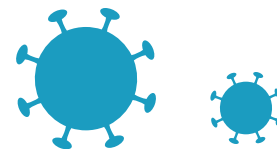


Microbiology - HLS

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Blood protozoa : Plasmodium & Babesia

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**Blue : not
mentioned in
018 video**

Overview

- ❖ A parasite is an organism that lives on or in a host organism and gets its food from or at the expense of its host.
- ❖ Protozoa are unicellular eukaryotes that form an entire kingdom.
- ❖ The protozoa that are infectious to humans can be classified into four groups based on their mode of movement **and mode of reproduction** :
 - Sarcodina – the amoeba **(They use pseudopods, asexual reproduction)**
 - Mastigophora – the flagellates, Giardia, Leishmania **(asexual reproduction)**
 - Ciliophora – the ciliates, e.g., Balantidium **(asexual reproduction)**
(asexual production = binary fission)
- Sporozoa – organisms whose adult stage is not motile (**gliding movement**) e.g., Plasmodium, Cryptosporidium undergo a complex life cycle with alternating **sexual and asexual** reproductive phases. The human parasites Cryptosporidium, Cyclospora, and Toxoplasma and the malarial parasites (Plasmodium species) **are all intracellular parasites.**

Epidemiology

- **Malaria is an intracellular protozoal infection and it's the most important parasitic disease. (the no.1 killer of all of them)**
- **Malaria is a vector-borne disease (the female anopheline mosquito). And the main targeted cells are RBCs.**
- Over 2 billion (41% world population) lives in malaria-risk area.
- Infects 300-500 million people per year, 90% of whom are in sub-Saharan Africa.
- Kills over 1 million people each year and some estimate as many as 2.5 million.
- Leading Infectious killer of children **in parasitic diseases**. Worldwide a child dies of malaria every 30 seconds.
- Disease Burden increasing due to: weakening public health, agricultural practices, global warming, lack of vaccine, drug resistance in parasite and vector, population growth in endemic areas, increased travel.

Plasmodium

- ***Plasmodium*** is a genus of parasitic alveolates, many of which cause malaria in their hosts.
- The parasite always has two hosts in its life cycle: Dipteran insect host and a vertebrate host.

- **Species:**

1. *P. falciparum* (most common , most serious , highest mortality rate)

2. *P. malaria* **causes Quartan Malaria** (classical malaria , common in old ages)

3. *P. vivax* (most common)

4. *P. ovale*

5. *Plasmodium knowlesi*

(Simian malaria)

- ***Plasmodium falciparum*** is the major species associated with deadly infections throughout the world. (associated with **Malignant Tertian malaria**)

❖ **Both *P. vivax* and *P. ovale* cause Benign Tertian malaria which has 48 hr cycles**

Mechanism of Infection

Two sub-life cycles : 1- intrahepatic (exoerythrocytic) 2- erythrocytic cycle

The **vector** for malaria is the female anopheline mosquito.

When the vector takes a blood meal, **sporozoites** contained in the salivary glands of the mosquito are discharged into the puncture wound.

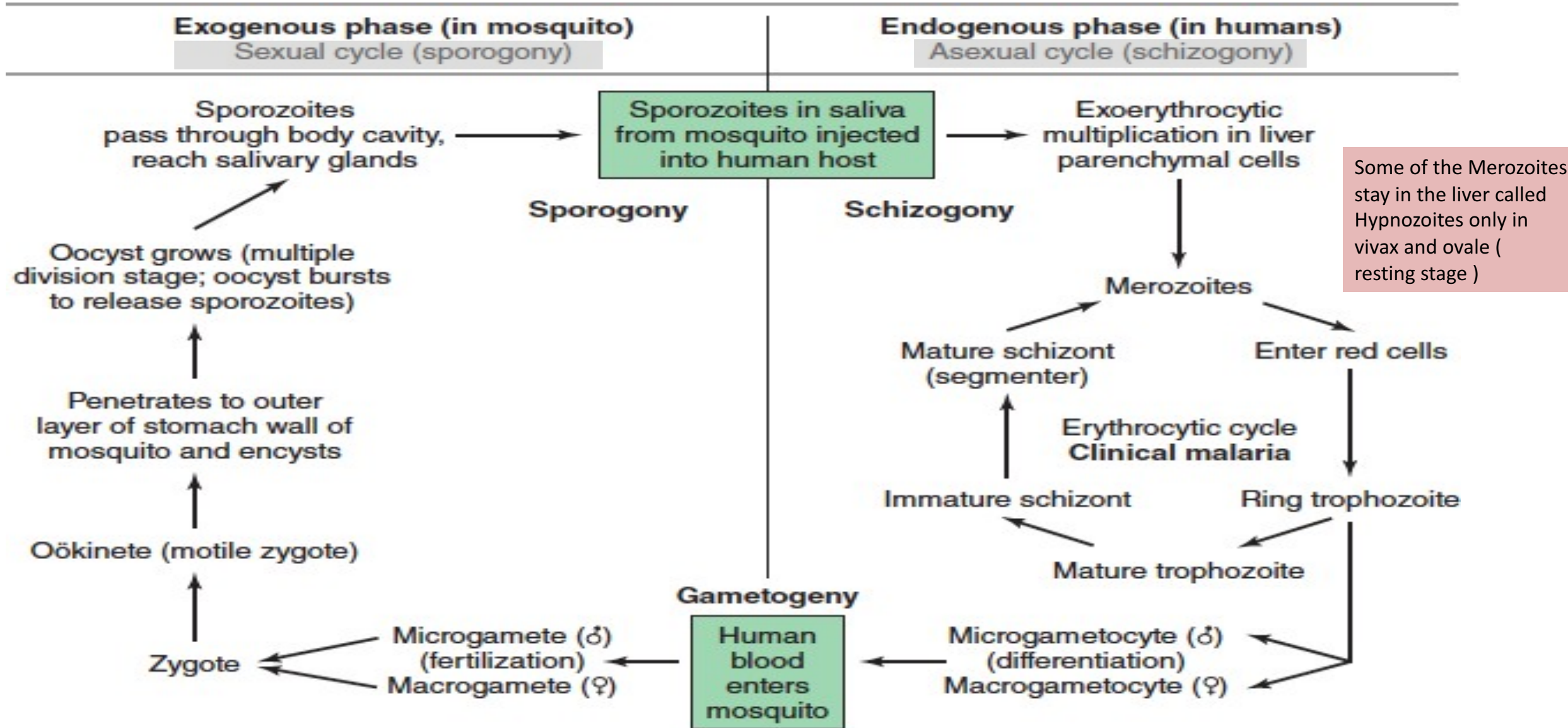
Within an hour, these infective **sporozoites** are carried via the blood to the liver, where they penetrate hepatocytes and begin to grow, initiating the **pre-erythrocytic** or **primary exoerythrocytic cycle**.

The sporozoites (**infective stage**) become round or oval and begin dividing repeatedly.

Schizogony results in (**asexual multiplication**) large numbers of exoerythrocytic merozoites

Once these **merozoites** leave the liver, they invade the red blood cells (RBCs), initiating the **erythrocytic cycle**.

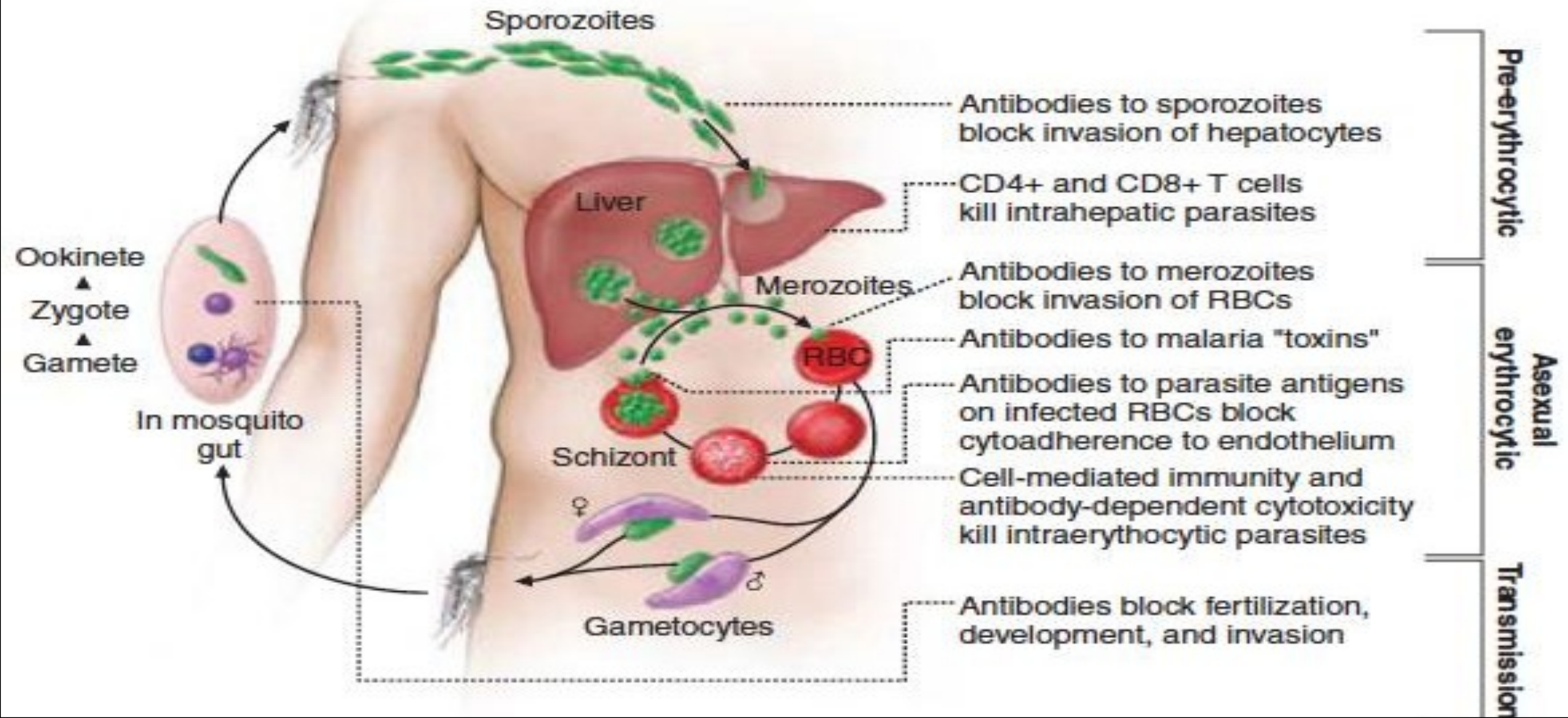
Malaria life cycle (important)



- **A dormant schizogony** may occur in *P. vivax* and *P. ovale* organisms, which remain quiescent in the liver. **(In extra-erythrocytic cycle)**
- These resting stages have been termed **hypnozoites** and lead to a true relapse **(sleeping plasmodium)** , often within 1 year or up to more than 5 years later.

- Once the RBCs and reticulocytes have been invaded, the parasites grow and feed on hemoglobin.
- Within the RBC, the merozoite (or young trophozoite) is vacuolated, ring shaped, more or less ameboid, and uninucleate.
- The excess protein and heme present from the metabolism of hemoglobin combine to form malarial pigment.
- Once the nucleus begins to divide, the trophozoite is called a **developing schizont**.
- The mature schizont contains merozoites (whose number depends on the species), which are released into the bloodstream.


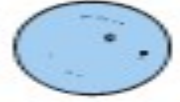



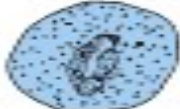











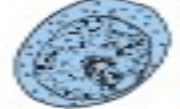
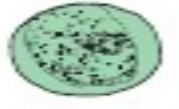


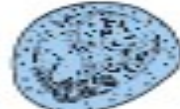


Malaria transmission cycle



Developmental stages of malarial parasites

P.ovale and P.vivax have granulation in the cytoplasm called Schüffner's dots

All plasmodia are intracellular

Stages	Parasites			
	<i>Plasmodium vivax</i>	<i>Plasmodium ovale</i>	<i>Plasmodium malariae</i>	<i>Plasmodium falciparum</i>
Ring stage				
Developing trophozoite				
Developing schizont				
Schizont				
Microgametocyte				
Macrogametocyte				

P.falciparum: Ring stage: double rings and each ring is double dotted.

Banana shaped gametocytes

PLASMODIUM VIVAX (BENIGN TERTIAN MALARIA)

Most wide spread malaria

After the incubation period the infection starts at the peripheral blood circulation which takes 48h

until مازينفجرو Merozoites from the RBCs the thing which effect the immune system causing fever

- *P. vivax* infects **only** the reticulocytes. (**benign state which means parasitemia will be low unlike *P. falciparum* ; symptoms less frequent and less severe**)
- ***Tertian cycle is caused by erythrocytic cycle ; cycle of fever is repeated every 48hrs. (1 day of fever followed by 2 days of being well [repeatedly].**
- **Splenomegaly** occurs during the first few weeks of infection, and the spleen will progress from being soft and palpable to hard, with continued enlargement during a chronic infection.
- If the infection is treated during the early phases, the spleen will return to its normal size.
- A secondary or **dormant schizogony** occurs in *P. vivax* and *P. ovale*, which remain quiescent in the liver.

The infected RBCs enlarge

- These resting stages have been termed **hypnozoites**.

- After a few days of irregular periodicity, a regular 48-hour cycle is established.
- ****The fever that comes on the 3rd to 4th day has 3 stages (cold stage with chills and rigors -> hot stage with fever - > sweating stage) then the next two days the infected person feels well, then the cycle is repeated and the fever attacks again .**

Type of Malaria	Characteristics
<i>Plasmodium vivax</i> (benign tertian malaria)	<ol style="list-style-type: none"> 1. 48-hour cycle 2. Tends to infect young cells 3. Enlarged RBCs 4. Schüffner's dots (true stippling) after 8-10 hours 5. Delicate ring 6. Very ameboid trophozoite 7. Mature schizont contains 12-24 merozoites

- ❖ Rely on the number of merozoites released from erythrocytes rupture in asexual reproduction to differentiate it from ovale
- ❖ (more than 8 merozoites can be observed within the mature schizont)

Plasmodium vivax

*** Intercellular
Oval in shape ***



trophozoite



schizont



gametocyte

Pathogenesis and Spectrum of Disease:

- In patients who have never been exposed to malaria:

Symptoms such as headache, photophobia, muscle aches, anorexia, nausea, and sometimes vomiting may occur before organisms can be detected in the bloodstream.

- In other patients with prior exposure to the malaria:

The parasites can be found in the bloodstream several days before symptoms appear.

PLASMODIUM OVALE

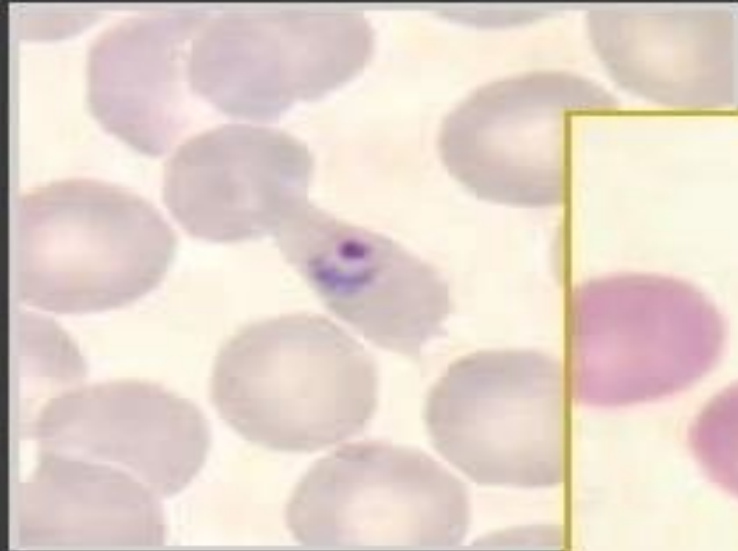
- Although *P. ovale* and *P. vivax* infections are clinically similar, *P. ovale* malaria is usually less severe, tends to relapse less frequently, and usually ends with spontaneous recovery.
- *P. vivax*, *P. ovale* infects only the reticulocytes.
 - ❖ **Note that both have the same treatment**
- After a few days of irregular periodicity, a regular 48-hour cycle is established. Over time, the paroxysms (**fever attacks**) become less severe and more irregular in frequency and then stop altogether.
- **P.ovale forms dormant schizogony (hypnozoites) in the liver**

*Plasmodium
ovale*

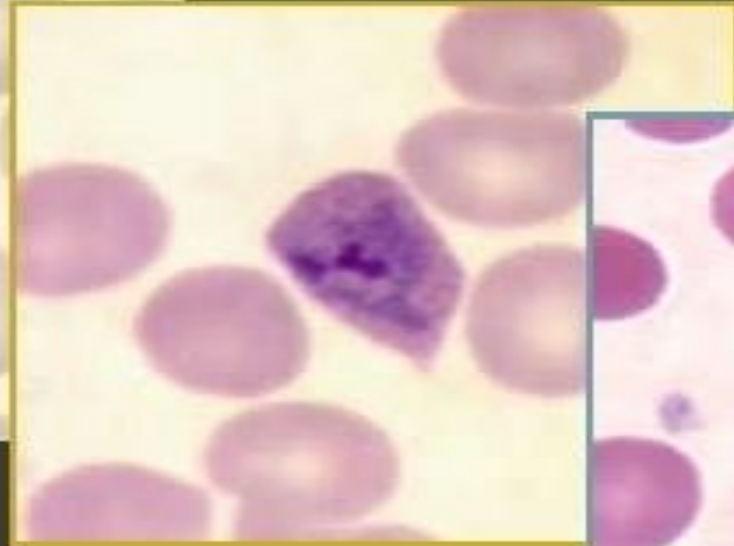
1. 48-hour cycle
2. Tends to infect young cells
3. Enlarged RBCs with fimbriated edges (oval)
4. Schüffner's dots appear in the beginning (in RBCs with very young ring forms, in contrast to *P. vivax*)
5. Smaller ring than *P. vivax*
6. Trophozoite less ameboid than that of *P. vivax*
7. Mature schizont contains an average of 8 merozoites

**Number of
merozoites released
is 8 or less**

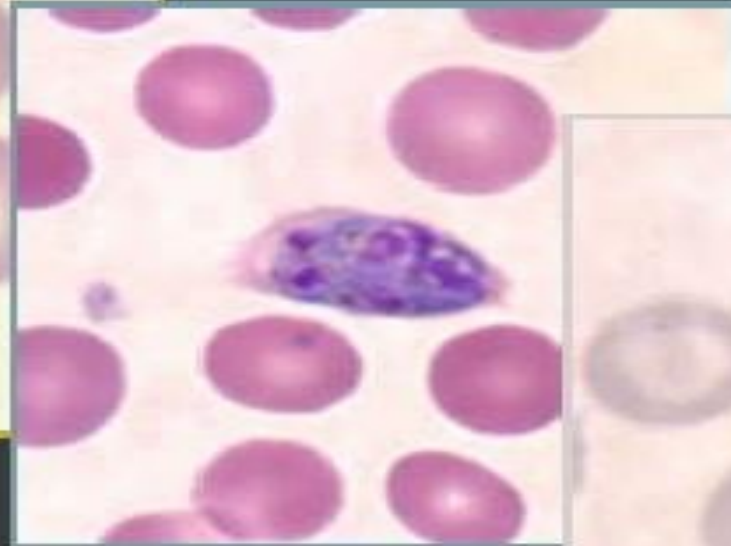
Plasmodium ovale



Ring



Trophozoite



Schizont



Gametocyte

Pathogenesis and Spectrum of Disease:

- The incubation period is similar to that for *P. vivax* malaria, but the frequency and severity of the symptoms are much less, with a lower fever and a lack of typical rigors.

PLASMODIUM MALARIAE (QUARTAN MALARIA)

*Plasmodium
malariae*
(quartan
malaria)

1. 72-hour cycle (long incubation period)
2. Tends to infect old cells **Malaria tends to infect all RBCs**
3. Normal size RBCs
4. No stippling **No schuffner's dots**
5. Thick ring, large nucleus
6. Trophozoite tends to form "bands" across the cell
7. Mature schizont contains 6-12 merozoites

Pathogenesis and Spectrum of Disease:

- **Proteinuria** is common in *P. malariae* infections and may be associated with clinical signs of nephrotic syndrome.
- With a chronic infection, kidney problems result from deposition within the glomeruli of circulating antigen antibody complexes.
- A membrane proliferative type of glomerulonephritis is the most common lesion seen in **quartan malaria**.
- Apply kidney functions test for patients with plasmodium malaria to check If there is protein in urine

PLASMODIUM FALCIPARUM (MALIGNANT TERTIAN MALARIA)

- *Plasmodium falciparum* invades all ages of RBCs **and sizes** . **[severe infections may result because the proportion of infected cells may exceed 50%]** .
- Schizogony occurs in the spleen, liver, and bone marrow rather than in the circulating blood.
- Ischemia caused by the obstruction of vessels within these organs by parasitized RBCs will produce various symptoms, depending on the organ involved.

**High level of
parasitaemia**

Two common complications associated with plasmodium falciparum:

1. cerebral malaria

2. Black water fever (in kidneys, intravascular hemolysis)

- A decrease in the ability of the RBCs to change shape(**projecting knobs**) when passing through capillaries or the splenic filter may lead to plugging of the vessels Also, only *P. falciparum* causes cytoadherence, a feature that is associated with severe malaria.

A decrease in the viscosity followed by ischemia, hypoxia then organ failure

- In *P. falciparum* infections, as the parasite grows, the RBC membrane becomes sticky and the cells adhere to the endothelial lining of the capillaries of the internal organs. (**spleen ,liver and kidneys**).
- Thus, only the ring forms and the gametocytes (occasionally mature schizonts) normally appear in the peripheral blood.

*Plasmodium
falciparum*
(malignant
tertian
malaria)

1. 36-48-hour cycle
2. Tends to infect any cell regardless of age, thus very heavy infection may result
3. All sizes of RBCs
4. No Schüffner's dots (Maurer's dots: may be larger, single dots, bluish)
5. Multiple rings/cell (only young rings, gametocytes, and occasional mature schizonts are seen in peripheral blood)
6. Delicate rings, may have two dots of chromatin/ring, appliqué or accolé forms
7. Crescent-shaped gametocytes

Pathogenesis and Spectrum of Disease

- Symptoms such as aches, pains, headache, fatigue, anorexia, or nausea. This stage is followed by fever, a more severe headache, and nausea and vomiting.
- v Severe or fatal complications can occur at any time and are related to the obstruction of vessels in the internal organs (liver, intestinal tract, adrenal glands, intravascular hemolysis/black water fever, and kidneys).
- v **Although childhood febrile convulsions may occur with any of the malarias, generalized seizures are specifically associated with P.falciparum malaria and it may cause the development of encephalopathy.**
- Blackwater fever is a complication of malaria that is a result of red blood cell lysis, releasing hemoglobin into the bloodstream and urine, causing discoloration.

- **Malignant Tertian Fever has 36-48hr cycle---**Extreme fevers, 41.7° C (107° F) or higher, may occur in an uncomplicated malaria attack or in cases of cerebral malaria. Without vigorous therapy, the patient usually dies.
- Cerebral malaria is considered to be the most serious complication and the major cause of death with *P. falciparum*.

P. falciparum



marginal form



ring form



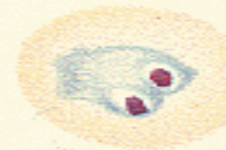
double dotted rings



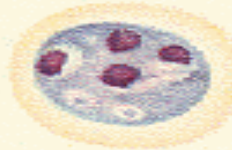
ring form



young trophozoite



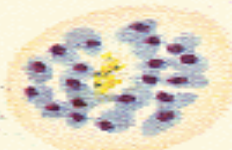
trophozoite



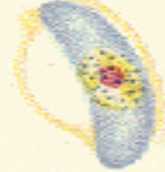
early schizont



schizont



mature schizont



female gametocyte



male gametocyte

"أنا بهمني"

"أنا بهمني"
Banana shaped

Large, single, bluish dots (Maurer's dots) instead of schuffner's dots. RBCs can be seen in all sizes after the infection.

Fever NEVER becomes regular sometimes.

Rings have 2 chromatin dots and show Applique'/Accole' forms (The ring attaches itself to the margin or the edge of erythrocytes).

Gametocytes are crescent 'banana' in shape

PLASMODIUM KNOWLESI

(SIMIAN MALARIA, THE FIFTH HUMAN MALARIA)

- *P. knowlesi* invades all ages of RBCs.
- The early blood stages of *P. knowlesi* resemble those of *P. falciparum*.
- Whereas the mature blood stages and gametocytes resemble those of *P. malariae*.
- Unfortunately, these infections are often misdiagnosed as the relatively benign *P. malariae*; however, infections with *P. knowlesi* can be fatal.

Plasmodium knowlesi (simian malaria)*

1. 24-hour cycle
 2. Tends to infect any cell regardless of age, thus very heavy infection may result
 3. All sizes of RBCs, but most tend to be normal size
 4. No Schüffner's dots (faint, clumpy dots later in cycle)
 5. Multiple rings/cell (may have 2-3)
 6. Delicate rings, may have two or three dots of chromatin/ring, appliqué forms
 7. Band form trophozoites commonly seen
 8. Mature schizont contains 16 merozoites, no rosettes
 9. Gametocytes round, tend to fill the cell
- Early stages mimic *P. falciparum*; later stages mimic *P. malariae*

Characteristic	Finding for Indicated Species ^a			
	<i>P. falciparum</i>	<i>P. vivax</i>	<i>P. ovale</i>	<i>P. malariae</i>
Duration of intrahepatic phase (days)	5.5	8	9	15
Number of merozoites released per infected hepatocyte	30,000	10,000	15,000	15,000
Duration of erythrocytic cycle (hours)	48	48	50	72
Red cell preference	Younger cells (but can invade cells of all ages)	Reticulocytes and cells up to 2 weeks old	Reticulocytes	Older cells
Morphology	Usually only ring forms ^b ; banana-shaped gametocytes	Irregularly shaped large rings and trophozoites; enlarged erythrocytes; Schüffner's dots	Infected erythrocytes, enlarged and oval with tufted ends; Schüffner's dots	Band or rectangular forms of trophozoites common
Pigment color	Black	Yellow-brown	Dark brown	Brown-black
Ability to cause relapses	No	Yes	Yes	No

- ❖ **we compare the RBC/hepatocyte released number**
- ❖ **blood sample or liver biopsy are used to count cells' numbers.**
- ❖ **Incubation period for all is from 1 week to 5 weeks**
- ❖ **Remember that *P.ovale* and *P.vivax* both have the ability to cause relapses**

CLINICAL FEATURES

- Malaria is a very common cause of fever in tropical countries. The first symptoms of malaria are nonspecific; the lack of a sense of wellbeing, headache, fatigue, abdominal discomfort, and muscle aches followed by fever are all similar to the symptoms of a minor viral illness.
- In some instances, a prominence of headache, chest pain, abdominal pain, cough, arthralgia, myalgia, or diarrhea may suggest another diagnosis. **[But are excluded by their specific symptoms]** .
- Although headache may be severe in malaria, the neck stiffness and photophobia seen in meningitis do not occur. While myalgia may be prominent, it is not usually as severe as in dengue fever, and the muscles are not tender as in leptospirosis or typhus. Nausea, vomiting, and orthostatic hypotension are common.
- **Then patterns start (cycles of 48-72 hrs); The fever that comes on the third to fourth day has 3 stages (cold stage with chills and rigors -----hot stage -----sweating stage) then there are two days of feeling well, then the fever attacks again.**

- The classic malarial paroxysms, in which fever spikes, chills, and rigors occur at regular intervals, are relatively unusual and suggest infection with *P. vivax* or *P. ovale*.
- The fever is usually irregular at first (that of *falciparum* malaria may never become regular); the temperature of nonimmune individuals and children often rises above 40°C in conjunction with tachycardia and sometimes delirium. Although childhood febrile convulsions may occur with any of the malarias, generalized seizures are specifically associated with *falciparum* malaria and may herald the development of encephalopathy (cerebral malaria).
- **Anemia can show (due to hemolysis) but mostly in *P. falciparum* due to high parasitemia**

LABORATORY DIAGNOSIS

(ALL SPECIES) **Definitive diagnosis by seeing plasmodium in the peripheral blood**

1. Routine Methods:

- Thick and thin blood films . **Thick – blood drop**
Thin – "مسحة" to see fine details
- At least 200 to 300 oil immersion fields should be examined on both films before a negative report is issued.
- **Stains:**
 1. Giemsa stain.
 2. Wright's stain.
 3. Fluorescent nucleic acid stains, such as acridine orange.
- Blood collected using (EDTA) anticoagulant.

2.Serologic Methods:

- Several rapid malaria tests (RMTs):

1. Some of which use monoclonal antibodies against the histidine-rich protein 2 (HRP2).
 2. Whereas others detect species-specific parasite lactate dehydrogenase (pLDH).
- These procedures are based on an antigen capture approach in dipstick or cartridge formats.

3. Molecular Diagnostics:

- Other methods include direct detection of the five species by using a specific DNA probe after PCR amplification of target DNA sequences.

4. Automated Instruments:

- Using automated flow cytometry hematology instruments, there are potential limitations related to the diagnosis of blood parasite infections.

THERAPY

All *falciparum* are resistance to Quinolines " هذا هو يلي بهمني "
Because it works on active motile producing form
-we use primaquines for hepnozoids

- Antimalarial drugs are classified according to the stage of malaria against which they are targeted.
- **QUINOLINES , ARTEMISININS**
- Tetracycline, doxycycline, and clindamycin are used increasingly in combination with other antimalarials to improve their efficacy
- **For prophylaxis you can take quinolones if you're travelling to endemic areas like mefloquine**
- **These drugs are referred to as :**
 1. Tissue schizonticides (which kill tissue schizonts).
 2. Blood schizonticides (which kill blood schizonts).
 3. Gametocytocides (which kill gametocytes).
 4. Sporonticides (which prevent formation of sporozoites within the mosquito).

CONTROL

Being a vector-borne disease means that the main mean of control is vector control.

Type of control	Measures
Personal protection	Insecticide treated mosquito nets; Mosquito proofing of dwellings; Repellents; Site selection
Environmental management	Drainage & water management; Land reclamation by filling and drainage
Chemical (Insecticides) control	Residual house spraying; larviciding; space spraying
Other measures	Biological control, Genetic control, Zooprophyllaxis



- ❖ **Avoid the feeding time of the mosquito (from dusk till dawn)**
- ❖ **A Vaccine was approved for [in the US in October 2021] and it shows mild to moderate effect. (Called RTS)**
- ❖ **The most commonly infected group in Jordan are Peacekeeping troops.**

Babesiosis **vector borne**

- Babesiosis is an emerging tick-borne infectious disease caused by protozoan parasites of the genus *Babesia* that invade and eventually lyse red blood cells (RBCs).
- Most cases are due to *Babesia microti*. *B. microti*, a parasite of small rodents, is the most common etiologic agent of human babesiosis
- The primary causative agent of human babesiosis in Europe is *B. divergens*, but *Babesia venatorum* and *B. microti* also have been reported.
- The infection typically is mild in young and otherwise healthy individuals but can be severe and sometimes fatal in persons >50 years of age and in immunocompromised patients. Sporadic cases have been reported in Europe and the rest of the world.

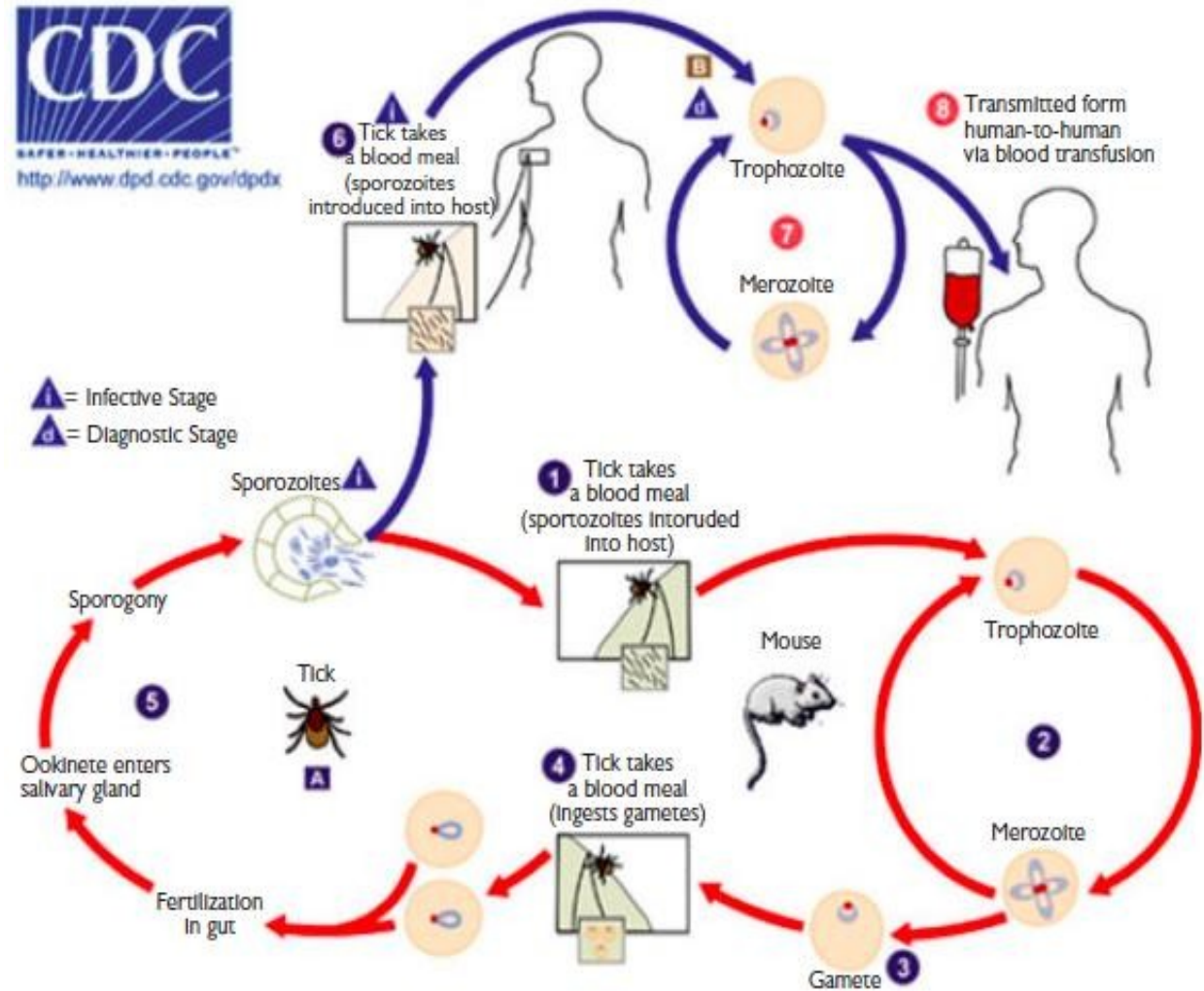
Modes of Transmission

- B. microti is transmitted to humans primarily by the nymphal stage of the deer tick (*Ixodes scapularis*), the same tick that transmits the causative agents of Lyme disease . **[No exo-erythrocytic cycle; directly to RBCs and their mainstay of pathogenesis is hemolysis]**
- The vectors for transmission of B. duncani and B. divergens– like organisms are thought to be *Ixodes pacificus* and *Ixodes dentatus*, respectively.

Affect animals mainly but accidentally humans

babesia life cycle

- ❖ **ASEXUAL** reproduction in humans
- ❖ Sexual reproduction in the definitive host.
- ❖ Reservoir for these parasites is white footed mouse.
- ❖ Humans get infected accidentally through blood transfusion.
- ❖ It has symptoms and signs very similar to malaria, but they differ in that there's no pattern in the fever (NO CYCLES)



RBCs are the target
Infective stage is sporozoites

CLINICAL MANIFESTATIONS

- Asymptomatic B. microti Infection: At least 20% of adults and 40% of children do not experience symptoms following B. microti infection. There is no evidence of long-term complications following asymptomatic infection; however, people who are asymptotically infected may transmit the infection when they donate blood. **[It mimics malaria starting with non-specific symptoms similar to viral illnesses and then later on fever becomes the chief complaint]**
- Mild to Moderate B. microti Illness Symptoms typically develop following an incubation period of 1–4 weeks after tick bite and 1–9 weeks (but as long as 6 months) after transfusion of blood products. Patients experience a gradual onset of malaise, fatigue, and weakness. Fever can reach 40.9C and is accompanied by one or more of the following: chills, sweats, headache, myalgia, arthralgia, nausea, anorexia, and dry cough.
- Severe B. microti Illness Severe babesiosis requires hospital admission and typically occurs in patients with one or more of the following: age of >50 years, neonatal prematurity, male gender, asplenia, HIV/AIDS, malignancy, hemoglobinopathy, and immunosuppressive therapy.

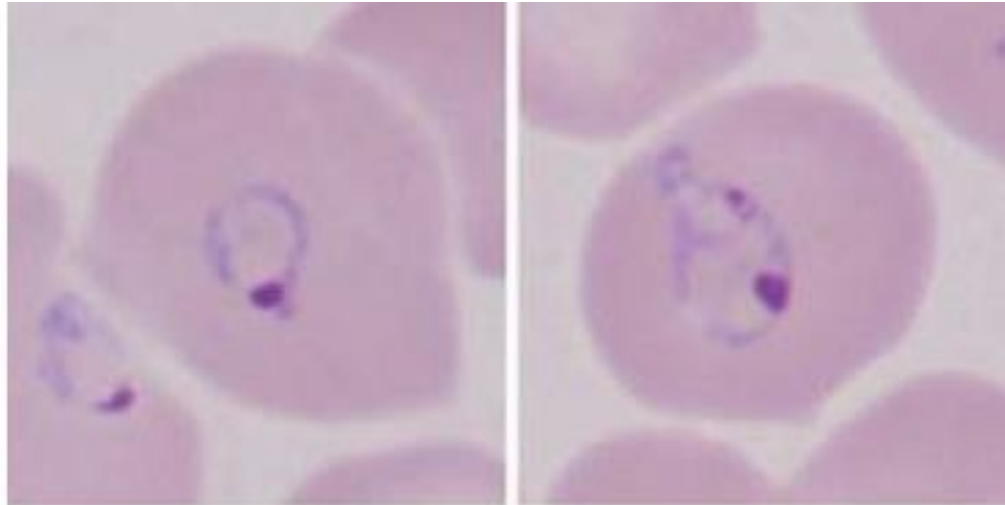
PATHOGENESIS

- Anemia is a key feature of the pathogenesis of babesiosis. Hemolytic anemia caused by rupture of infected RBCs generates cell debris that may accumulate in the kidney and cause renal failure.
- Anemia also results from the clearance of intact RBCs as they pass through the splenic red pulp and encounter resident macrophages.
- Babesia antigens expressed at the RBC membrane promote opsonization and facilitate uptake by splenic macrophages. In addition, RBCs are poorly deformable as a result of oxidation generated by the parasite and the host immune response and are filtered out as they attempt to squeeze across the venous vasculature. Bone marrow suppression due to cytokine production may also contribute to anemia.

DIAGNOSIS

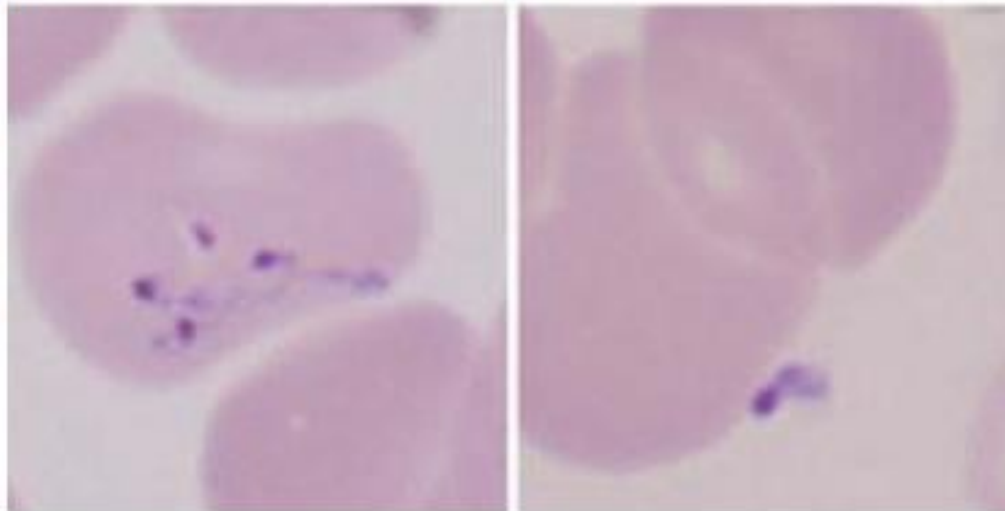
- Microscopic examination of Giemsa-stained thin blood smears
- Polymerase chain reaction (PCR)
- Serology can suggest or confirm the diagnosis of babesiosis. An indirect immunofluorescent antibody test for *B. microti* is most commonly used.

**Merozoites tend to
arrange in tetras
[maltese cross]
(pathognomonic)**



A

B



C

D

**extra cellular
merozoites unlike
malaria**

TREATMENT

- Atovaquone plus azithromycin is the recommended antibiotic treatment combination for mild to moderate babesiosis.
- Clindamycin plus quinones is the choice for severe infections.

Prevention

- Wear clothing that covers the lower part of the body, apply tick repellents (such as DEET) to clothing, and limit outdoor activities where ticks may abound from May through October.

The End