Paniker's Textbook of MEDICAL PARASITOLOGY

Revised & Edited by Sougata Ghosh

Foreword Jagdish Chander
Paniker’s Textbook of
MEDICAL PARASITOLOGY

EIGHTH EDITION

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Foreword
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The Health Sciences Publisher
New Delhi | London | Panama
HOW THIS BOOK IS USEFUL?

FEATURES

• Written as per the latest curriculum of MBBS and MD (Microbiology).
• Rearranges all the chapters in a sequential format and thoroughly updated and expanded to include new data.
• Adds the recent advances in each topic such as vaccine trials in Leishmania and Malaria, drug resistance in malaria. The new and fresh look reinvigorates the reading experience.
• Includes many new photographs, tables and flow charts.
• Rewrites the laboratory diagnosis, treatment protocols and prophylaxis completely.
• Describes important concepts for the preparations of postgraduate examination in separate boxes.
• Presents Key Points in a box format at the end of each chapter for last-minute revision.
• Arranges the whole content of the book in a bulleted format and uses subheads for increased readability.
This is a great pleasure to write the foreword to the eighth edition of Paniker’s Textbook of Medical Parasitology dealing with medically important parasites vis-a-vis human diseases caused by them.

The parasitic infections (protozoal and helminthic) are still major cause of high morbidity as well as mortality of substantial number of population residing in the developing world of tropical and subtropical regions. The clinical presentations of parasitic diseases have also significantly evolved with the passage of time. Malaria caused by *Plasmodium vivax* has never been life-threatening but now it is presenting with renal failure as well as acute respiratory distress syndrome (ARDS) thereby leading to fatal consequences. On the other hand, some of the infections such as dracunculiasis have been eradicated from India and others are the next targets being in the pipeline.

There are a number of novel diagnostic techniques, which are being designed for rapid diagnosis of various parasitic diseases and accurate identification of their causative pathogens. The non-invasive imaging techniques, both MRI and CT scans, are proving to be very useful tools for an early diagnosis thereby delineating the extent of disease in a particular patient. Therefore, to cope up with the changing epidemiological scenario and newer diagnostic modalities, medical students and professionals involved in the patient care need updates from time to time. Dr Sougata Ghosh (Editor), has done a remarkable job of going through the voluminous information and presenting it in a very lucid, concise and reproducible manner.

This edition will ideally be suited for medical students and resident doctors, who are preparing for various examinations and entrance tests. I feel the present edition will also be appreciated by students and teaching faculties in all disciplines of medicine. The chapter on pneumocystosis has been removed, however, on sporoza dealing with diseases caused by different species of microsporidia, traditionally retained in this edition, despite the fact that it has also been shifted now to the kingdom fungi like *Pneumocystis jirovecii*.

The unique feature of the textbook is that it has many illustrations, photographs of clinical specimens and photomicrographs with an easy-to-read and understand format. This will help the students to memorize the information given in the text easily as well as to use the same in medical practice. Each chapter has key points with a set of multiple choice questions (MCQs), which will help a student for better understanding and preparation before the examination. Although it is meant for medical graduates, recent advances mentioned in this book will also be useful for the postgraduates.

The original author, Professor CK Jayaram Paniker, was an experienced and enthusiastic medical teacher, and we recently lost him. Moreover, he was a legendary microbiologist and the author of numerous valuable textbooks, particularly co-author of *Ananthanarayan’s Textbook of Microbiology*. His name has been retained as such in the title of the eighth edition of this textbook is a great honor and real tribute to him thereby continuing his legacy to attain more heights in the field of medical parasitology even in his physical absence. I hope that this textbook will continue to benefit the medical students and faculties for many years as it has done during the last three decades.

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The previous editions of Paniker’s Textbook of Medical Parasitology have been widely accepted by the medical students and teachers across India and abroad for almost three decades.

Medical science is not a static art. Methods of diagnosis and treatment of parasitic infections change constantly. To keep pace with these developments, all the chapters of present edition have been thoroughly revised and expanded, providing up-to-date epidemiological data, new diagnostic methods and recent treatment guidelines of parasitic infections.

In the current edition, many new tables, flow charts and photographs of specimens and microscopic view pictures have been added for better comprehension of the subject.

Recent advances such as vaccinology of malaria and leishmaniasis, malarial drug resistance, new treatment protocols of different parasitic infections are the salient features of the book.

The aim of the contents of the book remains same in this edition, that is compact yet informative and useful for both graduate and postgraduate students.

Like the last edition, the present edition is also designed in a colorful format, which can be easily read and comprehended. Important points and terms have been highlighted by making them bold and italic. At the end of each chapter, the must-know facts are given as “Key Points” in box formats for quick recapitulation.

Important multiple choice questions (MCQs) and review questions from various university examinations’ papers have been added to test and reinforce understanding of the topics by the students.

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MALARIA

INTRODUCTION
Protozoan parasites characterized by the production of spore-like oocysts containing sporozoites were known as sporozoa.
- They live intracellularly, at least during part of their life cycle.
- At some stages in their life cycle, they possess a structure called the apical complex, by means of which they attach to and penetrate host cells.
- These protozoa are therefore grouped under the Phylum Apicomplexa.
- The medically important parasites in this group are the malaria parasites, Coccidia, and Babesia.
- The Phylum Apicomplexa includes two classes viz. (1) hematozoa and (2) coccidia and three orders—(1) eimeriida, (2) hemosporida and (3) piroplasmida (Table 1).

Note: Many minute intracellular protozoa formerly grouped as sporozoa have been reclassified because of some structural differences. These are now called microspora. They infect a large spectrum of hosts including vertebrates and invertebrates. Infection is mostly asymptomatic, but clinical illness is often seen in the immunodeficient.

CLASSIFICATION
Malaria parasite belongs to:
Phylum: Apicomplexa
Class: Sporozoa
Order: Hemosporida
Genus: Plasmodium.
- The genus Plasmodium is classified into two subgenera: (1) P. vivax, (2) P. malariae and P. ovale belong to the subgenus Plasmodium while P. falciparum belongs to subgenus Laverania because it differs in a number of aspects from the other three species.
- P. vivax, P. malariae and P. ovale are closely related to other primate malaria parasites. P. falciparum is more related to bird malaria parasites and appears to be a recent parasite of humans, in evolutionary terms. Perhaps for this reason, falciparum infection causes the most severe form of malaria and is responsible for nearly all fatal cases.
- P. knowlesi, a parasite of long-tailed Macaque monkeys may also affect man.

CAUSATIVE AGENTS OF HUMAN MALARIA
- Plasmodium vivax: Benign tertian malaria
- Plasmodium falciparum: Malignant tertian malaria
- Plasmodium malariae: Benign quartan malaria
- Plasmodium ovale: Benign tertian malaria.

MALARIA PARASITE
History and Distribution
Malaria has been known from ancient times. Seasonal intermittent fevers with chills and shivering, recorded in the religious and medical texts of ancient Indian, Chinese and Assyrian civilizations, are believed to have been malaria (Fig. 1).
Malaria and Babesia

The name malaria (mal: bad, aria: air) was given in the 18th century in Italy, as it was thought to be caused by foul emissions from marshy soil.

The specific agent of malaria was discovered in red blood cells (RBCs) of a patient in 1880 by Alphonse Laveran, a French army surgeon in Algeria.

In 1886, Golgi in Italy described the asexual development of the parasite in RBCs (erythrocytic schizogony), which therefore came to be called as Golgi cycle.

Three different species of malaria parasite infecting man: (1) *P. vivax*, (2) *P. malariae*, and (3) *P. falciparum* were described in Italy between 1886 and 1890. The fourth species, *P. ovale* was identified only in 1922.

The mode of transmission of the disease was established in 1897, when Ronald Ross in Secunderabad, India identified the developing stages of malaria parasites in mosquitoes. This led to various measures for the control and possible eradication of malaria by mosquito control. Both Ross (1902) and Laveran (1907) won the Nobel Prize for their discoveries in malaria.

Incidence of malaria is more in poor population in rural areas, also in urban areas having bad sanitary condition. An epidemic can develop when there are changes in environmental, economic and social conditions such as migrations and heavy rains following draughts.

The relative prevalence of the four species of malaria parasites varies in different geographical regions (Fig. 1):

1. *P. vivax* is the most widely distributed, being most common in Asia, North Africa, and Central and South America.
2. *P. falciparum*, the predominant species in Africa, Papua New Guinea and Haiti, is rapidly spreading in Southeast Asia and India.
3. *P. malariae* is present in most places but is rare, except in Africa.
4. *P. ovale* is virtually confined to West Africa where it ranks second after *P. falciparum* (Fig. 1).

Malaria parasite passes its life cycle in two hosts:

1. **Definitive host**: Female *Anopheles* mosquito.
2. **Intermediate host**: Man.

- The life cycle of malarial parasite comprises of two stages—(1) *an asexual phase* occurring in humans, which act as the intermediate host and (2) *a sexual phase* occurring in mosquito, which serves as a definitive host for the parasite (Fig. 2).

### Asexual Phase

- In this stage, the malaria parasite multiplies by division or splitting a process designated to as *schizogony* (from schizo: to split, and gone: generation).

### Vectors

Human malaria is transmitted by over 60 species of female *Anopheles* mosquito.

- The male mosquito feeds exclusively on fruits and juices, but the female needs at least two blood meals, before the first batch of eggs can be laid.
- Out of 45 species of *Anopheles* mosquito in India, only few are regarded as the vectors of malaria. These are *An. culicifacies*, *An. fluviatilis*, *An. stephensi*, *An. minimus*, *An. philippinensis*, *An. sundaicus*, etc.
Because this asexual phase occurs in man, it is also called the vertebrate, intrinsic, or endogenous phase.

In humans, schizogony occurs in two locations—(1) in the red blood cell (erythrocytic schizogony) and (2) in the liver cells (exoerythrocytic schizogony or the tissue phase).

Because schizogony in the liver is an essential step before the parasites can invade erythrocytes, it is called pre-erythrocytic schizogony.

The products of schizogony, whether erythrocytic or exoerythrocytic, are called merozoites (meros: a part, zoon: animal).

**Sexual Phase**

- Female *Anopheles* mosquito represents definitive host, in which sexual forms takes place. Although the sexual forms of the parasite (gametocytes) originate in human RBCs.
- Maturation and fertilization take place in the mosquito, giving rise to a large number of sporozoites (from sporos: seed). Hence, this phase of sexual multiplication is called sporogony. It is also called the invertebrate, extrinsic, or exogenous phase.

Thus, there is an alternation of hosts as the asexual phase takes place in humans followed by sexual phase in mosquito.

**Human Cycle (Schizogony)**

Human infection comes through the bite of the infective female *Anopheles* mosquito (Fig. 2).

- The sporozoites, which are infective forms of the parasite are present in the salivary gland of the mosquito.
- They are injected into blood capillaries when the mosquito feeds on blood after piercing the skin.
- Usually, 10–15 sporozoites are injected at a time, but occasionally, many hundreds may be introduced.
- The sporozoites pass into the bloodstream, where many are destroyed by the phagocytes, but some reach the liver and enter the parenchymal cells (hepatocytes).
Pre-erythrocytic (tissue) stage or exoerythrocytic stage: Within an hour of being injected into the body by the mosquito, the sporozoites reach the liver and enter the hepatocytes to initiate the stage of pre-erythrocytic schizogony or merogony.

- The sporozoites, which are elongated spindle-shaped bodies, become rounded inside the liver cells.
- They enlarge in size and undergo repeated nuclear division to form several daughter nuclei; each of which is surrounded by cytoplasm.
- This stage of the parasite is called the pre-erythrocytic orexoerythrocytic schizont or meront.

- The hepatocyte is distended by the enlarging schizont and the liver cell nucleus is pushed to the periphery.
- Mature liver stage schizonts are spherical (45–60 µm), multinucleate and contain 2,000–50,000 uninucleate merozoites.
- Unlike erythrocytic schizogony, there is no pigment in liver schizonts. These normally rupture in 6–15 days and release thousands of merozoites into the bloodstream.
- The merozoites infect the erythrocytes by a process of invagination.

Prepatent period: The interval between the entry of the sporozoites into the body and the first appearance of the parasites in blood is called the prepatent period.

- The duration of the pre-erythrocytic phase in the liver, the size of the mature schizont and the number of merozoites produced vary with the species of the parasite (Table 2).
- Latent stage: In P. vivax and P. ovale, two kinds of sporozoites are seen, some of which multiply inside hepatic cells to form schizonts and others persist and remain dormant (resting phase).

Relapse: The resting forms are called hypnozoites (hypnos: sleep). From time to time, some are activated to become schizonts and release merozoites, which go on infecting RBCs producing clinical relapse.

Recrudescence: In P. falciparum and P. malariae, initial tissue phase disappears completely, and no hypnozoites are found. However, small numbers of erythrocytic parasites persist in the bloodstream and in due course of time, they multiply to reach significant numbers resulting in clinical disease (short-term relapse or recrudescence).

Erythrocytic stage: The merozoites released by pre-erythrocytic schizonts invade the RBCs.

- The receptor for merozoites is glycophorin, which is a major glycoprotein on the red cells. The differences in the glycophorins of red cells of different species may account for the species specificity of malaria parasites.

- Merozoites are pear-shaped bodies, about 1.5 µm in length, possessing an apical complex (rhoptery). They attach to the erythrocytes by their apex and then the merozoites lie within an intraerythrocytic parasitophorous vacuole formed by red cell membrane by a process of invagination.

In the erythrocyte, the merozoite loses its internal organelles and appears as a rounded body having a vacuole in the center with the cytoplasm pushed to the periphery and the nucleus at one pole. These young parasites are, therefore called the ring forms or young trophozoites.

- The parasite feeds on the hemoglobin of the erythrocyte. It does not metabolize hemoglobin completely and therefore, leaves behind a hematin-globin pigment called the malaria pigment or hemozoin pigment, as residue (Box 1).

- The malaria pigment released when the parasitized cells rupture is taken up by reticuloendothelial cells. Such pigment-laden cells in the internal organs provide histological evidence of previous malaria infection.

- As the ring form develops, it enlarges in size becoming irregular in shape and shows ameboid motility. This is called the ameboid form or late trophozoite form.

- When the ameboid form reaches a certain stage of development, its nucleus starts dividing by mitosis followed by a division of cytoplasm to become mature schizonts or meronts.

- A mature, schizont contains 8–32 merozoites and hemozoin. The mature schizont bursts releasing the merozoites into the circulation.

- The merozoites invade fresh erythrocytes within which they go through the same process of development. This cycle of erythrocytic schizogony or merogony is repeated sequentially, leading to progressive increase in the parasitemia, till it is arrested by the development of host immune response.

### Table 2: Features of pre-erythrocytic schizogony in human malaria parasites

<table>
<thead>
<tr>
<th></th>
<th>P. vivax</th>
<th>P. falciparum</th>
<th>P. malariae</th>
<th>P. ovale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-erythrocytic stage (days)</td>
<td>8</td>
<td>6</td>
<td>15</td>
<td>9</td>
</tr>
<tr>
<td>Diameter of pre-erythrocytic schizont (µm)</td>
<td>45</td>
<td>60</td>
<td>55</td>
<td>60</td>
</tr>
<tr>
<td>No. of merozoites in pre-erythrocytic schizont</td>
<td>10,000</td>
<td>30,000</td>
<td>15,000</td>
<td>15,000</td>
</tr>
</tbody>
</table>

### Box 1: Appearance of malaria pigments in different species

- **P. vivax:** Numerous fine gold-brown dust-like particles
- **P. falciparum:** Few 1–3 solid blocks of black pigment
- **P. malariae:** Numerous coarse dark-brown particles
- **P. ovale:** Numerous blackish-brown particles.
The rupture of the mature schizont releases large quantities of pyrogens. This is responsible for the febrile paroxysms characterizing malaria.

The interval between the entry of sporozoites into the host and the earliest manifestation of clinical illness is the **incubation period** (Box 4). This is different from **prepatent period**, which is the time taken from entry of the sporozoites to the first appearance of malaria parasite in peripheral blood.

In *P. falciparum*, erythrocytic schizogony always takes place inside the capillaries and vascular beds of internal organs. Therefore, in *P. falciparum* infections, schizonts and merozoites are usually not seen in the peripheral blood.

The erythrocytic stages of all the four species of *Plasmodium* are shown in **Figure 3**.

<table>
<thead>
<tr>
<th></th>
<th><em>P. vivax</em></th>
<th><em>P. falciparum</em></th>
<th><em>P. malariae</em></th>
<th><em>P. ovale</em></th>
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<td><strong>Schizonts</strong></td>
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<td><strong>Mature</strong></td>
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<td><strong>Gametocytes</strong></td>
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</table>

**Fig. 3**: Malaria parasites—Erythrocytic stages of the four species (Giemsa stain. Magnification 2000X)
Gametogony

After a few erythrocytic cycles, some of the merozoites that infect RBCs do not proceed to become trophozoites or schizonts but instead, develop into sexually differentiated forms, the gametocytes.

- They grow in size till they almost fill the RBC, but the nucleus remains undivided.
- Development of gametocytes generally takes place within the internal organs and only the mature forms appear in circulation.
- The mature gametocytes are round in shape, except in *P. falciparum*, in which they are crescent-shaped.
- In all species, the female gametocyte is larger (macrogametocyte) and has cytoplasm staining dark blue with a compact nucleus staining deep red. In the smaller male gametocyte (microgametocyte), the cytoplasm stains pale blue or pink and the nucleus is larger, pale stained and diffuse. Pigment granules are prominent.
- Female gametocytes are generally more numerous than the male.
- Gametocyte appears in circulation 4–5 days after the first appearance of asexual form in case of *P. vivax* and 10–12 days in *P. falciparum*.
- A person with gametocytes in blood is a carrier or reservoir.
- The gametocytes do not cause any clinical illness in the host, but are essential for transmission of the infection.
- A gametocyte concentration of 12 or more per mm$^3$ of blood in the human host is necessary for mosquitoes to become infected.

The Mosquito Cycle (Sporogony)

When a female *Anopheles* mosquito ingests parasitized erythrocytes along with its blood meal, the asexual forms of malaria parasite are digested, but the gametocytes are set free in the midgut (stomach) of mosquito and undergo further development.

- The nuclear material and cytoplasm of the male gametocytes divides to produce eight microgametes with long, actively motile, whip-like filaments (exflagellating male gametocytes) (Fig. 4).
- At 25°C, the exflagellation is complete in 15 minutes for *P. vivax* and 15–30 minutes for *P. falciparum*.
- The female gametocyte does not divide but undergoes a process of maturation to become the female gamete or macrogamete. It is fertilized by one of the microgametes to produce the zygote (Fig. 4).
- Fertilization occurs in 0.5–2 hours after the blood meal. The zygote, which is initially a motionless round body, gradually elongates and within 18–24 hours, becomes a vermicular motile form with an apical complex anteriorly. This is called the ookinete (travelling vermicule).
- It penetrates the epithelial lining of the mosquito stomach wall and comes to lie just beneath the basement membrane.
- It becomes rounded into a sphere with an elastic membrane. This stage is called the oocyst, which is yet another multiplicative phase, within which numerous sporozoites are formed.
- The mature oocyst, which may be about 500 µm in size, bulges into body cavity of mosquito and when it ruptures, the sporozoites enter into the hemocoel or body cavity, from where some sporozoites move to the salivary glands.
- The mosquito is now infective and when it feeds on humans, the sporozoites are injected into skin capillaries to initiate human infection.

Extrinsic incubation period: The time taken for completion of sporogony in the mosquito is about 1–4 weeks (extrinsic incubation period), depending on the environmental temperature and the species.

Types of Malarial Parasites

*Plasmodium Vivax*

*P. vivax* has the widest geographical distribution, extending through the tropics, subtropics and temperate regions. It is believed to account for 80% of all malaria infections. It is the most common species of malaria parasite in Asia and America, but is much less common in Africa. It causes benign tertian malaria with frequent relapses.

- The sporozoites of *P. vivax* are narrow and slightly curved. On entering the liver cells, the sporozoites initiate two types of infection. Some develop promptly into exoerythrocytic schizonts, while others persist in the dormant state for varying periods as hypnozoites. There may be two distinct types of sporozoites: (1) the tachysporozoites (tachy: fast), which develops into the primary exoerythrocytic schizont and (2) the bradysporozoite (brady: slow) which becomes the hypnozoite.
- The pre-erythrocytic schizogony lasts for 8 days and the average number of merozoites per tissue schizont is 10,000.
- Merozoites of *P. vivax* preferentially infect reticulocytes and young erythrocytes.
- All stages of erythrocytic schizogony can be seen in peripheral smears (Fig. 5).
- The degree of parasitization is not generally heavy, each infected red cell usually having only one trophozoite and not more than 2–5% of the red cells being affected. Reticulocytes are preferentially infected.
- The trophozoite is actively motile, as indicated by its name *vivax*. The ring form is well-defined, with a prominent central vacuole. One side of the ring is thicker and the other side thin. Nucleus is situated on the thin side of the ring (Signet ring appearance). The ring is about 2.5–3 µm in diameter, about a third of the size of an erythrocyte. The cytoplasm is blue and the nucleus red in stained films. The ring develops rapidly to the ameboid form and accumulates malarial pigment (Figs 6 and 7).
- The infected erythrocytes are enlarged and show red granules known as Schuffner’s dots on the surface. They become irregular in shape, lose their red color, and present a washed out appearance. A few of the parasitized erythrocytes retreat into the blood spaces of the internal organs.
- The schizont appears in about 36–40 hours. It occupies virtually the whole of the enlarged red cell. The schizont matures in the next 6–8 hours, with the development of merozoites, each with its central nucleus and surrounding cytoplasm. The pigment granules agglomerate into a few dark brown collections at the center, and with the merozoites around it, this stage presents a rosette appearance. There are about 12–24 (usually 16) merozoites per schizont.
- Erythrocytic schizogony takes approximately 48 hours. The red cell, which now measures about 10 µm in diameter is heavily stippled and often distorted. It bursts to liberate the merozoites and pigment. The pigment is phagocytosed by reticuloendothelial cells.

**Fig. 5: Plasmodium vivax** (Giemsa stain, magnification 2000X)**

<table>
<thead>
<tr>
<th>Erythrocyte</th>
<th>Young ring stage</th>
<th>Older ring stage with Schuffner’s dots</th>
<th>Adult ring in enlarged cell, Schuffner’s dots marked</th>
</tr>
</thead>
<tbody>
<tr>
<td>Commencing chromatin division</td>
<td>Further chromatin division</td>
<td>Schizont</td>
<td>Schizont mature form prior to merozoite liberation</td>
</tr>
<tr>
<td>Female gametocyte early stage</td>
<td>Female gametocyte mature</td>
<td>Male gametocyte</td>
<td></td>
</tr>
</tbody>
</table>
The merozoites measure about 1.5 µm and have no pigment. Gametocytes appear early, usually within 4 days after the trophozoites first appear. Both male and female gametocytes are large, nearly filling the enlarged red cell. The macrogametocyte has dense cytoplasm staining deep blue and a small compact nucleus. The microgametocyte has pale-staining cytoplasm and a large diffuse nucleus. Pigment granules are prominent in the gametocytes.

**Plasmodium Falciparum**

The name *falciparum* comes from the characteristic sickle shape of the gametocytes of this species (*falc*: sickle, *parere*: to bring forth). This is the highly pathogenic of all the plasmodia and hence, the name malignant tertian or pernicious malaria for its infection. The disease has a high rate of complications and unless treated, is often fatal. The species is responsible for almost all deaths caused by malaria.

- The disease has a high rate of complications and unless treated, is often fatal. The species is responsible for almost all deaths caused by malaria.

- **Schizogony**: The sporozoites are sickle-shaped. The tissue phase consists of only a single cycle of pre-erythrocytic schizogony. *No hypnozoites occur*. The mature liver schizont releases about 30,000 merozoites.

- They attack both young and mature erythrocytes and so the population of cells affected is very large. Infected erythrocytes present a brassy coloration.

- **Ring form**: The early ring form in the erythrocyte is very delicate and tiny, measuring only a one-sixth of the red cell diameter. Rings are often seen attached along the margin of the red cell, the so-called **form appliqué** or accole. Binucleate rings (double chromatin) are common resembling **stereo headphones** in appearance. Several rings may be seen within a single erythrocyte. In course of time, the rings become larger, about a third of the size of the red cell and may have 1 or 2 grains of pigment in its cytoplasm (**Figs 8 and 9**).

- The subsequent stages of the asexual cycle—late trophozoite, early and mature schizonts—are not ordinarily seen in peripheral blood, except in very severe or pernicious malaria. The presence of *P. falciparum* schizonts in peripheral smears indicates a grave prognosis (**Box 2**).

- The mature schizont is smaller than in any other species and has 8–24 (usually 16) merozoites. The erythrocytic schizogony takes about 48 hours or less, so that the periodicity of febrile paroxysms is 36–48 hours.

- Very high intensity of parasitization is seen in falciparum malaria. In very severe infections, the rate of parasitized cells may even be up to **50%**.

- The infected erythrocytes are of normal size. They show a few (6–12) coarse brick-red dots which are called **Maurer’s clefts**. Some red cells show **basophilic stippling**.

- **Gametogony**: It begins after several generations of schizogony. Gametocytes are seen in circulation about 10 days after the ring stage first appears. The early gametocytes seldom appear in peripheral circulation. The mature gametocytes, which are seen in peripheral smears are curved oblong structures, described as **crescentic**, sickle, sausage, or banana-shaped. They are usually referred to as crescents (**Fig. 10**).

- The male gametocytes are broad and sausage-shaped or **kidney-shaped**, with blunt rounded ends as compared to the female gametocytes, which are thinner and more...
Paniker’s Textbook of Medical Parasitology

Box 2: Pathogenesis of malignant malaria

- Late stage schizonts of *P. falciparum* secrete protein on the surface of RBCs to form knob-like protuberances in erythrocyte’s cell membrane. These knobs produce specific adhesive *Plasmodium falciparum* erythrocyte membrane protein-1 (PfEMP-1) so that infected RBCs become sticky.
- Sometime inflammatory cytokines particularly IFN-γ produced by the malaria parasite upregulate the expression of endothelial cytoadherence receptors like thrombospondin, E-selectin, VCAM-1, ICAM-1 in capillaries in the brain, chondroitin sulfate B in placenta and CD36 in most other organs. The infected RBCs stick inside and eventually block capillaries and venules. This phenomenon is called cytoadherence. At the same stage these *P. falciparum* infected RBCs adhere to uninfected RBCs to form rosettes.
- This process of cytoadherence and rosetting causes capillary plugging and decrease microcirculatory flow in vital organs like brain, kidney, lungs, spleen, intestine, bone marrow and placenta resulting in serious complications such as cerebral malaria.
- Other virulence factors of *P. falciparum* are histidine-rich protein II (HRP II) and glycosylphosphatidylinositol (GPI).

Abbreviations: ICAM-1, intercellular adhesion molecule-1; IFN-γ, interferon gamma; RBCs, red blood cells; VCAM-1, vascular cell adhesion molecule-1
The development of the parasite, in man and mosquito is
occur
him. It causes
quartan malaria,
in which febrile paroxysms occur every 4th day, with 72 hours interval between the bouts.
• The disease is generally mild, but is notorious for its long persistence in circulation in undetectable levels, for 50 years or more. Recrudescence may be provoked by splenectomy or immunosuppression.
• The development of the parasite, in man and mosquito is much slower than with other species. Chimpanzees may be naturally infected with P. malariae and may constitute a natural reservoir for quartan malaria.
• P. malariae occurs in tropical Africa, Sri Lanka, Burma and parts of India, but its distribution is patchy.

- The sporozoites are relatively thick. Pre-erythrocytic schizogony takes about 15 days, much longer than in other species. Each schizont releases about 15,000 merozoites. Hypnozoites do not occur. The long latency of the infection is believed to be due to long time survival of few erythrocytic forms in some internal organs.
- P. malariae preferentially infects older erythrocytes and the degree of parasitization is low.
- The ring forms resemble those of P. vivax, although thicker and more intensely stained. The old trophozoites are sometimes seen stretched across the erythrocyte as a broad band. These band forms are a unique feature of P. malariae. Numerous large pigment granules are seen (Fig. 11).
- The schizonts appear in about 50 hours and mature during the next 18 hours. The mature schizont has an average of eight merozoites, which usually present a rosette appearance.
- The infected erythrocytes may be of the normal size or slightly smaller. Fine stippling, called Ziemann’s stippling, may be seen with special stains. The degree of parasitization is lowest in P. malariae.
- Erythrocytic schizogony takes 72 hours.
- The gametocytes develop in the internal organs and appear in the peripheral circulation when fully grown. Gametocytes occupy nearly the entire red cell. The male has pale blue cytoplasm with a large diffuse nucleus, while the female has deep blue cytoplasm and a small compact nucleus.

**Plasmodium Ovale**

This parasite produces a tertian fever resembling vivax malaria, but with milder symptoms, prolonged latency and fewer relapses.

- It is the rarest of all plasmodia infecting humans and is seen mostly in tropical Africa, particularly along the West Coast.
- The pre-erythrocytic stage extends for 9 days. Hepatocytes containing schizonts usually have enlarged nuclei. The mature liver schizont releases about 15,000 merozoites. Hypnozoites are present.
- The trophozoites resemble those in vivax malaria, but are usually more compact, with less ameboid appearance. Schuffner’s dots appear earlier and are more abundant and prominent than in vivax infection (Fig. 12).
- The infected erythrocytes are slightly enlarged. In thin films, many of them present an oval shape with fimbriated margins. This oval appearance of the infected erythrocyte is the reason for the name ovale given to this species.
- The schizonts resemble those of P. malariae, except that the pigment is darker and the erythrocyte is usually oval, with prominent Schuffner’s dots.
Fig. 11: *Plasmodium malariae* stages of erythrocytic schizogony (Giemsa stain, magnification 2000X)

<table>
<thead>
<tr>
<th>Stage Description</th>
<th>Image</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythrocyte</td>
<td><img src="image" alt="Erythrocyte" /></td>
</tr>
<tr>
<td>Ring form with eccentric nucleus</td>
<td><img src="image" alt="Ring form" /></td>
</tr>
<tr>
<td>Commencement of band form dividing chromatin pigment accumulation</td>
<td><img src="image" alt="Commencement" /></td>
</tr>
<tr>
<td>Band form Note: Chromatin on one side of band</td>
<td><img src="image" alt="Band form" /></td>
</tr>
<tr>
<td>Schizont, commencing daisy form</td>
<td><img src="image" alt="Schizont" /></td>
</tr>
<tr>
<td>Schizont, mature pigment centrally clumped daisy form</td>
<td><img src="image" alt="Schizont" /></td>
</tr>
<tr>
<td>Female gametocyte compact chromatin</td>
<td><img src="image" alt="Female gametocyte" /></td>
</tr>
<tr>
<td>Male gametocyte diffuse chromatin</td>
<td><img src="image" alt="Male gametocyte" /></td>
</tr>
</tbody>
</table>

Fig. 12: *Plasmodium ovale* stages of erythrocytic schizogony (Giemsa stain, magnification 2000X)

<table>
<thead>
<tr>
<th>Stage Description</th>
<th>Image</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythrocyte</td>
<td><img src="image" alt="Erythrocyte" /></td>
</tr>
<tr>
<td>Young ring stage</td>
<td><img src="image" alt="Young ring" /></td>
</tr>
<tr>
<td>Older ring stage</td>
<td><img src="image" alt="Older ring" /></td>
</tr>
<tr>
<td>Adult ring in enlarged oval erythrocyte Schuffner’s erythrocyte</td>
<td><img src="image" alt="Adult ring" /></td>
</tr>
<tr>
<td>Commencing chromatin division</td>
<td><img src="image" alt="Commencing" /></td>
</tr>
<tr>
<td>Further chromatin division</td>
<td><img src="image" alt="Further" /></td>
</tr>
<tr>
<td>Schizont oval form of erythrocyte persisting</td>
<td><img src="image" alt="Schizont" /></td>
</tr>
<tr>
<td>Merozoite development Note: Continued oval form and Schuffner’s dots</td>
<td><img src="image" alt="Merozoite" /></td>
</tr>
<tr>
<td>Daisy form of the parasite</td>
<td><img src="image" alt="Daisy" /></td>
</tr>
<tr>
<td>Female gametocyte</td>
<td><img src="image" alt="Female" /></td>
</tr>
<tr>
<td>Male gametocyte</td>
<td><img src="image" alt="Male" /></td>
</tr>
</tbody>
</table>
Mixed Infections

In endemic areas it is not uncommon to find mixed infections with two or more species of malaria parasites in the same individual.

- Mixed infection with *P. vivax* and *P. falciparum* is the most common combination with a tendency for one or the other to predominate.
- The clinical picture may be atypical with bouts of fever occurring daily.
- Diagnosis may be made by demonstrating the characteristic parasitic forms in thin blood smears.

The characteristics of the four species of plasmodia infecting man are listed in Table 3.

Pathogenesis

Clinical manifestations in malaria are caused by products of erythrocytic schizogony and the host’s reaction to them.

- The disease process in malaria occurs due to the local or systemic response of the host to parasite antigens and tissue hypoxia caused by reduced oxygen delivery because of obstruction of blood flow by the parasitized erythrocytes.
- Liver is enlarged and congested. Kupffer cells are increased and filled with parasites. Hemozoin pigments are also found in the parenchymal cells (Fig. 13). Parenchymal cells show fatty degeneration, atrophy and centrilobular necrosis.

### Table 3: Comparison of the characteristics of plasmodia causing human malaria

<table>
<thead>
<tr>
<th></th>
<th><em>P. vivax</em></th>
<th><em>P. falciparum</em></th>
<th><em>P. malariae</em></th>
<th><em>P. ovale</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypnozoites</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Erythrocyte preference</td>
<td>Reticulocytes</td>
<td>Young erythrocytes, but can infect all stages</td>
<td>Old erythrocytes</td>
<td>Reticulocytes</td>
</tr>
<tr>
<td>Stages found in peripheral blood</td>
<td>Rings, trophozoites, schizonts, gametocytes</td>
<td>Only rings and gametocytes</td>
<td>As in <em>vivax</em></td>
<td>As in <em>vivax</em></td>
</tr>
<tr>
<td>Ring stage</td>
<td>Large, 2.5 µm, usually single, prominent chromatin</td>
<td>Delicate, small, 1.5 µm, double chromatin, and multiple rings common, accole forms found</td>
<td>Similar to <em>vivax</em>, but thicker</td>
<td>Similar to <em>vivax</em>, more compact</td>
</tr>
<tr>
<td>Late trophozoite</td>
<td>Large irregular, actively ameboid, prominent vacuole</td>
<td>Compact, seldom seen in blood smear</td>
<td>Band form characteristic</td>
<td>Compact, coarse pigment</td>
</tr>
<tr>
<td>Schizont</td>
<td>Large filling red cell</td>
<td>Small, compact, seldom seen in blood smear</td>
<td>Medium size</td>
<td>Medium size</td>
</tr>
<tr>
<td>Number of merozoites</td>
<td>12–24 in irregular grape-like cluster</td>
<td>8–24 grape-like cluster</td>
<td>6–12 in daisy-head or rosette pattern</td>
<td>6–12 irregularly arranged</td>
</tr>
<tr>
<td>Microgametocyte (male gametocyte)</td>
<td>Spherical, compact, pale blue cytoplasm, diffuse nucleus</td>
<td>Sausage or banana-shaped pale blue or pink cytoplasm, large diffuse nucleus</td>
<td>As in <em>vivax</em></td>
<td>As in <em>vivax</em></td>
</tr>
<tr>
<td>Macrogametocyte (female gametocyte)</td>
<td>Large, spherical, deep blue cytoplasm, compact nucleus</td>
<td>Crescentic, deep blue cytoplasm, compact nucleus</td>
<td>As in <em>vivax</em></td>
<td>As in <em>vivax</em></td>
</tr>
<tr>
<td>Infected erythrocyte</td>
<td>Enlarged, pale, with Schuffner’s dots</td>
<td>Normal size, Maurer’s clefts, sometimes basophilic stippling</td>
<td>Normal, occasionally Ziemann’s stippling</td>
<td>Enlarged, oval fimbriated, prominent Schuffner’s dots</td>
</tr>
<tr>
<td>Duration of schizogony (days)</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Prepatent period (days)</td>
<td>8</td>
<td>5</td>
<td>13</td>
<td>9</td>
</tr>
<tr>
<td>Average incubation period (days)</td>
<td>14</td>
<td>12</td>
<td>30</td>
<td>14</td>
</tr>
<tr>
<td>Appearance of gametocyte after parasite latency (days)</td>
<td>4–5</td>
<td>10–12</td>
<td>11–14</td>
<td>5–6</td>
</tr>
<tr>
<td>Duration of sporogony in mosquito (25°C) (days)</td>
<td>9–10</td>
<td>10–12</td>
<td>25–28</td>
<td>14–16</td>
</tr>
<tr>
<td>Average duration of untreated infection (years)</td>
<td>4</td>
<td>2</td>
<td>40</td>
<td>4</td>
</tr>
</tbody>
</table>
The Kidneys

Abbreviations:

Box 3: Causes of anemia in malaria

- Destruction of large number of RBCs by complement-mediated and autoimmune hemolysis.
- Suppression of erythropoiesis in the bone marrow.
- Increased clearance of both parasitized and nonparasitized RBCs by the spleen.
- Failure of the host to recycle the iron bound in hemozoin pigment.
- Antimalarial therapy in G6PD deficient patients.

Abbreviations: G6PD, glucose-6-phosphate dehydrogenase; RBCs, red blood cells

Spleen

• Spleen is soft, moderately enlarged and congested in acute infection. In chronic cases, spleen is hard with a thick capsule and slate gray or dark brown or even black in color due to dilated sinusoids, pigment accumulation and fibrosis (Fig. 13).

• Kidneys are enlarged and congested. Glomeruli frequently contain malarial pigments and tubules may contain hemoglobin casts (Fig. 13).

• The brain in P. falciparum infection is congested. Capillaries of the brain are plugged with parasitized RBCs. The cut surface of the brain shows slate gray cortex with multiple punctiform hemorrhage in subcortical white matter.

• Anemia: After few paroxysms of fever, normocytic and normochromic anemia develops. Anemia is caused by destruction of large number of red cells by complement-mediated autoimmune hemolysis. Spleen also plays an active role by phagocytic removal of a large number of both infected and uninfected RBCs. Excess removal of uninfected RBCs may account for up to 90% of erythrocyte loss (Box 3).

Clinical Features

Benign Malaria

- Incubation period: 12–17 days (Box 4).
- The typical clinical feature of malaria consists of periodic bouts of fever with chill and rigor, followed by anemia, splenomegaly and hepatomegaly.

- The classic febrile paroxysm comprises of three distinct stages—(1) cold stage, (2) hot stage and (3) sweating stage.
  1. Cold stage: The patient feels intense cold with chill and rigor along with lassitude, headache and nausea. This stage lasts for 15 minutes to 1 hour.
  2. Hot stage: The patient feels intensely hot. The temperature mounts to 41°C or higher. Headache persists but nausea commonly diminishes. This stage lasts for 2–6 hours.
  3. Sweating stage: Profuse sweating follows the hot stage and the temperature comes down to normal. The skin is cool and moist. The patient usually falls asleep to wake up refreshed.

- The paroxysm usually begins in the early afternoon and lasts for 8–12 hours. The febrile paroxysm synchronizes with the erythrocytic schizogony.

- The periodicity is approximately 48 hours in tertian malaria (in P. vivax, P. falciparum and P. ovale) and 72 hours in quartan malaria (in P. malariae).

- Quotidian periodicity, with fever occurring at 24 hour intervals may be due to two broods of tertian parasites maturing on successive days or due to mixed infection.

- Regular periodicity is seldom seen in primary attack, but is established usually only after a few days of continuous,
remittent, or intermittent fever. True rigor is typically present in vivax malaria and is less common in falciparum infection.

- There can be both hypoglycemia or hyperglycemia in malaria.
- Sometimes, there may be hyperkalemia due to red cell lysis and fall in blood pH.
- Infection with P. vivax usually follows a chronic course with periodic relapses, whereas P. ovale malaria is generally mild. Although P. malariae malaria is less severe, but it may lead to renal complications. Relapse mainly occurs in inadequately treated cases after an interval of 8–40 weeks or more.

**Malignant Tertian Malaria**

**Incubation period:** 8–14 days.

The most serious and fatal type of malaria is malignant tertian malaria caused by P. falciparum. Falciparum malaria if not treated timely or adequately, severe life-threatening complications may develop. In severe falciparum malaria, parasitic load is very high and more than 5% red cells are affected. The term pernicious malaria also have been applied to these conditions that include cerebral malaria, blackwater fever, algid malaria and septicemic malaria (Box 5).

**Cerebral malaria:** It is the most common complication of malignant malaria.
- The initial symptoms are nonspecific with fever, headache, pain in back, anorexia and nausea.
- Anemia: The patient may be anemic and mildly jaundiced.
- Hepatosplenomegaly: Liver and spleen are enlarged and nontender.
- Thrombocytopenia is common.
- After 4–5 days of high fever, cerebral malaria is manifested by features of diffuse symmetric encephalopathy like headache, confusion, increased muscle tone, seizures, paralysis, slowly lapsing to coma.

**Box 5: Complications of falciparum malaria**

- Cerebral malaria
- Algid malaria
- Septicemic malaria
- Blackwater fever
- Pulmonary edema
- Acute renal failure
- Hypoglycemia (<40 mg/dL)
- Severe anemia (Hb<5 g/dL, PCV<15%)
- Hyperpyrexia
- Metabolic acidosis and shock
- Bleeding disturbances
- Hyperparasitemia.

Abbreviations: Hb, hemoglobin; PCV, packed cell volume

- Retinal hemorrhages may be seen in 15% of adults.
- Hypoglycemia is common in patients following quinine therapy or with hyperparasitemia.
- In 10% of cases renal dysfunction progressing to acute renal failure may occur.
- Other complications include metabolic acidosis, pulmonary edema and shock.
- Even with treatment, death occurs in 15% of children and 20% of adults who develop cerebral malaria.
- This occurs particularly when nonimmune persons have remained untreated or inadequately treated for 7–10 days after development of the primary fever.
- The basic pathogenesis of cerebral malaria is due to erythrocyte sequestration in microvasculature of various organs.

Late stage schizonts of P. falciparum secrete a protein on the surface of RBCs to form knob-like deformities. This knob produces specific adhesive proteins [Plasmodium falciparum erythrocyte membrane protein-1 (PfEMP-1)], which promote aggregation of infected RBCs to other noninfected RBCs and receptors of capillary endothelial cells. These sequestered RBCs cause capillary plugging of cerebral microvasculature, which results in anoxia, ischemia and hemorrhage in brain.

- **Blackwater fever:** A syndrome called blackwater fever (malarial hemoglobinuria) is sometimes seen in falciparum malaria, particularly in patients, who have experienced repeated past infections and inadequate treatment with quinine. An autoimmune mechanism has been suggested.
  - Patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency may develop this condition after taking oxidant drugs, even in the absence of malaria.
  - Clinical manifestations include fever, prostration and hemoglobinuria (black colored urine), bilious vomiting and prostration, with passage of dark red or blackish urine.
  - The pathogenesis is believed to be massive intravascular hemolysis caused by antikerthrocyte antibodies, leading to massive absorption of hemoglobin by the renal tubules (hemoglobinuric nephrosis) producing blackwater fever. Complications of blackwater fever include renal failure, acute liver failure and circulatory collapse.

- **Algid malaria:** This syndrome is characterized by peripheral circulatory failure, rapid thready pulse with low blood pressure and cold clammy skin. There may be severe abdominal pain, vomiting, diarrhea and profound shock.

- **Septicemic malaria:** It is characterized by high continuous fever with dissemination of the parasite to various organs, leading to multiorgan failure. Death occurs in 80% of the cases.
Merozoite-induced Malaria

Natural malaria is sporozoite-induced, the infection being transmitted by sporozoites introduced through the bite of vector mosquitoes. Injection of merozoites can lead to direct infection of red cells and erythrocytic schizogony with clinical illness. Such merozoite-induced malaria may occur in the following situations:

- **Transfusion malaria:** Blood transfusion can accidentally transmit malaria, if the donor is infected with malaria. The parasites may remain viable in blood bank for 1–2 weeks. As this condition is induced by direct infection of red cells by the merozoites, pre-erythrocytic schizogony and hypnozoites are absent. *Relapse does not occur and incubation period is short.*

  Table 4 enumerates the differences between mosquito-borne malaria and blood transfusion malaria.

- **Congenital malaria:** A natural form of merozoite-induced malaria, where the parasite is transmitted transplacentally from mother to fetus.

- **Renal transplantation** may lead to malaria if the donor had parasitemia.

- **Shared syringes** among drug addicts may be responsible.

Tropical Splenomegaly Syndrome

Tropical splenomegaly syndrome (TSS) or hyper-reactive malarial splenomegaly (HMS) is a benign condition seen in people of malaria endemic areas mainly tropical Africa, New Guinea and Vietnam.

*It happens from abnormal immunological response to repeated malaria infection.*

- Tropical splenomegaly syndrome is characterized by high level of immunoglobulin M (IgM) against malaria due to polyclonal activation of B-cells, decreased C3 and massive splenomegaly. Malaria parasite is **absent** in peripheral blood.

A normochromic normochromic anemia is present which does not respond to hematinics or anthelminthics.

- Spleen and liver are enlarged, congested, with dilated sinusoids and marked lymphocytic infiltration. Numerous pigment-laden Kupffer cells dot the liver. Changes are also seen in bone marrow, kidneys and adrenals.

- Tropical splenomegaly syndrome differs from various other types of splenomegalies seen in the tropics in its response to antimalarial treatment.

Immunity

Immunity in malaria could be two types: (1) **innate immunity** and (2) **acquired immunity**.

**Innate Immunity**

- It is the inherent, nonimmune mechanism of host resistance against malarial parasite.

  - **Duffy negative red blood cells:** The invasion of red cells by merozoites requires the presence of specific glycoprotein receptors on the erythrocyte surface. It has been found Duffy blood group negative persons are protected from *P. vivax* infection. Duffy blood group is absent in West Africa where *P. vivax* malaria is not prevalent.

  - **Nature of hemoglobin:** *Hemoglobin E* provides natural protection against *P. vivax*. *P. falciparum* does not multiply properly in sickled red cells containing HbS. Sickle cell anemia trait is very common in Africa, where *falciparum* malaria is hyperendemic and offers a survival advantage. *HbF* present in neonates protects them against all *Plasmodium* species.

  - **Glucose-6-phosphate dehydrogenase deficiency:** Innate immunity to malaria has also been related to G6PD deficiency found in Mediterranean coast, Africa, Middle East and India.

  - **Human leukocyte antigen-B53:** Human leukocyte antigen-B53 (HLA-B53) is protected from cerebral malaria associated with protection from malaria.

  - **Nutritional status:** Patients with iron deficiency and severe malnutrition are relatively resistant to malaria.

  - **Pregnancy:** *Falciparum* malaria is more severe in pregnancy, particularly in primigravida and may be enhanced by iron supplementation.

  - **Splenectomy:** The spleen appears to play an important role in immunity against malaria. Splenectomy enhances susceptibility to malaria.

**Acquired Immunity**

Infection with malaria parasite induces specific immunity involving both humoral and cellular immunity, which can
bring about clinical cure, but cannot eliminate parasites from the body.
- It can prevent superinfection, but is not powerful enough to defend against reinfection. This type of resistance in an infected host, which is associated with continued asymptomatic parasite infection is called premunition. This type of immunity disappears once the infection is eliminated.

**Humoral immunity:** Circulating antibodies (IgM, IgG and IgA) against asexual forms give protection by inhibiting red cell invasion and antibodies against sexual forms reduce transmission of malaria parasite.
- Acquired antibody-mediated immunity is transferred from mother to fetus across the placenta and is evident in endemic areas where infants below the age of 3 months are protected by passive maternal antibodies.
- Young children are highly susceptible to malaria. As they grow up, they acquire immunity by subclinical or clinical infections, so that incidence of malaria is low in older children and adults.

**Cellular immunity:** Sensitized T cells release cytokines that regulate macrophage activation and stimulate B cells to produce antibodies. The activated macrophages inside liver, spleen and bone marrow phagocytose both parasitized and nonparasitized RBCs.

**Clinical note:** Protective immunity against malaria is species specific, stage specific and strain specific.

**Recrudescence and Relapse**

**Recrudescence**

In *P. falciparum* and *P. malariae* infections after the primary attack, sometimes there is a period of latency, during which there is no clinical illness. But some parasites persist in some erythrocytes, although the level of parasitemia is below the fever threshold or sometimes below the microscopic threshold. Erythrocytic schizogony is repeated at a low level in the body when the number of parasites attain a significant level, fresh malarial attack develops. This recurrence of clinical malaria caused by persisting *P. falciparum* and *P. malariae* is called recrudescence. Recrudescence may be due to waning immunity of the host or possibly due to antigenic variation.

In *P. falciparum* infections, recrudescences are seen for 1–2 years, while in *P. malariae* infection, they may last for long periods, even up to 50 years (Table 5).

**Relapse**

It is seen in inadequately treated *P. vivax* and *P. ovale* infections. In both these species, two kinds of sporozoites are seen, some of which multiply inside hepatocytes promptly to form schizonts and others which remain dormant. These latter forms are called hypnozoites (from hypnos: sleep). Hypnozoites remain inside the hepatocytes as uninucleated forms, 4–5 µm in diameter, for long periods. Reactivation of hypnozoites leads to initiation of fresh erythrocytic cycles and new attacks of malarial fever. Such new attacks of malaria, caused by dormant erythrocytic forms, reactivated usually from 24 weeks to 5 years after the primary attack are called relapses (Table 5).

**Laboratory Diagnosis**

**Demonstration of Parasite by Microscopy**

Diagnosis of malaria can be made by demonstration of malarial parasite in the blood (Box 6).

Two types of smears are prepared from the peripheral blood. One is called thin smear and the other is called thick smear.

1. **Thin smears:** They are prepared from capillary blood of finger tip and spread over a good quality slide by a second slide held at an angle of 30–45° from the horizontal such that a tail is formed.
   - A properly made thin film will consist of an unbroken smear of a single layer of red cells, ending in a tongue, which stops a little short of the edge of the slide.
   - Thins smears are air dried rapidly, fixed in alcohol and stained by one of the Romanowsky stains such as Leishman, Giemsa, Field’s, or JSB stain (named after Jaswant Singh and Bhattacharjee).
   - Thins smears are used for detecting the parasites and determining the species.

2. **Thick smears:** They can be made on the same slide of thin smear or separately.
   - In a thick film, usually three drops of blood are spread over a small area (about 10 mm).
   - The amount of blood in thin smear is about 1–1.5 µL, while in a thick smear it is 3–4 µL.
   - The thick film is dried and kept in a Koplin jar for 5–10 minutes for dehemoglobinization.

**Table 5: Differences between recrudescence and relapse**

<table>
<thead>
<tr>
<th></th>
<th>Recrudescence</th>
<th>Relapse</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seen in</td>
<td><em>P. falciparum</em> and <em>P. malariae</em></td>
<td><em>P. vivax</em> and <em>P. ovale</em></td>
</tr>
<tr>
<td>Due to persistence</td>
<td>parasite at a subclinical level in circulation</td>
<td>Due to reactivation of hypnozoites present in liver cells</td>
</tr>
<tr>
<td>Occurs</td>
<td>within a few weeks or months of a previous attack</td>
<td>Occurs usually 24 weeks to 5 years after the primary attack</td>
</tr>
<tr>
<td>Can be prevented</td>
<td>by adequate drug therapy or use of newer antimalarial drugs in case of drug resistance</td>
<td>Can be prevented by giving primaquine to eradicate hypnozoites</td>
</tr>
</tbody>
</table>

See

07-07-2017 17:30:54
Box 6: Morphological feature of malaria parasites in blood smear

- In *P. vivax*, *P. ovale* and *P. malariae* all asexual forms and gametocytes can be seen in peripheral blood. In *P. falciparum* infection, only ring form alone or with gametocytes can be seen.
- Ring forms of all species appear as streaks of blue cytoplasm with detached nuclear dots. They are large and compact in *P. vivax*, *P. ovale*, and *P. malariae* and fine delicate with double chromatin (head-phone appearance). In *P. falciparum*, multiple rings with “accola” forms are seen.
- Gametocytes are banana-shaped (crescents) in *P. falciparum* and round in *P. vivax*, *P. ovale* and *P. malariae*.
- Enlarged red blood cells (RBCs) with intracellular coarse brick-red stippling (Schuffner’s dots) are characteristic in *P. vivax*. In *P. falciparum*, RBCs are normal in size with large red dots (Maurer’s dots) and sometimes, with basophilic stippling. Careful search in blood should be made for mixed infections.

Box 7: Quantification of parasites

Quantification of parasites can be done by thick smear. The counting of parasites are done to an approximate number in the following method:

- ++ = 1–10 parasite per 100 thick film fields
- +++ = 11–100 parasite per 100 thick film
- ++++ = 1–10 parasite per thick film
- ++++ = More than 10 parasite per thick film field.

- It is not fixed in methanol.
- Thick film is stained similar to thin film.
- The stained film is examined under the oil immersion microscope.
- The thick film is more sensitive, when examined by an experienced person, because it concentrates 20–30 layers of blood cells in a small area.
- Thick film is more suitable for rapid detection of malarial parasite, particularly when they are few (as low as 20 parasites/μL) (Box 7).
- The dehemoglobinized and stained thick film does not show any red cells, but only leukocytes, and, when present, the parasites. But the parasites are often distorted in form, and as the diagnostic changes in blood cells such as enlargement and stippling cannot be made out, species identification is difficult.
- Thin film is examined first at the tail end and if parasites are found, there is no need for examining thick film. If parasites are not detected in thin film, then thick film should be examined.
- It is recommended that 200 oil immersion fields should be examined before a thick film is declared negative (Fig. 14).

**Quantitative Buffy Coat, Smear**

The quantitative buffy coat (QBC) test is a novel method for diagnosing malaria, wherein a small quantity of blood (50–110 μL) of blood is spun in QBC centrifuge at 12,000 revolutions per minutes for 5 minutes.

**Microconcentration Technique**

In microconcentration technique, blood sample is collected in microhematocrit tube and centrifuged at high speed. The sediment is mixed with normal serum and smear is prepared. Though it increases the positivity rate, it changes the morphology of the parasite.

**Culture of Malaria Parasites**

- The original method of petridish culture employed a candle jar to provide an atmosphere of 3% oxygen and
10% carbon dioxide and a relatively simple self-culture medium (RPMI1640) supplemented with human, rabbit, or calf serum to maintain infected erythrocytes. Fresh red cells were added periodically for continuation of the growth and multiplication of plasmodia. The continuous flow method devised by Trager enables the prolonged maintenance of stock cultures.

- Computer-controlled culture systems, introduced subsequently, provide a steady abundant supply of parasites. Several culture lines have been established from blood of infected Aotus monkey or directly from human patients.
- Schizogony proceeds normally in culture. Gametocytes are formed infrequently. Pre-erythrocytic stages of some species have been obtained in tissue cultures. Plasmodia retain their infectivity in culture.
- Culture of plasmodia provides a source of the parasites for study of their antigenic structure, in seroepidemiologic surveys, drug sensitivity tests and studies in immunoprophylaxis.

Serodiagnosis

Serodiagnosis is not helpful in clinical diagnosis because they will not differentiate between an active and past infection. It is used mainly for seroepidemiologic survey and to identify the infected donors in transfusion malaria. The tests used are indirect hemagglutination (IHA), indirect fluorescent antibody (IFA) test and enzyme-linked immunosorbent assay (ELISA).

Newer Methods of Diagnosis (Box 8)

Fluorescence microscopy:
Kawamoto technique: Fluorescent dyes like acridine orange or benzothiocarboxy purine are used, which stain the parasites entering the RBCs but not white blood cells (WBCs). This is a method of differential staining.
- Acridine orange stains DNA as fluorescent green and cytoplasmic RNA as red.

- The stained slide is examined under fluorescent microscope.
- The method is mainly used for mass screening in field laboratory.

Rapid antigen detection tests: Rapid diagnostic test are based on the detection of antigens using immunochromatographic methods. These rapid antigen detection tests have been developed in different test formats like the dipstick, card and cassette bearing monoclonal antibody, directed against the parasite antigens. Several kits are available commercially, which can detect Plasmodium in 15 minutes (Fig. 15).

Parasite-F test: This test is based on detection of histidine rich protein-2 (HRP-2) antigen produced by the asexual stages of P. falciparum expressed on the surface of red cells.
- Monoclonal antibody produced against HRP-2 antigen (Pf band) is employed in the test strip.
- Advantage: It is widely popular and has high sensitivity (98%) and specificity.
  - The test is said to detect low asexual parasitemia of more than 40 parasites/μL.
  - The test can be performed within 10 minutes.
- Disadvantage: Plasmodium falciparum HRP-2 (PfHRP-2) antigen detection test cannot detect the other three malaria species.
  - It remains positive up to 2 weeks after cure.
  - In P. falciparum infection, PfHRP-2 is not secreted in gametogony stage. Hence in “carriers”, the Pf band may be absent.

Dual antigen test: The test detects parasite lactate dehydrogenase (pLDH) produced by trophozoites and gametocytes of all plasmodium species and PfHRP-2 antigen produced by P. falciparum simultaneously.
- Thus, one band (Pv band) is genus specific (Plasmodium specific) and other is Plasmodium falciparum specific (Pf band).

Fig. 15: Rapid ICT Kit for dual antigen
This test is a rapid two-site sandwich immunoassay used for specific detection and differentiation of *P. falciparum* and *P. vivax* malaria in areas with high rates of mixed infection. The "Pv" band can be used for monitoring success of antimalarial therapy in case of stained alone *P. vivax* infection as the test will detect only live parasites and therefore will be negative, if the parasite has been killed by the treatment. The disadvantage of the test is that it is expensive and cannot differentiate between *P. vivax*, *P. ovale*, and *P. malariae*.

**Molecular Diagnosis**

*Deoxyribonucleic acid probe*: Deoxyribonucleic acid probe is a highly sensitive method for the diagnosis of malaria. It can detect less than 10 parasites/µL of blood.

*Polymerase chain reaction*: Polymerase chain reaction (PCR) is increasingly used now for species specification and for detection of drug resistance in malaria.

- Chloroquine resistance in *P. falciparum* is due to mutation in the *Plasmodium falciparum* chloroquine resistance transporter (*PfCRT*), a transporter gene in the parasite.
- **Point mutation** in another gene *Plasmodium falciparum* multidrug resistance protein 1 (*PfMDR1*) is responsible for resistance in vitro.
- Pyrimethamine and sulfadoxine resistances are associated with point mutations in dihydrofolate reductase (*DHFR*) and dihydropteroate synthase (*DHPS*) genes, respectively.
- **Mutation in PfATPase gene** is associated with reduced susceptibility to artemisinin derivatives.

**Other Tests**

- Measurement of hemoglobin and packed cell volume (PCV), in case of heavy parasitemia, particularly in children and pregnant woman.
- Total WBC and platelet count in severe *falciparum* malaria.
- Measurement of blood glucose to detect hypoglycemia, particularly in young children and pregnant women with severe *falciparum* malaria and patients receiving quinine.
- Coagulation tests like measurement of antithrombin III level, plasma fibrinogen, fibrin degradation products (FDPs), partial thromboplastin time (PTT), if abnormal bleeding is suspected in *falciparum* malaria.
- Urine for free hemoglobin, if blackwater fever is suspected.
- Blood urea and serum creatinine to monitor renal failure.
- Glucose-6-phosphate dehydrogenase screening before treatment with an antioxidant drug like primaquine.

**Treatment**

Antimalarial drugs are used with various objectives like clinical cure, prevention of relapse, prevention of transmission and prophylaxis.

**Therapeutic**

Objective is to eradicate the erythrocytic cycle and clinical cure.

**Radical Cure**

Objective is to eradicate the exoerythrocytic cycle in liver to prevent relapse.

**Gametocidal**

Objective is to destroy gametocytes to prevent mosquito transmission and thereby reducing human reservoir.

**Chemoprophylaxis**

Objective is to prevent infections in nonimmune person visiting endemic areas. The most commonly used antimalarials are chloroquine, amodiaquine, quinine, pyrimethamine, doxycycline, sulfadoxine, proguanil and primaquine. Newer antimalarial like artemisinin, lumefantrine, mefloquine, halofantrine are now commonly used for multidrug-resistant *P. falciparum* infections.

**Treatment of Uncomplicated Malaria**

Positive *P. vivax*, *P. ovale* and *P. malariae* cases are treated with **chloroquine 25 mg/kg** divided over **3 days**.

- **Vivax** malaria relapses due to the presence of hypnozoites in the liver. The relapse rate of *vivax* malaria in India is about 30%.
- For prevention of relapse, **primaquine** is given in a dose of 0.25 mg/kg daily for **14 days** under supervision.
- Primaquine is contraindicated in G6PD deficiency patients, infants and pregnant women.
- **In case of chloroquine resistance**: **Quinine** is given in a dose of 600 mg 8 hourly for **7 days** along with doxycycline 100 mg/day.

**Treatment of Complicated (Falciparum) Malaria**

Due to emergence of drug resistance of *falciparum* malaria is based on area resistant or sensitive antimalarial drugs.

- **Artemisinin-based combination therapy**: According to revised malaria drug policy in India artemisinin-based...
combination therapy (ACT) (artemisinin + sulfadoxine – pyrimethamine) should be given to all microscopically positive falciparum cases for 3 days in all over India except North-eastern states. This is accomplished by single dose of primaquine 45 mg (0.75 mg/kg) on day 2 as gametocidal drug.

- In North-eastern states considering resistant to sulfadoxine – pyrimethamine drugs, Technical Advisory Committee on Malaria recommended artemether (20 mg + lumefantrine) as per age specific dose schedule.

**Note:** According to revised Malaria Drug Policy 2013, there is no scope for presumptive treatment. Production and sale of artemisinin as monotherapy has been banned in India as it can lead to development of parasite resistance to the drug.

**Drug resistance of malarial parasite:**

- A **drug resistant parasite** is defined as a parasite that will survive and multiply in a dosage that normally cures the infection. Such resistance may be relative (yielding to increased doses of the drug tolerated by the host) or complete (withstanding a maximum dose tolerated by the host).

- Resistance arises from spontaneous point mutations in the genome or gene duplications. The emergence of resistance can be prevented by use of combination of drugs with different mechanisms of action and different drug target.

- Three levels of resistance (R) are defined by the WHO:
  1. *R*: Following treatment, parasitemia clears but recrudescence occurs.
  2. *RI*: Following treatment, there is a reduction but not a clearance of parasitemia.
  3. *RII*: Following treatment, there is no reduction of parasitemia.

The earlier method of classifying resistance is based on counting trophozoites in blood film daily for 7 days after treatment and monitoring the patient for any subsequent recrudescence. All patients with a falciparum parasitemia of more than one trophozoite per high power field (++++ or over) in areas of suspected drug resistance, should be checked for a decrease and clearing of parasites following treatment.

**Prophylaxis**

**Chemoprophylaxis**

It is recommended for travelers going to endemic areas as short-term measure.

- Chloroquine (300 mg) or mefloquine (400 mg) weekly should be given 1 week and 2 weeks before travel to endemic area respectively.

- Alternatively doxycycline (100 mg) daily can be given from day 1 before travel.

**Malaria Vaccine**

Malaria vaccine is an area of intensive research. Over past decades, there has been a significant progress in malaria vaccine development. A completely effective vaccine is not yet available for malaria, although several vaccines are under development. *SPf66* (a cocktail of four antigens, three asexual blood stage antigens + circumsporozoite of Pf) was tested extensively in endemic areas in the 1990s, but clinical trials showed it to be insufficiently effective. Other vaccine candidates targeting the blood stage of parasite’s life cycle using merozoite surface protein 1 (MSP1), MSP2, MSP13 and ring-infected erythrocyte surface antigens (RESAs) have also been in insufficient on their own. Several potential vaccines targeting the pre-erythrocytic stage are being developed, with RTS.S/AS01 showing the most promising results. The RTS.S/AS01 (commercial name, mosquirix) was engineered using genes from the outer protein of *P. falciparam* and a portion of *hepatitis B virus*, plus a chemical adjuvant (AS01) to boost immune response.

**Vector Control Strategies**

- **Residual spraying:** Spraying of residual insecticides, e.g. dichlorodiphenyltrichloroethane (DDT), malathion and fenitrothion in the indoor surfaces of the house is highly effective against adult mosquitoes.

- **Space application:** Insecticidal formulation is sprayed into the atmosphere by ultra-low volume in the form of mist or fog to kill insects (pyrethrum extracts).

- **Individual protection:** Man-vector contact can be reduced by other preventive measures such as the use of repellants, protective clothing, bed net, preferably impregnated with long-acting repellant, mosquito coils and screening of house.

**Antilarval Measures**

- Old antilarval measures such as oiling the collection of standing water or dusting them with *Paris green* have now become promising with the increase of insecticide resistance.

- **Source reduction:** Mosquito breeding sites can be reduced by proper drainage, filling of land, water level management, intermittent irrigation, etc.

**Integrated Control**

In order to reduce too much dependence on residual insecticides, increasing emphasis is being put on integrated vector control methodology, which includes bioenvironmental and personal protection measures.
Malaria Control Programs

In India, the National Malaria Control Programme was introduced in 1953, with the objective of the ultimate eradication of the disease and operated successfully for 5 years, bringing down the annual incidence of malaria from 75 million in 1958 to 2 million.

- By 1961, the incidence dropped to an all-time low of 50,000 cases and no deaths. However, there have been setbacks from 1970 and by 1976, the incidence rose to 6.4 million cases. With the implementation of modified plan of operation in 1977, the upsurge of malaria cases dropped down to 2.1 million cases in 1984. Since then, the epidemiological situation has not shown any improvement.
- Malaria control added impetus as “roll-back malaria initiative” launched jointly by WHO, United Nations Children’s Fund (UNICEF), United Nations Development Programme (UNDP) and the World Bank in 1998. Accordingly, National Vector Borne Disease Control Programme (NVBDCP) is implemented by Directorate of Health Services jointly with Mission Directorate and National Rural Health Mission (NRHM). National goal established under the program is to reduce the number of cases and deaths recorded in 2000, by 50% or more in 2010 and by 75% or more by 2015.

BABESIA SPECIES

INTRODUCTION

Babesia is intraerythrocytic sporozoan parasites that morphologically resemble Plasmodium and cause tick-borne malaria-like illness in domestic and wild animals.

It causes opportunistic infection in humans.

CLASSIFICATION

Order: Piroplasmida
Family: Babesidaceae
Species: Medically important Babesia species are:

- B. microti (rodent strain)
- B. clivergens (cattle strain)
- B. bovis (cattle strain)

HISTORY AND DISTRIBUTION

Babesia is so named after Babes, who in 1888 described the intraerythrocytic parasite in the blood of cattle and sheep in Romania.

- In 1893, the parasite was shown to cause the tick-borne disease, Texas fever, an acute hemolytic disease of cattle in southern United States of America (USA).

- This was the first arthropod-borne disease to have been identified.
- In 2009, more than 700 cases were reported from endemic state of USA.
- Prevalence of B. microti is underestimated because young healthy individuals typically experience a mild, self-limiting disease and may not seek medical attention.

HABITAT

The parasite is present in erythrocytes and resembles the ring stage of P. falciparum.

MORPHOLOGY

Trophozoites are pleomorphic 2–5 µm in diameter found inside the red cells. The shape may be pyriform, ameboid, or spindle-like, usually in pairs and are often mistaken as ring form of Plasmodium (Fig. 16).

Merozoites may be spherical or oval or pyriform bodies, found in pairs.

LIFE CYCLE

Definitive Host

Ixodid ticks.

Intermediate Host

Man or other mammals.

 Infective Form

Sporozoites are the infective form for humans.

Mode of Transmission

Infection in vertebrate occurs through bite of the nympha stage of Ixodid ticks. Transmission occurs during May to

Fig. 16: Trophozoites of Babesia microti in human blood smear
Malaria and Babesia

September. Incubation period is 1–6 weeks. Babesiosis can also be transmitted via blood transfusion. Transovarian transmission in ticks also occurs.

- In their life cycle, merogony takes place in vertebrate hosts and sporogony in the invertebrates.
- Man acquires infection by bite of the infected ticks (definitive host).
- Sporozoites present in the salivary glands of tick are introduced in man or other mammals (intermediate host).
- Sporozoites change to trophozoites in the circulation, which then invade the erythrocytes and invade new erythrocytes.
- Some of the sporozoites grow slowly inside red cells and become folded like an accordion. These are thought be gametocytes.
- Female ticks become infected by feeding the host blood.
- In the digestive tract of tick, the gametocytes multiply sexually and later migrate to the salivary glands where they divide by multiple fission into smaller forms known as “vermicules”.
- Vermicules undergo secondary schizogony to produce sporozoites, which are the infective forms for human.

PATHOGENICITY AND CLINICAL FEATURES

Hemolysis of the infected erythrocytes is primarily responsible for many clinical manifestations.
- There is accumulation of parasites in the capillaries of liver, spleen and kidneys which leads to cellular degeneration and necrosis.
- The illness develops 1–6 weeks after the tick bite.
- This may be subclinical or mild self-limiting or acute illness, resembling malaria.
- In acute disease, there is malaise, fatigue, fever, myalgia, arthralgia, dry cough and anorexia. Fever exceeds 38°C and can reach 40.6°C accompanied by chill and sweat.
- Less common syndromes are neck stiffness, sore throat, abdominal pain, jaundice and anemia.
- Severe babesiosis is associated with parasitemia levels of more than 4% infected RBCs and requires hospitalization. Fatality rate is 5% among hospitalized cases but is higher (20%) among immunocompromised patients.
- Complications of acute babesiosis are renal failure, disseminated intravascular coagulation (DIC), acute respiratory distress syndrome (ARDS) and congestive cardiac failure (CCF).
- Risk factors for complication are severe anemia (<10 g%) and high levels of parasitemia.

LABORATORY DIAGNOSIS

Microscopy

Diagnosis of babesiosis is primarily done by examination of blood films stained with Leishman or Giemsa stain.
- Babesia appears as intraerythrocytic round or pyriform, or ring form simulating P. falciparum (Fig. 16).
- The ring forms are the most common and lacks the central hemozoin deposit, typical of P. falciparum.
- Other distinguishing features are the absence of schizonts and gametocytes and presence of tetrads (maltese crosses), which are pathognomonic of B. microti or B. duncani (Table 6).

Polymerase Chain Reaction

If parasite cannot be identified by microscopy, amplification of babesial 18S rRNA by PCR is recommended.

Serology

It is useful to confirm the diagnosis. An IFA for B. microti is available.
- Immunoglobulin M titer of more than 1:64 and IgG titer more than 1:1024, signify active or recent infection. Titer declines over 6–12 months.

Blood Picture

Parasitemia levels typically range from 1% to 20% in immunocompetent patients but can reach up to 85% in asplenic patients.

Table 6: Differential features of malaria and babesiosis

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Malaria</th>
<th>Babesiosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distribution</td>
<td>Worldwide</td>
<td>North America and Europe</td>
</tr>
<tr>
<td>Vector</td>
<td>Anopheles mosquito</td>
<td>Tick</td>
</tr>
<tr>
<td>Reservoir</td>
<td>Man</td>
<td>Rodent and cattle</td>
</tr>
<tr>
<td>No. of parasites per red blood cell (RBC)</td>
<td>1–3</td>
<td>1–12</td>
</tr>
<tr>
<td>Schizont</td>
<td>Present</td>
<td>Absent</td>
</tr>
<tr>
<td>Gametocyte</td>
<td>Present</td>
<td>Absent</td>
</tr>
<tr>
<td>Pigment in trophozoite</td>
<td>Present</td>
<td>Absent</td>
</tr>
<tr>
<td>Antigenic variation</td>
<td>None</td>
<td>Profound</td>
</tr>
<tr>
<td>Level of parasitemia</td>
<td>Correlate with severity of disease</td>
<td>Does not correlate with severity of disease</td>
</tr>
<tr>
<td>Animal inoculation</td>
<td>Negative</td>
<td>Positive</td>
</tr>
</tbody>
</table>
Reticulocyte count is elevated.
Thrombocytopenia is common.
White blood cell count may be normal or slightly decreased.

Other Tests
Liver function tests such as serum glutamic pyruvate transaminase (SGPT) and alkaline phosphatase yield elevated value.
Urine analysis may detect hemoglobinuria, excess urobilinogen and proteinuria.
In renal complications, increased blood urea nitrogen (BUN) and serum creatinine are found.

TREATMENT
B. microti infection appears to be mild and self-limiting. Most of the patients recover without any specific chemotherapy, with only symptomatic treatment.
In acute cases chemotherapy is required.
Atovaquone 750 mg twice daily, along with azithromycin 500 mg–1 g/day for a period of 7–10 days is effective. Alternatively, clindamycin (300–600 mg, 6 hourly) along with quinine (650 mg 6–8 hourly) may be given intravenously.
In fulminant cases, exchange transfusion is recommended.

PROPHYLAXIS
No vaccine is available at present. There is no role of chemotherapy. Individuals who reside or travel in endemic areas, should wear protective clothing and apply tick repellents.
Individuals with history of symptomatic babesiosis or with positive antibody titer should be indefinitely deferred from donating blood.

REVIEWS QUESTIONS
1. Describe briefly the life cycle and laboratory diagnosis of:
   a. Plasmodium vivax
   b. Plasmodium falciparum
2. Write short notes on:
   a. Clinical features of malaria
   b. Cerebral malaria
   c. Blackwater fever
   d. Malignant tertian malaria
   e. Prophylaxis of malaria
   f. Treatment of malaria
   g. Rapid detection test
   h. Babesiosis
3. Differentiate between:
   a. Different malarial parasites
   b. Recrudescence and relapse
   c. Malaria and Babesiosis
Malaria and Babesia

MULTIPLE CHOICE QUESTIONS

1. Old RBCs are preferentially infected by
   a. Plasmodium falciparum
   b. Plasmodium malariae
   c. Plasmodium vivax
   d. Plasmodium ovale

2. The infective form of the malaria parasite is
   a. Oocyst
   b. Sporozoite
   c. Bradyzoite
   d. Tachyzoite

3. Prolonged parasitism in malaria is due to
   a. Antigenic variation
   b. Intracellularity of parasite
   c. Immunosuppression
   d. Sequestration

4. Malaria pigment is formed by
   a. Parasite
   b. Bilirubin
   c. Hemoglobin
   d. All of the above

5. Schuffner’s dot in RBCs are seen in infection with
   a. Plasmodium vivax
   b. Plasmodium falciparum
   c. Plasmodium malariae
   d. Plasmodium ovale

6. Quartan malaria is caused by
   a. Plasmodium vivax
   b. Plasmodium falciparum
   c. Plasmodium malariae
   d. Plasmodium ovale

7. Schizonts of Plasmodium falciparum are not found in peripheral blood because
   a. Schizonts are absent in the life cycle
   b. Schizonts are killed by antibodies
   c. Schizonts develop only in capillaries of internal organs
   d. None of the above

8. Crescent-shaped or banana-shaped gametocytes are seen in infection with
   a. Plasmodium vivax
   b. Plasmodium falciparum
   c. Plasmodium malariae
   d. Plasmodium ovale

9. Malaria is not seen in patients with
   a. G6PD deficiency
   b. Sickle cell trait
   c. Duffy negative blood group
   d. All of the above

10. Which plasmodial infection is more often associated with nephritic syndrome
    a. Plasmodium vivax
    b. Plasmodium falciparum
    c. Plasmodium malariae
    d. Plasmodium ovale

11. Which is the treatment of choice for benign tertian malaria
    a. Sulfamethoxazole – pyrimethamine
    b. Quinine
    c. Mefloquine
    d. Chloroquine

12. Gametocidal pernicious malaria may occur in
    a. Plasmodium vivax
    b. Plasmodium falciparum
    c. Plasmodium malariae
    d. Plasmodium ovale

13. Babesiosis is transmitted by
    a. Ticks
    b. Mites
    c. Flea
    d. Mosquito

14. Maltose cross is a characteristic feature of
    a. Cryptococcus neoformans
    b. Babesia microti
    c. Blastomycosis
    d. Micrococcus

Answer