, Hawley W, Lal A, Nahlen B, Udhayakumar V, Campbell CC, Ruebush TK and McCormick JB. Longitudinal cohort study of the epidemiology of malaria infections in an area of intense malaria transmission I. Description of study site, general methodology, and study population. *Am J Trop Med Hyg* 1999;60:635-40.

26. Boareto AC, Muller JC, Bufalo, Botelho GG, de Araujo SL, Foglio MA, de Morais RN and Dalsenter PR. Toxicity of artemisinin [*Artemisia annua L.*] in two different periods of pregnancy in Wistar rats. *Reproductive Toxicology* 2008;25:239-46.

27. Boonma P, Christensen P, Suwanarusk R, R, Price RN, Russell B and Lek-Uthai U. Comparison of three molecular methods for the detection and speciation of *Plasmodium vivax* and *Plasmodium falciparum. Malaria Journal* 2007;6:124.

28. Bowie C, Purcell B, Shaba B, Makaula P and Perez M. A national survey of the prevalence of schistosomiasis and soil transmitted helminths in Malawi. *BMC Infectious Diseases* 2004;4:49.

29. Brabin B. Malaria in pregnancy: current issues. *Afr Health* 1997;19:19-20.

30. Brabin BJ. Epidemiology of Infection in Pregnancy. *Reviews of Infectious Diseases* 1985;7:579-603.

31. Brabin BJ. An analysis of malaria in pregnancy in Africa. *Bull World Health Organ* 1983;61:1005-16.

32. Brabin BJ, Ginny M, Sapau J, Galme K and Paino J. Consequences of maternal anaemia on outcome of pregnancy in a malaria endemic area in Papua New Guinea. *Ann Trop Med Parasitol* 1990;84:11-24.

33. Brabin BJ, Perrin L. Sickle cell trait and *Plasmodium falciparum* parasitaemia in pregnancy in Western Province, Kenya. *Trans R Soc Trop Med Hyg* 1985;79:733.

34. Brabin BJ, Eggelte TA, Parise M and Verhoeff F. Dapsone therapy for malaria during pregnancy: maternal and fetal outcomes. *Drug Saf* 2004;27:633-48.

35. Brabin BJ, Romagosa C, Abdelgalil S, Menéndez C, Verhoeff FH, McGready R, Fletcher KA, Owens S, D'Alessandro U, Nosten F and Fischer PR, Ordi J. The sick Placenta-the role of malaria. *Placenta* 2004;25:359-78.

36. Brabin L. The adolescent health gap in developing countries. *Ann Trop Paediatr* 2004;24:115-6.
37. Brabin L and Brabin BJ. Parasitic infections in women and their consequences. *Adv Parasitol* 1992;31:1-81.
38. Brabin L and Brabin BJ. The cost of successful adolescent growth and development in girls in relation to iron and vitamin A status. *Am J Clin Nutr* 1992;55:955-8.
39. Brabin L and Brabin BJ. HIV, malaria and beyond: reducing the disease burden of female adolescents. *Malaria Journal* 2005;4:2.
40. Brabin L and Brabin BJ. Malaria and glucose 6-phosphate dehydrogenase deficiency in populations with high and low spleen rates in Madang, Papua New Guinea. *Hum Hered* 1990;40:15-21.
41. Brabin L, Ikimalo J, Dollimore N, Kemp J, Ikokwu-Wonodi C, Babatunde S, Obunge O and Briggs N. How do they grow? A study of south-eastern Nigerian adolescent girls. *Acta Paediatr* 1997;86:1114-20.
42. Brabin L, Roberts C, Barr F, Agbaje S, Harper G and Briggs N. Sex hormone patterns and serum retinol concentrations in adolescent girls. *J Reprod Med* 2004;49:41-51.
43. Brabin L, Verhoeff FH, Kazembe P, Brabin BJ, Chimsuku L and Broadhead R. Improving antenatal care for pregnant adolescents in southern Malawi. *Acta Obstet Gynecol Scand* 1998;77:402-9.
44. Brook CGD. All about adolescence. John Wiley & Sons Ltd., 1985.
45. Brooks-Gunn J, Warren MP, Russo J and Gargiulo J. Validity of self-report measures of girls' pubertal status. *Child Dev* 1987;58: 829–841
46. Bruce-Chwatt L. A longitudinal survey of natural malaria infection in a group of West African adults. Part II. *West Afr Med J* 1963;12:199-217.
47. Butte NF, Garza C and de Onis M. Evaluation of the feasibility of International Growth Standards for school-aged children and adolescents. *Journal of Nutrition* 2007;137:153-7.
48. Caldwell JC. Education as a factor in mortality decline: an examination of Nigerian data. *Population Studies* 1979;33:395-413.
49. Caldwell JC. Routes to low mortality in poor countries. *Population and Development Review* 1986;12:171-220.
50. Camargo LM, dal Colletto GM, Ferreira MU, Gurgel Sde M, Escobar AL, Marques A, Krieger H, Camargo EP and da Silva LH. Hypoendemic malaria in Rondonia

(Brazil, western Amazon region): seasonal variation and risk groups in an urban locality. *Am J Trop Med Hyg* 1996;55:32-8.

51. Camargo LM, Ferreira MU, Krieger H, De Camargo EP and da Silva LP. Unstable hypoendemic malaria in Rondonia (western Amazon region, Brazil): epidemic outbreaks and work-associated incidence in an agro-industrial rural settlement. *Am J Trop Med Hyg* 1994;51:16-25.
52. Camargo LM, Noronha E, Salcedo JM, Dutra AP, Krieger H, Pereira da Silva LH and Camargo EP. The epidemiology of malaria in Rondonia (Western Amazon region, Brazil): study of a riverine population. *Acta Trop* 1999;72:1-11.
53. Caneiro IA, Smith T, Lusingu JPA, Malima R, Utzinger J and Drakeley CJ. Modeling the relationship between the population prevalence of *Plasmodium falciparum* malaria and anemia. *Am J Trop Med Hyg* 2006;75:82-9.
54. Carme B, Yombi B, Bouquety JC, Plassard H, Nzingoula S, Senga J, & Akani I. Child morbidity and mortality due to cerebral malaria in Brazzaville, Congo. A retrospective and prospective hospital-based study 1983-1989. *Trop Med Parasitol.* 1992;43:173-6.
55. Casalino E, Le Bras J, Chaussin F, Fichelle A and Bouvet E. Predictive factors of malaria in travelers to areas where malaria is endemic. *Arch Intern Med* 2002;162:1625-1630.
56. Centres for Disease Control (CDC). Epi Info. 2000. 2002.
57. Cernetich A, Garver LS, Jedlicka AE, Klein PW, Kumar N, Scott AL, Klein SL. Involvement of gonadal steroids and gamma interferon in sex differences in response to blood-stage malaria infection. *Infect Immun* 2006;74:3190-203.
58. Challis K, Osman NB, Cotiro M, Nordahl G, Dgedge M and Bergström S. Impact of a double dose of sulphadoxine-pyrimethamine to reduce prevalence of pregnancy malaria in southern Mozambique. *Trop Med Int Health* 2004;9:1066-73.
59. Chan NP, Sung RY, Kong AP, Goggins WB, So HK and Nelson EA. Reliability of pubertal self-assessment in Hong Kong Chinese children. *J Paediatr Child Health* 2008;44:353-8.
60. Chandramohan D, Jaffar S and Greenwood B. Use of clinical algorithms for diagnosing malaria. *Trop Med Int Health* 2002;7:45-52.

61. Chowdhury S, Shahabuddin AK, Seal AJ, Talukder KK, Hassan Q, Begum RA, Rahman Q, Tomkins A, Costello A and Talukder MQ. Nutritional status and age at menarche in a rural area of Bangladesh. *Ann Hum Biol* 2000;27:249-56.

62. Chung MK, Han SS, Roh JK. Synergistic embryotoxicity of combination pyrimethamine and folic acid in rats. *Reprod Toxicol* 1993;7:463-8.

63. Clark RL, White TE, Clode A, Gaunt I, Winstanley P and Ward SA. Developmental toxicity of artesunate and an artesunate combination in the rat and rabbit. *Birth Defects Res B Dev Reprod Toxicol* 2004;71:380-94.

64. Cleland JG, Van Ginneken JK. Maternal education and child survival in developing countries: the search for pathways of influence. *Soc Sci Med* 1988;27:1357-68.

65. Cordeiro LS, Lamstein, S., Mahmud, Z., and Levinson, F. J. Adolescence: a pivotal stage in the lifecycle. SCN News [31], 8-15. 2006. United Kingdom, Lavengham press. 19-7-2007.

66. Colbourne MJ. The effect of malaria suppression in a group of Accra school children. *Trans R Soc Trop Med Hyg* 1955;49:556-69.

67. Coleman RE, Sattabongkot J, Promstaporm S, Maneechai N, Tippayachai B, Kengluecha A, Rachapaew N, Zollner G, Miller RS, Vaughan JA, Thimasarn K and Khuntirat B. Comparison of PCR and microscopy for the detection of asymptomatic malaria in a *Plasmodium falciparum/vivax* endemic area in Thailand. *Malaria Journal* 2006;5:121.

68. Coly AN, Milet J, Diallo A, Ndiaye T, Bénéfice E, Simondon F, Wade S and Simondon KB. Preschool stunting, adolescent migration, catch-up growth, and adult height in young Senegalese men and women of rural origin. *Journal of Nutrition* 2006;136:2412-20.

69. Coutinho HM, Leenstra T, Acosta LP, Olveda RM, McGarvey ST, Friedman JF and Kurtis JD. Higher serum concentrations of DHEAS predict improved nutritional status in helminth-infected children, adolescents, and young adults in Leyte, the Philippines. *Journal of Nutrition* 2007;137:433-9.

70. Cox-Singh J, Mahayet S, Abdullah MS and Singh B. Increased sensitivity of malaria detection by nested polymerase chain reaction using simple sampling and DNA extraction. *Int J Parasitol* 1997;27:1575-7.

71. Cox-Singh J, Davis TME, Lee KS, Lee KS, Shamsul SS, Matusop A, Ratnam S, Rahman HA, Conway DJ and Singh B. *Plasmodium knowlesi* malaria in humans is

widely distributed and potentially life threatening. *Clinical Infectious Diseases* 2008;46:165-171.

72. Cruciani M, Nardi S, Malena M, Bosco O, Serpelloni G and Mengoli C. Systematic review of the accuracy of the ParaSight-F test in the diagnosis of *Plasmodium falciparum* malaria. *Med Sci Monit* 2004;10:MT81-MT88.

73. Cserti CM, Dzik WH. The ABO blood group system and *Plasmodium falciparum* malaria. *Blood* 2007;110:2250-8.

74. Cuzick J. A method for analysing case-control studies with ordinal exposure variables. *Biometrics* 1985;41:609-21.

75. D'Alessandro S, Gelati M, Basilico N, Parati EA, Haynes RK and Taramelli D. Differential effects on angiogenesis of two antimalarial compounds, dihydroartemisinin and artemisone: Implications for embryotoxicity. *Toxicology* 2007;241:66-74.

76. de Blas I de Blas I, Ortega C, Frankena K, Noordhuizen JP and Thrusfield M. Winepiscope 2.0. August 2000. http://www.clive.ed.ac.uk/cliveCatalogueItem.asp?id=B6BC9009-C10F-4393-A22D-48F436516AC4

77. de Onis M and Habicht JP. Anthropometric reference data for international use: recommendations from a World Health Organization Expert Committee. *Am J Clin Nutr* 1996;64:650-8.

78. Dellicour S, Hall S, Chandramohan D and Greenwood B. The safety of artemisinins during pregnancy: a pressing question. *Malaria Journal* 2007;6:15.

79. Dellicour S, Ter Kuile FO and Stergachis A. Pregnancy exposure registries for assessing antimalarial drug safety in pregnancy in malaria-endemic countries. *PLoS Med.* 2008 Sep 9;5(9):e187. [Epub ahead of print]

80. DeMaeyer E and els-Tegman M. The prevalence of anaemia in the world. *World Health Stat Q* 1985;38:302-16.

81. Deressa W. Treatment-seeking behaviour for febrile illness in an area of seasonal malaria transmission in rural Ethiopia. *Malaria Journal* 2007;6:49.

82. Desai MR, Terlouw DJ, Kwena AM, Phillips-Howard PA, Kariuki SK, Wannemuehler KA, Odhacha A, Hawley WA, Shi YP, Nahlen BL and Ter Kuile FO. Factors associated with hemoglobin concentrations in pre-school children in Western Kenya: cross-sectional studies. *Am J Trop Med Hyg* 2005;72:47-59.

83. Dewalt DA , Berkman ND, Sheridan S, Lohr KN and Pignone MP. Literacy and health outcomes: a systematic review of the literature. *J Gen Intern Med* 2004;19:1228-39.

84. Diagne N, Rogier C, Cisse B and Trape JF. Incidence of clinical malaria in pregnant women exposed to intense perennial transmission. *Trans R Soc Trop Med Hyg* 1997;91:166-70.

85. Dickson KE, Ashton J and Smith JM. Does setting adolescent-friendly standards improve the quality of care in clinics? Evidence from South Africa. *Int Journal for Quality in Health Care* 2007;19:80-9.

86. Dreizen S, Spirakis CN and Stone RE. A comparison of skeletal growth and maturation in undernourished and well-nourished girls before and after menarche. *J Pediatr* 1967;70:256-63.

87. Dunkel L, Alfthan H, Stenman UH, Selstam G, Rosberg S and Albertsson-Wikland K. Developmental changes in 24-hour profiles of leuteinizing hormone and follicle-stimulating hormone from prepuberty to midstages of puberty in boys. *J Clin Endocrinol Metab* 1992;74(4):890-7.

88. Edson F and Kayombo EJ. Knowledge on malaria transmission and its prevention among schoolchildren in Kyela District, south-western Tanzania. *Tanzan Health Res Bull* 2007;9:207-10.

89. Espino F and Manderson L. Treatment seeking for malaria in Morong, Bataan, The Philippines. *Soc Sci Med* 2000;50:1309-16.

90. Fazio-Tirrozzo G, Brabin L, Brabin B, Agbaje O, Harper G and Broadhead R. A community based study of vitamin A and vitamin E status of adolescent girls living in the Shire Valley, Southern Malawi. *Eur J Clin Nutr* 1998;52:637-42.

91. Flaherty A, Kipp W and Mehangye I. 'We want someone with a face of welcome': Ugandan adolescents articulate their family planning needs and priorities. *Tropical Doctor* 2005;35:4-7.

92. Fleming AF. Tropical obstetrics and gynaecology. 1. Anaemia in pregnancy in tropical Africa. *Trans R Soc Trop Med Hyg* 1989;83:441-8.

93. Fried M, Nosten F, Brockman A, Brabin BJ and Duffy PE. Maternal antibodies block malaria. *Nature* 1998;395:851-2.

94. Friedman JF, Kurtis JD, Mtalib R, Opollo M, Lanar DE and Duffy PE. Malaria is related to decreased nutritional status among male adolescents and adults in the setting of intense perennial transmission. *J Infect Dis* 2003;188:449-57.

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

96. Fuller B, Singer JD and Keiley M. Why do daughters leave school in southern Africa? Family economy and mothers' commitments. *Social Forces* 1995;74:657-81.

97. Gamble C, Ekwaru JP and ter Kuile FO. Insecticide-treated nets for preventing malaria in pregnancy. *Cochrane Database Syst Rev* 2006;CD003755.

98. Geissler PW, Meinert L, Prince R, Nokes C, Aagaard-Hansen J, Jitta J and Ouma JH. Self-treatment by Kenyan and Ugandan schoolchildren and the need for school-based education. *Health Policy and Planning* 2001;16:362-71.

99. Geissler PW, Nokes K, Prince RJ, Odhiambo RA, Aagaard-Hansen J and Ouma JH. Children and medicines: self-treatment of common illnesses among Luo schoolchildren in western Kenya. *Soc Sci Med* 2000;50:1771-83.

100. Giao PT, Vries PJ, Binh TQ, Nam NV and Kager PA. Early diagnosis and treatment of uncomplicated malaria and patterns of health seeking in Vietnam. *Trop Med Int Health* 2005;10:919-25.

101. Giha HA, Elbasit IE, Elgadir TME, Adam I, Berzins K, Elghazali G and Elbashir MI. Cerebral malaria is frequently associated with latent parasitemia among the semi-immune population of eastern Sudan. *Microbes and Infection* 2005;7:1196-203.

102. Gimnig JE, Kolczak MS, Hightower AW, Vulule JM, Schoute E, Kamau L, Phillips-Howard PA, ter Kuile FO, Nahlen BL and Hawley WA. Effect of permethrin-treated bed nets on the spatial distribution of malaria vectors in western Kenya. *Am J Trop Med Hyg* 2003;68:115-20.

103. Gordi T and Lepist EI. Artemisinin derivatives: toxic for laboratory animals, safe for humans? *Toxicology Letters* 2004;147:99-107.

104. Government of Malawi. Malawi Intergrated Household Survey. 1998. Lilongwe, Malawi.

105. Government of Malawi. Malawi and the Millennium development goals. 2005. Lilongwe, Malawi.

106. Government of Malawi, UNICEF. Mid-term evaluation of girl child education interventions, Country Programme 2002-2006. 2005. Lilongwe, Malawi.

107. Greenberg AE, Ntumbanzondo M, Ntula, N Mawa L, Howell J and Davachi F. Hospital-based surveillance of malaria-related paediatric morbidity and mortality in Kinshasa, Zaire. *Bull World Health Organ* 1989;67:189-96.

108. Greenwood B, Marsh K, Snow R. Why do some African children develop severe malaria? *Parasitol Today* 1991;7:277-81.

109. Guyer B (1998). Problem-solving in public health. In *Epidemiology and health services*. (eds. HK Armenian & S Shapiro) Oxford University Press, New York, p 15-26.

110. Greenwood BM , Fidock DA, Kyle DE, Kappe SH, Alonso PL, Collins FH and Duffy PE. Malaria: progress, perils and prospects for eradication. *J Clin Inv* 2008;118:1266-76.

111. Haas JD and Brownlie T. Iron Deficiency and Reduced Work Capacity: A Critical Review of the Research to Determine a Causal Relationship. *Journal of Nutrition* 2001;131:676S-690.

112. Hawley WA, Phillips-Howard PA, ter Kuile FO, Terlouw DJ, Vulule JM, Ombok M, Nahlen BL, Gimnig JE, Kariuki SK, Kolczak MS and Hightower AW. Community-wide effects of permethrin-treated bednets on child mortality and malaria morbidity in western Kenya. *Am J Trop Med Hyg* 2003;68 (Supplement 4):121-7.

113. Heald FP and Gong EJ. Diet, Nutrition and Adolescence. In: Shils ME, Olson JA, Shike M, Roos AC, eds. Modern Nutrition in Health and Disease. Maryland: Williams and Wilkins, 1999.

114. Hii JLK, Smith T, Vounatsou P, Alexander N, Mai A, Ibam E and Alpers MP. Area effects of bednet use in a malaria-endemic area in Papua New Guinea. *Trans R Soc Trop Med Hyg* 2001;95:7-13.

115. Hill AVS. Aspects of Genetic Susceptibility to Human Infectious Diseases. *Annual Review of Genetics* 2006;40:469-86.

116. Hill J, Lines J and Rowland M. Insecticide-treated nets. *Adv Parasitol* 2006;61:77-128.

117. Hoffman IF, Jere CS, Taylor TE, Munthali P, Dyer JR, Wirima JJ, Rogerson SJ, Kumwenda N, Eron JJ, Fiscus SA, Chakraborty H, Taha TE, Cohen MS and Molyneux ME. The effect of *Plasmodium falciparum* malaria on HIV-1 RNA blood plasma concentration. *AIDS* 1999;13:487-94.

118. Hogg A, Makwiza B, Mlanga S, Broadhead R and Brabin L. Finding a curriculum that works under trees: literacy and health education for adolescent girls in rural Malawi. *Development in Practice* 2005;15:655-67.

119. Holtz TH, Kachur SP, Marum LH, Mkandala C, Chizani N, Roberts JM, Macheso A and Parise ME. Care seeking behaviour and treatment of febrile illness in children aged less than five years: a household survey in Blantyre District, Malawi. *Trans R Soc Trop Med Hyg* 2003;97:491-7.

120. Hopkins H, Kambale W, Kamya MR, Staedke SG, Dorsey G and Rosenthal PJ. Comparison of HRP2- and pLDH-based rapid diagnostic tests for malaria with longitudinal follow-up in Kampala, Uganda. *Am J Trop Med Hyg* 2007;76:1092-7.

121. Hotchkiss DR and Gordillo A. Household health expenditures in Morocco: implications for health care reform. *Int J Health Plann Manage* 1999;14:201-17.

122. Howard SC, Omumbo J, Nevill CG, Some ES, Donnelly CA and Snow RW. Evidence for a mass community effect of insecticide treated bednets on the incidence of malaria on the Kenyan coast. *Trans R Soc Trop Med Hyg* 2000;94:357-60.

123. Job JC, Garnier PE, Chaussain JL and Milhaud G. Elevation of serum gonadotropins (LH and FSH) after releasing hormone (LH-RH) injection in normal children and in patients with disorders of puberty. *J Clin Endocrinol Metab* 1972;35:473-6.

124. Jonkman A, Chibwe RA, Khoromana CO, Liabunya UL, Chaponda ME, Kandiero GE, Molyneux ME and Taylor TE. Cost-saving through microscopy-based versus presumptive diagnosis of malaria in adult outpatients in Malawi. *Bull World Health Organ* 1995;73:223-7.

125. Kalanda BF, Verhoeff FH and Brabin BJ. Chronic malnutrition in pregnant adolescents in rural Malawi: an anthropometric study. *Acta Obstet Gynecol Scand* 2006;85:33-9.

126. Kantele A, Marti H, Felger I, Muller D and Jokiranta TS. Monkey malaria in a European traveler returning from Malaysia. *Emerg Infect Dis* 2008;14:1434-1436.

127. Kaseje DC, Spencer HC and Sempebwa EK. Usage of community-based chloroquine treatment for malaria in Saradidi, Kenya. *Ann Trop Med Parasitol* 1987;81 Suppl 1:111-5.

128. Kazembe L, Appleton C and Kleinschmidt I. Choice of treatment for fever at household level in Malawi: examining spatial patterns. *Malaria Journal* 2007;6:40.

129. Khan MA. Knowledge on *AIDS* among female adolescents in Bangladesh: evidence from the Bangladesh demographic and health survey data. *J Health Popul Nutr* 2002;20:130-7.

130. Kidane G and Morrow RH. Teaching mothers to provide home treatment of malaria in Tigray, Ethiopia: a randomised trial. *Lancet* 2000;356:550-5.

131. Killeen GF and Smith TA. Exploring the contributions of bednets, cattle, insecticides and excito-repellency to malaria control: A deterministic model of mosquito host-seeking behaviour and mortality. *Trans R Soc Trop Med Hyg* 2007;101(9):867-80.

132. Killeen GF, Smith TA, Ferguson HM, Mshinda H, Abdulla S, Lengeler C and Kachur SP. Preventing childhood malaria in Africa by protecting adults from mosquitoes with insecticide-treated nets. *PLoS Med* 2007;4:e229.

133. Kimani EW, Vulule JM, Kuria IW and Mugisha F. Use of insecticide-treated clothes for personal protection against malaria: a community trial. *Malaria Journal* 2006;5:63.

134. Klein, S. L. The effects of hormones on sex differences in infection: from genes to behaviuor. *Neurosci Biobehav Rev* 2000;24:627-638.

135. Klein, S. L. Hormonal and immunological mechanisms mediating sex differences in parasite infection. *Parasite Immunol* 2004;26:247-264.

136. Konradsen F, van der HW, Amerasinghe PH and Amerasinghe FP. Measuring the economic cost of malaria to households in Sri Lanka. *Am J Trop Med Hyg* 1997;56:656-60.

137. Koram KA and Molyneux ME. When is "malaria" malaria? The different burdens of malaria infection, malaria disease, and malaria-like illnesses. *Am J Trop Med Hyg* 2007;77:1-5.

138. Krucken J, Dkhil MA, Braun JV, Schroetel RM, El-Khadragy M, Carmeliet P, Mossmann H and Wunderlich F. Testosterone suppresses protective responses of the liver to blood-stage malaria. *Infect Immun.* 2005;73:436-43.

139. Kublin JG, Patnaik P, Jere CS, Miller WC, Hoffman IF, Chimbiya N, Pendame R, Taylor TE and Molyneux ME. Effect of *Plasmodium falciparum* malaria on concentration of HIV-1-RNA in the blood of adults in rural Malawi: a prospective cohort study. *Lancet* 2005;365:233-40.

140. Kurtis JD, Mtalib R, Onyango FK and Duffy PE. Human resistance to *Plasmodium falciparum* increases during puberty and is predicted by dehydroepiandrosterone sulfate levels. *Infect Immun* 2001;69:123-128.

141. Kurtis JD, Friedman JF, Leenstra T, Langdon GC, Wu HW, Manalo DL, Su L, Jiz M, Jarilla B, Pablo AO, McGarvey ST, Olveda RM and Acosta LP. Pubertal development predicts resistance to infection and reinfection with *Schistosoma japonicum*. *Clin Infect Dis* 2006;42:1692-8.

142. Kurz KM. Adolescent nutritional status in developing countries. *Proc Nutr Soc* 1996;55:321-31.

143. Kurz KM, Johnson-Welch C. The nutrition and lives of adolescents in developing countries: Findings from the nutrition of adolescent girls research program. 1994. Washington, ICRW.

144. Kurz KM, Galloway R. Improving adolescent iron status before childbearing. *Journal of Nutrition* 2000;130:437.

145. Lalloo DG, Olukoya P and Olliaro P. Malaria in adolescence: burden of disease, consequences, and opportunities for intervention. *Lancet Inf Dis* 2006;6:780-93.

146. Landgraf, B., H. Kollaritsch, G. Wiedermann, and W. H. Wernsdorfer. Parasite density of *Plasmodium falciparum* malaria in Ghanaian schoolchildren: evidence for influence of sex hormones? *Trans R Soc Trop Med Hyg* 1994;88:73-74.

147. Launiala A and Honkasalo ML. Ethnographic study of factors influencing compliance to intermittent preventive treatment of malaria during pregnancy among Yao women in rural Malawi. *Trans R Soc Trop Med Hyg* 2007;101:980-9.

148. Le Menach A, Takala S, McKenzie FE, Perisse A, Harris A, Flahault A and Smith DL. An elaborated feeding cycle model for reductions in vectorial capacity of night-biting mosquitoes by insecticide-treated nets. *Malaria Journal* 2007;6:10.

149. LEAD Technologies Inc. Statistical Package for Social Sciences (SPSS). [15.0]. 2006. Chicago, USA.

150. Leenstra T, Kariuki SK, Kurtis JD, Oloo AJ, Kager PA and ter Kuile FO. Prevalence and severity of anaemia and iron deficiency: cross-sectional studies in adolescent schoolgirls in western Kenya. *Eur J Clin Nutr* 2004;58:681-91.

151. Leenstra T, Petersen LT, Kariuki SK, Oloo AJ, Kager PA and ter Kuile FO. Prevalence and severity of malnutrition and age at menarche; cross-sectional studies in adolescent schoolgirls in western Kenya. *Eur J Clin Nutr* 2005;59:41-8.

152. Leenstra T, Phillips-Howard PA, Kariuki SK, Hawley WA, Alaii JA, Rosen DH, Oloo AJ, Nahlen BL, Kager PA and ter Kuile FO. Permethrin-treated bed nets in the prevention of malaria and anaemia in adolescent schoolgirls in western Kenya. *Am J Trop Med Hyg* 2003;68:86-93.

153. Leenstra T, ter Kuile FO, Kariuki SK, Nixon CP, Oloo AJ, Kager PA and Kurtis JD. Dehydroepiandrosterone sulfate levels associated with decreased malaria parasite density and increased hemoglobin concentration in pubertal girls from western Kenya. *J Infect Dis* 2003;188: 297-304.

154. Lengeler C. Insecticide-treated bednets and curtains for preventing malaria. *Cochrane Database Syst Rev* 2000;CD000363.

155. Lengeler C. Insecticide-treated bed nets and curtains for preventing malaria. *Cochrane Database Syst Rev* 2004;CD000363.

156. LeVine RA, LeVine SE, Rowe ML and Schnell-Anzola B. Maternal literacy and health behavior: a Nepalese case study. *Soc Sci Med* 2004;58:863-77.

157. Libonati RM, Cunha MG, Souza JM, Santos MV, Oliveira SG, Daniel-Ribeiro CT, Carvalho LJ and do Nascimento JL. Estradiol, but not dehydroepiandrosterone, decreases parasitemia and increases the incidence of cerebral malaria and the mortality in *Plasmodium berghei* ANKA-infected CBA mice. *Neuroimmunomodulation* 2006;13:28-35.

158. Longo M, Zanoncelli S, Manera D, Brughera M, Colombo P, Lansen J, Mazué G, Gomes M, Taylor WR and Olliaro P. Effects of the antimalarial drug dihydroartemisinin (DHA) on rat embryos in vitro. *Reproductive Toxicology* 2006;21:83-93.

159. Loscertales MP and Brabin BJ. ABO phenotypes and malaria related outcomes in mothers and babies in The Gambia: a role for histo-blood groups in Placental malaria? *Malaria Journal* 2006;5:72.

160. Luchavez J, Espino F, Curameng P, Espina R, Bell D, Chiodini P, Nolder D, Sutherland C, Lee KS and Singh B. Human Infections with *Plasmodium knowlesi*, the Philippines. *Emerg Infect Dis* 2008;14:811-813.

161. Lusingu JP, Vestergaard LS, Mmbando BP, Drakeley CJ, Jones C, Akida J, Savaeli ZX, Kitua AY, Lemnge MM and Theander TG. Malaria morbidity and immunity among residents of villages with different *Plasmodium falciparum* transmission intensity in north-eastern Tanzania. *Malaria Journal* 2004;3:26.

162. Luxemburger C, Thwai KL, White NJ, Webster HK, Kyle DE, Maelankirri L, Chongsuphajaisiddhi T and Nosten F. The epidemiology of malaria in a Karen population on the western border of Thailand. *Trans R Soc Trop Med Hyg* 1996;90:105-11.

163. Lyke KE, Dicko A, Dabo A, Sangare L, Kone A, Coulibaly D, Guindo A, Traore K, Daou M, Diarra I, Sztein MB, Plowe CV and Doumbo OK. Association of *Schistosoma haematobium* infection with protection against acute *Plasmodium falciparum* malaria in Malian children. *Am J Trop Med Hyg* 2005;73:1124-30.

164. MacDonald G. The epidemiology and control of malaria. Oxford University Press, London, 1957.

165. Magnussen P, Ndawi B, Sheshe AK, Byskov J and Mbwana K. Malaria diagnosis and treatment administered by teachers in primary schools in Tanzania. *Trop Med Int Health* 2001;6:273-9.

166. Malawi Meteorological Services. Climate of Malawi. http://www.metmalawi.com/climate/climate.php . 2007. 21-9-2007.

167. Maleta K, Virtanen SM, Espo M, Kulmala T and Ashorn P. Childhood malnutrition and its predictors in rural Malawi. *Paediatr Perinat Epidemiol* 2003;17:384-90.

168. Marsh K, Snow RW. Malaria transmission and morbidity. *Parasitologia* 1999;41:241-6.

169. Marsh K, Forster D, Waruiru C, Mwangi I, Winstanley M, Marsh V, Newton C, Winstanley P, Warn P and Peshu N. Indicators of life-threatening malaria in African children. *N Engl J Med* 1995;332:1399-404.

170. Marshall WA. Puberty. In: Falkner FT, Tanner JM, eds. Human growth: Postnatal growth. London: Bailliere Tindall, 1978:141-81.

171. Martorell R, Khan L and Schroeder DG. Reversibility of stunting: epidemiological findings in children from developing countries. *Eur J Clin Nutr* 1994;48:S45-S57.

172. Marx A, Pewsner D, Egger M, Nüesch R, Bucher HC, Genton B, Hatz C and Jüni P. Meta-analysis: accuracy of rapid tests for malaria in travelers returning from endemic areas. *Ann Intern Med* 2005;142:836-46.

173. Maxwell CA, Myamba J, Njunwa KJ, Greenwood BM and Curtis CF. Comparison of bednets impregnated with different pyrethroids for their impact on mosquitoes and on re-infection with malaria after clearance of pre-existing infections with chlorproguanil-dapsone. *Trans R Soc Trop Med Hyg* 1999;93:4-11.

174. Mayor A, Aponte JJ, Fogg C, Saúte F, Greenwood B, Dgedge M, Menendez C and Alonso PL. The epidemiology of malaria in adults in a rural area of southern Mozambique. *Malaria Journal* 2007;6:3.

175. Mbonye AK, Neema S and Magnussen P. Treatment-seeking practices for malaria in pregnancy among rural women in Mukono district, Uganda. *J Biosoc Sci* 2006;38:221-37.

176. Mbonye AK. Disease and health seeking patterns among adolescents in Uganda. *Int J Adolesc Med Health* 2003;15(2):105-12.

177. McCombie SC. Treatment seeking for malaria: a review of recent research. *Soc Sci Med* 1996;43:933-45.

178. McCombie SC. Self-treatment for malaria: the evidence and methodological issues. *Health Policy and Planning* 2002;17:333-44.

179. McElroy PD, ter Kuile FO, Lal AA, Bloland PB, Hawley WA, Oloo AJ, Monto AS, Meshnick SR and Nahlen BL. Effect of *Plasmodium falciparum* parasitemia density on hemoglobin concentrations among full-term, normal birth weight children in western Kenya, IV. The Asembo Bay Cohort Project. *Am J Trop Med Hyg* 2000;62:504-12.

180. McIntyre P. Adolescent friendly health services: An agenda for change. WHO. WHO/FCH/CAH/02.14. 2002. Geneva.

181. Millennium Project. Final Report to United Nations Secretary General. 356. 2005. United Nations.

182. Min-Oo G, Tam M, Stevenson MM and Gros P. Pyruvate kinase deficiency: Correlation between enzyme activity, extent of hemolytic anemia and protection against malaria in independent mouse mutants. *Blood Cells Mol Dis* 2007;39:63-9.

183. Min-Oo G and Gros P. Erythrocyte variants and the *Nature* of their malaria protective effect. *Cellular Microbiology* 2005;7:753-63.

184. Ministry of Health and Population. Government of Malawi. Malawi Roll Back Malaria Strategic Plan 2001-2005. 2001. Lilongwe, Malawi

185. Ministry of Health Government of Malawi. Malaria Prevention and Treatment Policy. 2003. Lilongwe, Malawi

186. Molineaux, L and Gramiccia G. The Garki project: research on the epidemiology and control of malaria in the Sudan Savannah of West Africa. 1979. World Health Organization, Geneva, Switzerland.

187. Molyneux CS, Mung'ala-Odera V, Harpham T and Snow RW. Maternal responses to childhood fevers: a comparison of rural and urban residents in coastal Kenya. *Trop Med Int Health* 1999;4:836-45.

188. Moody A. Rapid diagnostic tests for malaria parasites. *Clinical Microbiology Reviews* 2002;15:66-78.

189. Msyamboza KP, Savage, EJ, Chapotera Kalanda G, Kazembe PN, Gies, S, D'Alessandro U and Brabin BJ. Comparison of pregnancy outcomes in Malawian adolescent primigravidae by place of delivery. 2008. *Trop Med Int Health* Submitted for publication.

190. Mugisha F, Kouyate B, Gbangou A and Sauerborn R. Examining out-of-pocket expenditure on health care in Nouna, Burkina Faso: implications for health policy. *Trop Med Int Health* 2002;7:187-96.

191. Murray CK, Bell D, Gasser RA and Wongsrichanalai C. Rapid diagnostic testing for malaria. *Trop Med Int Hea* 2003;8:876-83.

192. Muula AS and Misiri HE. School children's accessibility to insecticide-treated bednets in peri-urban Blantyre, Malawi. *Afr J Health Sci* 2004;11:98-102.

193. Mwangi TW, Mohammed M, Dayo H, Snow RW and Marsh K. Clinical algorithms for malaria diagnosis lack utility among people of different age groups. *Trop Med Int Health* 2005;10:530-6.

194. Mwapasa V, Rogerson SJ, Molyneux ME, Abrams ET, Kamwendo DD, Lema VM, Tadesse E, Chaluluka E, Wilson PE and Meshnick SR. The effect of *Plasmodium falciparum* malaria on peripheral and placental HIV-1 RNA concentrations in pregnant Malawian women. *AIDS* 2004;18:1051-9.

195. Mzilahowa T. Malaria transimission intensity and the population structure of *Plasmodium falciparum* in the lower Shire valley, southern Malawi. 2005. University of Liverpool. PhD Thesis

196. National Statistical Office. Projected Population based on 1998 Malawi Population and Housing census. http://www.nso.malawi.net/ . 2004. Government of Malawi. 13-12-2004.

197. NCHS. Age at menarche United States. 133. 1974. Vital and Health statistics Series-11.

198. NCHS. Anthropometric reference data and prevalence of overweight- United States, 1976-80. 238. 1987. Hyattsville, Maryland. Vital and Health statistics- Series 11.

199. Neinstein LS. Adolescent health care: A practical guide. Baltimore, USA: Williams & Wilkins, 1996.

200. Nelson M. Anaemia in adolescent girls: effects on cognitive function and activity. *Proc Nutr Soc* 1996;55:359-67.

201. Nelson M, Bakaliou F and Trivedi A. Iron-deficiency anaemia and physical performance in adolescent girls from different ethnic backgrounds. *British Journal of Nutrition* 1994;72:427-33.

202. Ng OT, Ooi EE, Lee CC, Lee PJ, Ng LC, Pei SW, Tu TM, Loh JP and Leo YS. Naturally acquired human *Plasmodium knowlesi* infection, Singapore. *Emerg Infect Dis* 2008;14:814-816.

203. Ngwira B. The epidemiology and control of lymphatic filariasis and intestinal helminths in the lower Shire Valley - Chikwawa District, southern Malawi. 2005. University of Liverpool. PhD Thesis

204. Norgan NG. Long-term physiological and economic consequences of growth retardation in children and adolescents. *Proc Nutr Soc* 2000;59:245-56.

205. Nosten F, McGready R, d' Alessandro U, Bonell A, Verhoeff F, Menendez C, Mutabingwa T and Brabin B. Antimalarial drugs in pregnancy: a review. *Current Drug Safety* 2006;1:1-15.

206. Nsimba SE, Massele AY, Eriksen J, Gustafsson LL, Tomson G and Warsame M. Case management of malaria in under-fives at primary health care facilities in a Tanzanian district. *Trop Med Int Health* 2002;7:201-9.

207. Nsimba SE and Rimoy GH. Self-medication with chloroquine in a rural district of Tanzania: a therapeutic challenge for any future malaria treatment policy change in the country. *J Clin Pharm Ther* 2005;30:515-9.

208. Ntab B, Cisse B, Boulanger D, Targett G, Lines J, Alexander N, Trape JF, Simondon F, Greenwood BM and Simondon KB. Impact of intermittent preventive anti-malarial treatment on the growth and nutritional status of preschool children in rural Senegal (West Africa). *Am J Trop Med Hyg* 2007;77:411-7.

209. Nutbeam D. Health literacy as a public health goal: a challenge for contemporary health education and communication strategies into the 21st century. *Health Promotion International* 2000;15:259-67.

210. Nutbeam D and Kickbusch I. Advancing health literacy: a global challenge for the 21st century. *Health Promotion International* 2000;15:183-4.

211. Nwanyanwu OC, Redd SC, Ziba C, Luby SP, Mount DL, Franco C, Nyasulu Y and Chitsulo L. Validity of mother's history regarding antimalarial drug use in Malawian children under five years old. *Trans R Soc Trop Med Hyg* 1996;90:66-8.

212. Nyamongo IK. Health care switching behaviour of malaria patients in a Kenyan rural community. *Soc Sci Med* 2002;54:377-86.

213. O'Dempsey TJ, McArdle TF, Laurence BE, BE, Lamont AC, Todd JE and Greenwood BM. Overlap in the clinical features of pneumonia and malaria in African children. *Trans R Soc Trop Med Hyg* 1993;87:662-5.

214. Obasi AI, Cleophas B, Ross DA, Chima KL, Mmassy G, Gavyole A, Plummer ML, Makokha M, Mujaya B, Todd J, Wight D, Grosskurth H, Mabey DC and Hayes RJ. Rationale and design of the MEMA kwa Vijana adolescent sexual and reproductive health intervention in Mwanza Region, Tanzania. *AIDS Care* 2006;18:311-22.

215. Okanlawon AO, Ejiwunmi AB, Dada MO and Ashiru OA. Chloroquine-induced retardation of foetal lung maturation in rats. *Afr J Med Med Sci* 1993;22:61-7.

216. Okoko BJ, Enwere G and Ota MO. The epidemiology and consequences of maternal malaria: a review of immunological basis. *Acta Trop* 2003;87:193-205.

217. Onwujekwe O, Dike N, Ojukwu, Uzochukwu B, Ezumah N, Shu E and Okonkwo P. Consumers stated and revealed preferences for community health workers and other strategies for the provision of timely and appropriate treatment of malaria in southeast Nigeria. *Malaria Journal* 2006;5:117.

218. Onyango-Ouma W, agaard-Hansen J and Jensen BB. The potential of schoolchildren as health change agents in rural western Kenya. *Soc Sci Med* 2005;61:1711-22.

219. Pagnoni F, Convelbo N, Tiendrebeogo J, Cousens S and Esposito F. A community-based programme to provide prompt and adequate treatment of presumptive malaria in children. *Trans R SocTrop Med Hyg* 1997;91:512-7.

220. Perez EM, Hendricks MK, Beard JL, Murray-Kolb LE, Berg A, Tomlinson M, Irlam J, Isaacs W, Njengele T, Sive A and Vernon-Feagans L. Mother-infant interactions and infant development are altered by maternal iron deficiency anaemia. *Journal of Nutrition* 2005;135:850-5.

221. Plummer ML, Wight D, Wamoyi J, Nyalali K, Ingall T, Mshana G, Shigongo ZS, Obasi AI and Ross DA. Are schools a good setting for adolescent sexual health promotion in rural Africa? A qualitative assessment from Tanzania. *Health Educ Res* 2007;22:483-99.

222. Redd SC, Kazembe PN, Luby SP, Nwanyanwu O, Hightower AW, Ziba C, Wirima JJ, Chitsulo L, Franco C and Olivar M. Clinical algorithm for treatment of *Plasmodium falciparum* malaria in children. *Lancet* 1996;347:223-7.

223. Renkert S and Nutbeam D. Opportunities to improve maternal health literacy through antenatal education: an exploratory study. *Health Promotion International* 2001;16:381-8.

224. Ricke CH, Staalsoe T, Koram K, Akanmori BD, Riley EM, Theander TG and Hviid L. Plasma antibodies from malaria-exposed pregnant women recognize variant surface antigens on *Plasmodium falciparum*-infected erythrocytes in a parity-dependent manner and block parasite adhesion to chondroitin sulfate A. *Journal of Immunology* 2000;165:3309-16.

225. Rijken MJ, McGready R, Boel ME, Barends M, Proux S, Pimanpanarak M, Singhasivanon P and Nosten F. Dihydroartemisinin-Piperaquine rescue treatment of Multidrug-resistant *Plasmodium falciparum* malaria in pregnancy: A Preliminary Report. *Am J Trop Med Hyg* 2008;78:543-5.

226. Roberts CW, Walker W and Alexander J. Sex-associated hormones and immunity to protozoan parasites. *Clin Microbiol Rev* 2001;14:476-488.

227. Rodriguez AD, Penilla RP, Henry-Rodriguez M, Hemingway J, Francisco Betanzos A and Hernández-Avila JE. Knowledge and beliefs about malaria transmission and practices for vector control in southern Mexico. *Salud Publica Mex* 2003;45:110-6.

228. Rodulfo H, De Donato M, Mora R, Gonzblez L and Contreras CE. Comparison of the diagnosis of malaria by microscopy, immunochromatography and PCR in endemic areas of Venezuela. *Brazilian J Med Biol Res* 2007;40:535-43.

229. Rogerson SJ, van den Broek NR, Chaluluka E, Qongwane C, Mhango CG and Molyneux ME. Malaria and anemia in antenatal women in Blantyre, Malawi: a twelve-month survey. *Am J Trop Med Hyg* 2000;62:335-40.

230. Rogerson SJ, Hviid L, Duffy PE, Leke RF, Taylor DW. Malaria in pregnancy: pathogenesis and immunity. *Lancet Infectious Diseases* 2007;7:105-17.

231. Rogier C, Hviid L, Duffy PE, Leke RF and Taylor DW. *Plasmodium falciparum* clinical malaria in Dielmo, a holoendemic area in Senegal: no influence of acquired immunity on initial symptomatology and severity of malaria attacks. *Am J Trop Med Hyg* 1999;60:410-20.

232. Rogier C and Trape JF. Malaria attacks in children exposed to high transmission: who is protected? *Trans R Soc Trop Med Hyg* 1993;87:245-6.

233. Roll Back Malaria. What is malaria? World Health Organisation . http://www.rollbackmalaria.org 2004. 18-11-2004.

234. Roll Back Malaria Partnership. Scaling up insecticide treated netting programmes in Africa: a strategic framework for coordinated national action. World Health Organization, Geneva 2005.

235. Roll Back Malaria partnership. Roll Back Malaria: Looking forward. http://www.rollbackmalaria.org/docs/rbm_brochure.pdf . 2004. 17-1-2007.

236. Roshanravan B, Kari E, Gilman RH, Cabrera L, Lee E, Metcalfe J, Calderon M, Lescano AG, Montenegro SH, Calampa C and Vinetz JM. Endemic malaria in the Peruvian Amazon region of Iquitos. *Am J Trop Med Hyg* 2003;69:45-52.

237. Ross DA, Changalucha J, Obasi AI, Plummer ML, Cleophas-Mazige B, Anemona A, Everett D, Weiss HA, Mabey DC, Grosskurth H and Hayes RJ. Biological and behavioural impact of an adolescent sexual health intervention in Tanzania: a community-randomized trial. *AIDS* 2007;21:1943-55.

238. Ruebush TK, Kern MK, Campbell CC and Oloo AJ. Self-treatment of malaria in a rural area of western Kenya. *Bull World Health Organ* 1995;73:229-36.

239. Rush D. Nutrition and maternal mortality in the developing world. *Am J Clin Nutr* 2000;72:212S-40S.

240. Sandiford P, Cassel J, Montenegro M and Sanchez G. The impact of women's literacy on child health and its interaction with access to health services. *Population Studies* 1995;49:5-17.

241. Semba RD and Bloem MW. The anaemia of vitamin A deficiency: epidemiology and pathogenesis. *Eur J Clin Nutr* 2002;56:271-81.

242. Senga E, Loscertales MP, Makwakwa KE, Liomba GN, Dzamalala C, Kazembe PN and Brabin BJ. ABO blood group phenotypes influence parity specific immunity to *Plasmodium falciparum* malaria in Malawian women. *Malaria Journal* 2007;6:102.

243. Shapiro AE, Tukahebwa EM, Kasten J, Clarke SE, Magnussen P, Olsen A, Kabatereine NB, Ndyomugyenyi R and Brooker S. Epidemiology of helminth infections and their relationship to clinical malaria in southwest Uganda. *Trans R Soc Trop Med Hyg* 2005;99:18-24.

244. Simondon KB, Simon I and Simondon F. Nutritional status and age at menarche of Senegalese adolescents. *Ann Hum Biol* 1997;24:521-32.

245. Sizonenko PC. Physiology of puberty. *J Endocrinol Inv* 1989; 12(8 Suppl 3):59-63.

246. Slutsker L, Chitsulo L, Macheso A and Steketee RW. Treatment of malaria fever episodes among children in Malawi: results of a KAP survey. *Trop Med Parasitol* 1994;45:61-4.

247. Slutsker L and Marston BJ. HIV and malaria: interactions and implications. *Curr Opin Infect Dis* 2007;20:3-10.

248. Smith T, Genton B, Baea K, Gibson N, Taime J, Narara A, Al-Yaman F, Beck HP, Hii J and Alpers M. Relationships between *Plasmodium falciparum* infection and morbidity in a highly endemic area. *Parasitology* 1994;109(Pt 5):539-49.

249. Snow RW, Craig M, Deichmann U and Marsh K. Estimating mortality, morbidity and disability due to malaria among Africa's non-pregnant population. *Bull World Health Organ* 1999;77:624-40.

250. Snow RW, Guerra CA, Noor AM, Myint HY and Hay SI. The global distribution of clinical episodes of *Plasmodium falciparum* malaria. *Nature* 2005;434:214-7.

251. Snow RW and Gilles HM. The epidemiology of malaria. In: Warrell DA, Gilles HM, eds. *Essential malariology*. London: Arnold publishers, 2002:85-106.

252. Soe S, Khin SA, Htay A, Nay W, Tin A, Than-Swe, Roussilhon C, Pérignon JL and Druilhe P. Premunition against *Plasmodium falciparum* in a malaria hyperendemic village in Myanmar. *Trans R Soc Trop Med Hyg* 2001;95:81-4.

253. Sokhna C, Le Hesran JY, Mbaye P, Akiana J, Camara P, Diop M, Ly A and Druilhe P. Increase of malaria attacks among children presenting concomitant infection by *Schistosoma mansoni* in Senegal. *Malaria Journal* 2004;3:43.

254. Speroff L and Fritz MA. Abnormal puberty and growth problems. In *Clinical gynecologic endocrinology and infertility*. Lippincott Williams and Wilkins. 7th Revised edition, 2004:362-366.

255. Spiegel A, Tall A, Raphenon G, Trape J-F and Druilhe P. Increased frequency of malaria attacks in subjects co-infected by intestinal worms and *Plasmodium falciparum* malaria. *Trans R Soc Trop Med Hyg* 2003;97:198-9.

256. Stata Corporation. Stata. [8.0]. 2003. Texas, USA.

257. Steketee RW Wirima JJ, Slutsker L, Breman JG and Heymann DL. Comparability of treatment groups and risk factors for parasitemia at the first antenatal clinic visit in a study of malaria treatment and prevention in pregnancy in rural Malawi. *Am J Trop Med Hyg* 1996;55:17-23.

258. Stephensen CB. Burden of infection on growth failure. *Journal of Nutrition* 1999;129:534S-8S.

259. Stevenson MM and Riley EM. Innate immunity to malaria. *Nat Rev Immunol* 2004;4:169-80.

260. Stoltzfus RJ. Iron-deficiency anaemia: re-examining the nature and magnitude of the public health problem. Summary: implications for research and programs. *Journal of Nutrition* 2001;131:697S-700S.

261. Su Z, Segura M, Morgan K, Loredo-Osti JC and Stevenson MM. Impairment of protective immunity to blood-stage malaria by concurrent nematode infection. *Infection and Immunity* 2005;73:3531-9.

262. Sulcova J, Hill M, Hampl R and Starka L. Age and sex related differences in serum levels of unconjugated dehydroepiandrosterone and its sulphate in normal subjects. *Journal of Endocrinology* 1997;154:57-62.

263. Tamura T and Picciano MF. Folate and human reproduction. *Am J of Clin Nutr* 2006;83:993-1016.

264. Tanner JM. Growth at adolescence. Oxford: Blackwell Scientific Publications, 1962.

265. Tanner M and Vlassoff C. Treatment-seeking behaviour for malaria: A typology based on endemicity and gender. *Soc Sci Med* 1998;46:523-32.

266. Tarimo DS, Minjas JN and Bygbjerg IC. Perception of chloroquine efficacy and alternative treatments for uncomplicated malaria in children in a holoendemic area of Tanzania: implications for the change of treatment policy. *Trop Med Int Hea* 2001;6:992-7.

267. Tarimo DS, Urassa DP and Msamanga GI. Caretakers' perceptions of clinical manifestations of childhood malaria in holo-endemic rural communities in Tanzania. *East Afr Med J* 1998;75:93-6.

268. Taylor SJ, Whincup PH, Hindmarsh PC, Lampe F, Odoki K and Cook DG. Performance of a new pubertal self-assessment questionnaire: a preliminary study. *Paediatr Perinat Epidemiol.* 2001; 15: 88–94.

269. Taylor WR, Terlouw DJ, Olliaro PL, White NJ, Brasseur P and ter Kuile FO. Use of weight-for-age-data to optimize tablet strength and dosing regimens for a new fixed-dose artesunate-amodiaquine combination for treating falciparum malaria. *Bull World Health Organ* 2006;84:956-64.

270. ter Kuile FO, Parise ME, Verhoeff FH, Udhayakumar V, Newman RD, van Eijk AM, Rogerson SJ and Steketee RW. The burden of co-infection with human immunodeficiency virus type 1 and malaria in pregnant women in sub-Saharan Africa. *Am J Trop Med Hyg* 2004;71:41-54.

271. ter Kuile FO, Terlouw DJ, Kariuki SK, Phillips-Howard PA, Mirel LB, Hawley WA, Friedman JF, Shi YP, Kolczak MS, Lal AA, Vulule JM and Nahlen BL. Impact of permethrin-treated bed nets on malaria, anemia, and growth in infants in an area of intense perennial malaria transmission in western Kenya. *Am J Trop Med Hyg* 2003;68:68-77.

272. ter Kuile FO, Terlouw DJ, Phillips-Howard PA, Hawley WA, Friedman JF, Kolczak MS, Kariuki SK, Shi YP, Kwena AM, Vulule JM and Nahlen BL. Impact of permethrin-treated bed nets on malaria and all-cause morbidity in young children in an area of intense perennial malaria transmission in western Kenya: cross-sectional survey. *Am J Trop Med Hyg* 2003 Apr;68(4 Suppl):100-7.

273. Terlouw DJ, Nahlen BL, Courval JM, Kariuki SK, Rosenberg OS, Oloo AJ, Kolczak MS, Hawley WA, Lal AA and ter Kuile FO. Sulfadoxine-Pyrimethamine in treatment of malaria in Western Kenya: Increasing resistance and underdosing. *Antimicrobial Agents and Chemotherapy* 2003;47:2929-32.

274. The Partnership for Child Development. The health and nutritional status of schoolchildren in Africa: evidence from school-based health programmes in Ghana and Tanzania. *Trans R Soc Trop Med Hyg* 1998;92:254-61.

275. The World Bank Group. Health Nutrition and Population statistics: Demographic projections. devdata.worldbank.org/hnpstats/dp1.asp . 2007. 17-12-0007.

276. Thrusfield M, Ortega C, de Blas I, Noordhuizen JP and Frankena K. Win Episcope 2.0: improved epidemiological software for veterinary medicine. *Vet Rec.* 2001 May 5;148(18):567-72

277. Tolentino K and Friedman JF. An Update on Anemia in Less Developed Countries. *Am J Trop Med Hyg* 2007;77:44-51.

278. Toovey S. Are currently deployed artemisinins neurotoxic? *Toxicology Letters* 2006;166:95-104.

279. Toovey S. Safety of artemisinin antimalarials. *Clin Infect Dis* 2006;42:1214-5.

280. Trape JF. Malaria and urbanization in central Africa: the example of Brazzaville. Part IV. Parasitological and serological surveys in urban and surrounding rural areas. *Trans R Soc Trop Med Hyg* 1987;81 Suppl 2:26-33.

281. Trape JF, Quinet MC, Nzingoula S, Senga P, Tchichelle F, Carme B, Candito D, Mayanda H and Zoulani A. Malaria and urbanization in central Africa: the example of Brazzaville. Part V: Pernicious attacks and mortality. *Trans R Soc Trop Med Hyg* 1987;81 Suppl 2:34-42.

282. Uddenfeldt-Wort U, Warsame M and Brabin BJ. Birth outcomes in adolescent pregnancy in an area with intense malaria transmission in Tanzania. *Acta Obstet Gynecol Scand* 2006;85:949-54.

283. UNESCO. UNESCO Thesaurus. United Nations Educational Scientific and Cultural Organization. 1995. Paris.

284. UNESCO Institute for Statistics. Literacy and non-formal education section. http://www.uis.unesco.org/TEMPLATE/html/Exceltables/education/Literacy_National_July04.xls . 2004.

285. United Nations Population Fund. Adolescent realities in a changing world. www.unfpa.org/adolescents/about.htm . 2007. 17-1-2007.

286. Van Geertruyden JP, Mulenga M, Mwananyanda L, Chalwe V, Moerman F, Chilengi R, Kasongo W, Van Overmeir C, Dujardin JC, Colebunders R, Kestens L and D'Alessandro U. HIV-1 immune suppression and antimalarial treatment outcome in Zambian adults with uncomplicated malaria. *Journal of Inf Dis* 2006;194:917-25.

287. Vleugels MP, Brabin B, Eling WM and de Graaf R. Cortisol and *Plasmodium falciparum* infection in pregnant women in Kenya. *Trans R Soc Trop Med Hyg* 1989;83(2):173-7.

288. Vleugels MP, Eling WM, Rolland R and de Graaf R. Cortisol and loss of malaria immunity in human pregnancy. *Br J Obstet Gynaecol* 1987;94(8):758-64.

289. von Seidlein L, Clarke S, Alexander N, Manneh F, Doherty T, Pinder M, Walraven G and Greenwood B. Treatment uptake by individuals infected with *Plasmodium falciparum* in rural Gambia, West Africa. *Bull World Health Organ* 2002;80:790-6.

290. Wegmann TG, Lin H, Guilbert L and Mosmann TR. Bidirectional cytokine interactions in the maternal-fetal relationship: is successful pregnancy a TH2 phenomenon? *Immunol Today* 1993;14:353-6.

291. Wildling ES, Winkler PG, Kremsner C, Brandts LJ and Wernsdorfer WH. Malaria epidemiology in the province of Moyen Ogoov, Gabon. *Trop Med Parasitol* 1995;46:77-82.

292. White NJ (2008) Editorial Commentary: *Plasmodium knowlesi*: The fifth human malaria parasite. *Clin Inf Dis* 46, p 172-173.

293. White TE, Bushdid PB, Ritter S, Laffan SB and Clark RL. Artesunate-induced depletion of embryonic erythroblasts precedes embryolethality and teratogenicity in vivo. Birth Defects Res B Dev *Reprod Toxicol* 2006;77:413-29.

294. Whitworth J, Morgan D, Quigley M, Smith A, Mayanja B, Eotu H, Omoding N, Okongo M, Malamba S and Ojwiya A. Effect of HIV-1 and increasing immunosuppression on malaria parasitaemia and clinical episodes in adults in rural Uganda: a cohort study. *Lancet* 2000;356:1051-6.

295. WHO. Expert Committee on Malaria. Technical Report Series[892], 1-74. 2000.

296. WHO 2006. Guidelines for Core Population Coverage Indicators for Roll Back Malaria: To Be Obtained from Household Surveys. WHO

297. WHO. Malaria. http://www.who.int/mediacentre/factsheets/ . 2007.

298. Williams HA, Jones COH. A critical review of behavioral issues related to malaria control in sub-Saharan Africa: what contributions have social scientists made? *Soc Sci Med* 2004;59:501-23.

299. Winch PJ, Bagayoko A, Diawara A, Kané M, Thiéro F, Gilroy K, Daou Z, Berthé Z and Swedberg E. Increases in correct administration of chloroquine in the home and referral of sick children to health facilities through a community-based intervention in Bougouni District, Mali. *Trans R Soc Trop Med Hyg* 2003;97:481-90.

300. Winch PJ, Makemba AM, Makame VR, Mfaume MS, Lynch MC, Premji Z, Minjas JN and Shiff CJ. Social and cultural factors affecting rates of regular retreatment of mosquito nets with insecticide in Bagamoyo District, Tanzania. *Trop Med Int Hea* 1997;2:760-70.

301. Wirima JJ. A nation-wide malaria knowledge, attitudes and practices survey in Malawi: introduction. *Trop Med Parasitol* 1994;45:52-3.

302. Woodruff BA, Duffield A. Adolescents: assessment of nutritional status in emergency-affected situations. ACC/ SCN. 2000. Geneva.

303. World Health Organisation. Health promotion glossary. WHO/HPR/HEP/98.1. 1998. Geneva, Switzerland, World Health Organisation.

304. World Health Organization. Physical status: the use and interpretation of anthropometry: report of a WHO Expert committee. 1995. Geneva. WHO Technical report series.

305. World Health Organization. Global consultation on adolescent friendly health services - a consensus statement. WHO/FHC/CAH/02.18. 2001. Geneva, World Health Organization.

306. World Health Organization. Nutrition in adolescence - issues and challenges for the health sector: issues in adolescent health and development. 2005. http://www.who.int/child-adolescent-health/publications

307. World Health Organization. Adolescent pregnancy: unmet needs and undone deeds - a review of the literature and programmes. http://www.who.int/child-adolescent-health/publications/ [WHO discussion papers on adolescence]. 2007. 10-10-2007.

308. World Health Organization. Assessment of the safety of artemisinin compounds in pregnancy. 2003. Geneva, WHO.

309. World Health Organization. The World Health Report 1999. Making a difference. 25, 3-5. 1999. Health Millions.

310. World Health Organization, UNICEF. A healthy start in life. Report on the Global consultation on child & adolescent health and development. WHO/FCH/CAH/02.15. 2002. Geneva, World Health Organization. 1-12-2004.

311. Wunderlich F, Maurin W, Benten WP, & Schmitt-Wrede HP. Testosterone impairs efficacy of protective vaccination against *P. chabaudi* malaria. *Vaccine* 1993;11:1097-9.

312. Yoshida A, Maruyama H, Kumagai, Amano T, Kobayashi F, Zhang M, Himeno K and Ohta N. *Schistosoma mansoni* infection cancels the susceptibility to *Plasmodium chabaudi* through induction of type 1 immune responses in A/J mice. *International Immunology* 2000;12:1117-25.

APPENDICES

Appendix 1: List of survey villages

No.	Village name	Dry season	Wet season
1	Bestala	√	√
2	Chabuka		√
3	Chapepa	√	
4	Chigweshe		√
5	Chipakuza	√	
6	Dzilonzo		√
7	Joliji		√
8	Kutulo I	√	√
9	Kutulo II	√	√
10	Lunkhwe	√	
11	Mafale1		√
12	Mafale2		√
13	Malemia1		√
14	Malemia2		√
15	Matsukambiya		√
16	Mpoza	√	
17	Msanjama	√	
18	Mzangaya	√	
19	Nkhwazi		√
20	Nyamphota		√
21	Paiva	√	
22	Pangiresi	√	√
23	Robert		√
24	Salabeni	√	
25	Sekeni I	√	√
26	Sekeni II	√	
27	Thomu	√	
28	Tizola I		√
29	Tizola II		√

Appendix 2: Cross-sectional survey questionnaire

1. Identification

Code no. ☐☐☐☐☐☐ Cluster no. ☐☐

Village name (mudzi) T/A (mfumu yayikulu)

Interviewer initials Date

Name of participant (Dzina la mtsikana) Consent given ☐ Yes ☐ No

2. Individual characteristics

Age (zaka) ☐☐
(96 if unknown, lembani 96 ngati sakudzowa zaka)

If unknown, estimated age group: ☐
[1] = young adolescent -under 15 years, [2] = older adolescent -15 years and above
(ngati sakudziwa zaka, lembani [1] ngati muyerekeza kuti ndi wa zaka zochepera 15 ndipo lembani [2] ngati mukuyerekezera kuti ndi wa zaka 15 kapena kuposera apo)

Education level ☐☐
(lembani kalasi imene anasiyira sukulu)
(highest std 01 – 08; for Form 1 -4: 09-12; 13 if post-secondary)

Involvement in AGLIT programme: Yes ☐ No ☐
(lembani eya kapena ayi ngati napanga nawo kalasi za AGLIT)

Marital status:
- Never married (sanakwatiwepo) ☐
- Married (okwatiwa) ☐
- Cohabiting (kukhala limodzi ndi mamuna) ☐
- Separated/divorced (analekana ukwati) ☐
- No reply (sanayankhe) ☐

If married/ cohabiting (ngati ndi wokwatiwa kapena kukhala limodzi ndi mwamuna),
Spouse/ partner's age : ☐☐
(96 if unknown)
(zaka za mwamuna wawo (lembani 96 ngati sakuzidziwa)

Spouse/ partner's education level: ☐☐
(96 if unknown) (highest std 01 – 08; for Form 1 -4: 09-12, 13 if post-secondary)
(lembani kalasi yomwe mwamunayo anasiyira sukulu)

Main occupation:
(chochita cha mtsikana)

Spouse/ partner's occupation, if married
(chochita cha mwamuna wawo)

Ganyu ☐ ☐
Farming (ulimi) ☐ ☐
SUCOMA ☐ ☐
Tradesperson/ business (geni) ☐ ☐
Civil servant (ntchito yaboma) ☐ ☐
Student (ali pa sukulu) ☐ ☐
Other (zina) ☐ ☐

Have you earned any cash income in the past one year? Yes ☐
(Kodi mwapezako ndalama munjira in iliyonse chaka chapitachi) No ☐

Estimated amount (in kwachas) earned in one year (July 2004 to June 2005): K
(Insert zero – 0 if no income)
(tchulani kuchuluka kwa ndalama zomwe munapata chaka chapitachi mmakwacha)

When was the last time that you earned some cash income (kodi ndi liti munapezako ndalama komaliza)?

Currently earning (ndikupeza pakali pano) ☐
In the past 1 to 4 weeks (sabata imodzi mpaka zinayi zapitazi) ☐
In the past 1 to 3 months (mwezi mpaka miyezi itatu yapitayi) ☐
In the past 3 to 6 months (papita miyezi itatu mpaka isanu ndi umodzi) ☐
In the past 6 to 12 months (papita miyezi isanu ndi umodzi mpaka chaka) ☐
More than 12 months ago (papita chaka kapena kuposerapo) ☐
Not earning ☐

Frequency of cash income (choose one option that best describes):
[1] regular income (i.e. continuous form of income earning activity), [2] irregular income (e.g. seasonal, one-off), [3] no income

---

3. Household characteristics

Dwelling roof type: corrugated iron (yamalata) ☐
(Denga la nyumba) grass thatched (lamaudzu) ☐
not known (sakudziwa) ☐

Number of rooms (nambala ya zipinda mnyumba) ☐

Water source: Well (kuchitsime) ☐
(kotunga madzi) River (kumtsinje) ☐
Borehole (kumnjigo) ☐
Piped/ tap (pampope) ☐
Not known (sakudziwa) ☐

Fathers & mothers education level: Father (bamboo) ☐☐ Mother (mayi) ☐☐
(kalasi yomwe anafika makolo)
(96 if unknown)

(highest std 01 – 08; for Form 1 -4: 09-12,
13 if post-secondary)

Fathers & mothers main occupation: (ntchito ya makolo)	Father (bamboo)	Mother (mayi)
Ganyu	☐	☐
Farming (ulimi)	☐	☐
SUCOMA	☐	☐
Tradesperson/ business (geni)	☐	☐
Civil servant (ntchito yaboma)	☐	☐
Seeking employment (akufuna ntchito)	☐	☐
Other (zina)	☐	☐

4. Health data

Do you smoke any of these? (Kodi mumasuta fodya watchulidwayu?) (1 = yes, 2 = No)

commercial tobacco (fodya wogula)	☐
chingambwe	☐
ganja	☐
other (specify)	☐

If yes to any of the above, how often? ☐
(ngati mwayankha kuti eya, fotokozerani kuti pafupipafupi bwanji)
[1] = every day (tsiku lililonse), [2] = more than once a week (kuposera kamodzi pa sabata), [3] = once or less than once a week kapodzi kapena kuchepela pamodzi pa sabata)

Do you drink any alcohol? Yes ☐ No ☐
(Kodi mumwama mowa)

4.1 Medical history:

Do you have a health passport? Yes ☐ No ☐
(Muli ndi bukhu la kuchipatala)

Do you suffer or have you ever suffered from any of these conditions?
(Kodi mumadwala kapena munadwalapo matenda awa?)

TB (chifuwa chachikulu)	Yes	☐	Diabetes (matenda a suga)	Yes	☐
	No	☐		No	☐
Asthma (mphumu)	Yes	☐	STDs (matenda opatsirana muchiwerewere)	Yes	☐
	No	☐		No	☐
Hypertension (matenda othamanga magazi)	Yes	☐	Sickle cell (matenda a siko selo)	Yes	☐
	No	☐		No	☐
Urinary tract infection: (matenda a mmikodzo)	Yes	☐	Other (specify) (matenda ena)		
	No	☐			

Have you ever been tested or counselled for HIV? Yes ☐
(Kodi munayamba mwayezedwapo kapena kulandira uphungu wa kachirombo ka HIV) No ☐

Have you ever had immunisation against tetanus (TTV) Yes ☐
(Kodi munalandirako katemera woletsa matenda a kafumbata) No ☐

4.2 History of illness:

Did you have any fever in the past 3 days? Yes ☐
(Kodi mukumva kutentha thupi panopo kapena masiku atatu apitawo) No ☐

If yes (ngati eya),
Did you take any medication for the fever? Yes ☐
(Kodi munamwapo mankhwala ena aliwonse) No ☐

What illness do you think you had?
(Kodi mukuwona ngati mukudwala chiyani?)

What medication (please tick all that apply) (ngati mwamwa mankhwala, tchulani):

Aspirin ☐
Paracetamol/ Panado ☐
SP (Fansidar, Novidar) ☐
Quinine ☐
Chloroquine ☐
Halfan ☐
Bactrim ☐
Traditional (achikuda) ☐
Artesunate/ artemisinin ☐
Other (specify) (ena) .....

Sources of medication (please tick) (Tchulani komwe muapeza chithandizo cha ndalama):
Health facility (kuchipatala) ☐
Village grocer (kugolosale) ☐
Vendor (kwa mavenda) ☐
Friends/ relatives (anzanga/ abale) ☐
Other (specify) (kwina) .....

Are you ill currently/ history of any current illness? Yes ☐
(Panopo mukudwala matenda ena aliwonse) No ☐

Specify illness (tchulani matendawo):

If yes (ngati eya),
have you seen a doctor or nurse for the illness? Yes ☐
(munakawonana ndi adotolo kapena anamwino) No ☐

If no, why not?
(Ngati ayi, chifukwa chiyani?)

4.3 Gynaecological

Have you started having periods? Yes ☐
(Kodi munatha mnsinkhu/ munakula chinamwali) No ☐

If yes, at what age did you start having periods? (ngati eya, muli ndi zaka zingati?)

Age at menarche (zaka zothera mnsinkhu) ☐☐
Insert actual number of years and put 0.5 if less than 1 yr
(96 if unknown)

If unknown, estimated years since menarche: ☐
(Ngati sakudziwa, funsani mafunso othandiza kuyerezezera zakazo)
Insert actual number of years and put 0.5 if less than 1 yr

FOR GIRLS OVER 15 ONLY:

In your village, say out of 10 girls, how many do you think would have ever been pregnant: (Kodi kuyerekeza mwa atsikana 10, mukuganiza kuti ndi angati anakhalapo ndipakati ?

(a) Before age 15 (asanakwane zaka 15?)
(b) 15-19 years of age (a zaka 15 koma asanafike zaka 20?)

In your village, say out of 10 girls, how many do you think would have ever had an abortion? (Kodi kuyerekeza mwa atsikana 10, mukuganiza kuti ndi angati anayamba apitapo padera kapena kuchotsa mamba)

Are you currently pregnant? Yes ☐
(Kodi ndinu oyembekezera panopo?) No ☐

If YES, proceed with 4.3.1 and if NO, skip 4.3.1
(Ngati ndi woyembekezera, pitilizani 4.3.1 ndipo ngati siwoyembekezera mudumphe 4.3.1)

4.3.1 Obstetric history

FOR PREGNANT WOMEN ONLY

Do you have a health passport or antenatal card? Yes ☐
(Kodi muli ndi kadi yasikelo?) No ☐
(If available check information for some of the questions below) (ngati ali nayo, mupezemo mayankho ku mafunso ena ali mmusiwa)

Gravida: ☐ Para: ☐

When was your last menstrual period (LNMP): --/ --/ ---- Gestation (weeks)
(Kodi muzasamba liti komaliza, kusamba kwake kwabwino ngati kwa masiku onse)

Is this your first pregnancy? Yes ☐
(Kodi ndi mamba yoyamba) No ☐

Have you taken any antimalarial drugs during this pregnancy? Yes ☐
(Kodi munamwapo mankhwala a malungo mamba imeneyi?) No ☐

If yes, how many times have you taken antimalarial drugs? ☐
[0] = none and [#] insert number
(Ngati eya, mwamwa kangati?)

Are you well in your current pregnancy? Yes ☐
(Muli ndi umoyo wabwino chitengereni pathupipa?) No ☐

If NO, please specify illness
(Ngati ayi, nenani matendawa)

Do you think you have had malaria since you became pregnant? Yes ☐
(Kodi mukuwona ngati mwadwalapo malungo chitengereni pathupipa?) No ☐

---

5. Mosquito nets:

How many mosquito nets are there in your home/ dwelling unit? ☐
(Kodi kunyumba kwanu kulti masikito neti angati?)

What are the (estimated) costs of the following to the nearest kwacha? (tachulani mitengo ya zinthu izi?

Mosquito net (masikito neti)
Treatment/ re-treatment (kunyika neti mmankhwala)

Do you have a mosquito net over your sleeping place/ mat/ bed? Yes ☐
(Kodi muli ndi masikito neti pamalo/ pabedi pomwe mumagona?) No ☐

If yes, how did you acquire the net? (Ngati eya, kodi munapeza bwanji netiyi?) ☐
[1] health centre/ clinic/ hospital (kuchipatala/ kukiliniki)
[2] shop (kushopu)
[3] AGLIT
[4] NGO; specify
[5] other source, specify
[6] not applicable

After how long is a mosquito net supposed to be treated or re-treated? ☐
(Kodi masikito neti mumanyika mumankhwala patatha nthawi yayitali bwanji?)
[1] = 6 0r 12 months (miyezi 6/12), [2] = all wrong answers (mayankho ena onse), [3] = don't know (sakudziwa)

Who mends your net? (Kodi amakonza neti yanu ikawonongeka ndi ndani?) ☐
[1] = self (ndekha), [2] = spouse/ partner (wamuna), [3] = parent (kholo), [4] = siblings, [5] = friends/ peers (anzanga), [6] = other, specify (ena, tchulani)

If yes (ngati eya),
how many nights per week do you sleep under the net?
(kodi ndi masiku angati pasabata omwe mumagona mu netiyi?)
did you sleep under a mosquito net last night? Yes ☐
(kodi munagona mu neti usiku wathawu?) No ☐

If no (ngati ayi),
why do you not use the mosquito net? .....
(kodi ndi chifukwa chiyani simugona mu neti?)
would you use a mosquito net if you could afford to buy one? Yes ☐
(Kodi kapena mutakhala nayo neti, mungagonemo? No ☐
Why (or why not)? .....
(Chifukwa chiyani?)

Do other people in your bedroom use a mosquito net? Yes ☐
(Kodi anthu ena a muchipinda chomwe mumagonamo amagona mu neti?) No ☐

If yes, has it ever been treated and how long ago was it if treated? ☐
(Ngati eya, kodi netiyo inanyikidwapo mmankhwala, ndipo liti?)
[1] = never treated (sinanyikidwepo), [2] = treated in the past 3 months (mmiyezi itatu yapitayi), [3] = treated in the past 3- 6 months mmiyezi itatu mpaka 6 yapitayi), [4] treated more than 6 months ago (kuposera miyezi 6) [5] = don't know (sakudziwa)

Do you use of any other vector control measures? (tick those that apply)
(Kodi mumagwiritsapo ntchito njira zotetezera udzudzu izi, muchonge zonse zomwe atchula)

Coils (makoilo) ☐
Body repellents ☐
Sprays e.g. Doom ☐
Traditional methods ☐
Other (specify)

---

6. Physical examination

General: healthy looking ☐ ill-looking ☐
Temperature: Conjunctiva pallor: Yes ☐ No ☐

Height (cms) Weight (nearest 0.1 kg)
Mid-upper arm circumference (mm)

Breast development examination:
Description: .....
Tanner stage: 1☐ 2☐ 3☐ 4☐ 5☐

IF PREGNANT: Abdominal examination: fundal height (weeks)

Literacy tests

Attendance at AGLIT classes (Kodi munapangako makalasi a AGLIT?)? Yes ☐ No ☐

If yes, which year? (ngati eya, ndi chaka chiti?) ☐
[0] = no AGLIT attendance, [1] = 2005 i.e current, [2] = 2004, [3] = 2003, [4] = 2002

Can you recognise these (Kodi mungathe kuzizindikira izi?) (tick those that participant can read)

b ☐ u ☐ g ☐
h ☐ a ☐ e ☐

Can you please read the following (Kodi mungathe kuwerenga izi?) (tick those that participant can read):

Malungo ☐ Mafuta ☐ Kondomu ☐
Ofesi ☐ Madzi ☐

Can you please copy the following:
(mukupemphedwa kukopera izi?)

Kalata: _____

Specimen collection

Rapid malaria test done: Yes ☐
No ☐, specify why not: .....

Rapid malaria test result: Positive ☐
Negative ☐

Blood smear done: Yes ☐
No ☐, specify why not: .....

Urine collected Yes ☐
No ☐, specify why not: .....

Urine bloody Yes ☐ No ☐

Hb done: Yes ☐, please insert figure in g/dl
No ☐, specify why not: .....

Treatment for anaemia (Iron) Yes ☐
No ☐
Not applicable ☐

Treatment for malaria (sulfadoxine-pyimethamine) Yes ☐
No ☐
Not applicable ☐

Treatment for birharzia (praziquantel) Yes ☐
No ☐
Not applicable ☐

Ndikulola/ ndikukana kupanga nawo kafukufukuyu

Dzina: .....

Kusayina/ Chidindo .....

Tsiku: .....

Appendix 3: Information at village level

Kufotokozera za kafukufuku kwa anthu kumudzi

Moni a mfumu pamodzi ndi nduna zanu, amayi ndi abambo, anyamata ndi asungwana ndi ena nonse muli pano. Cholinga cha kafukufukuyu ameneyu tikufuna ndikufuna kudziwa zambiri za matenda a malungo mwa atsikana oyambira zaka khumi mpakana zaka khumi ndi mphambu zisanu ndi zinayi. Tikufuna kudziwitsitsa momwe matenda a malungowa amakhalira mthupi ndinso kudziwa momwe ndingawachepetsele, makamaka atsikana asanayambe kuchembeza. Pa chifukwa ichi, kafukufukuyu akhudza atsikana aang'ono, anamwali, achembere, atsikana osakwatiwa ndiponso amayi apabanja omwe ali a msikhu wochepera. Tikupanga kafukufukuyu mmidzi yowerengeka ku m'mboma lino la Chikwawa. Tidzakhala tikufunsa mafunso osiyanasiyana okhudza kunyumba kwathu, za umoyo makamaka zokhudza matenda a malungo. Moonjezera, adzakhala akupimidwa mthupi, kutenga magazi oyezera malungo monga momwe apangira ku chipatala tikadwala malungo, ndiponso kuyezedwa mikodzo. Zopima malungo tidzipimira mmidzi mommo ndipo zotsatira tidziwadziwitsa nthawi yomweyo ndipo zina tidzikapimira ku chipatala ndikubweretsa zotsatira patatha masiku angapo. Atsikana akavomera kukhala nawo mukafukufukuyu, tidzawapempha kusayina kapena kudinda kusonyeza chilolezo. Kutenga mbali mu kafukufukuyu sikowumilizidwa chifukwa palibe mphoto kapena cholowa chake, kungoti amene adzipezeka ndimatenda a malungo tikhoza kuwapatsa mankhwala konkuno koma matenda. Kafukufukuyu ali pa nyengo ziwiri, nyengo yopanda mvula pomwe malungo amakhala ochepera ndiponso nyengo ya mvula pomwe malungowa amakhala ochulukirapo. Midzi yoti tiyendemo timasankha monga ngati mwa lotale kotero pakhoza kupezeka midzi yoti yayanderedwa nyengo imodzi yokha kapena nyengo zonse.

Appendix 4: Consent form at village level

Kutenga chilolezo cha kafukufuku kwa atsikana/ azimayi *(consent form at village level for community-based cross sectional survey)*

Moni. Tikupanga kafukufuku wokhudzanan ndi maphunziro anga. Cholinga cha kafukufukuyu ameneyu tikufuna ndikufuna kudziwa zambiri za matenda a malungo mwa atsikana oyambira zaka khumi mpakana zaka khumi ndi mphambu zisanu ndi zinayi. Tidzakhala tikufuna kudziwa za mbiri yanu yokhudzana ndi za umoyo, makamaka zokhudza malungo. Tidzikuyesani makamakanso pa chifuwa ndi pamimba. Tikupemphani kupereka magazi a pachala kuti tikayese ngati muli ndi malungo ndinso magazi okwanira, ndinso mikodzo. Zotsatira za zoyesa zones tidzakudziwitsani ndipo ngati tingapeze kuti muli ndi malungo kapena kuperewera magazi mthupi tikupatsani mankhwala moyenerera. Kulola kukhala nawo mu kafukufukuyu zilibe malipiro an ndalama kapena zinthu. Mukalola kukhala nawo mu kafukufukuyu tikupemphani kusayina kapena kudinda kusonyeza kulola kwanu. Muli omasuka kulola kapena ayi ndipo zomwe mutasankhe sizikhudza momwe mungathandizidwile ndi anthu achipatala kwanu kuno. Mukhalenso omasuka kufunsa mafunso kuti mumvetse bwino.

Ndikulola/ ndikukana kupanga kafukufukuyu

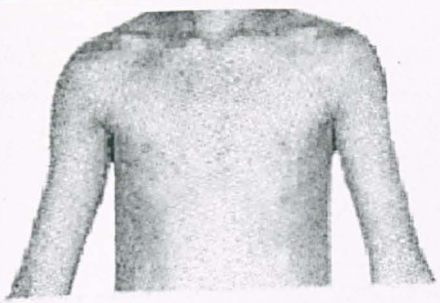
Dzina: .....

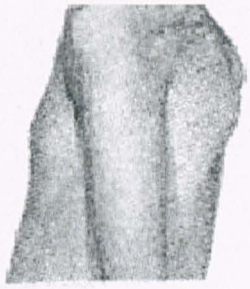
Kusayina/ Chidindo .....

Tsiku: .....

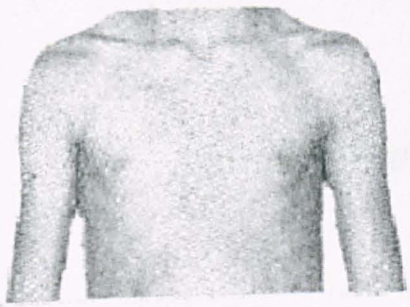
Appendix 5: Pictures of Tanner breast stages

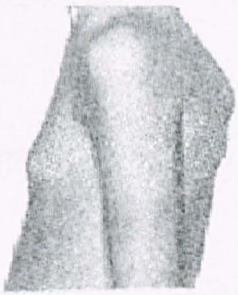
Breast Stages



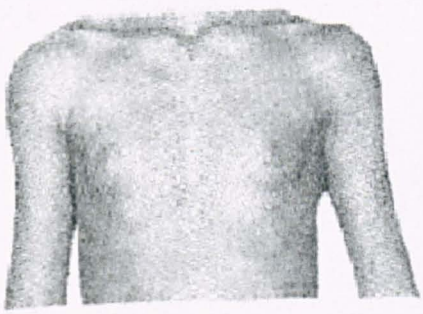


Stage One

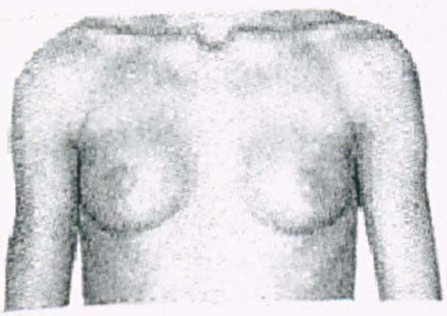


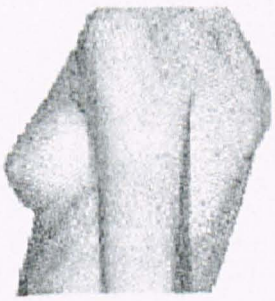


Stage Two

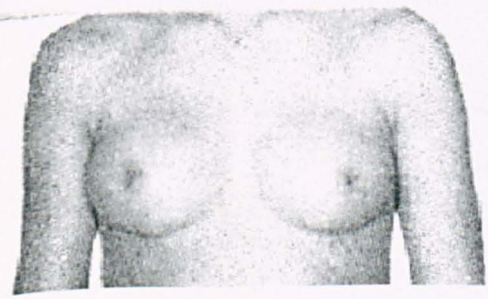


Stage Three





Stage Four



Stage Five

Appendix 6: AGLIT enrolment survey form

Assessment survey of adolescents girls literacy project (AGLIT)
Enrolment form – March 2003

Dzina la mtsikana
Mudzi
Date of interview
Dzina langa

Tsiku lobadwa ☐☐☐☐ Mtundu _____

Makolo Ali moyo Maphunziro Mpaka kalasi iti?

Mayi ☐ ☐ ☐☐

Bambo ☐ ☐ ☐☐

Wachingati mbanja

Nambala ya azichimwene ☐☐ azichemwali ☐☐

Ali pabanja muli nokha ☐ ndinu wamasiye ☐ munalekana ukwati ☐

Ali ndi ana angati ☐☐

Inde Ayi

Moyo wa sukulu Unapitapo kusukulu ☐ ☐ Mpaka kalasi iti? ☐☐

Mbiri ya umoyo wawo

Mwadwalapo kangati matenda otsatirawa pa miyezi itatu yapitayi?

Mwadwalapo kangati matenda otsatirawa mwezi wapitawu? In the last month?

Mutadwala munapita kuchipatala kapena kwa sing'anga?

	Inde	Ayi	(times)	Inde	Ayi	Inde	Ayi	Inde	Ayi
Malungo (Malaria)	☐	☐	☐☐	kuchipatala hospital/clinic	☐	☐	sing'anga? healer	☐	☐	ndekha* own	☐	☐
Kutsekula m'mimba (Diarrhoea)	☐	☐	☐☐	kuchipatala hospital/clinic	☐	☐	sing'anga? healer	☐	☐	ndekha own	☐	☐

* munazichiza nokha?

Musanapite kuchipatala china chimene munapanga ndi chiyani? _____

(before going t the hospital, what else did you do?

M'mene akuonekera (Appearance)

	Inde	Ayi
Kodi mtsikanayu wasamba? (Is the girl bathed?)	☐	☐
Zovala zake ndi zoyera? (Are her clothes clean?)	☐	☐
Wapesa tsitsi? (Is her hair combed?)	☐	☐
Kodi akuoneka wathanzi? Does she look healthy?	☐	☐
Ngati akudwala, lembani zizindikiro za matendawo If she is unhealthy, describe why (Please write in English)	☐	☐

Mid Upper Arm Circumference (cms) ☐☐ ☐ .

Health knowledge

Water

	Inde	Ayi	DK
Kodi kumwa madzi a mumtsinje kungabweretse bvuto lina lililonse? Is it possible to get diseases from drinking water from theriver?	☐	☐	☐

Ngati alipo ndi ati? (lembani zina zomwe a tchula)
Chongani (√) yankho loperekedwa. Ndipo lembani yankho lomwe laperekedwa koma silinalembedwe mbokosimu

Kutsegula m'mimba (diarrhoea)	☐
Kolera	☐
Malungo	☐
Kamwazi (dysentery)	☐

Breast feeding

	Inde	Ayi	DK
Kodi ndibwino kuyamwitsa mwana mkaka woyambirira (wachikasu oneka wa madzi) akangobadwa? (should you give calostrum?) (translated as the yellow and watery milk)	☐	☐	☐

Chifukwa chiyani (why?)

Kodi mwana ayenera kuyamba kudya phala ali ndi miyenzi ingati? ☐☐ or ☐
In what month should you first give a baby porridge?)

Kodi mwana ayenera kuyamba kumwa madzi ali ndi miyenzi ingati? ☐☐ or ☐
In what month should you first give a baby water?

Malaria

Kodi ndi mankhwala ati ochizira malungo? ☐
What is the best treatment to cure malaria?

Pogula asipirini ku sitolo (1) Pogula fansida (kapena kwinini) ku sitolo (2)
Pogula mankhwala ena a malungo ku sitolo (3) Njira zina (4)
Palibe njira (5)

Buy Aspirin from store (1), Buy Fansida (or quinine) from store (2) Buy other malaria treatment from store (3) Other (4) Nothing (5)

Sakudziwa

Kodi fansida mungamwe kwa masiku angati kuti muchize malungo? ☐☐ ☐
How many days do you need to take Fansidar tablets?

	Inde	Ayi	DK
Kodi muli ndi masikito neti kunyumba? Do you have a bed net at home?	☐	☐	☐
Kodi mumachapa neti yanun m'mankhwala a udzudzu? Do you wash it with insecticide?	☐	☐	☐
Ngati muli ndi masikito neti kodi mumagonamo tsiku ndi tsiku? If you have a bed net, do you sleep under it every night?	☐	☐	☐
Kodi ndinu oyembekezera? Are you pregnant?	☐	☐	☐
Ngati ndinu oyembekezera kodi munamwa mankhwala a malungo pa nthawi imene mumayembekezera? If you are pregnant/have been pregnant, have you taken any malaria treatment during your pregnancy?	☐	☐	☐
Kodi malungo angaopsyeze moyo wa mwana wanu yemwe mukuyembekezera? Can malaria harm your baby if you are pregnant?	☐	☐	☐
Kodi udzudzu ungafalitse malungo? Does mosquitoes give you malaria?	☐	☐	☐

Tchulani njira zochizira matenda awa (What treatment would you give to a patient with)

Kutsegula m'mimba?
Kumupatsa zamadzi ☐ Kuthamangira ku chipatala ☐

Diarrhoea?
Njira zina ☐
Give fluids/liquids Give medicines from hospital Other

Wagwidwa ndi mphere?
Kulandira mankhwala odzola ku chipatala ☐ Kusamba ndi sopo ☐
Masamba a Nimu ☐ Njira zina ☐
Use medicated fluid/cream from hospital Use soap and water Neem leaves other

Scabies?
Akusowa magazi mthupi?
Kumwa mapiritsi okhala ndi Ayironi ☐ Kuchiza malungo ☐
Kudya zakudya zokhala ndi Ayironi monga masamba obiliwira, liva ☐ Njira zina ☐
Take iron tablets Treat malaria/parasites Eating iron rich foods Other

Anaemia?
Kodi chofunikira kwambiri kuchita ndi chiyani pofuna kupewa (most important for prevention)

Malungo?
Kugona muneti ☐ Kupewa kulumidwa ndi udzudzu ☐
Kuyetsa ndowe ☐ Njira zina ☐
Use mosquito nets (1) - Avoid mosquito bites 2, burn dung (3) Other (4)

EDZI?
Kugwiritsa ntchito kondomu ☐ Kukana kugonana/Osachita chiwerewere ☐
Njira zina (3) ☐

AIDS Use condom - Avoid sex/promiscuity - Other

Kusowa kwa Vitamini A mthupi
Kudya zakudya zina zokhala ndi Vitamin A monga mango/papaya ☐
Kulandira kam'bulu ka Vitamini A ☐ Njira zina ☐
Eat foods rich in Vitamin A e.g. mango/papaya etc 7 (1); Take Vit. A tablets (2) Other (3)

Vitamin A deficiency

Mpempheni mtsikana ayankhe inde kapena ayi:

	Inde	Ayi	Sakudziwa
Tingatenge Edzi kudzera muchiwerewere? (Can you get AIDS through sex?)	☐	☐	☐
Mungadziwe kuti munthuyu ali ndi kachirombo koyambitsa edzi (translation?)	☐	☐	☐
Edzi tingaipewe? (is it preventable?)	☐	☐	☐

Kodi munthu angaziteteze bwanji ku nthenda ya Edzi pofuna kugonana

Chongani (√) yankho lopeekedwa. Ndipo lembani yankho lomwe laperekedwa koma silinalembedwe mbokosimu

How can you prevent HIV during sex

	(√)	Inde	Ayi	Sakudziwa
Tigwiritse ntchito kondomu (condom)	☐	☐	☐	☐
Osachita chiwerewere kapena tikhale ndi chibwenzi chimodzi (Not being promiscuous or having only One partner)	☐	☐	☐	☐
Tikhale ndi bwenzi loyezedwa magazi HIV -ve (Partner tested)	☐	☐	☐	☐
Osagonana ndi munthu wa Edzi (Not having sex with anybody suffering from AIDS)	☐	☐	☐	☐
Kupewa fisi ndi kuchotsa fumbi nthawi ya chinamwali (Not having sex during or after the initiation ceremonies)	☐	☐	☐	☐
Kugwiritsa ntchito jakiseni wowiritsa (Use sterilised needles)	☐	☐	☐	☐
Tikhale ndi bwenzi lokhulupirika (Trusted partner)	☐	☐	☐	☐
Kukwatiwa ndi munthu yemwe inu ndi a pabanja panu mukumudziwa bwino Marrying only a boy you or the family know	☐	☐	☐	☐
Tikwatire (marriage)	☐	☐	☐	☐
Kugonana ndi m'bale (sleeping with your relative)	☐	☐	☐	☐
Osamagonana pafupipafupi (Not having sex frequently)	☐	☐	☐	☐
Kugonana ndi munthu wathanzi (sleeping with a healthy looking person)	☐	☐	☐	☐
Kugonana cha mbali (Having sex laying on your side)	☐	☐	☐	☐
Mwamuna atayire mphamvu pansi (Withdraw of penis when ejaculating)	☐	☐	☐	☐

Kodi kolera tingaitenge bwanji?

How can you get cholera?

	(√)	Inde	Ayi	Sakudziwa
Kumwa madzi oyipa (contaminated water)	☐	☐	☐	☐
Kumwa madzi amumtsinje (river)	☐	☐	☐	☐
Kumwa madzi pachithaphwi (stagnant water)	☐	☐	☐	☐
Kudya chakudya choipa (contaminated food)	☐	☐	☐	☐
Kudya zakudya zaukhondo (clean food)	☐	☐	☐	☐
Kudzera ku mangos (from mangoes)	☐	☐	☐	☐
Kusasamba m'manja po chokera ku chimbudzi (not washing hands)	☐	☐	☐	☐
Zovala zakuda (having dirty cloths)	☐	☐	☐	☐
Kusasamala pa khomo (living in a dirty home)	☐	☐	☐	☐
Kupanda dzenje lotaira zinyalala (not having a rubbish pit)	☐	☐	☐	☐
Kusakwirira maenje ndi zithampwi (not filling holes and paddles)	☐	☐	☐	☐
Kudzera ku ntchentche (from flies)	☐	☐	☐	☐
Kudzera ku udzudzu (from mosquitoes)	☐	☐	☐	☐
Kugonana (having sex)	☐	☐	☐	☐
Kuyenda opanda nsapato (walking bare footed)	☐	☐	☐	☐

Appendix 7: AGLIT evaluation survey form

Adolescents Girls Literacy Project
Final assessment – November 2003

Dzina la mtsikana _____
Mudzi _____
Date of interview _____
Dzina langa _____

Kabweredwe ka mtsikana kusukulu (Attendance):

Ngati samajomba, amabwera kangati pa sabata? (If regular, how many days per week?) (1, 2, 3, 4 or 5) ☐

Ngati amajombajomba,	Inde	Ayi
-amajomba sabata zathunthu? (missed whole weeks?)	☐	☐
- amajomba miyezi yathunthu? (missed whole months?)	☐	☐
- Anasiya sukulu? (has she stopped attending?)	☐	☐

Mbiri ya umoyo wawo

Mwadwalapo kangati matenda otsatirawa mwezi wapitawu? In the last month?

	Inde	Ayi	(times)	Mutadwala munapita kuchipatala kapena kwa sing'anga? Inde	Ayi	Inde	Ayi	Inde	Ayi
Malungo (Malaria)	☐	☐	☐☐	kuchipatala hospital/clinic	☐	☐	sing'anga? healer	☐	☐	munazichiza nokha? own	☐	☐
Kutsekula m'mimba (Diarrhoea)	☐	☐	☐☐	kuchipatala hospital/clinic	☐	☐	sing'anga? healer	☐	☐	munazichiza nokha? own	☐	☐

Musanapite kuchipatala china chimene munapanga ndi chiyani? _____
(before going t the hospital, what else did you do?

M'mene akuonekera (Appearance)

	Inde	Ayi
Kodi mtsikanayu wasamba? (Is the girl bathed?)	☐	☐
Zovala zake ndi zoyera? (Are her clothes clean?)	☐	☐
Wapesa tsitsi? (Is her hair combed?)	☐	☐
Kodi akuoneka wathanzi? Does she look healthy?	☐	☐
Ngati akudwala, lembani zizindikiro za matendawo If she is unhealthy, describe why (Please write in English)	☐	☐

Mid Upper Arm Circumference (cms) ☐☐ . ☐

literacy assessment

Mungathe kuzindikila zilemba izi? (Chongani (√) zomwe angathe khwachani (X) zomwe alephera (letters read)

b	u	g	h	a	e
☐	☐	☐	☐	☐	☐

Mungathe kuwerenga mau awa?(Chongani mau omwe mtsikana angawerenge (√) Chongani mau omwe mtsikana alephera (X) (words read)

malungo	ofesi	Mafuta	madzi	kondomu
☐	☐	☐	☐	☐

Akutha kugwira pensulo bwinobwino (kuti alembere) (Can hold a pencil correctly (to write with) ☐ (√=1, X=2)

Akutha kukopela liu Kalata _____ ☐ (√=1, X=2)

Akutha kulemba mau payekha (edzi) _____ ☐ (√=1, X=2)

Werengani ziganizozi (Lembani mawu amene awerengedwa). (Please write the number of words read)	Nambala ya mawu (Number)
idyani mango (eat mango)	☐
mazira amamanga thupi (Eggs are a body building food)	☐
tigone mu neti kupewa malungo (We sleep under a net to avoid malaria)	☐
edzi imapha (AIDs kills)	☐
tipewe chiwerewere (Avoid promiscuity)	☐
malita popita ku Lilongwe anakwera minibasi (hen going to Lilongwe Malita took the minibus)	☐

Numeracy skills

Kodi mungathe kuzindikira manambala awa? Can recognise these numbers?

2 ☐ 5 ☐ 8 ☐ 10 ☐ 40 ☐ 60 ☐

70 ☐ 90 ☐ 100 ☐ 300 ☐ 250 ☐ 444 ☐

Kodi utapeleka K60 ndi Kugula sugar amene ali mtengo wa K35 anga kubwenzere ndalama zingati(a simple mental sum) ☐☐

Kodi malemba awa atanthauza chiyani? (Chongani (√) malemba onwe akutha khwachani (X) omwe alephera

15 Novembala 2000 ☐ 16.8.2002 ☐

20 Kg ☐ K 30 ☐

Health knowledge

Water — Inde Ayi DK

Kodi kumwa madzi a mumtsinje kungabweretse bvuto lina lililonse? ☐ ☐ ☐
Is it possible to get diseases from drinking water from theriver?

Ngati alipo ndi ati? (lembani zina zomwe a tchula)
Chongani (√) yankho loperekedwa. Ndipo lembani yankho lomwe laperekedwa koma silinalembedwe mbokosimu

Kutsegula m'mimba (diarrhoea) ☐

Kolera ☐

Malungo ☐

Kamwazi (dysentery) ☐

Breast feeding — Inde Ayi DK

Kodi ndibwino kuyamwitsa mwana mkaka woyambirira (wachikasu oneka wa madzi) akangobadwa? ☐ ☐ ☐
(should you give calostrum?) (translated as the yellow and watery milk)

Chifukwa chiyani (why? Please write in English))

Kodi mwana ayenera kuyamba kudya phala ali ndi miyenzi ingati? ☐☐ or ☐
In what month should you first give a baby porridge?)

Kodi mwana ayenera kuyamba kumwa madzi ali ndi miyenzi ingati? ☐☐ or ☐
In what month should you first give a baby water?

Malaria

Kodi ndi mankhwala ati ochizira malungo? Pogula asipirini/panadol ku sitolo (1) Pogula fansida (kapena kwinini) ku sitolo (2) ☐
What is the best treatment to cure malaria? Pogula mankhwala ena a malungo ku sitolo (3) Njira zina (4)
Palibe njira (5)
Buy Aspirin from store (1), Buy Fansida (or quinine) from store (2) Buy other malaria treatment from store (3) Other (4) Nothing (5)

Sakudziwa

Kodi fansida mungamwe kwa masiku angati kuti muchize malungo? ☐☐ ☐
How many days do you need to take Fansidar tablets?

	Inde	Ayi	DK
Kodi muli ndi masikito neti kunyumba? Do you have a bed net at home?	☐	☐	☐
Kodi mumachapa neti yanun m'mankhwala a udzudzu? Do you wash it with insecticide?	☐	☐	☐
Ngati muli ndi masikito neti kodi mumagonamo tsiku ndi tsiku? If you have a bed net, do you sleep under it every night?	☐	☐	☐
Kodi ndinu oyembekezera? Are you pregnant?	☐	☐	☐
Ngati ndinu oyembekezera kodi munamwa mankhwala a malungo pa nthawi imene mumayembekezera? If you are pregnant/have been pregnant, have you taken any malaria treatment during your pregnancy?	☐	☐	☐
Kodi malungo angaopsyeze moyo wa mwana wanu yemwe mukuyembekezera? Can malaria harm your baby if you are pregnant?	☐	☐	☐
Kodi udzudzu ungafalitse malungo? Does mosquitoes give you malaria?	☐	☐	☐

Tchulani njira zochizira matenda awa (What treatment would you give to a patient with)

Kutsegula m'mimba? Diarrhoea?
Kumupatsa zamadzi ☐ Kuthamangira ku chipatala ☐
Njira zina ☐
Give fluids/liquids Give medicines from hospital Other

Wagwidwa ndi mphere? Scabies?
Kulandira mankhwala odzola ku chipatala ☐ Kusamba ndi sopo ☐
Masamba a Nimu ☐ Njira zina ☐
Use medicated fluid/cream from hospital Use soap and water Neem leaves other

Akusowa magazi mthupi? Anaemia?
Kumwa mapiritsi okhala ndi Ayironi ☐ Kuchiza malungo ☐
Kudya zakudya zokhala ndi Ayironi monga masamba obiliwira, liva ☐ Njira zina ☐
Take iron tablets Treat malaria/parasites Eating iron rich foods Other

Kodi chofunikira kwambiri kuchita ndi chiyani pofuna kupewa (most important for prevention)

Malungo?
Kugona muneti ☐ Kupewa kulumidwa ndi udzudzu ☐
Kuyetsa ndowe ☐ Njira zina ☐
Use mosquito nets (1) - Avoid mosquito bites 2, burn dung (3) Other (4)

EDZI? AIDS Use condom - Avoid sex/promiscuity - Other
Kugwiritsa ntchito kondomu ☐ Kukana kugonana/Osachita chiwerewere ☐
Njira zina (3) ☐

Kusowa kwa Vitamini A mthupi Vitamin A deficiency
Kudya zakudya zina zokhala ndi Vitamin A monga mango/papaya ☐
Kulandira kam'bulu ka Vitamini A ☐ Njira zina ☐
Eat foods rich in Vitamin A e.g. mango/papaya etc (1); Take Vit. A tablets (2) Other (3)

Mpempheni mtsikana ayankhe inde kapena ayi:

	Inde	Ayi	Sakudziwa
Tingatenge Edzi kudzera muchiwerewere? (Can you get AIDS through sex?)	☐	☐	☐
Mungadziwe kuti munthuyu ali ndi kachirombo koyambitsa edzi (Can you tell by looking if somebody is infected?)	☐	☐	☐
Edzi tingaipewe? (Is it preventable?)	☐	☐	☐

Kodi munthu angaziteteze bwanji ku nthenda ya Edzi pofuna kugonana

Chongani (√) yankho lopeekedwa. Ndipo lembani yankho lomwe laperekedwa koma silinalembedwe mbokosimu

How can you prevent HIV during sex

	(√)	Inde	Ayi	Sakudziwa
Tigwiritse ntchito kondomu (condom)	☐	☐	☐	☐
Osachita chiwerewere kapena tikhale ndi chibwenzi chimodzi (Not being promiscuous or having only One partner)	☐	☐	☐	☐
Tikhale ndi bwenzi loyezedwa magazi HIV -ve (Partner tested)	☐	☐	☐	☐
Osagonana ndi munthu wa Edzi (Not having sex with anybody suffering from AIDS)	☐	☐	☐	☐
Kupewa fisi ndi kuchotsa fumbi nthawi ya chinamwali (Not having sex during or after the initiation ceremonies)	☐	☐	☐	☐
Kugwiritsa ntchito jakiseni wowiritsa (Use sterilised needles)	☐	☐	☐	☐
Tikhale ndi bwenzi lokhulupirika (Trusted partner)	☐	☐	☐	☐
Kukwatiwa ndi munthu yemwe inu ndi a pabanja panu mukumudziwa bwino Marrying only a boy you or the family know	☐	☐	☐	☐
Tikwatire (marriage)	☐	☐	☐	☐
Kugonana ndi m'bale (sleeping with your relative)	☐	☐	☐	☐
Osamagonana pafupipafupi (Not having sex frequently)	☐	☐	☐	☐
Kugonana ndi munthu wathanzi (sleeping with a healthy looking person)	☐	☐	☐	☐
Kugonana cha mbali (Having sex laying on your side)	☐	☐	☐	☐
Mwamuna atayire mphamvu pansi (Withdraw of penis when ejaculating)	☐	☐	☐	☐

Kodi kolera tingaitenge bwanji?

How can you get cholera?

	(√)	Inde	Ayi	Sakudziwa
Kumwa madzi oyipa (contaminated water)	☐	☐	☐	☐
Kumwa madzi amumtsinje (river)	☐	☐	☐	☐
Kumwa madzi pachithaphwi (stagnant water)	☐	☐	☐	☐
Kudya chakudya choipa (contaminated food)	☐	☐	☐	☐
Kudya zakudya zaukhondo (clean food)	☐	☐	☐	☐
Kudzera ku mangos (from mangoes)	☐	☐	☐	☐
Kusasamba m'manja po chokera ku chimbudzi (not washing hands)	☐	☐	☐	☐
Zovala zakuda (having dirty cloths)	☐	☐	☐	☐
Kusasamala pa khomo (living in a dirty home)	☐	☐	☐	☐
Kupanda dzenje lotaira zinyalala (not having a rubbish pit)	☐	☐	☐	☐
Kusakwirira maenje ndi zithampwi (not filling holes and paddles)	☐	☐	☐	☐
Kudzera ku ntchentche (from flies)	☐	☐	☐	☐
Kudzera ku udzudzu (from mosquitoes)	☐	☐	☐	☐
Kugonana (having sex)	☐	☐	☐	☐
Kuyenda opanda nsapato (walking bare footed)	☐	☐	☐	☐

Zotsatira (Feedback) Mukamaliza kumuyesa mtsikanayo, pangani chimodzi mwa izi malingana ndi momwe mtsikanayo wakhonzera (chongani bokosi lomwe mwasankha √)

☐ Wachita bwino mayeso ako ndipo tili osangalala. Kodi chakupangitsa kuti upambane ndi chani? (did well, why?)

☐ Mafunso ena wayankha bwino ndipo ena akuvuta kuyankha. Kodi ndi chifukwa chani zina zakuvuta? (some troubles, why?)

Appendix 8: Sample AGLIT lesson plans on malaria

LESSON PLANS Month 3: MALARIA (10 Lessons) Week 1: Lessons 1- 5

Lesson number	***Extra exercises/homework***
LESSON I: PARTICIPATORY STORY (Concentrate on treatment) Story 1: Aim: To find out girls/villagers' usual treatment for malaria. Story 2: Aim: Teach correct Fansidar doses for different age groups. Story 3: Aim: Teach importance of always giving ORS and how to make the solution	1. Read and copy keywords 2. Copy out all the Fansidar doses. (PE should write this on blackboard before lesson) Homework: Learn correct Fansidar doses and keywords.
LESSON 2: DISCUSSION (Concentrate on effects of malaria on people's lives) Revision: Read keywords. Recite Fansidar doses Discussion 1: Question 1: (Single) In what ways did your last bout of malaria disturb your daily activities? Question 2 (Pairs) In what ways does malaria in other members of your family disturb your lives? Question 3: How does malaria affect a whole community? Discussion 2: Question 1: What does your family do in the home to try to avoid malaria? Question 2: What does the village do communally to avoid malaria? What could it do? Question 3: How would life in a village improve if it had less malaria?	1. Read and copy keywords 2. Copy out instructions for ORS (Learn for homework) Homework: 1. Find out who had malaria in your household and .neighbouring household in past 2 months
LESSON 3: SURVEY (See sample survey) Revision" Read keywords. Recite ORS preparation NOTE: Group girls for survey (Group 1, Group 2 etc). Survey 1: How many cases of malaria in girls' households (out of total number of people in their households) in the past month? Survey 2: How did people treat their malaria? Survey 3: How many working days are lost by these cases of malaria? NOTE: Teacher to note down survey details in order to re-write it on board for next lesson.	▪ Copy survey into books ▪ Write sentences about survey. E.g. out of 60 people 20 had malaria in the past month. Homework: Show families the survey and discuss potential loss of earnings. Find out cost of bednet.
LESSON 4: ARITHMETIC Revision: Read and revise information from survey. Sample questions: If X earns K20 a day, how much money does he lose from 2 (3,4,.5 etc days lost through malaria) How much money could families from one group 1 lose from malaria in past month (roughly – if sum is too hard) How much is Fansidar? How much does a family spend for 2 (3,4 etc)cases of malaria How much is one bednet ? How much would 5 bednets cost?	NOTE: Each group can do own sums (not total for all groups) If sums are still too hard, PE can do the sum to show how much money is lost through malaria. Simple sums: Add cost of Fansidar + Aspirin
LESSON 5: READING (BOOK AND SONGS) Book : Discuss likely pictures and likely keywords first before reading. (See your Guide to Teaching reading) Songs: Can make up different songs about malaria (e.g. 1. Fansidar doses, 2. Effect of malaria on village)	Copy and read song words Homework : Revise all keywords and find out why pregnant women should especially avoid malaria)

NOTES TO TEACHERS: See Appendix for Curriculum sheet on Malaria

LESSON PLANS: MALARIA Week 2 : Lessons 6-10

Lesson number	Extra exercises/homework
LESSON 6: WRITING Revision: Reading keywords. Reciting key information Design posters 1. To warn pregnant women to avoid malaria (especially first pregnancy) Method: discuss effects of malaria on mother./baby 2. To encourage use of bednets Method: Discuss long term use of bednets/ how to treat with repellent Girls to draw pictures and write slogan in books first. Pass their books round. Girls choose best to make into A4 posters for village headman and office (to photocopy and send to donor)	NOTE: While some girls do the best posters, the others can role play poster messages. Homework: Show friends and family you poster message.
LESSON 7: MAPPING Revision: swap books and read each other's poster messages Ground map then blackboard map: 1. Danger areas in girls' homes where mosquitoes breed. 2. Communal danger areas in village where mosquitoes breed e.g. borehole 3. Copy maps into books	Homework: 1.Explain to family/friends the dangers. (Show your map) 2. Remedy danger areas in home and whole village(e.g. Cut grass, fill in water pools, air hut)
LESSON 8: PRACTICAL ACTIVITIES 1. Demonstrate dipping a bednet with mosquito repellent. 2. Remedy danger areas in community if not done for homework. 3. Read and write instructions on dipping bednet.	▪ Revise practical arithmetic on bednets Homework: Revise dipping bednet instructions.
LESSON 9: REVISION OF ALL MALARIA HEALTH KNOWLEDGE Methods: Stories, quizzes, hot money, drama to test Fansidar dose, ORS, bednet dipping, mosquito breeding areas etc. (chakuti,chakuti)	Homework: Any girl who does not know facts must learn them from one who does.
LESSON 10: REVISION OF READING KEY WORDS /ARITHMETIC. Book : Continue with book (Can do so for following weeks too) Songs: Revise the songs Arithmetic revision: Adapt sample questions from Lesson 4: If X earns K30 a day, how much money does he lose from 2 (3,4,.5 etc days lost through malaria) How much money could families from all class groups lose from malaria in past month (roughly – if sum is too hard) How much is Fansidar? How much does a family spend for 2 (3,4 etc)cases of malaria Which is cheaper buying 2 bednets or getting malaria often in a year?	Compare cost of losing money in a year and buying 1 (2,3-etc) bednets. Homework: Girls must notice what they eat for each meal over the week-end (If possible write down) Tell them this is in preparation for doing NUTRITION