Patterns of *Plasmodium falciparum* Infection and Morbidity in a Rural Community in Northern Ghana

INAUGURAL DISSERTATION

zur

Erlangung der Würde eines Doktors der Philosophie

vorgelegt der

Philosophisch-Naturwissenschaftlichen Fakultät der

Universität Basel

von

Seth Ogyebre Owusu-Agyei

aus Apesokubi (Ghana)

Basel, 2001

Genehmigt von der Philosophisch-Naturwissenschaftlichen Fakultät der Universität Basel auf Antrag der

Herren Prof. Dr. M. Tanner, PD Dr. T. Smith, und Prof. Dr. N.A. Weiss

Basel, den 22 November, 2001.

Prof. Dr. Andreas D. Zuberbühler
Dekan

dedicated to my:

parents: Christian & Comfort Owusu
& family: Mercy, Nana Yaw, Linda, and Joe

for your love, support, patience and tolerance.

TABLE OF CONTENTS

Acknowledgements

This thesis stems from research collaborations of the Navrongo Health Research Centre (NHRC) with Noguchi Memorial Institute for Medical Research (NMIMR), The Swiss Tropical Institute, Basel (STI), the US Naval Medical Research Center, Bethesda (NMRC) and the US Naval Medical Unit3 in Cairo (NAMRU-3).

My sincere thanks first and foremost go to the chiefs, elders and the community members of the Kassena-Nankana district whose participation made these studies and findings possible.

I acknowledge with thanks and appreciation the role that various people played to make this happen. Dr. Moses Adibo, the former Deputy Minister for Health who readily endorsed my application to WHO/TDR for this training fellowship award, Professor Francis Nkrumah, former Director of NMIMR, for his interest and role in my application to WHO/TDR. Others worked behind the scenes to make all these happen, Dr. Fred Binka, the former Director of NHRC and my longest associate in health research; I recall our times together in Noguchi and several years in Navrongo and wish to thank you for all your help and advice.

I wish to express my sincere thanks to Professor Marcel Tanner, Director of STI for accepting me into STI to study for my PhD. I am indebted to Dr. Tom Smith my principal supervisor, who took care of my entire academic and social needs. Special thanks also go to Drs Ingrid Felger and Hans-Peter Beck, for their patience in training me in molecular biology techniques; Professor Niklaus A Weiss for agreeing to co-referee this thesis and Professor Mitchell Weiss for his support.

My deep appreciation goes to all the staff of NHRC, both former and current as I attempt to list a few within the constraints of space: Dr. Abraham Hodgson, the current Acting Director who still recalls our experiences as students in STI; Dr. Alex Nazzar, the former Director of NHRC, for recommending me to WHO/TDR for training fellowship. Sincere appreciation to other staff members, Francis Anto, Martin Adjuik, Christine Clerk, Philomina Nyarko, Cornelius Depuur, Pierre Ngom, Lucas Amenga-Etego, Thomas Anyorigia, Victor Asoala, Cletus Tindana, Robert Addah, Timothy Awine, Kwame Amponsa Achiano, Rexford Oduro, Akum Aveika, Elizabeth Awini, Nathan Mensah, Peter Wontuo, Felix Kondayire, Julius Ayuure, Paulina Tindana, Salifu Abdul Rahman, Seidu Nchor, George Wak, Philip Adongo, Livesy Abotsi, Evelyn Sakeah, Everest Dery, Raymond Akparibo, Robert Alirigia, Sixtus Apaliya, Godwin Apaliya, Titus Tei, Margaret Bugase, Kalifa Bugri, Bawa Ayaga, and several others: fieldstaff, computing, canteen,

Administrative staff, drivers, watchmen and all fellow researchers. Sincere thanks to the following in the Ministry of Health: Drs. John Gyapong, Margaret Gyapong and Sam Adjei of the Health Research Unit; Dr Kofi Ahmed of the Onchocerciasis division; Drs Erasmus Agongo and Joseph Amankwa at the Upper East Regional Health Administration; Kweku Enos and Thomas Mensah-Afful KND Directors; and George Mumuni, the SMO in-charge of the War Memorial Hospital and Philip Amati. Steve Ntow, Seth Acquah and Said Hussein of HRD.

I thank Dr. Kwadwo Koram, Professors Edwin Afari and David Ofori-Adjei as well as Charles Attiogbe, Jonas Asigbee and John Fenteng all of NMIMR for your support and good wishes.

My sincere thanks and appreciation goes to Dr. Stephen Hoffman and the rest of the Navy team, especially, Drs Dave Fryauff, Joe Flanagan, Greg Utz, Kevin Baird, Braden Hale, Tom Richie, Martha Sedegah, Dan Carucci, and Bill Rogers for your warm collaboration.

My deep appreciation to Cornelia Naumann and Christine Walliser, to Drs Gerd Pluschke, Blaise Genton, Christian Lengeler, Jakob Zinsstag, Penelope Vounatsu, Jennifer and Paul Jenkins, Elisabeth Sedlmeier and Agnès Doré, Immler Heidi, Hug Nils, Tosun Mehtap, Michelle Dobler, Sylvia Steiger, Urs Hodel and Simon Roelly for all your support. This goes as well to fellow students (former/current): Lucy Ochola, Sébastien Gagneux, Armin Gemperli, Salim Abdulla, Hassan Mshinda, Happiness Minja, Regula Leuenberger, Felix Heckendorn, Ivo Müller, Esther Schelling, Ursula Kayali, Olivia Keiser, Beatrice Glinz, André Tiaden (Niggi), Regös Attila, Mirjam Kästli, Shubangi Parkar, Sohini Banarjee and Ernestina Mensah-Quainoo.

I am full of commendation for the immense support of: members of my extended family: Honourable Kwasi Owusu-Yeboa, Minister for the Volta Region of Ghana, Eben Owusu-Asiedu, Dan Owusu-Nyampong, Theophilus Owusu-Wiredu and Michael Bamfo-Tanor; the Christian brothers of the Navrongo Presbyterian Church: Rev. Abbey, Jacob Tetteh, Vida Donkor, Clement Fobi, Samuel Asiedu, Mathilda Achirobe and their families; Professor A.C. Kumah of Ameyi Chambers, and Honourable Gloria Akuffo, Deputy Attorney-General and Minister for Justice, Ghana; and Jim Philips of Population Council. I thank research colleagues, Diadier Diallo, Ricardo Thompson and Daniel Chandramohan for all your support and company.

Financial support for my studies came from the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR). I wish to thank Mr. Steven Wayling, Dr Fabio Zicker, and their secretaries for all their support.

SUMMARY

Malaria continues to be of major public health importance, especially in sub-Saharan Africa. At a minimum, 900 million acute febrile episodes occur yearly resulting in 1-3 million deaths yearly, mostly in African children below 5 years of age. Less than 20% of these deaths come to the attention of any formal health system. The World Bank (1993) ranks malaria as the leading cause of lost disability-adjusted life years (DALYs) in Africa with an estimated 35 million future life-years lost from disability and premature death.

Without effective malaria control programme(s), the massive burden of malaria morbidity and mortality is expected to at least double in the next 20 years due mainly to the growing spread of *Plasmodium falciparum* resistance to Chloroquine and other anti-malarials. Lack of development of new affordable drugs; the financial constraints on health services in most countries; and lack of expertise to plan properly for malaria control are also sources of worry. Hope has been re-kindled by the initiation of the Roll Back Malaria (RBM) whose goal is to reduce malaria by half in 10 years.

The goal of the present study is to characterise the epidemiology of *Plasmodium falciparum* parasites, patterns of seasonality in infection and morbidity and their relationship to radical cure in the Kassena-Nankana district (KND) in northern Ghana. Such information prior to clinical malaria intervention trials will be important for optimal design and implementation. In the KND, malaria transmission is holoendemic with seasonal peaks and troughs mirroring the wet and dry seasons in the district. It is the leading cause of both morbidity and mortality, accounting for 60% of hospital admissions, and 35% of all deaths

Cohorts of either non-immune children or semi-immune adults or all age groups were randomly selected using a cluster sampling approach that was facilitated by the availability of the Navrongo Demographic Surveillance System (NDSS). In total, between 200 and 300 inhabitants constituted the cohort for each of the studies. With all eligible people within the district included in the sample frame, and using the Stata program, 16 "index" compounds were randomly selected. Potential volunteers were recruited sequentially

from nearby compounds in order of proximity to the "index" compounds until the required number of volunteers was made up. Participants screened based on criteria pre-determined were enrolled to participate in the study. Those studied for incidence of infection and first clinical malaria were treated with anti-malarials to clear them of all malaria parasitaemia prior to follow-up. They were then followed prospectively for at least four months to determine re-infection, and clinical malaria. The selection procedure in those studies for parasite genotypes and severe anaemia were the same, but these studies were carried out as cross-sectional surveys.

The main findings were:

Malaria transmission was found to be intense throughout the year, with seasonal peaks and troughs. The overall prevalence of *P. falciparum* was 70% by microscopy and 82% by PCR with the highest parasite rates among 5-9 year olds and highest parasite density (geometric mean 1,922/µl blood) in 1-2 year olds. PCR-RFLP typing of the *P. falciparum msp2* gene revealed a mean multiplicity of 3.4 (range: 1 to 8) genotypes with the two *msp*2 allelic families (FC27 and 3D7) in almost equal proportions. The correlation between parasite density and *msp2* multiplicity was highest in infants, and decreased with age to a minimum by 10 years, then start to increase again from this age into adolescence and adulthood.

The incidence density of *P. falciparum* infection in both infants/young children and adults was the same (ID = 7.0 cases/person-year) in the wet season, and only decreased slightly (5.0 cases/person-year) in the dry season. The cumulative incidence of infection profiles in both age groups indicated the same rapid rise with over 90% re-infection rate within 12 weeks post-treatment.

The risk of developing febrile parasitaemia of >5,000/µl, >10,000/µl, >20,000/µl and >50,000/µl during the wet season was 1.92, 1.93, 2.45 and 4.33 times that of the dry season with p-values always less than 0.0025.

Malaria parasites were cleared prior to following up on the cohorts in order to determine incidence of *P. falciparum* infections. This resulted in 49% of volunteers in the treatment group experiencing clinical attacks of malaria compared to 38% in the untreated group [RR (95% CI)=1.29 (1.03-1.61)]. Clinical malaria in the treated group was associated with significantly more symptoms and lower parasitemia.

Severe anaemia defined as Hb<6.0g/dL, at the end of the wet season (November 1996) was 22.1% compared to 1.4% at the end of the dry season (April 1997), [OR (95% CI)=20.1 (7.1 – 55.3)]. Nutritional and hookworm anaemia appeared to have little impact upon this seasonal difference since anthropometric indices were comparable with no hookworm infection among 6-24 months aged children. A repeat survey in November 2000 confirmed that the proportion of severely anaemic children and overall mean haemoglobin levels, in the 2000 sample were significantly improved over those of the 1996 (17.5 % vs. 26.4%, P = 0.03; Hb 7.5 g/dL vs. 6.9 g/dL, P = 0.002). Relative to children with Hb≥6.0 g/dL, those with severe anaemia (Hb<6.0 g/dL) were older, more frequently parasitaemic [OR (95% CI)=1.60 (1.08-2.35)], more often febrile [OR (95% CI)= 2.44 (1.71-3.48)], and predominantly male [OR (95% CI)=1.50 (1.05-2.13)].

These findings bear upon the design of malaria drug and vaccine trials in holoendemic areas such as the KND. Optimal design of malaria intervention trials, ie sample sizes calculations and follow-up could be borne out of these findings. Changes in the multiplicity of infections based on *msp2* genotyping can be used for assessing the outcome of malaria clinical intervention trials. The evidence also suggests that dramatic peaks and troughs of severe anaemia are regular and possibly predictable events that may be used to assess malaria clinical intervention trials in areas similar to the KND. Evaluation of some clinical malaria intervention trials will require clearance of parasitaemia prior to follow-up. The interpretation of such intervention trials need to be carried out with a lot of caution as clinical malaria occurring after clearance of malaria parasitaemia may be distinctly different from "natural" disease and this may have significance for the design and interpretation of intervention trials.

ZUSAMMENFASSUNG

Die Malaria gehört nach wie vor zu einem der wichtigsten Gesundheitsprobleme, insbesondere in Afrika südlich der Sahara. Jährlich werden mindestens 900 Millionen akute Fieberepisoden und 1-3 Millionen Todesfälle verzeichnet, grösstenteils in Afrikanischen Kindern unter 5 Jahren. Weniger als 20% dieser Todesfälle werden von den formalen Gesundheitssystemen erfasst. Die Weltbank (1993) stuft die Malaria als die wichtigste Ursache für verlorene "Disability-Adjusted Life Years" (DALYs) in Afrika ein und schätzt die Anzahl der durch Behinderung und frühzeitigen Tod verlorener Lebensjahre auf 35 Millionen.

Es wird erwartet, dass sich die massive Last der Malaria Morbidität und Mortalität ohne effiziente Malaria-Kontrollprogramme in den nächsten 20 Jahren verdoppeln wird. Diese starke Zunahme ist vor allem auf die sich ausbreitende Resistenz von *Plasmodium falciparum* gegen Chloroquin und andere Malariamedikamente zurückzuführen. Beunruhigend ist auch, dass sich keine neuen preisgünstigen Medikamente in der Entwicklung befinden, dass in den meisten betroffenen Ländern die finanziellen Resourcen der Gesundheitssysteme stark begrenzt sind, und dass die Expertise zur Planung von effizienten Kontrollprogrammen oft unzureichend ist. Eine gewisse Hoffnung wurde durch die Initiative "Roll Back Malaria" (RBM) geweckt, die sich zum Ziel gesetzt hat, Malaria innerhalb der nächsten 10 Jahren um die Hälfte zu reduzieren.

Das Ziel der vorgelegten Studie ist es, die Epidemiologie der *Plasmodium falciparum* Parasiten zu charakterisieren, die Saisonalität von Infektion und Morbidität und deren Verhältnis zu Radikalbehandlungen im Kassene-Nankana Distrikt (KND) in Nord-Ghana zu untersuchen. Solche Informationen werden für die Planung und Durchführung künftiger klinischer Malaria Interventionsstudien von Bedeutung sein. Im KND ist die Malariatransmission holoendemisch und spiegelt durch saisonale Höhen und Tiefen die Regen- und Trockenzeiten im Distrikt wider. Malaria ist die wichtigste Ursache für Morbidität und Mortalität, verantwortlich für 60% der Spitalbesuche und 35% aller Todesfälle.

Kohorten von entweder nicht-immunen oder semi-immunen Erwachsenen oder von allen Altersklassen wurden randomisiert ausgewählt. Es wurde ein "cluster sampling" Ansatz verwendet, der durch das "Navrongo Demographic Surveillance System" (NDSS) ermöglicht wurde. Für jede Studie wurde eine Kohorte von 200 bis 300 Einwohnern rekrutiert. Alle in Frage kommenden Einwohner des Distriktes wurden in einer Stichprobengesamtheit zusammengefasst und 16 "Index"-Haushalte mit Hilfe des STATA Programmes randomisiert ausgewählt. Potentielle Freiwillige wurden aus den Nachbarhaushalte rekrutiert bis die gewünschte Anzahl Freiwillige erreicht war. Die ausgewählten Personen wurden nach Überprüfung hinsichtlich vorbestimmter Kriterien in die Studie aufgenommen. Die Personen, die an der Studie über Infektions-Inzidenz und erste klinische Malariaepisoden teilnahmen, wurden mit Malariamedikamenten behandelt, um sie vor den Nachfolgeuntersuchungen von allen Malariaparasiten zu befreien (Clearance). Sie wurden danach während mindestens 4 Monaten überwacht, um Neuinfektion und klinische Malaria zu erfassen. Das Auswahlverfahren in den Studien über Parasitengenotypen und schwere Anämie war dasselbe, ausser dass hierbei Querschnitts-Analysen durchgeführt wurden.

Die wichtigsten Resultate waren:

Mit saisonalen Höhen und Tiefen wurde eine hohe Malariatransmission während des ganzen Jahres beobachtet,. Die *P. falciparum* Prävalenz war 70% bei mikroskopischer Bestimmung und 82% bei PCR-Bestimmung. Die Prävalenz war bei 5-9 jährigen Kindern am höchsten und die höchsten Parasitendichten wurden bei 1-2 jährigen Kindern gefunden (geometrische Mittel 1.922/μl Blut). PCR-RFLP Typisierung des *P. falciparum msp2* Gens zeigte, dass Personen im Schnitt mit 3.4 (Umfang: 1 bis 8) verschiedenen Genotypen infiziert waren (Mulitplizität der Infektion). Die beiden *msp2* Allel-Familien FC27 und 3D7 waren zu beinahe gleichen Anteilen vertreten. Die Korrelation zwischen Parasitendichte und *msp2* Multiplizität war am höchsten bei Kindern, nahm mit dem Alter ab, erreichte ein Minimum bei den 10 jährigen und stieg dann mit dem Alter wieder an.

Die Inzidenz der *P. falciparum* Infektion war bei Kindern und Erwachsenen gleich, lag bei 7.0 Fällen pro Personen-Jahr in der Regenzeit und war in der Trockenzeit (5.0 Fälle pro Personen-Jahr) nur geringfügig reduziert. Auch die kumulative Inzidenz war in beiden Gruppen gleich und zeigte einen raschen Anstieg der Reinfektionsrate bis auf über 90% während den ersten 12 Wochen nach Behandlung.

Das Risiko, eine febrile Parasitämie mit >5‘000/μl, >10‘000/μl, >20‘000/μl und >50‘000/μl zu entwickeln, war in der Regenzeit jeweils 1.92, 1.93, 2.45 und 4.33 mal höher als in der Trockenzeit (alle p-Werte <0.0025).

Um die Inzidenz der *P. falciparum* Infektion zu bestimmen, wurden die Kohorten vor den Nachuntersuchungen von Malariaparasiten befreit. Dies führte dazu, dass 49% der Freiwilligen in der behandelten Gruppe klinische Malaria entwickelten im Vergleich zu 38% in der unbehandelten Gruppe [RR (95% CI)=1.29 (1.03-1.61)]. Klinische Malaria war in der behandelten Gruppe signifikant mit mehr Symptomen und geringerer Parasitendichten assoziiert.

Schwere Anämie, definiert als Hb<6.0g/dL, war am Ende der Regenzeit (November 1996) mit 22.1% weit häufiger [OR (95%)=20.1 (7.1-55.3)], als am Ende der Trockenzeit (1.4% im April 1997). Ernährungs- und Hakenwurminfektions-bedingte Anämien schienen bei diesem saisonalen Unterschied keine Rolle zu spielen, da die anthropometrischen Parameter in Hakenwurminfizierten-und nicht-infizierten 6-24 Monate alten Kindern vergleichbar waren. Eine Nachfolgestudie, die im November 2000 durchgeführt wurde, hat gezeigt, dass in der 2000er Kohorte der Anteil der schwer anämischen Kinder im Vergleich zu der 1996er Kohorte abgenommen und die Hämoglobinwerte signifikant zugenommen hatten (17.5% vs. 26.4%, p = 0.03; Hb 7.5g/dL vs. 6.9g/dL, p = 0.002). Im Vergleich zu Kindern mit Hb≥6.0g/dL waren die schwer anämischen Kinder (Hd<6.0g/dL) älter, hatten öfter eine Parasitämie [OR (95% CI)=1.60 (1.08-2.35)], hatten öfter Fieber [OR (95% CI)=2.44 (1.71-3.48)] und waren häufiger männlich [OR (95%)=1.50 (1.05-2.13)].

Diese Resultate haben wichtige Implikationen für die Planung von klinischen Studien zur Erprobung von Malariamedikamenten und -Impfstoffen in holoendemischen Gebieten wie dem KND. Solche Malaria Interventionsstudien sollten hinsichtlich der Teilnehmerzahl und der Festlegung von Nachfolgeuntersuchungen basierend auf diesen Ergebnissen entworfen werden. Durch *msp2* Genotypisierung nachgewiesene Änderungen in der Multiplizität der Infektionen können dazu verwendet werden, die Endpunkte von Malaria Interventionsstudien abzuschätzen. Die Resultate zeigen, dass die dramatischen Schwankungen in der Häufigkeit schwerer Anämien regelmässige und möglicherweise voraussagbare Ereignisse sind, die in der Auswertung von Malaria Interventionsstudien in Gebieten wie dem KND einbezogen werden müssen. Die Evaluierung gewisser Malaria Interventionsstudien wird die Eliminierung von Parasitämien vor den Nachfolgeuntersuchungen voraussetzten. Solche Interventionsstudien müssen mit grösster Sorgfalt interpretiert werden, da sich klinische Malaria, die nach dem Eliminieren der Parasitämie auftritt, sehr von der "natürlichen" Malaria unterscheiden kann. Dies kann für die Planung und Interpretation von Interventionsstudien von grosser Bedeutung sein.

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

DNA	De-oxyribonucleic acid
PCR-RFLP:	Polymerse Chain Reaction-Restriction Fragment Length Polymorphism
MSP2:	Merozoite Surface Protein 2
EIR:	Entomological Inoculation Rates
GMPD:	Geometric Mean Parasite Density
ID:	Incidence Density
CI:	Cumulative Incidence
NAI:	Naturally Acquired Immunity
G-6-PD:	Glucose-6-phosphate dehydrogenase deficiency
Hb:	Haemoglobin
PE:	Protective Efficacy
KND:	Kassena-Nankana District
NHRC:	Navrongo Health Research Centre
NDSS:	Navrongo Demographic Surveillance System
STI:	Swiss Tropical Institute
NMIMR:	Noguchi Memorial Institute for Medical Research
NMRC:	Naval Medical Research Centre
NAMRU-3:	Naval Medical Research Unit 3
WHO:	World Health Organisation
TDR:	Tropical Diseases Research

Chapter 1

INTRODUCTION

1.1 The burden of malaria

Malaria continues to be the most important parasitic disease of humans. It is of major public health importance in the tropics, especially in Africa south of the Sahara, where it remains a leading cause of morbidity and mortality. The current statistics leave close to half of the world's population at risk of being infected with this dreaded parasite (World Health Organisation, 1996a). Between 400 and 900 million acute febrile episodes occur yearly in African children under 5 years of age living in endemic areas (Breman, 2001). At a minimum, 0.7-2.7 million deaths occur yearly from malaria, about one million in children below 5 years (Breman, 2001), (World Health Organisation, 1996b). Over 75% of these deaths occur in sub-Saharan Africa (World Health Organisation, 1997a). In Africa, 25-30% of all deaths among children under 5 years of age are attributed to malaria (Molineaux, 1985); (Greenwood *et al.* 1987); (Payne *et al.* 1976); (Snow *et al.* 1999), but less than 20% of these deaths come to the attention of any formal health system.

Severe anaemia due to malaria is now believed to occur in between 1.5 to 6.0 million African children per year with case fatality rate of nearly 15% (Murphy *et al.* 2001). Respiratory distress, hypoglycaemia and overlapping conditions contribute another 1-2 million cases with mortality nearing 20% (Murphy *et al.* 2001). Malaria-related effects on pregnant women, their foetuses, and new-borns comprise an extremely large and often hidden burden (Steketee *et al.* 2001); (Murphy *et al.* 2001). Over 400,000 women develop severe anaemia during pregnancy annually as a result of malaria in sub-Saharan Africa.

The World Bank ranks malaria as the leading cause of lost disability-adjusted life years (DALYs) in Africa with an estimated 35 million future life-years lost from disability and premature death (World Bank, 1993). Malaria reduces the capacity for children to learn and the productivity of the work force and drains national treasuries. According to new research by Harvard, London School and the World Health Organisation, Africa's GDP would be up to $ 100 billion greater than it is currently if malaria had been eliminated years ago (World Health Organisation, 2000a). It has been documented that in areas

where malaria was eliminated, economic growth increased substantially over the following five years compared to growth in neighbouring countries (Gallup *et al.* 2001).

Without effective malaria control programme(s), the massive burden of malaria morbidity and mortality is expected to at least double in the next 20 years. One reason is the growing spread of *Plasmodium falciparum* resistance to Chloroquine and other anti-malarials. From data collected in Senegal, Trape (2001) demonstrated a two- to three-fold increase in hospital admissions and deaths, and a six-fold increase in child malaria mortality when Chloroquine resistance emerged in the late 1980s and early 1990s. Other reasons include lack of development of new affordable drugs; the financial constraints on health services in most countries; lack of expertise to plan properly for malaria control, and the wars in Africa that have and continue to disrupt health services in those countries.

Hope has been re-kindled by the recent initiation of the Roll Back Malaria (RBM) whose goal is to reduce malaria by half in 10 years (Nabarro *et al.* 1998); (Alnwick, 2000). Implementation of malaria control interventions is therefore expected to increase. The correct and timely application of current malaria control strategies (personal protection, drug use and vector control), can result in significant decrease in malaria-specific and overall mortality. Though it will take a while before a vaccine of public health importance is made available and affordable to people living in rural sub-Saharan Africa, the momentum for discovery of new and effective anti-malarial drugs as well as vaccines should be intensified. Funding of research towards the discovery of new anti-malarial drugs and vaccines should be made available in institutions with capabilities and in different epidemiological settings for testing of these drugs/vaccines. It is important to ensure that potential sites where such new vaccines and drugs are to be tested are well characterised, in terms of their malariometric indices, prior to testing.

1.2. Determinants of the malaria burden

To propagate malaria transmission, intrinsic factors related to humans, mosquitoes and human malaria parasites are required in the presence of suitable environmental conditions. Other contributing factors include social, economic and behavioural. The most important extrinsic factors include rainfall, economic conditions, social, behavioural, and political commitment as well as effectiveness of control and preventive efforts.

Parasite: Human malaria disease is caused by infections from 4 plasmodia species, *Plasmodium falciparum, P. vivax, P. malariae* and *P. ovale.* Of these, *P. falciparum* is the most virulent and the most widespread in Africa (Gilles, 1985). *P. vivax* is not common in Africa, especially West Africa because Duffy antigen, the receptor on the surface of red blood cell to which merozoites bind is rare in African populations. Transmission of *Plasmodium* parasites is from person to person through the bite of a female anopheles mosquito (Fig. 1.1:).

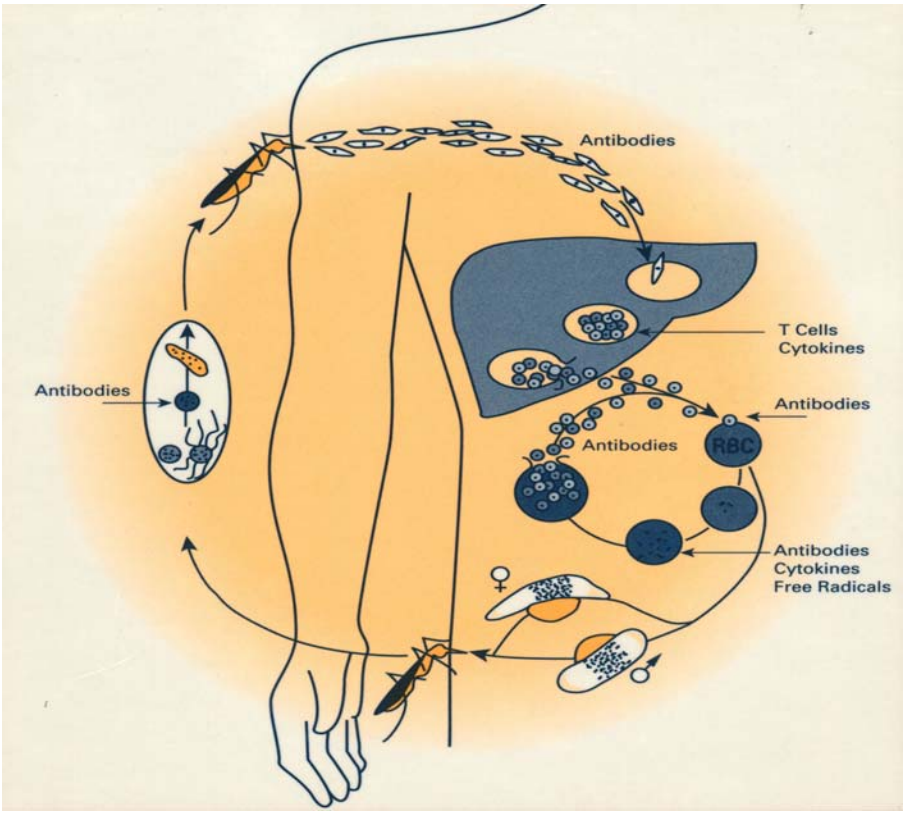


Figure 1.1. The life cycle of *Plasmodium* species. (By courtesy of Stephen Hoffman)

The life cycles of all human plasmodia species transmitted are the same with three reproductive phases. The species differ in the time taken to complete each phase. An initial stage consists of a single cycle of sexual reproduction that occurs in the female mosquito (sporogony), leading to production of sporozoites. At ambient temperature of 24^{o}C, sporogony takes 9 and 21 days in *P. falciparum* and *P. malariae* respectively. When the infected mosquito bites man, it injects the mosquito into the blood. The sporozoites then travel to the liver cells and develop the second phase, a single cycle asexual reproduction takes place within five to seven days. This "hepatic schizogony" or "pre-erythrocytic" phase results into merozoites that enter the blood stream, after bursting of the liver cells. The third and final stage "erythrocytic schizogony" phase takes place in the red blood cells and consists of several cycles (each lasting 48 hours in the case of *P. falciparum),* of asexual reproduction. New merozoites are produced in each cycle and these re-invade new red blood cells, starting the erythrocytic cycle again. Some of these merozoites, however, differentiate into male and female gametocytes that are taken up by the blood-sucking *Anopheles* to start the next sporogonic cycle. Each stage of these cycles is a target under investigation for potential malaria vaccines (Hoffman, 1996).

Vector: *Anopheles gambiae* ss and *Anopheles funestus* are the most efficient vectors for *Plasmodium falciparum* malaria transmission (Service, 1996). Their human biting habits and longevity makes them efficient vectors of malaria. The entomological inoculation rate (EIR), the number of sporozoite positive mosquito bites per year is the most common measure of malaria transmission. A high EIR results in stable and intense transmission, leaving young children as the vulnerable group, while a low EIR results in a wider age range of individuals being susceptible and developing severe infections and illness.

Host factors: Availability of human populations susceptible to malaria infections is a key factor. The genetic make up of humans can limit parasite multiplication and thus protect them from malaria. Sickle cell trait, Duffy blood factor, hereditary ovalocytosis,

Glucose-6-phosphate dehydrogenase deficiency, and thalassemia among others have been associated with decreased susceptibility to severe malaria (Luzzatto, 1979).

The immune status of the individual and of populations plays the most important role in the clinical response to infection and transmission. Maternal antibodies in new-borns are augmented by exposure later in life in highly endemic areas. Immunity is never complete, irrespective of the level of exposure, so malaria premunition (parasites and antibodies without symptoms) comes into play. Identification of specific immunologic determinants of protection will lead to development of the most promising vaccine candidates.

Extrinsic factors: These include environment, social, behavioural, political and economic factors. Climatic conditions in most tropical areas: warm temperature, heavy rainfall, high humidity is conducive to mosquito breeding and longevity and parasite sporogony. Engineering projects such as construction of dams for hydropower and irrigation, roads, industrial and residential centres usually result in water collections leading to increased mosquito breeding. Epidemics linked to rainfall, temperature, geography, and population susceptibility have been reported (Lepers *et al.* 1991); (Connor *et al.* 1999).

Improvements in education, economic and social conditions impact on malaria transmission (Koram *et al.* 1995). Filling in of swamps, elimination of open drainage ditches and other breeding sites, screening of windows and doors, widespread use of air conditioning and availability of rapid diagnosis and drugs for acute illnesses are related socio-economic status. The recent heads of states malaria conference in Abuja gave political support that raised the hope of commitment for malaria control (World Health Organisation, 2000b).

1.3. Manifestations of malaria

Malaria infections can result in asymptomatic parasitaemia, clinical malaria (febrile episodes, parasitaemia); severe malaria (anaemia, neurologic syndromes) and mortality (Breman *et al.* 2001).

The local epidemiologic profile of malaria in the context of other diseases is very important for diagnosing and quantifying accurately the cause of febrile illness (Rougemont *et al.* 1991). In its mild form, malaria presents as a febrile illness associated with other non-specific signs and symptoms. The fever may be periodic and interspersed with afebrile intervals. Malaria may be confirmed by the presence of the parasite in the peripheral blood. Parasitological diagnosis does not necessarily mean that malaria is the cause of the disease.

Acute severe illness can lead to cerebral malaria, with a high case fatality (World Health Organisation, 2000c); (Molyneux *et al.* 1989); with coma, severe anaemia, respiratory distress and hypoglycaemia being ominous. The debilitating acute and chronic effects of anaemia and neurologic, cognitive and developmental impairment are not well quantified (Slutsker *et al.* 1996); (Newton *et al.* 1998); (Brewster *et al.* 1990); (Van Hensbroek *et al.* 1997); (Verity *et al.* 1998). The effects of malaria on pregnant women and their foetus include severe malaria attacks on expectant mothers leading sometimes to death; low birth weight and subsequent increased infant and childhood mortality (Bloland *et al.* 1996); (Slutsker *et al.* 1996).

1.4 Rationale for the studies

The current malaria control and prevention measures include personal protection, drug use and vector control strategies. The appropriate and timely use of these strategies could result in significant decreases in malaria specific and overall mortality. It is, however, difficult to achieve and sustain this. Though the role of drug treatment is paramount among the control methods, the emergence/resurgence of resistance to anti-malarial drugs is still on the ascendancy. Chloroquine, which still remains the first-line drug in most African countries cures only a proportion of malaria infections. Studies

carried out with Sulphadoxine-Pyrimethamine (SP), the second line drug in these countries and currently the first line drug in a few other countries in Africa indicates that the malaria parasites are developing resistance against this drug at a faster rate than to Chloroquine. The few new anti-malarial drugs that have so far been discovered are beyond the pockets of the ordinary African family. It is therefore imperative that new anti-malarial drugs that will be cheap, affordable and effective (similar to Chloroquine) are discovered before the current ones become obsolete. This means that testing of new, potential anti-malarials in endemic areas will have to increase over the coming years.

The search for an effective vaccine against the disease will be another major strengthening of the malaria control process. Availability of the local epidemiologic profile of malaria prior to testing new drugs or vaccines will contribute to optimal design and evaluation of the study and generate reliable results that can be interpreted with confidence. Determination of malaria parasite genotypes and dynamics of the infection, attack rates, antigen variability, morbidity trends are essential for planning efficacious and effective vaccine and drug trials.

With the Kassena-Nankana district of northern Ghana earmarked as one of the potential sites for clinical studies including testing of new anti-malarial drugs and vaccines, an important first step is to characterise the malaria epidemiology in the area. Measurement of the incidence density (ID), which estimates the force of infection exerted by a pathogen upon a defined population, a true attack rate and the cumulative incidence (CI), which also estimates the risk or probability of infection during a defined period, are crucial for determination of sample sizes for trials. The ID and CI provide a good foundation for forecasting the frequency of new infections and disease and thus permit the application of optimal methods for formulation of strategies for interventions or the design and evaluation of drug or vaccine trials (Jones *et al.* 1994); (Beier *et al.* 1994).

Intervention trials of malaria vaccines or drugs may require radical cure of volunteers prior to follow-up for end points of interest. There has been no direct evaluation of the possible impact of radical cure on incidence and severity of subsequent symptomatic malaria. Does the elimination of parasitaemia render the host more susceptible to disease by parasitaemia introduced by re-infection? Does the drug(s) used for parasite clearance affect the immune system or interrupt premunition? Though the study of Pringle & Avery-Jones (1966) on the early course of untreated *falciparum* malaria in semi-immune African children following a short period of protection indicated that many of the new infections caused clinical symptoms of malaria and a parasitaemia that was significantly greater than had prevailed generally before treatment, there has not been any follow-up studies all these years to substantiate on this finding. This study concluded that even short period of a few weeks of drug protection against malarial infection had lowered the immunity of the children to an appreciable degree.

There has been a long-standing discussion on rebound effects following successful interventions (Snow *et al.* 1997), and the use of chemoprophylaxis or drug treatments (Menendez *et al.* 1997); (van den *et al.* 1996); (Greenwood *et al.* 1995). These studies reported significantly higher incidence of clinical attacks of malaria and severe anaemia after the interventions (medication/ bed net use) was stopped among children who had previously received antimalarials than among children who had previously received placebo; an impairment of the development of natural immunity. The question about the effects of completely clearing malaria parasites prior to assessing malaria interventions still remain relevant, especially to intervention trials where subtle differences in susceptibility affect end points of effective or ineffective outcomes.

A major strengthening of malaria control can be achieved through the availability of an effective vaccine against the disease. Though it has long been suggested that malaria vaccines should aim to mimic the effects of naturally acquired immunity (Alonso *et al.* 1996), we still do not understand some of the fundamental issues on the effects of long term exposure to malaria parasites on immunity. Does acquired immunity against pre-erythrocytic antigens results in a reduction in the incidence of new blood stage

infections, as the host grows older? It is still unclear how acquired immunity to blood stages affects the persistence of individual infections. We know however, that genetic diversity of parasites is frequent within species, patients and localities, due to recombination and selection. Such evolved parasites may alter clinical presentations and impact on the number of cases resulting in various age groups due, probably to the presence of more virulent forms (Jiang *et al.* 2000); (Gupta *et al.* 1994).

Studies of *Plasmodium falciparum* infections that people are exposed to gain a better understanding of the factors that impact on the development of immunity in different endemic areas will improve our knowledge of the use of molecular tools in the choice of new anti-malarial drugs and vaccine formulations. The design and evaluation of effective control measures against the parasite could improve with indepth knowledge of the molecular epidemiology in endemic settings. For example, the trial involving the SPf66 vaccine did not protect young Gambian children against clinical attacks of malaria. The knowledge about the genotypes in this area prior to testing the vaccine resulted in significantly fewer detectable *P. falciparum* genotypes than in control children (Haywood *et al.* 1999). Furthermore, knowledge of genes associated with disease or resistant to a drug or a vaccine in an area and the frequency of such genes are relevant as this could affect the outcome of interventions. Genetic diversity may account for the protective immunity and the frequent repeat infections and clinical episodes of malaria in persons, particularly young children, living in areas of intense and stable transmission, and this must be considered in the development of vaccines. Multiplicity of *falciparum* infections has been documented to reach high levels of up to 9 different parasite clones at a given time in a single asymptomatic host. This has been shown to be positively associated with protection against mild episodes of malaria in some circumstances (Smith *et al.* 1999a); (Al Yaman *et al.* 1997); (Beck *et al.* 1997); (Contamin *et al.* 1996); (Ntoumi *et al.* 1995). This further suggest that concurrent infections provide protection from super-infecting parasites and that concomitant immunity is, at least in part, a consequence of a response to the major merozoite surface proteins. The number, or multiplicity, of infection in a host may confer the degree of immunity against *P. falciparum,* and the risk of clinical malaria.

PCR-RFLP genotyping of parasites for *msp2* makes it possible to distinguish the individual parasite infections concurrently present in a blood sample (Felger *et al.* 1999). The possibility of tracing individual parasite clones over time allows detailed studies of infection dynamics. In immunological studies, genotyping can also provide important information on the diversity of antigenic challenge. Most importantly, genotyping makes it possible to determine the multiplicity of infection, which can be used as an outcome measurement of interventions such as drug trials, vaccine trials, or exposure-reducing interventions. The *msp2* of *P. falciparum* is not only an extremely polymorphic marker gene, but considered a prime candidate for inclusion in a sub-unit malaria vaccine against blood stage malaria (Engelbrecht *et al.* 1995). *Msp2,* as part of a sub-unit vaccine, entered into field trial in PNG (Genton et al., 1996); (Genton et al., 2001). Not only is *msp2* frequently and strongly recognised by the immune system of individuals exposed to malaria (Al Yaman *et al.* 1994); (Rzepczyk *et al.* 1990), but monoclonal antibodies against *msp2* prevent invasion of erythrocytes by merozoites *in vitro*. Two genotyping studies from PNG have already shown that the 2 allelic families of *msp2* are differently associated with morbidity. Parasites carrying the FC27-like genotype were twice as likely to be found in symptomatic malaria infections.

Infection, morbidity and/or mortality due to an infection are common end points of drug and vaccine trials. Malaria drug or vaccine trials with incidence of infection/re-infection as end point are not difficult to determine. This is however, not the case with drug or vaccine trials that have malaria morbidity and/or mortality as end point. In most endemic sites, malaria is normally one out of several common infectious diseases that occur. The presence of malaria parasitaemia with or without fever is not conclusive that the morbidity is due to malaria. It is therefore difficult to have a clear-cut definition for malaria morbidity in malarious areas. The disadvantages of malaria mortality are not only due to the difficulty in defining exclusively the attribution of malaria, but more importantly this is a rarer event that will require a very large population or long period of follow-up.

Severe anaemia, one of the manifestations of severe malaria, seems easier to assess as an end point of malaria vaccines when considering the control effects of malaria in endemic sites where severe anaemia is a regular annual phenomenon. In such circumstances, the contribution of other causes of severe anaemia, such as nutrition and worm infections in the age group considered needs to be properly quantified so that in the end the effect of the intervention on severe anaemia due to malaria can be reliably assessed. When it comes to the cause of malaria deaths, incidence of severe anaemia rather than mortality rates might serve as a measure of malaria vaccine or drug effect.

Chapter 2

Study Goal and Objectives

2.1 Study Goal

To characterise the epidemiology of *Plasmodium falciparum* parasites, patterns of seasonality in infection and morbidity, and their relationship to radical cure in a rural community in northern Ghana.

2.2 Specific Objectives

- To determine the age-dependent multiplicity of *Plasmodium falciparum* infections in the Kassena-Nankana district using the *msp2* gene assessed by PCR-RFLP.
- To establish the incidence rate of infections with *P. falciparum* during the wet season among semi-immune (18 to 55years) residents in the Kassena-Nankana district of northern Ghana.
- To establish the incidence rate of new infections with *P. falciparum* during the wet and dry seasons among infants/ young children (6-24 months) resident in the Kassena-Nankana district of northern Ghana.
- To determine if the densities/μL of blood of the incidence rate of first infection with *P. falciparum* is greater in the wet (high transmission) season than in the dry (low transmission) season.
- To determine if the incidence rate of axillary temperature $\geq 37.5^{o}$C and *P. falciparum* densities $\geq$ 20,000 parasites/μL of blood is greater in the wet season than in the dry season, and whether this impacts on more severe malarial infections.
- To determine if radical cure places individuals at increased risk of recurrent, symptomatic malaria during the next 6 months.
- To document the levels of severe anaemia in the wet (high) and dry (low transmission) seasons among infants/ young children (6-24 months) in the Kassena-Nankana District.
- To characterise severe anaemia in infants/ young children (6-24 months) in the Kassena-Nankana District of northern Ghana.

2.3 Study Area, Population and General Methodology

2.3.1 Study Area

The studies were carried out in the Kassena-Nankana district of northern Ghana. The district lies within the Guinea savannah woodlands between latitudes 10^0 $30^/$ and 11^0 $00^/$ North of the equator and between longitudes 1^0 $00^/$ and 1^0 $30^/$ West of the zero Meridian. It is located in the Upper East Region of Ghana, shares its northern boundary with Burkina Faso and covers an area of 1675 km^2. The 141,000 resident population is widely dispersed in roughly 14,000 compounds with a few living in concentrated settlements in the district capital. The residents are mainly subsistence farmers growing millet (the staple food), groundnuts, rice and vegetables. They also rear chicken, goats, sheep and cattle. A few engage in petty trading. There are two main seasons, a wet and a dry season, the wet season is short with an annual rainfall averaging 800-1000 mm per annum, occurring mainly between June and September when transmission of *Plasmodium species*, mostly *P. falciparum* by *Anopheles gambiae* and *An. funestus* peaks. The minimum and maximum daily temperatures range between 16^0C to 45^0C respectively. Previous malaria prevalence surveys in children (Binka *et al.* 1994) documented rates ranging from 53.3-76.5% at the end of the dry season to a peak of 84.5-94.2% during the wet season. The percentages of the different species of malaria parasites were 70.6% for *Plasmodium falciparum*, 16.9% *P. malariae*, 7.9% *P. ovale*, and 1.4% *P. vivax*.

The siting of a large irrigation project, which covers an area of 3,860 hectares and 42 kilometres of canals, allows for dry season farming of rice and tomato. There are roughly 90 dug out dams in addition to this that serve as water sources for the people as well as livestock during the long dry season. Other parasitic diseases including lymphatic filariasis and schistosomiasis of both *haematobium* and *mansoni* are therefore frequent and high.

The health facilities in the district comprise of a hospital, four health centres and three clinics. These are complemented by community-based service delivery. They provide

static services while the District Health Management Team (DHMT) operates outreach clinics, providing maternal and child health services within the district.

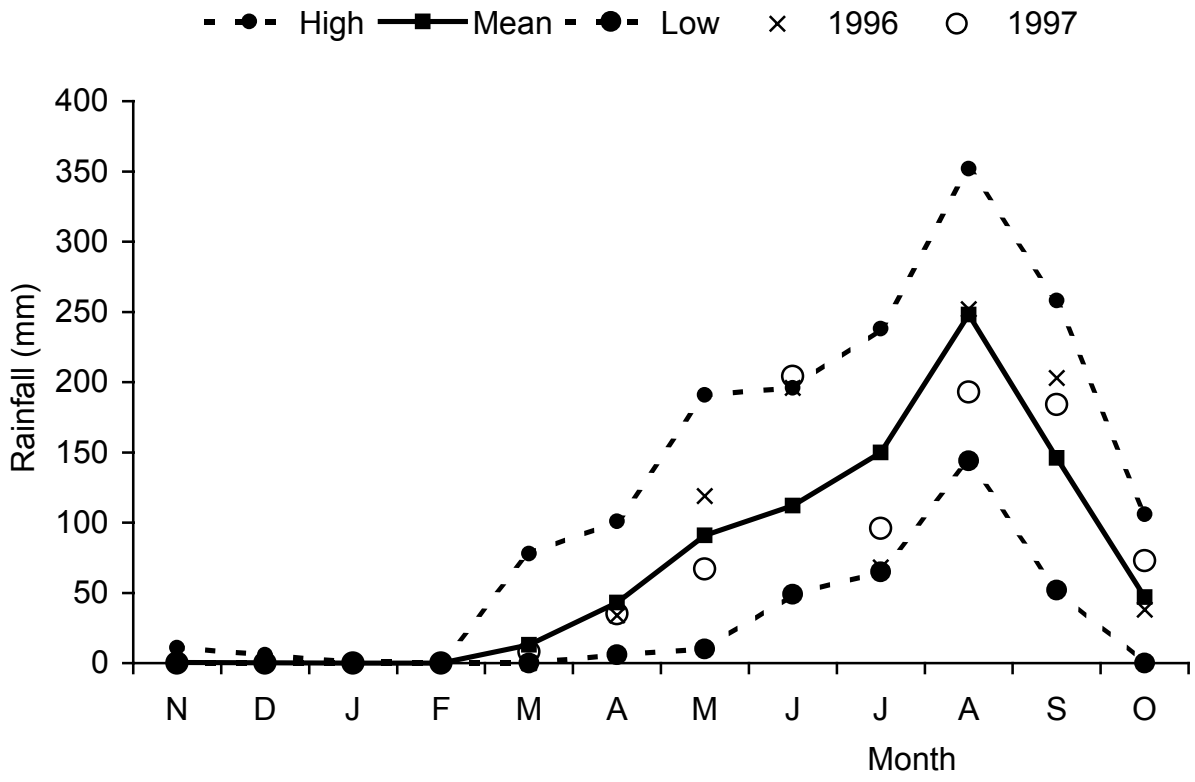


Figure 2.1 Maximum, minimum (solid dots, dotted lines) or mean (squares, solid line) monthly rainfall in the Kassena-Nankana District between 1978 and 1995, and monthly rainfall during 1996 (X) and 1997 (O).

Recent data from the district hospital showed the top four diseases prevalent to be malaria, acute respiratory infections, anaemia, and gastro-enteritis. Malaria is the leading cause of both morbidity and mortality, accounting for 60% of hospital admissions and 41% of hospital deaths. Data from verbal autopsies coded between 1993 and 1999, document malaria as responsible for 35% of all deaths in children aged less than five years and 9% of adult deaths. A further 18% of all the deaths are classified as anaemia deaths and can be attributed to malaria (Ngom, Personal communication).

2.3.2 Study population

The Navrongo Demographic Surveillance System (NDSS) implemented by the Navrongo Health Research Centre (NHRC), monitors the total population of about 141,000 people in the district through quarterly updates of births, deaths, in- and out-migrations, pregnancies and marriages. The educational attainment of all persons aged 6 years or above and the vaccination status of children less than two years of age are also collected annually. Adult educational attainment in the district is very low. Overall, about 34% of the adult population have ever been to school. Three-quarters (75%) of the women and 56% of the men have never been to school. Data from the NDSS goes to support studies of the determinants of morbidity and mortality and associated problems of high fertility in this rural Sahelian population.

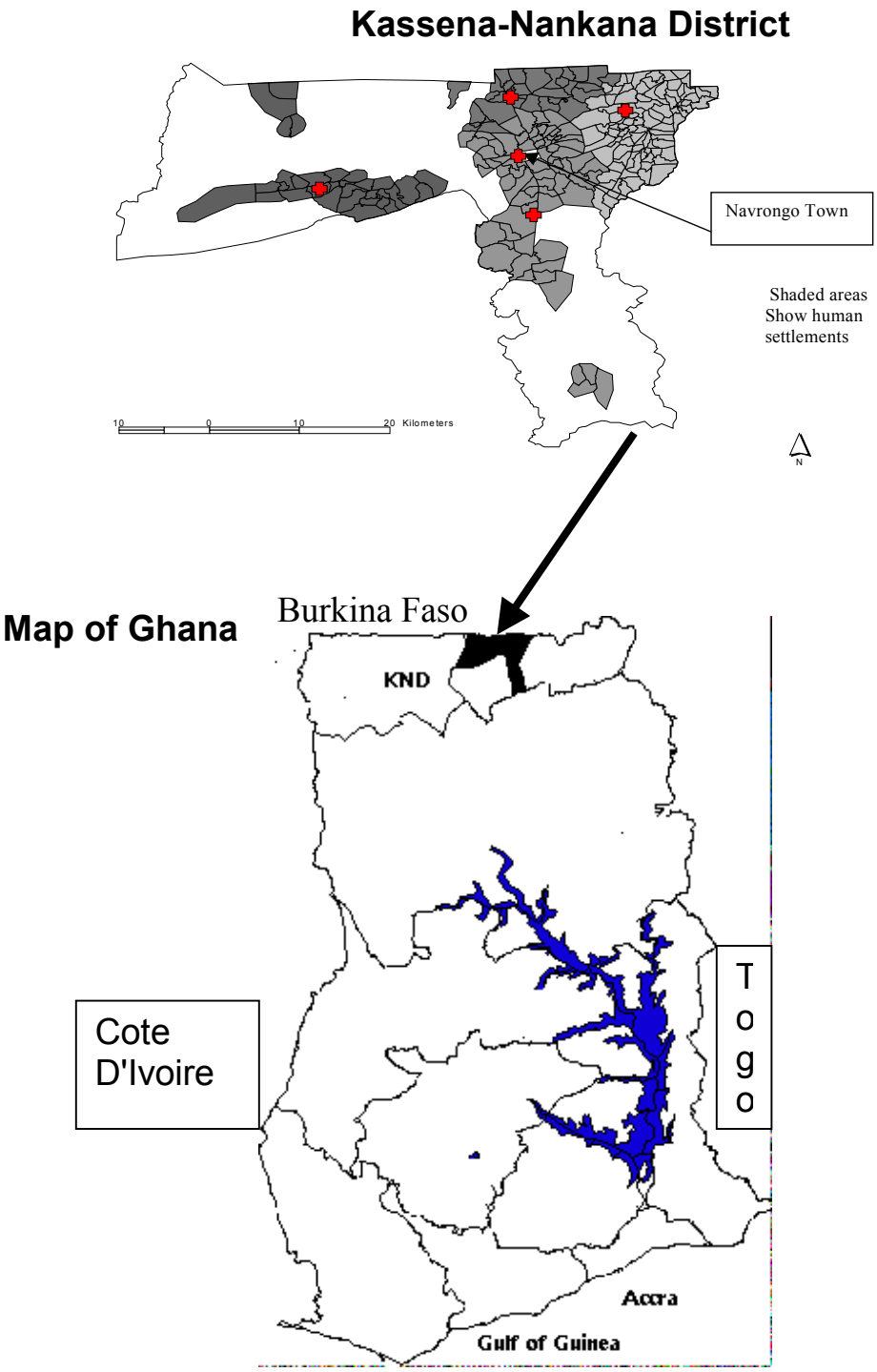
2.3.3 General Methodology for the studies carried out.

Cohorts of either infants/ young children 6-24 months or adults 18-55 years or all age groups were selected randomly using a cluster sampling approach (Bennett *et al.* 1991). In total between 200 and 300 inhabitants constituted the cohort for each of the studies. The cohorts were selected using the Navrongo Demographic Surveillance System (NDSS). All eligible people within the 4 geographic zones of the district (East, North, South, and West) were included in the sample frame. Using the Stata program (Stata Corporation, 1999), 16 "index" compounds were randomly selected from the 4 goegraphic zones of the district as determined by the NDSS, weighted by the size of each of the zones and the target population. The chiefs, elders and potential subjects were visited to seek their consent before beginning the recruitment exercise. Potential volunteers were then recruited sequentially from nearby compounds in order of proximity to the "index" compounds until the required number of volunteers was made up. Invited participants consenting to be part of the study were screened based on criteria pre-determined and those selected were enrolled to participate in the study. Those studied for incidence of infection and first clinical malaria were treated with anti-malarials to clear them of all malaria parasitaemia prior to follow-up. Once the radical cure had been completed, and blood immediately checked by microscopy and ascertained that there are no parasites, the assumption was that all volunteers were free of blood and liver parasite stages.

Participants were then followed prospectively for at least four months with routine bi-weekly (every other week) thick and thin smears as well as thrice-weekly field worker interviews of all subjects to determine symptoms or signs of illness. Those developing symptomatic malaria were treated and observed for clearance of parasitaemia and resolution of malarial illness.

The selection procedure in those studies for parasite genotypes and severe anaemia were the same, but they were carried out as cross-sectional surveys.

Figure 2 .2. Location of the Kassena-Nankana District



Chapter 3:

Molecular Epidemiology of *Plasmodium falciparum* infections among asymptomatic inhabitants of a holoendemic malarious area in northern Ghana.

S. Owusu-Agyei[1, 2], T. Smith[2,] H-P. Beck[2], L. Amenga-Etego[1], I. Felger[2]

[1]Navrongo Health Research Centre, Navrongo Ghana.

[2]Swiss Tropical Institute, Basel, Switzerland.

This article has been submitted to:
Tropical Medicine and International Health journal

3.1. Abstract

Age dependence of malaria infection was assessed in an age-stratified cluster sample of 308 individuals from the Kassena-Nankana district of northern Ghana during June/July 2000. Overall prevalence of *P. falciparum* by microscopy was 70%, with the maximum among 5-9 year olds. Parasite density was highest (geometric mean 1,922/µl blood) in 1-2 year olds. 82% of samples were positive by PCR, and RFLP typing of the *P. falciparum msp2* revealed a mean *msp2* multiplicity of 3.4 (range: 1 to 8) genotypes per PCR positive sample. Multiplicity increased with age until 5-9 years and then started to reduce again into adulthood. 49.3% of infections belonged to the *msp*2 FC27 allelic family and 50.7% to the 3D7 family. On the day of the survey, only 3.6% of the participants had fever (axillary temperature $\geq 37.5^0$C) and 2.3% had fever associated with parasitaemia. The correlation between parasite density and *msp2* multiplicity was 0.42; highest among infants, and decreased with age to a minimum among 5-9 year olds. Contrasting with results from Tanzania, this correlation increased with age in adolescents and adults. Parasite multiplicity is very high in this community, and the patterns of age dependence are similar to those in other holoendemic sites in Africa, validating the use of the age-multiplicity relationship as an indicator of malaria endemicity.

3.2. Introduction

In endemic areas, the number of clones of malaria parasites co-infecting a single host can be a useful indicator of the level of transmission and/or the immune status of the host. Increase in transmission levels (EIRs) is generally associated with progressive increases in the average number of malaria parasite clones per host (Arnot, 1998); (Babiker *et al.* 1999) and malariometric surveys have documented *falciparum* multiplicity of up to 9 different parasite clones at a given time in a single asymptomatic host (Felger *et al.* 1994); (Engelbrecht *et al.* 2000). This is almost certainly an underestimate because of technical limitations (Farnert *et al.* 2001).

In areas of very high transmission, multiplicity is age dependent, with the highest values reached in young children (Smith *et al.* 1999b); (Ntoumi *et al.* 1995), (Konate *et al.*

1999). However, studies in areas of lower endemicity: Ndiop in Senegal (Zwetyenga *et al.* 1998); Riboque in Sao Tome (Muller *et al.* 2001), The Gambia (Conway, 1991), Sudan (Babiker, 1998), reported little or no influence of age on infection complexity and allelic distribution in *P. falciparum* infections.

Multiplicity of infection also affects both the prevalence of parasite genetic markers such as those involved in resistance to anti-malarial drugs (Jelinek *et al.* 1999), (Schneider *et al.* 2001) and the risk of clinical disease. Inverse relationships between multiplicity of infection and clinical malaria (Al Yaman *et al.* 1997); (Beck *et al.* 1997); (Contamin *et al.* 1996) have been found in older children in holoendemic malarious areas, while positive relationships between multiplicity and clinical attacks have been observed in areas of lower transmission (Zwetyenga *et al.* 1998); (Roper *et al.* 1996); (Ofosu-Okyere *et al.* 2001).

The relationship between multiplicity of *P. falciparum* infection and parasite densities may also be a useful indicator of immune status, since it can indicate the extent to which different parasite clones are controlled independently of each other (Smith *et al.* 1999a). High correlations have been reported between parasite density and multiplicity of infection in infants and young children but not in older individuals (Ntoumi *et al.* 1995); (Smith *et al.* 1999b);(Engelbrecht *et al.* 2000).

The Kassena-Nankana district (KND) in northern Ghana has been well characterised as a highly endemic malarious area (Binka *et al.* 1994); (Owusu-Agyei et al., 2001), (Baird, 2001)], with plans to embark on clinical studies including testing of malaria vaccines. We have now carried out a study of the age dependence of multiple infections in KND and the patterns of relationship between multiplicity and other variables (age, sex, and parasite density), using the highly polymorphic merozoite surface protein 2 locus (*msp2*) of *P. falciparum* as marker gene. We then examined how the KND compares with other malaria endemic areas in terms of these variables. The results will be of value for the design of clinical trials using molecular typing results as outcomes.

3.3. Materials and Methods

Study site: This work was carried out in the Kassena-Nankana District (KND) located in the north-eastern part of Ghana, bordering Burkina Faso. The site is served by the Navrongo Health Research Centre (NHRC), which uses the Navrongo Demographic Surveillance System (NDSS) to monitor the population dynamics of the district (Binka et al., 1999). The district has a human population of about 141,000 living in roughly 14,000 compounds, mostly dispersed in rural areas. About 20,000 people live in Navrongo town, the administrative capital. Most people live in compounds of mud bricks, roofed with mud, thatch, or in a few cases, corrugated iron sheets. The main occupation is subsistence farming, with millet and sorghum grown around their compounds and small herds of livestock. The average annual rainfall is 850-950 mm, almost all in the months of May to September. The average annual temperature ranges from 16^{o}C to 45^{o}C. Water for mosquito breeding is available all year round due to the siting of a large irrigation project in the district (Irrigation Company of Upper Regions, 2001). Average *P. falciparum* malaria transmission levels (EIR) of 300 infective bites per person per annum have been documented. The main vectors incriminated are *Anopheles gambiae s.l*. (both *An. gambiae s.s.* and *An. arabiensis* and *An. funestus* (Appawu *et al.* 1994); (Binka et al., 1997). *P. falciparum* infection in KND shows seasonal peaks and troughs in prevalence (Binka *et al.* 1994) and clinical malaria incidence (Baird et al., 2001), with an incidence density of infection of 5 cases per person-year in the dry season to as high as over 7 cases per person-year in the wet season.

Study design: The malariological survey was carried out during June and July 2000, which mark the beginning of the rainy season. A cluster sample of the population of KND was drawn by selecting sixteen "index" compounds at random from the 14,000 within the district, making use of the NDSS. From each "index" compound, 2 people in each of the following age categories were selected; <1; 1-2; 3-4; 5-9; 10-19; 20-39; 40-59; 60+. Volunteers were recruited sequentially into each age category until the required number was made up. In some cases, additional people insisted on participating in the survey. Where necessary, participants were recruited from the nearby compounds in

order of proximity to the "index" compound. A period of one month was required to visit all the clusters.

Participants converged at the "index" compound of each of the clusters following an appointment. A signed/thumb-printed individual informed consent was obtained in their local language, followed by completing of a participant data form with a study identification number. Axillary temperature was recorded for all participants. Thick and thin blood films and DNA Isocode stix were prepared from all the participants, as well as an instant haemoglobin concentration determination using an automated haemocue machine (Haemocue® Lee Diagnostics Inc. system).

Laboratory methods: Blood slides were dried, the thin films fixed with methanol and both stained with Giemsa. These were later analysed by light microscopy. Parasite density was assessed by counting the number of parasites per 200 leukocytes in an oil immersed thick film (using a microscope with X100 objective and X 10 eyepiece). A sample was considered negative only after 200 high powered fields had been read without parasites. Parasite counts were converted to parasites per µl blood assuming an average of 8000 leukocytes per µl blood.

Deoxyribonucleic acid (DNA) was extracted from all samples collected on DNA-Isocode stix irrespective of microscopy results and the *msp2* gene of *P. falciparum* was genotyped by the Polymerase Chain Reaction- Restriction Fragment Length Polymorphism (PCR-RFLP) approach of (Felger *et al.* 1999). Restriction digests of the PCR products were performed with Hinf1 and Dde1, and the restriction fragments separated by gel electrophoresis. Individual clones were identified by allele-specific patterns. Up to 8 different clones can be distinguished by this method (Felger *et al.* 1999).

Statistical methods: Population parameters were estimated as weighted averages of the age-specific values weighed in proportion to the total district population in the age

group. The SAS GENMOD (SAS Institute Inc., 1996) procedure was used to compute confidence intervals, allowing for the clustering.

3.4. Results

Three hundred and eight residents of the Kassena-Nankana district participated in the survey. Their ages ranged from less than two weeks to 84 years. 53% of participants were female (Table 3.1).

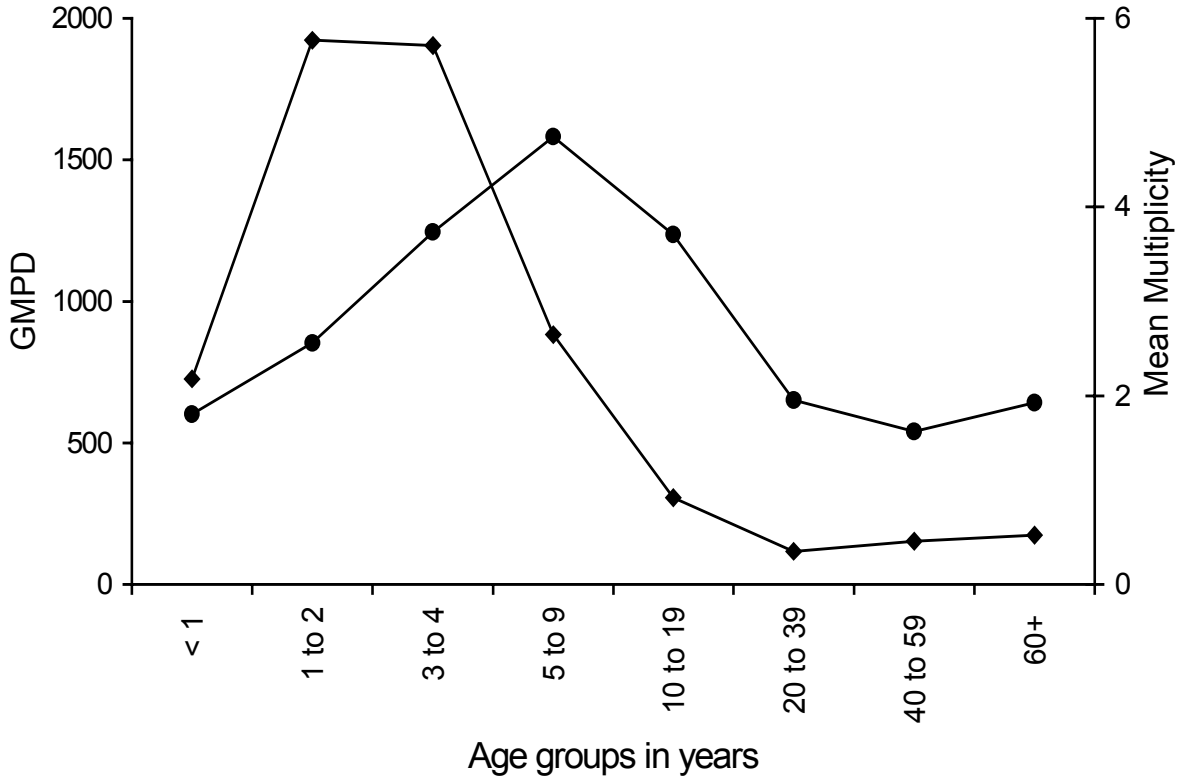
Table 3.1: Baseline Characteristics of participants

Age Group Years	Total Pop'n %	Cohort No (%)	Gender** No (%)	GMPD	Prevalence of *P. falciparum* Microscopy No. (%)	PCR No. (%)
< 1	4.0	31 (10.1)	14 (45.2)	726(CL:318-1659)	16 (51.6)	19 (61.3)
1-2	4.5	34 (11.0)	19 (55.9)	1922(CL:806-2901)	29 (85.3)	28 (82.4)
3-4	4.4	34 (11.0)	19 (55.9)	1903(CL:837-2999)	29 (85.3)	33 (97.1)
5-9	12.9	39 (12.7)	17 (43.6)	862(CL:518-1607)	34 (87.1)	38 (97.4)
10-19	23.3	51 (16.6)	30 (58.8)	306(CL:132-433)	39 (76.5)	48 (94.1)
20-39	23.0	42 (13.6)	26 (61.9)	117(CL:86-188)	29 (69.0)	31 (73.8)
40-59	17.6	37 (12.0)	17 (45.9)	153(CL:91-312)	18 (48.6)	25 (67.6)
60+	10.1	40 (13.0)	20 (50.0)	174(CL:104-243)	23 (57.5)	30 (75.0)

** Gender, female to male = (53%/47%) ; GMPD = Geometric Mean Parasite Density
PCR = Polymerase Chain Reaction

By microscopy, 217 (70.5%) of the samples were infected with *Plasmodium falciparum,* 16 (5.2%) with *P. malariae*, 1 (0.3%) *P. ovale* and 2 (0.6%) *P. vivax*. The prevalence of *P. falciparum* infection was highest among children 5-9 years, followed closely by those 3-4 years and 1-2 years of age, all these groups registering over 80% infection rates. The all age parasite rate of *P. falciparum* in the population, allowing for the age stratification of the sample was 69.1%. Infection rates generally decreased with age in individuals who were older than 10 years of age. The Geometric Mean Parasite Density (GMPD) (for microscopy positive slides only) was highest (1,922/µl blood; 95% CI: 806-2,901) in children 1-2 years of age, followed closely by children between 3-4 years

(1,903/µl blood; 95% CI: 837-2,999). A steady decrease was observed with increasing age to a GMPD of only 117/µl blood (95% CI: 86-188) in adults from 20 years of age onwards [Fig. 3.1]. There was a tendency of increasing GMPD in adults from 40 years of age and above.



GMPD (Geometric Mean Parasite Density) = graph with solid squares;
Mean multiplicity = graph with solid dots.

Figure 3.1. *P. falciparum* parasite GMPD and Mean Multiplicity of infection

By PCR analysis, 252 (81.8%) were positive for *Plasmodium falciparum*. Twelve of those classified as microscopy positive were negative by PCR. This gave a microscopy-

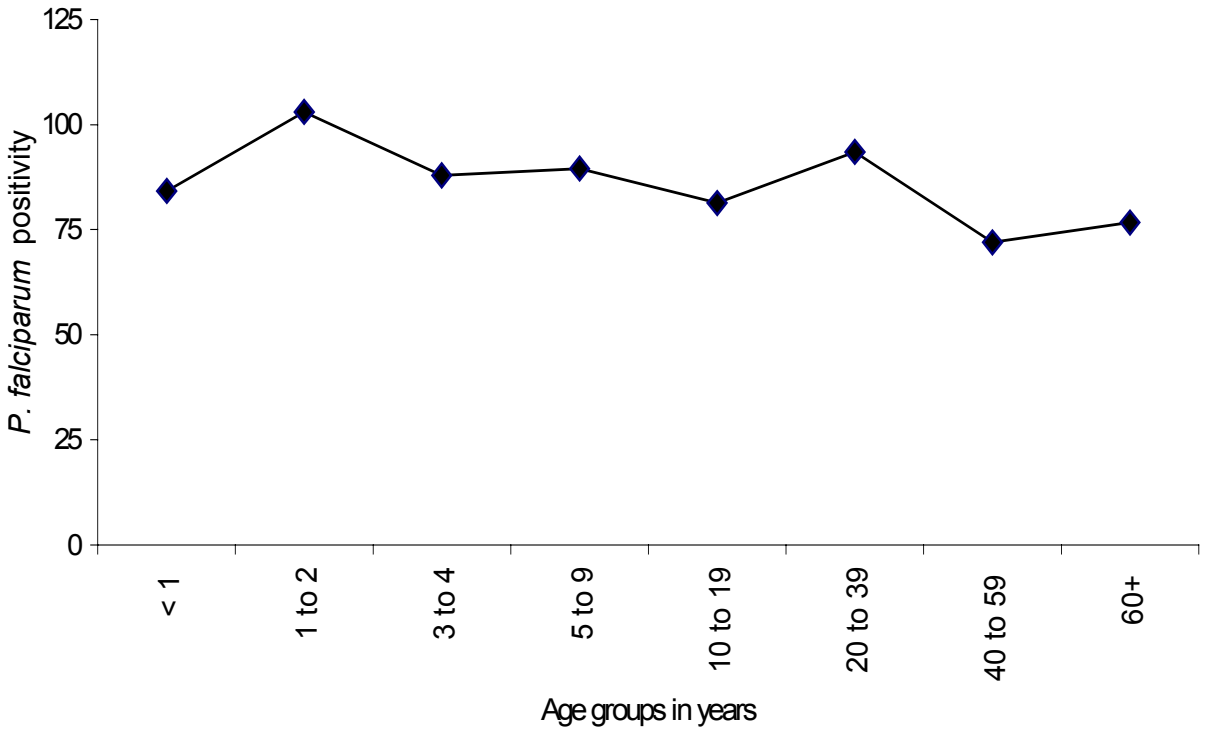


Figure 3.2. Sensitivity of microscopy

sensitivity of 81.3% and specificity of 78.6% compared with PCR (as the Gold standard). PCR positivity increased from a minimum of 61.3% in children under 1 year of age up to 97.4% in children between 5-9 years (Fig. 3.2) and decreased somewhat with age after the age of 19 years. The logistic regression indicated that the sensitivity of microscopy decreased with age (likelihood ratio $X^2_1 = 8.8$, p= 0.003).

All the 252 samples found to be PCR positive were analysed by RFLP. In total, 864 *P. falciparum* clones were detected; 426 (49.3%) belonged to the FC27 allelic family while

438 (50.7%) belonged to the 3D7 allelic family. The mean *msp2* multiplicity of infection was lowest among children less than one year of age. This increased steadily with age until 5-9 years and then started to reduce with age [Fig. 3.1]. The overall mean *msp2* multiplicity of these samples was 3.4, with a range of 1 to 8 clones per isolate (Fig. 3.3). Mean multiplicity of infection in isolates found to be microscopic positive was much higher (3.7, se = 0.1 clones per infection) than in isolates which were microscopically negative (2.4± 0.3 clones per infection). Only 16% of those with malaria infection had single infections. Of these, 38% were children < 5years and 58% were 18 years and above. Children between 5 and 17 years were almost exclusively infected with multiple clones.

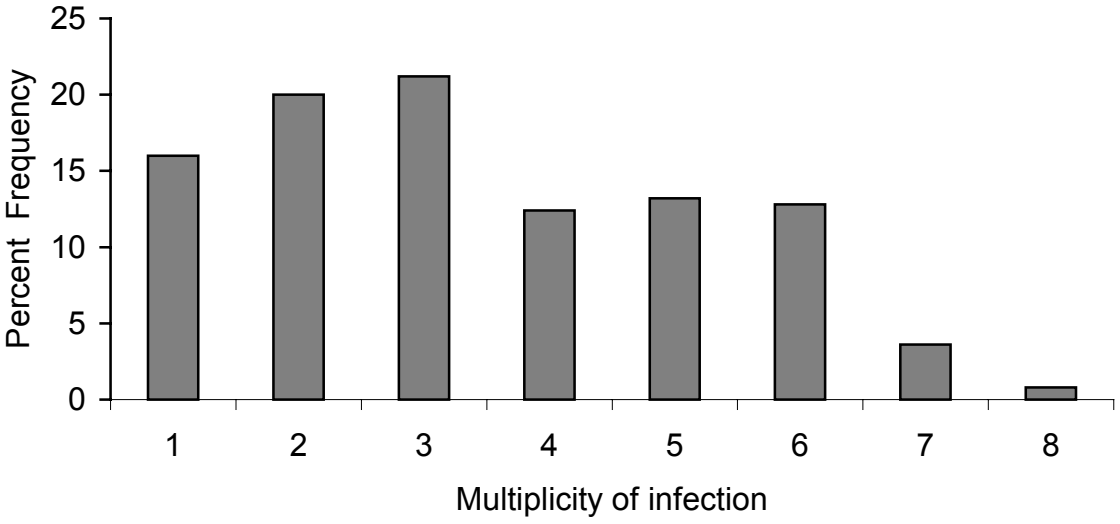


Figure 3.3. Frequency distribution of multiplicity of infection

Eleven (3.6%) of the participants had documented fever (axillary temperature ≥ 37.5^0C). Seven of these fevers were associated with parasitaemia, 6 of them detected by microscopy.

The Spearman's Rank Correlation between *P. falciparum* parasite density and *msp2* multiplicity of infection was 0.42. When this correlation was assessed separately by age group (Figure. 3.4), it was observed to be highest (0.66) among infants, decreasing gradually until it was lowest (-0.11) by the age of 5-9 years. A steady rise was observed again from 10-19 years and this continued to a high of 0.5 among the participants of age 60 years or more.

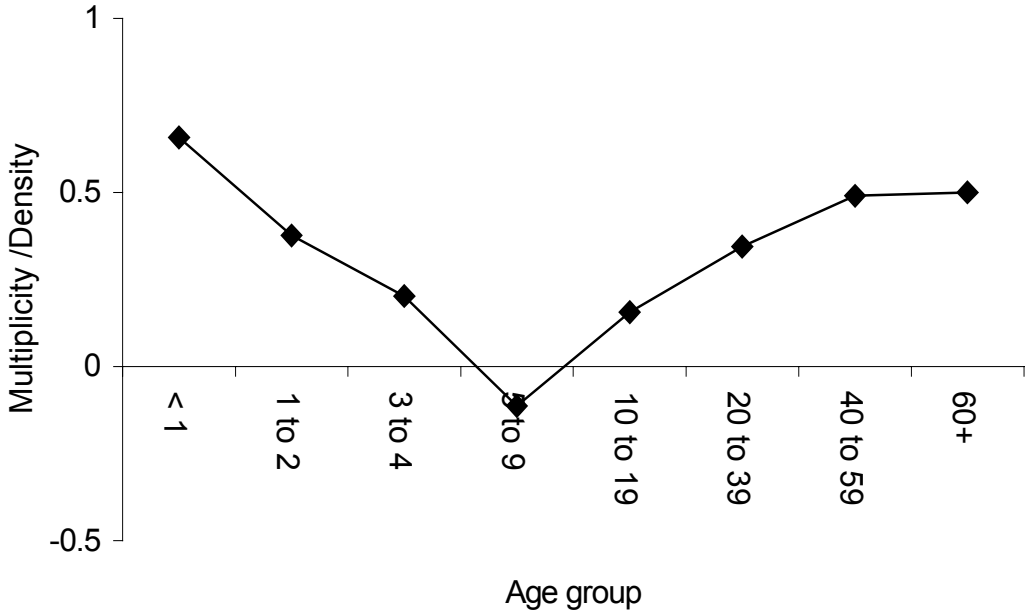


Figure 3.4. Correlation of *msp2* multiplicity of infection and *falciparum* parasite density

3.5. Discussion

Molecular analysis of parasites collected in this survey in the Kassena-Nankana (KND) demonstrates that parasite multiplicity is very large in this community, with most asymptomatics carrying multiple *P. falciparum* genotype infections. The multiplicity of infection seen in the KND was comparable with other holoendemic malarious areas such as in the Kilombero district in Tanzania (Hill *et al.* 1995); (Smith *et al.* 1999c)]; Agunu in Nigeria (Engelbrecht *et al.* 2000); Dielmo in Senegal (Ntoumi *et al.* 1995); (Trape *et al.* 1994). This multiplicity was higher than in areas with lower transmission, such as The Gambia (Conway, 1991); coastal Kenya (Kyes *et al.* 1997); (Mbogo *et al.* 1995); Darawesh in Sudan (Babiker, 1998). However, *P. falciparum* multiplicity is not directly proportional to intensity of transmission (as measured by EIR).

Table 3.2: Relationship of frequency of multiclonal infections of *P. falciparum* and entomological inoculation rate in comparison with other African countries.

Country	Entomological inoculation rate	No. of clones per isolates ✙	Frequency of multiclonal infection	References♥
Ghana (KND)	300	3.4 (1-8)✪	0.84	
The Gambia	2-4	2.34 (1-4)	0.5-0.6	1,2,3
Senegal	100-200	3.9-4.8 (1-6) ➸	0.82	4, 5
		1.9-2.3 (1-4)✻	0.15	6
Nigeria	-	3.9 (1-9)	0.18	7
Sao Tome	-	2.4 (1-6)		8
Tanzania	>500	3.29 (1-6)	0.85	9, 10
Kenya	0.1-26.7	2.1 (1-4)	0.70	11,12
Sudan	0.6	1.3 (1-3)	0.20	13

✙ Mean (range in parentheses)
✪ all ages (2 weeks to 84 yrs)
➸ children
✻ Adults
♥ References: 1. Carter & McGregor, 1973; 2. Hill & Babiker, 1995; 3. Lindsay et al., 1989; 4. Ntoumi et al., 1995; 5,6. Trape et al., 1994; 7. Engelbrecht et al., 2000; 8. Muller et al. 2001; 9. Hill et al., 1995; 10. Smith et al., 1993; 11. Kyes et al., 1997; 12. Mbogo et al., 1995; 13. Babiker et al., 1997.

The KND results fit in with a general pattern that incidence of infection appears to increase with EIR up to approximately one bite from a sporozoite-carrying mosquito per

adult per night but that higher levels of transmission do not result in a correspondingly higher incidence (Charlwood *et al.* 1998). Including the KND data in the plot relating multiplicity and EIR given by Arnot (1998) goes to support his view that increasing EIRs lead to progressively smaller increases in the average number of clones per host (Table 3.2).

The almost equal proportion of FC27 (49%) alleles compared with 3D7 (51%) alleles documented in the KND is similar to results of most other studies. Examples include studies in Kilombero, Tanzania (Felger *et al.* 1999), Ndiop in Senegal (Zwetyenga *et al.* 1998), The Gambia (Conway, 1991) and Papua New Guinea (Felger *et al.* 1994). The similarity in the diversity of *msp*2 in KND to that in other sites goes to confirm the suggestion by (Felger *et al.* 1999) that though the genetic diversity is extensive, it is limited by structural constraints or immune selection. However, different proportions of the two allelic families have been observed in studies carried out in Dielmo in Senegal (Ntoumi *et al.* 1995); (Ntoumi *et al.* 1997) and in Sao Tome (Muller *et al.* 2001). Ntoumi *et al.* (1997), attributed this difference to the high frequency of recombination in Dielmo. However, the levels of transmission are similar in Dielmo and KND, so there is no reason why recombination should be more frequent in Dielmo than in KND.

As in many other malarious areas, children under 10 years were the most infected. The highest GMPD in KND was in children under 4 years of age, similar to other holoendemic areas (Trape *et al.* 1994); (McElroy *et al.* 1994); (Smith *et al.* 1999b). The earlier peaks of malaria prevalence, 2-5 years and multiplicity 3-7 years, (Smith *et al.* 1999b), reported among Tanzanian children in Kilombero district compared to those in the KND (5-9 years) can be explained by the perennial nature of malaria transmission in the Tanzania site. As in KND, the peak in multiplicity was also found in the 5-9 year age group in Dielmo, Senegal, (Konate *et al.* 1999), where transmission is of similar seasonality and intensity to KND. The reduction in multiplicity of infection observed after age 10 in this study was observed in both these other holoendemic sites (Ntoumi *et al.* 1995); (Ntoumi *et al.* 1997); (Smith *et al.* 1999b). One possibility is that this could reflect specific anti-parasite immunity acquired after about 10-15 years of age in

holoendemic areas (Ntoumi *et al.* 1997). However it could also be due to lower detectibility by PCR at low densities.

The peaks in multiplicity and density in KND are in different age groups, just as they are in Dielmo and in Kilombero. Moreover there is no peak in multiplicity in lower transmission areas although parasite densities can show age dependence there (e.g. Muller *et al.* 2001).). The mechanisms controlling multiplicity of infection and parasite densities follow different age-profiles and must therefore be different. Further evidence for this is provided by the age profile of the correlation between parasite density and multiplicity. In Kilombero there was a gradual loss of the correlation between parasite density and multiplicity with age (Smith *et al.* 1999b). This age profile matched that of the acquisition of clinical immunity. In contrast, correlation between multiplicity and parasite density was observed in all age groups in Sao Tome (Muller *et al.* 2001), where there is slower acquisition of clinical immunity. In a meso-endemic area in Uganda this correlation showed different age patterns with *msp*1 from that with typing of the allelic families of *msp*2 (Peyerl-Hoffmann, 2001).

In KND the pattern in children less than 10 years old matched that in the Tanzanian study. We suggested in the Tanzanian study that the loss in correlation between multiplicity and density could be explained by gradual acquisition of pan-specific immunity controlling parasite densities. In older individuals in KND however, we observed a steady and gradual increase (from this minimum) in the correlation between multiplicity and parasite density after the age of 10 until over 60 years of age. One explanation for this could be that, given the very low densities observed in older participants and the diversity of the parasites, the multiplicity might simply be counting the number of individual parasites in the sample. The existence of a correlation between multiplicity and density could then be an indication of accurate quantitation of low-density parasitaemia.

Considering the fact that the two methods were applied independently and blind, there was also a very high level of agreement between microscopy and PCR for positivity for

P. falciparum. This validates the parasitological data reported in the past in studies carried out in the Kassena-Nankana district in northern Ghana using the same microscopy techniques. As in other studies (Trape, 1985); (Muller *et al.* 2001), the positive samples that were missed by the microscopists had significantly lower mean multiplicity of infection than the average and were predominantly in older individuals who generally have very low parasite densities.

A further strength of the study is that it was based on a random cluster sample of the whole district population, drawn from the NDSS. This made it possible for us to ensure that adequate numbers of individuals were allocated to each of the age groups and also to make estimates of population parameters allowing for the different sampling fraction in each age group.

Malaria transmission in the KND can be stratified into areas close to and under the direct influence of the rice irrigation project and areas further away and indirectly influenced by the irrigation project. Though a slightly higher multiplicity was observed among the individuals living in communities closer to the irrigation project, this difference was not significant. This tendency can be explained by the higher inoculations experienced in the irrigated areas where mosquito-man contact is high all year round compared to non-irrigated areas in the dry season. Non-significant differences in multiplicity between individuals close to the irrigated areas and those far can also be explained with the belief that increasing EIRs lead to progressively smaller increases in the average number of clones per host.

The present study constitutes the baseline of a longitudinal study designed to collect parasitological data at eight weeks intervals from individuals of all age groups over a period of one year. At the beginning of the wet season, the data from the Kassena-Nankana district in northern Ghana has demonstrated one of the highest multiplicity with age dependence patterns similar to those in other holoendemic sites in Africa, thus validating the use of the age-multiplicity relationship as an indicator of malaria endemicity. However, the age-patterns of multiplicity could differ significantly in the dry

season especially in the non-irrigated areas of the district. Data from the additional surveys will allow us to build upon this baseline and contribute to our knowledge and understanding of the seasonal dynamics and multiplicity of infections in all age groups in this area of highly seasonal holoendemic malaria.

3.6. Acknowledgement

Sincere thanks to the community members of the KND, especially the participants and the parents of the children consenting on their behalf. Special thanks go to Dr. Fred Binka who facilitated the initiation of the study. We wish to express our thanks to Mr. Charles Attiogbe, for reading all the slides, our field assistants, especially Victor Asoala, Cletus Tindana and Timothy Awine. We also thank Elizabeth Awini for agreeing to help with the data management and Francis Anto for his assistance in field supervision. We thank the Director of Navrongo Health Research Centre, and his team for the congenial atmosphere in which field data collection took place. Sincere thanks to Professor Marcel Tanner, for his support and helpful comments on this paper.

Financial support: Funding was provided by the Jubilee Foundation of the Swiss Tropical Institute and a WHO-TDR Training Fellowship to the principal investigator.

Chapter 4

Incidence of symptomatic and asymptomatic *Plasmodium falciparum* infection following curative therapy in adult residents of northern Ghana.

S. Owusu-Agyei[1], KA Koram[2], JK Baird[3], GC Utz[4], FN Binka[1], FK Nkrumah[2], DJ Fryauff[3] and SL Hoffman[5]

[1]*Navrongo Health Research Centre, Navrongo, Ghana*
[2]*Noguchi Medical Research Institute, Legon, Ghana*
[3]*US Naval Medical Research Center, Silver Spring, Maryland, USA*
[4]*US Naval Medical Research Unit #3, Cairo, Egypt*
[5]*Celera Genomics, 45 West Gude Drive, Rockville, MD 20850*

This article has been published in:
American Journal of Tropical Medicine and Hygiene (2001), 65(3): 197-203

4.1. Abstract

Adult residents of holoendemic malaria regions in Africa have a naturally acquired immunity (NAI) to malaria that renders them more resistant to new infections, limits parasitaemia, and reduces the frequency and severity of illness. Given such attributes, it is not clear how one might evaluate drug or vaccine efficacy in adults without serious confounding. To determine symptomatic and asymptomatic malaria attack rates in adults of northern Ghana, 197 men and women underwent curative therapy for any existing malaria infection at the start of the wet (high transmission) season. They were monitored for first parasitaemia and first clinical episode of infection by *Plasmodium falciparum* over 20 weeks (May-Oct., 1996). The cumulative incidence of primary infection by *Plasmodium falciparum* was 0.98 and incidence density of infection was calculated to be 7.0 cases/person-year. Symptoms were reported by 19.5% of individuals at the time of first recurrent parasitaemia. Incidence of infection, parasite density, and the frequency of symptoms were comparable in males and females. The results show that NAI provided these adults with no defence against rapid re-infection and suggest that this population-infection design could serve to demonstrate the efficacy of a drug or vaccine in preventing parasitaemia.

4.2 Introduction:

Malaria is one of the leading causes of morbidity and mortality in the tropics, affecting individuals of all ages, but primarily very young children and pregnant women (World Health Organisation, 1997a). Integrated approaches against both the parasites and vectors are required to control this disease with vaccines offering the best hope for sustained control in areas of intense transmission (Miller *et al.* 1998); (Hoffman, 1996).

Malaria morbidity in adult Africans has long been considered of minor importance (Wilson, 1950) because adult residents of holoendemic areas have usually developed high levels of protective immunity as a result of their long and continuous exposure to malaria infections. The two forms of immunity described are (i) an anti-disease immunity, that allows one to carry parasite loads without symptoms; and (ii) an anti-parasite immunity, that is responsible for a marked reduction of parasite densities after

a certain age (Daubersies *et al.* 1996); (Trape *et al.* 1994); (Day *et al.* 1991). Malaria slides from adults in highly endemic areas are usually positive regardless of the clinical context (Trape, 1985) and adults are known to become rapidly re-infected with malaria parasites after treatment (Hoffman *et al.* 1987). Appropriate criteria for deciding when adults should receive treatment for malaria have never been agreed upon. It has been variably suggested that treatment *s*hould be based on parasite density threshold, (Rogier *et al.* 1996); febrile condition, (Rougemont *et al.* 1991); (Smith *et al.* 1995); symptom history, (Bassett *et al.* 1991) *or* an association between symptom history and parasitaemia (Mkawagile *et al.* 1986); (Stein *et al.* 1985).

The present study was conducted to measure the incidence and features of symptomatic and asymptomatic infection by *Plasmodium falciparum* in malaria-immune adult Ghanaians that had been radically cured of all pre-existing *Plasmodium* infections. Effort was made to study newly incident infections in comparably sized cohorts of males and females, 18-55 years old, who were lifetime residents of the Kassena-Nankana district of northern Ghana. This study was conducted during the peak malaria transmission season in order to characterise malaria in the area and to provide the foundation data needed for valid and efficient design of malaria vaccine and anti-malarial drug trials in the region.

4.3 Subjects and Methods

Study Site and Population: The Kassena-Nankana district (KND) lies within the Guinea savannah woodlands between latitudes 10º 30' and 11º 00' north and between longitude 10º 00' and 10º 30' west . It is located in the upper Northeast region of Ghana at its border with Burkina Faso. The KND has an area of 1,675 km^2 and a population of 141,000 living in roughly 14,000 dispersed compounds. The main occupation is subsistence farming of predominantly millet and livestock. The KND has a mean monthly temperature range of 16ºC to 45ºC and rainfall averages 800-1,000 mm per annum, occurring mainly between May and October. Malaria transmission by *Anopheles gambiae* and *An. funestus* peaks at the close of wet season, during October-November. Malaria parasite prevalence among children <5 years in the KND was

significantly greater in the wet season [May - Oct.] than in the dry season [Nov. - Apr.] (Binka *et al.* 1994). Malaria is the leading diagnosis of illness in the district hospital, accounting for 60% of all admissions and 41% of hospital deaths (Ministry of Health, 1996).

Subject Selection and Consent: A representative sample population of adult men and non-pregnant women between the ages of 18 and 55 years was sought. The sample was randomly selected using the Navrongo Demographic Surveillance System (NDSS) a longitudinal census of all district residents that is updated every 3 months (Binka et al., 1999). We tallied the total number of eligible adults (48,032) within the 5 geographic zones of the district and randomly selected 16 "index" compounds, with selections weighted by population size. Chiefs, elders, and potential subjects were then visited, the research work explained and discussed, and opinions sought before enrolment. Translation, narration, discussion, and communal consent were the essential elements of the informed consent process.

Enrolment and Screening: The screening process involved clinical history, vital signs, physical examination by a physician, collection of 1.0 ml venous blood for malaria smears, and haematology screening. Urine was collected from all women for β-HCG pregnancy testing. Laboratory screening involved microscopy of Giemsa-stained thick and thin blood smears, haemoglobin testing, and qualitative testing for Glucose-6-phosphate dehydrogenase deficiency (G-6-PD). Remaining blood and plasma were archived for future laboratory work. Specific exclusion criteria were pregnancy, allergic reaction to antimalarial drugs, and illness or a condition requiring hospitalisation.

Curative Therapy of Malaria: Just prior to the start of the rainy season (Apr. 1996) radical curative therapy was employed to achieve complete clearance of any patent, dormant, or incubating malaria infection from blood and liver. Volunteers were treated with oral quinine sulphate (650 mg three times daily for four days) followed by doxycycline, (100mg twice daily for 10 days). Subjects testing normal for G-6-PD (NADP+ spot test, Sigma Chemical Co., St. Louis, MO) received primaquine (0.5 mg

base per kilogram body weight once daily for 14 days. Nineteen (19) subjects who tested G-6-PD deficient received only quinine and doxycycline treatment. All doses were supervised and directly observed over the 18 days of radical curative or 14 days of curative therapy.

Surveillance and Referral: Routine blood films were obtained on day 15 of the initial treatment and were repeated at two week intervals for the duration of the study (20 weeks) by trained fieldworkers permanently residing in each of the 16 compound clusters. Fieldworkers visited subjects three times weekly at their homes to inquire of their general health and check for fever by measuring axillary temperature. Each visit began with a direct question: "Have you been sick since the last visit?" If the answer was no, the interview ended. If the answer was yes the subject was asked to describe his or her symptoms and these were recorded. If a subject complained of current illness to the fieldworker or had an axillary temperature $\geq 37.5\ ^{0}C$, a non-routine blood film was obtained. Subjects were transported to the district hospital where the blood smear was examined by an expert malaria microscopist. Based upon presenting signs, symptoms and microscopy results, physicians decided upon a treatment plan.

Study end points, treatment and follow-up: The two primary outcome variables in this study were: (1) time to primary post-cure parasitaemia, and (2) time to first episode of parasitaemia associated with clinical symptoms. Blood slides were prepared as Giemsa-stained thick and thin smears examined by 1000 X light microscopy using oil immersion. Parasitaemia were scored per microlitre of blood by counting the number of asexual parasites per 200 white blood cells, assuming 8,000 white blood cells per microlitre, and multiplying the parasite count by 40. A slide was considered negative if no parasites were observed within 200 high power fields.

Our working diagnosis of clinical malaria was a patent asexual stage parasitaemia accompanied by any one or more of the following symptoms: fever, headache, chills, myalgia, dizziness, nausea, diarrhoea. All symptomatic subjects with microscopy-confirmed malaria were promptly treated with Chloroquine diphosphate (25mg base/kg

body weight over 48 hours) and monitored for outcome. Chloroquine failures were treated with 3 tablets of Fansidar® (Sulphadoxine 500 mg + Pyrimethamine 25mg, Hoffman-La Roche).

Data handling and analysis: Cumulative Incidence (CI), expressed as the risk of an outcome and/or probability of infection during the 20 weeks, and Incidence Density (ID), which estimates new infections per unit of person-time at risk were used as quantitative tools in determining the frequency of newly incident *Plasmodium* infections in this cohort. Owing to the pre-erythrocytic stage incubation period of the parasite, the first 10 days following curative therapy were excluded from analyses as risk-free person-time. To identify predictors of a positive blood slide, and to determine whether the use of specific complaints or combinations of complaints significantly increased the likelihood of correctly predicting a positive blood smear, we used logistic regression models that fitted parasite positivity against symptoms recorded.

4.4 Results

The study plan is chronologically depicted as a flow diagram in Figure 4.1. During the initial radical or curative therapy stage, 98.7% of the 16,758 drug doses were administered and witnessed with only one volunteer dropping out. The baseline characteristics of the enrolled study population that completed radical or curative treatment and contributed person-time and/or study endpoints are presented in Table 4.1. The mean age, weight, and haemoglobin concentration among females was significantly different from that of males; older age possibly an artefact that arose from the necessity of restricting female enrolment to those not pregnant or nursing. Patent malaria infections were identified in 110 of 197 (55.8%) subjects at enrolment and *Plasmodium falciparum* accounted for 102 of the 110 (92.7%) malaria infections, the remaining 7.3% due to infections by *P. malariae* (7) and *P. ovale* (1). Gametocytes were observed in 29.6% of these subjects.

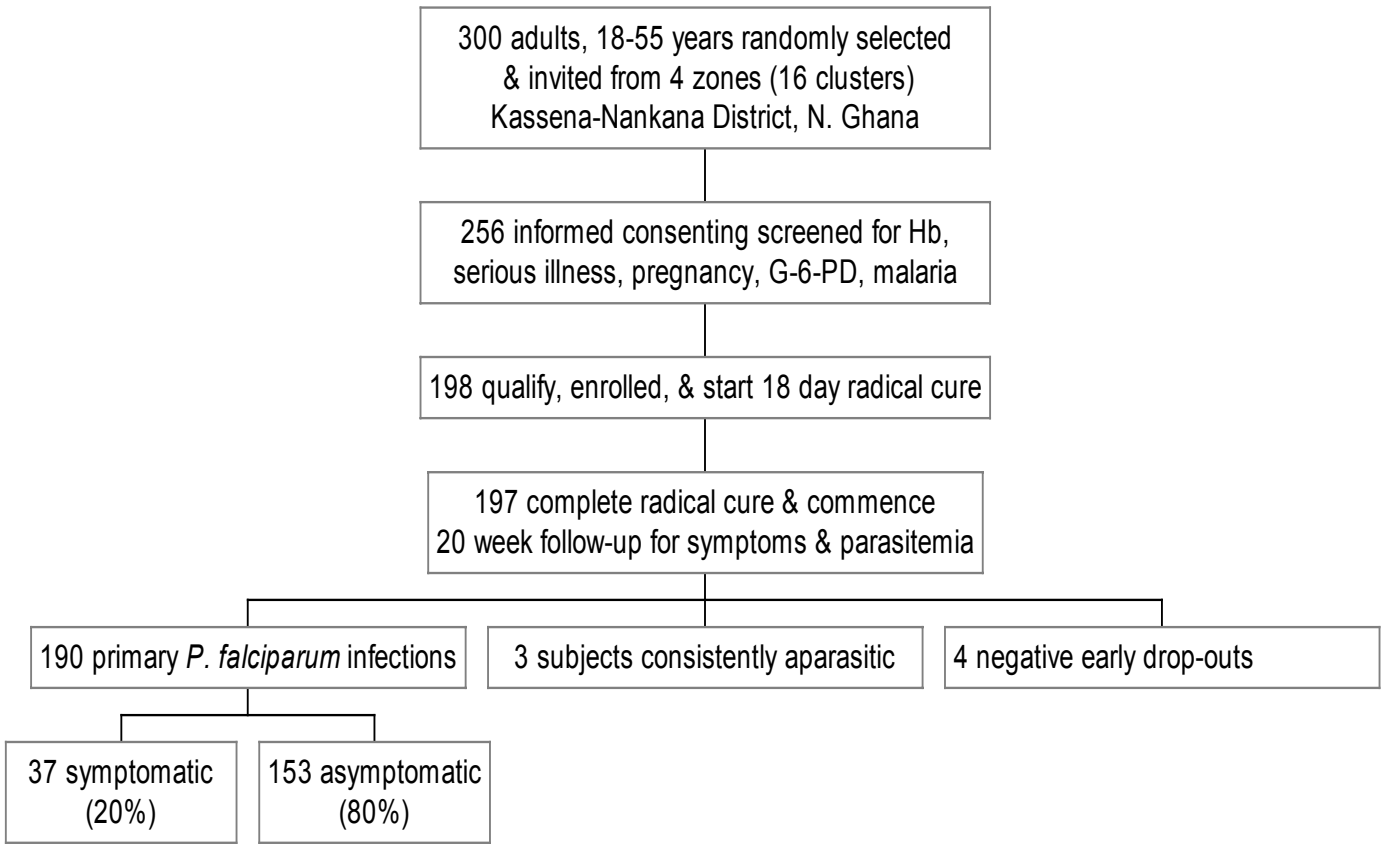

300 adults, 18-55 years randomly selected & invited from 4 zones (16 clusters) Kassena-Nankana District, N. Ghana
256 informed consenting screened for Hb, serious illness, pregnancy, G-6-PD, malaria
198 qualify, enrolled, & start 18 day radical cure
197 complete radical cure & commence 20 week follow-up for symptoms & parasitemia
190 primary P. falciparum infections
3 subjects consistently aparasitic
4 negative early drop-outs
37 symptomatic (20%)
153 asymptomatic (80%)

Figure 4.1: Study Plan.

Glucose-6-phosphate dehydrogenase deficiency (G-6-PD) was detected in 19 of the 197 (9.6%) enrolled subjects (7.1% of women vs 11.6% of men, *P* = 0.28). Baseline malaria prevalence was significantly greater among males (58% vs. 38%, *P* = 0.01). All subjects belonged to the two main ethnic groups (Kassim, Nankam), in near balanced numbers, and had lived their entire lives in the area.

Blood smear screening on day 15 following the start of radical or curative therapy demonstrated parasite clearance in all subjects. The first infections thereafter were identified during week 3 of post-cure follow-up.

Table 4.1. Baseline characteristics of the Ghanaian study population*.

Characteristic	Males	Females	*P* value
No. subjects (%)	112 (56.8)	85 (43.1)	-----
Mean Age (yrs) (95% CI)	35.4 (33.3 - 37.5)	43.3 (41.3 – 45.2)	< 0.01
Mean Weight (kg) (95% CI)	57.4 (56.0 – 58.8)	50.3 (49.0 – 51.6)	< 0.01
Mean Hb level (g/dL) (95% CI)	12.1 (11.8 – 12.4)	10.8 (10.3 – 11.2)	< 0.01
No. *P. falciparum* positive (%)	64 (57.1)	33 (38.8)	0.01
GM parasitaemia/μL (95%CI)	135 (103 - 177)	98 (78 - 123)	0.07
No. Febrile (%)	2 (1.0)	1 (0.5)	-----

* CI = confidence interval; Hb = haemoglobin; GM = geometric mean.

The cumulative incidence of primary *P. falciparum* infection over the 20 weeks following cure are plotted for males and females in Figure 4.2. Both sexes demonstrated the most profound rise in new infections during weeks 5 to 10, when the CI rose from 0.22 in males and 0.21 in females to respectively 0.81 and 0.77. Three subjects remained

consistently aparasitic to the 20-week endpoint. Four others dropped out of the study, negative, at various earlier time points. The collective 20-week CI for *P. falciparum* infection in adults was 0.98. The respective 20 week wet season IDs for infection in males and females was 7.2 and 6.7 (RR = 1.07, 95% CI: 0.70, 1.24) infections/person-year. The CI of illness coincident with parasitaemia during this period was 0.42 among males and 0.49 among females (Fig. 4.2). Analysis by location (Fig. 4.3) found the acquisition of primary infection to be most rapid in the western compounds and slowest in the south (mean time to infection: West, 7.2 weeks vs. South, 8.7 weeks, $P = 0.03$). Mean time to the first episode of confirmed symptomatic malaria was similar among the four locations studied.

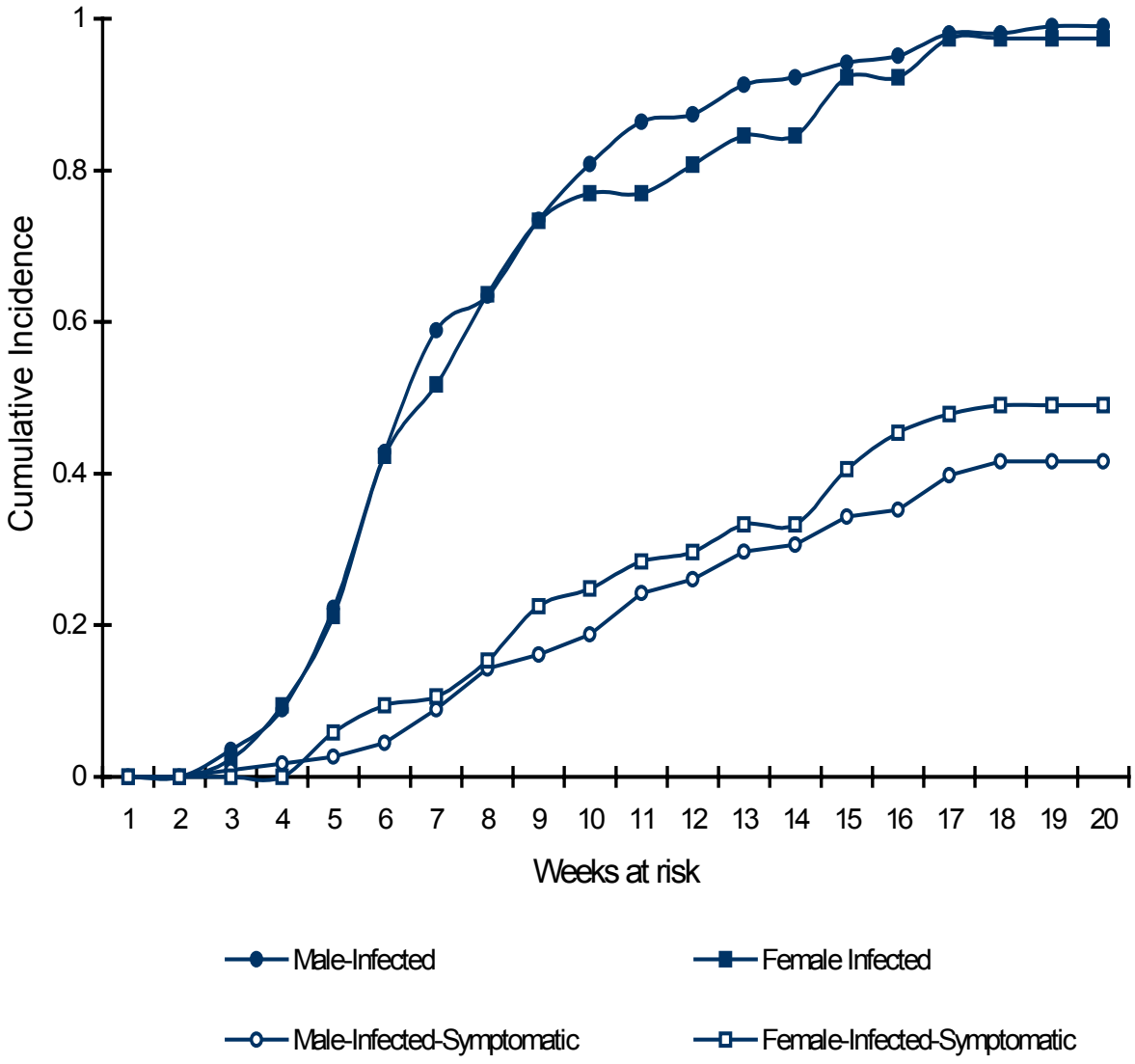


Figure 4.2. Cumulative incidence of post-curative primary infection and primary symptomatic infection by *P. falciparum* in adult males and females. Wet season (May-Dec., 1996), northern Ghana.

Table 4.2 presents the characteristics of these primary infections in male and female subjects over the 20-week period. There were no statistically significant differences between sexes for either CI, ID, the frequency of symptomatic primary infections, or the GM density of parasitaemia. Irrespective of sex, adults experienced a 20 week wet season ID of 7.0 (95% CI: 5.1, 6.8) new *P. falciparum* infections per person-year of exposure. There were 4 females (4.7%) and 3 males (3.6%) who did not develop a detectable parasitaemia during their 8 to 20 week participation. All were G-6-PD normal.

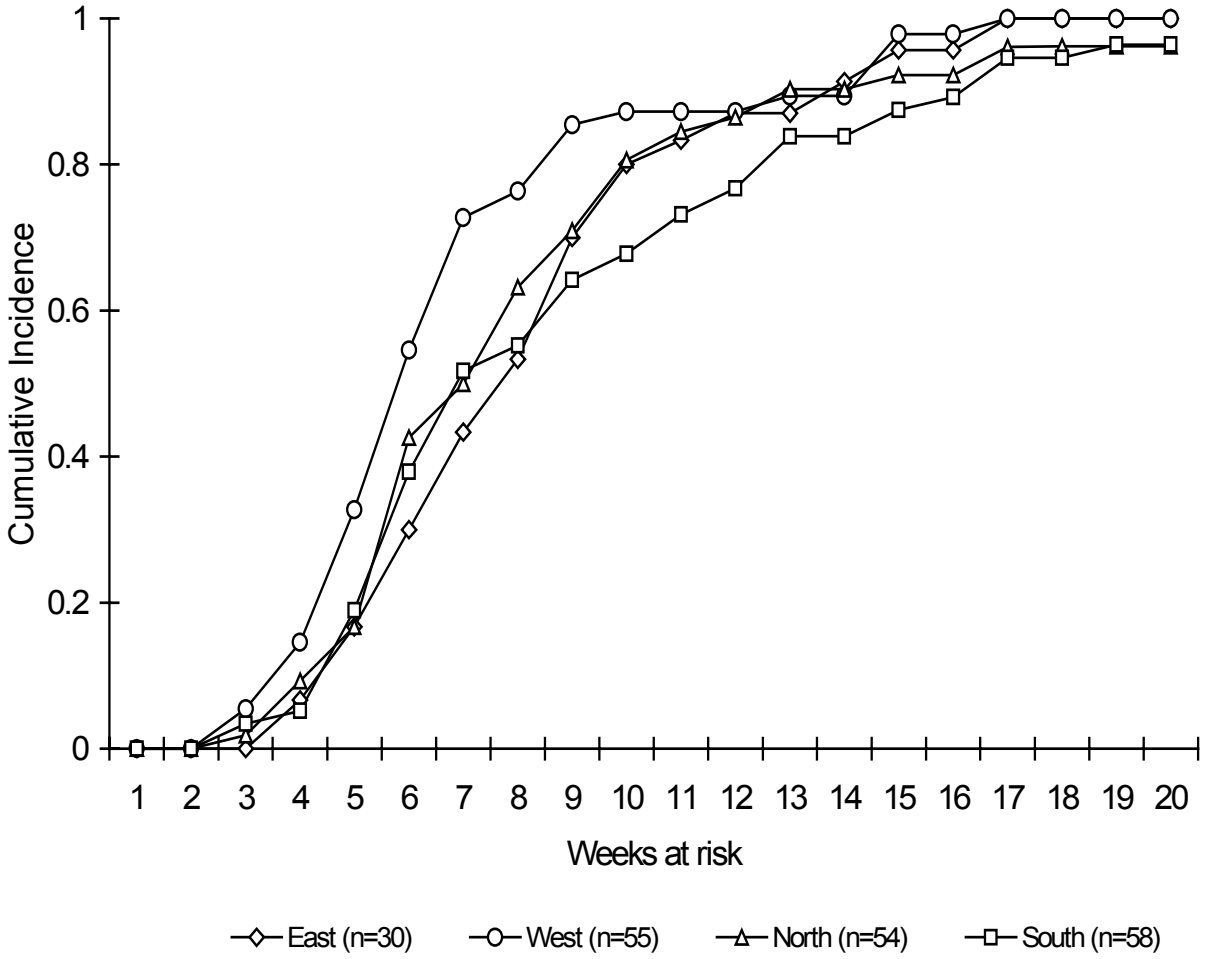


Figure 4.3. Cumulative incidence after radical cure of primary *Plasmodium falciparum* infection by location in the Kassena-Nankana District of northern Ghana during the wet season (May-Dec., 1996).

One of these subjects was infected with *P. falciparum* at enrolment, two others with *P. malariae*, and 6 of the 7 made voluntary clinic visits for diagnosis and treatment of illness during the study period. Analysis by age, which divided the population into two near equal groups, < 40 years (n = 100) and $\geq$ 40 years (n = 97), revealed that mean times to parasitaemia and symptomatic parasitaemia in the older age group were both significantly delayed ($P < 0.01$) relative to those of the younger group (Fig. 4.4). As a result, wet season ID in the older group was 5.8 infections/person-year compared to 7.1 in the younger group ($P = 0.17$). Parasite densities at infection and at the time of symptoms were similar in the two groups.

Table 4.2. Characteristics of first infections with *P. falciparum* after radical cure in adult male and female study subjects during the wet season (May-December, 1996) in northern Ghana*.

Characteristic	Males	Females	*P* value
Person-weeks at risk	777	629	-----
No. with primary infections/total subjects (%)	109/112 (97.3)	81/85 (95.3)	0.45
No symptomatic at 1st +ve slide/total +ve subjects (%)	22/109 (20.2)	15/81 (18.5)	0.77
Cumulative Incidence/20 wks wet season	0.99	0.97	-----
Incidence Density/ person-year	7.3	6.7	0.56
GMPD/μL 1st appearance, asymptomatic (95% CI)	129(96, 169)	162(118, 223)	0.27
GMPD/μL 1st appearance, symptomatic (95% CI)	451(171, 1190)	347(131, 921)	0.72

*GMPD = Geometric mean parasite density; CI = Confidence Interval; 1st = first; wks = weeks; +ve = positive

There were 190 primary, post-cure infections by *P. falciparum* during the 20 weeks following cure. Symptoms and physical complaints characterised the first appearance of parasites in 37 of these 190 (19.5%) infections. Comparable proportions of males and females were symptomatic at this first parasitaemia. (males: 22/109 = 20.2%; females: 15/81 = 18.5%). Fever was present in 8 of the 37 (21.6%) primary infections that presented with clinical illness, but was not associated with the level of parasitaemia. Among individuals with asymptomatic primary parasitaemia who were not treated, asexual stage parasites were variably present in subsequent follow-up slides. Parasitaemia was detected in 65% of 566 follow-up slides from 113 representative subjects and usually not detected over more than 3 consecutive examination dates. During the course of study time applied to the 153 infected-asymptomatic volunteers, there were 242 occasions, judged to be clinical malaria on the basis of signs and symptoms, that prompted blood smear examination. Asexual stage parasites of *P. falciparum* were present at 62 of these 242 occasions and those of *P. malariae* were present in one case. Asexual stages of *P. falciparum* were thus associated with 99 of 279 cases of malaria-like illness in these previously cured adults.

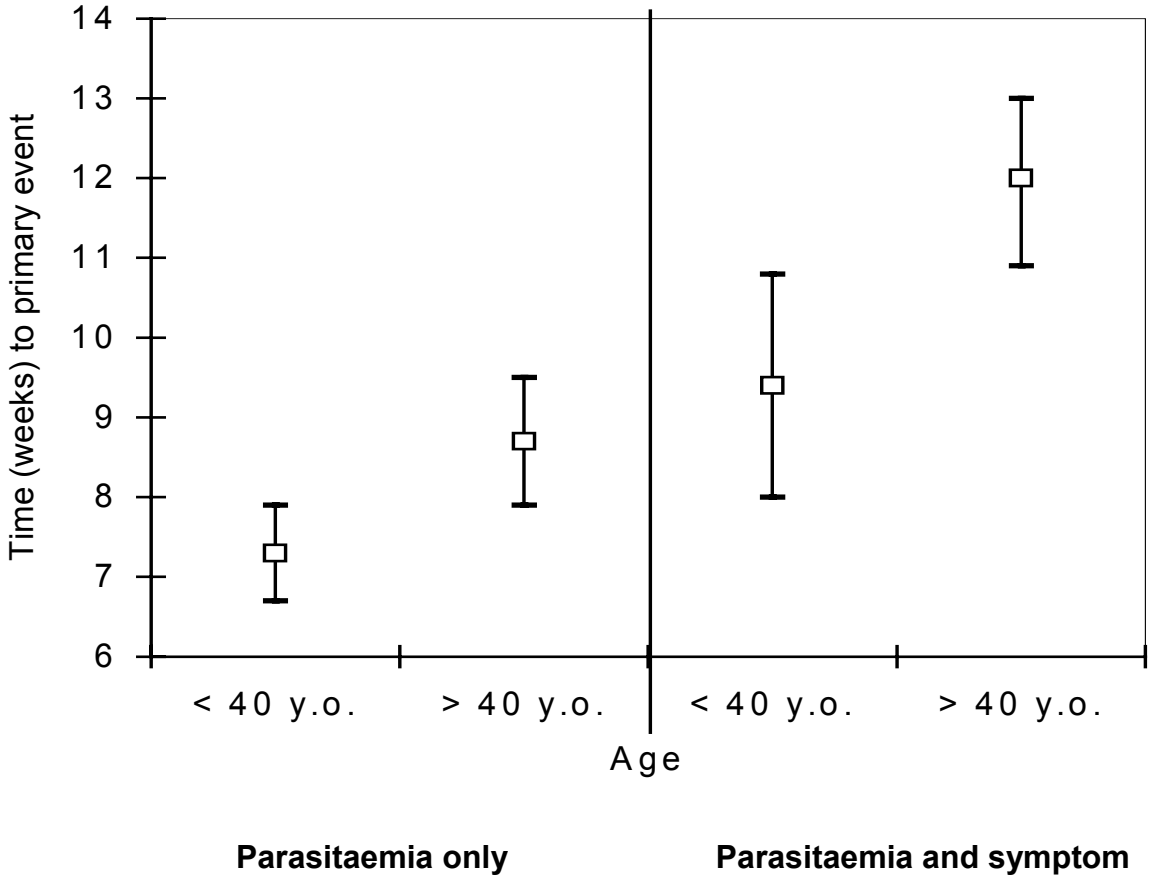


Figure 4.4. Mean time to primary infection by *P. falciparum* and primary symptomatic infection by age group in adult Ghanaians. Error bars indicate 95% confidence intervals

The frequencies of specific complaints/symptoms associated with these infections are plotted against those of 180 other cases where parasites were undetected (Fig. 4.5). The frequency of vertigo, chills, and headache were significantly greater among those with a confirmed parasitaemia.

Stepwise logistic regression that was applied to the data revealed dizziness and myalgia as potential ($P < 0.1$) predictors of a positive blood slide, while diarrhoea was unrelated. This approach showed dizziness and myalgia to be directly linked to parasitaemia, with diarrhoea independent of all other variables.

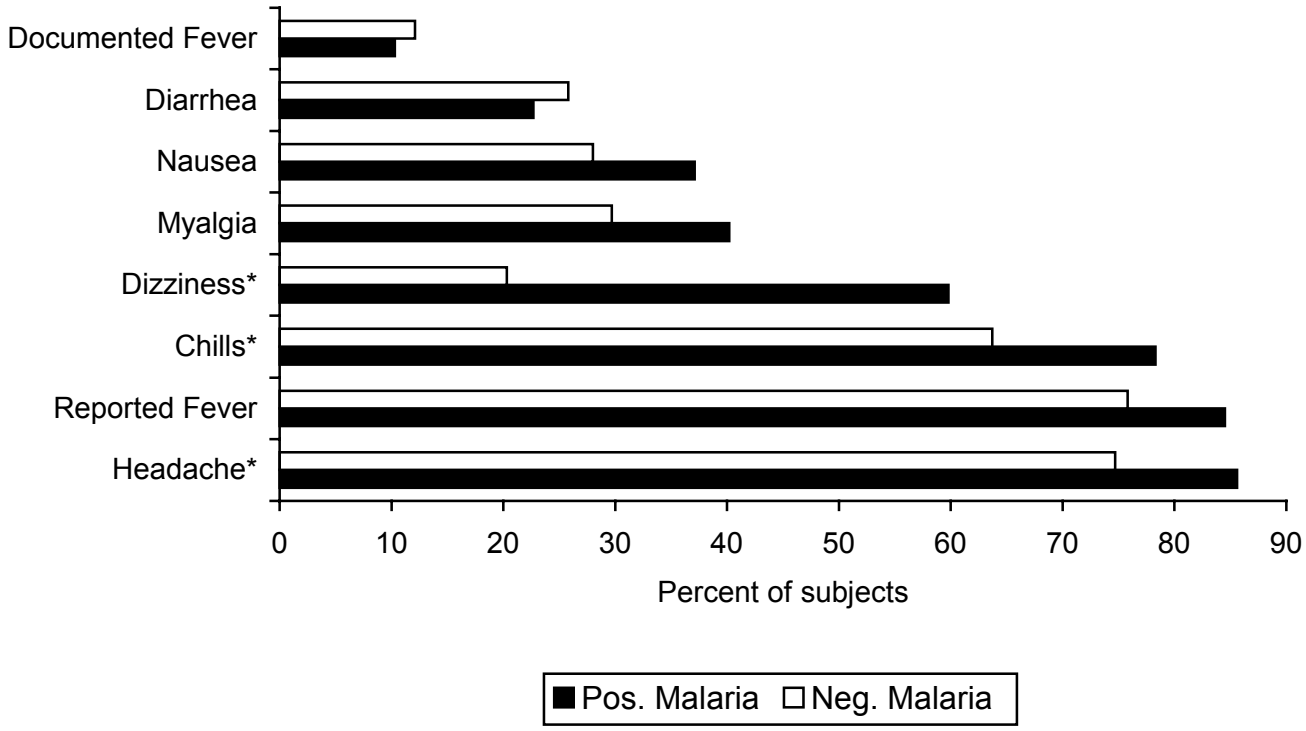


Figure 4.5. Comparative physical complaints/symptoms reported by adult Ghanaians with positive or negative malaria blood smears. * Indicates a statistically significant difference between groups.

The other reported symptoms; chills, nausea, headaches and fever independent of the parasitaemia given a presentation of dizziness and/or myalgia. Patients who reported chills or nausea were likely to also report dizziness or myalgia but rarely both. Headache and fever were independent of the other symptoms given the presence of chills.

Table 4.3 presents the range and geometric mean densities of primary post-cure *P. falciparum* parasitaemia that were measured in symptomatic and asymptomatic cases. Relatively low-density parasitaemia characterised more than 85% of the infections and were associated with physical complaints. The density of asymptomatic parasitaemia at first appearance of infection was no different than the density of parasitaemia later associated with the first occasion of clinical illness following curative treatment. There were no significant differences between sexes, or between symptomatic and asymptomatic cases in the frequency of high parasitaemia or in the GM densities of parasitaemia.

Table 4.3. Range and geometric mean densities of first *Plasmodium falciparum* infections after radical cure in adult Ghanaians.

Parasitaemia Range/μL of blood	Asymptomatic		Symptomatic	
	Males	Females	Males*	Females‡
< 2,000/	82	64	40	46
2,000 – 9,999/μL	3	1	3	5
10,000 – 19,999/μL	2	0	2	0
> 20,000/μL	0	1	2	1
Combined	87	66	47	52
GM parasitaemia/μL (range)	129(40-12,560)	162(40-31,640)	199(40-97,680)	200(40–22,000)

*Males: 22 symptomatic at first positive slide; 25 of 87 others (initially asymptomatic-positive) later became symptomatic-positive); ‡Females: 15 symptomatic at first positive slide; 37 of 66 others (initially asymptomatic-positive) later became symptomatic-positive)

4.5 Discussion

The results of this study show that wet season malaria transmission in the Kassena-Nankana district of northern Ghana is intense and that the incidence of infection and clinical illness associated with malaria in adults is virtually identical in males and females. Despite a natural immunity derived from a lifetime of intense exposure, our results demonstrate that adults become rapidly re-infected and parasitaemic following an effective curative treatment. Neither genetic resistance nor long-acquired immunity appears to be sufficiently strong or widely present in this population to completely inhibit parasitaemia and its symptoms.

Rapid re-infection and patency among treated malaria-immune adults was recognised more than a decade ago in western Kenya (Hoffman *et al.* 1987). This general susceptibility and rapid appearance of low density blood stage infections may result from the ineffectiveness of anti-sporozoite and anti-liver stage immunity or to an asexual blood stage effect coupled with a pre-erythrocytic stage immunity that reduces but does not completely eliminate all infected hepatocytes (Hoffman *et al.* 1987); (Hoffman, 1996).

In spite of comparable malaria attack rates measured in adult males and females, the relatively small size and ecological uniformity of the KND study area, and seasonal climatic conditions that impinge evenly, thereby smoothing over ecological and hydrological differences, our analysis identified a significant difference between locations in the mean time to infection. This difference may relate to the geographic concentration of well-established irrigation systems in the western compounds. If this was not due to chance, it is likely that even more highly significant differences between locations will exist in the KND during dry season conditions. This observation may be supported by location analysis of data from the wet and dry season infant cohorts. Drug and vaccine efficacy trials might be intentionally focused within particular areas of the KND in order to achieve rapid endpoints and tight confidence intervals.

Contrary to expectations, the ID of primary parasitaemia in this adult cohort was virtually identical to that of a location-matched cohort of infants/young children, 6-24 months old, who were curatively treated and followed through 20 weeks of the wet season the following year [Adult ID 7.03 infections/person-year, vs. Infant/young children ID 7.06 infections/person-year; Relative Risk (95% CI) = 0.99 (0.82 - 1.19) (Baird, 2001). The cumulative incidence profiles of adult and infant cohorts indicate the same rapid rise and plateau effect. Among infants, the most rapid rise in incidence occurred between 5 and 10 weeks with a plateau reached at week 10 that incremented only slightly more by the 20 week endpoint. In an analogous manner, new infections among adults rose most steeply from weeks 5 (Male CI = 0.22, Female CI = 0.21) to 10 (Male CI = 0.81; Female CI = 0.77). Among infants, only 18 new infections occurred in weeks 11 to 20, leaving 2.3% of the wet season cohort parasite-free. In a similar manner, adult CI attained 0.88 in week 13 and only 18 additional subjects (9.5% of the infected cohort) developed patent infections during the ensuing 7 weeks of study.

We assumed that adult life-long residents of Navrongo, in contrast to their young children, would manifest a lower or delayed incidence of patent re-infection, lighter parasitaemia at the time of re-infection, and mild or negligible clinical manifestations.

We found lighter parasitaemia and decreased clinical manifestations in adults, but a near identical rate of re-infection following radical cure. A lower attack rate in adults might have been observed had both cohorts been studied simultaneously, but was not apparent in our comparison of wet season ID's for adults during 1996 and for infants during 1997. Heightened transmission impacting upon adults, or reduced transmission experienced by the infant cohort may have obscured real differences attributable to age dependent immunity.

Interestingly, in contrast to the near equal incidence density seen in adults and children, we observed among adults that the > 40 years old age group had a lower ID of re-infection as compared to those < 40 years old, suggesting an effective age-dependent, naturally acquired immunity. Under identical conditions of risk, an anti-parasite immunity capable of barring certain genotypes and suppressing parasitaemia to sub-clinical levels in adults would be expected to prevent or delay the patency of primary infections relative to those sustained by infants.

As expected, adults experienced parasite densities and febrile episodes that were considerably reduced from those recorded in the infant cohorts (Scheller *et al.* 1995); (Baird et al, 2001). It was interesting to note that many of the infected Ghanaian adults in the present study experienced episodes of illness that corresponded with, and may have arisen from, their parasite infections. We concede that the exclusive role of the parasitaemia as a cause of their illness cannot be ascertained with certainty since these same individuals reported signs and symptoms more often by these same individuals in the absence of parasites. However, the 37 occasions of illness that corresponded precisely with the first appearance of parasites following radical cure are most probably the true result of infection. Literature on the subject of adult malaria implies that adults with lifelong exposure to malaria do not become ill when they become re-infected with malaria parasites (Wilson, 1950); (Smith *et al.* 1995). Our data suggest that this may not be the case when parasites have been completely cleared. Interesting recent consideration has been given to the impact of liver schizonticidal radical therapy upon protective immunity (Smith *et al.* 1999a) and to the potential loss of premunition in

clinically immune adults who have undergone such curative therapy to eliminate their “benign” chronic malaria infections.

None of the adult patients became severely ill, but they reported physical complaints that limited their capacity to function normally, prompting them to interrupt their usual routine and seek medical care. Most of the symptoms observed were rather non-specific. There were however significant differences between parasitaemic and non-parasitaemic patients that may not have been due entirely to chance. Although we did not undertake or power this investigation to test a specific hypothesis of adult clinical predictors of parasitaemia, we found dizziness and myalgia to be the best multivariate predictors of parasitaemia. This pattern was confirmed when multivariate interactions between different symptoms and parasitaemia were investigated. Parasitaemia was directly linked only to dizziness and myalgia, which in turn were likely to occur in combination with chills or nausea. This is in contrast to other studies that did not find symptom reports useful in diagnosing clinical malaria (Trape, 1985). Our preliminary findings suggest that adults presenting with dizziness or myalgia in association with chills and headache are likely to be parasitaemic.

The incidence data herein reported for adult males and females provides the necessary foundation for rational design of trials to evaluate drug or vaccine effects. Although African infants and pregnant women will certainly be the target population for vaccination when an effective product becomes deliverable, adults and school children will probably be the earliest recipients, in whom safety, tolerance and infection-blocking qualities can be readily assessed. In this regard, it was important to demonstrate near universal susceptibility to re-infection and its rapid manifestation as patent parasitaemia in adult Ghanaian males and females. Additionally important in planning such studies is our documentation of radical cure compliance and dropout rates, the proportion of radically-cured subjects who dropped out prior to yielding an endpoint, and the frequency and nature of illness that developed coincident with re-infection. Applying our findings of an 80% attack rate within 12 weeks to a prospective12 week trial of a drug/vaccine with predicted 75% protective efficacy (PE), and an 80% probability

(power) of having the lower 95% confidence limit for that PE ≥70%, we would require a population of 1,004 adults. With these variables kept the same, but with the lower 95% confidence limit relaxed to ≥60% for the PE, we would need only to enrol 151 subjects.

In conclusion, this study has shown that Ghanaian adults of the northern Kassena-Nankana District experience a seasonal ID of infection by *P. falciparum* that was comparable to that of infants living in the same communities. Within the 20 week wet season virtually all adults had manifested a patent primary infection and many reported illness that coincided with parasitaemia. Adults experienced predominantly low-density infections, characterised by mild clinical illness. Dizziness, myalgia, chills, fever, and headache were the most commonly reported symptoms that prompted health clinic visits seeking medical care. This study provides the foundation for designing efficient, practical clinical trials and preventive strategies against malaria in this holoendemic region.

4.6 Acknowledgements

We thank the people of the Kassena-Nankana District in northern Ghana for their co-operation, which made this study possible. Sincere appreciation also goes to the field and laboratory staff of the Navrongo Health Research Centre and Noguchi Memorial Institute for Medical Research, and especially to Mr. Charles Attiogbe, for technical assistance. We also thank Drs Kweku Enos and Thomas Mensah Afful of the War Memorial Hospital, Navrongo. Sincere thanks also go to Dr. Trevor Jones of the U.S. Naval Medical Research Center and Drs. Thomas Smith and Ivo Muller of the Swiss Tropical Institute for their helpful comments and statistical advice.

Financial support: The work was supported by the Naval Medical Research Center independent research initiative funds and the STO F6.1 work unit 61102AA0101.BXF.1431 of the Military Infectious Disease Research Program.

Disclaimer: The views of the authors expressed herein do not purport to reflect those of the Ghanaian Ministry of Health, the U.S. Navy, or the U.S. Department of Defence.

Chapter 5

Seasonal Malaria Attack Rates in Infants and Young Children in Northern Ghana

J. Kevin Baird[1], S. Owusu-Agyei[2], Gregory C. Utz[3], Kwadwo Koram[4], Mazie J. Barcus[1], Trevor R. Jones[1], David J. Fryauff[1], Fred N. Binka[2], Stephen L. Hoffman[5], and Francis N. Nkrumah[4]

[1]*Naval Medical Research Center, Silver Spring, Maryland*
[2]Navrongo Health Research Centre, Navrongo, Ghana
[3]*U.S. Naval Medical Research Unit No. 3, Cairo, Egypt*
[4]*Noguchi Memorial Institute for Medical Research, Accra, Ghana*
[5]Celera Genomics, 45 West Gude Drive, Rockville MD 20850

This article is In Press:
American Journal of Tropical Medicine and Hygiene

5.1 Abstract

The incidence density of infection and disease caused by *Plasmodium falciparum* in infants/young children aged 6 to 24 months living in the holoendemic Sahel of northern Ghana was measured during the dry season of 1996 and wet season of 1997. At the beginning of each season a cohort composed of 259 and 277 randomly selected children received supervised curative therapy with quinine and Fansidar®, and primaquine for those with normal glucose-6-phosphate dehydrogenase activity. The 20 weeks of post-therapy follow-up consisted of 3 home visits weekly and examination of Giemsa-stained blood films once every two weeks. Blood films were also taken from children brought to clinic with illness. The incidence density of parasitaemia after radical cure was 4.7 infections/person-year during the dry season and 7.1 during the wet season [relative risk (95% confidence interval) = 1.51 (1.25, 1.81), p=0.00001]. Although the mean parasitaemia count at time of re-infection in the dry season (3,310/μl), roughly equalled that in the wet season (3,056/μl; p=0.737), the risk ratio at 95% CI for parasitaemia >20,000/μl during the wet season was [1.71 (1.2, 2.4); p=0.0025]. The risk ratio at 95 CI for parasitaemia >20,000/μl with fever during the wet season was [2.45 (1.5,4.1); p=0.0002]. The risk ratio for anaemia (haemoglobin <8g/dl) at first post-radical cure parasitaemia showed no difference between seasons [RR (95%CI) = 1.0 (0.73, 1.4); p=0.9915]. We did not see seasonal differences in anaemia known to exist in this region, probably because the longitudinal cohort design using first parasitaemia as an end point prevented the subjects from developing the repeated or chronic infections required for anaemia induction. These findings bear upon the design of malaria drug and vaccine trials in holoendemic areas.

5.2 Introduction

The risk of infection by *Plasmodium falciparum* within endemic regions varies widely across geographic and seasonal parameters. If one gauges risk as the time to reach a proportion of 1.0 (i.e., 100% of those at risk have been infected), holoendemic areas of sub-Saharan Africa often yield estimates of just 8 to 12 weeks (Pull *et al.* 1974); (McElroy *et al.* 1997); (Beier *et al.* 1994). This reflects an incidence density of approximately 5 infections/person-yr. In contrast, the time to 100% risk in most of the

endemic regions of the Amazon basin range between 5 and 10 years, or an incidence density of approximately 0.1 infections/person-year (Pan American Health Organisation, 1997).

Risk of infection has been linked to patterns of morbidity and mortality caused by *P. falciparum* (Delacollette *et al.* 1993); (Snow *et al.* 1997); (Alles *et al.* 1998); (Gupta *et al.* 1999); (Modiano *et al.* 1998). Severe anaemia represents the primary cause of malaria-related death where the highest risk of infection prevails, while death due to cerebral malaria predominates in areas where infection risk is lower. Naturally acquired immunity in older children and adults in holoendemic Africa effectively prevents either outcome in those groups, but degree of exposure in infants and young children defines risk of specific disease outcomes. Protection from cerebral malaria requires the relatively heavy, uninterrupted exposure such as occurs through the Sahel of sub-Saharan Africa, but this carries risk of severe anaemia.

The threshold of exposure for protection from cerebral malaria versus that for significant risk of severe anaemia is not known. The argument has been made that diminishing risk of severe anaemia by reducing exposure to infection, e.g., with mosquito bed nets, may increase susceptibility to cerebral malaria (Snow *et al.* 1997). However, the basis of that argument lies in empirical observations. It remains possible that an exposure level ideal for maintaining protection against cerebral malaria lies well below the exposure of risk for severe anaemia. This represents a critical question in the context of control strategies intended to diminish disease and death caused by malaria, especially in sub-Saharan Africa. Few studies in the region have linked disease risks to quantitatively measured incidence density of infection. Thus it is difficult to begin to assign optimal exposure thresholds, i.e., incidence densities that induce protective immunity from cerebral malaria but below those leading to severe anaemia.

We measured the incidence density of infection by *P. falciparum* in infants and young children residing in holoendemic northern Ghana, where seasonal severe anaemia is the major cause of malaria-related morbidity (Koram *et al.* 2000). Incidence density

was measured as the post-radical cure attack rate for *P. falciparum* parasitaemia. This work provides relatively precise measures of incidence that may be linked to established patterns of disease risk in the region, i.e., high risk of severe anaemia among infants and young children. These studies provide a foundation for planning intervention trials in the region, and contribute to the process of assigning risk to defined degrees of exposure to infection.

5.3 Materials and Methods

Study site: The Kassena-Nankana District in the Upper East Region of northern Ghana in West Africa (Fig. 2.1) served as the study site. Approximately 141,000 people reside in this district of typical Guinea savannah with sharply distinct wet and dry seasons (Fig. 2. 2). Most residents practice subsistence farming, predominantly raising livestock and growing millet. They live within traditional compounds of extended family groups. An on-going census of the population conducted by the Navrongo Health Research Centre tracks births, deaths, migration, and other features on a quarterly basis (the Navrongo Demographic Surveillance System, NDSS). Malaria is holoendemic (Binka *et al.* 1994) and is the most important cause of morbidity and mortality (Binka *et al.* 1995).

Study Subjects: Two cohorts were recruited, one at the beginning of the dry season in November 1996 and another at the beginning of the wet season in May 1997. Infants/ young children between the ages of 6 months and 24 months were eligible for participation. They were selected for recruitment by stratification of the study site followed by a randomised selection of 16 "index" clusters of family compounds among the four geographic regions of the district recognised by the NDSS. The number of subjects recruited from each zone represented a sample weighted according to the proportional contribution to the entire population of the district.

Meetings were held with tribal and family group leaders representing the selected "index" compounds. The rationale, methods, risks and benefits of the study were explained and questions addressed. The assent of these leaders represented a key element of the process of informed consent within the cultural context of northern

Ghana. We nonetheless repeated the process with individual volunteers at enrolment in accordance with U.S. Navy regulations concerning the use of human subjects of medical research (SECNAVINST 3900.39B) and those of the Ministry of Health, Republic of Ghana. The verbal explanation and the written informed consent forms were in the native languages of the subjects (Kassim or Nankam).

Each "index" cluster had a target of 19 study subjects. However, variability in the number of eligible subjects per compound required a uniform strategy for reaching beyond the randomly identified "index" cluster. When the "index" compound failed to yield the target of 19 eligible subjects, the closest compound to the east served as a secondary target for recruitment. If necessary, the process was repeated in adjacent compounds to the north, west, and south, always in that order. The population-weighted random selection of "index" compounds among zones assured all eligible children in the district had an equal probability of selection.

Mothers indicating a willingness to participate in the study were transported with eligible children to an enrolment station. During the two week enrolment phase of each cohort, approximately 40 subjects were screened daily by the research team. Children with peripheral blood haemoglobin concentrations >6.0g/dl and free of disease requiring hospitalisation were enrolled. Among the 349 children screened at the beginning of the dry season (end of wet season), 94 were excluded due to severe anaemia. These severely anaemic children were taken to the district hospital for treatment. Among the 286 children screened at the beginning of the wet season, 275 were enrolled; only 4 of 11 exclusions had severe anaemia. The report by Koram *et al.* (2000) details the marked differences in prevalence of severe anaemia between these two randomly selected cohorts. Table 5.1 lists the statistical summary of each cohort.

Radical Cure: Radical cure started on the day following enrolment. Subjects received quinine sulphate (Cox Pharmaceuticals or Royce Laboratories; 10 mg/kg, 3 times daily for 4d), followed by Fansidar® (Hoffman La Roche Pharmaceuticals; 1/2 tablet for 5kg to 10kg body weight, and 1 tablet for >10kg, single dose). Subjects testing normal for

Glucose-6-phosphate dehydrogenase (G-6-PD, assay by NADP+ spot test; Sigma Chemical Co., St. Louis, MO) received 0.3mg of primaquine base per kg body weight (primaquine phosphate, Sanofi Winthrop Pharmaceuticals, New York, NY) once a day for 14 days. A licensed pharmacist prepared all of these medications in flavoured syrups. A trained field health worker employed by the research team used a syringe to dispense volumes of medicated syrup prescribed by the team physician. Only 6 of 536 subjects (1.1%) missed 2 or more of the 27 doses. In the dry season cohort 18 of 259 subjects (6.9%) and in the wet season cohort 27 of 277 subjects (9.7%) tested positive for G-6-PD deficiency and primaquine was withheld from their radical cure.

Laboratory: We used the qualitative NADP+ spot test with modifications to the procedure recommended by the manufacturer (Sigma Chemical Co.). Batches of 30 specimens were tested using a single positive control spot (G-6-PD-deficient control from Sigma Chemical Co.). Prescribed mixtures of blood (10μl), buffer, and G-6-PD substrate were incubated at room temperature (approx. 30°C) for 10 min, blotted onto filter paper, air dried and read in a darkened room under ultraviolet light. We did not use a 37°C water bath or readings at timed intervals as prescribed by the kit manufacturer. Subjects diagnosed as G-6-PD-deficient produced blots that failed to fluoresce to the same extent as most other blots (i.e., the negative control), and to the same extent as the known G-6-PD-deficient sample on the same blotter (the positive control).

Haemoglobin levels were measured using the Haemocue® (Lee Diagnostics Inc.) system. A micro-cuvette preloaded with stable reagents drew in approx. 10 microlitres of blood from the site of puncture. It was immediately placed into a portable spectro-photometric instrument. Stable control cuvettes provided by the manufacturer confirmed proper operation of the instrument. Axillary temperatures were obtained using digital thermometers placed under the arm.

Thick and thin blood films were stained with Giemsa reagents in the usual manner and were read by an experienced malaria microscopist on the day collected. 200 optical

fields under 1000X oil immersion magnification were examined before a film was recorded as negative. Counts of parasites in the thick film were based on the number of asexual trophozoites per 200 white blood cells (WBC). This number was converted to parasites per μl whole blood using a conversion multiple of 40 (assumes 8,000 WBC/μl).

Follow up: A trained field health worker with at least a secondary school education was assigned to each "index" compound for the 18 days of radical cure and the 20 week follow-up. The field worker visited each compound within his or her area 3 times per week to query mothers/guardians of the health of their child. Complaint of illness prompted transport of the mother and child to the district hospital for examination by a physician, including a blood film examination for plasmodia. Every 2 weeks a blood film was collected whether or not the mother complained of illness in her child. Children with malaria were treated with Lariam® mixed in syrup (mefloquine HCl; Hoffman La Roche, 20mg base/kg body weight in a single dose). Whether administered after an unscheduled visit to hospital or after a routine blood film examination, therapy with mefloquine marked the endpoint of participation in this study. The efficacy of mefloquine for therapy in these cohorts was virtually complete (Baird et al., ASTMH conference, 1997).

Clinical Analysis: Children presenting with signs of disease (fever, anaemia) and patent parasitaemia were considered to be ill with malaria. Although other diseases certainly occur in the district, and we have not yet estimated the risk attributable to malaria among febrile or anaemic children, malaria infection overwhelmingly dominates the burden of disease in these communities. An important exception to this rule is epidemic meningococcal meningitis, which did not occur during these studies. Evidence of disease reported by the mother, including fever, rigors, or vomiting, when coupled with patent parasitaemia were attributed to malaria. Although the lower limit for permissible haemoglobin at enrolment was 6.0g/dl we used haemoglobin of <8.0g/dl to define anaemia as a disease endpoint in the presence of parasitaemia.

Epidemiological Analysis:

Incidence Density. Incidence density estimates the force of infection, or new infections per unit person-time. The administration of radical cure allowed classification of post-treatment parasitaemia as a new infection. In the absence of cure, recrudescence and relapse confounds the estimate. We used the direct method to measure incidence density; the number of post-treatment parasitaemia divided by the sum of person-time at risk. Subjects no longer contributed person-time to the denominator after becoming parasitaemic or when lost to follow-up.

The incidence density of other endpoints was measured. Parasitaemia with fever (axillary temperature $\geq 37.5^0$C) or with anaemia (haemoglobin <8.0g/dl) at parasitaemia densities of >5,000/μl, >10,000/μl, >20,000/μl, and >50,000/μl constituted these endpoints. Subjects having parasitaemia without the specified outcome variable were designated as dropouts in these estimates, i.e. contributing person-time to the denominator up to the time of unqualified parasitaemia.

We used incidence densities to calculate the risk of an outcome in one group relative to the risk experienced by another group using SPSS (version 8.0, Chicago, IL). A relative risk of 1.0 reflected no difference in the risk to the two groups, and the distance from 1.0 (in either direction) represented the degree of difference between the rates. We calculated 95% confidence intervals around the relative risks as measure of the precision of our estimates. The significance of the difference between the rates was determined using Fisher's exact or a chi square test depending on number of events within groups.

Cumulative Incidence: Cumulative incidence expresses the risk of an outcome over a defined period as a proportion. The primary risk evaluated in this study was first parasitaemia after radical cure. We also calculated risk of high-density parasitaemia (>20,000/μl). The first 10 days following radical cure were considered to be free of risk, and were excluded from the denominator of the estimate of cumulative incidence. The life table method using weekly intervals estimated cumulative incidence. When

calculating the risk of developing a parasitaemia >20,000/μl, subjects developing parasitaemia <20,000/μl were treated as withdrawals, i.e., contributing to the denominator up to the interval in which they became parasitaemic (assuming a mid-interval event for all subjects).

5.4 Results

Radical Cure: The prevalence of parasitaemia at enrolment in the cohorts was 69% and 52% at the end of the wet and end of the dry seasons respectively, and this probably accounted for much of the vomiting that occurred during radical cure. Repeated vomiting (3 or more episodes) occurred in 4.7% and 3.6% of subjects taking quinine at the beginning of the dry and wet seasons respectively ($p>0.1$). After the single dose of Fansidar®, 0.8% and 6.2% of subjects reported vomiting in the dry and wet season cohorts ($p<0.001$). During the administration of primaquine at the start of the dry season and wet season cohorts, 4.6% and 6.8% of subjects had repeated vomiting ($p>0.1$). Despite the risk of vomiting, 531 of 537 subjects (98.9%) beginning radical cure completed the regimen, and 530 had negative blood films on day 15 of radical cure (>99% efficacy).

Point Measures: Enrolment: The two cohorts were virtually identical with respect to composition by sex, age, weight, and frequency of G-6-PD deficiency (see Table 5.1). The prevalence of parasitaemia at enrolment was significantly higher among the cohort at the beginning of the dry season (end of wet season) than among the cohort at the beginning of the wet season (end of dry season); 69% vs. 52%, $P<0.0001$.

Table 5.1. Comparison of dry and wet season cohorts at enrolment of infants and young children in the Kassena-Nankana District of northern Ghana.

Feature	Dry Season Cohort	Wet Season Cohort	P value
Sample Size	259	277	---
Male:Female	0.9923	1.022	0.865
Mean Age (months)	14.98	14.83	0.759
Mean Weight (kg)	7.97	7.80	0.211
%G-6-PD deficient	6.95	9.71	0.247

Parasitaemia	69%	52%	0.001

However, there was no difference either overall or within specific age groups in geometric mean parasite densities between wet and dry season cohorts at enrolment (see Table 5.2A).

First Parasitaemia: The density of first parasitaemia after radical cure was also similar among age groups between the wet and dry seasons (Table 5.2B). Infants <12 months old in the dry season cohort exhibited a mean parasite density after radical cure that approached being significantly higher compared to those in the wet season cohort (P=0.061, see Table 5.2B).

The prevalence of fever with first parasitaemia was 31% in the dry season cohort and 34% in the wet season (relative risk = 0.91, 95%CI=0.69, 1.18, p=0.464). Only children aged 12 months to 18 months had a slightly higher risk of fever during the wet season [relative risk (95%CI) =0.67 (0.42, 1.07), p=0.083].

Table 5.2. Age-specific geometric mean *P. falciparum* parasite densities (95% CI) in infants and young children in the Kassena-Nankana District in northern Ghana.

A. Enrolment.

Age Group (months)	Dry Season Cohort	Wet Season Cohort	P value
<12	1515 (949-2418)	925 (528-1620)	0.194
12 to 18	811 (470-1401)	1169 (645-2120)	0.376
>18	1084 (696-1688)	1150 (706-1875)	0.860
All ages	1112 (842-1470)	1099 (801-1507)	0.911

B. Post-Radical Cure.

Age Group (months)	Dry Season Cohort	Wet Season Cohort	P value
<12	3796 (2161-6667)	1704 (970-2991)	0.061
12 to 18	2580 (1461-4555)	5131 (2629-10013)	0.127
>18	3564 (2111-6015)	3716 (2174-6353)	0.913
All ages	3310 (2409-4547)	3056 (2177-4290)	0.737

Incidence Density: Figure 5.1 illustrates the incidence density of infection among "index" compounds representing the sample evaluated in the wet and dry season cohorts.

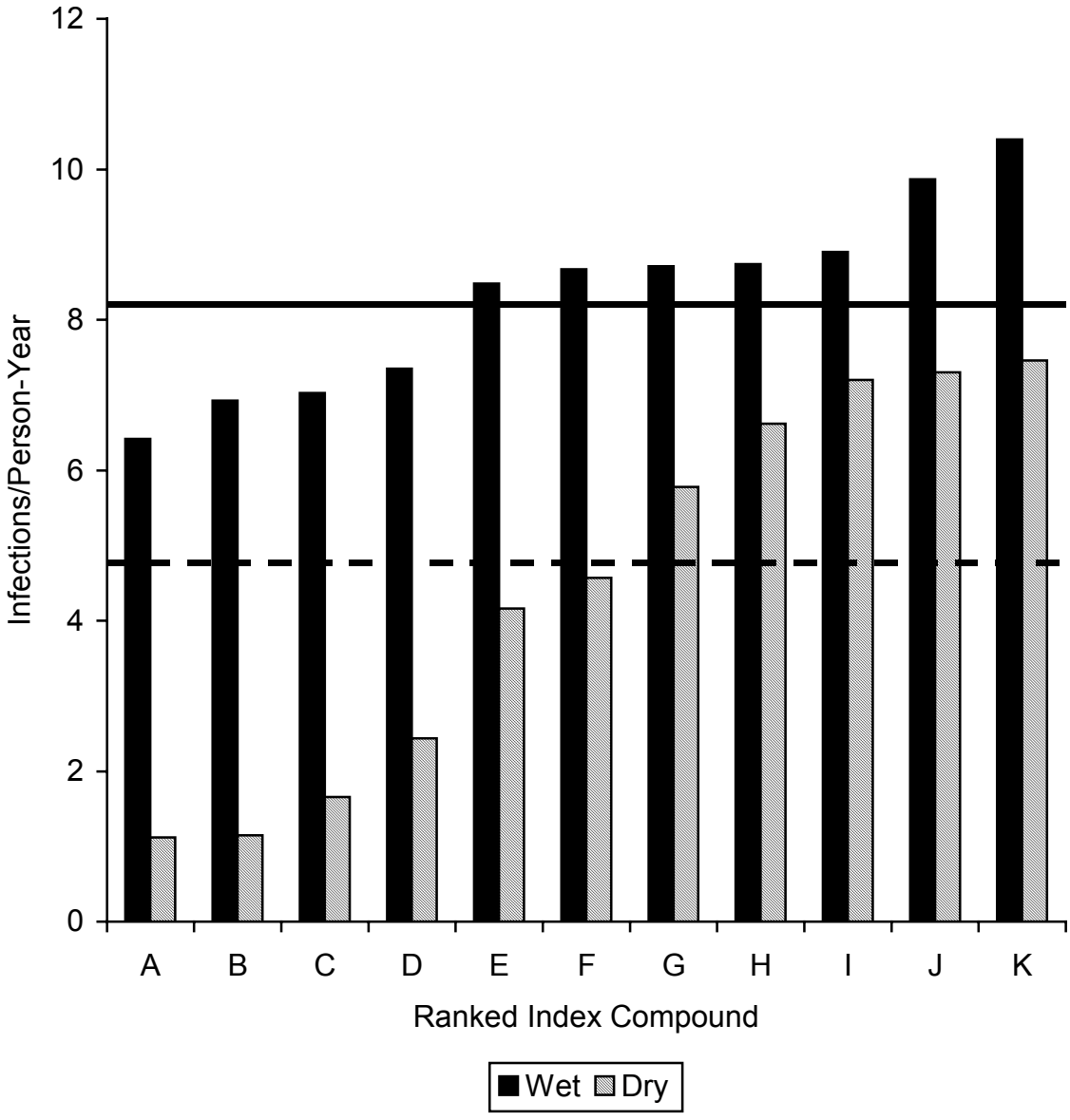


Figure 5.1. Incidence density among index compounds representing the sample population for the wet (solid bars) and dry (dashed bars) season cohorts. Variance from the mean incidence density in the wet season (solid line) and dry season (dashed line) among index compounds was not significant ($p>0.10$ and $p>0.10$) by the Mantel-Cox log rank test.

The data do not include 21 subjects that could not be retrospectively assigned to an index compound. Only 11 of 15 "index" compounds (A through K in Fig. 5.1) appear in this analysis because proximate compounds were lumped together due to uncertainty with original "index" assignment. All 15 "index" compounds were represented among the 11 clustered compounds illustrated in Figure 5.1. "Index" compounds in the wet season cohort had incidence density ranging from 6.42 to 10.4 per person-year (Mantel-Cox log rank test, p>0.10). "Index" compounds in the dry season cohort had incidence density ranging from 1.12 to 7.29 per person-yr (p>0.10). These data illustrate the relatively uniform incidence of new infection among the randomly selected "index" compounds in both the wet and dry season cohorts.

Table 5.3. Incidence density of *Plasmodium falciparum* parasitaemia post-radical cure in infants and small children in the Kassena-Nankana District of northern Ghana.

Endpoint	Dry Season		Wet Season		RR^{o}	95%CI†	P value‡
	N	Incidence Density*	N	Incidence Density*			
Any parasitaemia							
+/-fever, anaemia	200	4.71	254	7.11	1.51	1.25, 1.81	0.00001
$^{\$}$with fever	62	1.46	87	2.43	1.66	1.20, 2.31	0.00225
$^{£}$with anaemia	73	1.72	63	1.76	1.02	0.73, 1.44	0.99147
Parasitaemia >5,000/μl							
+/-fever, anaemia	90	2.18	121	3.39	1.56	1.18, 2.04	0.00146
$^{\$}$with fever	37	0.89	61	1.71	1.92	1.27, 2.87	0.00221
$^{£}$with anaemia	33	0.80	27	0.76	0.95	0.57, 1.58	0.89733
Parasitaemia >10,000/μl							
+/-fever, anaemia	78	1.89	103	2.88	1.53	1.14, 2.05	0.00458
$^{\$}$with fever	33	0.80	55	1.54	1.93	1.25, 2.97	0.00264
$^{£}$with anaemia	30	0.73	25	0.70	0.96	0.57, 1.64	1.00000
Parasitaemia >20,000/μl							
+/-fever, anaemia	55	1.33	81	2.27	1.71	1.21, 2.40	0.00249
$^{\$}$with fever	23	0.56	49	1.37	2.45	1.50, 4.05	0.00022
$^{£}$with anaemia	20	0.48	18	0.50	1.04	0.55, 1.97	1.00000
Parasitaemia >50,000/μl							
+/-fever, anaemia	18	0.44	53	1.48	3.36	2.00, 5.82	0.00000
$^{\$}$with fever	10	0.24	37	1.04	4.33	2.13, 8.61	0.00001
$^{£}$with anaemia	7	0.17	10	0.28	1.65	0.63, 4.35	0.33789

*outcomes per person-yr; RR^{o} = Relative Risk = the ratio of wet season rates over dry season;

†The confidence intervals are Sato limits; ‡The p-value is Fisher's exact (2-tail) chi-square;
[$]Fever = axillary temperature >37°C; [£]Anaemia = haemoglobin <8g/dl

Table 5.3 lists the incidence densities of new infection in the wet and dry season cohorts. The table stratifies incidence density according to parasite density and accompanying illnesses, i.e., with fever or anaemia.

Incidence density of any parasitaemia in the wet season was significantly higher than in the dry season; 7.11 vs. 4.71 infections/person-yr, [relative risk ((95%CI) =1.51 (1.25, 1.81), p=0.00001].

The relative risk was still higher when fever was included in the endpoint; 1.46 vs. 2.43 infections/person-yr, [relative risk (95%CI) =1.66 (1.20, 2.31), p=0.0023]. However, for parasitaemia with anaemia, there was no difference in incidence density between wet and dry season cohorts (relative risk = 1.02, p=0.9915).

The pattern of significant differences in incidence density between any parasitaemia, or those with fever but not anaemia in the wet and dry season cohorts, appeared at all strata of parasite density (Table 5.3). In general, the greater the parasite density, the greater the relative risk for that outcome in the wet season cohort. Compared to the dry season, the risk of having a febrile parasitaemia of any density was 1.66 times higher than that in the wet season. Similarly, the risk of developing febrile parasitaemia of >5,000/μl, >10,000/μl, >20,000/μl, and >50,000/μl during the wet season was 1.92, 1.93, 2.45, and 4.33 times that in the dry season. The 95% confidence intervals for all of these relative risks did not include 1.0, and the p-values were always <0.0025. In contrast, the relative risk of parasitaemia with anaemia never produced 95% confidence intervals that excluded 1.0, and the p-values were never less than 0.33.

Cumulative Incidence: Figure 5.2 illustrates the cumulative incidence of all parasitaemia and parasitaemia >20.000/μl in the two cohorts. The cumulative incidence of parasitaemia after radical cure increased more slowly during the first 3 weeks of risk in the wet season cohort compared to the same period in the dry season cohort (cumulative incidence of 0.19 vs. 0.04; p=0.0025).

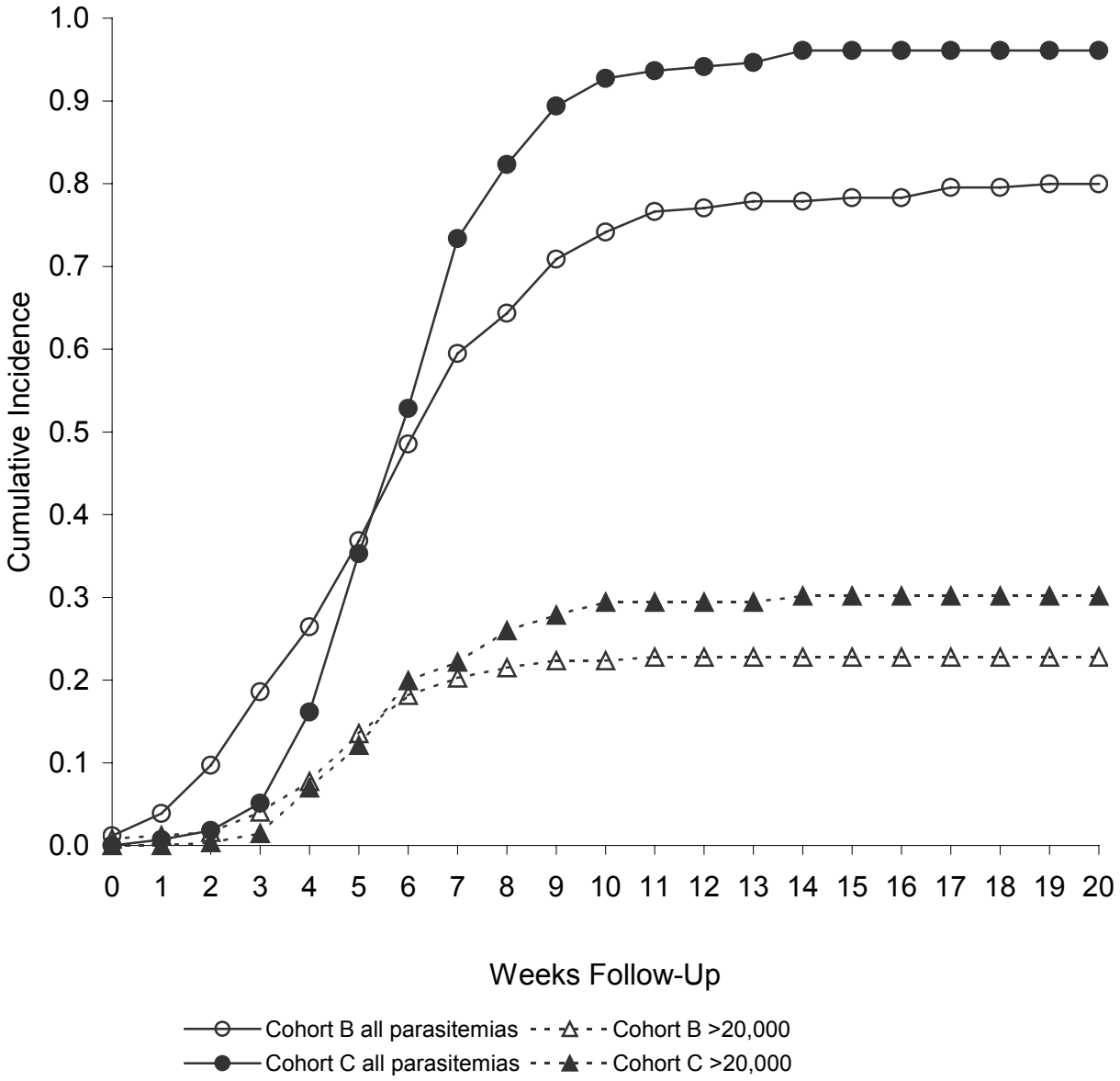


Figure 5.2. Cumulative incidence of any parasitaemia by *P. falciparum* in infants and young children during the wet (solid circles) and dry (hollow circles) seasons, along with cumulative incidence of high density parasitaemia (>20,000/ul) in the wet (solid triangles) and dry (hollow triangles) seasons in the Kassena-Nankana District, northern Ghana.

The 20wk cumulative incidence of parasitaemia in the dry season cohort reached a plateau at 0.76 at the end of 12 weeks, leaving about one quarter of the cohort virtually risk-free for the remaining 2 months of follow-up. In contrast, the wet season cohort reached a plateau cumulative incidence of 0.93 at the end of 10 weeks. During the remaining 10 weeks of presumably high risk, only an additional 3.5% of subjects became infected.

A total of 6 subjects remained free of infection throughout the study, and these accounted for the 3.5% risk of not being infected. The 20-week cumulative incidence of infection by *P. falciparum* was 96.5%. The risk of parasitaemia <20,000/ul peaked at about 25% in both cohorts at about the same time, at the end of nine weeks.

5.5 Discussion

Intense transmission of *P. falciparum* occurred from May to February in the Kassena-Nankana District of northern Ghana. The incidence density of infection decreased moderately during the dry season that commenced in October. Transmission abruptly dropped to very low levels only during the last two to three months of the dry season (Fig. 5.2). No significant differences appeared among children of any age in the dry and wet season cohorts with respect to geometric mean density of parasitaemia at either enrolment or first parasitaemia (Table 5.2A and 5.2B). Appreciable differences between seasons appeared only when the data were stratified by parasite count and fever (Table 5.3). The relative risk of parasitaemia with fever at any density, >5,000/μl, >10,000/μl, >20,000/μl, and >50,000/μl for the wet season cohort relative to the dry season cohort was 1.66, 1.92, 1.89, 2.45, and 4.33, respectively. Risk of parasitaemia with fever increased in proportion to the density of parasitaemia during the wet season. In both cohorts the incidence density of these parasitaemia outcomes diminished sharply with increasing counts. The outcomes of greatest relative risk during the wet season represented low frequency events, e.g., parasitaemia >50,000/μl with fever was four times more likely to occur during the wet season, but occurred less frequently than the lower parasitaemia levels with fever (1.04 vs. 1.71 events per person-yr).

Despite the relatively subtle parasitological and clinical distinctions between the dry and wet season cohorts, the statistical power of vaccine trials applying endpoints of high parasitaemia and fever would be enhanced by execution framed to capture wet season risks. The example of risk of parasitaemia >50,000/ul with fever measured in these studies best illustrates this point. If one assumes a vaccine efficacy of 85%, and the operational requirement to have an 80% probability of having a 95% confidence interval that excludes 70% (i.e., the lower limit of acceptability for efficacy), then the dry season incidence rate of 0.24 events per person year for this outcome demands a sample size of 2055. Applying the same parameters to the 1.04 events per person year incidence rate in the wet season gives a sample size estimate of just 363 subjects. Relatively subtle distinctions in seasonal end point incidence densities may profoundly impact sample size requirements in intervention trials.

Anaemia is an important feature of morbidity caused by malaria in the Kassena-Nankana District. The infants and young children in these studies showed dramatic distinctions with respect to the prevalence of severe anaemia as seen at enrolment at the end of the dry and wet seasons (Koram *et al.* 2000). In effect, an epidemic of severe malarial anaemia occurred in these children as the wet season progressed: apparently starting at 1% in May and rising to 24% in October. Given the fact that we have now documented that intense transmission continues at least to February, the prevalence of severe anaemia may even worsen relative to the 25% rate measured in October. The relatively brief respite in transmission between February and May appeared sufficient to allow recovery of most severe anaemia to above the 6.0g/dl level. The work reported here and by (Koram *et al.* 2000) demonstrates the need for longitudinal studies of seasonal risk of severe anaemia in northern Ghana.

The relatively low risk of anaemia among our subjects with first parasitaemia and the absence of any differences between the dry and wet season risks of anaemia is a product of the experimental design. Chronic infection causes anaemia in malaria (Koram *et al.* 2000); (Miller *et al.* 1994). Measuring the incidence density of infection necessitated radical cure and, thus, the interruption of chronic parasitaemia. Moreover,

we could not enrol the 25% of screened children with severe anaemia, so that children in the dry and wet season cohorts began radical cure with similar anaemia profiles, parasitological features, and chemotherapy. Thus it was not surprising that the first parasitaemia of the dry season had no less risk of anaemia compared to that in the wet season. This study has demonstrated that intervention trials applying an endpoint that includes severe anaemia should allow for a chronic course of parasitaemia.

The failure of 2.2% of subjects in the wet season cohort to develop parasitaemia (accounting for a 3.5% residual risk) raises potentially important questions. Given the 10-week plateau in the face of intense transmission, it is difficult to assign these residual aparasitaemic subjects to chance. These children were somehow protected. Unknown genetic factors may have precluded parasitaemia in these children, e.g. HLA or cytokine promoter alleles (Hill, 1999); (McGuire *et al.* 1999). However, in an adult cohort from the same gene pools and treated and followed through the wet season in the same manner (Owusu-Agyei et al., 2001), all developed parasitaemia. Possible explanations for the minority of child subjects failing to develop parasitaemia include consistently applied and extraordinarily effective protective measures by the family (e.g., mosquito netting, repellants, or antimalarials), and genetic or immunogenetic factors that protect children but not adults from parasitaemia.

The geometric mean parasitaemia densities at enrolment were significantly lower than at first parasitaemia post-radical cure. At enrolment, the dry and wet season cohorts had geometric mean densities of 1112 and 1099/μl, whereas these densities were 3310 3056/μl at first parasitaemia ($p<0.001$ for each). Many explanations for these differences are possible, but we consider the effect of radical cure as most likely. Although it is difficult to rule out drug-mediated interference with immune effectors that limit parasite densities as an explanation, we instead favour diminished effectiveness of premunition as a consequence of eradication of parasitaemia by radical curative therapy. Premunition is the protection from heavy parasite burdens imbued by lesser parasite burdens. We think the elimination of chronic, relatively low-grade parasitaemia may have established susceptibility to acute, relatively high-grade parasitaemia. This

carries important implications with regard to experimental design in intervention studies and to control strategies aimed at delivering effective therapies in holoendemic Africa. Studies designed to address this important question should be carried out.

In summary, these studies have documented intense malaria transmission with an incidence density between 5 and 7 infections/person-yr through most of the year among children aged 6 to 24 months living in the Kassena-Nankana District of northern Ghana. A period of 2 to 3 months at the end of the dry season had very low risk of infection, and marked a dramatic fall in the prevalence of severe anaemia. Post-radical cure infections carried risk of relatively high-density parasitaemia during dry or wet seasons.

5.6 Acknowledgements

The authors wish to express thanks to the Kassena-Nankana community, especially the volunteers in this study. We also acknowledge the team of health workers attached to the MARS (Malaria Attack Rate Study) project at the Navrongo Health Research Centre; their excellent work made this report possible. In particular, we acknowledge Mr. Charles Attiogbe of Noguchi Medical Research Institute for his input as the study microscopist; Cletus Tindana, Salifu Abdul Rahman and Paulina Tindana as field supervisors. Special thanks goes to Drs. Kweku Enos and Mensah-Afful, the study physicians; and especially Dr. Alex Nazzar for essential support and advice. We gratefully acknowledge the support and assistance of Mr. Troy Fitrell, Vice Consul at the American Embassy, Accra.

Financial support: This work was supported by the Naval Medical Research Center independent research initiative funds and the STO F6.1 work unit 61102AA0101.BXF.1431 of the Military Infectious Disease Research Program.

Disclaimer: The views of the authors expressed herein are their own and do not purport to reflect those of the Ghanaian Ministry of Health, the U.S. Navy or the Department of Defence.

Chapter 6

Does Radical Cure of Asymptomatic *Plasmodium falciparum* place Adults in Endemic Areas at Increased Risk of Recurrent Symptomatic Malaria?

S. Owusu-Agyei[1, 4], F. Binka[2], K. Koram[3], F. Anto[1], M. Adjuik[1], F. Nkrumah[3], T. Smith[4]

[1]Navrongo Health Research Centre, Navrongo Ghana.
[2]School of Public Health, University of Ghana. Legon.
[3]Noguchi Memorial Institute for Medical Research, Legon. Ghana
[4]Swiss Tropical Institute, Basel, Switzerland.

This article has been submitted to:
Tropical Medicine and International Health journal

6.1 Abstract

In malaria vaccine and drug trials, chemotherapy is often used to eliminate parasites in the blood and liver prior to intervention so that time to infection can be studied. The impact of such radical treatment on the incidence and severity of subsequent malaria infections in semi-immune people is unclear. In May 1996, a cohort of 197 volunteers aged 18-55 years were randomly recruited for malaria incidence studies in the Kassena-Nankana District of northern Ghana, radically cured, and their clinical status followed for 20 weeks through the peak malaria transmission season. A further 202 adults were sampled and followed up in the same way one year later without being treated. Forty-nine percent (49%) of volunteers in the treated group experienced clinical attacks associated with parasitaemia in the follow-up compared with 38% in the untreated group [log-rank test for equality of survivor functions X^2=4.42; p= 0.035, RR (95% CI)=1.29 (1.03-1.61). Clinical malaria in the treated group was associated with significantly more symptoms, although the parasitaemia densities on presentation were lower. All cases in the treated group were sensitive to Chloroquine compared with a 9% Chloroquine failure rate at day 7 in the untreated group. Clinical malaria occurring after radical cure may be distinctly different from "natural" disease and this may have significance for the design and interpretation of intervention trials.

6.2 Introduction:

The desire to avert 1-2 million deaths due to malaria of which over 90% occur in Africa, south of the Sahara (Snow *et al.* 1999); (World Health Organisation, 1999) is manifested in research to develop new anti-malarial drugs and malaria vaccines that can be deployed in endemic sites (Miller *et al.* 1998). In intervention trials of malaria vaccines and anti-malarial drugs, a common endpoint is incidence of parasitaemia (symptomatic or asymptomatic) in the interval after the intervention is applied. Several trials of insecticide treated mosquito nets (Stich *et al.* 1994); (Msuya *et al.* 1991) have administered drugs to eliminate parasites prior to studying the effects of the nets. The same is for testing of new anti-malarial drugs (Taylor *et al.* 1999) and vaccines (Alonso *et al.* 1994) and is recommended for vaccine trials by the (World Health Organisation, 1997b). This facilitates subsequent study of the time to infection. There has, however,

been little evaluation of the possible impact of radical therapy on the incidence and severity of subsequent malaria episodes in semi-immune adults and children in endemic countries. Does the elimination of parasitaemia render the host more susceptible to disease caused by new inoculations? Can the 'cocktail' of drugs lead to important side effects or immune suppression? It is important to consider these questions from the points of view both of the consequences for the volunteers and of the relevance of the results of trials to situations where parasites are not cleared.

The Kassena-Nankana District in northern Ghana, where malaria is holoendemic, is a possible site for malaria vaccine trials. To estimate malaria incidence a cohort of 197 adults between the ages of 18 to 55 years, was followed through the peak malaria transmission period (May to October) of 1996 (Owusu-Agyei et al., 2001). A cohort of children aged 6-24 months was also followed one year later, during the high transmission period from May to October 1997 (Baird et al., 2001). In both these cohorts, a radical antimalarial therapy was administered to all volunteers to eliminate malaria parasites in the blood stream and the liver so that the incidence of infection and of clinical malaria could be studied. During the 1997-study period, malaria-related morbidity was also recorded in a further cohort of 202 adults who were not pre-treated. We report here an analysis of the effects of radical therapy on incidence of clinical episodes of malaria by comparing the patterns of malaria morbidity in the two adult cohorts.

6.3 Methodology:

Study site and population: The Kassena-Nankana district lies within the Guinea Savannah woodlands between latitudes 10^0 30' and $11^0$00' north of the equator and between longitudes $1^0$00' and $1^0$30' west of the zero meridian, in the Upper East Region of Ghana, bounded on the north by part of the international boundary between Ghana and Burkina Faso. It has a population of 141,000 people as determined by a 90-day cycle demographic surveillance [NDSS] (Binka et al., 1999). Malariological surveys over half a century have consistently reported that the area is holoendemic for *P. falciparum* malaria (Coulbourne *et al.* 1955); (Binka *et al.* 1994)

Subject Selection, Consent and Enrolment: The same sampling procedure, using the Navrongo Demographic Surveillance System (NDSS) was used for both cohorts. Sixteen (16) "index" compounds were randomly selected from 4 geographical zones of the district with the number of compounds per zone proportional to the 18-55 year population. Potential volunteers were then selected by visiting compounds radially in concentric circles around each of the "indexed" compounds until a pre-determined number of subjects (approximately 20) were recruited to form a cluster. Verbal informed consent was sought with the chiefs, opinion leaders and the community members (communal consent), prior to recruitment.

In 1996, 202 adults were enrolled into the study following a screening process that involved written and signed informed consent, taking of vital signs, clinical history, physical examination by a physician, collection of blood for microscopy and G-6-PD status, and urine from all the women for pregnancy tests. Those enrolled received therapy designed to clear all malaria infections. All drugs were given under direct observation at the homes of the volunteers by fieldworkers. The 18-day regimen consisted of oral quinine sulphate (650 mg, three times daily for four days); followed by doxycycline (100mg, twice daily for 10 days). Subjects testing normal for G-6-PD (NADP + spot test, Sigma Chemical Co., St. Louis, MO) received in addition to quinine sulphate and doxycycline, primaquine (0.5mg base per kilogram body weight once daily for 14 days), given concurrently with the doxycycline. The success of the treatment was confirmed by microscopic examination of a blood film collected on day 15. All but five subjects completed therapy, and were found to have cleared all parasites by microscopy.

The second cohort of adults was enrolled using the same procedures, with the exceptions that G-6-PD status and pregnancy tests were not carried out as there was no plan to treat this cohort. No treatment was given prior to follow-up.

Follow-up: Fieldworkers paid thrice weekly visits to the volunteers at their homes to inquire of their general health and to detect fever by measuring axillary temperature.

Blood films were collected every two weeks for the 20 weeks duration of the study, or if the subject reported any signs of illness or had an axillary temperature $\geq 37.5\ ^{0}C$. In the latter case, an interview was conducted, information solicited on a detailed list of signs and symptoms of malaria, and the subject was referred to a project physician in the district hospital.

Blood slides were prepared as Giemsa-stained thick and thin smears examined by 1000X light microscopy using oil immersion. Parasitaemias were scored per microlitre of blood by counting the number of asexual parasites per 200 white blood cells and multiplying the parasite count by 40, assuming 8,000 white blood cells per microlitre. A slide was considered negative if no parasites were observed within 200 high power fields. A 10% random sample of all slides was re-examined for quality control.

All symptomatic (any one or more of the following: reported/documented fever, headache, chill, nausea, dizziness, myalgias) subjects with microscopy-confirmed parasitaemia were classified as clinical malaria cases and promptly treated with Chloroquine Diphosphate (25mg/kg body weight over 48 hours). Treatment was the same for patients in the two cohorts; each sick patient seen by the study physician was prescribed with 25mg/kg weight of Chloroquine, the standard practice in the ministry of health of Ghana. The first dose of 600 mg was taken in the presence of the doctor's assistant and the remaining 6 tablets were (except in-patients admitted to the hospital) packed for the patient to take home. , Patients were advised to take a further 600 mg (4 tablets the next day) and 300mg (2 tablets on the third day). Subjects with malaria parasites in a blood smear collected routinely at one of the 2-week samples but who had no clinical symptoms or signs of malaria were not treated.

Patients treated with Chloroquine were clinically evaluated again on days 2 and 7 after initiation of therapy and blood films were taken at each of these visits. Those who did not respond to the treatment were treated with three tablets of Fansidar® (Sulphadoxine 500 mg + Pyrimethamine 25 mg per tablet, Hoffman - La Roche), and followed up until smear-negative.

Data analysis: The primary outcome variable for the study was the time to the first episode of clinical malaria. Owing to the pre-erythrocytic stage incubation period of the parasite, the first ten days following curative therapy among the treated group were excluded from analyses as risk-free period. Observation for clinical malaria among the untreated cohort started from the day of recruitment; no days were excluded in the analyses as risk-free period since they had no treatment prior to follow-up.

6.4 Results

The two cohorts had a similar age distribution (Table 6.1). Forty-four percent (44%) of those who received radical therapies were female compared with 52% of the untreated cohort (Table 6.1).

Table 6.1: Characteristics of adults in the two cohorts.

	Radical Therapy group	No Radical Therapy group.
Mean age (Range)	38.6 (18 – 55 years)	36. 8 (18 – 54 years)
Male	56%	48%
Parasitaemic at enrolment	56%	28%
Geometric Mean Parasite density at enrolment (95% CI)	116 (90, 150)	161 (114, 226)
Geometric Mean Parasite density at First Malaria Clinical episode	201 (125, 325)	669 (516, 869)

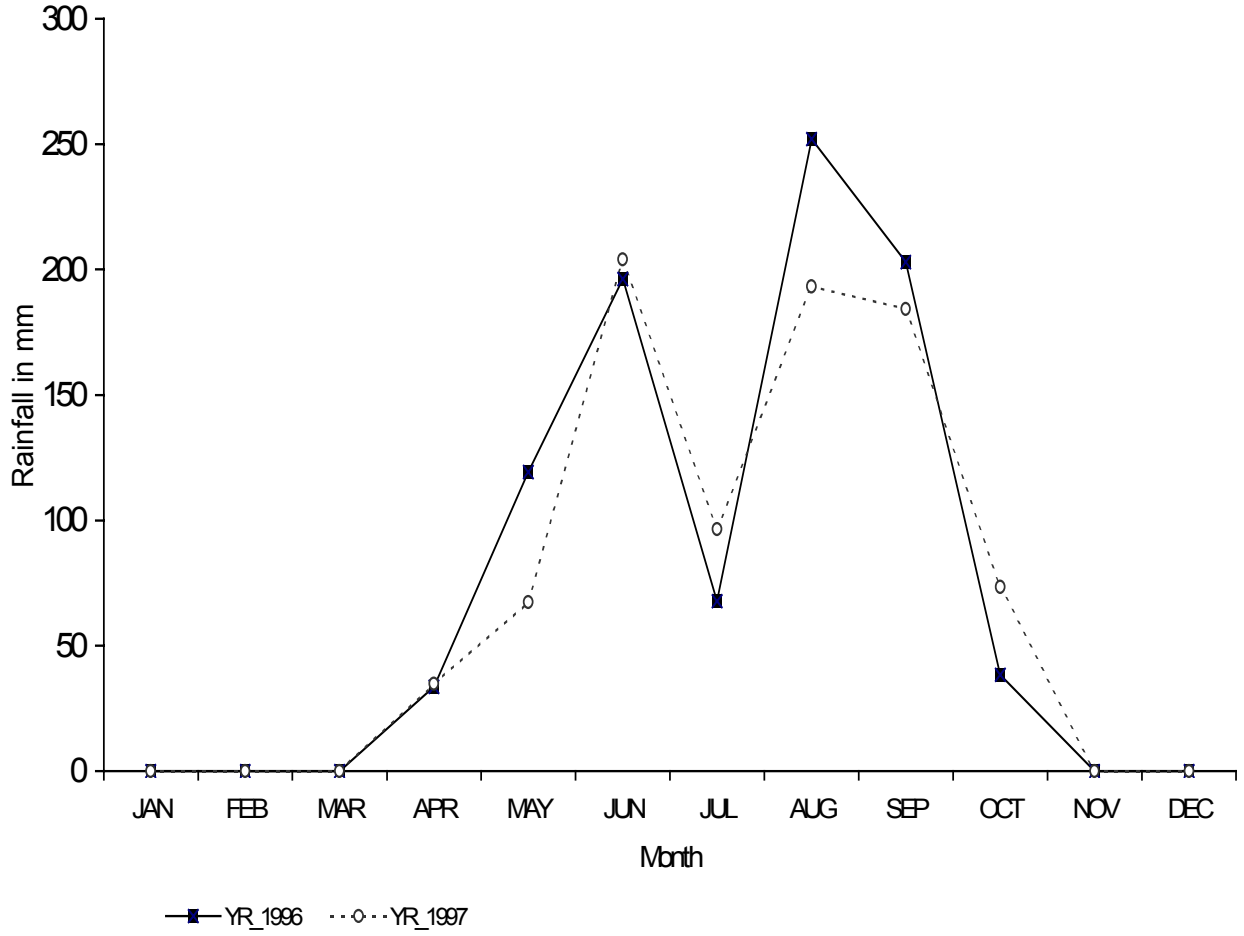


Figure 6.1:Monthly Rainfall for 1996 and 1997 in the Kassena-Nankana district.

The main difference at enrolment was that 56% of the volunteers who underwent a radical therapy were parasitaemic by microscopy compared to only 28% among the cohort that did not receive any radical therapy, due, presumably, to different levels of transmission during the preceding dry season. Though the parasite densities at enrolment were similar in the two cohorts.

Environmental conditions relevant for malaria transmission were similar during the two years. The rainfall from January 1996 to December 1997, recorded in the district (Irrigation Company of Upper Regions, 2001) is shown in Figure 6.1. During 1996, the total rainfall was 910 mm, quite similar to that of 1997 (861 mm) and also similar to the mean rainfall for the previous 20 years (885 mm). In both years, the rainy season started in April and ended in October with similar monthly rainfall (Fig. 6.1).

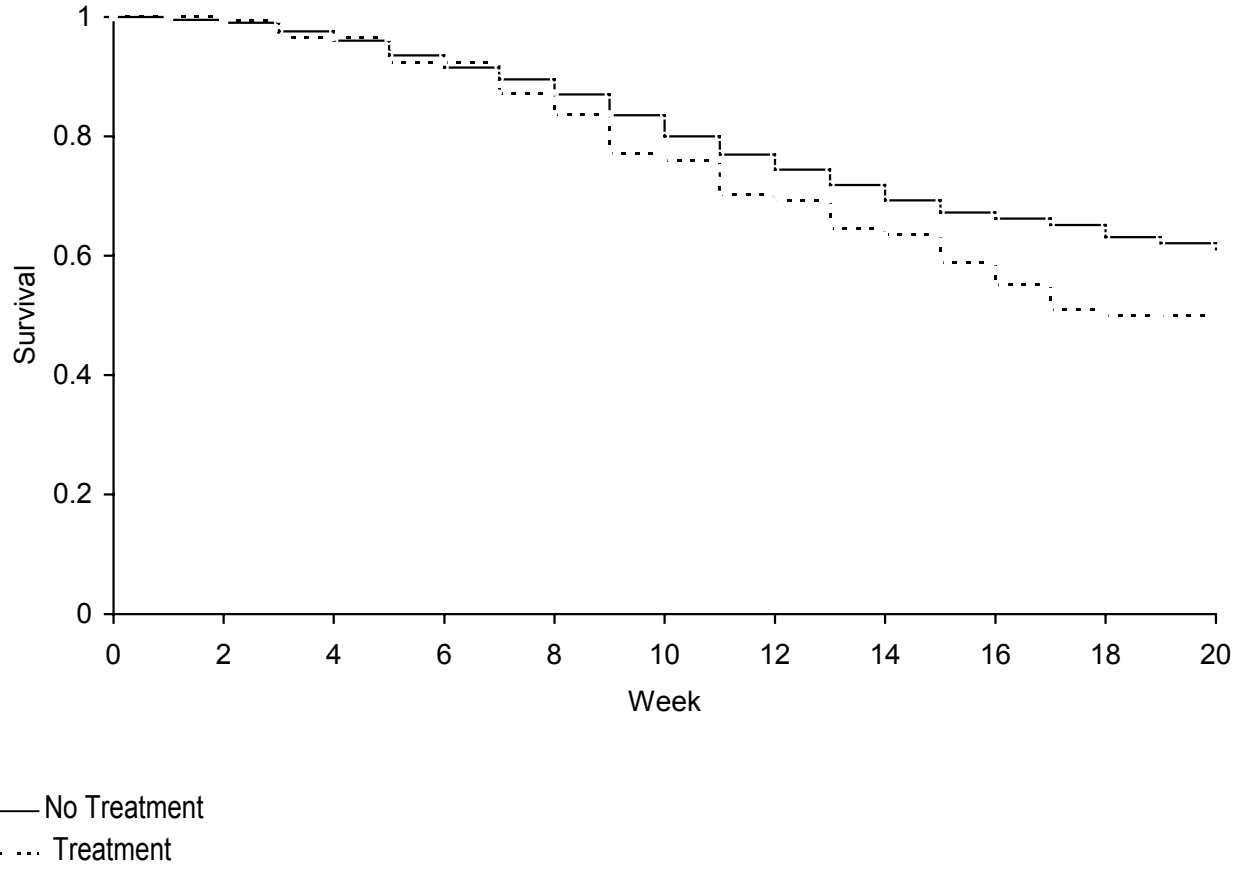


Figure 6.2: KM survival curve of clinical malaria development between the two cohorts

Clinical malaria attack rates in the 20 weeks follow-up on the two cohorts are presented as Kaplan-Meier curves in Figures 6.2 and 6.3. In Figure 6.2, the curve for the radically cured cohort falls below that for the untreated cohort, indication that the tendency is for the former to have higher incidence of clinical episode. Further evidence for the protective property of individuals with parasitaemia is demonstrated in the curves in Figure 6.3. The average risk of developing clinical malaria is presented in Table 6.2. Forty-nine percent (97/197) of volunteers in the treatment group developed clinical malaria in the 20 weeks, compared with 38% (77/202) in the group that were not treated prior to observation [log-rank test of equality of survivor functions, X^2=4.42, p= 0.035; RR (95% CI)=1.29 (1.03-1.61)]. This corresponded to a 22% increase in the risk of developing a clinical episode of malaria in the treated cohort. When we further separated the untreated cohort into those who started with initial parasitaemia (56) and

those who had no parasitaemia at enrolment (146), 33.9% (19/56) and 39.7% (58/146) respectively had developed clinical malaria within the 20 week period (Log-rank test X^2=0.71, p= 0.4; RR (95% CI) = 1.17 (0.77–1.78). The curve for the untreated individuals without parasitaemia at enrolment falls below that for the untreated cohort with parasitaemia at enrolment, indication that the tendency is for those without parasitaemia to have higher incidence of clinical episode.

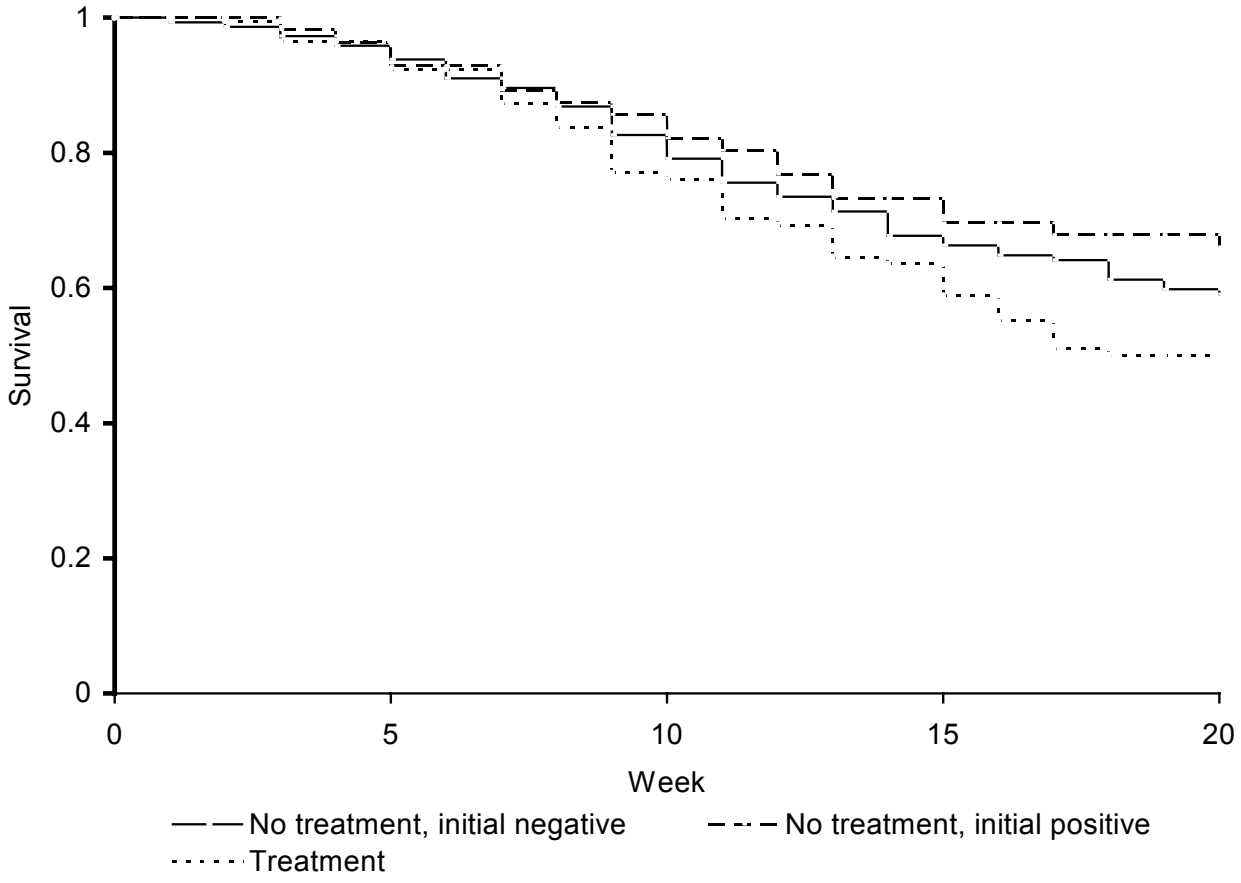


Figure 6.3: KM survival curve among adults developing clinical malaria

Table 6.2A: Risk of developing clinical malaria in the treated and untreated cohorts

Cohort	Proportion with Clinical Malaria	Statistic
Treated Adults	97/197	
Untreated Adults	77/202	RR (95% CI) = 1.29 (1.03–1.61) χ^2 = 4.42; p-value = 0.035

Table 6.2B: Risk of developing clinical malaria in the untreated cohort

Enrolment Microscopy	Proportion with Clinical Malaria	Statistic
POSITIVE	58/146	
NEGATIVE	19/56	RR (95% CI) = 1.17 (0.77–1.78) χ^2 = 0.71; p-value = 0.4

Though the geometric mean parasite densities at enrolment were similar in both cohorts, at first episode of clinical malaria, the geometric mean density was three times as high in the untreated group as in the treated group (Table 6.1). However, substantially more symptoms were associated with each episode among the treated group than the untreated (Table 6.3).

Table 6.3: Comparison of symptom reports between the two adult cohorts

Symptom Histories	Treated	Not Treated	Pearson χ^2	*p*-value
Documented Fever	10/97	1/77	5.88	0.01
Reported Fever within 48 hrs	82/97	42/77	18.85	0.00
Chill	76/97	30/77	27.81	0.00
Headache	83/97	55/77	5.20	0.02
Nausea	36/97	13/77	21.52	0.00
Myalgias	39/97	1/77	36.49	0.00

All volunteers who received treatment prior to follow-up and were diagnosed with clinical malaria during follow-up, cleared all parasitaemia by Day 7 when they were treated with a full dose of Chloroquine. Nine percent (9%) of those who did not receive treatment at enrolment failed to clear their malaria parasites by Day 7 when they were similarly treated with full dose of Chloroquine.

6.5 Discussion

While young children are highly susceptible to both malaria infection and disease, adults who have lived in endemic areas and exposed to malaria transmission throughout their lives, have a high level of immunity (Day *et al.* 1991) and rarely suffer from clinical malaria (Trape *et al.* 1994). We were therefore surprised when of 197 volunteers who received treatment prior to being followed-up for incidence of malaria infection, 20% had clinical attacks at first re-infection (Owusu-Agyei et al., 2001). Within the first 20 weeks of follow-up, 49% of the subjects in this cohort had experienced a clinical malaria attack. The disease rates were thus unexpectedly high for adults who have lived all their lives in a holoendemic area (Trape *et al.* 1994). The significantly higher incidence of clinical attacks among this treated cohort raise the question of whether clearance of malaria parasitaemia can increase the risk of clinical attacks in adults when they subsequently become re-infected. It has long been suggested that the presence of malaria parasites may protect against superinfection (Sergent *et al.* 1935) or consequent clinical episodes (Smith *et al.* 1999a); (Al Yaman *et al.* 1997). Could radical therapy lead to removal of this premunition?

The study of the initial cohort was not designed to investigate the effects of radical therapy on semi-immune adults living in holoendemic malarious area. The untreated group was studied only because of the apparently unusual responses in the first year. This imposes some limitations on the ways in which the findings can be interpreted, and a properly randomised study with concurrent controls is needed to determine if these are true effects or an artefact of having made the observations in two different time periods.

However, the study design, selection of volunteers, treatment of subjects in the treatment arm and follow-up in the two cohorts, observation techniques, data collection and clinical monitoring of patients in the two cohorts were as near as possible identical. Field data collection was carried out by the same field workers, slide reading by the same technician and clinical diagnosis by the same physicians in the two cohorts.

We believe that the intensity of infection and mix of parasites circulating were similar in the two years. The cumulative incidence curve for adults who were radically cured prior to follow-up in the peak transmission season of 1996 was similar to that for the infants who were radically cured and identically followed-up in the same period as the control cohort (Fig. 6.4).

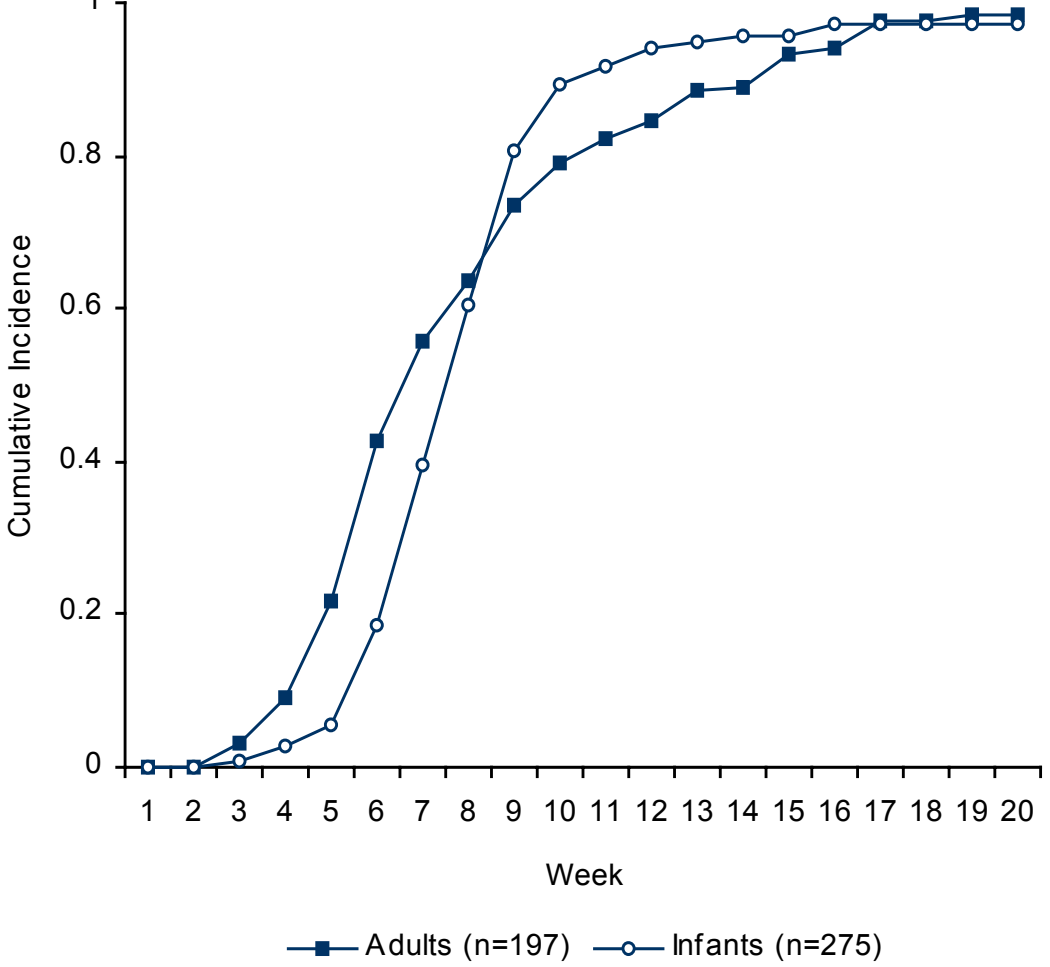


Figure 6.4: Cumulative Incidence of infection among adults and children

The rainfall patterns in the two years were also very similar (Fig. 6.1), although there was a significant difference in the parasite prevalence rate (56% and 28%) in the two cohorts at enrolment. The variation is within what has been reported previously in this area (Owusu-Agyei, unpublished data).

If pre-existing parasitaemia were protective against clinical malaria, then we would expect those individuals lacking parasites at baseline to be more at risk than parasitaemic individuals in the untreated cohort. The tendency, which we observed, was in this direction though the effect was not statistically significant. It is possible that this analysis was affected by misclassification of individuals with very low parasite densities since parasite densities at baseline were very low. We may argue that the role and significance of parasite retention may have been shown if we used PCR methods which is more sensitive and would have detected sub-patent parasitaemia among a lot more subjects who were classified negative by microscopy (Tirasophon *et al.* 1994).

The most obvious sources of bias would act to decrease the relative risk of episodes in the treated cohort. Many of the clinical episodes may have been caused by other pathogens but were classed as clinical malaria because of incidental parasitaemia (Smith, 1996). This could have happened in both cohorts, but we would expect less over-diagnosis of clinical malaria in the treated than in the untreated group, since the former were parasitaemic for much more of the time at risk. Similarly, the fact that the treated cohort became sick after less time at risk than the controls is remarkable since parasites were present in the controls from the start of follow-up. For these reasons it could even be the case that the effect of radical therapy on disease incidence is substantially greater than we estimated.

The fact that the density at first clinical malaria among the untreated group is much higher than the enrolment density is an evidence that these are mainly malaria episodes. The similarities between the densities at first clinical malaria and enrolment in the treated cohort might be used to argue that these are not really malaria episodes. However, since these episodes occurred disproportionately at the same time as parasites re-appeared, we believe that the explanation for the low densities is more likely to be that the treatment group became sick at lower parasite densities as a result of a loss of parasite tolerance.

The responses to Chloroquine treatment are also of interest. Though completion of the dose schedule was not directly observed, there was no reason to suspect a difference in compliance between the cohorts. The 9% parasitological failure rate is comparable with other studies carried out in the sub-region (Afari *et al.* 1992). Untreated individuals in holoendemic areas often harbour many clones of parasites (Ntoumi *et al.* 1995); (Beck *et al.* 1997),(Rougemont *et al.* 1991) and so the chances of a Chloroquine resistant strain being present is higher than the resistance rate for single infections. The absence of resistance among the treated group is probably due to these patients being re-infected with only one or a few strains of parasites.

Clinical malaria occurring after radical therapy may be distinctly different from the "natural" disease, and the implications of treating asymptomatic individuals may be very different in adults than for children. Intermittent treatment in a highly exposed population has been shown to be of benefit in the first year of life (Schellenberg *et al.* 2001). As radical cure is a part of the standard strategy for evaluating malaria vaccines [8] these findings may have significance for the design and interpretation of such trials. Clinical malaria as an outcome needs to be evaluated and such trials must provide adequate facilities to treat patients who become ill.

6.6 Acknowledgements

We thank the Kassena-Nankana community in northern Ghana for their co-operation and willingness to participate in this study. We are also grateful to Professor Peter Smith for his invaluable advice and comments on the manuscript. Sincere appreciation also goes to the field and laboratory staff of the Navrongo Health Research Centre and Noguchi Memorial Institute for Medical Research for technical assistance. We also thank Drs Kweku Enos and Thomas Mensah Afful of the War Memorial Hospital, Navrongo as well as Robert Alirigia of Navrongo Health Research Centre.

This research was approved by scientific and ethical review boards of the Ministry of Health of Ghana and was conducted in accordance with regulations governing the protection of human subjects in medical research.

The Navrongo Health Research Centre, Ministry of Health of Ghana, mainly supported this work. We acknowledge the core support of the "MARS" study (on which this study was piggy-backed) by the US Naval Medical Research Center independent research initiative funds and the STO F6.1 work unit 61102AA0101.BXF.1431 of the Military Infectious Disease Research Program.

Chapter 7

Severe Anaemia in Young Children after High and Low Malaria Transmission seasons in the Kassena-Nankana District of Northern Ghana

K. A. Koram[1], S. Owusu-Agyei[2], G. Utz[3], F. Binka[2] , K. Baird[4], S. L. Hoffman[4], and F. K. Nkrumah[1]

[1]Noguchi Memorial Institute for Medical Research, University of Ghana, Legon, Ghana
[2]Navrongo Health Research Centre, Ministry of Health, Navrongo, Ghana
[3]US Naval Medical Research Unit #3, Cairo, Egypt
[4]Naval Medical Research Center, Rockville, Maryland, USA

This article has been published in:
The American Journal of Tropical Medicine and Hygiene (2000), 62 (6), 670-674

7.1 Abstract

Malaria and anaemia accounted for respectively 41% and 18% of hospital deaths in the Kassena-Nankana district of northern Ghana during 1996. We measured haemoglobin (Hb), malaria prevalence, and anthropometric indices of 6-24 months old infants and young children randomly selected from this community at the end of the high (May – October, n = 347) and low (November – April, n = 286) malaria transmission seasons. High transmission season is characterised by rainfall (800 – 900 mm/yr.); while the remaining months receive less than 50 mm/yr. Severe anaemia, defined as Hb < 6.0g/dL, was 22.1% at the end of the high transmission season compared to 1.4% at the end of the low transmission season, [OR (95% CI) = 20.1 (7.1 – 55.3)]. Parasitaemia was 71% and 54.3% at these time points [OR (95% CI) = 2.1 (1.5 - 2.9)]. Nutritional anaemia appeared to have little impact upon this seasonal difference since anthropometric indices were comparable. Although the relative contributions of other causes of severe anaemia were not assessed, repeated malaria infections may be a primary determinant of severe anaemia among infants and young children during the high transmission season.

7.2 Introduction

Anaemia is one of the leading causes of childhood morbidity and mortality world-wide (World Health Organisation, 1996c). In malaria endemic areas, anaemia is among the leading causes of morbidity and mortality in hospitalised patients. It has been observed that in malaria endemic areas, the incidence of severe anaemia and age specific rates of anaemia are strongly correlated with the intensity of *Plasmodium falciparum* transmission (Clark *et al.* 1949) and significant improvements in haematological indices have been seen after malaria control trials (Alonso *et al.* 1991); (Greenwood *et al.* 1989). While there may be several causes for the anaemia, *P. falciparum* infection is believed to be a major contributory factor to the aetiology of severe anaemia seen in malaria endemic areas. This may be as a result of direct destruction of parasitised red blood cells, through immune mechanisms that destroy both parasitised and non-parasitised red cells, or suppression of the bone marrow as a result of the infection. Kurtzhals and colleagues have recently reported that some of these mechanisms may

operate in asymptomatic infections in Ghanaian school children (Kurtzhals *et al.* 1997). We report here the prevalence of severe anaemia (haemoglobin concentration < 6.0g/dL) seen among infants and young children aged 6 to 24 months at the end of the high and low malaria transmission seasons in the Kassena-Nankana District. This study was part of a larger study that investigated the incidence of new malaria infections among the population, which will be reported separately.

7.3 Methods

Study Area: The study was conducted in the Kassena-Nankana district in northern Ghana, an area that has been demographically well characterised previously (Binka *et al.* 1994). In summary, the district lies in the sub-Sahelian region of West Africa and is bounded on the north by part of the international border between Ghana and Burkina Faso. Annual rainfall averages approximately 850 mm almost all of which occurs in the wet months of May – September, the rest of the year being dry. One of the main features of the area is a large reservoir in the centre of the district that provides water for irrigation. Malaria transmission occurs throughout the year although there is very little transmission in the district towards the end of the dry season, except for compounds sited near the few permanent bodies of water (Binka *et al.* 1994).

Study Population: The district population of approximately 141,000 has been followed up regularly since 1993. The children who participated in the present study were randomly selected from enumeration clusters of the Navrongo Demographic Surveillance System (NDSS). Two cross sectional surveys were conducted at the end of the low and high malaria transmission seasons to select children for a malaria incidence study. Infants/ children were eligible to enter the study if they were aged between 6 and 24 months at the beginning of the study, were not suffering from any acute or chronic illness at the time of enrolment, were going to reside in the area for the following 6 months and their parent(s) or guardian(s) agreed to their participation in the study. These criteria were applied for entry into the malaria incidence study. Investigation review boards at Naval Medical Research Centre, NAMRU #3 and the Ministry of Health, Ghana approved the study protocol.

At enrolment, all the children were weighed, examined physically for signs of illness, and their axillary temperature taken. A blood smear was made for the identification and quantification of malaria parasites and haemoglobin estimated using a Hemocue® photometer (Leo Diagnostics, Sweden). They were also screened for the presence of G-6-PD deficiency using a qualitative visual fluorescence method for the presence of G-6-PD in whole blood (Sigma Diagnostics, USA). The test is used to distinguish normal from grossly deficient samples. Haemoglobin genotype was determined by gel electrophoresis of a filter paper blot at NMIMR. All children who qualified whose parents gave informed consent to take part in the study were then enrolled. Children with haemoglobin concentration less than 6g/dL were excluded from the associated malaria incidence study but are included in this report. All blood slides were stained with Giemsa stain and examined under oil immersion with a light microscope (ocular magnification 10X and objective 100X). All microscopic examinations were done the same day by the study microscopist. Parasite densities were estimated by determining the number of parasites per 200 white blood cells and multiplying by 40. A slide was declared negative after examination of 200 high power fields.

Data analysis: Differences between the two child cohorts (high and low transmission seasons) with respect to malariometric indices were tested using χ^2 for proportions and Student's *t*-distribution for means. Parasite counts were log transformed [log(x+1)] and densities reported as geometric mean densities [antilog of Σlog(x+1)/n) where x is the parasite count per μl of blood and n is the total number of children with parasitaemia]. Statistical significance is reported at $p < 0.05$.

7.4 Results

A total of 347 children were screened at the end of the high transmission season in November, 1996 and 286 children were screened at the end of the low transmission season in May 1997. The two child cohorts were comparable in terms of age, sex and anthropometric characteristics (Table 7.1). This was expected as each child cohort was selected randomly from the database of the Navrongo Demographic Surveillance System (NDSS). The prevalence of Glucose 6 Phosphate Dehydrogenase (G-6-PD)

deficiency and haemoglobinopathies were also similar in the two groups of children and no episode of hemolysis was observed following administration of primaquine for radical cure.

Table 7.1. General characteristics of study children

	First child cohort (end of high transmission Season); n = 347	2nd child cohort (End of low transm. Season); n = 286
Mean age months (95% CI)	15.0 (14.4, 15.6)	14.8 (14.1, 15.4)
Sex		
Males	51.9%	50.2%
Females	48.1%	49.8%
Mean WFA-z-Score (95% CI)	-2.20 (-2.3140, -2.0264)	-2.19 (-2.3471, -2.0366)
% with G-6-PD deficiency	7.1%	10.05%
Hb phenotype (%)		
A	66.2%	69.6%
AS/AC	29.7%	26.9%
SS/SC/CC	4.1%	3.5%

Table 7.2 shows the results of univariate analysis comparing children seen at the end of the high transmission season with those seen at the end of the low transmission season. An arbitrary haemoglobin concentration of 6.0g/dL was used to define eligibility into the malaria incidence study on the grounds of anaemia. Based on this criterion, more than one out of every 5 children (22.1%) screened at the end of the high transmission season was found to be ineligible.

Table 7.2. Comparison of the cohorts seen at the end of the high and low transmission seasons in the Kassena-Nankana District.

Characteristic	First child cohort. (end of high transmission season)(N = 347)	Second child cohort. (end of low transmission season). (N = 286)	OR (95% CI)	P value
Parasite rate	70.0%	54.3%	2.1 (1.5, 2.9)	0.001
Parasite Density[a]	1074 (40 - 103,990)	1118 (40 - 39,520)	__	NS [b]
Proportion with Hb. < 6.0g/dL	22.1%	1.4%	20.1 (7.1, 55.3)	0.0001
Mean Hb. g/dL (95% CI)	7.2 (7.0, 7.4)	8.9 (8.7, 9.1)	__	0.00001 [b]
Proportion of children with Hb. < 6.0g/dL and parasitaemia	81.8% (n = 66)	100% (n = 4)	0 (0, 8.11)	*NS* [c]
Proportion of children with Hb. ≥ 6.0g/dL and parasitaemia	67.9% (n = 259)	53.6% (n = 278)	1.84 (1.27, 2.65)	*0.001*
Proportion of children with fever	10.8%	3.3%	3.56 (1.69, 7.52)	*0.0001*
Proportion of febrile children with severe anaemia(Hb<6.0g/dL)	43.2% (n = 37)	0% (n = 9)	__	0.02 [c]
Proportion of febrile children with parasitaemia	71.4% (n = 37)	77.8% (n = 9)	__	*NS*

OR = odds ratio; CI = confidence interval; Hb = haemoglobin; [a]Geometric Mean Parasite Density (range); [b]Student's t-test; [c]Fisher's exact test; NS = Not significant at P < 0.05

This figure was significantly lower at the end of the low transmission season (1.4%); [OR (95% CI) = 20.1 (7.1, 55.3); p < 0.0001]. At the end of the high transmission season, the mean haemoglobin concentration was 7.2 g/dL (7.0, 7.4) compared to 8.9 (8.7, 9.1) at the end of the low transmission season (p < 0.0001). Figure 7.1 shows the distribution of haemoglobin values seen among the two child cohorts. Almost all

haemoglobin values below 6 g/dL were seen at the end of the high transmission season while haemoglobin values above 12 g/dL were mainly seen among children at the end of the low transmission season.

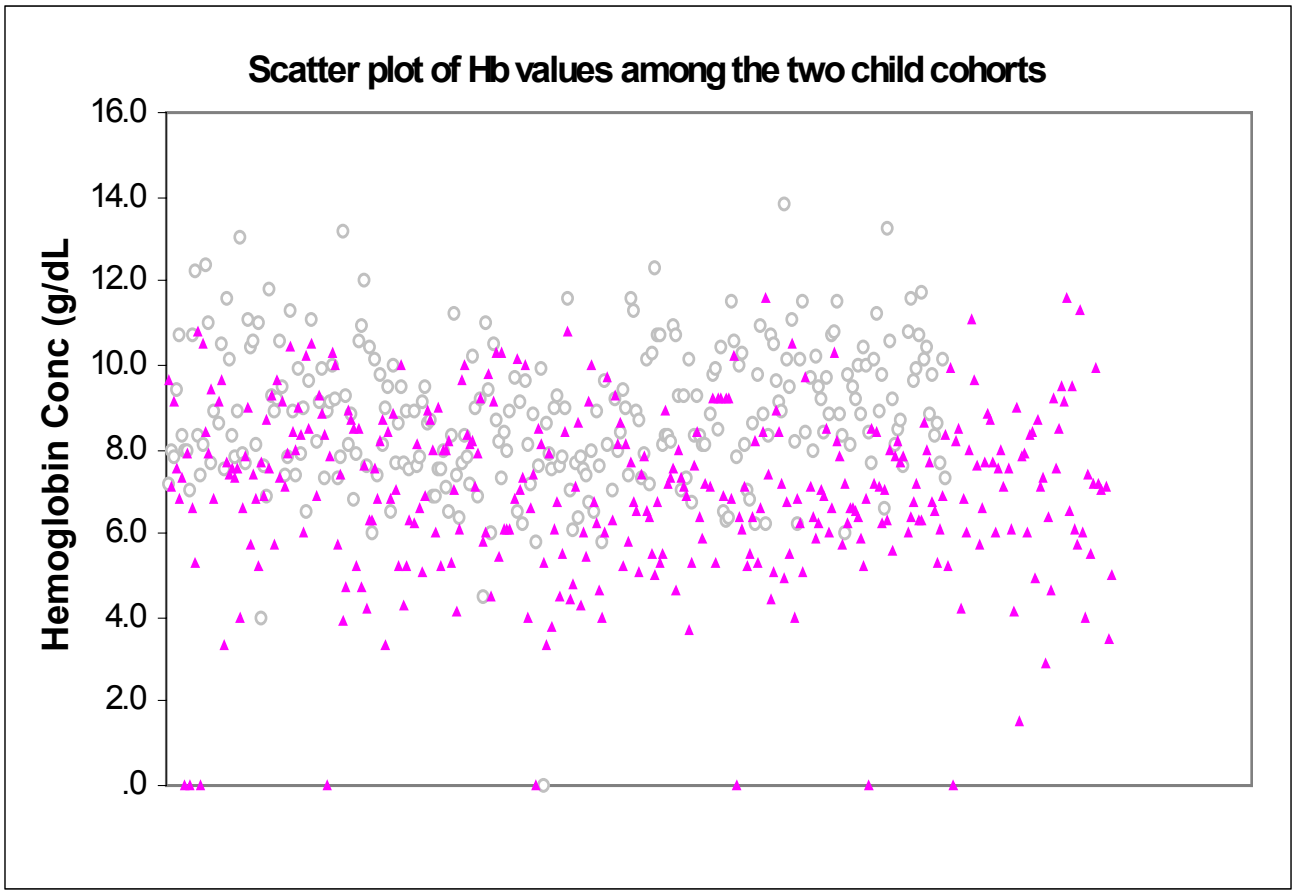


Figure 7.1: Scattergram showing the distribution of haemoglobin values by age (6-24 months) seen among the two cohorts, the first child cohort seen at the end of the high transmission season (▲) and the second child cohort (○) seen at the end of the low transmission. Many more children seen at the end of the high transmission season had haemoglobin values below 6 g/dL while all of those with Hb > 12 g/dL were seen after the low transmission season.

The prevalence of fever (axillary temperature ≥ 37.5^{o} C) was significantly higher (10.8%) at the end of the high transmission season (range, 37.5 ^{o} C – 39.7 ^{o} C; mean 38.1 ^{o} C) than at the end of the low transmission season (3.3%) [OR (95% CI) = 3.56 (1.69, 7.52), p < 0.001]; (range, 37.5 ^{o} C – 39.2 ^{o} C, mean 37.9 ^{o} C). Forty three percent (16/37) of febrile children seen at the end of the high transmission season were also severely

anaemic while none of the nine febrile children at the end of the low transmission season were anaemic. The mean Hb of febrile children at the end of the high transmission season was 6.3 (5.7, 7.0) g/dL compared to 8.3 (7.2, 9.4) g/dL for febrile children seen at the end of the low transmission season [p = 0.005]. Indeed, even for afebrile children the mean haemoglobin level was significantly lower at the end of the high transmission season [mean Hb = 7.3 (7.1, 7.5)] than that at the end of the low transmission season [mean Hb = 8.9 (8.7, 9.1), p < 0.001]. At the end of the high transmission season the mean (Hb) was lower in those with fever than those without fever [6.3 (5.7, 7.0) g/dL vs. 7.3 (7.1, 7.5), p = 0.002. This difference was not found at the end of the low transmission season.

Infants/ young children seen at the end of the high transmission season were more likely to have *P. falciparum* infection (70.1%) than children seen at the end of the low transmission season (54.3%); [OR (95% CI) = 2.1 (1.5, 2.9), p < 0.001]. Infections were almost entirely due to *P. falciparum*, which accounted for more than 98% of infections seen. Other infections were due to *P. malariae* and *P. ovale*. The geometric mean parasite densities were similar at the end of both transmission seasons [high transmission season, mean = 1111, range (40 – 103,900) and low transmission season, mean = 1118, range (40 – 39,520)]. However, heavy infections (parasite densities $\geq$ 20,000 /μl) were more prevalent at the end of the high transmission season (5.1%) compared to the low transmission season (2.6%) but this difference was not statistically significant [OR (95% CI) = 2.01 (0.59, 8.72)]. Febrile children tended to have higher parasite densities than afebrile children. At the end of the high transmission season the geometric mean parasite densities among febrile and afebrile children were 1879 (857, 4124) and 1026 (795, 1326) respectively, (p = 0.13). The respective mean values seen at the end of the low transmission season were 2631 (290, 23,845) and 996 (720, 1377), (p = 0.20).

The prevalence of anaemia was also analysed with respect to parasite densities. At similar intensities of parasitaemia children seen at the end of the high transmission season were more likely to have lower Hb than children seen at the end of the low

transmission season (Fig. 7.2). The differences in the mean Hb concentrations were significantly different at the lower parasite densities but not at the higher parasite densities. However, Hb concentration was still considerably less in children seen at the end of the high transmission season even at the higher parasite densities.

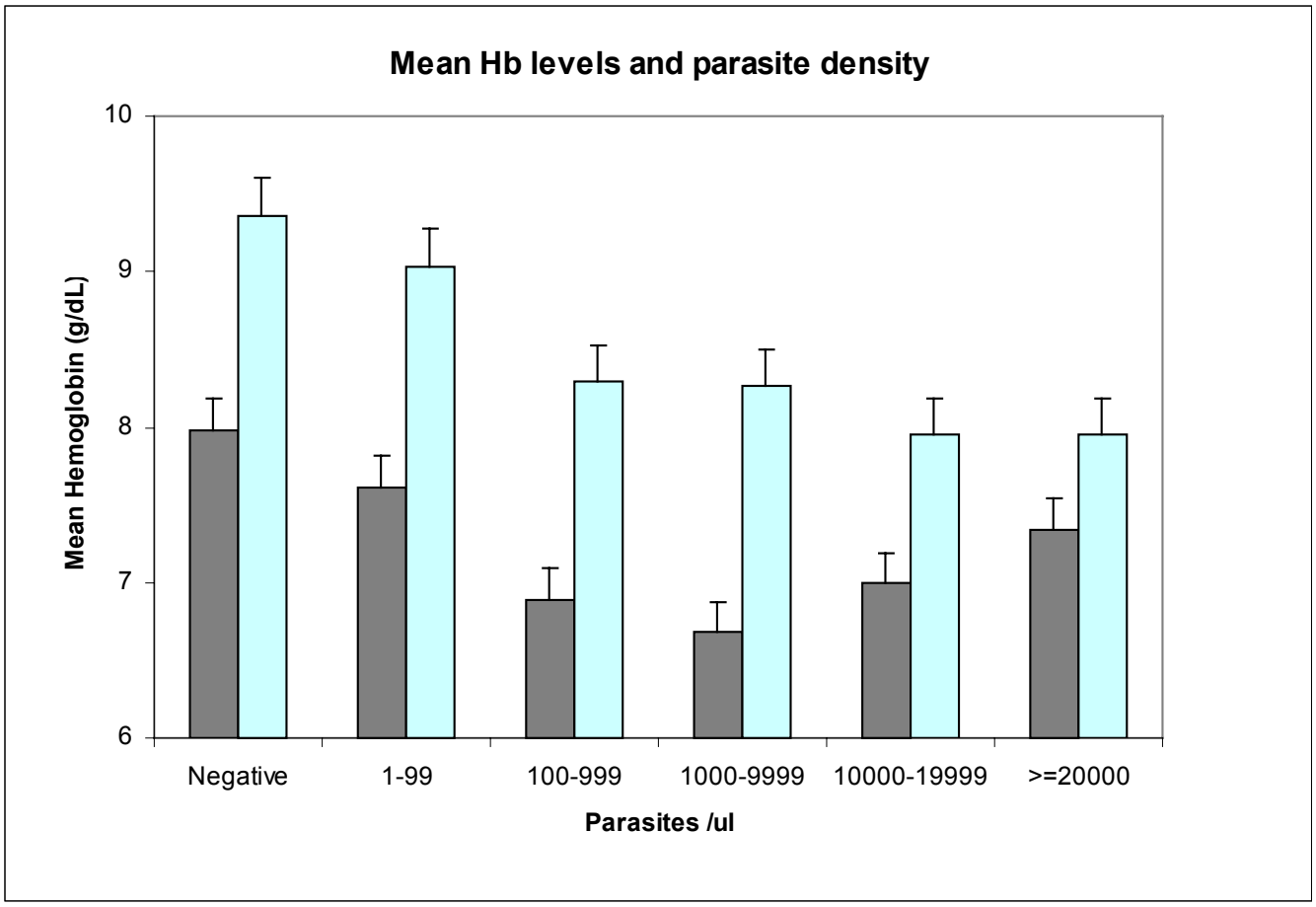


Figure 7.2: Chart showing the mean haemoglobin levels and average parasite density among children seen at the end of the high transmission season (dark bars) and at the end of the low transmission season (lighter bars). In all categories of parasite densities, mean haemoglobin values were higher at the end of the low transmission season compared to the high transmission season. Error bars are standard errors of the mean.

7.5 Discussion

The primary objective of the main study was to determine the incidence of *P. falciparum* infections in infants/ young children aged 6-24 months in the Kassena-Nankana District. In this paper we have reported data acquired at the time of enrolment into the longitudinal study. A very high prevalence of anaemia was observed at the end of the

wet season, corresponding to the high malaria transmission season in the district (Binka *et al.* 1994). Among this randomly selected cohort of children, the prevalence of anaemia (haemoglobin < 6.0g/dL) was 22.1% at the end of the high malaria transmission season compared to 1.4% among children seen at the end of the low transmission season. The prevalence of parasitaemia was 70% at the end of the high transmission season and 54.3% at the end of the low transmission season. However, there was no difference between the intensity of infection as measured by geometric mean parasitaemia in those with parasites seen at the end of both transmission seasons. There was also no seasonal variation in nutritional status as measured by anthropometric indices. Febrile episodes were more common at the end of the high transmission season and a higher proportion of febrile children had coincident parasitaemia.

Anaemia in children is a commonly reported problem from hospitals and clinics in malaria endemic countries. In the Kassena-Nankana District it accounted for approximately 20% of all admissions in the year preceding the commencement of this study. In a recent study in the same area (Binka *et al.* 1994), Binka and colleagues reported significant malaria morbidity among infants/ young children of 6–24 months of age. In that study the authors reported that children in this age group suffer most from the effects of *P. falciparum* infection, although parasite rates in this population peak at a later age. Young children aged 6-24 months had the lowest mean haemoglobin levels, the highest geometric mean parasite densities and the highest prevalence of fever coincident with *P. falciparum* infection.

Infection with *P. falciparum* is known to be one of the major causes of childhood anaemia in malaria endemic areas. In epidemiological studies a strong correlation has been reported between the incidence of severe anaemia, age specific rates of anaemia and the intensity of *P. falciparum* transmission and also malaria control trials have usually been followed by marked improvements in haematological indices in children (Alonso *et al.* 1991); (Greenwood *et al.* 1989); (Molyneux *et al.* 1980). Malaria transmission in this area is intense and seasonal with most of the infections occurring

during the rainy season. In the main study, the malaria attack rate was 8 infections per child per year during the rainy season compared to 5.2 per child per year during the dry season. And during the dry season almost all infections occurred during the first part of the season (attack rate = 5.1 per child per year) compared to the latter part when the attack rate was only 0.9 infections per child per year (Baird et al, 2001). Children were therefore very likely to have suffered from repeated attacks of malaria during this period. If the attacks were frequent such that there was not enough time for complete recovery from one infection, a marked drop in haemoglobin levels would have resulted by the end of the transmission season.

The mean haemoglobin level at the end of the high transmission season was significantly lower than that reported in the same age group of children from the previous study by (Binka *et al.* 1994). With the spread of Chloroquine resistance in sub-Saharan Africa it is likely that the efficacy of Chloroquine treatment will be reduced considerably in most areas resulting in poor haematological recovery, especially in children in areas of intense malaria transmission. Such a situation has been reported from sites in East Africa, where there is high prevalence of Chloroquine resistant *P. falciparum* (Bloland *et al.* 1993), and recently in Cote d'Ivoire with only moderate level of Chloroquine resistant *P. falciparum* (Henry *et al.* 1996). In a recent study of the situation in the country, only 60% of infections in the northern part of the country were found to be sensitive to Chloroquine treatment with the prevalence of RI responses as high as 31% (Koram, unpublished data). Thus, the frequent infections during the high transmission season coupled with a probable reduction in the efficacy of Chloroquine treatment, the drug of choice in the area, are likely to have contributed to the 22% prevalence of severe anaemia seen at the end of the high transmission season.

In this study, the parasite densities were similar at the end of both transmission seasons. The degree of anaemia associated with *P. falciparum* infection has not always been proportional to the severity of parasitaemia seen and anaemia has often been reported to be found at times when the parasitaemia has waned (Jilly & Nkrumah,. 1964); (Nkrumah, 1973). This could be due to the fact that, most of the reported studies

determined the level of anaemia and the intensity of infection at the same time. Presumably, the level of anaemia is a cumulative effect of several episodes of infection and the intensity of infection determined at the same time as the haemoglobin concentration is not a good indicator of the haemoglobin concentration. The report presented here is also the results of two cross sectional studies and would have suffered from the same deficiencies. However, in our larger incidence study, the frequency of infections was significantly higher in the wet season than in the dry season (Baird et al., 2001).

Malarial anaemia has been postulated to be secondary to direct destruction of both infected and non-infected rbc's and also to suppression of bone marrow function as a result of the infection (Kurtzhals *et al.* 1997); (Topley, 1968) in The Gambia showed that the seasonal fall in haemoglobin values in adults could be prevented if malaria parasitaemia was cleared or reduced by administration of Sulphadoxine-Pyerimethamine prophylaxis. The relatively high prevalence of anaemia observed at the end of the high transmission season opens up the possibility of using incidence of anaemia as a primary outcome variable for the effects of novel intervention tools such as vaccines, at least in areas of high malaria transmission.

7.6 Acknowledgements

We are grateful to the mothers and children of the Kassena-Nankana district who kindly allowed us into their compounds to carry out the study, our field workers at the Navrongo Health Research Centre for help with the field work, Mr. Charles Attiogbe of NMIMR for technical assistance and the staff of the Navrongo Health Research Centre for technical support, and Dr. Tom Richie of the US Naval Medical Research Center for suggestions regarding the manuscript.

Financial support: This work was supported by US Naval Medical Research and Development Command independent research initiative funds and STO F6.1 work unit 61102AA0101.BXF. 1431 of the Military Infectious Diseases Research Program. The

views of authors expressed herein are their own and do not purport to reflect those of the US Navy or the US Department of Defence or the Ghanaian Ministry of Health.

Chapter 8:

Characteristics of severe anaemia and its association with malaria in young children of the Kassena-Nankana District of northern Ghana.

Seth Owusu-Agyei[1], David J. Fryauff[2] Daniel Chandramohan[3], Kwadwo A. Koram[4], Fred N. Binka[5], Francis K. Nkrumah[4], Greg C. Utz[6], Kevin J. Baird[2] and Stephen L. Hoffman[7]

[1]Navrongo Health Research Centre, Ministry of Health, Navrongo, Ghana;
[2]Naval Medical Research Center, Silver Spring, MD, USA
[3]London School of Hygiene and Tropical Medicine, UK
[4]Noguchi Memorial Institute for Medical Research, University of Ghana, Legon,
[5]School of Public Health, University of Ghana, Legon, Ghana
[6]United States Naval Medical Research Unit No. 3, Cairo, Egypt
[7]Celera Genomics, Rockville, MD, USA;

This article has been submitted to
The American Journal of Tropical Medicine and Hygiene

8.1. Abstract

We sought to elucidate factors associated with severe anaemia in young Ghanaian children, 6-24 months old, by analysing two studies separated by 4 years, but conducted in the same community and at the same seasonal time point. Age-adjusted comparison confirmed that the proportion of severely anaemic children and overall mean Haemoglobin (Hb) levels, in the 2000 sample were significantly improved over those of the 1996 (17.5 % vs. 26.4%, P = 0.03; Hb 7.5 g/dL vs. 6.9 g/dL, P = 0.002). Weight for age z-scores also indicated a significant improvement in the 2000 sample (-1.93 vs. –2.20, P < 0.05). Independently, each survey identified statistically significant associations between severe anaemia and age, parasite rate, fever, and sex. Relative to children with Hb ≥ 6.0 g/dL, those with severe anaemia (Hb < 6.0 g/dL) were older, more frequently parasitaemic [OR (95% CI) = 1.60: (1.08-2.35)], more often febrile [OR: (95% CI) = 2.44 (1.71-3.48)], and predominantly male [OR (95% CI) = 1.50 (1.05-2.13)]. Evidence suggests that dramatic peaks and troughs of severe anaemia are regular and possibly predictable events that may be used to gauge the health and survival of young children in this area.

8.2. Introduction

Severe anaemia may be the principal underlying cause of malaria death in areas of intense seasonal malaria transmission such as the holoendemic Kassena-Nankana District (KND) of northern Ghana (Binka *et al.* 1994; Koram *et al.* 2000). A recent malaria/anaemia study of young children 6 to 24 months old in this community revealed that 22% of those sampled at the end of the wet season (November, 1996), a time corresponding to agricultural and nutritional abundance, had haemoglobin (Hb) concentrations below 6.0 g/dL. In contrast, survey of the same age cohort six months later, at the end of the dry season, and at a time of food scarcity found that only 1% of the children fell into this category of severe anaemia (Koram *et al.* 2000). With entomological inoculation rates in non-irrigated and irrigated sectors calculated to be, respectively, 72 and 800 infective bites per person-year (Binka et al., 1997), and a clear pattern of malaria deaths that mirrored rainfall (Binka *et al.* 1994) **,** it was reasoned that anaemia trends in this vulnerable age group were primarily influenced by the intensity of

malaria transmission and that dramatic troughs and peaks of severe anaemia are regular seasonal events. If this is the case, then proportions of young children with severe anaemia, rather than proportions dying, might serve as a more conservative and accessible measure of malaria vaccine effect. Towards this objective we sought to elucidate factors associated with or influencing severe anaemia in young children of this district by re-examining the previously collected data and by expanding the database with newly acquired results. We hypothesised that a repeat malaria-anaemia study of the same age groups and locations as previously studied, but at a time corresponding to the end of the 2000 wet season, would again show large numbers of young children with severe anaemia. Relative to the cohort with non-critical Hb, we also hypothesised that severe anaemia would be associated with parasitaemia, higher parasite density, more febrile illness, and residence proximal to perennial breeding sites of anopheline mosquitoes. Owing to natural decline by six months of maternally transferred protection (Akanmori *et al.* 1995), and the greater vulnerability to infection than malnutrition, low birth weight, and stunting conceivably impart, we further expected that severe anaemia might be more prevalent among older children in the 6-24 month range studied, and would be associated with higher rates of parasitaemia, greater parasite densities and more febrile illness.

8.3. Materials and Methods

Subjects and informed consent process. The study site and population has been described in published reports (Ghana VAST Study Team, 1993); (Binka *et al.* 1994). As with previous studies, use was made of the Navrongo Demographic Surveillance System (NDSS) a continually updated database that records virtually all births, deaths, and movements in the district's population of 141,000. An accurate name, age, and home location listing was made from this database of young children 6-24 months old on the first of November, 1996, and again in November, 2000. Respective parents and community leaders were identified, contacted, and given a detailed explanation of the study plan. Informed parents wishing to have their children tested for malaria and Hb gave their assent in writing or thumb print and brought their infants to a central location for registration and testing. This research was approved by the scientific and ethical

review boards of the Ghanaian Ministry of Health and the US Navy and was conducted in accordance with regulations governing the protection of human subjects in medical research.

Sample collection and screening. Children were assigned a study number based upon their consecutive order of appearance and given a brief physical examination. Axillary temperature was recorded and temperatures ≥ 37.5 were designated febrile. A sterile lancet was used to prick the heel or toe sufficient to make thick and thin blood films on a Snow clean-labelled slide. Approximately 5-µL additional blood was obtained for haemoglobin determination by means of the Haemocue photometer (Leo Diagnostics, Sweden). Haemoglobin readouts were transcribed into the child's record. Parents were informed when their child's Hb readout was below 6.0 g/dL and treatment was initiated. Based upon physical condition and Hb level, children were either administered daily treatment with oral ferrous sulphate solution or typed in preparation for transfusion by a malaria-free family member. Oral Chloroquine (25 mg/kg) was provided for all presumed cases of clinical malaria. Malaria slides were stained with Giemsa and examined under high power 100x oil immersion microscopy for the presence of malaria parasites. Parasite species were identified by morphology and parasite density per microlitre of blood was estimated from counts per 200 white blood cells and an assumption of 8000 lymphocytes/µL. Two hundred thick film fields were examined before assigning a negative malaria diagnosis.

Blood transfusion records. Daily record of blood transfusions performed in the single district hospital during the 18 month period of October 1999 to March 2001 were evaluated as a complementary "index" of severe anaemia in the district population. This activity recorded each recipient's name, address, age, sex, blood type, malaria status, and Hb level, as well as the identity and results of laboratory screening performed on donors.

8.4. Results

Full data set comparisons between times and cohorts. Severe anaemia was significantly more prevalent at the time of the 1996 survey than in 2000 (22% vs. 12.5%; $p < 0.0002$) (Table 8.1). The ages of children in both the Hb < 6.0 and Hb ≥ 6.0 cohorts of the 1996 survey were significantly greater than those of their respective 2000 cohorts ($P < 0.0001$), and mean Hb levels in these two 1996 cohorts, were significantly lower ($P = 0.005$) than their 2000 counterparts.

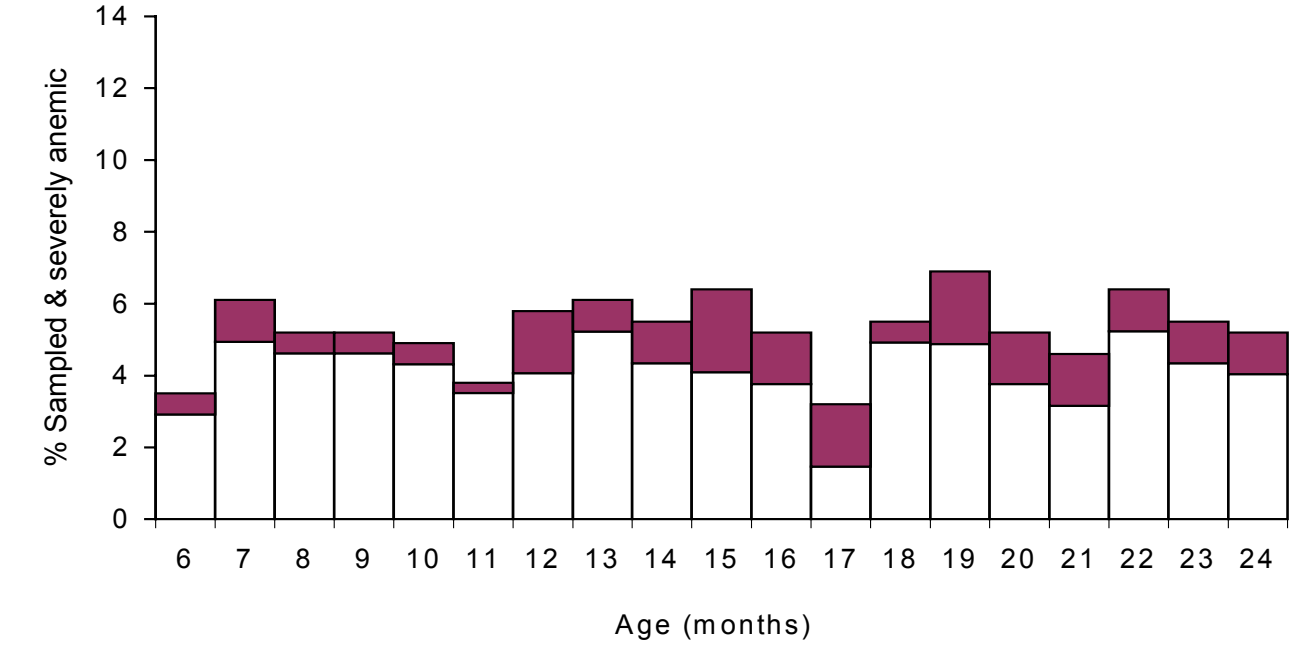
Table 8.1: Characteristics of severe anaemia (Hb < 6.0) and non-critical anaemia (Hb≥ 6.0) among two populations of Ghanaian children, 6-24 months old, surveyed in November 1996 November 2000.

Characteristic	November 1996		November 2000	
	Hb < 6.0 g/dL	**Hb ≥ 6.0 g/dL**	**Hb < 6.0 g/dL**	**Hb ≥ 6.0 g/dL**
Number (%)	75 (22.0)	266	87 (12.5)	608
- Males	45 (25.4)	132	51 (14.6)	299
- Females	30 (18.3)	134	36 (10.4)	309
Mn. Age (mos.) ± 95% CI	16.0 ± 1.9	15.0 ± 0.7	12.8 ± 0.7	12.0 ± 0.3
- Males	16.4 ± 1.5	15.3 ± 0.2	12.5 ± 1.0	12.1 ± 0.5
- Females	15.4 ± 0.4	14.6 ± 0.9	13.8 ± 1.2	11.8 ± 0.5
Mn. Hb (g/dL) ± 95% CI	4.8 ± 0.2	7.8 ± 0.2	5.2 ± 0.1	8.2 ± 0.1
- Males	4.9 ± 0.3	7.8 ± 0.2	5.3 ± 0.2	8.1 ± 0.1
- Females	4.8 ± 0.2	7.9 ± 0.2	5.1 ± 0.2	8.3 ± 0.1
No parasitaemic (%)	55 (76.0)	181 (68.0)	49 (65.3)	296 (55.9)
- Males	32/45 (71.1)	90/132 (68.2)	30/47 (63.8)	141/256 (55.1)
- Females	23/30 (83.3)	91/134 (67.9)	19/28 (67.8)	155/273 (56.8)
GMPD/uL (95% CI)	1288 (860 – 1928)	1106 (842 – 1452)	1644 (1062 – 2547)	1312 (1066 – 1614)
- Males	1248 (737 – 2115)	904 (614 – 1330)	2203 (1202 – 4036)	1306 (955 – 1977)
- Females	1345 (709 – 2551)	1349 (920 – 1977)	1037 (601 – 1791)	1315 (984 – 1758)
Febrile (%)	16/76 (21.0)	22/265 (8.3)	18/74 (24.3)	62/528 (11.7)
Febrile-parasitaemic (%)	12/55 (21.8)	15/181 (8.3)	12/48 (25.0)	39/295 (13.2)
- Males	7/32 (21.9)	7/90 (7.8)	7/30 (23.3)	20/141 (14.2)
- Females	5/23 (21.7)	8/91 (8.8)	5/18 (27.8)	19/154 (12.3)
Mn. Weight (kg) ± 95% CI	7.9 ± 0.3	8.0 ± 0.2	7.7 ± 0.3	7.6 ± 0.1
- Males	8.2 ± 0.5	8.3 ± 0.3	8.1 ± 0.4	7.9 ± 0.1
- Females	7.4 ± 0.4	7.6 ± 0.3	7.1 ± 0.4	7.3 ± 0.1

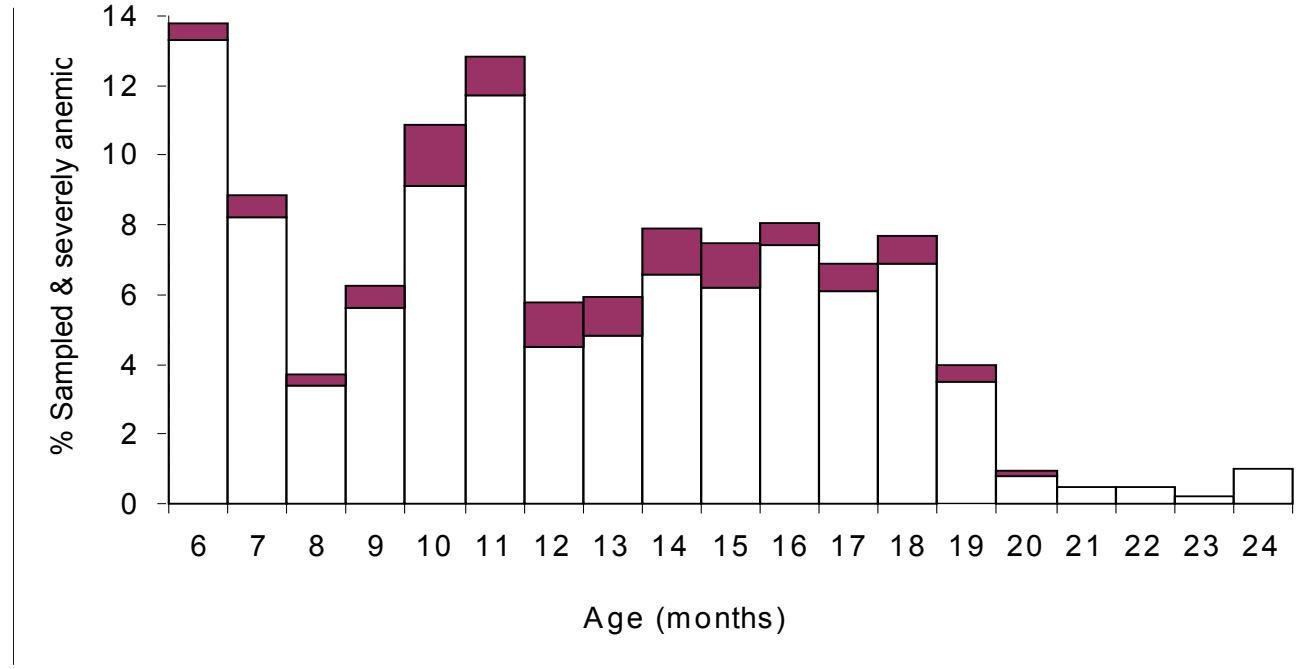
Moreover, significantly greater numbers of children, overall (69.2% vs. 57.1%, P < 0.001), as well as in the two Hb cohorts, were parasitaemic in the 1996 survey. Analysis of each sample population subdivided into severely anaemic (Hb < 6.0 g/dL), moderately anaemic (Hb 6.0-7.9 g/dL), and normal (Hb ≥ 8.0 g/dL) groups, revealed that severely anaemic children in both surveys were older (1996: 16.0 months. Vs 14.3 months, P = 0.03; 2000: 12.6 months vs. 11.4 months, P = 0.02) and contained fewer females than normal Hb groups (1996: 39.5% vs. 53%, P = 0.0002; 2000: 38.7% vs. 54%, P = 0.01). Relative to the normal Hb group, point prevalence of parasitaemia was significantly higher in both the moderately (P < 0.001) and severely anaemic (P = 0.05) children.

Age-stratified analysis. Figure 8.1, which plots the frequency distribution of ages sampled and proportions of severely anaemic children by age, shows uniformity in sampling over age groups in 1996, and no indication of declining rates of severe anaemia in the 18-24 month age range.

The 2000 survey, by contrast, sampled preferentially in the 6-11 month range. Children of 20-24 months, who comprised 27% of the 1996 sample and 29% of its severely anaemic cohort, comprised only 3% of the 2000 survey. To determine whether this bias may have been responsible for differences in prevalence of Hb between the 1996 and 2000 surveys, analysis was reapplied, but only to children 12-18 months of age in the two survey populations. This age-adjusted comparison confirmed that the proportion of severely anaemic children and overall mean Hb levels, in the 1996 sample were significantly different from those of the 2000 sample (26.4% vs. 17.5%, P = 0.03; 6.9 g/dL vs. 7.5 g/dL, P = 0.002). Differences between survey times for rates of parasitaemia in children 12-18 months old (69.2 % vs. 57.1%) and the GM parasitaemia of those with patent infections were not statistically significant.



A



B

Figure 8.1. Comparative frequency distributions of ages sampled and the proportions of severely anaemic (Hb < 6.0 g/dL) children detected by age group in two community-wide surveys of malaria-anaemia, **A**) November, 1996, and **B**) November, 2000.

Comparisons between males and females. Despite uneven age group sampling, nearly equal numbers of males and females were screened in the two surveys. No statistically significant differences were seen in either survey between males and females with regard to any measured characteristic except that of body weight.

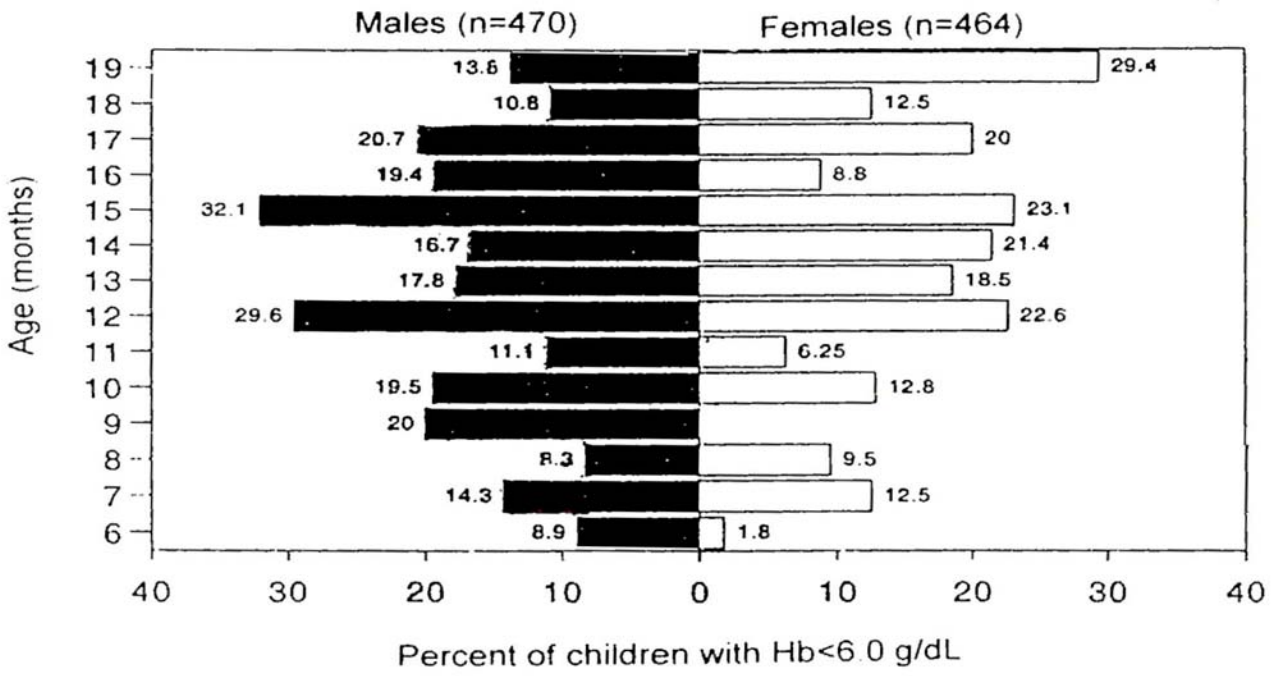


Figure 8.2. Paired frequency histogram of the combined 1996 and 2000 survey populations comparing proportions by age of severely anaemic (Hb < 6.0 g/dL) male and female children, 6-24 months old.

Female infants in both non-critical and severely anaemic cohorts weighed significantly less ($P < 0.02$) than their male counterparts. Interestingly, males accounted for the majority of severely anaemic children in both surveys, a difference that attained statistical significance when the two study populations were combined (males 18.2% vs. females 12.9%, $P = 0.02$). Figure 8.2, a paired frequency histogram of this combined population, comparing proportions by age of severely anaemic male and female children, shows similar profiles and the suggestion of age-relatedness; lowest proportions of severe anaemia in males and females were measured in the younger, under 12 month age groups. A negative correlation coefficient between age and Hb in the combined 1996 and 2000 population attests to this relationship ($r = -0.19$, $P < 0.01$).

Effect of irrigation and sector of residence. Analysis of the 1996 and 2000 survey populations was stratified according to whether children lived in irrigated or non-irrigated communities of the KND. Children residing in irrigated communities accounted for 57% of the 1996 survey population but comprised only 29% of the 2000 sample ($P < 0.0001$). Unexpectedly, no statistically significant differences were seen between survey years in malaria point prevalence (66.2% vs. 62.2%), frequency of severe anaemia (19.9% vs. 10.9%, $P = 0.11$), mean Hb levels (7.3 g/dL vs. 7.7 g/dL), or GM parasitaemia (891/μL vs. 1,349/μL) of children living in irrigated communities. Significant differences were seen in comparing between 1996 and 2000 "non-irrigated" cohorts of children; the expected higher frequencies in 1996 of parasitaemia (72% vs. 54.5%, $P = 0.0003$) and severe anaemia (25.3% vs. 13.8%, $P = 0.05$), and the overall mean Hb level (7.0 g/dL vs. 7.8 g/dL, $P < 0.05$). Analysis between the 1996 irrigated and non-irrigated cohorts revealed a significantly lower mean Hb level among severely anaemic children of the irrigated cohort (4.6 g/dL vs. 5.0 g/dL, $P = 0.03$) and significantly greater GM parasitaemia among those of the non-irrigated cohort (1,549/μL vs. 891/μL, $P = 0.02$). Measured characteristics of children in the November 2000 irrigated and non-irrigated cohorts were comparable.

Analysis by sector was performed to determine whether severe anaemia and malaria infection in these children might be associated with residence in a particular geographic zone within the district (North, South, East, West, and Central). Consistent differences were seen between sectors in the prevalence of severe anaemia with highest levels in both surveys associated with residence in the north (Fig. 8.3). Differences between zones in the prevalence of severe anaemia were most pronounced and statistically significant in the 2000 survey. Mean Hb levels of severely anaemic children did not differ appreciably among zones but in both surveys Hb levels for the larger cohorts of non-critical ($Hb \geq 6.0$ g/dL) children followed the same zonal pattern seen with the severely anaemic cohorts (Fig. 8.4).

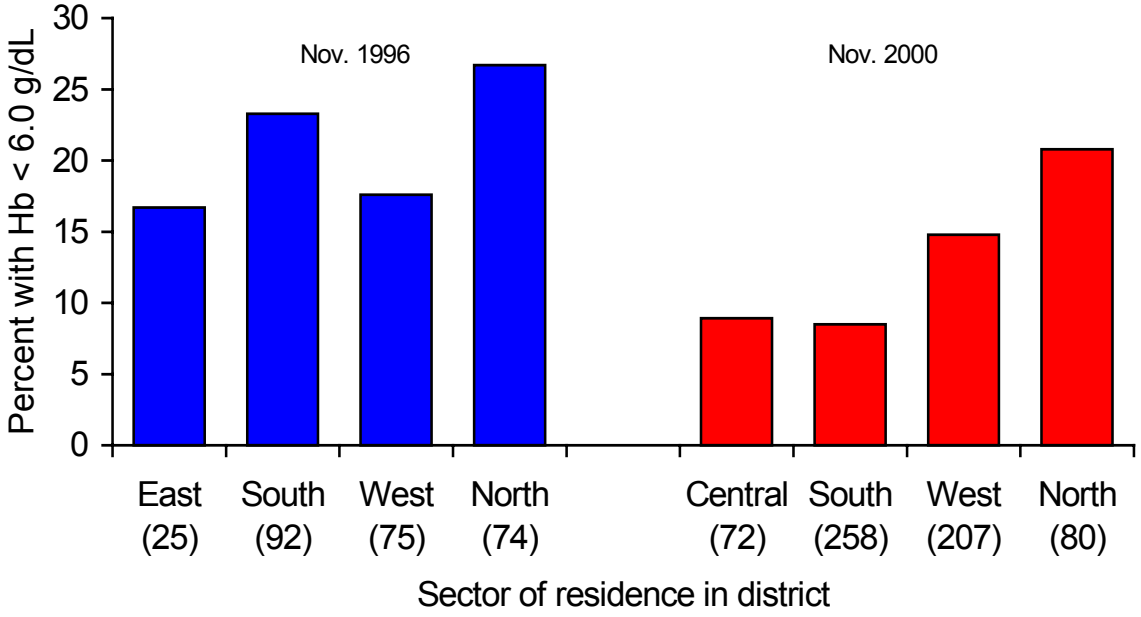


Figure 8.3. Point prevalence of severe anaemia (Hb < 6.0 g/dL) in young children according to geographic sector of residence within the Kassena-Nankana District, northern Ghana: comparison of 1996 and 2000 survey results.

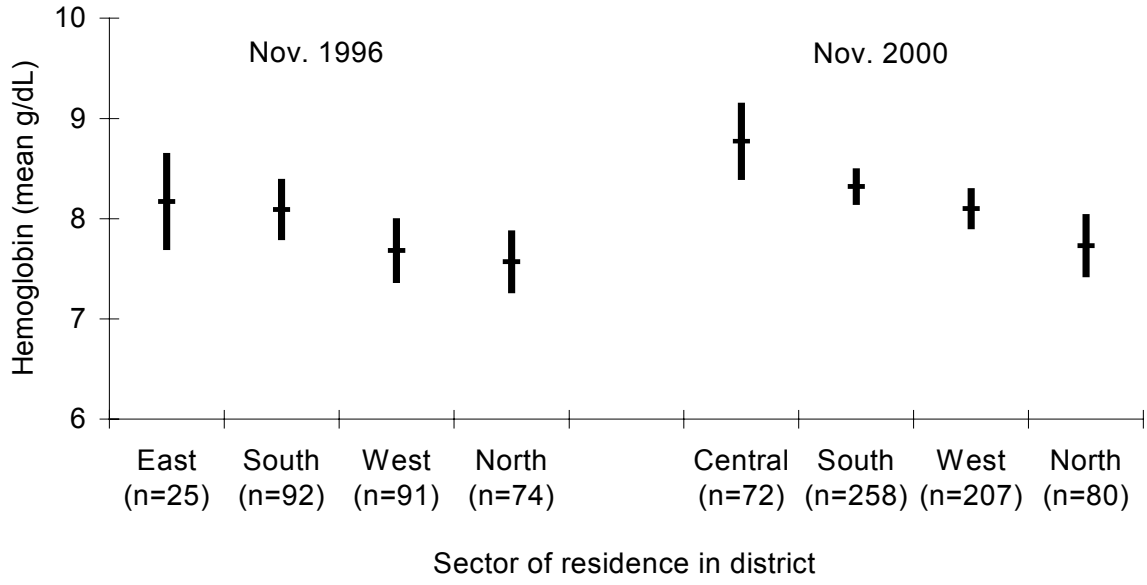


Figure 8.4. Mean haemoglobin levels in young, non-critically anaemic (Hb ≥ 6.0 g/dL) children according to geographic sector of residence within the Kassena-Nankana District, northern Ghana: comparison of 1996 and 2000 survey results

Blood transfusions as plausible marker of severe anaemia. Figure 8.5, which plots the monthly numbers of young paediatric (age < 60 months) transfusions and rainfall, shows that most transfusions went to infants under 25 months of age (381/485=78.6%) and there was a distinctly seasonal pattern in the frequency of occurrence. The monthly transfusion profile during 2000 for children < 2 years old appears to follow the very sharply demarcated period of rains with transfusions peaking one month after the month of greatest rainfall. As with severe anaemia, males accounted for the majority of young children receiving transfusions (57.5%); the frequency of transfusion for young female children being significantly lower than that expected under conditions of equality (50% vs. 42.5%, P = 0.01). There was no difference between sexes in the age and Hb levels of those receiving blood.

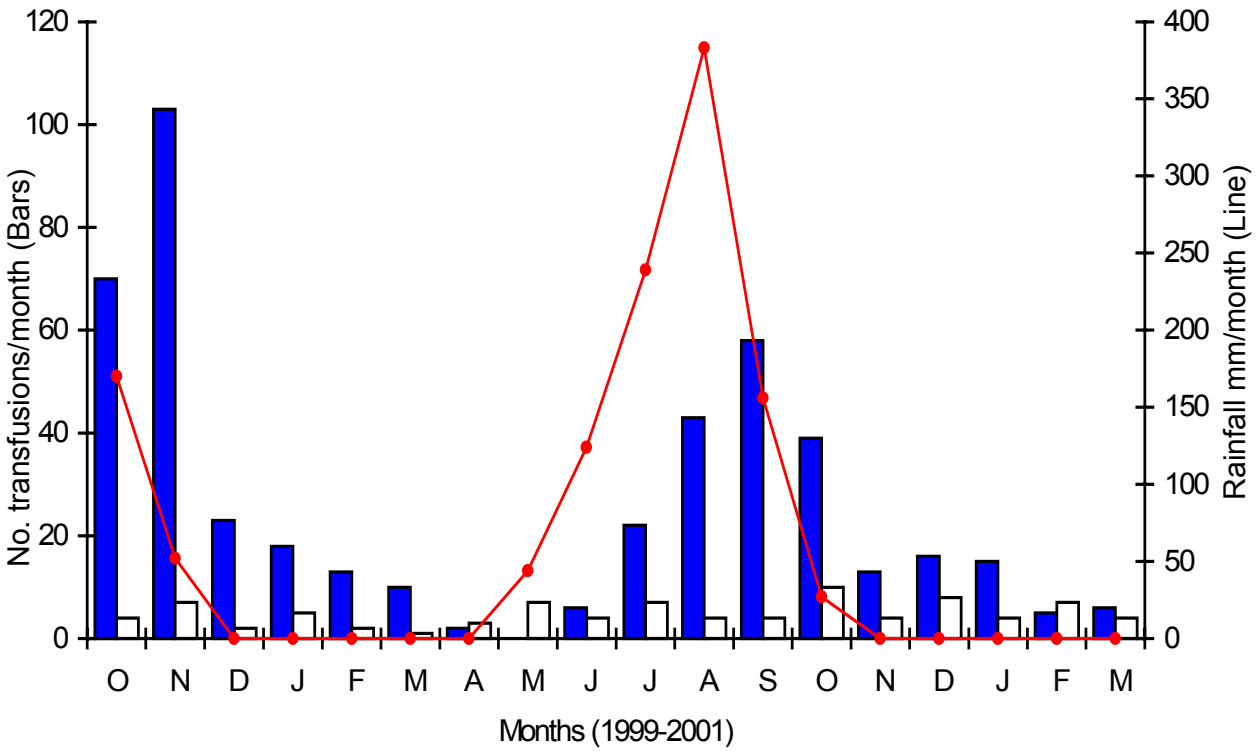


Figure 8.5. Monthly distribution of paediatric (0-5 years) transfusions during October 1999 to March 2001; separated into age ≥ 24 months (shaded bars) and 25-60 months (open bars).

Pair-wise comparison by age between male and female children (Fig. 8.6) shows that the majority of transfusion recipients in both sexes were of 5-12 months age and that relatively few children over 36 months of age were transfused.

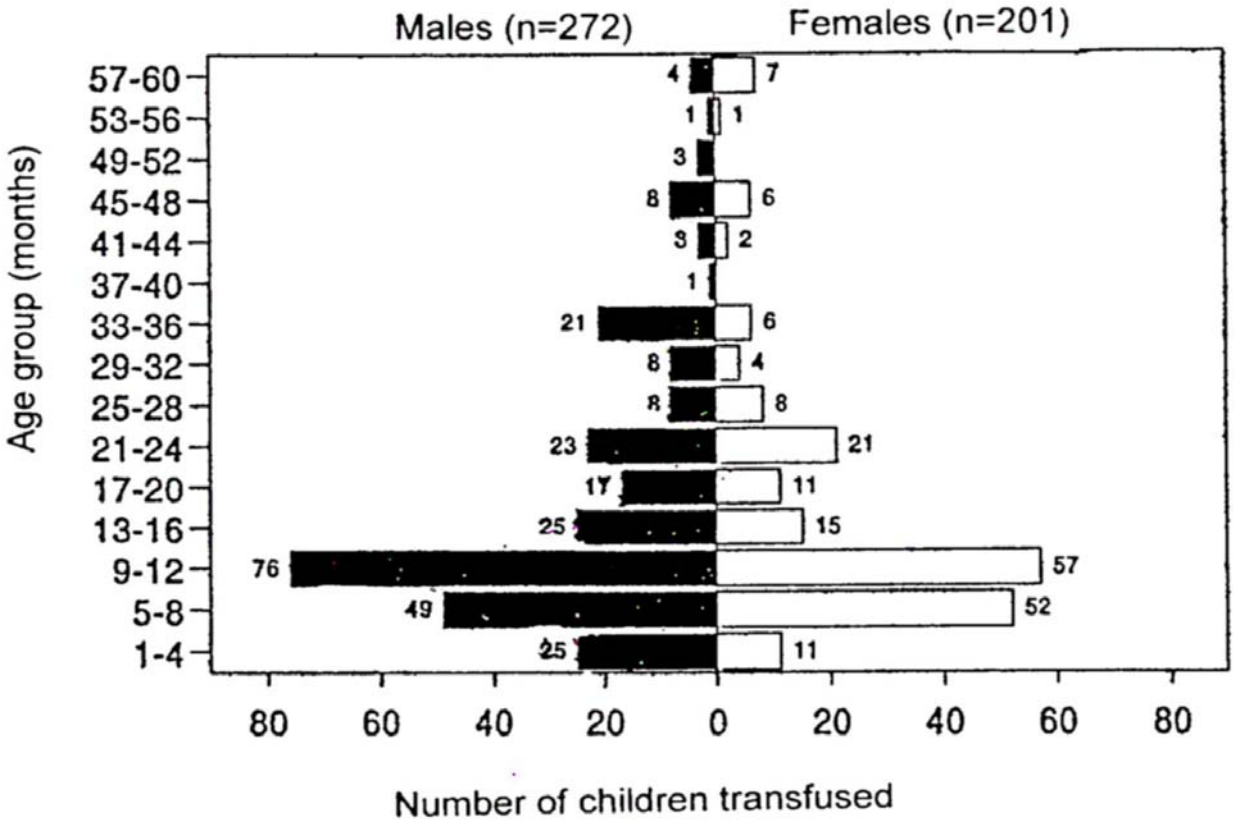


Figure 8.6. Pair-wise comparison by age between male and female children under 5 years old receiving at least 1 blood transfusion, for any reason, in the Kassena-Nankana District Hospital during October, 1999 to March, 2001.

8.5. Discussion

Two malaria-anaemia surveys, separated by 4 years, but conducted in the same community and at the same November time point corresponding to early dry season in the northern Ghana Sahel, both document alarmingly high rates and levels of anaemia in young children 6 to 24 months old. Independently, each survey identified associations between severe anaemia and age, parasite rate, fever, and sex. Relative to children with Hb $\geq$ 6.0 g/dL, those with severe anaemia were older, more frequently parasitaemic, more often febrile, and predominantly male. Our initial hypotheses that severe anaemia was associated with higher parasitaemias was not born out by analysis, nor was evidence obtained to suggest that severe anaemia was associated with younger, lower weight, female children.

Our classification of severe anaemia, less stringent than some investigations (Snow *et al.* 1998); (Tomashek *et al.* 2001); (McElroy *et al.* 2000), yet more stringent than others (Fleming, 1987), was pragmatic, and based on Ghanaian national health policy calling for clinical intervention in cases of Hb < 6.0 g/dL. By this classification, children diagnosed with Hb 5.0 to 5.9 g/dL received oral ferrous sulphate while those with Hb < 5.0 g/dL were transfused. Although more than 98% of our surveyed children fit the clinical definition of anaemia (Hb < 11.0 g/dL) and 12.9% (75/581) of the parasitaemias exceeded 10,000/μL, only 0.6% (6/945) fit the restrictive WHO classification of severe malaria anaemia[SMA] (Warrell *et al.* 1990). This low prevalence of SMA is in contrast with rates of 5.2% reported under conditions of fluctuating malaria in Malawi (Slutsker *et al.* 1994) and rates exceeding 30% from areas of intense perennial transmission (Lackritz *et al.* 1997); (Dorward *et al.* 1990). Notably, however, these high rates refer to predominantly male hospital admissions while our rate is derived from a random cross-section of households with equal composition of boys and girls.

The unfortunate fact of “household-level gender bias” that exists over much of the malarious world, is assumed to account for the reduced weight, health, and survival of infant girls, and the predominance of male hospital admissions for all causes (Schellenberg *et al.* 1999); (Slutsker *et al.* 1994). Hospital based studies, for this

reason, are unsuited and unable to detect important natural differences between males and females. In this regard, we are impressed by random survey data indicative of a lower risk of severe anaemia among infant girls where we had anticipated just the opposite. If this difference was not due simply to chance, one might speculate that the reduced nutritional state of females renders them less capable or less suitable hosts for parasite superinfection and density-related pathology of these infections. Such a protective mechanism may compensate in a small way for the greatly increased risks of death they will later face from malaria in pregnancy (Matteelli *et al.* 1994).

Sampling bias was initially considered to be the major cause underlying differences between our studies. There was evidence from the 1996 study that severe anaemia continued undiminished from 18 to 24 months, and beyond, but the 2000 population was not structured to sample these age groups and focused preferentially on children under 12 months, where severe anaemia rates had been lower. The 1996 population had also been balanced in terms of children residing in irrigated and non-irrigated communities, whereas the 2000 population sampled predominantly children from non-irrigated compounds. However, even after correcting for age bias, differences remained highly significant, suggesting that rates of severe anaemia, moderate anaemia, and parasitaemia may have improved from 1996. Monthly rainfall profiles for the KND show remarkable similarity over 20 years of recording, suggesting no aberrant pattern or volume as reasons for reduced malaria and anaemia in 2000. In fact, total rainfall in 2000 exceeded that of 1996 and closely followed the classic profile of the 20-year average for the district. It is possible that the findings of vitamin A supplement trials (Ghana VAST Study Team, 1993) and treated studies (Binka *et al.* 1996; Binka *et al.* 1997; Binka *et al.* 1998) may have become policy and widespread practice by November 2000. The 1996 Ghanaian Ministry of Health Policy of free-medical care and medicines for pregnant women and children under 5 years old also may have reduced malaria morbidity and mortality in children of the KND by November, 2000.

Twenty years of rainfall records show consistently that the July-August-September quarter is the high point of the KND wet seasons each year. Detailed studies in the

KND have identified a corresponding pattern of child mortality in which rates increased four-fold from April, the last month of dry season to August, the month of greatest rainfall. Rates of febrile illness and parasitaemia, and mean Hb levels were similarly correlated with rainfall; infants of 6-24 months age being most affected (Binka *et al.* 1994). However, the regularity of the rainfall pattern does not translate over to an invariable pattern of malaria morbidity and death. Young children studied over a three year period in the KND experienced highest death rates in the first two years (1992 & 1993), during the July-September quarter, but in 1994, during the early dry season quarter of October-December (Binka *et al.* 1996). Blood transfusions performed on young children during two consecutive years appear to follow a pattern closely dictated by rainfall and malaria transmission. However, despite very comparable rainfall profiles in the two years, November stood out in 1999, as opposed to September in 2000, as the peak month for paediatric transfusions. We concede that other reasons may account for a portion of the transfusions done each month, but we believe that malaria-induced anaemia is the dominant factor underlying paediatric transfusions. Further to this, we speculate that longitudinal profiling of infant deaths as well as rates of severe anaemia would closely mirror the transfusion profile each year.

We obtained no evidence of positive or negative correlation between parasite density and age, weight, or Hb in either individual or combined survey populations, but did identify a relatively weak, yet statistically significant, negative correlation between age and Hb. Previous study of children 0-5 years old at this location had reported a significant negative correlation between GM parasite density and Hb level (Binka *et al.* 1996). This relationship was not apparent in our surveys, and may have resulted from focusing upon younger children of 6-24 months. Longitudinal study of Kenyan infants from birth also determined an association between concurrent parasitaemia and lower mean Hb, but required multiple slide readings over 90 days to show that significantly lower mean Hb levels were related to significantly higher mean parasite densities (McElroy *et al.* 2000).

It is well recognised that *P. falciparum* causes red cell destruction far in excess of that explained by parasitaemia (Greenwood *et al.* 1987); (Menendez *et al.* 2000). Low level parasitaemia and chronic asymptomatic malaria, such as that seen in our study population, is hypothesised to produce a frank suppression of bone marrow (Hviid *et al.* 1997); (Kurtzhals *et al.* 1999) and/or dysfunctional erythropoeisis (Abdalla *et al.* 1980); (Weatherall *et al.* 1983). African children adjust physiologically to the borderline state of moderately severe and moderate anaemia produced and maintained by such chronic infection but may rapidly decompensate when faced with the heightened parasitaemia of a new infection (Newton *et al.* 1998) or resurgence following a partially effective treatment (Bojang *et al.* 1997).

We hypothesised that residence in an irrigated area would be a risk factor for malaria and severe anaemia owing to increased density of infective mosquitoes and perennial transmission, but no clear pattern of increased risk was apparent in our analysis. This result is suggestive of a uniform condition of malaria transmission over the entire KND, virtually equalising irrigated and non-irrigated communities for a time, hence our failure to detect any November differences between irrigated and non-irrigated cohorts. Alternatively, malaria transmission affecting young children in irrigated sectors may be so much more intense and regular as to induce an earlier, broader, and stronger immunity than that triggered only periodically by seasonal malaria in the non-irrigated sectors. Such an edge, or disproportionate immunity in the irrigated cohort might originate from immediately protective higher titre maternal antibodies transferred during gestation and breast feeding, foetal haemoglobin, and subsequently, by self-made cellular and humoral responses to a continuum of infections (McGregor, 1965); (Pasvol *et al.* 1977); (Snow *et al.* 1998). Although we failed to detect clear differences between irrigated and non-irrigated cohorts in either survey, we observed that anaemia was more pronounced among children of the northern sector. As this was seen in both surveys and manifested in multiple factors (proportions of severely anaemic children, mean Hb levels among non-critical children), we consider these differences to be valid and suggestive of increased risk. Greater risk of severe anaemia in the north may derive from 1) distance from medical treatment facility, 2) less effective or delayed

protective immunity, and 3) nutritional and economic stress. Based upon these initial observations we hypothesise that differences between locations would be even more apparent during the truly dry months of December to April.

In summary, severe anaemia in the northern Ghana Sahel appears to be a regular and near-predictable seasonal event that is akin to a grim "rite of passage" for young children of rural Africa. Under starkly seasonal conditions that rigidly delimit malaria transmission, we identified high rates of severe anaemia among males and older children in the infant age group studied, and obtained evidence associating anaemia severity with residence in the northern sector of the KND. We believe that the daily record of blood transfusions performed at the district hospital is a simple, valuable index that is reflective of the intensity of malaria, malaria-induced anaemia, and malaria death in this district. From recent demonstrations of success against the high level of severe malaria anaemia that occurs under conditions of continuous heavy transmission (Menendez *et al.* 1997); (Schellenberg *et al.* 2001); (Tomashek *et al.* 2001), we are inclined to advocate a similar attack against infant anaemia as it occurs in the KND. This strategy, based on provision of oral iron syrup to mothers and simple presumptive intermittent treatment of young children with Sulphadoxine-Pyrimethamine (SP), both timed in accordance with childhood immunisations and given through the existing infrastructure of the WHO Expanded Program of Immunisation (EPI) could improve health and save many young lives.

8.6. Acknowledgements:

We thank the chiefs and people of the Kassena-Nankana District for their Cupertino in making this study possible. Sincere appreciation also goes to the Navrongo Health Research Centre (NHRC) Teams who carried out the fieldwork and data management, especially Dr. Kwame Amponsa-Achiano, Timothy Awine and Nathan Mensah. We also thank Dr. George Mumuni, Senior Medical Officer in charge of the Navrongo War Memorial District Hospital and Mr. Philip Amati of the hospital laboratory for daily recording of transfusion data. We thank Dr. Abraham Hodgson, Acting NHRC Director

for his role in facilitating this work. Kalifa Bugri and Charles Attiogbe are commended for their good work in malaria microscopy.

Financial support: The work was supported by the Navrongo Health Research Centre, the Noguchi Memorial Institute of Medical Research, and US Naval Medical Research Center Independent Research Initiative funds and the STO F6.1 work unit 61102AA0101.BXF.1431 of the Military Infectious Disease Research Program and the London School of Hygiene and Tropical Medicine.

Disclaimer: The views of the authors expressed herein do not purport to reflect those of the Ghanaian Ministry of Health, the US Navy, or the US Department of Defense.

CHAPTER 9

General Discussion, Conclusions and Recommendations

Chapter 9: General Discussion, Conclusions and Recommendations

The main goal of the work presented here is to contribute to the understanding of the epidemiology of *Plasmodium falciparum* parasites, the patterns of seasonality in infection and morbidity and their relationship to radical cure in the Kassena-Nankana district (KND) of northern Ghana. The findings from these studies are discussed in detail in each of the chapters. The general discussion of the design, methodology and the contribution of the results from these studies to our knowledge of *Plasmodium falciparum* infections and future control of the disease through clinical interventions have been addressed in this chapter.

9.1. Study population, Design and Methodology of the studies

All the studies presented here were carried out in the Kassena-Nankana district (KND) in northern Ghana. The district has been described in detail in section 2.3.

Selection of participants: Cluster randomisation was used in sampling participants in all these studies. Though cluster randomisation is considered to be less efficient and has weaker statistical power relative to a design individually randomising the same number of subjects, we had to adopt it mainly because of logistical and cost constraints. This did not compromise the quality of the study and the inferences drawn because, we tallied the total number of eligible members of the community within the 4 geographic zones of the district and randomly selected 16 "index" compounds. Using the Stata programme the selection was stratified by zone, based on population parameters, estimated as weighted averages of the age-specific values, weighed in proportion to the total district population in the age group. The SAS GENMOD (SAS Institute Inc., 1996) procedure was used to compute confidence intervals, allowing for the clustering.

The selection was facilitated by the availability of the Navrongo Demographic Surveillance System (NDSS), a longitudinal census of all district residents that is updated every 3 months. With the target population already on the NDSS database, the eligible individuals of the target group of each of the "index" compounds followed by nearby compounds were recruited into the study, sufficient to allow for cluster sampling

analysis. We involved all age groups (1 week to 85 years) in the cohort used for the molecular epidemiological studies, ensuring that each age group was adequately represented in the cohort. In the incidence studies, we studied a cohort of semi-immune inhabitants (18-55 years) at the end of the peak transmission season (May-October) of 1996. This was followed by the recruitment of a cohort of non-immune infants/young children (6-24 months of age) in November 1996, a time coinciding with low malaria transmission. We followed this with the recruitment of a second cohort of infants/ young children of the same age group in May 1997, exactly one year after the adult cohort to coincide with the high transmission season of 1997. In studying the two infant/child cohorts, haemoglobin levels were determined using automated haemocue (details in the chapters). The striking difference in severe anaemia levels in November 1996 (a period following the high transmission season of 1996) and May 1997 (a period following the low transmission season) led to a repeat survey in November 2000. This was to establish whether the peak in anaemia levels in 1996 is a regular annual event.

Laboratory procedures:

Microscopy: All blood slides were stained using the standard Giemsa method and the procedure for reading and assessing parasite densities was the same. The details are found in the various chapters. Parasite counts were converted to parasites per microlitre of blood assuming an average of 8000 leukocytes per µl blood. This standard procedure has been used all these years in malariometric surveys carried out in this region as it is internationally accepted. It will be a useful exercise, however, to check the number of leukocytes per microlitre of blood in one of the future surveys to re-assure malariologists of the validity of this standard conversion. Most of the slides from these studies were read by a microscopist with considerable experience in this field of specialisation. Re-reading of a blinded 10% random selected microscopic slides showed very high agreement between the two readers. We had the opportunity to compare the latest microscopy readings with PCR results carried out in the most recent survey (refer chapter 3).

Considering the fact that the two methods were applied independently and blind, there was a very high level of agreement between microscopy and PCR for positivity for *Plasmodium falciparum* (Figure 3.2). This validates the parasitological data reported in the past in studies carried out in the KND. As in other studies (Muller *et al.* 2001), the positive samples that were missed by microscopy were predominantly in older individuals who generally have low parasite densities and significantly lower mean multiplicity of infection than the average in the community.

PCR: Deoxyribonucleic acid (DNA) on DNA-Isocode stix was added directly to the PCR mix. Both primary and nested PCR were performed with each sample to optimise sensitivity. The *msp2* gene of *P. falciparum* was genotyped by the Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) approach of (Felger *et al.* 1999). This method was preferred to the other major method used for *msp2* genotyping i.e. analysis of size polymorphism with subsequent hybridisation with family-specific probes (Foley *et al.* 1992); (Carter *et al.* 1973); (Ntoumi *et al.* 1995). In order to study the infection dynamics of individual genotypes and to be able to follow a single parasite clone over time, the use of an extremely polymorphic marker is an essential pre-condition. Despite the views of (Babiker *et al.* 1999) that the use of one marker, no matter how polymorphic, would miss variation at other polymorphic loci and thus almost certainly underestimate the magnitude of multiple infections, only the *msp2* marker was analysed in this study. A comparison of multiplicity obtained from one locus (by PCR-RFLP) versus two loci genotyping indicated that additional information was gained only in 2.6% of infections, all being infections with a single parasite clone (Felger *et al.* 1999). To use two less polymorphic marker genes instead cannot fulfil this task because the marker genes are not genetically linked. For genotyping blood samples from Ghana, with a mean-multiplicity of 3.4 (range 1-8) genotypes per PCR-positive sample, it was therefore concluded that the PCR-RFLP technique using a single locus only, was the method of choice to address the questions that are of interest to us. *Msp2* genotyping makes it possible to determine the multiplicity of infection, which can be used as an outcome measurement of interventions such as drug/vaccine trials, or exposure-reducing interventions. Restriction digests of the PCR products were

performed with Hinf I for 2 hours and then run on a 10% PAA gel. When a higher discriminatory power of the RFLP pattern was desired, usually in the case of new alleles or ambiguities, an additional Dde1 digest was performed. We identified individual clones by allele-specific patterns; up to 8 different clones were distinguished (Felger *et al.* 1999).

9.2. General Discussion of the findings from these studies

This section is divided into four main parts, the first part discusses incidence of infection and disease among non-immune children (6-24 months) and in semi-immune adults (18-55 years) and application of the results for optimal design of clinical intervention trials, including testing of malaria vaccines. This is followed by a discussion of the molecular epidemiology of *P. falciparum* infections in the locality and how such knowledge could be used in the assessment clinical malaria intervention trials. A discussion of the effects of clearing malaria parasites on incidence and severity of morbidity at re-infection is covered in the third section. The fourth section discusses malaria morbidity trends in the Kassena-Nankana district (KND), focusing on the seasonal characteristics of severe anaemia. This has been discussed in the context of using severe malaria anaemia as possible end points in the evaluation of malaria clinical studies, including malaria vaccine studies.

Incidence data and applicability to optimal design of malaria intervention studies: The results from these studies show that malaria transmission in the Kassena-Nankana district (KND) is intense throughout the year but with seasonal peaks and troughs. The incidence density (ID) of infection which estimates the force of *Plasmodium falciparum* infection in infants and young children who were non-immune was the same as in semi-immune adults during the wet season (ID = 7 cases/person-year). This only decreased slightly among the non-immunes during the dry season when malaria transmission is usually low in KND (ID = 5.0 cases/person-year). Despite a natural immunity derived from a lifetime of intense exposure, these adults studied became rapidly re-infected following an effective curative treatment. Neither genetic resistance nor long-acquired immunity appears to be sufficiently strong or widely present in this population to

completely inhibit parasitaemia. This observation suggests that antisporozoite antibody is poorly developed under natural conditions and appears not to protect against development of malaria infection, in support of the finding of (Carter *et al.* 1973). (Hoffman *et al.* 1987) in their study in western Kenya wondered whether any level of naturally acquired antibodies to the circumsporozoite protein can predict resistance to *Plasmodium falciparum* malaria. The data from KND supports the views of (Hoffman *et al.* 1987), that prevention of malaria infection is unlikely, no matter the immune status of an individual; unless these individuals are immunised with sporozoite vaccines that induce antibodies superior to the CSP antibodies existing in these individuals. This general susceptibility and rapid appearance of low density blood stage infections may result from the ineffectiveness of anti-sporozoite and anti-liver stage immunity or to an asexual blood stage effect coupled with a pre-erythrocytic stage immunity that reduces but does not completely eliminate all infected hepatocytes (Hoffman, 1996).

The cumulative incidence profiles of adult and infant cohorts in the wet season in the KND indicate the same rapid rise and plateau effect with over 90% re-infection rate within twelve weeks post-treatment (Fig. 6.4). These incidence data are available for use in estimating appropriate sample sizes for intervention trials, drugs and vaccines inclusive. For example, using the results from these studies as a hypothetical case for non-immune children, the statistical power of a vaccine trial applying endpoints of high parasitaemia (>50,000/ul) and fever can best be illustrated. If one assumes a vaccine efficacy of 85%, and the operational requirement to have an 80% probability of having a 95% confidence interval that excludes 70% (i.e., the lower limit of acceptability for efficacy), then the dry season incidence rate of 0.24 events per person year for this outcome demands a sample size of 2055. Applying the same parameters to the 1.04 events per person year incidence rate in the wet season for the same non-immunes gives a sample size estimate of just 363 subjects. In the case of the semi-immune adults, applying the finding of an 80% attack rate to a prospective12 week trial of a drug/vaccine with predicted 75% protective efficacy (PE), and an 80% probability (power) of having the lower 95% confidence limit for that PE $\geq$70%, we would require a population of 1,004 adults. With these variables kept the same, but with the lower 95%

confidence limit relaxed to ≥60% for the PE, we would need only to enrol 151 subjects. These clearly demonstrate that subtle distinctions in seasonal end point incidence densities could profoundly impact sample size requirements in intervention trials.

The results from these incidence studies were applied in the design of a randomised, double blind, placebo-controlled dose-ranging chemoprophylaxis drug trial in 1998 in the KND. A cluster random sample of roughly 500 semi-immune adults in this community were studied to determine the minimum effective weekly dose of Tafenoquine (TQ) for prevention of infection by *Plasmodium falciparum* (Table 9.1).

Table 9.1. Primary outcomes: Incidence and protective efficacy (PE) relative to placebo

	Placebo	TQ 25 mg	TQ 50 mg	TQ 100 mg	TQ 200 mg	MF 250 mg
No. Subjects	94	93	91	94	91	46
No. positive malaria smears	86	58	13	11	12	6
Total person-weeks*	505	801	1059	1132	1087	555
Cumulative Incidence/12 wks	0.93	0.65	0.16	0.13	0.14	0.13
Incidence Density/person-yr	8.9	3.8	0.6	0.5	0.6	0.6
%Protective Efficacy**	N/A	56.9	92.8	94.3	93.5	93.7
Lower 95% CI	N/A	39.9	87.1	89.3	88.1	88.1
Upper 95% CI	N/A	69.1	96.0	96.9	96.5	97.2

CI = Cumulative incidence; wks = weeks; yr = years; *Follow-up time until positive blood smear, negative early drop-out, or negative at the 12 week endpoint. **% Protective efficacy = [1-(drug incidence density/placebo incidence density)] x 100
MF = Mefloquine, TQ = Tafenoquine, mg = milligrams.

The study was designed to end by 12 weeks of follow-up and a sample size based on the cumulative incidence (CI) results from the incidence studies. By the end of the 12-week follow-up period, all the four Tafenoquine doses in this blinded study demonstrated highly significant protection from malaria infection relative to the placebo and Mefloquine groups. The conclusion from this study was that Tafenoquine doses of

50, 100, and 200 mg/wk were safe, well tolerated, and as effective as Mefloquine at 250 mg/wk in this rural African population (Hale et al., 2000).

Molecular epidemiology of Plasmodium falciparum infections and relevance to assessment of clinical malaria interventions: Molecular analysis of parasites collected in this survey in the Kassena-Nankana district (KND) demonstrates that parasite multiplicity is very large in this community, with most asymptomatics carrying multiple *P. falciparum* genotype infections. The multiplicity of infection seen in the KND was comparable with other holoendemic malarious areas (Hill *et al.* 1995); (Smith *et al.* 1999c); (Engelbrecht *et al.* 2000); (Ntoumi *et al.* 1995). The two *msp*2 allelic families were of almost equal proportions in KND [FC27 (49%) and 3D7 (51%)], supporting the suggestion by (Felger *et al.* 1999), that though the genetic diversity at this locus is extensive, it is limited by structural constraints or immune selection.

The age pattern in multiplicity and parasite density among children in the KND revealed results similar to observations made in other holoendemic sites (Ntoumi *et al.* 1995); (Smith *et al.* 1999b). The peaks of multiplicity of infection and parasite densities were in different age groups, which gives an indication that the mechanisms for multiplicity of infection and parasite densities are not the same. Further evidence for this is provided by the age profile of the correlation between parasite density and multiplicity in Kilombero where a gradual loss of the correlation between parasite density and multiplicity with age was observed (Smith *et al.* 1999b). This age profile matched that of the acquisition of clinical immunity. This could reflect specific anti-parasite immunity acquired after about 10-15 years of age in holoendemic areas. Antigenic polymorphism in malaria appears to be one of the important mechanisms deployed by the parasite to evade immune responses (Mendis *et al.* 1991). Knowledge of how to counteract this immune evasion will provide guidance to antigens that will stimulate highest quantity of effective antibodies and the best conditions under which immunisation can be conducted in malaria vaccine trials.

The steady and gradual increase in the correlation between multiplicity and parasite density after the age of 10 observed until over 60 years of age in KND was however, unexpected. Given the very low densities observed in older participants and the diversity of the parasites, it could be that multiplicity might simply be counting the number of individual parasites in the sample. The existence of a correlation between multiplicity and density could then be an indication of accurate quantitation of low-density parasitaemia.

Parasites carrying the FC27-like genotypes have been found to be more frequently associated with symptomatic malaria patients compared with asymptomatic ones (Engelbrecht *et al.* 1995); (Al Yaman *et al.* 1997)**.** The association between the allelic family type and the frequency of malaria disease could not be discussed as only 3.6% of the participants had documented fever (axillary temperature $\geq 37.5^{o}C$) and 2.3% of participants had fever associated with malaria parasitaemia. The findings from this study presented in chapter 3, constitutes the baseline of a longitudinal study designed to collect parasitological data at eight weeks intervals from individuals of all age groups over a period of one year. It is envisaged that the links between allelic families and symptomatology can be determined when the data from the repeated surveys are pulled together and the allelic family types of the symptomatic ones are re-analysed in comparison with the asymptomatic ones. Mention could be made of an intervention trial that is ongoing in the KND, in the same communities where these genotyping studies were carried out, to evaluate the effects of treatment with Artesunate suppositories on child mortality (Gyapong et al., 2000). Severely ill (nil per Os) malaria patients are the target for this study. In order to address questions related to the multiplicity of infection and it relationship to severe-complicated clinical malaria, blood samples are currently being collected on filter paper samples (Owusu-Agyei, protocol 2001) from all the severely ill children identified within the context of this trial for genotyping later on. The relationship between allele types and severity of malaria will be established from this add on study.

At the beginning of the wet season, the data from the KND demonstrates one of the highest multiplicity with age dependence patterns similar to those in other holoendemic sites in Africa, thus validating the use of the age-multiplicity relationship as an indicator of malaria endemicity. However, the age-patterns of multiplicity could differ significantly in the dry season especially in the non-irrigated areas of the district. Data from the additional surveys will allow us to build upon these baseline results and contribute to our knowledge and understanding of the seasonal dynamics and multiplicity of infections in all age groups in this area of highly seasonal holoendemic malaria. The availability of genotyping data will contribute to evaluating intervention programmes by way of studying the changes in multiplicity with time (ie reduction of parasite clones following an effective intervention), the role and dynamics of disease causing alleles, ie how alleles appear and disappear, which ones appear or not at the time of clinical malaria and the effects of dominant genes in clinical malaria attacks.

Clearance of parasitaemia and effects of subsequent infections in intervention trials: It remains unclear whether it is a good idea to clear malaria parasitemia prior to an intervention so that the effect of the intervention can be evaluated. Over 30 years ago, (Pringle & Avery-Jones, 1966) concluded from studies carried out in western Kenya that even short period of a few weeks of drug protection against malarial infection lowered the immunity of the children studied appreciably. This resulted in clinical symptoms of malaria and a parasitaemia that was significantly greater than had prevailed generally before treatment. Up to date, the question about the effects of completely clearing malaria parasites prior to assessing malaria interventions still remain relevant, especially to intervention trials where subtle differences in susceptibility affect end points of effective or ineffective outcomes. A direct evaluation of the possible impact on incidence and severity of subsequent symptomatic malaria is required.

In chapter 4, 20% of the adult volunteers, who received treatment prior to being followed-up for incidence of malaria infection, developed clinical malaria attacks at first re-infection (Owusu-Agyei, 2001). Furthermore, close to half of these volunteers had experienced a clinical malaria attack by the end of the 20 weeks follow-up period.

These disease rates in adults raise the question of whether clearance of malaria parasitaemia could have increased the risk of clinical attacks when they subsequently became re-infected.

The tendency for the incidence of clinical malaria to be lower among untreated volunteers who had parasitaemia at enrolment than among those who had no parasitaemia at enrolment as reported in our studies, further suggests that the retention of parasites among semi-immune adults may be protective. Similarly, the fact that the treated cohort became sick after less time at risk than the controls is remarkable since parasites were present in the controls from the start of follow-up. Furthermore, within the control cohort, those who were microscopically positive at enrolment took longer time to develop clinical malaria as compared with those who were microscopically negative at enrolment. For these reasons it could even be the case that the effect of radical therapy on disease incidence is substantially greater than we estimated.

The doubling of parasite densities in both adults and children at first clinical malaria following radical therapy compared to their enrolment parasite densities ($p<0.001$ for each cohort) can be attributed to the effect of radical cure. Pringle & Avery-Jones (1966) reported similar results, a significantly higher malaria parasitaemia was observed among semi-immune children following parasite clearance. Although it is difficult to rule out drug-mediated interference with immune effectors that limit parasite densities as an explanation, diminished effectiveness of premunition (ie the presence of established infections in an individual offering cross-protection against invading clones either by preventing superinfecting clones from becoming established or via tolerance of the new infections), as a consequence of eradication of parasitaemia by radical curative therapy is favoured. The elimination of chronic, relatively low-grade parasitaemia may have established susceptibility to acute, relatively high-grade parasitaemia. This is supported by the 100% response to Chloroquine treatment among the radical therapy cohort compared to the 9% parasitological failure rate among the untreated cohort. Untreated individuals in holoendemic areas often harbour many clones of parasites (Ntoumi *et al.* 1995); (Beck *et al.* 1997; Rougemont *et al.* 1991) and so the chances of a Chloroquine

resistant strain being present is higher than the resistance rate for single infections. This carries important implications with regard to experimental design in intervention studies and to control strategies aimed at delivering effective therapies in holoendemic Africa. There has also been long-standing discussion on rebound effects following successful interventions such as s (Snow *et al.* 1997) and the use of chemoprophylaxis or drug treatments (Menendez *et al.* 1997); (van den *et al.* 1996); (Greenwood *et al.* 1995). The evidence so far suggests that clinical malaria occurring after clearing of parasites may be distinctly different from the "natural" disease. As radical cure is a part of the standard strategy for evaluating malaria vaccines (World Health Organisation, 1997a) these findings may have significance for the design and interpretation of such trials. Though the work that we carried out came up with findings supporting earlier work related to clearance of parasitaemia, the treatment and control cohorts in our study were not randomised and followed within the same time. Drawing concrete conclusions become quite difficult. There is therefore the need to carry out a double blind, randomised placebo-controlled treatment trial.

Trends of severe anaemia in KND & the relevance in assessment of intervention trials: Anaemia is an important feature of morbidity caused by malaria in the Kassena-Nankana District (KND). Two malaria-anaemia surveys, separated by 4 years, but conducted in the same community and at the same November time point corresponding to early dry season in northern Ghana, both document alarmingly high rates and levels of anaemia in infants and young children 6 to 24 months old. Independently, each survey identified associations between severe anaemia and age, parasite rate, fever, and sex. Relative to children with Hb $\geq$ 6.0 g/dL, those with severe anaemia were predominantly male, older, more frequently parasitaemic, and more often febrile. These randomly selected cohorts of infants and young children showed dramatic distinctions with respect to the prevalence of severe anaemia as seen at enrolment at the end of the wet and dry seasons (Koram *et al.* 2000). In effect, an epidemic of severe malarial anaemia occurred in these children as the wet season progressed: apparently starting at 1% in May and rising to 22% by October. The relatively brief respite in transmission

between February and May appeared sufficient to allow recovery of most severe anaemia to above the 6.0g/dl level.

The contribution of nutrition and worm load to the anaemia status of these young children was investigated to see if they play any significant role. Though the nutritional status of these infants/young children demonstrated that there was general wasting and stunting in their growth as measured by anthropometric indices (Koram *et al.* 2000); (Owusu-Agyei et al., 2001a), there was no seasonal variation in nutritional status in these young children. The time of the wet season surveys (November 1996 and November 2000) is a period that coincides with high food stores and if anything this should have impacted positively on the nutritional status of these young children. We see the children worse off at this time in terms of their anaemia levels, clearly indicating that nutrition is generally a problem in the KND, but was not the cause of the sharp seasonal difference in haemoglobin levels observed. In diarrhoea studies carried out in the KND from 1998 to 2000, involving infants/young children of the same age group (6-24 months old), only one child out of over 1500 children examined by microscopy of faeces was reported to have been infected with hookworm (Anto, personal communication). Other helminth eggs were absent in the stools of these infants/young children.

Infection with *P. falciparum* is known to be one of the major causes of childhood anaemia in malaria endemic areas (Orago *et al.* 2001); (Menendez *et al.* 2000); (McElroy *et al.* 2000). A strong correlation has been reported between the incidence of severe anaemia, the age-specific rates of anaemia and the intensity of *P. falciparum* transmission. Malaria control trials have usually been followed by marked improvements in haematological indices in children (Alonso *et al.* 1991); (Greenwood *et al.* 1987); (Molyneux *et al.* 1980). With such intense malaria transmission in the KND, and the seasonal peaks and troughs in anaemia levels, there is no doubt that *P. falciparum* infections are responsible for the severe anaemia in the age group that we studied in this district. Children suffer from repeated attacks of malaria during the wet season. The frequent attacks do not give these children enough time for complete recovery from

one infection, as a result, a marked drop in haemoglobin levels result by the end of the high transmission season.

Anaemia in children is a commonly reported problem from hospitals and clinics in malaria endemic countries. In the KND, it accounted for 22% of all hospital admissions in the year 2000 (Owusu-Agyei et al., 2001b). Blood transfusions performed on young children during two consecutive years appear to follow a pattern closely dictated by rainfall and malaria transmission, and peaked around the November month. We speculate that longitudinal profiling of infant deaths as well as rates of severe anaemia would closely mirror the transfusion profile each year. This assessment will be made using the Navrongo Demographic Surveillance System (NDSS) mortality data collected over the years for such analyses.

Severe anaemia in the northern Ghana appears to be a regular and near-predictable seasonal event among the infants and young children. This relatively high prevalence of anaemia observed as a regular annual event that follows the high transmission season each year, opens up the possibility of using incidence of anaemia as a primary outcome variable for the effects of novel intervention tools such as vaccines, at least in areas of high malaria transmission such as the Kassena-Nankana district in northern Ghana.

The recent demonstration of Sulphadoxine-Pyrimethamine (SP) success against the high level of severe malaria anaemia that occurs under conditions of continuous heavy transmission (Menendez *et al.* 1997); (Schellenberg *et al.* 2001); (Tomashek *et al.* 2001), can be used in making a case for evaluating malaria vaccines using anaemia as an end point. This strategy, based on provision of oral iron syrup to mothers and intermittent treatment of infants and young children with SP, both timed in accordance with childhood immunisations and given through the existing infrastructure of the WHO Expanded Program of Immunisation (EPI) could improve health and save many young lives.

9.3. Conclusions and Recommendations

In conclusion,

Malaria transmission was found to be intense throughout the year, with seasonal peaks and troughs. The overall *P. falciparum* microscopy prevalence was 70%, with the highest parasite rates among 5-9 year olds and highest parasite density (geometric mean 1,922/µl blood) in 1-2 year olds..

The incidence studies have documented intense malaria transmission with incidence densities between 5 and 7 infections/person-year occur through most of the year among non-immune infants/young children aged 6 to 24 months and semi-immune adults (18-55 years of age) living in the Kassena-Nankana District of northern Ghana. Within the 20-week wet season virtually all and infants/young children and adults had manifested a patent primary infection and many reported illnesses that coincided with parasitaemia. Adults experienced predominantly low-density infections, characterised by mild clinical illness while infants/young children have high-density infections with more severe forms of clinical illness. The findings can be used for optimal design of malaria clinical interventions.

Evaluation of some malaria clinical intervention trials will require clearance of parasitaemia prior to follow-up. The interpretation of such intervention trials need to be carried out with a lot of caution as clinical malaria occurring after radical therapy may be distinctly different from the “natural” disease, and the implications of treating asymptomatic individuals may be very different in adults than for children. Results from these studies also support the view that clearance of parasitaemia may put individuals at increased risk of subsequent clinical malaria in terms of duration and severity.

The genotyping study also demonstrated 82% *P. falciparum* prevalence by PCR with a high multiplicity of infection in this community. PCR-RFLP typing of the *P. falciparum msp2* gene revealed a mean *msp2* multiplicity of 3.4 (range: 1 to 8) genotypes with the two *msp*2 allelic families (FC27 and 3D7) in almost equal proportions. The correlation

between parasite density and *msp2* multiplicity was highest in infants, and decreased with age to a minimum by 10 year, then start to increase again from this age to adulthood. The patterns of age dependence are similar to those in other holoendemic sites in Africa, validating the use of the age-multiplicity relationship as an indicator of malaria endemicity. Results from repeat surveys designed for seasonality will soon be available to build up an all-year round molecular epidemiology of *P. falciparum* infections in the KND. The availability of these data will contribute significantly in evaluating intervention programmes. The changes in multiplicity with time (ie reduction of parasite clones following an effective intervention), the role and dynamics of disease causing alleles, and the effects of dominant genes in clinical malaria attacks.

The evidence suggests that dramatic peaks and troughs of severe anaemia are regular and possibly predictable events that may be used to gauge the health and survival of young children in this area. The high prevalence of anaemia observed at the end of the high transmission season opens up the possibility of using severe anaemia as a primary outcome variable for the effects of novel intervention tools such as vaccines, at least in areas of high malaria transmission.

These studies provide the foundation for designing efficient, practical clinical intervention trials and preventive strategies against malaria in this holoendemic region. The studies can also be evaluated with end points that have been documented from our findings.

Recommendation:

The question about the effects of completely clearing malaria parasites prior to assessing malaria interventions still remain relevant, especially to intervention trials where subtle differences in susceptibility affect end points of effective or ineffective outcomes. As the study of the initial cohort was not designed to investigate the effects of radical therapy, the untreated group was studied a year after the treated cohort. Studies designed to address this important question about the effects of radical cure on incidence and severity of clinical malaria needs to be investigated in a more scientific manner in which the treatment and placebo groups are randomised and followed up concurrently.

In studying the effects of radical therapy on incidence and severity of morbidity, the opportunity should be taken to collect blood samples for DNA studies such as determining whether treatment affects rates of acquisition and the duration of individual *P. falciparum* infections by genotyping *msp*1 and *msp*2 gene markers. Other research areas include, comparing the rates of acquisition and the duration of individual *P. falciparum* clones in non-immune children with those in semi-immunes. Examining whether morbidity is invariably associated with the acquisition of new parasite clones, or related to prior low multiplicity and whether multiplicity is a function of long-term immune status of an individual.

It is recommended that questions related to the multiplicity of infection and its relationship to severe/complicated clinical malaria are addressed. Blood samples are currently being collected on filter paper samples (Owusu-Agyei, protocol, 2001) from all the severely ill children identified within the context of the Artesunate suppository trial currently ongoing in the KND for genotyping later on. It is expected that the relationship between allele types of *msp1* and *msp2* and severity of *Plasmodium falciparum* malaria will be established from this add on study. We will test whether a particular allelic family will be dominantly found in severe cases using quantitative allele family specific PCR.

The findings reported in chapters 7 and 8 calls for longitudinal studies of seasonal risk of severe anaemia in northern Ghana. The relatively brief respite in transmission between February and May each year appears to be sufficient to allow recovery of most severe anaemia to above the 6.0g/dl level. The process of recovery from their low haemoglobins following the wet season needs to be investigated.

We speculate that longitudinal profiling of infant deaths as well as rates of severe anaemia would closely mirror hospital transfusion profile each year. This assessment will be made using the Navrongo Demographic Surveillance System (NDSS) mortality data collected over the years for such analyses.

Severe anaemia in the KND appears to be a regular and near-predictable seasonal event among the infants/young children, and follows the high malaria transmission season each year. It therefore opens up the possibility of using incidence of anaemia as a primary outcome variable for the effects of malaria vaccine trials. This needs to be explored for future malaria intervention studies.

Reference List

Abdalla S, Weatherall DJ, Wickramasinghe SN, Hughes M(1980) The anaemia of *P. falciparum* malaria. *Br.J.Haematol.* **46** 171-183.

Afari EA, Akanmori BD, Nakano T, Ofori-Adjei D(1992) *Plasmodium falciparum*: sensitivity to Chloroquine in vivo in three ecological zones in Ghana. *Trans.R.Soc.Trop.Med.Hyg.* **86** 231-232.

Akanmori BD, Afari EA, Sakatoku H, Nkrumah FK(1995) A longitudinal study of malaria infection, morbidity and antibody titres in infants of a rural community in Ghana. *Trans.R.Soc.Trop.Med.Hyg.* **89** 560-561.

Al Yaman F, Genton B, Anders RF, Falk M, Triglia T, Lewis D, Hii J, Beck HP, Alpers MP(1994) Relationship between humoral response to *Plasmodium falciparum* merozoite surface antigen-2 and malaria morbidity in a highly endemic area of Papua New Guinea. *Am.J.Trop.Med.Hyg.* **51** 593-602.

Al Yaman F, Genton B, Reeder JC, Anders RF, Smith T, Alpers MP(1997) Reduced risk of clinical malaria in children infected with multiple clones of *Plasmodium falciparum* in a highly endemic area: a prospective community study. *Trans.R.Soc.Trop.Med.Hyg.* **91** 602-605.

Alles H, Mendis K, Carter R(1998) Malaria mortality rates in South Asia and in Africa: Implications for malaria control. *Parasitol.Today* **14** 369-375.

Alnwick D(2000) Roll back malaria--what are the prospects? *Bull.World Health Organ* **78** 1377.

Alonso PL, Lindsay SW, Armstrong JR, Conteh M, Hill AG, David PH, Fegan G, de Francisco A, Hall AJ, Shenton FC, .(1991) The effect of insecticide-treated bed nets on mortality of Gambian children. *Lancet* **337** 1499-1502.

Alonso PL, Smith T, Schellenberg JR, Masanja H, Mwankusye S, Urassa H, Bastos DA, I, Chongela J, Kobero S, Menendez C, .(1994) Randomised trial of efficacy of SPf66 vaccine against *Plasmodium falciparum* malaria in children in southern Tanzania. *Lancet* **344** 1175-1181.

Alonso PL, Tanner M(1996) Malaria vaccine. *Lancet* **348** 1378.

Appawu MA, Baffoe-Wilmot A, Afari EA, Nkrumah FK, Petrarca V(1994) Species composition and inversion polymorphism of the Anopheles gambiae complex in some sites of Ghana, west Africa. *Acta Trop.* **56** 15-23.

Arnot D(1998) Unstable malaria in Sudan: the influence of the dry season. Clone multiplicity of *Plasmodium falciparum* infections in individuals exposed to variable levels of disease transmission. *Trans.R.Soc.Trop.Med.Hyg.* **92** 580-585.

Babiker HA(1998) Unstable malaria in Sudan: the influence of the dry season. *Plasmodium falciparum* population in the unstable malaria area of eastern Sudan is stable and genetically complex. *Trans.R.Soc.Trop.Med.Hyg.* **92** 585-589.

Babiker HA, Ranford-Cartwright LC, Walliker D(1999) Genetic structure and dynamics of *Plasmodium falciparum* infections in the Kilombero region of Tanzania. *Trans.R.Soc.Trop.Med.Hyg.* **93 Suppl 1** 11-14.

Baird JK Owusu-Agyei S, Utz GC, Koram KA, Barcus MJ, Jones TR, Fryauff DJ, Binka FN, Hoffman SL, and Nkrumah FK(2001) Seasonal malaria attack rates in infants and young children in northern Ghana. *Am.J.Trop.Med.Hyg.* **In Press**.

Bassett MT, Taylor P, Bvirakare J, Chiteka F, Govere E(1991) Clinical diagnosis of malaria: can we improve? *J.Trop.Med.Hyg.* **94** 65-69.

Beck HP, Felger I, Huber W, Steiger S, Smith T, Weiss N, Alonso P, Tanner M(1997) Analysis of multiple *Plasmodium falciparum* infections in Tanzanian children during the phase III trial of the malaria vaccine SPf66. *J.Infect.Dis.* **175** 921-926.

Beier JC, Oster CN, Onyango FK, Bales JD, Sherwood JA, Perkins PV, Chumo DK, Koech DV, Whitmire RE, Roberts CR, (1994). *Plasmodium falciparum* incidence relative to entomologic inoculation rates at a site proposed for testing malaria vaccines in western Kenya. *Am.J.Trop.Med.Hyg.* **50** 529-536.

Bennett S, Woods T, Liyanage W, Smith D(1991) A simplified general method for cluster-sample surveys of Health in developing countries. *Rapp.Trimest.Stat.Sanit.Mond.* **44** 98-106.

Binka FN(1997) Impact and determinants of permethrin impregnated s on child mortality in northern Ghana. Swiss Trop. Inst, pp. 58-74. PhD Thesis/Dissertation

Binka FN, Ngom P Phillips JF Adazu K MacLeod BB(1999). Assessing population dynamics in a rural African society: the Navrongo Demographic Surveillance System. J. Biosoc. Sci. **31**, 375-391. 1999.

Binka FN, Adongo P(1997) Acceptability and use of insecticide impregnated s in northern Ghana. *Trop.Med.Int.Health* **2** 499-507.

Binka FN, Indome F, Smith T(1998) Impact of spatial distribution of permethrin-

impregnated bed nets on child mortality in rural northern Ghana. *Am.J.Trop.Med.Hyg.* **59** 80-85.

Binka FN, Kubaje A, Adjuik M, Williams LA, Lengeler C, Maude GH, Armah GE, Kajihara B, Adiamah JH, Smith PG(1996) Impact of permethrin impregnated s on child mortality in Kassena-Nankana district, Ghana: a randomised controlled trial. *Trop.Med.Int.Health* **1** 147-154.

Binka FN, Maude GH, Gyapong M, Ross DA, Smith PG(1995) Risk factors for child mortality in northern Ghana: a case-control study. *Int.J.Epidemiol.* **24** 127-135.

Binka FN, Morris SS, Ross DA, Arthur P, Aryeetey ME(1994) Patterns of malaria morbidity and mortality in children in northern Ghana. *Trans.R.Soc.Trop.Med.Hyg.* **88** 381-385.

Bloland P, Slutsker L, Steketee RW, Wirima JJ, Heymann DL, Breman JG(1996) Rates and risk factors for mortality during the first two years of life in rural Malawi. *Am.J.Trop.Med.Hyg.* **55** 82-86.

Bloland PB, Lackritz EM, Kazembe PN, Were JB, Steketee R, Campbell CC(1993) Beyond Chloroquine: implications of drug resistance for evaluating malaria therapy efficacy and treatment policy in Africa. *J.Infect.Dis.* **167** 932-937.

Bojang KA, Palmer A, Boele VH, Banya WA, Greenwood BM(1997) Management of severe malarial anaemia in Gambian children. *Trans.R.Soc.Trop.Med.Hyg.* **91** 557-561.

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

Breman JG, Egan A, Keusch GT(2001) The intolerable burden of malaria: a new look at the numbers. *Am.J.Trop.Med.Hyg.* **64** iv-vii.

Brewster DR, Kwiatkowski D, White NJ(1990) Neurological sequelae of cerebral malaria in children. *Lancet* **336** 1039-1043.

Carter R, McGregor IA(1973) Enzyme variation in *Plasmodium falciparum* in the Gambia. *Trans.R.Soc.Trop.Med.Hyg.* **67** 830-837.

Charlwood JD, Smith T, Lyimo E, Kitua AY, Masanja H, Booth M, Alonso PL, Tanner M(1998) Incidence of *Plasmodium falciparum* infection in infants in relation to exposure to sporozoite-infected anophelines. *Am.J.Trop.Med.Hyg.* **59** 243-251.

Clark H, Tomlison W (1949) Malariology. WB Saunders, Philadelphia, *P.* 845.

Connor SJ, Thomson MC, Molyneux DH(1999) Forecasting and prevention of epidemic malaria: new perspectives on an old problem. *Parassitologia* **41** 439-448.

Contamin H, Fandeur T, Rogier C, Bonnefoy S, Konate L, Trape JF, Mercereau-Puijalon O(1996) Different genetic characteristics of *Plasmodium falciparum* isolates collected during successive clinical malaria episodes in Senegalese children. *Am.J.Trop.Med.Hyg.* **54** 632-643.

Conway, DJ Greenwood BM and McBride JS(1991). The epidemiology of multiple-clone *Plasmodium falciparum* infection in The Gambia. *Parasitology.* 1103, 1-6.

Coulbourne M, Wright F(1955) Malaria in the Gold Coast. *West Afr.J.Med.* **4** 161-174.

Daubersies P, Sallenave-Sales S, Magne S, Trape JF, Contamin H, Fandeur T, Rogier C, Mercereau-Puijalon O, Druilhe P(1996) Rapid turnover of *Plasmodium falciparum* populations in asymptomatic individuals living in a high transmission area. *Am.J.Trop.Med.Hyg.* **54** 18-26.

Day KP, Marsh K(1991) Naturally acquired immunity to *Plasmodium falciparum*. *Immunol.Today* **12** A68-A71.

Delacollette C, Van Der SP(1993) High parasitaemia incidence rates can be used to estimate malaria morbidity rates. *Ann.Trop.Med.Parasitol.* **87** 537-539.

Dorward J, knowles J, Dorward I(1990) Treatment of severe anaemia in children in a rural hospital. *Trop.Doctor* **19** 155-158.

Engelbrecht F, Felger I, Genton B, Alpers M, Beck HP(1995) *Plasmodium falciparum*: malaria morbidity is associated with specific merozoite surface antigen 2 genotypes. *Exp. Parasitol.* **81** 90-96.

Engelbrecht F, Togel E, Beck HP, Enwezor F, Oettli A, Felger I(2000) Analysis of *Plasmodium falciparum* infections in a village community in Northern Nigeria: determination of msp2 genotypes and parasite-specific IgG responses. *Acta Trop.* **74** 63-71.

Farnert A, Arez AP, Babiker HA, Beck HP, Benito A, Bjorkman A, Bruce MC, Conway DJ, Day KP, Henning L, Mercereau-Puijalon O, Ranford-Cartwright LC, Rubio JM, Snounou G, Walliker D, Zwetyenga J, do R, V(2001) Genotyping of *Plasmodium falciparum* infections by PCR: a comparative multicentre study. *Trans.R.Soc.Trop.Med.Hyg.* **95** 225-232.

Felger I, Irion A, Steiger S, Beck HP(1999) Genotypes of merozoite surface protein 2 of *Plasmodium falciparum* in Tanzania. *Trans.R.Soc.Trop.Med.Hyg.* **93 Suppl 1** 3-9

Felger I, Tavul L, Kabintik S, Marshall V, Genton B, Alpers M, Beck HP(1994) *Plasmodium falciparum*: extensive polymorphism in merozoite surface antigen 2 alleles in an area with endemic malaria in Papua New Guinea. *Exp. Parasitol.* **79** 106-116.

Fleming A(1987) The aetiology of severe anaemia in pregnancy in Ndola, Zambia. *Ann.Trop.Med.Parasitol.* **83** 37-49.

Foley M, Ranford-Cartwright LC, Babiker HA(1992) Rapid and simple method for isolating malaria DNA from fingerprick samples of blood. *Mol.Biochem.Parasitol.* **53** 241-244.

Gallup JL, Sachs JD(2001) The economic burden of malaria. *Am.J.Trop.Med.Hyg.* **64** 85-96.

Genton B., Al-Yaman F. Reber R. Anders R. Brown G. Saul A. Mai A. Stuerchler D. & Alpers M. Safety and immunogenicity of a three-component blood-stage vaccine against *Plasmodium falciparum* malaria in adults living in a highly endemic area of Papua New Guinea (PNG). XIVth International Congress for Tropical Medicine & Malaria, **76**. 1996. Nagasaki, Japan.

Genton B. Betuela I, Al-Yaman F, Anders RF, Saul A, Rare L, Baisor M, Lorry K, Brown GV, Pye D, Irving DO, Felger I, Beck H-P, Smith TA, Alpers MP (2001) Safety and pilot-efficacy of a three component blood-stage malaria vaccine (MSP1, MSP2, RESA) against *Plasmodium falciparum* in Papua New Guinea children. *J.Infect.Dis.* **In Press** .

Ghana VAST Study Team(1993) Vitamin A supplementation in northern Ghana: effects on clinic attendances, hospital admissions and child mortality. *Lancet* **342** 7-12.

Gilles HM (1985) The malaria parasites. In Bruce-Chwatt's Essential malariology. Edward Arnold, London, 12-34 pp.

Greenwood BM, Bradley AK, Greenwood AM, Byass P, Jammeh K, Marsh K, Tulloch S, Oldfield FS, Hayes R(1987) Mortality and morbidity from malaria among children in a rural area of The Gambia, West Africa. *Trans.R.Soc.Trop.Med.Hyg.* **81** 478-486.

Greenwood BM, David PH, Otoo-Forbes LN, Allen SJ, Alonso PL, Armstrong S, Jr.,

Byass P, Hurwitz M, Menon A, Snow RW(1995) Mortality and morbidity from malaria after stopping malaria chemoprophylaxis. *Trans.R.Soc.Trop.Med.Hyg.* **89** 629-633.

Greenwood BM, Greenwood AM, Snow RW, Byass P, Bennett S, Hatib-N'Jie AB(1989)

The effects of malaria chemoprophylaxis given by traditional birth attendants on the course and outcome of pregnancy. *Trans.R.Soc.Trop.Med.Hyg.* **83** 589-594.

Gupta S, Hill AV, Kwiatkowski D, Greenwood AM, Greenwood BM, Day KP(1994) Parasite virulence and disease patterns in *Plasmodium falciparum* malaria. *Proc.Natl.Acad.Sci.U.S.A* **91** 3715-3719.

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

Hale BR., Owusu-Agyei S. Koram KA Fryauff DJ Adjuik M Oduro AR Prescott RW Baird

JK Nkrumah FK Franke ED Binka FN Horton J and Hoffman SL. A Randomised, Double-blind, Placebo-controlled Dose-ranging Trial of Tafenoquine for Weekly Prophylaxis against *Plasmodium falciparum*. Conference Proceeding. *Am. Soc. Trop. Med. Hyg*. (**49**). 2000.

Haywood M, Conway DJ, Weiss H, Metzger W, D'Alessandro U, Snounou G, Targett G,

Greenwood B(1999) Reduction in the mean number of *Plasmodium falciparum* genotypes in Gambian children immunised with the malaria vaccine SPf66. *Trans.R.Soc.Trop.Med.Hyg.* **93 Suppl 1** 65-68.

Henry MC, Eggelte TA, Watson P, Docters VL, Bakker DA, Kluin J(1996) Response of childhood malaria to Chloroquine and Fansidar in an area of intermediate Chloroquine resistance in Cote d'Ivoire. *Trop.Med.Int.Health* **1** 610-615.

Hill AV(1999) The immunogenetics of resistance to malaria. *Proc.Assoc.Am.Physicians* **111** 272-277.

Hill WG, Babiker HA, Ranford-Cartwright LC, Walliker D(1995) Estimation of inbreeding

coefficients from genotypic data on multiple alleles, and application to estimation of clonality in malaria parasites. *Genet.Res.* **65** 53-61.

Hoffman SL, Oster CN, Plowe CV, Woollett GR, Beier JC, Chulay JD, Wirtz RA, Hollingdale MR, Mugambi M(1987) Naturally acquired antibodies to sporozoites do not prevent malaria: vaccine development implications. *Science* **237** 639-642.

Hoffman, SL Editor. Malaria Vaccine Development: A Multi-Immune Response Approach. 1996. ASM Press, Washington DC.

Hviid L, Kurtzhals JA, Goka BQ, Oliver-Commey JO, Nkrumah FK, Theander TG(1997)

Rapid re-emergence of T cells into peripheral circulation following treatment of severe and uncomplicated *Plasmodium falciparum* malaria. *Infect.Immun.* **65** 4090-4093.

Irrigation Company of Upper Regions (ICOUR). Monthly rainfall statidtics 1981-2000.

Jelinek T, Kilian AH, Westermeier A, Proll S, Kabagambe G, Nothdurft HD, von Sonnenburg F, Loscher T(1999) Population structure of recrudescent *Plasmodium falciparum* isolates from western Uganda. *Trop.Med.Int.Health* **4** 476-480.

Jiang G, Daubenberger C, Huber W, Matile H, Tanner M, Pluschke G(2000) Sequence diversity of the merozoite surface protein 1 of *Plasmodium falciparum* in clinical isolates from the Kilombero District, Tanzania. *Acta Trop.* **74** 51-61.

Jilly P, Nkrumah F(1964) A survey of anaemia in children in the Korle Bu Hospital with special reference to malaria. *Ghana Med.J.* **3** 118-124.

Jones TR, Baird JK, Bangs MJ, Annis BA, Purnomo, Basri H, Gunawan S, Harjosuwarno S, McElroy PD, Hoffman SL(1994) Malaria vaccine study site in Irian Jaya, Indonesia: *Plasmodium falciparum* incidence measurements and epidemiologic considerations in sample size estimation. *Am.J.Trop.Med.Hyg.* **50** 210-218.

Konate L, Zwetyenga J, Rogier C, Bischoff E, Fontenille D, Tall A, Spiegel A, Trape JF, Mercereau-Puijalon O(1999) Variation of *Plasmodium falciparum* msp1 block 2 and msp2 allele prevalence and of infection complexity in two neighbouring Senegalese villages with different transmission conditions. *Trans.R.Soc.Trop.Med.Hyg.* **93 Suppl 1** 21-28.

Koram KA, Bennett S, Adiamah JH, Greenwood BM(1995) Socio-economic determinants are not major risk factors for severe malaria in Gambian children. *Trans.R.Soc.Trop.Med.Hyg.* **89** 151-154.

Koram KA, Owusu-Agyei S, Utz G, Binka FN, Baird JK, Hoffman SL, Nkrumah FK(2000) Severe anemia in young children after high and low malaria transmission seasons in the Kassena-Nankana district of northern Ghana. *Am.J.Trop.Med.Hyg.* **62** 670-674.

Kurtzhals JA, Addae MM, Akanmori BD, Dunyo S, Koram KA, Appawu MA, Nkrumah

FK, Hviid L(1999) Anaemia caused by asymptomatic *Plasmodium falciparum* infection in semi- immune African schoolchildren. *Trans.R.Soc.Trop.Med.Hyg.* **93** 623-627.

Kurtzhals JA, Rodrigues O, Addae M, Commey JO, Nkrumah FK, Hviid L(1997) Reversible suppression of bone marrow response to erythropoietin in *Plasmodium falciparum* malaria. *Br.J.Haematol.* **97** 169-174.

Kyes S, Harding R, Black G, Craig A, Peshu N, Newbold C, Marsh K(1997) Limited spatial clustering of individual *Plasmodium falciparum* alleles in field isolates from coastal Kenya. *Am.J.Trop.Med.Hyg.* **57** 205-215.

Lackritz EM, Hightower AW, Zucker JR, Ruebush TK, Onudi CO, Steketee RW, Were JB, Patrick E, Campbell CC(1997) Longitudinal evaluation of severely anemic children in Kenya: the effect of transfusion on mortality and hematologic recovery. *AIDS* **11** 1487-1494.

Lepers JP, Fontenille D, Rason MD, Chougnet C, Astagneau P, Coulanges P, Deloron P(1991) Transmission and epidemiology of newly transmitted *falciparum* malaria in the central highland plateaux of Madagascar. *Ann.Trop.Med.Parasitol.* **85** 297-304.

Luzzatto L(1979) Genetics of red cells and susceptibility to malaria. *Blood* **54** 961-976.

Matteelli A, Donato F, Shein A, Muchi JA, Leopardi O, Astori L, Carosi G(1994) Malaria and anaemia in pregnant women in urban Zanzibar, Tanzania. *Ann.Trop.Med.Parasitol.* **88** 475-483.

Mbogo CN, Snow RW, Khamala CP, Kabiru EW, Ouma JH, Githure JI, Marsh K, Beier JC (1995) Relationships between *Plasmodium falciparum* transmission by vector populations and the incidence of severe disease at nine sites on the Kenyan coast. *Am.J.Trop.Med.Hyg.* **52** 201-206.

McElroy PD, Beier JC, Oster CN, Beadle C, Sherwood JA, Oloo AJ, Hoffman SL(1994) Predicting outcome in malaria: correlation between rate of exposure to infected mosquitoes and level of *Plasmodium falciparum* parasitemia. *Am.J.Trop.Med.Hyg.* **51** 523-532.

McElroy PD, Beier JC, Oster CN, Onyango FK, Oloo AJ, Lin X, Beadle C, Hoffman SL(1997) Dose- and time-dependent relations between infective Anopheles inoculation and outcomes of *Plasmodium falciparum* parasitemia among children in western Kenya. *Am.J.Epidemiol.* **145** 945-956.

McElroy PD, Kuile FO, Lal AA, Bloland PB, Hawley WA, Oloo AJ, Monto AS,

Meshnick SR, Nahlen BL(2000) 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.* **62** 504-512.

McGregor I(1965) The passive transfer of human immunity. *Am.J.Trop.Med.Hyg.* **13** 237-261.

McGuire W, Knight JC, Hill AV, Allsopp CE, Greenwood BM, Kwiatkowski D(1999) Severe malarial anemia and cerebral malaria are associated with different tumor necrosis factor promoter alleles. *J.Infect.Dis.* **179** 287-290.

Mendis KN, David PH, Carter R(1991) Antigenic polymorphism in malaria: is it an important mechanism for immune evasion? *Immunol.Today* **12** A34-A37.

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

Menendez C, Kahigwa E, Hirt R, Vounatsou P, Aponte JJ, Font F, Acosta CJ, Schellenberg DM, Galindo CM, Kimario J, Urassa H, Brabin B, Smith TA, Kitua AY, Tanner M, Alonso PL(1997) Randomised placebo-controlled trial of iron supplementation and malaria chemoprophylaxis for prevention of severe anaemia and malaria in Tanzanian infants. *Lancet* **350** 844-850.

Miller LH, Good MF, Milon G(1994) Malaria pathogenesis. *Science* **264** 1878-1883.

Miller LH, Hoffman SL(1998) Research toward vaccines against malaria. *Nat.Med.* **4** 520-524.

Ministry of Health. Report of the War Memorial Hospital, Kassena-Nankana District of Ghana. 1996.

Mkawagile DS, Kihamia CM(1986) Relationship between clinical diagnosis of malaria and parasitaemia in adult patients attending Mwananyamala dispensary, Dar es Salaam. *Cent.Afr.J.Med.* **32** 2-5.

Modiano D, Sirima BS, Sawadogo A, Sanou I, Pare J, Konate A, Pagnoni F(1998) Severe malaria in Burkina Faso: influence of age and transmission level on clinical presentation. *Am.J.Trop.Med.Hyg.* **59** 539-542.

Molineaux L(1985) The pros and cons of modelling malaria transmission. *Trans.R.Soc.Trop.Med.Hyg.* **79** 743-747.

Molyneux L, Gramiccia G (1980) The Garki Project: Research on the Epidemiology and

Control of Malaria in the Sudan Savanna of West Africa. World Health Organisation, Geneva.

Molyneux ME, Taylor TE, Wirima JJ, Borgstein A(1989) Clinical features and prognostic indicators in paediatric cerebral malaria: a study of 131 comatose Malawian children. *Q.J.Med.* **71** 441-459.

Msuya FH, Curtis CF(1991) Trial of pyrethroid impregnated s in an area of Tanzania holoendemic for malaria. Part 4. Effects on incidence of malaria infection. *Acta Trop.* **49** 165-171.

Muller DA, Charlwood JD, Felger I, Ferreira C, do R, V, Smith T(2001) Prospective risk of morbidity in relation to multiplicity of infection with *Plasmodium falciparum* in Sao Tome. *Acta Trop.* **78** 155-162.

Murphy SC, Breman JG(2001) Gaps in the childhood malaria burden in Africa: cerebral malaria, neurological sequelae, anemia, respiratory distress, hypoglycemia, and complications of pregnancy. *Am.J.Trop.Med.Hyg.* **64** 57-67.

Nabarro DN, Tayler EM(1998) The "roll back malaria" campaign. *Science* **280** 2067-2068.

Newton CR, Taylor TE, Whitten RO(1998) Pathophysiology of fatal *falciparum* malaria in African children. *Am.J.Trop.Med.Hyg.* **58** 673-683.

Nkrumah F(1973) Severe anaemia associated with malaria in children. *Ghana Med.J.* **12** 17-21.

Ntoumi F, Contamin H, Rogier C, Bonnefoy S, Trape JF, Mercereau-Puijalon O(1995) Age-dependent carriage of multiple *Plasmodium falciparum* merozoite surface antigen-2 alleles in asymptomatic malaria infections. *Am.J.Trop.Med.Hyg.* **52** 81-88.

Ntoumi F, Mercereau-Puijalon O, Ossari S, Luty A, Reltien J, Georges A, Millet P(1997) *Plasmodium falciparum*: sickle-cell trait is associated with higher prevalence of multiple infections in Gabonese children with asymptomatic infections. *Exp.Parasitol.* **87** 39-46.

Ofosu-Okyere A, Mackinnon MJ, Sowa MP, Koram KA, Nkrumah F, Osei YD, Hill WG, Wilson MD, Arnot DE(2001) Novel *Plasmodium falciparum* clones and rising clone multiplicities are associated with the increase in malaria morbidity in Ghanaian children during the transition into the high transmission season. *Parasitology* **123** 113-123.

Orago AS, Wattimah DN, Aloka PL, Gitau CW, Orago MA, Onyango TG(2001) An analysis of haematological parameters in patients and individual residents of a *Plasmodium falciparum* malaria holoendemic area of western Kenya. *Microbios* **106 Suppl 2** 117-132.

Owusu-Agyei S Fryauff DJ, Chandramohan D, Koram KA, Binka FN, Nkrumah FK, Utz GC, Baird JK and Hoffman SL(2001a) Characteristics of severe anemia and its association with malaria in young children of the Kassena-Nankana District of Northern Ghana. *Am.J.Trop.Med.Hyg.* **In Press.**

Owusu-Agyei S, Awini E, Anto F, Mensah-Afful T, Afari E, and Binka F(2001b) Situation analysis of Malaria control activities in the Kassena-Nankana District in northern Ghana: Monitoring and Evaluating through the Roll Back Malaria. *Trop.Med.Int.Health* . **In Press**

Owusu-Agyei S, Koram KA, Baird JK, Utz GC, Binka FN Nkrumah FK, Fryauff DJ and Hoffman SL(2001c) Incidence of symptomatic and asymptomatic *Plasmodium falciparum* infection following curative therapy in adult residents of northern Ghana. *Am.J.Trop.Med.Hyg.* **65(3)** 197-203.

Pan American Health Organisation. Status of malaria programs in the Americas. XLV. 1997. Washington DC.

Pasvol G, Weatherall D, Wilson RM(1977) Effects of foetal haemoglobin on susceptibility of red cells to *Plasmodium falciparum*. *Nature* **270** 171-173.

Payne D, Grab B, Fontaine RE, Hempel JH(1976) Impact of control measures on malaria transmission and general mortality. *Bull.World Health Organ* **54** 369-377.

Peyerl-Hoffmann G, Jelinik T, Kilian A, Kabambe G, Metzger W,G, von Sonnenurg F(2001) Genetic diversity of *Plasmodium falciparum* and its relationship to parasite density in an area with different malaria endemicities in West Uganda. *Trop.Med.Int.Health* **6** 607-613.

Pringle G & Avery-Jones S(1966) Observations of early course of untreated *falciparum* malaria in semi-immune African children following a short period of protection. *Bull.World Health Organ* **34** 269-272.

Pull J, Grab B(1974) A simple epidemiologic model for evaluating the malaria inoculation rate and the risk of infection in infants. *Bull.World Health Organ* **51** 507-516.

Rogier C, Commenges D, Trape JF(1996) Evidence for an age-dependent pyrogenic threshold of *Plasmodium falciparum* parasitemia in highly endemic populations. *Am.J.Trop.Med.Hyg.* **54** 613-619.

Roper C, Elhassan IM, Hviid L, Giha H, Richardson W, Babiker H, Satti GM, Theander TG, Arnot DE(1996) Detection of very low level *Plasmodium falciparum* infections using the nested polymerase chain reaction and a reassessment of the epidemiology of unstable malaria in Sudan. *Am.J.Trop.Med.Hyg.* **54** 325-331.

Rougemont A, Breslow N, Brenner E, Moret AL, Dumbo O, Dolo A, Soula G, Perrin L(1991) Epidemiological basis for clinical diagnosis of childhood malaria in endemic zone in West Africa. *Lancet* **338** 1292-1295.

Rzepczyk CM, Csurhes PA, Baxter EP, Doran TJ, Irving DO, Kere N(1990) Amino acid sequences recognized by T cells: studies on a merozoite surface antigen from the FCQ-27/PNG isolate of *Plasmodium falciparum*. *Immunol.Lett.* **25** 155-163.

SAS Institute Inc. (1996) SAS/STAT Software. Changes and Enhancements for Release. SAS Institute Inc., 6.12 Cary, NC, USA.

Schellenberg D, Menendez C, Kahigwa E, Aponte J, Vidal J, Tanner M, Mshinda H, Alonso P(2001) Intermittent treatment for malaria and anaemia control at time of routine vaccinations in Tanzanian infants: a randomised, placebo- controlled trial. *Lancet* **357** 1471-1477.

Schellenberg D, Menendez C, Kahigwa E, Font F, Galindo C, Acosta C, Schellenberg JA, Aponte JJ, Kimario J, Urassa H, Mshinda H, Tanner M, Alonso P(1999) African children with malaria in an area of intense *Plasmodium falciparum* transmission: features on admission to the hospital and risk factors for death. *Am.J.Trop.Med.Hyg.* **61** 431-438.

Scheller LF, Azad AF(1995) Maintenance of protective immunity against malaria by persistent hepatic parasites derived from irradiated sporozoites. *Proc.Natl.Acad.Sci.U.S.A* **92** 4066-4068.

Schneider, AG, Premji, Z, Felger, I, Smith, T, Abdulla, S, and Beck, HP and Mshinda H. A point mutation in codon 76 of pfcrt of *Plasmodium falciparum* is positively selected for by Chloroquine treatment in Tanzania. *Molecular Epidemiology and Evolutionary Genetics of Infectious Diseases.* 2001 In Press.

Sergent E, Parrot L(1935) L'immunite, la premunition et al. resistance innee. *Archives* **13** 279-319.

Service MW (1996) Medical entomology for students. Chapman & Hall, London UK, pp 36-53 .

Slutsker L, Bloland P, Steketee RW, Wirima JJ, Heymann DL, Breman JG(1996) Infant and second-year mortality in rural Malawi: causes and descriptive epidemiology. *Am.J.Trop.Med.Hyg.* **55** 77-81.

Slutsker L, Taylor TE, Wirima JJ, Steketee RW(1994) In-hospital morbidity and mortality due to malaria-associated severe anaemia in two areas of Malawi with different patterns of malaria infection. *Trans.R.Soc.Trop.Med.Hyg.* **88** 548-551.

Smith T(1996) Distinction between parasitologic and clinical efficacy of antimalarial agents. *J.Infect.Dis.* **173** 275-276.

Smith T, Beck HP, Kitua A, Mwankusye S, Felger I, Fraser-Hurt N, Irion A, Alonso P, Teuscher T, Tanner M(1999b) Age dependence of the multiplicity of *Plasmodium falciparum* infections and of other malariological indices in an area of high endemicity. *Trans.R.Soc.Trop.Med.Hyg.* **93 Suppl 1** 15-20.

Smith T, Felger I, Kitua A, Tanner M, Beck HP(1999c) Dynamics of multiple *Plasmodium falciparum* infections in infants in a highly endemic area of Tanzania. *Trans.R.Soc.Trop.Med.Hyg.* **93 Suppl 1** 35-39.

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

Smith T, Hurt N, Teuscher T, Tanner M(1995) Is fever a good sign for clinical malaria in surveys of endemic communities? *Am.J.Trop.Med.Hyg.* **52** 306-310.

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

Snow RW, Nahlen B, Palmer A, Donnelly CA, Gupta S, Marsh K(1998) Risk of severe malaria among African infants: direct evidence of clinical protection during early infancy. *J.Infect.Dis.* **177** 819-822.

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

Stata Corporation (1999) Stata Statistical Software: Release 6.0.. Stata Press, College Station.

Stein CM, Gelfand M(1985) The clinical features and laboratory findings in acute *Plasmodium falciparum* malaria in Harare, Zimbabwe. *Cent.Afr.J.Med.* **31** 166-170.

Steketee RW, Nahlen BL, Parise ME, Menendez C(2001) The burden of malaria in pregnancy in malaria-endemic areas. *Am.J.Trop.Med.Hyg.* **64** 28-35.

Stich AH, Maxwell CA, Haji AA, Haji DM, Machano AY, Mussa JK, Matteelli A, Haji H, Curtis CF(1994) Insecticide-impregnated bed nets reduce malaria transmission in rural Zanzibar. *Trans.R.Soc.Trop.Med.Hyg.* **88** 150-154.

Taylor WR, Richie TL, Fryauff DJ, Picarima H, Ohrt C, Tang D, Braitman D, Murphy GS,

Widjaja H, Tjitra E, Ganjar A, Jones TR, Basri H, Berman J(1999) Malaria prophylaxis using azithromycin: a double-blind, placebo- controlled trial in Irian Jaya, Indonesia. *Clin.Infect.Dis.* **28** 74-81.

Tirasophon W, Rajkulchai P, Ponglikitmongkol M, Wilairat P, Boonsaeng V, Panyim S(1994) A highly sensitive, rapid, and simple polymerase chain reaction-based method to detect human malaria (*Plasmodium falciparum* and *Plasmodium* vivax) in blood samples. *Am.J.Trop.Med.Hyg.* **51** 308-313.

Tomashek KM, Woodruff BA, Gotway CA, Bloland P, Mbaruku G(2001) Randomized intervention study comparing several regimens for the treatment of moderate anemia among refugee children in Kigoma Region, Tanzania. *Am.J.Trop.Med.Hyg.* **64** 164-171.

Topley E(1968) Common anaemia in rural Gambia. 3. A spontaneously remitting anaemia possibly precipitated by malarial parasitaemia. *Trans.R.Soc.Trop.Med.Hyg.* **62** 602-606.

Trape JF(1985) Rapid evaluation of malaria parasite density and standardization of thick smear examination for epidemiological investigations. *Trans.R.Soc.Trop.Med.Hyg.* **79** 181-184.

Trape JF(2001) The public health impact of Chloroquine resistance in Africa. *Am.J.Trop.Med.Hyg.* **64** 12-17.

Trape JF, Rogier C, Konate L, Diagne N, Bouganali H, Canque B, Legros F, Badji A, Ndiaye G, Ndiaye P, .(1994) The Dielmo project: a longitudinal study of natural malaria infection and the mechanisms of protective immunity in a community living in a holoendemic area of Senegal. *Am.J.Trop.Med.Hyg.* **51** 123-137.

Van den HJ, Dalderop E, Smith Y(1996) Does iron therapy benefit children with severe malaria-associated anaemia? A clinical trial with 12 weeks supplementation of oral iron in young children from the Turiani Division, Tanzania. *J.Trop.Pediatr.* **42** 220-227.

Van Hensbroek MB, Palmer A, Jaffar S, Schneider G, Kwiatkowski D(1997) Residual neurologic sequelae after childhood cerebral malaria. *J.Pediatr.* **131** 125-129.

Verity CM, Greenwood R, Golding J(1998) Long-term intellectual and behavioral outcomes of children with febrile convulsions. *N.Engl.J.Med.* **338** 1723-1728.

Warrell DA, Molyneux M, Beales P(1990) Severe and complicated malaria. *Trans.R.Soc.Trop.Med.Hyg.* **84(Suppl. 2)** 1-65.

Weatherall DJ, Abdalla S, Pippard MJ(1983) The anaemia of *Plasmodium falciparum* malaria. *Ciba Found.Symp.* **94** 74-97.

Wilson DB(1950) A review of hyperendemic malaria. *Trop.Dis.Bull.* **47** 677-698.

World Bank. World Development Report: Investing in health. **329**. 1993. New York, Oxford University Press.

World Health Organisation. Ad hoc Committee on Health Research Relating Future Intervention options. Investing in health research and development**. TDR/Gen/96.1**. 1996. Geneva, Switzerland.

World Health Organisation. The World Health Report. Fighting disease, fostering development. **137**. 1996. Geneva, WHO.

World Health Organisation. World Malaria Situation. Weekly Epidemiological Record. **3**, 17-22. 1996. Geneva.

World Health Organisation. Guidelines for the evaluation of *Plasmodium falciparum* vaccines in populations exposed to natural infections. **TDR/MAL/VAC/97**. 1997. Geneva, Switzerland.

World Health Organisation. Tropical Diseases Research Progress 1995-96. Thirteenth Program Report UNDP/World Bank/WHO Special Program for Research and Training in Tropical Diseases (TDR). **1997**. WHO/TDR.

World Health Organisation. The World Health Report **1999**: making a difference. 1999.

World Health Organisation.

World Health Organisation. Economic costs of malaria are many times higher than previously estimated. **WHO/28**. 2000. Geneva, WHO.

World Health Organisation. Roll Back Malaria, The Abuja Declaration. Part III. **2000**. Abuja, Nigeria.

World Health Organisation(2000c) Severe and complicated malaria. *Trans.R.Soc.Trop.Med.Hyg.* **94(suppl.)** 51-90.

Zwetyenga J, Rogier C, Tall A, Fontenille D, Snounou G, Trape JF, Mercereau-Puijalon O(1998) No influence of age on infection complexity and allelic distribution in *Plasmodium falciparum* infections in Ndiop, a Senegalese village with seasonal, mesoendemic malaria. *Am.J.Trop.Med.Hyg.* **59** 726-735.

CURRICULUM VITAE:

Personal Data

Name:	Seth Ogyebre Owusu-Agyei
Date & Place of Birth:	December 25, 1960, Apesokubi. Ghana
Marital status:	Married to Mercy Owusu-Agyei
Nationality:	Ghanaian

EDUCATION /TRAINING

1965 - 1970:	Presbyterian Primary School, Apesokubi
1970 - 1973:	Local Authority Middle School, Apesokubi
1973 - 1978:	Presbyterian Boys Secondary School, Legon (GCE O' Level)
1978 - 1980:	Bishop Herman Secondary School, Kpando (GCE A' Level)
1982 - 1986:	University of Ghana, Legon. BSc Zoology/ Chemistry
1993 - 1994:	Liverpool School of Tropical Medicine, UK. (MSc Medical Parasitology & Entomology
1996 - 2001:	Swiss Tropical Institute, Basel. PhD in Epidemiology

Professional Experience:

1987 - 1989:	Research Assistant, Noguchi Memorial Institute for Medical Research. Legon. Ghana.
1989 - 1992:	Principal Research Assistant, Ghana Vitamin A Supplementation Trial, Navrongo.
1992 - Present:	Senior Health Research Scientist, Navrongo Health Research Centre.

Publications:

Afari, E.A., Akanmori, B.D., Nakano, T., Ofori-Adjei, D., **Owusu-Agyei**, S., Gyan, B. and Adjei, A. (1989): In-vivo and in-vitro sensitivity status of Plasmodium falciparum to chloroquine in three communities in Ghana. *Ghana Medical Journal*: 23, 233-236.

Akanmori, B.D., Afari, E.A., Nakano, T., Ofori-Adjei, D., Gyan, B., **Owusu-Agyei, S**. and Adjei, A. (1989): In vivo and In vitro sensitivity status of Plasmodium falciparum to chloroquine, Ammodiaquine, Quinine and Sulfadoxine/Pyrimethamine in three communities in Ghana. *Ghana Medical Journal*: 23, 227-232.

Afari, E.A., Nakano, T., Binka, F., **Owusu-Agyei, S**. and Asigbee, J. (1993): Seasonal characteristics of malaria infection in under-five children of a rural community in southern Ghana: *West African Journal of Medicine: 12, 39-42*.

K. A. Koram, **S. Owusu-Agyei**, G. Utz, F. Binka , K. Baird, S. L. Hoffman, and F. K. Nkrumah: Severe Anaemia in Young Children after High and Low Malaria Transmission seasons in the Kassena-Nankana District of northern Ghana. *American Journal of Tropical Medicine & Hygiene* 62(6) 2000, pp. 670-674.

S Owusu-Agyei, KA Koram, JK Baird, GC Utz, FN Binka, FK Nkrumah, DJ Fryauff, and SL Hoffman: Incidence of symptomatic and asymptomatic .*P. falciparum* infection following curative therapy in adult residents of northern Ghana: *American Journal of Tropical Medicine & Hygiene* 65(3) 2001, pp 197-203.

J. Kevin Baird, **S. Owusu-Agyei**, Gregory C. Utz, Kwadwo Koram, Mazie J. Barcus, Stephen L. Hoffman, and Francis N. Nkrumah: Seasonal Malaria Attack rates in Infants and Young Children in Northern Ghana. *American Journal of Tropical Medicine and Hygiene* 65(4). 2001. (In Press).

Owusu-Agyei S Fryauff DJ, Chandramohan D, Koram KA, Binka FN, Nkrumah FK, Utz GC, Baird JK and Hoffman SL (2001) Characteristics of severe anemia and its association with malaria in young children of the Kassena-Nankana District of Northern Ghana. Submitted to *American Journal of Tropical Medicine and Hygiene.*

S. Owusu-Agyei Fred N. Binka, K. A. Koram, F. Anto, and M.Adjuik: Does Radical Cure of Asymptomatic *P. falciparum* place Adults in Endemic Areas at Increased Risk of Recurrent Symptomatic Malaria? Submitted to *Tropical Medicine & International Health 2001*

S. Owusu-Agyei, T. Smith, HP. Beck, L. Amenga-Etego, I. Felger: Molecular Epidemiology of *Plasmodium falciparum* infections among asymptomatic inhabitants of a holoendemic malarious area in northern Ghana. Submitted to *Tropical Medicine & International Health 2001*

Owusu-Agyei S, Awini E, Anto F, Mensah-Afful T, Afari E, and Binka F(2001) Situation analysis of Malaria control activities in the Kassena-Nankana District in northern Ghana: Monitoring and Evaluating through the Roll Back Malaria. Submitted to *Tropical Medicine & International Health*.