قسم التقنيات الاحيائية

مادة الاحياء المجهرية٢ (العملي)

المرحلة الثانية

الكورس الثاني

أ. م.د. عائدة حسين ابراهيم

م.د. علياء محمد هادي

م.د. رند مناف عبد الرحمن

م.م. نور الهدى فخري

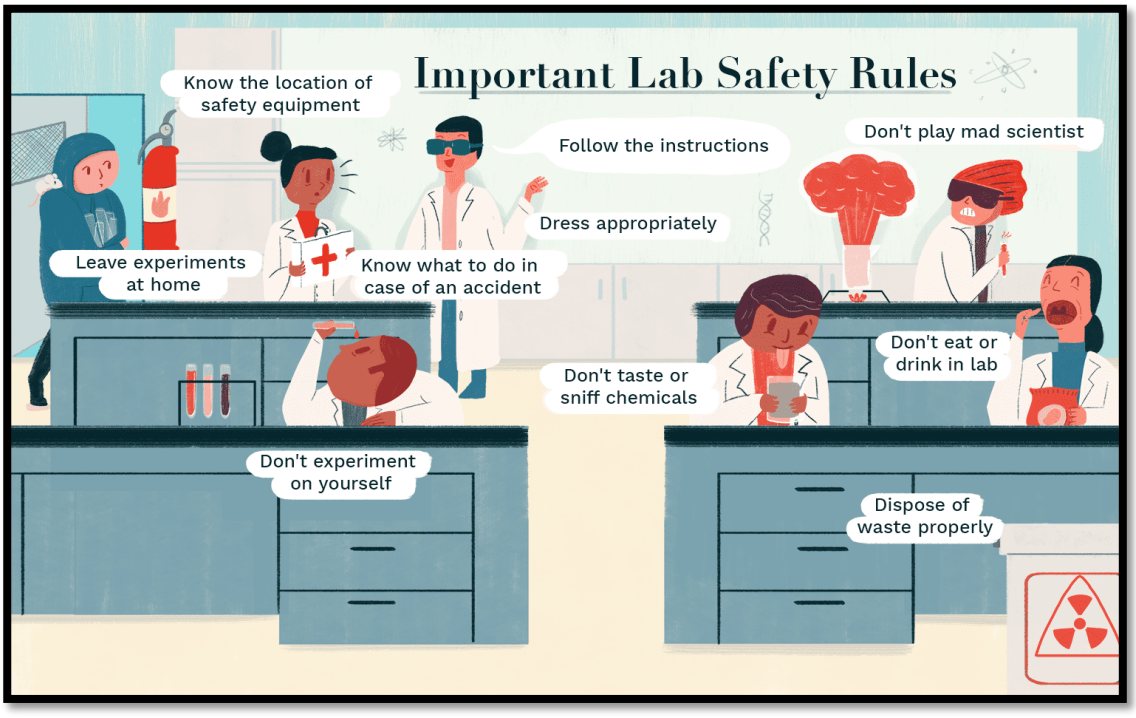
Lab. one

Instructions for the laboratory work and learn how to make the blood smear

Lab safety is one of the most important concerns when you are working with medical supplies, hazardous chemicals, and heavy-duty equipment. Because accidents can easily occur when working in the lab, it is critical to pay attention to the proper care and usage of the supplies you use in the lab. Working in a lab is a high-risk environment, and the lab technicians and experts will have frequent trainings to keep up-to-date on the proper safety precautions they need to take on a daily basis. From wearing protective equipment to properly caring for toxic chemicals, there are many safety rules you must follow when you are working in the lab.

Five Lab Safety Rules You Must Follow :

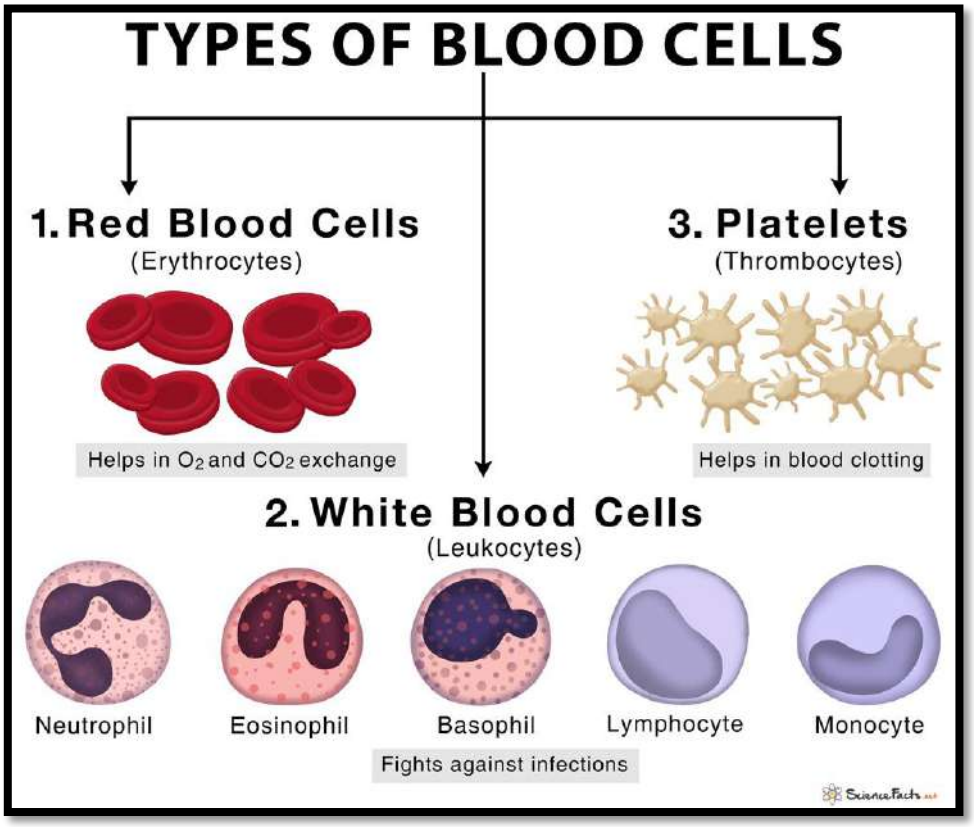
- Wear Proper Lab Clothing. ...
- Handle Chemicals with Care. ..
- Properly Care for the **Equipment**. ...
- Always Locate Emergency **Equipment**. ...
- Keep Food and Drink Out of the Lab.



What is a blood smear?

A blood smear is a blood test used to look for abnormalities in blood cells. The three main blood cells that the test focuses on are:

- Red cells, which carry oxygen throughout your body.
- White cells, which help your body fight infections and other inflammatory diseases
- Platelets, which are important for blood clotting.



The test provides information on the number and shape of these cells, which can help doctors diagnose certain blood disorders or other medical conditions.

Irregularities in the number or shape of your red blood cells can affect how oxygen travels in your blood. These abnormalities are often caused by a mineral or vitamin

deficiency, but they can also be caused by inherited medical conditions, such as sickle cell anemia.

White blood cells are an integral part of your body's immune system, which is a network of tissues and cells that help your body fight infection. Having too many or too few white blood cells can indicate a blood disorder. Disorders affecting these cells often result in the body's inability to eliminate or control infections or other inflammatory problems.

Abnormalities in the shape or number of white blood cells may be signs of a platelet disorder. Platelet disorders affect your blood's ability to clot, which can lead to excessive or prolonged bleeding or blood clotting. They often occur when the body produces too many or too few platelets.

Why is a blood smear done? The blood smear test is often done to diagnose conditions that are causing:

- unexplained jaundice.
- unexplained anemia (low levels of normal red blood cells).
- abnormal bruising.
- persistent flu-like symptoms.
- sudden weight loss.
- unexpected or severe infection.
- skin rashes or cuts.
- bone pain.

Lab two

Preparation of the blood smear:

A. Blood collection for thick or thin blood smears:

1. Label pre – cleaned slides (preferably frosted-end) with patient's name (or other identifier), date and time of collection.

2. Wear gloves.

3. Clean slides with 70 to 90% alcohol and allow to dry. Do not touch the surface of the slide where the blood smear will be made.

4. Select the finger to puncture, usually the middle or ring finger. In infants, puncture the heel.

5. Clean the area to be punctured with 70% alcohol; allow to dry.

6. Puncture the ball of the finger, or in infants puncture the heel.

7. Wipe away the first drop of blood with clean gauze.

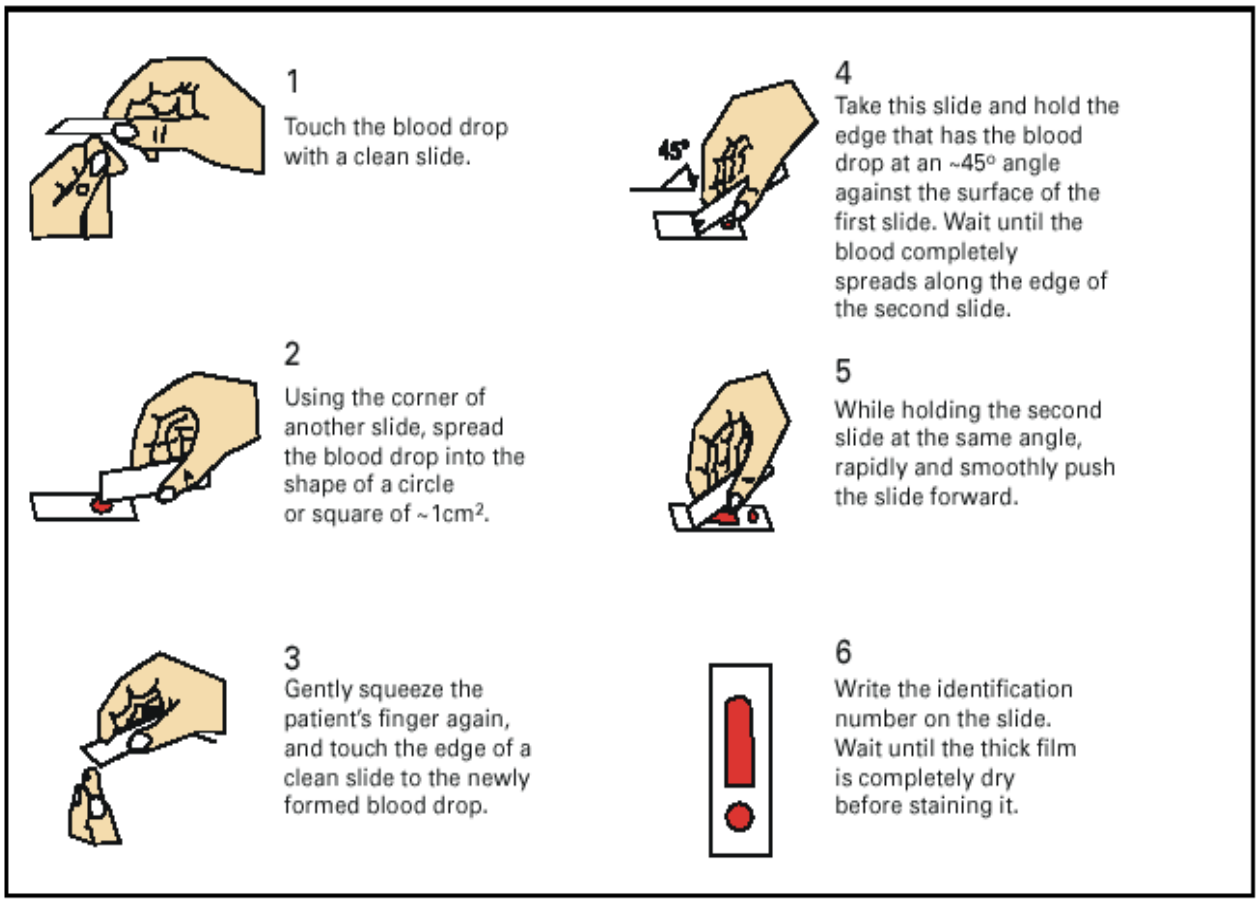
8. Touch the next drop of blood with a clean slide. Repeat with several slides (at least two thick and two thin smears should be made). If blood does not well up, gently squeeze the finger.

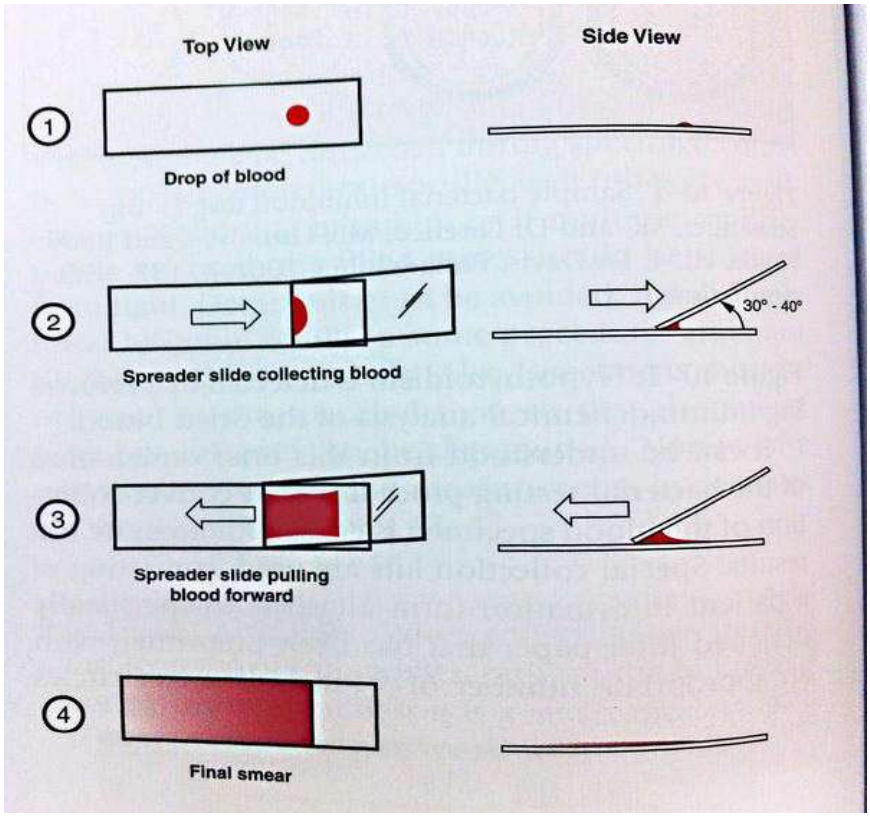
Making thick and thin blood smears

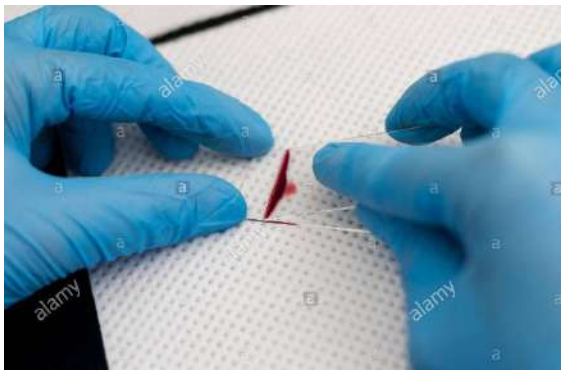
1. Whenever possible, use separate slides for thick and thin smears. 2. Thin film (a): Bring a clean spreader slide, held at a 45° angle, toward the drop of blood on the specimen slide. 3. Thin film (b): Wait until the blood spreads along the entire width of the spreader slide. 4. Thin film (c): While holding the spreader

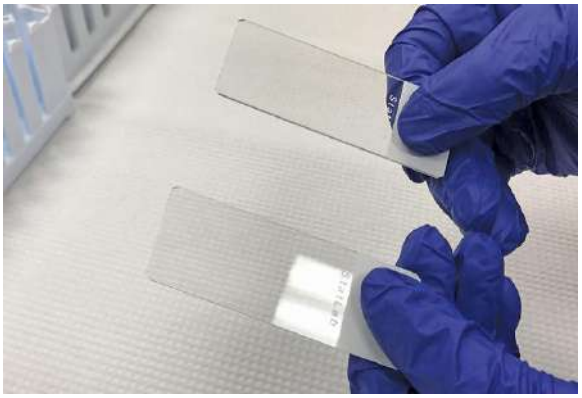
slide at the same angle, push it forward rapidly and smoothly. 5. Thick film: Using the corner of a clean slide, spread the drop of blood in a circle the size of a dime (diameter 1-2 cm). Do not make the smear too thick or it will fall off the slide. (You should be able to read newsprint through it.) 6. Wait until the thin and thick films are completely dry before staining. Fix the thin film with methanol (100% or absolute) and let it dry completely before staining. The thick film should not be fixed. 7. If both thin and thick films need to be made on the same slide, fix only the thin film with methanol. The thick film should not be fixed.

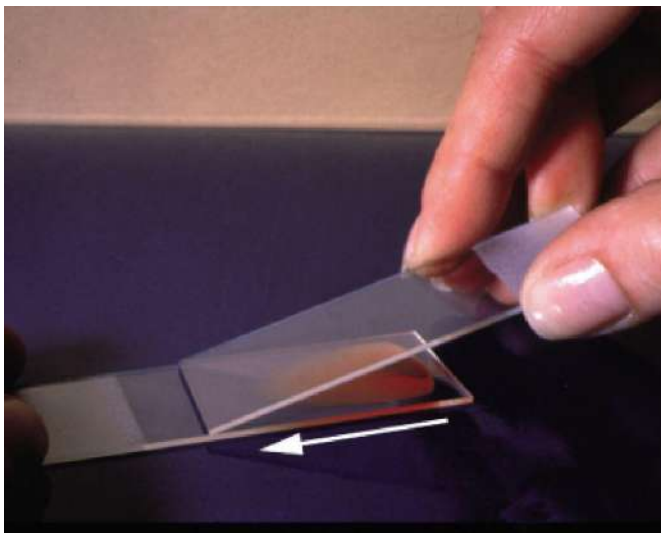
Figure A-2. Preparation of a thin and a thick blood film on the same slide.




Top View
Side View
1
Drop of blood
2
30° - 40°
Spreader slide collecting blood
3
Spreader slide pulling blood forward
4
Final smear







Fixing of the blood smear :

Before staining, the blood films need to be fixed with acetone free methyl alcohol for up to 1 minute in order to prevent hemolysis when they come in contact with water when water has to be added subsequently. Alcohol denatures the proteins and hardens the cell contents. For wright s stain and leishman s stain no pre-fixation is required as these contain acetone free methyl alcohol but for Giemsa s stain pre-fixation is must because the alcohol content is only 5% in the ready to use stain.

Stain preparation and staining:

Routine analysis of blood in medical laboratories is usually performed on blood films stained with Wright's stain or Giemsa stain.

1. Cover the slide with stain (Gimza or Wright's stain for 1-2 minutes taking care that it do not dry on the slide.
2. Now dilute this with equal amount of buffer water.
3. Diluted stain is allowed to act for 3-5 minutes and then flooded off with buffer (or top water).
4. Shake excess buffer away and dry in the air.

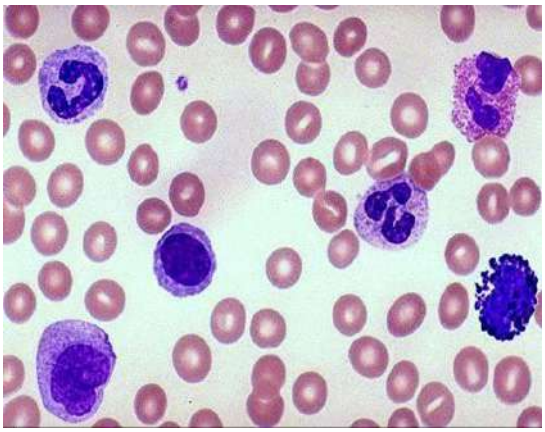
Examination of blood Film

There are several necessary steps in the examination of a peripheral blood smear.

a. Low –power (x10) Scan :

b. High – Power (x 40) Examination

c. Oil immersion (x100) Examination



Lab three

Laboratory diagnosis methods of parasites:

Laboratory diagnosis is an important part of identifying the parasite causing the disease, as it confirms the clinical diagnosis and provides unquestionable evidence of its presence.

The responsibility for accurate diagnosis requires high skill and the ability to know the parasite and distinguish it from the impurities and foreign substances present, as well as provide the needs of necessary devices and materials.

Methods for the diagnosis of parasitic infections have stagnated in the past three decades. Currently, the detection and diagnosis of parasite infections rely on several laboratory methods in addition to clinical symptoms, clinical history, travel history, and geographic location of patient. The primary tests currently used to diagnose many parasitic diseases have changed little since the development of the microscope in the 15th century by Antonie van Leeuwenhoek. Furthermore, most of the current tests cannot distinguish between past, latent, acute, and reactivated infections and are not useful for following response to therapy or for prognosis.

Kinds of tests are used to diagnose parasitic infections:

- **A fecal (stool) exam, also called an ova and parasite test (O&P)**
  This test is used to find parasites that cause diarrhea, loose or watery stools, cramping, flatulence (gas) and other abdominal illness. This test looks for ova (eggs) or the parasite . Specimens not collected in special container and don't use a preservative fluid but should be refrigerated, not frozen, until delivered to the lab .
- **Endoscopy/Colonoscopy**
  Endoscopy is used to find parasites that cause diarrhea, loose or watery stools, cramping, flatulence (gas) and other abdominal illness. This test is used when stool exams do not reveal the cause of your diarrhea. This test is a procedure in which a tube is inserted into the mouth (endoscopy) or rectum (colonoscopy) so that the

doctor, usually a gastroenterologist, can examine the intestine. This test looks for the parasite or other abnormalities that may be causing your signs and symptoms.

- **Blood tests** :

Some, but not all, parasitic infections can be detected by testing your blood. Blood tests look for a specific parasite infection; there is no blood test that will look for all parasitic infections. There are two general kinds of blood tests that your doctor may order:

  - *Serology* This test is used to look for antibodies or for parasite antigens produced when the body is infected with a parasite and the immune system is trying to fight off the invader.
  - *Blood smear* This test is used to look for parasites that are found in the blood. By looking at a blood smear under a microscope, parasitic diseases such as filariasis, malaria, or babesiosis, can be diagnosed. This test is done by placing a drop of blood on a microscope slide. The slide is then stained and examined under a microscope.

- **X-ray, Magnetic Resonance Imaging (MRI) scan, Computerized Axial Tomography scan (CAT) :** These tests are used to look for some parasitic diseases that may cause lesions in the organs.

1. Diagnostic tools and instruments for the detection of parasitic agents:

a. Microscopy (light compound microscope): For many years, microscopy has been the only tool available for the detection of parasites through inspection of blood smears, tissue specimens, feces, lymph node aspirates, bone marrow, and even cerebrospinal fluid. However, sample preparation for direct observation is time-consuming, labour intensive, and proper diagnosis depends on qualified laboratory technicians. In reality, all major intestinal helminth infections are still solely dependent on microscopy for diagnosis. As for other parasite infections, many are

confirmed by the use of microscopy in conjunction to other methods of diagnosis including serology-based assays and more recently molecular-based assays.

b. Dissecting microscope ; incubator; drying oven ; centrifuge; Autoclave; microtome; Bunsen burner; syringes; water bath; hot plate.

2. **Reagents and chemicals: includes** :

- **10% FORMALIN FIXATIVE IN WATER :** This preservative is a good overall fixative and will fix both ova and cysts although it only preserves the internal morphology of the cysts for up to 6 months, after which the cytoplasm of the organism becomes granular with poor nuclear definition.
- **SODIUM ACETATE ACETIC ACID FORMALIN (SAF) :** SAF fixed material is suitable for direct examination, concentration (Formalin/Ethyl Acetate) and permanent staining.
- **MERTHIOLATE-IODINE FORMALIN (MIF) :**Formol - Ethyl Acetate concentration methods can be performed on samples preserved in MIF.
- **POLYVINYL ALCOHOL (PVA) :** This method will preserve ova, larvae and trophozoites well, but cysts may show some distortion. However, some ova and cysts do not concentrate well when preserved in PVA.
- **MAYER'S GLYCERIN-ALBUMIN :** Mayer's Glycerin-Albumin is used when preparing slides for staining. The albumin helps to ensure that the specimen adheres to the slide and the glycerin retains sufficient moisture to prevent distortion or disruption of organisms on drying.
- **TRITON X-100 SOLUTION :** Used to emulsify parasites in faeces,
- **ETHYL ACETATE :** A solvent that removes fat from faeces.
- **ACETONE :** Solvent**.**
- **NEUTRAL RED :** Aqueous solution and dye, can be used in the Gram's technique.

3. Parasitology Stains :

- **EOSIN/SALINE :** This stain is useful for the detection of motile trophozoites of Entamoeba species.
- **ACRIDINE ORANGE (ACETATE BUFFERED):** The addition of acridine orange to a faecal concentrate highlights the chromidial bars of Entamoeba coli, Entamoeba histolytica / dispar and Entamoeba hartmanni, which fluoresce bright green.
- **AURAMINE PHENOL (LEMPERT)**
- **GIEMSA STAIN RAPID :** Giemsa stain can also be used to stain films of unformed faeces, faecal exudate, duodenal aspirates etc.
- **LUGOL'S IODINE – AQUEOUS :** Temporary stain for protozoa.
- **IRON HAEMATOXYLIN – SOLUTION A AND SOLUTION B.**
- **TRICHROME FOR PROTOZOA.**
- **MALACHITE GREEN.**

Selection, Collection, and Submission of Samples

Parasitology Submission Guidelines

1. **Fresh Feces**

- Fresh fecal samples are less than 48 hours old.
- Samples should be placed in individual sealed containers labeled with the animal number/name and the date collected.
- Acceptable containers include plastic containers with lids, WhirlPaks, and ziplock bags. Please do not send feces in rubber gloves or OB sleeves.
- Refrigerate samples as soon as possible after collection, but do not freeze.
- Submit samples as soon as possible in a Styrofoam container with frozen gel packs via any of the 24 to 48 hour transport services.
- See individual test for sample size. Most tests require 5 grams of feces for accurate results.

- For Baermann exams: fecal samples must be no more than 1-2 hours old and unrefrigerated. Larvae are fragile! These samples must be submitted to the lab before noon due to the test run time of 4 hours.

2. **Whole Blood**

- 1 mL of whole blood submitted in a purple top tube (EDTA tube).

3. **Serum**

- Serum samples need to be at least .5mL.

4. **Skin Scrapings/ Hair**

- If possible submit samples in a glass tube. Red top blood collecting tubes work well as specimen containers.
- If necessary or if sample size is small, samples can be submitted on a glass slide if the cover slip is sealed with nail polish or VasPar.
- Please do not use scotch tape as a cover slip as it obscures vital details of the sample.

Parasites

- Ectoparasites can be transported in 70% alcohol in a sealed container. Red top blood collecting tubes work well.
- Intestinal parasites need to be transported in water in a sealed container. Intestinal parasites received in formalin cannot be identified.
- Please label container with host and location where the parasite was found.
- Level of Parasite Identification is determined by lab personnel when the sample arrives in the laboratory to be identified.

Soil

- Soil samples need to be at least 30 grams.
- Collect soil from 2-3 inches below the surface from 3 places in the area to be tested.

- Please send sample in a sealed plastic container or ziplock bag.

Direct Smear

- Direct fecal smears are most useful for the diagnosis of protozoal parasites which have motiletrophozoite stages that are passed in the feces. Cysts and oocysts of coccidia and *Giardia* sp. can be seen on direct smears; however, these non-motile stages are more likely to be recovered when concentrated using a flotation technique.
- In order to be diagnostic, direct smears MUST be performed using fresh feces. Fresh feces means BODY TEMPERATURE (usually less than five or ten minutes old!). As the specimen cools, trophozoites lose their motility and their diagnostic features become less recognizable. In preparing the smear, use saline. Water will rupture some trophozoites, rendering them unrecognizable.
- **Indications:** Motile protozoa trophozoites (feces must be body temperature).
- **Limitations:** Small sample size (sample size is so small that if you see nothing, it may not mean that the animal has no parasites; it just means there aren't enough to show up in a direct smear).

Lab. four

Parasitology: is the science that's study of morphology, taxonomy, life-cycles, pathogenesis, epidemiology, ecology, physiology, biochemistry, genetics and molecular biology, diagnosis, immunology and treatment of parasites infections.
Parasite : is the organism that lives on host with benefits food , habitat and protection.
Host : is the organisms that harmed from the presences of parasite . Relationships among the organisms are:
1.Parasitism : is the relationship between two organisms one of the organism benefits food and protection(parasite) and the other is harmed (the host) .

2. **Commensalism** : One organism benefits and the other is unharmed

3. **Mutualism**: is the relationship between two organisms and both of them are useful.

Types of parasites:

a. Types of Parasites according to the specialization

1. Obligate Parasite is completely dependent on its host
2. Facultative parasite can exist free living or parasitism.
3. Incidental Parasite is parasite that enter the wrong host.

b. Types of Parasites according to the presence period of parasite on a host:

1. Temporary parasite that s only visits host for meals e.g. Ticks.

2. Permanent parasite that's completely lives on its host e.g. lice.

3. Opportunistic parasite that can exists in immunodeficient patient such as toxoplasma.

c. Types of Parasites according to the habitat :

1. Ectoparasite is attached to skin e.g. mites , lice.

2. Endoparasite lives inside body of host e.g. worms and protozoa

d. **Types of Parasites according to the number of hosts which parasite required**

1. Monoxenous parasite : which has one a host)

2. **Heteroxenous parasite** : which have more than one a host)

Types of Hosts:

1. Definitve Host :that harbors adult sexually mature stage of the parasites(human , animal) .

2. Intermediate Host: that harbors immature larvae (unsexual). **3. Reservoir Host** : that harbors all stages of parasites .

4. **Vector host** : is the host that transmits parasite from one host to another.

Taxonomy : Parasites of humans are classified in six major divisions.

These include the:

1. Protozoa (amebae, flagellates, ciliates, sporozoans,),
2. Nematoda or roundworms.
3. Platyhelminthes or flatworms (Cestodes, Trematodes).
4. Pentastomids or tongue worms (may be grouped with the arthropods), the
5. Acanthocephala or thorny-headed worms.
6. Arthropoda (e.g., insects, spiders, mites, ticks).

1. Protozoa: The protozoa are the simplest and most primitive animals, usually defined as "unicellular" animals, the protozoa are classified into four classes on the basis of the structures they possess for locomotion:

1. **Class: Sarcodina**: protozoa with locomotion by means of pseudopodia. Example: ***Amoeba*** spp.
2. **Class: Mastigophora**: protozoa with locomotion by means of flagella e.g. *Giardia lamblia.*

3. **Class: Sporozoan**: parasitic protozoa without locomotion structure but move by gliding. Example ***Plasmodium spp.***

4. **Class: Ciliate**: protozoa with locomotion by means of cilia. Example ***paramecium***

Class: Sarcodina.

Genus: *Amoeba* : *Amoeba* is a genus of protozoa that moves by means of temporary projections called pseudopods, the ***amoeba*** is Greek word meaning (change).The most famous species, ***Amoeba proteus,*** is 700-800 μm in length but the species ***Amoeba dubia*** is as large as a millimeter, and visible to the naked eye. ***Amoeba*** eats algae, bacteria, other protozoan, and tiny particles of dead plant or animal matter and reproduce by a process called binary fission.

*The pseudopodia: is a part of the amoeba's body that it can stretch out and pull itself with. Or, to eat, the amoeba stretches out the pseudopodia, surrounds a piece of food.

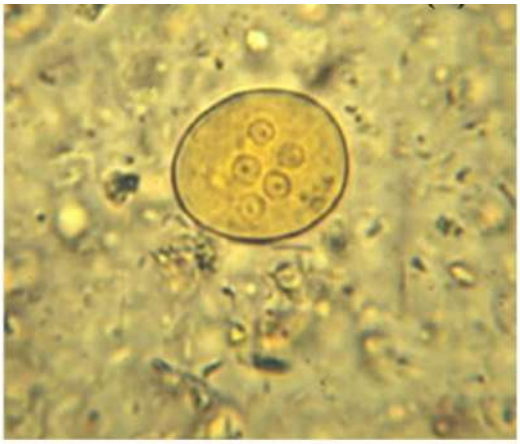
Lab. Five

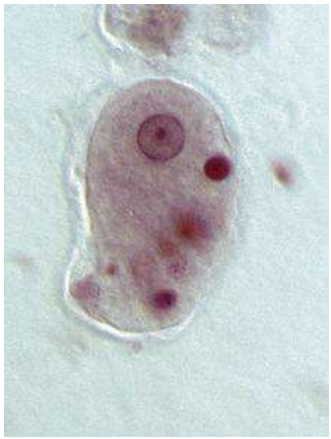
a. Intestinal amoebae e.g. *Entamoeba histolytica:*

It is pathogenic for human and responsible for amoebiasi.

Morphology: It is occurs in two stages; cyst (non motile stage) and trophozoite (food with vacule) (motile stage) (figure 1).

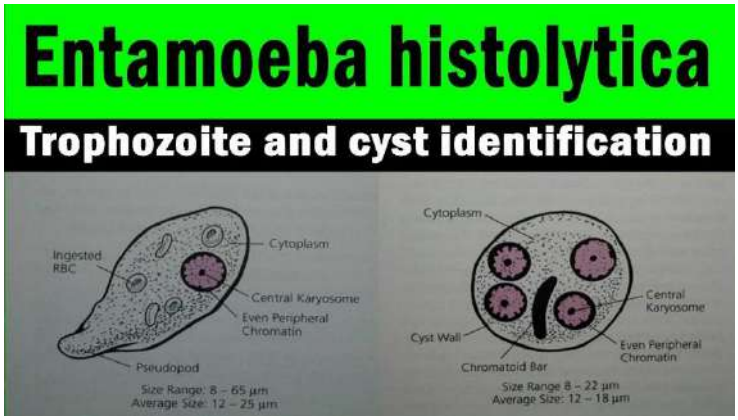
Infective stage: Cyst(with 4 nuclei).





Cyst of Entamoeba histolytic

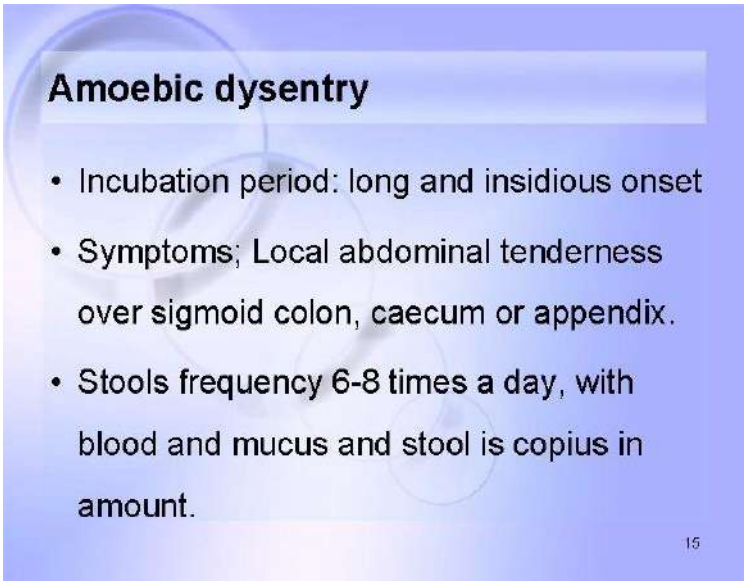
Trophoziotes of Entamoeba histolytica:

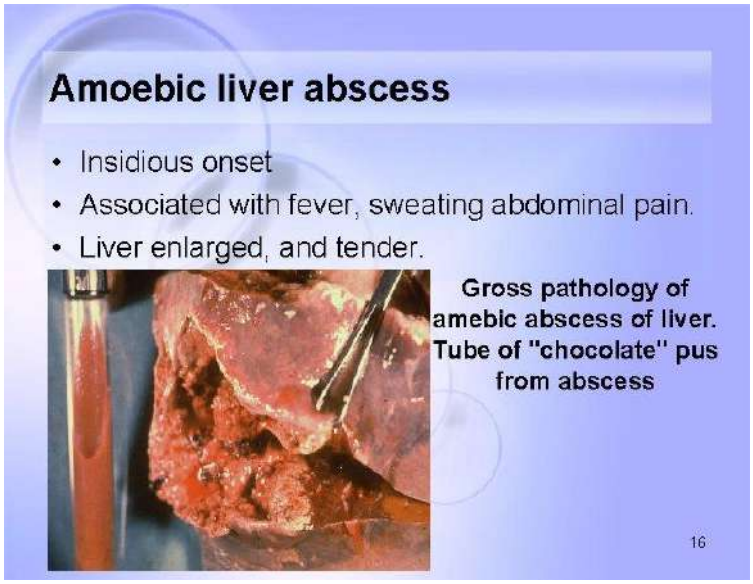


Mode of transmission : Ingestion of the infective stage (cyst) by fecal oral rout by contaminated foods and water.

Pathogenesis: The trophozoites adhere to mucus lining of intestine by lectins and secretes proteolytic enzymes then enter to sub mucosa layer which causes necrosis and flask shaped amoebic ulcer, the parasites may be carried with blood circulation to other organs such as liver, heart, brain and produces ulcer.

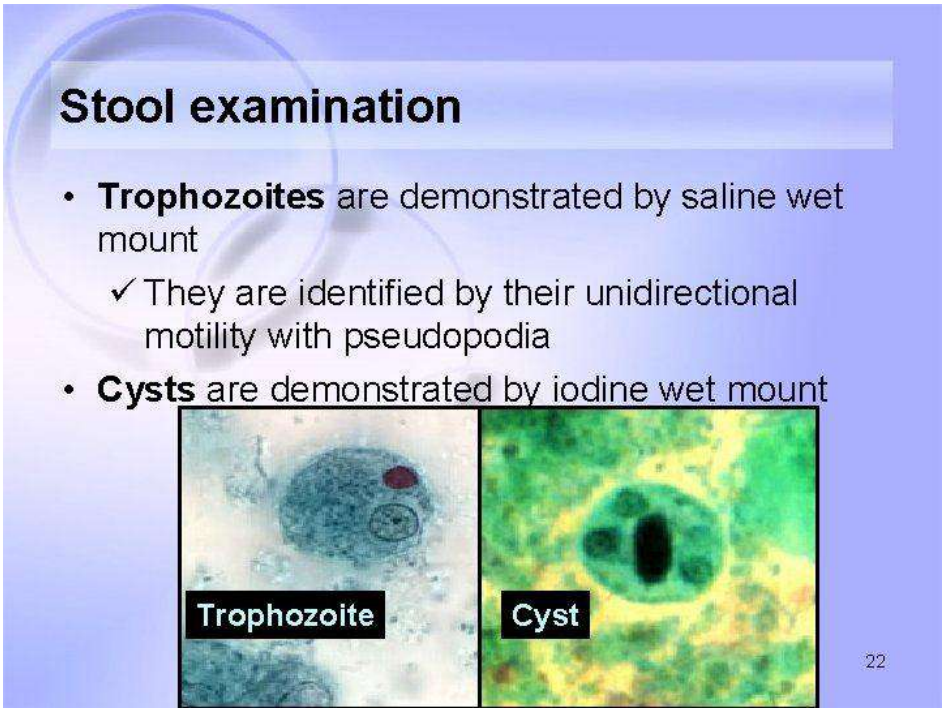
Clinical symptoms:





Diagnosis:

1. Stool examination made by wet mount preparation for finding either motile stage (trophozoites) in the diarrheal stool or the cyst in the formed stool.



Comparison between amoebic dysentery and bacillary dysentery (bacterial).

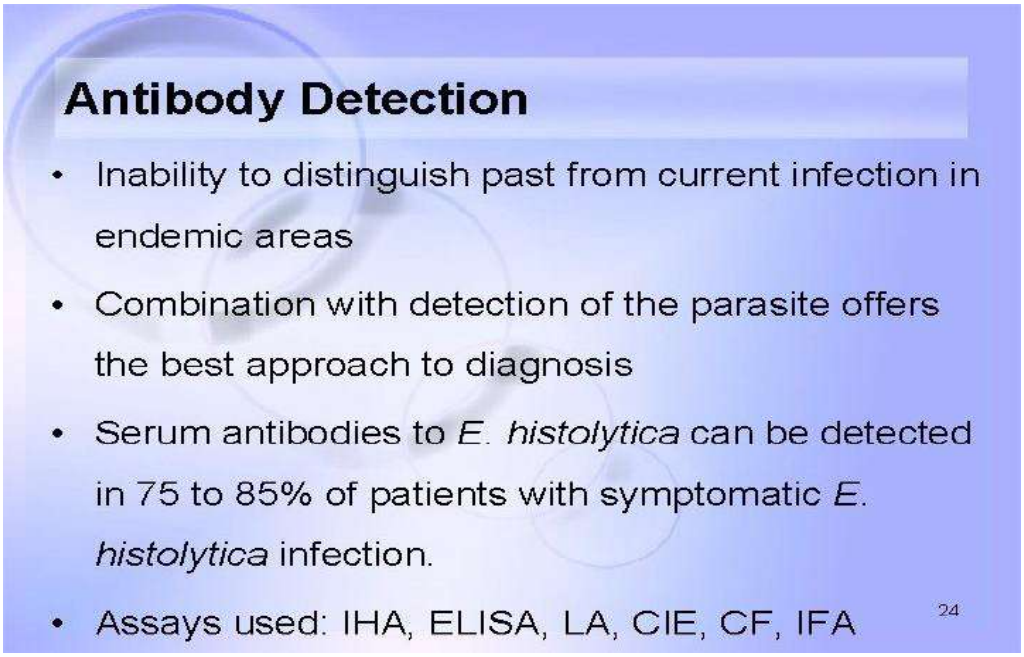
Character	Amoebic dysentry	Bacillary dysentry
Macroscopic examination		
Blood and mucus	Stool consists of blood and mucus along with formed stool	Stool consists of blood and mucus hardly any faecal matter
Volume	copious	small
Frequency	6-8 times/day	>10 times/day
Tenesmus	Usually absent	Severe

18

Character	Amoebic dysentry	Bacillary dysentry
Microscopic examination		
Pus cells	Few	Plenty
RBCs	Agglutinated in clumps	Discrete
Parasites	Trophozoites with ingested erythrocytes seen	Amoebic trophozoites absent
Charcot- Leyden crystals	Present	Absent

19

2. In the case of liver abscesses biopsy samples are required for diagnosis.
3. Serology : such as :



4. PCR (poly chain reaction) and Radiological assay.

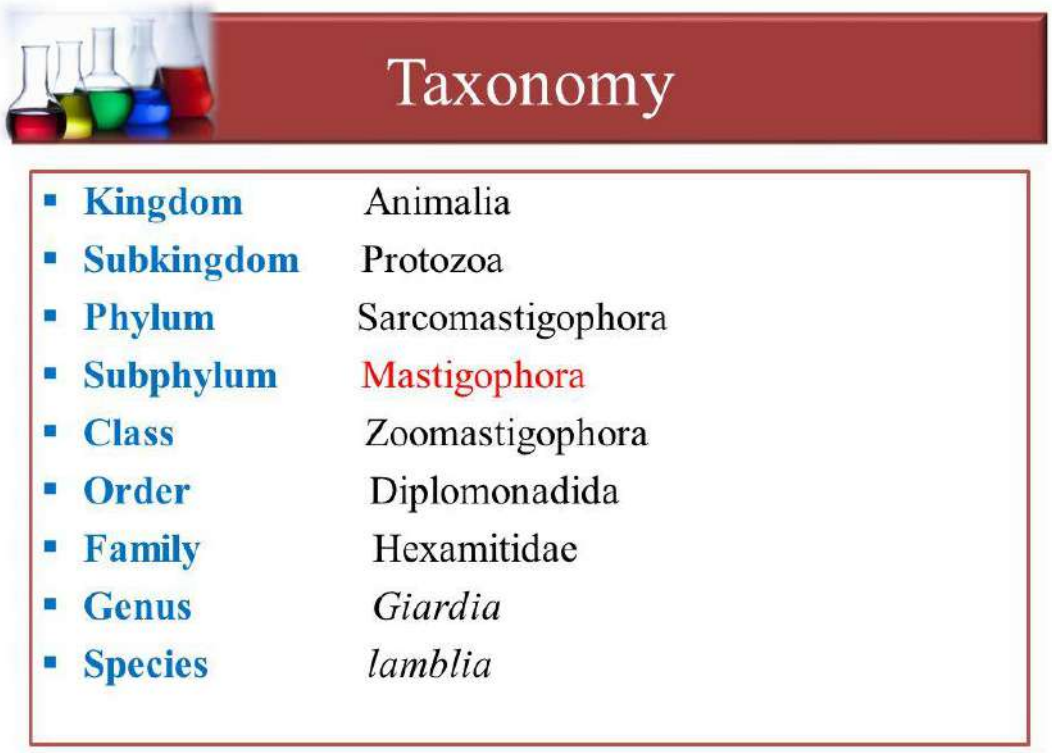
Treatment: metronidazole (flagyl).

Lab. six

***Giardia lamblia* :**

- *Giardia lamblia* is also known as *intestinilis* or *G.duodenalis.*
- It was first observed by Antony von Leewenhoek (1681) while examining his own stool and Lambi (1859) describe the parasite and named it as Giardia labmlia
- Giardia is the only intestinal flagellate known to cause endemic and epidemic diarrhea in human.

Classification of Giardia Lambelia :



Habitat:

- Inhabits the small intestine of human.

Morphology : *G. lamblia* exists in two morphological form- trophozoite and cyst

1. Cyst :

a. It is an infective stage of parasite.

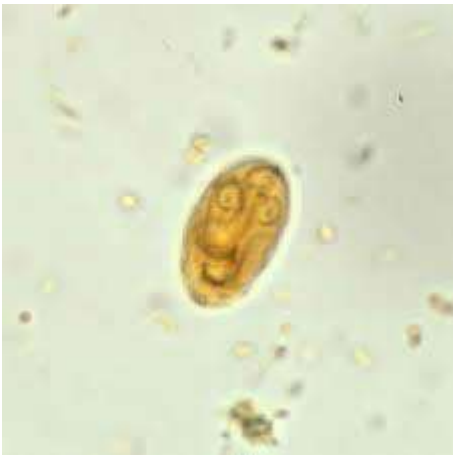
b. A fully mature cyst is oval or ellipsoidal in shape and measures 8-12µm in length and 7-10 µm in breadth.

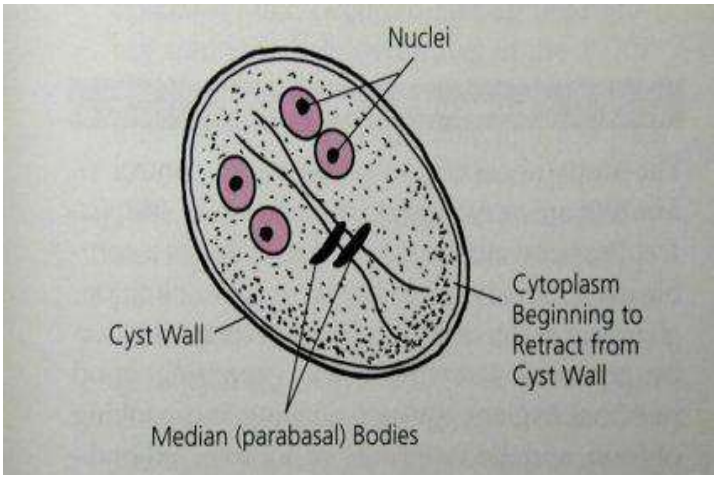
c. Cyst is surrounded by a thick cyst wall. Cytoplasm is granulated and is separated from the cyst wall by clear space.

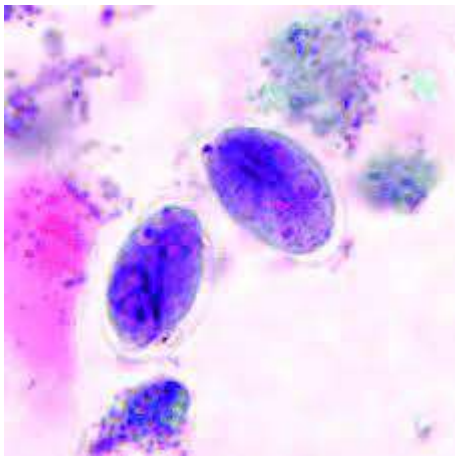
d. The axostyle lies more or less diagonally.

e. A cyst contains 4 nuclei.

f. The remaining of flagella and the margins of sucking disc may be seen inside the cytoplasm.







2. Trophozoites :

a. It is the active feeding stage of parasite which is responsible for colonization in intestine.

b. The shape of trophozoite is pear shape or tennis racket shape with broad round anterior end and a tapering posterior end.

c. It measures 9-21 µm in length and 5-5µm in breadth.

d. The dorsal surface is convex while ventral surface is concave with a sucking disc (adhesive disc) which acts as an organ for attachment.

e. Behind the adhesive disc lies a pair of large curved and transverse median bodies, unique to

f. It is bilaterally symmetrical and all organs of body are paired. They have two median bodies, two axostyle, two nuclei and four pairs of flagella.

g. Each nucleus consists of large central karyosome giving a characteristic face like appearance to the parasite in stained preparation.

h. Cytoplasm is uniform and finely granulated.

i. Motility shown typical ‘fallling leaf type’ motility.

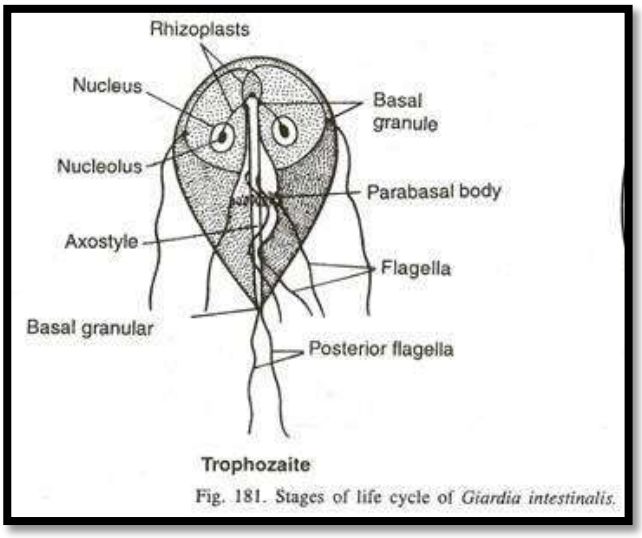
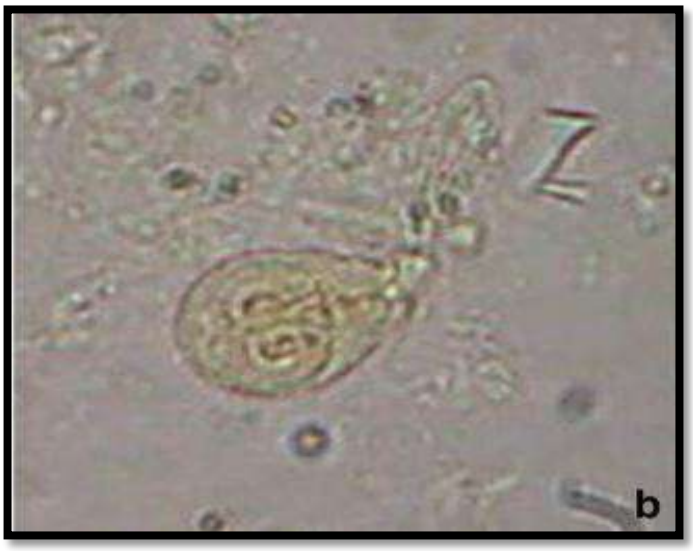
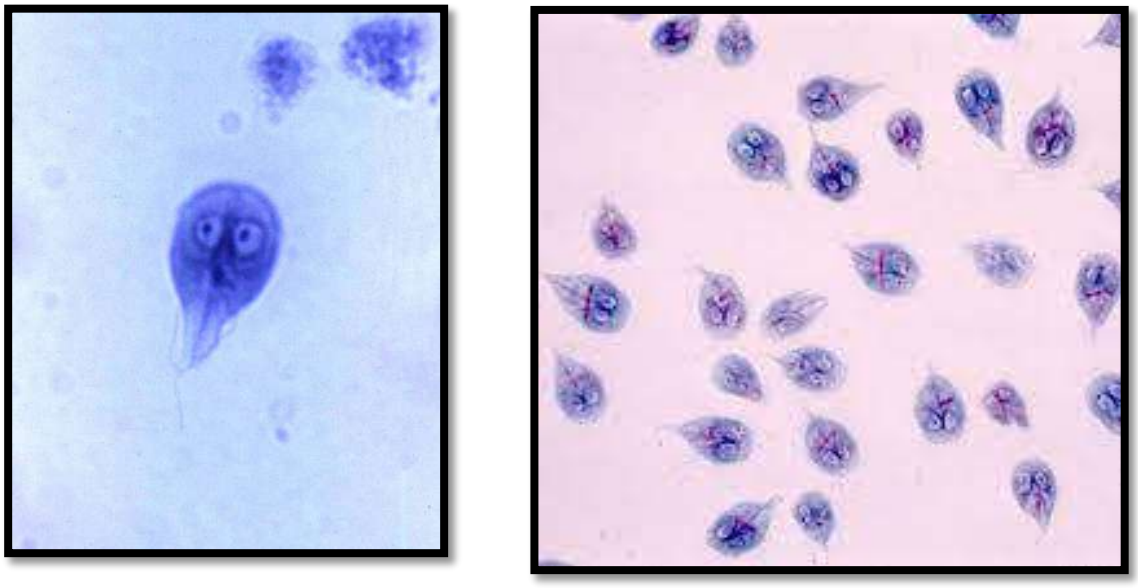


Fig. 181. Stages of life cycle of *Giardia intestinalis*.





Trophozoites of *Giardia lambelia*

Life cycle :

Upon ingestion, the infective *g. lamblia* cysts enter the stomach. The digestive juices, particularly gastric acid, stimulate the cysts to excyst in the duodenum. The resulting trophozoites become established and multiply approximately every 8 hours via longitudinal binary fission. The trophozoites feed by attaching their sucking discs to the mucosa of the duodenum. trophozoites may also infect the common bile duct and the gallbladder. Changes resulting in an unacceptable environment for trophozoite multiplication stimulate encystation, which occurs as the trophozoites migrate into the large bowel. The cysts enter the outside environment via the feces and may remain viable for as long as 3 months in water.

Mode of transmission:

- Human is the main reservoir of Giardia.
- Infection is acquired due to-
    - Ingestion of contaminated food and water

- Person to person transmission due to poor hygiene in day care centers, nursing homes, mental asylums
- Sexual transmission-oral-anal and oral-genital sex

- Immunocompromised individuals such as AIDS patients, X-linked gammaglobulinaemia, patients with protein energy malnutrition are more susceptible for giardiasis

Virulence factors:

- **Cytoskeleton:**
  - Giardia contains microtubules (MT) cytoskeletion which is essential for motility, attachment, intracellular transport, cell division and encystation/excystation.
- **Cysts:**
  - Cysts are resistant and responsible for transmission of parasite

Clinical manifestation of *Giardia lamblia*:

- Incubation varies from 1-3 weeks
- In majority of cases infection remains asymptomatic.
- Symptomatic infection is more common in children than adults because of their lower immunity.

1. **Acute giardiasis:**

- It is characterized by acute watery diarrhea, abdominal cramp, bloating and flatulence. Occasionally nausea, vomiting, fever, rashes or constipation in some.
- Pus, blood and mucus are not seen in stool.
- The condition lasts for 5-7 days.

2. **Chronic giardiasis:**

- Symptoms includes chronic diarrhea with malabsorption of fat (steatorrhoea) and malabsorption of vitamin A, protein and D-xylose, weight loss, malaise, nausea, anorexia
- Protuberance of abdomen, spindly extremities and stunted growth are most common sign in children.
- It lasts for several weeks

3. Extra-intestinal are rare and sometimes urticarial and reactive arthritis are seen in rare case

4. Complication:

- In adults, malabsorption syndrome and weight loss
- In children, growth retardation, delayed milestones achievements
- Giardiasis is self-limited disease and progression to chronic state is only 5% of infected people and death is rare.

Laboratory diagnosis of *Giardia lamblia*:

- **Specimen:**
  - Stool, duodenal contents, bile stained mucus, duodenal/jejunal biopsy
- **Stool examination:**
  - **Microscopy:**
    - Direct wet mount preparation: trophozoites are identified by their characteristic falling leaf motility
    - Iodine wet mount preparation: cyst can be observed
  - Examination of stained stool smear for demonstration of trophozoitse.
  - Concentration method: formalin-ethyl acetate and zinc sulfate concentration method is used to concentrate stool and increase parasite yield for microscopy
  - Stool antigen detection: ELISA, IFA.

- **Stool culture**
- **Entero-test:**
    - In entero-test, a gelatin capsule containing a nylon string with a weight attached to it is swallowed by patients.
    - When it reaches to stomach, the gelatin capsule is diddolved and nylon string moves down to duodenum and jejunum due to its attached weight.
    - The string is allowed to remain there for 4-6 hours or overneight.
    - After removal of string, bile stained mucus is collected on glass slide and examined for living trophozoites.
- **Serology**
- **Molecular methods**

Treatment for giardiasis:

- Metronidazole, trinidazole, nitroimidazole derivatives
- Nitrofurans- furazolidine
- * metronidazole is drug of choice. Dose- orally 250mg, 3 times daily for adults, 15mg/kg /day in three divided dose for children for 7 days

Prevention of giardiasis:

- Improve water supply
- Proper disposal of human faeces
- Maintenance of good and proper personal hygiene
- Health education at individual as well as community levels
- Identifying the source of infection, particularly in outbreak situation.

Lab. seven

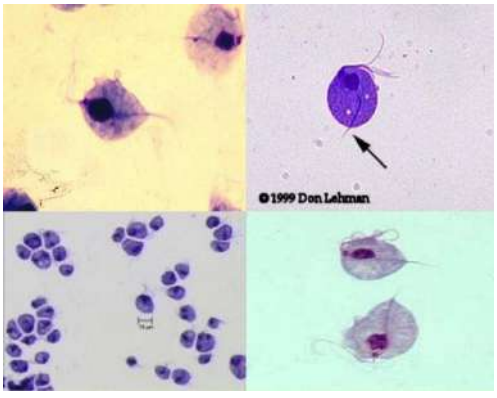
Trichomonas vaginalis:

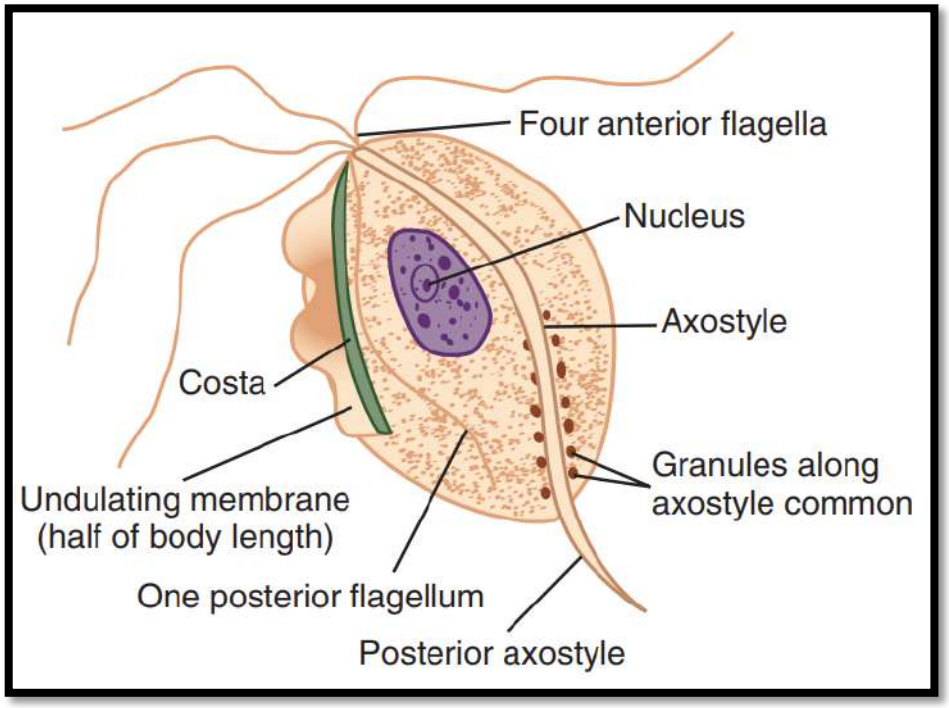
Trichomonas vaginalis is a urogenital flagellate protozoan parasite .This parasite has important medical implications because infected women during pregnancy are predisposed to premature rupture of the placental membranes, and low birth-weight infants. Trichomoniasis, which is caused by *T vaginalis*, is the most common sexually transmitted infection (STI) today, with an annual incidence of more than 170 million cases worldwide.

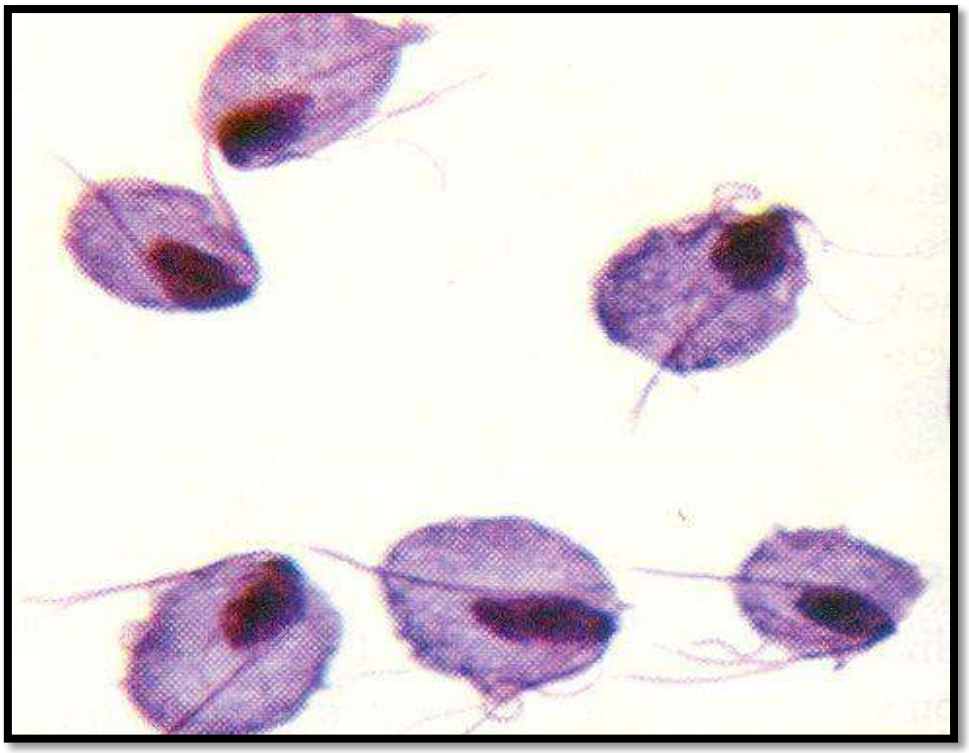
Classification:

• Domain	: Eukarya
• Kingdom`	: Protista
• Phylum	: Metamonada
• Class	: Parabasilia
• Family	: Trichomonadida
• Genus	: Trichomonas
• Species	: Trichomonas vaginalis

Morphology: It is exists only in trophozoite form, *T vaginalis* trichomonads are approximately has the same size of white blood cells (about 10-20 µm long and 2-14 µm wide), although this may vary. Trichomonads have 4 flagella that project from the organism's anterior and 1 flagellum that extends backward across the middle of the organism, forming an undulating membrane. An axostyle, a rigid structure, extends from the organism's posterior.



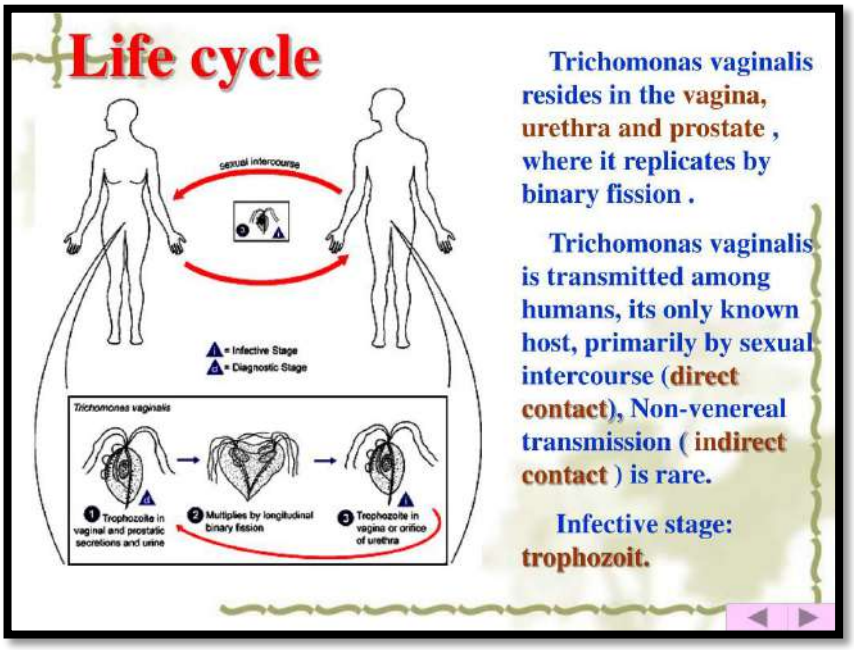




Habitat: This parasite lives in the vagina andurethra of woman and in the urethra, prostate and seminal vesicle of man. Life cycle : *T .vaginalis* exist only as a trophozoite and lacks a cystic stage and generally reproduce by longitudinal binary fission .

Transmission: Infection is transmitted by sexual intercourse.

Life cycle:



Clinical Presentation: In female, it causes vaginitis, cervicitis, hemolysis. In male, it causes urethritis, prostitis. Treatment: Metronidazole or tinidazole.

Diagnostic Tests:

1. **Microscopy: By using wet mount preparation:**

The diagnosis of trichomoniasis has traditionally depended on the microscopic observation of motile protozoa from vaginal or cervical samples and from urethral or prostatic secretions. *T vaginalis* can be differentiated on the basis of its characteristic jerky movements. On occasion, flagellar movement can also be noted. The sensitivity of this test varies from 38% to 82% and is dependent on the inoculum size because fewer than 104 organisms/mL will not be seen.

This wet mount examination is clearly the most cost-effective diagnostic test, but the lack of sensitivity contributes to the under diagnosis of the disease. Because viable organisms are required, delay in transport and evaporation of moisture from the specimen reduces motility and, consequently, diagnostic sensitivity.

2. **Culture:**

Broth culture technique has been the gold standard for *T vaginalis* for the past 40 years. The inoculum size required is only in the range of 102 organisms /mL and

the growth of the organism is easy to interpret. The standard broth is Diamond's TYI medium in glass tubes. Incubation periods ranging from two to seven days are required to identify *T vaginalis* in culture. Contamination with bacteria is a major problem, even with broth cultures spiked with antibiotics to eliminate vaginal flora. Passage of the culture after two to three days to reduce the bacterial contamination may be required to definitively identify the *T vaginalis* culture.

3. Nucleic acid detection:

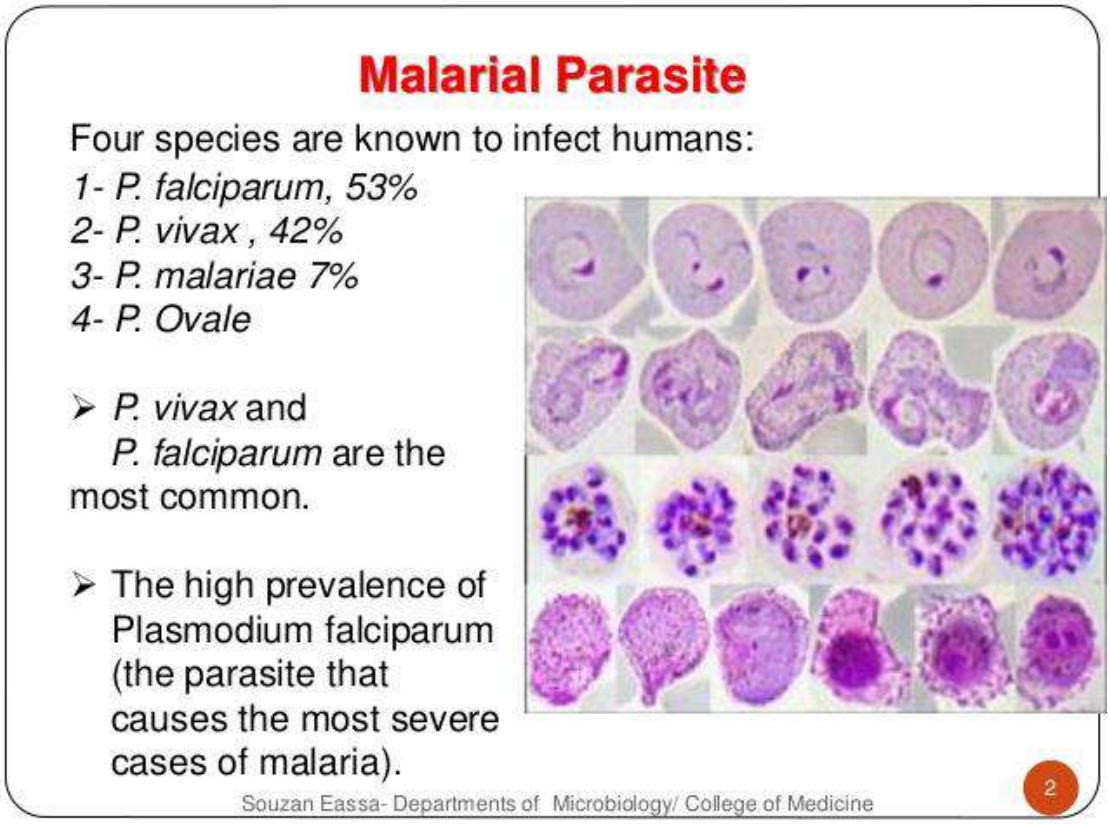
Recombinant DNA technology has been adapted over the past decade as a diagnostic tool.

4. Antibody based technique

T vaginalis has an estimated eight serotypes and, in immunoblot studies, a wide variety of antigenic markers have been seen. A variety of techniques, including complement fixation, hemagglutination, gel diffusion, fluorescent antibody and ELISA, have been used to determine the presence of trichomonal antibodies.

Lab. eight

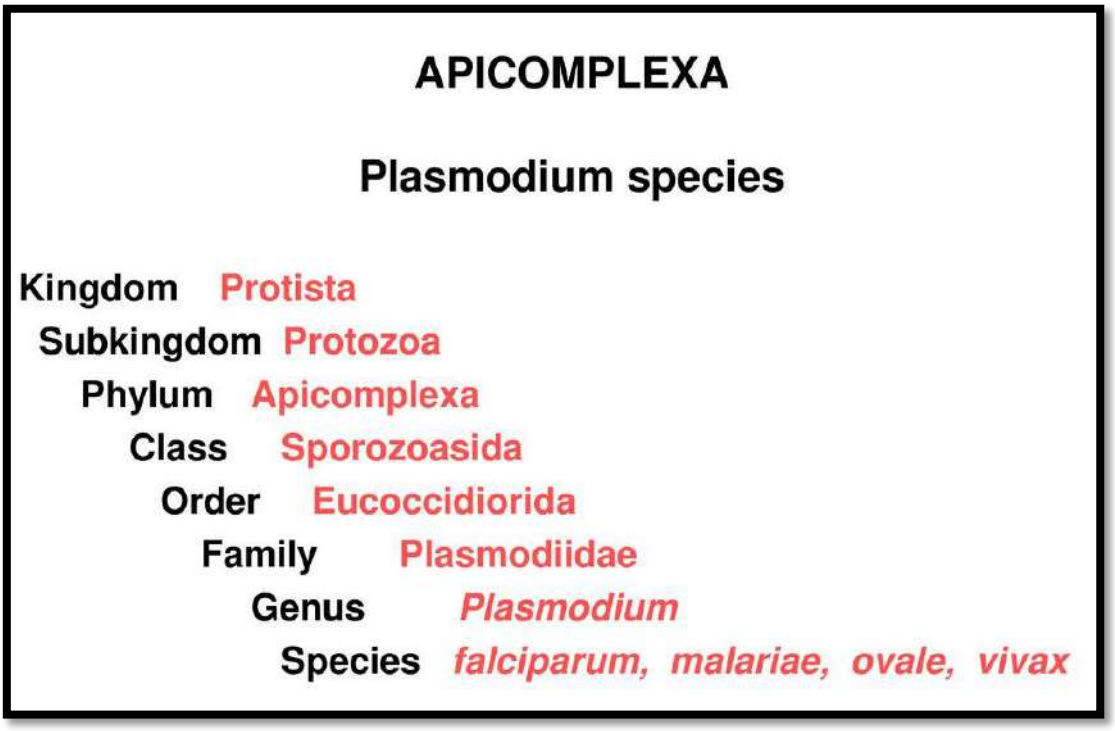
Plasmodium spp. : Plasmodium : is a eukaryote intercellular protozoan parasite. Scientific name of disease : Malaria in humans.



Transmission : Transmitted by bite the female of Anopheles mosquito.

Malaria : Malaria is a serious and sometimes fatal **disease** caused by a parasite that commonly infects a certain type of mosquito which feeds on humans. People who get **malaria** are typically very sick with high fevers, shaking chills, and flu-like illness.

Classification of plasmodium spp:



- **Habitat**: Various stages of malarial parasites are found inside the parenchymal cellsof liver and inside RBCs of Human.

Morphology *of Plasmodium falciparum*:

Following are the diagnostic forms of parasite found in human

1. **Ring form:**
    - This is the young trophozoite found inside RBCs. The name ring is derived from the morphological appearance of the stage resembling a ring like structure.
    - It consists of central vacuole and nucleus present at the center in the cytoplasm. Often two or more rings forms of the parasite are found inside a single RBC.
    - In stained smear, ring shaped cytoplasm surrounds central blue colored vacuole with red colored nucleus on it.
2. **Trophozoites:**
    - The trophoizoites ae vacuolated more or less amoeboid and uninucleated.

- They are small, delicate and measures 1.25 -1.5 µm in size
- In a stained preparation, they show a thin ring of blue cytoplasm and darkish stained nucleus.
- In heavy infection, growing forms assume the shape of compact form.
- Single large mass of pigment colored yellow to black called haemozoin are present.

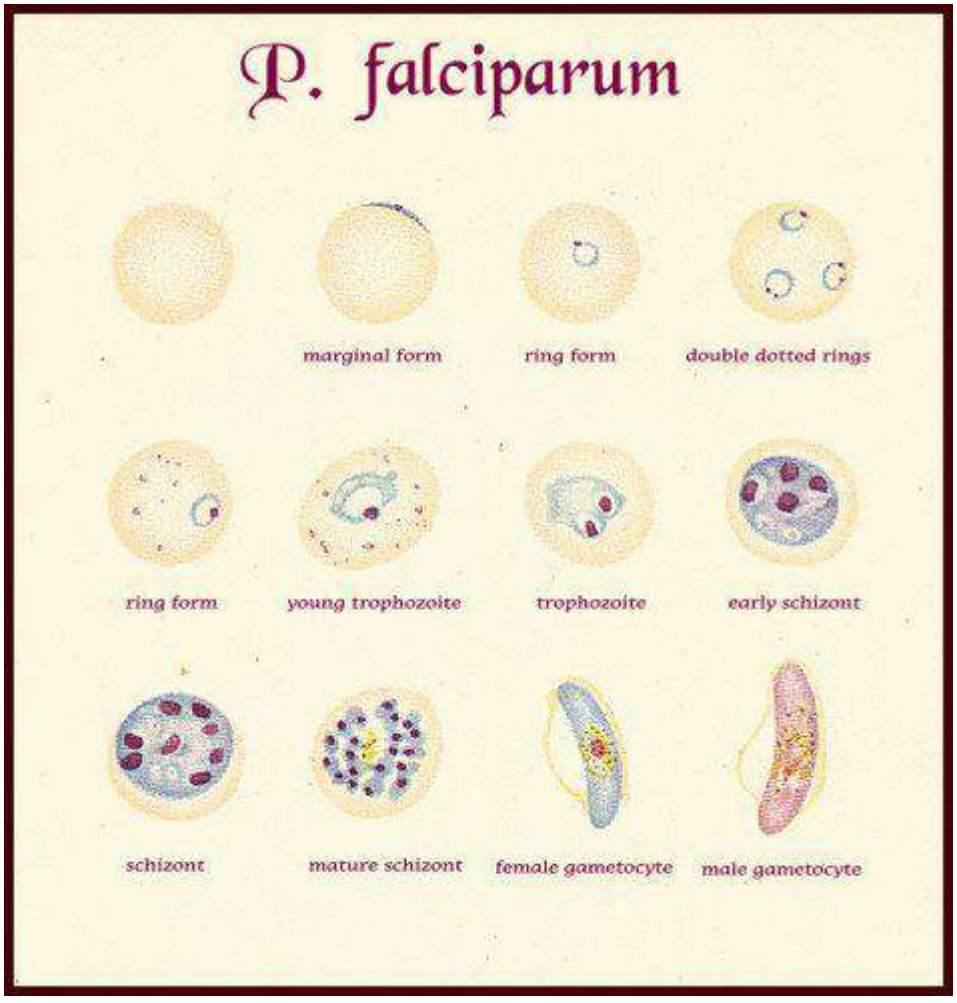
3. Schizonts:

- They are small, immobile, asexual and dividing form of parasite.
- They measure 4.5-5 µm in diameter and occupy about $2/3^{rd}$ of the infected RBC.
- Each schizont contains two or four merozoites and an aggregate of dark stained pigments
- On maturation schizont contains 10-36 merozoites arranged in grape like cluster.
- Each merozoite measure 5-10µm in length.
- Schizonts are very rarely seen in peripheral blood smear. Presence of schizonts in peripheral blood suggests severe infection.

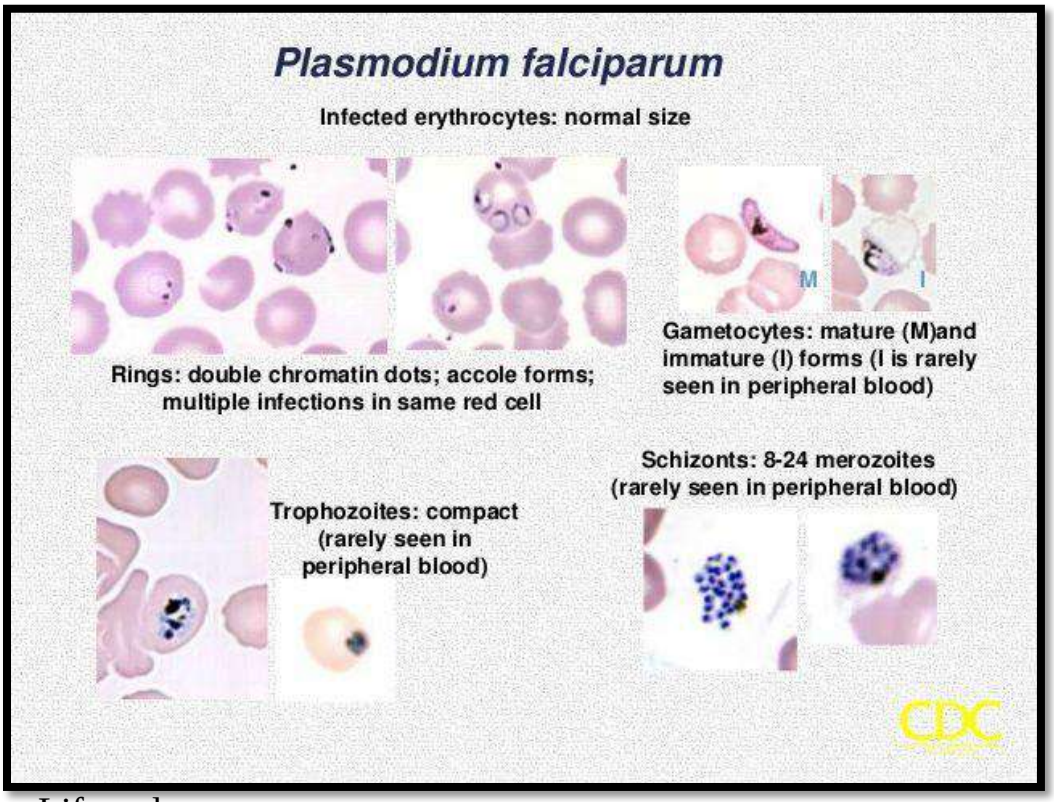
4. Gametocytes:

- Gametocytes are sexual and erythrocytic stage of parasite and are infectious to mosquitoes.
- They are typically crescent (banana) shape with round or pointed ends.
- Size of mature gametocyte is about one and half time larger than RBC.
- There are two types of gametocytes.
    - Microgamete: male form
    - Macrogamete: female form

5. **Sporozoites (infective stage):**
    - The sporozoites are the infective form and are infectious to human
    - They are found in infected mosquitoes.
    - Sporozoites : are single nucleated, sickled shaped structure with equally pointed ends. They have complex structure and a thick pellicle.
    - Pellicle consists of a thin outer membrane, a two layered membrane and a layer of subpericular microtubules. They contain 3 polar rings and a mitochondrion present at posterior end.
    - The peripheral fibres serves as organ of locomotion.
    - They measures 10-15 µm in length
6. **Ookinete and Oocyst:**
    - They are other form found in infected mosquitoes.



Morphology *of Plasmodium falciparum*:



Lifecycle

The natural history of malaria involves cyclical infection of humans and female *Anopheles* mosquitoes. In humans, the parasites grow and multiply first in the liver cells and then in the red cells of the blood. In the blood, successive broods of parasites grow inside the red cells and destroy them, releasing daughter parasites ("merozoites") that continue the cycle by invading other red cells.

Pathogenicity: Uncomplicated malaria caused by all four species and characterized by periodic fever and chills, mild anemia and splenomegaly. complicated malaria is caused by *P. falciparum* infections characterized by hyper anemia .

Laboratory diagnosis of malarial parasite:

1. Specimen: blood

- Blood is collected from finger tips or ear lobe in older children and adults. In case of infants blood is collected from great toe.
- Smear should be examined at least twice daily until parasite is detected.

2. Methods of examination:

a. Light microscopy:

- After blood collected from capillary, smear is prepared and stained with Giemsa stain.
- Thick smear is used for detecting the parasite, quantitating parasitaemia, demonstrating malarial pigments.
- Thin smear is used for detecting parasites and also for determining the species.
- **Diagnostic methods of falciparum is :**
    - Detection of multiple rings in a single RBC with accole form.
    - Presence of maurer's dots in RBC containing large ring.
    - Presence of characteristics banana shaped gametocyte.

b. Antigen Detection

Various test kits are available to detect antigens derived from malaria parasites. Such immunologic ("immunochromatographic") tests most often use a dipstick or cassette format, and provide results in 2-15 minutes. These "Rapid Diagnostic Tests" (RDTs) offer a useful alternative to microscopy in situations where reliable microscopic diagnosis is not available.

c. **Molecular Diagnosis**

PCR is most useful for confirming the species of malarial parasite after the diagnosis has been established by either smear microscopy or RDT.

d. **Serology**

Serology detects antibodies against malaria parasites, using either indirect immunofluorescence (IFA) or enzyme-linked immunosorbent assay (ELISA). Serology does not detect current infection but rather measures past exposure.

Treatment: A number of drugs have been developed to treat Plasmodium infection; however, the parasites have evolved resistance to each drug developed. Chloroquine is the drug of choice for acute malaria.

Prevention:

Malaria can be prevented by: 1) Avoiding mosquito bites by using mosquito repellants, mosquito nets .

2) Chemoprophylaxis – 2 weeks before entering to 4 weeks after leaving an endemic area Chemoprophylaxis is defined as the use of medications or chemical agents to prevent a disease.

Lab. nine

Toxoplasma gondii:

Is an obligate intracellular parasitic one-celled eukaryote, This protozoan parasite is a zoonotic parasite infects human and other warm-blooded animals and causes the disease toxoplasmosis.

Morphology : This parasite presents at a three main stages, tachyzoites, bradyzoites and sporozoites.

1. **Tachyzoites** : Crescent or oval in shape, Blunted at posterior end and tapered at anterior end. Reside in a vacuole, fast replicating and highly invasive and destructive for tissues **can be eliminated by drug treatment because it represent the acute infection figure (1).**

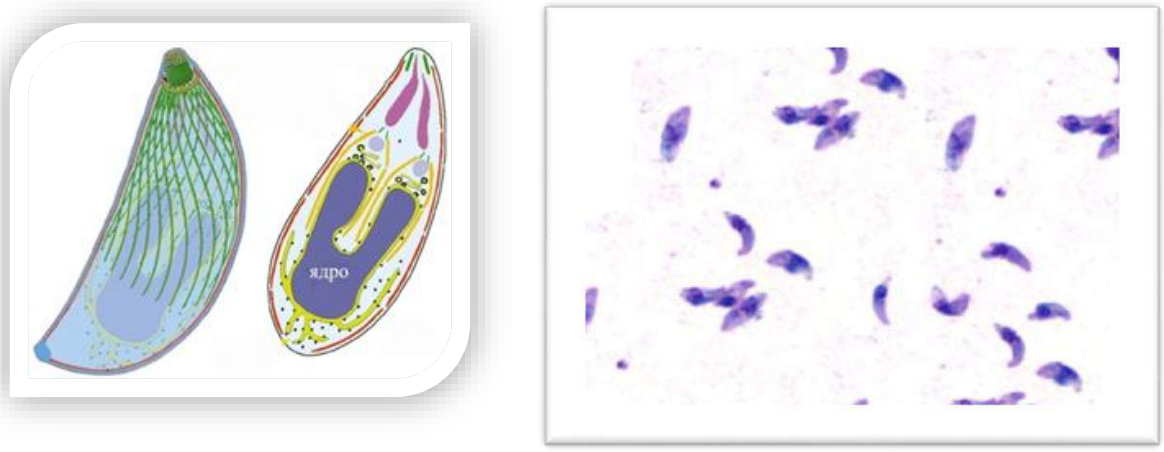
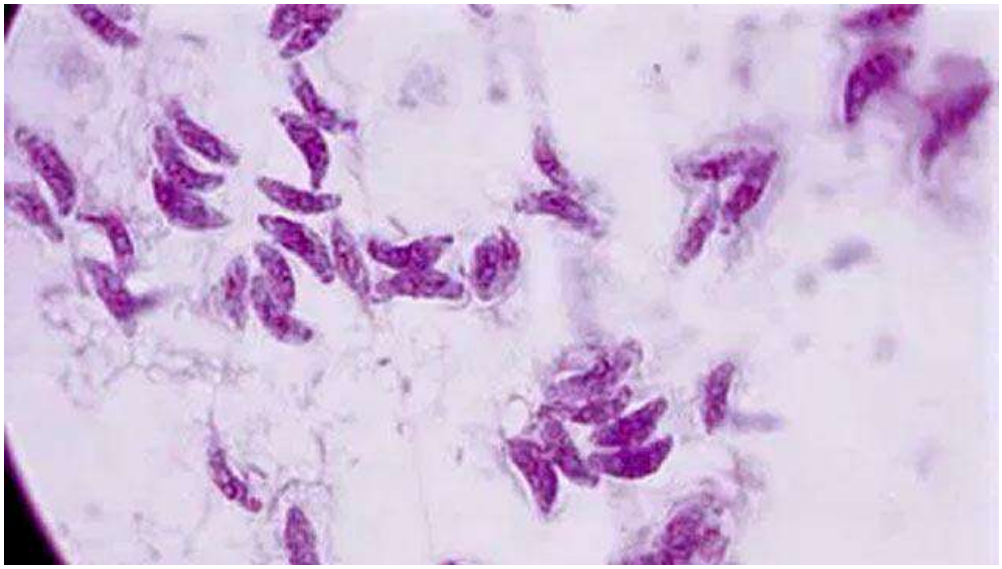
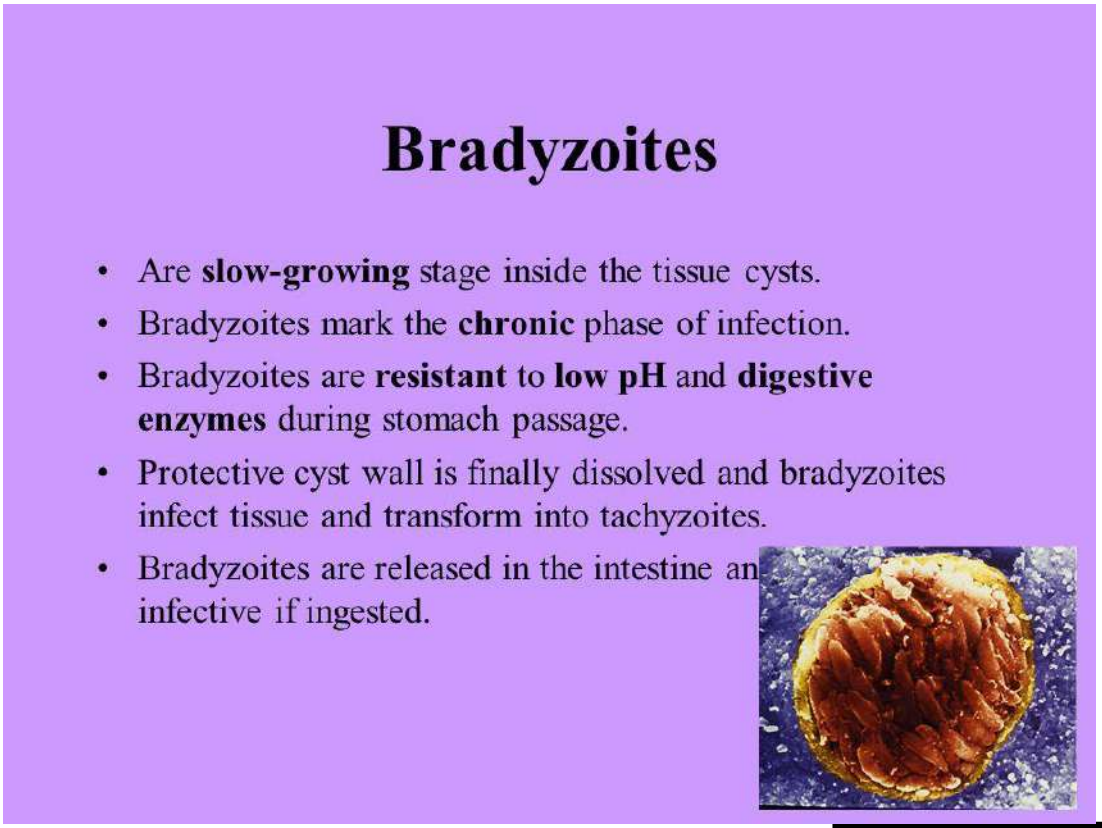
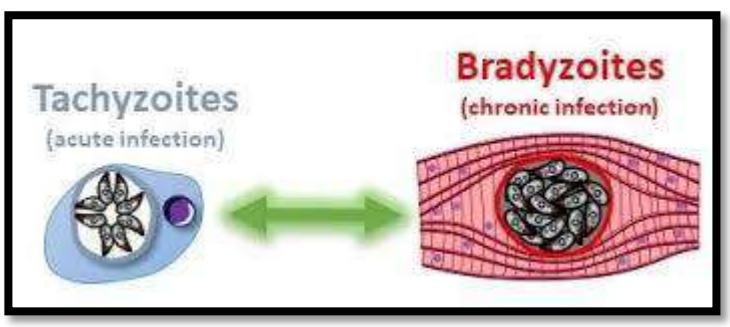


Figure 1: Tachzyoites of *T.*



2. **Bradyzoites** : Reside in a tissue cyst characterized by slow growth and persistent in host, **resistant to current drug treatment because it represent the chronic infection figure 2.**





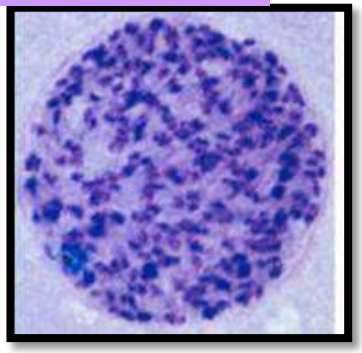
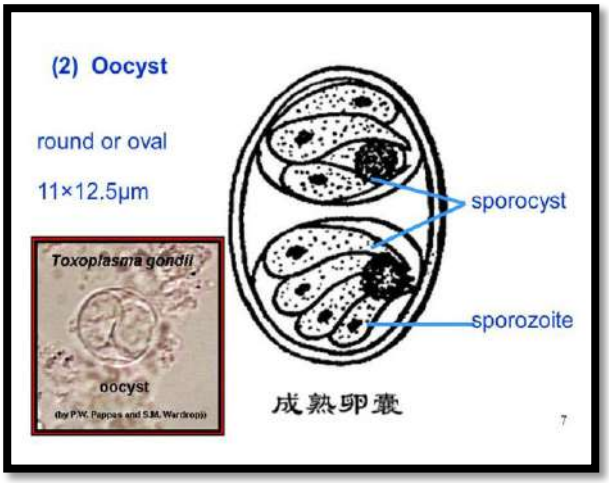
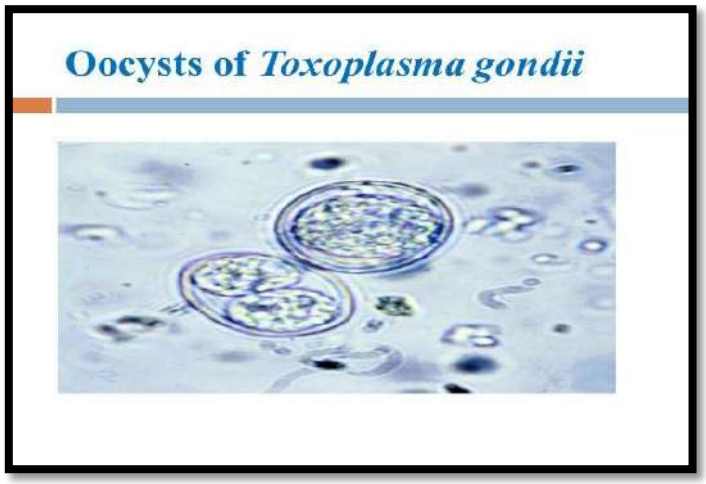


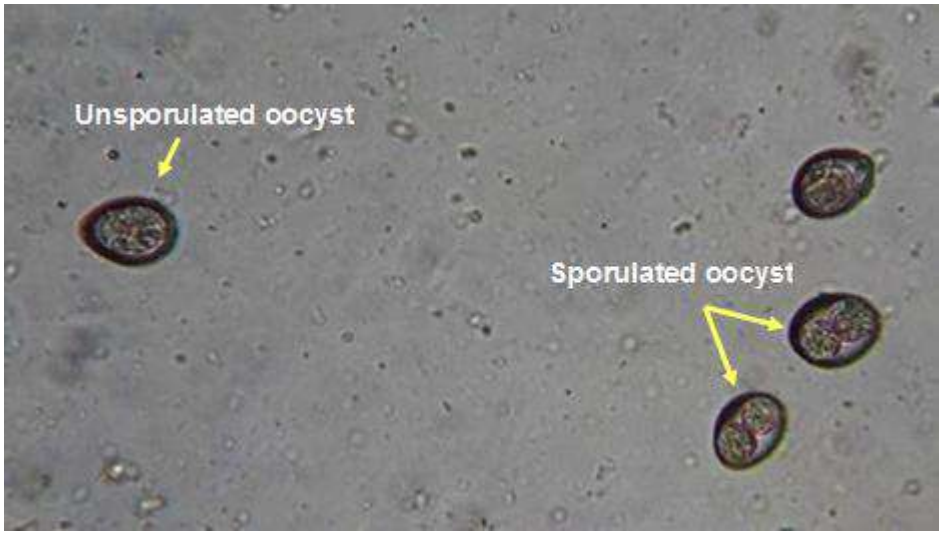
Figure 2: Bradyzoites of *T.gondii*

3. Oocyst :

- The oocyst is non infectious before sporulation .
- Unsporulated oocysts are subspherical to spherical.
- Sporulated oocysts are sub- spherical to ellipsoidal .
- Each oocyst has two ellipsoidal sporocysts (figure 3).
- Each sporocysts contains four sporozoites.
- Shedding occurs 3-5 days after ingestion of tissue cysts.
- Sporulated oocysts remain infective for months.







Geographical distribution:

T. gondii is a cosmopolitan parasite with a variable frequency rate worldwide. It is estimated that T.gondii infects one third of world population.

Classification of *toxoplamsa gondii:*

Habitat:

The most common location is the brain, eye , lungs , heart and skeletal muscles. This parasite presents at a three main stages, tachyzoites, bradyzoites and sporozoites.

Life cycle.

The life cycle of T.gondii is complex. It comprises a phase of sexual reproduction in definitive hosts, especially cats. It also comprises a phase of asexual reproduction that occurs in intermediate hosts (birds and mammals) as well as in definitive hosts (Figure 4).

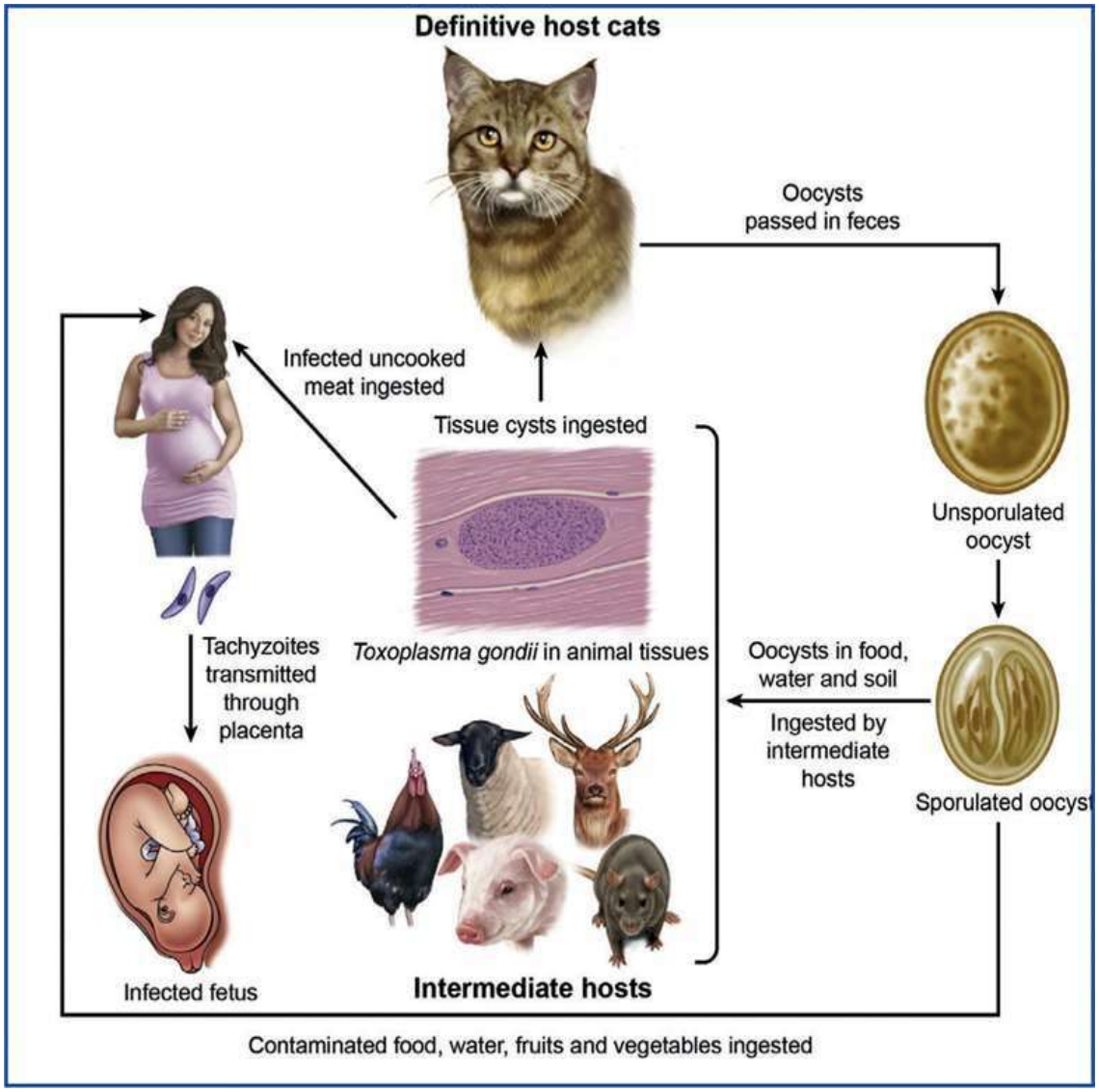


Figure 4: life cycle of *T. gondii*

How do humans become infected?

The possible ways of contamination are:

1. Ingestion of tissue cysts by eating raw meat, undercooked or insufficiently frozen (lamb, pork, cow, beef, chicken, horse,...)

2. Ingestion of oocysts present in an environment contaminated by cat feces: plants (fruit, vegetable from the garden...), water, soil (gardening or farming activities), animal fur …

3. Direct contamination by cat by handling dropping litter in the absence of a proper hygiene.

5. Contamination through blood transfusion or organ transplant is quite possible although much infrequent.

6. Transplacental transmission leading to a congenital infection of fetus when a woman acquires infection during pregnancy.

- **Clinical features of toxoplasmosis**

Symptomatic features classically associate fever, lymphadenopathy and asthenia. The patient will have a slight fever for a few days or weeks that will spontaneously disappear. The weakness can persist for several months.

In immunocompetent patients may lead to an ocular impairment with uveitis and retinochoroiditis.

Congenital toxoplasmosis: It derives from the contamination of the foetus during pregnancy and can lead to the abortion or to more or less severe symptoms according to the period of infection during pregnancy.

- **Laboratory diagnosis of *toxoplasma gondii* :**

The diagnosis of *T. gondii* infection or toxoplasmosis may be established by:

1. Demonstration of the *Toxoplasma gondii* organism in blood, body fluids, or tissue.
2. Detection of Toxoplasma gondii antigen in blood or body fluids by enzyme-linked immunosorbent assay (ELISA) technique
3. **The Sabin-Feldman dye test**: is a sensitive and specific.
4. **Neutralization test.** It measures IgG antibody and is the standard reference test for toxoplasmosis. High titters suggest acute disease.
5. **Serologically**: IgM fluorescent antibody test detects IgM antibodies within the first week of infection, but titters fall within a few months.
6. **Amplification of specific nucleic acid sequences (**i.e., **Polymerase Chain Reaction** :on body fluids, including CSF, amniotic fluid, and blood) .
7. **Skin test results** : showing delayed skin hypersensitivity to *Toxoplasma gondii* antigens. Antibody levels in aqueous humor or CSF may reflect local antibody production and infection.
8. **Animal inoculation:** inoculation of suspected infected tissues into experimental animals

9. **Isolation of the organism**: inoculation of suspected infected tissues into tissue culture.
10. **Histologic demonstration of the parasite (tachyzoites) and /or its antigens (i.e., immunoperoxidase stain).**

- **Treatment :**

- Pyrimethamine and sulfadiazine combination is quite useful.
- Spiramycin as such or in combination with sulfadiazine may be tried.

- **Prevention:**

To prevent risk of toxoplasmosis and other infections from food we should cook food to safe temperatures.

Freeze meat for several days at sub-zero (0° F) temperatures before cooking to greatly reduce chance of infection.

Lab. Ten

Leshmania spp:

Leishmania : a genus of trypanosomes that are responsible for the disease leishmaniasis.

Transmission : Leishmaniasis is transmitted by the bite of infected female phlebotomine sandflies . Their primary hosts are vertebrates; *Leishmania* commonly infects hyraxes, canids, rodents, and humans.

Epidemiology

Leishmania currently affects 6 million people in 98 countries. About 0.9-1.6 million new cases occur each year, and 21 species are known to cause disease in humans.

Structure

Leishmania species are unicellular eukaryotes having a well-defined nucleus and other cell organelles including kinetoplasts and flagella. Depending on their life

cycle there are two stages of leishmania including Promastigote (infective stage) and amastigote (figure 1)

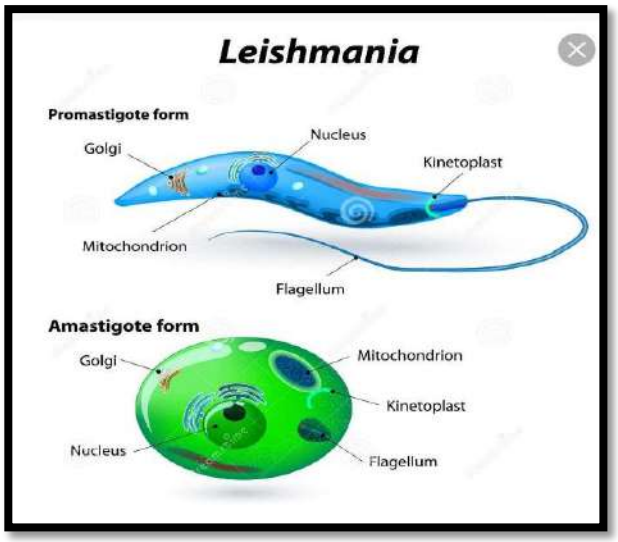


Figure 1: leimanial stages

Life cycles : The sandflies inject the infective stage (i.e., promastigotes) from their proboscis during blood meals . Promastigotes that reach the puncture wound are phagocytized by macrophages and other types of mononuclear phagocytic cells. Promastigotes transform in these cells into the tissue stage of the parasite (i.e., amastigotes) , which multiply by simple division and proceed to infect other mononuclear phagocytic cells . Sandflies become infected by ingesting infected cells during blood meals .In sandflies, amastigotes transform into promastigotes, develop in the gut (in the hindgut for leishmanial organisms in the *Viannia* subgenus; in the midgut for organisms in the *Leishmania* subgenus), and migrate to the proboscis .

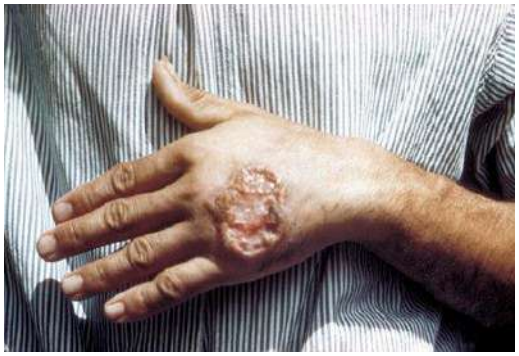
Clinical signs :

Type	Pathogen	Location
1. *Cutaneous leishmaniasis* (localised and diffuse) infections appear as obvious skin reactions.	The most common is the *Oriental Sore* (caused by Old World species *L. major*, *L. tropica*,.	Cutaneous infections are most common in Iraq (Baghdad boils) Iran, Peru, Saudi Arabia and Syria.
2. *Mucocutaneous leishmaniasis* infections start off as a reaction at the bite, and can go by metastasis into the mucous membrane and become fatal.	*L. braziliensis*	Mucocutaneous infections are most common in Bolivia, Brazil and Peru.
3. *Visceral leishmaniasis* (*kala azar*)	Caused exclusively by species of the *L. donovani*	visceral infections are most common in Bangladesh, Brazil, India, Nepal, and Sudan.

Diagnosis : We recommend using multiple diagnostic approaches to maximize the likelihood of a positive *Leishmania* result, using methods such as visualization of the characteristic amastigote in smears or tissue (histopathology); parasite isolation by *in vitro* culture; molecular detection of parasite DNA; and, for VL, serologic testing . Simultaneous testing for other diagnoses (e.g., by histopathology and culture) should be considered**.**

Treatment :

The skin sores of **cutaneous leishmaniasis** usually heal on their own, even without treatment. But this can take months or even years, and the sores can leave ugly scars.

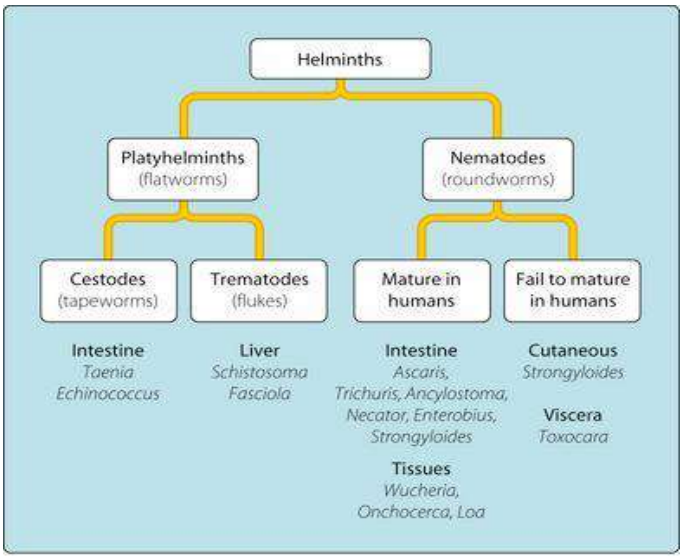


Prevention : The best way to prevent leishmaniasis is to avoid the bite of the sand fly. Simple insect precautions, including protective clothing (long sleeves, shirts tucked into pants, long pants, and socks) and insect repellents containing N, N-diethylmetatoluamide (DEET) reduce the risk of bites.

Helminthes

The helminths are worm-like parasites. The clinically relevant groups are separated according to their general external shape and the host organ they inhabit. There are both hermaphroditic and bisexual species. The definitive classification is based on the external and internal morphology of egg, larval, and adult stages. The helminths are invertebrates characterized by elongated, flat or round bodies. In medically oriented schemes the flatworms or platyhelminths (platy from the Greek root meaning "flat") include flukes and tapeworms. Roundworms are nematodes (nemato from the Greek root meaning "thread"). These groups are subdivided for convenience according to the host organ in which they reside, e.g., lung flukes, extraintestinal tapeworms, and intestinal roundworms. This chapter deals with the structure and development of the three major groups of helminths.

Classification of helminthes



Flukes (Trematodes)

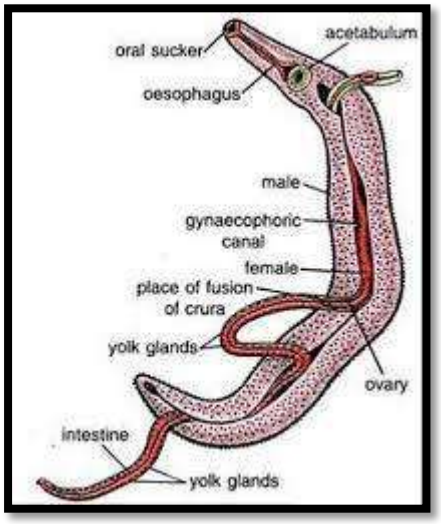
Adult flukes are leaf-shaped flatworms. Prominent oral and ventral suckers help maintain position in situ. Flukes are hermaphroditic except for blood flukes, which are bisexual. The life-cycle includes a snail intermediate host such as schistosoma spp. And fasciola spp.

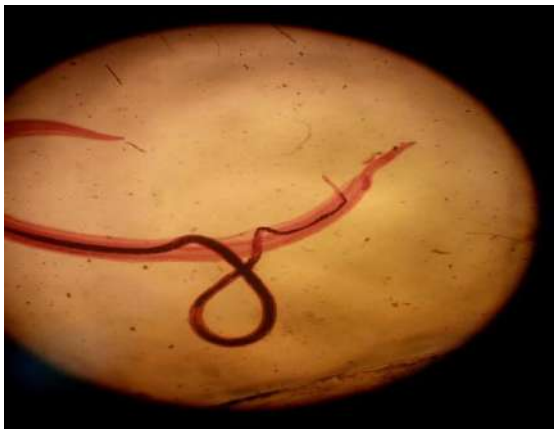
Schistosoma spp.

Schistosoma is a genus of trematodes, commonly known as **blood flukes**. They are parasitic flatworms responsible for a highly significant group of infections in humans termed *schistosomiasis*, which is considered by the World Health Organization as the second-most socioeconomically devastating parasitic disease (after malaria), with hundreds of millions infected worldwide. The genus *Schistosoma* contains six species that are of major pathological importance to man, *Schistosoma haematobium* (*S. haematobium*), *S. mansoni*, *S. japonicum*, *S. mekongi*, *S. intercalatum*, and *S. guineensis*. The species differ in their final location in the human host, the species of the intermediate (snail) host they use in their life cycle, the pathology they induce, and the number, size and shape of the eggs they produce. This *Monograph* is restricted to *S. haematobium.*

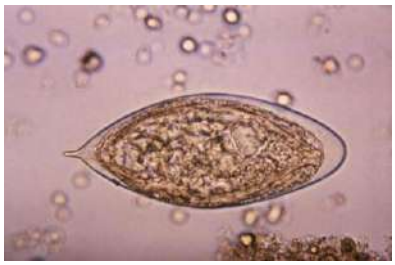
Transmission : via penetration wound skin by infective stage (cercariae) which released by snails.

Structure : Unlike all other pathologically important trematodes, schistosomes are not hermaphroditic, but have separate sexes. The adult worms are 1–2 cm long with a cylindrical body that features two terminal suckers, a complex tegument, a blind digestive tract, and reproductive organs. The male's body forms a groove or gynaecophoric channel, in which it holds the longer and thinner female. As permanently embraced couples, the schistosomes live within the perivesical (*S. haematobium*) or mesenteric (other species) venous plexus. Schistosomes feed on blood particles through anaerobic glycolysis





Adult worm



eggs of *sch. Haematobium* it has terminal spine

Prevalence, geographic distribution

Human schistosomiasis is endemic in large areas of the (sub)tropics. It has been estimated that over 700 million people in 74 countries are exposed to the risk of schistosomal infection

life cycle : The female of *S. haematobium* worm produces hundreds of eggs per day throughout her life. By the terminal spine penetrate through the bladder wall where they are excreted with urine. Each ovum contains a ciliated larva (miracidium), which secretes proteolytic enzymes that help the eggs migrate into the lumen of the bladder. About half of the eggs produced do not reach the vesical lumen, and are carried away with the bloodstream, and/or trapped in the tissues. These retained eggs provoke a granulomatous inflammatory response, which is the main cause of pathology in the human host. The excreted eggs hatch if they come into contact with water, and release the miracidium. These remain viable for up to 48 hours and are able to locate a suitable freshwater snail host (i.e. *Bulinus spp.* for *S. haematobium*) using external stimuli such as light and snail-derived chemicals. In the snail, asexual multiplication takes place, and several generations of multiplying larvae (sporocysts) develop. Eventually, these sporocysts produce large numbers of infective larvae with a typical bifurcated tail (cercariae). These leave the snail at a rate of thousands per day after a period of weeks. Shedding of these cercariae can continue for months. The cercariae survive for up to 72 hours and use water turbulence and skin-derived chemicals to locate the human host. They attach to and penetrate the human skin within 3–5 minutes.

Clinical signs:

- **Fever**.
- **Abdominal pain** (liver/spleen area)
- Bloody **diarrhea** or blood in the stools.
- **Cough**.

- **Malaise**.
- **Headache**.
- **Rash**.
- Body aches

Diagnosis : Serology is the most sensitive and useful test for screening. Among individuals living in endemic areas, the parasite burden should be determined by microscopy for egg detection and antigen detection. The infecting species can be determined via microscopy and molecular tests (polymerase chain reaction [PCR]), although these are less sensitive in the setting of early infection (<3 months).

TREATMENT

Drug of choice: Praziquantel.

PREVENTION and CONTROL

Infection by Schistosomes is acquired when the aquatic cercariae penetrate the skin. Thus, the infection can be prevented by avoiding contact with water known to harbor infected snails shedding the cercariae. Proper sanitation that eliminates contamination with bathing water or other communal water sources can aid in control by preventing snails from becoming infected.

Tapeworms (Cestodes)

Adult tapeworms are elongated, segmented, hermaphroditic flatworms that inhabit the intestinal lumen. Larval forms, which are cystic or solid, inhabit extraintestinal tissues.

Roundworms (Nematodes)

Adult and larval roundworms are bisexual, cylindrical worms. They inhabit intestinal and extraintestinal sites.