BLOOD PARASITES

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Parasites Found in Blood

- Plasmodium species (Malaria)
- Babesia
- Trypanosoma spp
- Microfilariae
- Leishmania (Visceral)
Plasmodium (Malaria)

- Four species are considered true parasites of humans, as they utilize humans almost exclusively as a natural intermediate host: P. falciparum, P. vivax, P. ovale and P. malariae. P. knowlesi, a simian malaria parasite, has also been reported from humans.

- All stages in humans occur within red blood cells. The size and shape of the infected red blood cell as well as the characteristics of the organism inside the cell are used to identify the species present.

- Microscopy (morphologic analysis) continues to be the "gold standard" for malaria diagnosis. Parasites may be visualized on both thick and thin blood smears stained with Giemsa, Wright, or Wright-Giemsa stains. Giemsa is the preferred stain, as it allows for detection of certain morphologic features (e.g. Schüffner’s dots, Maurer’s clefts, etc.) that may not be seen with the other two. Ideally, the thick smears are used to detect the presence of parasites while the thin smears are used for species-level identification.
Infected Red Blood Cells

- Note the size, shape and color of the infected RBCs
- Note the Presence or absence Schuffner’s stippling/dots
- Note the percentage of RBCs (% parasitemia)
Size, shape and color of the infected RBCs

- **P. vivax** – Infected RBCS are 1.5 to 2X larger than normal RBCs, the shape is normal to oval and the cytoplasm is pale.

- **P. ovale** – Infected RBCs are paler, 60% are larger than normal and 20% have irregular or fimbriated edges.

- **P. malariae** – Infected RBCs are normal shape and color and the size is normal to slightly smaller

- **P. falciparum** – Infected RBCs are of a normal shape and size with the cytoplasm a normal or occasionally slightly bluish color.
Presence or absence Schuffner’s stippling/dots

- *P. vivax* – Schuffner’s dots usually present in all but early ring stages

- *P. ovale* – Schuffner’s dots present in all stages, including early ring forms. Dots are usually larger and darker than *P. vivax*

- *P. malariae* - No Schuffner’s dots

- *P. falciparum* – Not Schuffner’s dots but may rarely have Maurer’s clefts (comma or triangular shaped)
Percentage of infected RBCs

- P. vivax and P. ovale infect only reticulocytes so parasitemia is limited to 2-5%.

- P. malariae infect only older smaller RBCs so parasitemia is limited to 2-5%.

- P. falciparum is able to infect all stages of RBCs so percentage of parasitemia can be very high and even exceed 50%. This is also true of P. knowlesi.
Characteristics of the Organism within the RBCs

- Rings
- Developing Trophozoites
- Mature Schizonts
- Gametocytes
- Pigment
Ring stages

- **P. vivax** – ring is about 1/3 the diameter of the RBC with a heavy chromatin dot. Occasional double ring infections occur.

- **P. ovale** – ring is usually larger and thicker than in P. vivax but otherwise similar. Occasional double ring infections occur.

- **P. malariae** – ring is smaller than that of P. vivax with a heavy chromatin dot occasionally in the center of the ring. Double ring infections are rare.

- **P. falciparum** – rings are delicate with a small chromatin dot, frequently two (headphone forms). Accole forms are present. Multiple infections of a single RBC are common.
P. falciparum - rings
Developing Trophozoites

- P. vivax – developing trophozoites become diffuse or amoeboid and may appear stringy

- P. ovale – developing trophozoites are similar to P. vivax but less amoeboid

- P. malariae – developing trophozoites remain in a solid compact form. Basket or band forms may be present.

- P. falciparum – developing trophozoites are not present as development of all stages following the ring form occur in the capillaries of the viscera.
P. vivax - Trophozoites
P. ovale – Trophozoites
P. malariae – Trophozoites
Mature Schizonts

- P. vivax – 12-24 merozoites which fill the entire RBC
- P. ovale – 6-16 merozoites in irregular clusters and occasional rosette.
- P. malariae - 6-12 merozoites in rosettes or irregular clusters with a central arrangement of pigment
- P. falciparum – 8 - 24 merozoites but not seen in peripheral blood.
P. Vivax - Mature Schizonts
P. ovale – Mature Schizonts
P. malariae – Mature Schizonts
Gametocytes

- P. vivax, P. ovale and P. malariae – gametocytes are rounded or oval and resemble one another.

- P. falciparum – Gametocytes are banana shaped and diagnostic. They appear 7 to 10 days post infection.
P. vivax - Gametocytes
P. ovale - Gametocytes
P. malariae - Gametocyte
P. falciparum - Gametocytes
Pigment

- *P. vivax* – pigment is fine, brown in color and usually present
- *P. ovale* – pigment is a dark brown and usually not abundant
- *P. malariae* – pigment is dark brown, coarse and usually present
- *P. falciparum* – pigment is black and usually present
Plasmodium knowlesi

1. Developing trophozoites resemble those of *P. malariae* as they are compact with occasional band forms.
2. Schizonts contain up to 16 merozoites which are large and clustered around pigment.
3. Gametocytes resemble those of *P. malariae*.
4. Rings resemble *P. falciparum* as they are delicate with 1-2 chromatin dots and occasional accolé forms occur.
Plasmodium knowlesi
Ookinete of *P. vivax* in a thin blood smear.
Babesia

- Babesiosis is caused by parasites of the genus, Babesia. While more than 100 species have been reported, only a few have been identified as causing human infections, including B. microti, B. divergens, B. duncani, and a currently un-named strain designated MO-1.

- In Europe, most reported cases are due to B. divergens and occur in splenectomized patients. In the United States, B. microti is the agent most frequently identified (Northeast and Midwest), and can occur in nonsplenectomized individuals. Babesia duncani has been isolated in patients in Washington and California. MO-1 has been isolated from patients in Missouri. Most infections are probably asymptomatic, as indicated by serologic surveys.

- Manifestations of disease include fever, chills, sweating, myalgias, fatigue, hepatosplenomegaly, and hemolytic anemia. Symptoms typically occur after an incubation period of 1 to 4 weeks, and can last several weeks. The disease is more severe in patients who are immunosuppressed, splenectomized, and/or elderly. Infections caused by B. divergens tend to be more severe (frequently fatal if not appropriately treated) than those due to B. microti, where clinical recovery usually occurs.

- Diagnosis
  - Microscopic examination of thick and thin blood smears stained with Giemsa. Repeated smears may be needed.
    - It is important to be able to distinguish Babesia from Plasmodium species especially P. falciparum
  - Antibody detection tests are useful for detecting infected individuals with very low levels of parasitemia (such as asymptomatic blood donors in transfusion-associated cases), for diagnosis after infection is cleared by therapy, and for discrimination between Plasmodium falciparum and Babesia infection in patients whose blood smear examinations are inconclusive and whose travel histories cannot exclude either parasite.
  - PCR
• Babesia morphology in blood smears stained with Giemsa, Wright Giemsa etc.

- Red Blood Cells are normal in size and only rings are present within the RBCs.
- All stages of RBCs are infected so levels of parasitemia can become quite high.
- Rings have delicate cytoplasm are often pleomorphic and may be vacuolated or pyriform.
- Red Blood cells often multiply infected with up to 10 organisms within a cell.
- Tetrad forms (Maltese Cross) may be present.
- Pigment is lacking.
- Extra cellular forms may be present.
Babesia sp. in a thin blood smear stained with Giemsa.
Note the tetrads (Maltese Cross), a dividing form characteristic for Babesia.

*Babesia* sp. in a thin blood smear stained with Giemsa.
Note the triad, a form characteristic for *Babesia*. 
*Babesia* sp. in a thin blood smear stained with Giemsa. Note the clumped extracellular forms indicative of *Babesia*.

*Babesia* MO-1 in a thin blood smear stained with Giemsa. *Babesia* sp. cannot be identified to the species level by morphology alone; additional testing, such as PCR, is always recommended. Note the vacuolated parasites (black arrows) in the image.
*Babesia* in a thin blood smear stained with Giemsa.

Rings of *P. falciparum* in a thin blood smear.
Trypanosomatidae

- **Trypanosoma cruzi** - **American Trypanosomiasis or Chagas Disease** - transmitted by a triatomine bug.
  - Acute phase - lasts for about 2 months after infection and symptoms are mild or absent.
    - A high number of parasites (trypomastigotes) circulate in the blood
  - Chronic indeterminate phase - Most people are unaware of their infection and may remain asymptomatic for life. 20 to 30% of patients eventually suffer from cardiac or from GI disorders
    - Parasites (amastigotes) are found in heart and digestive mucosa
    - Few or no Trypomastigotes are found in the blood during the acute stage

- **Trypanosoma brucei** - **African Sleeping sickness** - transmitted by tsetse flies.
  - Trypanosoma brucei gambiense - accounts for 98% of reported sleeping sickness and causes a chronic infection.
  - Trypanosoma brucei rhodesiense - accounts for 2% of disease and causes an acute infection. Parasite load is much higher than in T.B. gambiense infections
  - Diagnosis is made through laboratory methods as the clinical feature of the disease are not sufficiently specific
    - Trypomastigotes may be found in blood, body fluid CNS, lymph node fluid, chancre fluid
    - Serological testing not available in the US
*T. cruzi* trypomastigote in a thin blood smear stained with Giemsa. A typical trypomastigote has a large, subterminal or terminal kinetoplast, a centrally located nucleus, an undulating membrane, and a flagellum running along the undulating membrane, leaving the body at the anterior end. Trypanosomes measure from 12 to 30 µm in length and typically form a C shape.

Higher magnification of above.

*Trypanosoma cruzi* trypomastigotes are the only stage found in the blood of an infected person. Motile circulating trypomastigotes are readily seen on slides of fresh anticoagulated blood in acute infection but are rarely detectable by microscopy in chronic *T. cruzi* infection.
Trypanosoma brucei ssp. in a thin blood smear stained with Giemsa. The trypomastigotes have a small posterior kinetoplast and long undulating membrane are pleomorphic in size ranging from 16-42µm in length by 1-3µm in width. They occur as elongate slender dividing forms (with long free flagellum) or stumpy non-dividing infective (metacyclic) forms (with no free flagellum).

Trypanosoma brucei ssp. in a thin blood smear stained with Giemsa. The trypomastigote is beginning to divide; dividing forms are seen in African trypanosomes, but not in American trypanosomes.
Microfilariae

- Loa Loa
- Wuchereria bancrofti
- Brugia malayi and Brugia timori
- Mansonella ozzardi and Mansonella perstans
Loa Loa

- Loiasis is often asymptomatic. Episodic angioedema (Calabar swellings) and subconjunctival migration of an adult worm can occur.
- West and Central Afric where it is transmitted by Chrysops (fly)
- Diurnal
- Sheath present but does not stain well with Giemsa
- Microfilariae 231 to 250 microns with nuclei to tip of tail

Wuchereria bancrofti - Lymphatic filariasis

- Tropics and subtropic worldwide
- Transmitted by mosquitos
- Nocturnal
- Sheath present but does not stain well with Giemsa
- Microfilariae 244 to 269 microns with no nuclei in tail

Brugia malayi and Brugia timori - Lymphatic filariasis

- Southeast Asia and Indian subcontinent. B.timori in some islands of the Indonesian archipelago such as Timor
- Transmitted by mosquitos
- Nocturnal
- Sheath present. Sheath of Brugia malayi stains well with Giemsa but B. timori does not
- Microfilariae of B. malayyi are 177- 230 microns and those of B. timori are 265 -325 micron with terminal and subterminal nuclei widely spaced
- **Mansonella ozzardi**
  - *Mansonella ozzardi* can cause symptoms that include arthralgias, headaches, fever, pulmonary symptoms, adenopathy, hepatomegaly, and pruritus.
  - Caribbean, Central and South America
  - Transmitted by biting midges (Culicoides) and black flies (Simulium)
  - Aperiodic
  - **No sheath present**
  - Adults in the subcutaneous tissues
  - Microfilariae are 163 - 203 microns with **no nuclei in the long slender pointed tail**

- **Mansonella perstans**
  - Infections by *Mansonella perstans*, while often asymptomatic, can be associated with angioedema, pruritus, fever, headaches, arthralgias, and neurologic manifestations.
  - Africa and South America
  - Transmitted by biting midges (Culicoides)
  - Aperiodic
  - **No sheath present**
  - Adults in mesenteries and connective tissue of abdominal organs
  - Microfilariae are 190- 200 microns with **nuclei to the tip of a bluntly rounded tail**.
Microfilaria of *L. loa* in a thin blood smear, stained with Giemsa. Nuclei extend to tip of tail.
Microfilaria of *W. bancrofti* in a thick blood smear stained with Giemsa. They have a gently curved body, and a tail that is tapered to a point.

Close-up of the posterior end of *W. bancrofti* in a thick smear stained with Giemsa. The nuclear column is loosely packed; the nuclei can be visualized individually and do not extend to the tip of the tail.
Microfilaria of *B. malayi* in a thick blood smear, stained with Giemsa. The tail is tapered, with a significant gap between the terminal and subterminal nuclei. Microfilaria circulate in the blood.

Microfilaria of *B. malayi* in a thin blood smear, stained with Giemsa.
Microfilaria of *M. perstans* in a thick blood smear stained with Giemsa. The tail is blunt and nuclei extend to the tip of the tail.

Microfilaria of *M. ozzardi* in a thick blood smear, stained with Giemsa. The tail tapers to a point and the nuclei end well before the end of the tail.
Leishmaniasis is a vector-borne disease that is transmitted by sandflies and caused by obligate intracellular protozoa of the genus *Leishmania*. Human infection is caused by about 21 of 30 species that infect mammals. These include the **L. donovani complex** with 3 species (*L. donovani*, *L. infantum*, and *L. chagasi*), the **L. mexicana complex** with 3 main species (*L. mexicana*, *L. amazonensis*, and *L. venezuelensis*); **L. tropica; L. major; L. aethiopica**; and the subgenus **Viannia** with 4 main species (**L. (V.) braziliensis**, **L. (V.) guyanensis**, **L. (V.) panamensis**, and **L. (V.) peruviana**).

The different species are morphologically indistinguishable, but they can be differentiated by isoenzyme analysis, molecular methods, or monoclonal antibodies.

Human Leishmaniasis encompasses multiple clinical syndromes, most notably visceral, cutaneous, and mucosal forms. Infections can result in two main forms of disease, cutaneous leishmaniasis and visceral leishmaniasis (kala-azar). Muco-cutaneous leishmaniasis is less common. Visceral Leishmaniasis is caused by the species, *Leishmania donovani*, *Leishmania infantum/chagasi*.

Persons who have visceral leishmaniasis usually have fever, weight loss, and an enlarged spleen and liver (usually the spleen is bigger than the liver). Some patients have swollen glands. Certain blood tests are abnormal. For example, patients usually have low blood counts, including a low red blood cell count (anemia), low white blood cell count, and low platelet count. Some patients develop post kala-azar dermal leishmaniasis. Visceral leishmaniasis is becoming an important opportunistic infection in areas where it coexists with HIV.

**Diagnosis**
- Giemsa stains of smears
  - In visceral Leishmaniasis it may be possible to see WBC containing amastigotes in the blood.
- Culture followed by isoenzyme analysis
- Molecular methods
- Monoclonal antibodies
*Leishmania* amastigotes in a Giemsa-stained tissue scraping. Amastigotes of *Leishmania* are spherical to ovoid and measure 1-5 µm long by 1-2 µm wide. They possess a large nucleus, a prominent kinetoplast, and a short axoneme, the last of which is rarely visible by light microscopy. The organisms reside in macrophages of the host and can be found throughout the body.

Leishmania amastigotes are being freed from a rupturing macrophage.
**Ehrlichia**

- *Ehrlichia* are small, gram-negative bacteria, round or ellipsoidal in shape. They preferentially invade mononuclear phagocytes, such as monocytes and macrophages, and in some cases neutrophils. In all of these cell types they occupy cytoplasmic vacuoles, usually in bacterial microcolonies known as morulae. *Ehrlichia* cycle in nature between ticks and mammals, and can cause disease in many mammalian species.
- First recognized in the US in 1986. Ehrlichiosis became a reportable disease in 1999 and is considered an merging zoonotic disease according to the CDC.
- **Laboratory Diagnosis**
  - IFA on paired serum samples to demonstrate a 4x rise in antibody titers
  - PCR - most sensitive during first week of illness
  - Morulae - During the first week of illness these may be seen in the cytoplasm of white cells in up to 20% of patients.
    - E. chaffeensis - infects monocytes
    - E. ewingii - infects granulocytes
- Several forms of infection are found in humans
  - Ehrlichia bacterium not yet named- referred to E. muris-like
  - Ehrlichia chaffeensis
    - Transmitted by ticks such as *Amblyomma americanum* (Lone Star Tick)
  - Ehrlichia ewingii
    - Transmitted by *Amblyomma americanum*
  - Ehrlichia sennetsu - Sennetsu fever
    - Vector not determined but may be from ingestion of raw fish.
    - Cases limited to Western Japan and Malaysia
E. chaffeensis morulae in the cytoplasm of a human monocyte.

Image showing E. ewingii morulae in the cytoplasm of a neutrophil.
Anaplasma phagocytophilium (formerly Ehrlicia phagocytophilium) is a small gram negative intracellular bacterium of granulocytes. First identified in 1990 in humans though known to cause veterinary disease since 1932.

- Casuative agent of human granulocytic anaplasmosis.
  - Anaplasmosis can be a serious illness that can be fatal (< 1%) if not treated correctly, even in previously healthy people. Severe clinical presentations may include difficulty breathing, hemorrhage, renal failure or neurological problems.
  - Patients who are treated early may recover quickly on outpatient medication, while those who experience a more severe course may require intravenous antibiotics, prolonged hospitalization or intensive care.

- Transmission via bite of a tick – Ixodes scapularis or Ixodes pacificus.

- Laboratory Diagnosis
  - IFA using acute and convalescent phase serum to look for a 4x increase in antibody titer
  - PCR - most sensitive during the first week of illness
  - Wright or Giemsa stained blood smears to detect morulae in granulocytes. During the first week of illness a microscopic examination of blood smears (known as a peripheral blood smear) may reveal morulae in the cytoplasm of white blood cells in up to 20% of patients. However, the observance of morulae in a particular cell type cannot conclusively identify the infecting species.
Morulae detected in a granulocyte on a peripheral blood smear, associated with A. phagocytophilum infection.
Borrelia

- Borrelia burgdorferi
  - Spirochete bacteria that is the causative agent of Lyme disease
  - Transmitted via the bite of an Ixodes tick
- Diagnosis
  - Two-tiered testing recommended by CDC
    - EIA or IFA followed by IgM and IgG Western blot if EIA or IFA is positive
  - Blood smears - not recommended but occasionally ordered.
    - Spirochetes stain well with Giemsa and can be seen in blood smears.