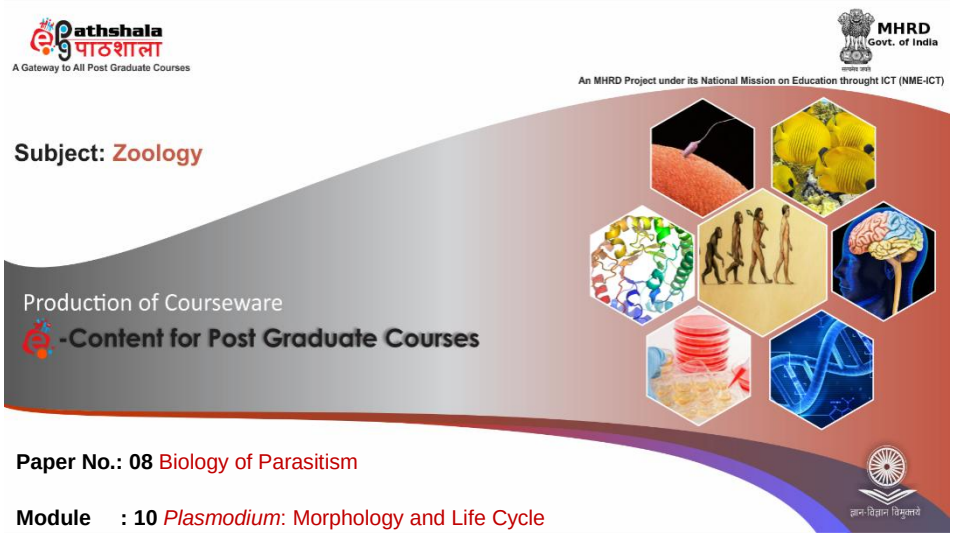


Paper No.: 08 Biology of Parasitism

Module : 10 *Plasmodium*: Morphology and Life Cycle

Development Team

Principal Investigator:	**Prof. Neeta Sehgal** **Head, Department of Zoology, University of Delhi**
Paper Coordinator:	Dr. Pawan Malhotra ICGEB, New Delhi
Content Writer:	**Dr. Gauri Mishra** **Swami Shraddhanand College, University of Delhi**
Content Reviewer:	**Prof. Virender Kumar Bhasin** **Department of Zoology, University of Delhi**

Description of Module	
Subject Name	ZOOLOGY
Paper Name	Biology of Parasitism
Module Name/Title	*Plasmodium*: Morphology and Life Cycle
Module Id	10; Morphology and Life Cycle
Keywords	*Plasmodium*, Infection, Species, Parasite, Erythrocytic

Contents

1. Learning Outcomes

The module has been designed to make you understand:

- General aspect of *Plasmodium*
- Spectrum of *Plasmodium* infection
- Species of Malaria parasite afflict human beings
- Morphology of different stages
- Life Cycle of *Plasmodium*

2. Introduction

Plasmodium, an intracellular endoparasitic protozoan which passes on to human beings by female *Anopheles* mosquito, is responsible for causing Malaria. It is commonly known as the **malaria parasite**.

Malaria is one of the most dreaded diseases of tropical countries and remains as an epidemic in more than 100 countries (Figure 1). Deadly fevers-probably malaria-have been recorded since the beginning of the written word and references can also be found in Vedic writings in India. The word "malaria" comes from the Italian *mala aria* means **"bad air".** When the term was coined, it was commonly believed that malaria was caused by breathing in bad air or gas above marsh or swamps. In 1880, French Army physician, Charles-Louis-Alphonse Laveran discovered *Plasmodium*, the causative organism for malaria and subsequently in the year of 1897, Sir Ronald Ross discovered malaria cysts in the stomach wall of *Anopheles* mosquito. For the same he was honored the Nobel Prize in 1902. In 1898, malaria transmission through the mosquito was established by an Italian scientist Giovanni Batista Grassi. These discoveries transformed the face of malaria research.

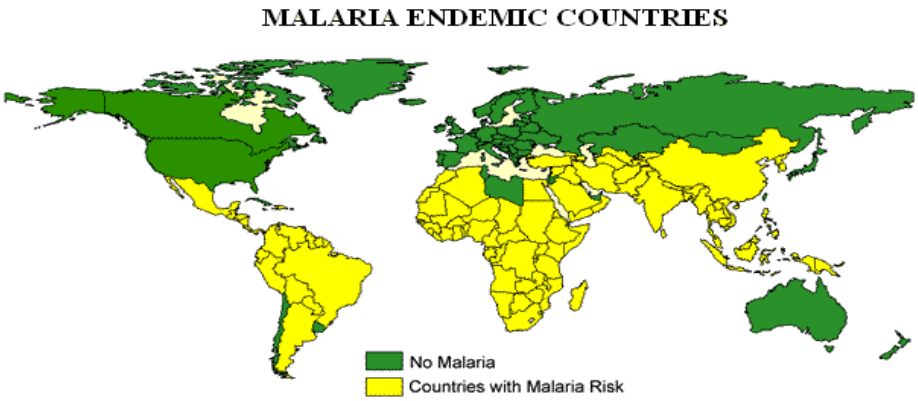


Figure 1: Global distribution of Malaria

The systematic position of malaria parasite described by Mhelhorn and Walldorf (1988) is as follows:

Kingdom	Protista
Sub Kingdom	Protozoa
Phylum	Apicomplexa
Class	Sporozoa
Sub Class	Coccidia
Order	Haemosporida
Sub Order	Aconoidina
Family	Haemosporidae
Genus	*Plasmodium*

3. Spectrum of *Plasmodium* Infection

There are more than 120 species of *Plasmodium* which are known to infect various groups of vertebrates. Few of them are as follows:

Human parasites: There are four species of *Plasmodium* which are known to infect humans; *Plasmodium falciparum, P. vivax, P. ovale* and *P. malariae*. Out of four species mentioned, *P. falciparum* is the major cause of morbidity and mortality. Recently, *P. knowlesi* has also been reported to cause infection in human beings which is known to infect monkeys.

Rodent parasites: *P. berghei, P. yoelii, P. vinckei* and *P. chabaudi* are the four species known to infect rodents which can be differentiated on the basis of their erythrocytic cycle synchronicity and preference to infect differently aged RBCs.

Simian parasites: There are twenty species of *Plasmodium* which are believed to infect monkeys or apes with a range of similar biological characteristic to that of human malaria parasites. *e.g. P.cynomolgi, P. fragile, P. fieldi, P. hylobati, P. simiovale* etc.

Avian parasites: *P. gallinaceum, P. lophurae, P. elongatum, P. relictum* and *P. fallax* are the most common avian malarial parasites. These species differ from the mammalian parasites in having different site of pre-erythrocytic schizogony. The site of pre-erythrocytic schizogony in avian parasites is mesoderm while in mammals it is liver parenchyma.

Reptilian parasites: *P. wenyoni* is the only species which infects the snakes. Malaria parasites are very common in lizards while absent in crocodiles.

3.1. Species of Malaria Parasite Afflict Human Beings

Various species of *Plasmodium* infect humans to produce different symptoms and periodicity which are as follows:

Plasmodium vivax

P. vivax causes benign tertiary malaria. Primarily, the symptom includes headache, nausea, anorexia and vomiting. Other symptoms include perspiration, shivers and very high temperature.

Plasmodium ovale

P. ovale causes ovale malaria. The symptoms are comparable to benign tertiary malaria. If left untreated, it can last for about one year.

Plasmodium malariae

P. malariae causes quaternary malaria. The bouts of temperature are of 72 hour periodicity. The symptoms are much similar to benign tertiary malaria. Untreated cases can last about 20 years.

Plasmodium falciparum

P. falciparum causes malignant tertiary malaria which is the lethal form of malaria. Symptoms are similar to flu with daily shivers, temperature, intense nausea, vomiting and diarrhoea. Crises reappear every 36 to 48 hours. In this, brain is more frequently affected. The unique property of causing agent is sequestration with endothelial wall of capillaries causing brain haemorrhage. This eventually leads to either coma or death of the infected individual. Renal lesions are found in infected individuals. Because of vomiting and diarrhoea liver is also affected which causes rapid dehydration.

4. Morphology of *Plasmodium*

The blood-stages of human *Plasmodium* species exhibit different morphology and modification in the host erythrocyte. These differences can be used to distinguish the four species (Table 1).

P. falciparum blood stages are characterized by the presence of slightly smaller and numerous ring stages than the other species. Erythrocytes having multiple infections are seen more often in *P. falciparum* than in the other species. Distinct crescent-shaped gametocytes of *P. falciparum* appear late in the infection.

P. vivax with enlarged infected erythrocytes and granules 'Schüffner's dots', over the erythrocyte cytoplasm, manifests at caveola-vesicle complexes that form on the erythrocyte membrane. The trophozoite of *P. vivax* has an ameboid appearance. The schizonts can have more than 20 merozoites.

P. ovale also exhibits Schüffner's dots with an enlarged erythrocyte. It is difficult to distinguish the infection from *P. vivax*. In general, *P. ovale* is a more compact parasite than *P. vivax*. This insistence is most evident in the growing trophozite stage. Merozoites are fewer per schizont. Elongated host erythrocytes are found in case of *P. ovale*.

P. malariae exhibit compact stages and does not modify the host erythrocyte except few elongated trophozoites which stretch across the erythrocyte to form a band like structure. Schizonts will typically have 8-10 merozoites, often arranged in a rosette pattern, with a clump of pigment in the center.

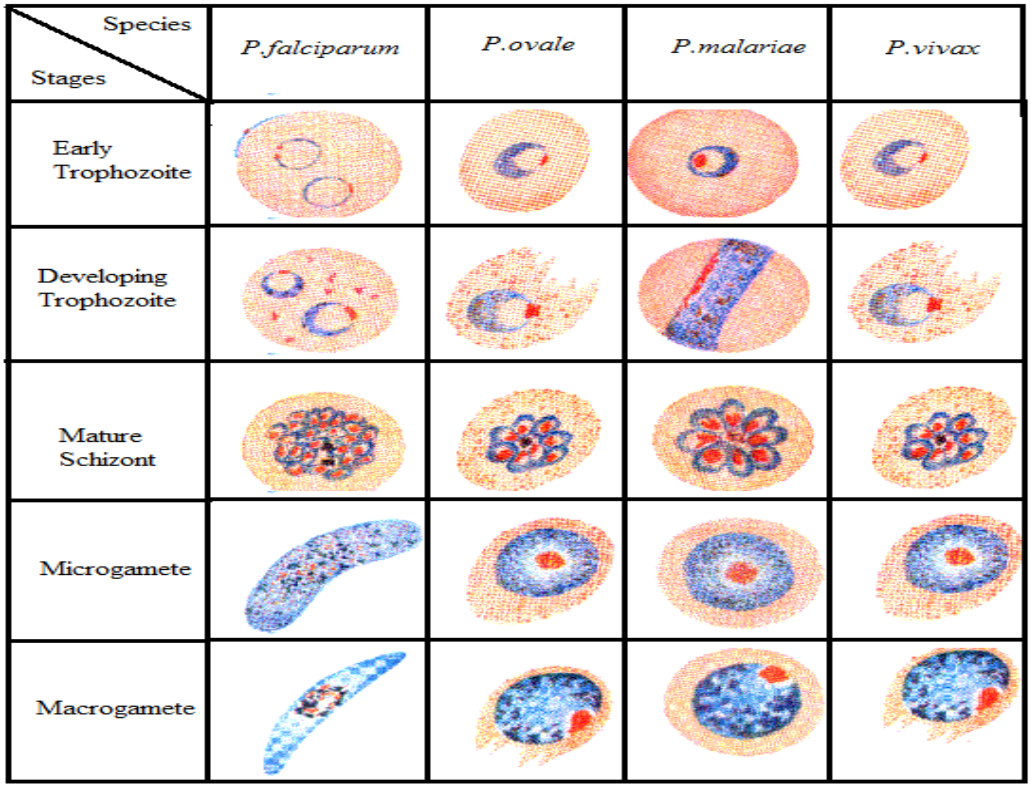
A comparative account of various stages (Table 1) and the diagrammatic figures (Table 2) clearly represents the disparity in the morphological appearance of the four *Plasmodium* species.

Table 1: Characteristics of *Plasmodium* parasites

Species	***P. falciparum***	***P. vivax***	***P. malariae***	***P. ovale***
Host Cell				
Size	Not enlarged	Enlarged	Not enlarged	Enlarged
Shape	Round	Round or oval	Round	Round or oval,
Color	Normal & may turn dark	Pale	Normal	Normal
Stippling	Large red spots Maurer's dots	Small red dots Schüffner's dots	few tiny dots Ziemann's dots	Numerous small red dots James's dots
Pigment	Usually black or dark brown	Golden brown granules	Black or brown coarse granules	As of *P. malariae*
Parasite				
General features	Small, compact dark, staining parasite. Multiple infections of single RBC	Large light staining amoeboid parasite. Many trophozoites	Regular shape Strong tendency to form a band across the infected RBC	Regular shape. Size in between *P. vivax* and *P. malariae*
Common Stages found in smear	Only rings and gametocytes	Trophozoites, Schizonts, Gametocytes	As in *P. vivax*	As in *P. vivax*
Ring stage	Delicate, small, 1.5 µm Double chromatin and multiple rings common.	Large 2.5 µm, usually single. Prominent thicker chromatin	Similar to *P. vivax* but thicker	Similar to *P. vivax*, but compact
Trophozoite	Compact, small, vacuole inconspicuous, seldom seen in smear	Large, irregular vacuole prominent Chromatin as dots or threads	Characteristic band form, vacuole inconspicuous	Compact rough pigment, large irregular clumps of chromatin
Schizont	Small, compact rarely seen in	Large, filling the RBC, segmented,	Nearly fills RBC, segmented,	Fills three fourth of RBC, segmented, pigment dark yellow

	blood smear	yellow brown pigment	pigment is dark brown	brown
Micro-gametocyte	Larger than RBC, kidney shaped with blunt round ends, cytoplasm reddish blue, many fine granules in smear	Fills enlarged RBC, round or oval, compact cytoplasm, pale blue, profuse brown granules	Smaller than RBC, very few in peripheral blood film, round compact. Pale blue cytoplasm. Pigment and chromatin as in *P. vivax*	Same of RBC, round, compact very few in peripheral blood film, cytoplasm pale blue, chromatin and pigment as in *P. vivax*
Macro-gametocyte	Slender, nucleus small, compact, pigment granules closely aggregated	Large, loose and ill-defined mass of chromatin and smaller mass	Same as *P.vivax*, low numbers appear after 12–14 days.	Same as *P.vivax*, low numbers appear after 12–14 days.

Table 2: Diagrammatic illustration of the morphology of the different stages of the *Plasmodium sps.* life cycle in thin blood films



Source: http://www.phsource.us/PH/PARA/Diagnosing_Medical_Parasites.pdf

Structure of *Plasmodium* Merozoite

Merozoite is an ovoid cell and measures approximately 1.5 micron in length and 1 micron in width. The apical end is like truncated cone-shaped projection demarcated by the polar rings. Three types of membrane-bound organelles, Rhoptries (two prominent pear-shaped), micronemes (ovoid bodies) and dense granules (spheroid vesicles) are present at the anterior end of the Merozoite (Figure 2). The function of these organelles is related to the binding and entry of the Merozoite into the host cells. Merozoites are basically short-lived and need to invade a new host erythrocyte just after the release. A trilaminar pellicle surrounds the merozoite, which is composed of a plasma membrane and two closely aligned inner membranes. Beneath this inner membrane complex is a row of subpellicular Microtubules, radiating posteriorly from the polar ring of the apical end. The inner membrane complex and subpellicular Microtubules function as a cytoskeleton giving rigidity to the Merozoite. Mitochondrion are generally acristae or with very few cristae. The 'apicoplast' (plastid) is believed to be the evolutionary homologue of the plant chloroplast. A single vesicular nucleus with a centrally located nucleolus is also present in Merozoite.

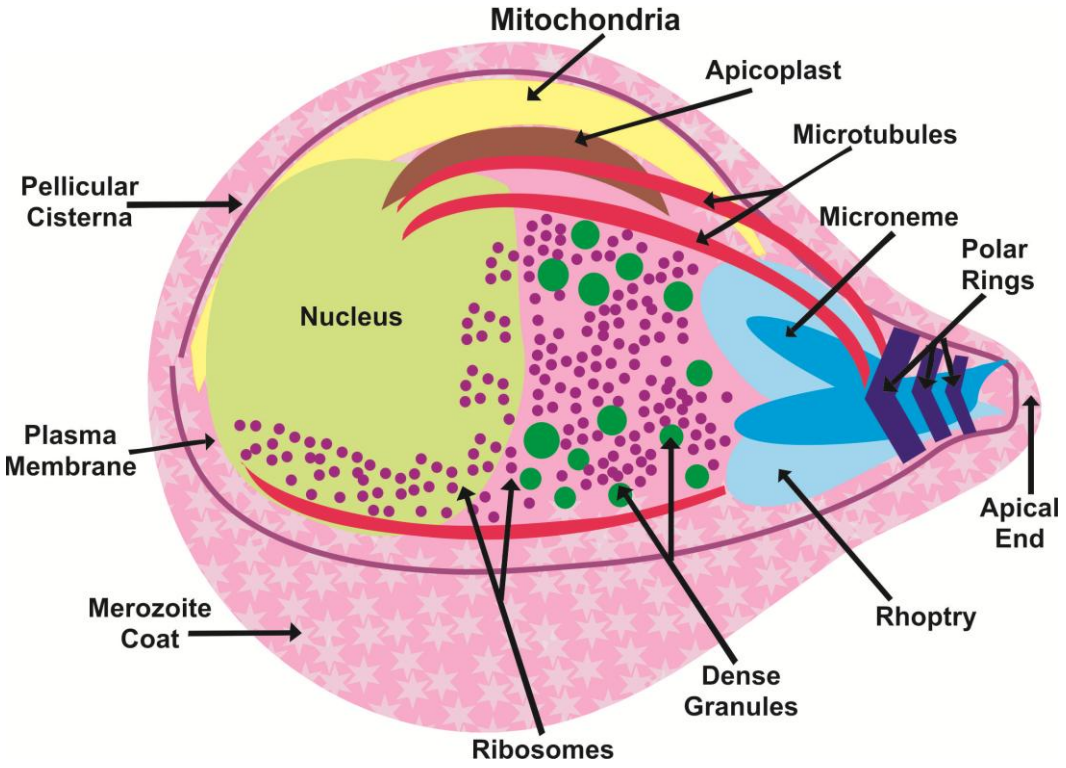


Figure 2: Merozoite of *Plasmodium*

The Infective Stage: Sporozoite

The most versatile of the invasive stages of the *Plasmodium* life cycle are the Sporozoites. During their passage from the mosquito vector to the vertebrate host, sporozoites exhibit diverse behaviors,

including gliding locomotion, invasion, migration and egress from target cells. These functions are performed by rhoptries and micronemes, which are the only secretory organelles around the apical cap (Figure 3). Finally the sporozoites invade hepatocytes and transform into exoerythrocytic stages, continuing the cycle to the erythrocytic part in RBC.

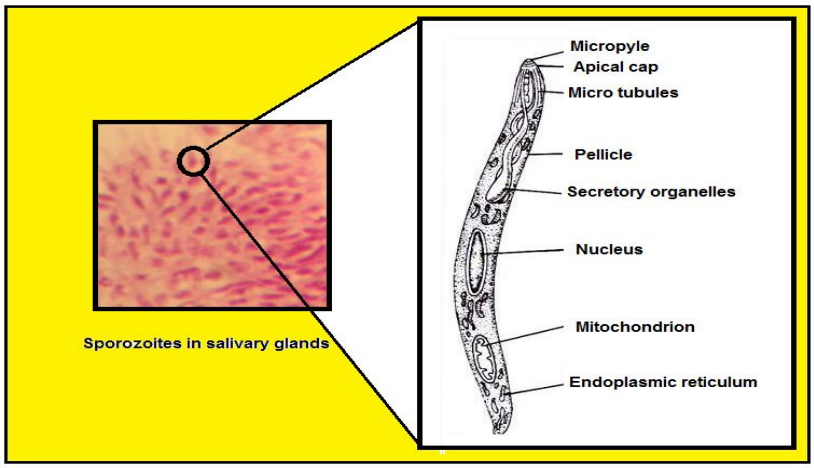


Figure 3: Typical structure of sporozoite of *Plasmodium*
Source: http://www.kullabs.com

5. Life Cycle of the Malaria Parasite

The life cycle of malaria parasite is very complex. It's a digenetic parasite, i.e. it requires two hosts to complete its cycle (Figure 4). The life cycle of all species of human malaria parasites is essentially the same. It comprises an exogenous sexual phase (sporogony) with multiplication in certain Anopheles mosquitoes and an endogenous asexual phase (schizogony) with multiplication in the vertebrate host. The latter phase includes the development cycle in the red cells (erythrocytic schizogony) and the phase taking place in the parenchyma cells in the liver (pre-erythrocytic schizogony). The vertebrate act as the intermediate (secondary) host for the parasite, while the mosquito is considered to be the definitive (primary) one as the sexual reproduction takes place in mosquito.

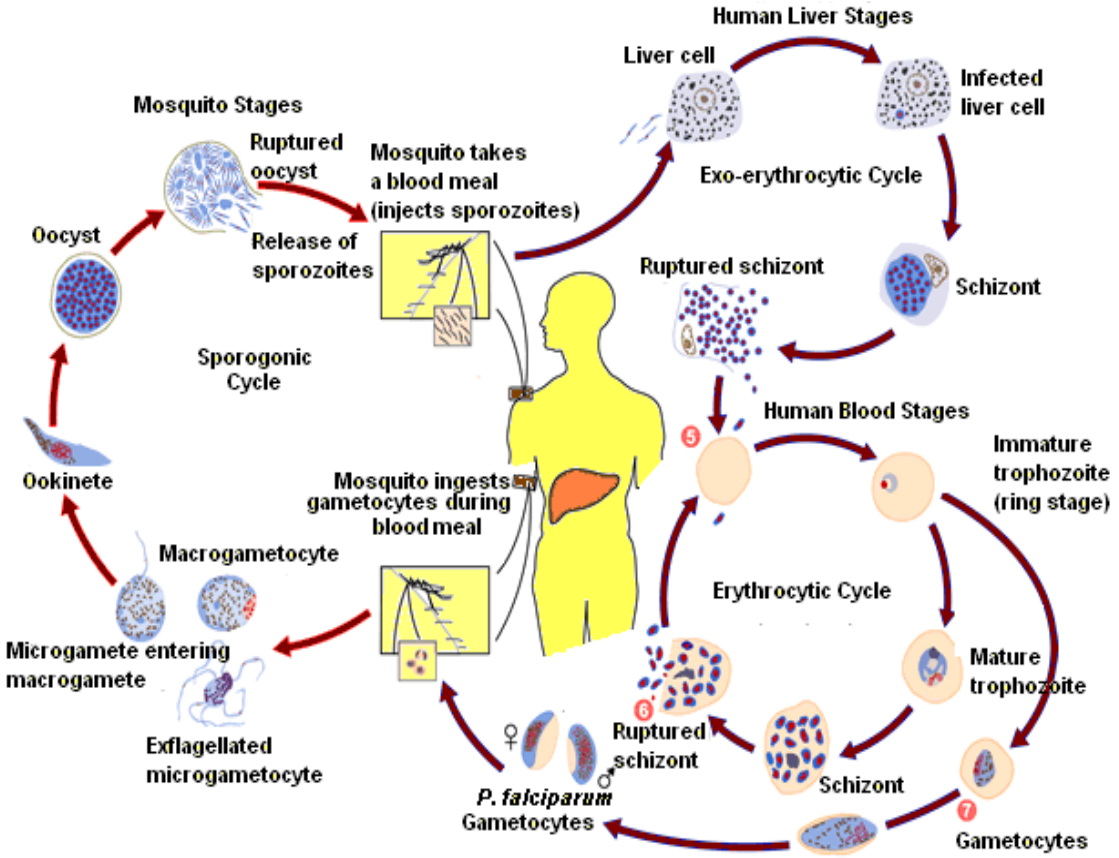


Figure 4: Life cycle of malaria parasite (*P. falciparum*)
Source: http://www.cdc.gov/parasites

5.1. Exo-Erythrocytic Stages of Human Malaria Parasites

Sporozoites are the infective stage. Malaria infection in the human starts with their injection into the blood stream during a blood meal by an infectious mosquito. The circulatory period of sporozoites is short for about 60 minutes, after which they actively enter the liver of the host. The sporozoites die shortly after invasion in the Kuppfer cells of liver, as these are resistant to sporozoites. Most of the sporozoites enter the hepatocytes to begin the asexual exo-erythrocytic schizogonic cycle. The liver trophozoite, a mononucleated round body, divides asexually to form a mature multinucleated schizont which finally releases a large number of merozoites. The number of merozoites produced at the end of the cycle is also dependent on the species of the parasite. The merozoites are released into the sinusoids of the liver by the rupture of liver schizonts. Released merozoites invade red blood cells. Two species of human malaria parasite show relapses are *P. vivax* and *P. ovale* where some of the liver trophozoites immediately start the exo-erythrocytic schizogony while others remain in a dormant stage and are termed as hypnozoites.

5.2. Erythrocytic Stages of Human Malaria Parasites

The intricate and varying spectrum of symptoms characterizing the disease in humans is due to Erythrocytic stages of malaria parasite. The recognition of parasites in the blood of a patient allows the diagnosis of the infection and the differentiation of the various species. The time required to complete the erythrocytic cycle is a fixed characteristic of the parasite species (Table 3). Merozoites initiate the blood phase of the life-cycle on rupture and discharge of liver schizonts into the circulation. The merozoites possess a single nucleus and adjacent cytoplasm with a diameter of 1 µm. It invades immediately an erythrocyte to develop in trophozoite stage. The young trophozoite feeds on erythrocyte, produces a vacuole which assumes the characteristic ring form. This stage is referred to as **signet ring stage**. Approximately in 18 hours expansion of cytoplasm and disappearance of vacuole takes place slowly, and there is an appearance of a characteristic parasitic pigment within the cytoplasm. Pigment, known as **haemozoin** (Yellowish brown malarial pigment, haemozoin), is formed due to the parasite ingestion of haemoglobin and decomposition of the same into protein and haematin. Protein is used as food whereas unused haematin forms toxic.

Mature trophozoite has a single nucleus, a large cytoplasm without vacuole and inconsistent amount of pigment. *P. falciparum*, *P. vivax* and *P. ovale* takes approximately 30 hours to start nuclear division after invasion while *P. malariae* takes approximately 40 hours. Nuclear division leads to the production of the schizont stage. Nuclear division continues until an appropriate number of merozoites are produced (Table 3).

Table 3: Characteristic features of four species of human *Plasmodia*

Species	***P. vivax***	***P. ovale***	***P. malariae***	***P. falciparum***
Pre-erythrocytic cycle (days)	8	9	13	5-6
Pre-patent period (days)	11-13	10-14	15-16	9-10
Incubation period (days)	13	17	28	12
Number of merozoites per tissue schizont	10,000	15,000	2,000	40,000
Hypnozoites	present	present	absent	absent
Erythrocytic cycle (hours)	48	50	72	48

A typical malaria paroxysm is determined by erythrocytic rupture to release the merozoites into the blood stream. Merozoites released into the circulation invade new erythrocytes to repeat the schizogony until the process is inhibited by the specific immune response or by chemotherapy. Some of the merozoites differentiate into sexual forms (the gametocytes) in the course of erythrocytic schizogony which remain inside the RBCs (Figure 4.). Gametocytes become visible approximately from the third generation in case of *P. vivax*, *P. ovale*, and *P. malariae* while in case of *P. falciparum* it requires approximately 10 generations for appearance of gametocytes which probably reflects the slow maturation and the sequestration of the immature stages in this species. Gametocytes of *P. vivax*, *P. ovale* and *P. malariae* are

morphologically similar to the late trophozoite while *P. falciparum* gametocytes present a crescent shape. In the peripheral blood two types of gametocytes, the female macro-gametocytes and the male micro-gametocytes are present. They can be differentiated on the basis of nuclear material which is dispersed in male parasite (preparing to ex-flagellation) while condensed in female parasite.

5.3. Sexual Cycle in the Mosquito

Protein, required for egg formation of female *Anopheles* mosquitoes, comes through the blood meal. Oviposition continues throughout the life of the female mosquito which requires repeated contacts with the vertebrate host for blood meal. These subsequent feedings allow malaria parasite's multiplication, maturation and transmission to other individuals. The sexual cycle starts with the ingestion of mature female and male gametocytes by a suitable species of *Anopheles* during a blood meal. In the midgut of insect, the female gametocytes get rid of the cover of red blood cell to remain free in the extra-cellular space as macrogamete. The male gametocyte nucleus divides into eight sperm like flagellated micro-gametes through ex-flagellation which arrive at midgut and actively move to fertilize a macrogamete. After fertilization a zygote is formed, which develops into slowly motile ookinete in approximately 15 hours. The ookinete ruptures the peritrophic membrane and the epithelium of the midgut to inhabit below the basal lamina of the outer gut wall. In 24-72 hours after the blood meal the ookinete develops into a non motile oocyst. Narrow and curved sporozoites are produced from oocyst on maturation which are actively motile and about 15 µm in length. The sporozoites migrate and reach the salivary glands by making small perforations in the wall of cyst. Sporozoites penetrate the basal membrane of salivary gland and settle into the salivary duct. During the mosquito blood feeding, the salivary fluid content (which has anti-clotting properties) with sporozoites are actively injected into the human host to start another asexual cycle. Mosquito, a poikilothermic host, affect the speed of the cycle since it strongly depends on the temperature and other climatic factors.

6. Summary

Plasmodium is an intracellular endoparasitic protozoan and causes Malaria. Malaria is one of the most dreaded diseases of tropical countries. There are more than 120 species of *Plasmodium* which are known to infect various groups of vertebrates. Among those, *Plasmodium falciparum*, *P. vivax*, *P. ovale* and *P. malariae* can infect human beings. The malignant tertiary malaria caused by *P. falciparum* is the lethal one. The blood-stages of human *Plasmodium* species exhibit different morphology and modification in the host erythrocyte. A fifth species, *Plasmodium knowlesi*, has recently been identified as a clinically significant pathogen in humans. This is an emerging infection in South East Asia. Merozoite is an ovoid cell with an apical truncated cone-shaped projection demarcated by the polar rings. Three types of membrane-bound organelles, Rhoptries (two prominent pear-shaped), micronemes (ovoid bodies) and dense granules (spheroid vesicles) are present at the anterior end of the Merozoite. The function of these organelles is related to the binding and entry of the Merozoite into the host cells. The most versatile and infective stage of the *Plasmodium* life cycle

is the Sporozoite. The life cycle of malaria parasite is very complex. It's a digenetic parasite, i.e. it requires two hosts to complete its cycle. It comprises an exogenous sexual phase (sporogony) with multiplication in certain Anopheles mosquitoes and an endogenous asexual phase (schizogony) with multiplication in the vertebrate host. The vertebrate act as the intermediate (secondary) host for the parasite, while the mosquito is considered to be the definitive (primary) one as the sexual reproduction takes place in mosquito. Two species of parasite *P. vivax* and *P. ovale* show relapses where dormant stage, hypnozoites, are present. Erythrocytic stages include signet ring stage, mature trophozoite (feeding stage) and schizonts. The female macro-gametocytes and the male micro-gametocytes can be differentiated on the basis of nuclear material which is dispersed in male parasite (preparing to ex-flagellation in mosquito) while condensed in female parasite. Mosquito, a poikilothermic host, affect the speed of the cycle since it strongly depends on the temperature and other climatic factors. Sporozoites, infective stage, find way to get accumulated in the salivary gland of *Anopheles* mosquito for next cycle in human.