Lab(3)

Kingdom: Protista
Subkingdom: Protozoa
Phylum: Sarcomastigophora
2-Subphylum: Ciliophora
Class: Ciliata
Order: Euciliata
Genus: Balantidium coli

3- Subphylum: Mastigophora (Flagellates)
Class: Zoomastigophora
Order: Diplomonadida
Genus: Giardia lamblia, Trichomonas vaginalis

**Balantidium coli**

**Disease name:** Balantidiasis, Balantidil dysentery

**Site of infection:** Large intestine, cecum and terminal ileum

**Morphology of trophozoite**

They are covered in cilia and have boring or rotary motility. *Balantidium coli* is known for being the largest protozoan parasite of humans, the two nuclei are clearly visible. The macronucleus is long and kidney-shaped, and the spherical micronucleus is nestled next to it.
**Cyst**

Cysts are smaller than trophozoites, measuring Cysts are round and have a tough, heavy cyst wall made of one or two layers. Usually only the macronucleus and perhaps cilia and contractile vacuoles are visible in the cyst.

**Life cycle**

**Infective stage:** Cyst  
**Diagnostic stage:** Cyst, trophozoite
Symptoms:

who are infected with B. coli remain asymptomatic. trophozoites can invade the mucosa of the large intestine (cecum and colon) and cause ulcerations. Other bacteria in the intestine may enter the ulcer leading to secondary infections. Common symptoms chronic diarrhea, occasional dysentery (diarrhea with passage of blood or mucus), nausea, foul breath, colitis (inflammation of the colon), abdominal pain, weight loss, deep intestinal ulcerations, and possibly perforation of the intestine.

Diagnosis

Examination of stool samples, looking for trophozoites and cysts. Trophozoites are readily identified because of their large size and the fact that B. coli is the only ciliate that parasitizes humans.

*Giardia lambilia*

Disease name: Giardiasis

Site of infection: small intestine

Morphology:
Trophozoite: It is flattened pear shaped with two nuclei, two slender axostyles and eight flagella (four pairs of flagella). They attach themselves to the surface of jejuna or duodenal mucosa by their disc like suckers which are found on their ventral surface.

Cyst: It is ellipsoid in shape, contain four nuclei. Longitudinal fibrils consisting of the remains of axonesmes and parabasal bodies may also seen.
*Giardia lamblia* cyst stained with iodine

**life cycle**
Symptoms: are abdominal pain, flatulence and watery diarrhea no blood no mucus is normally seen.

Laboratory diagnosis

1- Trophozoites and cyst are found by examination of saline wet preparation of fresh diarrheic stool.

2- duodenal or jejuna aspirate

3- Elisa to detect IgM in serum provides the evidence of current infection.

4- Biopsy from the upper intestine

3- *Trichomonas vaginalis*

It exists only in trophozoite form

Transmission is by sexual intercourse

Disease name: Trichomoniasis
Site of infection: the urethra & vagina of women and the urethra & prostate gland of man

Morphology:

Pear shaped organism with central nucleus and four anterior flagella and

Undulating membrane extends about two-thirds of its length.

![Trichomonas vaginalis trophozoite](image)

**Trichomonas vaginalis** trophozoite

Life cycle:

![Trichomonas vaginalis life cycle](image)

Symptoms:

Women

- A **vaginal discharge** is common. This is typically greeny-yellow and may be 'frothy'. The discharge usually has an unpleasant smell.
- Vagina itching and uncomfortable.
- It may be sore when you pass urine.
• No symptoms occur in some women. However, they can still pass on the infection even if you have no symptoms.

Men

• Discharge from the penis is common.
• It may be sore when you pass urine.
• You may pass urine frequently (due to irritation inside the penis).
• No symptoms occur in most infected men. However, you can still pass on the infection even if you have no symptoms.

Diagnosis:
cell cultures
antigen tests (antibodies bind if the *Trichomonas* parasite is present, which causes a color change that indicates infection)
tests that look for *Trichomonas* DNA
examining samples of vaginal fluid (for women) or urethral discharge (for men) under a microscope
**Blood and Tissue flagellates:** it include flagellated protozoa that contain

1-flagella  
2-nucleus  
3-kinetoplast

**Morphology of tissue and blood flagelates**

1-Amastigote  
2-Promastigote  
3-Epimastigote  
4-Trypomastigote

Kingdom: Protista

Subkingdom: Protozoa

Phylum: Sarcomastigophora

Class: Zoomastigophora

Order: Kinetoplastida

Family: Trypanosomatidae


2- *Trypanosoma* spp.

*Leishmania* spp.
1- *Leishmania tropica*

2- *Leishmania donovani*

3- *Leishmania braziliensis*

**Disease name:**

- *Leishmania tropica* causes Baghdad boil (cutaneous leshmaniasis).

- *Leishmania donovani* causes kalaazar or black fever (visceral leshmaniasis).

- *Leishmania braziliensis* causes subcutaneous (mucocutaneous leshmaniasis).

**Site of infection:**

- *Leishmania tropica* (skin)

- *Leishmania donovani* (liver, spleen, lymph node, bone marrow)

- *Leishmania braziliensis* (mucocutaneous tissue of skin, nose, mouth)

**Definitive host**: human

**Intermediate host (vector)**: *Phlebotomus* female (sand fly)

**Morphology:**

*Leishmania* exist as flagellated extracellular Promastigotes in the sandfly vector and as a flagellar obligate intracellular Amastigotes within mononuclear phagocytes of their vertebrate hosts. The various species are not distinguishable morphologically from one another. When stained with Giemsa stain, amastigotes appear as round or oval bodies ranging from 2-3 micrometer in diameter with a well defined nucleus and kinetoplast, a rod shaped specialized mitochondrial structure. The flagellated Promastigote form is spindle shaped, measuring (10-20) micrometer in length, not including the length of the flagellum. As in the Amastigote form a nucleus and kinetoplast are clearly visible.
**Life cycle:**

All forms of infection starts when a female sand fly (*Phlebotomus sp.*) takes a blood meal from an infected host. Small amounts of blood, lymph and macrophages infected with *Leishmania* amastigotes are ingested. Once ingested the amastigotes transform to promastigotes in the sandfly, the non-infective promastigotes divide and develop into infective metacyclic promastigotes. These are formed in the midgut of the sandfly and migrate to the proboscis. When the sandfly bites, the extracellular inoculated promastigotes at the site of the bite are phagocytosed by macrophages. After phagocytosis, transformation to dividing amastigotes occurs within 24 hours. Reproduction at all stages of the lifecycle is believed to occur by binary fission. No sexual stage has been identified.
**Diagnosis:**

1- Direct smear of blood and lymph.

2- Serology.

3- Cuturing in N.N.N. (Nove MacNeal-Nicole).

4- Biopsy from liver, Spleen and bone marrow.
**Lab(5)**

*Trypanosoma* *spp.*

Trypanosomes are hemoflagellates and three species of the genus *Trypanosoma* are responsible for disease in humans

1-**African trypanosomiasis (sleeping sickness)**

There are two clinical forms of African trypanosomiasis

A – *Trypanosoma brucei gambiense* causes (Gambian trypanosomiasis chronic sleeping sickness).

B- *Trypanosoma brucei rhodesiense* causes (Rhodesians trypanosomiasis acute sleeping sickness).

**Site of infection:** blood, lymph, spleen, liver, cerebrospinal fluid

**Vector Tsetse genus:** *Glossina* *spp.*

The parasite undergo several forms depending on the host

In vertebrate host (human) which is definitive host: trypomastigote

In invertebrate host (insect) which is intermediate host: trypomastigote and epimastigote

**Morphology**

The parasite is an elongated cell with single nucleus which usually lies near the centre of the cell, single flagellum which appears to arise from a small granule kinetoplast. Two forms of trypomastigote can be seen in peripheral blood: one is long slender, 30 μm in length, and is capable of multiplying in the host, the other is stumpy, not dividing, 18 μm in length.
**Life Cycle**

Transmission from one vertebrate to another is carried out by blood-sucking invertebrates, usually an insect (vector). Metacyclic (infective) trypomastigotes are inoculated through the skin when a tsetse fly takes a blood meal. The parasites develop into long slender trypomastigotes which multiply at the site of inoculation where ulceration occurs. The trypanosomes continue to develop and then may invade the lymphatic tissues, the heart, various organs and in later stages, the central nervous system. Trypomastigotes are taken up by the tsetse fly (male and female) during a blood meal. The parasites develop in the midgut of the fly where they multiply. 2-3 weeks later the trypomastigotes move to the salivary glands transforming from epimastigotes into metacyclic S-shape (infective) trypomastigotes. These are known as salivarian trypanosomes as they complete their development in the salivary system (anterior portion of the vector). The tsetse fly remains infective for life i.e. about three months.
**Symptoms:**

The early stages of African trypanosomiasis may be asymptomatic and there is a low grade parasitaemia. This period may last for several weeks to several months. The disease may terminate untreated at this stage or go on to invade the lymph glands. Invasion of the lymph glands is usually accompanied by a high irregular fever with shivering, sweating and an increased pulse rate. Trypanosomes may invade the central nervous system giving symptoms of meningoencephalitis, confusion, apathy, excessive sleeping and incontinence.

**Laboratory diagnosis of African Trypanosomiasis is by:**
- Examination of blood for the parasites
- Examination of aspirates from enlarged lymph glands for the parasites
- Examination of the CSF for the parasite
- Detection of trypanosomal antibodies in the serum

2-**American trypanosomiasis**

*Trypanosoma cruzi* causes (chagas disease).

**Site of infection:** muscular muscle, kidneys, thyroid gland, sexual organs

**Vector Bug genus:** *Tritoma*

**Morphology:**

*Trypanosoma cruzi* has a single form (monomorphic), about 20μm in length, and characteristically curved (C-shape). The kinetoplast is large, considerably larger than the *Trypanosoma brucei* species. The flagellum is medium in length. *Trypanosoma cruzi* in man only occurs in the amastigote phase in muscular tissue and cells.
Lifecycle of *Trypanosoma cruzi*

Symptoms:
In an acute infection there may be fever, malaise, increased pulse rate, and enlargement of lymph glands, liver, and possibly spleen. Chronic infection include signs of cardiac muscle damage leading to heart failure.

**Laboratory diagnosis** of South American trypanosomiasis is by:

- Examination of blood.
- Xenodiagnosis
- Blood culture
- Serology
Lab (6)

**Kingdom:** Protista

**Subkingdom:** Protozoa

**Phylum:** Sarcomastigophora

**Class:** Ampicomplexa (sporozoa)

**Order:** Eucoccida

**Family:** Plasmodidae

**Genus:** *Plasmodium vivax* (benign tertian malaria).

- *Plasmodium malaria* (quartian malaria).
- *Plasmodium ovale* (ovale tertian malaria).
- *Plasmodium falsiparm* (malignant tertian malaria).

**Disease name:** Malaria

**Site of infection:** Red blood cell and Liver tissue cell

*Plasmodium requires two hosts:*

- **Definitive invertebrate host:** (vector Anopheles mosquitoes female).
- **Intermediate vertebrate host:** (mammals, birds and lizards).
**Morphology:**

<table>
<thead>
<tr>
<th></th>
<th><em>P. falciparum</em></th>
<th><em>P. malaria</em></th>
<th><em>P. vivax</em></th>
<th><em>P. ovalae</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Trophozoite</strong></td>
<td>ring forms</td>
<td>band form</td>
<td>amoeboid form</td>
<td>compact rings in cells</td>
</tr>
<tr>
<td><strong>Schizont</strong></td>
<td>(8 – 36) merozoites.</td>
<td>(6–12) large merozoites</td>
<td>(16). merozoites</td>
<td>(6-12) merozoites</td>
</tr>
<tr>
<td><strong>Microgametocyte</strong></td>
<td>Larger than RBC, kidney shaped with blunt round ends</td>
<td>Smaller than RBC, round compact</td>
<td>Fills enlarged RBC, small round or oval, compact with central nucleus</td>
<td>Of the size of RBC round, compact</td>
</tr>
<tr>
<td><strong>Macrogametocyte</strong></td>
<td>more slender and longer than the male</td>
<td>Round or oval with peripheral nucleus</td>
<td>large round or oval with peripheral nucleus</td>
<td>Round or oval with peripheral nucleus</td>
</tr>
</tbody>
</table>
**Life cycle:**

**sexual cycle**

occurs in mosquito (9-21 days), fusion of micro and macrogametes are infective for mosquito → zygote → ookinete (~24 hours) → oocyst

Asexual replication (sporogony) → sporozoites released → migrate through hemocoel → invade salivary glands

**Asexual cycle**

1-**Exoerythrocytic schizogony (Liver stage)**

Sporozoites injected during mosquito feeding → merozoites (uninucleate asexual stages) invade liver cells. After this initial replication in the liver (exo-erythrocytic schizogony) the parasites undergo asexual multiplication in the erythrocytes.

2-**Erythrocytic schizogony (Blood stage)**

Merozoites infect red blood cells and forms the ring stage in it and transform into trophozoites that mature into schizonts ( multinucleated asexual stages) , which rupture releasing merozoites. Some parasites differentiate into asexual erythrocytic stages (gametocytes) repeated erythrocytic schizogony (48hr) in Pf., Pv., Po. and (72 hr) in Pm.
Symptoms:

- **Fever**: Often irregular. The regular pattern of fever does not occur until the illness has continued for a week or more.
- **Anemia**: The anemia is hemolytic in type. It is more severe in infections with *P. falciparum* because in this infection cells of all ages can be invaded.
- **Splenomegaly**: The spleen enlarges early in the acute attack of malaria.
- **Jaundice**: A mild jaundice due to hemolysis may occur in malaria.

Diagnosis:

1. Thin blood films stained with Giemsa stain.
2. Antibody test.

Lab (7)
Kingdom: Protista

Subkingdom: Protozoa

Phylum: Sarcomastigophora

Class: Ampicomplexa (sporozoa)

Order: Eucoccidia

Genus:
1- Toxoplasma gondii
2- Cryptosporidium parvum
3- Isospora belli

1- Toxoplasma gondii
Disease name: Toxoplasmosis
Site of infection: brain, eye, skeletal muscle, neural tissue
Definitive host: Cats

Morphology:
1- Tachyzoite: pear shaped organisms
2- Bradyzoites
3- Oocysts: contain 4 sporozoites (infective stage)

Life cycle:

Infection occur by ingestion of Oocyst from contaminated hands or food, sporozoites released from oocyst in the small intestine penetrate the intestinal mucosa and find their way into macrophages where they divide very rapidly (hence the name tachyzoites) and form a cyst which may occupy the whole cell. The infected cell burst and release the tachyzoites to enter muscle and nerve cells where they are protected from the host immune system and multiply slowly (bradyzoites). these cysts are infectious to carnivores (including man). cats get infected by ingestion of cysts in flesh. Decystation occurs in the small intestine the organism penetrates the submucosal epithelial cell where they undergo mitosis, resulting micro (male) and macro (female) gametocytes. Fertilized macrogametocytes develop into Oocyst that are discharged into the gut lumen and excreted.
Symptoms

Abortion, Hydrocephalus or Microcephaly, Blindness

Laboratory Diagnosis
1. Serological Techniques
2. Isolation parasites techniques.
3. Direct identification of the parasite from peripheral blood, amniotic fluid or in tissue section.

2-Cryptosporidium parvum

Disease name: Cryptosporidiosis
Site of infection: Epithelial cells of the small intestine
Infective stage: Oocyst

Life cycle
Symptoms
Persistent watery offensive diarrhea accompanied with abdominal pain, nausea, vomiting and anorexia

Diagnosis:
- Demonstration of oocyst in the stool.
- intestinal fluid or small bowel biopsy specimens
- Antigen in stool (ELISA)
- Molecular methods (PCR)
- Serological test

3-Isospora belli
Disease name: Isosporiasis
Site of infection: Epithelial cells of the small intestine.
Infective stage: Oocyst

Life Cycle
This organism can be acquired by the ingestion of sporulated oocysts found in contaminated food or water. The oocyst are thin walled, transparent, ovoid in shape and much larger than the oocysts of Cryptosporidium parvum. Oocysts of I. belli can survive for years in the environment.
Symptoms

In chronic infections, severe non-bloody diarrhea with cramp-like abdominal pain can last for weeks and result in fat malabsorption and weight loss. Eosinophilia may be present (atypical of other protozoa infections).

Laboratory Diagnosis:
Oocysts can be detection in stool samples. Alternatively, oocysts can be seen in a fecal smear stained by a modified Ziehl-Neelsen method, where they stain a granular red color against a green background, or by phenolauramine.