LAB-8-
PARASITOLOGY

Submitted by

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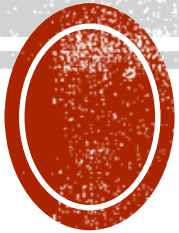


Plasmodium Spp.
Toxoplasma gondi

Plasmodium Spp. (malaria)

There are four species infecting humans, namely,

- Plasmodium falciparum(Malignant tertian malaria)
- Plasmodium vivax (Benign malaria)
- Plasmodium ovale (Ovale tertian Malaria)
- Plasmodium malaria (Quartun Malaria)

- The life cycle of malaria is passed in two hosts (alternation of hosts) and has sexual and asexual stage.

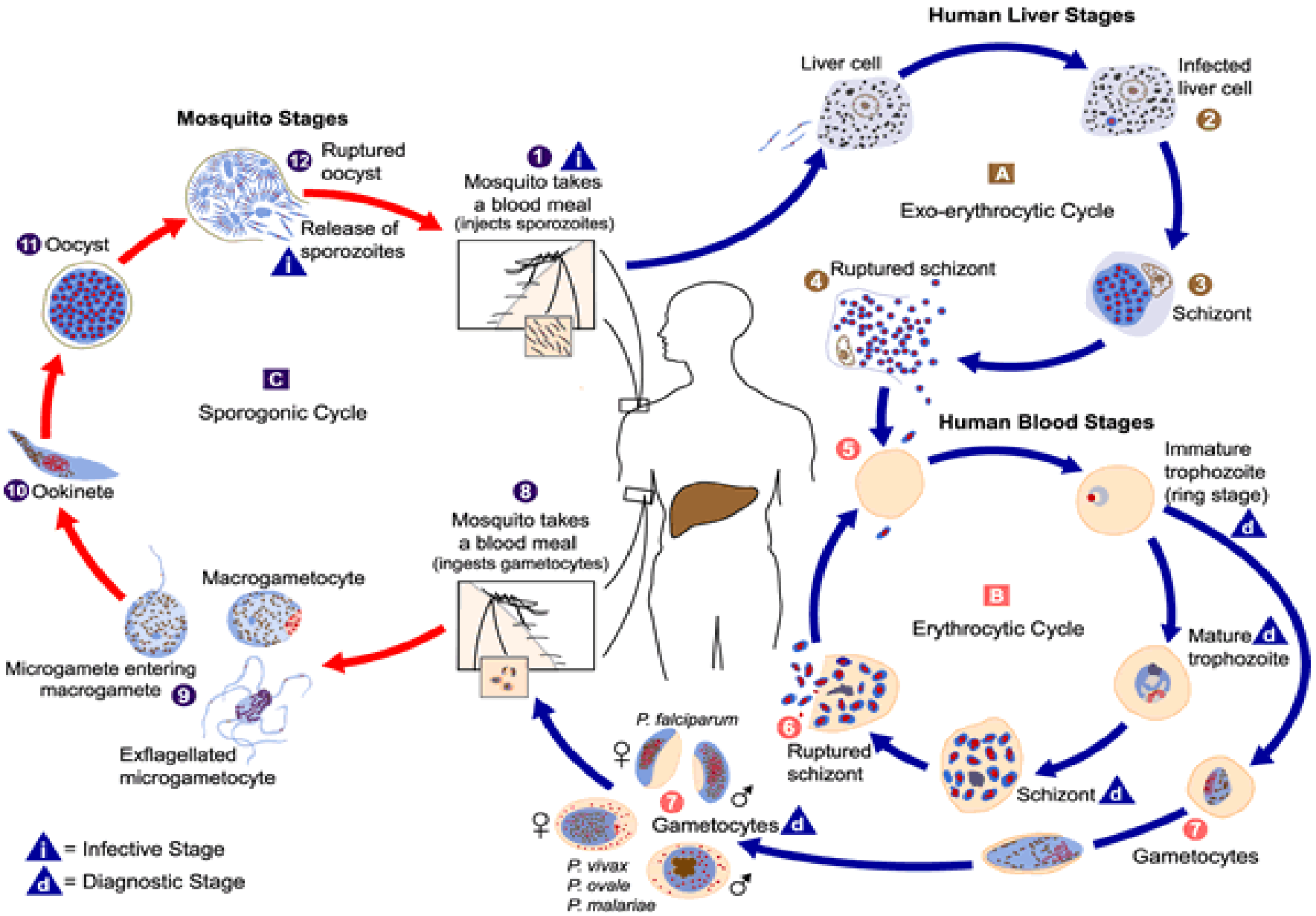
  (alternation of generations).
- Vertebrate host - man (intermediate host), where the asexual cycle takes place.
- The parasite multiplies by schizogony and there is formation of male and female gametocytes (gametogony).
- Invertebrate host - mosquito (definitive host) where the sexual cycle takes place. Union of male and female gametes ends in the formation of sporozoites (sporogony).
- **The life cycle passes in three stages:**
- in man:- Pre or Exo - erythrocytic schizogony,

  - Erythrocytic schizogony
- One in mosquito - Sporogony

- when an infective female Anopheles mosquito bites man, it inoculates saliva containing sporozoites (infective stage).
- Pre-Erythrocytic Schizogony Sporozoites reach the blood stream and within30 minutes enter the parenchymal cells of the liver, initiating a cycle of schizogony. Multiplication occurs in tissue schizonts, to form thousands of tiny merozoites.
- Merozoites are then liberated on rupture of schizonts about 7^{th} – 9^{th} day of the bites and enter into the blood stream. These merozoites either invade the RBC's or other parenchymal liver cells. In case of *P. falciparum* and *P. malariae*, all merozoites invade RBC's without liver cells.
- *P. vivax* and *P. ovale*, some merozoites invade RBC's and some re-invade liver cells initiating further *Exo-erythrocytic* schizogony, which is responsible for relapses. Some of the merozoites remain dormant (hypnozoite) becoming active later on

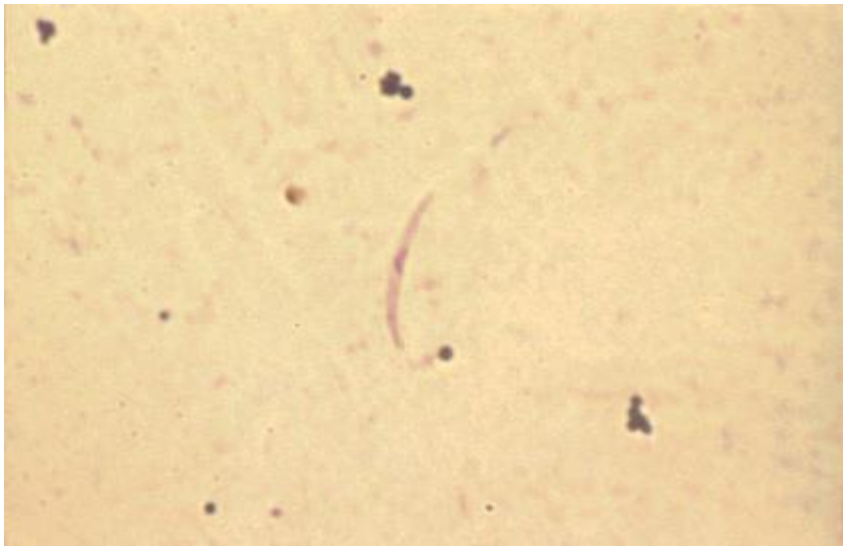
- Erythrocytic schizogony (blood phase) is completed in 48 hrs in *P. vivax, P. ovale,* and *P. falciparum*, and 72 hrs in *P. malariae*. The merozoites reinvade fresh RBC's repeating the schizogony cycles
- Erythrocytic merozoites do not reinvade the liver cells. So malaria transmitted by blood transfusion reproduces only erythrocytic cycle
- **Gametogony**
- Some merozoites that invade RBC's develop into sexual stages (male and female gametocytes). These undergo development until taken by the mosquito.

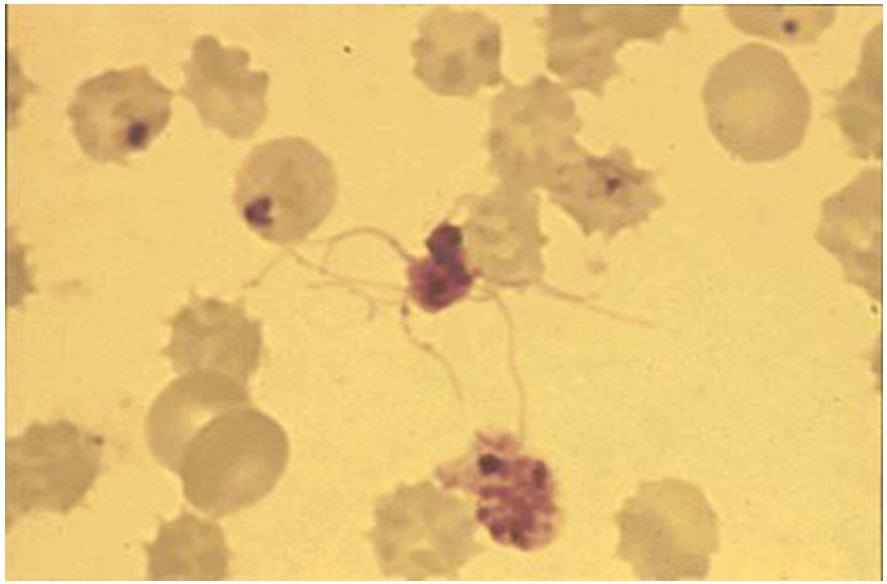
- ***Sporogony***
- When a female Anopheles mosquito vector bites an infected person, it sucks blood containing the different stages of malaria parasite. All stages other thangametocytes are digested in the stomach.
- The microgametocyte (male)undergoes ex-flagellation. the microgametes, are actively motile and separate from the gametocyte.
- The macrogametocyte by reduction division becomes a macrogamete.
- Fertilization occurs by entry of a micro gamete into the macro gamete forming a zygote.The zygote changes into a worm like form, the ookinete, which penetrates thewall of the stomach to develop into a spherical oocyst between the epitheliumand basement membrane. The oocystes increase in size. Thousands ofsporozoites develop inside the oocysts. Oocysts rupture and sporozoites areliberated in the body cavity and migrate everywhere particularly to the salivaryglands.
- Now the mosquito is infectiveThe sporogonous cycle in the mosquito takes 8-12 days depending onTemperature



life cycle of *Plasmodium spp.*

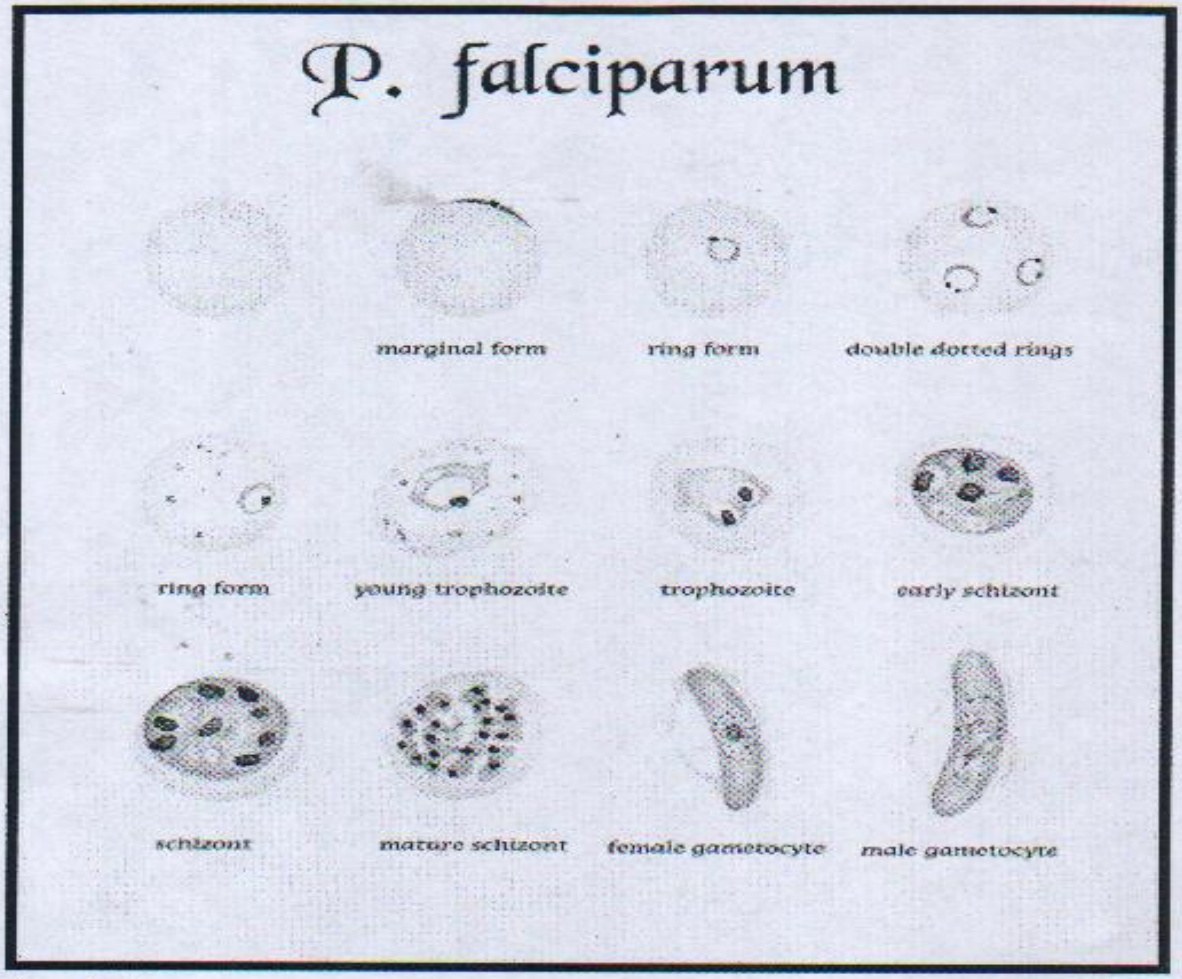
Fig : Anopheles Female (Mosquito)
The vector of malaria





Plasmodium spp Exflagellation

Fig : some stages of Plasmodium spp in insect



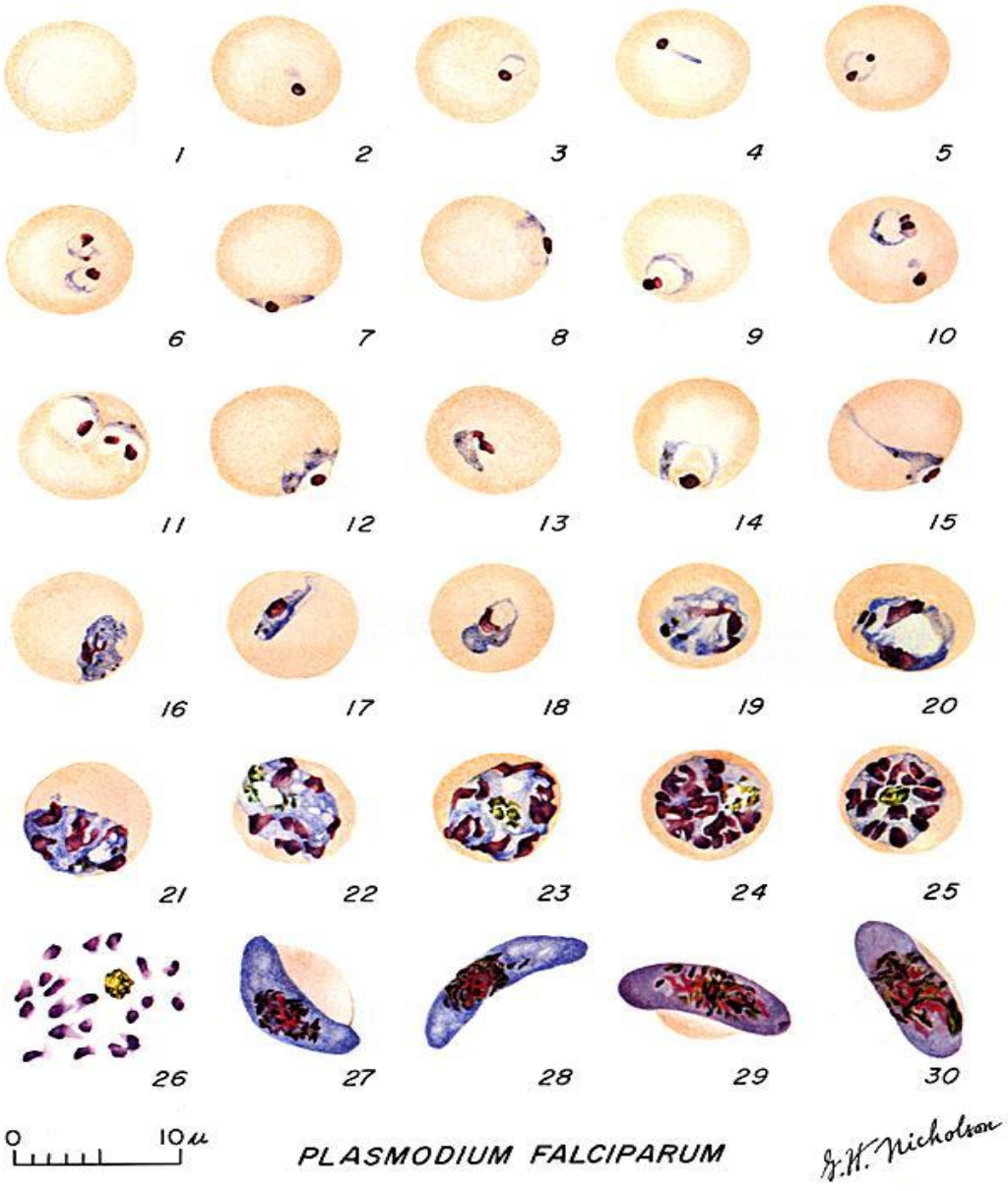
Diagnostic points:-

1. Red Cells are not enlarged.
2. Rings appear fine and delicate and there may be several in one cell.
3. Some rings may have two chromatin dots.
4. Presence of marginal or applique forms.
5. It is unusual to see developing forms in peripheral blood films.
6. Gametocytes have a characteristic crescent shape appearance.
   However, they do not usually appear in the blood for the first four weeks of infection.
7. Maurer's dots may be present.

- **Clinical features**
- *P.falciparum* rapidly produces daily chills and fever as well as severe nausea, vomiting an ddiarrhea. Involvement of the brain (cerebral malaria) is most often seen in *P.falciparum* infection. Capillary plugging from an adhesion of infected red blood cells with each other and endothelial linings of capillaries causes hypoxic injury to the brain that can result in coma and death.
- Kidney damage is also associated with *P.falciparum* malaria, resulting in an illness called “black water” fever. Intravascular hemolysis with rapid destruction of red blood cells produces a marked hemoglobinuria and can result in acute renal failure, tubular necrosis, nephrotic syndrome, and death. Liver involvement is characterized by abdominal pain, vomiting of bile, hepatosplenomegally, severe diarrhea, and rapid dehydration.

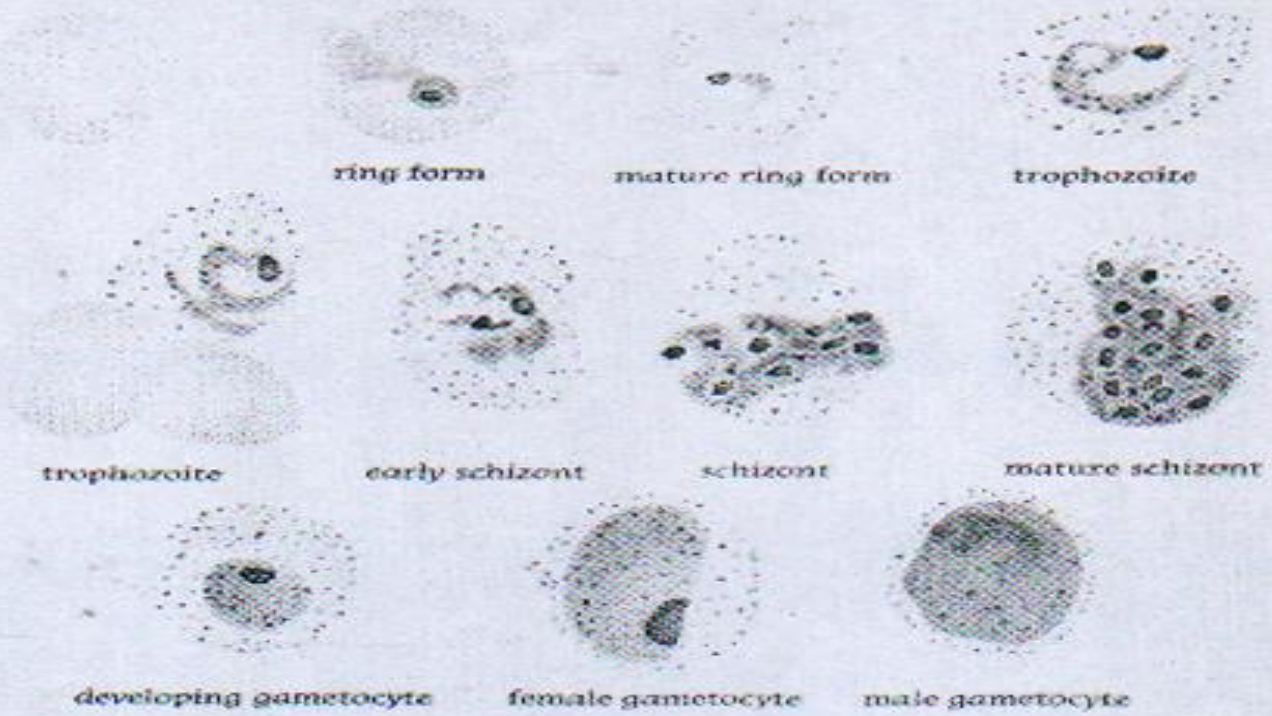
Normal red cell;

- Figs. 2-18: Trophozoites
- Figs. 2-10 correspond to ring-stage trophozoites)
- Figs. 19-26: Schizonts
- Fig. 26 is a ruptured schizont
- Figs. 27, 28: Mature macrogametocytes (female);
- Figs. 29, 30: Mature microgametocytes (male)



- **Epidemiology**
- *P.falciparum* occurs in tropical and subtropical regions. Weather (rainfall, temperature & humidity) ,To date, abnormal weather conditions are also important causes of significant and widespread epidemics. Moreover, drug-resistant infection of *P.falciparum* is the commonest challenge in many parts of the world. In Ethiopia,
- even though all the four species of plasmodium infecting man have been recorded,

P. vivax

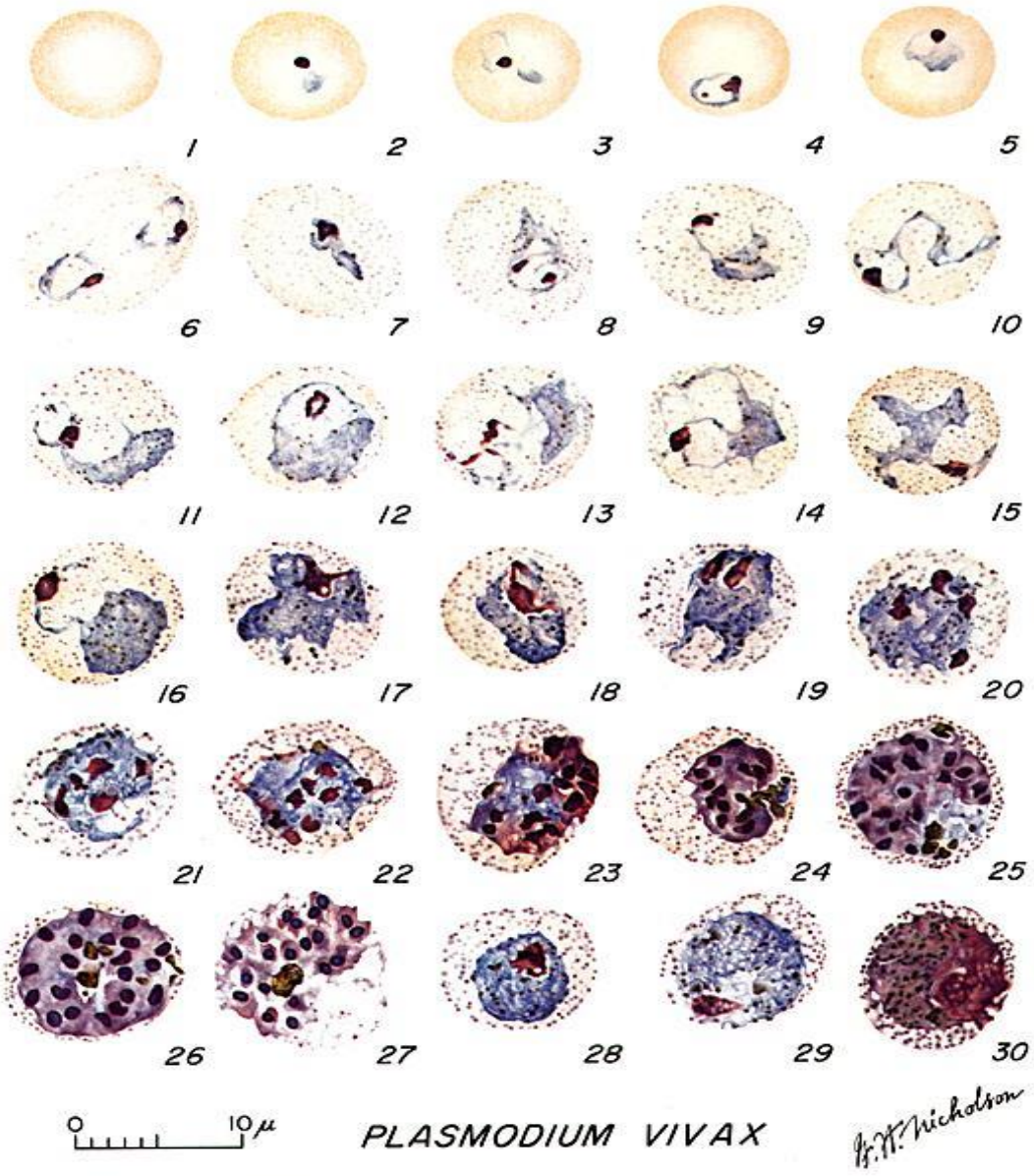


Diagnostic points:-

1. Red cells containing parasites are usually enlarged.
2. Schuffner's dots are frequently present in the red cells as shown above.
3. The mature ring forms tend to be large and coarse.
4. Developing forms are frequently present.

Normal red cell;

- Figs. 2-6: Young trophozoites (ring stage parasites);
- Figs. 7-18: Trophozoites;
- Figs. 19-27: Schizonts;
- Figs. 28 and 29: Macrogametocytes (female);
- Fig. 30: Microgametocyte (male)

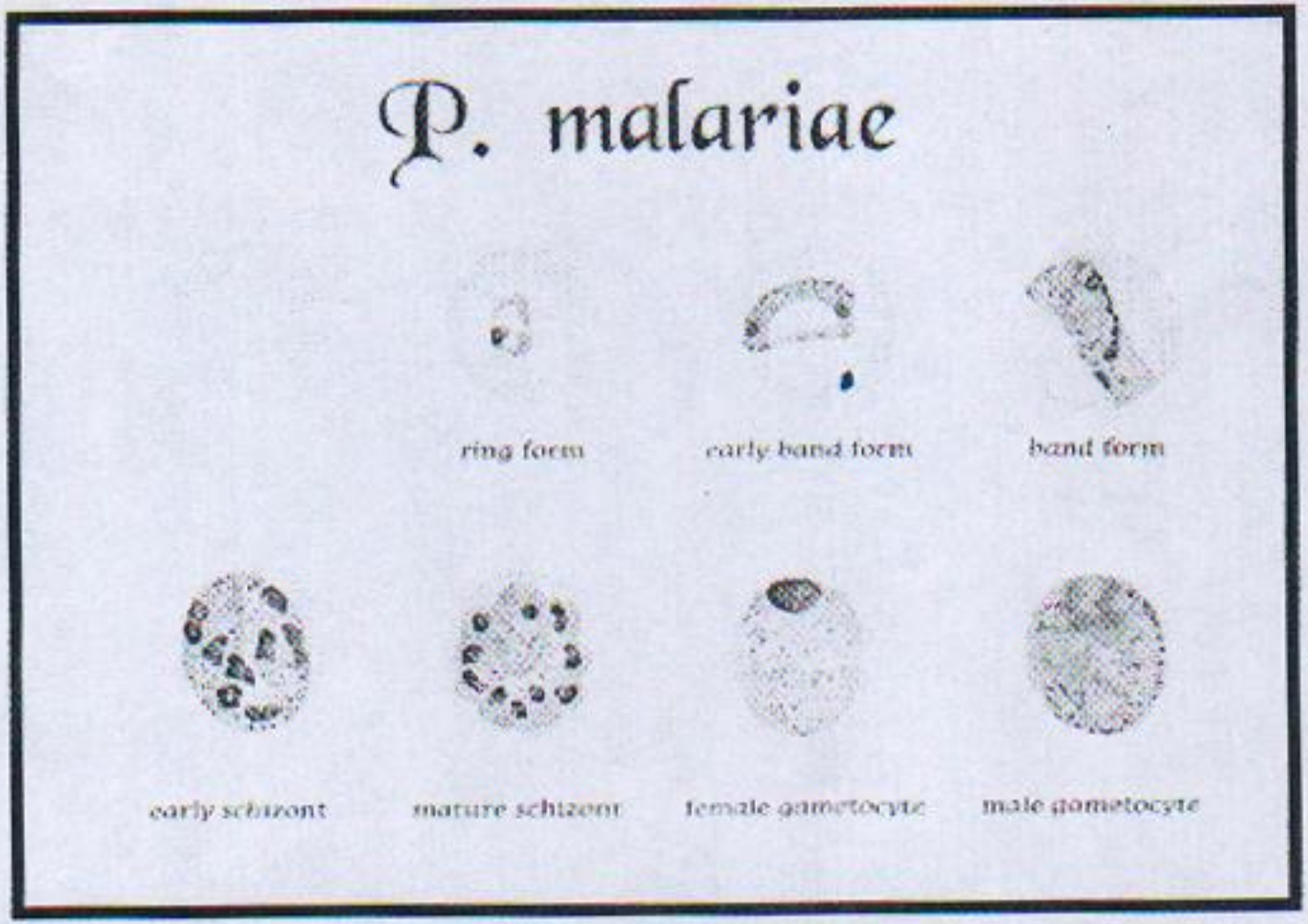


- **Clinical features**
- the patient experiences vague flu-like symptoms, such as headache, muscle pains, photophobia, anorexia,nausea and vomiting. increased numbers of rupturing erythrocytes liberate merozoites as well as toxic cellular debris and hemoglobin in to circulation.
- In combination, these substances produce the typical pattern chills,

  fever and malarial rigors. These paroxysms usually reappear periodically (generally every 48 hours) as the cycle of infection, replication,
- The paroxysms may remain relatively mild or may progress to severe attacks, with hours of sweating, chills, shaking persistently, high temperatures .
- Since *P.vivax* infects only the reticulocytes, the
- Parasitemia is usually limited to around 2 to 5% of the available RBCs.

- **Epidemiology**
- *P. Vivax* is the most prevalent of the human plasmodia with the widest geographic distribution, including the tropics, subtropics, and temperate regions. However,
- it is the second most prevalent in Ethiopia following *P. falciparum*

P. malariae

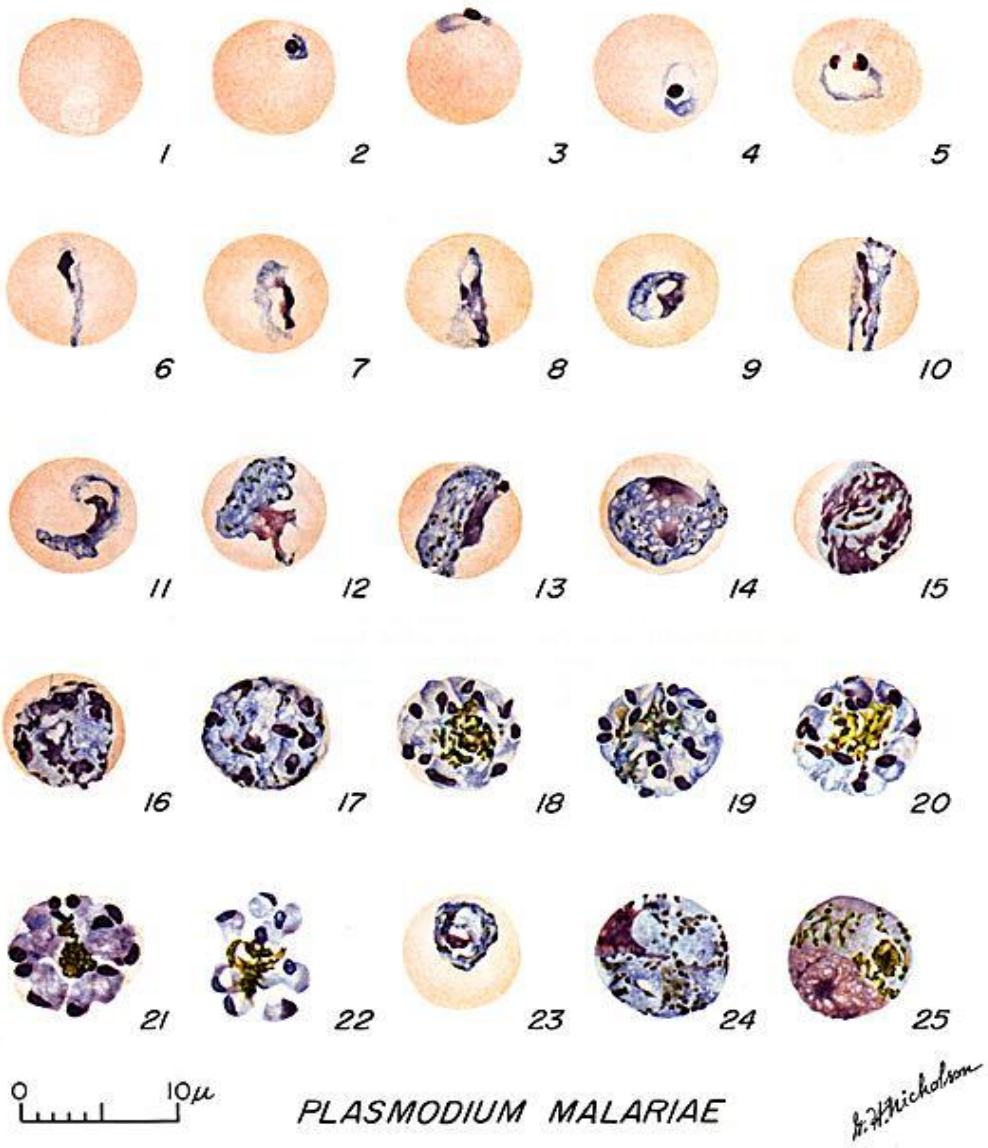


Diagnostic points :-

1. Ring forms may have a squarish appearance.
2. Band forms are a characteristic of this species.

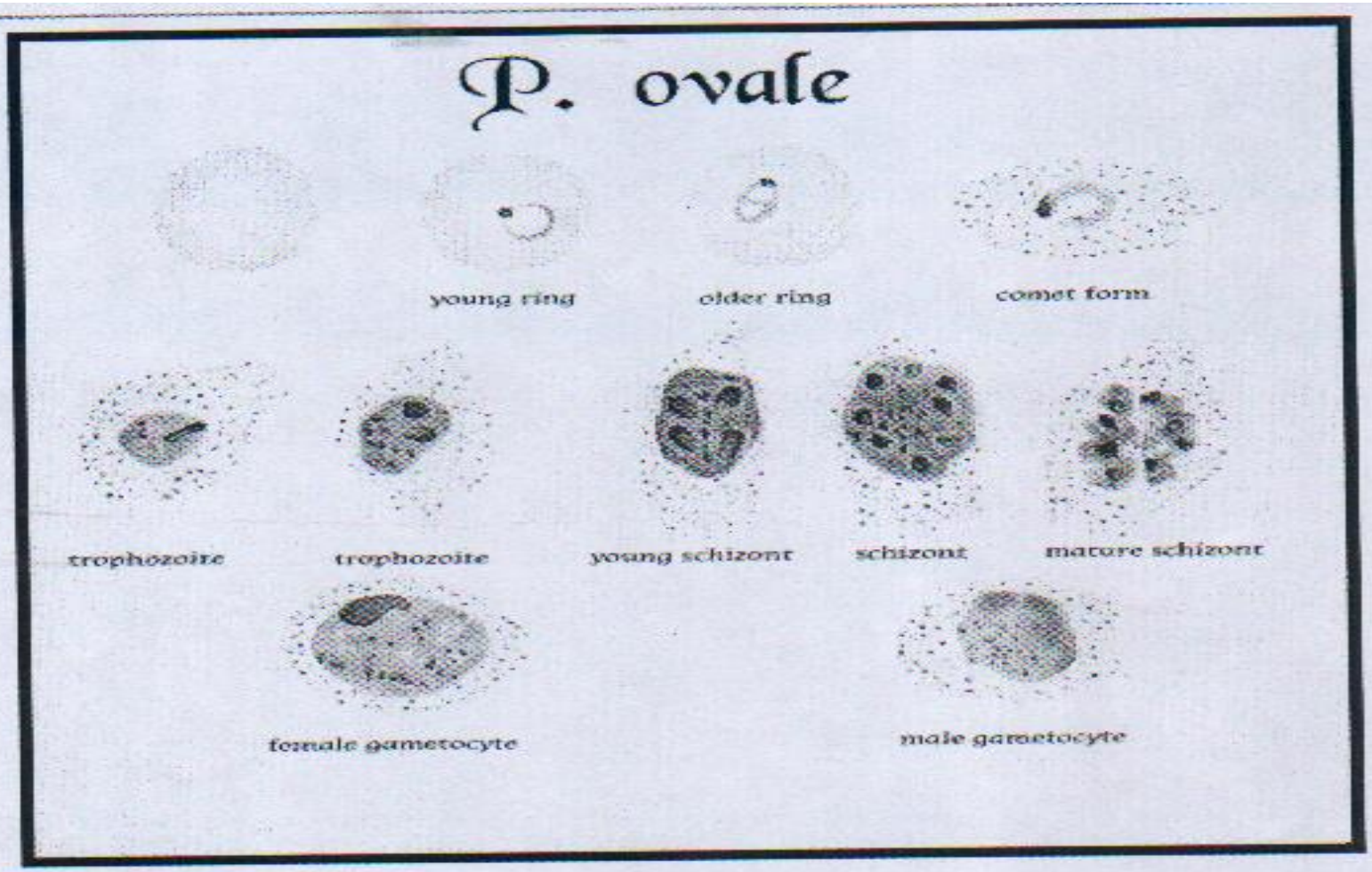
Normal red cell;

- Figs. 2-5: Young trophozoites (rings);
- Figs. 6-13: Trophozoites;
- Figs. 14-22: Schizonts;
- Fig. 23: Developing gametocyte.
- Fig. 24: Macrogametocyte (female);
- Fig. 25: Microgametocyte (male).



- **Clinical features**
- The early symptoms are flu-like with fever patterns of 72 hours (quartan or malarial) in periodicity.

- **Epidemiology**
- *P. malariae* infection occurs primarily in the same sub-tropical and temperateregions as infections with the other plasmodia but is less prevalent

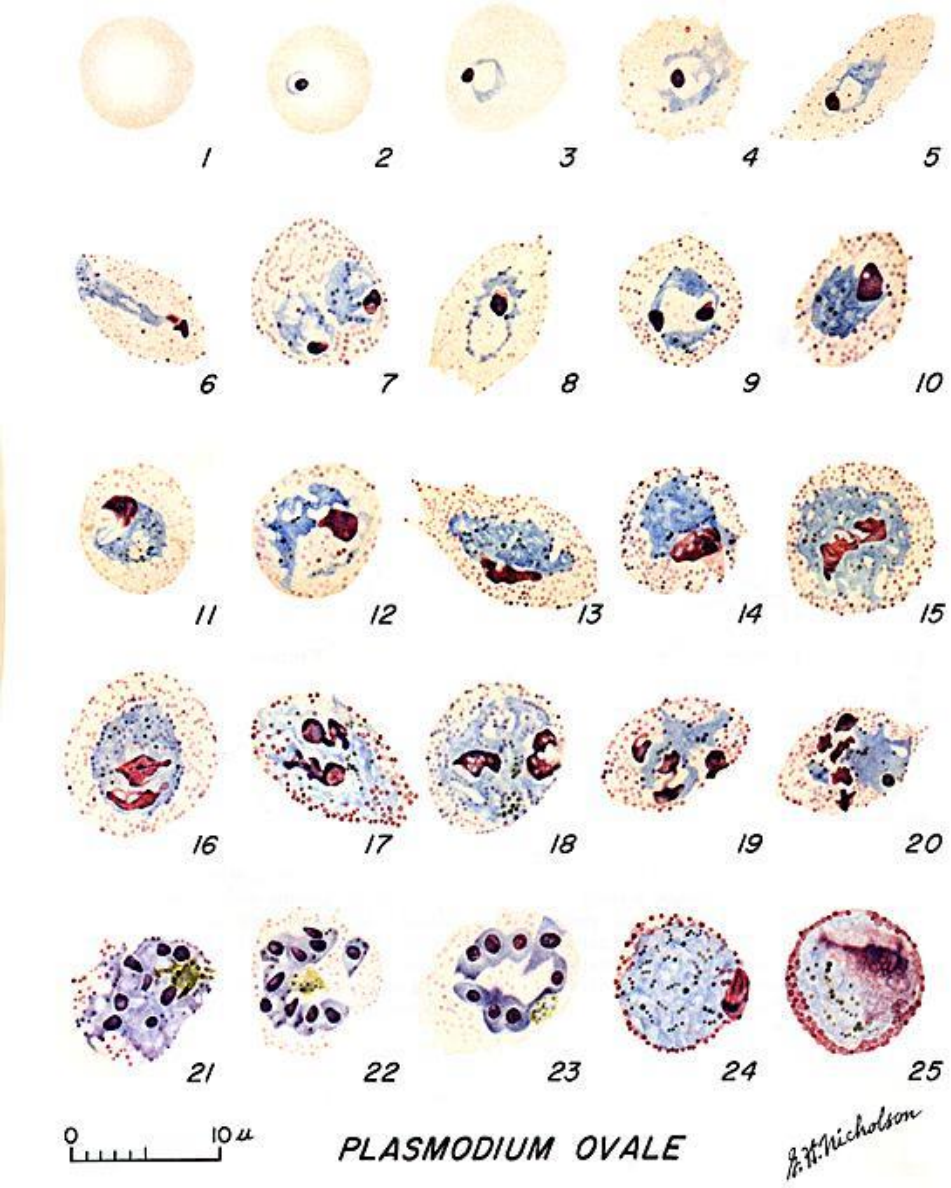


Diagnostic points :-

1. Red cells enlarged.
2. Comet forms common (top right)
3. Rings large and coarse.
4. Schaffer's dots, when present, may be prominent.
5. Mature schizonts similar to those of P. *malariae* but larger and more coarse

Normal red cell;

- Figs. 2-5: Young trophozoites (Rings);
- Figs. 6-15: Trophozoites;
- Figs. 16-23: Schizonts;
- Fig. 24: Macrogametocytes (female);
- Fig. 25: Microgametocyte (male)

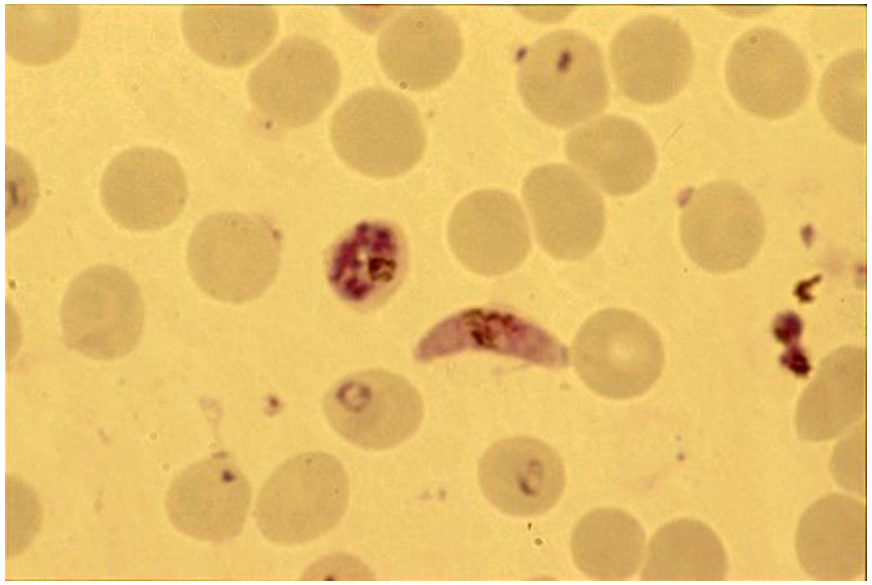


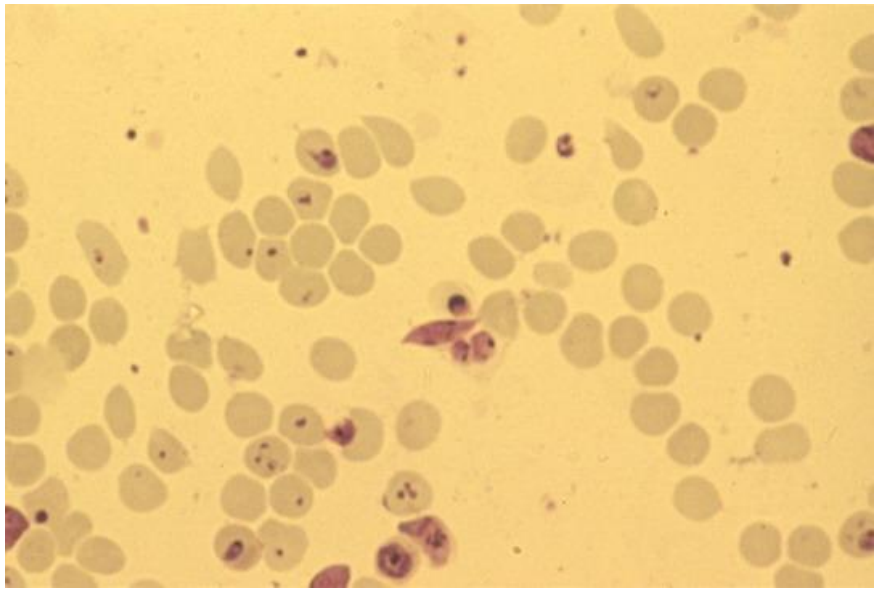
- **Clinical features**
- Clinically, ovalemalaria resembles vivax malaria with attacks recurring every 48-50 hours. There are however, fewer relapses with P.ovale. Less than 2% of RBCs usually become infected.
- **Epidemiology**
- *P.ovale* is distributed primarily in tropical Africa. It is also found in Asia and South America.

Table 3 . Selected Characteristics of the Four Species of Human Malaria

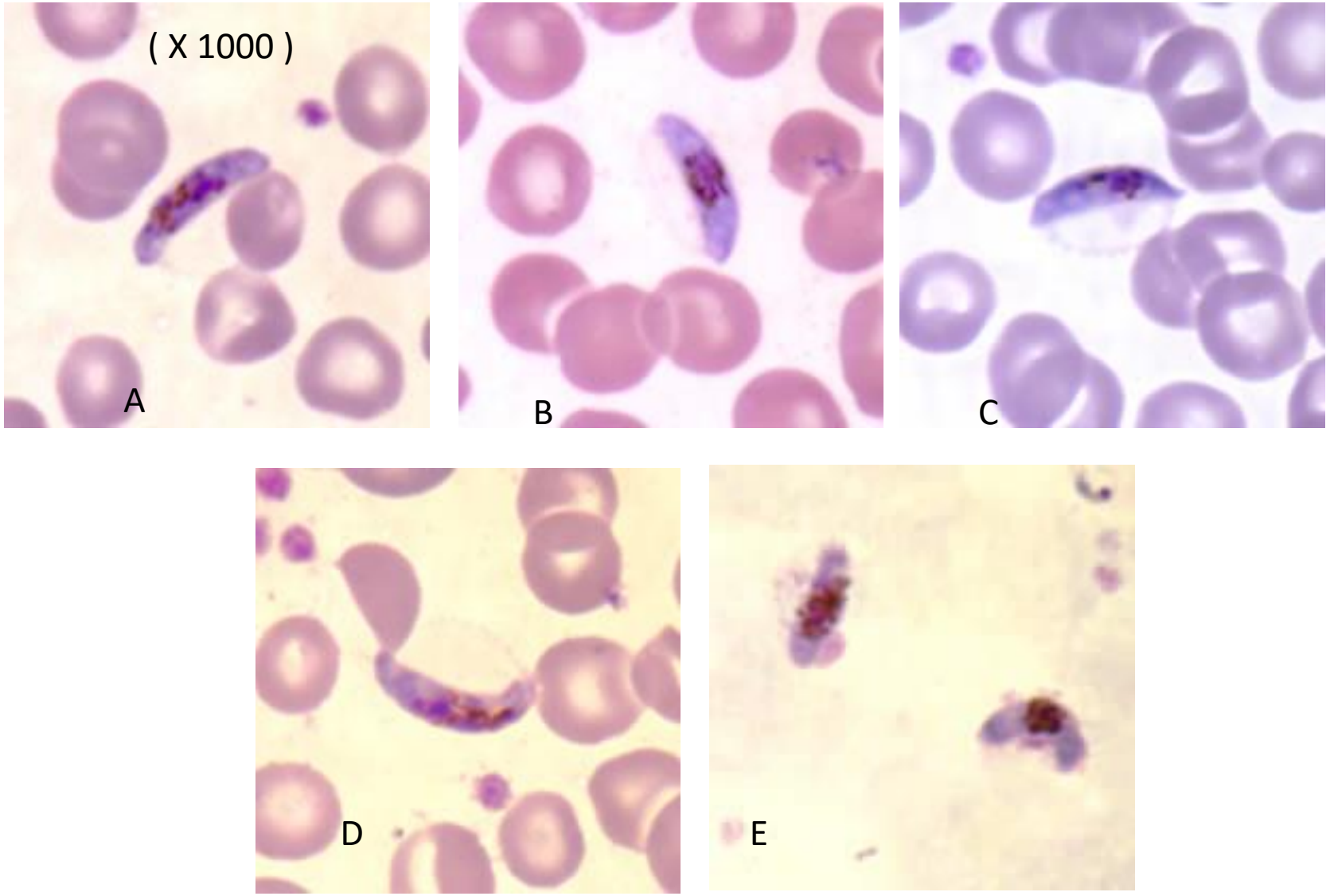
	P. falciparum	P. vivax	P. ovale	P. malariae
Incubation days (range)	12 (9-14)	13 (12-17) or up to 6-12 months	17 (16-18) or longer	28 (18-40) or longer
Exoerythrocytic cycle (days)	5.5-7	6-8	9	12-16
No. of merozoites per liver cell	40,000	10,000	15,000	2,000
Erythrocytic cycle (hours)	48	42-48	49-50	72
Red blood cell preference	younger cells, but can invade cells of all ages	Reticulocytes	Reticulocytes	Older cells
Relapses	No	Yes	Yes	No
Fever periodicity (hours)	none	48	48	72
Febrile paroxysm length (hours)	16-36 or longer	8-12	8-12	8-10
Severity of primary attack	severe in non-immune	mild to severe	mild	mild
Drug Resistance	++	+	—	—

- **Laboratory diagnosis**
- Microscopic examination of thick and thin films of blood is the method of choice for confirming the clinical diagnosis of malaria and identifying the specific speciesresponsible for disease.
- Malaria parasites in thick and thin blood films are best stained at pH 7.1 – 7.2 using a Romanowsky stain (contains azure dyes and eosin).
- The thick film is a concentration method that may be used to detect the presence of organisms. The thin film is most useful for establishing species identification.
- Serologic procedures are available but they are used primarily for epidemiological surveys or for screening blood donors.





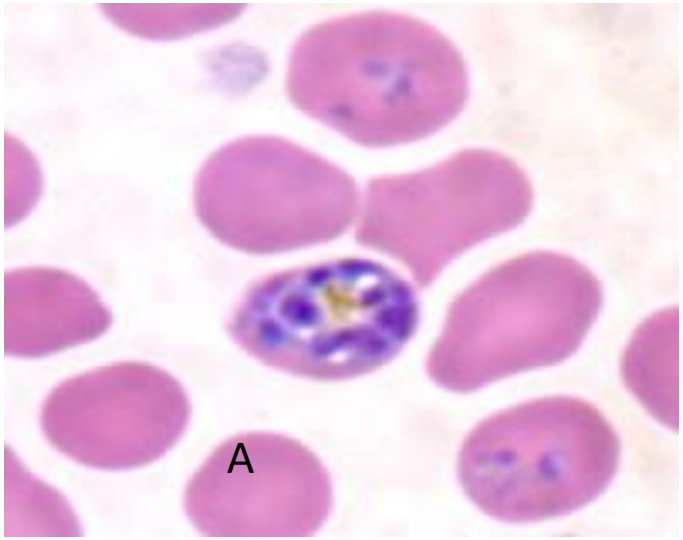
Plasmodium falciparum Blood Film

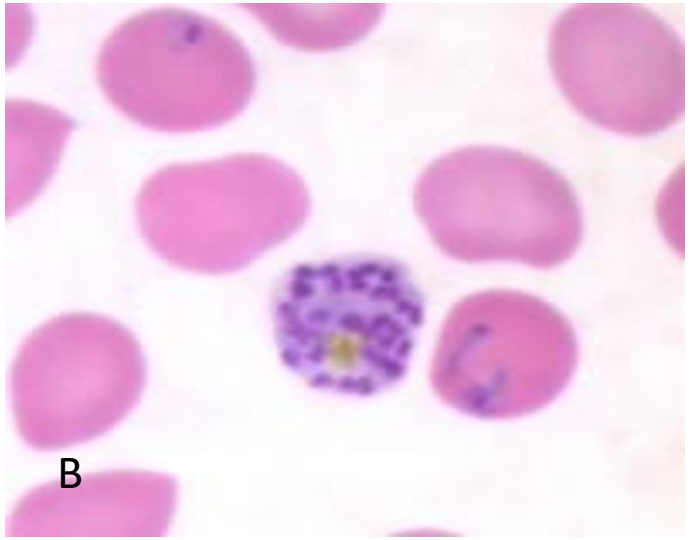


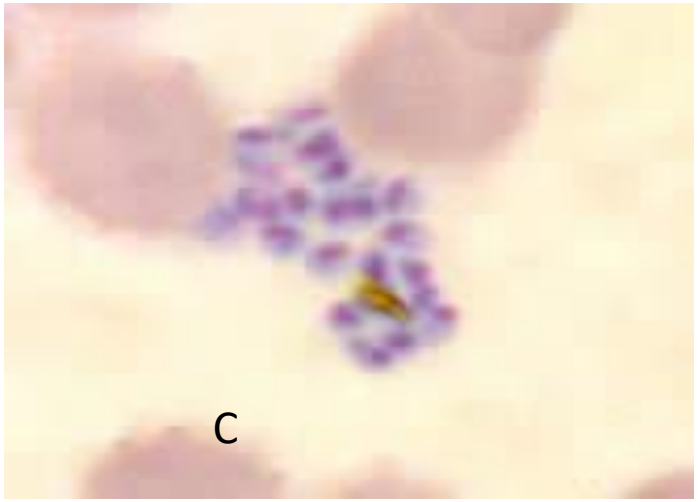
A, B, C, D: Gametocytes of P. falciparum in thin blood smears. Note the presence of a “Laveran’s bib”, which is not always visible

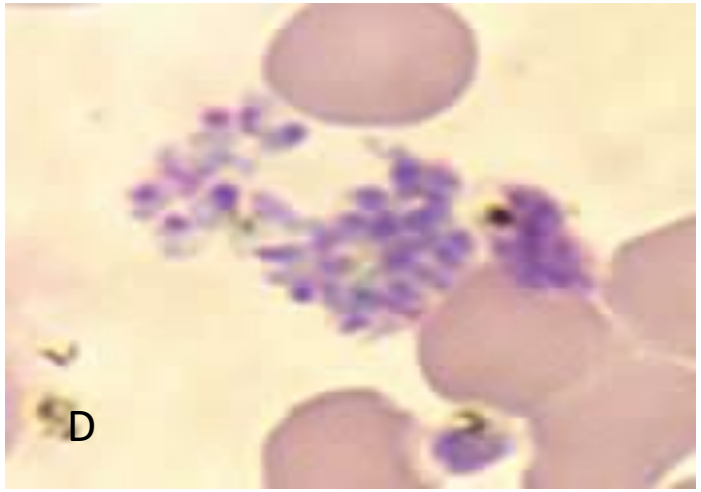
E: Two gametocytes captured from a thick blood smear

P. falciparum (X 1000)

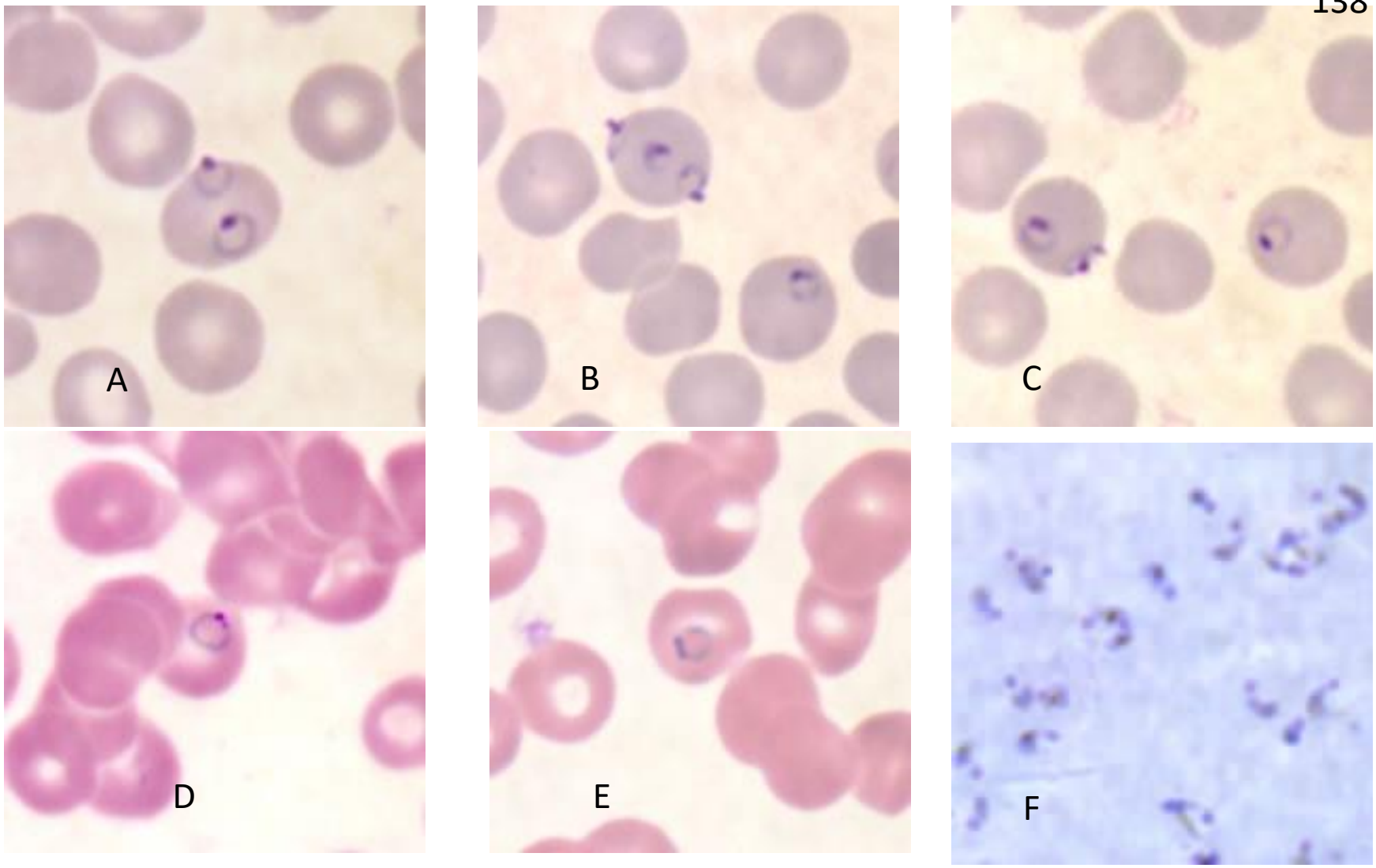








A: Immature schizont in a thin blood smear.
B: Mature schizont
C, D: Ruptured schizonts in a thin blood smear



A, B, C: Multiply infected red blood cells with appliqué forms in thin blood smears
D: Signet ring form.
E: Double chromatin dot
F: A thick blood smear showing many ring forms of P. falciparum (X 1000)