International Journal of Biomedical and Pharmaceutical Sciences ©2007 Global Science Books

Malaria: Cause and Control

Ibrahim Hassan Garba[1*] • Donatien Gatsing[2]

[1] Industrial Chemistry Programme, School of Science, Abubakar Tafawa Balewa University, P. M. B. 0248 Bauchi, Nigeria
[2] Department of Biochemistry, Faculty of Science, University of Dschang, P.O.Box 67 Dschang, Cameroon

Corresponding author: * **ihgarba2002@yahoo.com**

ABSTRACT

Malaria is an endemic tropical disease caused by the haemosporidian blood parasite of the genus *Plasmodium*. The human variant of this infection is caused by four members of this genus: *P. falciparum*, *P. vivax*, *P. ovale* and *P. malariae*, with *P. falciparum* malaria infection responsible for the most lethal form of the disease. The disease accounts for over 500 million acute clinical episodes annually and over 1 million deaths, particularly among children below the age of four years; with other debilitating effects in pregnant women. The economic impact of malaria infection has been put conservatively at over US$1.8 billion annually and it is the eighth most important disease in terms of lost disability adjusted life years (DALYS). This review addresses the current state of knowledge on the epidemiology/epidemiological patterns, economic impacts of malaria on populations and communities, vector ecology and transmission dynamics, pathogenesis and pathogenic outcomes of this disease and global malaria eradication and control efforts. It also recommends the way forward in the unending battle between man, his environment and a determined parasite that has so far eluded the human quest to conquer and eradicate it.

Keywords: control, *falciparum*, health, vector

CONTENTS

INTRODUCTION

Malaria is a disease caused by members of the genus *Plasmodium* and *Haemoproteus* within the order *Haemosporidiidea* and spread by the female *Anopheles* mosquito (Clark *et al*. 2004). It is one of the most common parasitic diseases causing significant morbidity and mortality, particularly in the tropics (Nand *et al*. 2001). Various species of malaria parasites are responsible for causing malaria infection depending on the host (Gautret *et al*. 1996; Hofmann *et al*. 1997; le Moyec *et al*. 1997; Fallon *et al*. 2005). Among the numerous species of *Plasmodium* so far identified, the following four are known to be responsible for causing human malaria infection: *P. vivax*, *P. malariae*, *P. ovale* and *P. falciparum* (Moody *et al*. 1998). Worldwide, 300-500 million acute, febrile cases are reported yearly (WHO 2005), with about 90% of such cases occurring in Africa. The disease kills up to 1.5-2 million persons annually, with over half of such fatalities involving children less than five years of age, a figure which accounts for about 25% of all childhood deaths (Amador and Patarroyo 1996; Samba 1997).

Falciparum malaria infection is the commonest malaria infection because of its ability to cause fatal disease. Therefore, except otherwise specified, the references to malaria in this review are to *falciparum* malaria. From an economic point of view, malaria is a costly disease. An estimated 1.8 billion $US is spent annually on both direct costs such as lost productivity, time costs and other direct costs and losses. This is approximately equal to the entire gross domestic product (GDP) of some countries such as Malawi, Benin, or Togo (Foster 1998).

Received: 27 February, 2007. Accepted: 13 April, 2007.

Invited Review

EPIDEMIOLOGY

Malaria is one of the most devastating infectious diseases of our time, rivalling HIV and tuberculosis as a killer disease (Barry 2005). It occurs in over 100 countries, but is mainly confined to poor tropical Africa, Asia and Latin America (Tourre and Oduola 2004). According to the World Health Organization, 3.2 billion people (> 40 percent of the global population) are at risk of malaria infection each year (WHO 2005) with around 500 million preceeding to clinical disease and 2-3 million deaths occurring annually (Snow *et al*. 2005). Well over 90% of these deaths occur in sub-Saharan Africa, with the burden of morbidity and mortality significantly biased towards children under five years, pregnant women and travellers (IAMAT 2002; Snow *et al*. 2005; WHO 2005; IAMAT 2006). Malaria varies dramatically in its epidemiological pattern. In some instances it appears as massive epidemics affecting people of all ages and causing temporary social disruption. This pattern which alternates between large epidemics and scarce malaria is called unstable or epidemic malaria (Bradley 1992). Unstable malaria is characterised by a parasite population that is less genetically diverse, due to clonal expansion of single genomes in the absence of immune pressure (Arez *et al*. 1999; Laserson *et al*. 1999). It may also be associated with diverse parasites where infections have been able to persist sub-patently in the host between transmission seasons (Barbiker 1998; Barry 2005). At the other extreme is very stable (endemic) malaria with continuous or regular seasonal transmission and everyone being infected and re-infected y mosquitoes. Stable and unstable malaria differ not only in their mortality and public health effects, they also differ in their response to attempts at control (Cohen 1988; Bradley 1991, 1992). In this context a clear understanding of the major differences between these epidemiological patterns can be expected to have a significant impact on control and eradication measures. A malaria epidemic simply refers to a sharp increase in the frequency of malaria transmission that exceeds by far the inter-seasonal variation normally experienced (Anthony and Awash 2004). Such epidemics occur in areas where environmental conditions are marginal for mosquito vector and parasite development such as highlands or semi-arid regions (Packard 1986; Bradley 1992; Crowe 1997). Epidemics can also occur when people who have had little exposure of infection move into endemic regions as refugees, in resettlement programmes or situations where there are changes in the landscape, breakdown in intervention programmes or insecticide resistance (Bradley 1992; Anthony and Awash 2004; Barry 2005). Malaria epidemiology in areas of stable transmission (endemic malaria) on the other hand is typified by an age-dependent pattern of non-sterilizing immunity in the human host (Molyneaux and Gramiccia 1980) and is generally characterised by a genetically diverse parasite population (Barry 2005). Because of the complex interplay between history, environment, vector, host and parasite, we are confronted with a global spectrum of varying epidemiological patterns between households, locally (within a community or village), within continents and countries (Barry 2005; Snow *et al*. 2005; Guerra *et al*. 2006) each with its own unique pathological and socio-economic outcomes. These diverse epidemiological scenarios have contributed to making this disease one of man's most formidable foes and it is on the resurge unless some more drastic action is taken to mitigate it.

ECONOMIC IMPACT OF MALARIA

Several approaches have been used to estimate the global economic cost of malaria infection. Current estimates of the cost of this disease are derived from both microeconomic and macroeconomic perspectives. In spite of this, the overall picture arising from these estimates are a gross understatement of the economic burden of this disease due to a combination of factors aptly summarised by Brehman (2001) as 'ears of the Hippopotamus'. However despite the identified deficiencies in the nature of the data/data sourcing methodologies aimed at finding a realistic estimate of the global economic burden of malaria and their limitations, conclusions drawn from such studies are providing critical insights towards the formulation of an action agenda and relevant health policies aimed at controlling the scourge of this disease. A recent study has estimated that the annual economic growth rate of countries with severe malaria are 1.3% lower even after controlling for other factors known to influence economic growth (Gallups and Sachs 2001) such as savings rates, economic and political institutions and literacy levels of the population (Goodman *et al*. 2000). In addition, the per capita Gross Domestic Product (GDP) (adjusted for differences in purchasing power) in highly malarious countries is on average one one-fifth of non-endemic countries (Gallups and Sachs 2001; Sachs and Goodman 2002). In Africa, hardest hit by the disease, many African families spend a quarter of their annual income on malaria treatment, with efforts at malaria control and treatment cutting aggregate economic growth by about 1.3% per annum (WHO 2005). Malaria also slows down long term economic growth through its impact on the accumulation of human and physical capital (Goodman *et al*. 2000). At the household level, malaria places significant burdens on households that have a sick family member (Konrandsen *et al*. 1997) particularly in developing countries (Russel 1987). These burdens include work time lost by the sick individual, caregiving time spent by other family members, lost productivity, cost of seeking treatment (transportation and medical care inclusive) and premature mortality (Laxminarayan 2004). At the community level, malaria imposes a cost on the entire community by modifying social and economic decisions taken in response to the perceived risk of infection, including effects on crop choice, trade, investment and fertility; with significant negative effects on economic productivity and growth (Goodman *et al*. 2000). Recent estimates put the total costs for universal coverage in both prevention and treatment in Africa alone at US$3.2 billion per year, with US$850 million going to operational and health support costs (WHO 2005). Average spending levels, going by 2004 estimates is barely a fifth of this amount. This implies that a more concerted effort is required from African governments in particular, through the formulation of tested workable strategies, government-private sector partnerships and a significant increase in the quantum of research spending, backed by committed political will for the tide this resurging scourge to be stemmed.

VECTOR CYCLE AND TRANSMISSION DYNAMICS

Approximately 70 species of *Anopheles* have been implicated in malaria transmission worldwide. Among these, over 40 species are responsible for transmitting human malaria, all of which differ in their transmission potential. In Africa, the major vectors are *Anopheles gambiae* which is considered the most important in most regions, *Anopheles arabiensis*, which of the preceding complex with distinct characteristics, and *Anopheles fenestus*, which is often reported as the second most important species in terms of malaria transmission, and more particularly, is considered the end-of-rainy season vector that sustains the parasite (Depinay *et al*. 2004). During the life time of a typical mosquito it passes through four distinct stages: egg, larva, pupa and adult. The egg are laid in or near water where hatch into larvae within a few days.

Most of the time, eggs are laid in bunches in distinct raft-like structures, but they may also be laid singly. In the process of development, the larvae feed on microscopic plant life, molt several times as they grow, and become pupae, that subsequently turn into adults. In some species of mosquito, this entire process can occur within 7-10 days. The major determinant effecting the presence and continued activity of malaria parasite vectors is climate. Tropical areas of the world have been reported to have the best combi-

nation of adequate rainfall, temperature and humidity which allows for breeding and survival of *Anophelines* (WHO 2005). Specifically, temperature influences *Anopheline* mosquito feeding intervals, population density and longevity (Small *et al*. 2003) as well as the reproductive potential of the plasmodium parasite. This malaria transmission dynamics is further complicated by a complex interaction of several factors related not only to the vector and parasite densities and behaviour, but includes also land use strategies, public health control programmes, human migration and drug resistance (Hay *et al*. 1998; Small *et al*. 2003; Talisuna *et al*. 2004).

THE PLASMODIUM LIFE CYCLE

The life cycle of all species of human malaria parasites is essentially the same as the one shown in **Fig. 1**.

The life cycle of the human malaria parasite comprises an exogenous sexual phase (sporogony) with multiplication in certain *Anopheles* mosquitoes, (2 and 3) and an endogenous asexual phase (schizogony) with multiplication in the vertebrate host (4 and 5). The latter phase includes the development cycle in red blood cells (erythrocytic schizogony) (5) and the cycle taking place in the liver cell parenchyma (exo-erythrocytic schizogony) (4). *P. falciparum* and, to a much lesser extent, *P. vivax* are the main infecting species and causes of disease and death from malaria. The stage that infects man, the lance-shaped sporozoites reside in the mosquito's salivary gland (Sinden 1984). Mosquitoes inject parasites (sporozoites) into the subcutaneous tissues, and much less frequently directly into the bloodstream as shown in **Fig. 1**. Recent evidence indicates that sporozoites pass through several hepatocytes before invasion is followed by parasite development (Miller *et al*. 2002). The co-receptors on sporozoites that mediate invasion involve in part, the thrombospondin domains on the circumsporozoite protein and on thrombospondin-related adhesive protein (TRAP). These domains bind specifically to heparin sulphate proteoglycans on hepatocytes in the region in apposition to sinusoidal endothelium and Kuppfer cells. Inside the hepatocytes, the parasite undergoes a complex series of transformations until it eventually transforms into a giant multi-nucleate stage known as the schizont, which further divides into roughly small spherical merozoites (Bruce-Chwatt 1985). The result is an enormous amplification of parasites: a liver cell infected by one sporozoite releases into the blood stream from 5000-10,000 merozoites. Each merozoite invades a red blood cell, as indicated in (5) **Fig. 1** where it multiplies asexually until the cell bursts and releases from 10-20 new merozoites that go on to invade more red cells. The sequence of invasion is probably similar for all *Plasmodium* species. The parasite must engage binding receptors on the red blood cells and undergo apical reorientation, junction formation and signalling. The parasite induces a vacuole derived from the red blood cell's plasma membrane and enters the vacuole by a moving junction. After invading the red cell, *P. falciparum* initiates a remarkable process of secreting proteins into the surrounding erythrocyte cytoplasm and plasma membrane (Adisa *et al*. 2001) leading to the progressive transformation of the infected red cells into non-self cells (Giribaldi *et al*. 2001). One of these exported proteins, the knob-associated histidine-rich protein (KAHRP), is reported to be essential for micro-vascular sequestration (Wickham *et al*. 2001), a strategy whereby infected red cell adhere via knob structures to capillary walls and thus avoid being eliminated by the spleen. It is the periodic lyses of the red blood cells with concomitant release of merozoites and toxic waste products that cause the regular chills and fevers in malaria infection. Some merozoites develop into male and female gametocytes, thus initiating the parasite's sexual cycle (Willamson *et al*. 1996). Disease begins only once the asexual parasite multiplies in the red blood cells. This is the only gateway to disease.

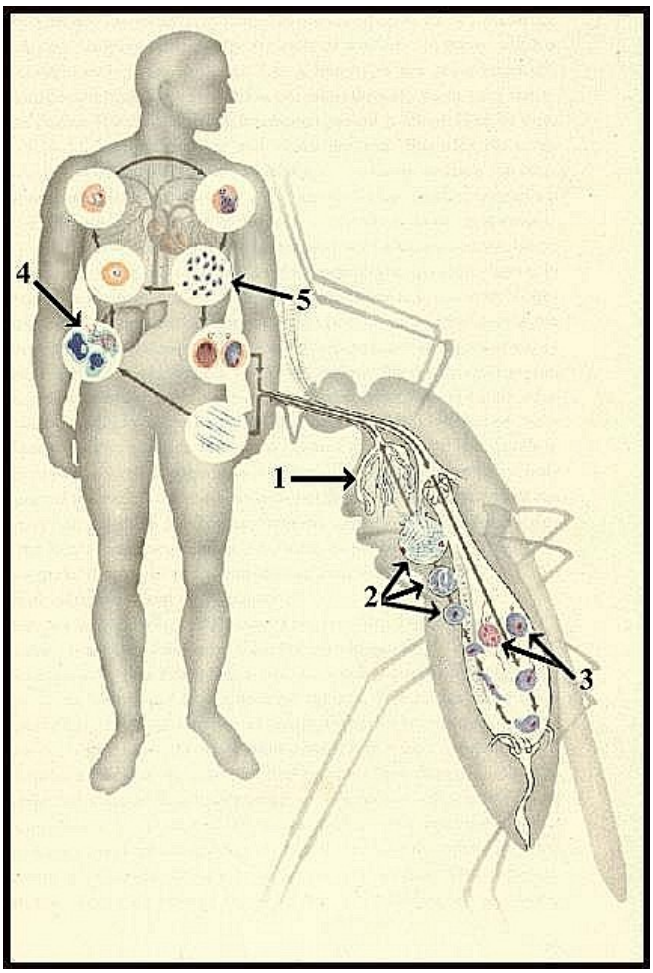

Fig. 1 Life cycle of the human malaria parasite in its mosquito and human hosts. Kindly provided, with permission, from Davis and Icke 2001, Royal Perth Hospital, Australia.

SYMPTOMS AND CLINICAL FEATURES

Uncomplicated malaria is a febrile illness associated with headache, muscular discomfort, weakness and malaise. These are non-specific features resembling influenza, and are common to the four human malarias caused by *P. falciparum*, *P. vivax*, *P. malariae* and *P. ovale*. As the untreated infection becomes synchronized, the fever becomes periodic with pyrexial spikes every one, two or three days, associated with chills or rigors (White and Ho 1992). If the disease progresses, the headache, limb pains and general malaise increases (Buyse *et al*. 1996). Anxiety and mental confusion are common at this stage. Fever becomes irregular without distinct periodicity. Hyperpyrexia, with temperatures well above 40 degrees centigrade is not uncommon (Long *et al*. 2001) together with convulsion and consequent aspiration pneumonia (Bruce-Chwatt 1985). The pulse and respiration rate are more rapid. Nausea, vomiting and diarrhea increase in intensity with an associated pulmonary involvement, leading to cough. There is hepatomegaly and splenomegaly with slight jaundice (Srivastava *et al*. 1996). Occasionally, *P. falciparum* malaria presents with encephalopathy and fever mimicking fulminant hepatic failure (Abisheganaden *et al*. 1996).

PATHOGENESIS OF HUMAN MALARIA INFECTION

The basic pathological process in malaria infection is the invasion of red blood cells, which follow the pre-erythrocytic stage of the parasite life cycle (Molyneaux 1996; Williamson *et al*. 1996). Marked alterations in the structure of erythrocytes ensue following invasion by *P. falciparum* malaria parasites (Riggione *et al*. 1996). Apart from enabling the *P. falciparum* parasites to avoid clearance by the spleen via

adhesion to endothelial cells, the alterations lead to changes in the red cell membrane permeability. These alterations facilitate movement of nutrients into and parasite-derived proteins out of the cells to meet the needs of the growing parasites (Garnham 1984).

Maegraith (1981), in postulating the 'rheological hypothesis' to explain this phenomenon suggested that the plugging of small vessels arises because the infected red cells are non-malleable and their changes in shape lead to sheer resistance to flow. Dietsch and Wellems (1996) have consequently postulated that antigenic variations of this protein allow parasitized erythrocytes to vary their phenotype and produce a sustained and chronic malaria infection. Anaemia usually develops in *P. falciparum* malaria infection (Bruce-Chwatt 1985). The type of anaemia is hemolytic, normochromic and normocytic, and in the acute attack there may be a striking fall in hemoglobin values of the blood (Abisheganaden *et al*. 1996). Anaemia causes significant morbidity particularly in children with *falciparum* malaria. During the infection, increased reactive O_2^- species (ROS) are generated which may contribute to erythrocyte damage and anaemia (Griffiths *et al*. 2001). In this line, a significant correlation has been found between parasitaemia and a depression of the following anti-oxidants: vitamin A, α/β-carotene, vitamin E, lycopene and lutein/zeathanthin by Metzger *et al*. (2001) who postulated that higher plasma lycopene could be associated with rapid clearance of malaria parasitaemia. Considering the release of toxic oxidant compounds, which occurs during erythrocytic merogony, Garba *et al.* (2004) examined the effect of *falciparum* malaria infection on total serum levels of ascorbic acid, one of the major aqueous phase anti-oxidants. The high total serum ascorbate that they discovered during the acute phase of the disease provided some evidence for the significant role of ascorbic acid in the pathogenesis of this disease in adults, particularly the role of this vitamin in neutralizing the oxidant compounds released during erythrocytic merogony and the boosting of the activity of the host humoral immune function. Menendez *et al*. (2001) have reported that there is a significant increase in soluble transferrin receptors during the disease, while Odunukwe *et al*. (2001) reported an association between infection and elevated plasma transferrin levels.

Additionally, a significant percentage of malaria patients present with thrombocytopenia, hyponatremia and liver dysfunction. The probable factors involved in hepatic dysfunction in acute *falciparum* malaria are local circulatory failure associated with centrilobular cellular damage (Maegraith 1981). Serum concentration of laminin and basic fibroblast growth factor (FGF) is also increased. These increased values are believed to be the consequence of endothelial and basement membrane damage induced by sequestration of the parasites (Burgman *et al*. 1996). The resulting inflammation arising from the above damage may account for the significant increase in interleukin-18, and the slight elevation in gamma-interferon observed in this disease (Torre *et al*. 2001). Plasma concentration of nitrite and nitrate (reactive nitrogen intermediates) reflecting high nitric oxide production somewhere in the body can be high in patients with cerebral malaria; but even higher in symptom-free parasitized individuals who are termed malaria-tolerant. Clark *et al*. (1996) have concluded from an earlier work that the high nitric oxide causing high serum levels of reactive nitrogen intermediates in malaria-tolerant individuals is generated in macrophages during the establishment and maintenance of malaria tolerance, and makes autoimmune disease rare in many tropical rural populations by minimizing proliferation of auto-reactive T cells.

In several studies aimed at assessing the changes in host-specific enzyme activities in malaria infection, Garba and Ubom (2005a) reported an over two-fold increase in the serum activity of lactate dehydrogenase (LDH) in adult *falciparum* malaria patients. The high total serum LDH was postulated to arise as a consequence of the combination of acute hepatocellular injury and red cell haemolysis induced by the invading merozoites. Apart from its significance in helping to understand the pathogenesis of this disease, the changes in serum LDH activity also hold a potential as a valuable enzymatic marker of acute, uncomplicated *falciparum* malaria infection, particularly in the absence of other complicating diseases known to be associated with an above-normal serum LDH activity (Garba and Ubom 2005a). In a related work involving serum alkaline phosphatase, the enzyme was found to be significantly elevated in *falciparum* malaria patients relative to a control group of age-matched adults. Since alkaline phosphatase is found localized on the membranes of the host hepatocytes drainage pathways, it was concluded that the enzyme is a potentially important biomarker for assessing the integrity of the hepatic drainage system in acute *falciparum* malaria infection (Garba and Ubom 2005b). Similarly, the total serum activities of the tissue-specific enzyme acid phosphatase and two of its isoenzymes; tartrate-resistant acid phosphatase (TRAP) and erythrocyte-specific acid phosphatase were found to be significantly elevated in malaria infection. This finding suggests that measurement of serum acid phosphatase activity, particularly the erythrocyte isoenzymes could be potentially used as biomarker of acute *falciparum* malaria infection (Garba *et al.* 2006a). Garba *et al.* (2006b) have also found malaria infection to be associated with an over 30% decline in serum copper levels, which could lead to a compromised superoxide dismutase (Cu-SOD) activity and an ineffective immune response. In contrast, magnesium was found to increase by a three-fold magnitude during malaria infection. This increase was attributed to haemolysis arising from erythrocytic merogony since the red blood cells contain a high concentration of this metal. Garba and Ubom (2006c) concluded that the increased serum magnesium has potential application as a metallic biomarker of acute *falciparum* malaria infection in adults.

MALARIA CONTROL

Malaria control is too complex to be addressed by a simple approach, and any attempt to do so is fraught with danger. It is important to tailor the strategy to the prevailing ecological and epidemiological conditions (Mauchet and Carnavale 1998; Shiff 2002). In addition, malaria control is a scientific and technical activity that requires skilled and dedicated staff with training in epidemiology, entomology, mapping and planning, and manpower management. Since much of the control work is in the field, it requires personnel prepared to undertake field work. It is not an arena for pure clinicians or laboratory scientists, and although they all play a role in the fight against malaria, the control operations are the realm of malariologists (Gusmão 1999). The major tools in malaria control consist of properly trained personnel with the requisite authority to coordinate and carry out their scientific work (Roberts *et al*. 2000). Once this foundation has been firmly set, then malaria control efforts can come in any of the following forms.

Malaria vector control

Significant insights made into the understanding of the dynamics of malaria epidemiology and life cycle have yielded important clues on how best to approach the issue of malaria control from a vector point of view. There are six groups of major control options.

Biological control

Biological control is defined as the action of predators, parasites or pathogens in maintaining the density of another organism at a lower average than would occur in their absence (Ghosh *et al*. 2005). There is a lot of inclination to use biocontrol agents rather than chemical insecticides due chiefly to the residual effects of chemical insecticides, widespread resistance in target insects, and soaring price of chemical insecticides and most importantly, the environmental

friendliness of the method (WHO 1984). Fish are one of the major biocontrol agents for controlling malaria vectors in many countries. Although most fish are potential predators of mosquito larvae, the most promising species are the *Gambusia affinis* and *Poecilia reticulate*. The bacteria *Bacillus thuringiensis israelensis* (Bti) and *Bacillus sphaericus* (Bs) are naturally occurring bacteria that have been used extensively as biocontrol agents against mosquito larvae (Wu *et al*. 1997). The larvicidal activity is dependent on the crystals of an endotoxin produced by the bacteria during sporulation. The toxin acts by hydrolysing the epithelial cell of the gut of mosquito larvae, leading to their death within 24 hours. Bti and Bs have a highly specific mode of action and are safe to most of the non-target aquatic vertebrates and invertebrates. In addition, some species of entomopathogenic fungi like *Lagenidium gigantum*, *Tolypocladium cylindrosporum*, *Metarrhizium anosopliae* and *Chrysosprium lobatum* have also generated a lot of interest as potential biocontrol agents of mosquito larvae (Federici 1981; Mohanty and Prakash 2002).

Source reduction through environmental management

This involves the modification of the environment to make it unfavourable for the vectors to breed. It includes draining or filling up of ponds, burrow pits and gutters. Other measures include intermittent draining of irrigated areas and maintenance of irrigated channels and construction of drainage channels.

This method can be used in urban settings effectively to control mosquito breeding (www.cdc.gov/ncidod/dvbid/westnile/index.htm.). The advantage of this method is that it can be applied any where, where breeding sites are well-defined, limited in number and accessible and it entails a multi-sectoral approach involving all concerned partners in health, agriculture, environment, planning and water resources.

Reduction of human-anopheline vector contact

The use of insecticide-(pyrethroid)-treated nets (ITNs) is rapidly gaining grounds as an important method of malaria vector control. The residual action of the pyrethroids has been shown to lead to increased vector control effectiveness by adding to the barrier effect of the net, the killing action of the insecticide. Specifically, the repellent effect of the pyrethroids prevents feeding through the net and the penetration of mosquitoes through holes in the net (Zaim *et al*. 2000; N'Guessau *et al*. 2001).

Space and indoor residual spraying

The main effect of space-spraying is the rapid reduction of vector density and a resulting increase in adult mosquito mortality, leading to rapid reduction in transmission (Nejara and Zaim 2001). This control method has been used to fight epidemics of mosquito-borne diseases and as a complimentary measure against exophilic malaria vectors. Indoor residual spraying on the other hand includes all methods of indoor spraying with residual insecticides. The killing effect is usually targeted to house-resting malaria vectors, and it constitutes a most efficient way of using the insecticide to kill malaria transmitting vectors (WHO 2002).

Genetic control

A different approach to the control of vector-borne disease is the use of genetic methods to either reduce the density of the vector population or replace competent vectors with genetically modified counterparts that have been made refractory to parasite infection or development and no longer can transmit target pathogens or parasites, and consequent loss of vectorial capacity (Boete and Koella 2003). Genetic methods are usually directed against adult mosquitoes. Sterile-male release techniques are being studied in some countries for obtaining a high proportion of infertile insemination (UNDP/WHO/World Bank 1991). Mosquitoes that are refractory to infection with malaria are released for replacing the naturally susceptible ones, leading to dilution of malaria-susceptible genes in the mosquito population by males carrying the refractoriness genes (Ito *et al*. 2002). Along this line, the mosquito, *Anopheles stephensi* Liston has been successfully transformed so that the binding of the malaria parasite *Plasmodium berghei* to the mosquito midgut membrane and sporozoite passage across the epithelium of the salivary glands were significantly reduced (Ito *et al*. 2002). Promising as this control method sounds, several reports on the fitness of genetically transformed mosquitoes relative to their wild counterparts and how they will eventually fare in the natural environment (Clark 2002; Boete and Koella 2003; Catteruccia *et al*. 2003) have shown that controlling malaria with transgenic mosquitoes is still in its early phase of development (Catteruccia *et al*. 2000; Ito *et al*. 2002).

Other methods

Other protective methods include house protection by use of nettings to screen windows and doors, the use of repellents through direct application on skin as lotions, creams or aerosols or on clothes (WHO 2002). This should be used to compliment the use of bed nets and house protection methods. Fumigant insecticide dispensers are also widely used in the tropics for individual protection, usually in the form of mosquito coils and, in urban areas, electrically heated dispensers (WHO 2002).

ANTI-MALARIAL CHEMOTHERAPY

One of the main components of the global malaria control strategy aimed at preventing mortality and reducing morbidity is early diagnosis and prompt treatment (Talisuna *et al*. 2004). The latter involves the use of anti-malarial drugs. Anti-malarial chemotherapy dates back to antiquity. Early natural products include the bark of the cinchona tree and extracts of the worm wood plant (Rosenthal 1998). Extensive efforts involving the screening of hundreds of compounds led to the development of a number of effective anti-malarial drugs, with the most important being chloroquine, which has been the mainstay of anti-malarial chemotherapy for over 50 years (Coatney 1963). Chloroquine eliminates parasites rapidly, has minimal toxicity and is widely available at low cost worldwide (Olliaro *et al*. 1996).Other important anti-malarials are quinine (Panisko and Keystone 1990), amodiaquine and mefloquine (Palmer *et al*. 1993) and fansidar, which is a combination of sulfadoxine and pyrimethamine (Luzzi and Peto 1993). The widespread development of resistance to these agents has led to the introduction of a new class of anti-malarials which include halofantrine (White 1996) and artemisinin and its derivative compounds (Talisuna *et al*. 2004). The major threat to the effectiveness of anti-malarial chemotherapy is the emerging resistance to virtually all known anti-malarials, with the exception of artemisinin in several countries (Bloland *et al*. 1993; Bloland 2000). This underscores the need for investment into the search for new, easily affordable anti-malarial drugs or combination therapies, and studies aimed at understanding the mechanistic basis of anti-malarial drug resistance with a view to designing circumvention strategies.

MALARIA VACCINES AS CONTROL TOOLS

The development and implementation of a vaccine against malaria is critical to finding a long term solution to this age-old killer disease (Hoffman 1996). Effective malaria vaccines have the potential of saving millions of lives and could be more cost-effective relative to anti-malarial chemotherapy. To this end, significant advances have been made in the understanding of the nature of the immune

mechanisms during malaria infection and identification of potential vaccine targets (Sherman 1998; Lingelbach *et al*. 2004). One of the major areas where great advances have been made in malaria vaccinology is the work on transmission-blocking vaccines. Functionally, these vaccines consist of antibodies which are ingested by the mosquito with the blood meal and interfere with parasite development (Riehle *et al*. 2003). Several proteins expressed on the surface of gametes and ookinetes have been tested as potential transmission-blocking vaccine candidates (Duffy and Kaslow 1997; Carter 2001). However, it is worth noting that transmission-blocking vaccines do not protect the immunized individual but act by preventing infection of people in the surrounding community, hence they may have to be incorporated into conventional vaccines which target the vertebrate stages of the parasite (Riehle *et al*. 2003). Another approach to vaccine development which shows some potential for success is the targeting of the parasite's hepatic life cycle. The hepatic life cycle is an ideal target for vaccine-induced protective immunity, because this stage lasts for at least 5.5 days and most significantly, it is not associated with pathology (Hoffman and Doolan 2000). Such a vaccine would be able to effectively prevent both the clinical symptoms of malaria and malaria transmission (Daubersies *et al*. 2000). Another vaccine strategy currently under serious study a polyepitope DNA vaccine primarily encoding CD 8^+ T cell epitopes (Gilbert *et al*. 1997). However, in order to significantly reduce mortality and morbidity due to malaria which affects hundreds of millions and results in the death of over a million children annually in sub-Saharan Africa, a vaccine which induces immune response against sporozoites, hepatic stages and erythrocytic stages are required (Miller and Hoffman 1998). Looking at the challenges and prospects for an effective malaria vaccine, the ideal anti-malarial vaccine may be the multi-antigen DNA-based vaccines that are currently in clinical trials (Hoffman *et al*. 1998). The present challenge is to adequately test these vaccines in the field, while working and investing in the development of improved and perhaps more complex vaccines (Hoffman and Doolan 2000).

CONCLUSION

An effective and sustainable war against malaria requires a multi-sectoral and concerted effort. There is the need for research and investment into the development of novel environmentally-friendly insecticides for insecticide treated net application and indoor/outdoor spraying, and biocontrol. However, the search and eventual adoption of biocontrol methods must also consider the all important issue of maintaining an ecological equilibrium in favour of both the predatory biocontrol agents and the immediate ecological niche. Vector-human contact prevention strategies and polyvalent vaccines capable of inducing protective immunity to all the blood stages of the parasite's life cycle will also go a long way towards strengthening the global armamentarium against this killer disease. Equally important in the global fight against malaria is action-backed political will from governments and the various levels of health services, particularly in malaria-endemic countries, a will that goes beyond mere pronouncements and highly politicised promises to act at glamorous, Klieg light-infested international conferences and meetings.

REFERENCES

Abisheganaden J, Rajasoorya C, Tari PK, Chaw LS (1996) Malaria: an audit of 56 cases admitted to a hospital. *Singapore Medical Journal* **37**, 153-156

Adisa A, Albano FR, Reeder J, Folley M, Tillet M (2001) Evidence for a role for *Plasmodium falciparum* homologue of Sec31p in the export of proteins to the surface of malaria parasite-infected erythrocytes. *Journal of Cell Science* **11**, 3377-3386

Amador R, Patarroyo ME (1996) Malaria vaccines. *Journal of Clinical Immunology* **16**, 183-189

Anthony EK, Awash T (2004) A review of the clinical and epidemiological burdens of epidemic malaria. *American Journal of Tropical Medicine and Hygiene* **71**, 128-135

Arez AP, Snounou G, Pinto J, Sousa CA, Modiano D (1999) A clonal *Plasmodium falciparum* in an isolated outbreak of malaria in the Republic of Cabo Verde. *Parasitology* **118**, 347-355

Babiker HA (1998) Unstable malaria in Sudan: the influence of the dry season. *Plasmodium falciparum* population in the unstable malaria area of eastern Sudan is stable and genetically complex. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **92**, 585-589

Barry AE (2005) Malaria epidemiology: Insights from the genome of the malaria parasite. *Journal of Molecular and Genetic Medicine* **1**, 76-86

Bloland P (2001) Drug resistance in malaria. WHO monograph WHO/CDS/CSR/DRS/2001.4. World Health Organization, Geneva Switzerland

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

Boete C, Koella JC (2003) Evolutionary ideas about genetically manipulated mosquitoes and malaria control. *Trends in Parasitology* **19**, 32-38

Bradley DJ (1992) Malaria: old infections, changing epidemiology. *Health Transition Review* **2**, 137-152

Brehman JG (2001) The ears of the hippopotamus: manifestations, determinants, and estimates of the malaria burden. *American Journal of Tropical Medicine and Hygiene* **64**, 1-11

Bruce-Chwatt LJ (1985) *Essential Malariology* Oxford University Press, Oxford, 529 pp

Burgman H, Looaresuwan S, Viravan C, Vanijanonta S, Zedwitz LK, Vorbatch H, Graninger W (1996) Serum laminin and basic fibroblast growth factor concentration in patients with complicated plasmodium *falciparum* malaria. *Journal of Clinical Immunology* **16**, 278-282

Buyse D, van den Ende J, Vervoorti T, van den Ende E (1996) Sleeping sickness as an import pathology following a stay in Zaire. *Acta Clinica Belgica* **51**, 409-411

Carter R (2001) Transmission blocking malaria vaccines. *Vaccine* **19**, 2309-2314

Catteruccia F, Godfray HC, Crisanti A (2003) Impact of genetic manipulation on the fitness of *Anopheles stephensi* mosquitoes. *Science* **299**, 1225-1227

Catteruccia F, Nolan T, Loukeris TG, Blass C, Savakis C, Kafatos CS, Crisanti A (2000) Stable germline transmission of the malaria mosquito *Anopheles stephensi*. *Nature* **405**, 959-962

Clark IA, Alleva LM, Mills AC, Cowden WB (2004) Pathogenesis of malaria and clinically similar conditions. *Clinical Microbiology Reviews* **17**, 509-539

Clark IA, Al-Yaman FM, Cowden WB, Rockett KA (1996) Does malarial tolerance, through nitric oxide explain the low incidence of autoimmune diseases in tropical Africa? *Lancet* **348**, 1492-1494

Clark IA, Rockett KA, Burgner D (2003) Genes, nitric oxide and malaria in African children. *Trends in Parasitology* **19**, 335-337

Clarke T (2002) Mosquitoes minus malaria. *Nature* **415**, 670-672

Coatney GR (1963) Pitfalls in a discovery: the chronicle of chloroquine. *American Journal of Tropical Medicine and Hygiene* **12**, 121-128

Cohen JE (1988) Estimating the effect of successful malaria programmes on mortality. *Population Bulletin of the United Nations* **25**, 6-26

Crowe S (1997) Malaria outbreak hits refugees in Tanzania. *Lancet* **350**, 41

Daubersies P (2000) Protection against *plasmodium falciparum* malaria in chimpanzees by immunization with conserved pre-erythrocyte antigen, SLA-3. *Nature Medicine* **6**, 1258-1263

Davis R, Icke G (2001) Malaria: An Information Resource from Royal Perth Hospital, Abbott Diagnostics Division, CD ROM. Available online: http://www.rph.wa.gov.au/malaria.html

Depinay JO, Mbogo CM, Killeen G, Knols B, Beier J, Carlson J, Dushoff J, Bilingsway P, Mwambi H, Githure J, Toure AM, McKenzie FE (2004) A simulation model of African *Anopheles* ecology and population dynamics for the analysis of malaria transmission. Available online: www.malariajournal.com/content/3/1/29 *Malaria Journal* **3**

Dietsch KW, Wellems TE (1996) Membrane modifications in erythrocytes parasitized by plasmodium *falciparum*. *Molecular Biochemistry and Parasitology* **76**, 1-10

Duffy PE, Kaslow DC (1997) A novel malaria protein, pfs28, and pfs25 are genetically linked and synergistic as *falciparum* malaria transmission-blocking vaccines. *Infection and Immunity* **65**, 1109-1113

Fallon SM, Bermingham E, Ricklefs RE (2005) Host specialization and geographic localization of avian malaria parasites: A regional analysis in Lesser Antilles. *The American Naturalist* **165**, 466-480

Federici BA (1981) Microbial control of pests and plant diseases 1970-1980. In: Burges HD (Ed) Academic Press, London, pp 555-572

Foster S (1998) Economics and its contribution to the fight against malaria. *Annals of Tropical Medicine and Parasitology* **92**, 391-398

Gallups JL, Sachs JD (2001) The economic burden of malaria. *American Journal of Tropical Medicine and Hygiene* **64**, 85-96

Garba IH, Ubom G, Haruna M (2004) Serum ascorbic acid concentration in patients with acute *falciparum* malaria infection: Possible significance. *Brazilian Journal of Infectious Diseases* **8**, 378-381

Garba IH, Ubom GA (2005a) Total serum lactate dehydrogenase activity in acute *Plasmodium falciparum* malaria infection. *Singapore Medical Journal*

46, 632-634

Garba IH, Ubom GA (2005b) Serum alkaline phosphatase activity as a potential biomarker for the integrity of the hepatic drainage system in acute *falciparum malaria* infection. *The Internet Journal of Infectious Diseases* **4**, Available online: http://www.ispub.com/ostia/index.php?xmlFilePath=journals/ijid/vol4n2/malaria.xml

Garba IH, Gatsing D, Ubom G (2006a) Elevated total and isoenzyme forms of acid phosphatase in *falciparum* malaria. *Comptes Rendus Biologies* **329**, 75-78

Garba IH, Ubom GA, Ejiogu NB (2006b) Serum copper concentration in adults with acute, uncomplicated *falciparum* malaria infection. *Biological Trace Elements Research* **113**, 125-130

Garba IH, Ubom GA (2006c) Potential role of serum magnesium measurements as a biomarker of acute *falciparum* malaria infection in adult patients. *Biological Trace Elements Research* **114**, 115-120

Garnham PCC (1984) The present state of malaria research. *Experientia* **40**, 1305-1445

Gautret P, Gantier JC, Baccam D, Miltgen F, Saulai M, Chabaud AG, Landau I (1996) The gametocytes of *Plasmodium vinkei* Petteri, their morphological stages, periodicity and infectivity. *International Journal of Parasitology* **26**, 1095-1101

Ghosh A, Mandal S, Bhattacharjee I, Chandra G (2005) Biological control of vector mosquitoes by some common exotic fish predators. *Turkish Journal of Biology* **29**, 67-171

Gilbert SC (1997) A protein particle vaccine containing multiple malaria epitopes. *Nature Biotechnology* **15**, 1280-1284

Giribaldi G, Ulliers D, Mannu F, Arese P, Turrini F (2001) Growth of plasmodium *falciparum* induces stage-dependent hemicrome formation, oxidative aggregation of band 3, membrane deposition of compliment and antibodies, and phagocytosis of parasitized erythrocytes. *British Journal of Haematology* **133**, 492-499

Goodman C, Coleman P, Mills AB (2000) *Economic analysis of malaria control in Sub-Saharan Africa*, Global Forum for Health Research, Strategic Research Series. Available online: www.globalforumhealth.org

Griffiths MJ, Ndungu F, Baird KL, Muller DP, Marsh K, Newton CR (2001) Oxidative stress and erythrocyte damage in Kenyan children with severe plasmodium *falciparum* malaria. *British Journal of Haematology* **113**, 486-491

Guerra CA, Snow RW, Hay SI (2006) Defining the global spatial limits of malaria transmission in 2005. *Advances in Parasitology* **62**, 157-179

Gusmao RD (1999) Overview of malaria control in the Americas. *Parasitologia* **41**, 355-360

Hay SI, Snow RW, Rogers DJ (1998) From predicting mosquito habitat to malaria seasons using remotely sensed data: Practice, problems and perspectives. *Parasitology Today* **14**, 306-312

Hoffman SL (1996) *Malaria Vaccine Development*, ASM Press, Washington, DC, 325 pp

Hoffman SL (1998) From genomics to vaccines: malaria as a model system. *Nature Medicine* **4**, 1351-1353

Hoffman SL, Crutcher JM, Puri SK, Ansari AA, Villinger F, Franke ED, Singh PP, Finkelman F, Gately, MK, Dutta, GP, Sedegal M (1997) Sterile protection of monkeys against malaria after administration of interleukin-12. *National Medicine* **3**, 80-83

Hoffman SL, Doolan DL (2000) Malaria vaccines – targeting hepatocytes. *Nature* **6**, 1281-1219

International Association for Medical Assistance to Travellers (IAMAT) (2006) How to protect yourself against malaria. Available online: www.iamat.org

International Association for Medical Assistance to Travellers (IAMAT) (2002) World malaria risk chart. Available online: www.iamat.org

Ito J, Ghosh A, Moreira LA, Wimmer EA, Jacobs-Lorena M (2002) Transgenic anopheline mosquitoes impaired in transmission of a malaria parasite. *Nature* **417**, 452-455

Laserson KF, Petralanda I, Almera R, Barker RH (1999) Genetic characterization of an epidemic of *Plasmodium falciparum* malaria among Yanomami Amerindians. *Journal of Infectious Diseases* **180**, 2081-2085

Laxminaraya R (2004) Does reducing malaria improve household living standards? *Tropical Medicine and International Health* **9**, 267-272

le Moyec L, Ekwalanga M, Eugene M, Bouanga JC, Bauza G, M'Bengue A, Mazier D, Gentilini M (1997) Brain metabolites in mice coinfected with *Plasmodium berghei* - ANKA and LP-BM5 virus: Assessment by proton magnetic resonance spectroscopy. *Experimental Parasitology* **85**, 296-298

Lingelbalch K, Kirk K, Rogerson S, Langhorne J, Carucci DJ, Waters A (2004) Molecular approaches to malaria. *Molecular Microbiology* **54**, 575-587

Long HY, Lell B, Dutz K, Kremsner PG (2001) Plasmodium *falciparum*: *in vitro* growth inhibition by febrile temperatures. *Parasitology Research* **87**, 553-555

Luzzi GA, Peto TEA (1993) Adverse effects of antimalarials: an update. *Drug Safety* **8**, 259-311

Maegraith B (1981) Aspects of the pathogenesis of malaria. *Southwest Asian Journal of Tropical Medicine and Public Health* **12**, 251-267

Menendez C, Quinto LL, Kahigwa E, Alvarez ZL, Fernandez R, Gimenez N, Metzger A, Mukasa G, Shankar AH, Ndeezi G, Melikran G, Samba RD (2001) Antioxidant status and acute malaria in children in Kampala, Uganda. *American Journal of Tropical Medicine and Hygiene* **65**, 115-119

Miller LH, Baruch DI, Marsh K, Doumbo OK (2002) The pathogenic basis of malaria. *Nature* **415**, 673-679

Miller LH, Hoffman SL (1998) Research towards vaccines against malaria. *Nature Medicine* **4**, 520-524

Mohanty SS, Prakash S (2002) Efficacy of *Chrysosporium lobatum* against larvae of malaria vector, *Anopheles stephensi* in the laboratory. *Current Science* **83**, 1585-1588

Molineaux L (1996) Plasmodium *falciparum* malaria: some epidemiological implications of parasite and host diversity. *American Journal of Tropical Medicine and Parasitology* **90**, 375-393

Molyneaux L (1988) The epidemiology of human malaria as an explanation of its distribution, including some implications for its control. In: Wernsdorfer WH, McGregor I (Eds) *Malaria Principles and Practice of Malariology*, Churchill Livingston, London, UK, pp 913-999

Molyneaux L, Gramiccia G (1980) The Garki Project: Research on the epidemiology and control of malaria in the Sudan Savannah of West Africa. Geneva, World Health Organization, 311 pp

Molyneaux ME, Looareesuwan S, Menzies IS, Greinger SL, Phillips RE, Wattanagoon Y, Thomson RP, Warrell DA (1989) Reduced hepatic blood flow and intestinal mal-absorption in severe falciparum malaria. *American Journal of Tropical Medicine and Hygiene* **40**, 470-476

Moody AH, John SM, Sudarsanam A, Sitarram U (1998) Evaluation of Optimal, a dipstick test for the diagnosis of malaria. *Annals of Tropical Medicine and Parasitology* **92**, 621-622

Mouchet J, Carnavale P (1998) Entomological biodiversity of malaria in the world. *Research Reviews in Parasitology* **51**, 189-195

N'Guessan R, Darriet F, Doannio JMC, Chandre F, Carnavale P (2001) Olyset net efficacy against pyrethroids-resistant *Anopheles gambiae* and *Culex quinquefasciatus* after 3 years' field use in Cote d'Ivoir. *Medical and Veterinary Entomology* **15**, 97-104

Nand N, Aggarwal H, Sharma M, Singh M (2001) Systemic manifestations of malaria. *Journal of the Indian Academy of Clinical Medicine* **2**, 190-193

Nejara JA, Zaim M (2001) Malaria vector control. Insecticides for residual spraying. Geneva, WHO, document WHO/CDS/WHOPES/2001.3, 102 pp

Nejara JA, Zaim M (2003) Malaria vector control. Decision-making criteria and procedures for judicious use of insecticides. WHO/CDS/WHOPES/2002.5 Rev. 1, 105 pp

Odunukwe NN, Salako LA, Okanny C, Ahmed OA, Made AG, Efinemokwu C, Raheem TY (2001) Serum ferritin and other haematologocal measurements in apparently healthy children with malaria parasitaemia in Lagos, Nigeria. *West African Journal of Medicine* **20**, 42-45

Olliaro P, Cattani J, Wirth D (1996) Malaria, the submerged disease. *Journal of the American Medical Association* **275**, 230-233

Olliaro P, Nevill C, LeBras J, Ringwald P, Mussano P, Garner P, Brasseur P (1996) Systematic review of amodiaquine treatment in uncomplicated malaria. *Lancet* **348**, 1196-1201

Packard RM (1984) Maize, cattle and mosquitoes: the political economy of malaria epidemics in colonial Swaziland. *Journal of African History* **25**, 189-212

Pal KK, Gardener BM (2006) Biological control of plant pathogens. *The Plant Health Instructor* **DOI:10.1094/PHI-A-2006-1117-02**, 1-25

Palmer KJ, Holliday SM, Brogden RN (1993) Mefloquine: a review of its antimalarial activity, pharmacokinetic properties and therapeutic efficacy. *Drugs* **45**, 430-475

Panisko DM, Keystone JS (1990) Treatment of malaria. *Drugs* **39**, 160-189

Riehle MA, Srinivasan P, Moreira CK, Jacobs-Lorena M (2003) Towards genetic manipulation of wild mosquito populations to combat malaria: advances and challenges. *Journal of Experimental Biology* **206**, 3809-3816

Riggione F, Pulido M, Woya O (1996) Plasmodium *falciparum:* surface modifications of infected erythrocytes from clinical isolates. Evidence of antigenic diversity using Venezuelan human cerebral malarial sera. *Parasitology Research* **82**, 490-496

Roberts D, Manguin S, Mouchet J (2000) DDT house spraying and re-emerging malaria. *Lancet* **356**, 330-332

Rosenthal PJ (1998) Proteases of malaria parasites: New targets for chemotherapy. *Emerging Infectious Diseases* **4**, 49-57

Russel RC (1987) Seasonal abundance, longevity and population age composition of potential malaria vectors in Northern and Southern Australia. *Australian Journal of Zoology* **35**, 289-306

Sachs J, Malaney P (2002) The economic and social of malaria. *Nature* **415**, 680-685

Samba EM (1997) The burden of malaria in Africa. *Africa Health* **19**, 17-18

Schellenberg D, Aponte JJ, Tanner M, Alonso PL (2001) Effect of malaria on soluble transferrin receptor levels in Tanzanian infants. *American Journal of Tropical Medicine and Hygiene* **65**, 138-142

Sherman IW (1998) *Malaria: Parasite Biology, Pathogenesis and Protection*, ASM Press, Washington, DC, 700 pp

Shiff C (2002) Integrated approach to malaria control. *Clinical Microbiology Review* **15**, 278-293

Sinden RE (1984) The biology of plasmodium in the mosquito. *Experientia* **40**,

1330-1343

Small J, Goetz SJ, Hay SI (2003) Climatic suitability for malaria transmission in Africa, 1911-1995. *Proceedings of the National Academy of Sciences USA* **100**, 15341-15345

Snow RW, Guerra CA, Noor AM, Myint HY, Hay SI (2005) The global distribution of clinical episodes of *Plasmodium falciparum* malaria. *Nature* **434**, 214-217

Srivastava A, Khanduri A, Lakhtakia S, Pandey R, Choudhuri G (1996) *Falciparum* malaria with acute renal failure. *Tropical Gastroenterology* **17**, 172-174

Talisuna AO, Bloland P, D'Alessandro U (2004) History, dynamics and public health importance of malaria parasite resistance. *Clinical Microbiology Reviews* **17**, 235-254

Torre D, Giola M, Spenza F, Matteeli A, Basilico C, Biondi G (2001) Serum levels of interleukin-18 in patients with uncomplicated plasmodium *falciparum* malaria. *European Cytokine Network* **12**, 316

Toure YT, Oduola A (2004) Malaria. *Nature Reviews Microbiology* **2**, 276-277

UNDP/WORLD BANK/WHO (1991) Report of the meeting 'Prospects for malaria control by genetic manipulation of its vectors'. UNDP/WORLD BANK/WHO Special Programme for Research and Training in Tropical Diseases (TDR), Geneva, Switzerland, *TDR/BCV/MAL-ENT/91.3*, 105 pp

White JJ (1996) The treatment of malaria. *New England Journal of Medicine* **335**, 800-806

White NJ, Ho M (1992) The pathophysiology of malaria. *Advances in Parasitology* **31**, 83-166

Wickham ME, Rug M, Ralph SA, Klonis N, McFadden GZ, Tilley L, Cowman AF (2001) Trafficking and assembly of the cytoadherence complex in plasmodium *falciparum*-infected human erythrocytes. *The EMBO Journal* **20**, 5636-5649

Williamson KC, Fujioka H, Aikawa M, Kaslow C (1996) Stage-specific processing of pfs 230, a plasmodium *falciparum* transmission-blocking vaccine candidate. *Molecular Biochemistry and Parasitology* **78**, 161-169

World Health Organization (WHO) (1984) Report of the WHO Expert Committee on vector biology, control and chemistry and speciation of parasites. *Technical Report Series* **669**, 120 pp

World Health Organization (WHO) (1990) Severe and complicated malaria. *Transactions of the Royal Society for Tropical Medicine and Hygiene* **84**, 1-65

World Health Organization (WHO) (2005) World malaria report, Available online: www.rbm.who.int/wmr2005/pdf/adv_e.pdf, 326 pp

Wu XQ, Vennison J, Liu HR, Ben-Dov E, Zaritsky A, Boussiba S (1997) Mosquito larvicidal activity of transgenic *Anabaena* PCC 7120 expressing combinations of genes from *Bacillus thuringiensis sp israelensis*. *Applied and Environmental Microbiology* **6312**, 4971-4975

Zaim M, Aitio A, Nakashima NY (2000) Safety of pyrethroid-treated mosquito nets. *Medical and Veterinary Entomology* **14**, 1-5