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This book discussed on biology of malaria parasite, epidemiology of malaria, life cycle pathology, pathogenesis, diagnosis and control of malaria. This book give basic knowledge to medical students about malaria infections which enable them to deal properly with cases of this fatal disease. Also in this book you will find brief information about vaccination strategy against malaria parasite which is the most dangerous parasitic disease.

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978-620-2-05168-2

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Cover image: www.ingimage.com

Publisher:
LAP LAMBERT Academic Publishing
is a trademark of
International Book Market Service Ltd., member of OmniScriptum Publishing Group
17 Meldrum Street, Beau Bassin 71504, Mauritius

Printed at: see last page
**ISBN: 978-620-2-05168-2**

# Essential Malariology

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**DEDICATION**

**To all good people in the world**

**ACKNOWLEDGEMEN:**

***We want to give many thanks to all people who encouraged us from our birth till now.***

## Introduction:

Recognizable descriptions of malaria were recorded in Chinese, Indian, Egyptian and Mesopotamian texts as early as 5,000 years ago. Evidence from human DNA sequences shows the effects of malaria to be far older still, influencing human evolution across tens of thousands of years. It is no exaggeration to say that malaria has played a crucial role in human history, determining the fates of armies and empires. Malaria brought down Alexander the Great and saved Rome from Attila's hordes. Dubbed the 'King of Diseases' in the Vedas, its modern name comes from the Italian peninsula, where mal'aria or 'bad air' was thought to cause the debilitating paroxysmal tertian or quartan (three- or four-day) fevers and febrile deaths that ravaged the populace every year for millennia. (1)

Malaria is a mosquito-borne disease caused by a parasite. People with malaria often experience fever, chills, and flu-like illness. Left untreated, they may develop severe complications and die. In 2015 an estimated 212 million cases of malaria occurred worldwide and 429,000 people died, mostly children in the African Region. About 1,700 cases of malaria are diagnosed in the United States each year. The vast majority of cases in the United States are in travelers and immigrants returning from countries where malaria transmission occurs, many from sub-Saharan Africa and South Asia. (2)

Malaria can occur if a mosquito infected with the *Plasmodium* parasite bites you. An infected mother can also pass the disease to her baby at birth. This is known as congenital malaria. Malaria is transmitted by blood, so it can also be transmitted through:

- ✓ an organ transplant
- ✓ Blood transfusion
- ✓ Use of shared needles or syringes. (3)

### Malaria history

Malaria parasites have been with us since the dawn of time. They probably originated in Africa (along with mankind), and fossils of mosquitoes up to 30 million years old, show that the malaria vector, the malaria mosquito, was present well before the earliest history. Hippocrates, a physician born in ancient Greece, today regarded as the "Father of Medicine", was the first to describe the manifestations of the disease, and relate them to the time of year and to where the patients lived. Before this, the supernatural was blamed. The association with stagnant waters (breeding grounds for the Anopheles mosquito) led the Romans to begin drainage programs, the first intervention against malaria.

The first recorded treatment dates back to 1600, when the bitter bark of the Cinchona tree in Peru was used by the native Peruvian Indians. By 1649, the bark was available in England, as "Jesuits powder," so that those suffering from "agues" might benefit from the chemical substance quinine, which it contained. Not until 1889 was the protozoal (single celled parasite) cause of

malaria discovered by Alphonse Laveran working in Algeria, and only in 1897 was the Anopheles mosquito demonstrated to be the vector for the disease by Ronald Ross.

When Alphonse Laveran, in 1879, began his research at the military hospital of Bône in Algeria, he only set himself the task of explaining the role of the particles of black pigment found in the blood of people suffering from malaria. After 1850, when these particles, called melanins, were discovered, methods had been discussed in determining whether they were only to be found in patients suffering from malaria, or were present in other diseases as well. Laveran first set about solving this problem, which was particularly important to the diagnosis of malaria. During his investigations, Laveran not only found the particles he had been looking for: he also found some entirely unknown bodies with certain characteristics which led him to suppose that parasites were involved. His initial investigations were carried out on fresh blood without using chemical reactions or any staining process. He was nonetheless successful, using this primitive method of examination, in distinguishing and describing most of the more important forms adopted by these new bodies, which varied so much in their appearance. (4)

**Malaria facts :**

- Malaria is a life-threatening disease caused by parasites that are transmitted to people through the bites of infected female *Anopheles* mosquitoes.
- In 2015, 91 countries and areas had ongoing malaria transmission.
- Malaria is preventable and curable, and increased efforts are dramatically reducing the malaria burden in many places.
- Between 2010 and 2015, malaria incidence among populations at risk (the rate of new cases) fell by 21% globally. In that same period, malaria mortality rates among populations at risk fell by 29% globally among all age groups, and by 35% among children under 5.
- The WHO African Region carries a disproportionately high share of the global malaria burden. In 2015, the region was home to 90% of malaria cases and 92% of malaria deaths. (5)

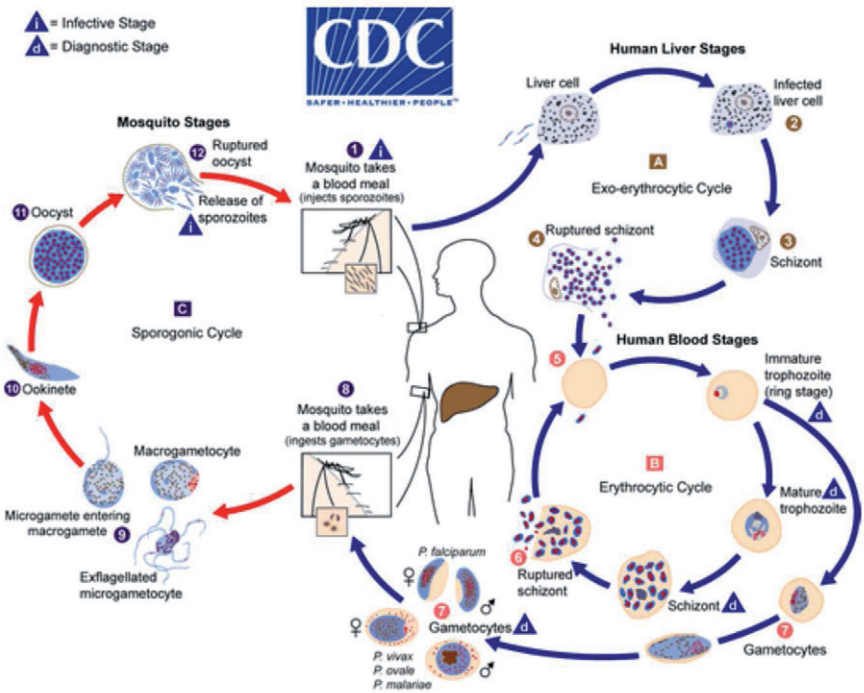
**Biology of Malaria parasite**

The natural ecology of malaria involves malaria parasites infecting successively two types of hosts: humans and female *Anopheles* mosquitoes. In humans, the parasites grow and multiply first in the liver cells and then in the red cells of the blood. In the blood, successive broods of parasites grow inside the red cells and destroy them, releasing daughter parasites ("merozoites") that continue the cycle by invading other red cells.

The blood stage parasites are those that cause the symptoms of malaria. When certain forms of blood stage parasites ("gametocytes") are picked up by a female *Anopheles* mosquito during a blood meal, they start another, different cycle of growth and multiplication in the mosquito.

After 10-18 days, the parasites are found (as "sporozoites") in the mosquito's salivary glands. When the *Anopheles* mosquito takes a blood meal on another human, the sporozoites are injected with the mosquito's saliva and start another human infection when they parasitize the liver cells.

Thus the mosquito carries the disease from one human to another (acting as a "vector"). Differently from the human host, the mosquito vector does not suffer from the presence of the parasites.

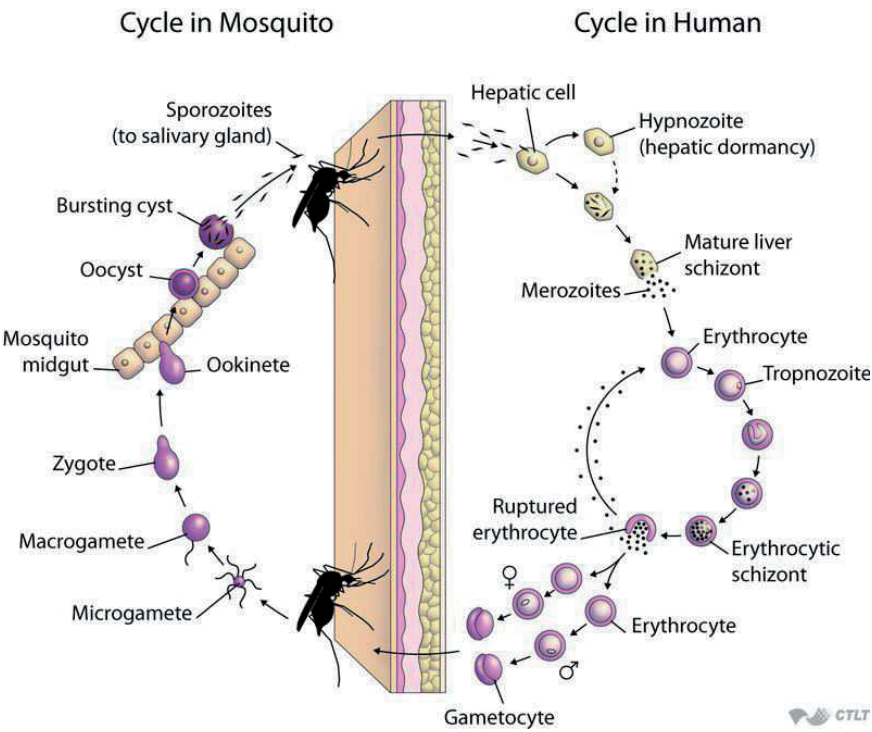



The malaria parasite life cycle involves two hosts. During a blood meal, a malaria-infected female *Anopheles* mosquito inoculates sporozoites into the human host❶. Sporozoites infect liver cells ❷and mature into schizonts❸, which rupture and release merozoites❹. (Of note, in *P. vivax* and *P. ovale* a dormant stage [hypnozoites] can persist in the liver and cause relapses by invading the bloodstream weeks, or even years later.) After this initial replication in the liver (exo-erythrocytic schizogonyⒶ), the parasites undergo asexual multiplication in the erythrocytes (erythrocytic schizogonyⒷ). Merozoites infect red blood cells❺. The ring stage trophozoites mature into schizonts, which rupture releasing merozoites. Some parasites differentiate into sexual erythrocytic stages (gametocytes) ❼. Blood stage parasites are responsible for the clinical manifestations of the disease.

The gametocytes, male (microgametocytes) and female (macrogametocytes), are ingested by an *Anopheles* mosquito during a blood meal❽. The parasites' multiplication in the mosquito is known as the sporogonic cycleⒸ. While in the mosquito's stomach, the microgametes penetrate the macrogametes generating zygotes❾. The zygotes in turn become motile and elongated

(ookinetes) ⑩which invade the midgut wall of the mosquito where they develop into oocysts⑪. The oocysts grow, rupture, and release sporozoites⑫, which make their way to the mosquito's salivary glands. Inoculation of the sporozoites ①into a new human host perpetuates the malaria life cycle. [6]

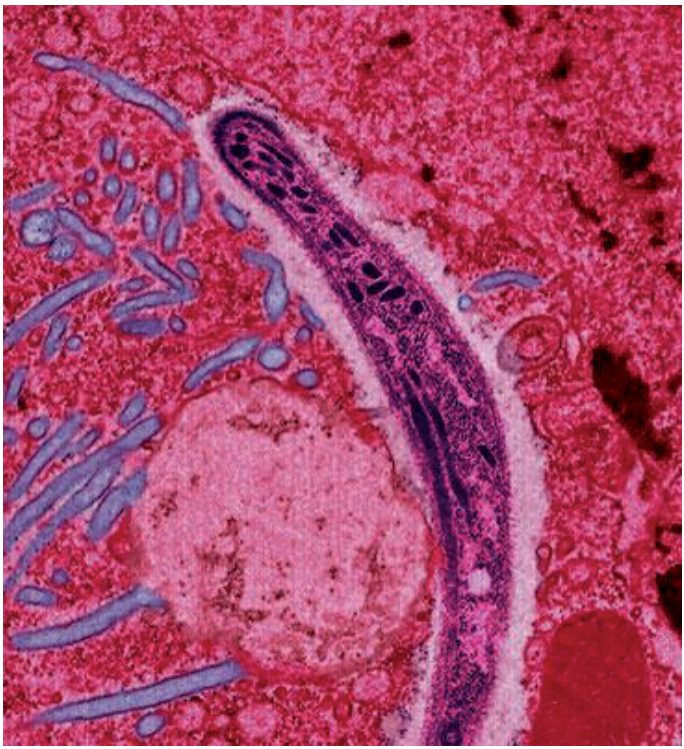
The malaria parasite has a complex, multistage life cycle occurring within two living beings, the vector mosquitoes and the vertebrate hosts. The survival and development of the parasite within the invertebrate and vertebrate hosts, in intracellular and extracellular environments, is made possible by a toolkit of more than 5,000 genes and their specialized proteins that help the parasite to invade and grow within multiple cell types and to evade host immune responses. The parasite passes through several stages of development such as the sporozoites (Gr. *Sporos* = seeds; the infectious form injected by the mosquito), merozoites (Gr. *Meros* = piece; the stage invading the erythrocytes), trophozoites (Gr. *Trophes* = nourishment; the form multiplying in erythrocytes), and gametocytes (sexual stages) and all these stages have their own unique shapes and structures and protein complements. The surface proteins and metabolic pathways keep changing during these different stages that help the parasite to evade the immune clearance, while also creating problems for the development of drugs and vaccines.




**Sporogony within the Mosquitoes:**

Mosquitoes are the definitive hosts for the malaria parasites, wherein the sexual phase of the parasite's life cycle occurs. The sexual phase is called ***Sporogony*** and results in the development of innumerable infecting forms of the parasite within the mosquito that induce disease in the human host following their injection with the mosquito bite.

When the female *Anopheles* draws a blood meal from an individual infected with malaria, the male and female gametocytes of the parasite find their way into the gut of the mosquito. The molecular and cellular changes in the gametocytes help the parasite to quickly adjust to the insect host from the warm-blooded human host and then to initiate the sporogonic cycle. The male and female gametes fuse in the mosquito gut to form zygotes, which subsequently develop into actively moving ookinetes that burrow into the mosquito midgut wall to develop into oocysts. Growth and division of each oocyst produces thousands of active haploid forms called sporozoites. After the sporogonic phase of 8–15 days, the oocyst bursts and releases sporozoites into the body cavity of the mosquito, from where they travel to and invade the mosquito salivary glands. When the mosquito thus loaded with sporozoites takes another blood meal, the sporozoites get injected from its salivary glands into the human bloodstream, causing malaria infection in the human host. It has been found that the infected mosquito and the parasite mutually benefit each other and thereby promote transmission of the infection. The *Plasmodium*-infected mosquitoes have a better survival and show an increased rate of blood-feeding, particularly from an infected host.



**Colored TEM of malaria sporozoites in a Anopheles mosquito gut**

### Schizogony in the human Host:

Man is the intermediate host for malaria, wherein the asexual phase of the life cycle occurs. The sporozoites inoculated by the infested mosquito initiate this phase of the cycle from the liver, and the latter part continues within the red blood cells, which results in the various clinical manifestations of the disease.

**Pre-erythrocytic Phase – Schizogony in the Liver:**

With the mosquito bite, tens to a few hundred invasive sporozoites are introduced into the skin. Following the intradermal deposition, some sporozoites are destroyed by the local macrophages, some enter the lymphatics, and some others find a blood vessel. The sporozoites that enter a lymphatic vessel reach the draining lymph node wherein some of the sporozoites partially develop into exo-erythocytic stages and may also prime the T cells to mount a protective immune response.

The sporozoites that find a blood vessel reach the liver within a few hours. It has recently been shown that the sporozoites travel by a continuous sequence of stick-and-slip motility, using the thrombospondin-related anonymous protein (TRAP) family and an actin–myosin motor .The sporozoites then negotiate through the liver sinusoids, and migrate into a few hepatocytes, and then multiply and grow within parasitophorous vacuoles. Each sporozoite develop into a schizont containing 10,000–30,000 merozoites (or more in case of *P. falciparum*). The growth and development of the parasite in the liver cells is facilitated by a favorable environment created by the circumsporozoite protein of the parasite. The entire pre-erythrocytic phase lasts about 5–16 days depending on the parasite species:] on an average 5-6 days for *P. falciparum*, 8 days for *P. vivax,* 9 days for *P. ovale,* 13 days for *P. malariae* and 8-9 days for *P. knowlesi* The pre-erythrocytic phase remains a "silent" phase, with little pathology and no symptoms, as only a few hepatocytes are affected. This phase is also a single cycle, unlike the next, erythrocytic stage, which occurs repeatedly.

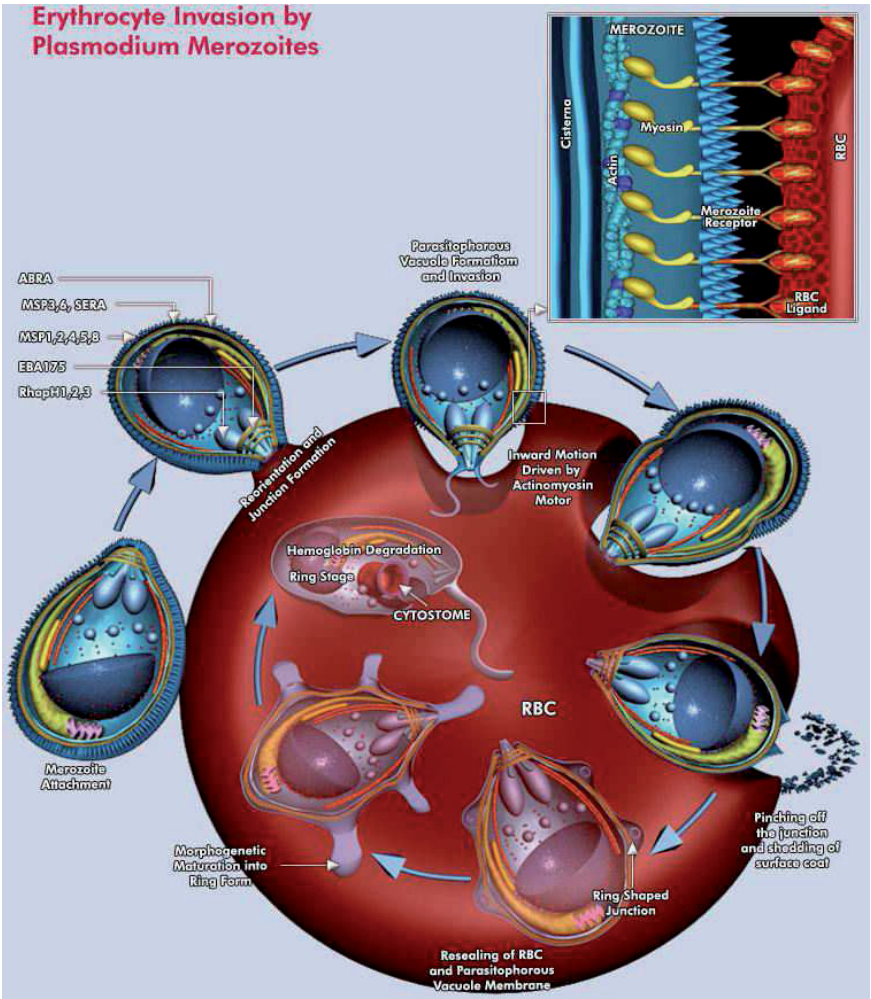
The merozoites that develop within the hepatocyte are contained inside host cell-derived vesicles called merosomes that exit the liver intact, thereby protecting the merozoites from phagocytosis by Kupffer cells. These merozoites are eventually released into the blood stream at the lung capillaries and initiate the blood stage of infection thereon.

In *P. vivax* and *P. ovale* malaria, some of the sporozoites may remain dormant for months within the liver. Termed as hypnozoites, these forms develop into schizonts after some latent period, usually of a few weeks to months. It has been suggested that these late developing hypnozoites are genotypically different from the sporozoites that cause acute infection soon after the inoculation by a mosquito bite, and in many patients cause relapses of the clinical infection after weeks to months.

**Erythrocytic Schizogony**

Red blood cells are the 'centre stage' for the asexual development of the malaria parasite. Within the red cells, repeated cycles of parasitic development occur with precise periodicity, and at the end of each cycle, hundreds of fresh daughter parasites are released that invade more number of red cells.

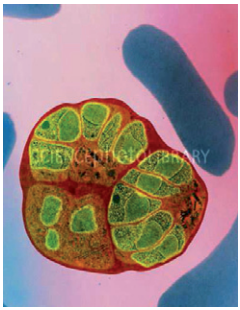
The merozoites released from the liver recognize, attach, and enter the red blood cells (RBCs) by multiple receptor–ligand interactions in as little as 60 seconds. This quick disappearance from the circulation into the red cells minimizes the exposure of the antigens on the surface of the parasite, thereby protecting these parasite forms from the host immune response. The invasion of the merozoites into the red cells is facilitated by molecular interactions between distinct ligands on the merozoite and host receptors on the erythrocyte membrane. *P. vivax* invades only Duffy blood group-positive red cells, using the Duffy-binding protein and the reticulocyte homology protein, found mostly on the reticulocytes. The more virulent *P. falciparum* uses several different receptor families and alternate invasion pathways that are highly redundant. Varieties of Duffy binding-like (DBL) homologous proteins and the reticulocyte binding-like homologous proteins of *P. falciparum* recognize different RBC receptors other than the Duffy blood group or the reticulocyte receptors. Such redundancy is helped by the fact that *P. falciparum* has four Duffy binding-like erythrocyte-binding protein genes, in comparison to only one gene in the DBL-EBP family as in the case of *P. vivax*, allowing *P. falciparum* to invade any red cell.



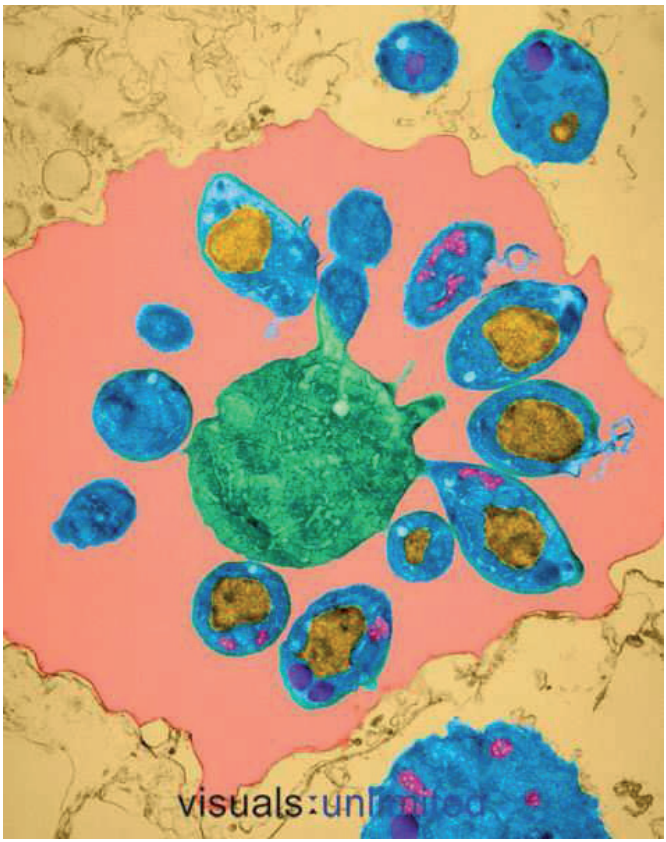

**Process of Invasion of Red Cells by Merozoites [© 2009 QIAGEN, all rights reserved]**

The process of attachment, invasion, and establishment of the merozoite into the red cell is made possible by the specialized apical secretory organelles of the merozoite, called the micronemes, rhoptries, and dense granules. The initial interaction between the parasite and the red cell stimulates a rapid "wave" of deformation across the red cell membrane, leading to the formation of a stable parasite–host cell junction. Following this, the parasite pushes its way through the erythrocyte bilayer with the help of the actin–myosin motor, proteins of the thrombospondin-related anonymous protein family (TRAP) and aldolase, and creates a parasitophorous vacuole to seal itself from the host-cell cytoplasm, thus creating a hospitable environment for its development within the red cell. At this stage, the parasite appears as an intracellular "ring".

Within the red cells, the parasite numbers expand rapidly with a sustained cycling of the parasite population. Even though the red cells provide some immunological advantage to the growing parasite, the lack of standard biosynthetic pathways and intracellular organelles in the red cells tend to create obstacles for the fast-growing intracellular parasites. These impediments are overcome by the growing ring stages by several mechanisms: by restriction of the nutrient to the abundant hemoglobin, by dramatic expansion of the surface area through the formation of a tubovesicular network, and by export of a range of remodeling and virulence factors into the red cell. Hemoglobin from the red cell, the principal nutrient for the growing parasite, is ingested into a food vacuole and degraded. The amino acids thus made available are utilized for protein biosynthesis and the remaining toxic heme is detoxified by heme polymerase and sequestrated as hemozoin (malaria pigment). The parasite depends on anaerobic glycolysis for energy, utilizing enzymes such as pLDH, plasmodium aldolase etc. As the parasite grows and multiplies within the red cell, the membrane permeability and cytosolic composition of the host cell is modified. These new permeation pathways induced by the parasite in the host cell membrane help not only in the uptake of solutes from the extracellular medium but also in the disposal of metabolic wastes, and in the origin and maintenance of electrochemical ion gradients. At the same time, the premature hemolysis of the highly permeabilized infected red cell is prevented by the excessive ingestion, digestion, and detoxification of the host cell hemoglobin and its discharge out of the infected RBCs through the new permeation pathways, thereby preserving the osmotic stability of the infected red cells.



**Colored TEM of a human red blood cell infected with merozoites (green) [Source: LONDON SCHOOL OF HYGIENE / SCIENCE PHOTO LIBRARY]**




**TEM of *P. falciparum* schizont (X2810) [Credit: Dennis Kunkel Microscopy, Inc. /Visuals Unlimited, Inc.]**

The erythrocytic cycle occurs every 24 hours in case of *P. knowlesi,* 48 h in cases of *P. falciparum, P. vivax* and *P. ovale* and 72 h in case of *P. malariae.* During each cycle, each merozoite grows and divides within the vacuole into 8–32 (average 10) fresh merozoites, through the stages of ring, trophozoite, and schizont. At the end of the cycle, the infected red cells

rupture, releasing the new merozoites that in turn infect more RBCs. With sunbridled growth, the parasite numbers can rise rapidly to levels as high as $10^{13}$ per host.

A small proportion of asexual parasites do not undergo schizogony but differentiate into the sexual stage gametocytes. These male or female gametocytes are extracellular and nonpathogenic and help in transmission of the infection to others through the female anopheline mosquitoes, wherein they continue the sexual phase of the parasite's life cycle. Gametocytes of *P. vivax* develop soon after the release of merozoites from the liver, whereas in case of *P. falciparum*, the gametocytes develop much later with peak densities of the sexual stages typically occurring 1 week after peak asexual stage densities. [7]

**Malaria pathology**

The most pronounced changes related to malaria involve the blood and the blood-forming system, the spleen and the liver. Secondary changes can occur in all the other major organs, depending on the type and severity of the infection. The pathological changes are more profound and severe in case of *P. falciparum* malaria. Severe malaria is a complex multisystem disorder with many similarities to sepsis syndromes.

**Red blood cells:** Red blood cells are the principal sites of infection in malaria. All the clinical manifestations are primarily due to the involvement of red blood cells.

The growing parasite consumes and degrades the intracellular proteins, mainly hemoglobin. The transport properties of the red cell membrane are altered, cryptic surface antigens are exposed and new parasite derived proteins are inserted. The red cell becomes more spherical and less deformable. In *P. falciparum* infection, membrane protuberances appear on the red cell surface in the second 24-hour of the asexual cycle. Accretions of electron-dense, histidine-rich parasite proteins are found under these 'knobs'. These knobs extrude a strain specific, adhesive variant protein of high molecular weight that mediates red cell attachment to receptors on venular and capillary endothelium, causing *cytoadherence*. *P. falciparum* infected red cells also adhere to uninfected red cells to form *rosettes*. Cytoadherence and rosetting are central to the pathogenesis of *P. falciparum* malaria, resulting in the formation of red cell aggregates and intra vascular sequestration of red cells in the vital organs like the brain and the heart. This further interferes with the microcirculation and metabolism and allows parasite development away from the principal host defense, splenic processing and filtration. As a result, in *P. falciparum* malaria, only younger forms of the parasite are found in the peripheral circulation and the peripheral parasitemia is usually an underestimate of the true parasite load. Mature forms of *P. falciparum* are rarely seen in the peripheral blood and when found, indicate severe infection. Sequestration does not occur in cases of *P. vivax* and *P. malariae* infections and therefore, all stages of the parasite can be seen in the peripheral blood and complications are very rare.

**Hypovolaemia** is a major feature of severe malaria and, when further exacerbated by anaemia and microvascular obstruction from sequestered parasites, is likely to lead to decreased delivery of oxygen to tissues, anaerobic metabolism and lactic acidosis.

**Immunopathogenic processes** are now recognized as having a central role in severe malaria, with proinflammatory cytokine cascades leading to complex downstream metabolic changes. As in sepsis, cytokine-induced failure of oxygen utilization is likely to play an important role. Proinflammatory cytokines and anti-inflammatory cytokines, such as interleukin-10 (IL-10), have been proposed to have a protective or counter-regulatory role. Tumour necrosis factor (TNF) is raised in those with severe malaria and has been implicated in the pathogenesis of murine cerebral malaria. TNF is also raised in placental malaria and is associated with low birth weight.

**Nitric oxide** (NO) seems to offer protection from severe malaria. NO synthesis requires extracellular arginine, and recent studies found an association between hypoargininaemia and severe malaria and death in children. Immunohistochemistry of cerebral tissue postmortem revealed increased inducible NOS expression and markers of NO production in severe malaria. NO has been implicated in the pathogenesis of severe sepsis, and it has been suggested that NO could alternatively play a role in the pathogenesis of severe disease.

Recent studies have provided strong evidence supporting a role for **perforin** in the pathogenesis of severe murine malaria, through disruption of the blood–brain barrier. Mice deficient in perforin appear to be resistant to cerebral and severe complications of malaria. CD8C T cells have been implicated in the pathogenesis of murine CM and might be a source of perforin, as might NKT cells. Changes in prostaglandin synthesis and expression of chemokines have also been implicated in disease pathogenesis in mice and to a lesser extent in a protective role in humans. It remains to be established how these changes relate to one another in the causal pathway, and to what extent these processes contribute to human severe malaria.

The triggers that lead to excess proinflammatory cytokines are not well understood, but glycosylphosphatidylinositol (GPI) of Plasmodium falciparum has been implicated in several studies. GPI can stimulate TNF production by macrophages and increase iNOS expression.

**Sequestration** of parasitized RBCs (pRBCs) within the small vessels of many tissues have been found on post-mortem examinations of people who have died from *P. falciparum* infection. Although it may contribute to high total body parasitaemia, establishing a direct cause-and-effect relationship between sequestration and cerebral malaria has proven difficult. During pregnancy, pRBCs typically sequester in the placenta. Maternal health also suffers through the development of maternal anaemia and the resultant increased likelihood of maternal death.

Sequestration occurs principally during the second half of the intra-erythrocytic asexual growth phase of the parasite, following the adherence of mature parasites to endothelial cells through electron-dense knobs on the pRBC surface (cytoadherence). In vitro studies have identified several cell-surface molecules as potential receptors for pRBC binding, including thrombospondin (TSP), CD36, intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule, E-selectin, chondroitin sulphate A (CSA), CD31 and hyaluronic acid (HA). In addition to adhering to endothelial cells and syncytiotrophoblasts, mature-stage pRBCs can also adhere to non-infected RBCs, forming **rosettes**, and to other pRBCs, forming clumps (with platelets) or autoagglutinates.

Linking specific cytoadherence phenotypes to clinical syndromes has proved difficult. A plausible case can be made for ICAM-1 as a key host receptor in the brain: it is widely distributed on cerebral vessels, is upregulated by cytokines including TNF-a and was co-localized with pRBCs in brains of patients dying of cerebral malaria.

Another major endothelial receptor, CD36, is not detected on human cerebral vasculature, but is ubiquitously expressed in lung, kidney, liver and muscle vasculature. Most parasite isolates causing clinical disease in non-pregnant individuals can bind to CD36. The relationship between CD36 binding and pathogenesis is not clear.

Most cytoadherence phenomena appear to be mediated by PfEMP-1, a high-molecular-weight protein of approximately 240 kDa, and inserted into the erythrocyte membrane between 16 and 20 h after invasion. PfEMP-1 has been shown to bind to many host receptors.

**Anemia** is a fairly common problem encountered in malaria and it poses special problems in pregnancy and in children. It can be due to multiple causes. Repeated hemolysis of infected red cells is the most important cause for a reduction in hemoglobin levels. Anemia depends on the degree of parasitemia, duration of the acute illness and the number of febrile paroxysms. It may occur even after 3-5 febrile paroxysms. *P. vivax* predominantly invades young red cells and the number of parasites infected rarely exceeds 2%. *P. malariae* develops mostly in mature red cells and the parasitemia is rarely greater than 1%. The pathogenesis of malarial anaemia is complex and undoubtedly involves multiple processes relating to both the destruction of erythrocytes and their reduced production. *P. falciparum* affects red cells of all ages and the parasitemia can be as high as 20-30% or more. Massive destruction of red cells accounts for rapid development of anemia in *P. falciparum* malaria. Non parasitized RBCs are also removed from the circulation by complement-mediated lysis and phagocytosis resulting from immune complex deposition and complement activation.[Claire LM, 2004] Increased splenic clearance of parasitized as well as non-parasitized red cells, reduction of red cell survival even after disappearance of parasitemia, dyserythropoeisis in the bone marrow, drug induced hemolysis etc. also contribute to the anemia. During *P. falciparum* infections, reticulocyte levels are inappropriately low, reflecting suppression of the normal response of erythropoietin (EPO). Some of these mechanisms may perpetuate anemia even after completion of the treatment.

Anemia of malaria is usually normocytic hypochromic with increase in the number of reticulocytes and polychromatophils. Rarely, atypical manifestations like macrocytic anemia or pseudoaplastic picture with pancytopenia may be seen. Anemia may be associated with hyperbilirubinemia of the indirect type, due to the hemolytic process. Splenomegaly may also be seen.

Leukocyte count is usually low to normal in most cases of malaria. Increased leukocyte count indicates either a severe infection or secondary bacterial infection. Reduction in the leukocyte count is attributed to hypersplenism or sequestration in the spleen. Relative lymphocytosis, monocytosis, eosinopenia, presence of stab neutrophils are observed with prolonged duration of the illness.

**Thrombocytopenia** is also fairly common in malaria. It has been observed that the platelet count shows a moderate decline during the paroxysms of fever. Thrombocytopenia may be related to the sequestration of the platelets in the spleen. Severe thrombocytopenia however indicates severe infection and may herald bleeding syndromes.

**Erythrocyte Sedimentation Rate** is usually elevated in malaria up to 30-50 mm in one hour. Prolonged malaria, severe anemia and severe malaria are usually associated with a higher ESR.

**Bone marrow**

Bone marrow may show evidence of dyserythropoeisis, iron sequestration and erythrophagocytosis in the acute phase of falciparum malaria. Maturation defects may be present in the marrow for 3 weeks after the clearance of parasitemia. Large, abnormal looking megakaryocytes have been found in the marrow and the circulating platelets may also be enlarged, suggesting dysthrombopoeisis.

**Spleen**

Spleen plays an important role in the immune response against malarial infection and splenectomy invariably activates a latent infection. Enlargement of the spleen is one of the early and constant signs of malarial infection. Spleen may become palpable as early as the first paroxysm.

Spleen may be palpable at the early stages of infection in the right lateral position or even in supine position. Its edge is usually round and hard to palpate and it may be tender. As the disease progresses, the spleen becomes harder, less sensitive and readily palpable. In falciparum malaria, spleen may not be palpable if the patient presents very early (due to severity). Otherwise, splenomegaly is common in all types of malaria.

The early enlargement of the spleen is due to engorgement, oedema of the pulp and later due to lymphoid and reticulo-endothelial hyperplasia with an increased hemolytic and phagocytic function of the organ. Frequent relapses and re-infections lead to pulp sclerosis and dilated sinuses.

Following treatment, spleen regresses in size, usually completely, within two weeks. In cases of large, fibrotic spleen due to repeated malaria, regression is slower, but complete involution with treatment is common.

Rapid and considerable enlargement of spleen may sometimes result in splenic rupture, which is a serious complication of malaria. This is more common in primary attack of malaria. Due to fibrosis and perisplenitis, rupture is less likely in case of chronic splenomegaly.

A small proportion of adults in Africa and India and a high proportion of adults from New Guinea have been found to suffer from huge enlargement of the spleen. This condition has been termed as the Tropical Splenomegaly Syndrome. Its nature still remains unclear. It is characterized by marked enlargement of the spleen whose weight may reach 2000-4400 g. The splenic sinuses are dilated and there is marked lymphoid hyperplasia. There is increased phagocytosis of red and white blood cells. The liver is also enlarged and shows lymphoreticular infiltration of the sinusoids. High levels of IgG and IgM antibodies against malaria have been demonstrated in these patients. These patients also have anemia, leucopenia, and thrombocytopenia with fairly well maintained general health. Prolonged anti-malarial treatment may reduce the size of the spleen in these patients.

**Liver**

Enlargement of the liver also occurs early in malaria. The liver is enlarged after the first paroxysms, it is usually firm and may be tender. It is oedematous, colored brown, grey or even black as a result of deposition of malaria pigment. Hepatic sinusoids are dilated and contain hypertrophied Kupffer cells and parasitized red cells. Small areas of centrilobular necrosis may be seen in severe cases and these may be due to shock or disseminated intravascular coagulation. Prolonged infection may be associated with stromal induration and diffuse proliferation of fibrous connective tissue. However, changes of cirrhosis are not seen. In falciparum malaria, in addition to the involvement of the mesenchyma, the hepatocytes may also be involved, causing functional changes as well (malarial hepatitis).

Malarial hepatitis is characterized by hyperbilirubinemia with elevation of conjugated bilirubin, increased levels of transaminases and alkaline phosphatase. Being part of the severe falciparum infection, it may be associated with renal failure, anemia or other complications of falciparum malaria. Liver involvement in severe falciparum malaria is due to impairment of local microcirculation associated with hepatocellular damage.

In patients with repeated attacks of malaria, liver also enlarges significantly along with a large and hard spleen. However, there is no functional abnormality of the liver in these patients. Malaria is not a proven cause for cirrhosis of the liver.

**Lungs**

Involvement of the lungs occurs in *P. falciparum* malaria and is secondary to the changes in the red blood cells and the microcirculation. Acute pulmonary oedema is an infrequent but nearly fatal complication of P. falciparum malaria, largely due to capillary endothelial lesions and perivascular oedema. Fluid overload and blood transfusion may also contribute to this problem. Pulmonary capillaries and venules are packed with inflammatory cells and parasitized red cells. The vascular endothelium is oedematous with narrowing of the lumen. Interstitial oedema and hyaline-membrane formation is also seen. Focal or lobar pneumonia and bronchopneumonia can also complicate malaria.

**Cardiovascular system**

Malaria is commonly associated with cardiovascular function abnormalities. The most frequent changes during a paroxysm include decrease in blood pressure, tachycardia, muffled heart sounds, transient systolic murmur at the apex and occasional cardiac dilation. Also there is peripheral vasodilation, leading to postural hypotension.

In *P. falciparum* malaria, there could be microcirculatory changes in the coronary vessels. The myocardial capillaries are congested with parasitized red cells, pigment laden macrophages, lymphocytes and plasma cells.

Malaria may aggravate a pre-existing cardiac dysfunction and may prove fatal to patients already suffering from significant cardiac failure or valvular obstruction.

**Gastro-intestinal tract**

Malaria is often accompanied by nausea and vomiting, mainly central in origin. In the acute phase, patient may have anorexia, abdominal distention, and pain in the epigastrium. Sometimes the abdominal colics may be so severe as to mimic acute abdomen or appendicitis. Some patients may have watery diarrhea and the condition may mimic gastro-enteritis or cholera.

Acute colitis may be associated with malaria. Bacillary dysentery, amoebiasis, etc. may complicate malaria.

In falciparum malaria, involvement of splanchnic microcirculation can lead to ischaemia of the gut, mucosal oedema, necrosis and ulceration. This may hamper absorption. Further these changes in the gut may also lead absorption of toxins, precipitating septic shock.

**Kidneys**

Malaria can cause varied problems in the kidneys. During the acute attack, albuminuria may be seen commonly. Acute diffuse malarial nephritis with hypertension, albuminuria and oedema may also be seen rarely.

In *P. malariae* infection, nephrotic syndrome may be seen (Quartan malaria nephropathy). This immune complex mediated nephropathy develops weeks after the malarial illness and is characterized by albuminuria, oedema and hypertension. It may be progressive and may require treatment with steroids or immunesuppressants.

In severe *P. falciparum* malaria, acute renal failure may develop in 0.1-0.6% of the patients. Microcirculation disorders, anoxia and subsequent necrosis of the glomeruli and renal tubules are responsible for this serious complication. Disseminated intravascular coagulation also may cause or aggravate this problem.

**Central nervous system**

Central nervous system manifestations in malaria could be due to pathological involvement of the brain, paroxysms of fever or due to the side effects of antimalarial drugs.

The febrile paroxysms are usually accompanied by headaches, vomiting, delirium, anxiety and restlessness. These are as a rule transient and disappear with normalization of the temperature.

Antimalarial drugs like chloroquine, quinine, mefloquine and halofantrine can cause various symptoms like dizziness, vertigo, tinnitus, restlessness, hallucinations, confusion, delirium or even frank psychosis, convulsions etc. Quinine can induce hypoglycemic coma. Artemisinin derivatives are known to cause brain stem dysfunction in animal studies. These factors should always be kept in mind while managing cases of malaria.

Nervous system gets involved predominantly in *P. falciparum* malaria and only very rarely in the other forms. Decreased deformability, increased cytoadherence and rosetting of red cells, occlusion of the microcirculation by the red cell rosettes and their thrombosis- all these result in cerebral anoxia, development of malaria granulomas and punctate hemorrhages leading to

malarial encephalitis and meningoencephalitis. At autopsy, the brain is found to be oedematous; small blood vessels are congested with parasitized red cells; the surface of the brain appears leaden or plum colored while the cut surface has a slatey-grey hue. Up to 70% of the red cells in the brain may be found to be parasitized, and many mature forms of the parasite including schizonts could be seen. In larger vessels, the parasites form a layer along the endothelium, called as 'margination'. The vascular endothelium shows pseudopodial projections, which may be in close apposition to the 'knobs' on the surface of the parasitized red cells. Numerous petechial hemorrhages are found in the white matter, proximal to the occlusive plugs in the end arterioles. Dürck's granulomata, small collections of microglial cells surrounding an area of demyelination may be seen at the site of these hemorrhages. [8]

**Symptoms of malaria**

**Symptoms of malaria can develop as quickly as seven days after you're bitten by an infected mosquito.**

Typically, the time between being infected and when symptoms start (incubation period) is 7 to 18 days, depending on the specific parasite you're infected with. However, in some cases it can take up to a year for symptoms to develop.

The initial symptoms of malaria are flu-like and include:

- a high temperature (fever)
- headache
- sweats
- chills
- vomiting

These symptoms are often mild and can sometimes be difficult to identify as malaria.

With some types of malaria, the fever occurs in 48-hour cycles. During these cycles, you feel cold at first with shivering. You then develop a fever, accompanied by severe sweating and fatigue. These symptoms usually last between 6 and 12 hours.

Other symptoms of malaria can include:

- muscle pains
- diarrhea
- generally feeling unwell

The most serious type of malaria is caused by the Plasmodium falciparum parasite. Without prompt treatment, this type could lead to you quickly developing severe and life-threatening complications, such as breathing problems and organ failure. [9]

**Complications of malaria**

Malaria can be a very serious illness and is potentially fatal. The falciparum parasite causes the most severe malaria symptoms and the most deaths.

**Anaemia**

The destruction of red blood cells by the malaria parasite can cause severe anaemia. This is a condition where the red blood cells are unable to carry enough oxygen, which leaves you feeling drowsy, weak and faint.

**Cerebral malaria**

In some rare cases of malaria, the infected red blood cells can block the small blood vessels leading to the brain, stopping blood flow and leading to a shortage of oxygen. This is known as cerebral malaria.

Cerebral malaria can cause your brain to swell and, in some cases, may lead to permanent brain damage. It can also cause you to have a seizure or fall into a coma.

**Other complications**

Other complications of a severe case of malaria can include:

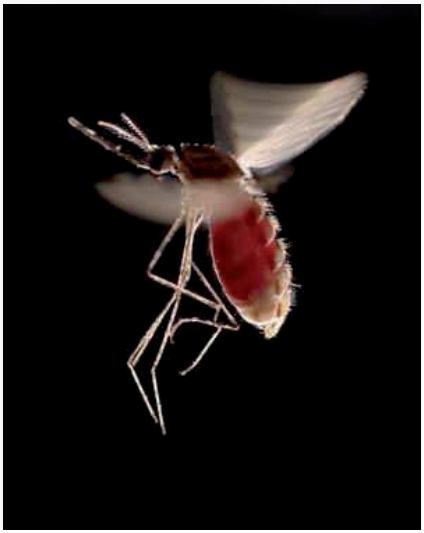
- breathing problems (such as fluid in your lungs)
- liver failure and jaundice (a yellow discoloration of the skin)

- shock (sudden drop in blood flow)
- spontaneous bleeding
- abnormally low blood sugar
- kidney failure
- swelling and rupturing of the spleen
- dehydration

Complications of severe malaria can appear within hours or days of your first symptoms, so it is important to get urgent medical help as soon as you think you have malaria.

Malaria is usually more severe in pregnant women, babies, young children and older people. [10]

**Malaria vector**



Malaria is transmitted among humans by female mosquitoes of the genus Anopheles. Female mosquitoes take blood meals to carry out egg production, and such blood meals are the link between the human and the mosquito hosts in the parasite life cycle. The successful development of the malaria parasite in the mosquito (from the "gametocyte" stage to the "sporozoite" stage) depends on several factors. The most important is ambient temperature and humidity (higher temperatures accelerate the parasite growth in the mosquito) and whether the Anopheles survives long enough to allow the parasite to complete its cycle in the mosquito host ("sporogonic" or "extrinsic" cycle, duration 10 to 18 days). Differently from the human host, the mosquito host does not suffer noticeably from the presence of the parasites.

There are approximately 3,500 species of mosquitoes grouped into 41 genera. Human malaria is transmitted only by females of the genus Anopheles. Of the approximately 430 Anopheles species, only 30-40 transmit malaria (i.e., are "vectors") in nature.

**Geographic Distribution**

Anophelines are found worldwide except Antarctica. Malaria is transmitted by different Anopheles species, depending on the region and the environment.

Anophelines that can transmit malaria are found not only in malaria-endemic areas, but also in areas where malaria has been eliminated. The latter areas are thus constantly at risk of re-introduction of the disease.

**Life Stages**

Like all mosquitoes, anophelines go through four stages in their life cycle: 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. The adult females can live up to a month (or more in captivity) but most probably do not live more than 1-2 weeks in nature.

**Eggs**

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.

**Larvae**

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. Larvae swim either by jerky movements of the entire body or through propulsion with the mouth brushes.

Larvae develop through 4 stages, or instars, after which they metamorphose into pupae. At the end of each instar, the larvae molt, shedding their exoskeleton, or skin, to allow for further growth.

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. Others prefer habitats that have none. 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.

**Pupae**

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. 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.

**Adults**

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 odors as well as odors 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 in captivity) 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 defenses. [12]

**Malaria parasite species**

Malaria is caused by protozoan parasites of the genus *Plasmodium* – single-celled organisms that cannot survive outside of their host(s).

**Plasmodium falciparum** is responsible for the majority of malaria deaths globally and is the most prevalent species in sub-Saharan Africa. The remaining species are not typically as life threatening as P. falciparum.

**Plasmodium vivax**, is the second most significant species and is prevalent in Southeast Asia and Latin America. P. vivax and **Plasmodium ovale** have the added complication of a dormant liver stage, which can be reactivated in the absence of a mosquito bite, leading to clinical symptoms.

P. ovale and **Plasmodium malariae** represent only a small percentage of infections.

A fifth species **Plasmodium knowlesi**– a species that infects primates – has led to human malaria, but the exact mode of transmission remains unclear. [13]

**Plasmodium falciparum**

*Plasmodium falciparum* is a protozoan parasite, one of the species of Plasmodium that cause malaria in humans. It is transmitted by the female Anopheles mosquito. Malaria caused by this species (also called malignant or falciparum malaria) is the most dangerous form of malaria, with the highest rates of complications and mortality. As of 2006, there were an estimated 247 million human malarial infections (98% in Africa, 70% being 5 years or younger). It is much more prevalent in sub-Saharan Africa than in many other regions of the world; in most African countries, over 75% of cases were due to P. falciparum, whereas in most other countries with malaria transmission, other, less virulent plasmodial species predominate. Almost every malarial death is caused by P. falciparum.

A HUMAN PARASITE CAUSING THE MALIGNANT FORM OF TERTIAN (PERNICIOUS OR MALIGNANT) MALARIA, FOUND IN ALL CONTINENTS.

Malaria is caused by an infection with protozoa of the genus Plasmodium. The name malaria, from the Italian mala aria, meaning "bad air", comes from the linkage suggested by Giovanni Maria Lancisi (1717) of malaria with the poisonous vapours of swamps. This species name comes from the Latin falx, meaning "sickle", and parere meaning "to give birth". The organism itself was first seen by Laveran on November 6, 1880 at a military hospital in Constantine, Algeria, when he discovered a microgametocyte exflagellating. Patrick Manson (1894) hypothesized that mosquitoes could transmit malaria. This hypothesis was experimentally confirmed independently by Giovanni Battista Grassi and Ronald Ross in 1898. Grassi (1900) proposed an exerythrocytic stage in the life cycle, later confirmed by Short, Garnham, Covell and Shute (1948), who found Plasmodium vivax in the human liver.

Around the world, malaria is the most significant parasitic disease of humans, and claims the lives of more children worldwide than any other infectious disease. Since 1900, the area of the world exposed to malaria has been halved, yet two billion more people are presently exposed. Morbidity, as well as mortality, is substantial. Infection rates in children in endemic areas are of the order of 50%: Chronic infection has been shown to reduce school scores by up to 15%. Reduction in the incidence of malaria coincides with increased economic output.

While there are no effective vaccines for any of the six or more species that cause human malaria, drugs have been employed for centuries. In 1640, Huan del Vego first employed the tincture of the cinchona bark for treating malaria; the native Indians of Peru and Ecuador had been using it even earlier for treating fevers. Thompson (1650) introduced this "Jesuits' bark" to England. Its first recorded use there was by Dr John Metford of Northampton in 1656. Morton (1696) presented the first detailed description of the clinical picture of malaria and of its treatment with cinchona. Gize (1816) studied the extraction of crystalline quinine from the cinchona bark, and Pelletier and Caventou (1820) in France extracted pure quinine alkaloids, which they named quinine and cinchonine.

## PLASMODIUM LIFE CYCLE

The life cycle of all Plasmodium species is complex. Infection in humans begins with the bite of an infected female Anopheles mosquito. Sporozoites released from the salivary glands of the mosquito enter the bloodstream during feeding, quickly invading liver cells (hepatocytes). Sporozoites are cleared from the circulation within 30 minutes. During the next 14 days in the case of P. falciparum, the liver-stage parasites differentiate and undergo asexual multiplication,

resulting in tens of thousands of merozoites that burst from the hepatocyte. Individual merozoites invade red blood cells (erythrocytes) and undergo an additional round of multiplication, producing 12-16 merozoites within a schizont. The length of this erythrocytic stage of the parasite lifecycle depends on the parasite species: irregular cycle for P. falciparum, 48 hours for P. vivax and P. ovale, and 72 hours for P. malariae. The clinical manifestations of malaria, fever, and chills are associated with the synchronous rupture of the infected erythrocytes. The released merozoites go on to invade additional erythrocytes. Not all of the merozoites divide into schizonts; some differentiate into sexual forms, male and female gametocytes. These gametocytes are taken up by a female Anopheles mosquito during a blood meal. Within the mosquito midgut, the male gametocyte undergoes a rapid nuclear division, producing eight flagellated microgametes that fertilize the female macrogamete. The resulting ookinete traverses the mosquito gut wall and encysts on the exterior of the gut wall as an oocyst. Soon, the oocyst ruptures, releasing hundreds of sporozoites into the mosquito body cavity, where they eventually migrate to the mosquito salivary glands.

## PATHOGENESIS

Plasmodium falciparum causes severe malaria via a distinctive property not shared by any other human malaria, that of sequestration. Within the 48-hour asexual blood stage cycle, the mature forms change the surface properties of infected red blood cells, causing them to stick to blood vessels (a process called cytoadherence). This leads to obstruction of the microcirculation and results in dysfunction of multiple organs, typically the brain in cerebral malaria.

## KNOWN VECTORS

Anopheles gambiae (Principal vector)

→ Anopheles albimanus

→ Anopheles freeborni

→ Anopheles maculatus

→ Anopheles stephensi. [14]

**Plasmodium vivax**

**Plasmodium vivax** is a protozoal parasite and a human pathogen. The most frequent and widely distributed cause of recurring (Benign tertian) malaria, P. vivax is one of the five species of malaria parasites that commonly infect humans. It is less virulent than Plasmodium falciparum, the deadliest of the five, but vivax malaria can lead to severe disease and death due to splenomegaly (a pathologically enlarged spleen). P. vivax is carried by the female Anopheles mosquito, since it is only the female of the species that bites.

Epidemiology:

Plasmodium vivax was found mainly in the United States, Latin America, and in some parts of Africa. More recently it became a plague of low- and middle-income countries, except those in sub-Saharan Africa, where the P. vivax map has a conspicuous hole. Overall it accounts for 65% of malaria cases in Asia and South America. It is logical that plasmodium vivax is found there where humans and mosquito population are high. It is uncommon in cooler areas.

As overall malaria rates fall in a region, the proportion of vivax cases increases. It has been estimated that 2.5 billion people are at risk of infection with this organism.

Although the Americas contribute 22% of the global area at risk, high endemic areas are generally sparsely populated and the region contributes only 6% to the total population at risk. In Africa, the widespread lack of the Duffy antigen in the population has ensured that stable transmission is constrained to Madagascar and parts of the Horn of Africa. It contributes 3.5% of global population at risk. Central Asia is responsible for 82% of global population at risk with high endemic areas coinciding with dense populations particularly in India and Myanmar. South East Asia has areas of high endemicity in Indonesia and Papua New Guinea and overall contributes 9% of global population at risk.

P. vivax is carried by at least 71 mosquito species. Many vivax vectors live happily in temperate climates—as far north as Finland. Some prefer to bite outdoors or during the daytime, hampering the effectiveness of indoor insecticide and bed nets. Several key vector species have yet to be grown in the lab for closer study, and insecticide resistance is unquantified.

**Clinical presentation**

Pathogenesis results from rupture of infected red blood cells, leading to fever. Infected red blood cells may also stick to each other and to walls of capillaries. Vessels plug up and deprive tissues of oxygen. Infection may also cause the spleen to enlarge.

Unlike *P. falciparum*, *P. vivax* can populate the bloodstream with sexual-stage parasites—the form picked up by mosquitoes on their way to the next victim—even before a patient shows symptoms. Consequently, prompt treatment of symptomatic patients doesn't necessarily help stop an outbreak, as it does with falciparum malaria, in which fevers occur as sexual stages develop. Even when symptoms appear, because they are usually not immediately fatal, the parasite continues to multiply.

The parasite can go dormant in the liver for days to years, causing no symptoms and remaining undetectable in blood tests. They form what are called hypnozoites (the name derives from "sleeping organisms"), a small form that nestles inside an individual liver cell. The hypnozoites allow the parasite to survive in more temperate zones, where mosquitoes bite only part of the year.

A single infectious bite can trigger six or more relapses a year, leaving sufferers more vulnerable to other diseases. Other infectious diseases, including falciparum malaria, appear to trigger relapses.

**Complications**

Serious complications for malaria are dormant liver stage parasites, organ failures such as acute kidney failure. More complications of leprosy can also be impairment of consciousness, neurological abnormalities, hypoglycemia and low blood pressures caused by cardiovascular

collapse, clinical jaundice and or other vital organ dysfunctions and coagulation defects. The most serious complication ultimately being death. [15]

**Plasmodium malariae**

Description and significance

*Plasmodium malariae* is a malaria-causing parasite that colonizes the blood of a human host. Malaria is a disease that is both preventable and curable, but still continues to cause hundreds of thousands of deaths annually. A recent inquiry conducted by the World Health Organization (WHO) showed that in 2009, 781,000 deaths could be attributed to malaria, and a majority of the victims were African children. This is one of the reasons that studying the species of the genus *Plasmodium* is so important. Although *Plasmodium malariae* is one of the less virulent strains of the genus, it is still one of the few species that use a human as a host. It is the study of the group

of organisms that infect humans that could lead to new drugs that may be more readily available, easier to produce, cheaper, or that can combat drug-resistant strains (WHO).

## Genome structure

Not much is known about the genome of the species *Plasmodium malariae* specifically, because it is among the species for which the entire genome has not been sequenced yet. However, there are some important characteristics about its genome can be determined from the genome sequences of other species in the *Plasmodium* genus. It is estimated that the genome of an organism in the genus *Plasmodium* contains anywhere from 23 million to 27 million base pairs, in the form of 14 linear chromosomes. These 14 chromosomes code for about 5,500 genes, many of which function in invading the host immune system. Many of the *Plasmodium* genomes that have been sequenced show that the species of this genus have DNA that is A+T rich. This is especially true for the species *P. falciparum* that is almost 79.6% A+T. This high A+T content is often known to affect recombination frequencies in other species, but seems to have no effect on the virulence of any particular species. Research shows that 77% of the proteins coded for by the genome of *Plasmodium* are conserved across the different species of the genus. Some additional research shows that the mitochondrial genomes of *Plasmodium* are highly conserved throughout the genome as well.

## Cell structure and metabolism

The parasite Plasmodium malariae, takes on several structures during its 78 hour life cycle. We shall begin observing it starting with the structure it has in the salivary glands of the anopheles mosquito before it infects its mammalian host. It starts its cycle at this point as a Sporozoite, with Sporo meaning seed and zoite coming from the Greek word zoo which means animal. A sporozoite (which can also be known as a falciform body), is an elongated nucleated cell. Its ability to glide on solid substrates, thus invading host cells is based on the help it receives from the trans membrane protein TRAP ((thrombospondin-related anonymous protein). Another protein that assists the Sporozoite with its attachment to its hosts cells and the sporozoite development is a multifunctional protein named circumsporozoite protein (CSP). This protein has been observed on the immature oocysts capsule (found in the mosquito host), but rarely in its cytoplasm.

Once sporozoites enter the mammalian hosts body, they quickly travel to the liver. At the liver they differentiate into merozoites, which is the cell stage that binds via ligands to the host's red blood cells. The P. malariae characteristically causes "spikes" in its host's cell, with dimensions of a mean height of 7·59 nm and a mean diameter: 52·95 nm. This is a different morphology

than that that other Plasmodium infections cause Red Blood Cells to undertake, and can be used to differentiate amongst the different infections.

Another characteristic of P. malariae is that it does not appear to enlarge its host's cell. Infection is observed by the fact that upon entrance to the cell, the merozoites appear to quickly take over, up to one third of the cell. As the invasion progresses, the cell begins to become segmented, with the merozoites filling up the cell, and the cells pigment darkening. The merozoites symmetrically arrange themselves in the cell wall, with the nucleus and cytoplasm separating. At some point, the cell bursts, and the merozoites enter the blood stream. They then take on the developmental stage where they are called trophozoites. The next stage is a schizont, and following the burst of the schizont in the mammalian host, we have merozoites again. In the mosquito host, the developmental stages vary slightly, with the uptake of gametocytes, which later develop into ookinetes, and then oocysts, which then rupture to release sporozoites. The sporozoites can be then injected into a mammalian host.

## Ecology

Malaria is a disease that is commonly found throughout most tropical and subtropical areas in the world. There are four malarial parasites from the *Plasmodium* genus that infect humans and cause symptoms indicative of the disease.

Infections of P. malariae generally coincide with those of P. falciparum. The presence of P. malariae tends to go unnoticed unless PCR techniques are employed to reveal the infection. P. malariae is widely found in sub-Saharan Africa, Southeast Asia, and islands in the western Pacific. Cases have also been reported in the Amazon basin of South America, and in recent history, in Europe and southern parts of the United States.

Plasmodium malariae is primarily found in one of the two host species it infects. It is transmitted by bites from the Anopheles mosquitos, and causes malaria symptoms when it infects humans. Not just limited to mosquitoes and humans, scientists at Osaka University in Japan have discovered strains of P. malariae in imported chimpanzees from Africa. Despite being infected, the chimps have not shown symptoms of disease.

## Pathology

P. malariae is one of the four species of the genus Plasmodium that uses humans as a primary host. The other three species are P. falciparum, P. vivax, and P. ovale. The primary mode of

transmission from host to host by these four species uses a female Anopheles mosquito as a vector. While the symptoms resulting from the different species are different, the life cycle only has minor differences. The life cycle is initiated when the mosquito vector injects **sporozoites** into the human hosts during a blood-meal. The sporozoites then migrate to the liver, where they reproduce asexually and produce **merozoites**. These merozoites then enter the bloodstream and infect erythrocytes, becoming **trophozoites**. The period of time that the trophozoites are enlarging is called the trophic period, and this ends when several divisions occur, but none of these cycles go through the cytokinesis stage, forming what is called a **schizont**. The erythrocyte then lyses, introducing new merozoites into the blood cycle and starting the cycle over again, until an uninfected Anopheles mosquito takes a blood-meal from the infected host, and transmits the infection to another host. [16]

**Plasmodium ovale**

*Plasmodium ovale* is one of the five described *Plasmodium* species that cause malaria in humans (with *Plasmodium falciparum* generally causing the most severe disease and most of the mortality from the disease)

Plasmodium ovale is generally thought of as having a relatively limited distribution, with endemic transmission traditionally described as being limited to areas of tropical Africa, New Guinea, the eastern parts of Indonesia, and the Philippines. However, P. ovale infections have also been reported from the Middle East, the Indian subcontinent, and parts of Southeast Asia. In West Africa (and to a lesser extent Central Africa), age specific prevalence (based on light microscopy, LM) of >10% has been observed. However, in most places where P. ovale is observed, it is relatively uncommon and its prevalence (as detected by LM) rarely exceeds 3–5%. As is the case for P. malariae and P. falciparum, P. ovale infections in West African populations tend to be most common in children under ten years of age. Little is known about the potential for interaction between P. ovale and other malaria infections. However, the fact that P. ovale has been found to be most prevalent in areas of West Africa where P. vivax is nearly absent because of the virtual absence of the Duffy blood-group-antigen, which P. vivax requires to invade red blood cells, might indicate a negative interaction between these two species Recent work indicates that there are likely two distinct Plasmodium species that have both been referred to as P. ovale.

Like *P. vivax*, *P. ovale* has long been thought to have a dormant stage "hypnozoites" stage that can persist in the liver and cause relapses by invading the bloodstream weeks (or even years) later. [(17)]

**History**

This species was first described in 1914 by Stephens in a blood sample taken in the autumn of 1913 from a patient in the sanitarium of Pachmari in central India and sent by Major W. H. Kenrick to Stephens (who was working in Liverpool).

**Clinical features**

In humans, symptoms generally appear 12 to 20 days after the parasite has entered the blood. In the blood, the parasite's replication cycle lasts approximately 49 hours, causing tertian fever which spikes approximately every 49 hours as newly replicated parasites erupt out of red blood cells. Mean maximum parasite levels have been found to be 6,944/µl for sporozoite-induced infections and 7,310/µl for trophozoite-induced infections.

In some cases, relapse may occur up to 4 years after infection. [18]

**Plasmodium knowlesi**

Plasmodium knowlesi is a malaria parasite that is found in nature in long-tailed and pig-tailed macaques. Naturally acquired human infections were thought to be extremely rare until a large focus of human infections was reported in 2004 in Sarawak, Malaysian Borneo. Human infections have since been described throughout Southeast Asia, and P. knowlesi is now

recognized as the fifth species of Plasmodium causing malaria in humans. The molecular, entomological, and epidemiological data indicate that human infections with P. knowlesi are not newly emergent and that knowlesi malaria is primarily a zoonosis. Human infections were undiagnosed until molecular detection methods that could distinguish P. knowlesi from the morphologically similar human malaria parasite P. malariae became available. P. knowlesi infections cause a spectrum of disease and are potentially fatal, but if detected early enough, infections in humans are readily treatable. In this review on knowlesi malaria, we describe the early studies on P. knowlesi and focus on the epidemiology, diagnosis, clinical aspects, and treatment of knowlesi malaria. We also discuss the gaps in our knowledge and the challenges that lie ahead in studying the epidemiology and pathogenesis of knowlesi malaria and in the prevention and control of this zoonotic infection. [19]

In 1932, when Knowles and Das Gupta succeeded in transmitting to humans the monkey malaria they had discovered, it appeared that a new agent for malaria therapy had been discovered. Since the Nobel Prize-winning research of Julius Wagner-Jauregg, malaria therapy had become widely used for the treatment of general paralysis of the insane (neurosyphilis), one of the main reasons for admission to psychiatric institutions. But it soon became apparent that this infection could rapidly become uncontrollable, and after several fatalities, its use was largely discontinued in favor of the less virulent human parasite Plasmodium vivax. Malaria parasites are generally rather choosy, both about their mammalian, avian, or reptilian hosts and their respective mosquito vectors. Transmission of Plasmodium knowlesi, for malaria therapy, from human to human was by blood passage. So initially, it was uncertain whether natural infection could take place and, thus, whether this could be a zoonosis. In 1960, Eyles et al. demonstrated the first experimental mosquito transmission of a simian malaria organism to humans (Plasmodium cynomolgi), and in 1967, Chin et al. showed that P. knowlesi could also be transmitted from monkeys to humans. The mosquitoes used were Anopheles balabacencis (part of the Anopheles leucosphyrus group, which has undergone extensive taxonomic revision in recent years). This is an important vector of human malaria in forested areas of Southeast Asia, where the natural hosts of P. knowlesi—the long-tailed and pig-tailed macaques (Macaca fasicularis and Macaca nemestrina, respectively)—normally live. But the zoonotic potential of P. knowlesi has, until recently, seemed limited, with only sporadic case reports of human infection.

The studies of the Kuching group, led by Balbir Singh and Janet Cox-Singh, have changed this view radically. Investigating what appeared initially to be an unusually high incidence of Plasmodium malariae infection, they have shown conclusively that P. knowlesi is a major cause

of malaria in Malaysia—particularly on the island of Borneo. Younger stages of these 2 parasites appear very similar under light microscopy, but, although P. malariae multiplies every 3 days (quartan) and never reaches dangerously high densities in the blood, P. knowlesi has a daily (quotidian) cycle and, if unchecked, can rapidly reach potentially lethal densities. In this issue, the Kuching group retrospectively reviews the recent Malaysian experience with P. knowlesi infection and describes 4 fatal cases. There are several important practical lessons from this experience. Humans can and do acquire some monkey malarias if they share the same habitat (the reverse is also true). Molecular techniques are very useful in identifying the infection, in describing the epidemiology, and in characterizing mixed infections, which are otherwise underreported. This discovery resulted from good clinical and laboratory investigation, combined with an efficient malaria-control program. Presumably, these P. knowlesi infections were acquired from their natural reservoirs, forest-dwelling macaques—but it remains possible that some may have derived from other human infection. If so, Ciuca's observation, from his malaria therapy practice in Romania, that serial passage of P. knowlesi enhanced virulence may be relevant. There are also potential insights into pathological processes of relevance to severe Plasmodium falciparum malaria. P. knowlesi does not sequester significantly in the microcirculation, but once high parasite densities have been reached, P. knowlesi is rapidly and predictably lethal in the Rhesus monkey (Macaca mulatta). This has been among the most studied of animal models. A fatal outcome in the monkey is associated with very high parasite loads and rapid development of anemia, jaundice, and renal failure—all of which are features of severe P. falciparum malaria in adults, although the clinical picture is unlike cerebral malaria. This unique clinicopathological syndrome seems specific to severe P. falciparum malaria. Terminally ill monkeys are obtunded but not comatose. Thus, although there is no satisfactory animal model of human cerebral malaria, severe P. knowlesi infection in the Rhesus monkey and in humans may have important similarities. Although details in this retrospective study are limited, there are several interesting features of the 4 fatal cases reported, in this issue of Clinical Infectious Diseases, by Cox-Singh et al. The patients were relatively old (age, 39–69 years), and each presented with abdominal pain and fever. Two patients were not anemic despite high parasite counts, although they may have been dehydrated and hemoconcentrated (patient 2 had a perforated gastric ulcer); each had renal impairment and jaundice, which are ominous signs in severe P. falciparum malaria. All had platelet counts <30,000 platelets/uL, and 3 patients had

leukocytosis. This small series raises many questions. How similar is severe disease in humans and Rhesus monkeys (in which the pathophysiology has been investigated extensively)? Do the abdominal symptoms reflect gut ischemia? What is the relationship between parasite biomass and disease severity? Additional studies of severe P. knowlesi malaria in humans to assess metabolic acidosis, exclude concomitant bacteremia, and assess the response to antimalarial treatment would be informative. Microcirculatory studies in Rhesus monkeys conducted >60 years ago showed sludging of RBCs in the capillaries and venules, so there is clearly more to learn about microvascular dysfunction. Cox-Singh et al. provide important advice; in Asia, high parasite loads with what appears to be P. malariae should be regarded as the potentially lethal P. knowlesi malaria and should be managed carefully to prevent a fatal outcome. Despite its simian preference, it is legitimate to claim P. knowlesi to be the fifth human malaria parasite. [20]

**Evolution**

Based on a Bayesian coalescent approach the most probable time of evolution of *P. knowlesi* is 257,000 years ago (95% range 98,000–478,000).

**Life cycle**

Plasmodium knowlesi parasite replicates and completes its blood stage cycle in 24-hour cycles resulting in fairly high loads of parasite densities in a very short period of time. This makes it a potentially very severe disease if it remains untreated. Life cycle: merozoite → trophozoites → schizont → merozoite. These stages of Plasmodium knowlesi are microscopically indistinguishable from Plasmodium malariae and the early trophozoites are identical to those of Plasmodium falciparum.

Mosquito stages: A mosquito ingests gametocytes, which have been formed in the mammalian host. These are either microgametocytes (which are male gametocytes) or macrogametocytes (which are female gametocytes). These gametocytes mature into microgametes and macrogametes respectively, and then fertilize to form zygotes within the midgut of the mosquito. The zygotes mature into ookinetes, then into oocysts. Finally, the oocysts mature to release sporozoites which move to salivary gland of the mosquito.

**Summary**: gametocytes → (microgamete or macrogamete) → zygote → ookinete → oocyst → sporozoites.

**In man**: exo- erythrocytic stage (in the liver): The sporozoites are injected into humans when the mosquito bites and they travel to the liver through blood stream and undergo asexual reproduction to become merozoites through schizonts in the liver cell. Hypnozoites in the liver has not yet been found.

**Summary**: sporozoites → schizonts → merozoites.

**In man**: erythrocytic stage (in the blood): Merozoites are unleashed into the blood stream to infect erythrocytes constituting one asexual cycle of infection of the erythrocytes. Within the red blood cells some merozoites develop into trophozoites, which in turn mature into schizonts that rupture to release merozoites, while others develop into microgametocytes or macrogametocytes. These gametocytes remain in the blood to be ingested by mosquitoes.

**Summary**: Merozoite → trophozoite → schizont → merozoites.

**Vectors**

Theoretically there are four modes of transmission: from an infected mosquito to another monkey, from an infected monkey to a human, from an infected human to another human and from an infected human back to a monkey. In practice human malaria appears to be almost entirely due to monkey to human transmission.

The known vectors belong to the genus Anopheles, subgenus Cellia, series Neomyzomyia and group Leucosphyrus. Mosquitoes of this group are typically found in forest areas in South East Asia but with a greater clearing of forest areas for farmland, humans are increasingly becoming exposed to these vectors.

Within the monkey population in Peninsular Malaysia, *Anopheles hackeri* is believed to be the main vector of *P. knowlesi*: although *A. hackeri* is capable of transmitting malaria to humans, it is not normally attracted to humans and seems unlikely to be an important vector for transmission to humans.

Anopheles latens is attracted to both macaques and humans and has been shown to be the main vector transmitting P. knowlesi to humans in the Kapit Division of Sarawak, Malaysian Borneo.

Anopheles cracens has also been reported as a vector of P. knowlesi. Both species of mosquitoes have been shown to contain as many as 1,000 sporozoites suggesting that they may be efficient vectors.

A study of potential vectors in Malayasia suggests that Anopheles cracens may be an important vector of P. knowlesi.

**Clinical presentation:**

Two possible modes of transmission to humans have been proposed: either from an infected monkey to a human or from an infected human to another human.

Symptoms typically begin approximately 11 days after an infected mosquito has bitten a person and the parasites can be seen in the blood between 10 – 12 days after infection. The parasite may multiply rapidly resulting in very high parasite densities that may be fatal.

Although the current infection rate with Plasmodium knowlesi is relatively low, one risk it presents is misdiagnosis with other forms of malarial parasites such as P. malariae especially when microscopy is used. P. knowlesi can only be accurately distinguished from P. malariae using PCR assay and/or molecular characterization.

Symptoms of *P. knowlesi* in humans include headache, fever, chills and cold sweats. Singh *et al.* (2004) showed clinical symptoms in 94 patients with single species *P. knowlesi* infection at Kapit Hospital, Sarawak, and Malaysian Borneo. Symptoms included fever, chills, and rigor in 100% of patients, headache in 32%, cough in 18%, vomiting in 16%, nausea in 6%, and diarrhea in 4%. Asexual cycle of the parasite in humans and its natural host macaque is about 24 hours. Hence the disease may be called quotidian malaria, in concert with designation of tertian malaria and quartan malaria. In addition to a lab diagnosis using PCR assay, knowlesi malaria may also present itself with elevated levels of C-reactive protein and thrombocytopenia.

This parasite causes non-relapsing malaria due to lack of hypnozoites in its exo- erythrocytic stage.

While infection with this organism is normally not serious, life-threatening complications or even death may occur in a minority of cases. The most common complications are respiratory distress, abnormal liver function including jaundice and renal failure. Mortality in one series of cases was about 2%. [(21)]

**Diagnosis of malaria**

Malaria, sometimes called the "King of Diseases", is caused by protozoan parasites of the genus *Plasmodium*. The most serious and sometimes fatal type of malaria is caused by *Plasmodium*

*falciparum*. The other human malaria species, *P. vivax*, *P. ovale*, *P. malariae*, and sometimes *P. knowlesi* can cause acute, severe illness but mortality rates are low. Malaria is the most important infectious disease in tropical and subtropical regions, and continues to be a major global health problem, with over 40% of the world's population exposed to varying degrees of malaria risk in some 100 countries. It is estimated that over 500 million people suffer from malaria infections annually, resulting in about 1-2 million deaths, of whom 90% are children in sub- Saharan Africa. The number of malaria cases worldwide seems to be increasing, due to increasing transmission risk in areas where malaria control has declined, the increasing prevalence of drug resistant strains of parasites, and in a relatively few cases, massive increases in international travel and migration. The need for effective and practical diagnostics for global malaria control is increasing, since effective diagnosis reduces both complications and mortality from malaria. Differentiation of clinical diagnoses from other tropical infections, based on patients' signs and symptoms or physicians' findings, may be difficult. Therefore, confirmatory diagnoses using laboratory technologies are urgently needed. This review discusses on the currently available diagnostic methods for malaria in many settings, and assesses their feasibility in resource-rich and resource-poor settings.

Prompt and accurate diagnosis is critical to the effective management of malaria. The global impact of malaria has spurred interest in developing effective diagnostic strategies not only for resource-limited areas where malaria is a substantial burden on society, but also in developed countries, where malaria diagnostic expertise is often lacking. Malaria diagnosis involves identifying malaria parasites or antigens/products in patient blood. Although this may seem simple, the diagnostic efficacy is subject to many factors. The different forms of the 5 malaria species; the different stages of erythrocytic schizogony, the endemicity of different species, the interrelation between levels of transmission, population movement, parasitemia, immunity, and signs and symptoms; drug resistance, the problems of recurrent malaria, persisting viable or non-viable parasitemia, and sequestration of the parasites in the deeper tissues, and the use of chemoprophylaxis or even presumptive treatment on the basis of clinical diagnosis, can all influence the identification and interpretation of malaria parasitemia in a diagnostic test.

Malaria is a potential medical emergency and should be treated accordingly. Delays in diagnosis and treatment are leading causes of death in many countries. Diagnosis can be difficult where malaria is no longer endemic for healthcare providers unfamiliar with the disease. Clinicians may forget to consider malaria among the potential diagnoses for some patients and not order the necessary diagnostic tests. Technicians may be unfamiliar with, or lack experience with, malaria, and fail to detect parasites when examining blood smears under a microscope. In some areas, malaria transmission is so intense that a large proportion of the population is infected but remains asymptomatic, e.g., in Africa. Such carriers have developed sufficient immunity to protect them from malarial illness, but not infection. In such situations, finding malaria parasites in an ill person does not necessarily mean that the illness is caused by the parasites. In many malaria-endemic countries, the lack of resources is a major barrier to reliable and timely diagnosis. Health personnel are undertrained, underequipped, and underpaid. They often face excessive patient loads, and must divide their attention between malaria and other equally severe infectious diseases, such as tuberculosis or HIV/AIDS.

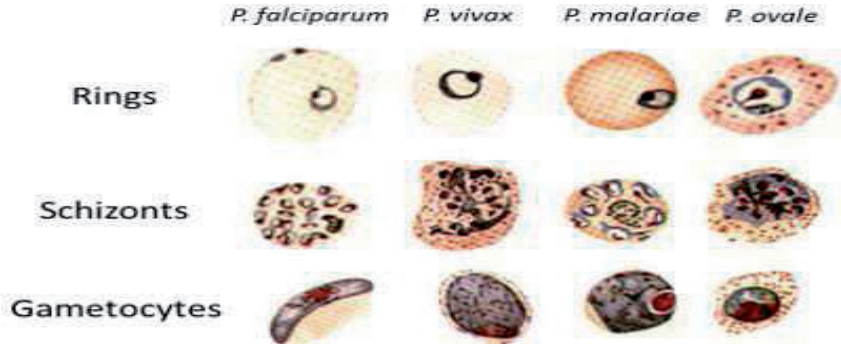
**CLINICAL DIAGNOSIS OF MALARIA**

A clinical diagnosis of malaria is traditional among medical doctors. This method is least expensive and most widely practiced. Clinical diagnosis is based on the patients' signs and symptoms, and on physical findings at examination. The earliest symptoms of malaria are very nonspecific and variable, and include fever, headache, weakness, myalgia, chills, dizziness, abdominal pain, diarrhea, nausea, vomiting, anorexia, and pruritus A clinical diagnosis of malaria is still challenging because of the non-specific nature of the signs and symptoms, which overlap considerably with other common, as well as potentially life-threatening diseases, e.g. common viral or bacterial infections, and other febrile illnesses. The overlapping of malaria symptoms with other tropical diseases impairs diagnostic specificity, which can promote the indiscriminate use of antimalarial and compromise the quality of care for patients with non-malarial fevers in endemic areas. The Integrated Management of Children Illness (IMCI) has provided clinical algorithms for managing and diagnosing common childhood illnesses by minimally trained healthcare providers in the developing world having inappropriate equipment for laboratory diagnosis. A widely utilized clinical algorithm for malaria diagnosis, compared with a fully trained pediatrician with access to laboratory support, showed very low specificity (0-9%) but 100% sensitivity in African settings. This lack of specificity reveals the perils of distinguishing malaria from other causes of fever in children on clinical grounds alone. Recently, another study showed that use of the IMCI clinical algorithm resulted in 30% over-diagnosis of malaria. Therefore, the accuracy of malaria diagnosis can be greatly enhanced by combining clinical-and parasite-based findings.

**LABORATORY DIAGNOSIS OF MALARIA**

Malaria is conventionally diagnosed by microscopic examination of stained blood films using Giemsa, Wright's, or Field's stains. This method has changed very little since Laverran's original discovery of the malaria parasite, and improvements in staining techniques by Romanowsky in the late 1,800s. More than a century later, microscopic detection and identification of *Plasmodium* species in Giemsa-stained thick blood films (for screening the presenting malaria parasite), and thin blood films (for species' confirmation) remains the gold standard for laboratory diagnosis .Malaria is diagnosed microscopically by staining thick and thin blood films on a glass slide, to visualize malaria parasites. Briefly, the patient's finger is cleaned with 70% ethyl alcohol, allowed to dry and then the side of fingertip is picked with a sharp sterile lancet and two drops of blood are placed on a glass slide. To prepare a thick blood film, a blood spot is stirred in a circular motion with the corner of the slide, taking care not make the preparation too thick, and allowed to dry without fixative. After drying, the spot is stained with diluted Giemsa (1: 20, vol/vol) for 20 min, and washed by placing the film in buffered water for 3 min. The slide is allowed to air-dry in a vertical position and examination using a light microscope. As they are unfixed, the red cells lyse when a water-based stain is applied. A thin blood film is prepared by immediately placing the smooth edge of a spreader slide in a drop of blood, adjusting the angle between slide and spreader to 45° and then smearing the blood with a swift and steady sweep along the surface. The film is then allowed to air-dry and is fixed with absolute methanol. After drying, the sample is stained with diluted Giemsa (1: 20, vol/vol) for 20 min and washed by briefly dipping the slide in and out of a jar of buffered water (excessive washing will decolorize the film). The slide is then allowed to air-dry in a vertical position and examined under a light microscope. The wide acceptance of this technique by laboratories all around the world can be attributed to its simplicity, low cost, its ability to identify the presence of parasites, the infecting

species, and assess parasite density-all parameters useful for the management of malaria. Recently, a study showed that conventional malaria microscopic diagnosis at primary healthcare facilities in Tanzania could reduce the prescription of antimalarial drugs, and also appeared to improve the appropriate management of non-malarial fevers. However, the staining and interpretation processes are labor intensive, time consuming, and require considerable expertise and trained healthcare workers, particularly for identifying species accurately at low parasitemia or in mixed malarial infections. The most important shortcoming of microscopic examination is its relatively low sensitivity, particularly at low parasite levels. Although the expert microscopists can detect up to 5 parasites/µl, the average microscopists detects only 50-100 parasites/µl. This has probably resulted in underestimating malaria infection rates, especially cases with low parasitemia and asymptomatic malaria. The ability to maintain required levels of in malaria diagnostics expertise is problematic, especially in remote medical centers in countries where the disease is rarely seen. Microscopy is laborious and ill-suited for high-throughput use, and species determination at low parasite density is still challenging. Therefore, in remote rural settings, e.g. peripheral medical clinics with no electricity and no health-facility resources, microscopy is often unavailable.




**QBC technique**

The QBC technique was designed to enhance microscopic detection of parasites and simplify malaria diagnosis. This method involves staining parasite deoxyribonucleic acid (DNA) in micro-hematocrit tubes with fluorescent dyes, e.g. acridine orange, and its subsequent detection

by epi-fluorescent microscopy. Briefly, finger-prick blood is collected in a hematocrit tube containing acridine orange and anticoagulant. The tube is centrifuged at 12,000 g for 5 min and immediately examined using an epi-fluorescent microscope. Parasite nuclei fluoresces bright green, while cytoplasm appears yellow-orange. The QBC technique has been shown to be a rapid and sensitive test for diagnosing malaria in numerous laboratories settings. While it enhances sensitivity for *P. falciparum*, it reduces sensitivity for non-falciparum species and decreases specificity due to staining of leukocyte DNA. Recently, it has been shown that acridine orange is the preferred diagnostic method (over light microscopy and Immunochromatographic tests) in the context of epidemiologic studies in asymptomatic populations in endemic areas, probably because of increased sensitivity at low parasitemia. Nowadays, portable fluorescent microscopes using light emitting diode (LED) technology, and pre-prepared glass slides with fluorescent reagent to label parasites, are available commercially. Although the QBC technique is simple, reliable, and user-friendly, it requires specialized instrumentation, is more costly than conventional light microscopy, and is poor at determining species and numbers of parasites.

**Rapid diagnostic tests (RDTs)**

Since the World Health Organization (WHO) recognized the urgent need for new, simple, quick, accurate, and cost-effective diagnostic tests for determining the presence of malaria parasites, to overcome the deficiencies of light microscopy, numerous new malaria-diagnostic techniques have been developed. This, in turn, has led to an increase in the use of RDTs for malaria, which are fast and easy to perform, and do not require electricity or specific equipment. Currently, 86 malaria RDTs are available from 28 different manufacturers. Unlike conventional microscopic diagnosis by staining thin and thick peripheral blood smears, and QBC technique, RDTs are all based on the same principle and detect malaria antigen in blood flowing along a membrane containing specific anti-malaria antibodies; they do not require laboratory equipment. Most products target a P. falciparum-specific protein, e.g. histidine-rich protein II (HRP-II) or lactate dehydrogenase (LDH). Some tests detect P. falciparum specific and pan-specific antigens (aldolase or pan-malaria pLDH), and distinguish non-P. falciparum infections from mixed malaria infections. Although most RDT products are suitable for P. falciparum malaria diagnosis, some also claim that they can effectively and rapidly diagnose P. vivax malaria. Recently, a new RDT method has been developed for detecting P. knowlesi. RDTs provide an opportunity to extend the benefits of parasite-based diagnosis of malaria beyond the confines of light microscopy, with potentially significant advantages in the management of febrile illnesses in remote malaria-endemic areas. RDT performance for diagnosis of malaria has been reported as excellent; however, some reports from remote malaria-endemic areas have shown wide variations in sensitivity. Murray and co-authors recently discussed the reliability of RDTs in an "update on rapid diagnostic testing for malaria" in their excellent paper. Overall, RDTs appears a highly valuable, rapid malaria-diagnostic tool for healthcare workers; however it must currently be used in conjunction with other methods to confirm the results, characterize infection, and monitor treatment. In malaria-endemic areas where no light microscopy facility exists that may benefit from RDTs, improvements are required for ease of use, sensitivity for non-falciparum infection, stability, and affordability. The WHO is now developing guidelines to ensure lot-to-lot quality control, which is essential for the community's confidence in this new diagnostic tool.

Because the simplicity and reliability of RDTs have been improved for use in rural endemic areas, RDT diagnosis in non-endemic regions is becoming more feasible, which may reduce time-to-treatment for cases of imported malaria.

**Serological tests**

Diagnosis of malaria using serological methods is usually based on the detection of antibodies against asexual blood stage malaria parasites. Immunofluorescence antibody testing (IFA) has been a reliable serologic test for malaria in recent decades. Although IFA is time-consuming and subjective, it is highly sensitive and specific. The literature clearly illustrates the reliability of IFA, so that it was usually regarded as the gold standard for malarial serology testing IFA is useful in epidemiological surveys, for screening potential blood donors, and occasionally for providing evidence of recent infection in non-immunes. Until recently, it was a validated method for detecting *Plasmodium*-specific antibodies in various blood bank units, which was useful for screening prospective blood donors, so avoiding transfusion-transmitted malaria. In France, for example, IFA is used as a part of a targeted screening strategy, combined with a donor questionnaire. The principle of IFA is that, following infection with any *Plasmodium* species, specific antibodies are produced within 2 wks. of initial infection, and persist for 3-6 months after parasite clearance. IFA uses specific antigen or crude antigen prepared on a slide, coated and kept at -30℃ until used, and quantifies both IgG and IgM antibodies in patient serum samples. Titers > 1: 20 are usually deemed positive, and < 1: 20 unconfirmed. Titers > 1: 200 can be classified as recent infections. In conclusion, IFA is simple and sensitive, but time-consuming. It cannot be automated, which limits the number of sera that can be studied daily. It also requires fluorescence microscopy and trained technicians; readings can be influenced by the level of training of the technician, particularly for serum samples with low antibody titers. Moreover, the lack of IFA reagent standardization makes it impractical for routine use in blood-transfusion centers, and for harmonizing inter-laboratory results.

**MOLECULAR DIAGNOSTIC METHODS**

As mentioned above, traditional malaria diagnostic methods remain problematic. New laboratory diagnostic techniques that display high sensitivity and high specificity, without subjective variation, are urgently needed in various laboratories. Recent developments in molecular biological technologies, e.g. PCR, loop-mediated isothermal amplification (LAMP), microarray, mass spectrometry (MS), and flow cytometric (FCM) assay techniques, have permitted extensive characterization of the malaria parasite and are generating new strategies for malaria diagnosis.

**PCR technique**

PCR-based techniques are a recent development in the molecular diagnosis of malaria, and have proven to be one of the most specific and sensitive diagnostic methods, particularly for malaria cases with low parasitemia or mixed infection. The PCR technique continues to be used extensively to confirm malaria infection, follow-up therapeutic response, and identify drug resistance. It was found to be more sensitive than QBC and some RDTs. Concerning with the gold standard method for malaria diagnosis, PCR has shown higher sensitivity and specificity than conventional microscopic examination of stained peripheral blood smears, and now seems

the best method for malaria diagnosis. PCR can detect as few as 1-5 parasites/µl of blood (≤ 0.0001% of infected red blood cells) compared with around 50-100 parasites/µl of blood by microscopy or RDT. Moreover, PCR can help detect drug-resistant parasites, mixed infections, and may be automated to process large numbers of samples. Some modified PCR methods are proving reliable, e.g., nested PCR, real-time PCR, and reverse transcription PCR, and appear to be useful second-line techniques when the 96 Korean J Parasitology. Vol. 47, No. 2: 93-102, June 2009 results of traditional diagnostic methods are unclear for patients presenting with signs and symptoms of malaria; they also allow accurate species determination .Recently, the PCR method has become widely accepted for identifying *P. knowlesi* infections. Although PCR appears to have overcome the two major problems of malaria diagnosis-sensitivity and specificity- the utility of PCR is limited by complex methodologies, high cost, and the need for specially trained technicians. PCR, therefore, is not routinely implemented in developing countries because of the complexity of the testing and the lack of resources to perform these tests adequately and routinely. Quality control and equipment maintenance are also essential for the PCR technique, so that it may not be suitable for malaria diagnosis in remote rural areas or even in routine clinical diagnostic settings.

**LAMP technique**

The LAMP technique is claimed to be a simple and inexpensive molecular malaria-diagnostic test that detects the conserved 18S ribosome RNA gene of P. falciparum. Other studies have shown high sensitivity and specificity, not only for P. falciparum, but also P. vivax, P. ovale and P. malariae .These observations suggest that LAMP is more reliable and useful for routine screening for malaria parasites in regions where vector-borne diseases, such as malaria, are endemic. LAMP appears to be easy, sensitive, quick and lower in cost than PCR. However, reagents require cold storage, and further clinical trials are needed to validate the feasibility and clinical utility of LAMP.

**Microarrays**

Publication of the *Plasmodium* genome offers many malaria-diagnostic opportunities. Microarrays may play an important role in the future diagnosis of infectious diseases. The principle of the microarrays technique parallels traditional Southern hybridization. Hybridization of labeled targets divided from nucleic acids in the test sample to probes on the array enables the probing of multiple gene targets in a single experiment. Ideally, this technique would be miniaturized and automated for point-of-care diagnostics. A pan-microbial oligonucleotide microarray has been developed for infectious disease diagnosis and has identified *P. falciparum* accurately in clinical specimens. This diagnostic technique, however, is still in the early stages of development.

**FCM assay**

Flow cytometry has reportedly been used for malaria diagnosis. Briefly, the principle of this technique is based on detection of hemozoin, which is produced when the intra-erythrocytic

malaria parasites digest host hemoglobin and crystallize the released toxic heme into hemozoin in the acidic food vacuole. Hemozoin within phagocytotes can be detected by depolarization of laser light, as cells pass through a flow-cytometer channel. This method may provide a sensitivity of 49-98%, and a specificity of 82-97%, for malarial diagnosis as are its labor intensiveness, the need for trained technicians, costly diagnostic equipment, and that false-positives may occur with other bacterial or viral infections. Therefore, this method should be considered a screening tool for malaria.

**Automated blood cell counters (ACC)**

An ACC is a practical tool for malaria diagnosis, with 3 reported approaches. The first used a Cell-Dyn® 3500 apparatus to detect malaria pigment (hemozoin) in monocytes, and showed a sensitivity of 95% and specificity of 88%, compared with the gold-standard blood smear. The second method also used a Cell-Dyn® 3500, and analyzed depolarized laser light (DLL) to detect malaria infection, with an overall sensitivity of 72% and specificity of 96%. The third technique used a Beckman Coulter ACC to detect increases in activated monocytes by volume, conductivity, and scatter (VCS), with 98% sensitivity and 94% specificity. Although promising, none of the 3 techniques is routinely available in the clinical laboratory; further studies are required to improve and validate the instrument and its software. The accuracy these methods promise, for detecting malaria parasites, mean ACC could become a valuable and routine malaria-diagnostic laboratory method.

**Mass spectrophotometry**

A novel method for in vitro detection of malaria parasites, with a sensitivity of 10 parasites/µl of blood, has been reported recently. It comprises a protocol for cleanup of whole blood samples, followed by direct ultraviolet laser desorption mass spectrometry (LDMS). For malaria diagnosis, the principle of LDMS is to identify a specific biomarker in clinical samples. In malaria, heme from hemozoin is the parasite-specific biomarker of interest. LDMS is rapid, high throughput, and automated. Compared with the microscopic method, which requires a skilled microscopist and up to 30-60 min to examine each peripheral blood smear, LDMS can analyze a sample in < 1 min. However, the remote rural areas without electricity are inhospitable for existing high-tech mass spectrometers. Future improvements in equipment and techniques should make this method more practicable.

Recently, other reliable malaria-diagnostic tests have been developed and introduced, and some tests are commercially available, for example, enzyme linked immunosorbent assay (ELISA)/enzyme immunoassay (EIA), latex agglutination assay, and cultivation of live malaria parasites. Post-mortem organ diagnoses, by investigating malaria parasites in tissue autopsy, e.g. liver and spleen .kidney] and brain have also been described. However, parasite culture, molecular techniques, serology techniques and pathobiological diagnostic techniques, although sometimes useful in research laboratories, are not practical or appropriate for the routine clinical diagnosis of malaria. [(22)]

**Malaria culture**

Cultivation of both human and non-human species of Plasmodium spp., the causal agent of malaria, has been a major research success, leading to a greater understanding of the parasite. Efforts at cultivating the organisms in vitro are complicated by the parasites' alternating between a human host and an arthropod vector, each having its own set of physiological, metabolic, and nutritional parameters. Life cycle stages of the four species that infect humans have been established in vitro. Of these four, P. falciparum remains the only species for which all stages have been cultured in vitro; different degrees of success have been achieved with the other human Plasmodium spp. The life cycle includes the exo -erythrocytic stage (within liver cells), the erythrocytic stage (within erythrocytes or precursor reticulocytes), and the sporogonic stage (within the vector). Culture media generally consist of a basic tissue culture medium (e.g., minimal essential medium or RPMI 1640) to which serum and erythrocytes are added. Most of the efforts have been directed toward the stage found in the erythrocyte. This stage has been cultivated in petri plates or other growth vessels in a candle jar to generate elevated $CO_2$ levels or in a more controlled $CO_2$ atmosphere. Later developments have employed continuous-flow systems to reduce the labor-intensive nature of medium changing. The exo-erythrocytic and sporogonic life cycle stages have also been cultivated in vitro. A number of avian, rodent, and simian malarial parasites have also been established in vitro. Although cultivation is of great help in understanding the biology of Plasmodium, it does not lend itself to use for diagnostic purposes.

On a global scale, malaria has been and remains a major public health concern. The disease is caused by parasitic protozoa of the genus Plasmodium. The life cycle of this organism is complex, with the parasite alternating between sexual reproduction in an invertebrate (mosquito) host and asexual reproduction in a vertebrate host. In addition to mammals as vertebrate hosts, birds and reptiles also serve as hosts for malarial parasites. The portion of the life cycle in the mosquito is the sporogonic phase, leading to formation of sporozoites which are injected by the vector into the vertebrate host at time of feeding. Sporozoites give rise to the schizogonic phase, with proliferation of the parasites in erythrocytic and exo-erythrocytic sites. The parasite is extracellular during its sporogonic phase, shifting to an intracellular location during the schizogonic stages of development. In vitro cultivation of the parasite requires simulating conditions in the mosquito vector for the sporogonic phase of the life cycle and, for the schizogonic phase, conditions promoting growth in exo-erythrocytic and erythrocytic locations of the vertebrate hosts. In this review, we attempt to bridge some of the earlier literature to recent developments relating to in vitro growth of Plasmodium spp. Both human and non-human malarial parasites are dealt with in this section, even though the latter are not of clinical significance. Non-human Plasmodium spp. share nutritional characteristics with their human counterparts, and have often served as models for cultivation of species infecting humans. Since this review does not deal in detail with the extensive literature on cultivation of Plasmodium spp., the interested reader is referred to the more comprehensive treatments of cultivation of the various stages in the parasite life cycle.

When introduced into the bloodstream of a vertebrate host by a mosquito, sporozoites enter into the exo-erythrocytic phase of development. In mammals, this occurs in hepatic cells, while in avian hosts, the parasites invade cells of the reticuloendothelial system. In mammalian malarias, the invasive stage for erythrocytes, the merozoite, passes through a developmental sequence beginning with a characteristic ring stage and leading to formation of a multinucleate schizont. Further development of the schizont leads to formation of multiple merozoites, which upon

rupture of the infected host cell, invade other erythrocytes. In avian malarias, after exiting from cells of the reticuloendothelial system, the merozoites can either reinvade reticuloendothelial cells or enter erythrocytes. Parasites can be recovered from the peripheral bloodstream of the vertebrate host, where they go through a cycle of repeated invasion of erythrocytes with characteristic synchrony to increase the level of parasitemia in the host bloodstream. Humans are infected by four species of malaria parasites: P. falciparum, P. vivax, P. ovale, and P. malariae. To a greater or lesser extent, all four species have been cultured or maintained in vitro; P. falciparum, however, is the only species for which all life cycle stages have been established in culture.

Differences exist between strains of Plasmodium. Some strains are readily established in vitro, while others are refractory to cultivation. Isolates undergo change once in culture, perhaps due to selection. In P. falciparum, for example, gametocyte formation is typical of recently cultured strains but is lost with prolonged cultivation. In this regard, it is important to note that cryopreservation of isolates can maintain those characteristics in a strain that may be lost on prolonged cultivation. Also, the use of cloned cell lines for experiments and the characterization of laboratory strains based on proteins and DNA is another consideration.

## HUMAN MALARIAS

### Exo-erythrocytic Stages:

When the parasite is first introduced into the bloodstream of its vertebrate host by the mosquito vector, the sporozoite stage of *P. falciparum* invades hepatic cells. Aspects of exo-erythrocytic cultivation have been reviewed by Hollingdale), Jensen, and Trager and Jensen.

In the case of P. falciparum, the parasite is probably taken up first by the Kupffer cells of the liver sinusoids in their passage to liver hepatocytes. Mazier et al have successfully infected hepatocytes prepared from human liver biopsy specimens in minimal essential medium (MEM) with sporozoites of P. falciparum, as determined by indirect immunofluorescence staining. Invading sporozoites developed into schizonts in host cells, with modest yields of about 650 schizonts/35-mm-diameter culture dish. Addition of human erythrocytes to infected hepatocyte cultures resulted in appearance of ring-stage organisms in the blood cells, indicating production of merozoites infective for erythrocytes. Calvo-Calle et al. (observed that P. falciparum developed within human hepatoma cell lines huH-1 and huH-2 but not consistently (one out of three experiments). A different human hepatoma cell line (HHS-102) allowed development of liver stages of P. falciparum and formation of ring stages in co-cultured erythrocytes.

The pattern of relapse that occurs with the human malarias caused by P. vivax and P. ovale is associated with the presence of dormant parasites termed hypnozoites that survive in the liver parenchymal cells of the host. Hollingdale et al. compared dividing and nondividing stages of P. vivax in hepatoma cells (HepG2-A16). They found that ca. 1 in $10^4$ sporozoites was infective for hepatoma cells, with merozoite release occurring on day 9 in vitro, compared to release on day 5 to 6 in vivo. They observed a population of smaller, nondividing parasites in infected hepatoma

cells, which they believed to be hypnozoites. Primary human hepatocytes successfully supported transformation and maturation of exo-erythrocytic stages of P. ovale, while other cell types (HepG2 and rat hepatocytes) would support transformation only. Developmental liver stages of P. malariae were produced in primary hepatocytes from the chimpanzee.

**Erythrocytic Stages**

The greatest amounts of effort and time have been invested in cultivation of the erythrocytic stages in the *Plasmodium* life cycle, this being the stage most often associated with the pathogenesis of malaria and a major target for vaccine development. A significant accomplishment in this area was defining in vitro conditions for continuous cultivation of *P. falciparum*, the most important and deadly of the human malarial parasites. This was accomplished by Trager and Jensen using HEPES-buffered RPMI 1640, a tissue culture medium developed for in vitro cultivation of leukocytes, supplemented with human serum, erythrocytes, and sodium bicarbonate. Parasites were cultured initially in petri plates placed in a candle jar that provided an atmosphere of 3% $CO_2$-17% $O_2$ or in vials that allowed for continuous flow of medium into culture vessels with an atmosphere of 7% $CO_2$-1% $O_2$-92% $N_2$. Later efforts gave rise to various continuous-flow devices, as well as suspension cultures for improved control and yields.

**Serum as medium supplement.**

The growth system functions best with 10 to 15% human serum as a supplement. For reasons that include cost, reproducibility, and possible presence of inhibitory immune factors and antimalarial drugs, there is interest in substituting other types of mammalian sera (bovine, monkey, horse, goat, sheep, rabbit, or swine) for human serum or even developing a serum-free medium for parasite cultivation. In comparing horse, swine, and lamb sera, horse serum was superior to the others but not as good as human serum. Fetal bovine serum, generally less effective for growth than human serum, when freshly obtained from fetuses of different breeds of cattle produced good parasite growth initially but led to a decline in numbers of parasites over 30 days. Rabbit serum (5 to 10%) was used in place of human serum but required a 2- to 3-week period of adaptation of cultures. Jensen compared percentages of growth of P. falciparum in sera of different animal origins. With fresh human serum as a standard at 100%, the following sera were rated as indicated: fresh fetal bovine, 35%; adult bovine, 7%; newborn bovine, 1%; horse, 19%; swine, 14%; and sheep, 2%. Ifediba and Vanderberg reported replacement of human serum by neopeptone and Proteose Peptone no. 3; bovine serum albumin (5 g/liter) was reported to replace serum by Ofulla et al. Dialyzed human serum lost its ability to support growth of parasites, and commercial samples of human serum supported growth at about one-quarter that of control cultures. Heat-inactivated, semi-immune human plasma from a region of malaria endemicity was used successfully for continuous cultivation of primary isolates of P. falciparum.

**Serum replacements.**

Freshly prepared human high-density lipoprotein fraction (concentration range of 0.25 to 0.50 mg/ml) was used to support growth of *P. falciparum*, with results comparable to those obtained using human serum. Other lipoprotein fractions, low- and very-low-density lipoproteins, produced little or no growth. Growth-promoting factor GF 21 (containing an ammonium sulfate

fraction of adult bovine serum plus insulin, transferrin, and sodium selenite) was used with Daigo's T basal medium for serum-free growth of *P. falciparum*. RPMI 1640 was supplemented with adenosine, unsaturated $C_{18}$ fatty acids, and fatty acid-free bovine serum albumin for serum-free growth, but growth rates of parasites were lower than those in plasma-containing medium. Pooling sera minimized variations in growth-promoting properties of serum samples obtained from different humans and rabbits.

**Commercial serum replacements.**

Linguae et al. used a commercially available serum replacement preparation, Nutridoma-SR (4%), to support the growth of several strains of P. falciparum from different global locations, with a resulting parasitemia of about 10% within 3 to 4 days. Flores et al. had better results using a lower concentration of Nutridoma-SR (1%) combined with Albumax I (0.5%), a purified serum albumin preparation. Cultures were maintained for 30 to 50 days, with parasitemias of 10%, compared to parasitemias of >15% obtained with human serum. They found that cultures raised in higher concentrations of Nutridoma-SR (2 or 4%) were nonviable or gave lower levels of parasitemia (parasitemia being the level of infection of blood cells). Binh et al. also used Albumax for cultivation of P. falciparum, with parasitemias reaching as high as 85% after 7 days with continuously passaged plasmodia. Cranmer et al., using Albumax II (0.5%) for growth of P. falciparum, achieved parasitemias of about 6 and 12% for two different malaria strains. They found that it was necessary to add hypoxanthine to the growth medium in order to obtain these levels of parasitemia. Plasma, without prior heat treatment, has been used for large-scale growth of P. falciparum; clotting was avoided by use of plastic culture vessels or siliconized glassware.

**Basal medium.**

The tissue culture medium RPMI 1640 remains the medium of choice, not only for *P. falciparum* but also for most other *Plasmodium* spp. that have been cultured in vitro. Better consistency for parasite growth is obtained by preparing the medium from a powdered preparation rather than using the liquid form available from most suppliers .The medium is supplemented with hypoxanthine as a purine source. RPMI 1640 has been supplemented with additional glucose, hypoxanthine, and reduced glutathione to improve parasite yield. Divo et al. produced a semidefined growth medium containing hypoxanthine as the preferred purine source; calcium pantothenate; and the amino acids cystine, glutamate, glutamine, isoleucine, methionine, proline, and tyrosine. Glucose could not be replaced by other sugars: ribose, mannose, fructose, galactose, and maltose. Antimetabolites of riboflavin, nicotinamide, pyridoxine, and thiamine were inhibitory to growth, expressed as incorporation of [$^3$H] hypoxanthine, in semidefined medium.

**Role of the erythrocyte in the culture system.**

Red blood cells are essential for development of the parasite, providing not only a location for asexual reproduction but also a source of nutrients for the parasite over and above that present in supplemented RPMI 1640. In vivo or in vitro, the parasite enters into the erythrocyte across the membrane and is enclosed in a vacuole, the parasitophorous vacuole that forms in part from the membrane of the red blood cell. The malaria parasite takes in nutrients and develops into a multinucleate schizont which undergoes fission to produce a characteristic number of merozoites

which, upon rupture of the parasitized blood cell, invade other blood cells and repeat the growth cycle. Human blood cells of all groups are suitable for growth of P. falciparum. Type O cells are useful because of their compatibility with serum or plasma of all other blood groups. Trager has used a combination of type A cells and serum because of availability. Type AB serum is compatible with any type of red blood cells. Citrated red blood cells may be stored for up to 5 weeks, at which time they become too fragile to use in cultures. Citrated red blood cells are washed and prepared as a 50% erythrocyte suspension which remains usable for 4 days at 4°C. Saline-adenine-glucose-stored blood cells were found to improve parasite yield. Chimpanzee, but not rhesus monkey or guinea pig, red blood cells supported development of P. falciparum.

### Culture systems

Plate cultures are prepared with a 5% hematocrit and about 1% parasitemia. The lower the initial parasitemia is, the greater the increase in numbers of parasites that will occur during in vitro growth is. Trager obtained 20- to 50-fold increase in parasite numbers with a starting parasitemia of 0.1%. Parasitemia of cultures can be increased to about 20% by changing medium in cultures every 8 h Monitoring of parasitemia is accomplished by preparing blood films, staining with Giemsa stain following methanol fixation, and counting infected red blood cells microscopically.

While the simplest system for cultivation of parasites uses petri or Linbro plates in a candle jar, this system is labor-intensive, requiring constant attention and daily changes of medium in order to maintain parasite growth. In this static system infected erythrocytes settle out to form a layer, producing microenvironments high in lactic acid in the region of the proliferating parasites. This may lead to conditions unfavorable for schizont development and penetration of merozoites into uninfected erythrocytes. Lactic acid production taxes the buffering capacity of the medium and leads to a drop in pH, which is detrimental to the growth of *Plasmodium*. Optimal parasite yields occurred with an extracellular pH of 7.2 to 7.45 and a lactate concentration below 12 µm; higher lactate concentrations were postulated to cause negative feedback of glycolysis. Glucose diffusion into the cell layer also becomes limiting.

Several devices to semiautomate cultivation of plasmodia have been described previously. These devices allow for continuous flow of medium through growth vessels, with a controlled gas phase of 2 to 5% $CO_2$, 3 to 18% $O_2$, and the remainder being $N_2$. Semiautomated devices reduce the amount of time spent on maintenance of stock cultures of the parasite. Although the malarial parasite is found in red blood cells, it is microaerophilic in its oxygen preference .Taylor-Robinson used a commercial system designed for growth of *Campylobacter* spp. to generate the low $O_2$ and high $CO_2$ levels favored by *P. falciparum*. Anaerobic jars and gas-generating envelopes were employed to grow parasites in tissue culture flasks or microtiter plates in an atmosphere of 6%$O_2$-8% $CO_2$-86% $N_2$. Suspension cultures of parasites have also been tried. Zolg et al.) Reported improved yields with shaking of cultures, but these claims have been disputed. Fragility of the infected red blood cells is an important factor in agitated cultures, but this has been countered to some extent by the use of methylcellulose in agitated cultures. Mons et al. have used a culture flask with a stirring bar for cultivation of the rodent parasite *P. berghei*.

**Induction of synchrony in vitro.**

In its human host, P. falciparum exhibits a synchrony of about 48-h duration. Blood sampled at any one time from an infected host will reveal a parasite population at the same stage in its developmental cycle, i.e., mostly ring stages or mostly schizonts, etc. This synchrony is in part a response to the circadian rhythmicity of the host's body. Synchrony can be artificially imposed in vitro upon developing malaria parasites by one of several methods. Most popular is the use of sorbitol or mannitol treatment of infected erythrocytes. Infected cells are treated with 5% sorbitol, which causes lysis of erythrocytes containing late stages and preferentially selects for red blood cells with early ring stages. The effect is not osmotic, but has to do with the permeability of infected cells and the sensitivity of the parasites to sorbitol. Treatment can be repeated at 34 h to further select for young stages and improve on the synchrony. Other techniques involve the separation of late-stage parasites, as by sedimentation in Plasmagel or gelatin. Most strains of P. falciparum produce knobs over the surface of the host erythrocyte when the parasite reaches the late trophozoite to schizont stage. Such red cells do not form rouleaux in the Plasmagel, as do uninfected red cells or those containing ring-stage organisms. As a result, the cells with late-stage parasites remain in suspension while the uninfected ones and those with rings settle out. If the late-stage parasites are then mixed with fresh red cells and put under culture conditions, merozoites formed by them invade new cells. If one allows invasion to go on for 3 h and then treats the cells with sorbitol to kill all late-stage parasites, one gets a population of rings just 0 to 3 h old. Such a tightly synchronized culture will remain quite synchronous through about three cycles. Shifting cultures of P. falciparum from 37 to 28°C has been used to delay the asexual cycle for 12 to 16 h.

Awad-El-Kariem et al. have used feeder cells, either mouse peritoneal macrophages or the flagellated protozoon Crithidia fasciculata, for establishment of P. falciparum in vitro with a success rate better than 80%. While the role of Crithidia in facilitating cultivation of malaria parasites is not clear, the authors suggest that it might involve lowering the redox potential of the culture medium, thereby protecting parasites from damage due to reactive oxygen intermediates.

It is worth noting that cultured P. falciparum retains its infectivity and immunogenicity. In a laboratory accident, a puncture wound resulted in inoculation of cultured parasites leading to human infection with a strain that had been maintained in vitro for approximately 4 years. Trager noted instances in which cultured material was used for vaccine development, indicating retention of immunogenicity.

***Plasmodium* spp. other than *P. falciparum*.**

Although the in vitro system has worked well for P. falciparum, P. vivax has not been amenable to cultivation using the same methods. Brockelman et al. used SCMI 612 medium for schizogonic stages of P. vivax and found that this medium worked better than either RPMI 1640 or Weymouth media. They reported that a higher glucose level (3 mg/ml) was needed for P. vivax than for P. falciparum. Unlike P. falciparum, which develops in erythrocytes, P. vivax invades developing erythrocytes, the reticulocytes. Thus, in order to maintain this species in vitro, a large supply of reticulocytes is needed for development. Mons et al. used RPMI 1640

medium with an enriched reticulocyte fraction from owl monkeys (Aotus sp.), noting that P. vivax preferentially infects these immature erythrocytes. However, levels of parasitemia were low. Not only were reticulocytes necessary for parasite development, but agitation of the culture medium was needed to establish contact between parasite and host cell. Agitation of cultures led to decrease in the numbers of parasites due to fragility of the parasitized Aotus blood cells. A variety of supplements including hypoxanthine, ascorbic acid, choline, biotin, $B_{12}$, and $MgCl_2$ had little or no effect on growth and schizogony. The need for young erythrocytes and reticulocytes was noted by Lanners, who used a continuous-flow system. Methylcellulose (0.1%) was added to reduce breakage of erythrocyte membranes. Other supplements included glucose, spermidine, and antioxidants.

Golenda et al. achieved stable in vitro cultivation of *P. vivax* for eight growth cycles by use of enriched populations of reticulocytes from humans with hemochromatosis. Using McCoy's 5A medium supplemented with glutamine and 20% human serum, they cultured the parasite initially in a candle jar until schizogonic stages were produced. The reticulocytes were then added and the culture was transferred to a shaker, which promoted contact between parasites and host cells. They obtained 85% ring-stage organisms after about 12 h under these conditions. Gametocytes did not develop, although the parasites remained infective for owl monkeys.

Lingnau et al. observed development over a 6-day period of erythrocytic stages of *P. malariae* in RPMI 1640 medium supplemented with glutamine, hypoxanthine, and 20% human serum. The variety of stages seen in red blood cells suggested that merozoites were produced in vitro and invaded noninfected erythrocytes.

**Cell-Free Development of Erythrocytic Stages**

While in vitro growth of plasmodia is a significant accomplishment in its own right, growth of the parasites under a completely cell-free or axenic condition would allow examination of the parasites' nutritional needs, biochemical and molecular properties, and sensitivity to antimicrobial agents, in the absence of those of the host erythrocyte. Towards this end, Trager and coworkers were successful in obtaining merozoite development of *P. falciparum* to ring stages under cell-free conditions. In an early study, Trager and Lanners cultured *P. falciparum* merozoites to ring and trophic stages in HEPES-buffered RPMI 1640 in which NaCl was replaced by KCl, in the presence of a serum-supplemented, frozen-thawed 33% erythrocyte extract; dipotassium ATP (1.6 μM); and pyruvate (3.6 μM). Ultrastructural evidence indicated absence of the parasitophorous membrane in these extracellular forms, yet development occurred, suggesting that factors present in the erythrocyte but not necessarily the intracytoplasmic location of the parasite were needed for development. In a later study, it was found that sonicated erythrocyte preparations (about 50% erythrocyte extract), with ATP (2 μM) and pyruvate (5 μM) added, supported better development of extracellular *P. falciparum* than did frozen-thawed preparations, with about 30% of the merozoites developing to later stages. These extracellular forms react to the same monoclonal antibodies that the intracellular forms respond to, suggesting a similar pattern of molecular differentiation.

**Biphasic cultivation system**

Further refinement of the technique for axenic cultivation involved the use of a biphasic system with merozoites embedded in a Matrigel substrate in a well containing a fluid medium overlay. Coenzyme A, added as a supplement to the basic medium (0.15 μM), may enhance formation of later stages in the life cycle, though its role was not clear from an earlier study. The fluid medium could be changed with minimal disturbance to the parasites developing within the Matrigel layer. Ring-stage parasites developing within Matrigel attained larger sizes and showed motility, though only about 1% of the merozoites inoculated completed the asexual cycle in vitro. Gametocytes were observed in cultures after 36 to 44 h of incubation .Trager et al. postulated that contact between parasite and spectrin in the erythrocyte sonicate may be an important factor for development. More recently, Williams et al. have increased the yields of schizonts forming extracellularly by addition of erythrocyte ghosts obtained by osmotic lysis, to double the amount of membrane present. These results support the critical role of the erythrocyte membrane in the development of the malarial parasite. The role of Matrigel, a preparation of solubilized tissue basement membrane, may be simply to provide the parasite with a substrate that approximates the cytoplasmic matrix in which the merozoites begin to develop. Matrigel has also been an important factor in cultivation of the mosquito stages of the P. falciparum life cycle.

**Gametocytogenesis**

Gametocytes are the precursors of the macrogamete (female gamete) and the sperm cell which fuse to form a motile, banana-shaped zygote, the ookinete, in the wall of the gut of the mosquito. In vivo, gametocyte development occurs within erythrocytes in the peripheral circulatory system of the vertebrate host. These stages are picked up by the blood-sucking mosquito, in which they complete the sexual cycle of the malaria parasite. In vitro, variation exists among different strains of P. falciparum with respect to gametocyte formation, and even among different clones from the same strain of parasite. As an example of the latter, Bhasin and Trager isolated three clones of a Honduran strain of P. falciparum, of which two developed gametocytes and one did not.

**Induction of gamete formation**

Gametocyte formation in cultures can be enhanced by changing the growth medium without providing fresh erythrocytes. Culture conditions also affect gametocytogenesis of P. falciparum. Strains recently isolated are more likely to form gametocytes than are strains that have been in culture over long periods of time. Ifediba and Vanderberg reported that hypoxanthine (50 μg/ml) was necessary for induction and maturation of gametocytes forming in cultures of P. falciparum; without hypoxanthine, mosquitoes feeding on gametocyte-containing cultures would not develop oocyst infections. Others have reported that some ammonium compounds (ammonium carbonate or ammonium bicarbonate, but not ammonium chloride or ammonium acetate) with or without concanavalin A trigger gametocyte formation on the third day following treatment. Evidence that signal transduction is involved in gametocytogenesis comes from several studies in which secondary messengers, or compounds affecting them, have been shown to enhance gametocyte formation. Cyclic AMP (cAMP) and dibutyryl cAMP (1 mM) increased gamete formation in P. falciparum). Trager and Gill) employed phorbol compounds (phorbol 12-myristate-13-acetate

and phorbol dibutyrate) and a phosphodiesterase inhibitor (8-bromo cAMP) to promote gametocyte production by 50% or more in cultured P. falciparum; they found, however, that cAMP and forskolin were without effect on gametocyte differentiation. Lingnau et al.have examined the role of several hormones in P. falciparum gametocytogenesis in serum-free medium. Exflagellation, the differentiation and development of male gametes from a male gametocyte, was accomplished in vitro by Carter and Beach by washing gametocytes in fetal bovine or human serum at pH 8. No evidence of gametocyte formation was seen in the short-term cultivation of P. malariae.

**Problems with induction**

Unlike the asexual division cycle that occurs within erythrocytes over a 48-h period, maturation of gametocytes of P. falciparum requires about 2 weeks. Nutrients present within the infected erythrocyte would be exhausted by the developing parasite over this prolonged time period and may be a factor in the difficulty of inducing gametocyte formation in vitro. Immature erythrocytes (reticulocytes) supported better gametocyte formation than did mature erythrocytes. Other factors affecting gametocyte formation may include variations in nutrients present in serum supplements of growth medium, absence of essential activating factors in RPMI 1640, and selection of non-gametocyte-forming populations under in vitro conditions. Trager concluded that none of the techniques currently available for inducing gametocytogenesis is of practical value, largely due to the difficulties in obtaining gametocytes in quantity. Reviews of gametocytogenesis are available.

**Sporogonic Stages**

The mosquito acquires gametocytes from the blood of a vertebrate. Development of the gametocytes into male and female gametes is initiated within the gut of the mosquito. The fused gametes form an ookinete, which develops into the oocyst in the hemocoel of the insect. Sporozoites develop within the oocyst and migrate to the salivary glands of the mosquito, to be injected into the next vertebrate host that the insect bites. A different set of growth conditions is needed for the successful cultivation of these stages of the *P. falciparum* life cycle. Warburg and Schneider successfully produced sporozoites of *P. falciparum* in a complex culture of *Drosophila melanogaster* cells and a Matrigel substrate, to which ookinetes adhered. Development of sporozoites took 12 to 16 days. Wheat germ agglutinin was used to enhance transformation of zygotes into retorts and ookinetes, by providing a modifying environment approximating conditions in the mosquito stomach for development of the sexual stages. [23]

**Malaria control**

Malaria is a difficult disease to control largely due to the highly adaptable nature of the vector and parasites involved. While effective tools have been and will continue to be developed to combat malaria, inevitably, over time the parasites and mosquitoes will evolve means to circumvent those tools if used in isolation or used ineffectively. To achieve sustainable control

over malaria, healthcare professionals will need a combination of new approaches and tools, and research will play a critical role in development of those next-generation strategies.

Malaria has a significant impact on the health of infants, young children, and pregnant women worldwide. More than 800,000 African children under the age of five die of malaria each year. Malaria also contributes to malnutrition in children, which indirectly causes the death of half of all children under the age of five throughout the world. Fifty million pregnant women throughout the world are exposed to malaria each year. In malaria-endemic regions, one-fourth of all cases of severe maternal anemia and 20 percent of all low-birth weight babies are linked to malaria. Scientists are working to better understand how malaria uniquely affects children and pregnant women and to develop new research tools, methods, and products appropriate for these populations.

The development of a safe and effective vaccine against malaria will be critical in malaria control, prevention, and eradication efforts. Currently, no licensed vaccine against malaria (or any parasitic disease that afflicts humans) exists. The complexity of the *Plasmodium* parasite and the lack of understanding of critical processes, such as host immune protection and disease pathogenesis, have hampered vaccine development efforts.

Antimalarial drugs, in combination with mosquito control programs, have historically played a key role in controlling malaria in endemic areas, resulting in significant reduction of the geographic range of malarial disease worldwide. Over the years, however, the emergence and spread of drug-resistant parasites has contributed to a reemergence of malaria, turning back the clock on control efforts. The need for new, effective drugs for malaria has become a critical priority on the global malaria research agenda. [24]

Prevention of malaria is currently based on two complementary methods: chemoprophylaxis and protection against mosquito bites. While several malaria vaccines are under development, none is available yet.

**Chemoprophylaxis**

In Europe, malaria chemoprophylaxis is only for travelers to malaria endemic countries, which are classified in three (or four) groups, to determine which drug is recommended for chemoprophylaxis. The choice of drugs depends on the travel destination, the duration of potential exposure to vectors, parasite resistance pattern, level and seasonality of transmission, age and pregnancy. In endemic countries, chemoprophylaxis could also be recommended for autochthonous young children and pregnant women, depending on endemicity level and seasonality of transmission.

**Personal protection measures against mosquito bites**

Because of the nocturnal feeding habits of most of *Anopheles* mosquitoes, malaria transmission occurs primarily at night. Protection against mosquito bites include the use of mosquito bed nets (preferably insecticide-treated nets), the wearing of clothes that cover most of the body, and use

of insect repellent on exposed skin. Type and concentration of repellents depend on age and status. [24]

**Biological resistance to malaria**

**Human genetic resistance to malaria** refers to inherited changes in the DNA of humans which increase resistance to the disease and result in increased survival of individuals with the genetic

change. Evolutionarily, the existence of these genotypes are likely due to pressure from evolving alongside the parasites that cause malaria (of the genus *Plasmodium*). Since malaria infects red blood cells, these genetic changes are most commonly alterations to molecules essential for red blood cell function (and therefore parasite survival), such as hemoglobin or other cellular proteins or enzymes of red blood cells. These alterations generally protect red blood cells from invasion by *Plasmodium* parasites or replication of parasites within the red blood cell.

**Genetic resistance to parasitic infection**

Microscopic parasites (like viruses, protozoans that cause malaria, and others) cannot replicate on their own. They replicate by invading the hosts' cells, and usurping the cellular machinery to replicate themselves. Eventually, unchecked replication causes the cells to burst, releasing the infectious organisms into the bloodstream. There they spread and infect other cells. As cells die and toxic products of invasive organism replication accumulate, disease symptoms appear.

The process of invading the host cell, hijacking the cellular machinery, replication and final release is a complicated set of steps. Very specific proteins coded by the DNA of the infectious organism as well as the host cells allow those steps to happen. Even a very small change in a critical protein might make infection difficult or impossible. Such changes might arise by a process of mutation in the gene that codes for the protein. If the change is in the gamete, that is, the sperm or egg that join to form a zygote that grows into a human being, the protective mutation will be inherited. Since lethal diseases kill many persons who lack protective mutations, in time, many persons in regions where lethal diseases are endemic come to inherit protective mutations.

Mutations may have detrimental as well as beneficial effects, and any single mutation may have both. Infectivity of malaria depends on specific proteins present in the cell walls and elsewhere in red blood cells. Protective mutations alter these proteins in ways that make them inaccessible to malaria organisms. However, these changes also alter the functioning and form of red blood cells that may have visible effects, either overtly, or by microscopic examination of red blood cells. These changes may impair the function of red blood cells in various ways that have a detrimental effect on the health or longevity of the individual. However, if the net effect of protection against malaria outweighs the other detrimental effects, the protective mutation will tend to be retained and propagated from generation to generation.

These alterations which protect against malarial infections but impair red blood cells are generally considered blood disorders, since they tend to have overt and detrimental effects. Their protective function has only in recent times, been discovered and acknowledged. Some of these disorders are known by fanciful and cryptic names like sickle-cell anemia, thalassaemia, glucose-6-phosphate dehydrogenase deficiency, ovalocytosis, elliptocytosis and loss of the Gerbich antigen and the Duffy antigen. These names refer to various proteins, enzymes, and the shape or function of red blood cells.

**Innate resistance**

The potent effect of genetically controlled innate resistance is reflected in the probability of survival of young children in malarious environments. It is necessary to study innate immunity in the susceptible age group, younger than four years; in older children and adults the effects of innate immunity are overshadowed by those of adaptive immunity. It is also necessary to study populations in which random use of antimalarial drugs does not occur.

**Mechanisms of protection**

The mechanisms by which erythrocytes containing abnormal hemoglobins, or are G6PD deficient, are partially protected against *P. falciparum* infections are not fully understood, although there has been no shortage of suggestions. During the peripheral blood stage of replication malaria parasites have a high rate of oxygen consumption and ingest large amounts of hemoglobin. It is likely that HbS in endocytic vesicles is deoxygenated, polymerizes and is poorly digested. In red cells containing abnormal hemoglobins, or which are G6PD deficient, oxygen radicals are produced, and malaria parasites induce additional oxidative stress. This can result in changes in red cell membranes, including translocation of phosphatidylserine to their surface, followed by macrophage recognition and ingestion. The authors suggest that this mechanism is likely to occur earlier in abnormal than in normal red cells, thereby restricting multiplication in the former. In addition, binding of parasitized sickle cells to endothelial cells is significantly decreased because of an altered display of *P. falciparum* erythrocyte membrane protein-1 (PfMP-1). This protein is the parasite's main cytoadherence ligand and virulence factor on the cell surface. During the late stages of parasite replication red cells are adherent to venous endothelium, and inhibiting this attachment could suppress replication.

Sickle hemoglobin induces the expression of heme oxygenase-1 in hematopoietic cells. Carbon monoxide, a byproduct of heme catabolism by heme oxygenase-1(HO-1), prevents an accumulation of circulating free heme after *Plasmodium* infection, suppressing the pathogenesis of experimental cerebral malaria Other mechanisms, such as enhanced tolerance to disease mediated by HO-1 and reduced parasitic growth due to translocation of host micro-RNA into the parasite, have been described.

**Types of innate resistance**

Evidence has accumulated that the first line of defense against malaria is provided by genetically controlled innate resistance, mainly exerted by abnormal hemoglobins and glucose-6-phosphate dehydrogenase deficiency. The three major types of inherited genetic resistance - sickle cell disease, thalassemias, and G6PD deficiency - were present in the Mediterranean world by the time of the Roman Empire.

**Hemoglobin abnormalities**

**Hb S**

This was the first time a genetic disease was linked to a mutation of a specific protein and Pauling introduced his fundamentally important concept of sickle cell anemia as a genetically transmitted molecular disease.

The molecular basis of sickle cell anemia was finally elucidated in 1959, when Ingram perfected the techniques of tryptic peptide fingerprinting. In the mid-1950s, one of the newest and most reliable ways of separating peptides and amino acids was by means of the enzyme trypsin, which split polypeptide chains by specifically degrading the chemical bonds formed by the carboxyl groups of two amino acids, lysine and arginine. Small differences in hemoglobin A and S will result in small changes in one or more of these peptides . To try to detect these small differences, Ingram combined paper electrophoresis and the paper chromotagraphy methods. By this combination he created a two-dimensional method that enabled him to comparatively "fingerprint" the hemoglobin S and A fragments he obtained from the tryspin digest. The fingerprints revealed approximately 30 peptide spots, there was one peptide spot clearly visible in the digest of haernoglobin S which was not obvious in the haemoglobin A "finger print". The Hb S gene defect is a mutation of a single nucleotide (A to T) of the β-globin gene replacing the amino acid glutamic acid with the less polar amino acid valine at the sixth position of the β chain.

HbS has a lower negative charge at physiological pH than does normal adult hemoglobin. The consequences of the simple replacement of a charged amino acid with a hydrophobic, neutral amino acid are far ranging, Recent studies in West Africa suggest that the greatest impact of Hb S seems to be to protect against either death or severe disease—that is, profound anemia or cerebral malaria—while having less effect on infection per se. Children who are heterozygous for the sickle cell gene have only one- tenth the risk of death from falciparum as do those who are homozygous for the normal hemoglobin gene. Binding of parasitized sickle erythrocytes to endothelial cells and blood monocytes is significantly reduced due to an altered display of *Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP-1), the parasite's major cytoadherence ligand and virulence factor on the erythrocyte surface. Protection also derives from the instability of sickle hemoglobin, which clusters the predominant integral red cell membrane protein (called band 3) and triggers accelerated removal by phagocytic cells. Natural antibodies recognize these clusters on senescent erythrocytes. Protection by HbAS involves the enhancement of not only innate but also of acquired immunity to the parasite. Prematurely denatured sickle hemoglobin results in an up regulation of natural antibodies which control erythrocyte adhesion in both malaria and sickle cell disease. Targeting the stimuli that lead to endothelial activation will constitute a promising therapeutic strategy to inhibit sickle red cell adhesion and vasco-occlusion.

P. Brain also while working in Northern Rhodesia suggested that while homozygotes for the sickle cell gene suffered from several problems heterozygotes might be protected against malaria.

**Thalassemias**

It has long been known that a kind of anemia, termed thalassemia, has a high frequency in some Mediterranean populations, including Greeks and southern Italians. The name is derived from the Greek words for sea (thalassa), meaning the Mediterranean sea, and blood (haima). Vernon Ingram deserves the credit for explaining the genetic basis of different forms of thalassemia as an imbalance in the synthesis of the two polypeptide chains of hemoglobin.

In the common Mediterranean variant, mutations decrease production of the β-chain (β-thalassemia). In α-thalassemia, which is relatively frequent in Africa and several other countries, production of the α-chain of hemoglobin is impaired, and there is relative over-production of the β-chain. Individuals homozygous for β-thalassemia have severe anemia and are unlikely to survive and reproduce, so selection against the gene is strong. Those homozygous for α-thalassemia also suffer from anemia and there is some degree of selection against the gene.

The lower Himalayan foothills and Inner Terai or Doon Valleys of Nepal and India are highly malarial due to a warm climate and marshes sustained during the dry season by groundwater percolating down from the higher hills. Malarial forests were intentionally maintained by the rulers of Nepal as a defensive measure. Humans attempting to live in this zone suffered much higher mortality than at higher elevations or below on the drier Gangetic Plain. However, the Tharu people had lived in this zone long enough to evolve resistance via multiple genes. Medical studies among the Tharu and non-Tharu population of the Terai yielded the evidence that the prevalence of cases of residual malaria is nearly seven times lower among Tharus. The basis for resistance has been established to be homozygosity of α-Thalassemia gene within the local population.[27] Endogamy along caste and ethnic lines appear to have prevented these genes from being more widespread in neighboring populations.

**HbC and HbE erythroids**

There is evidence that the persons with α-thalassemia, HbC and HbE have some degree of protection against the parasite. Hemoglobin C (HbC) is an abnormal hemoglobin with substitution of a lysine residue for glutamic acid residue of the β-globin chain, at exactly the same ß-6 position as the HbS mutation. The "C" designation for HbC is from the name of the city where it was discovered—Christchurch, New Zealand. People who have this disease, particularly children, may have episodes of abdominal and joint pain, an enlarged spleen, and mild jaundice, but they do not have severe crises, as occur in sickle cell disease. Haemoglobin C is common in malarious areas of West Africa, especially in Burkina Faso. In a large case–control study performed in Burkina Faso on 4,348 Mossi subjects, that HbC was associated with a 29% reduction in risk of clinical malaria in HbAC heterozygotes and of 93% in HbCC homozygotes. HbC represents a ‘slow but gratis’ genetic adaptation to malaria through a transient polymorphism, compared to the polycentric ‘quick but costly’ adaptation through balanced polymorphism of HbS. HbC modifies the quantity and distribution of the variant antigen *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) on the infected red blood cell surface and the modified display of malaria surface proteins reduces parasite adhesiveness (thereby avoiding clearance by the spleen) and can reduce the risk of severe disease.

Hemoglobin E is due to a single point mutation in the gene for the beta chain with a glutamate-to-lysine substitution at position 26. It is one of the most prevalent hemoglobinopathies with 30

million people affected. Hemoglobin E is very common in parts of Southeast Asia. HbE erythrocytes have an unidentified membrane abnormality that renders the majority of the RBC population relatively resistant to invasion by P falciparum. [25]

**Malaria vaccines**

Malaria parasites are transmitted by the bite of female *Anopheles* mosquitoes. The *Plasmodium falciparum* parasite is responsible for most malaria infections and almost all deaths caused by the disease worldwide. Most of the previous vaccines which have been tried involved the use of individual molecules found in the pathogen. However, they were unable to provide sufficient immunity to the disease. The Tuebingen study involved 67 healthy adult test persons, none of whom had previously had malaria. The best immune response was shown in a group of nine test persons who received the highest dose of the vaccine three times at four-week intervals. At the end of the trial, all nine of these individuals had 100 percent protection from the disease.

"That protection was probably caused by specific T-lymphocytes and antibody responses to the parasites in the liver," Professor Peter Kremsner explained. The researchers analyzed the bodies' immune reactions and identified protein patterns which will make it possible to further improve malaria vaccines, Kremsner added. The researchers injected live malaria parasites into the test subjects, at the same time preventing the development of the disease by adding chloroquine -- which has been used to treat malaria for many years. This enabled the researchers to exploit the behavior of the parasites and the properties of chloroquine.

Once the person is infected, the *Plasmodium falciparum* parasite migrates to the liver to reproduce. During its incubation period there, the human immune system could respond; but at this stage, the pathogen does not make the person sick. On top of that, chloroquine does not take effect in the liver -- so it is unable to prevent the parasite from reproducing. Malaria only breaks out when the pathogen leaves the liver, entering the bloodstream and going into the red corpuscles, where it continues to reproduce and spread. As soon as the pathogen enters the bloodstream, however, it can be killed by chloroquine -- and the disease cannot break out.

"By vaccinating with a live, fully active pathogen, it seems clear that we were able to set of a very strong immune response," said study leader Benjamin Mordmueller, "Additionally, all the data we have so far indicate that what we have here is relatively stable, long-lasting protection." In the group of test persons who demonstrated 100 percent protection after receiving a high dose three times, Mordmueller said, the protection was reliably still in place after ten weeks -- and remained measurable for even longer. He added that the new vaccine showed no adverse effects on the test persons. The next step is to further test the vaccine's effectiveness over several years in a clinical study in Gabon funded by DZIF. Malaria is one of the biggest health threats in the African nation. The University of Tuebingen has worked with the Albert Schweitzer Hospital in the Gabonese town of Lambaréné and with the neighboring research institute, the Centre de Recherches Médicales de Lambaréné, for many years.

Malaria is one of the deadliest infectious diseases worldwide. The World Health Organization reports that some 214 million people became infected with malaria in the year 2015 alone. Approximately 438,000 died of the disease. Around 90 percent of those malaria deaths were in Africa. Nearly three-quarters of those who succumb to the disease are children under five. The search for a vaccine has been going on for more than a century. An effective vaccine would make it easier to control malaria; vaccination campaigns could be conducted in severely affected areas to eliminate the pathogen. Such a vaccine could also help to stop the spread of resistance to the treatment, and to better protect travelers [(26)].

**References:**

1-http://www.vivaxmalaria.com/template_disease.htm

2-https://www.cdc.gov/malaria/

3- http://www.healthline.com/health/malaria#overview

4- https://www.nobelprize.org/educational/medicine/malaria/readmore/history.html

5- http://www.who.int/mediacentre/factsheets/fs094/en/

6- https://www.cdc.gov/malaria/about/biology/

7- http://www.malariasite.com/life-cycle/

8- http://www.malariasite.com/pathology/

9-http://www.nhs.uk/Conditions/Malaria/Pages/Symptoms.aspx

10-https://www.hse.ie/eng/health/az/M/Malaria/Complications-of-malaria.html

11-https://www.cdc.gov/malaria/about/biology/mosquitoes/

12-https://www.cdc.gov/malaria/about/biology/mosquitoes/

13-https://www.mmv.org/malaria-medicines/five-species?gclid=CjwKCAjw2s_MBRA5EiwAmWIacyuPWDQEKyYQgaz6pvfDZ1lQGOR8_NU93purYG53jCPCmkr0GfP0uxoCYwoQAvD_BwE

14-http://scientistsagainstmalaria.net/parasite/plasmodium-falciparum

15- https://en.wikipedia.org/wiki/Plasmodium_vivax

16-https://microbewiki.kenyon.edu/index.php/Plasmodium_malariae

17-http://eol.org/pages/10408875/overview

18-https://en.wikipedia.org/wiki/Plasmodium_ovale

19-https://www.ncbi.nlm.nih.gov/pubmed/23554413

20-https://academic.oup.com/cid/article/46/2/172/454007/Plasmodium-knowlesi-The-Fifth-Human-Malaria

21-https://en.wikipedia.org/wiki/Plasmodium_knowlesi

22-https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2688806/

23-http://cmr.asm.org/content/15/3/355.full

24-https://ecdc.europa.eu/en/malaria/prevention-and-control

25- https://en.wikipedia.org/wiki/Human_genetic_resistance_to_malaria

26-https://www.sciencedaily.com/releases/2017/02/170215131606.htm