The Epidemiology and the burden of malaria in Mozambique

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This work is dedicated to my family

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ABBREVIATIONS

µl	Microlitre
AIDS	Acquired Immunodeficiency Syndrome
AMA	Apical membrane antigen
An	*Anopheles*
B.C.	Before Christ
bp	base pairs
CFM	Mozambique Railways
CISM	Centro de Investigaçao em Saúde da Manhiça.
CSP	Circumsporozoite protein
DDT	Dichlorodiphenyltrichloroethane
DHFR	Dehydropholate Reductase
DHPS	Dehydrophosphate sinthetase
dl	decilitre
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleotide triphosphate
DSS	Demographic Surveillance Sites
EDTA	Ethylenediamine tetra-acetic acid
G6PD	Glucose-6-Phosphatase
GHG	Greenhouse gases
gr	gram
HBsAG	Hepatitis B surface antigen
HCH or BCH	Hexachlorocyclohexane
Hgb	haemoglobin
HIV	Human Immunodeficiency Virus
HLA	Human Antigen
IL	Interleukin
Kg	kilogram
Km^2	square kilometre
l	litre
LM	Lourenço Marques city
M	Molar
Ml	Millilitre

Mm	Millimetres
MSP	Merosoite surface protein
NMCP	National Malaria Control Programme
º C	Degree Celsius
º	Degree
P. falc.	*Plasmodium falciparum*
PCR	Polymerase Chain Reaction
P*f*EMP	*P. falciparum* erythrocyte membrane protein
pH	Potential Hydrogen
PVC	Packed Volume Concentration
TNF	Tumour Necrotic Factor
UVR	Ultra Violet Radiation
WHO	World Health Organization

ABSTRACT

Malaria occurs mostly in the tropical regions of the world. Sub-Saharan Africa is the area most affected. The occurrence of a very efficient mosquito vectors *Anopheles gambiae* complex and *Anopheles funestus* group sustain high transmission of the *Plasmodium falciparum*, the most predominant and deadly malaria parasite species. Local weather conditions are appropriate and often transmission occurs throughout the year.

Limited resources and socio-economic instability constitute the major factors impeding efficient malaria control activities.

The worldwide malaria eradication programme carried out during the 1950's focused mainly on insecticide residual spraying with DDT, anti-malarial drug treatment, and surveillance.

Regional eradication of the disease was achieved, nevertheless, in many endemic regions of sub-Saharan Africa excluded from the eradication campaign, the disease is still afflicting their inhabitants.

The malaria disease burden estimation in tropical Africa relies on mortality and morbidity data collected by the health system information.

Conservative estimates of the burden of disease claim for more than 300 million clinical episodes and 1 – 3 million deaths every year and young children harbour the largest and most important portion of this bulk.

Currently, the situation is deteriorating, increasing malaria-related morbidity and mortality have been reported. The rapid development and widespread of parasites strains resistant to almost all anti-malarial drugs, and vector resistance are the major contributing factors.

In addition, global climate change is affecting the health of human populations, including changes in the transmission and seasonality of vector-borne diseases. The range of factors affecting transmission and distribution of vector-borne diseases, particularly malaria, include those related to temperature, humidity and precipitation.

In Mozambique, malaria is endemic throughout the country, due to a multitude of factors such as climatic/environmental (favourable temperatures and rain patterns, abundant breeding sites) and socio-economical (poverty related improper housing/shelter, unaffordable preventive means). Transmission is

perennial, with peaks during and after rainy seasons. The intensities of transmission may vary depending on the amount of rain and air temperature. However, at present there is a lack of good quality and updated information on the endemicity levels in the country.

The country-wide malaria survey carried out between 2002 and 2003 aimed to determine the prevalence and intensity of *Plasmodium* infections, the prevalence and the severity of anaemia in children under 10 years of age and in pregnant women across different ecological settings, in order to characterize the malaria transmission intensities and to estimate the disease burden in Mozambique.

The last comprehensive malaria survey in the country was carried out in 1952. For that reason, this survey was an unique opportunity to perform a sound methodological assessment of the current epidemiological malaria situation in the country.

Based on altitude and on geographical region differences samples were collected from stratified areas distinguished as coastal, plateau and highland strata, in the northern, centre-northern, central and southern regions. For sampling at community level, in each of those stratified areas, a modified cluster sampling method with 30 clusters, used by WHO for evaluation of coverage of the Expanded Programme of Immunization was adopted.

The study consisted of house-to-house survey, in 24 districts randomly selected. A total of 12,002 subjects including children less than ten years of age and pregnant women were enrolled. The malariometric survey consisted of finger pricking and blood collection to prepare thick and thin film for malaria parasite species identification, and respective density and determination of haemoglobin concentration. Measurement of axillary temperature and in those with fever a rapid enzyme test for malaria diagnosis was performed.

The entomologic survey consisted of pyrethrum spray knock down mosquito collections. In total 6,557 female anopheline mosquitoes caught in 1,440 dwellings, were analysed for sporozoite infection using PCR techniques and the entomological inoculation rates were determined for each strata across regions.

Malaria in children

In general, 58.9% (5,190/8,816) of blood smears children were positive for malaria parasites. The majority, 46.5% (4,098/8,816) were *Plasmodium falciparum* infection, 3.6% (321/8,816) were *Plasmodium malariae* and 2.9% (253/8,816) were mixed infections of *Plasmodium falciparum* and *Plasmodium malariae*. Gametocytes only for *Plasmodium falciparum* were recorded in 5.9% (518/8,816). Of all infections *Plasmodium falciparum* accounted for 92.7% (4,098/4,419).

The prevalence of malaria infection showed variations throughout various regions in the country, decreasing from north-to-south. The highest prevalence of *Plasmodium falciparum* infection was recorded in both northern and the central-northern regions 54.8% (1,313/2,387) and 58.7% (992/1,929), respectively.

Across strata, there was a significant decrease of *Plasmodium falciparum* infection prevalence from the coastal stratum to the highland stratum in both central ($p=0.02$) and southern ($p=0.004$) regions, while in the northern region ($p=0.247$) and in the northern-central region ($p=0.470$), the variations observed were not statistically significant.

High mean parasite density of 2,058 parasites/µl (95% CI, 1,836 – 2,306) was recorded in children across the central-northern region, and young children aged below 12 months old harbour the maximum load of parasite density 3,494 parasites/µl, (95% CI 2,641 – 4,621). Whereas, low mean parasite density of 891 parasites/µl (95% CI, 799 – 994) was recorded among children in central region. The overall mean parasite density in the northern and southern regions was 1,077 parasites/µl (95% CI, 965 – 1,200) and 1,193 parasites/µl (95% CI, 1,025 – 1,388), respectively.

Generally, in both northern and centre-northern regions, mean parasite density peaked during the first 12 months of age, while in central and southern regions the peak was recorded later among children in the 12 – 23 months age group. Though, in all regions, parasite mean densities were markedly age-dependent, and decreased sharply with age ($p<0.0001$).

Overall, the prevalence of fever among children was 9.4% (766/8,816), and fever associated with *Plasmodium falciparum* infections was 5.7% (498/8,816).

The overall attributable fraction of fever was 37.8% (95% CI, 31.9 – 43.6). The highest attributable fraction of fever of 43.5% (95% CI, 25.8 – 61.2) was recorded among children less than 12 months of age. Children in the age group 12 – 59 months of age had an attributable fraction of 39.6% with 95% CI, 30.3 – 48.9) and in children aged above 5 years old the attributable fraction was 21.5% (95% CI, 11.6 – 31.4).

Overall mean haemoglobin concentration was 9.9 g/dl (95% CI 9.5 – 10.2), and showed insignificant differences across regions. Overall the prevalence of anaemia was 69.8% (6.257/8.816) and among anaemic children 11.5% (743/6.257) were severely anaemic.

The highest anaemia prevalence was recorded in the northern and central-northern regions 77.9% and 79.4% respectively. There were not significant differences on anaemia prevalence across strata, except in the northern region where the coastal stratum had high proportion of anaemia.

There was a significant association between prevalence of anaemia and malaria parasite infections.

Malaria in pregnancy

Among pregnant women, 34.7% (478/1,531) of blood smears were positive for malaria parasites. The majority of blood smears, 33.6% (465/1,531) were purely *Plasmodium falciparum* parasites. Gametocytes only for *Plasmodium falciparum* were recorded in 1.4% (28/1,531) of blood smears. There were no records of infections by *Plasmodium ovale* or *plasmodium vivax* parasites. *Plasmodium falciparum* parasites accounted for 97.3% (465/478) of all malaria infections.

Pregnant women living in the northern region had the highest prevalence of asexual *Plasmodium falciparum* parasites 40.0% (75/203). The lowest prevalence was recorded in the southern region 24.6% (127/470).

Overall, younger pregnant women had a higher risk to malaria parasites infection compared to older pregnant women. The prevalence of *Plasmodium falciparum* infection and mean parasite density decreased with increasing parity.

The prevalence of clinical malaria among pregnant women was very low 1.2%.

Mean haemoglobin concentrations ranged from 1.9 to 17.7 g/dl, and overall mean estimation was 10.3 g/dl (95% CI, 9.9 – 10.6). The prevalence of anaemia

was very high 62.5% (975/1,531), and was significantly associated with *Plasmodium falciparum* malaria parasite infection (p=0.003).

Entomological Inoculation Rates

An. gambiae s.l. and *An. funestus* are the most prevalent vectors. Overall, the sporozoite rate of 0.7% (46/6,557) obtained, exhibited significant regional variations. The gradient of entomological inoculation rates showed both north-to-south and low-to-highlands decrease. Entomological inoculation rate of 33.4 infective bites/person/year was recorded in the central-northern region. Conversely, in the southern region was recorded the lowest entomological inoculation rate of 2.6 infective bites/person/year. In the coastal stratum the entomological inoculation rate was 19.0 infective bites/person/year, while in the highland stratum the entomological inoculation rate was 2.0 infective bites/person/year.

Conclusion

This large country-wide survey confirms that malaria, especially that caused by *Plasmodium falciparum,* remains endemic throughout the country and therefore represents a large public health problem in Mozambique.

Transmission is due to *Anopheles gambiae s.l.* and *Anopheles funestus*.

There is substantial variation in the intensity of malaria transmission across different geographical and ecological settings throughout the country. This heterogeneity is reflected in the large variation in the prevalence of *Plasmodium falciparum* infection, and is partly a consequence of the variations in the estimated entomological inoculation rates.

Young children and pregnant women bear the brunt of the infection and this implies exposure to intense malaria transmission.

The prevalence of malaria infection during pregnancy is high, particularly among young women in their first pregnancy.

In general, along the coastline and in the flat terrains, malaria transmission can be categorized as hyperendemic. The inland strata across central and southern regions can be categorized as mesoendemic.

This survey highlights the huge burden of anaemia in the country.

Among children, the prevalence of severe anaemia is high during the first two years of life. Among pregnant women, the risk of both mild and severe anaemia is high during the first and second trimester of gestation.

Given that anaemia is a key risk factor for survival and cognitive development, its control should become a Public Health priority for the country.

Despite the multi-factorial aetiology of anaemia, malaria infection is bound to be one of the key determinants of this high prevalence.

Across the country approximately more than2,6 million children less than ten years of age are infected with *Plasmodium falciparum* malaria parasites at any time and more than 3,8 milliion are anaemic.

Across the country more than 666,000 pregnant women are infected with *Plasmodium falciparum* malaria parasites at any time and more than 1.2 million are anaemic.

Maternal malaria infection and anaemia are major contributing factors to the global burden of infant mortality. Interventions to prevent maternal malaria and anaemia are essential, not only for improving maternal health status, but also, for preventing child mortality and improving infant health and survival.

The burden of malaria disease and anaemia-related malaria during pregnancy and childhood constitutes a major public health problem and warrant integrated and collaborative interventions to its control. Intermittent preventive treatment, insecticide treated nets, mass de-worming, iron and vitamin A supplementation programmes have already proven to be cost-effective interventions, particularly in areas lacking adequate health care services. Moreover, in future malaria vaccines may also contribute to improved control.

Estimates of the disease burden caused by malaria are crucial for planning cost-effectively malaria control interventions, monitoring and advocacy.

Both entomological inoculation rates and malaria prevalence should be used to characterize malaria endemicity and guide planning and implementation of appropriate control interventions.

ABSTRACT (Spanish)

La malaria se encuentra preferentemente en las regiones tropicales del mundo, siendo África sub-sahariana el área con más afectación. La gran eficiencia de los mosquitos vectores *Anopheles gambiae* complex y *Anopheles funestus* favorece una gran transmisión del *Plasmodium falciparum*, el parásito más predominante y más maligno de las especies causante de malaria. Las condiciones ambientales locales son apropiadas y a menudo la transmisión se da a lo largo todo el año.

Los recursos limitados y la inestabilidad socio-económica constituyen los principales factores que impiden la total eficacia de las estrategias de control de la malaria.

El programa de erradicación mundial de la malariase se llevó a cabo durante los años 50 focalizándose básicamente en la fumigación con DDT, el tratamiento con fármacos anti-maláricos y la vigilancia.

La erradicación regional de la enfermedad se conseguió en muchas regiones endémicas del África sub-sahariana, no obstante en zonas excluidas de la campaña de erradicación, la enfermedad continua afectando a sus habitantes.

La estimación de la carga de enfermedad por malaria en el África tropical se obtiene de los datos de mortalidad y morbilidad recogidos por el sistema de informació de la salud.

Los datos conservadores estiman que la carga de esta enfermedad causa más de 300 millones de episodios clínicos y entre 1 – 3 millones de muertes cada año, siendo los niños los más afectados.

En la actualidad, la situación se esta deteriorando, observándose un incremento de la morbilidad y mortalidad por malaria. El rápido desarrollo, la propagación de cepas del parásito resistentes ante todos los fármacos anti-maláricos y la presencia de vectores resistentes son los factores que más han contribuido a este incremento .

A demás, el cambio climático global está afectando la salud de las poblaciones, incluyendo cambios en la transmision y estacionalidad de las enfermedades mediadas por vectores. Los factores que afectan la transmisión y la distribución de este tipo de enfermedades, particularmente la malaria, incluyen los relacionados con la temperatura, la humedad y las precipitaciones.

En Mozambique, la malaria es una enfermedad endémica en todo el país, debido a la multitud de factores, como las climáticos/medioambientales (temperaturas favorables y patrones de precipitación, abundantes espacios para la reproducción) y socio-económicos (pobreza relacionada con vivienda inadecuada, medios preventivos inasequibles). La transmisión es perenne, con picos durante y después de la estación de lluvias. Las intensidades de transmisión pueden variar dependiento de la cantidad de precipitación y la temperatura en el ambiente. Sin embargo, actualmente, hay una falta de información actualizada y de calidad sobre los niveles de endemicidad del país.

El estudio llevado acabo por todo el país entre los años 2002 y 2003 tenía como objetivo determinar la prevalencia y la intensidad de las infecciones por *Plasmodium*, la prevalencia y la severidad de la malaria en niños menores de 10 años de edad y en mujeres embarazadas a través de diferentes nichos ecológicos, para poder caracterizar la intensidad de transmisión por malaria y estimar la carga de esta enfermedad en Mozambique.

El último estudio exahustivo de estas características en el país fue realizado en 1952. Por esta razón, este estudio era una oportunidad única para realizar un asesoramiento metodológico profundo de la situación epidemiológica actual de la malaria el país. Las muestras fueron recogidas basándose en la altitud y diferencias geográfica de cada región. Las areas estratificadas se clasificaron en: estrato costero, plateau y de montaña, y según la región en: norte, centro-norte, central y sur. Para el muestreo a nivel de comunidad, en cada una de esas áreas estratificadas, se utilizó un método por muestreo modificado por grupos con 30 grupos, ya usado por la OMS para la evalución de la cobertura del Programa Ampliado de Vacunación.

El estudio consisitió en visitar casa por casa en 24 distritos seleccionados aleatóriamente. Se incluyeron un total de 12,002 individuos, tanto niños menores de 10 años de edad como mujeres embarazadas. El estudio malariométrico consistió en recoger sangre por punción en el dedo para preparar una lámina delgada y otra gruesa para la identificación de la especie del parásito de la malaria y una estimación de la densidad, y para la determinación de la concentración de hemoglobina. Se tomó la tempreatura axilar y en presencia de fiebre se realizaba un test enzimático rápido para el diagnóstico de malaria.

El estudio entomológico consistió en la recogida de mosquitos rociados con piretrum. En total 6,557 mosquitos anófeles hembra de 1,440 viviendas fueron analizados para infeción de esporozoito usando técnicas de PCR, y la tasa de inoculación entomológica fue determinada para cada estrato a través de las diferentes regiones.

Malaria en niños

En general, el 58.9% (5,190/8,816) de frotis de sangre de niños dieron positivo para parásitos de la malaria. La mayoría, el 46.5% (4,098/8,816) eran infecciones por *Plasmodium falciparum,* el 3.6% (321/8,816) eran causadas por *Plasmodium malariae* y un 2.9% (253/8,816) eran infecciones causadas por ambos, *Plasmodium falciparum* y *Plasmodium malariae*. Sólo los gametocitos de *Plasmodium falciparum* fueron registrados en un 5.9% (518/8,816). De todas las infecciones, *Plasmodium falciparum* representó un 92.7% (4,098/4,419).

La prevalencia de infección por malaria mostró variaciones según las diferentes regiones del país, disminuyendo de norte a sur. La prevalencia más alta de infección por *Plasmodium falciparum* se encontró en la región norte y centro-norte, con un 54.8% (1,313/2,387) y un 58.7% (992/1,929), respectivamente.

A través de los estratos, se encontró una disminución significativa de la prevalencia de la infección por *Plasmodium falciparum* del estrato costero al estrato montañoso en ambas regiones, la central (p=0.02) y en la region sur (p=0.004), mientras que en la región norte (p=0.247) y en la centro-norte (p=0.470), las variaciones que se observaron no eran estadísticamente significativas.

Se observó una densidad media-alta de parásito, 2,058 parásitos/µl (95% CI, 1,836 – 2,306), en niños a través de la región centro-norte, siendo los niños menores de 12 meses los que presentaban una carga mayor de densidad de parásito, 3,494 parásitos/µl, (95% CI 2,641 – 4,621). Sin embargo, una densidad parasitaria media-baja, 891 parásitos/µl (95% CI, 799 – 994), se encontró entre los niños de la región central. La media global de densidad parasitaria en las regiones norte y sur fue de 1,077 parásitos/µl (95% CI, 965 – 1,200) y 1,193 parásitos/µl (95% CI, 1,025 – 1,388), respectivamente.

En general, en ambas regiones del norte y centro-norte, la densidad parasitaria media alcanzó el punto más alto durante los primeros 12 meses de edad, mientras que en las regiones central y del sur se presentó más adelante, en niños entre 12 – 23 meses de edad. Aunque, en todas regiones, la media de la densidad parasitaria era altamente edad-dependiente, y disminuía claramente con la edad (p<0.0001).

En conjunto, la prevalencia de fiebre entre los niños fue de 9.4% (766/8,816), y de fiebre asociada a infecciones con *Plasmodium falciparum* un 5.7% (498/8,816).

La fracción general atribuida a fiebre fue de 37.8% (95% CI, 31.9 – 43.6). La fracción más alta atribuible a fiebre, un 43.5% (95% CI, 25.8 – 61.2), fue presentada en niños menores de 12 meses de edad. Los niños en el grupo de edad entre los 12 – 59 meses de edad tenían una fracción atribuible de 39.6% (95% CI, 30.3 – 48.9) y en niños mayores de 5 años de edad, la fracción atribuible era de 21.5% (95% CI, 11.6 – 31.4).

La media general de concentración de hemoglobinaral fue de 9.9 g/dl (95% CI 9.5 – 10.2), y no hubieron diferencias significativas a lo largo de las regiones. La prevalencia de anemia en general fue de 69.8% (6.257/8.816) y entre niños anémicos un 11.5% (743/6.257) tenían anemia severa.

La prevalencia más alta de anemia se presentó en las regiones norte y centro-norte 77.9% y 79.4%, respectivamente. No hubieron diferencias significativas en la prevalencia de anemia a través de los estratos, a excepción de la zona norte, donde el estrato costa presentaba una proporción más alta de anemia.

Hubo una asociación importante entre la prevalencia de anemia e infecciones por parásitos de la malaria.

Malaria en embarazo

Entre las mujeres embarazadas, el 34.7% (478/1,531) de los frotis de sangre dieron positivo para parásitos de la malaria. La mayoría de los frotis, el 33.6% (465/1,531) eran puramente *Plasmodium falciparum*. Sólo los gametocitos de *Plasmodium falciparum* fueron observados en 1.4% (28/1,531), de los frotis de sangre. No se observaron infecciones por *Plasmodium ovale* o *Plasmodium vivax*. Los parásitos de *Plasmodium falciparum* representaron un 97.3% (465/478) de todas las infecciones de malaria.

Las mujeres embarazadas de la zona norte tuvieron una más alta prevalencia del parásito asexual de *Plasmodium falciparum* 40.0% (75/203). La prevalencia más baja fue registrada en la región sur 24.6% (127/470).

En general, las mujeres jóvenes embarazadas tenían mayor riesgo de infección por malaria comparado con las embarazadas de edad más avanzada. La

prevalencia de infecciones por *Plasmodium falciparum* y la densidad parasitaria media disminuían con un incremento en la paridad.
La prevalencia de malaria clínica en mujeres embarzadas era muy baja 1.2%.
La concentración media de hemoglobina osclilaba entre 1.9 y 17.7 g/dl, y la estimación media fue de 10.3 g/dl (95% CI, 9.9 – 10.6). La prevalencia de anemia fue muy alta 62.5% (975/1,531), y estaba significativamente asociada a la infección por *Plasmodium falciparum* (p=0.003).

Tasa de inoculación entomológica

An. gambiae s.l. y *An. funestus* son los vectores más prevalentes. En general, la tasa de esporozoito que se obtuvo, un 0.7% (46/6,557), mostraba unas variaciones regionales significativas . El gradiente de la tasa de inoculación entomológica indicó una disminución tanto de norte a sur como de estrato bajo a alto/montañosode. Se observó una tasa de inoculación entomológica de 33.4 picaduras infectivas/persona/año en la región centro-norte. Y a la inversa, en la región sur se observó la tasa de inoculación entomológica más baja, de 2.6 picaduras infectivas/persona/año. En el estrato costero, la tasa de inoculación entomolgica fue de 19.0 picaduras infectivas/persona/año, mientras que en el estrato montañoso, la tasa de inoculación entomológica fue de 2.0 picaduras infectivas/persona/año.

Conclusión

Este extenso estudio a través de todo el país confirma que la malaria, especialmente la causada por *Plasmodium falciparum,* sigue siendo endémica a través del país, y por lo tanto, representa un gran problema de salud pública en Mozambique.
La transmisión es debida a *Anopheles gambiae s.l.* y *Anopheles funestus*.
Hay una variedad sustancial en la intensidad de la transmisión de malaria a través de las diferentes nichos ecológicos y geográficos del país. Esta heterogenidad se refleja en la gran variación de la prevalencia de infección por *Plasmodium falciparum,* y en parte es consecuencia de las variaciones en las tasas de inoculación entomológicas estimadas.
Los niños pequeños y las mujeres embarazadas representan el grupo más susceptible a la infección y esto implica una mayor exposición a la malaria.

La prevalencia de la infección de malaria durante el embarazo es alta, particularmente en mujeres jóvenes primigrávidas.

En general, a lo largo del litoral y en los terrenos llanos, la transmisión de malaria puede ser categorizada como hiperendémica. El estrato interior a través de las regiones central y sur puede definirse como mesoendemica.

Este estudió destaca la gran carga de anemia en el país.

Entre los niños, la prevalencia de anemia severa es alta durante los dos primeros años de vida. Entre mujeres embarazadas, el riesgo de anemia moderada y severa es alta durante el primer y el segundo trimestre de gestación.

Dado que la anemia es un factor clave para la supervivencia y el desarrollo cognitivo, su control debería ser una prioridad de Salud Pública en el país.

A pesar de que la etiología de la anemia es multi-factorial, la infección de malaria está ligada a uno de estos determinantes claves de esta alta prevalencia.

A lo largo del país aproximadamente más 2,6 millones de niños menores de diez años estan infectados con *Plasmodium falciparum* y más de 3.8 millones presentan anemia.

A lo largo del país, más de 660,000 mujeres embarazadas están infectadas por *Plasmodium falciparum* y más de 1,2 millones presentan anemia.

La presencia de malaria materna y anemia son los factores que más contribuyen a la mortalidad infantil globalmente. Las intervenciones para prevenir la malaria materna y la anemia son esenciales, no sólo para mejorar la salud maternal, sino también para prevenir la mortalidad infantil y mejorar la salud y la supervivencia infantil.

La carga por enfermedad malárica y la anemia relacionada con la malaria durante el embarazo y la infancia constituyen un importante problema de salud pública que requiere de colaboraciones e intervenciones para su control. El tratamiento preventivo intermitente, las redes mosquiteras impregnadas de insecticida, la desparasitación masiva, los programas de suplemento de hierro y de vitamina C han demostrado ser ya intervenciones costo-eficaces, particularmente en areas donde no hay unos servicios asistencia sanitaria adecuados. Además, en un futuro las vacunas contra la malaria pueden contribuir a mejorar su control. La estimación de la carga de la enfermedad

causada por malaria es crucial para planear una intervención de control coste-efectividad, y su control y apoyo.

La tasa de inoculación entomológica y la prevalencia de malaria deberían ambas ser usadas para caracterizar la endemicidad de la malaria y la estrategia para la apropiada implementación de intervenciones de control.

1 Introduction

1.1 The History of Malaria

The Antiquity of Malaria Infections

“Whatever the hypothesis of the nature of malaria as the prehistoric zoonosis of the Old World, there is little doubt that one of the greatest steps in human civilization, that of transition from a food gathering to a food-production economy (related to the invention of agricultural tools, the development of social life and increase of the size of settled human group), must have been of paramount importance to the epidemiology of communicable disease”.

“L J Bruce-Chwatt, in Malaria, Principles and Practice of Malariology”

Undoubtedly malaria is one of the ancient ailments afflicting the humankind.

In the general histories of medicine, prehistoric data on the occurrence of malaria-related illnesses is well elucidated. Enlarged spleens, suggesting probably occurrence of malaria infection, have been found in Egyptian mummies more than 3,000 years old. Splenomegaly, fever and large number of supposedly curative remedies are mentioned in the Ebers Papyrus, 1570 B.C. Writings found in the library of Ashurbanipal (2000 B.C.), mention cyclic deadly fevers in the region between the Tigris and Euphrates rivers.

Writings from the Vedic period (1500 – 800 B.C.) mention autumnal fevers as the “king of diseases” and again enlarged spleens are described, suggesting the occurrence of malaria infection in India.

Malaria was well known in China long before the Christian era. The Chinese medical classic, “Nei Ching”, prepared in 2700 B.C. by the Emperor Huang Ti, also mentions the associations of different types of fevers with spleen enlargement and the mythology description of the disease attributes the symptoms of headache, chills, and fevers to three demons-one carrying a hammer, another with a pail of water, and the third with a stove.

Various indigenous Chinese herbal remedies used by Chinese physicians including Ch´ang Shan (*Dichroa febrifuga*) and Qinghaosu (*Artemisia annua*), the later nowadays recognised by its potent anti-malarial activity, were described in a treatise "Fifty Two Remedies" of 168 B.C.

The earlier contact between the European population and those of Africa (via the Nile Valley) and Asia Minor could have facilitated the introduction of malaria infection in the Mediterranean and Southern Europe. In Greece, by the end of the sixth century B.C. descriptions of intermittent fevers in the writings of Aristophanes (445-385 B.C.), Aristotle (384-322 B.C.), Plato (428-347 B.C.), and Sophocles (496-406 B.C.) indicate the presence of malaria infection. Hippocrates (460-370 B.C.) in his *Book of Epidemics* had characterised febrile ailments resembling that caused by *Plasmodium malariae* and *Plasmodium vivax*. He recognized the seasonal pattern on its occurrence; late summer and autumn, and that the quartan fever was the less dangerous. Also, established the relationship between enlarged spleens and marshes, even though he never hypothesized the causal elements involved in the origin of those illnesses.

In Italy among the ancient Etruscans, after 200 B.C., the disease was well known in the Roman Republic, especially occurring near the marshes. In the medical literature is referred as "Roman fever", and the Italian expression *mal'aria*, meaning "bad air", was used to explain that vapours emanating from marshes was the origin of the illness.

By the 12th century, malaria was reported to reach as far west as Spain, and as far East as Poland and Russia. By the 15th century, in Eastern Europe intermittent fevers were commonly reported in marshy areas. Throughout the 17th and 18th centuries, imported malaria, mainly by returning expeditions from India and Africa, was recorded in England (Bruce-Chwatt, 1988).

In the Americas, it is not well known when malaria infection was introduced. However, it is assumed that when the European explorers and colonists landed on its shores, they brought *Plasmodium malariae* and *Plasmodium vivax* into the Americas, and latter *Plasmodium falciparum* malaria was introduced from Africa in the advent of African slaves trading (Bruce-Chwatt, 1988).

By the 19th century, malaria infection had already expanded worldwide to include Northern Europe, North America, Russia and transmission in Southern

Europe was intense (Bruce-Chwatt, 1988; White, 2003). Although, successful eradication campaigns carried out in these regions, had contributed in the interruption of transmission of the disease, in the tropical regions the burden of the disease is still astonishing, particularly in sub-Saharan Africa (White, 2003).

1.2 The discovery era and initial attempts to malaria control

Although the disease was spreading worldwide, the causal agents and conditions of transmission of malaria infection were still unknown, until later 19th century. The remarkably and revolutionary event of the history of malaria infection in human population was the discovery of malaria parasites by Laveran in 1880. Later in 1897, Ronald Ross, working in Sierra Leone, discovered malaria parasites in wild-caught mosquitoes (Bruce-Chwatt, 1988; Gilles & Lucas, 1998). The description of sporogonic cycle of human malaria parasite by Grassi, Marchiafava, Bignami and Ross, culminated with the description of the malaria transmission cycle, and the conditions responsible for disease dissemination.

Since that time a general approach to eliminate the factors contributing to multiplication and dissemination of malaria parasites was adopted, particularly in most southern Europe. Initial attempts to malaria control in Africa were carried out in Sierre Leone. Treatment of fever cases with quinine and larviciding was first introduced in British troop camps. From 1930 to 1950, the same approach was adopted by Anglo-American mining companies in South Africa and Zambia former Northern-Rhodesia (Utzinger et al. 2002), malaria control in and around the copper-belt mines, consisted of extensive environmental modification, larviciding, patient treatment and individual protection by house screening.

Before World War II, activities for malaria control through environmental management; larviciding and treatment of breeding sites were successfully implemented in vast areas of Europe, Asia, and the Americas (Ault, 1994; Najera, 2001). Those large-scale programmes have reduced or eliminated suitable conditions for malaria vectors proliferation, in proximity to vulnerable

human population and consequently halted malaria infection transmission (Ault, 1994).

Despite that in 1874, dichlorodiphenyltrichloroethane (DDT) was already synthesized; its insecticidal properties were discovered in 1939, by Paul Muller (Smith, 1991). Large-scale production started in 1943, and was extensively (worldwide) used just after World War II, in malaria vector control and in Agricultural pests control. Consequently, during the early 1950's DDT spraying campaigns were followed by interruption of malaria transmission in Venezuela, Italy, Greece, Guyana, Ceylon and the USA (Bruce-Chwatt, 1985; Gramiccia & Beales, 1988).

Simultaneously, the development of synthetic anti-malarial drugs (i.e., chloroquine, etc) and other synthetic compounds with insecticidal action, hexachlorocyclohexane (BHC or HCH) and Dieldrin, gave more impetus to the improvement of malaria control techniques.

1.3 Global Eradication Campaign

The early 1950's were characterized by hope and optimism for malaria eradication worldwide. The first large-scale "Malaria Eradication Program", was carried out by World Health Organization (W.H.O.) during 1955 – 1969 (Molineaux & Gramiccia, 1980; Wernsdorf, 1988). The main goal was to eradicate malaria in vast areas across the word by vector control.

The malaria eradication programme aimed at cessation of transmission of malaria and elimination of the reservoir of infected cases in a campaign limited in time, carried to such degree of accomplishment that, malaria would become a disease of the past.

Eradication efforts focused mainly on insecticide residual spraying with DDT, anti-malarial drug treatment, and surveillance. The programme was designed to be carried out in four successive steps as follows:

1. The preparatory phase; Devoted to geographical reconnaissance of the area, training of field personnel, identification and numbering of all

sprayable premises and assessment of all logistics required (equipment, transport, etc.).

2. The attack phase; Application of residual insecticide, covering all premises and areas, to ensure the elimination of vector population. To supplement residual spraying, chemotherapy was recommended. The decrease of malaria transmission was followed by case detection surveillance.

3. The consolidation phase; Begins when the surveillance activity shows that the annual parasite incidence is below 0.1 per 1,000 inhabitants. Complete coverage by residual spraying is stopped when there is no more transmission of malaria throughout the region. The surveillance system should be effective to eliminate any remaining foci of infection. Nevertheless, receptivity and vulnerability to introduced infections must be taken into account.

4. The maintenance phase; Begins at the end of consolidation phase, after a period of three consecutive years with no evidence of malaria transmission. The preventive activities during this phase are known as “vigilance” consisting of alertness for any occurrence of any imported or indigenous cases of malaria, and application of appropriate measures.

The achievements of the campaign included malaria transmission eradication in vast temperate climates areas of Europe. In some other countries of the Americas, northern of Africa and Middle East regional eradication of the disease was accomplished. Nevertheless, in countries such as India and Sri Lanka, the sharp reductions in the number of cases, was followed by increases to substantial levels after efforts ceased. While, negligible progress was attained in countries such as Indonesia, Afghanistan, Haiti, and Nicaragua (Bruce-Chwatt, 1987).

Some nations, most of sub-Saharan Africa, Papua New Guinea and some of the islands of Indonesia were excluded completely from the eradication campaign (Molineaux & Gramiccia, 1980).

Lack of adequately trained local personnel, scanty infrastructures and financial resources, have prevented the implementation of malaria eradication activities in many countries in the African region.

Only in a few urban and peri-urban settlements in sub-Saharan Africa, with adequate infrastructure development and resources to support the campaigns, malaria eradication efforts were initiated. Those activities consisted of insecticide residual spraying campaigns and mass drug prophylaxis using chloroquine, pyremethamine, or proguanil through maternal health centres, dispensaries or schools (Wernsdorf, 1988; Payne, 1988). However, the expected results of indoor spraying with residual insecticides alone or combined with mass drug administration, were not achieved, with the exception of the islands of Mauritius and Réunion, where eradication was successfully accomplished (Kouznetsov, 1977), and in the upper southern Egypt, where the *Anopheles gambiae* was eradicated (Shousha, 1948; Utzinger et al. 2002).

It was intended that other African countries would begin eradication when infrastructure and resources were in place. Regrettably, before this could happen, the development of malaria parasite resistance to chloroquine, vector resistance to DDT and loss of confidence in the campaign resulted in the abandonment of the malaria eradication effort.

Although administrative, technical, financial and ecologic difficulties were the main reasons to exclude many parts of sub-Saharan Africa, lack of solid knowledge on several factors (biological and natural determinants) related to malaria dynamic and transmission, hampered appropriate planning and effective implementation of eradication measures in different epidemiological settings across tropical Africa.

1.4 The Garki and Kisumu projects

In the wake of insufficient understanding of the dynamics of malaria transmission, a comprehensive, multidisciplinary and longitudinal field research with a strong focus on epidemiology and entomology, was conducted from 1971 to 1980. The Garki district, in northern Nigeria and Kisumu in Kenya, both areas of intensive malaria transmission in sub-Saharan Africa savannah were chosen to perform such field research. These projects aimed at what could be achieved with adequate financial and technical assistance (Molineaux et al., 1980; Payne et al., 1976). Secondly to collect baseline epidemiological data, to evaluate the impact of indoor spraying with an effective residual insecticide, alone or in combination with mass drug administration and to the development and testing of mathematical models, pertinent for an understanding of the dynamic of malaria transmission. Such comprehension, would guide strategic planning of future malaria control activities programmes on a large scale in different epidemiological settings. Those projects provided a unique opportunity to study a set of sero-immunological tests before, during and after malaria control interventions (Molineaux & Gramiccia, 1980).
The details of the results of the project are described elsewhere (Molineaux & Gramiccia, 1980; WHO, 1988), briefly an outline of the main findings.

- Malaria in tropical Africa is characterised by very high levels of transmission. Transmission intensity oscillations were observed within one setting, from one setting to another, and from season to season. The pattern of malaria endemicity is maintained by highly effective vectors, which produce very high rates of entomological inoculation.

- The infection by all existing parasite species in the area occurs early in life, and differences in immune responses between individuals is associated with the degree of exposure to malaria infection.

- The infant mortality rate was very high before interventions. Oscillations on infant mortality rate were observed among years, and it was associated with infant's risk to acquiring malaria infection. However, infant mortality rate was reduced significantly after malaria control interventions.

Despite high coverage rates of residual spraying with an effective insecticide against the mosquito vectors, combined with mass drug administration at high frequency and coverage, reduction on malaria transmission to low levels was observed, nevertheless, interruption of malaria transmission could not be attained (Molineaux & Gramiccia, 1980). Similar results were reported from repeated cross-sectional survey data collected from one historical trial of indoor residual spraying against malaria vector in two contiguous districts in Tanzania-Kenya (Pare-Taveta project) carried out in 1954 (Sama et al., 2004).

The MacDonald models showed basic elements of practical relevance to malaria control or eradication programmes. In that model the reduction of the basic reproduction rate is pertinent to the interruption of malaria transmission, as the first step to malaria control or eradication.

Vector longevity in determining transmission is clearly important and focuses control measures on the adult mosquito. Therefore the survivorship of adult female Anopheles is the key element in the chain of malaria transmission (McKenzie et al., 2004). Moreover, the MacDonald model emphasizes the role of immunity as a regulating mechanism of transmission (Bruce-Chwatt, 1986).

An expanded model developed by Dietz and Molineaux, as a result of the Garki project included other variables such as the endemic levels in relation to the whole range of vectorial capacity involved in transmitting *Plasmodium falciparum* parasites. The main output variable was the prevalence of *Plasmodium falciparum* parasitaemia as a function of the season, and of the age group of the population. It was fitted to the data obtained after one year of baseline observation in the field and after two years of insecticide residual spraying (Molineaux & Gramiccia, 1980; Bruce-Chwatt, 1987)

Despite the existing entomological methods, the practical measurement of vectorial capacity in the field is still a challenging assignment. Nevertheless, in the Garki project a set of entomological data pertinent to assessing vectorial capacity was collected, (e.g. vector species composition, age-grading, infectivity, to mention only the most important). It was recognized that the daily rate of survival of the vector is the most crucial component of vectorial capacity.

The epidemiology of malaria is complex and may vary considerably even within relatively small geographic areas. Malaria transmission to man depends on several interrelated factors (Cattani et. al., 1986; Molineaux et. al., 1988). The most important pertain to the anopheline mosquito vector and, in particular, its longevity. As sporogony (development of sporozoite parasites in the vector) takes over a week (depending on ambient temperatures), the mosquito must survive for longer than this after feeding on a gametocyte-carrying human, if malaria is to be transmitted (Wernsdorf, 1988).

MacDonald gave the following formula for the likelihood of infection based on sporozoite rates (Molineaux et al., 1988) i.e. the proportion of anopheline mosquitoes with sporozoites in their salivary glands:

a) $S=P^{n}ax/ax-\log_{e}P$

Where ***P***=the probability of mosquito survival through one day; ***n***=the duration, in days, of the extrinsic cycle of the parasite in the mosquito; **a**=average number of blood meals on man per day, and ***x***=the proportion of infective bites to man. The probability of a mosquito surviving ***n*** days is given by:

b) $Pn/-\log eP$

The incubation rate, or the mean daily number of bites (***h***) received by sporozoite-bearing mosquitoes is given by

c) $h=mabs$

Where ***m***=anopheline density in relation to man, and ***b***=proportion of bites that are infectious.

The reproductive rate of the infections ($\boldsymbol{R_0}$) or the number of secondary cases resulting from a primary case is then given by:

d) $R_0 = ma^2bP^n / -z\log_e P$ X (1ax / ax-$\log_e P$)

Where ***z*** is the recovery rate, or the reciprocal of the duration of human infectivity. This is usually estimated at 80 days for *Plasmodium falciparum* in a non-immune subject, i.e. ***z***=0.0125. The term:

e) 1-ax / ax-$\log_e P$

Refers to the proportion of anopheline mosquitoes "not yet infected". When transmission is very low (i.e. ***x*** approaches to zero) then the basic reproductive rate ($\boldsymbol{R_0}$) reduces to

f) $\boldsymbol{R_0} = ma^2bP^n / -z\log_e P$

Since the malaria parasites are carried by female anopheline mosquitoes, and transmission to the host depends on the proportion and frequency of female *Anopheles* feeding on man, their oviposition interval and mean duration of life. On the other hand, the host/parasite relationship is governed environmental factors, such as the ambient temperature, which is relevant to the sporogony, proportion of infective *Anopheles* and number of their oocysts and sporozoites. The proportion of the population of mosquitoes which live long enough for the development of malaria parasites depends on the daily mortality, and this has and important bearing on the probability of transmission (Bruce-Chwatt, 1987). The probability of survival of a proportion of the anopheline vector population through one day and through the extrinsic period of the development of the parasite forms an important element in the expression of the reproduction rate.

The foundation of the understanding of the dynamics of transmission of the infection is the basic reproduction rate or the number of secondary infections that would originate from a single primary case of malaria it there had been no suppressive effect of the immune response of the human host enhanced by the possibility of super-infection (Bruce-Chwatt, 1987).

Thus, the control of transmission could be attained through the decrease of the reproduction rate, so that each successive number of cases would be

progressively smaller until the disease eventually fades out. The aim of malaria eradication is to reduce the reproduction rate below one and to maintain it persistently below this critical level.

The attack on the vector using residual insecticides has a rapid effect on transmission because it drastically reduces the probability of anopheline survival, consequently decreasing the longevity of Anopheles vectors. The effect of insecticide residual spraying on the vector can be assessed by measuring the vectorial capacity, a term that expresses the mean number of probable inoculations transmitted from on case of malaria in a unit of time (Bruce-Chwatt, 1987).

At very high levels of transmission, with high basic reproduction rates, large reductions in transmission would reduce malaria by a negligible amount (e.g., a reduction in transmission of 90% from 300 infectious bites per year to 30 bites per year will make very little difference to the prevalence of malaria), however as the basic reproduction rate approaches the critical value of one (below which the disease fades out), small reductions in the reproduction rate will have a very large impact on the amount of malaria. In such conditions, malaria control programmes can be successful and eradication accomplished, as it certainly happened in many areas of Europe where the basic reproduction rates were relatively low. The precise values of the basic reproduction rates has been much debated, although there is good reason to believe it was fairly low (White, 2003).

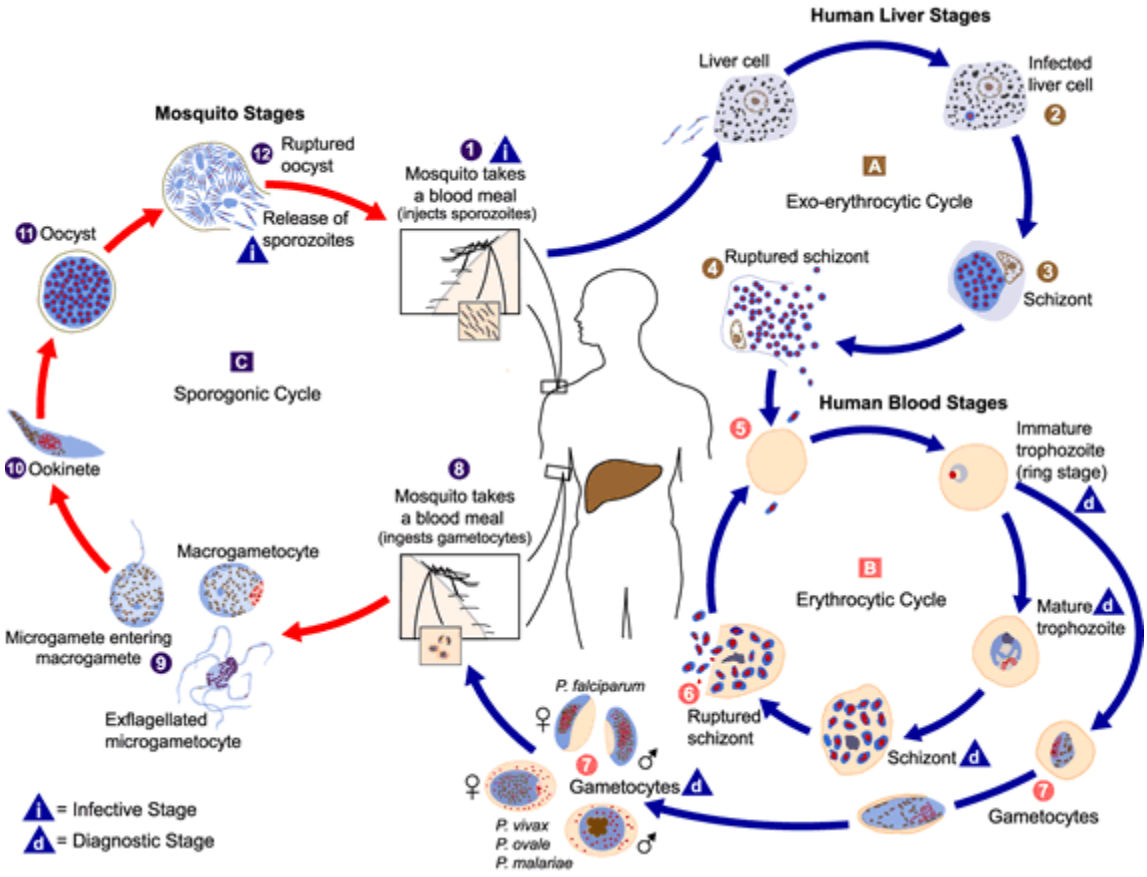
1.5 The Life Cycle of Human Malaria Parasites

The life cycle of parasites of the family Plasmodidae is characterized by two multiplication's phases. The *schizogony*, an asexual phase, occurring in the vertebrate host, and *sporogony* a single sexual multiplication taking place in the invertebrate host, a mosquito of *Anopheles* species.

The life cycle is illustrated in figure 1. When an infected *Anopheles* mosquito bites, a number of Plasmodia sporozoites are introduced into subcutaneous tissue of the vertebrate host. After a short period of time the sporozoites reach

the liver. The mechanisms by which the sporozoites enter the hepatic cells are not clear, although it has been suggested that hepatic cells receptors to the principal surface protein on the sporozoite, play an important role. Inside the hepatic cells, the sporozoites develop and transform into schizonts, containing thousands of merozoites. In some species of malaria, the sporozoites (e.g. plasmodium vivax) may remain as latent hypnozoites in the liver for a long period. The merozoites are released into the bloodstream through the sinusoids of the liver after 6-15 days, depending on the *Plasmodium* spp. The circulating merozoites infect erythrocytes within a few second and begin the asexual multiplication. Within the erythrocytes, the parasites develop into trophozoites stages before the production of the erythrocyte schizont. With the rupture of the infected erythrocytes, about 20 merozoites are produced from each matured schizont. The merozoites released are then free to infect more red cells and thus perpetuating the asexual multiplication. Therefore, the asexual cycle persists until infection is controlled, either by the host's immune response or chemotherapy, or until the host dies. In the course of this process, some merozoites within the erythrocytes differentiate themselves into immature forms, called gametocytes. For the sexual reproduction of plasmodia to take place, these gametocytes must be taken up into the alimentary tract of an anopheles mosquito. On biting an infected vertebrate host the mosquito ingests blood containing erythrocytes parasitized including gametocytes (the sexual forms of the parasite), and subsequently the sexual stage of the cycle continues in the mosquito. The gametocytes are released from the ingested erythrocytes and transform into male and female gametes. The male form (microgamete) undergoes flagellation and fertilises the female form (macrogamete), forming a zygote. Within 24 hours the zygote develops into an ookinete, which penetrates the midgut wall of the mosquito, forming an oocyst between the midgut epithelium and the basal lamina. Many sporozoites are then formed asexually within the oocyst. Approximately 7 to18 days after gametocytes ingestion, the maturity is accomplished, the oocyst bursts to release the sporozoites, and they migrate to the mosquito's salivary glands where they can be transmitted through a bite into another vertebrate host.

Figure 1. *The life cycle of human malaria parasites, illustrating the Sporogonic, Exo-erythrocytic and Erythrocytic cycles*



Source: http://www.dpd.cdc.gov/dpdx - 20th November 2005

1.6 Pathogenesis and Clinical Manifestations of Malaria

Of all parasitic diseases affecting humans, malaria infection, particularly by *Pasmodium falciparum*, is the only one causing impairment and dysfunction of vital organs such as brain, liver, kidneys, placenta, and lungs in a single infection. On the other hand, the progression and severity of the clinical manifestations are distinct respectively to age, the degree of exposure, the pattern of disease transmission, the immune status of the individual and during pregnancy.

Therefore, most concepts and understanding of human malaria infection are based on *Plasmodium falciparum* infection studies conducted either during the malariotherapy era or on animal experiments. In addition, *Plasmodium falciparum* is the most common species found in the tropical world, causing severe disease with fatal outcome if not treated. Knowledge on other malaria species infection has been gained, as well, particularly with *Plamodium vivax* infection commonly found outside tropical Africa and *Plasmodium malariae* which occurs alongside *Plasmodium falciparum*.

In general, the pathogenesis of malaria infection is very complex. Reflects the interaction of both human host and parasite factors, including the involvement of immunological mechanisms, which are related to the pathogenesis of clinical manifestations of the disease (Houba, 1988).

The host genotype determines in part the host specific immune response (Hill and Greenwood, 1991) and the intensity of the inflammatory response (McGuire et al., 1994). The host's age is an important determinant of the pattern and severity of vital organ dysfunction (White, 2003).

The whole process is initiated when *Plasmodium* sporozoites enter the hepatic cells of the vertebrate host soon after being inoculated into the bloodstream. Within a few days, the invaded sporozoites develop into schizonts, which eventually rupture and release a massive quantity of erythrocyte-infective merozoites into the bloodstream, with immediate invasion of red blood cells. Initially, the host is not aware of the expanding infection or may complain a non-specific symptoms and starts mobilizing non-specific and specific immune responses.

The generalised circulatory disturbances arising from changes on the invaded red blood cells and their destruction, and the hostile effects of parasite products, host cellular material, hemozoin, and antibody complexes stimulate cells of the macro-phage-monocyte series, and possibly endothelium to release pro-inflammatory cytokines (White, 2003).

In severe malaria there is a cascade of cytokine profile secretion. The early production of tumour necrotic factor (TNF), interleukin-1β (IL-1β) and gamma Interferon induces a cascade release of other pro-inflammatory cytokines including interleukin-8 (IL-8), interleukin-12 (IL-12) and interleukin-18 (IL-18).

Simultaneously, as an auto-regulatory system, anti-inflammatory cytokines interleukin-6 (IL-6) and interleukin-10 (IL-10) are released (Keller et al., 2004; Good et al., 2005). Cytokines are responsible for many of the symptoms and signs of malaria, particularly the "paroxysms," such as shivering, cool extremities, headache, chills, fever, and sometime rigors followed by sweating, vasodilatation, and defervescence. Also, cytokines are probably involved in placental dysfunction, suppression of erythropoiesis, hepatic dysfunction and inhibition of gluconeogenesis. Cytokines are as well important mediators of parasite killing by activating leukocytes, and possibly other cells, to release nitric oxide, generating parasiticidal lipid peroxides, and causing fever (Luty et al., 2000; White, 2002).

On the other hand the phenomenon of cytoadherence, rosetting, sequestration and aggregation, play an important role on the progress and severity of clinical manifestations: Cytoadherence is a phenomenon characterized by adhesion of erythrocytes containing mature forms of *Plasmodium falciparum* into the vascular endothelium. However, this process is rarely observed in infections caused by the other human malaria parasites. The process is not fully understood, however, it has been elucidated the effect of parasite-derived proteins termed *Plasmodium falciparum* erythrocyte membrane protein 1 or P*f*EMP1, causing humps or knobs on the surface of the parasitized red cell, which are the points of attachment to vascular endothelium. In addition the presence of different sticky proteins on the surface of vascular endothelium has been shown to facilitate binding of parasitized red blood cells (White, 2003). As a consequence of cytoadherence, parasitized red cells disappear from the circulation, a phenomenon known as sequestration (Benedict et al., 1994; Cooke et al., 1995). The phenomenon of sequestration is considered to be important in the pathogenesis of falciparum malaria. It occurs predominantly in the venules of vital organs. It is more prominent in the brain, particularly in the white matter, and least prominent in the heart, medullary vessels of the kidney, intestines and adipose tissue (MacPherson et al., 1985; Sein et al., 1993).

Additionally, red blood cells containing mature parasites also adhere to uninfected erythrocytes. This process leads to another phenomenon named "rosetting" due to the formation of rosettes, in which an uninfected red cell is

surrounded by parasitized cells. Consequently, the cytoadherence, rosetting and sequestration of *Plasmodium falciparum* malaria-infected erythrocytes in the capillaries and venules of vital organs obstruct the microcirculatory, resulting in a reduced oxygen and substrate supply, leading to anaerobic glycolisis, a reduced pH and lactic acidosis (White & Ho, 1992). Recently disease severity in African children has been associated with a new adherence property, characterized by platelet aggregation of parasitized erythrocytes, and also contributing to vascular occlusion (White, 2003).

1.7 Clinical manifestations of malaria

The spectrum of clinical manifestations of malaria is very wide and, varies largely in different age groups (White, 2003 in Manson's). The development and the severity of the clinical manifestations are significantly influenced by the level of individual's immune status acquired in different epidemiological settings (Bruce-Bruce-Chwatt, 1986; Harinasuta & Bunnag, 1988; Snow et al., 1997; Bloland, 1999; White, 2003).

In areas of stable and intense P. falciparum malaria transmission, severe malaria illness is common amongst younger children, between 6 months and 2 to 3 years of age, becoming however, less frequent in older children (Slutsker et al., 1994; Snow & Marsh, 1998; Snow et al., 1997; White, 2003), and adults usually harbour asymptomatic low parasitaemias (White, 2003).

During pregnancy, malaria is frequently associated with severe anaemia, reduction in birth weight and it is the main cause of prematurity (McGregor, et al., 1983; Menendez, et al., 2000).

In areas of stable but of lower intensity transmission pattern, severe malaria episodes are seen evenly throughout the entire childhood period. Although severe anaemia is common in the early years, cerebral malaria turn out to be the most prominent presentation of severe malaria disease during childhood (Snow & Marsh, 1998; White, 2003). Adults may develop mild symptoms. Pregnant women are at risk of severe disease, particularly in the second and third trimesters, anaemia is a common finding (White, 2003). Malaria may also

be implicated in abortion, premature delivery and low birth weight (Nosten et al., 1991).

In areas of unstable or sporadic pattern of transmission, symptomatic and/or severe disease is seen in all age groups (White, 2003).

Severe malaria does not occur with *Plasmodium vivax, Plasmodium* ovale or *Plasmodium malariae* but acute infection in non-immune individuals may result into a serious illness.

1.7.1 The incubation and pre-patent period

The incubation period and the duration of pre-patent period are strongly influenced by the degree of immunity. Effective immunity prolongs both pre-patent period and the incubation period (White, 2003). *Plasmodium falciparum* has the shortest incubation period varying between 8 and 15 days, the longest incubation period is observed with *Plasmodium malariae* infections.

1.7.2 Uncomplicated malaria

The clinical manifestations of uncomplicated malaria infection are common to all human plasmodium species. Both, *Plasmodium malariae* and *Plasmodium ovale* have a more trivial onset than *Plasmodium vivax*.

The clinical manifestations of uncomplicated falciparum-malaria infection consist of bouts of fever accompanied by non-specific symptoms including headache, muscular ache, vague abdominal discomfort, lethargy, lassitude, dysphoria and loss of appetite. The temperature rises erratically, with shivering, mild chills, worsening headache, alternating with asymptomatic periods (Harinasuta & Bunnag, 1988).

Children are irritable, lethargic and anorexic, and abdominal discomfort is the primary complaint. A dry cough could be present, but not prominent, however, the respiratory rate may be raised, particularly in younger children. If the infection is left untreated may evolve to severe and/or complicated life-threatening illness.

1.7.3 Clinical manifestations of severe malaria

Of all human malaria species only *Plasmodium falciparum* cause a potentially life-threatening infection. There is insufficient information in relation to severe manifestations caused by Plasmodium vivax. The symptoms may progress suddenly from uncomplicated to a severe and lethal illness. In young children presenting severe anaemia and/or cerebral malaria a history of a very short onset period of the illness episode is commonly reported. Severe falciparum malaria infection is rarely observed in infancy (White, 2003). Adults with severe disease usually have a history of being ill for several days, and particularly in pregnancy severe anaemia is often associated.

1.7.4 Clinical manifestations of severe malaria in children

Data on the pattern of clinical disease in children outside Africa is scarce (White, 2003). Existing data gathered in sub-Saharan Africa, suggests that in settings with very high inoculation rates throughout the year, severe anaemia affecting mainly infants and very young children is the commonest presentation, while in areas with less intense transmission, cerebral malaria is frequently seen in relatively older children (Snow et al., 1994; Snow & Marsh, 1998). Therefore severe anaemia and cerebral malaria are the main manifestations of severe malaria in African children. Additionally, recent studies have mentioned that clinical syndrome of respiratory distress resulting from metabolic acidosis is as well, an important manifestation of severe malaria in children (Marsh et al., 1995).

High fever (temperatures ranging from 36°C to 41°C or even higher) is a common sign of *falciparum* malaria in children with cerebral malaria and dehydration is present in many cases (Waller et al., 1995; English et al., 1996). Generalized or focal convulsions may occur in children of any age and at any level of body temperature (Molineaux et al., 1989). Most patients may have tachycardia, rapid and deep breathing suggesting acidosis.

Hypoglycaemia is particularly common in young children (bellow 3 years old) with cerebral malaria (White et al., 1987a; Taylor et al., 1988), often associated

with convulsions and deep breathing (Taylor et al., 1993; Marsh et al., 1995; Waller et al., 1995).

Neurological features in children with profound coma, corneal reflexes and oculocephalic reflexes may be abnormal. Retinal haemorrhages and exudates are found relatively commonly in cerebral malaria (Kayembe et al., 1980; Lewallen et al., 1996, 1999). Plantar and abdominal reflexes are abnormal. In some children extreme opisthotonos and bruxism (grinding of teeth) could be observed. Muscular hypotonia and decerebrate or decorticate postures, are seldom seen (Molineaux et al., 1989; Mabeza et al., 1995; Waller et al., 1995).

By definition severe malaria includes one or more of the following clinical features, in the presence of *Plasmodium falciparum* infection:

Clinical manifestations

- Cerebral malaria – Unrousable coma not attributable to any other cause in a patient presenting with *falciparum* malaria.
- Severe anaemia – Paleness of the conjunctive mucosae or tongue.
- Renal failure – Urine output less than 400 ml/24 hours in adults and less than 12 ml/Kg body weight in 24 hours in children.
- Pulmonary oedema or adult respiratory distress syndrome.
- Repeated generalized convulsions – more than two in 24 hours.
- Impairment of consciousness less marked than unrousable coma – Assessed by Blantyre coma scale in children and Glasgow coma scale for adults
- Prostration – inability to sit in a child who is normally able to do so or inability to feed in a child not old enough to sit.

* Modified WHO definition, proposed in 2000

Laboratory findings of severe malaria

- Hyperparasiatemia – The relation parasitaemia and severity of disease is different in different populations and age groups, in general parasitaemia ≥ 20 % is associated with severe disease.
- Acidaemia – Arterial or capillary pH < 7.35
- Hypoglycaemia – Whole blood glucose concentration < 2.2 mol/l
- Severe anaemia – Normocytic anaemia with haematocrit < 15% or haemoglobin less than 5 gr/dl in the presence parasitaemia > 10.000/µ.
- Renal failure – Serum creatinine > 265 µmol/l.
- Macroscopic haemoglubinuria – When associated with acute malaria infection not as a result of oxidant anti-malarial drugs in patients with G6PD deficiency.
- Post-mortem confirmation of diagnosis – In fatal cases a diagnosis of severe *falciparum* malaria can be confirmed by histological examination of the brain.

* Modified WHO definition, proposed in 2000

Summary of severe malaria features in children, based on severity

Group I . Children at high risk of dying if effective treatment is not given

a) Prostrated children (refer to Blantyre coma scale)

(i) Prostrate but fully conscious
(ii) Prostrate with impaired consciousness but not in deep coma
(iii) Coma (Inability to localize a painful stimulus)

b) Respiratory distress (deep breathing)

(i) Mild – sustained nasal flaring and/or mild intercostals indrawing
(ii) Severe – presence of either marked indrawing of the bony structure of the lower chest wall or deep breathing

Group II.	Children at risk of clinical deterioration
	a) Children with haemoglobin level < 5 g/dl or haematocrit <15%
	b) Children with 2 or more convulsions within a 24 hours period
	c) Children with persistent vomiting
	d) Children with hyperpyrexia

Modified from WHO 2000 definition of severe *falciparum* malaria

1.7.5 Clinical manifestations of severe malaria in adults

In adults, features of severe disease usually precede a period of 3-7 days of mild symptoms, exception for non-immune adults, sometime dying within 24 hours of the onset of symptoms (White, 2003).

The common presentation for severe malaria habitually starts with generalized convulsion followed by persisting unconsciousness.

Mild neck stiffness is not uncommon, photophobia and hyperextension of the neck may occur in severely ill adults. Progression to coma is common and is associated with hypoglycaemia. Other neurological signs include tooth grinding (bruxism) and absence of primitive reflexes such as the grasp, abdominal and cremasteric reflex.

Jaundice is common in adult patients with malaria. Acute renal failure consequent to tubular necrosis is a common complication of severe *falciparum* malaria and is often lethal (Habte, 1990). It occurs most exclusively in adults and older children.

Other life-threatening features of severe malaria in adults are pulmonary oedema, metabolic acidosis and the classical symptoms of hypoglycaemia may be present i.e. anxiety, breathlessness, a feeling of coldness, tachycardia and sweating or “goose-flesh”.

1.7.6 Clinical manifestations of severe malaria in pregnancy

Pregnancy increases the risk that *Plasmodium falciparum* infection will develop into severe disease. In pregnancy susceptibility to malaria is very high in primigravidae and secondigravidae (McGregor, et al, 1983). Possibly, due to suppression of systemic and placental cell-mediated immune response (White, 2003).

Pregnant women are more vulnerable to malaria infection and more likely to have higher parasitaemias than non-pregnant women of the same age (Stekettee & Wirima, 1996).

The clinical manifestations of malaria in pregnancy are similar of those observed in adults. However, the principal complications to which pregnant women are particularly susceptible are severe anaemia, hypoglycaemia and acute pulmonary oedema.

Placental malaria is associated with low birth weight (McGregor et al., 1983). Low birth weight associated with placental malaria has been reported in the first pregnancies, decreasing with increasing parity (McGregor et al., 1983).

Definition of unrousable coma in adults: modified Glasgow coma scale

	Score[a]
Eyes open	
Spontaneously	……4
To speech	……3
To pain	……2
Never	……1
Best verbal response	
Oriented	
Confused	……5
Inappropriate words	……4
Incomprehensible sounds	……3
None	……2

Best motor response	
Obeys commands	……1
Localize pain	……5
Flexion to pain	……4
Extension to pain	……3
None	……2
	……1

[a] Total score can range from 3 to 14; 9 or less indicates “unrousable coma”

1.8 Main pathologic features in vital organs

A common feature observed in malaria infection is the darkness of almost all vital organs resulting from the parasite pigment.

In severe disease, the brain presents slightly swollen with multiple small petechial haemorrhages throughout the white matter. In many capillaries and venules sequestration of erythrocytes containing mature forms of the parasite is observed. The sequestration is prominent in the white matter, although the tissue is much less vascular than the grey matter (Boonpcknavig & Boonpcknavig, 1988; White, 2003).

The myocardial microvasculature is normal. However, some petechial epicardial haemorrhages are commonly observed, with marked interstitial oedema and local infiltrations of mononuclear inflammatory cells (White, 2003; Boonpcknavig & Boonpcknavig, 1988).

Pulmonary oedema is commonly observed among adults as a direct complication of *falciparum* malaria (White, 2003; Boonpcknavig & Boonpcknavig, 1988). Other pulmonary complications may result from coexistent bacterial infections (Boonpcknavig & Boonpcknavig, 1988).

Enlarged liver due to congestion of the centrilobular capillaries with sinusoidal dilatation is common. In addition, sequestration of parasitized erythrocytes is associated with swelling of the hepatocytes and perivenous ischaemic changes with centrizonal necrosis. In uncomplicated malaria hepatic histology is often

normal. Hypoglycemia is related to increased requirements of glucose consequence of increased metabolic demands of the febrile illness, a hypermetabolic state with increased glycolysis, the obligatory metabolic demands of the parasites that use glucose, aggravated by relative failure of hepatic gluconeogenesis and glycogenolysis (Fletcher & Gilles, 1988; Scheibel & Sherman, 1988; White 2003).

The spleen is enlarged, soft and friable, and fully packed of erythrocytes containing mature and immature parasites, with clear evidence of reticular hyperplasia and architectural reorganization (Boonpcknavig & Boonpcknavig, 1988; White, 2003).

Acute glomerulonephrites is often associated with *Plasmodium falciparum* infection, while chronic and progressive kidney injuries are observed in *P. malariae* infections (Boonpcknavig & Boonpcknavig, 1988). In acute forms the kidneys are slightly swollen. The sequestration is much more marked, particularly in the glomerular capillaries and sometimes mesangial and endothelial cell prolipherative changes are observed. Renal dysfunction is more commonly observed in adults with severe *falciparum* malaria, due to acute tubular necrosis. The process is probably originated by microvascular obstruction consequent of sequestration observed in the medullary vessels, hypoxia and probably progress to ischaemia and tubular necrosis. The role of local cytokines release and altered regulation of renal microvascular flow are unclear (White, 2003).

There is an intense sequestration in the gut and visceral ischaemia may explain the acute abdominal pain that sometimes occurs in severe malaria.

In the bone marrow macrophages containing malaria pigment and erythrophagocytosis may be seen. Dyserythropoetic changes are evident in acute malaria.

In the placenta, intense sequestration leads to the thickening of the syncitiotrophoblast and anomalous uteroplacental blood flow. The intervillous spaces show mononuclear inflammatory infiltration associated with placental insufficiency, responsible of foetal growth reduction (Menendez et al 2000; White, 2003).

2 The Epidemiology of Malaria

2.1 Distribution and Determinants of Malaria

Presently malaria is found throughout the tropics and subtropics. It was established in Europe, Northern Asia, and North America, but it has long been eradicated from those areas.

The distribution of human plasmodium species is not equal across malarious regions of the world. In sub-Saharan Africa, Papua New Guinea and Haiti, the foremost predominant species is *Plasmodium falciparum*, while, in the Central and parts of South America, North Africa, the Middle East and the Indian subcontinent *Plasmodium vivax* is the prevailing species. *Plasmodium malariae* and *Plasmodium ovale* are rarely found outside sub-Saharan Africa (White, 2003).

In those areas where malaria occurs, the intensity of transmission varies significantly with natural environment, and climatic conditions, the species of malaria vectors present, the biological factors of man including behavioural, social and economic factors, and specific malaria control measures available (Molineaux, 1988).

2.1.1 The Natural Environment

The natural environment has a significant effect on parasite and vector development

Climatic conditions are critical for the development of both malaria parasites and their mosquito vectors. The most important climatic conditions include temperature, humidity of the air, type and abundance of breeding places

2.1.1.1 Temperature

The temperature variations influence the development of both aquatic stages and geotropic maturation in different vector species.

The range of temperatures between 20° C and 30° C offer an optimal window for the development of most malaria vectors (Molineaux, 1988). Within that range, high temperatures tend to increment the growth rate, by shortening the minimum generation time of vector populations.

In some species, e.g. *An. gambiae sl*, at high temperatures, the minimum generation time, is as short as 10-11 days, while in lower temperatures is prolonged up to 21 days (Gilles & De Meillon, 1968).

The duration of sporogony (development of parasites in the vector) varies from one species to another and is strongly affected by environmental temperatures. Various studies have suggested that cessation of the development of parasites in the vector may occur in temperatures below 15° C, and above it the duration of sporogony decreases with increasing temperatures (Molineaux, 1988).

2.1.1.2 Humidity of the air

A high relative humidity, preferably at least over 60%, lengthens the life span of adult vectors (Molineaux, 1988), thus, making it possible to transmit the infection to as many as possible human hosts.

2.1.1.3 Rainfall and breeding places

The malaria vectors differ on their breeding habits. In general, each vector species breed specifically in a certain type of water surface. However, several vector species have adapted perfectly to changes in the type of water surface available (Molineaux, 1988).

Rain pools are favoured by certain species (*An. arabiensis, An. gambiae*) while others species breed in salt-water or in swamps. In addition, some species vectors are affected by the sunlight or by shade or emerging vegetation around the breeding sites (Molineaux, 1988).

For example in South-East Asia mosquitoes of the *anopheles dirus* complex are important causes of ‘forest fringe’ malaria. They breed in tree collections of water and *A. stephensi*, the principal vector in the Indian subcontinent, breeds in wells or stagnant waters (White et al; 2003).

Other natural environmental condition affecting the availability of breeding places is the amount and distribution of rainfall throughout the year.

Permanent pools with water bearing plants, favoured by some species of anopheline, are typically found in places with abundant rainfall. Hence, *Anopheline* mosquitoes breed all year around and transmission is perinneal. However, excessive rainfall and flooding may have a negative effect. On other hand, droughts reduce the availability of breeding sites.

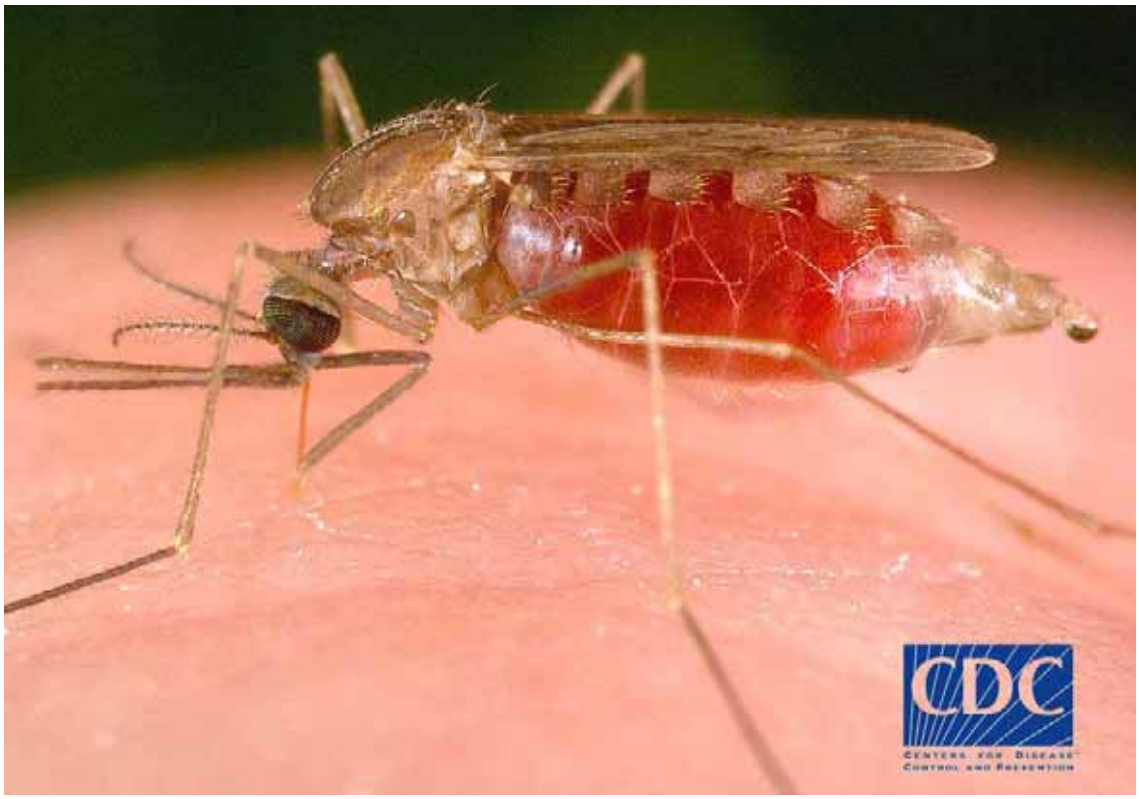
FIGURE 2. *An overview of a common breeding site*

2.1.2 The Vectors

Malaria is transmitted by different *Anopheles* species, depending on the region and the environment. Of the nearly 3,000 mosquitoes species recorded worldwide, 400 species are *anopheline* mosquitoes, many of which are species complexes. Approximately 60 are considered to be important vectors of malaria parasites (Gilles, 1988; Molineaux et al., 1988).

In Africa, the major vectors are *Anopheles gambiae, Anopheles funestus, Anopheles nili* and *Anopheles moucheti*. The *Anopheles gambiae* complex contains the most efficient malaria vectors species (Coetzee et al., 2004; Levine et al., 2004). The *Anopheles gambiae* complex is mainly responsible for approximately 80% of malaria morbidity and mortality that occurs in sub-Saharan Africa (Breman et al., 2001).

Figure 3. *A feeding anopheline mosquito*



Source: http://www.dpd.cdc.gov/dpdx - 20th November 2005

The *Anopheles gambiae* sl complex consist of six named and one unnamed morphological species (Hunt et al., 1998). *An. gambiae ss, An. arabiensis, An. quadriannulatus, An. merus, An. melas,* and *An. quadriannulatus B* (recently described in Ethiopia). Differences in malaria vector competence among members of the complex have been recognized and are attributed primarily to

preferences for feeding on humans versus animals, tendency to enter houses, and ability to recover in number after dry seasons (White GB, 1974).

An. gambiae and *An. arabiensis* are the major members of the complex responsible for malaria transmission.

Anopheles funestus complex contains nine named species (*An. funestus* ss, *Anopheles rivulorum* Leeson, *An. leesoni* Evans, *Anopheles vaneedeni* Gilles & Coetzee, *An. parensis* Gillies, *An. confusus* Evans & Leeson, *An. aruni* Sobti, *An. fuscivenosus* Leeson, and *An. brucei* Service) that are morphologically very similar and can only be distinguished at specific stages of their development (Gilles & Coetzee, 1987). The mainly endophilic and anthropophilic *An. funestus* is considered a major malaria human vector in Africa.

2.1.2.1 Life cycle of *anopheline* mosquitoes

Like all mosquitoes, the life cycle of *anopheline* mosquitoes has four stages: egg, larva, pupa, and adult. The first three stages are aquatic and last 5-14 days, depending on the species and the ambient temperature. The adult stage is when the female Anopheles mosquito acts as malaria vector.

1. Egg stage

Adult females lay 50-200 eggs per oviposition. Eggs are laid singly directly on water and are unique in having floats on either side. Eggs are not resistant to drying and hatch within 2-3 days, although hatching may take up to 2-3 weeks in colder climates.

2. Larvae stage

Mosquito larvae have a well-developed head with mouth brushes used for feeding, a large thorax and a segmented abdomen. They have no legs. In contrast to other mosquitoes, Anopheles larvae lack a respiratory siphon and for this reason position themselves so that their body is parallel to the surface of the water.

Larvae breathe through spiracles located on the 8th abdominal segment and therefore must come to the surface frequently. The larvae spend most of their

time feeding on algae, bacteria, and other microorganisms in the surface microlayer. They dive below the surface only when disturbed.

The larvae occur in a wide range of habitats but most species prefer clean, unpolluted water. Larvae of *Anopheles* mosquitoes have been found in fresh- or salt-water marshes, mangrove swamps, rice fields, grassy ditches, the edges of streams and rivers, and small, temporary rain pools. Many species prefer habitats with vegetation. Some breed in open, sun-lit pools while others are found only in shaded breeding sites in forests. A few species breed in tree holes or the leaf axils of some plants

3. Pupae stage

The pupa is comma shaped when viewed from the side. The head and thorax are merged into a cephalothorax with the abdomen curving around underneath. As with the larvae, pupae must come to the surface frequently to breathe, which they do through a pair of respiratory trumpets on the cephalothorax.

4. Adult stage

After a few days as a pupa, the dorsal surface of the cephalothorax splits and the adult mosquito emerges.

The duration from egg to adult varies considerably among species and is strongly influenced by ambient temperature. Mosquitoes can develop from egg to adult in as little as 5 days but usually take 10-14 days in tropical conditions

Like all mosquitoes, adult *anophelines* have slender bodies with 3 sections: head, thorax and abdomen.

The head is specialized for acquiring sensory information and for feeding. The head contains the eyes and a pair of long, many-segmented antennae. The antennae are important for detecting host odours as well as odours of breeding sites where females lay eggs. The head also has an elongate, forward-projecting proboscis used for feeding, and two sensory palps.

The thorax is specialized for locomotion. Three pairs of legs and a pair of wings are attached to the thorax.

The abdomen is specialized for food digestion and egg development. This segmented body part expands considerably when a female takes a blood meal.

The blood is digested over time serving as a source of protein for the production of eggs, which gradually fill the abdomen.

Anopheles mosquitoes can be distinguished from other mosquitoes by the palps, which are as long as the proboscis, and by the presence of discrete blocks of black and white scales on the wings. Adult *Anopheles* can also be identified by their typical resting position: males and females rest with their abdomens sticking up in the air rather than parallel to the surface on which they are resting.

Adult mosquitoes usually mate within a few days after emerging from the pupal stage. In most species, the males form large swarms, usually around dusk, and the females fly into the swarms to mate.

Males live for about a week, feeding on nectar and other sources of sugar. Females will also feed on sugar sources for energy but usually require a blood meal for the development of eggs. After obtaining a full blood meal, the female will rest for a few days while the blood is digested and eggs are developed. This process depends on the temperature but usually takes 2-3 days in tropical conditions. Once the eggs are fully developed, the female lays them and resumes host seeking.

The cycle repeats itself until the female dies. Females can survive up to a month or longer, but most probably do not live longer than 1-2 weeks in nature. Their chances of survival depend on temperature and humidity, but also their ability to successfully obtain a blood meal while avoiding host defences.

2.1.3 The Parasites

The evolutionary theory of malaria parasites evokes the adaptation of Coccidia of the intestinal epithelium to some tissues of the internal organs. The following step was the invasion of red blood cells, and blood-sucking arthropods, the transitional invertebrate hosts, would have facilitated transmission of malaria parasites to a wide range of vertebrates (Bruce-Chwatt, 1986).

Approximately 120 species of Plasmodia have been described, infecting a large variety of hosts, including mammals, rodents, birds and reptiles. Within the sub-genus *Plasmodium* there are four species infecting humans namely:

Plasmodium falciparum, Plasmodium malariae, Plasmodium vivax and *Plasmodium ovale* (Bruce-Chwatt, 1986; Eldryd Parry, 2004).

In the past, due to the periodicity of clinical manifestations (every three days of *Plasmodium malariae, Plasmodium falciparum* and *P. ovale* or every four days for *Plasmodium vivax*, colloquial names have been attributed to the illness caused by those parasites. At that time, *Plasmodium falciparum* was recognised causing severe disease with fatal outcome, hence the name 'pernicious or malignant tertian'. *Plasmodium vivax* was known as 'tertian benign' or 'simple tertian', Plasmodium ovale as 'ovale tertian' and 'quartan' for *Plasmodium malariae*. Currently, those clinical pictures are uncommonly observed. The availability of effective anti-malarial drugs and the susceptibility of the host to develop clinical symptoms tend to limit such clinical pictures; hence colloquial names have been abandoned.

2.1.3.1 The distribution of malaria parasites

Human malaria species are found in malarious areas of tropic and subtropical regions. However, climatic conditions, existing species of invertebrate host and other human genetic factors, such as, absence of Duffy factor, or haemoglobin traits play an important role in the distribution and occurrence of certain type of malaria species in a given area (Eldryd Parry, 2004).

Following is a brief description of some important characteristics and distribution of human malaria parasite species:

2.1.3.1.1 Plasmodium vivax

Plasmodium vivax is a malaria parasite species predominantly found in temperate zones. It may occur in tropical areas; however, it is less common in tropical Africa (Bruce-Chwatt, 1988; White, 2003). It is hardly found in populations without Duff blood group antigen (Eldryd Parry et al 1988). Therefore, *Plasmodium vivax* is the main cause of malaria infection in subtropical regions, particularly Central and South America, the Indian Subcontinent and eastern Asia (Garnham, 1988).

The sporozoites of this species are characterized by their differentiation either into tissue schizonts responsible for the primary attack, or into hypnozoites, a dormant type of parasites causing recurrent relapses. Some strains (*P. vivax* hibernans), in the northern hemisphere (China and neighbouring countries), have a long incubation period, and thus the first clinical symptoms may occur 8-10 months after an infective bite (Bruce-Chwatt, 1988; White, 2003).

Tropical strains show a relatively short period of incubation and cause erratic relapses in a short period of time after the primary attack, however, some intermediate sub-tropical strains have been found producing primary attack or relapses even after 9 months (Bruce-Chwatt 1988).

The erythrocytic (asexual) cycle of development of *Plasmodium vivax* takes approximately 48 hours and all blood forms can be found in the circulation, even though, the parasite density rarely exceed 50.000 per µl of blood.

The parasitized erythrocytes usually are enlarged, discoloured and present small reddish granules know as Schuffner's dots (Bruce-Chwatt, 1988; White, 2003).

2.1.3.1.2 Plasmodium ovale

Plasmodium ovale is a malaria parasite species confined to tropical Africa (Bruce-Chwatt 1988), and rarely found outside West Africa (White, 2003). The tertian pattern type of fever caused by *Plasmodium ovale* infection resembles that of *P. vivax* malaria. However, infection by *P. ovale* generally has a prolonged latency and tends to produce mild clinical manifestations and fewer relapses (Eldryd Parry, 2004; Bruce-Chwatt 1988).

The main distinction which characterise *P. ovale* infection is the oval distortion of the parasitized erythrocytes, accompanied by heavy and early Schuffner's dots.

The asexual erythrocytic extends over 50 hours, while the pre-erythrocytic stage has general period of nine days to reach maturity (Bruce-Chwatt, 1988; Garnham, 1988).

2.1.3.1.3 Plasmodium malariae

Despite the world-wide distribution of *Plasmodium malariae*, its occurrence is erratic across in both tropical and subtropical regions. It is commonly reported in West and East Africa, Guiana and parts of India (Bruce-Chwatt, 1988; Garnham, 1988; White, 2003). Natural infection occurs in chimpanzees and they become potential reservoirs, especially in West Africa (Bruce-Chwatt, 1988).

The development of *Plasmodium malariae* is markedly slow. The pre-erythrocytic schizonts (hepatic development) maturation is accomplished in 15 days and the asexual erythrocytic cycle shows a 72 hours periodicity (Bruce-Chwatt, 1986; Garnham, 1988).

The course of the disease is characterized by low parasitaemias, rarely reaching 30 000 parasites per µl and gametocytes are not frequent. Severe disease is infrequently observed (Bruce-Chwatt, 1988; Garnham, 1988; White, 2003), however, the infection has the tendency to persist for a long period of time, in some cases may last for lifetime which is responsible for the recrudescence observed in *Plasmodium malariae* infection (Bruce-Chwatt, 1986; Garnham, 1988).

2.1.3.1.4 Plasmodium (Laverania) falciparum

Plasmodium falciparum is the commonest human malaria species found throughout the tropics and subtropics. It is prevalent in tropical Africa, Papua New Guinea and Haiti (White, 2003). The distinctive feature of *Plasmodium falciparum* comparatively to others human malaria species is its greater virulence and it is responsible for much morbidity and mortality attributed to malaria infection (Bruce-Chwatt, 1986; Garnham, 1988; White, 2003).

The asexual development of *Plasmodium falciparum* is completed in 48 hours, however, irregular and not synchronised broods of parasites, always yield erratic periodicity of symptoms and therefore the tertian pattern of symptoms is not observed.

The infection caused by *Plasmodium falciparum* may arise to exceed 300,000 parasites per µl of blood. Nevertheless, subsequent stages of the asexual

erythrocytic cycle usually do not occur in the peripheral blood-steam, are confined to the capillaries and sinusoids of internal organs (Garnham, 1988). The presence of maturing or matured schizonts of *Plasmodium falciparum* in the peripheral circulation is suggestive of severe disease (Bruce-Chwatt, 1986; Garnham, 1988).

2.1.3.1.5 Mixed infections

Mixed infections, although neglected, they are commonly seen in areas where two or more malaria species prevail, particularly in endemics areas. However, one species tends to suppress the co-existing infections, as is the case of *Plasmodium falciparum* over *Plasmodium malariae* and *Plasmodium ovale*, or *Plasmodium vivax* over *Plasmodium malariae*. In tropical Africa, double infections commonly seen are of *Plasmodium falciparum* and *Plasmodium malariae* or *Plasmodium falciparum* and *Plasmodium ovale*, conversely, in Asia the most predominant combination is *Plasmodium vivax* and *Plasmodium malariae* (Harinasuta and Bunnag, 1988).

The clinical implications of co-infections are the relapses caused by the suppressed co-infection, after a period of apparent recovery from former infection.

2.2 The Human Host

2.2.1 Biological factors

One part of the parasite's life cycle occurs in the blood stream of the human host. The differentiation and development of gametocytes, a process which guarantee the maintenance and the transmission of the infection, occurs within the human host.

Several genetic factors related to the human host, may well affect the development of the parasite's life cycle of the parasite. Moreover the occurrence and geographical distribution of different species are certainly influenced by genetic factors of the human host.

Genetic factors

The invasion of red blood cells by some parasite species is dependant on the presence of certain antigens on the surface of the erythrocyte. A host with Duffy-negative genotype, their red blood cells will resist to the invasion by *Plasmodium vivax.* Hence in areas or regions where the general populations harbour Duffy negative blood group, the absence of *Plasmodium vivax* infection will be notorious in that particular region or area, as is the example of absence of *P. vivax* from West Africa.

The HLA complex and the genetic control of the immune response, are implicated in the development of antibodies against *Plasmodium falciparum* and play an important role in the distribution of the malaria parasites among the human host.

Other genetic factors playing an important role in the frequency and distribution of different parasite species include the sickle-cell trait or haemoglobin S, which gives partial protection against *Plasmodium falciparum*; The Glucose-6-Phosphatase Deficiency (G6PD) is associated with lower prevalence and density of *Plasmodium falciparum*. On the other hand the epidemiological association between malaria and human hosts carrying some abnormal haemoglobins, i.e., haemoglobin C, D, K, O, and foetal haemoglobin or thalassemias has been documented.

Other human biological factors include the age and the maturity of the immune system, pregnancy and nutritional status.

3 Malaria in Africa

3.1 The epidemiology of malaria in Africa

Malaria infection and poverty are geographically specific, and restricted to the tropical and sub-tropical zones of the globe. Therefore, the devastating effects of malaria infection have been linked to a malicious cycle of poverty and ill-health, particularly in areas of low economic growth (Sachs & Malaney, 2002).

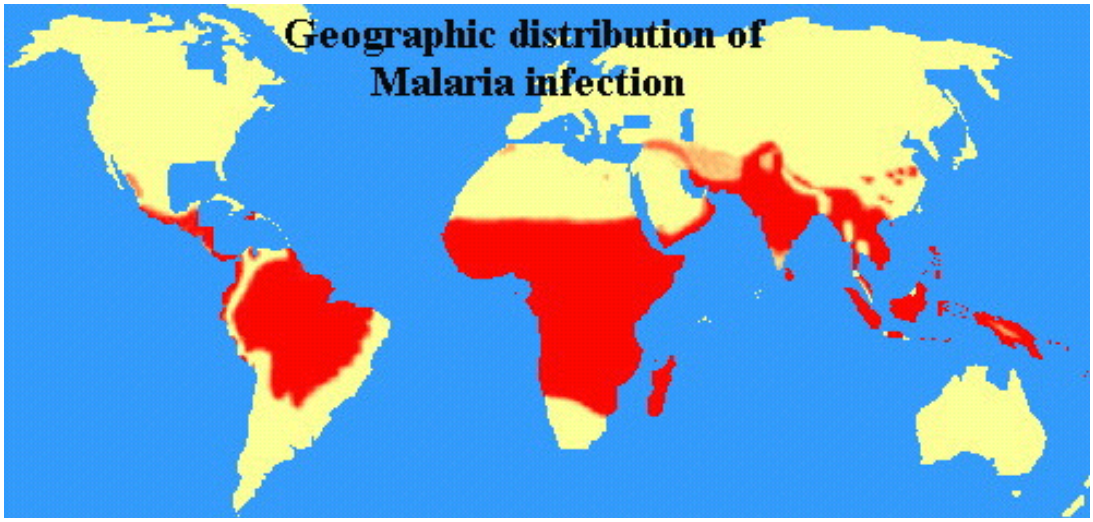
Malaria situation in Africa is worst particularly in the poorest tropical countries of the continent, comparatively with the northern and southern extremes, in which, malaria is free or negligible.

Similarly, the poorest countries in the tropics outside Africa also have a significant malaria problem (Gallup & Sachs, 2001).

Transmission occurs through exposure to the infective bites of female Anopheles mosquitoes. The *Anopheles gambiae* complex and *Anopheles funestus* group, include the most efficient mosquito vectors of human malaria, implicated in malaria infection transmission across several regions of tropical Africa (Hunt RH, et al. 1998; Breman JG, et al. 2001, Coetzee M, 2004; Levine R et al. 2004).

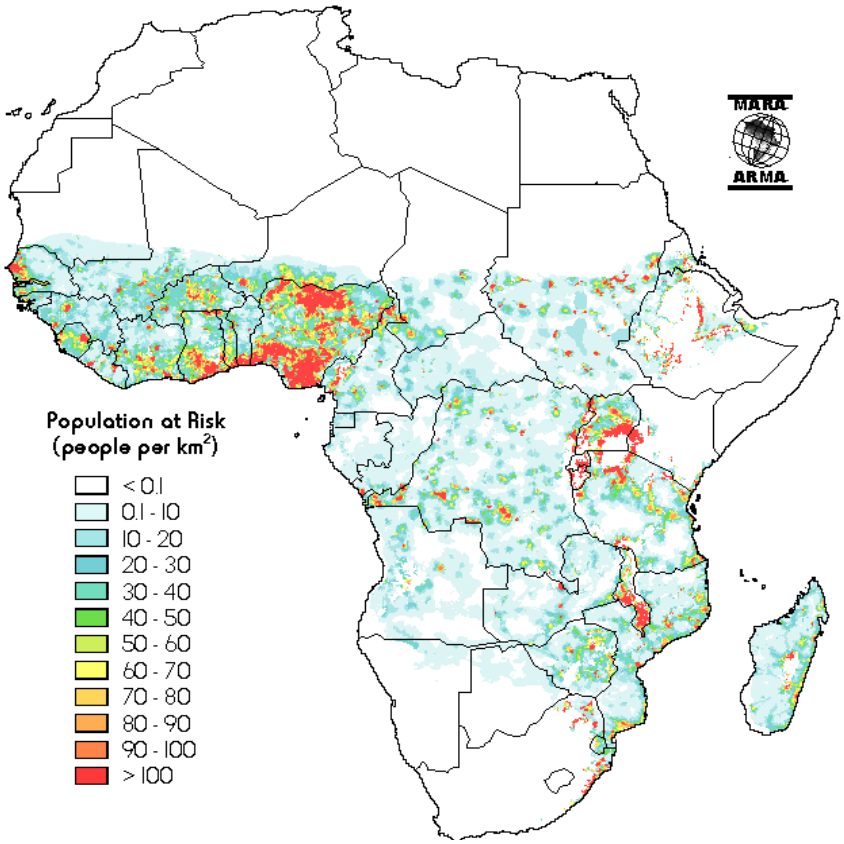
The intensity of malaria transmission fluctuates largely along with variations on geophysical characteristics, climatic, environment conditions, malaria mosquito vectors and parasite species and the socio-economic status, behaviour and distribution of human populations (Molineaux L et al. 1988).

FIGURE 4. Geographic distribution of malaria infection in the world



Source: http://who.int/Malaria 2003_map.pdf

FIGURE 5. Distribution of population at risk of malaria infection in Africa



Source: MARA/ARMA, http://www.mara.org.za

In general, the wide range of the amount and/or the severity of malaria infection can be categorized in two broad situations: UNSTABLE and STABLE malaria.

Unstable malaria is characterised by its unpredictable occurrence over a given period of time. The exposure to malaria infection is inconsistent, and consequently, an effective and long-lasting level of collective immunity is not acquired. All age groups are vulnerable to infection. Areas of unstable malaria are prone to epidemic outbreaks.

In stable malaria situation, conditions are favourable for long and persistent periods of transmission, with little variations related to seasonal and climatic changes. The amount of malaria is enormous, reflecting the presence of highly effective vectors (Bloland et al. 1999a) and high exposure to the infection. Epidemic outbreaks are unlikely to occur in the indigenous population, due to high collective immunity in the population.

In this situation, malaria is described as endemic and the intensity of transmission can be graded into four different endemic levels.

The methods commonly used to estimate the levels of malaria endemicity are based on the proportion of palpable enlarged spleens and the relative degree of splenomegaly that is “Spleen Rate”, or based on the results of blood smear examination for malaria parasite presence, the “Parasite Rate”. To estimate the degree of exposure and the intensity of the malaria infection transmission was estimated in indigenous population aged between 2 and 10 years old (Molineaux, 1988)

The endemicity levels and intensity of transmission are classified as follow:

(1). HYPOENDEMIC: The intensity of transmission is low, the spleen rate or parasite rate do not exceed 10%.

(2). MESOENDEMIC: The intensity of transmission varies depending on local conditions, the maximum incidence of malaria infection occurs in childhood and adolescence and the spleen rate or parasite rate oscillation is between 11% and 50%

(3). HYPERENDEMIC: The intensity of transmission is high and seasonal, the maximum incidence of malaria infection occurs by late infancy or early

childhood and the spleen rate or parasite rate is constantly over 50%. The spleen rate in adults is also high (over 25%).
(4). HOLOENDEMIC: The transmission is intense and perennial; individuals acquire malaria infection in early infancy, resulting in a substantial degree of immunity in all age groups, particularly in the adults. The spleen rate or parasite rate is constantly over 75%, but the spleen rate in adults is low.

For unknown reasons, in areas of intense malaria transmission, despite the considerably amount of immunity acquired, adults still present some degree of splenomegaly.
In hypo-endemic and meso-endemic malaria, the parasite rates may increase with seasonal increase of malaria infection transmission.
The method of enlarged spleen examination, was first introduced in India by Dempster in 1848, it is not an accurate measurement of the degree of exposure to the malaria infection; enlarged spleens are seen also in other parasitic diseases, for instance intestinal leishmaniasis or Manson's schistosomiasis.

3.2 The burden of malaria in Africa

Malaria especially that caused by *Plasmodium falciparum*, is one of the most important pathogenic disease afflicting inhabitants of endemic tropical Africa. Remains a leading cause of mortality and morbidity in impoverished communities across sub-Saharan Africa (Snow et al., 1999; WHO 2002). Conservative estimates of the burden of disease claim for more than 300 million clinical episodes and 1 – 3 million deaths every year and, young children harbour the large and most important portion of this toll (Stuerchler, 1989; WHO 2002; Snow et al., 2003). Nevertheless, the true magnitude and impact of the disease remain imprecise (Alonso et al 1996; Snow et al, 1998).
The malaria disease burden estimation in tropical Africa relies primarily on mortality and morbidity data collected by the health system information.
Reports currently available provide an extremely variable morbidity and mortality estimates. The imprecision is evidenced by the wide variability on

morbidity and mortality estimates as shown in table 1, reflecting different methods of estimation.

Table 1. *Clinical malaria cases and deaths estimates in Africa and in the world*

Author	Year	Clinical Cases *		Deaths *	
		World	Africa	World	Africa
Snow et al.	1999		80-449		0.74-1.3
WHO	1997	300-500	90	1.5-2.7	1
Murray & Lopez	1996				856.000
Brinkmann and Brinkmann	1991		189		No data
Sturchler	1989	234	190.1	2.3	1.9
Breman and Campbell	1988				685.000
Baudon et al.	1987		35		No data
Bruce-Chwatt	1952	250		2.5	1

* total number of cases and deaths expressed in million

Traditionally, estimates of morbidity and mortality are derived from historical maps of the geographical extent of malaria. Various methods, each applicable to particular circumstances, can be used for base map production. These maps are based on population at risk of malaria infection, determined from retrospective modelling climate data and population projection, as is the example of MARA (Mapping Malaria Atlas in Africa). The MARA maps are useful if well understood, they reflect the extension of the disease, resulting from a correlation between climatic, environmental conditions favourable to the occurrence of malaria infection and the existing population in a given region or area. Similarly, the model described by MacDonald, malaria transmission is directly proportional to the density of the vectors, the number of times that mosquito feeds on a gametocyte-carrying person and the probability of the

mosquito surviving. These concepts are crucial if the amount and severity of malaria transmission is to be predicted. However, there are some limitations, since do not take into account all elements involved in the complex dynamic of the malaria infection, especially, the determinants of disease outcome (severity and duration of clinical manifestations, sequelae, death); the crucial elements required to estimate the overall malaria disease burden.

On the other hand, the most reliable and complete epidemiological features of the malaria situation and its socio-economic impact have been underestimated due to insufficient surveillance and inaccurate statistics collected and reported on the disease in Tropical Africa.

Additionally in most endemic malaria affected countries, the existing health information systems routinely collect information based on febrile reported histories and clinical signs, and definitive diagnosis invariably is made without laboratory confirmation of the disease. Moreover, the health system network, do not cover the immense rural areas where the majority of the populations live, and therefore a substantial number of malaria cases and deaths, take place outside the formal health system.

In many rural areas, 80% or more of childhood deaths occur at home and are not reported in either hospital statistics or national vital statistics (Mung'ala and Snow, 1994; Rustein, 2000)

Verbal autopsy data from demographic surveillance undertaken between 1931 and 1997 have already been used to obtain a median estimate of malaria mortality across Africa of 7.3 per 1000 chid-years, or about 800.000 deaths among children in 1995 (Snow et al, 1999). However, no adjustments were made in this earlier study for differences in mortality between areas of malaria transmission risk, for the diagnostic limitations of verbal autopsy, or for possible temporal changes since 1931.

Attribution of cause of death during household surveys can be achieved only by soliciting histories from their relatives on symptoms and clinical signs observed during the fatal illness.

Current estimates of the malaria mortality burden in Africa are largely based on observations made in demographic surveillance sites (DSS). The DSS have been established to record prospectively disease and deaths, to investigate

epidemiological determinants of child survival, and to provide a platform on which to undertake large-scale community intervention trials (Sankoh et al, 2002).

Most of DSS sites have adopted verbal autopsy to attribute cause of death.

With the current global information available, certainly the burden of the disease would be an underestimation, however an outline of the situation could be drawn.

Recent trends in malaria deaths in children under five years of age is obtained from demographic surveillance system, which measure deaths and possible causes prospectively over time in populations of known size and composition.

Recently, data from 1982-1998 were analysed across 28 DSS sites, adjusting for the specificity and sensitivity of verbal autopsies that were used to attribute deaths to malaria (Korenromp et al, 2003). Malaria mortality in children under five years old almost doubled in eastern and southern Africa over the period 1990-1998 compared with 1982-1989. It is known that the prevalence of malaria infections caused by chloroquine-resistant parasites increased substantially from the late 1980s in these same areas.

Throughout Africa south of Sahara, the decrease in all-cause under-5 mortality that was apparent during the 1970s and 1980s stabilized in the 1990s, perhaps partially as a result increased malaria mortality. Some of the important factors that may have contributed to the increasing malaria burden in these African settings include: Spread of falciparum malaria-resistant, emergence of HIV/AIDS, climate and environmental change and more critically, the breakdown of control programmes.

3.3 The Economic and Social Impact of Malaria in Africa

Malaria occurs mostly in poor, tropical and subtropical areas of the world. The area most affected is Africa south of the Sahara, where an estimated 90% of the deaths due to malaria occur (Snow et al., 1998). This is due to a combination of factors:

Anopheles gambiae, a very efficient mosquito vector (Levine, 2004; Coetzee, 2004) assuring high transmission of the *Plasmodium falciparum*, the most predominant and deadly malaria parasite species. Local weather conditions are appropriate and often transmission to occur throughout the year. Moreover, limited resources and socio-economic instability constitute the major factors impeding efficient malaria control activities.

3.3.1 Social and Economic Toll

Although malaria imposes substantial costs to both individuals and governments, information on the socio and economic burden of malaria in Africa is scarce. The definition and measurement of the health burden of malaria is challenging, hence in turn is very difficult to quantify (Breman 2001). Several studies conducted in Africa, rely on febrile illness to estimate the overall cost. Thus, the impact of uncomplicated febrile illness is overestimated, but the impact of severe disease and mortality is underestimated. Additionally other malaria-related and debilitating manifestations and the impairment of intellectual development are not taken into account (Chima et al., 2003).

Direct costs are generally defined as expenditure on prevention and treatment of malaria by households and health services (Chima et al., 2003)

The costs to households include: expenses for travel to, and treatment at, dispensaries and clinics, purchase of drugs for treating, lost days of work, absenteeism from school and expenses for preventive measures.

The costs to governments include: maintenance of health infrastructures; acquisition of drugs and provisions; planning and implementation of preventive interventions against malaria; lost days of work with resulting loss of income; and lost opportunities for joint economic ventures and tourism, which in turn is an important obstacle to economic development (Sachs & Malaney 2002; Utzinger et al., 2002).

It has been estimated in a retrospective analysis that economic growth per year of countries with intensive malaria was 1.3% lower than that of countries without malaria (Gallup & Sachs 2001; Sachs & Malaney 2002).

It is well known that of the most important determinants of the economic cost is the indirect cost of productive labour time lost due to illness (Utzinger 2001). The explanation for this emphasis is provided by human capital theory, which regards investment in health improvement as similar to investment in physical assets, with the benefits to be measured in increased output in the economy. The scope of the indirect costs included varies; some studies measure only the time spent seeking treatment, most include additional morbidity time, and a few also incorporate the cost of mortality, in terms of life-time income foregone.
Most studies of indirect costs are based on the salary rate method, which uses estimates of the time lost, multiplied by some value of a day's work. The time cost is measured as the sum of the opportunity cost of time foregone by the sick individual due to illness, and the opportunity cost of healthy household members' time spent treating or attending to the sick person, or accompanying them for treatment. The opportunity cost of time is defined as the marginal product of labour. There is considerable variation between studies in the methods used for measuring and valuing time lost which can have a significant impact on the indirect cost estimations.

4 Malaria Drug Resistance

The global death toll from malaria is rising, and this is attributed directly to drug resistance. Drug pressure, anti-malarial drugs misuse and selection of resistant parasite strains are the major contributing factors in the emergence of anti-malarial drugs resistance (Wernsdorfer, 1994). During decades Chloroquine was the first-line treatment for uncomplicated malaria. However, *Plasmodium falciparum* has developed resistance to all classes of anti-malarial drugs with the exception of the artemisinin derivatives (White, 1992; Brockman et al, 2000, Bloland, 2001).
Chloroquine-resistant *Plasmodium falciparum* was first reported from Southeast Asia in 1957 (Harinasuta et al., 1965), and during the early 1960's was reported from South America (Moore et al., 1961). Later in 1978 chloroquine-resistant *Plasmodium falciparum* was recorded in Africa (Fogh et al., 1979).

In Oceania and parts of Indonesia significant *Plasmodium vivax* resistance to chloroquine has been reported (Rieckmann et al, 1989; Baird et al, 1996).

The appearance and dramatic widespread of chloroquine-resistant *Plasmodium falciparum* during the 1970's, have been associated with increasing malaria-related morbidity and mortality, particularly in the sub-Saharan Africa (Trape et al, 1998). Additionally, Pyremethamine resistance has also worsened rapidly, and the loss of the synergistic combination with sulphonamides (Sulphadoxine-Pyremethamine) has been more rapid, as predicted.

Resistance to other anti-malarial drugs emerged immediately in areas where the drugs have been introduced (Talisuna et al., 2004). In Peninsular Malaya resistance to the type 2 antifolate proguanil was recorded in 1948, a year after its introduction, while sulphadoxine-pyremethamine resistance was observed in 1967, the same year of its deployment in Thailand (Peters, 1987). Resistance to mefloquine was first reported five years after it had been introduced in 1982 (Nosten et al., 1991).

Since 1988 mefloquine resistance has developed rapidly in Southeast Asia (Nosten et al, 1988; Fontanet et al, 1993), while sensitivity to quinine has declined progressively. In 1994, the combination of artesunate and mefloquine was introduced in Thailand (Nosten et al, 1994). Although, *Plasmodium falciparum* was resistant to mefloquine, the combination proved to be effective. During the subsequent years cure rates have remained high (Brockman et al, 2000; Nosten et al, 2000) and the incidence of *falciparum malaria* declined considerably. These results and the dramatic decline in malaria mortality associated with artemisinin deployment in Vietnam, led to a global initiative to evaluate anti-malarial drug combinations based on the artemisinin derivatives. These combinations have proved safe and effective (White, 1999), and it is now widely accepted that such combinations should replace existing monotherapy for the treatment of malaria to ensure sustained efficacy and prevent the emergence of resistance.

5 Vector Resistance

Vector control using DDT and other synthetic insecticides during the 1950´s led to a significant decrease in the prevalence of malaria infection worldwide. Since then, insecticide-based control measures have been the cornerstone strategy to controlling malaria vectors. However, after prolonged exposure to insecticides over several generations, mosquitoes like other insects, develop the ability to tolerate the contact with an insecticide. This biological phenomenon develops as a result of selection pressure by the relevant insecticidal compound or its analogue. On the other hand, insects may acquire the ability to avoid contact with the insecticide, a phenomenon termed “behaviouristic resistance” or “insecticide avoidance” (Bruce-Chwatt, 1986).

The use of an insecticide until resistance becomes a limiting factor is rapidly eroding almost all categories of insecticides available for insect control. Currently, there are over 125 mosquito species with documented resistance to one or more insecticides. Resistance of *Anopheles gambiae s.s*. to pyrethroids and DDT, has been reported from West Africa (Chandre et at., 1999), and East Africa vector population (Ranson et al., 2000). Recently, *An. funestus* resistance to pyrethroids has been reported from Mozambique (Brooke et al, 2001; Casimiro et al., 2006 unpublished paper).

The use of insecticides in agriculture has often been implicated as a contributing factor to resistance development in mosquito populations.

6 Global Climatic and Environmental Changes

The global population rising, rapid industrialization (through use of fossil fuels) and increased agricultural production over the last century led to the accumulation of “greenhouse gases” (GHG’s) in the troposphere. The atypical weather patterns of the last two decades, characterised by increasing global mean surface temperature, extreme weather events (droughts, storms and floods) denote the beginning of a long-term process of world’s climate changes.

It has been thought that, by altering local weather patterns and by disturbing life-supporting natural systems and processes, global climate change, would affect the health of human populations, including changes in the transmission and seasonality of vector-borne diseases (McMichael et al., 2001; Martin B., 2002).

The range of factors affecting transmission and distribution of vector-borne diseases, particularly malaria, include those related to climate (temperature, humidity and the amount of precipitation).

The development of malaria parasites in the mosquito is determined not only by genetic factors of both parasite and mosquito, but also, variations on ambient temperature play an important role. Low temperatures are the most important limiting factor to the development of malaria parasites, and therefore will affect the duration of incubation period in the vector. On the other hand, the minimum generation time of human malaria, i.e. the minimum time necessary to generate a secondary infective case from a primary infective case, varies from species to another but is influenced by the temperature variations.

Environmental factors, in particular temperature and humidity, can also have an effect on the longevity and geographical distribution of the anopheline mosquitoes. Therefore, variations of temperatures, will determine the survival rates of both vector and parasite, while the amount of rainfall will have an effect on the abundance of breeding sites and vector densities (Molineaux 1988).

The geographical distribution of malaria depends on factors other than temperature and humidity. However, attempts to estimates the impacts of climate change on the potential transmission of malaria have been made using mathematical models. Average temperatures and rainfall are the major variables considered to generate the likely scenarios in the models. Obviously, there will be limitations, since other variables affecting malaria transmission, such as local demographic dynamics, previous exposure and immunity-related factors, and other technical aspects of malaria control already in place, socio-economic development, are not incorporated in the models. Nevertheless, some scenario-based studies conducted in Africa to forecast the health impacts of climate change, especially on malaria, estimates a potential increase of 16% to 28% in person-month of exposure, due to a prolonged transmission, resulting

from a combined effect of increase in both temperature and precipitation (Tanser et al. 2003).

In addition to the extreme whether events (temperature and precipitation) as part of global environmental changes, the depletion of stratospheric ozone is reducing the shield of earth's surface against incoming solar Ultra Violet Radiation (UVR). Direct exposure to UVR may have harmful effects on living organisms. Continued exposure to UV-B and UV-C is damaging to amphibian eggs, plants and marine phytoplankton (Holm-Hasen et al., 1993; Blaustein et al., 1994).

In experimental studies, both UV-B and UV-C are absorbed and cause direct damage to DNA. In humans, irradiation of skin even at low levels is associated with development of skin cancer and eye lesions, and probably some systemic immunossupression (Noonan & DeFabo EC., 1992; Jeevan & Kripke, 1993).

Climate changes are likely to have diverse range of effects on human health. The magnitude and extent of this impact it is imprecise, and some of the effects of global climate change will emerge slowly over time, currently, the knowledge gained from limited past experience of environmental-health relationships is fragmentary.

7 The Epidemiology of Malaria in Mozambique

Malaria continues to be one of the major public health problems in Mozambique due to a multitude of factors such as climatological/environmental (favourable temperatures and rain patterns, abundant breeding sites) and socio-economical (poverty related improper housing/shelter, unaffordable preventive means). Major portions of the population in Mozambique live in malaria high-risk areas. During the last five to ten years, the number of malaria cases has steadily increased throughout the country, particularly in the rural regions, where approximately 73% of the Mozambican population lives.

Malaria is endemic throughout the country, varying between mesoendemic and hyperendemic areas. Transmission is perennial, with peaks during and after rainy seasons (January to April). However the intensities of transmission may

vary depending on the amount of rain and air temperatures observed in each year and also depending on the specific local conditions. At present there is a lack of good quality and updated information on the endemicity levels in the country.

7.1 Disease burden

Malaria is a very common presentation to health care facilities. It is also the most common cause of admission to wards. Severe malaria presents as cerebral malaria or as malaria with severe anaemia, often requiring life saving blood transfusion. Malaria accounts for an estimated 44% of all outpatient attendance, 57% of paediatric admissions and 29% of all hospital deaths in rural and provincial hospitals. It also contributes to the high maternal mortality observed (1.500 per 100.000 births). The case fatality rate is thought to vary between 1.8% and 9.9%, depending on level of health facility. *Plasmodium falciparum* is responsible for over 90% of all malaria cases (NMCP, 2002).

Incidence of clinical malaria established through weekly active case detection suggests that the risk of clinical malaria is highest between the age of one and three years when children experience an average of more than 2 episodes per year. The risk of malaria drops sharply after the age of 6 years; Based on a continuous demographic surveillance system and verbal autopsies – (Centro de Investigaçao em Saúde da Manhiça).

Malaria is also a major problem in pregnant women in rural areas. Approximately 20% of women are parasitaemic, and among them prim-gravid shows the highest prevalence 31%. A recent study in rural southern Mozambique, revealed a prevalence of maternal anaemia associated with microscopic parasitaemia to be 59% (Saute et al., 2002).

The real burden of malaria and its impact on the society and economy is not known due to lack of detailed studies. However, data from sentinel sites shows that malaria is a very common presentation to health care facilities. It is also the most common cause of admission to wards. Severe malaria presents as

cerebral malaria or as malaria with severe anaemia, often requiring life saving blood transfusion.

There is malaria all year round but transmission is highest between the months of November and April, with peaks following a few weeks after the anopheline mosquito density peak.

Malaria is also a major problem in pregnant women in rural areas. Approximately 20% of women are parasitaemic, and among them prim-gravid shows the highest prevalence 31%. Anaemia, often associated with malaria is a major problem and 68% have a PVC <33%. Malaria is the main cause of morbidity in children under five accounting for over 30% of reported deaths in the community; data based on a continuous demographic surveillance system and verbal autopsies.

The economic loss due to malaria in Mozambique has never been estimated through carefully designed investigations. However general references such as episodes of illness due to malaria contribute to a loss of industrial labour, school absenteeism and poor agricultural productivity (Wernsdorfer, 1988). Studies on this aspect are very important when industrial labour and agricultural productivity are the main source of income and potential economic gains for the majority of rural population.

7.2 Malaria and Past Control Activities in Mozambique

The first attempt to characterize the ailments afflicting Mozambican inhabitants dates from the early 1846, entitled “Draft on the illnesses of the African eastern cost”, was described by Jacques de Salis, a Swiss physician working for his majesty the king of Portugal at the “Ilha de Mozambique” the first colonial capital city of the Portuguese province of Mozambique, (Santos Reis, 1982).

The pathogenesis of various infectious diseases was yet not well understood at that time, however, the author remarkably stressed the relationship between the illnesses and the environment conditions and the type of soil.

When describing the region’s climate, the author emphasized the alternation between dry and rainy seasons, hot temperatures, predominant southern winds

and the lower altitude that are typical characteristics of the Mozambican costal line. Fevers were the most important ailments, always referred to as endemic fevers and believed to emanate from swamps. The treatment of those fevers consisted of infusions, including lemon syrups, cooked barley and other seasonings containing sulphured quinine. Other ailments described were dysenteries, hepatitis, tick bite fever, flegmasiasis, elephantiasis, ulcers, tetanus, etc. The author had characterized the region as “unhealthy and flooded” (Santos Reis, 1982).

At the time no specific anti-malarial activities were carried out with the exception of the symptomatic treatment of fever cases.

In Mozambique as in many other places around the world, the history of malaria control began during the early 1900s, with the discovery of synthetic anti-malarial drugs and the implementation of ant-larval activities. Later, the discovery of the insecticidal effect of DDT, BHC and dieldrin has increased malaria control activities worldwide (Molineaux & Gramiccia, 1980).

The scarce information available of malaria reflects the weakness of the surveillance system and the absence of a national plan for malaria control during the colonial period. However, some statistics compiled in the main hospital in Lourenço Marques (LM) city, during the period between 1900 and 1909, showed an increased number of fever cases admitted in the hospital. The second most important disease was anaemia. Clearly malaria and malaria-related anaemia were the main cause of admission at the LM Hospital between that period (Firmino Sant’Anna. 1910). Not surprisingly, the case fatality rates were very high, given that the treatment of fever cases was palliative.

The first anti-malarial activities were initiated in May 1907, in LM city and consisted of environment management (elimination of breeding places and application of larvicides such as residual oils (Serrão de Azevedo, 1910).

Lack of both human and financial resources was the most important obstacle for expansion to a larger scale implementation of the anti-malaria activities to the rest of the territory. In addition, in those areas where these activities were carried out, local communities were refractory to comply with the health authorities’ recommendations concerning anti-malarial actions (Serrão de Azevedo, 1910).

Table 2. *Distribution of malaria-related cases, deaths and case fatality rates observed at the L.M. city hospital between 1901 and 1907.*

	1901	1902	1903	1904	1905	1906	1907
Intermitent Fever	2,321	1,091	1,370	2,090	960	1,089	1,782
Remittent Fever	33	4	22	11	3	9	30
Pernicious Fever	41	19	39	26	17	17	47
Bilious Fever	101	70	56	89	29	47	99
Anaemia	139	264	68	130	92	195	46
Total Cases	2,635	1,448	1,555	2,346	1,101	1,357	2,004
Total Deaths	504	367	703	642	579	597	612
Case Fatality Rate	19.1	25.3	45.2	27.3	52.6	54.8	30.5

Source: 1908 Report - Arquivo de Patologias Exóticas. Vol **3**:19-42.

Between 1935 and 1939, a first large-scale control of rural malaria using pyrethrum spraying was successfully implemented in South Africa.

The positive results achieved within neighbouring territories of the Republic of South Africa influenced the sanitary entities of the bordering regions, particularly in the southern part of Mozambique, and consequently, during subsequent year's entomologist from Transvaal - South Africa, jointly with Mozambican authorities, initiated several studies towards malaria vectors identification, behaviour and habit characterization to provide elements to plan anti-malarial interventions in Maputo region (Paiva Martins, 1941).

The first epidemiological study to categorize the endemicity levels in Mozambique was carried out in Maputo region southern of the Umbeluzi River. The study started in January 1937 and was completed in March 1938, (Paiva Martins, 1941). The study subjects included children less than 15 years of age, born in the study area. Pyrethrum spray mosquito collections were performed in selected dwellings in one locality.

Microscopy examination of blood-slide for *plasmodium* species identification, density determination and enlarged spleen rates was the methodology used to determine the endemicity levels of malaria infection in the Maputo region (Paiva Martins, 1941).

Briefly the results obtained:

7.2.1 Parasite prevalence

Of the observed children 84% had parasites. The 1 – 5 years age group had the highest malaria parasite prevalence 92.1 %. Infections caused by *Plasmodium falciparum* were between 76.6 % and 93.7%, while *Plasmodium vivax* infections were between 1.6% and 19.5%. Mixed infections (*Plasmodium falciparum* and *Plasmodium vivax*) were between 3% and 7.3%. Infections by *Plasmodium malariae* and *Plasmodium ovale* were not observed in the study areas (Paiva Martins, 1941).

Table 3. *Distribution of parasite prevalence infection and enlarged spleen rate, by age group in children resident in Maputo region during 1937 - 1938 survey.*

Age-groups	Parasite prevalence (%)	Spleen rate (%)
< 1 year	79.9	56.0
1 - 5 years	92.1	69.3
5 - 10 years	83.2	52.5
10 - 15 years	72.0	37.7

Predominantly enlarged spleens were type I and type II and the 1 – 5 years age group had the highest frequency 69.3%. The average spleen rate was 56.7%. (Paiva Martins, 1941).

Table 3.1. *Distribution of enlarged spleen by category, associated with parasitaemia in children observed during 1937-1938 survey in Maputo region – Southern Mozambique*

Spleen Category	Total Observed	With Parasitaemia	Enlarged Spleen plus parasitaemia
B0	3070	2566	83.5 %
BI	1796	1556	86.6 %
BII	1384	1098	79.3 %
BIII	597	547	91.3 %
BIV	252	202	80.0 %

7.2.2 Malaria Vectors

Anopheles funestus and *Anopheles gambiae* complex were the two main mosquito species captured in the dwellings visited in the study areas. The majority exhibited anthropophagic feeding habits and low sporozoite infection rates. The sporozoite indexes were lower and the feeding habit was anthropophagic (Paiva Martins, 1941).

Table 4. *Prevalence of sporozoite and oocyst infections in mosquitoes collected in three localities of Maputo – southern Mozambique during 1937 – 1938 surveys*

	Total mosquitoes dissected	Oocyst positive (%)	Sporozoite positive (%)
March			
Bela Vista	120	17.8	5.6
Salamanga	120	18.5	6.0
Catembe	110	20.2	6.1
April			
Bela Vista	135	-	5.9
Salamanga	140	-	6.1
Catembe	155	-	6.3

Simultaneously, between May and September 1937, another study was carried out in some primary schools of Lourenço Marques city. Samples from students were grouped in three age categories (under 10 years; 10 –15 years; and over 15 years old), and included all racial groups blacks and coloured (Europeans and Asiatic, mainly Indians) (Alberto Soeiro, 1941).

The results obtained are summarized as follow:

Children from various schools in Lourenço Marques city were observed and grouped in two major racial categories: African and European origins. The African origin group showed greater parasite prevalence and spleen rates as compared to the similar Europeans age group. In both racial groups infections by *Plasmodium falciparum* were higher, closer to 98% followed by *Plasmodium malariae* infections, about 2% in Africans and 1% in Europeans. Infections by *Plasmodium vivax* were not observed in the European group, while in Africans this was observed in 0.25%. In both groups were not observed mixed infections (Soeiro, 1941).

Table 5. *Distribution of parasite prevalence infection by species in indigenous children resident in Lourenço Marques city, during 1937 malaria survey*

Age-group	**Overall Parasite Prevalence**	**Prevalence of *P. falciparum***	**Prevalence of *P. malariae***	**Prevalence of *P. vivax***
1 – 10 years	49.2	96.88	2.08	1.04
10 – 15 years	43.3	97.27	2.19	0.54
> 15 years	31.9	100.00	-	-
Total	46.16	97.7	2.05	0.25

Table 6. *Distribution of parasite prevalence infection by species in Europeans children resident in Lourenço Marques city, during 1937 malaria survey*

Age-group	Overall Parasite Prevalence	Prevalence of *P. falciparum*	Prevalence of *P. malariae*	Prevalence of *P. vivax*
1 – 10 years	6.38	100.00	-	
10 – 15 years	5.38	98.92	1.08	
> 15 years	0.90	100.00	-	
Total	4.22	99.30	0.7	

Table 7. *Distribution of parasite prevalence infection and spleen rate in Indigenous and Europeans children resident in Lourenço Marques city, during 1937 malaria survey*

Age-groups	Indigenous children		European children	
	Parasite Prevalence	Spleen Rate	Parasite Prevalence	Spleen Rate
1 – 10 years	45.94	29.7	5.79	0.0
10 – 15 years	43.35	48.88	5.58	3.74
> 15 years	31.89	12.93	0.90	0.90

The difficulty on the diagnosis of different parasite species must be taken into account in this study. Slides were coloured using the Leishmann method, thus, species identification requires much more time, however, the fast nature of the survey, did not allow enough time for accurate species identification (Soeiro, 1941).

Other important studies to characterize the endemcity of malaria infection in Mozambique were carried out in the central and northern region of the country. The urbanization and the need of expansion of cities or establishment of new

economical centres were preceded by epidemiological studies, to characterize malaria endemicity in those areas.

The project of construction of the Beira-Tete railway road, which facilitated the resettlement in the Zambezi valley, an area for potential economic growth (construction of Cahora Bassa Dam, agriculture, livestock and mining) was preceded by malariometric studies along the proposed route (Rebelo, 1938). Similarly, the establishment of Quelimane town was preceded by epidemiological studies of malaria infection and formulation of respective anti-malarial measures (Rebelo, 1948). The urbanization and expansion of Nampula and Niassa districts, followed malaria surveys (Soeiro e Morais, 1959).

The results from various studies conducted, showed higher endemicity levels of malaria infection, especially in peri-urban and rural areas. In addition, high admission rates due to malaria in main hospitals reflected the magnitude and severity of the problem. Despite these facts, malaria was still a non compulsory notified disease and anti-malaria activities were restricted to a few areas in the southern region of the Country, lack of financial, technologic and human resources was the main constraint (Soeiro, 1959).

7.2.3 Anti-malarial activities

The first large scale anti-malarial interventions started in LM city and its environs by 1942, and consisted in the application of pesticides, using kerosene and Pyrethrum and larviciding, applying oils in all identified permanent breeding places. In semi-urban areas, breeding sites were treated using residual oils. These activities were later expanded to Beira city in 1946. A complementary measure, included spatial fogging with Tifa® machine, and was carried out weekly in LM city and suburbs, to decrease the vector density not only of mosquitoes that transmit malaria but also other insects. In addition anti-larval brigades were responsible for house-to-house treatment and control campaigns of in-house breeding sites i.e. water containers for domestic purposes (Soeiro, 1959).

The use of DDT and BHC began in 1946, in the LM city area and surroundings, and later expanded to João Belo, Inhambane towns and to the Limpopo valley, in the southern region of Mozambique.

Special remark for one locality in southern Mozambique, precisely Ressano Garcia, a small village around the main and very important railway station in the border with South Africa, the activities were successfully implemented with support of the health services of Mozambique Railways (C.F.M.) Company (Soeiro, 1959).

DDT solution was used to spray western style houses, while local dwellings were sprayed using BHC.

By 1948, in Beira the second largest city, situated in the central region of the country, DDT was added to other previously existing anti-malarial activities. Progressively the use of DDT was expanded to other towns such as Quelimane, António Enes, Nampula and Porto Amélia, in the northern region of the country.

The plan was to establish anti-malarial centres in each city and municipality, however, and once again shortage of trained manpower and financial resources were the major impeding factors (Soeiro, 1959).

The expansion of anti-malarial control activities to the various regions of the country, was not consequent of neither increased knowledge of malaria epidemiology nor the understanding of the dynamic of malaria transmission in those areas, rather a response to an international appeal to apply general recommended measures, such as vector control using available insecticides (DDT, HCH, dieldrin) and larviciding in malaria endemic areas.

The few studies carried out, yielded fragmentary information on the epidemiology of malaria in the country.

Lack of information on vectors distribution, behaviour and habit was notorious. The prevalence and parasite species distribution was available only in a limited number of localities; hence the pattern of malaria transmission and its endemicity was not well known in a large extension of the country.

The first countrywide and comprehensive study, which described the basic factors implicated in malaria occurrence; natural environmental conditions, the parasites and vectors species prevalence and distribution, demographic and

social factors, was conducted by Soeiro and his collaborators between 1949 and 1951. Detailed results are described elsewhere (Soeiro, 1952)

A brief outline of the main findings

Based on altitude four stratum were identified: The costal line, characterized by low lands and altitude below 200 metres above sea level, flat terrains with altitude between 200 – 600 metres above sea level and high grounds sited between 600 – 1,000 meters above sea level and high land/mountains located 1,000 metres above sea level.

The climate was described into four main categories, as tropical marginal, tropical costal, tropical monsoons and sub-tropical de altitude.

Two main seasons: rainy season from December to March and dry season from May to November.

In the southern region rainy season are much more prolonged as compared to the northern region.

Mean temperatures varied from 22°C in south and 26°C to the north.

Population was estimated to be 5,732,767 (1950 census). Population density varied from 11.57 inhabitants per Km^2 in Zambezia province (with the highest population density) and 4.57 inhabitants per Km^2 in Sofala-Manica region.

Malaria endemicity across the country was considered moderate to highly endemic, and there were no malaria free zones.

The peak of malaria cases was observed between January and April, decreasing between July and September.

The parasite and spleen indexes varied between different strata and varied in different age groups, in average the costal line showed the lowest indexes.

In many areas in the costal region anti-malarial activities were already underway, using DDT and other insecticide.

Table 8. *Average parasite and spleen indexes in children under 10 years old, during surveys conducted in 1949-1951*

STRATUM	PARASITE INDEX	SPLEEN INDEX
Costal line (< 200 m)	68.2 %	48.2%
Flat terrains (200–600 m)	68.0 %	46.6%
High grounds (600–1,000 m)	53.6 %	50.8%
Highland/mountains >1,000 m)	27.2 %	47.0%

Parasite species prevalence and distribution

The parasite indexes varied in different stratum and in different age groups.

In children less than one year of age the parasite index varied between 33.5% in southern region and 80% in the Zambezia province, and in average was 58.5%. In the age group of 2-10 years old varied between 65.9% the southern region and 100% in Zambezia province and average was 92.5%. Adults in average had parasite index of 17.9%.

In general high parasite indexes were observed in the northern region as compared to the southern region.

A total of 4,742 indigenous children between (1-12 years old) were examined, 2,331 were found to be parasitaemic, average 49.2%. *Plasmodium falciparum* gametocytes were observed in 2.02% of all examined children.

The distribution of different plasmodium species was as follows.

Table 9. *Prevalence of malaria parasites by species in children less than 10 years of age*

Species	Prevalence
Plasmodium falciparum	42.1%
Plasmodium malariae	5.5%
Plasmodium vivax	1.0%
Mixed infections	0.6%

Among the positives, the percentage of various species was: *Plasmodium falciparum* 85.5%; *Plasmodium malariae* 11.1%; *Plasmodium vivax* 2.1%; Mixed infections 1.3%.

Malaria vectors

In Mozambique were identified 20 species of anopheline and 5 sub-species (Botha de Meillon & Mario Pereira). However, only *A. gambiae* and *A. funestus* were reported to be the main vectors of malaria parasites. Both vectors were found to be highly anthropophylic and endophylic.

The sporozoite oocyst indexes varied between 4.3 and 22.0% for Anopheles funestus while for anopheles gambiae varied between 0.1 to 14.7%.

In conclusion (Soeiro et al 1952) characterized the country as moderate to highly endemic malaria area. The peak of malaria incidence was between January and April.

The coastal plains and regions with altitude below 600 meters above sea level had high enlarged spleen indexes, while the lowest enlarged spleen indexes were recorded in regions (highlands/mountains) above 1,000 meters of sea level.

The highest parasite indexes were recorded in the northern regions while in the southern region were recorded the lowest parasite indexes. *Plasmodium falciparum* was the main prevailing species, accounting for more than 85 % of all malaria parasite infections. *Anopheles funestus* and *Anopheles gambiae* complex were the two major mosquito vectors.

7.2.4 Some anti-malarial trials carried out in Mozambique

During the early 1970´s, trials to evaluate the efficacy of anti-malarial drug were conducted in the southern region of Mozambique. The first trial was conducted in the Limpopo valley, and area considered as mesoendemic where two cycles of spraying with DDT had already took place. The trial involved 186 immune individuals with confirmed *Plasmodium falciparum* infection, treated with Maloprin® an association of 12.5 mg of Pyremethamine and 100 mg of dapsone tablets, kindly provided by “Burroughs Wellcome & Co”, given in a single dose

during the maximum transmission season. Other trials were conducted in Taninga village, using Camoprim (containing 300 mg of amodiaquine and 30 mg of primaquine) and Fanasil-pyremethamine" 500 mg of fanasil plus 25 mg of pyremethamine per tablet, kindly provided by "Hoffman-Roche". The results showed an efficacy of 100% in both study areas (Botelho, 1973).

8 The Current National Malaria Control Strategies

The national malaria control programme was established in the early 1980´s, after the worldwide change from malaria eradication campaigns to malaria prevention and control strategies.

The strategy adopted consists of three basic technical elements:

(1) To provide early diagnosis, prompt and effective treatment, through health care services.

(2) Implementation of selective preventative measures to reduce man-vector contact; Insecticide residual spraying is the backbone of the vector control interventions. The use of insecticide treated nets through social marketing scheme is encouraged.

(3) Community health education and social mobilization; to improve health awareness in the community.

8.1.1 Malaria Control Current Status and Recommendations

Implementation of such a control strategies was hampered by various problematic situations which eventually led to the breakdown of health services. Furthermore, the widespread of parasites resistant strains to the available anti-malarial drugs, resulted in a deficient case management, particularly at the periphery of health systems where a significant proportion of people are exposed to malaria parasites.

In addition, the widespread use of insecticides (in malaria control, agriculture and other pest control) led to a selection of resistant mosquito vectors, which

became a challenge to the vector control interventions, especially insecticide residual spraying.

Community health education and social mobilization were the basic elements to improve good health practices. However, to reach remote communities in rural settlements with health information and education materials is still a challenging task.

In light of past achievements, it is noteworthy to re-examine the existing tools for malaria control to improve their effectiveness in an integrated and coordinated approach. The complexity of the dynamic of malaria transmission warrants the development and deployment of specific control strategies for specific ecological settings.

The interventions should focus on the most afflicted groups (children, pregnant women, immuno-deficient and elderly), in areas of risk, mainly rural settlements.

Integrated vector management approach should include indoor residual spraying, insecticide treated nets and environmental management.

Expansion of the health net work, trained health workers for an accurate diagnosis and effective anti-malarial drugs for prompt treatment is crucial.

Monitoring and evaluation are important tools to inform planning and advocacy process.

Adequate and persistent funding is an important component for the success of any malaria control interventions.

8.2 Challenges and Controversies in the Field of:

8.2.1 Vaccines

Evidences of protective immunity to malaria came from experiment using attenuated X-irradiated sporozoites in monkeys (Collins et al., 1972), and later in human volunteers (Rieckmann et al., 1979; Hoffman et al., 2002). Those findings provided concepts leading to malaria vaccine development. During the later 1980's decade, development of a pioneer asexual blood stage malaria vaccine Spf66 (Moreno & Pararroyo, 1989) generated much enthusiasm and optimism on vaccine development. Unfortunately, initial field trials showed that

Spf66 vaccine failed to establish long-term protection in areas of high transmission, and efficacy was limited to certain period of time (Alonso et al., 1994) and did not reduce the risk of clinical malaria among study population (Acosta, et al., 1999).

The understanding of the immune mechanism involved in the protection against malaria parasites, gained during the past decades, has made a crucial progress and many vaccine candidate antigens have been identified. Since then, a variety of promising malaria vaccine candidates targeting various antigens exhibited in each stage of the complex parasite's life have been studied.

8.2.2 Parasite life cycle and vaccine strategies and targets

In each stage of the parasite life cycle different antigens are being expressed, which offer potential opportunities for interruption.

Pre-erythrocytic stage:

Sporozoites injected into the circulation of the host by a feeding anopheline female mosquito migrate to liver within few minutes of biting. After invasion of liver cells, sporozoites maturation into liver-stage trophozoites and then into schizonts takes place over 6-7 days. Before rupturing of the infected liver cells, and release into the circulation, about 20,000 – 40,000 merozoites are produced through asexual multiplication (Jones & Hoffman, 1994; Webster & Hill 2003). This is the end of pre-erythrocytic stage.

Pre-erythrocytic vaccines:

Pre-erythrocytic vaccines are designed to target both sporozoites before invasion of hepatocytes or schizont-infected hepatocytes and thus prevent the release of primary merozoites from infected hepatocytes. Interrupting the parasite cycle at this stage would prevent manifestation of clinical disease and block the transmission of malaria parasites.

Evidence from pre-clinical studies, in rodents and humans immunized with radiation-attenuated sporozoites indicated that antibodies targeting sporozoites could abort their ability to infect liver cells or kill parasite-infected hepatocytes

before they can release infectious merozoites (Rieckmann et al., 1979; Hoffman et al., 2002).

The target antigen of the malaria parasite in the pre-erythrocytic stage is the circumsporozoite (CS) protein. The primary structure and variability of this sporozoite membrane-associated protein, has been very well characterized across a large number of parasite strains, and various vaccine constructs targeting the CS protein have been developed.

Recently, remarkable progress on development of promising malaria vaccine candidates has been made. A significant number of malaria candidate vaccines, namely RTS,S/AS02A and MVA-ME TRAP are being entered in clinical trials in several settings (Ballou et al., 2004).

Asexual or blood stage:

Rupture of infected hepatocytes and release of merozoites into the systemic circulation mark the beginning of blood or asexual stage of the parasite life cycle. This stage is characterized by a continuous cycle of invasion of red blood cells, followed by parasite asexual multiplication, burst of parasitized cells and invasion of other red blood cells. During this stage parasite components released into the systemic circulation stimulate host responses that induce the symptoms and signals of the disease.

Blood stage vaccines:

An effective blood-stage vaccine is one that would prevent the invasion of erythrocytes by merozoites. Therefore the development of a blood-stage malaria vaccine is to target immune responses against the asexual stage (blood stage) of the parasites. Although antibodies directed against the blood-stage parasites are crucial to trigger immune responses, acquisition of immunity to malaria infection involvement of cell-mediated mechanisms may be critical (Good, 2001).

The rationale for development of blood-stage vaccines is based on observations that: the majority of individuals living in malaria-endemic areas acquire the ability to control parasite replication to levels below those that result in clinical disease, and 3) hyperimmune globulin prepared form the sera of individuals

chronically infected with malaria enhance clearance of parasitized red blood cells from infected individuals (Cohen et al., 1961; Ballou et al., 2004).

During invasion, the membranes of both parasite and red blood cell fuse, to allow parasite invasion without damaging the red blood cell. This complex process involves a number of parasite proteins that are located on the surface of the merozoites which become temporarily accessible to circulating antibodies.

Most of the development of blood-stage vaccines has been focused on targeting the surface protein1 (MSP), a protein synthesized during the development of the schizont and present on the surface of the merozoites as a complex of proteolytic fragments (Blackman et al., 1990; Egan, AF et al., 1996; Weiss et al., 1998; Egan, AF et al., 1999). The most well characterized surface antigens are: MSP-1, MSP-2, MSP-3, and apical membrane antigen 1 (AMA-1). Antibodies to these molecules are reported to block invasion of merozoites, except MSP-3, in which they trigger a monocyte-mediated effect. MSP-1, AMA-1, and MSP-3 have been produced as candidate vaccines and have been shown to protect non-human primates from uncontrolled asexual stage parasitaemia when administered with Freund's complete adjuvant. Moreover antibodies to MSP-3 can reproducibly transfer protection in a new mouse model of *Plasmodium falciparum* malaria.

Sexual stage:

Some of the blood-stage merozoites do not undergo asexual multiplication; instead, they differentiate into male or female gametocytes that are subsequently taken up by a feeding mosquito. Exflagellation and fertilization that occur inside mosquito midgut, complete the life cycle of malaria parasites.

Transmission-Blocking vaccines:

Over the past decades, several pre-clinical studies have clearly demonstrated that antibodies directed against sexual stage antigens are able to halt the process of exflagellation and fertilization of the gametocytes and thus preventing formation of infectious sporozoites in the salivary glands of Anopheles mosquitoes (Vermeulen et al., 1985; Kaslow et al., 1991; Duffy &

Kaslow, 1997). The most promising transmission-blocking vaccines, inducing monoclonal antibodies, targeting a 25-kDa protein found on surface of Plasmodium falciparum zygotes and ookinetes, are currently in a Phase 1 of clinical trials (Carter, 2001; Ballou et al., 2004).

Currently, in the GlaxoSmithKline Biologiclals (GSKBio) (Rixensart, Belgium) the most advanced malaria vaccine candidate is RTS´S/AS02A. A pre-erythrocytic stage vaccine, based on the circum-sporozoite (CS) protein of the 3D7 clone of *Plasmodium falciparum*. This vaccine candidate has two polypeptides (RTS and S) that are expressed simultaneously in *Saccharomyces cerevisiae*. RTS is a single polypeptide chain corresponding to amino acids 207-395 of the CS protein fused into the amino terminus of the hepatitis B surface antigen (HBsAg; adw serotype). S is a polypeptide of 226 amino acids that corresponds to HBsAg. Each RTS molecule includes 19 copies of the tetra peptide repeat motif (NANP) fused to the C-terminal region of the protein (minus the hydrophobic anchor sequence). During purification, the two polypeptides spontaneously assemble to form composite particulate structures (RTS´S) that constitute the vaccine antigen (Gordon et al., 1995). The adjuvant AS02A consists of an oil-in-water emulsion that incorporates the immunostimulants monophosphoryl lipid A and the saponin derivative QS21. The formulation induces high levels of CS repeat-specifics antibodies and stimulates Th-1 cellular immune responses characterized by antigen-specific production of interferon-γ (IFN-γ). Presumably, these responses constitute an important component of the protection observed in clinical trials (Lalvani et al., 1999).

Results from field trials with the most advanced pre-erythrocytic vaccine candidate (RTS,S/AS02A), revealed considerable protection from infection and indicated to have potential trend towards reducing clinical malaria episodes over two malaria seasons (Bojang et al., 2001).

Recently, clinical development trials aiming to evaluate the RTS,S/AS02A candidate vaccine, towards its implementation through the EPI scheme, a step-down age de-escalation and dose escalation trials were carried out in children age 6-11 years old and in children aged 1-5 years old. The results showed that the RTS,S/AS02A vaccine was safe at all dose levels and all doses were highly immunogenic for anti-CSP and anti-HBsAg antibodies. Furthermore, data from

these studies were used to a proof of concept efficacy study in children aged 1-4 years (Bojang et al., 2005). Results in children age 1-4 years old, showed that the prevalence of *Plasmodium falciparum* infection was 37% lower in the vaccine group compared to control group, the efficacy against severe disease was 58% and vaccine efficacy for extending time to first infection was 45% after a 6-month follow-up period (Alonso et al., 2004).

After the eighteen-month period extended follow-up, the RTS,S/AS02A vaccine efficacy was 35% and the efficacy for severe disease was 49%, showing a significant positive impact in reducing the risk of clinical malaria and severe malaria (Alonso et al., 2005).Trials in young infants are currently underway in Mozambique.

Development of a malaria vaccine that impact on the clinical and severe malaria, will contribute to the reduction of the scourge of malaria in many malaria endemic countries.

9 Objectives

9.1 General Objective

To carry out a country-wide malaria survey in Mozambique that can contribute to improve planning and evaluation of malaria control activities.

9.2 Specific Objectives

- To determine the prevalence and intensity of *Plasmodium* infections in children under 10 years of age and in pregnant women across different ecological settings.

- To describe the prevalence and the severity of anaemia in children under 10 years of age and pregnant women.

- To establish a case definition of malaria and to examine its relation to age and epidemiological settings.

- To estimate the prevalence of clinical malaria in children under 10 years of age and in pregnant women.

- To describe the relationships between malaria infections (parasite prevalence and clinical malaria) and the transmission intensities in different epidemiological settings.

10 Material and Methods

10.1 Study Area

The survey was conducted in Mozambique, a country located in Southern Africa region, between the parallels 10º 27´ and 26º 52´ South latitude and 30º 12´ and 40º 51´ longitude East (Map 1).

The most remarkable attribute of its natural feature is the enormous mosaic of the geography and ecology backgrounds.

The country is stretched north-south, over a land surface of approximately 799.380 Km^2, embraces rainforest and mountains, flat and arid terrains, marshlands, valleys, lakes, rivers crossing the country from the mountains in the west into the Indian Ocean in the east, and coastal swamps.

The estimated population of 18 million inhabitants (1997 census) comprises more than thirty ethnic groups, with immense cultural and linguistic diversity.

10.1.1 Geographic, climatic and demographic characteristics

The country could be stratified mainly into three distinct geographical zones:

The coastal plain, which comprises about 40% of the total size of the land with a long maritime coastal line of approximately 2,400 km.

In this stratum, the maximum altitude is below 200 meters above sea level.

The coastal stratum is highly populated, with population densities varying between 9.6 and 43.1 inhabitants per Km^2, exception for Maputo the capital city, which is in average the highest in the country about 2.920 inhabitants per Km^2.

The majority of urban centres are situated along the coastal line.

The vegetation is mostly steppes type, and with permanent marshy lands. The fertile lowlands are mainly used for agriculture (rice, maize, sugar cane, and a large variety of vegetables).

FIGURE 6. *Geographic location of Mozambique, the study area*

Source: Atlas Geográfico de Moçambique, 2002

Interior zones, with two distinct land types:

The flat terrains with altitude ranged between 200 and 600 meters. In this zone the population densities varies between 10.4 and 14.8 inhabitant Km^2. The vegetation varies from semi-forested and shrubs areas, with a wide range of valleys and lakes. Agriculture and livestock are the main activities in the rural communities.

The highlands, with altitude above 600 meters, rarely found in the southern region, but a common feature of central and northern regions of the country. In this zone, population density varies between 4.6 and 8 inhabitants Km^2. The vegetation type is tropical rainforest, with various microclimate areas all along the mountains.

10.1.2 The Climate

The climate of Mozambique is tropical and humid. Generally is influenced by the monsoons from the Indian Ocean and the hot current of the Mozambique channel.

There are predominantly two climatic seasons, one hot and wet from September/October to April/May characterised by tropical rainstorm, high temperatures and high relative humidity. The dry and cold season characterised by windy weather and relatively low temperatures.

The dry season last for about four to six months in the central and northern regions, while in southern region the dry tropical season lasts much longer between six and nine months.

Temperature variations are not significant within the country. In areas of high altitude the annual average temperature varies between 18º C and 20º C, whilst the coastal plain in the north and the great Zambezi valley in central regions, the annual average temperatures varies between 26º C and 28º C, and in the coastal plains in the south the annual average temperatures varies between 20º C and 22º C. In the Plateau zones, the average temperatures are much higher, generally above 28º C.

10.1.3 The rainfall pattern

The annual average precipitation is around 1000 mm. It decreases from north to south. The coastal zone is under the influence of tropical depression and, therefore the occurrence of tropical rainstorms and some cyclones throughout the year. This fact leads to a continuous presence of water bodies all along the inlands, particularly in the flat terrain areas, and swamps along the coastal line.
The country is crossed by several rivers, running from upper inlands to the shorelines, therefore, mild flooding of the alluvial plains of the rivers are common, and during the drought seasons, infinite small ponds are found along the course of those rivers.

10.2 Design and Sampling Methods

10.2.1 Stratification

Based on geographic differences the country was divided into to four main regions: The Northern, Northern-Centre, Centre and southern regions. In relation to altitude differences, the country was stratified into three different strata: The coastal stratum with altitude below 200 meters above sea level; the plateau stratum with altitude between 200 and 600 meters above sea level and the highland stratum situated 600 meters above sea level. Each geographic region encompass the three stratum described above.

10.2.2 Cluster Sampling Method

A modified cluster sampling method with 30 clusters, used by the World Health Organization for the evaluation of the Expanded Programme of Immunization coverage was adopted.
A cluster unit consisted of a total of eight congregated households chosen randomly and, in each district a total of thirty cluster units were designed.
The primary sampling units were the districts, therefore the list of all districts categorized based on stratum, i.e., Coastal, Plateau and Highland, was first entered into a excel database. From each stratum eight districts were randomly

selected using the excel random list generator operator; altogether 24 districts were selected (Table 10). In each district chosen, the list with estimated number of households for all Administrative Units of the district was produced from the 1997 census data.

The number of clusters to be sampled in each Administrative Units was calculated based on the sampling with probability proportional to the size of the Administrative Unit, as described below.

From the list of all Administrative Units, in a given district, the sampling interval (k) was obtained dividing the total number of estimated households by the number of cluster units required for each the district (30).

Randomly, an initial number between zero and the sampling interval was chosen using a table of random numbers. By adding the sampling interval to the initial random number *n* times as possible, the cumulative number of clusters fitting in each Administrative Unit was then worked out.

In each Administrative Unit, in order to make up the respective clustering units, a total of 8 households were chosen at random manner for sampling, at a turn of a pencil point follow up until 8 households were sampled.

The centre of each Administrative Unit was chosen as a starting point. If the limit of the locality did not allow the sampling of 8 households, the team revert to the centre of the locality and started the same procedure until 8 households were sampled.

In case of branching of the direct line path, a pencil or bottle was rotated to choose the line of path. This process was carried out as many times as required to obtain the total number of cluster units previously calculated for that particular Administrative Unit. If the selected family was absent during the day of sampling, the team returned another day to sample. If the family refuse to participate or consistently absent during the sample, then was excluded.

10.3 Study population

All children aged bellow ten years old and pregnant women, living in the selected household (family unit), were eligible for the study.

Within each cluster two households were randomly selected for mosquito's collections.

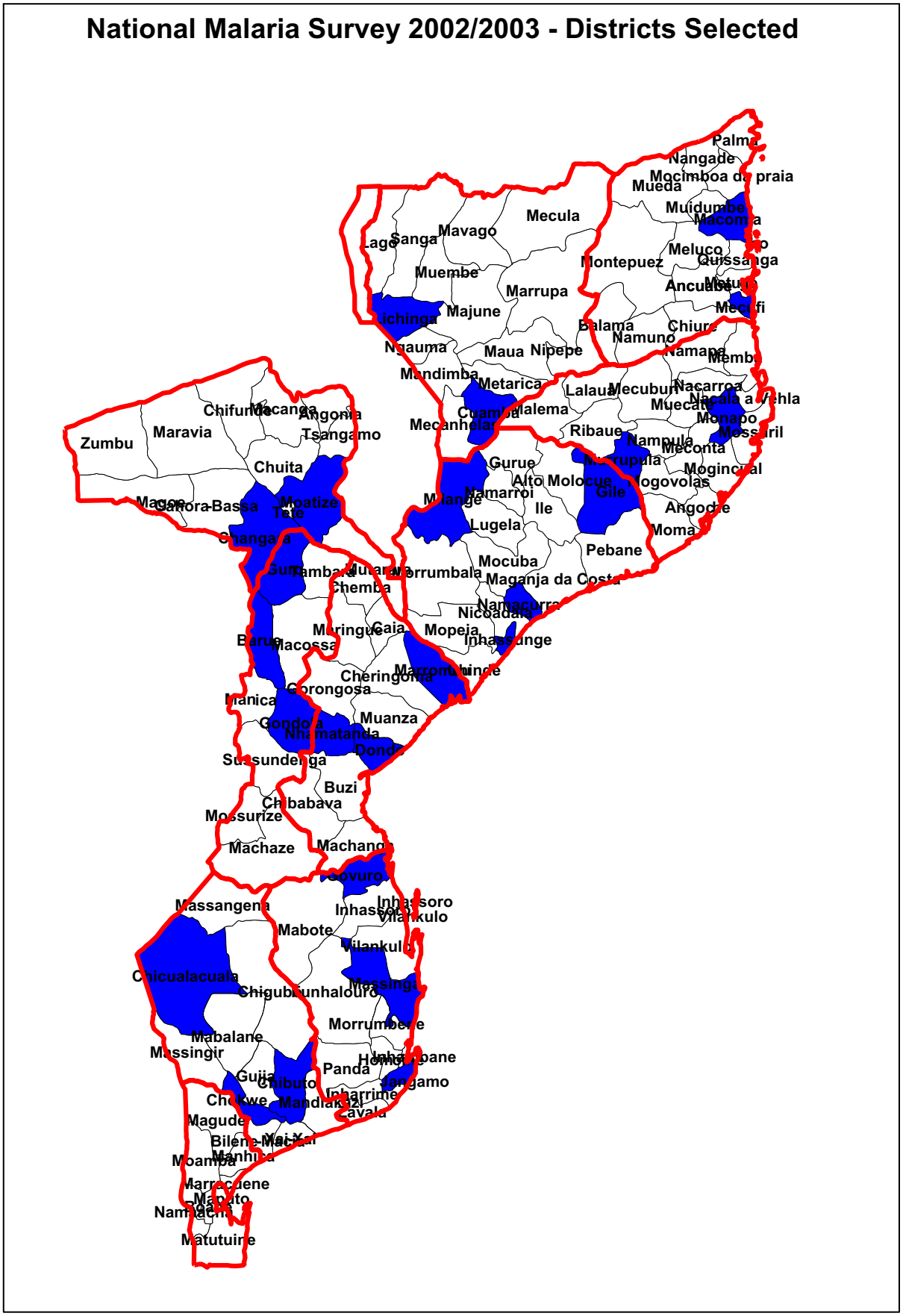
Altogether (12,002) subjects were enrolled for the malariometric survey.

The entomologic surveys were carried out in 1,440 dwellings, and consisted of pyrethrum spray knock down mosquito collections. In total 6,557 *anopheline* mosquitoes were collected.

Table 10. - *Districts selected for surveys, by regions and strata*

REGIONS (STRATA)	ESTRATUM	DITRICTS
NORTH	Costal	Mecufi, Macomia
	Plateau	Murrupula, Monapo
	Highlands	Cuamba, Lichinga
NORTH-CENTRE	Costal	Inhassunge
	Plateau	Namacura
	Highlands	Milange, Gile
CENTRE	Costal	Dondo, Marromeu
	Plateau	Nhamatanda, Barue
	Highlands	Gondola, Moatize, Changara
SOUTH	Costal	Jangamo, Massinga, Govuro
	Plateau	Chokwe, Chibuto,
	Highlands/Arid	Chicualacuala

FIGURE 7. *The districts selected for the national malaria survey 2002-2003*



10.4 The Cross Sectional Survey

10.4.1 Field Work Procedures

Before the beginning of actual sampling, local authorities and respective Administrative Units chiefs in each selected districts were consulted and briefed on the project activities and their consent and cooperation was seek.
However, in every sampled individuals consent was seek from the parent or guardians of the child, from the pregnant women and from the owner of the house for mosquito collections.

The surveys consist of house-to-house surveys in which from all selected subjects axillary temperature was measured and blood samples were collected, to prepare thick and thin blood films, haemoglobin values determination and rapid malaria test.

All individuals living in the same family aggregate were eligible for the survey.
Identification of subjects fulfilling the inclusion criteria i.e. under ten years old and pregnant women, followed by filling individual forms, coding and labelling of blood slides and reading of rapid malaria test.

Finger prick – and blood were collected to prepare:

(i) Thick and thin film for parasite presence, density and species
(ii) Filter paper for DHFR and DHPS mutations (data not presented)
(iii) Haemoglobin determination
(iv) Rapid Enzyme Test for diagnosis of malaria in individuals with body temperature $\geq 37{,}5^{o}C$.

1 - Measurement and record of body temperature
Axillary temperature was measured in participating subjects, using electronic digital thermometer (Microlife-Switzerland).

2 - Preparation of blood smears – thick and thin films

To prepare blood smears new microscope slides, without scratches, clean and free from grease were used. To ensure good quality of preparation, microscope slides were cleaned, by soaking in clean water, into which a liquid detergent was previously added. Thereafter and individually were air dried and polished up with a clean cotton cloth. Lastly, the slides were wrapped in paper in batches of about ten, sealed with rubber band and packaged in plastic bags to protect them from dust.

Sterile and disposable needles with a sharp point and a cutting edge were used to finger pricking. Routinely, the third finger of left hand was chosen for pricking. First it was carefully cleaned with a cotton swab moistened with 70% spirit and then with a dried one to remove any residual spirit left on the finger.

The pricking act was made, with the selected finger squeezed gently in between the thumb and index fingers of the field worker just below the apical joint. The first drop of blood was swabbed off. From subsequent drops of blood squeezed out by continuous gentle pressure by fingers of the operator, the smears were prepared; with the microscope slide held only by their edges, its surface was then brought in contact with the top of the blood globule only. Both thin and thick blood smears were prepared in the same microscope slide.

To prepare the thin blood smear, the drop of blood was placed just before the centre of the microscope slide on which the smear is to be made. A second slide, the spreader, was then brought in contact with the surface of the slide and held at an angle of between 30 and 40 from the horizontal and then drawn back until its lower edge contacts the blood drop. The spreader was then pushed steadily down the surface of the slide, drawing the blood behind it not lifted until the smear was completely formed.

To prepare the thick blood smear, the blood drop was placed at about the centre of the second half of the microscope slide and it was spread with the corner of another microscope slide to form a rough circle.

Freshly prepared blood smears, after labelled, were kept flat in a slide tray until thick smears were relatively dried, and afterwards were placed in a slide box during at least 12 hours before they are stained.

3 - Haemoglobin determination

Blood drops from were used as well for haemoglobin determination. Haemoglobin concentration was measured using the HemoCue System (HemoCue, Anglholm, Sweden). The HemoCue haemoglobin microcuvettes contain an exact quantity of dry reagent, which automatically yields a chemical reaction when in contact with blood. The reaction in the cuvette is a modified azidemethemoglobin reaction.

Absorbency values are measured at two wave lengths and the result was obtained after one minute. Haemoglobin readouts were recorded in individual forms previously elaborated for each participating subject.

4 - Rapid enzyme Test for malaria diagnosis

Rapid malaria test based on the detection of HRPII antigen of *Plasmodium falciparum* (Parasite – F) was performed for all participating subjects presenting with fever (temperature $\geq$ 37.5^{o}C). All malaria positive participants received a treatment dose of chloroquine. Those with a recent history of chloroquine intake received a treatment of pyremethamine/sulphadoxine, accordingly to the national policy. If signs and/or symptoms of severe illness were observed medical consultation was encouraged.

5 – Mosquito knock down catch

If the household was indicated for entomological survey, mosquito pyrethrum spray sheet collections were carried out.

Field-collected adult mosquitoes preserved in silica gel, were held in Petri Plate, labelled for location, date and type of catch and transferred to the entomology laboratory at the National Institute of Health in Maputo. Species identification was carried out using morphological characters (Gilles & De Meillon, 1968; Gilles & Coetzee, 1987).

All data obtained, were recorded in individual forms and tables for mosquito collection previously made available for the survey (appendix 1).

At the end of each day survey all forms were revised, for coding and/or labelling errors. Second visit were planed to absent families or to collect missing information.

10.5 Laboratorial Methods

10.5.1 Blood smears – staining methods

Blood smears were stained with Giemsa standard methods. A mass technique was used due to the large quantity of microscope slides to be stained in a short period of time. For this purpose 100 ml of stain solution consisting of 3% of Giemsa stain in a buffer solution of pH 7.0 – 7.2 per staining jar of 20 slides were used. All blood films were previously fixed with methyl alcohol.

The microscope slides were placed back to back with the smears facing outwards in staining jars. Then the stain solution was overflowed into the staining jars until the microscope slides were covered completely.

After 30 minutes staining, all microscopes slides were smoothly rinsed, by flooding the staining jar with tap water to get rid of the surface scum and finally by dipping each slide several times in a container of tap water. To drain and dry out the microscope slides were placed in a drying rack.

10.5.2 Characteristics and quality of stained blood smears

Blood smears stained accurately exhibited clearly the blood elements. Cells should be arranged in a way that their edges almost touch each other or with minimal overlapping.

The colour of erythrocytes should be almost neutral, varying from pale straw to light grey. Other staining colours are unacceptable. The nuclei of leucocytes should be stained dark blue or purple with lighter blue cytoplasm, except for monocytes it may be mottled blue/grey. The stippling of neutrophils (polymorphonuclear leucocytes) should be small, clearly defined dots and a mixture of blue and pink, while the granules of eosinophils may be either dark or red. Blood platelets may stain blue or purple.

The cytoplasm of parasites presents colours varying from light to medium blue, depending not only on the characteristics of the stain used, but also on the species and age of the parasite. The nuclear chromatin should be dark purple/red and when stippling is present on the envelope of the erythrocyte, it should be pink or red. The presence of clearly defined stippling in infections of *Plasmodium falciparum* is a good indicator of satisfactory staining.

Electric supplied binocular microscopes (Olympus CH21) were used to screen all blood films. The thick smears were observed under a 10 times magnifier objective lens, for a rapid malaria diagnosis. Then thin blood films were screened for species identification and parasite counting to estimate parasite density. Observations were made on 100 microscope fields, using a 100 times oil immersion objectives.

Identification of species, mature schizonts and sexual parasite forms was carried out using WHO standard templates.

The number of asexual Plasmodium falciparum parasites per 500 leukocytes were counted and a final density was calculated using an assumed leukocyte count of 8.000/mm3.

10.5.3 Mosquito species identification and sporozoite detection

Polymerase Chain Reaction (PCR) was performed for the identification of members of *An. gambiae* complex, *An. funestus* group and for the presence of sporozoites.

To identify species of *An. gambiae* complex, DNA was extracted using a slightly modified method described by Collins *et al*. (1988). A second method described by Boom et al. (1990) with minor modifications, was performed to extract DNA to identification species belonging to *An. funestus* group and to detect the presence of sporozoite in mosquitoes.

10.5.4 Main steps for DNA-Polymerase Chain Reaction (PCR)

For method described by Collins et al. (1988), only the abdomen and legs of individual mosquito was used for identification. Main steps were DNA extraction with potassium acetate and DNA precipitation with 95% ethanol. DNA pellets

from each sample were resuspended in 400 µl sterile dH_2O. Aliquots (1µl) from each DNA sample were used in the PCR reaction and for method by Boom *et al*. (1990) only head and thorax were used. The segments of specimens were homogenised by lysis in buffer L6, and 50 µl of silica was then added. The silica-NA pellet was subsequently washed; twice with washing buffer L2; twice with ethanol 70%; once with acetone and finally the pellet were resuspended in 75 µl sterile dH_2O.

10.5.5 Species identification of the *An. gambiae* complex by PCR

DNA extraction

From all specimens preserved in silica gel, DNA was extracted according to the PCR technique protocol described by Scott and others with minor modifications (Scott et. al., 1993). All reagents used were obtained as a kit from Promega and the reactions were carried out using the PELTIER – THERMAL CYCLE 100 machines.

The PCR technique was performed in 24 µl total volume containing: 2.5 µl 10 X PCR buffer (Promega); 0.625 µl of a solution containing 1.2nmol of each dNTP (dATP, dCTP, dGTP and dTTP); 0.8 µl of each of the primers (6.25ng of primer GA, 12.5 ng of primers UN and MR, 18.75 ng of primer AR, and 25 ng of primer QD): 0.035 µl (0.625 units) Taq DNA polymerase; 3.0 µl MgC12 (25mM); 15.04 µl distilled water and 1 µl of 400 µl of the mosquito DNA sample.

All specimens were individually assigned to a species of the *An. gambiae* complex using rDNA probes in an rDNA-PCR diagnostic assay. Species identification was done using only the abdomen and legs. The thorax and head were kept for other assays. Each segment of specimens were placed individually in Eppendorf tubes and homogenized in 100 ml of distilled water.

To perform the PCR, reaction vessels were placed in the thermal cycler machine and ran over a programme consisting of 30 cycles for denaturation at 94ºC for 60 seconds, annealing at 50ºC for 30 seconds, and extension at 72ºC for 30 seconds.

For each PCR reaction performed three controls were used: one negative control with the PCR mix and without any DNA template; one positive control for *An. gambiae s.s.* and one positive control for *An. Arabiensis.*

The final amplified product (13µl) was then electrophoresed in 1.5% agarose-Tris-borate-EDTA gel containing ethidium bromide.

All amplified segments were visualized over a UV transilluminator. Bromophenol blue was used as a dye front indicator, and the fragment size was estimated by comparison with size markers (pGEM, Promega).

In the presence of appropriate template DNA, characteristic fragments were produced: *An. arabiensis*, 313 base pairs (bp); *An. quadrianulutus*, 150 bp; *An. gambiae s.s.* 390 bp; *An. merus* 464 bp.

10.5.6 Species identification of the *An. funestus* group by PCR

Members of the *An. funestus* group were identified to species level using a modified PCR-SSCP (Koekemoer *et al.* 2002). Based on species-specific primers in the ITS2 region on the rDNA, this PCR is able to identify *An. funestus* (≈505 bp), *An. vaneedeni* Gilles and Coetzee (≈587), *An. rivulorum* Leeson (≈411bp), *An. leesoni* Evans (≈146 bp) and *An. parensis* Gilles (≈252 bp).

The PCR mixture consisted of 2.5 (1 of 10x reaction buffer (500 mM KCl of each primer, 200 (M of each dNTP, 2 U thermos table taq DNA Polymerase. Amplifications were carried out in a programme consisting of 30 cycles for denaturation at 94 ºC during 30s, annealing at 40 ºC during 30s, and two extension, the first at 72ºC during 30s, and the last one at 72ºC during 10 min. (one of the product was electrophoresed on 2% agarose gel. The remaining PCR product was later used for SSCP analyses. Two controls were included, one negative control with the PCR mix and without any DNA template and one was positive for the SSCP electrophoresis was obtained by amplifying extracted DNA from *An. funestus*.

All amplified segments were visualized over a UV transilluminator.

10.5.7 *Plasmodium* sporozoites detection in *Anopheles* mosquitoes and estimation of infective rate

The head and thorax of all *Anopheline* caught were separated from the abdomen and tested for the presence of *P. falciparum*, *P. ovale* and *P. malariae* CSP using a slightly modified Nestled PCR method, described by Snounou et al. (1993).

All PCR reactions were carried out in a total volume of 20 µl total volume containing: 7.4µl PCR dH2O; 2µl 10X PCR buffer; 1.6µl MgC12); 2 µl of each primer (Plu5, Plu 6); 0.63 µl of a solution containing 50.0 mmol of each of dATP, dCTP, dGTP and dTTP; 0.1 µl of Gelatin (2%); 0.2 µl Taq DNA polymerase and 4 µl of DNA sample.

The amplification programme was as follows: step 1: 95ºC for 5 minutes; step 2: annealing at 55 ºC (rPF1/rPF2 and rPV1/rPV2) or 52 ºC (rPM1/rPM2 and rPO1/rpLU6) for 2 minutes; step 3: extension at 72 ºC for 1 minute; repeat step 4: denaturation at 94 ºC for 1 minute; repeat steps 2-4 39 times, then step 2, and finally step 3 for 5 minutes. On termination of the amplification reaction, the temperature was reduced to 20 ºC.

One µl of the product obtained was then used as a template in a second amplification reaction in which the presence of each parasite species was individually assayed.

The nested primer pairs used for detection of P. falciparum, P. vivax, P. malariae and P. ovale were rFAL1/rFAL2 (205 base bp), rVIV1/rVIV2 (120 bp), rMAL1/rMAL2 (144bp) and rOVA1/rOVA2 (c.800 bp) respectively.

All amplified segments were visualized over a UV transilluminator.

In all products, Bromophenol blue was used as a dye front indicator, and the fragment size was estimated by comparison with size markers (pGEM, Promega).

10.6 Data Management

Data collect during the survey has been double-entered by two data entry clerks using a Data Management for Field Trials (DMFFT2) run over a Windows NT network. All data was stored and processed in a dedicated secure directory on a central server.

A data manager performed daily cross-checking routines to compare data entries of the two data entry clerks. With this procedure it was possible to detect and correct any discrepancies between the two entries. Discordances detected at this point were recorded in a log file permitting quality control of the data checking process.

Checks for duplicate records, completeness of the databases (all records/questionnaires from the field were entered into a database record), range (all variables have a maximum and a minimum value acceptable within a given span of expected values, for example body temperature range is between 34º C and 42º C; the categorized answers for methods of malaria prevention ranged between 1 and 6, therefore only values confined between those limits are acceptable), consistency (defined as rational flow of questions as a corollary of answers obtained throughout the interview) and referential integrity (defined as a logical linkage between the three different database records) were also performed.

The cleaned and locked database files were than used for final analysis.

10.6.1 Data analysis

Statistical analyses were performed using STATA version 8.1 (Stata Corporation, College Station, TX, USA).

The study population, pregnant women and children under ten years old were analysed separately. Children under years old were grouped into different age categories: less than 12 months old; 12 to 23 months old; 24 to 59 months old; 5 to less than 7 years old and from 7 to less than 10 years old. Pregnant women were categorized as follows: less than 20 years old; from 20 to less than 30 years old; 30 years old or above.

The study profile consisted on number of districts selected in each stratum and in each region, and the number of subjects enrolled in each stratum and in each region, including their age groups distribution, and total number of withdrawals.
The summary measures of all categorical variables consisted of means, the percentages, the 95% confidence interval (95% CI) and standard deviations for continuous variables.
Overall parasite density for each age group is shown as geometric density parasite mean, after $\log_{10}$ transformation.

10.6.2 Accounting for the sample design

There are three factors arising from the design of data collection procedure, namely: Sampling Weights, Clustering and Stratification.

- Sampling Weights - Observations were selected through a random process, hence may have different probability of being selected.

- Clustering - Observations were sampled as a group (clusters), therefore not independently, and

- Stratification - The stratum categorization was made in advance and sampling was done independently across each strata. Consequently, strata and regions are statistically independent and therefore can be analysed as such.

Adjustments to the weights were done and the estimators obtained were approximately unbiased for all point prevalence estimated.
Clustering and the stratification of the survey design was considered and estimates of standard errors, valid p-values, and confidence intervals whose true coverage are close to 95% were attained. Additionally to handling Clustering and Stratification effects, the design effects to measure how the survey design affects variance estimates were calculated.
All survey mean estimations were adjusted for sampling weights, stratification and clustering. The design effect “deff” was computed automatically.

Comparisons between proportions (in different age groups) were carried out using the Chi-square test ($\chi 2$ test) of Pearson or Fisher exact test if any expected frequency is lower than 5.
The relationship between age, stratum or region and fever prevalence, parasite prevalence, parasite density and anaemia was determined using linear regression method.

10.6.3 Clinical malaria – establishment of case definition

A classic method proposed by Smith et al (1994), was performed to estimate the proportion of fever cases attributed to malaria parasites infection, using a logistic regression of fever on a monotonic function of the parasite density. The sensitivity and specificity of the estimated attributable fraction of fever in different parasite density cut-off was estimated to establish a case definition of clinical malaria. Using bootstrap facilities, confidence intervals for the attributable fraction of fever and the sensitivity and specificity for the cut-off definition of one or more parasites and 2,500 or more parasites were estimated.

10.6.4 Entomological inoculation rates

The entomological inoculation rate (EIR) is the proportion at which people are bitten by infectious mosquitoes in a given unit of time (Smith et al., 2004). It is widely used to estimate the level of exposure of human to Plasmodium falciparum-infected mosquitoes for assessing malaria endemicity and transmission intensity (Burkot & Graves, 1995). The impact of malaria control interventions aiming to reduce human-vector contact can be evaluated by EIR assessments (Drakeley, et al., 2003). Classically the EIR derived from the density of man-biting *anopheline* mosquitoes, the sporozoite rates within that mosquito population and the human blood index. The human biting catch is considered the most accurate method for assessing man-biting rates, although this technique has serious ethical and logistics constraints. Light trap catches and pyrethrum spray catches represent viable alternatives and have been used against human bite catches (Mbogo et al., 1993; Mathenge et al., 2005)

Two methods were used to estimate the overall annual EIR, for each region as follows:

- Standard Method

Number of sporozoite-positive PCR / number of mosquitoes tested X number of mosquitoes collected/number of catches X 365 days.

- Alternative Method

Number of sporozoite-positive PCR / number of catches X 365 days.

10.6.5 Definitions

- Fever - Axillary temperature ≥ 37.5º Celsius.
- Anaemia - Haemoglobin/Hematocrit concentration value below the age specific level used (Newton et al., 1979), refer to the table below.

Age group	Haemoglobin (below g/dl)	Hematocrit (below %)
≤ 59 months of age	11.0	33
5 to 11 years	11.5	34
12 to 14 years	12.0	36
Non pregnant women (Above 15 years)	12.0	36
Pregnant women	11.0	33
Men (above 15 years)	13.0	39

- Clinical malaria, the definition may be modified for different age groups depending on the analysis of the age specific sensitivity and specificity. Therefore, it will be defined as the presence of asexual malaria parasites in a given parasite density cut-off point and the presence of fever.
- Malaria infection, detection of *Plasmodium* asexual malaria parasites in the peripheral blood film.
- Gametocytes, sexual forms of malaria parasites.

- Primigravidae, women during their first pregnancy
- Multigravidae, women during their second, third or fourth pregnancies
- Grand multigravidae, women during their fifth or more pregnancies.

11 Results

11.1 The study profile

The national malaria survey was carried out in 24 districts randomly selected across different geographical regions of Mozambique, between February/March 2002 and March/April 2003. A total of 5,760 households were selected from 720 clusters, each enclosing 8 households units.

The study aimed to enrol about 11, 200 subjects, to include 9,600 children aged below 10 years old and 1,600 pregnant women. Both female and male children and pregnant women living in selected family unit (household) were eligible for the survey. Informed consent was obtained from 12,002 subjects.

Of the 11,792 blood films obtained during the cross-sectional survey, 11,480 (97.4%) were included for parasite examination and the remaining 312 (2.6%) were dropped out due to bad quality staining result.

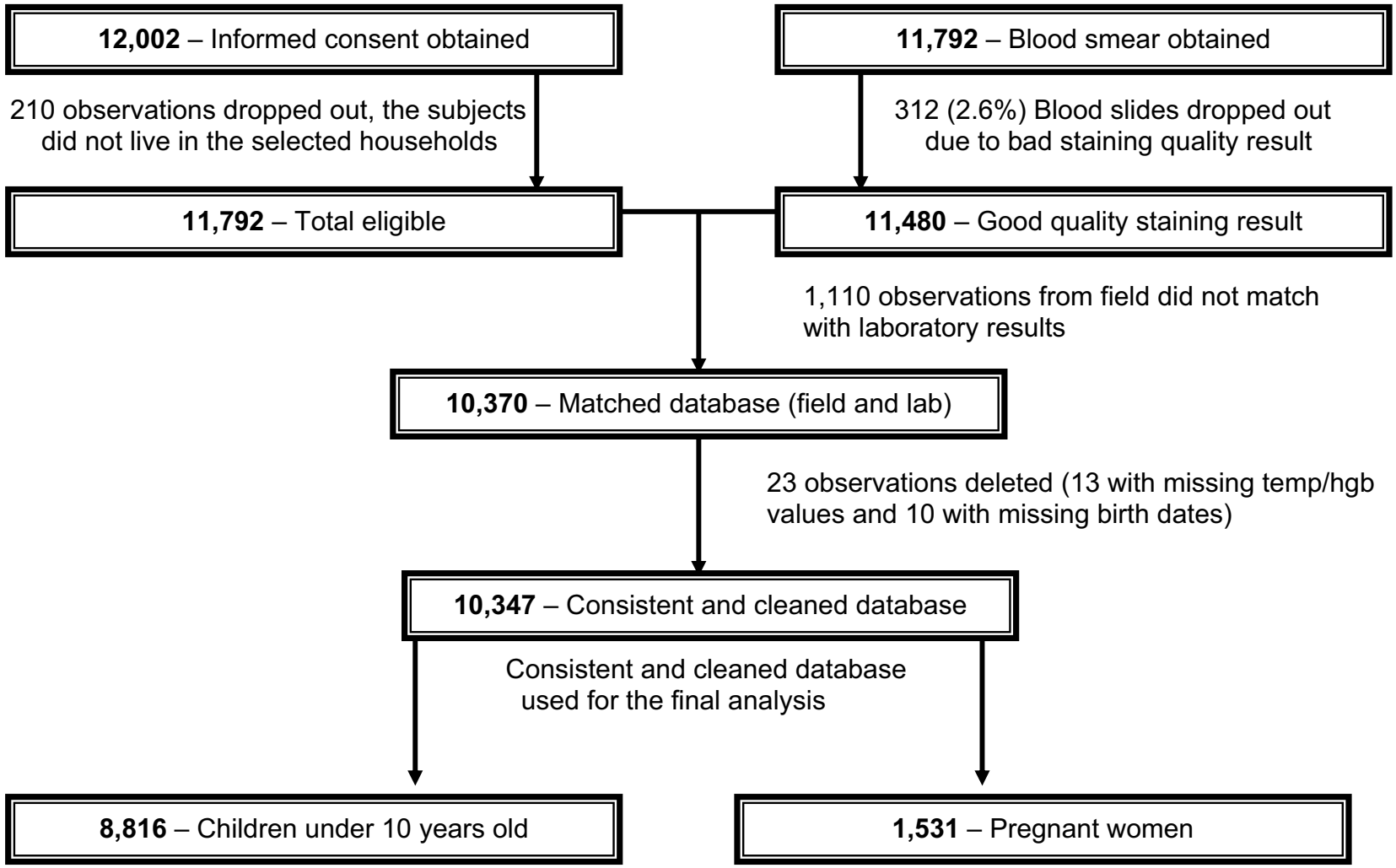
Both database (one recording findings from the field survey and the second recording results of blood smears, carried in the laboratory) were merged into one single database, using the individual identification number. In total 1,110 (9.7%) individual identification numbers of the blood smears database did not match with the corresponding field survey identification numbers, and therefore were dropped out.

A database containing 10,370 subjects with both field and laboratory matching identification numbers was then obtained. Twenty-three records (0.2%) with missing birth date, or haemoglobin and temperature values out of range were dropped out.

The final database (with referential integrity between the two databases, consistent and cleaned) included 10,347 subjects.

In total 1,531 pregnant women and 8,816 children less than ten years old were separately included for the final analysis (see study profile, Table 11).

Table 11. *Study Profile*



11.2 Demographic characteristics of children

Demographic characteristics of children are illustrated in table 12. Of the 8,816 children aged bellow 10 years old, 47% (4,143/8, 8816) were male and 53% (4,763/8,816) were female. The mean age was 42 months (range, minimum 3 months and maximum 9 years and 10 months - SD 30 months). The age groups were stratified into five categories as follows: Less than 12 months; 12 months to 23 months old; 24 months to 59 months old; 5 years to less than 7 years old; and from 7 years to less than 10 years old. Overall, the age group of 24 – 59 months was the largest category, included 39.9% (3,515/8,816) of all children, and the age group of 7years to less than 10 years 10.8% (951/8,816) was the smallest category.

Table 12. *Characteristics of children less than ten years of age across different regions and strata in the study area*

	% (n/N)
Mean age	**42** months std.dev. 30 months Range: [3 months – 9 years +10 months]
Female	**53 %** 4,763/8,816
Male	**47 %** 4,143/8,816
Age groups composition	
< 12 months	**17.2%** 1,517/8,816
12 – 23 months	**18.3%** 1,609/8,816
24 – 59 months	**39.9%** 3,515/8,816
5 – < 7 years	**13.9%** 1,224/8,816
7 – < 10 years	**10.8%** 951/8,816

Across regions, the distribution was as follows: The northern and the central regions had 27.1% (2,387/8,816) and 33.2% (2,930/8,816) respectively, while the southern region had the smaller sample of children 17.8% (1,570/8,816).
In the northern region, their distribution within the stratum coastal, plateau and highlands was 32.3 %(771/2,387); 33.3 %(796/2,387) and 34.3 %(820/2,387) respectively. The distribution according to age groups, in each region and stratum is illustrated in table 13.

Table 13. *Distribution of children less than ten years of age across different regions and strata in the study area*

	Coastal (%)	**Plateau** (%)	**Highland** (%)	**Total** (%)
North	**771** (32.3)	**796** (33.3)	**820** (34.3)	**2,387** (100.0)
NorCentre	**860** (44.6)	-	**1,069** (55.4)	**1,929** (100.0)
Centre	**770** (26.3)	**895** (30.5)	**1,265** (43.2)	**2,930** (100.0)
South	**713** (45.4)	**521** (33.2)	**336** (21.4)	**1,570** (100.0)
Total	**3,114** (35.3)	**2,212** (25.1)	**3,490** (39.6)	**8,816** (100.0)

In the central-northern region, the highland stratum recorded 55.4% (1,069/1,929), while the coastal stratum recorded about 44.6% (860/1,929).
The central region recorded 26.3% (770/2,930); 30.5% (895/2,930) and 43.2 % (1,265/2,930) in the coastal, plateau and highland strata respectively. Across the southern region were recorded 45.5% (713/1,570) in coastal stratum, 33.2% (521/1,570) in the plateau stratum and 21.4 % (336/1,570) in the highland stratum.
With respect to strata distribution, the highland stratum had 39.6% (3,490/8,816), the coastal and plateau strata had 35.2% (3,114/8,816) and 25.1% (2,212/8,816) respectively.

11.3 Prevalence and intensity of malaria infection and anaemia

In children less than 10 years of age

Laboratory and clinical findings are summarized in table 14 (appendix 5). Overall, 58.9% (5,190/8,816) of blood smears obtained from participating children were positive for malaria parasites. The majority of blood smears, 46.5% (4,098/8,816) exhibited a pure *Plasmodium falciparum* infection, 3.6% (321/8,816) were *Plasmodium malariae* and 2.9% (253/8,816) were mixed infections of *Plasmodium falciparum* and *Plasmodium malariae*. Gametocytes (sexual forms) only for Plasmodium falciparum were recorded in 5.9 % (518/8,816) of all blood smears. There were no records of infections by other parasite species, namely *Plasmodium ovale* or *plasmodium vivax*. *Plasmodium falciparum* accounted for 92.7% (4,098/4,419) of all malaria parasite infections.

The overall geometric mean parasite density only for *Plasmodium falciparum* asexual parasites was 1,211 parasites/µl (95% CI, 1,141 – 1.286).

Axillary temperature ranged between 35.1° C and 40.5° C (SD 0.67). The mean temperature was 36.7° C (95% CI 36.6° C – 36.9° C).

Haemoglobin concentrations ranged from 1.5 to 19.7 g/dl, and overall mean estimation was 9.9 g/dl (95% CI 9.5 – 10.2). The prevalence of anaemia was 69.8% (6.257/8.816) and among anaemic children 11.5% (743/6.257) were severely anaemic.

11.3.1 Overall prevalence of malaria parasite and geometric mean parasite density

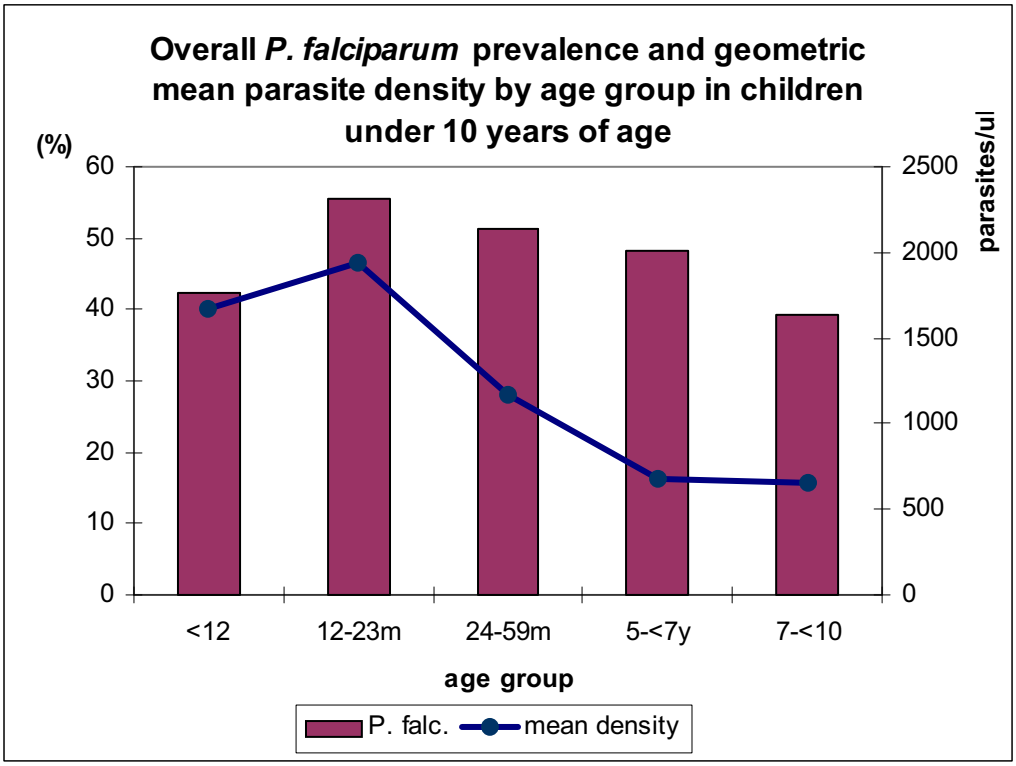
Although the occurrence of other malaria parasites, i.e., *Plasmodium malariae* and mixed infections between *Plasmodium falciparum* and *Plasmodium malariae* was recorded, the study area is markedly endemic for *Plasmodium falciparum*, hence attention is restricted to *Plasmodium falciparum* infections.

The overall prevalence of *Plasmodium falciparum* (mean estimation) was 48.6% (95% CI, 40.0% – 57.3%). Figure 8, depicts the distribution of malaria infection prevalence among children under ten years old. There was a significant variation between age groups (p=0.0002). It increased with age from 42.2% in

children less than 12 months old to reach a peak of 55.4% among children aged between 12 – 23 months old, and thereafter it decreased progressively to the lowest prevalence of 39.3% among older children in the 7 years to less than 10 years old age group. In relation to gender, the proportions of *Plasmodium falciparum* infection among boys and girls were not significantly different (p=0.746).

Overall, mean parasite density increased during the first year of life from 1,671 parasites/µl (95% CI 1,425.21 – 1,961.83), peaking among the children 12 – 23 months age group to 1,939 parasites/µl (95% CI 1,698 – 2,213). The distribution among age groups showed significant differences (p=0.0001). Despite, the presence of relatively high prevalence of *Plasmodium falciparum* parasites among older children, low mean parasite densities were confined to older children. Based on age-specific densities, mean parasite density showed also an age-dependent variation (p=0.0001), decreasing dramatically with age, as illustrated in figure 8.

FIGURE 8. *Overall Plasmodium falciparum prevalence and mean parasite density in children less than ten years of age in Mozambique*

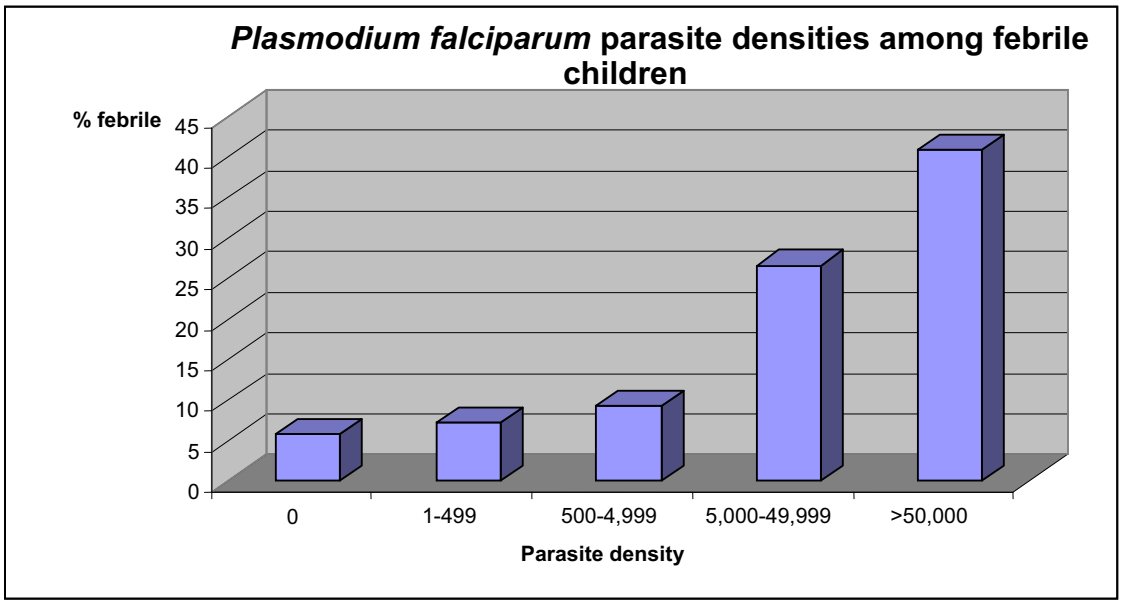


11.3.2 Prevalence of fever and fever associated with malaria parasites infection

Overall, fever prevalence (Axillary temperature ≥ 37.5°C) among children was 9.4% (766/8,816). The prevalence of fever peaked among children during the first 12 months of life [15.1% (206/1,517)]. The lowest fever prevalence of 5.9% (67/1,224) was recorded among children in the 5 years to less than 7 years old age group. Similarly to malaria parasite density, mean fever prevalence decreased rapidly with age.

Prevalence of fever associated with *Plasmodium falciparum* infections (asexual forms) accounted for 5.7% (498/8,816). In a total of 766 fever episodes, *Plasmodium falciparum* parasite was the most prevalent species associated with fever 65.0% (498/766), comparatively to 3.9% (30/766) of *Plasmodium malariae* parasites infections associated with fever.

FIGURE 9. *Distribution of febrile children and risk of fever according to parasite density*



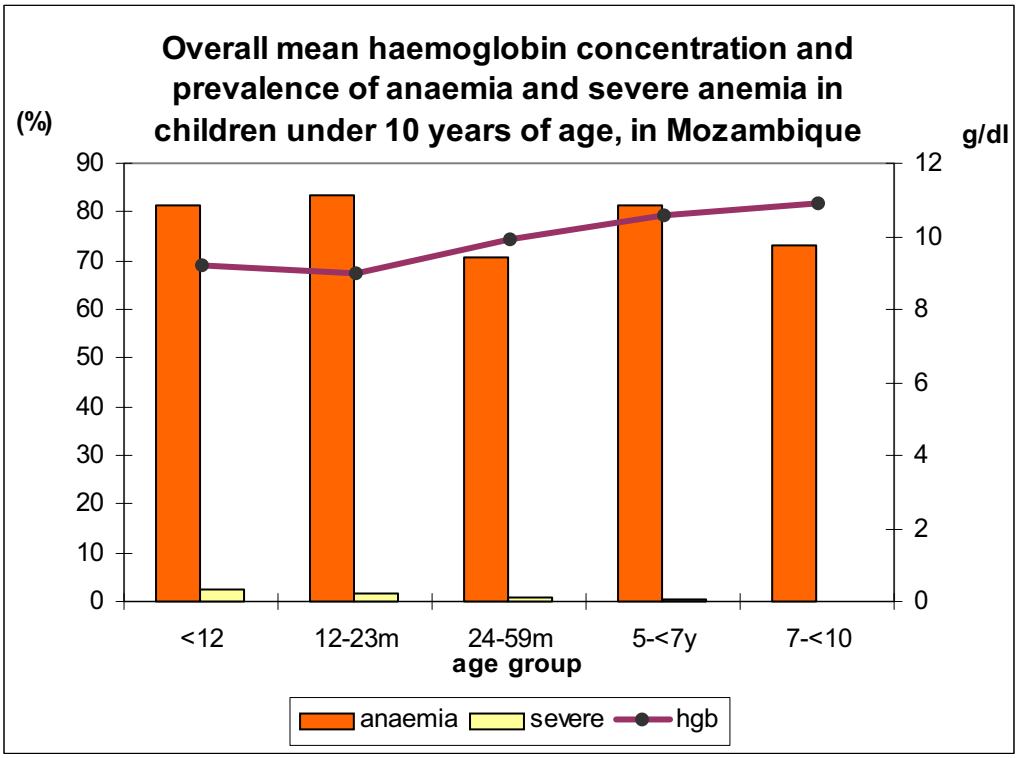
High *Plasmodium falciparum* parasite densities were significantly associated with fever ($p<0.05$), and the risk of being febrile increased with increasing parasite density, particularly from parasite density category equal or higher than 5,000 parasites/µl) figure 9. According to age group, the risk of fever among

parasitaemic children increased during the first 12 months of age, thereafter decreased significantly with age (p<0.0001).

11.3.3 Overall mean haemoglobin and prevalence of anaemia

Overall, mean haemoglobin concentration was 9,9 g/dl (95% CI, 9.5 – 10.2), the prevalence of anaemia using the 11.0 g/dl altitude adjusted race-specific WHO cut off was 69.8%, and for severe anaemia (haemoglobin less than 5 g/dl) was 1.2%.

FIGURE 10. *Overall haemoglobin concentration and prevalence of anaemia and severe anaemia in children 10 years of age*.



There was considerable variation in the prevalence of anaemia among age groups (p<0.0001). In general, all age groups had low haemoglobin concentration, and consequently high levels of anaemia prevalence. However, children during the second year of life had the lowest haemoglobin concentration. Thereafter, haemoglobin concentration increased with increased age as is illustrated in figure 10. There were no significant differences between

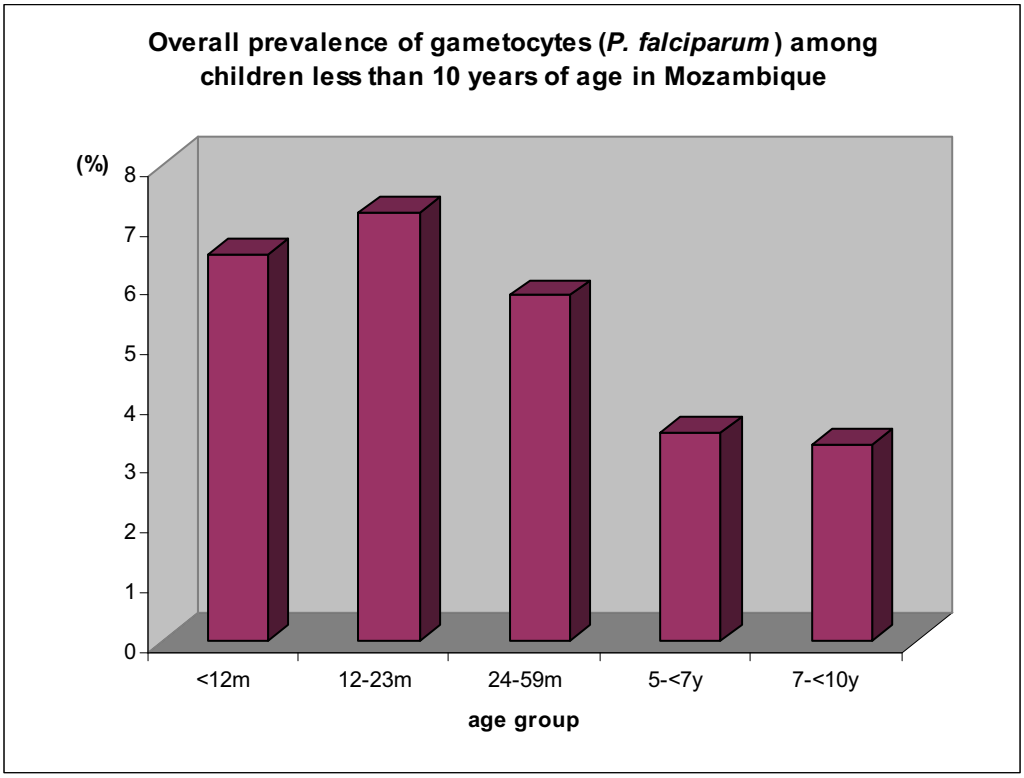
male and female children in mean haemoglobin concentration or prevalence of anaemia at any level (p=0.554).

Approximately half of anaemic children had *Plasmodium falciparum* parasites infection associated, while *Plasmodium malariae* infections were recorded in 4.5%, and mixed infections by *Plasmodium falciparum* and *Plasmodium malariae* accounted for 3.6% among anaemic children.

11.3.4 Overall prevalence of *P. falciparum* sexual forms

The overall prevalence of *Plasmodium falciparum* gametocytes was 5.6% (95% CI, 3.6% – 7.5%). The highest prevalence of gametocytes (7.2%) was recorded among children in the 12 – 23 months age group. Thereafter the prevalence of gametocytes decreased with age (figure 11). Although, there was a significant variation between age groups (p=0.029), there were no significant differences between boys and girls (p=0.158).

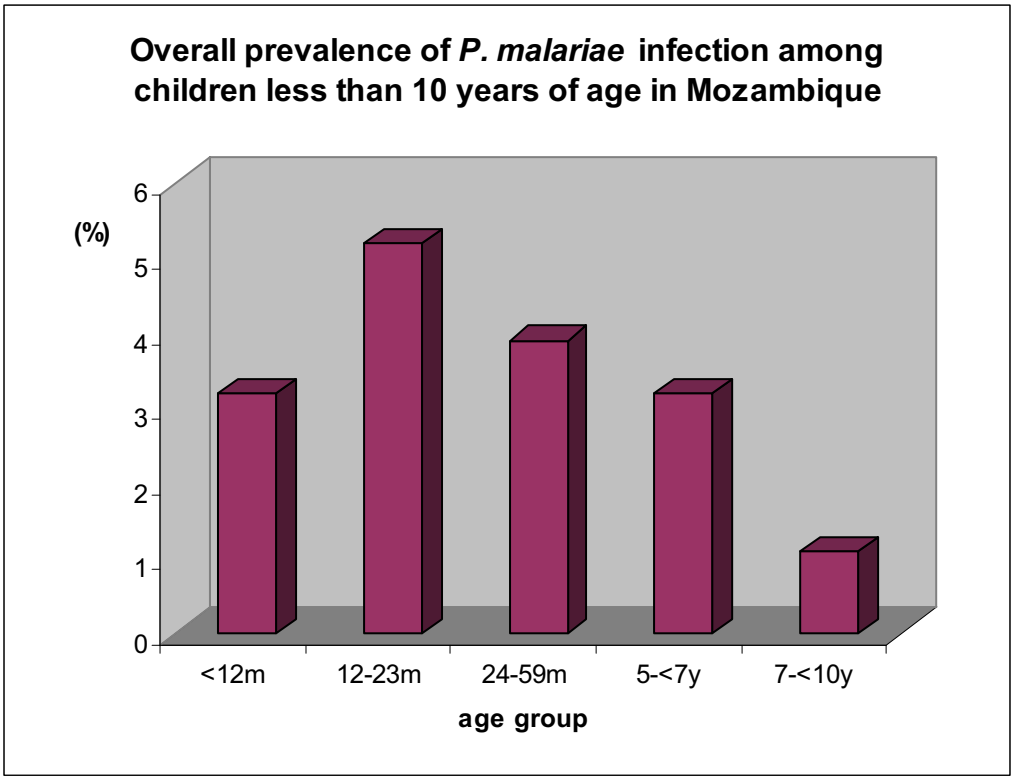
FIGURE 11. *Overall prevalence of gametocytes, only for Plasmodium falciparum among children under ten years of age in Mozambique*.



11.3.5 Prevalence of *Plasmodium malariae*

The overall prevalence of *Plasmodium malariae* was 3.6% (95% CI, 1.3% – 5.9%). It increased from 3.2% during the first 12 months of life, peaking among children in the 12 – 23 months age group, afterwards decreased rapidly with age (figure 12). Variations on distribution among age groups were statistically significant (p=0.017), but no significant differences were observed between male and female children (p=0.435).

FIGURE 12. *Overall prevalence of gametocytes, only for Plasmodium malariae among children under ten years of age in Mozambique*.



11.3.6 Prevalence of mixed infection

The overall prevalence of mixed infection was 2.9% (95% CI, 0.6% – 5.3%). The highest prevalence (4.9%) was recorded among children aged between 12 and 23 months. Rarely mixed infection were recorded in older children, and there were no significant differences between boys and girls (p=0.313).

11.3.7 Attributable fraction of fever, clinical malaria case definition and its relation to age

The attributable fractions and respective confidence intervals for different age groups across regions and strata are presented in figure 18 (appendix 6). Overall, the estimated attributable fraction of fever was 37.8% (95% CI, 31.9 – 43.6), for parasite cut off point of one parasite/µl had 100% sensitivity and the specificity was 56.2% (95% CI, 54.5 – 7.9). For parasite density cut off point of 2,500 parasites/µl the sensitivity was 75.0% (95% CI, 71.9 – 79.1) and specificity was 83.3 (95% CI, 82.4 – 84.3).

Estimating attributable fraction in different age groups generated the following results:

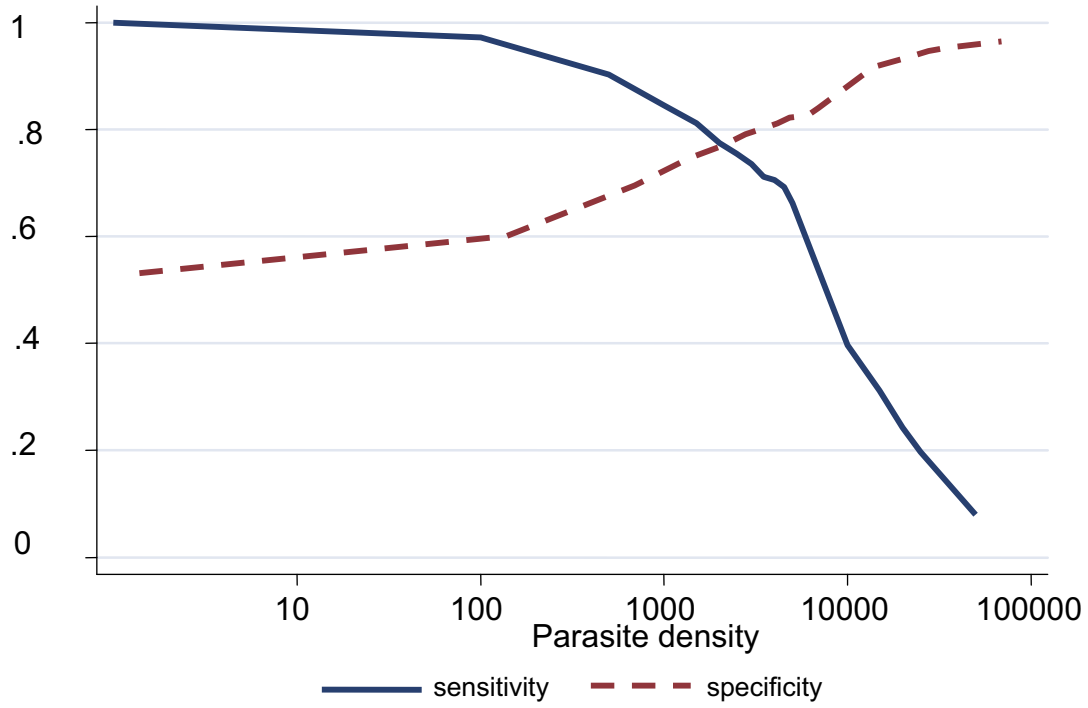
In the age group less than 12 months attributable fraction was 43.5% (95% CI, 25.8 – 61.2). Mean parasite cut off point of one parasite/µl had 100% sensitivity and the specificity was 61.8% with 95% CI, 58.9 – 64.7). For parasite density cut off point of 2,500 parasites/µl the sensitivity was 72.9% (95% CI, 62.2 – 83.6) and specificity was 83.0 (95% CI, 79.7 – 86.4)

In children in the age group 12 – 59 months of age the attributable fraction was 39.6% with 95% CI, 30.3 – 48.9). For mean parasite cut off point of one parasite/µl had 100% sensitivity and the specificity was 52.0% with 95% CI, 48.7 – 55.4). For parasite density cut off point of 2,500 parasites/µl the sensitivity was 77.4% (95% CI, 72.5 – 82.3) and specificity was 80.9 with 95% CI, 79.9 – 82.0)

In the age group 5 years old and above the attributable fraction was 21.5% (95% CI, 11.6 – 31.4). Mean parasite cut off point of one parasite/µl had 100% sensitivity and the specificity was 61.1% with 95% CI, 56.5 – 65.6). For parasite density cut off point of 2,500 parasites/µl the sensitivity was 68.2% (95% CI, 45.5 – 90.9) and specificity was 89.2 (95% CI, 85.1 – 93.3).

Figure 13 illustrates the overall sensitivity and specificity of case definition based on different cut off points of parasite density in the country.

FIGURE 13. *Overall Sensitivity and Specificity of malaria case definition, in children less than ten years of age*



Attributable fraction of clinical malaria: 37.8%

Parasite Density Cut-off	Sensitivity	Specificity
1	100.0	56.2
100	97.3	63.2
500	90.3	72.6
1,000	85.6	77.7
1,500	81.2	80.1
2,000	77.6	82.2
2,500	75.5	83.3
3,000	73.6	84.3
3,500	71.2	85.3
4,000	70.6	85.6
4,500	69.3	86.1
5,000	62.3	87.2
10,000	39.7	94.7
15,000	31.2	96.5
20,000	24.2	97.7
25,000	19.8	98.4
50,000	07.9	99.6

11.4 Variations on the prevalence and intensity of malaria infection and anaemia across regions

11.4.1 Overall parasite prevalence and geometric mean parasite density

Overall, the prevalence of malaria infection showed variations throughout various regions in the country, decreasing from north-to-south. The highest overall prevalence of *Plasmodium falciparum* infection was recorded in both northern and the central-northern regions 54.8% (1,313/2,387) and 58.7% (992/1,929), respectively. Whereas the lowest overall prevalence of 36.8% (1,180/2930) and 44.6% (613/1,570) corresponding to central and southern regions (figure 14 and 15).

FIGURE 14. *Overall prevalence of Plasmodium falciparum among children less than 10 years of age, variations across different regions in Mozambique*

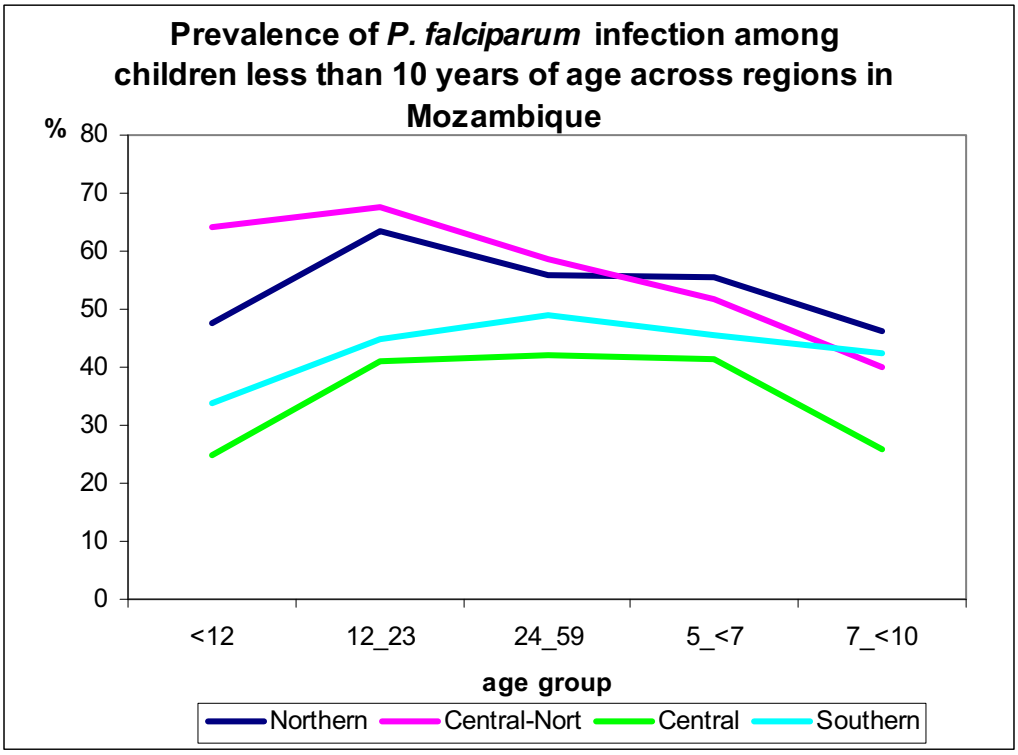
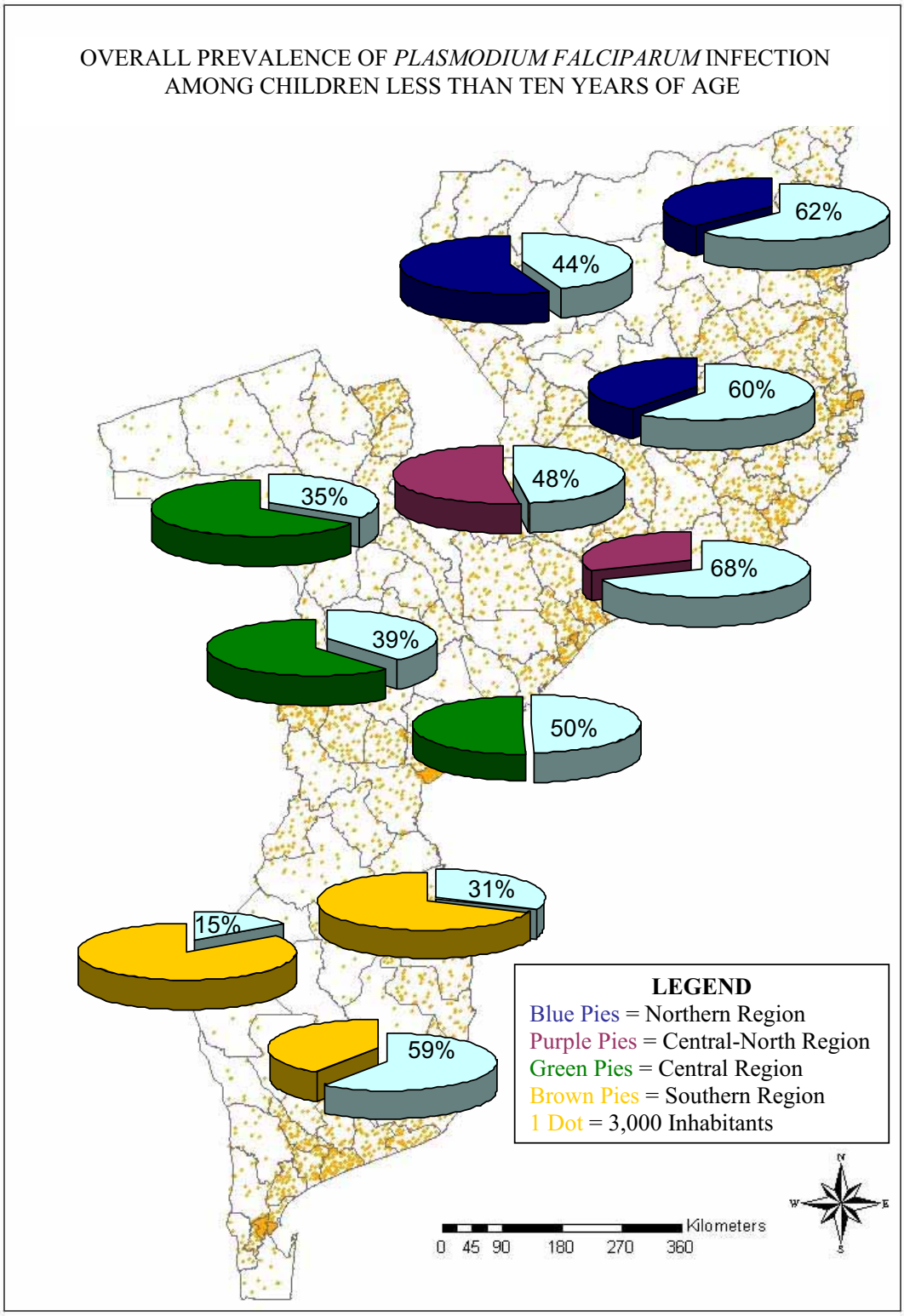


FIGURE 15. *Overall prevalence of Plasmodium falciparum infection in children less than ten years of age across different regions and strata in the country*



In the northern and central-northern regions, the prevalence of P. falciparum infection, increased during the first 12 months of life from 47.5% and 64.3%, peaking to 63.3% and 67.7%, respectively among the 12 – 23 months age group

children, and thereafter decreased progressively with age. Only in the central-northern region, significant differences in age group variation were observed (p=0.042). Within the same age groups, the peak observed of 41.2% and 44.9% across central and southern regions respectively, was much lower. Thereafter the prevalence decreased slightly with very little variation among older children. Differences in the distribution between age groups were observed only for the central region (p<0.0001).

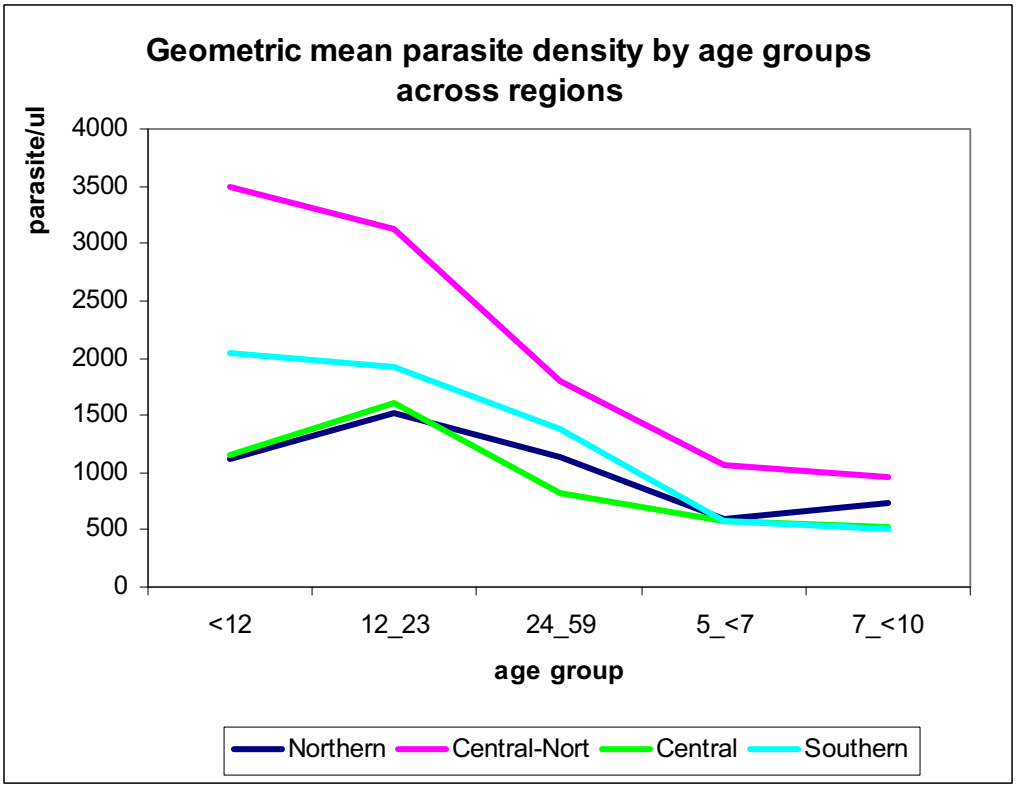
Across strata, there was a significant decrease of *Plasmodium falciparum* infection prevalence from the low lands of the coastal stratum to the highland stratum in both central (p=0.02) and southern (p=0.004) regions, while the variations observed across strata in the northern region (p=0.247) and in the northern-central region (p=0.470) were not statistically significant.

Similarly, the overall mean parasite density showed a considerable regional variation (p=0.046). Figure 16, depicts the distribution of mean parasite density among age groups in various regions.

High mean parasite density of 2,058 parasites/µl (95% CI, 1,836 – 2,306) was recorded in children across the central-northern region, and young children aged below 12 months old harbour the maximum load of parasite density 3,494 parasites/µl, (95% CI 2,641 – 4,621). Comparatively, low mean parasite density of 891 parasites/µl (95% CI, 799 – 994) was recorded among children in central region. The overall mean parasite density in the northern and southern regions was 1,077 parasites/µl (95% CI, 965 – 1,200) and 1,193 parasites/µl (95% CI, 1,025 – 1,388), respectively.

Generally, in both northern regions, mean parasite density peaked during the first 12 months of age, while in central and southern regions the peak was recorded later among children in the 12 – 23 months of life. Nonetheless, in all regions, parasite mean densities were markedly age-dependent, and decreased sharply with age (p<0.0001).

FIGURE 16. *Geometric mean parasite density by age groups in different regions*



11.4.2 Prevalence of fever and fever associated with malaria parasites infection

Across regions the mean axillary temperature decreased slightly from 37.1º (95% CI, 36.9º C – 37.2º C) within the northern region to 36.7º C (95% CI 36.6º C – 36.8º C) in the other three regions. Overall, high fever prevalence were recorded in the northern and central-northern regions 12.8% (287/2,387) and 10.8% (187/1,929), while in the central and southern region were recorded the lowest fever prevalence of 6.9% (185/2,930) and 7.2% (107/1,570) respectively. Although the prevalence of fever showed significant regional variations (p=0.019), declining from north to south following the same pattern of malaria parasites infection distribution, there were no significant differences within strata

across the regions. In all regions fever prevalence decreased with age, though a slight increase were observed among children aged 7 years old and above, except in the central-northern region.

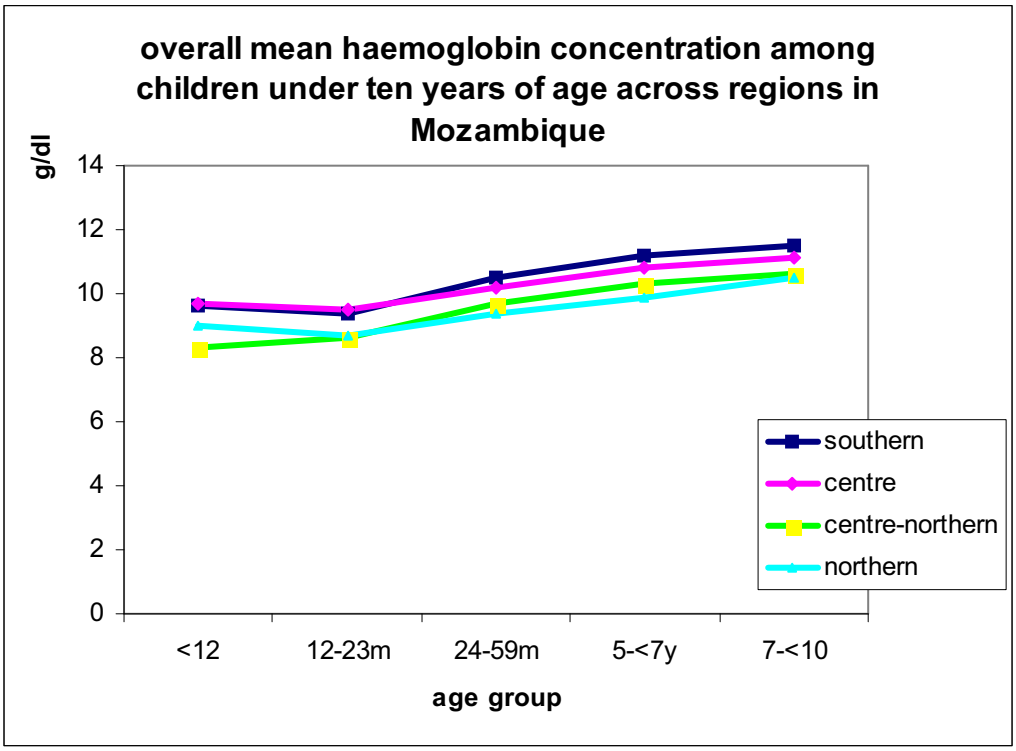
Fever associated with malaria parasites was markedly high in the northern and central-northern regions; 9.1% (199/2,387) and 8.4% (139/1,929), respectively. The central region registered the lowest prevalence of fever associated with parasites 6.9% (101/2,930).

In spite of high variations of the proportions of fever associated with malaria parasites, the differences across regions were not statistically significant (p=0.108).

11.4.3 Overall mean haemoglobin and prevalence of anaemia

Mean haemoglobin concentration showed insignificant differences between regions as illustrated in figure 17.

FIGURE 17. *Overall mean haemoglobin concentration among children less than ten years of age across various regions of Mozambique*

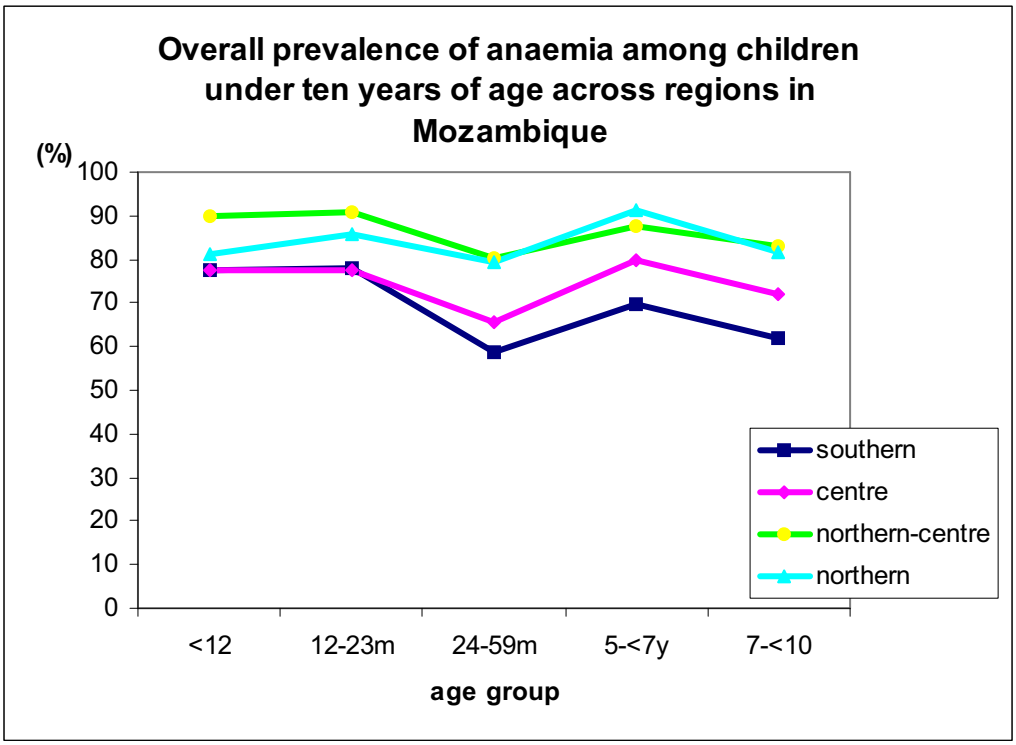


In general, there was some degree of variations on mean haemoglobin concentration across regions. The northern and central-northern regions recorded mean haemoglobin of 9.4 g/dl (95% CI, 8.3 – 10.4) and 9.4 g/dl (95% CI, 8.5 – 10.3), respectively. A slightly higher mean haemoglobin concentration of 10.2 g/dl (95% CI, 9.8 – 10.7) and 10.4 g/dl (95% CI, 9.8 – 10.9) were recorded in the central and in the southern regions, respectively.

According to age groups, mean haemoglobin concentration, increased significantly with age only within the central ($p<0.05$) and southern ($p<0.005$) regions. In the northern regions also increased, but without significant differences among age groups.

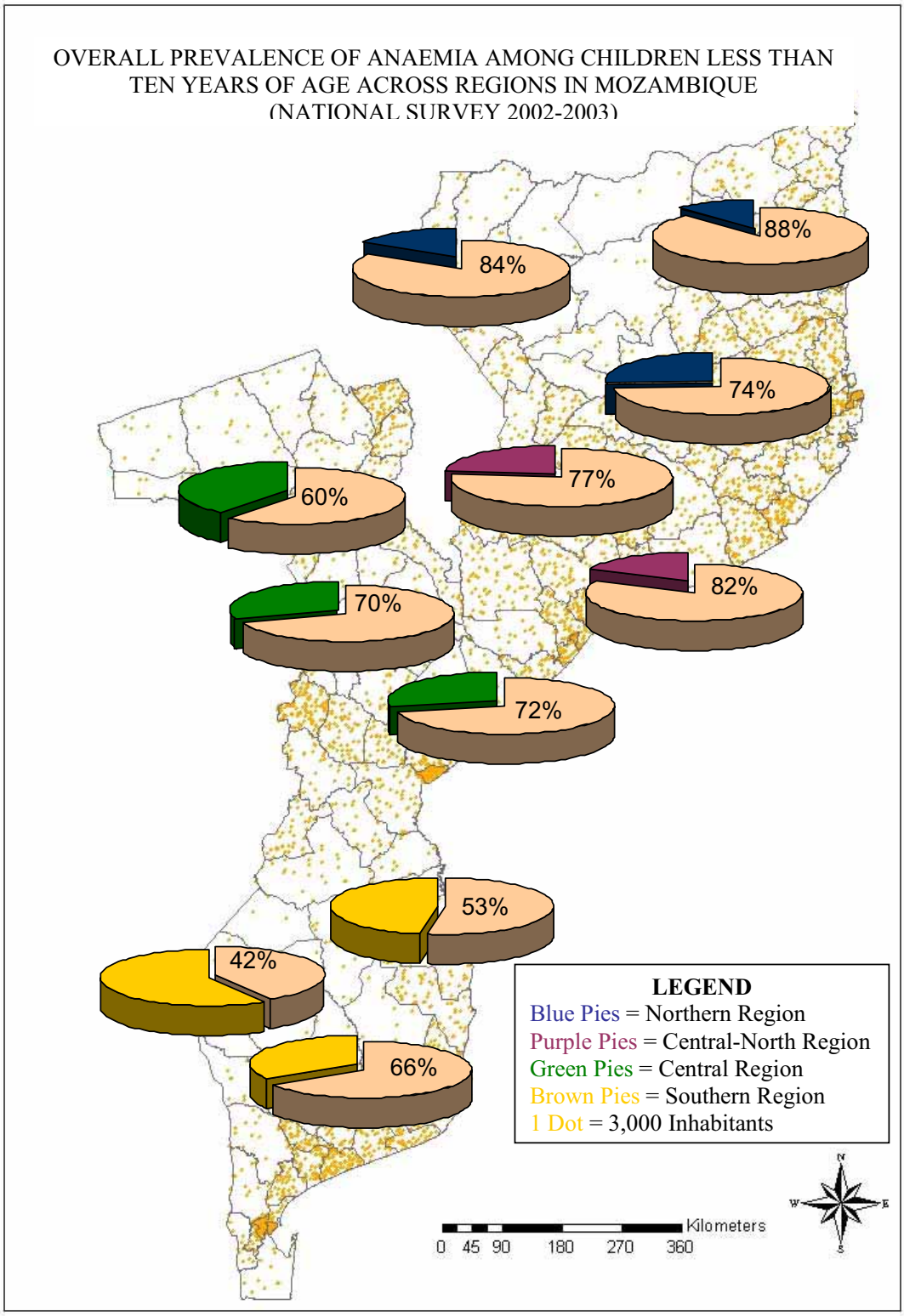
Not surprisingly, the prevalence of anaemia was very high in all regions as is illustrated in figures 18 and 19.

FIGURE 18. *Overall prevalence of anaemia among children under ten years old across different regions of Mozambique.*



Overall, the highest levels of anaemia prevalence were recorded in the northern and central-northern regions 77.9% and 79.4% respectively. Although significant regional differences on the levels of anaemia (p=0.0002), coincidently, in all regions the prevalence of anaemia rose dramatically among children less than 12 months of age to peak in children in the 12 – 23 months age group. In northern and central-northern regions increased from 81.2% and 89.8%, respectively, during the first 12 months of life, peaking at 86.5% and 91.0% among children in the 12 – 23 months age group. While in the central and southern regions the peak was 77.6% and 77.9% respectively, among children in the 12 – 23 months age group. Thereafter it decreased progressively with age. There were not significant differences on anaemia prevalence across.

FIGURE 19. *Overall prevalence of anaemia in children less than ten years of age across different regions and strata in the country*



strata, except within the northern region where the coastal stratum had high proportion of anaemia and significantly different from other strata (p=0.049).

The levels of severe anaemia were higher among children in the northern and central northern regions 2.2% and 1.6%. The lowest prevalence of severe anaemia was recorded in the southern region (0.3%), and the differences observed between regions were statistically significant (p=0.031). Overall, the peak was recorded among children in the 12 – 23 months age group. However, the northern region recorded the highest peak (3.9%) of severe anaemia prevalence among children aged below 12 months. Unlike prevalence of anaemia, severe anaemia decreased sharply with age. In the southern region there were no records of severe anaemia among children aged 5 years and above.

Comparison among anaemic and non-anaemic subjects in all regions, revealed a significant association between prevalence of anaemia and malaria parasite infections. Across the northern region among anaemic children 58.3% had *Plasmodium falciparum* parasites, while among non-anaemic was 42.7%, the difference was statistically significant (p=0.012). In the central-northern region among anaemic children the presence of *Plasmodium falciparum* infection was recorded in 63.0%, against 42% among non-anaemic (p=0.05). In the central region among anaemic children 40.9% had *Plasmodium falciparum* infection, while among non-anaemic the presence of malaria parasites was 29.5% (p=0.006). Across the southern region the presence of *Plasmodium falciparum* was recorded in 53.9% among anaemic children, while in non-anaemic was 36.3%, the difference was statistically significant (p<0.0001).

11.4.4 Overall prevalence of *P. falciparum* sexual forms

There was a significant regional variation on the prevalence of *Plasmodium falciparum* gametocytes (p=0.002). Nevertheless, no significant differences were observed across strata of all regions (p=0.55). Overall the central and central-northern regions recorded the highest prevalence of gametocytes 7.3% (131/2,387) and 8.2% (136/1,929), respectively. The lowest prevalence was recorded in the southern region 2.0% (29/1,570).

Regions with low prevalence showed an erratic distribution of gametocytes, according to age groups. However, across regions with high gametocytes prevalence, it increased among children less than 12 months of age, and peaking among children in the 12- 23 months of age. In the central-northern region the peak was observed earlier during the first 12 months of age. Thereafter, the prevalence of gametocytes decreased considerably with age (p=0.029).

11.4.5 Prevalence of *Plasmodium malariae*

The prevalence of Plasmodium malariae parasites was relatively low in the study area, accounting for 3.6% (518/8,816) of malaria infections. Overall, the prevalence of *Plasmodium malariae* infection showed significant regional variations (p=0.013). The highest was recorded in the central-northern regions 7.4% (124/1,929). While the central region recorded the lowest prevalence 1.4% (59/2,930). Across strata, there was a significant decrease of *Plasmodium malariae* infection prevalence from 6.8% in the low lands of the coastal stratum to 1.7% in the highland stratum (p=0.026).

In general, the prevalence of *Plasmodium malariae* infection varied significantly with age (p=0.02). The peak of infection was recorded among younger children aged between 12 – 23 months old, and thereafter decreased gradually with age. No sex differences were observed.

11.4.6 Prevalence of mixed infection

The rare episodes of mixed infections were recorded predominantly in the central-northern region 7.0% (117/1,929) and in the northern region 3.1% (82/2,387). The occurrence of mixed infections in both central 0.9% (41/2,930) and southern 0.8% (13/1,570), regions were negligible.

Variations in the distribution of mixed infection showed significant differences among age groups. The peak of mixed infections was recorded among children in the 24 – 59 months age group.

11.5 Estimated number of children harbouring malaria parasite infection and anaemia across the country

In order to estimate the absolute population of children infected with *Plasmodium falciparum* malaria parasites and/or bearing anaemia at a position in time in Mozambique, we have carried out some simple calculations.
According to 1997 population census, there are approximately 5,674,904 children less than ten years of age expected by 2005 in Mozambique.
Their distribution by region and strata is illustrated in table 15.

Table 15. *Estimated number of children less than ten years of age living in the study area per region and strata*

Region/stratum	Coastal	Plateau	Highland	Total
Northern	797,107	699,899	447,158	1,944,164
Centre-northern	442,421	395,850	325,994	1,164,265
Central	407,071	552,454	494,301	1,453,826
Southern*	534,071	489,566	89,012	1,112,649
Total	2,180,670	2,137,769	1,356,465	5,674,904

*excluding Maputo City

If we apply the age regional and stratum specific prevalence rates for anaemia and/or malaria infection, we can estimate absulote numbers accordingly.

Table 16 depicts the distribution of expected number of children less than ten years of age harbouring *Plasmodium falciparum* malaria parasites across regions and strata in the country.

Table 16. *Number of children less than ten years of age expected to harbouring Plasmodium falciparum malaria parasites infection per region and strata*

Region/stratum	Coastal	Plateau	Highland	Total
Northern	495,801	391,244	197,645	1,084,690
Centre-northern	303,058		156,151	459,209
Central	201,500	214,905	170,534	586,939
Southern*	166,096	291,292	13,530	470,918
Total	1,166,455	897,441	537,860	2,601,756

*excluding Maputo City

Table 17 show the distribution of expected anaemia cases in the country.

Table 17. *Estimated number of children less than ten years of age with haemoglobin concentration below age specific value per region and strata*

	Coastal	Plateau	Highland	Total
Northern	699,860	516,526	374,271	1,590,657
Centre-northern	362,343		250,363	612,706
Central	292,277	384,508	299,052	975,837
Southern*	281,989	322,624	37,118	641,731
Total	1,636,469	1,223,658	960,804	3,820,931

*excluding Maputo City

11.6 Characteristics of pregnant women

The characteristics of pregnant women are illustrated in table 18. A total of 1,531 pregnant women included for the final analysis had mean age of 24.9 years, ranging from 12.2 years to 44.2 years (SD – 6.2).

According to age they were categorized into three age groups as follows: Less than 20 years; between 20 and 30 years; 30 years and above. Overall, 50.2% (769/1,531) of pregnant women were in the 20 – 30 years old age group. Less than 20 years and 30 years and above age groups were 25.3% (388/1,531) and 24.4% (374/1,531), respectively.

Table 18. *Characteristics of pregnant women in the study area*

	% (n/N)
Mean age (years)	24.9 std.dev. 6.1 Range: [12.2 - 44.2]
Mean gestational age (weeks)	23.5 std.dev. 0.4 95% CI 22.8 - 24.3
Parity	
Primigravidae	**17.4** (266/1,531)
Multigravidae	**58.2** (891/1,531)
Grand multigravidae	**24.4** (374/1,531)
Age groups composition	
< 20 years	**25.3** (388/1,531)
20 – < 30 years	**50.2** (769/1,531)
≥ 30 years	**24.4** (374/1,531)

According to the number of pregnancies, 17.4% (266/1,531) were primigravidae, 58.2% (891/1,531) were multigravidae; 24.4% (374/1,531) were Grandmultigravidae.

Among pregnant women in the less than 20 years old age group, 68.6% (266/388) were primigravidae, while 31.4% (122/388) were in the multigravidae

category. All pregnant women in 30 years and above age group were grandmultigravidae. Age and parity were strongly correlated (r=0.8; p<0.0001). Overall, 39.1% (598/1,531) of women were in the third trimester of pregnancy, while 23.5% (360/1,531) and 37.4% (573/1,531) were in the first and second trimesters of pregnancy, respectively.

Their distribution in each region and stratum is presented in table 19.

Table 19. *Distribution of pregnant women across different regions and strata in the study area*

	Coastal (%)	**Plateau** (%)	**Highland** (%)	**Total** (%)
North	88 **(43.4)**	70 **(34.5)**	45 **(27.2)**	203 **(100.0)**
NorCentre	225 **(65.0)**		121 **(34.9)**	346 **(100.0)**
Centre	173 **(33.8)**	144 **(28.1)**	195 **(38.1)**	512 **(100.0)**
South	163 **(34.7)**	157 **(33.4)**	150 **(31.9)**	470 **(100.0)**
Total	649 **(42.4)**	371 **(24.2)**	511 **(33.4)**	1,531 **(100.0)**

11.7 Prevalence and intensity of malaria infection and anaemia

During pregnancy

Prevalence of malaria, parasite infection intensity and the degree of anaemia in pregnant women across regions are shown in table 20 (appendix 7) and figure 21, shows the prevalence of malaria infection across the country.

Overall, 34.7% (478/1,531) of blood smears obtained from participating pregnant women were positive for malaria parasites. The majority of blood smears, 33.6% (465/1,531) were purely *Plasmodium falciparum* parasites, while *Plasmodium malariae* and mixed infections of *Plasmodium falciparum* and *Plasmodium malariae* were only recorded in 0.6% (7/1,531) and 0.5% (6/1,531), respectively. Gametocytes (sexual forms) only for *Plasmodium falciparum* were recorded in 1.4% (28/1,531) of blood smears. There were no records of infections by *Plasmodium ovale* or *plasmodium vivax* parasites. *Plasmodium*

falciparum parasites accounted for 97.3% (465/478) of all malaria infections. Figure 20, depicts overall infection among pregnant women in different regions across the country.

Geometric mean parasite density only for asexual forms of *Plasmodium falciparum* was 446 parasites/µl (95% CI, 381 – 521).

The mean temperature recorded was 36.7° C (95% CI 36.6° C – 36.7° C), with axillary temperature ranging from 35.2° C to 40.6° C. Fever prevalence was 4.4% (53/1,531), and prevalence of fever associated with parasites was 1.9% (19/1,531).

Haemoglobin concentrations ranged from 1.9 to 17.7 g/dl, and overall mean estimation was 10.3 g/dl (95% CI, 9.9 – 10.6). The prevalence of anaemia, based on the 11.0 g/dl altitude adjusted race-specific WHO cut off was 62.5% (975/1,531), and among anaemic pregnant women, the prevalence of severe anaemia (haemoglobin less than 5 g/dl) was 1.1% (13/1,531).

FIGURE 20. *Overall parasite and anaemia prevalence among pregnant women in different regions of Mozambique*

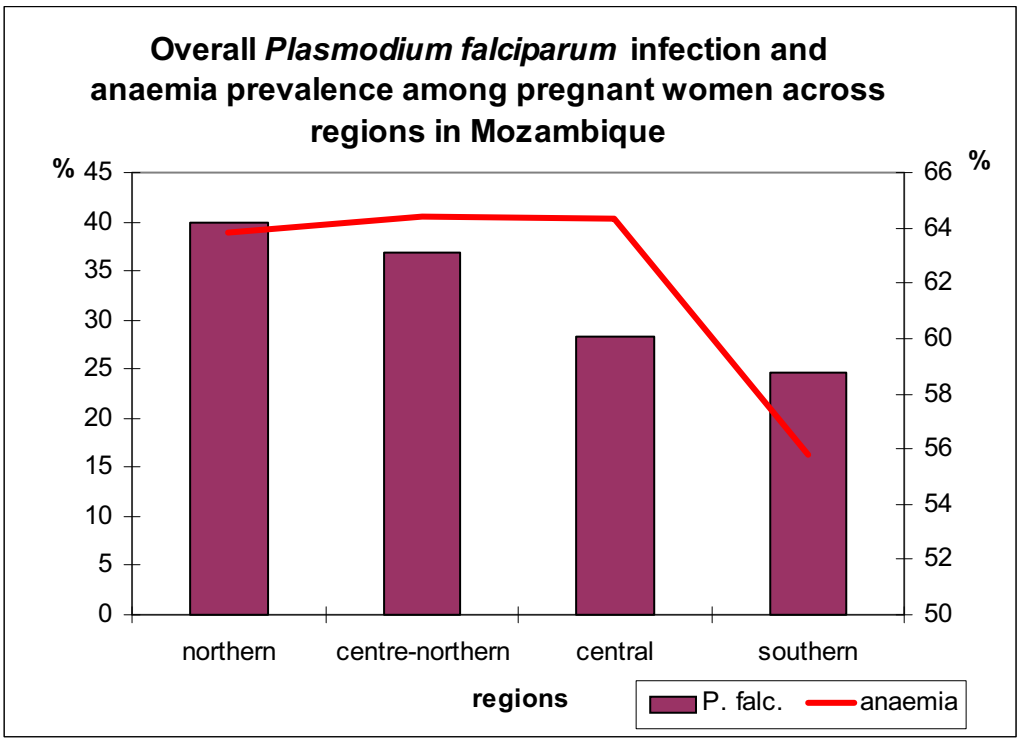
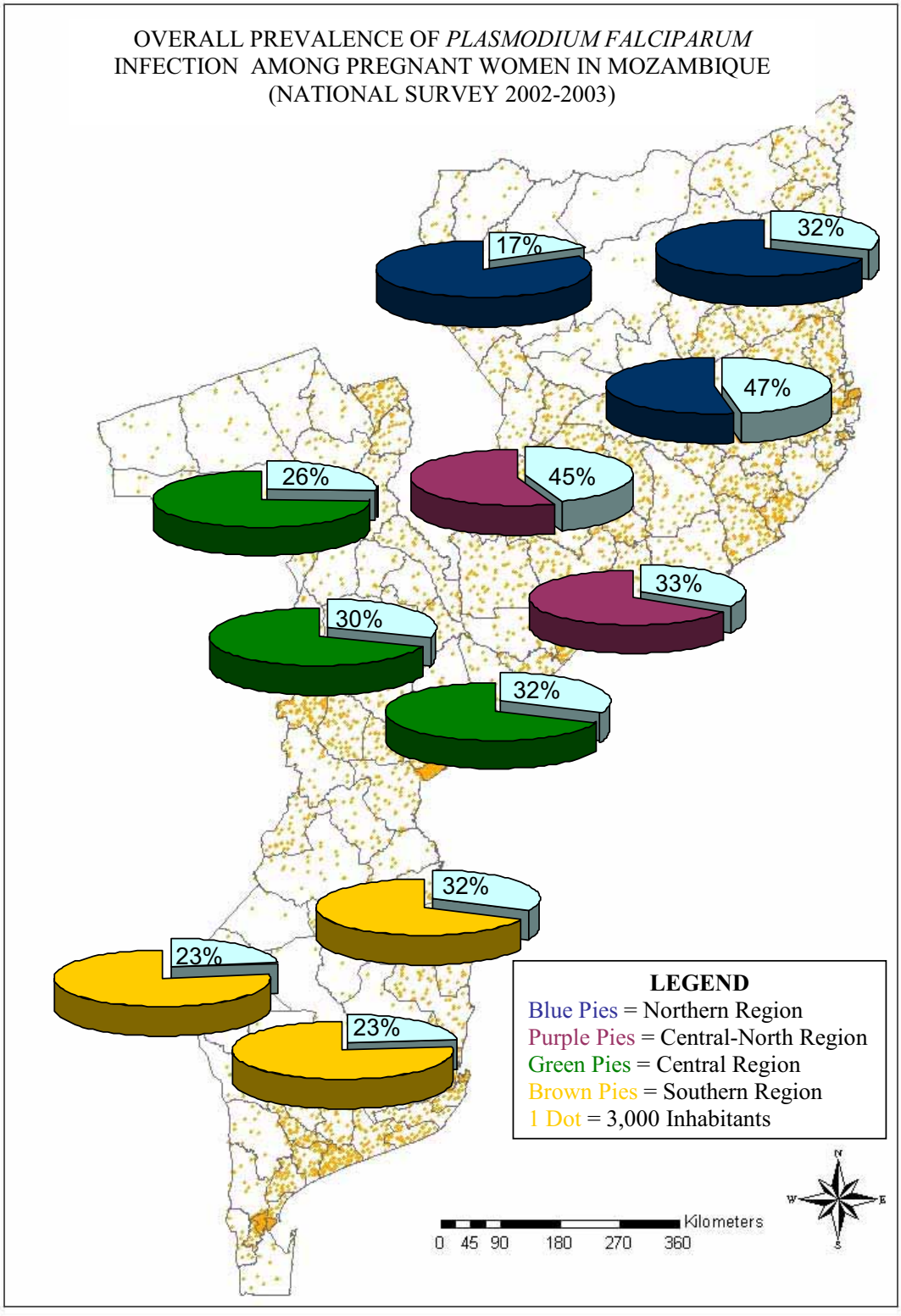


FIGURE 21. *Prevalence of malaria infection among pregnant women across regions in the country*



11.7.1 Risk factors for malaria infection in pregnancy

The prevalence of malaria infection among pregnant women showed significant differences according to age group distribution (p=0.0001). Among younger pregnant women aged below 20 years old the prevalence of *Plasmodium falciparum* parasites was higher, 44.4% (155/388), conversely older pregnant women in the age group 30 years old and above had the lowest prevalence of *Plasmodium falciparum* parasites of 25.9% (88/374). Among pregnant women aged between 20 and 30 years old, the prevalence of malaria parasites was 30.6% (222/769). The risk factors for parasitaemia are summarized in table 21.

In relation to parity, the group of women with at least one pregnancy had the highest malaria parasite infection prevalence of 43.0% (102/266). In the group of 1-4 pregnancies the prevalence of malaria infection was 33.1% (275/891) and in the group with five or more pregnancies the prevalence of malaria parasites infection was 25.9% (88/374). Although, the prevalence of *Plasmodium falciparum* infection, decreased with increasing parity, and differences observed were statistically significant (p=0.03), multiple regression analysis showed age of pregnant women to be significantly associated with parasitaemia. Younger pregnant women had a higher risk to malaria parasites infection compared to older pregnant women; however there was not a significant association with the parity.

Geometric mean parasite density was also high in the primigravidae group 595 parasites/µ (95% CI, 431 – 823), while among grand multigravidae pregnant women the geometric mean parasite density was very low 302 parasites/µ (95% CI, 212 – 432). Mean parasite density decreased with increasing parity (p=0.08) and age (p=0.08). Although, young and primigravidae women had the highest mean parasite density, while old and multigravidae women had the lowest mean parasite density, the differences observed were not statistically significant.

The prevalence of malaria parasites was different through different gestational age, and in different categories of parity. In general the prevalence decreased with increasing gestational age. During the first and third trimester, high prevalence of malaria parasite infections was recorded among primigravidae women. The multigravidae women category had high prevalence of malaria parasites during the first trimester. While pregnant women in the grand

multigravidae category showed high prevalence of malaria parasites during the second trimester. Despite the variations on the distribution of malaria parasites prevalence among pregnant women in different gestational age, there was no association between prevalence of malaria parasites and gestational age. The differences observed were not statistically significant.

The prevalence of clinical malaria among pregnant women was very low 1.2% (19/1,531). Among pregnant women aged bellow 20 years old, 4.4% had fever and *Plasmodium falciparum* parasites associated. The risk of clinical malaria among younger women was 6.5 times high compared to pregnant women in the 30 years old and above age group.

Table 21. *Potential risk factors for parasitaemia during pregnancy*

	% (n/N)	**Unadjusted OR** (95% CI)	**Adjusted OR** (95% CI)
Age (years)			
< 20	**44.4** (155/388)	**1**	**1**
20 – 29	**30.6** (222/769)	**0.6** (0.55-0.65)	**0.9** (0.8-1.3)
≥ 30	**25.9** (88/374)	**0.5** (0.46-0.54)	**0.8** (0.6-1.1)
Parity			
Primigravidae	**43.0** (102/266)	**1**	**1**
Multigravidae	**33.1** (275/852)	**0.8** (0.57-0.63)	**0.8** (0.6-0.9)
Grand multigravidae	**25.9** (88/374)	**0.5** (0.6-0.8)	**1.5** (0.9-2.5)
Trimester			
Third	**31.8** (167/598)	**1**	**1**
Second	**33.4** (170/573)	**1.1** (0.77-0.83)	**0.9** (0.7-1.2)
First	**36.8** (128/360)	**1.5** (0.66-0.74)	**0.8** (0.6-1.1)

11.7.2 Risk factors for anaemia in pregnancy

The overall prevalence of anaemia was 62.5%. The risk factors for anaemia are summarized in table 22.

Table 22. *Potential risk factors for anaemia during pregnancy*

	% (n/N)	**Unadjusted OR** (95% CI)	**Adjusted OR** (95% CI)
Age (years)			
< 20	**70.1** (267/388)	**1**	**1**
20 – 29	**59.4** (473/769)	**0.7** (0.67-0.63)	**1.2** (0.9-1.1)
≥ 30	**59.4** (235/374)	**0.8** (0.76-0.84)	**0.9** (0.6-1.1)
Parity			
Primigravidae	**70.5** (185/266)	**1**	**1**
Multigravidae	**60.8** (555/852)	**0.8** (0.67-0.63)	**0.9** (0.7-1.2)
Grand multigravidae	**59.4** (235/374)	**0.7** (0.5-0.7)	**1.1** (0.6-1.8)
Trimester			
Third	**61.3** (376/598)	**1**	**1**
Second	**64.8** (385/573)	**1.2** (1.34-1.5)	**0.8** (0.6-0.9)
First	**60.6** (214/360)	**0.8** (1.16-1.24)	**1.7** (1.1-2.6)
Parasitaemia			
Positive	**70.2** (333/465)	**1**	**1**
Negative	**58.6** (642/1,066)	**0.6** (1.6-1.8)	**0.6** (0.5-0.8)
Density			
≤ 499	**58.6** (642/1,066)	**1**	**1**
500 - 4,999	**68.1** (296/420)	**1.7**	**1.9** (1.6-2.1)
≥ 5,000	**88.1** (37/45)	**2.9**	**2.7** (2.4-3.1)

Anaemia was significantly associated with *Plasmodium falciparum* malaria parasite infection (p=0.003).

Among parasitaemic pregnant women 70.2% (333/465) were anaemic and 2.2% (6/465) were severely anaemic. Among non parasitaemic 58.6% (642/1,066) were anaemic.

Pregnant women harbouring *Plasmodium falciparum* parasites were 1.7 times more likely to have anaemia, which increased significantly with increasing mean parasite density (p=0.006). Mean haemoglobin concentration increased significantly with increasing parity categories (p=0.003), similarly the same trend was observed in relation to age.

Pregnant women in the age group 30 years old and above had high mean haemoglobin concentration when compared to pregnant women aged bellow 20 years old (p=0.02). The prevalence of anaemia was higher in the age group less than 20 years old 70.1% (267/388).

Among older pregnant women in the 20 – 30 years old and 30 years old and above was 59.4% (473/769) and (235/374), respectively. Despite variations in the prevalence of anaemia between younger pregnant women and others, the differences were not statistically significant (p=0.134). According to parity, primigravidae had the highest prevalence of anaemia 70.5% (185/266). It decreased with increasing parity, the multigravidae category had 60.8% (555/852) and the grand multigravidae category the prevalence was 59.4% (235/374). However, the differences observed were not significant (p=0.0781).

In relation to gestational age, overall high prevalence of anaemia 64.8% (214/360) was recorded during the second trimester. During the first and third trimesters the prevalence was 60.6% (385/573) and 61.3% (376/598), respectively. However, the variations were not statistically significant (p=0.498).

Among anaemic pregnant women 6.2% (12/333) were febrile and had *Plasmodium falciparum* parasites simultaneously. Conversely, amongst non-anaemic pregnant women 4.4% (7/132) had fever associated with *Plasmodium falciparum* parasites. The differences were not significant (p=0.450). However, febrile pregnant women carrying malaria parasites were 1.5 times more likely to be anaemic comparatively to afebrile pregnant parasite carriers.

11.8 Regional variations across different strata

Overall, the prevalence of malaria infection among pregnant women showed significant regional variations (p=0.020), figure 16.

The northern region had the highest prevalence of asexual *Plasmodium falciparum* parasites 40.0% (75/203) with 95% CI, 30.9 – 49.1. The lowest prevalence was recorded in the southern region 24.6% (127/470) with 95% CI, 21.7 – 27.6. The central-northern and central regions had 36.9% (119/346), (95% CI, 28.0 – 45.8) and 28.3% (144/512) with 95% CI, 25.9 – 30.5, respectively.

Across strata the variations were not statistically significant (p=0.945). High prevalence was recorded in the stratum plateau 34.6% (114/371), (95% CI, 22.5 – 46.6); in the stratum coastal the prevalence was 32.7% (212/649), (95% CI, 29.5 – 35.9) and in the stratum highland was 22.8% (139/511), (95% CI, 20.6 – 46.9).

In all regions, the prevalence of malaria parasites infection, decreased significantly with age in the central-northern and central regions, exception for the southern and northern regions where the differences were not statistically significant. Across strata, there was an increasing from the coastal stratum to the highland, although without significant differences.

Overall the prevalence of *Plasmodium falciparum* infection decreased with increasing parity. The primigravidae category was associated with high prevalence of malaria parasites, thereafter it decreased with parity. Though across regions there were not significant differences.

The first trimester is associated with high malaria prevalence, only in the central-northern region the highest prevalence was recorded during the second trimester. Nonetheless, the differences observed were not statistically significant.

The mean haemoglobin concentration was similar across regions (10.2 g/dl). Therefore, the prevalence of anaemia showed insignificant regional variations, figure 22, although across strata increased from 57.1% in the low lands of the

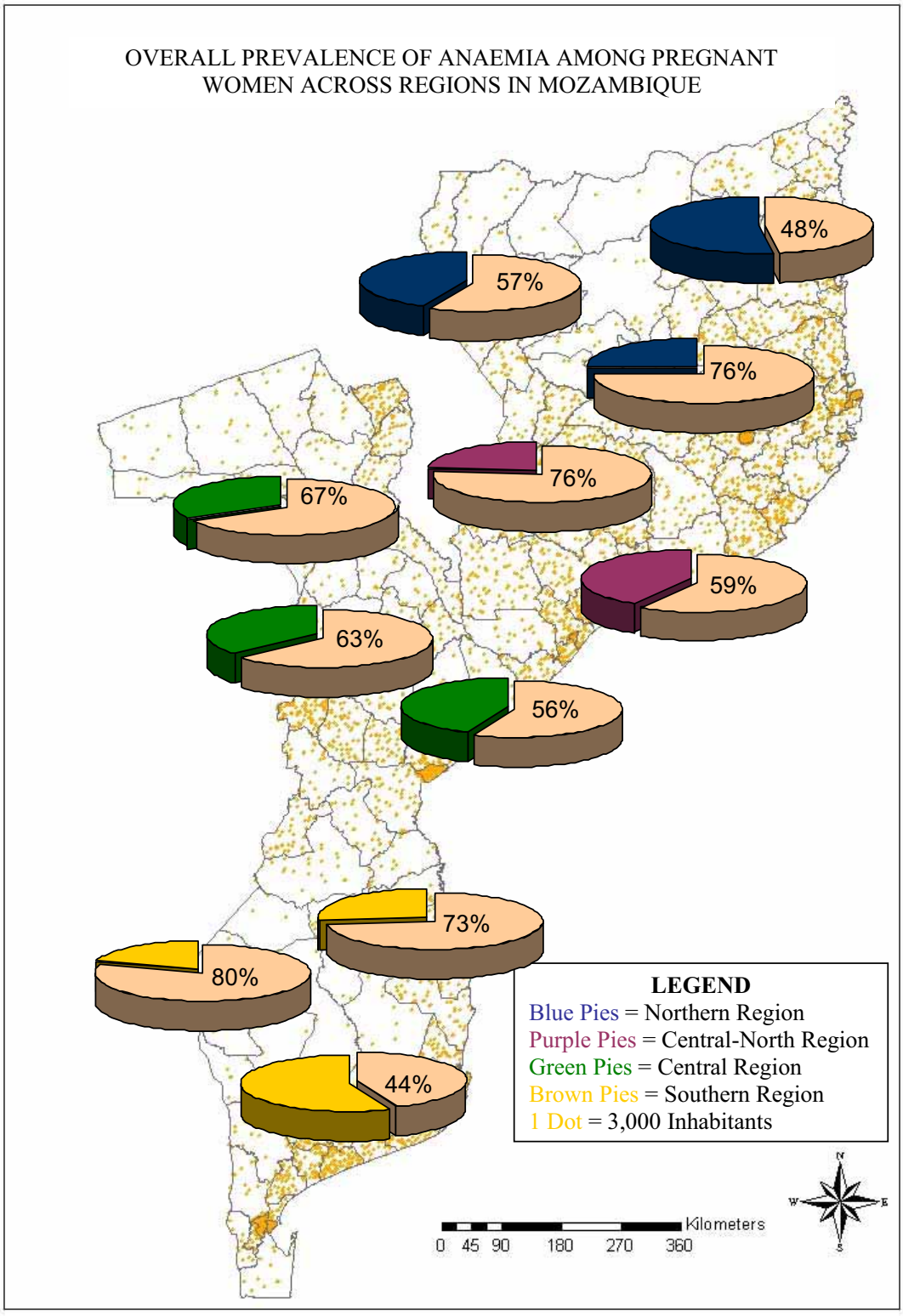
coastal stratum to 72.7% in the highland stratum, however without significant differences (p=0.203).

The pattern of anaemia was variable in relation to parity. Across central-northern and central regions decreased with increasing parity, while in the southern region increased in the primigravidae category to peak among multigravidae women, thereafter decreased with parity, although with a slight increase among grand multigravidae category. In the northern region it increased among primigravidae category to peak among multigravidae and thereafter decreased significantly among grand multigravidae category.

Within the southern and central regions, the second trimester of pregnancy was associated with high prevalence of malaria parasites, while in the northern and central-northern regions was the third and first trimesters, respectively. All differences observed in the prevalence of anaemia were not statistically significant.

There was a strong association of febrile pregnant women harbouring *Plasmodium falciparum* parasites and prevalence of anaemia in southern region. Although the same trend was observed in other regions, yet the association was not statistically significant.

FIGURE 22. *Overall prevalence of anaemia among pregnant women across different regions and strata in Mozambique*



11.9 Estimated number of women in bearing age harbouring malaria parasite infection and anaemia across the country

In order to estimate the absolute population of pregnant women infected with *Plasmodium falciparum* malaria parasites and/or bearing anaemia at a position in time in Mozambique, we have carried out some simple calculations.
According to 1997 population census, there are approximately 2,120,499 pregnant women expected by 2005 in Mozambique. Their distribution by region and strata is shown in table 23.

Table 23. *Estimated number of pregnant women age per region and stratum*

	Coastal	Plateau	Highland	Total
Northern	280,133	245,953	157,137	683,223
Centre-northern	157,065	140,531	115,732	413,328
Central	137,999	182,284	167,571	487,854
Southern*	218,925	200,682	36,487	536,094
Total	794,122	849,450	476,927	2,120,499

*excluding Maputo City

If we apply the regional and stratum specific prevalence rates for anaemia and/or malaria infection, absolute numbers can be estimated accordingly.
Table 24 shows the distribution of expected absolute number of pregnant women infected by malaria parasites across regions and strata in the country.

Table 24. *Estimated number of pregnant women harbouring Plasmodium falciparum malaria parasites infection per region and stratum*

	Coastal	Plateau	Highland	Total
Northern	88,802	115,844	35,356	240,002
Centre-northern	52,303	51,856	51,501	155,660
Central	44,032	55,059	44,239	143,321
Southern*	70,275	46,960	8,356	125,591
Total	255,412	269,719	139,452	664,583

*excluding Maputo City

Table 25 shows the distribution of expected anaemia cases among pregnant women across regions and strata in the country.

Table 25. *Estimated number of pregnant women with haemoglobin concentration below age specific value per region and stratum*

	Coastal	Plateau	Highland	Total
Northern	204,777	108,957	125,395	439,129
Centre-northern	92,197	89,799	88,419	270,415
Central	77,417	114,657	111,770	303,844
Southern*	104,865	151,716	20,652	277,233
Total	479,256	465,129	346,236	1,290,621

*excluding Maputo City

11.10 Entomological inoculation rate

The total number and composition of mosquitoes species caught, by region and strata and respective entomological inoculation rates are presented in table 26 (appendix 8).

In all regions *An. gambiae s.l.* followed by *An. funestus* were the most prevalent vectors. Other vectors included *An. arabiensis, An. merus* and *An. quadrianulatus*.

The overall sporozoite rate of 0.7% (46/6,557) obtained, exhibited significant regional variations. The gradient of entomological inoculation rates showed both north-to-south and low-to-highlands decrease.

Regional variations were demonstrated by a maximum of entomological inoculation rate of 33.4 infective bites/person/year recorded in the central-northern region. While in the southern region was recorded the lowest entomological inoculation rate of 2.6 infective bites/person/year.

Across northern and central regions the entomological inoculation rates were 19.3 infective bites/person/year and 6.1 infective bites/person/year, respectively.

Across the low lands of coastal stratum an entomological inoculation rate of 19.0 infective bites/person/year was recorded. While, the minimum entomological inoculation rate of 2.0 infective bites/person/year was recorded in the highland stratum.

The number of anopheline tested, the proportion positive for CSP analysis and respective entomological inoculation rates by region are presented in table 27.

Table 27. *Overall Entomological Inoculation Rates by regions*

	No. of mosquitoes tested by PCR	No. sporozoite positive	Proportion sporozoite positive	No. of catches	EIR standard method	EIR alternative method
North	2,508	19	0.0076 **(19/2,508)**	360	19.3	19.3
North-Centre	1,496	16	0.011 **(16/1,496)**	180	33.4	32.4
Centre	1,896	8	0.0042 **(8/1,896)**	480	6.1	6.1
South	657	3	0.0046 **(3/657)**	420	2.6	2.6
Overall	6,557	46	0.007 **(46/6557)**	1440	11.6	11.7

12 Discussion

It is well known that children and pregnant women living in many parts of Sub-Saharan Africa have an increased risk of malaria infection. However, available epidemiological data have been reporting the prevalence of malaria parasites infection in limited areas of southern region of the country.

The goal of the present study was to assess the malaria disease burden, by estimating the prevalence and intensity of Plasmodium infections in children less than 10 years of age and in pregnant women across different ecological settings in Mozambique.

12.1 Prevalence of malaria parasites and parasite density

In children

Overall, 58.9% of children aged less than 10 years old harbour malaria parasites, the most common species was *Plasmodium falciparum* 48.6%. The prevalence of malaria infection showed a marked age-dependence, increasing during the first year of life, before reaching a plateau and maximum peak prevalence among children aged between 12 – 59 months old, revealing a relative slow build-up of anti-malarial immunity, which requires repeated exposure to infecting parasites (Molineaux and Gramiccia, 1980). Similarly, mean parasite density increased during the first year of life with maximum peak among children during the second year of life, however, decreased dramatically with increasing age, suggesting that an effective anti-parasite immunity is acquired very early in the childhood. As described previously in other endemic areas, the prevalence of malaria infection and parasite density among children is age-dependent (Molineaux & Gramiccia, 1980; Marsh & Snow, 1997; Bloland et al., 1999). Therefore, as children grow up, they acquire an ability to limit the occurrence of high density infection, and to reduce the occurrence of any parasite density infection (Bloland et al., 1999). Across different ecological settings in the country, after adjusting for covariates (age, region and stratum),

variations were observed on the prevalence of malaria infection and mean parasite densities, decreasing from northern to southern regions and (with altitude) from low lands in the coastal and plateau strata to the highlands stratum.

The regional variations reflect the differences on the intensity of malaria transmission and may well guide to a classification of the malaria endemicity levels across different epidemiological settings in the country, as described the method proposed by Metselaar and van Thiel, based on parasite rate in children. This method has been routinely used as a “border line” marker of malaria endemicity levels across Sub-Saharan Africa (Bruce-Chwatt, 1987; Molineaux, 1988).

The prevalence of gametocytes (only for asexual *Plasmodium falciparum*) showed the same pattern of distribution of *Plasmodium falciparum* parasites. Other malaria species, namely *Plasmodium malariae* and mixed infections *(Plasmodium falciparum and Plasmodium malariae)* according to age exhibited an inconsistent distribution pattern in different epidemiological settings across the country.

The study conducted by Soeiro et al. in 1952, showed similar trend, the overall prevalence of malaria infections was 58.5%, and varied between 33.5% in the Sul-do-Save district (southern region) and 80% in the Zambezia district (centre-northern region).

The variations observed in prevalence of malaria infections and its intensity within regions could be explained by the regional variations on the amount of rainfall, average air temperatures, and humidity and also on the human population distribution (Bruce Chwatt, 1987).

The coastal stratum, particularly in the northern and centre-northern regions are characterised by high annual rainfalls throughout the year, and the relatively high average air temperatures and humidity, adequate conditions to support *anopheline* populations (Service, 1978). On the other hand, the majority of human population is concentrated in the rural of coastal and plateau strata. Moreover, health services are scarce in rural areas and preventive and control measures only cover the main urban and peri-urban settlements. Conversely, the lower annual rainfalls, relatively low average air temperature, associated

with desertification phenomenon and low density population particularly in the highland stratum for the most part of the southern and central regions, are conditions not favourable for the occurrence of intense malaria infections.

12.2 Prevalence of anaemia

The low levels of haemoglobin concentration among children are clearly reflected by the severity of anaemia in the study area. Although, markedly regional variations were observed, with northern regions carrying the heavy burden of anaemia, a significant age-dependence was a common observation across regions. Prevalence of anaemia rose dramatically among very young children, peaking during the second year of life thereafter decreased gradually. These findings are in agreement with results from studies in other malaria-endemic areas (Schellenberg et al., 2003).

It is well established, that the *Plasmodium falciparum* malaria is regarded as the most important cause of malaria-related anaemia, the mechanism involving destruction and decreased production of red blood cells (Menendez 1995). Not surprisingly, the finding that parasitized children carried a strikingly high burden of anaemia compared to non-parasitized children is in agreement with other community surveys carried out in malaria-endemic areas (McElroy et al., 2000; Ekvall et al., 2001; Schellenberg et al., 2003).

Along these lines, the results of this study strongly support the findings that malaria plays a key role in the burden of anaemia (Premji et al., 1995; Bloland et. al., 1999; Menendez et al., 2000). However, during childhood other risk factors such as nutritional deficiencies (mainly iron and folate deficiencies), intestinal parasitic diseases (bilharzias, intestinal helmintics) have been associated with high prevalence of anaemia (Greenwood et al., 1987; Hedberg et al., 1993)

12.3 Prevalence of clinical malaria and establishment of case definition

The association between malaria infection and body temperature varies significantly among children. Despite, that the definition of clinical malaria have been related to fever episode and presence of parasites in the blood stream, in endemic-malaria areas, manifestations of clinical malaria have a wide spectrum (Bruce-Chwatt, 1986;) and the parasite density required to trigger fever differs significantly from one individual to another (Rogier et al., 1996). Indeed, the majority of parasitized children were asymptomatic carriers, and not all fever episodes were associated with malaria parasites, hence very few fever episodes associated with asexual *Plasmodium falciparum* infections were observed. Moreover, the risk of fever among parasitized children was age-dependent, and increasing with mean parasite density. Therefore, the proportion malaria-attributable fraction, the sensitivity and specificity of clinical malaria definition was age-specific. These findings corroborate with results from other studies in *Plasmodium falciparum* highly endemic populations (Rogier et al., 1996).

Overall, the proportion attributable fraction to malaria parasitaemia was 37.8%. However, when adjusted for age showed variations, the highest was recorded among younger children (43.5 %) and decreased with age to a low of 21.5% among older children. After adjusting for other covariates stratum and region, the proportion attributable fraction showed significant regional differences. The highest proportion attributable was recorded in the northern regions 48.2% and the lowest in the southern region 31.1%, and according to stratum, the highest was recorded in the stratum plateau 41.4% and in the stratum highland was 38.5%. Differences on the attributable fraction among children have been reported from community-based surveys in other endemic-malaria areas (Smith et al., 1995). These figures provide an insight on the proportion of febrile morbidity that would have been removed if malaria infections were eliminated among children in various settings in the study area. Moreover, the findings highlight the changing patterns of the relationship between malaria parasites and the host (Rogier et al., 1996; Bloland et al., 1999). Therefore, the outcome

or the risk of developing clinical manifestations decreased with increasing age, due to acquired anti-parasite immunity. On the other side reflect the burden of other fever attributable conditions (more than 60%) usually underestimated in many malaria-endemic settings, where malaria diagnosis is often presumptive.
Based on attributable fractions it was possible to establish a case definition by modelling the relationship between the risk of fever and parasite density in a given cut off point (Armstrong-Schellenbreg et al., 1994; Smith et al., 1994).
Based on the analysis of the age specific sensitivity and specificity confidence intervals for the attributable fraction, the sensitivity and specificity for the cut-off-points definition for one parasite/µl and 2,500 parasites/µl were obtained for different age groups. It was crucial to determine age specific sensitivity and specificity, otherwise lack of specificity would result in a biased estimate of case definition and lack of sensitivity would result in a loss of power.
As anticipated the sensitivity and the specificity of malaria case definition, showed age-dependence variations across regions and strata. In all settings, the overall sensitivity was 100% for parasite density cut off point of one parasite/µl; however the specificity decreased significantly with age, from 61.8% in children less than 12 months of age to 21.5% in children aged 5 years and above. For the parasite cut off point of 2,500 parasites/µ or higher the sensitivity was 77.5% and the specificity was 83.3%. Although the sensitivity did not show significant variations the specificity decreased from 89.2% in older children aged 5 years and above to 72.9% among children than 12 months of age.

12.4 Prevalence of malaria parasites and parasite density

During pregnancy

It is well established that in malaria-endemic areas, women are more susceptible to malaria infection during pregnancy (Riley et al. 1994; McGregory, 1984; Steketee, et al., 1996) and the impact of the infection on low birth weight (Guyatt & Snow, 2004). Despite the high prevalence of P. falciparum in Mozambique, there are limited epidemiological data reporting the burden of malaria related disease during pregnancy. Not surprisingly, the prevalence of

malaria infection and anaemia in pregnancy was high in the study area. Findings from this study corroborate with results reported from similar studies carried out in other areas elsewhere in sub-Saharan Africa (Steketee, et al., 2001; Guyatt and Snow, 2001).

In relation to spatial distribution, overall, women in the northern regions had the highest malaria infection prevalence; however, after adjusting for covariates, the risk of malaria infection among pregnant women was high in the northern regions compared to southern region.

The prevalence of malaria was high among young pregnant women, aged bellow 20 years. After adjusting for others factors (Parity, gestational age and region), they were 1.6 more times more likely to have malaria infection then pregnant women aged ≥ 30 years old. Although the prevalence of malaria infection decreased with increasing parity, after adjusting for other factors, there was no association between parity and parasitaemia. This finding has been recently reported from other studies. The pregnant women's acquired ability to limit P. falciparum is impaired when co-infected by HIV (Steketee et al., 1996; Verhoeff et al., 1999; van Eijk et al., 2003). Thus, HIV infection during pregnancy yields a confounding effect on the association between malaria infection and parity, altering the parity pattern of malaria infection (ter Kuile et al., 2004). Despite limited studies on HIV prevalence in Mozambique, it is well-established that the prevalence of HIV infection across Sub-Saharan Africa is high in southern Africa.

The highest risk period for malaria infection was observed later in the first trimester and early in the second trimester.

The prevalence of clinical malaria was very low (1.9 %) among pregnant women. Overall, the occurrence of malaria infections associated with fever episodes decreased significantly with increasing age and parity. It appears that the ability to tolerate malaria parasites without developing fever increased with age and parity. However, when adjusted to covariates the association with was not significant.

Evidence from this study suggests that the prevalence of anaemia among pregnant women followed the same pattern of malaria infection distribution. Surprisingly, when adjusted for covariates, parasitized pregnant women had

high risk to have anaemia compared to non-parasitized. This finding is in agreement with results from a review of 26 studies across Africa (Guyatt and Snow, 2001). There was an apparent association between mean haemoglobin concentration and malaria infection; however, there was no evidence to support decreasing mean haemoglobin concentration with increasing malaria transmission intensity, among pregnant women exposed to different levels of malaria transmission. Similar findings were recorded by Saute et al. 2002, in a study conducted in a rural settlement of southern Mozambique. This could be explained by the multi-factorial origin of anaemia, although the role of malaria parasite infection in endemic-areas is crucial other risk factors such as nutritional status, micronutrient deficiencies, HIV infection are also important contributor factors.

The prevalence of anaemia decreased with increasing age and parity; however, after adjusting for covariates it showed that neither age nor parity categories were strongly associated with the severity of anaemia. The highest prevalence of anaemia was during the third trimester. This finding substantiated the fact that the risk of malaria infection occurs during the second trimester and its contribution to the anaemia-malaria related could be expressed later in the pregnancy.

Recently, a review of all pregnancy-related maternal deaths occurred at the central hospital in Maputo city, revealed that 15.5% were attributed to malaria infection mostly associated to severe anaemia and predominantly occurred among adolescent primigravidae (Granja et al., 1998).

These estimates are derived from a hospital-based study in an urban settlement; however, suggest the high burden of malaria and anaemia-malaria related during pregnancy. In rural settlement, the impact of malaria related disease in pregnancy and its possible contribution to infant mortality could be enormous.

13 Conclusion

- This large country-wide survey confirms that malaria, especially that caused by *Plasmodium falciparum,* remains endemic throughout the country and therefore represents a large public health problem in Mozambique.
- Transmission is mostly due to *Anopheles gambiae s.l.* and *Anopheles funestus*.
- There is substantial variation in the intensity of malaria transmission across different geographical and ecological settings throughout the country. This heterogeneity is reflected in the large variation in the prevalence of *Plasmodium falciparum* infection, and is partly a consequence of the variations in the estimated entomological inoculation rates.
- Young children and pregnant women bear the brunt of the infection and this implies exposure to intense malaria transmission.
- The prevalence of malaria infection during pregnancy is high, particularly among young women in their first pregnancy.
- In general, along the coastline and in the flat terrains, malaria transmission can be categorized as hyperendemic. The inland strata across central and southern regions can be categorized as mesoendemic.
- This survey highlights the huge burden of anaemia in the country.
- Among children, the prevalence of severe anaemia is high during the first two years of life. Among pregnant women, the risk of both mild and severe anaemia is high during the first and second trimester of gestation.
- Given that anaemia is a key risk factor for survival and cognitive development, its control should become a Public Health priority for the country.
- Despite the multi-factorial aetiology of anaemia, malaria infection is bound to be one of the key determinants of this high prevalence.

- Across the country we estimate that more than 2.6 million children less than ten years of age are infected with *Plasmodium falciparum* malaria parasites at any time and more than 3.8 million are anaemic
- Across the country more than 660,000 pregnant women are infected with *Plasmodium falciparum* malaria parasites at any time and more than 1.2 million are anaemic
- Maternal malaria infection and anaemia are major contributing factors to the global burden of infant mortality. Interventions to prevent maternal malaria and anaemia are essential, not only for improving maternal health status, but also, for preventing child mortality and improving infant health and survival.
- The burden of malaria disease and anaemia-related malaria during pregnancy and childhood constitutes a major public health problem and warrant integrated and collaborative interventions to its control. Intermittent preventive treatment, insecticide treated nets, mass de-worming, iron and vitamin A supplementation programmes have already proven to be cost-effective interventions, particularly in areas lacking adequate health care services. Moreover, in future malaria vaccines may also contribute to improved control.
- Estimates of the disease burden caused by malaria are crucial for planning cost-effectively malaria control interventions, monitoring and advocacy.
- Both entomological inoculation rates and malaria prevalence should be used to characterize malaria endemicity and guide planning and implementation of appropriate control interventions.

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Appendix 1

Forms used for data collection (in children and pregnant women)

Individual form for data collection during the national survey 2002-2003

INQUÉRITO MALARIOMÉTRICO
FICHA INDIVIDUAL

1. DATA |dia| / |mês| / |ano| |___|___| / |___|___| / |___|___|___|___|

2. CÓDIGO |___|___| . |___|___| . |___|___| |___| . |___|___|

3. NOME |___|___|___|___|___|___|___|___|___|___|___|___|___|___|___|___|___|___|___|___|

4. DATA DE NASCIMENTO |mês|/|ano| |___|___| / |___|___|___|___|

5. SEXO (1=Masculino 2=Femenino) |___|

6. GRÁVIDA (1=Sim 2=Não) |___|

7. SE SIM QUANTOS MESES |___|

8. FEBRE NAS ÚLTIMAS 24 HORAS (1=Sim 2=Não) |___|

9. FEBRE NAS ÚLTIMAS 2 SEMANAS (1=Sim 2=Não) |___|

10. TEMPERATURA AXILAR (^{o}C) |___|___| . |___|

11. FEZ A LÂMINA (1=Sim 2=Não) |___|

12. TESTE RÁPIDO (se temperatura >= 37,5^{o}C) |___|
1=Positivo 2=Negativo 9=não feito

13. HEMOGLOBINA |___|___| . |___|
99.9= não feito

14. NÚMERO DA LÂMINA/PAPEL DE FILTRO (colar aqui o rótulo)

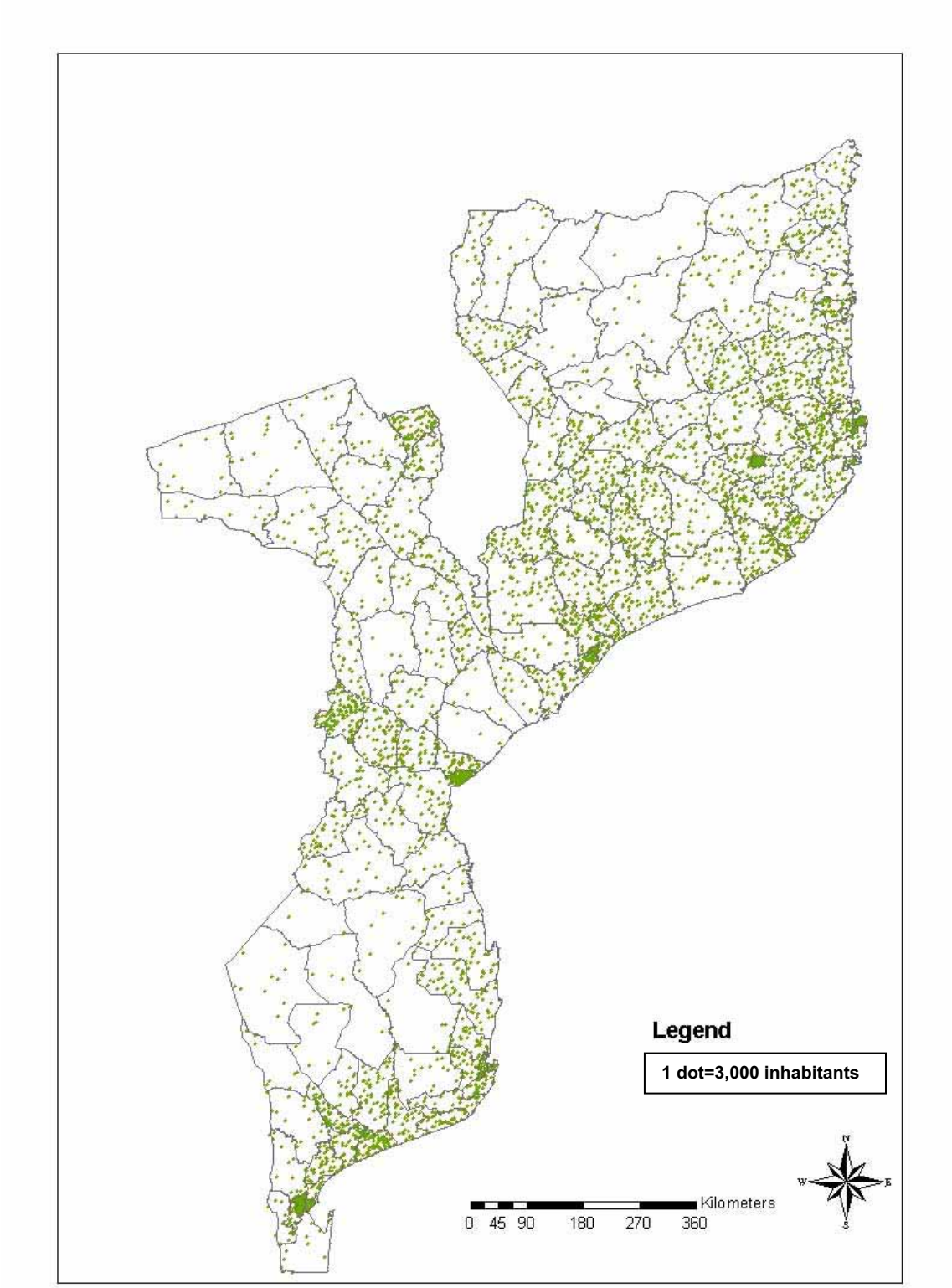
15. OBSERVAÇÕES_____
_____
_____
_____
_____

16. CÓDIGO DO INQUERIDOR |___|___|___|___|

Appendix 2

Map of Mozambique showing population density, across regions

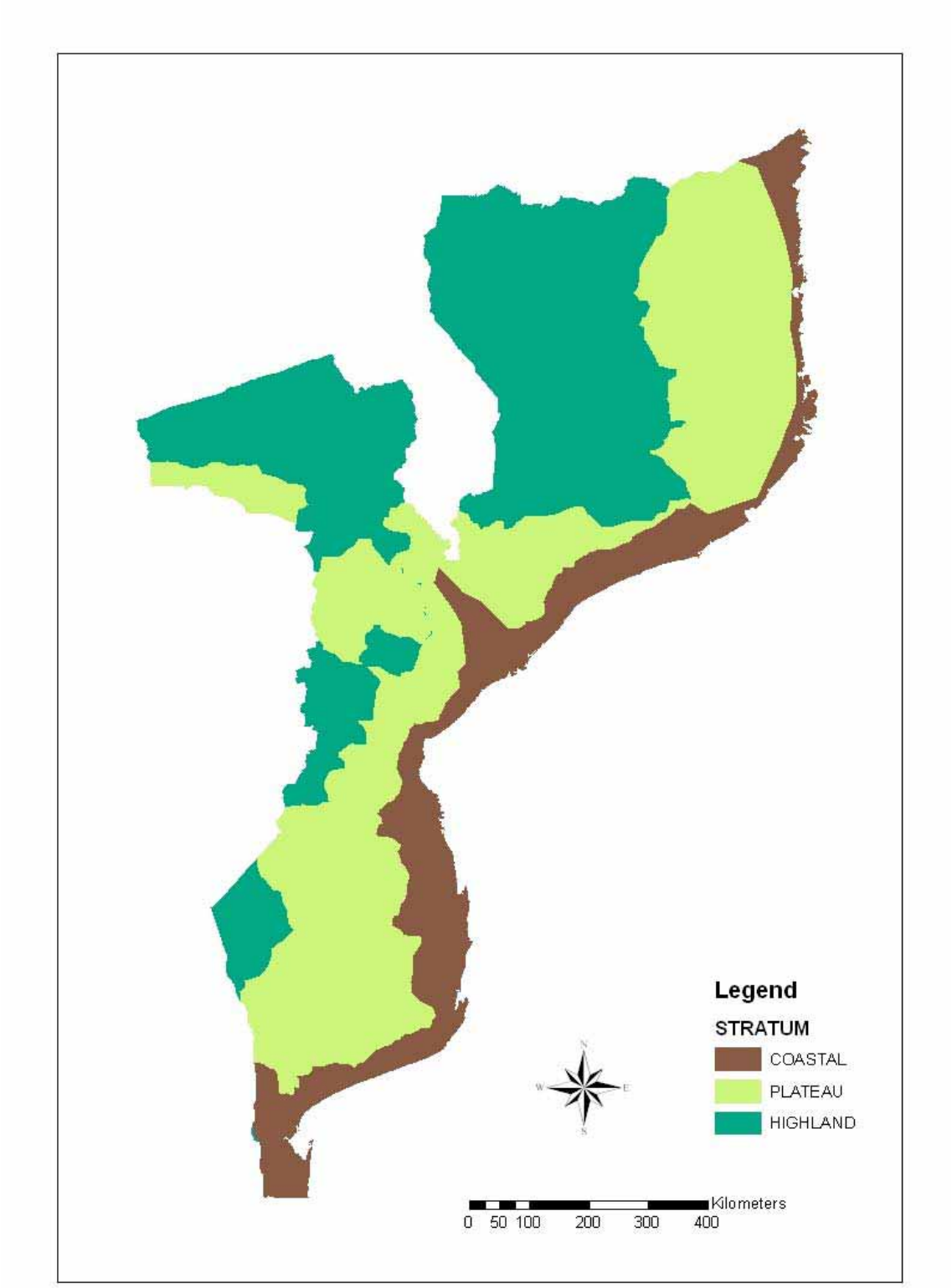
Map of Mozambique showing population density, according to 1997 census



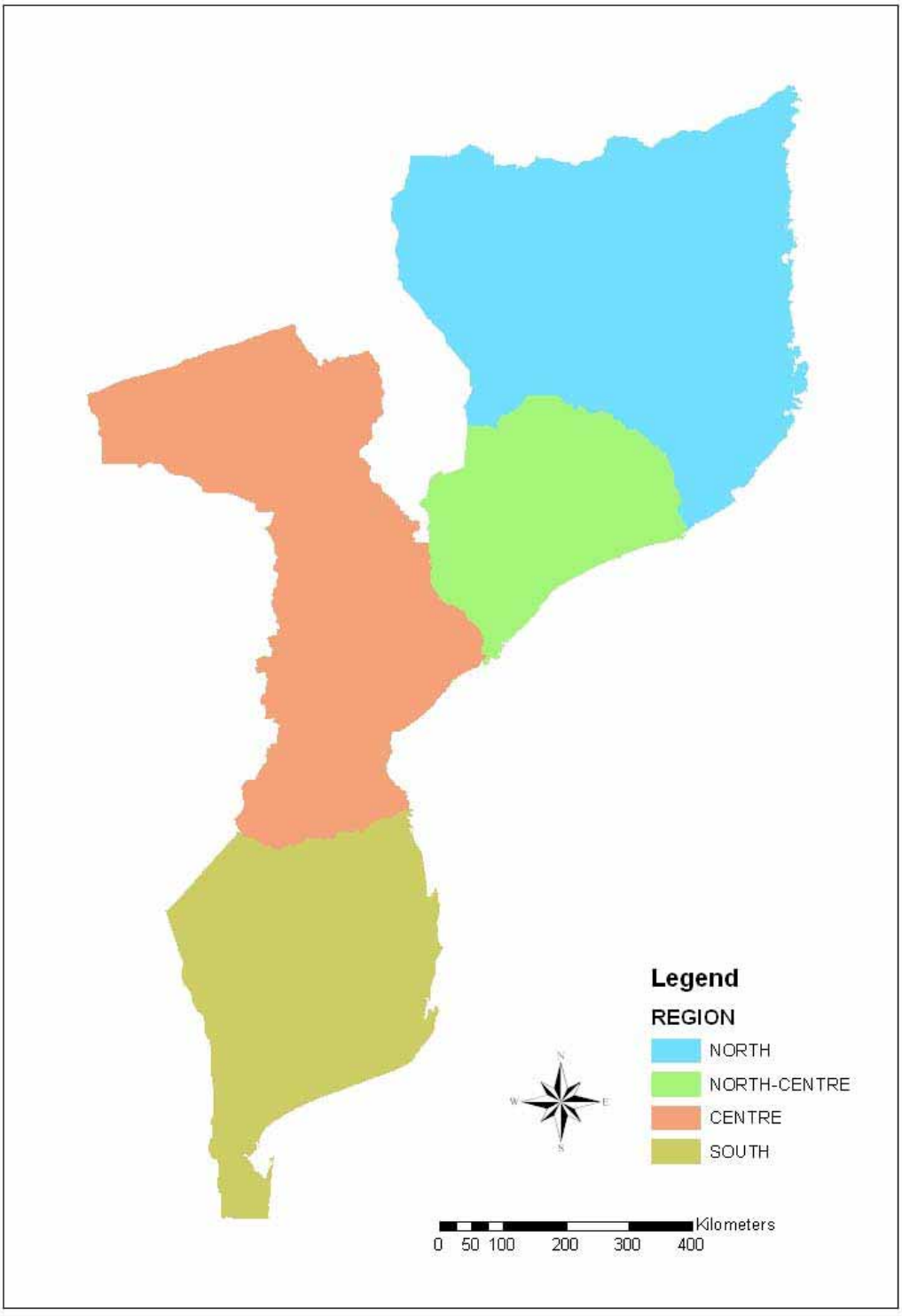
Appendix 3

Maps of Mozambique showing geographical regions and strata acocording to altidude

Map of Mozambique showing the three strata according to altitude



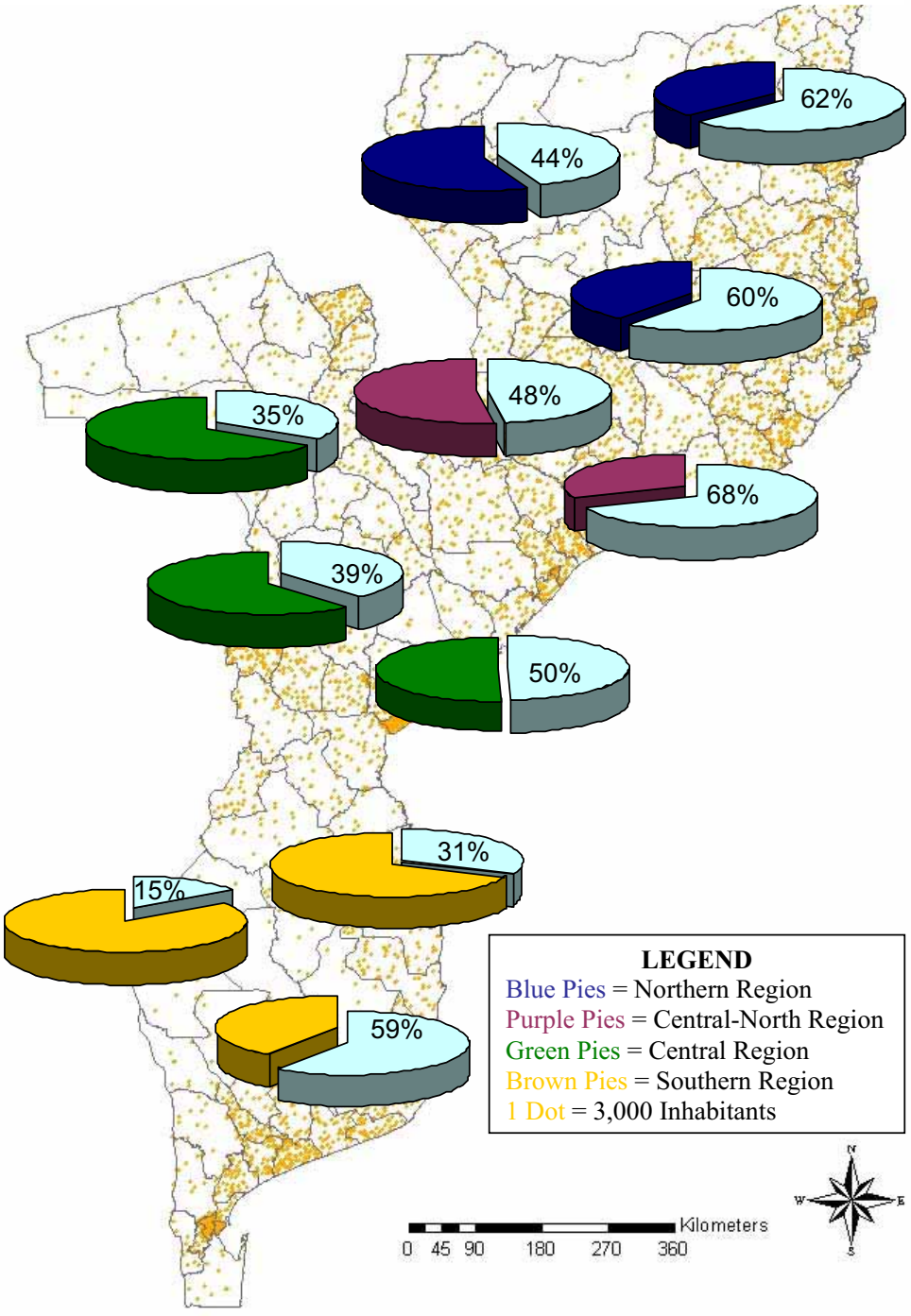
Map of Mozambique showing the four geographical regions



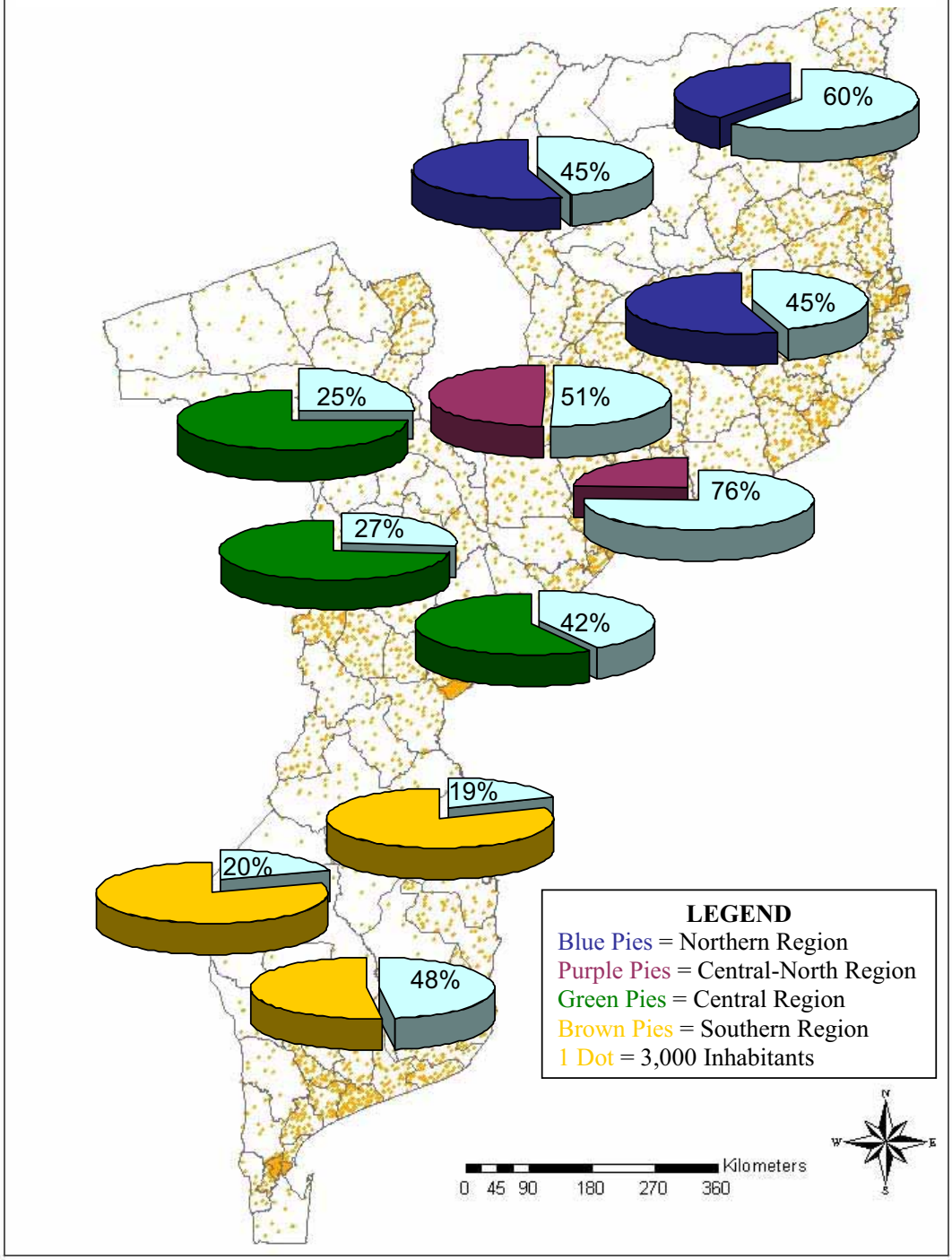
Appendix 4

Maps of malaria infection and anaemia prevalence in children less than ten years of age and in pregnant women across regions in Mozambique

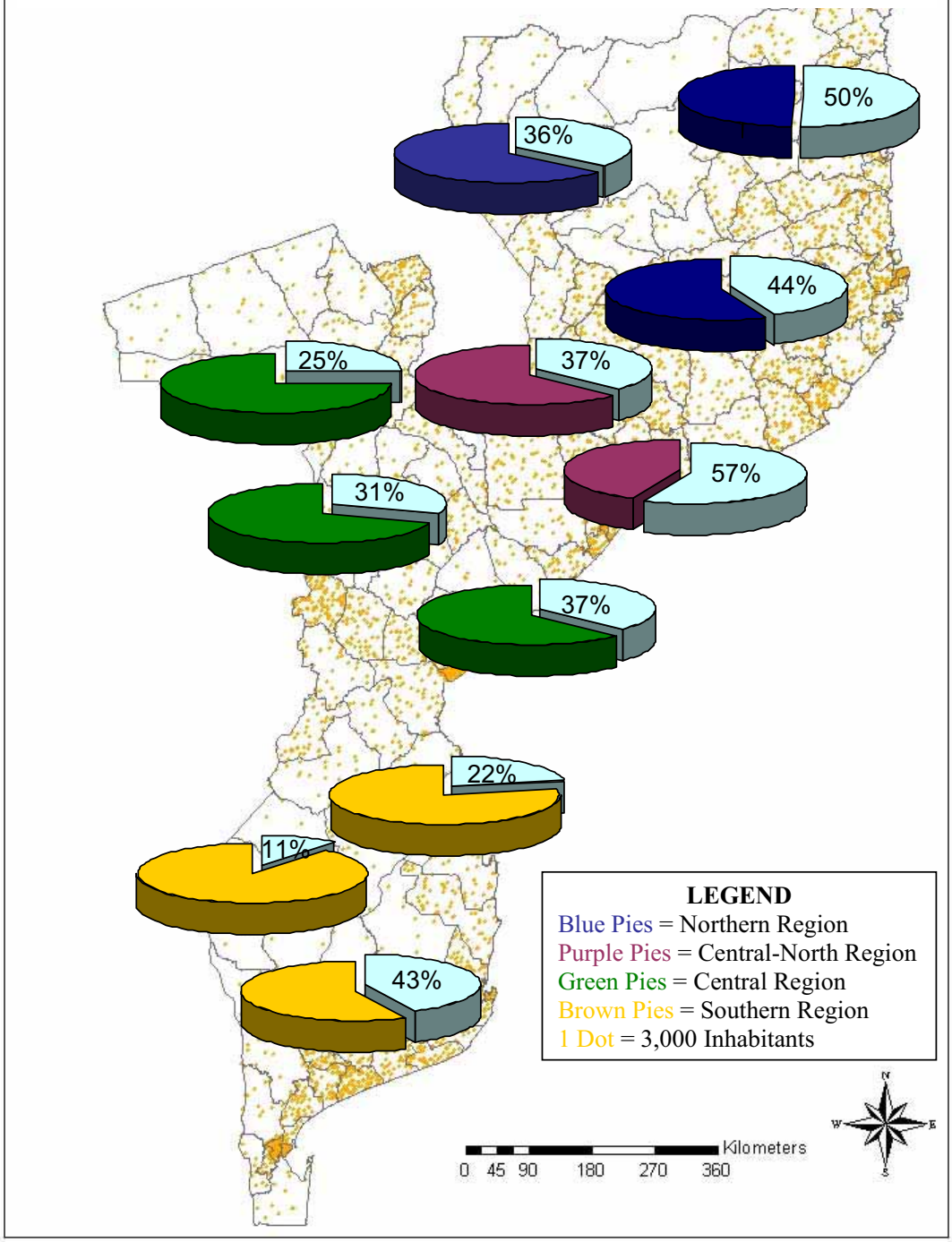
OVERALL PREVALENCE OF *PLASMODIUM FALCIPARUM* INFECTION AMONG CHILDREN LESS THAN TEN YEARS OF AGE IN MOZAMBIQUE (NATIONAL SURVEY 2002 – 2003)



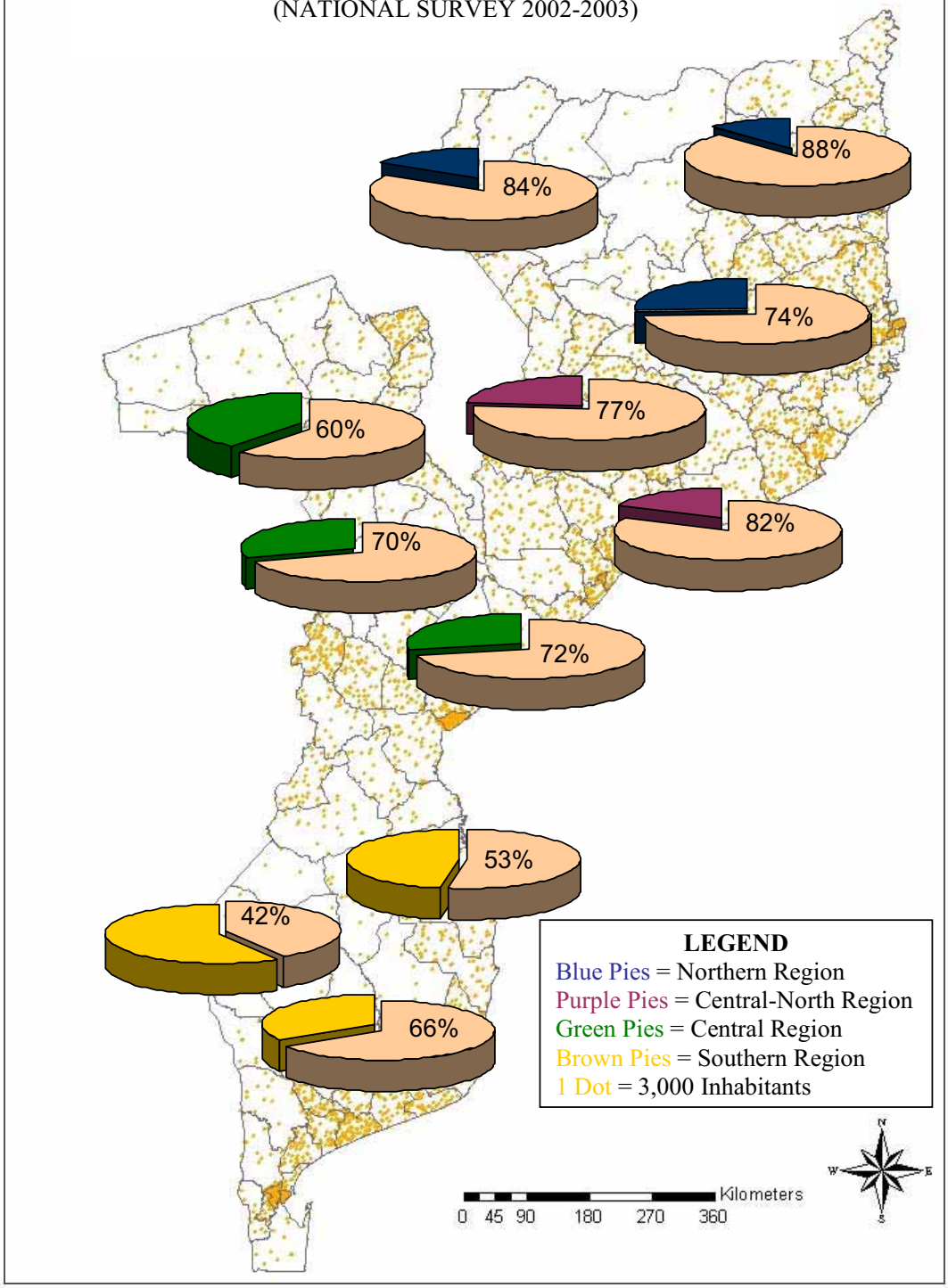
PREVALENCE OF *PLASMODIUM FALCIPARUM* INFECTION AMONG CHILDREN LESS THAN TWELVE MONTHS OF AGE IN MOZAMBIQUE (NATIONAL SURVEY 2002-2003)



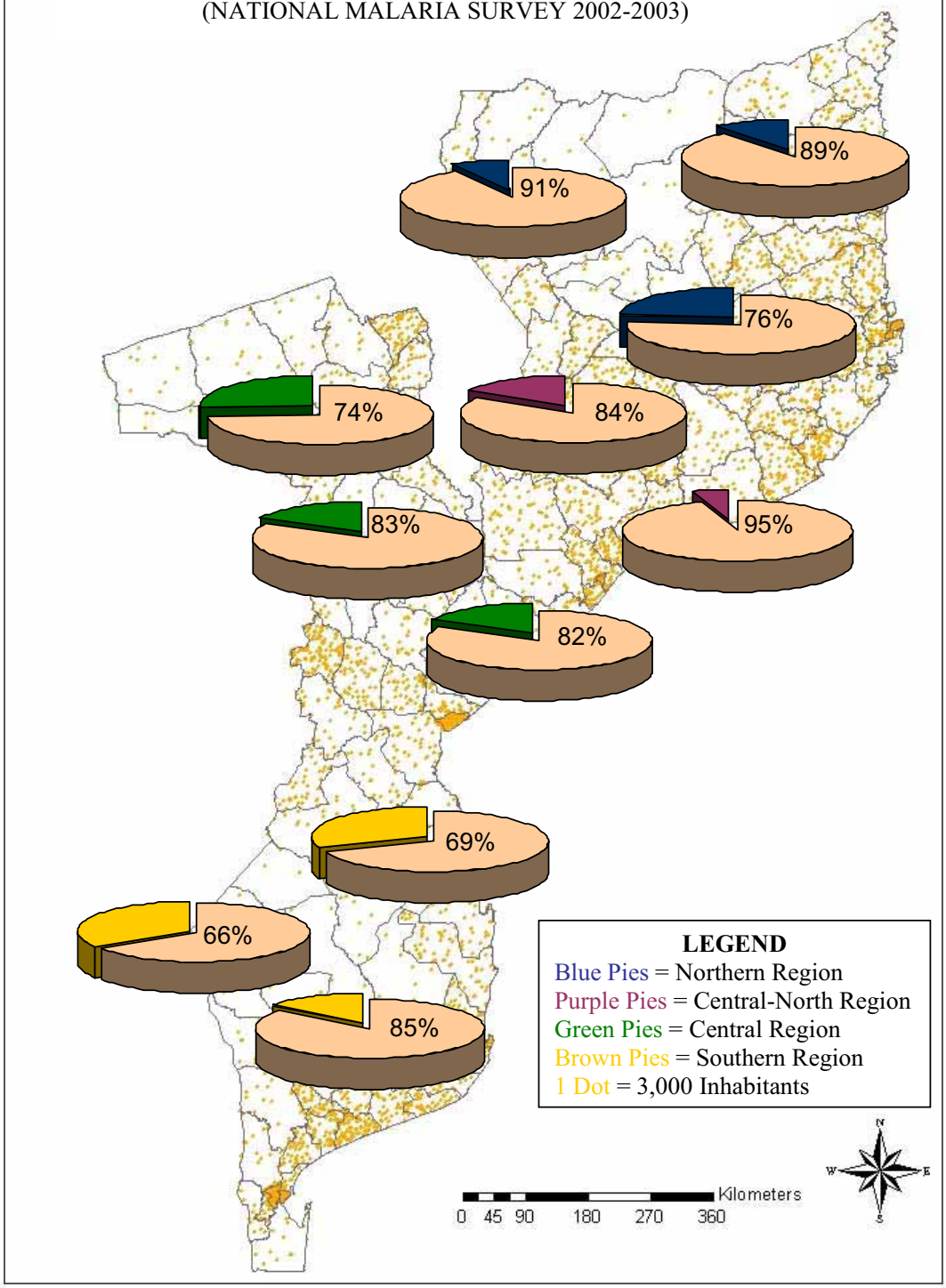
PREVALENCE OF *PLASMODIUM FALCIPARUM* INFECTION AMONG CHILDREN LESS THAN FIVE YEARS OF AGE ACROSS REGIONS IN MOZAMBIQUE (NATIONAL SURVEY 2002-2003)



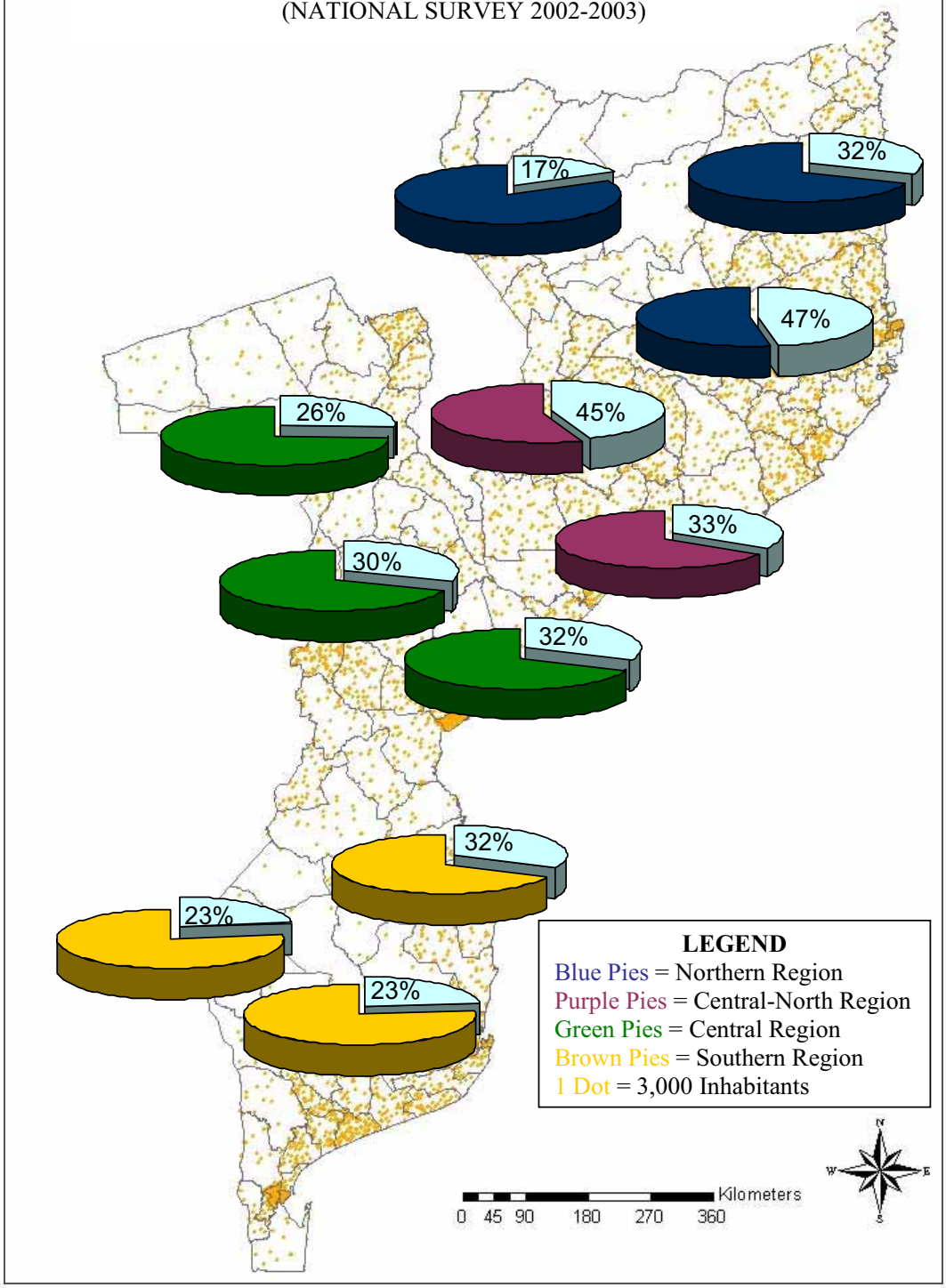
OVERALL PREVALENCE OF ANAEMIA AMONG CHILDREN
LESS THAN TEN YEARS OF AGE IN MOZAMBIQUE
(NATIONAL SURVEY 2002-2003)



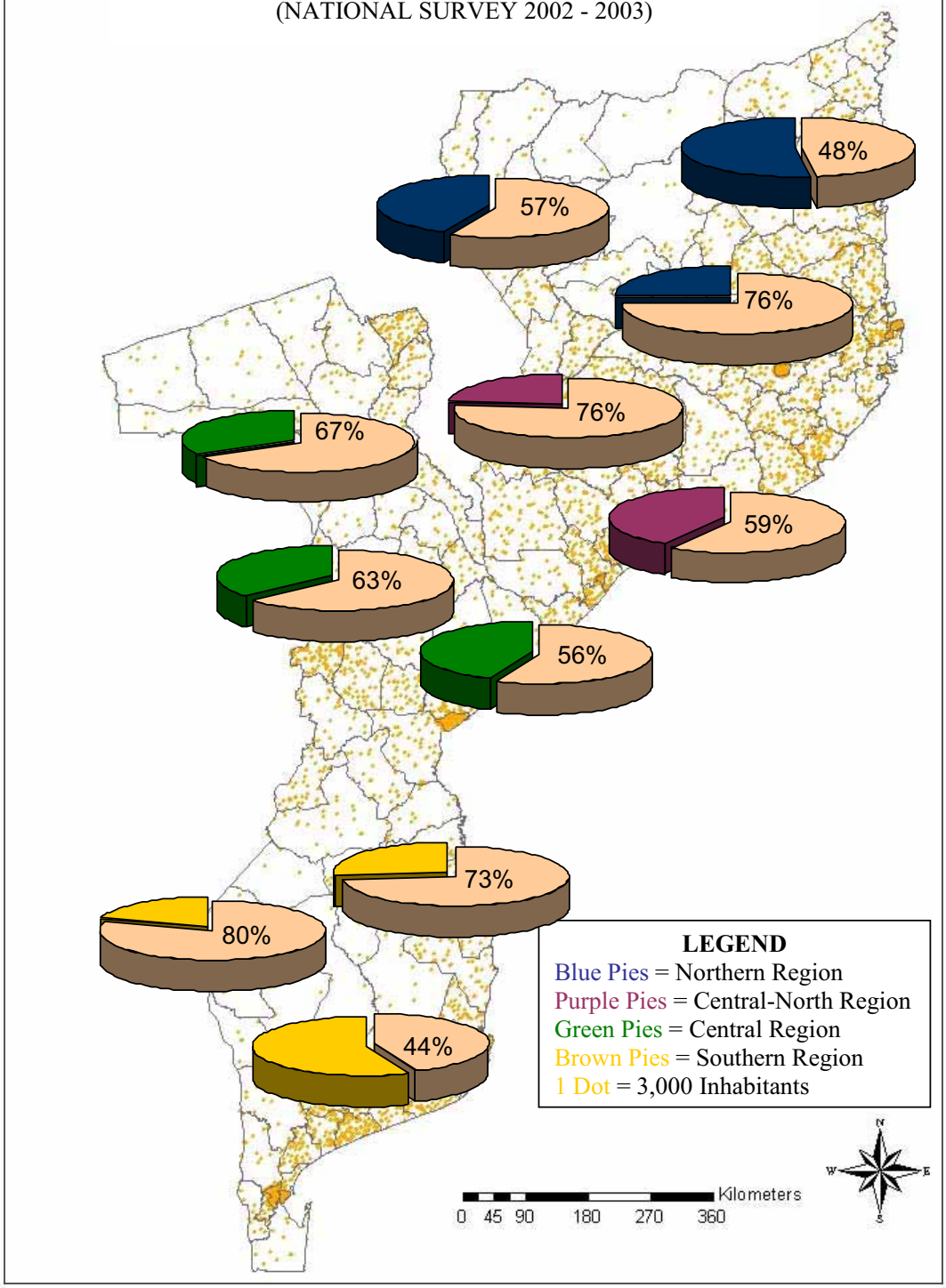
PREVALENCE OF ANAEMIA AMONG CHILDREN LESS THEN TWELVE MONTHS OF AGE IN MOZAMBIQUE
(NATIONAL MALARIA SURVEY 2002-2003)



OVERALL PREVALENCE OF *PLASMODIUM FALCIPARUM* INFECTION AMONG PREGNANT WOMEN IN MOZAMBIQUE (NATIONAL SURVEY 2002-2003)



OVERALL PREVALENCE OF ANAEMIA AMONG
PREGNANT WOMEN IN MOZAMBIQUE
(NATIONAL SURVEY 2002 - 2003)



Appendix 5

Tables of malariometric indicators in children less than ten years of age (and in different age groups) across regions in Mozambique

Table 14. ***Overall distribution of malariometric indicators by age groups among children in Mozambique***

	Overall	<12m	12–23m	24–59m	5 – <7y	7 – <10y	p-Value
P. falciparum (%)	48.6	42.2	55.4	51.3	48.1	39.3	0.0002
Parasite density (CI 95%)	1,211 (1,141-1,286)	1,671 (1,422-1,963)	1,939 (1,698-2,213)	1,172 (1,072-1,281)	673 (577-785)	650 (538-784)	
Fever prevalence	9.4	15.1	13.2	7.1	5.9	6.5	0.0000
Prevalence of fever & parasites	6.3	10.6	10.3	4.6	3.2	3.3	0.0000
Mean hgb g/dl (CI 95%)	9.9 (9.5-10.2)	9.2 (8.6-9.8)	9.0 (8.7-9.4)	9.9 (9.7-10.3)	10.6 (10.3-10.9)	10.9 (10.7-11.3)	
Anaemia (%)	69.8	81.2	83.6	70.7	53.4	46.5	0.0000
Severe anaemia (%)	11.5	20.6	16.7	7.4	3.8	3.8	0.0000
Gametocytes (%)	5.6	6.5	7.2	5.8	3.5	3.3	0.0285
P. malariae (%)	3.6	3.2	5.2	3.9	3.1	1.1	0.0172
Mixed infection (%)	2.9	2.6	4.9	3.0	2.6	0.3	0.0003

P. faciparum = Asexual forms; **Gametocytes** = Sexual forms only for *P. falciparum*; **Mixed infection** = Concomitant presence of *P. falciparum* and *P. malariae*; **Parasite Density** = Geometric mean parasite density only for *P. falciparum*, after log10 transformation (expressed as asexual parasites/μl; **Fever** = Axilar temperature ≥ 37.5 °C; **Fever & Parasites** = Fever associated with one or more asexual forms of *P. falciparum* parasites; **Anaemia** = Haemoglobin < 11 g/dl; **Severe Anaemia** = Haemoglobin < 7 g/dl.

Table 14.*1* ***Overall distribution of malariometric indicators by age groups among children across coastal stratum in Mozambique***

	Overall	<12m	12–23m	24–59m	5 – <7y	7 – <10y	p-Value
P. falciparum (%)	51.5	47.9	57.5	51.3	52.3	44.7	0.3284
Parasite density (CI 95%)	1,449 (1,324-1,586)	1,980 (1,573-2,492)	2,535 (2,114-3,039)	1,366 (1,191-1,567)	707 (547-912)	669 (497-901)	
Fever prevalence	8.9	12.9	13.2	6.5	5.7	8.2	0.0275
Prevalence of fever & parasites	6.1	8.9	10.4	3.9	3.7	3.7	0.1020
Mean hgb g/dl (CI 95%)	9.8 (8.9-10.7)	8.8 (7.2- 10.4)	8.9 (8.0-9.8)	9.9 (9.3-10.7)	10.8 (10.4-11.4)	11.2 (10.5-11.9)	
Anaemia (%)	70.2	82.6	84.1	69.8	50.4	44.9	0.0000
Severe anaemia (%)	13.6	28.6	17.7	7.8	1.2	1.6	0.0035
Gametocytes (%)	6.4	8.2	9.3	6.2	2.5	2.4	0.0220
P. malariae (%)	6.8	6.3	10.8	5.8	6.6	3.3	0.0654
Mixed infection (%)	5.7	5.4	10.3	4.7	5.6	0.9	0.0012

P. faciparum = Asexual forms; **Gametocytes** = Sexual forms only for *P. falciparum*; **Mixed infection** = Concomitant presence of *P. falciparum* and *P. malariae*; **Parasite Density** = Geometric mean parasite density only for *P. falciparum*, after log10 transformation (expressed as asexual parasites/μl; **Fever** = Axilar temperature ≥ 37.5 °C; **Fever & Parasites** = Fever associated with one or more asexual forms of *P. falciparum* parasites; **Anaemia** = Haemoglobin < 11 g/dl; **Severe Anaemia** = Haemoglobin < 7 g/dl.

Table 14.2 ***Overall distribution of malariometric indicators by age groups among children across plateau stratum in Mozambique***

	Overall	<12m	12–23m	24–59m	5 – <7y	7 – <10y	p-Value
P. falciparum (%)	53.8	42.9	60.4	58.6	54.0	45.9	0.0426
Parasite density (CI 95%)	1,077 (959-1,210)	1,694 (1,208-2,375)	1,591 (1,230-2,057)	1,152 (969-1,368)	554 (420-732)	467 (328-664)	
Fever prevalence	9.9	15.9	12.5	8.1	6.7	6.3	0.0647
Prevalence of fever & parasites	7.2	12.3	10.0	5.8	2.9	3.6	0.0420
Mean hgb g/dl (CI 95%)	9.8 (9.4-10.2)	9.2 (8.3-10.1)	8.9 (8.2-9.6)	9.9 (9.4-10.4)	10.6 (9.8-11.4)	11.0 (10.6-11.5)	
Anaemia (%)	70.1	81.1	85.2	71.1	53.1	45.6	0.0156
Severe anaemia (%)	13.5	21.2	19.3	9.5	5.1	4.6	0.0331
Gametocytes (%)	4.4	5.4	5.9	4.4	2.3	2.3	0.3565
P. malariae (%)	2.7	2.3	2.9	3.5	2.3	0.6	0.3383
Mixed infection (%)	1.9	1.7	2.8	2.4	1.8	0.0	0.4250

P. faciparum = Asexual forms; **Gametocytes** = Sexual forms only for *P. falciparum*; **Mixed infection** = Concomitant presence of *P. falciparum* and *P. malariae*; **Parasite Density** = Geometric mean parasite density only for *P. falciparum*, after log10 transformation (expressed as asexual parasites/μl; **Fever** = Axilar temperature ≥ 37.5 °C; **Fever & Parasites** = Fever associated with one or more asexual forms of *P. falciparum* parasites; **Anaemia** = Haemoglobin < 11 g/dl; **Severe Anaemia** = Haemoglobin < 7 g/dl.

Table 14.3 ***Overall distribution of malariometric indicators by age groups among children across highland stratum in Mozambique***

	Overall	<12m	12–23m	24–59m	5 – <7y	7 – <10y	p-Value
P. falciparum (%)	40.2	35.3	46.2	44.0	38.6	28.8	0.0143
Parasite density (CI 95%)	1,056 (948-1,177)	1,282 (949-1,731)	1,531 (1,162-2,018)	981 (837-1,150)	754 (576-987)	863 (619-1,203)	
Fever prevalence	9.1	16.2	14.3	6.6	5.5	5.7	0.0131
Prevalence of fever & parasites	5.7	10.0	10.6	3.9	2.9	2.7	0.0017
Mean hgb g/dl (CI 95%)	10.0 (9.5-10.6)	9.6 (9.1-10.0)	9.4 (8.8-9.9)	10.0 (9.5-10.5)	10.5 (9.9-11.1)	10.8 (10.2- 11.6)	
Anaemia (%)	68.9	79.9	80.9	71.1	56.3	48.5	0.0000
Severe anaemia (%)	7.2	10.1	11.9	4.8	4.7	4.2	0.3490
Gametocytes (%)	6.1	6.1	6.4	6.7	5.4	5.0	0.7718
P. malariae (%)	1.7	1.2	1.6	2.6	1.0	0.4	0.0586
Mixed infection (%)	1.3	0.8	1.2	2.0	0.8	0.3	0.0807

P. faciparum = Asexual forms; **Gametocytes** = Sexual forms only for *P. falciparum*; **Mixed infection** = Concomitant presence of *P. falciparum* and *P. malariae*; **Parasite Density** = Geometric mean parasite density only for *P. falciparum*, after log10 transformation (expressed as asexual parasites/μl; **Fever** = Axilar temperature ≥ 37.5 °C; **Fever & Parasites** = Fever associated with one or more asexual forms of *P. falciparum* parasites; **Anaemia** = Haemoglobin < 11 g/dl; **Severe Anaemia** = Haemoglobin < 7 g/dl.

Table 14.4 ***Overall distribution of malariometric indicators by age groups among children across northern region in Mozambique***

	Overall	<12m	12–23m	24–59m	5 – <7y	7 – <10y	p-Value
P. falciparum (%)	54.8	47.5	63.3	55.9	55.5	46.2	0.2422
Parasite density (CI 95%)	1,077 (965-1,200)	1,114 (851-1,458)	1,516 (1,194-1,925)	1,133 (959-1,338)	589 (424-819)	734 (523-1,029)	
Fever prevalence	12.8	15.9	16.1	11.4	9.3	10.1	0.2213
Prevalence of fever & parasites	9.1	11.8	13.4	7.7	6.2	4.6	0.2128
Mean hgb g/dl (CI 95%)	9.4 (8.3-10.4)	9.0 (6.9-11.0)	8.7 (7.0-10.4)	9.4 (8.5-10.3)	9.9 (9.5-10.3)	10.5 (10.1-10.8)	
Anaemia (%)	77.9	81.2	85.6	79.4	68.2	61.8	0.0930
Severe anaemia (%)	17.1	24.9	24.4	13.4	8.4	7.6	0.0870
Gametocytes (%)	4.9	3.9	5.4	5.5	4.3	4.9	0.4736
P. malariae (%)	4.2	5.9	5.3	4.3	2.8	1.9	0.4766
Mixed infection (%)	3.1	5.3	4.9	3.2	1.3	0.4	0.2088

P. faciparum = Asexual forms; **Gametocytes** = Sexual forms only for *P. falciparum*; **Mixed infection** = Concomitant presence of *P. falciparum* and *P. malariae*; **Parasite Density** = Geometric mean parasite density only for *P. falciparum*, after log10 transformation (expressed as asexual parasites/μl; **Fever** = Axilar temperature ≥ 37.5 °C; **Fever & Parasites** = Fever associated with one or more asexual forms of *P. falciparum* parasites; **Anaemia** = Haemoglobin < 11 g/dl; **Severe Anaemia** = Haemoglobin < 7 g/dl

Table 14.5 ***Overall distribution of malariometric indicators by age groups among children across coastal stratum in northern region of Mozambique***

	Overall	<12m	12–23m	24–59m	5 – <7y	7 – <10y	p-Value
P. falciparum (%)	62.2	60.2	67.6	64.3	46.7	66.5	p=0.2661
Parasite density (CI 95%)	1,016 (852-1,212)	999 (674-1,482)	1,823 (1,245-2,669)	1,068 (813-1,404)	350 (183-666)	656 (396-1,088)	
Fever prevalence	12.6	16.6	17.4	11.1	6.7	9.0	0.4057
Prevalence of fever & parasites	8.5	9.5	12.8	8.5	2.1	5.2	0.4078
Mean hgb g/dl (CI 95%)	8.9 (4.1-13.6)	8.5 (5.8-11.3)	8.3 (1.1-15.5)	8.7 (2.8-14.7)	9.9 (4.8-15.1)	9.9 (8.1-11.7)	
Anaemia (%)	87.8	89.1	94.4	89.9	74.8	78.8	0.3720
Severe anaemia (%)	19.2	27.6	24.5	19.3	4.0	4.9	0.2282
Gametocytes (%)	5.6	4.0	11.2	4.9	2.1	5.2	0.3161
P. malariae (%)	5.1	3.3	7.0	6.8	1.0	2.6	0.1485
Mixed infection (%)	3.8	1.9	7.0	4.9	0.0	1.3	0.5007

P. faciparum = Asexual forms; **Gametocytes** = Sexual forms only for *P. falciparum*; **Mixed infection** = Concomitant presence of *P. falciparum* and *P. malariae*; **Parasite Density** = Geometric mean parasite density only for *P. falciparum*, after log10 transformation (expressed as asexual parasites/μl; **Fever** = Axilar temperature ≥ 37.5 °C; **Fever & Parasites** = Fever associated with one or more asexual forms of *P. falciparum* parasites; **Anaemia** = Haemoglobin < 11 g/dl; **Severe Anaemia** = Haemoglobin < 7 g/dl.

Table 14.6 ***Overall distribution of malariometric indicators by age groups among children across plateau stratum in northern region of Mozambique***

	Overall	<12m	12–23m	24–59m	5 – <7y	7 – <10y	p-Value
P. falciparum (%)	55.9	44.9	65.9	57.7	62.2	42.6	0.3445
Parasite density (CI 95%)	1,162 (961-1,404)	1,451 (838-2,514)	1,428 (966-2,111)	1,317 (984-1,764)	670 (395-1,138)	593 (329-1,068)	
Fever prevalence	13.5	17.1	16.6	11.6	9.9	11.1	0.4460
Prevalence of fever & parasites	9.9	13.9	14.7	7.8	6.4	4.5	0.3288
Mean hgb g/dl (CI 95%)	9.6 (4.4-14.8)	9.3 (2.1-20.7)	8.9 (0.5-17.3)	9.6 (4.9-14.3)	9.9 (8.3-11.6)	10.8 (8.4-13.1)	
Anaemia (%)	73.8	76.2	81.8	75.6	66.5	55.9	0.3291
Severe anaemia (%)	17.1	25.4	24.7	12.8	8.6	6.7	0.3415
Gametocytes (%)	4.6	3.6	4.3	5.6	4.3	3.7	0.5496
P. malariae (%)	4.2	5.4	5.1	4.3	2.8	1.5	0.6481
Mixed infection (%)	3.2	4.1	4.7	3.4	1.4	0.0	0.6444

P. faciparum = Asexual forms; **Gametocytes** = Sexual forms only for *P. falciparum*; **Mixed infection** = Concomitant presence of *P. falciparum* and *P. malariae*; **Parasite Density** = Geometric mean parasite density only for *P. falciparum*, after log10 transformation (expressed as asexual parasites/μl; **Fever** = Axilar temperature ≥ 37.5 °C; **Fever & Parasites** = Fever associated with one or more asexual forms of *P. falciparum* parasites; **Anaemia** = Haemoglobin < 11 g/dl; **Severe Anaemia** = Haemoglobin < 7 g/dl.

Table 14.7 ***Overall distribution of malariometric indicators by age groups among children across highland stratum in northern region of Mozambique***

	Overall	<12m	12–23m	24–59m	5 – <7y	7 – <10y	p-Value
P. falciparum (%)	44.2	45.3	49.0	43.0	39.9	42.2	0.5177
Parasite density (CI 95%)	1,057 (862-1,296)	972 (598-1,581)	1,296 (787-2,134)	1,025 (753-1,396)	849 (488-1,476)	1,190 (555-2,549)	
Fever prevalence	10.6	11.1	12.6	10.6	9.3	6.2	0.6954
Prevalence of fever & parasites	6.9	6.2	8.5	6.2	9.4	4.9	0.5419
Mean hgb g/dl (CI 95%)	9.0 (6.1-11.9)	8.5 (6.2-10.8)	8.3 (3.5-13.1)	9.2 (6.1-12.3)	9.9 (5.2-14.7)	9.5 (8.4-10.6)	
Anaemia (%)	83.7	91.3	92.8	82.4	68.3	73.9	0.1166
Severe anaemia (%)	15.3	20.9	23.2	9.3	11.9	14.3	0.1727
Gametocytes (%)	5.9	5.2	4.6	5.8	6.5	10.6	0.5850
P. malariae (%)	3.5	4.9	4.6	2.4	4.1	3.1	0.5719
Mixed infection (%)	2.2	3.1	3.6	1.3	2.0	1.2	0.4830

P. faciparum = Asexual forms; **Gametocytes** = Sexual forms only for *P. falciparum*; **Mixed infection** = Concomitant presence of *P. falciparum* and *P. malariae*; **Parasite Density** = Geometric mean parasite density only for *P. falciparum*, after log10 transformation (expressed as asexual parasites/μl; **Fever** = Axilar temperature ≥ 37.5 °C; **Fever & Parasites** = Fever associated with one or more asexual forms of *P. falciparum* parasites; **Anaemia** = Haemoglobin < 11 g/dl; **Severe Anaemia** = Haemoglobin < 7 g/dl.

Table 14.8 ***Overall distribution of malariometric indicators by age groups among children across central-northern region of Mozambique***

	Overall	<12m	12–23m	24–59m	5 – <7y	7 – <10y	p-Value
P. falciparum (%)	58.7	64.3	67.7	58.5	51.8	39.9	0.0420
Parasite density (CI 95%)	2,058 (1,836-2,306)	3,494 (2,641-4,621)	3,129 (2,497-3,922)	1,791 (1,501-2,137)	1,059 (779-1,439)	966 (634-1,472)	
Fever prevalence	10.8	22.8	18.0	5.2	6.2	4.9	0.0078
Prevalence of fever & parasites	8.4	18.4	14.6	3.8	4.2	2.8	0.0311
Mean hgb g/dl (CI 95%)	9.4 (8.5-10.3)	8.3 (6.2-10.3)	8.6 (8.5-8.7)	9.7 (8.6-10.8)	10.4 (9.1-11.6)	10.6 (8.5-12.7)	
Anaemia (%)	79.4	89.8	91.0	80.4	62.8	57.7	0.0577
Severe anaemia (%)	12.9	32.8	17.5	5.3	3.8	2.5	0.0407
Gametocytes (%)	8.2	12.1	11.2	7.6	4.9	2.6	0.1556
P. malariae (%)	7.4	7.4	12.4	6.6	6.4	0.9	0.0635
Mixed infection (%)	7.0	7.4	11.6	6.3	5.9	0.9	0.0911

P. faciparum = Asexual forms; **Gametocytes** = Sexual forms only for *P. falciparum*; **Mixed infection** = Concomitant presence of *P. falciparum* and *P. malariae*; **Parasite Density** = Geometric mean parasite density only for *P. falciparum*, after log10 transformation (expressed as asexual parasites/μl; **Fever** = Axilar temperature ≥ 37.5 °C; **Fever & Parasites** = Fever associated with one or more asexual forms of *P. falciparum* parasites; **Anaemia** = Haemoglobin < 11 g/dl; **Severe Anaemia** = Haemoglobin < 7 g/dl.

Table 14.9 ***Overall distribution of malariometric indicators by age groups across coastal stratum in central-northern region of Mozambique***

	Overall	<12m	12–23m	24–59m	5 – <7y	7 – <10y	p-Value
P. falciparum (%)	68.5	75.8	74.9	65.3	63.8	51.7	0.2707
Parasite density (CI 95%)	2,288 (1,977-2,648)	3,860 (2,751-5,417)	3,309 (2,539-4,313)	2,029 (1,602-2,571)	1,063 (710-1,590)	651 (351-1,204)	
Fever prevalence	11.3	20.2	17.6	5.7	7.5	5.9	0.1494
Prevalence of fever & parasites	9.1	17.5	14.4	4.1	6.0	1.8	0.3064
Mean hgb g/dl (CI 95%)	9.1 (6.2-12.0)	7.3 (1.4-13.2)	8.3 (7.7- 8.9)	9.6 (5.3-13.9)	10.6 (10.1-11.1)	10.8 (6.3-15.2)	
Anaemia (%)	81.9	94.9	93.2	80.6	58.6	59.1	0.1145
Severe anaemia (%)	18.4	48.7	21.6	6.9	0.0	0.0	0.1098
Gametocytes (%)	10.6	15.6	12.8	10.1	4.4	3.7	0.3970
P. malariae (%)	12.5	13.5	18.9	9.5	12.8	1.9	0.2300
Mixed infection (%)	11.8	12.9	18.0	8.9	11.9	1.8	0.2606

P. faciparum = Asexual forms; **Gametocytes** = Sexual forms only for *P. falciparum*; **Mixed infection** = Concomitant presence of *P. falciparum* and *P. malariae*; **Parasite Density** = Geometric mean parasite density only for *P. falciparum*, after log10 transformation (expressed as asexual parasites/μl; **Fever** = Axilar temperature ≥ 37.5 °C; **Fever & Parasites** = Fever associated with one or more asexual forms of *P. falciparum* parasites; **Anaemia** = Haemoglobin < 11 g/dl; **Severe Anaemia** = Haemoglobin < 7 g/dl.

Table 14.10 ***Overall distribution of malariometric indicators by age groups among children across highland stratum in central-northern region of Mozambique***

	Overall	<12m	12–23m	24–59m	5 – <7y	7 – <10y	p-Value
P. falciparum (%)	47.9	50.6	55.7	51.2	40.5	34.2	0.2646
Parasite density (CI 95%)	1,766 (1,473-2,118)	2,926 (1,774-4,827)	2,777 (1,800-4,284)	1,541 (1,180-2,012)	1,054 (647-1,718)	1,258 (707-2,237)	
Fever prevalence	10.3	25.9	18.9	4.7	4.9	4.4	0.1086
Prevalence of fever & parasites	7.6	19.5	14.8	3.4	2.5	3.3	0.1716
Mean hgb g/dl (CI 95%)	9.8 (5.1-14.5)	9.4 (8.7-10.2)	9.2 (6.9-11.5)	9.8 (5.2-14.3)	10.1 (3.5-16.7)	10.5 (1.7-19.4)	
Anaemia (%)	76.8	83.7	87.3	80.1	66.7	56.9	0.2985
Severe anaemia (%)	6.6	11.2	10.1	3.6	6.9	3.8	0.2155
Gametocytes (%)	5.7	7.8	8.4	4.9	5.5	2.1	0.2864
P. malariae (%)	1.8	0.9	1.2	3.5	0.4	0.5	0.2564
Mixed infection (%)	1.8	0.9	0.9	3.5	0.4	0.5	0.2866

P. faciparum = Asexual forms; **Gametocytes** = Sexual forms only for *P. falciparum*; **Mixed infection** = Concomitant presence of *P. falciparum* and *P. malariae*; **Parasite Density** = Geometric mean parasite density only for *P. falciparum*, after log10 transformation (expressed as asexual parasites/μl; **Fever** = Axilar temperature ≥ 37.5 °C; **Fever & Parasites** = Fever associated with one or more asexual forms of *P. falciparum* parasites; **Anaemia** = Haemoglobin < 11 g/dl; **Severe Anaemia** = Haemoglobin < 7 g/dl.

Table 14.11 ***Overall distribution of malariometric indicators by age groups among children across central region of Mozambique***

	Overall	<12m	12–23m	24–59m	5 – <7y	7 – <10y	p-Value
P. falciparum (%)	36.8	24.8	41.2	41.9	41.4	25.7	0.0005
Parasite density (CI 95%)	891 (799-994)	1,152 (825-1,607)	1,609 (1,242-2,085)	827 (706-969)	580 (446-753)	517 (361-740)	
Fever prevalence	6.9	9.3	9.2	6.2	4.8	5.9	0.0176
Prevalence of fever & parasites	3.7	4.6	5.9	3.6	1.5	2.6	0.0131
Mean hgb g/dl (CI 95%)	10.2 (9.8-10.7)	9.7 (9.4-10.1)	9.5 (9.1-9.9)	10.2 (9.7-10.7)	10.8 (10.4-11.3)	11.1 (10.9-11.3)	
Anaemia (%)	64.0	77.4	77.6	65.4	48.9	42.3	0.0001
Severe anaemia (%)	7.1	9.5	10.7	6.0	2.5	3.7	0.1437
Gametocytes (%)	7.3	6.6	9.1	8.2	5.4	5.2	0.3923
P. malariae (%)	1.4	0.4	0.9	2.6	1.0	0.1	0.0457
Mixed infection (%)	0.9	0.1	0.8	1.6	0.8	0.1	0.0955

P. faciparum = Asexual forms; **Gametocytes** = Sexual forms only for *P. falciparum*; **Mixed infection** = Concomitant presence of *P. falciparum* and *P. malariae*; **Parasite Density** = Geometric mean parasite density only for *P. falciparum*, after log10 transformation (expressed as asexual parasites/μl; **Fever** = Axilar temperature ≥ 37.5 °C; **Fever & Parasites** = Fever associated with one or more asexual forms of *P. falciparum* parasites; **Anaemia** = Haemoglobin < 11 g/dl; **Severe Anaemia** = Haemoglobin < 7 g/dl.

Table 14.12 ***Overall distribution of malariometric indicators by age groups among children across coastal stratum in central region of Mozambique***

	Overall	<12m	12–23m	24–59m	5 – <7y	7 – <10y	p-Value
P. falciparum (%)	49.5	41.7	48.7	52.6	51.5	49.2	0.3419
Parasite density (CI 95%)	1,101 (916-1,325)	1,252 (721-2,176)	2,186 (1,493-3,202)	1,017 (776-1,334)	569 (345-937)	675 (349-1,305)	
Fever prevalence	4.9	6.7	4.6	5.6	1.3	5.6	0.3747
Prevalence of fever & parasites	2.8	3.9	2.6	3.4	0.0	3.2	0.3426
Mean hgb g/dl (CI 95%)	9.8 (9.4-10.2)	9.4 (8.2-10.7)	9.0 (8.2-9.8)	9.8 (9.2-10.4)	10.6 (10.1-11.2)	10.9 (8.3-13.6)	
Anaemia (%)	71.8	82.1	85.1	74.9	51.9	41.3	0.1088
Severe anaemia (%)	10.7	13.5	19.1	5.1	8.3	7.7	0.2248
Gametocytes (%)	9.7	11.9	15.3	8.1	6.9	3.9	0.2471
P. malariae (%)	4.2	0.8	0.9	7.8	4.3	3.2	0.3823
Mixed infection (%)	3.0	0.8	0.7	5.8	1.7	3.2	0.3921

P. faciparum = Asexual forms; **Gametocytes** = Sexual forms only for *P. falciparum*; **Mixed infection** = Concomitant presence of *P. falciparum* and *P. malariae*; **Parasite Density** = Geometric mean parasite density only for *P. falciparum*, after log10 transformation (expressed as asexual parasites/μl; **Fever** = Axilar temperature ≥ 37.5 °C; **Fever & Parasites** = Fever associated with one or more asexual forms of *P. falciparum* parasites; **Anaemia** = Haemoglobin < 11 g/dl; **Severe Anaemia** = Haemoglobin < 7 g/dl.

Table 14.13 ***Overall distribution of malariometric indicators by age groups among children across plateau stratum in central region of Mozambique***

	Overall	<12m	12–23m	24–59m	5 – <7y	7 – <10y	p-Value
P. falciparum (%)	38.9	27.3	41.9	43.3	45.6	27.6	0.2098
Parasite density (CI 95%)	926 (758-1,131)	1,450 (792-2,653)	1,623 (1,012-2,601)	876 (657-1,168)	586 (367-935)	368 (176-771)	
Fever prevalence	5.1	7.2	6.4	4.4	3.9	4.1	0.5187
Prevalence of fever & parasites	2.9	5.3	2.8	2.7	0.0	3.4	0.3663
Mean hgb g/dl (CI 95%)	9.9 (5.6-14.1)	9.4 (7.8-10.9)	9.2 (8.9-9.6)	9.9 (4.3-15.5)	10.6 (2.6-18.6)	10.9 (8.6-13.2)	
Anaemia (%)	69.6	82.7	84.2	68.3	53.8	46.8	0.1336
Severe anaemia (%)	9.9	12.6	11.0	9.2	6.6	7.6	0.7062
Gametocytes (%)	7.7	7.8	13.1	7.4	4.5	2.8	0.4099
P. malariae (%)	1.3	0.0	0.9	2.7	0.7	0.0	0.2394
Mixed infection (%)	0.9	0.0	0.9	1.5	0.7	0.0	0.3612

P. faciparum = Asexual forms; **Gametocytes** = Sexual forms only for *P. falciparum*; **Mixed infection** = Concomitant presence of *P. falciparum* and *P. malariae*; **Parasite Density** = Geometric mean parasite density only for *P. falciparum*, after log10 transformation (expressed as asexual parasites/μl; **Fever** = Axilar temperature ≥ 37.5 °C; **Fever & Parasites** = Fever associated with one or more asexual forms of *P. falciparum* parasites; **Anaemia** = Haemoglobin < 11 g/dl; **Severe Anaemia** = Haemoglobin < 7 g/dl.

Table 14.14 ***Overall distribution of malariometric indicators by age groups among children across highland stratum in central region of Mozambique***

	Overall	<12m	12–23m	24–59m	5 – <7y	7 – <10y	p-Value
P. falciparum (%)	34.5	21.6	39.5	40.3	38.8	23.5	0.0168
Parasite density (CI 95%)	720 (600-865)	848 (460-1,562)	1,125 (677-1,870)	674 (518-878)	584 (381-895)	553 (321-953)	
Fever prevalence	8.0	10.6	11.7	7.1	5.6	6.7	0.0727
Prevalence of fever & parasites	4.1	4.4	8.3	3.9	2.1	2.2	0.0705
Mean hgb g/dl (CI 95%)	10.4 (9.9-10.9)	9.9 (9.5-10.4)	9.8 (8.9-10.8)	10.4 (9.9-10.7)	10.9 (10.7-11.2)	11.2 (11.0-11.3)	
Anaemia (%)	60.5	74.3	72.4	62.9	46.8	40.7	0.0055
Severe anaemia (%)	5.1	7.4	8.9	4.5	0.0	1.8	0.0759
Gametocytes (%)	6.9	5.4	5.6	8.6	5.5	6.2	0.6018
P. malariae (%)	1.1	0.4	0.8	2.1	0.8	0.0	0.3171
Mixed infection (%)	0.7	0.0	0.8	1.3	0.8	0.0	0.6502

P. faciparum = Asexual forms; **Gametocytes** = Sexual forms only for *P. falciparum*; **Mixed infection** = Concomitant presence of *P. falciparum* and *P. malariae*; **Parasite Density** = Geometric mean parasite density only for *P. falciparum*, after log10 transformation (expressed as asexual parasites/μl; **Fever** = Axilar temperature ≥ 37.5 °C; **Fever & Parasites** = Fever associated with one or more asexual forms of *P. falciparum* parasites; **Anaemia** = Haemoglobin < 11 g/dl; **Severe Anaemia** = Haemoglobin < 7 g/dl.

Table 14.15 ***Overall distribution of malariometric indicators by age groups among children across southern region of Mozambique***

	Overall	<12m	12–23m	24–59m	5 – <7y	7 – <10y	p-Value
P. falciparum (%)	44.6	33.7	44.9	49.1	45.4	46.8	0.0695
Parasite density (CI 95%)	1,193 (1,025-1,388)	2,037 (1,305-3,179)	1,918 (1,291-2,848)	1,374 (1,105-1,708)	578 (410-815)	515 (342-776)	
Fever prevalence	7.2	12.6	7.9	5.9	4.5	5.5	0.1970
Prevalence of fever & parasites	4.4	7.9	5.7	3.6	1.6	3.4	0.1153
Mean hgb g/dl (CI 95%)	10.4 (9.8-10.9)	9.6 (8.4-10.9)	9.4 (8.1-10.8)	10.5 (10.1-10.9)	11.2 (10.9-11.4)	11.5 (11.2-11.8)	
Anaemia (%)	58.9	77.4	77.9	58.6	38.5	32.1	0.0018
Severe anaemia (%)	7.6	14.1	11.7	4.3	0.0	0.0	0.2143
Gametocytes (%)	2.0	4.2	2.7	1.8	0.0	0.9	0.2760
P. malariae (%)	1.6	0.4	0.7	2.4	2.2	1.7	0.2670
Mixed infection (%)	0.8	0.0	0.7	1.1	1.9	0.0	0.3892

P. faciparum = Asexual forms; **Gametocytes** = Sexual forms only for *P. falciparum*; **Mixed infection** = Concomitant presence of *P. falciparum* and *P. malariae*; **Parasite Density** = Geometric mean parasite density only for *P. falciparum*, after log10 transformation (expressed as asexual parasites/μl; **Fever** = Axilar temperature ≥ 37.5 °C; **Fever & Parasites** = Fever associated with one or more asexual forms of *P. falciparum* parasites; **Anaemia** = Haemoglobin < 11 g/dl; **Severe Anaemia** = Haemoglobin < 7 g/dl.

Table 14.16 ***Overall distribution of malariometric indicators by age groups among children across coastal stratum in southern region of Mozambique***

	Overall	<12m	12–23m	24–59m	5 – <7y	7 – <10y	p-Value
P. falciparum (%)	31.1	18.8	24.7	34.5	41.8	34.2	0.1402
Parasite density (CI 95%)	1,484 (1,185-1,895)	2,660 (1,289-5,485)	2,757 (1,459-5,210)	1,543 (1,109-2,148)	912 (514-1,619)	705 (332-1,494)	
Fever prevalence	5.9	5.7	5.4	5.9	4.1	9.6	0.6303
Prevalence of fever & parasites	2.8	1.3	3.7	2.6	2.3	4.4	0.2784
Mean hgb g/dl (CI 95%)	10.7 (9.9-11.5)	10.3 (9.6-11.0)	10.2 (9.5-10.9)	10.7 (9.8-11.6)	11.3 (10.5-11.9)	11.8 (11.3-12.3)	
Anaemia (%)	52.8	69.2	63.9	53.5	34.9	27.6	0.0636
Severe anaemia (%)	3.6	5.3	3.5	3.9	0.0	0.0	0.3536
Gametocytes (%)	1.8	2.1	1.4	2.8	0.0	0.6	0.3059
P. malariae (%)	1.7	0.9	0.0	1.9	1.8	4.4	0.3638
Mixed infection (%)	0.4	0.0	0.0	0.6	0.9	0.0	0.6088

P. faciparum = Asexual forms; **Gametocytes** = Sexual forms only for *P. falciparum*; **Mixed infection** = Concomitant presence of *P. falciparum* and *P. malariae*; **Parasite Density** = Geometric mean parasite density only for *P. falciparum*, after log10 transformation (expressed as asexual parasites/μl; **Fever** = Axilar temperature ≥ 37.5 °C; **Fever & Parasites** = Fever associated with one or more asexual forms of *P. falciparum* parasites; **Anaemia** = Haemoglobin < 11 g/dl; **Severe Anaemia** = Haemoglobin < 7 g/dl.

Table 14.17 ***Overall distribution of malariometric indicators by age groups among children across plateau stratum in southern region of Mozambique***

	Overall	<12m	12–23m	24–59m	5 – <7y	7 – <10y	p-Value
P. falciparum (%)	59.5	47.7	64.2	70.6	50.7	56.7	0.1445
Parasite density (CI 95%)	1,149 (926-1,426)	2,394 (1,285-4,461)	1,906 (1,135-3,201)	1,355 (991-1,854)	401 (262-613)	436 (244-778)	
Fever prevalence	8.6	18.6	10.2	5.9	5.1	2.9	0.2080
Prevalence of fever & parasites	6.3	13.8	7.7	5.2	1.3	2.9	0.2360
Mean hgb g/dl (CI 95%)	10.0 (9.7-10.4)	9.0 (4.5-13.6)	8.7 (5.3-12.1)	10.2 (8.1-12.4)	11.1 (10.6-11.6)	11.3 (10.7-11.9)	
Anaemia (%)	65.9	85.4	90.7	66.9	42.1	36.0	0.1052
Severe anaemia (%)	10.9	20.9	16.9	4.8	0.0	0.0	0.3225
Gametocytes (%)	2.3	6.3	3.8	0.8	0.0	0.8	0.3968
P. malariae (%)	1.6	0.0	1.3	3.1	2.5	0.0	0.3626
Mixed infection (%)	1.2	0.0	1.3	1.7	2.5	0.0	0.4156

P. faciparum = Asexual forms; **Gametocytes** = Sexual forms only for *P. falciparum*; **Mixed infection** = Concomitant presence of *P. falciparum* and *P. malariae*; **Parasite Density** = Geometric mean parasite density only for *P. falciparum*, after log10 transformation (expressed as asexual parasites/μl; **Fever** = Axilar temperature ≥ 37.5 °C; **Fever & Parasites** = Fever associated with one or more asexual forms of *P. falciparum* parasites; **Anaemia** = Haemoglobin < 11 g/dl; **Severe Anaemia** = Haemoglobin < 7 g/dl.

Table 14.18 ***Overall distribution of malariometric indicators by age groups among children across highland stratum in southern region of Mozambique***

	Overall	<12m	12–23m	24–59m	5 – <7y	7 – <10y	p-Value
P. falciparum (%)	15.2	19.7	11.0	15.2	10.4	21.6	0.3839
Parasite density (CI 95%)	469 (294-746)	416 (109-1,585)	309 39-2,441)	692 (311-1,539)	324 (73-1,427)	419 (154-1,141)	
Fever prevalence	4.8	15.5	3.6	3.3	0.0	2.3	0.1543
Prevalence of fever & parasites	1.2	5.3	1.7	0.0	0.0	0.0	0.2986
Mean hgb g/dl (CI 95%)	11.1 (6.3-15.9)	10.4 (7.6-13.2)	10.3 (9.2-11.5)	11.3 (6.7-15.9)	11.6 (8.8-14.4)	11.9 (7.3-16.6)	
Anaemia (%)	41.7	66.1	64.3	35.7	27.0	14.7	0.1480
Severe anaemia (%)	2.3	2.5	2.9	2.6	0.0	0.0	0.7586
Gametocytes (%)	0.9	0.0	1.9	0.0	0.0	4.9	0.4797
P. malariae (%)	0.6	0.0	0.0	0.8	1.8	0.0	0.6670
Mixed infection (%)	0.3	0.0	0.0	0.0	1.8	0.0	0.6185

P. faciparum = Asexual forms; **Gametocytes** = Sexual forms only for *P. falciparum*; **Mixed infection** = Concomitant presence of *P. falciparum* and *P. malariae*; **Parasite Density** = Geometric mean parasite density only for *P. falciparum*, after log10 transformation (expressed as asexual parasites/μl; **Fever** = Axilar temperature ≥ 37.5 °C; **Fever & Parasites** = Fever associated with one or more asexual forms of *P. falciparum* parasites; **Anaemia** = Haemoglobin < 11 g/dl; **Severe Anaemia** = Haemoglobin < 7 g/dl.

Appendix 6

Figures showing sensivity and specificity of malaria case definition in children less than ten years of age (and in different age groups) across regions in Mozambique

Figure 18. Sensitivity (solid lines) and Specificity (dashed lines) of malaria cases definition by age category.
A.-Overall – countrywide, children under 10 years of age

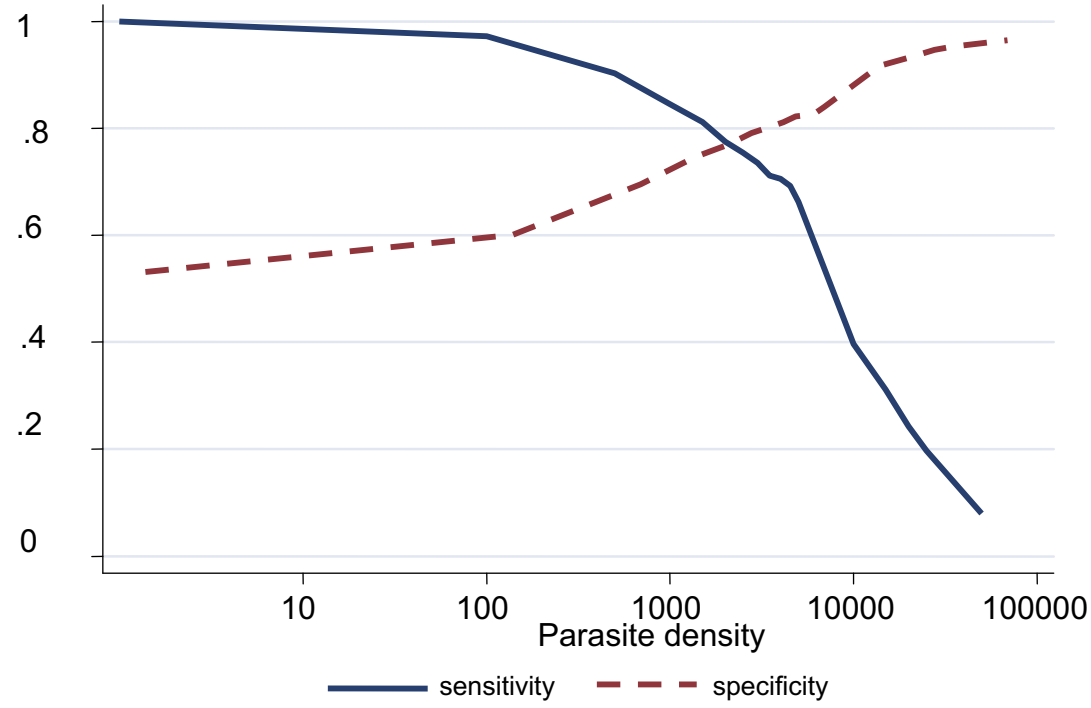


Figure 18.1
B.-Overall – countrywide, children less than 12 months of age

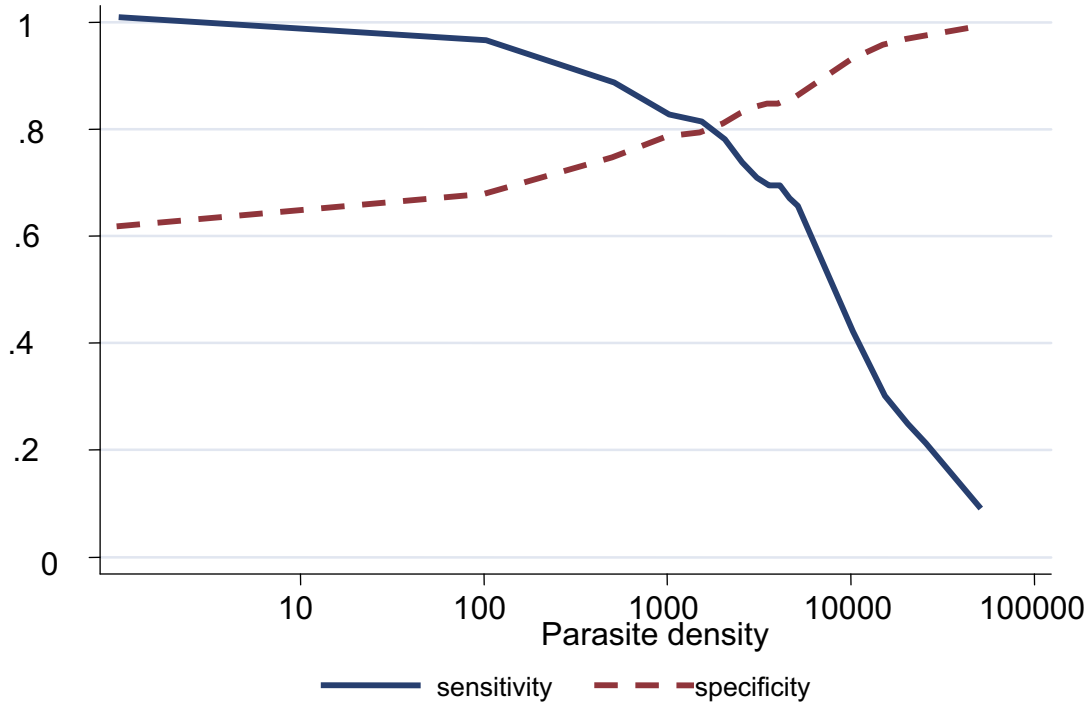


Figure 18.2 Sensitivity (solid lines) and Specificity (dashed lines) of malaria cases definition by age category

A.-*Overall – countrywide, between 12 and 59 months of age*

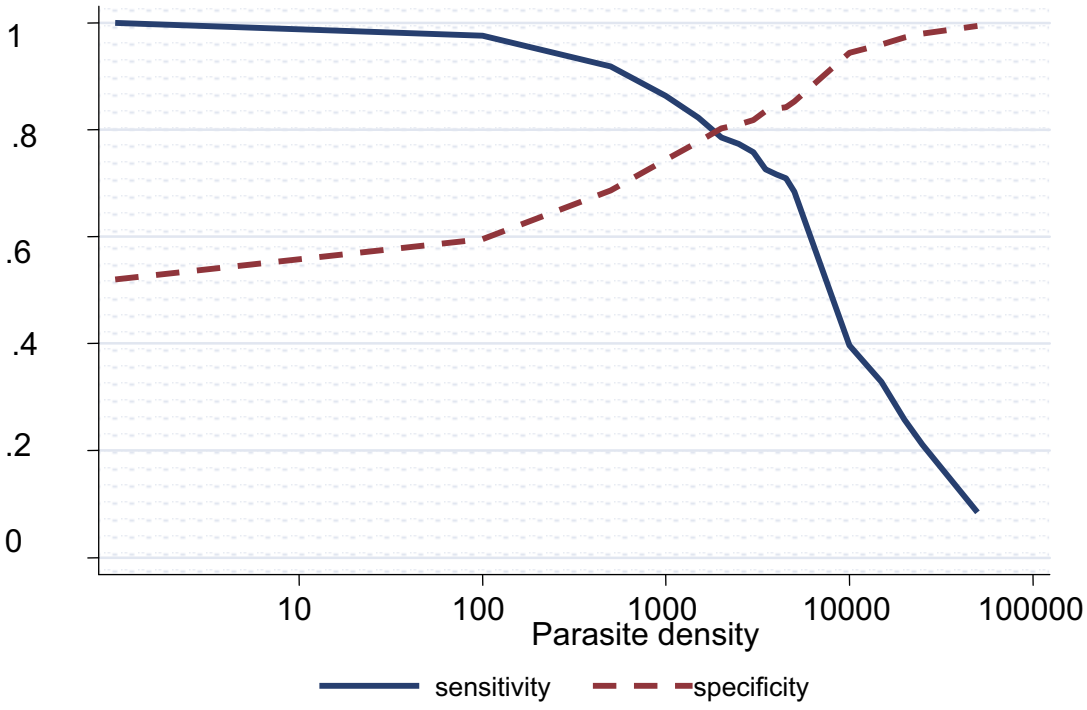


Figure 18.3

B.-Overall – countrywide, children aged 5 years of age and above

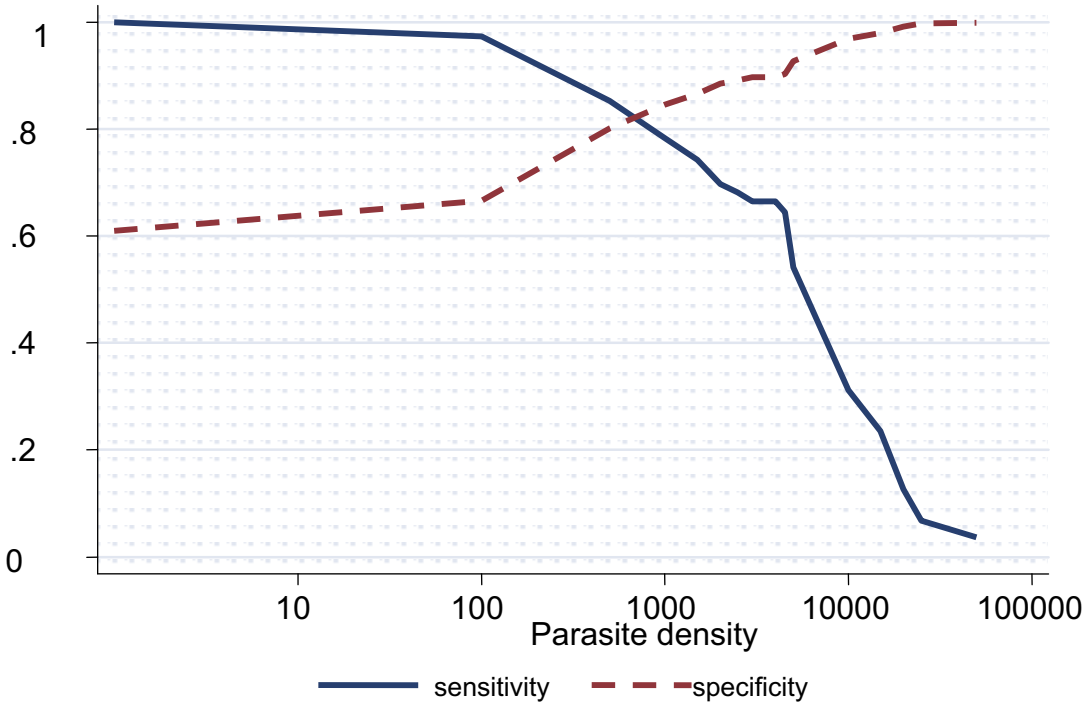


Figure 18.4 Sensitivity (solid lines) and Specificity (dashed lines) of malaria cases definition by region

A.-*Overall – Coastal Stratum, children less than 10 years of age*

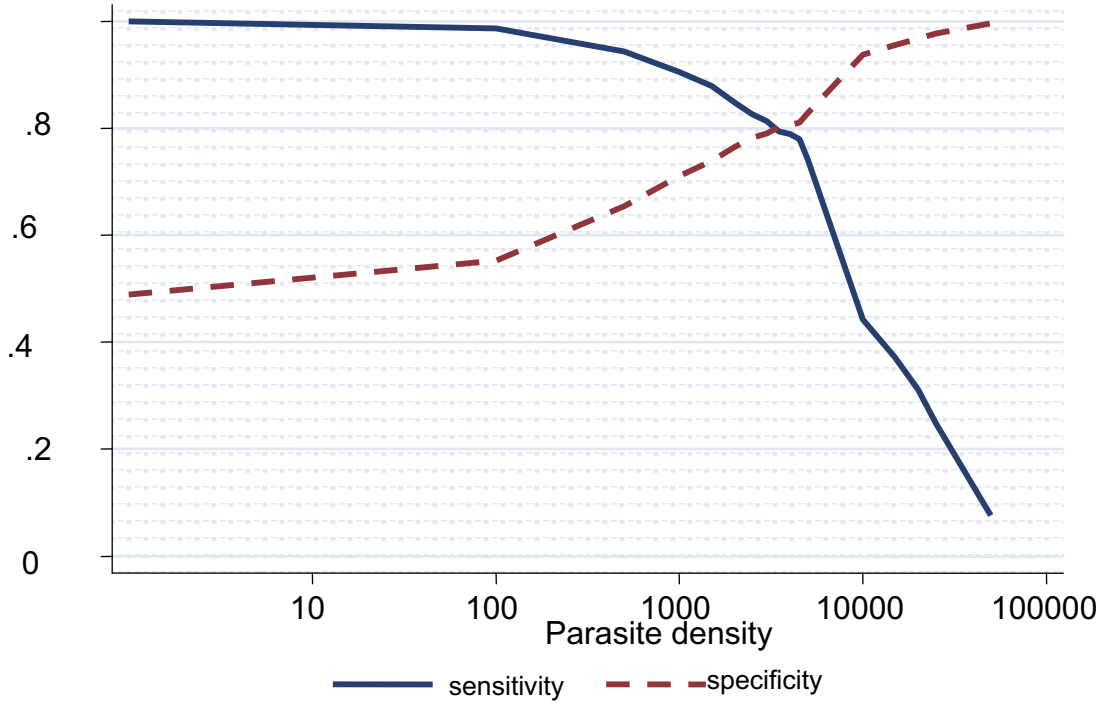


Figure 18.5

B.- Overall – Plateau Stratum, children under 10 years of age

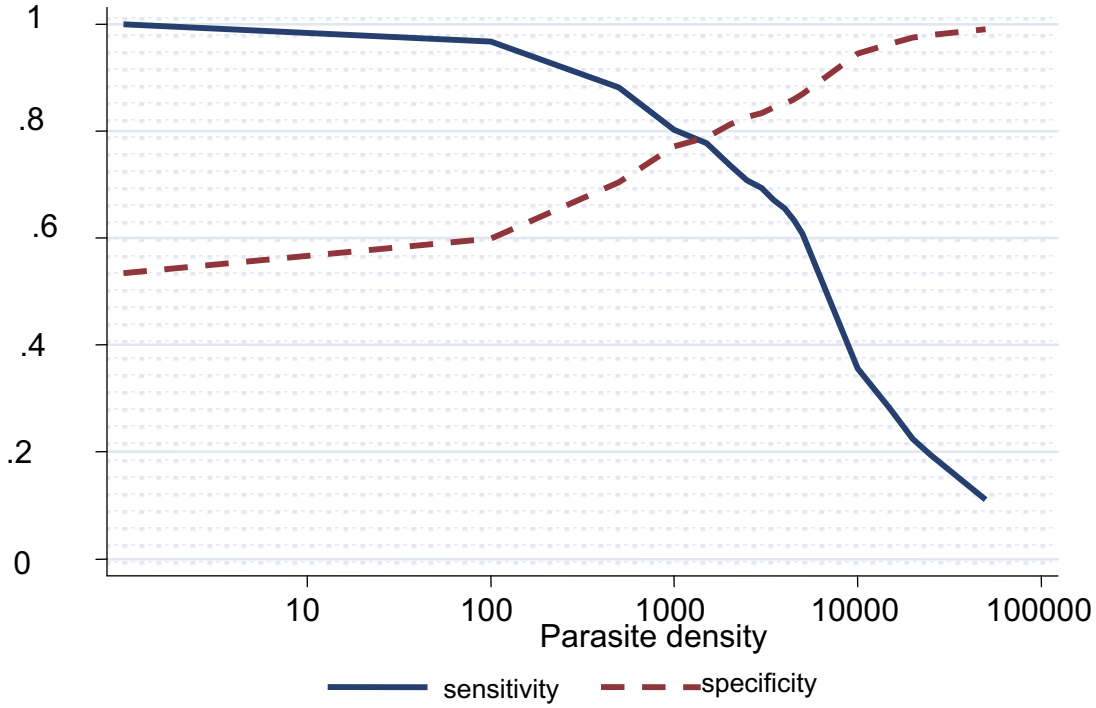


Figure 18.6 Sensitivity (solid lines) and Specificity (dashed lines) of malaria cases definition by region

A.-*Overall – Highland Stratum, children under 10 years of age*

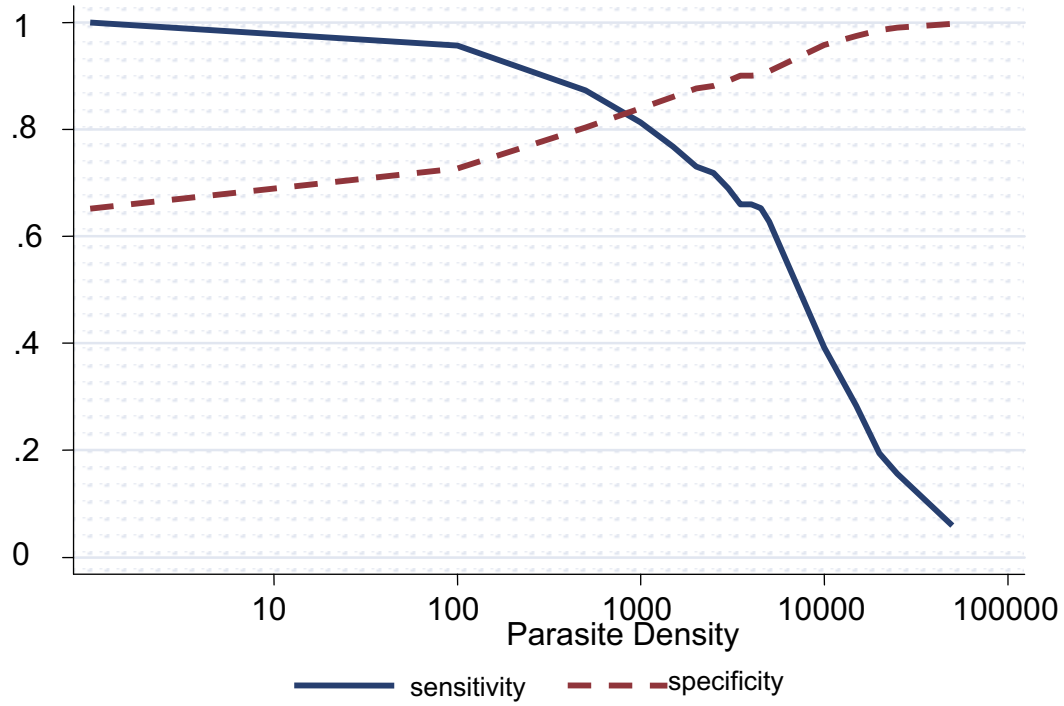


Figure 18.7
B.-Overall – North Region, children under 10 years of age

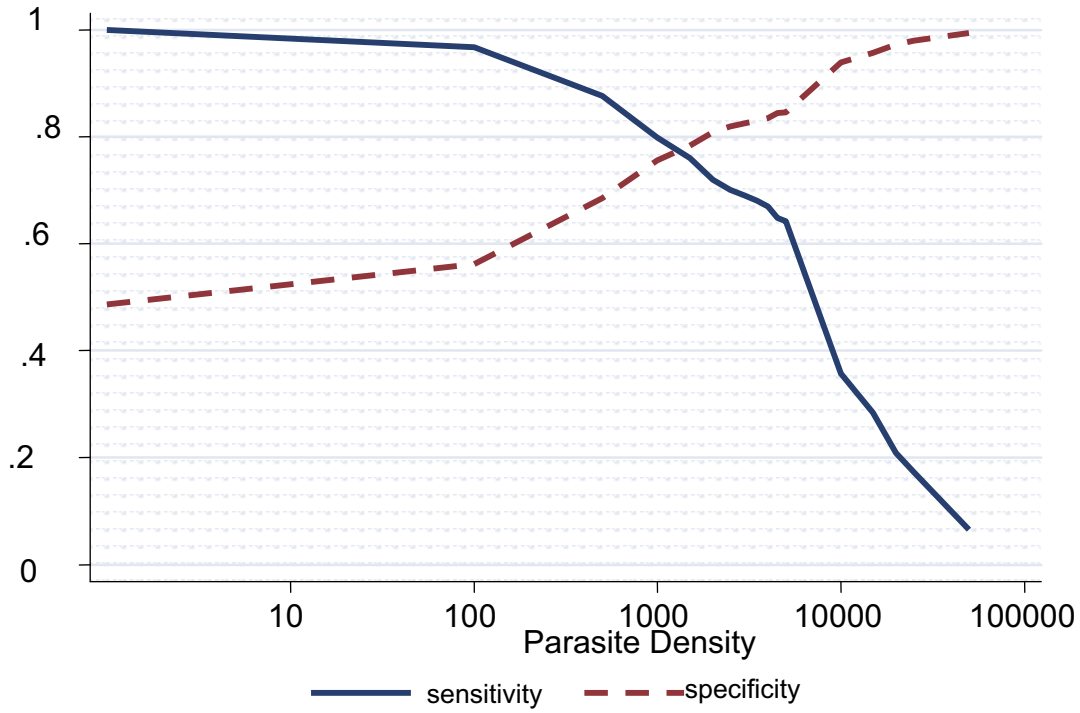


Figure 18.8 Sensitivity (solid lines) and Specificity (dashed lines) of malaria cases definition by age category
A.- Overall – Centre-Northern region, children under 10 years of age

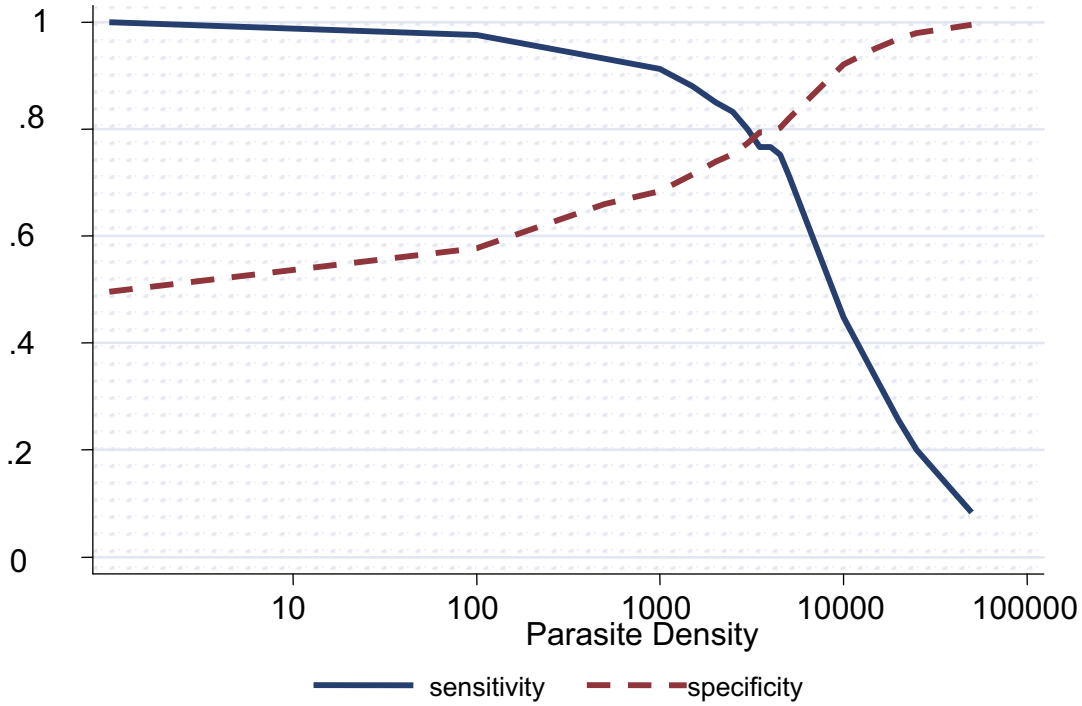


Figure 18.9
B.-*Overall – Centre Region, children under 10 years old*

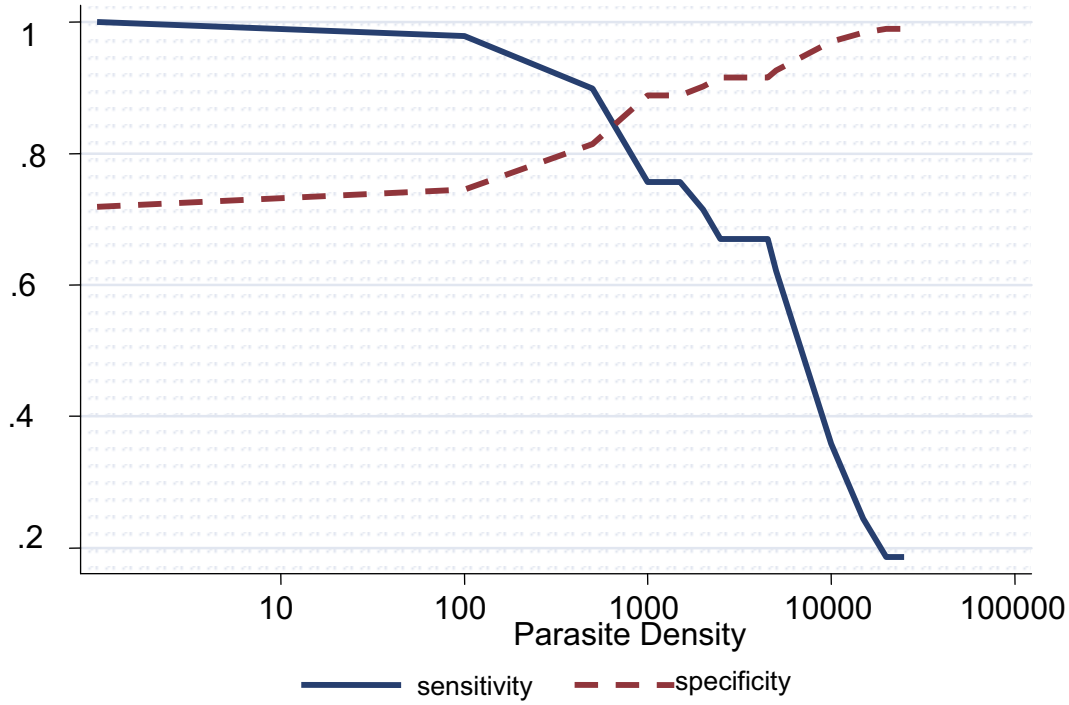
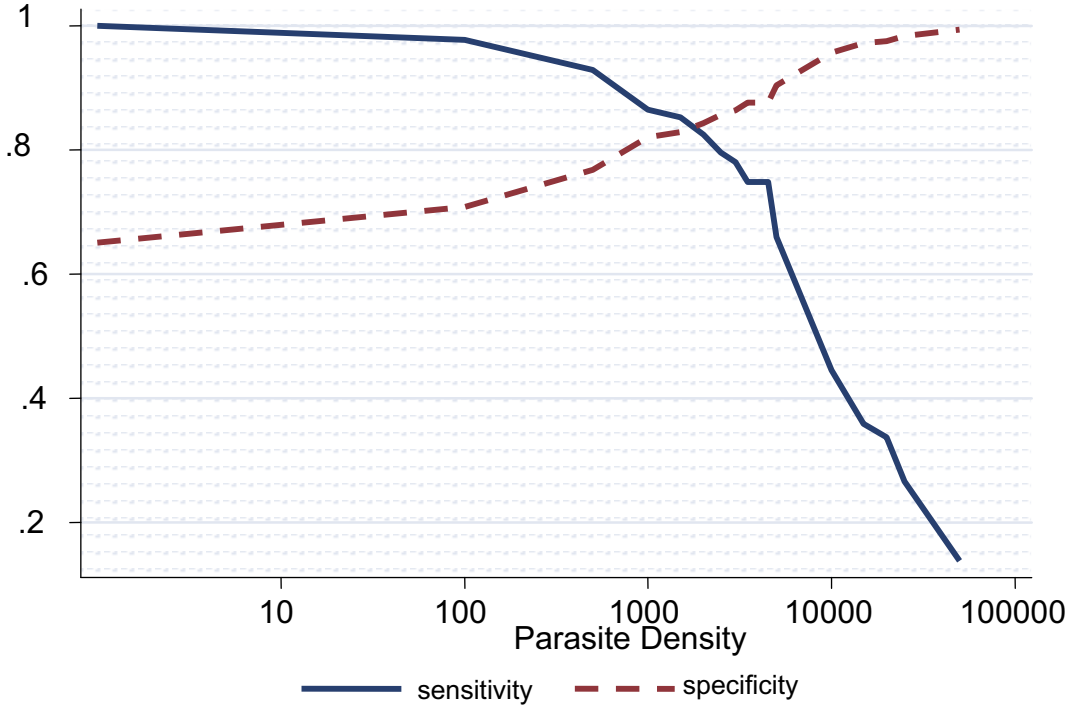


Figure 18.10 Sensitivity (solid lines) and Specificity (dashed lines) of malaria cases definition by age category
A.-Overall – Southern Region, children under 10 years old


1
.8
.6
.4
.2
10
100
1000
10000
100000
Parasite Density
sensitivity
specificity

Appendix 7

Tables of malariometric indicators among pregnant women across regions in Mozambique

Table 20. ***Overall distribution of malariometric indicators by age groups among pregnant women in Mozambique***

	Overall	< 20 years	20 – 29 years	≥ 30 years	p-Value
P. falciparum (%)	33.6	44.4	30.6	25.9	0.0001
Parasite density (CI 95%)	446 (381-521)	509 (394-657)	473 (374-600)	302 (212-432)	
Fever prevalence	4.4	7.3	2.7	4.6	0.007
Prevalence of fever & parasites	1.9	4.4	0.5	1.8	0.0025
Mean hgb g/dl (CI 95%)	10.3 (9.9-10.6)	9.9 (9.5-10.3)	10.4 (10.1-10.8)	10.5 (10.0-11.0)	
Anaemia (%)	62.5	70.1	59.4	59.4	0.1343
Severe anaemia (%)	6.6	7.1	7.2	4.4	0.5550
Gametocytes (%)	1.4	1.9	1.3	0.9	0.3926
P. malariae (%)	0.6	0.8	0.4	0.5	0.5920
Mixed infection (%)	0.5	0.8	0.4	0.3	0.6079

P. faciparum = Asexual forms; **Gametocytes** = Sexual forms only for *P. falciparum*; **Mixed infection** = Concomitant presence of *P. falciparum* and *P. malariae*; **Parasite Density** = Geometric mean parasite density only for *P. falciparum*, after log10 transformation (expressed as asexual parasites/μl; **Fever** = Axilar temperature ≥ 37.5 °C; **Fever & Parasites** = Fever associated with one or more asexual forms of *P. falciparum* parasites; **Anaemia** = Haemoglobin < 11 g/dl; **Severe Anaemia** = Haemoglobin < 7 g/dl.

Table 20.1 ***Overall distribution of malariometric indicators by age groups among pregnant women across northern region of Mozambique***

	Overall	< 20 years	20 – 29 years	≥ 30 years	p-Value
P. falciparum (%)	40.0	47.4	37.6	34.3	0.2120
Parasite density (CI 95%)	512 (372-705)	660 (193-2,248)	607 (399-922)	351 (199-617)	
Fever prevalence	1.7	2.4	0.1	5.5	0.1530
Prevalence of fever & parasites	1.0	0.2	0.0	5.5	0.0611
Mean hgb g/dl (CI 95%)	10.2 (9.4-11.0)	10.2 (9.5-10.9)	10.2 (9.3-11.1)	10.3 (9.3-11.3)	
Anaemia (%)	63.8	57.3	68.2	61.9	0.2650
Severe anaemia (%)	7.8	8.1	7.3	8.9	0.8328
Gametocytes (%)	0.4	0.2	0.7	0.0	0.6136
P. malariae (%)	0.4	0.0	0.7	0.0	0.4595
Mixed infection (%)	0.4	0.0	0.7	0.0	0.4595

P. faciparum = Asexual forms; **Gametocytes** = Sexual forms only for *P. falciparum*; **Mixed infection** = Concomitant presence of *P. falciparum* and *P. malariae*; **Parasite Density** = Geometric mean parasite density only for *P. falciparum*, after log10 transformation (expressed as asexual parasites/μl; **Fever** = Axilar temperature ≥ 37.5 °C; **Fever & Parasites** = Fever associated with one or more asexual forms of *P. falciparum* parasites; **Anaemia** = Haemoglobin < 11 g/dl; **Severe Anaemia** = Haemoglobin < 7 g/dl.

Table 20.2 ***Overall distribution of malariometric indicators by age groups among pregnant women across coastal stratum in the northern region of Mozambique***

	Overall	< 20 years	20 – 29 years	≥ 30 years	p-Value
P. falciparum (%)	31.6	44.3	27.8	22.4	0.1414
Parasite density (CI 95%)	563 (343-925)	734 (0.76-7,037)	542 (298-986)	583 (207-1,643)	
Fever prevalence	1.7	5.8	0.0	0.0	0.0306
Prevalence of fever & parasites	0.0	0.0	0.0	0.0	
Mean hgb g/dl (CI 95%)	10.8 (9.7-11.8)	10.4 (8.5-12.3)	10.6 (9.5-11.7)	11.8 (10.6-13.0)	
Anaemia (%)	47.9	48.6	56.3	19.8	0.2656
Severe anaemia (%)	3.8	8.7	1.9	0.0	0.4164
Gametocytes (%)	0.9	0.0	1.6	0.0	0.5558
P. malariae (%)	0.9	0.0	1.5	0.0	0.5558
Mixed infection (%)	0.9	0.0	1.6	0.0	0.5558

P. faciparum = Asexual forms; **Gametocytes** = Sexual forms only for *P. falciparum*; **Mixed infection** = Concomitant presence of *P. falciparum* and *P. malariae*; **Parasite Density** = Geometric mean parasite density only for *P. falciparum*, after log10 transformation (expressed as asexual parasites/μl; **Fever** = Axilar temperature ≥ 37.5 °C; **Fever & Parasites** = Fever associated with one or more asexual forms of *P. falciparum* parasites; **Anaemia** = Haemoglobin < 11 g/dl; **Severe Anaemia** = Haemoglobin < 7 g/dl.

Table 20.3 ***Overall distribution of malariometric indicators by age groups among pregnant women across plateau stratum in the northern region of Mozambique***

	Overall	< 20 years	20 – 29 years	≥ 30 years	p-Value
P. falciparum (%)	47.1	50.0	46.7	43.1	0.7668
Parasite density (CI 95%)	466 (235-926)	451 (7-28,820)	673 (296-1,531)	178 (37-849)	
Fever prevalence	1.7	0.0	0.0	9.5	0.1196
Prevalence of fever & parasites	1.7	0.0	0.0	9.5	0.3178
Mean hgb g/dl (CI 95%)	9.8 (8.1-11.5)	10.0 (8.3-11.7)	9.8 (6.8-12.8)	9.2 (6.7-11.8)	
Anaemia (%)	75.6	63.2	78.2	90.5	0.3230
Severe anaemia (%)	9.9	8.3	10.7	10.5	0.7181
Gametocytes (%)	0.0	0.0	0.0	0.0	
P. malariae (%)	0.0	0.0	0.0	0.0	
Mixed infection (%)	0.0	0.0	0.0	0.0	

P. faciparum = Asexual forms; **Gametocytes** = Sexual forms only for *P. falciparum*; **Mixed infection** = Concomitant presence of *P. falciparum* and *P. malariae*; **Parasite Density** = Geometric mean parasite density only for *P. falciparum*, after log10 transformation (expressed as asexual parasites/μl; **Fever** = Axilar temperature ≥ 37.5 °C; **Fever & Parasites** = Fever associated with one or more asexual forms of *P. falciparum* parasites; **Anaemia** = Haemoglobin < 11 g/dl; **Severe Anaemia** = Haemoglobin < 7 g/dl.

Table 20.4 ***Overall distribution of malariometric indicators by age groups among pregnant women across highland stratum in the northern region of Mozambique***

	Overall	< 20 years	20 – 29 years	≥ 30 years	p-Value
P. falciparum (%)	22.5	37.3	13.2	17.4	0.4184
Parasite density (CI 95%)	493 (286-849)	839 (232-3,033)	660 (242-1,797)	321 (145-707)	
Fever prevalence	4.0	5.5	3.9	0.0	0.4233
Prevalence of fever & parasites	2.0	5.5	0.0	0.0	0.5011
Mean hgb g/dl (CI 95%)	10.8 (6.6-15.2)	10.9 (8.7-13.1)	10.9 (7.8-14.0)	10.7 (4.6-16.0)	
Anaemia (%)	56.6	53.8	56.6	65.3	0.8027
Severe anaemia (%)	0.0	0.0	0.0	0.0	
Gametocytes (%)	2.0	5.5	0.0	0.0	0.2552
P. malariae (%)	0.0	0.0	0.0	0.0	
Mixed infection (%)	0.0	0.0	0.0	0.0	

P. faciparum = Asexual forms; **Gametocytes** = Sexual forms only for *P. falciparum*; **Mixed infection** = Concomitant presence of *P. falciparum* and *P. malariae*; **Parasite Density** = Geometric mean parasite density only for *P. falciparum*, after log10 transformation (expressed as asexual parasites/μl; **Fever** = Axilar temperature ≥ 37.5 °C; **Fever & Parasites** = Fever associated with one or more asexual forms of *P. falciparum* parasites; **Anaemia** = Haemoglobin < 11 g/dl; **Severe Anaemia** = Haemoglobin < 7 g/dl.

Table 20.5 ***Overall distribution of malariometric indicators by age groups among pregnant women across central-northern region of Mozambique***

	Overall	< 20 years	20 – 29 years	≥ 30 years	p-Value
P. falciparum (%)	36.8	52.5	27.2	33.1	0.0211
Parasite density (CI 95%)	445 (323-613)	587 (373-924)	389 (221-684)	251 (109-577)	
Fever prevalence	7.5	10.8	4.4	9.5	0.0278
Prevalence of fever & parasites	3.6	8.1	0.9	2.3	0.1422
Mean hgb g/dl (CI 95%)	10.2 (9.8-10.5)	9.6 (7.9-11.2)	10.5 (9.6-11.4)	10.5 (10.3-10.7)	
Anaemia (%)	64.4	80.3	55.6	57.5	0.0784
Severe anaemia (%)	5.4	7.6	5.1	0.0	0.6162
Gametocytes (%)	1.4	2.6	0.8	0.3	0.3088
P. malariae (%)	0.9	0.1	0.8	0.0	0.3419
Mixed infection (%)	0.9	0.1	0.8	0.0	0.3419

P. faciparum = Asexual forms; **Gametocytes** = Sexual forms only for *P. falciparum*; **Mixed infection** = Concomitant presence of *P. falciparum* and *P. malariae*; **Parasite Density** = Geometric mean parasite density only for *P. falciparum*, after log10 transformation (expressed as asexual parasites/μl; **Fever** = Axilar temperature ≥ 37.5 °C; **Fever & Parasites** = Fever associated with one or more asexual forms of *P. falciparum* parasites; **Anaemia** = Haemoglobin < 11 g/dl; **Severe Anaemia** = Haemoglobin < 7 g/dl.

Table 20.6 ***Overall distribution of malariometric indicators by age groups among pregnant women across coastal stratum in the central-northern region of Mozambique***

	Overall	< 20 years	20 – 29 years	≥ 30 years	p-Value
P. falciparum (%)	33.2	53.6	25.2	29.7	0.0171
Parasite density (CI 95%)	331 (226-483)	504 (293-867)	245 (127-475)	214 (81-568)	
Fever prevalence	0.7	10.9	4.3	8.2	0.0295
Prevalence of fever & parasites	3.3	10.9	0.0	2.7	0.1369
Mean hgb g/dl (CI 95%)	10.3 (10.2-10.3)	9.2 (3.4-14.9)	10.7 (9.8-11.6)	10.6 (9.4-11.7)	
Anaemia (%)	58.7	79.7	50.9	53.6	0.2139
Severe anaemia (%)	7.3	14.5	5.0	0.0	0.3809
Gametocytes (%)	0.6	0.0	1.1	0.0	0.6188
P. malariae (%)	0.6	0.0	1.1	0.0	0.6188
Mixed infection (%)	0.6	0.0	1.1	0.0	0.6188

P. faciparum = Asexual forms; **Gametocytes** = Sexual forms only for *P. falciparum*; **Mixed infection** = Concomitant presence of *P. falciparum* and *P. malariae*; **Parasite Density** = Geometric mean parasite density only for *P. falciparum*, after log10 transformation (expressed as asexual parasites/μl; **Fever** = Axilar temperature ≥ 37.5 °C; **Fever & Parasites** = Fever associated with one or more asexual forms of *P. falciparum* parasites; **Anaemia** = Haemoglobin < 11 g/dl; **Severe Anaemia** = Haemoglobin < 7 g/dl.

Table 20.7 ***Overall distribution of malariometric indicators by age groups among pregnant women across highland stratum in the central-northern region of Mozambique***

	Overall	< 20 years	20 – 29 years	≥ 30 years	p-Value
P. falciparum (%)	44.5	51.4	33.2	50.6	0.2427
Parasite density (CI 95%)	781 (440-1,387)	740 (319-1,714)	1.011 (378-2,698)	434 (26-7,124)	
Fever prevalence	8.9	10.5	4.8	16.2	0.2235
Prevalence of fever & parasites	4.2	5.3	3.8	0.0	0.3108
Mean hgb g/dl (CI 95%)	9.9 (9.7-10.2)	9.9 (8.9-10.9)	9.8 (8.9-10.8)	10.1 (9.6-10.6)	
Anaemia (%)	76.3	80.8	69.6	77.4	0.1948
Severe anaemia (%)	2.3	0.9	5.4	0.0	0.4940
Gametocytes (%)	3.0	5.3	0.0	2.1	0.3054
P. malariae (%)	1.6	2.9	0.0	0.0	0.1808
Mixed infection (%)	1.6	2.9	0.0	0.0	0.1808

P. faciparum = Asexual forms; **Gametocytes** = Sexual forms only for *P. falciparum*; **Mixed infection** = Concomitant presence of *P. falciparum* and *P. malariae*; **Parasite Density** = Geometric mean parasite density only for *P. falciparum*, after log10 transformation (expressed as asexual parasites/μl; **Fever** = Axilar temperature ≥ 37.5 °C; **Fever & Parasites** = Fever associated with one or more asexual forms of *P. falciparum* parasites; **Anaemia** = Haemoglobin < 11 g/dl; **Severe Anaemia** = Haemoglobin < 7 g/dl.

Table 20.8 ***Overall distribution of malariometric indicators by age groups among pregnant women across central region of Mozambique***

	Overall	< 20 years	20 – 29 years	≥ 30 years	p-Value
P. falciparum (%)	28.3	33.7	29.3	14.7	0.0124
Parasite density (CI 95%)	288 (223-372)	325 (230-459)	322 (210-494)	130 (77-217)	
Fever prevalence	2.9	5.4	1.7	1.5	0.1009
Prevalence of fever & parasites	1.4	3.6	0.4	0.0	0.0443
Mean hgb g/dl (CI 95%)	10.2 (9.5-10.9)	10.0 (9.1-10.9)	10.3 (9.6-11.1)	10.2 (8.8-11.6)	
Anaemia (%)	64.3	69.0	60.9	64.7	0.4659
Severe anaemia (%)	7.9	6.9	8.6	8.5	0.7286
Gametocytes (%)	2.4	3.6	1.9	1.4	0.3474
P. malariae (%)	0.3	0.0	0.0	1.8	0.1052
Mixed infection (%)	0.3	0.0	0.0	1.8	0.1052

P. faciparum = Asexual forms; **Gametocytes** = Sexual forms only for *P. falciparum*; **Mixed infection** = Concomitant presence of *P. falciparum* and *P. malariae*; **Parasite Density** = Geometric mean parasite density only for *P. falciparum*, after log10 transformation (expressed as asexual parasites/μl; **Fever** = Axilar temperature ≥ 37.5 °C; **Fever & Parasites** = Fever associated with one or more asexual forms of *P. falciparum* parasites; **Anaemia** = Haemoglobin < 11 g/dl; **Severe Anaemia** = Haemoglobin < 7 g/dl.

Table 20.9 ***Overall distribution of malariometric indicators by age groups among pregnant women across coastal stratum in the central region of Mozambique***

	Overall	< 20 years	20 – 29 years	≥ 30 years	p-Value
P. falciparum (%)	31.9	37.8	30.7	24.7	0.1703
Parasite density (CI 95%)	260 (173-390)	226 (145-351)	462 (200-1,068)	94 (67-131)	
Fever prevalence	0.3	0.0	0.0	1.5	0.5300
Prevalence of fever & parasites	0.3	0.0	0.0	1.5	0.4310
Mean hgb g/dl (CI 95%)	10.6 (9.7-11.4)	10.4 (7.5-13.4)	10.6 (9.9-11.2)	10.7 (9.0-12.4)	
Anaemia (%)	56.1	62.2	52.6	52.8	0.4900
Severe anaemia (%)	3.8	6.5	1.6	2.9	0.4540
Gametocytes (%)	4.1	5.2	3.5	3.4	0.5898
P. malariae (%)	0.0	0.0	0.0	0.0	
Mixed infection (%)	0.0	0.0	0.0	0.0	

P. faciparum = Asexual forms; **Gametocytes** = Sexual forms only for *P. falciparum*; **Mixed infection** = Concomitant presence of *P. falciparum* and *P. malariae*; **Parasite Density** = Geometric mean parasite density only for *P. falciparum*, after log10 transformation (expressed as asexual parasites/μl; **Fever** = Axilar temperature ≥ 37.5 °C; **Fever & Parasites** = Fever associated with one or more asexual forms of *P. falciparum* parasites; **Anaemia** = Haemoglobin < 11 g/dl; **Severe Anaemia** = Haemoglobin < 7 g/dl.

Table 20.10 ***Overall distribution of malariometric indicators by age groups among pregnant women across plateau stratum in the central region of Mozambique***

	Overall	< 20 years	20 – 29 years	≥ 30 years	p-Value
P. falciparum (%)	30.2	35.1	30.8	21.1	0.5125
Parasite density (CI 95%)	336 (210-537)	404 (195-837)	337 (158-716)	216 (55-842)	
Fever prevalence	1.9	3.5	1.7	0.0	0.5824
Prevalence of fever & parasites	0.9	3.5	0.0	0.0	0.4334
Mean hgb g/dl (CI 95%)	10.2 (1.6-18.9)	9.9 (2.9-17.0)	10.5 (1.2-19.7)	9.9 (6.0-19.3)	
Anaemia (%)	63.2	70.2	59.8	63.1	0.4742
Severe anaemia (%)	8.2	9.9	5.7	12.5	0.5704
Gametocytes (%)	2.4	3.5	1.7	2.6	0.7656
P. malariae (%)	0.9	0.0	0.0	5.3	0.2221
Mixed infection (%)	0.9	0.0	0.0	5.3	0.2221

P. faciparum = Asexual forms; **Gametocytes** = Sexual forms only for *P. falciparum*; **Mixed infection** = Concomitant presence of *P. falciparum* and *P. malariae*; **Parasite Density** = Geometric mean parasite density only for *P. falciparum*, after log10 transformation (expressed as asexual parasites/μl; **Fever** = Axilar temperature ≥ 37.5 °C; **Fever & Parasites** = Fever associated with one or more asexual forms of *P. falciparum* parasites; **Anaemia** = Haemoglobin < 11 g/dl; **Severe Anaemia** = Haemoglobin < 7 g/dl.

Table 20.11 ***Overall distribution of malariometric indicators by age groups among pregnant women across highland stratum in the central region of Mozambique***

	Overall	< 20 years	20 – 29 years	≥ 30 years	p-Value
P. falciparum (%)	26.3	32.3	28.0	7.1	0.0659
Parasite density (CI 95%)	281 (175-453)	400 (200-801)	228 (110-473)	125 (1-8,254)	
Fever prevalence	4.1	7.3	2.1	2.5	0.2180
Prevalence of fever & parasites	1.9	4.4	0.6	0.0	0.1952
Mean hgb g/dl (CI 95%)	10.1 (8.9-11.3)	9.9 (8.1-11.9)	10.2 (9.6-10.8)	10.3 (6.7-13.9)	
Anaemia (%)	66.7	70.1	63.2	69.7	0.6899
Severe anaemia (%)	8.6	5.6	11.6	7.5	0.4583
Gametocytes (%)	2.0	3.3	1.7	0.0	0.2190
P. malariae (%)	0.0	0.0	0.0	0.0	
Mixed infection (%)	0.0	0.0	0.0	0.0	

P. faciparum = Asexual forms; **Gametocytes** = Sexual forms only for *P. falciparum*; **Mixed infection** = Concomitant presence of *P. falciparum* and *P. malariae*; **Parasite Density** = Geometric mean parasite density only for *P. falciparum*, after log10 transformation (expressed as asexual parasites/μl; **Fever** = Axilar temperature ≥ 37.5 °C; **Fever & Parasites** = Fever associated with one or more asexual forms of *P. falciparum* parasites; **Anaemia** = Haemoglobin < 11 g/dl; **Severe Anaemia** = Haemoglobin < 7 g/dl.

Table 20.12 ***Overall distribution of malariometric indicators by age groups among pregnant women across southern region of Mozambique***

	Overall	< 20 years	20 – 29 years	≥ 30 years	p-Value
P. falciparum (%)	24.6	21.6	28.9	19.6	0.1159
Parasite density (CI 95%)	249 (1,633-3,021)	846 (445-1,608)	752 (442-1,278)	939 (338-2,607)	
Fever prevalence	3.5	8.9	3.8	1.1	0.1101
Prevalence of fever & parasites	0.3	0.01	0.6	0.04	0.4044
Mean hgb g/dl (CI 95%)	10.7 (9.4-11.9)	10.5 (9.4-11.6)	10.7 (9.3-12.2)	10.7 (9.5-11.9)	
Anaemia (%)	55.8	59.8	53.8	57.3	0.3490
Severe anaemia (%)	6.2	2.5	9.3	3.4	0.1867
Gametocytes (%)	1.7	0.002	2.2	1.7	0.5321
P. malariae (%)	0.4	1.5	0.0	0.7	0.3764
Mixed infection (%)	0.2	1.5	0.0	0.0	0.4023

P. faciparum = Asexual forms; **Gametocytes** = Sexual forms only for *P. falciparum*; **Mixed infection** = Concomitant presence of *P. falciparum* and *P. malariae*; **Parasite Density** = Geometric mean parasite density only for *P. falciparum*, after log10 transformation (expressed as asexual parasites/μl; **Fever** = Axilar temperature ≥ 37.5 °C; **Fever & Parasites** = Fever associated with one or more asexual forms of *P. falciparum* parasites; **Anaemia** = Haemoglobin < 11 g/dl; **Severe Anaemia** = Haemoglobin < 7 g/dl.

Table 20.13 ***Overall distribution of malariometric indicators by age groups among pregnant women across coastal stratum in the southern region of Mozambique***

	Overall	< 20 years	20 – 29 years	≥ 30 years	p-Value
P. falciparum (%)	32.1	23.4	36.1	26.8	0.5626
Parasite density (CI 95%)	954 (576-1,580)	1,384 (655-2,922)	855 (407-1,797)	532 (21-13,142)	
Fever prevalence	3.8	0.0	5.2	2.2	0.5735
Prevalence of fever & parasites	0.5	0.0	4.3	3.1	0.6483
Mean hgb g/dl (CI 95%)	9.8 (9.6-9.9)	10.4 (6.9-13.8)	9.6 (8.7-10.6)	9.8 (8.0-11.6)	
Anaemia (%)	73.1	53.2	73.5	77.3	0.0498
Severe anaemia (%)	11.0	0.0	15.4	5.0	0.2392
Gametocytes (%)	2.1	0.0	1.8	3.4	0.6571
P. malariae (%)	0.0				
Mixed infection (%)	0.0				

P. faciparum = Asexual forms; **Gametocytes** = Sexual forms only for *P. falciparum*; **Mixed infection** = Concomitant presence of *P. falciparum* and *P. malariae*; **Parasite Density** = Geometric mean parasite density only for *P. falciparum*, after log10 transformation (expressed as asexual parasites/μl; **Fever** = Axilar temperature ≥ 37.5 °C; **Fever & Parasites** = Fever associated with one or more asexual forms of *P. falciparum* parasites; **Anaemia** = Haemoglobin < 11 g/dl; **Severe Anaemia** = Haemoglobin < 7 g/dl.

Table 20.14 ***Overall distribution of malariometric indicators by age groups among pregnant women across plateau stratum in the southern region of Mozambique***

	Overall	< 20 years	20 – 29 years	≥ 30 years	p-Value
P. falciparum (%)	23.4	18.5	29.4	16.7	0.2817
Parasite density (CI 95%)	685 (379-1,238)	601 (162-2,229)	602 (253-1,427)	1,262 (313-5,088)	
Fever prevalence	3.5	10.8	3.3	0.0	0.2468
Prevalence of fever & parasites	0.4	10.7	2.5	0.0	0.4553
Mean hgb g/dl (CI 95%)	11.3 (6.3-16.2)	10.7 (7.4-13.9)	11.4 (6.4-16.5)	11.4 (4.3-18.4)	
Anaemia (%)	44.3	56.9	41.0	42.9	0.4242
Severe anaemia (%)	1.7	0.0	1.8	2.7	0.5775
Gametocytes (%)	1.3	0.0	1.8	1.2	0.6634
P. malariae (%)	0.4	0.0	0.0	1.2	0.5807
Mixed infection (%)	0.0	0.0	0.0	0.0	

P. faciparum = Asexual forms; **Gametocytes** = Sexual forms only for *P. falciparum*; **Mixed infection** = Concomitant presence of *P. falciparum* and *P. malariae*; **Parasite Density** = Geometric mean parasite density only for *P. falciparum*, after log10 transformation (expressed as asexual parasites/μl; **Fever** = Axilar temperature ≥ 37.5 °C; **Fever & Parasites** = Fever associated with one or more asexual forms of *P. falciparum* parasites; **Anaemia** = Haemoglobin < 11 g/dl; **Severe Anaemia** = Haemoglobin < 7 g/dl.

Table 20.15 ***Overall distribution of malariometric indicators by age groups among pregnant women across highland stratum in the southern region of Mozambique***

	Overall	< 20 years	20 – 29 years	≥ 30 years	p-Value
P. falciparum (%)	22.9	53.1	20.2	22.3	0.2614
Parasite density (CI 95%)	876 (237-3,242)	643 (82-5,007)	1,346 (5-303,344)	1,552	
Fever prevalence	3.4	0.0	4.1	2.9	0.6149
Prevalence of fever & parasites	0.0				
Mean hgb g/dl (CI 95%)	9.5 (5.7-13.4)	8.6 (2.3-14.9)	9.4 (7.4-11.5)	9.7 (3.1-16.3)	
Anaemia (%)	79.7	100.0	78.3	79.0	0.3159
Severe anaemia (%)	10.8	19.2	17.1	3.4	0.4271
Gametocytes (%)	2.9	0.0	4.1	2.1	0.5553
P. malariae (%)	0.9	19.2	0.0	0.0	0.1360
Mixed infection (%)	0.9	19.2	0.0	0.0	0.1360

P. faciparum = Asexual forms; **Gametocytes** = Sexual forms only for *P. falciparum*; **Mixed infection** = Concomitant presence of *P. falciparum* and *P. malariae*; **Parasite Density** = Geometric mean parasite density only for *P. falciparum*, after log10 transformation (expressed as asexual parasites/μl; **Fever** = Axilar temperature ≥ 37.5 °C; **Fever & Parasites** = Fever associated with one or more asexual forms of *P. falciparum* parasites; **Anaemia** = Haemoglobin < 11 g/dl; **Severe Anaemia** = Haemoglobin < 7 g/dl

Appendix 8

Tables of entomological inoculation rates by region and strata, composition of mosquitoes species and respective proportion CSP-sporozoite positive

Table 26. ***Overall Entomological Inoculation Rates by regions***

	No. of mosquitoes tested by PCR	No. sporozoite positive	Proportion sporozoite positive	No. of catches	EIR standard method*	EIR alternative method** (95%CI)
North	2,508	19	0.0076 **(19/2,508)**	360	19.3	19.3
North-Centre	1,496	16	0.011 **(16/1,496)**	180	33.4	32.4
Centre	1,896	8	0.0042 **(8/1,896)**	480	6.1	6.1
South	657	3	0.0046 **(3/657)**	420	2.6	2.6
Overall	6,557	46	0.007 **(46/6557)**	1440	11.6	11.7

* Standard Method: Number of sporozoite-positive PCR/number of mosquitoes tested X number of mosquitoes collected/number of catches X 365 days

** Alternative Method: Number of sporozoite-positive PCR/number of catches X 365 days

Table 26.1 ***Overall Entomological Inoculation Rates across strata***

	No. of mosquitoes tested by PCR	No. sporozoite positive	Proportion sporozoite positive	No. of catches	EIR standard method*	EIR alternative method** (95%CI)
Coastal	2,963	28	0.0095 **(28/2,963)**	540	19.0	18.9
Plateau	1,714	15	0.0088 **(15/1,714)**	360	15.3	15.2
Highland	1,880	3	0.0016 **(3/1,880)**	540	2.0	2.0
Overall	6,557	46	0.007 **(46/6,557)**	1440	11.6	11.7

* Standard Method: Number of sporozoite-positive PCR/number of mosquitoes tested X number of mosquitoes collected/number of catches X 365 days

** Alternative Method: Number of sporozoite-positive PCR/number of catches X 365 days

Table 26.2 ***Entomological Inoculation Rates across northern region***

	No. of mosquitoes tested by PCR	No. sporozoite positive	Proportion sporozoite positive	No. of catches	EIR standard method*	EIR alternative method** (95%CI)
Coastal	868	10	0.012 **(10/868)**	120	31.7	30.4
Plateau	1,278	8	0.0062 **(8/1,278)**	120	24.1	24.3
Highland	362	1	0.0028 **(1/362)**	120	3.1	3.0
Overall	2,508	19	0.0076 **(19/2,508)**	360	57.9	57.8

Table 26.3 ***Entomological Inoculation Rates across north-central region***

	No. of mosquitoes tested by PCR	No. sporozoite positive	Proportion sporozoite positive	No. of catches	EIR standard method*	EIR alternative method** (95%CI)
Coastal	1,398	16	0.011 **(16/1,398)**	90	62.4	64.9
Highland	98	0	0.0 **(0/98)**	90	-	-
Overall	1,496	16	0.011 **(16/1,496)**	180	33.4	32.4

Table 26.4 ***Entomological Inoculation Rates across central region***

	No. of mosquitoes tested by PCR	No. sporozoite positive	Proportion sporozoite positive	No. of catches	EIR standard method*	EIR alternative method** (95%CI)
Coastal	429	2	0.0046 **(2/429)**	120	6.0	6.1
Plateau	123	4	0.032 **(4/123)**	180	7.9	8.1
Highland	1,344	2	0.0015 **(2/1,344)**	180	4.1	4.1
Overall	1,896	8	0.0042 **(8/1,896)**	480	6.1	6.1

Table 26.5 ***Entomological Inoculation Rates across southern region***

	No. of mosquitoes tested by PCR	No. sporozoite positive	Proportion sporozoite positive	No. of catches	EIR standard method*	EIR alternative method** (95%CI)
Coastal	268	0	0.0 **(0/268)**	320	-	-
Plateau	313	3	0.0096 **(3/313)**	180	6.1	6.1
Highland	76	0	0.0 **(0/76)**	60	-	-
Overall	657	3	0.0046 **(3/657)**	480	2.3	2.3

Table 26.6 ***Composition of mosquitoes species (Knock-Down catch):***

By region	AR	FUN	GA	MR	NEG	QD	TOTAL
Northern	42	130	2,223	4	108	48	2,555
Centre-Nothern	24	72	1,400	0	0	0	1,496
Centre	208	188	1,464	0	60	16	1,936
Southern	38	210	397	0	12	0	657
TOTAL	312	600	5,484	4	180	64	6,644
By stratum	AR	FUN	GA	MR	NEG	QD	TOTAL
Coastal	88	220	2,611	0	44	40	3,003
Plateau	52	130	1,451	4	76	8	1,721
Highland	172	250	1,422	0	60	16	1,920
TOTAL	312	600	5,484	4	180	64	6,644

Table 26.7 ***Proportion of mosquitoes with CSP-sporozoite positive/negative for Plasmodium falciparum, by species***

	NEGATIVE (%)	POSITIVE (%)
AR	**310** (99.4)	**2** (0.6)
FUN	**600** (100.0)	**0** (0.0)
GA	**5,440** (99.2)	**44** (0.8)
MR	**4** (100.0)	**0** (0.0)
QD	**64** (100.0)	**0** (0.0)
NEG	**93** (100.0)	**0** (0.0)
TOTAL	**6,511** (99.3)	**46** (0.7)

AR (*Anopheles arabiensis, ss*); FUN (*Anopheles funestus, sl*); GA (*Anopheles gambiae, sl*); MR (*Anopheles merus, ss*); QD (*Anopheles quadrianulatus, ss*); NEG (Negative)