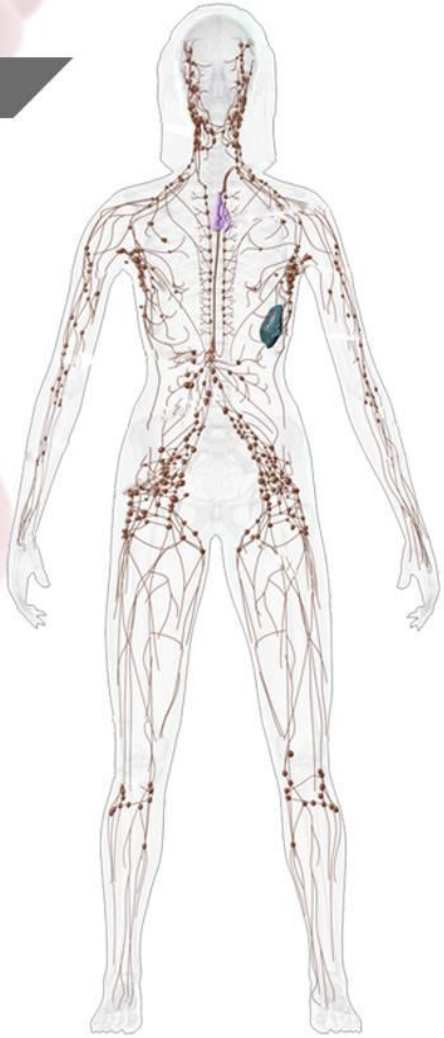
Sheet 2

Hematology and Lymphatic system



Subject | Microbiology

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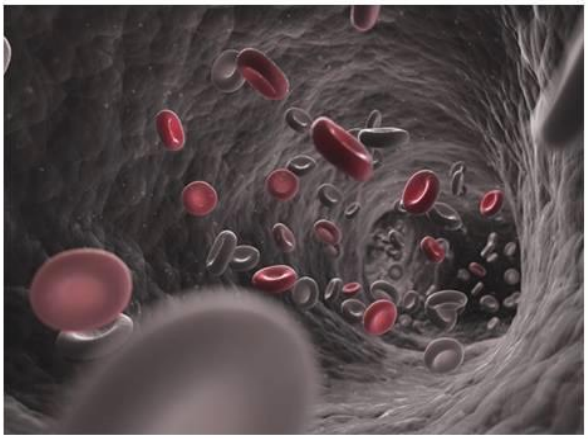


In this sheet, I underlined what the doctor said is important.

Malaria

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.
- There are **three** main classes of parasites that can cause disease in humans: protozoa, helminths, and ectoparasites.
- 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**:

  1- Sarcodina, e.g. Amoeba.

  2- Flagellates (Mastigophora), e.g. Giardia, Leishmania.

  3- Ciliates (Ciliophora), e.g. Balantidium.

  4- Sporozoites (non-motile): they are organisms whose **adult** stage is **not motile**. They undergo a **complex** life cycle with alternating **sexual** and **asexual** reproductive phases, e.g. **Plasmodium** *(which we will discuss* and Cryptosporidium.

  The human Sporozoites are Cryptosporidium, Cyclospora, Toxoplasma and the malarial parasites *(Plasmodium species)*. They are all **intracellular** infectious parasites.

Epidemiology of Malaria *(caused by protozoa of the genus Plasmodium)*

- Over 2 billion (41% world population) lives in malaria-risk areas.
- 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**. Worldwide a child dies of malaria every 30s.
- 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 species induce **Anemia** and have a characteristic feature in causing **periodic fevers** (recurrent episodes of fever).
- **Vectors**: **female** anopheles mosquitoes.
- **Tropism**: RBCs.

Plasmodium

- Plasmodium is a genus of parasitic alveolates *(characterized by the presence of sacs of fluid under the cell membrane),* they cause malaria in their hosts.
- The parasite always has **two** hosts in its life cycle: Dipteran insect host *(sexual cycle)* and a vertebrate host *(asexual cycle).*
- Malaria is a febrile disease caused by 5 species of Plasmodium:

1- P. falciparum

⇨ Most common plasmodium associated with deadly infections throughout the world with the highest fatality rates. It causes Malignant Tertian malaria.

2- P. malariae

⇨ Quartan malaria.

3- P. vivax

4- P. ovale

⇨ Both cause Benign Tertian malaria.

5- P. knowlesi

⇨ Simian Malaria.

Plasmodium Life Cycle:

'It is not hard, just understand. Refer to the slides for plasmodium's life cycle pictures'

1- Thin and **motile forms** of the Plasmodia are called '**Sporozoites'**. The **female** anopheline mosquito (vector) carries the Sporozoites within its **salivary glands** and injects them into humans' bloodstream while it feeds.

2- Sporozoites in the bloodstream reach the **liver** and settle in **hepatocytes**.

⇨ *This marks the **beginning** of the **pre-erythrocytic cycle** in the **liver** (or primary exo-erythrocytic cycle), so-named because this cycle occurs before the erythrocytes are invaded.*

3- The sporozoite **rounds** up within the **liver** cell forming a '**Trophozoite'**.

4- The Trophozoite begins **dividing** repeatedly forming **thousands** of new nuclei in a process called **Schizogony**. This big mass is now a cell with thousands of nuclei, called a '**Schizont'**.

5- A cytoplasmic membrane then forms around each nucleus, creating thousands of small bodies called '**merozoites**'.

⇨ *This marks the **end** of the **pre-erythrocytic cycle**.*

⇨ *It is the **asexual** part of the plasmodium's life cycle, called the '**Schizogony**' cycle.*

⇨ *It results in the formation of large numbers of **merozoites**.*

6- The new overloaded liver cell **bursts** open, releasing the **merozoites** into the **liver** and **bloodstream**:

a- Some will **re-infect** other **hepatocytes**, where merozoite will **round** up to form a **trophozoite** as the sporozoite did initially, repeating the same cycle.

b- Other merozoites will enter the **bloodstream** and invade red blood cells and reticulocytes starting the **erythrocytic cycle**. The parasites grow and feed on **hemoglobin**, the excess protein and hematin produced from the metabolism of hemoglobin combine to form **malarial pigmen**t.

7- In the **red cells**, the rounded merozoites *(young trophozoite)* are vacuolated, **ring-shaped**, more or less amoeboid, and uni-nucleated. The young trophozoite *(or 'ring' stage because of its morphology)* grows before undergoing Schizogony *(divisions).*

Repeatedly, nuclear division occurs with the formation of a large multinucleated '**developing schizont**'. Cytoplasm surrounds each nucleus to form **new merozoites** within the schizont.

8- The **mature schizont** contains **merozoites**, whose number depends on the plasmodium species. **Red cell lysis** occurs with the release of these merozoites into the bloodstream predisposing to **Anemia**.

9- The released merozoites stimulate an **immune response**, manifested as fevers, chills, and sweats.

10- **Sporogony (sexual cycle):** Other merozoites in the bloodstream may change into male and female **gametocytes**. These cells circulate and will be taken up by a **biting** Anopheles mosquito where the male and female gametocytes **fuse** inside it *(fertilization)* forming an '**oocyst**'. The oocyst divides into many **sporozoites**, which disseminate within the mosquito. They may find their way into the mosquito salivary gland and will be injected into the human for **another asexual** reproduction.

⇨ *Plasmodia undergo **sexual** division (**Sporogony**) in the Anopheles **mosquito** and **asexual** division (**Schizogony**) in the human **liver** and **red cells**.*

⇨ ***Schizogony** cycle is what causes the symptoms in humans.*

⇨ *Male gametes are **microgametocytes**, while female gametes are **macrogametocytes**.*

Note: P. vivax and P. ovale have a **dormant stage** in the human **liver**, where after the sporozoites enter the hepatocytes **not** all of them will divide and form **schizonts**, but some remain as **hypnozoites** (resting stage). These hypnozoites can **reactivate**, 1 year or up to more than 5 years later, undergoing a **secondary exo-erythrocytic** cycle infecting RBCs with the released merozoites causing a **relapsing malaria**.

Summary:

Exposure: Sporozoites from mosquitoes' saliva invade the bloodstream →Liver.

Pre-erythrocytic / asexual / primary exo-erythrocytic cycle: Sporozoites round up forming Trophozoites → Schizogony (Divisions) → Schizont → Membrane forms around each nucleus → Small merozoites within the Schizont → Hepatocytes burst to release merozoites.

Merozoites either:

1- Re-infect other hepatocytes repeating the cycle **(exo-eryhrrocytic cycle).**

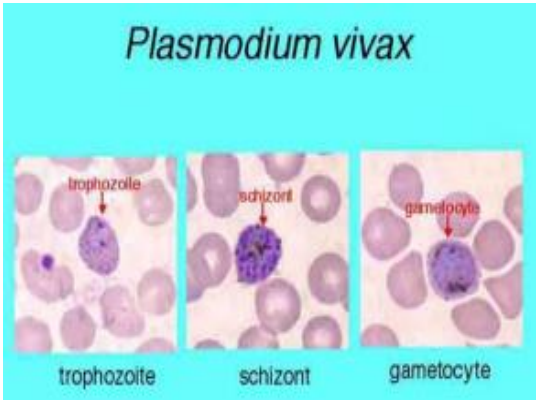
2- Enter the bloodstream and invade RBCs **(erythrocytic cycle):**
Merozoites are rounded (young Trophozoites 'ring stage') → Undergoes schizogony (Divisions) → Developing schizont → Membrane forms around each nucleus → Mature schizont containing merozoites → Blood cell lysis → Induces immune response.

3- Change into male and female gametocytes **(sexual / sporogony cycle inside the mosquito)**:
They fuse inside the mosquito → Oocyst → Divides → Sporozoites.

'Now to discuss each plasmodium specie in details'

1- Plasmodium Vivax

- P. vivax tends to infect the **reticulocytes** (young cells); thus parasitaemia is low. RBCs are **enlarged**; because their schizonts are large.
- Forms **hypnozoites** as discussed before.
- **Shows Schüffner's dots (stippling) after 8-10hrs:** refers to a hematological finding that is associated with malaria, exclusively found in infections caused by Plasmodium ovale or Plasmodium vivax.
- The young trophozoite **'ring stage'** is very ameboid and **delicate**. Mature schizont contains **12-24 merozoites (>8)**.
- **Benign Tertian Malaria**: after a few days of irregular periodicity, a regular 48-hour cycle is established.

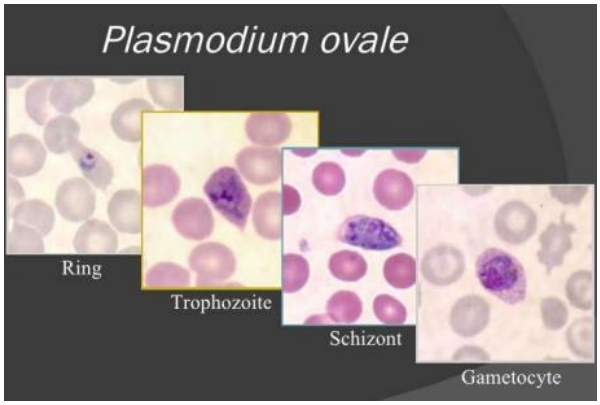


Pathogenesis:

- **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 malaria:** the parasites can be found in the bloodstream several days before symptoms appear.
- **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 chronic infection. If the infection is treated during the early phases, the spleen will return to its normal size.

2- Plasmodium Ovale

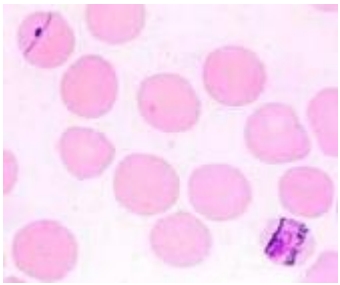
- P. ovale, like P. vivax, infects the **reticulocytes**. RBCs are **enlarged** with fimbriated edges (**oval**).
- Forms **hypnozoites** as discussed before.
- **Shows Schüffner's dots (stippling)** from the beginning, unlike P. vivax.
- Smaller **ring** (young trophozoite) compared to P. vivax.
- A mature schizont contains **merozoites less** than P. vivax (usually <8).
- **Benign Tertian Malaria**: a regular 48-hour cycle. Over time, the paroxysms (attacks) become less severe and more irregular in frequency and then stop altogether.



- Although P. ovale and P. vivax infections are clinically similar, P. ovale malaria is usually **less common**, **less severe**, tends to **relapse less frequently**, and usually ends with spontaneous recovery.
- The incubation period is similar to that for P. vivax malaria *(the period between exposure to an infection and the appearance of the first symptoms)*, but with **lower** fever and may lack typical rigors *(cold and shivering with a rise in temperature).*

3- Plasmodium Malariae

- It tends to infect **old** RBCs, unlike P. vivax and ovale. RBCs are normal in size.
- **Shows no Schüffner's dots (**no stippling**).**
- The **young trophozoite** (ring) is **thick** with a **large nucleus**. Trophozoite tends to form '**bands**' across the cell. Mature schizont contains **6-12 merozoite**.
- **Quartan Malaria**: 72-hour cycle with a long incubation period.

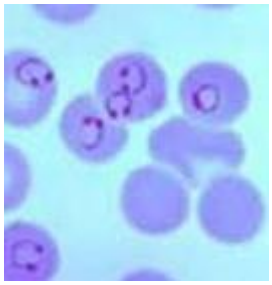


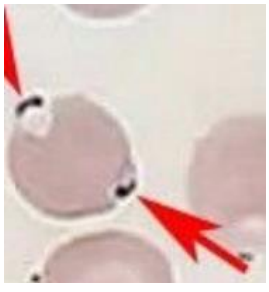
Pathogenesis:

- **Proteinuria** is the most common complication of P. malariae.
- With 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.

4- Plasmodium Falciparum

- After leaving the liver, P. falciparum tends to invade **all ages** of RBCs, and the proportion of infected cells may exceed 50%, thus severe infections may result.
- RBCs are seen in **all** sizes.
- **Malignant Tertian malaria**: It has a 36-48 hour cycle.
- Shows **no Schüffner's dots** (no stippling)**.** Large, single, bluish dots may show later on (**Maurer's dots**).
- Rings are **delicate**, with **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.





Pathogenesis and Spectrum of Disease:

- One feature of mature Plasmodium falciparum-infected erythrocytes leading to the development of severe malaria is thought to be:

  1- **Cytoadherence**: as the parasite grows, the RBC membrane becomes sticky and adhere to the endothelial lining of the capillaries of the internal organs. Thus, only the ring forms, the gametocytes, and occasionally mature schizonts normally appear in the peripheral blood.

  2- **Blockage of the microvasculature**: Schizogony occurs in the internal organs (spleen, liver, bone marrow, etc.) rather than in the circulating blood. Parasitized RBCs have a decreased ability to change shape when passing through capillaries or the splenic filter which may lead to plugging of the vessels of these organs. Ischemia caused by the plugging of vessels within these organs by masses of parasitized RBCs will produce various symptoms, depending on the organ involved.

- Symptoms show such as aches, pains, headache, fatigue, anorexia, or nausea. This stage is followed by fever, a more severe headache, nausea, and vomiting.
- **Black-water fever:** is a complication of malaria that is a result of red blood cell lysis, releasing hemoglobin into the bloodstream and urine, causing discoloration.
- **Cerebral malaria**: is considered to be the **most** serious **complication** and the major cause of death with P. falciparum.
- Extreme fevers, 41.7° C (107° F) or higher, may occur in an uncomplicated malaria attack or cases of **cerebral malaria**. Without vigorous therapy, the patient usually dies.

5- Plasmodium Knowlesi

- Also known as the Simian Malaria or the 5th human Malaria.
- All sizes of RBCs are seen, but most tend to be normal. It has a 24-hour cycle.
- P. knowlesi infects any RBC **regardless of age**, thus heavy infections may result. Mature schizont contains **16 merozoites.**
- Shows **no Schüffner's dots** (no stippling). Faint, clumpy dots may show later in the cycle.
- The early blood stages of P. knowlesi mimic **P. falciparum**:
  ⇨ Rings are **delicate**, with **2-3** dots of chromatin and **applique'** forms.
- The later blood stages mimic **P. malariae**:
  ⇨ **Band** form trophozoite is commonly seen.

- Gametocytes are **round** and tend to fill the cell.
- Unfortunately, these infections are often **misdiagnosed** as the relatively benign P. malariae; however, infections with P. knowlesi can be **fatal**.

'To recap…'

Characteristics	P. Vivax	P. Ovale	P. Malaria	P. Falciparum	P. Knowlesi
Malaria	**Benign Tertian Malaria** (48-hr cycle)	**Benign Tertian Malaria** (48-hr cycle)	**Quartan Malaria** (72-hr cycle)	**Malignant Tertian malaria** (36-48hr cycle)	**Simian Malaria** (24hr cycle)
Morphology	- Schüffner's dots. - RBCs are **enlarged**	- Schüffner's dots. - RBCs are **enlarged** with fimbriated edges (oval).	- **No** Schüffner's dots. - RBCs are **normal** in size.	- **No** Schüffner's dots. - Large, single, bluish dots may show later on *(Maurer's dots).* - RBCs are seen in **all** sizes. - Gametocytes are **crescent**.	- **No** Schüffner's dots. - Faint, clumpy dots may show later. - RBCs are **mostly** normal. - Gametocytes are **round**.
Merozoites	**12-24**	**6-12**	**6-12**	**Up to 36**	**Up to 16**
Ring stage	- Ameboid and delicate.	- Smaller than P. vivax.	- Thick with a large nucleus. - Tend to form **'bands'**	- Delicate, with **2** chromatin dots, and show Applique'/ Accole' forms.	- **Early:** Rings are delicate, with **2-3** dots of chromatin and applique' forms - **Late:** Band form trophozoite.
Relapse 'hypnozoites'	**Yes**	**Yes**	**No**	**No**	**No**
RBC preference	**Reticulocytes** (young cells)	**Reticulocytes** (young cells)	**Old cells**	**Any age**	**Any age**
Pathogenesis	- **Splenomegaly**	- Symptoms are much **less** severe and may lack rigors. - Usually ends with **spontaneous** recovery.	- Long incubation period. - **Proteinuria**.	- Cytoadherence - Blockage of vessels. - Black-water fever - Cerebral malaria	- Misdiagnosed as P. malariae. - Infections can be **fatal**.

Malaria's Clinical Features

- Incubation periods of different Plasmodium species **vary** from 1-5 weeks.
- 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**. These **prodromal** symptoms are followed by **paroxysms**.

Malaria Paroxysms consist of 3 stages:

1- **Cold**: rigors (chills and shivering).
2- **Hot**: fever.
3- **Sweating**: drop in temperature with profuse sweating.

These three stages are repeated regularly depending on the type of malaria:

1- **P. vivax and ovale**: every 48hrs (3^{rd} day).
2- **P. falciparum:** every 36-48hrs (irregular).
3- **P. malaria:** every 72hrs (4^{th} day).
4- **P. knowlesi**: every 24hr.

In the period between the paroxysms, the patients **feels well**.

- In some instances, a prominence of headache, chest pain, abdominal pain, cough, arthralgia, myalgia, or diarrhea may suggest another diagnosis:
  - **a-** Although headache may be **severe** in malaria, the neck stiffness and photophobia seen in meningitis do **not** occur.
  - **b-** While **myalgia** (muscle pain) may be **prominent**, it is not usually as severe as in dengue fever, and the muscles are not as tender as in leptospirosis or typhus.
- Nausea, vomiting, and orthostatic hypotension are **common** symptoms.
- The **fever** is usually **irregular** at first *(that of falciparum malaria may never become regular)*; the temperature of **non-immune** individuals and children often rises **above** 40 degrees with **tachycardia** and sometimes **delirium** *(restlessness, illusions, and incoherence).*
- Although childhood **febrile convulsions** may occur with **any** of the malarias, generalized seizures are specifically associated with **falciparum** malaria and may sign the development of **encephalopathy** (cerebral malaria).
- **Anemia** is a complication of Malaria, but it is mostly seen as a result of **P. Falciparum**.

Malaria Species Diagnosis

1- **Routine Methods:**

- Blood is collected and (**EDTA**) anticoagulant is used.
- **Thick and thin blood films** tests are performed. At least 200 to 300x oil immersion fields should be examined in both films before a report is issued.
- **Stains** used could be: Giemsa stain. // Wright's stain. // Fluorescent nucleic acid stains, such as acridine orange.

2- **Serologic Methods 'faster':**

- Several rapid malaria tests (**RMTs**):
  - **a-** Some of which use monoclonal antibodies against the histidine-rich protein (**HRP2**).
  - **b-** Whereas others detect species-specific parasite lactate dehydrogenase (**pLDH**).
- **Dipstick and cartridge** format is an example of an RMT that uses antibodies to detect unique Plasmodium **antigen**.

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**:

Flow cytometry-based automated hematology analyzers have been used for malaria diagnosis. It is a laboratory method used to detect and identify specific cells. However, there are potential **limitations** related to it.

Malaria's Therapy

- Antimalarial drugs are classified according to the stage of malaria against which they are targeted. 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 the formation of sporozoites within the mosquito).

- **Artemisinins** *(mainly for P. falciparum)* and **Quinolones** *(drug of choice for other P. species).*
- Treatment of P. vivax and ovale require additional drugs *(primaquine)* to kill hypnozoites ***(Quinolones alone are not enough for them).***
- Tetracycline, doxycycline, and clindamycin are used increasingly in combination with other antimalarial drugs to improve their efficacy.

Malaria's Control

- There are no vaccines yet.

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, Zooprophylaxis

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Babesiosis

- Babesiosis is an emerging **tick-borne** infectious disease caused by protozoan parasites of the genus **Babesia** that invade and eventually **lyse** red blood cells.
- **Babesia microti** is a parasite of **small rodents** and is the **most common** causative agent of human Babesiosis worldwide.
- **Reservoir** *(the host that does not experience the symptoms of disease)*: **white-footed mouse**. **Accidental host**: **Human**.

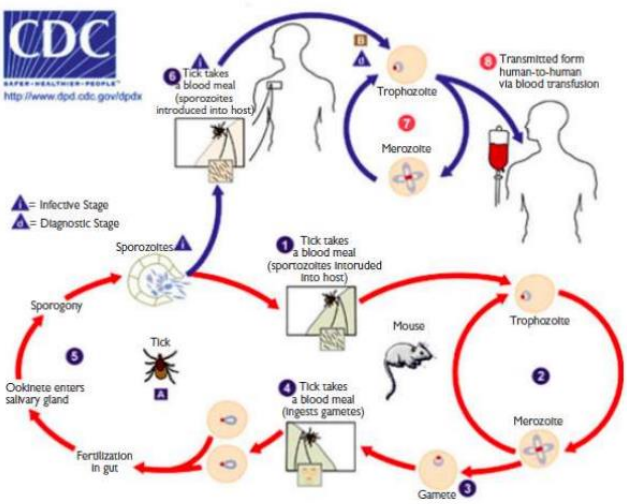
  The primary causative agent of human Babesiosis in **Europe** is **B. divergens.** However, B. venatorum and B. microti also have been reported.
- The infection typically is **mild** in young and otherwise **healthy** individuals but can be **severe** and sometimes **fatal** in persons **>50 years** of age and **immune-compromised** patients. **Sporadic** cases have been reported in Europe and the rest of the world.

- Although Babesiosis is similar to malaria clinically, it differs in:
  1- There are more than 100 species of Babesia, mostly causing disease in animals.
  2- Babesia is spread by **tick bites**, not mosquito bites.
  3- They do **not** affect liver cells *(there is **no exo-erythrocytic phase**).*
  4- There are **no periodic fevers**.

Modes of Transmission:

- **B. microti** is transmitted to humans primarily by the nymphal stage (immature) of the **deer tick** (Ixodes scapularis), it is the same tick that transmits the causative agents of Lyme disease.
- The **vectors** for transmission of **B. duncani** and **B. divergens** are thought to be **Ixodes pacificus** and **Ixodes dentatus**, respectively.

Babesia Life Cycle: *'just understand'*



1- Like Plasmodium, Babesia ticks (definitive host) take a blood meal from a rodent (intermediate host) where sporozoites slide out of tick salivary glands and into the bloodstream.
2- The sporozoites invade erythrocytes and form a ring-shaped trophozoite.
3- Trophozoites asexually divide into 4 merozoites that stick together, forming a cross **'Maltese cross'**.
4- Merozoites develop into Gametes.
5- These gametes may get ingested by another tick where fertilization takes place re-forming sporozoites *(sexual cycle inside the tick)* which may be introduced into another intermediate host *(e.g. human).*

⇨ Human-Human transmission via blood transfusion is possible.

Clinical Manifestations:

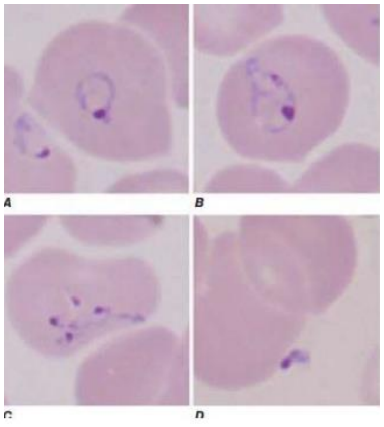
- **Asymptomatic B. microti Infection:** At least 20% of adults and 40% of children do not experience symptoms following B. microti infection. There is no evidence of long-term complications following asymptomatic infection; however, people who are asymptomatically infected may transmit the infection when they donate blood.
- **Mild to Moderate B. microti Infection:** Symptoms typically develop following an incubation period of 1–4 weeks after a 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 Infection**: 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 the 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. Besides, 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:

- Babesia differs from malaria species characteristically:
  - **a-** In Babesiosis, extracellular protozoa can be seen, but **never** in Malaria ‘pic d’.
  - **b-** **The Maltese cross** is a feature of Babesia.
- **Thin Giemsa-stained** smears are preferred to thick 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.

Treatment:

- Atovaquone + azithromycin → for mild to moderate Babesiosis.
- Clindamycin + Quinones → 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.

Good Luck