

Subphylum Sporozoa - Malaria

A. Introduction

Four species of malaria parasites infect humans, *Plasmodium vivax*, *P. ovale*, *P. malariae*, and *P. falciparum*. All are transmitted by female *Anopheles* sp. mosquitoes. Recent years have seen a resurgence in numbers of cases of malaria due to increased travel to malarious regions, political and civil unrest, development of drug resistance by malaria parasites, resistance to insecticides by mosquitoes, and government's failure to adequately fund control measures.

B. Life cycle:

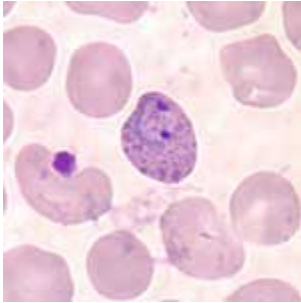
The mosquito injects sporozoites while feeding; these enter hepatocytes within 30 minutes and multiply asexually forming merozoites. Upon rupture of the infected hepatic cell, merozoites invade red blood cells and begin an erythrocytic cycle - rings to trophozoites to schizonts or gametocytes. Schizonts continue the erythrocytic cycle, whereas, gametocytes are picked up by female *Anopheles* spp. mosquitoes. In the mosquito, sexual reproduction takes place. Gametes are fertilized; the resulting motile ookinete penetrates the mosquito's stomach wall forming a zygote on the hemocele side of the stomach. Sporozoites develop within the zygote; this structure ruptures and escaping sporozoites invade the salivary gland of the mosquito.

C. Terms:

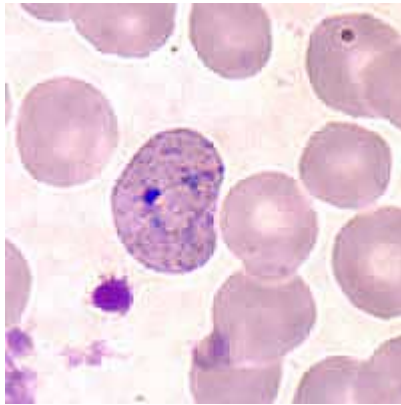
1. Ring stage - a young trophozoite, it still contains a vacuole.
2. Developing trophozoite – the vacuole is filled in as the organism feeds, adding to its bulk of cytoplasm; pigment begins to show up.
3. Pigment - product of the digestion of haemoglobin to hematin.
4. Schizont - the stage in which asexual reproduction takes place with the production of merozoites. Characterized by nuclear division and differentiation of cytoplasm around the nuclei.
5. Gametocyte - the stage picked up by the mosquito vector. This stage initiates sexual reproduction in the mosquito.
6. Sporozoite - the infective stage, a product of sexual reproduction; is present in the salivary gland of the vector mosquito.
7. Schuffner's stippling - a fine, granular eosinophilic stippling seen on the infected red blood cell. Seen only in *P. vivax* and *P. ovale* infections.
8. Maurer's clefts - heavy, clefts of eosinophilic stippling sometimes seen on infected red blood cells in *P. falciparum* infections. They are sparse in number.
9. Prepatent period – time from infection until first discovery of parasites in a blood film.
10. Incubation period – time from infection until first symptoms appear

- D. Components of the Malaria Parasite:
1. Cytoplasm - light blue staining component of parasite.
 2. Chromatin - reddish to purplish red staining nuclear material.
 3. Pigment - does not stain. Product of digestion of haemoglobin to hemozoin. Color varies with species present, generally brown to black.
- E. Diagnostic Features seen in Thin Blood Films:
1. Appearance of the Red Blood Cell:
 - a. Size - normal or enlarged.
 - b. Stippling - present or absent.
 2. Appearance of the Parasite:
 - a. Outline of the developing trophozoite - smooth and rounded or irregular (amoeboid).
 - b. Density of cytoplasm - compact and dark staining, or diffuse and light staining.
 - c. Amount & color of pigment - black, brown, etc.; heavy, light.
 - d. Number of merozoites in the mature schizont- a species-specific number.
 - e. Stages present - what stages are present; same stages or a variety.
- F. Diagnostic Features seen in Thick Blood Films:
1. Appearance of the Parasite: generally similar to that seen on thin films; the infected RBC has been lysed. The observer should still note the following:
 - a. Stages present - i.e., rings, trophozoites, schizonts, gametocytes, all.
 - b. Outline of the developing trophozoite – smooth and round or irregular.
 - c. Density of cytoplasm - diffuse or compact.
 - d. Amount & color of pigment – dense, sparse; black, brown.
 - e. Number of merozoites in the mature schizont – 9; 16; 24?
 - f. Shape of the gametocyte – round or banana shaped.

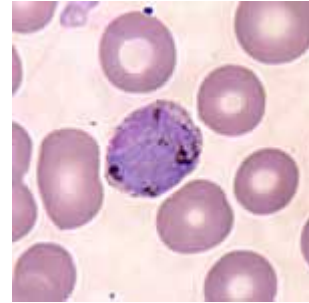
G. Characteristics of *Plasmodium vivax* ("benign tertian malaria")



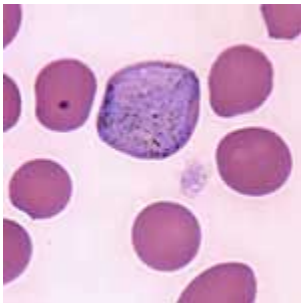
Plasmodium vivax old ring



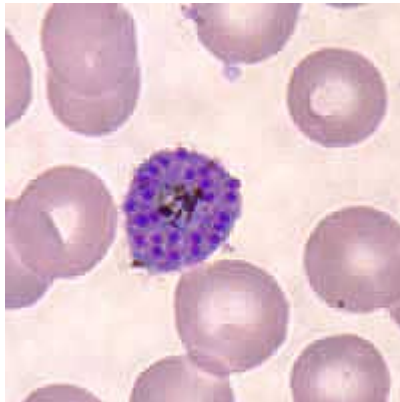
P. vivax developing trophozoite



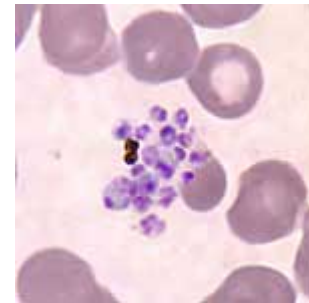
P. vivax gametocyte (female)



P. vivax gametocyte (male)



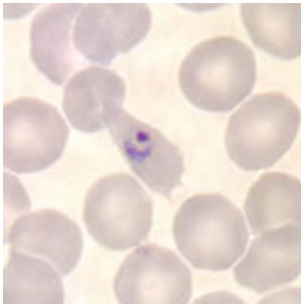
P. vivax mature schizont



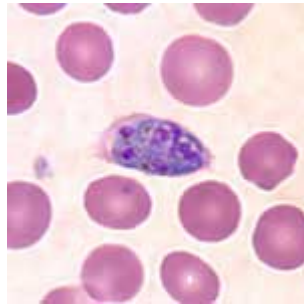
P. vivax schizont (breaking up)

1. Stages present in peripheral blood films - all can be present.
2. Size of infected red blood cells - usually enlarged.
3. Stippling - usually present if stained at a pH of 7.0 - 7.2.
4. Density of cytoplasm - diffuse, light staining
5. Pigment - usually present in older organisms, brownish-black to yellowish-black.
6. Number of merozoites in the mature schizont - average of 16.
7. Length of asexual cycle - 48 hours.
8. Prepatent period – 8 to 17 days.
9. Incubation period – 14 days.

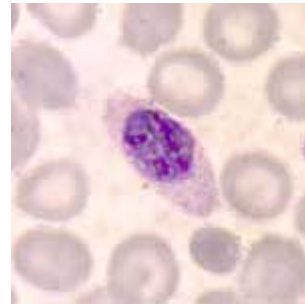
H. Characteristics of *Plasmodium ovale* (benign tertian malaria)



Plasmodium ovale ring



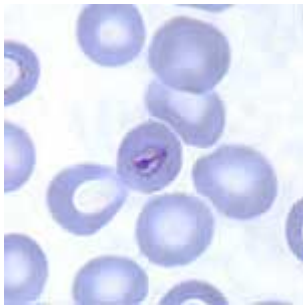
P. ovale immature schizont



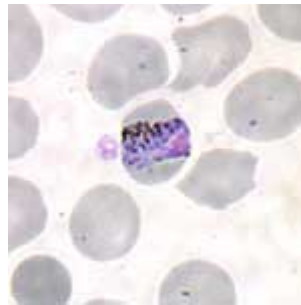
P. ovale immature schizont

1. Stages present in peripheral blood films - all can be present, but one stage usually is predominant.
2. Size of infected red blood cells - usually enlarged, often oval with fimbriated edges.
3. Stippling - usually present if stained at pH 7.0 - 7.2.
4. Density of cytoplasm - rounded and compact, therefore dark staining.
5. Pigment - usually present in older organisms, black to brownish-black.
6. Number of merozoites in the mature schizont - average of 9.
7. Length of asexual cycle - 48 hrs.
8. Prepatent period – 8 to 17 days.
9. Incubation period – 14 days.

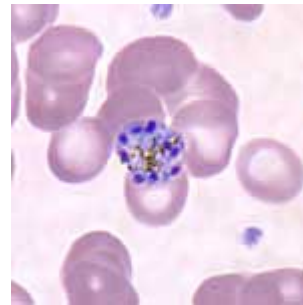
I. Characteristics of *Plasmodium malariae* (Quartan malaria)



Plasmodium malariae ring



P. malariae trophozoite (band)

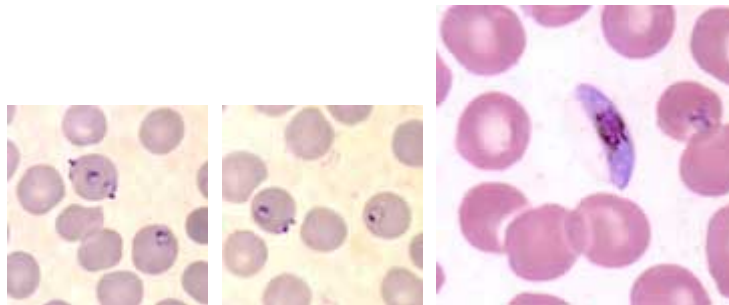


P. malariae mature schizont

1. Stages present in peripheral blood films - all can be present, but one stage usually is predominant.
2. Size of infected red blood cells - normal or slightly smaller.
3. Stippling - none.

4. Density of cytoplasm - rounded and compact, therefore dark staining.
5. Pigment - usually present in older organisms, dark black.
6. Number of merozoites in the mature schizont - average of 9.
7. Length of asexual cycle - 72 hrs.
8. Prepatent period – 14 to 30 days.
9. Incubation period – 30 days.

J. Characteristics of *Plasmodium falciparum* (Malignant tertian malaria)



Plasmodium falciparum rings

P. falciparum gametocyte

1. Stages present in peripheral blood films - usually only rings and gametocytes; rarely other stages.
2. Size of infected red blood cells - normal.
3. Stippling - rarely, Maurer's clefts can be seen.
4. Density of cytoplasm - compact.
5. Pigment - black.
6. Number of merozoites in the mature schizont - average of 24.
7. Shape of the gametocyte - banana or sausage shaped.
8. Length of asexual cycle - 48 hrs.
9. Prepatent period – 5 to 15 days.
10. Incubation period – 12 days.

K. Collection of Blood for Malaria Confirmation:

1. Venous - acceptable, but a "red-top" tube is best and smears need to be made as close to the time of collection as possible.
2. Finger stick - the preferred specimen. Wipe off first drop of blood in order to avoid "tissue juices". Avoid making contact of slide with finger.

3. Anticoagulants - can alter morphology somewhat, but can be used. EDTA (purple-top) is best.
4. Timing of collection - midway between fever peaks, if infection is synchronized; every six to eight hours otherwise. Collection at the fever peak may reveal more organisms, but they will be immature and difficult to speciate.

L. Mixed Infections: (These are possible, but are much more rare than single infections)

1. Dominant infection - one species will usually be predominant.
2. Confirmation of other species - based upon identifying a stage uniquely different from the same stage of the dominant species present.

M. Epidemiology of Malaria:

1. Geographical distribution - malaria is present worldwide in tropical and subtropical areas.
2. Transmission - naturally acquired infections are via the bite of infected female *Anopheles* mosquitoes. Malaria is also transmitted via blood transfusion, sharing of contaminated needles among IV drug abusers, and congenital transmission also has been documented.
3. Relapse versus Recrudescence - *P. vivax* is, traditionally, the “relapsing” malaria. All others can recrudescence (“bloom”) due to incomplete therapy. Relapse is due to dormant stages (hypnozoites) in liver cells. These are from the initial infection. Only sporozoite induced malaria can relapse.
4. Prevention - detect and treat infected individuals; mosquito control.
5. Immunity - incomplete immunity follows infection. Some persons get reinfected over and over.
 - a. Sickle cell trait - the malaria parasite is not successful at utilizing the “S” haemoglobin. This trait does not confer immunity to infection, but does offer resistance to infection.
 - b. Duffy factor - represents the “portal of entry” antigen for *P. vivax*. Persons without the factor are immune to this species (but not the others).

N. Pathology/Pathogenesis:

1. Prodromal stage – a time period which can include premonitory symptoms such as headache, myalgia, anorexia & nausea prior to the paroxysm.
2. Paroxysm - period of chills & fever followed by profuse sweating.
3. Blackwater Fever – a complication of *P. falciparum* malaria. Hemolysis and hematuria are due to a severe immune reaction.

O. Treatment: (Often complicated by an increasing resistance of the parasite to drugs)

1. Prophylaxis - administration of drug(s) prior to entering a malarious region.
2. Therapeutic - administration of drug(s) to cure an infection.
3. Drug resistance - so far, only *P. falciparum* has shown this, although strains of *P. vivax* on Papua, New Guinea have been reported to be developing resistance.