# Understanding Human Malaria: Further Review on the Literature, Pathogenesis and Disease Control

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**Abstract:** Human malaria is caused by single-celled parasites from the genus Plasmodium. More than one hundred different species of Plasmodium exist and produce malaria in many types of animals including birds and humans. A further review on the four different species of plasmodium parasites implicated in human malaria in the tropics was undertaken. The pathogenesis, laboratory diagnosis; public health significance, treatment and control strategies including the new and holistic approaches to fighting the ancient scourge was highlighted. All the manifestations of malarial illness are caused by the infection of red cells by the asexual forms of the malaria parasite, and this makes malaria a potentially multisystem disease, as every organ of the body is reached by the blood. The parasite damages red blood cells using plasmepsin enzymes which are aspartic acid proteases that degrade hemoglobin. Understanding the molecular biology of the parasite and the sporogonic cycle to aid in re- engineering of the anopheline mosquito and improved entomological field will help in controlling the disease. The protection of the host from the bite of mosquito using netting, repellants and elimination of the vector through biological control measures, by introducing organisms that will feed on their larvae into the site of breeding coupled with prophylactic treatment with anti-malaria drugs for exposed persons, in theory, would eradicate the disease.
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**Key words:** Anopheline mosquito, plasmodium parasites, human malaria, pathogenesis, public health significance, holistic approaches, anti-malaria drugs, ancient scourge, multisystem disease, malarial illness, red blood cells.

## 1. Introduction

Plasmodium is a Eukaryote, an organism whose cells have a nucleus, but with unusual features. Chavatt *et al.* (2007) reported that all the species examined have 14 chromosomes, one mitochondrion and one plastid (Martinsen *et al*., 2007). The plastid, unlike those found in algae, is not photosynthetic but there is some evidence that it is involved in reproduction (Collins *et al.*, 2008). Malaria was first described in 1885 by Marchiafava and Celli.

It now contains about 200 species divided into several subgenera. Current theory suggests that the genera *Plasmodium, Haemocystis, Haemoproteus* and *Hepatocystis* evolved from leukocytozoon species (Chavatt *et al,* 2007). Parasites of the genus Leukocytozoan are known to infect white blood cells and are transmitted by 'black flies' (*Simulium species*), a large genus of flies related to the mosquitoes (Telfold, 1988).

The parasite is thought to have originated from *Dinoflagellates*, a large group of photosynthetic protozoa (Laudau *et al.*, 2003). Mosquitoes of the genera Culex (*Culex fatigans*), Anopheles (*Anopheles albimanus*), Mansonia (*Mansonia crassippes*) and Aedes (*Aedes aegypti*) may act as vectors. However, it was reported by Giovanni Battista Grassi in 2003 that human malaria could only be transmitted by the female Anopheles mosquitoes.

Every year 300 to 500 million people suffer from malaria, causing an estimated 1 to 2.7 million deaths (Gallup and Sachs, 2001). Report has it that 90 percent of these deaths occur in sub-Saharan Africa, mostly among children younger than five (Sherman, 1998; Philip, 2011). Plasmodium, the malaria-causing protozoan belonged to the class sporozoa, family Plasmodiidae, order Haemosporidia, and phylum Apicomplexa (Telfold, 1988). Organisms in the class, sporozoa are all obligate parasites; therefore, there are no free-living representatives (John *et al.*, 2006; Hay *et al*., 2009). Plasmodium lack locomotive organelles and multiply by spore formation.

Malaria accounts for 9 to10 percent of Africa's entire disease burden with severe economic consequences (Craig *et al.*, 1999; Anyido *et al.*, 2010). Countries with a high incidence of malaria can suffer a 1.3 percent annual loss of economic growth. A Harvard/World Health Organization study suggests that if malaria had been eliminated 35 years ago, Sub-Saharan Africa's gross domestic product could be $100 billion greater (Gallup and Sachs, 2001).

Malaria thrives in the tropical areas of Asia, Central and South America, where it strikes millions of people (Cohen *et al.*, 2009; Decastro *et al.*, 2004; Bomblies *et al.*, 2008). Each year 350 to 500 million cases of malaria occur worldwide. The understanding of malaria pathogenesis can inform how best to defeat malaria and contain the rising cases of infant mortality and morbidity associated with the ancient global scourge.

The present review aims at creating a flexible framework for a better understanding of the human malaria. A further review on the stages and development of plasmodium parasites coupled with possible new tools in the control, treatment and eradication of the disease was re-examined.

**1.1. Disease transmission**

Malaria is transmitted by the blood feeding of infectious female Anopheles mosquitoes. There are about 100 species of Anopheles genus, but only 50–60 can transmit malaria. Examples include *Anopheles stephensi* and *A. gambiae* (Clement, 2000). In rare cases, a person may contract malaria through contaminated blood, or a fetus may become infected by its mother during pregnancy.

Because the malaria parasite is found in red blood cells, malaria can also be transmitted through blood transfusion, organ transplant, or the shared use of needles or syringes contaminated with blood (Kileen *et al*., 2006). Malaria also may be transmitted from a mother to her fetus before or during delivery. This is known as "congenital" malaria.

Malaria is endemic to over 100 nations and territories in Africa, Asia, Latin America, the Middle East, and the South Pacific. At least 10 species of *Plasmodium* infect humans; other species infect other animals, including birds, reptiles and rodents, while 29 species infect non-human primates (Chavatt *et al.,* 2007; Yotoko and Elisei, 2006). The only known host of *P. falciparium* and *P. malaria* is humans. *P. vivax*, however, has been reported to infect chimpanzees and orangutans (Reid *et al*., 2006).

*P. ovale* are said to have an unusual distribution, being found in Africa, the Philippines and New Guinea and can be transmitted to chimpanzees and other animals (Sullivan and Galland, 1994) as well.

**1.2. Species of plasmodium that infect humans**

Four species of *Plasmodium* commonly infect humans. They include *Plasmodium falciparum* (the cause of malignant tertian malaria), *Plasmodium malariae* (the cause of benign quartian malaria), *Plasmodium ovale* (the other, less frequent, cause of benign tertian malaria), and *P*lasmodium *vivax* (the most frequent cause of benign tertian malaria). *Plasmodium (P.) falciparum* is by far the deadliest of the four human malarial species; *P. vivax* is the most widespread. *P. malariae* and *P. ovale*, although also significant, cause fewer cases and less severe forms of the disease (Depinay *et al.*, 2004; Nester *et al*., 1998; Sullivan and Galland, 1994).

*Plasmodium ovale* is rare, can cause relapses, and generally occurs in West Africa. Nearly all human deaths from malaria are caused by *Plasmodium falciparum,* mainly in sub-Saharan Africa (Obi, 2013). In addition to being the deadliest form of malaria, *P. falciparum* destroys red blood cells, which can cause acute anemia. Compared to *P.vivax*, *P. falciparum* is less widespread.

Adherence to cells in certain tissues may cause problems within those organs, such as the lungs, kidneys and brain (Baier, 1998). *Plasmodium vivax*, the most geographically widespread of the species, produces less severe symptoms. Relapses, however, can occur for up to three years, and chronic disease is debilitating.

*Plasmodium malariae* infections not only produce typical malaria symptoms but also can persist in the blood for very long periods, possibly decades, without ever producing symptoms (Yotoko and Elisei, 2006). A person with asymptomatic (no symptom) *P. malariae*, however, can infect others, either through blood donation or mosquito bites. Although, *P. malariae* has been wiped out from temperate climates, it persists in African sub-region.

**1.3. Life Cycle**

All types of malaria have a similar life cycle .The malaria parasite exhibits a complex life cycle (Richard, 2007) involving two very different hosts: an insect vector (mosquito) and a vertebrate host (human) as shown in Figure 1. They have a sexual cycle, in which spores are formed, and an asexual cycle. The sexual cycle takes place in the gut and abdominal wall of the female of some species of mosquito in the genus *Anopheles* while the asexual cycle takes place in the liver and erythrocytes of humans and causes the symptoms of the disease (Obi, 2013).

During the sexual cycle, in the mosquito's stomach a "male" gametocyte fertilizes a "female" to form an egg, or oocyst, which matures into thousands of sporozoites that swim to the mosquito's salivary glands to be injected into another human at the next bite (Trager *et al.*, 197). An organism such as the malaria parasite (Plasmodium) must alternate sexual and asexual cycles (alternation of generation) in order to continue to exist (Sacci *et al.*, 2006). Both the intracellular as well as extracellular stages must be accomplished in both the human host and mosquito vector, respectively.

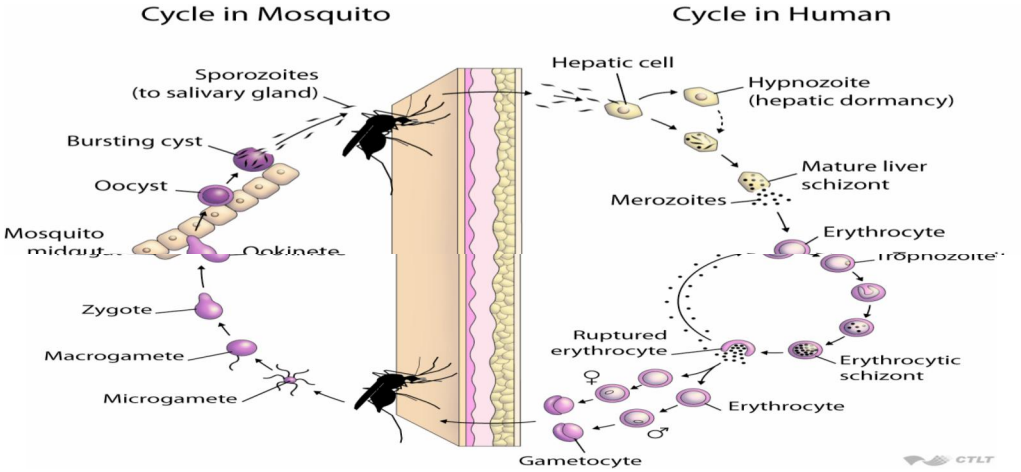


**Figure 1.** Life cycle of *Plasmodium* parasite (Source: Richard, 2007)

**1.4. Pathogenesis**

The disease remains one of the major killers of humans worldwide, threatening the lives of more than one-third of the world's population (Smith and McKenzie, 2004). Humans contact malaria from the bite of a plasmodial-infected female *Anopheline* mosquito (Killeen *et al.*, 2000). As the mosquito inserts its proboscis into a human to take its blood meal, it injects the plasmodial sporozoite at the same time through its saliva (Killeen *et al.*, 2006). The sporozoite begins the asexual cycle by the pre-erythrocytic development of merozoites in the parenchymal cells of the liver (Tsuji *et al.*, 1994). The merozoites can repeat the pre-erythrocytic cycle in the liver cells, or they can enter the erytrocytic cycle. Once the merozoites penetrate the erythrocytes, the parasite undergoes several morphological changes, as shown in Figure 2. First, a ring form develops, which enlarges to become a mature amoeboid trophozoite filling most of the parasitized red blood cell (Philip, 2011). Next, asexual multiplication takes place by the splitting of nuclear material and cytoplasm of the amoeboid-appearing parasite to form more merozoites.

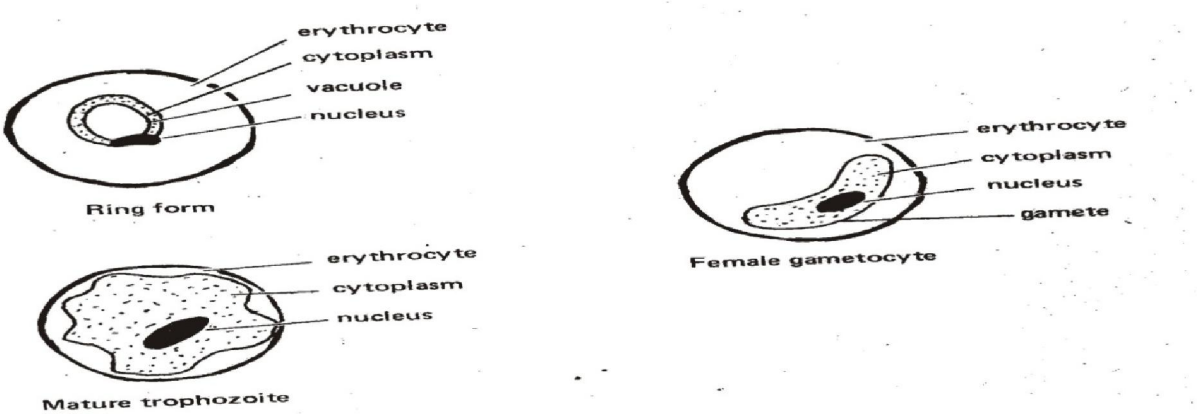


**Figure 2.** Stages of the plasmodium life cycle in blood smears (Source: Sherman, 1998)

Depending on the species, this multiple fission (schizogony) results in 6 to 36 new merozoites per parasitized erythrocyte. As the erythrocyte ruptures, the merozoites are freed into the blood plasma to infect many other erythrocytes. During the erythrocytic cycle, some merozoites differentiate as male and female gametocytes (Sortt *et al*., 1951; Zhu and Hollingdale, 1991).

Figure 3 is a simplified presentation of the asexual cycle as seen in humans. Each merozoite invades a red blood cell, and for two days multiplies into more merozoites. The red blood cell full of merozoites ruptures to release more merozoites. It is this stage of the life cycle that causes disease and, too often, death. Some merozoites change into the form called gametocytes, which do not cause disease but remain in the blood until they are cleared by drugs or the immune system, or taken up by the bite of a mosquito. For the sexual cycle to evolve, the gametocytes of both sexes must be ingested in the blood meal of another female *Anopheles* mosquito, as shown in Figure 4. In the gut of the mosquito, the male gametocyte forms spermatozoa, and the female forms an ovum. Fertilization of the ovum takes place, and the resting zygote changes shape, becomes motile, and invades the gut wall.

Next, in the tissues of the gut wall, sporogony of the parasite takes place. That is, there is multiple fission of parasitic content, and numerous sporozoites are formed. The sporozoites migrate through the tissues of the mosquito to the salivary glands where they wait to be injected into another unsuspecting human host when the mosquito takes its next blood meal (Saul *et al.*, 1990; WHO, 1999; Clement, 2000). The asexual cycle begins again, and malaria is established in a new host.

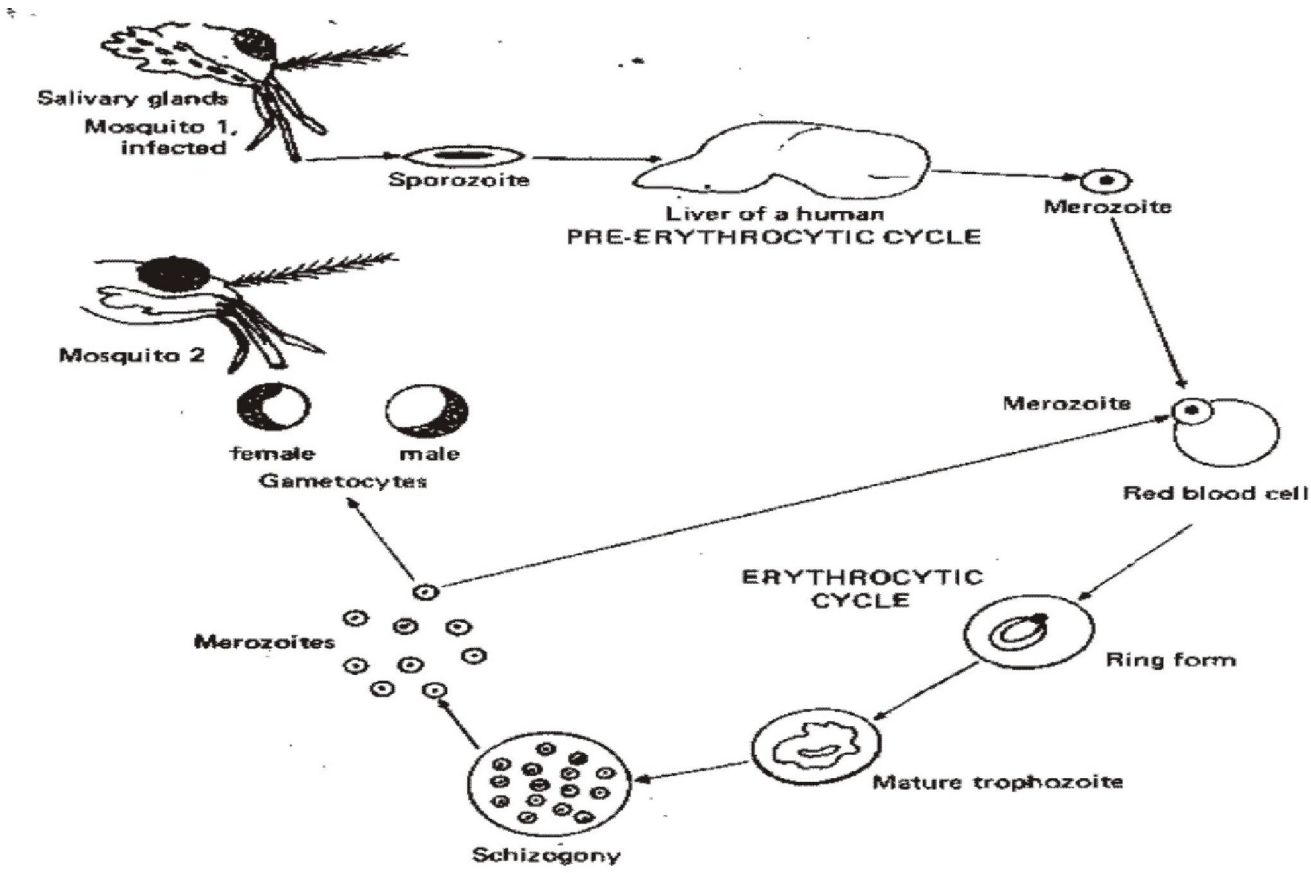


**Figure 3.** Asexual cycle in humans (Source: Sullivan and Galland, 1994)

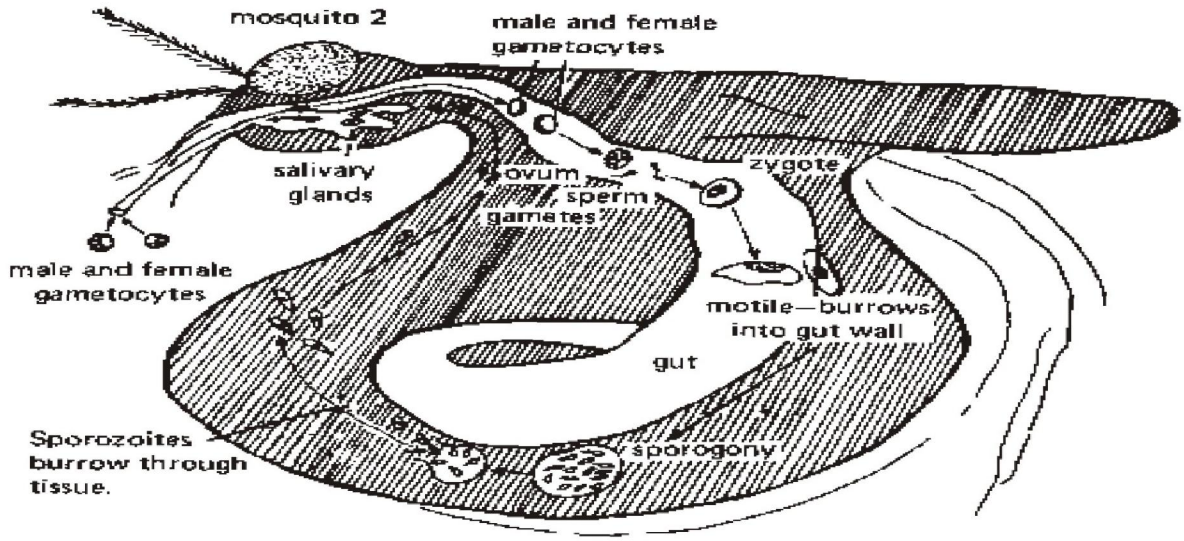


**Figure 4.** Sexual cycle in gut of mosquito (Source: Beier, 1998)

At the completion of schizogony within the red blood cells, each cycle lasting 24 to 72 hours depending on the species of the infecting parasite, newly developed merozoites are released by the lyses of infected red cells accompanied by waste substances, such as red cell membrane products, hemozoin pigment, and other toxic factors such as glycosylphosphatidylinositol (GPI). These products, particularly the GPI, activate macrophages and endothelial cells to secrete cytokines and inflammatory mediators such as tumor necrosis factor (TNF), IFN-γ, interleukin 1 (IL-1), IL-6, IL-8, macrophage colony-stimulating factor, and lymphotoxin, as well as superoxide and nitric oxide (Nardin *et al.,* 1982; Obi, 2013). The GPI tail was reported to be common to several merozoite surface proteins such as MSP-1, MSP-2, and MSP-4, as a key parasite toxin (Clement, 2000).

### 1.5. Geographical classification of malaria

Geographical areas classified by intensity of transmission (based upon percent of children, age 2–9, with enlarged spleens and malaria parasitemia) are as presented in Table 1.

**Table 1: GEOGRAPHICAL AREAS CLASSIFIED BY INTENSITY OF TRANSMISSION**

| S/NO. | Geographical areas | Intensity of transmission |
|---|---|---|
| 1 | Holo-endemic | Intense transmission with continuing high EIRs where everyone is infected with malaria parasites all the time. In older children and adults, parasites difficult to detect because of high levels of immunity, but sufficient search will generally reveal the presence of parasites. *Criteria:* Spleen and parasite rates of over 75%. |
| 2 | Hyper-endemic | Regular, often seasonal transmission to all, but immunity in some does not confer continuing protection at all times. *Criteria:* Spleen and parasite rates from 50–75%. |
| 3 | Meso-endemic | Transmission fairly regularly but at much lower levels. Danger is occasional epidemics involving those with little immunity resulting in fairly high mortality. *Criteria:* Spleen and parasite rates from 10–50%. |
| 4 | Hypo-endemic | Limited malaria transmission and population with little or no immunity. Danger is severe malaria epidemics involving all age groups. *Criteria:* Spleen and parasite rates less than 10%. |

Source: Richard (2007)

**1.6. Signs and symptoms**

Human shows symptoms, usually about 10 days after being bitten by the infected mosquito (Murphy and Breman, 2004). Malaria typically produces a string of recurrent attacks, or paroxysms, each of which has three stages such as chills, followed by fever, and then sweating. The chill and fever symptoms of malaria are associated with the almost simultaneous release of many merozoites into the bloodstream. The chill may last as long as one hour.

The patient usually experiences headache, fever, nausea and vomiting, diarrhea, anorexia, tiredness, aching joints and muscle, thrombocytopenia, immunosuppression, coagulopathy, and central nervous system manifestations during this time. These systemic manifestations have been largely attributed to the various cytokines released in response to the parasite and red cell membrane products (Hoffman, 1996).

**1.7. Laboratory diagnosis**

Health care providers should suspect malaria in anyone who has been in the tropics recently or received a blood transfusion, and who develops a fever and other signs that resemble the flu. Laboratory examination of the malaria parasite is usually done through microscopic inspection of stained blood smear for the different stages of the *Plasmodium* parasite. The thick film is often stained with Giemsa stained (Cheesbrough, 2005) and examined using x100 objective to view the inside of the erythrocytes (Sacci *et al.*, 2006). The common features used to identify the malaria parasite on Giemsa stained blood smears are dark red chromatin bodies, pale purplish-blue cytoplasm, black pigments, and rings that are usually in marginal location. The ring form is the most common stage. The three structures of the ring form of the parasite that are commonly seen are the nucleus, cytoplasm and vacuole. The infected erythrocytes contain a developing trophozoite with a distinctive chromatin dot.

**1.8. Public health significance**

Public health significance of a disease equals the incidence and consequent disability and death. In low-transmission areas this is a useful formulation but in high-transmission holo-endemic Africa, however, everyone is infected all the time and neither incidence nor prevalence has much meaning (Decastro *et al*., 2004). Malaria has continued to be a major health problem in many parts of the world, with over 2400 million people in about 100 countries at risk of infection.

Nearly all the people who live in endemic areas are exposed to infection repeatedly. Those who survive malaria in childhood gradually build up some immunity (U.S. Department of Health and Human Services, National Institutes of Health, National Institute of Allergy and Infectious Diseases, 2007). In other areas, where the infection rate is low, people do not develop immunity because they rarely are exposed to the disease. The enormous loss of life, days of labour, absenteeism in schools; the cost of treatment of patients and the negative impact of the disease on the socio-economic growth of a nation makes malaria a major social and economic burden (WHO, 1995; Hermsen *et al*., 2004).

**1.9. Control strategies**

Control strategies geared toward prevention of the disease include general infrastructure, community and household empowerment including the role of vector control and environmental improvements to reduce breeding. Residual insecticide (role for DDT), bed-nets impregnated and personal protection. Household use of anti-malarial for under-fives by mothers; intermittent preventive therapy for pregnant women and for under-fives.

Monitoring for anti-malarial resistance everywhere; the protection of the host from the bite of the mosquito using netting and repellants. Treatment and prophylaxis using quinine, quinidine, chloroquine, amodiaquine, and relatives; Pyrimethamine. Others are Proquanil and chlorproguanil, Mefloquine, Halofantrine, Artemisinin and derivatives (qinghaosu). Antibiotics such as tetracycline, clindamycin, rifampicin and Primaquine are recommended. Traditional first-line treatments using choloroquine, Sulphadoxine and /or Pyrimethamine have lost much of their effectiveness in many countries. This has led to the need for new and more expensive antimalarial drugs, including artemisinin combination therapy–ACT, now being introduced by some governments (Okell *et al.*, 2008; Hoffman, 1996).

**1.10. Holistic approach to fighting malaria**

Key challenges right now are to produce clinical-grade vaccines against malaria. Vaccine development, especially asexual phase, but perhaps sporozoite with new developments. Several different malaria vaccine approaches, using the latest advances in technology, are now in human clinical trials in

Africa, Asia, Europe, and the United States (Prescott *et al*., 2005; Philip, 2011). Malaria vaccines could save millions of lives and are likely to be hugely cost-effective.

Much progress has been made in understanding the immune mechanisms and in identifying potential vaccine targets. Although vaccine development is reaching maturity, it is still a challenge and could be ten years before an effective vaccine could be licensed and introduced. Methods for better understanding of micro-epidemiological variation and factors that contribute to its spread and better diagnostic tests that rapidly and inexpensively indicate drug resistance are necessary.

The new tools included drug development and acceleration of those in the pipeline, understanding of the molecular biology of the parasite. Better understanding of the sporogonic cycle to aid in re- engineering of the anopheline, improved entomological field methods for better understanding of micro-epidemiological variation and understanding of the mechanism of drug resistance and factors that contribute to its spread coupled with better diagnostic tests that rapidly and inexpensively indicate drug resistance.

**Conclusion**

A major turning point in recent years is the development and implementation of a vaccine against malaria which are critical to the long-term solution to this age-old killer. Vaccines are directed against sporozoites (plus), asexual forms (patarroyo) and gametocytes (plus). This is referred to as "transmission-blocking" Campaigns must address the ecology and behaviour of local mosquito populations in order to ensure that sufficient resources with broad enough effects for all relevant components of the local mosquito populations are introduced. A one-size-fits-all campaign is not optimal, being wasteful in some circumstances and insufficient in others; local tailoring and design are important.

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**Reference**

1. Anyido, A.E., V.O. Agbata; P.U. Umeaneto, M. O. Obiukwu and E.S. Amadi (2010). Malaria burden and vector abundance in a sub-urban community in the rainforest zone of Nigeria. *Nig. J. Microbiol.* **24 (1):** 2224-2230.
2. Beier, J.C. (1998). Malaria parasite development in mosquitoes. *Ann. Rev. Entomol.*43:519-543.
3. Bomblies, A., J-B. Duchemin and E.A.B.Eltahir(2008).Hydrology of malaria: Model development and application to a Sahelian village. *Water Resour. Res.* 44:W12445.
4. Bertani and G. Snounou (2003). Taxonomic status and re-description of *Plasmodium relictum* (Grassi et Feletti, 1891), *Plasmodium* major Raffaele, 1931, and description of *P. biguetin.* Sp. in sparrows. *Parasitologia,* 45 (3-4): 119-23.
5. Cheesbrough, M. (2005). District Laboratory Practice in Tropical Countries, Part 1, Cambridge University Press UK, 2005 Ed.191.
6. Collins, W.E., J.S. Sullivan, D. Nace, T. Williams, A. Williams and J.W. Barnwell (2008). Observations on the sporozoite transmission of *Plasmodium vivax* to monkeys. *J. Parasitol.* 94 (1): 287-8.
7. Craig, M.H., R.W. Snow and D. le Sueur (1999). A climate-based distribution model of malaria transmission in sub-Saharan Africa. *Parasitol. Today*, 15:105-111.
8. Clements, A.N. (2000). *The Biology of Mosquitoes,* Volume 1: Development, Nutrition, and Reproduction New York: CABI Publishing.
9. Chavatt, J.M., F. Chiron, A. Chabaud ad I. Landau (2007). Probable speciations by host-vector fidelity: 14 species of *Plasmodium* from magpies. *Parasite (in Frence)*, 14 (1): 21-37.
10. Cohen, J.M, D.L. Smith, A. Vallely, G. Taleo, G. Malefoasi, and O. Sabot (2009). Holding the Line. In Shrinking the Malaria Map:

A Prospectus on Malaria Elimination. Edited by: Feachem, R.G.A., A.A. Phillips, G.A. Targett, M.E. Group. San Francisco: The Global Health Group: *UCSF Global Health Sciences*, 40-60.

11. Depinay, J-M., C. Mbogo, G. Killeen, B. Knols, J. Beier, J. Carlson, J. Dushoff, P. Billingsley, H. Mwambi, J. Githure, A.M. Toure and F.E. McKenzie (2004). A simulation model of African Anopheles ecology and population dynamics for the analysis of malaria transmission. *Malar J.* 3:29.
12. De Castro, M.C., Y. Yamagata, D. Mtasiwa, M. Tanner, J. Utzinger, J. Keiser and B. H. Singer (2004). Integrated urban malaria control: a case study in Dar Es Salaam, Tanzania. *Am. J. Trop. Med. Hyg.*71:103-117.
13. Gallup, J.L., and J. D. Sachs (2001). The Economic Burden of Malaria. *Am. J. Trop. Med. Hyg*. **64(1-2)**:85-96.
14. Hay, S.I., C.A. Guerra, P.W. Gething, A.P. Patil, A.J. Tatem, A.M. Noor, C.W. Kabaria, B.H. Manh, I.R. Elyazar, S. Brooker, D.L. Smith, R.A. Moyeed and R.W. Snow (2009). A world malaria map: *Plasmodium falciparum* endemicity in 2007. *PLoS Med*. 6:e1000048.
15. Hoffman, S.L. (1996). *Malaria Vaccine Development*. Washington, DC: ASM Press.
16. Hermsen, C.C., S.J. De Vlas, G.J.A. Van Gemert, D.S.C. Telgt; D.F. Verhage, and R.W. Sauerwein (2004). Testing vaccines in human experimental malaria: statistical analysis of parasitemia measured by a quantitative real-time polymerase chain reaction. *Am. J. Trop. Med. Hyg.* 71:196–201.
17. Killeen, G., F. McKenzie, B. Foy, C. Schieffelin, P. Billingsley and J. Beier (2000). A simplified model for predicting malaria entomologic inoculation rates based on entomologic and parasitologic parameters relevant to control. *Am. J. Trop. Med. Hyg.* 62:535-544.
18. Killeen, G.F., A. Ross and T. Smith (2006). Infectiousness of malaria-endemic human populations to vectors. *Am. J. Trop. Med. Hyg.* 75:38-4.
19. Laudau, I., A.G. Chabaud, S. Murphy, S.C., and J.G. Breman (2014). Gaps in the Childhood Malaria Burden in Africa: Cerebral Malaria, Neurological Sequelae, Anemia, Respiratory Distress, Hypoglycemia, and Complications of Pregnancy. *Am. J. Trop. Med. Hyg*. 64(1–2):57–67.
20. Martinsen, E.S., J.L. Waite and J.J. Schall (2007). Morphologically defined subgenera of Plasmodium from avian hosts: test of monophyly by phylogenetic analysis of two mitochondrial genes. *Parasitol.* 134 (4): 483-90.
21. Nardin, E.H., V. Nussenzweig; R.S Nussenzweig, W.E. Collins; K.T. Harinasuta, P. Tapchaisri and Y. Chomcharn (1982). Circumsporozoite proteins of human malaria parasites *Plasmodium falciparum* and *Plasmodium vivax*. *J. Exp. Med.* 156:20–30.
22. Nester, E.W, C. E. Roberts; N.N. Pearsall; D. G. Anderson and M.T. Nester (1998). *Microbiology: A human perspective*. 2nd ED. WCB/McGraw- Hill. USA.
23. Okell, L., C. Drakeley, A. Ghani, T. Bousema and C. Sutherland (2008). Reduction of transmission from malaria patients by artemisinin combination therapies: a pooled analysis of six randomized trials. *Malar J.* 7:125.
24. Obi, R, K. (2013). Pathogenesity of severe malaria. In An Annual Newsletter Published

by the Nigerian Society for Microbiology (NSM), 6 (1): 23.
25. Philip, A. E. (2011). A malaria transmission-directed model of mosquito life cycle and ecology. *Eckhoff Malaria Journal,* 10:303.
26. Prescott, L.M., J.P. Harley and D.A. Klein (2005). *Microbiology,* 6th Ed. McGraw Hill Co. New York, U.S.A.
27. Richard, M. (2007). *Epidemiology and control of Malaria.* John Hopkins Bloomberg School of Public Health, the John Hopkins University and Richard Morrow, USA.
28. Reid, M.J., R. Ursic and D. Cooper (2006). Transmission of human and macaque *Plasmodium* spp. to ex-captive orangutans in Kalimantan, Indonesia. *Emerging Infect, Dis*. 12 (12): 1902-8.
29. Sacci, J.B.; A. Uzma, D. Donna; L. Jamie, D; J.T. Lorne; A. F. Azad, and M.K.Norman (2006). *Plasmodium falciparum* infection and exoerythrocytic development in mice with chimeric human livers. *Int. J. Parasitol*. 36:353–360.
30. Shortt, H.E., N.H. Fairley; G. Covell; P.G. Shute and P.C.C. Garnham (1951).The pre-erythrocytic stage of *Plasmodium falciparum*. *Trans. R. Soc. Trop.Med. Hyg*. 44: 405–419.
31. Sullivan, J.S., and G.G. Galland (1994). Further studies on the sporozoite transmission of the Salvador 1 strain of *Plasmodium vivax. J. Parasitol.* 80 (4): 512-7.
32. Sherman, I.W (1998). *Malaria: Parasite Biology, Pathogenesis and Protection*. Washington, DC: ASM Press.
33. Smith, D.L. and F.E. McKenzie (2004). Statics and dynamics of malaria infection in Anopheles mosquitoes. *Malar J.* 3:13.
34. Saul, A.J., P.M. Graves and B.H. Kay (1990). A cyclical feeding model for pathogen transmission and its application to determine vectorial capacity from vector infection rates. *J. Appl. Ecol*. 27:123-133.
35. Trager, W., and J.B. Jensen (1976). Human malaria parasites in continuous culture. *Science,* 193: 673–675.
36. Tsuji, M., D. Mattei, R.S. Nussenzweig; D. Eichinger; and F. Zavala (1994). Demonstration of heat-shock protein 70 in the sporozoite stage of malaria parasites. *Parasitol. Res*. 80: 16.
37. Telfold, S. (1988). A contribution to the systematic of the reptilian malaria parasites, family Plasmodiidae (Apicomplexa: Haemosporina). *Bulletin of the Florida State Museum Biological Sciences,* 34 (2): 64-96.
38. U.S. Department of Health and Human Services, National Institutes of Health, National Institute of Allergy and Infectious Diseases (2007). Understanding Malaria: Fighting an Ancient Scourge. *NIH Publication No*. 07-7139.
39. World Health Organization (1995). *The malaria* manual: Guidelines for the rapid Assessment of social, economic and cultural aspects of malaria. TGR/Ser/MSR/95.1,Geneva.10.
40. Yotoko, K.S.C. and C. Elisei (2006). Malaria parasites (Apicomplexa, Haematozoea) and their relationships with their host: is there an evolutionary cost for the speciation? *J. Zoo. Syst. Evol. Res*. 44(4): 265-273.
41. Zhu, J., and M.R. Hollingdale (1991). Structure of Plasmodium falciparum liver stage antigen-Mol. *Biochm, Parasitol.* 48:223–226.