Protozoa

Parasites of medical importance come under the kingdom called protista and animalia . The microscopic single-celled eukaroytes(having true nuclear membrane) known as protozoa. In contrast, helminthes are macroscopic, multicellular worms possessing well differentiated tissues and complex organs belonging to the kingdom animalia.

Four major groups (Subphylum)of protozoa are recognized:-

- **Flagellates**(or Mastigophora) :moved by flagellum.
- **Amoebae**(or Sarcodina) moved by pseudopodium.
- **Sporozoans**(or Sporozoa, Apicomplexa) have no locomotion organelle
- **ciliatess** (or Ciliophora) moved by cilia .

Amoebae (Sarcodina)

-Amoeboid shape (irregular shape)

-Moved by pseudopodium which considers a temporary projection of cytoplasm.These help not only in locomotion but also in in ingestion of food.

-Reproduction by binary fission.

-There are trophozoite and cyst stages in their life cycle.

Pathogenic Intestinal Amoeba.Ex: *Entamoeba histolytica*

Non pathogenic Intestinal Amoeba.Ex: *Entamoeba dispar*

Entamoeba coli

Entamoeba hartmanni

Endolimax nana

Iodamoeba bütschlii

Entamoeba histolytica

A single-celled protozoan parasite, the causative agent of intestinal amebiasis(old name is Amebic Dysentery).. This parasite is endemic (Belonging or restricted to a particular locality or region) in most tropical and subtropical areas of the world, where it causes millions of cases of dysentery each year. Infected persons display a wide range of disease severity, that range from asymptomatic, discomfort, diarrhea, dysentery, liver abscess and amoebic encephalitis, reflecting the contributions of the:-

- patient's immune.
- nutritional status
- the infective dose
- pathogenic potential of the infecting organism.

Habitat: caecum and sigmoido rectal region of man.

Definitive hosts: Humans

Infective stage: Quadrinucleate cyst

Mode of infection:-

- ❖ Eating raw vegetables (salad)
- ❖ Drinking water.
- ❖ Flies and food handlers (cyst passer).
- ❖ Faeco-oral(Autoinfection).

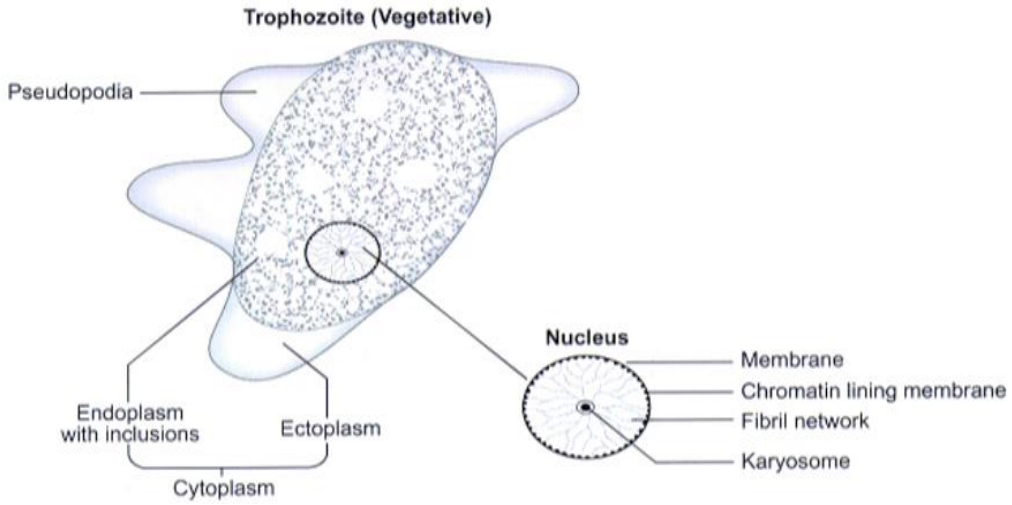
Morphology of Trophozoite (vegetative form):

- Active, feeding stage,are 15-30 micrometers in diameter .
- Motility is rapid, progressive and directional, through pseudopods
- Cytoplasm is clearly differentiated into:

  Ectoplasm: is clear with well developed pseudopodia.

  Endoplasm: dense & fine granular enclosing,few ingested bacteria or debris in vacuoles , white blood cell ,red blood cell seen in side cytoplasm.

  Nucleus: is spherical and characterized by evenly arranged chromatin on the nuclear membrane and the presence of a small, compact, centrally located karyosome.



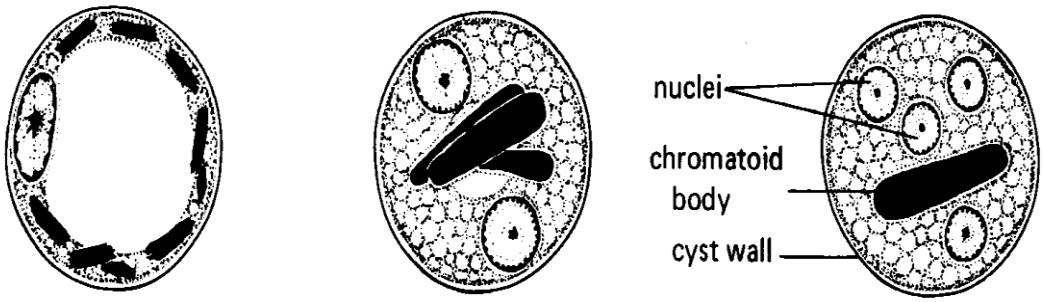
Morphology of cyst:-

Precyst stage:-

- 10-60X15-30 m average(15-20 m)
- Round or oval with a blunt pseudopodia
- Smaller than the trophozoite but larger than cyst
- Absent cyst wall.
- Single nucleus present.

Cyst stage:-

- Spherical ,10-20 m average (15 m).
- The cystic stage of *E. histolytica* is either mature or immature , the maturity of cyst depend on the number of nuclei found in the cyst.The immature cyst includes uni and bi –nucleated cyst while the mature cyst is quadrinucleated cyst
- Glycogen mass and chromatoid bodies are present in immature cysts – may disappear
- –less conspicuous in mature ones.



A. Uninucleate B. Binucleate C. Quadrinucleate

Reproduction

Various modes of reproduction seen in these organism include **Excystation, Encystation and Multiplication.**

Excystation is the process of transformation of cyst in to trophozoite and occurs only in small intestine of the susceptible host. During excystation a quadrinucleate cyst gives rise to eight amoeba, each one of which is capable of developing into a trophozoite.

Encystation is the process transformation of trophozoite into cyst, which occurs in the lumen of an infect individual.

Multiplication is occurs only in the trophozoite stage,it occurs by simple binary fission first of the nucleus and then of cytoplasm.

Intestinal Disease

Patients with intestinal disease may exhibit a number of symptoms including profuse diarrhea with blood and mucus, fever ,dehydration, weight loss& abdominal pain. Amebic ulcers may develop in the large colon and can also be found in the rectal area. Trophozoites produce histolytic enzyme that produce

necrosis of mucosa leading to the formation of flask-shaped ulcer with a small opening on the mucosal surface and a larger area below the surface.

Hepatic Disease(Extraintestinal manifestations)

Trophozoites are transported from the intestine to the liver and liver disease is characterized with abdominal pain, fever, hepatomegaly and tenderness. If the abscess ruptures, there is spreading to the brain, pericardium and other sites. If left untouched the abscess will grow
normally until it reaches a surface where it can discharge, e.g. the skin, the peritoneum, the pleural cavity or the pericardium. The stretching of the liver is presumably the main source of the pain.

Laboratory Diagnosis (Intestinal amoebiasis):-

1- stool examination: Trophozoites are found in diarrhoeic stool. Cysts are found in formed stool.

- Wet preparation

- Iodine stained

Permanent stain with iron haematoxylin or trichrome.

2- Concentration techniques for cysts.

3-Immunodiagnosis (ELISA, IFAT and latex agglutination).

4- Molecular analysis by PCR

5 -Sigmoidoscopy: - to visualize the ulcer, scrap, aspirate or take biopsy to see the trophozoites.

Diagnosis (Extraintestinal amoebiasis)

Clinical :according to the organ affected.

Laboratory:

Diagnosed by the use of scanning procedures for liver and other organs.

Specific serologic tests, together with microscopic examination of the abscess material, can confirm the diagnosis.

Treatment:-

- **Metronidazole, Tinidazole** Very effective in killing amoebas in the wall of the intestine, in blood and in liver abscesses.
- **Diluxanide furoate** kills trophozoites and cysts in the lumen of the intestine.

Entamoeba coli

- Non pathogenic amoeba that very closely resembles *Entamoeba histolytica*
- has a worldwide distribution
- Feeds on bacteria and any other cells available to it
- does not invade tissues
- common inhabitant of the lumen of the cecum and colon of man and other animals
- Has the typical Entamoeba nucleus

Morphology of Trophozoite:-

- Usually 15-25 μm in diameter (range 10-50 μm) ,perhaps a little larger than the trophozoite of *Entamoeba histolytica*
- Cytoplasm:

More vacuolated or granular endoplasm with bacteria and debris but no RBCs

Pseudopodia: broad short pseudopodia and little locomotion

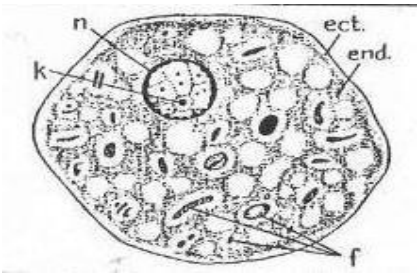
- function more to ingest food

– sluggish, non-directional motility

1. Nucleus

1 nucleus

–Thicker, irregular, coarsely granular peripheral chromatin with a large eccentric karyosome



Morphology of cyst:-

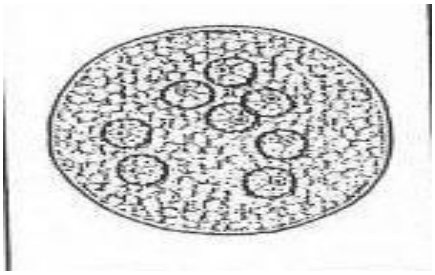
-size: 10-35 μm

-Nucleus

- Usually spherical
- mature cyst: 8 nuclei
- Immature cyst: 2 or more nuclei
- Karyosome is large, may/may not be compact and/or eccentric

-Cytoplasm: coarsely granular

-chromatoidal bodies: Splinter-shaped or broom-shaped with rough, pointed end.



Laboratory diagnosis:-

Routine microscopic examination of stool is sufficient for diagnosis.

Flagellates Protozoa

Sub-phylum: Mastigiphora

Class: Zoomastigophora

Suborder 2. Diplomonadina

INTRODUCTION

Flagellates are unicellular microorganisms. Their locomotion is by lashing a tail-like appendage called a flagellum or flagella and reproduction is by simple binary fission. **There are three groups of flagellates:**

a-**Flagellates of digestive tract:**(**Lumen dwelling flagellates**)

-*Giardia lamblia* ,

-*Chilomastix mesnili* ,

b-**Flagellates of genital organs: (Lumen dwelling flagellates)**

-*Trichomonas vaginalis.*

c-Hemoflagellates(Flagellates of blood and tissue.)

-Trypanosoma species.

-Leishmania species.

- -The flagellates protozoa are distinguished by having in their trophozoite stage one to several thread-like extensions of the ectoplasm(flagella) ; arising from a complex system of axonemes extending along the midline which arising from a basal body .

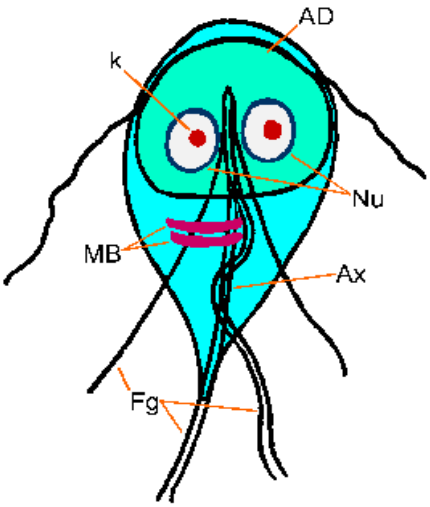
Giardia lamblia

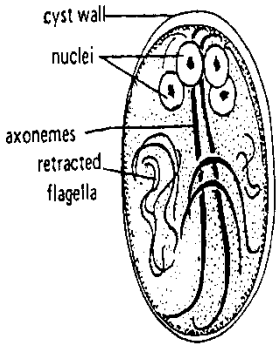
Giardia lamblia is a flagellate of world-wide distribution. It is more common in warm climates than temporal climates . It is the most common flagellate of the intestinal tract, causing Giardiasis. Humans are the only important reservoir of the infection.. *Giardia* inhabits the crypts of the duodenum and upper jejunum. Giardiasis is an infection of the upper small bowel, which may cause diarrhea.

Disease: **Giardiasis, Lambliasis, steatorrhea**.

Morphology

The parasite has both a trophic and cystic stage. The trophozoites of *G. lamblia* is pear shaped, with a broad anterior and much attenuated posterior . It is 10 -12μm long and 5-7 μm wide, bilaterally symmetrical, and has two nuclei with central karyosomes . It is also relatively flattened with a large sucking disck on the anterior ventral side, which serves as the parasites method attachment to the mucosa of the host.The trophozoite also has two median bodies the function of the median bodies is not known, but most believe they are somehow involved with the adhesive disk and its formation, and four pairs of flagella .The fibrils are called axonemes (Ax) or (axostyles). They attach themselves to the surface of the jejunal or duodenal mucosa by their disc-like suckers which are found on their ventral surface. They multiply in the gut by binary fission.





Trophozoite of *G. lamblia* cyst of G. *lamblia*

Morphology of Cysts

It is ovoid in shape; 8-12 µm long x 7-10 µm wide and have thin cyst wall Four nuclei present, often concentrated at on end. Flagella shorten and are retracted within cyst, axonemes provide internal support and parabasal bodies may also seen.

MODE OF TRANSMISSION

- ✓ Infection is occurred by ingestion of cyst in contaminated food & water.
- ✓ Direct transmission from person to person may occur in children, , mentally ill persons.

CLINICAL FEATURES

1-**Silent cases without any symptoms**.
2-**Intestinal :which include the following:-**
1. Malabsorption syndrome (Steatorrhoea)
It has been suggested that the coating of large surface areas of upper small bowel by giardia may act as a barrier to fat absorption and thus cause the steatorrhea (fatty or oil diarrhea)
2. Mucus diarrhea

3. Dull epigastric pain

4. Flatulence

5. Chronic enteritis

6. Acute enterocolitis

Incubation period : about 2 weeks

3. General :

1. Fever

2. Anaemia

3. Weight loss

4. Allergic manifestations.

4. Chronic cholecystopathy

- Note: Stool containing a large amount of mucus and fat and no blood.
- However 50% of *G. lamblia* infections are symptomless, although severe infections may develop in immunocompromised hosts..

Laboratory Diagnosis

1-Stool Examination

- Identification of cysts in formed stool and trophozoites & cysts in diarrhoeic stool or after a purgative.
- In asymptomatic carriers only cysts are seen.
- Macroscopy : offensive odour, pale coloured & fatty stool.
- Microscopy : salaine & iodine wet preparations.
- Multiple specimens need to be examined.
- Concentration techniques like formal ether or zinc acetate are used.

2- Enterotest (STRING TEST)

Method for obtaining duodenal specimen (upper part of small intestine)

Procedure :

-A coiled string with a small weighted gelatin capsule is swallowed by the patient & the free end of the string is attached to the side of the patient's face.

-The capsule dissolves in the stomach & the string which is weighted at its distal end, passes into the duodenum.

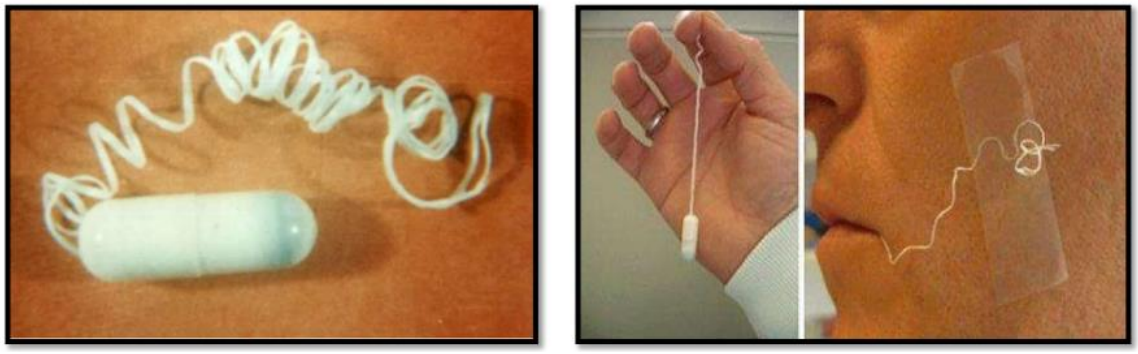
- After 2-4 hrs the string is withdrawn & placed in a saline with mechanical shaking.

-The centrifuged deposit of saline is examined by wet mount technique to detect the presence of motility of the organism or specific morphological forms of trophozoites of Giardia (and larvae of Strongyloides stercoralis).

When the test should performed ???

-Entero-test is performed when a physician suspects a parasitic infection, but no parasites were found in stool sample.

-As its sensitivity is comparable to duodenal aspirate, it eliminates the need of duodenal intubation.



Enterotest (String test)

3- Serological test

Serodiagnosis

A-Antigen detection in feces –

1- ELISA

2- IIF (Indirect immunofluroscent tests)

3- Immuno-chromatographic strip test

-Antigen present – active infection.

-Giardia specific Ag 65 (GSA 65) detection by ELISA kits.

-Sensitivity - 95% Specificity – 100% compared to microscopy.

-Tests are not for routine purpose.

-It is for epidemiological & control purposes

B-Antibody detection –

1- IIF

2- ELISA

-Tests can't differentiates between recent & past infection.

- Lack sensitivity & specificity.

-Antibody detection (anti Giardia IgG Ab) is useful for epidemiological & pathophysiological studies.

-The presence of anti Giardia secretory IgA Ab in breast milk protects breast fed infants from giardiasis

4-Molecular method

DNA based techniques are available now.

They are used to demonstrate the genome of the parasite.

- PCR

- DNA probe

Treatment

-Metronidazole – 250mg x 3 times daily x 5 days. (Cure rate -95%)

-Tinidazole – 2 g single dose. (More effective)

-Furazolidone
-Nitazoxamide → Children (less adverse effects)

Pregnant female → Parmomycin

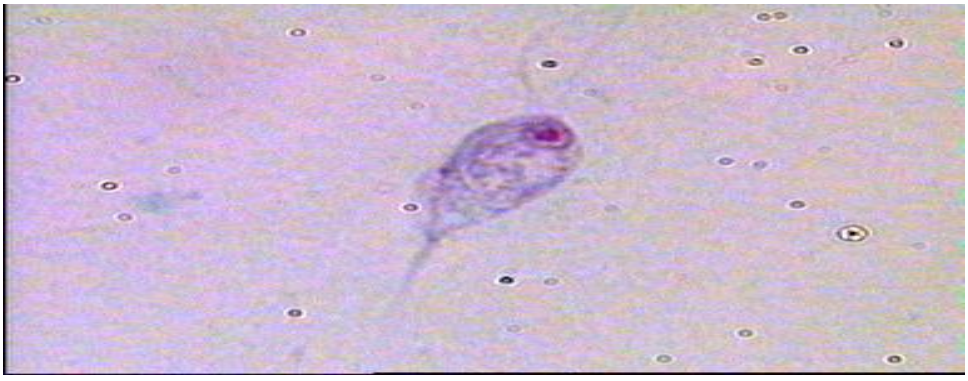
Chilomastix mesnili

It is thought to be non-pathogenic although the trophozoite has been associated with diarrheic stool. This is the largest flagellate found in man. The natural habitat of *Chilomastix mesnili* is the colon.

Morphology of trophozoite:

The parasite has both a trophic and cystic stage. The trophozoite of *C. mesnili* is pear shaped and measure 6-20μm in length. It is unsymmetrical rounded anteriorly and spirally twisted posteriorly. It has one large nucleus

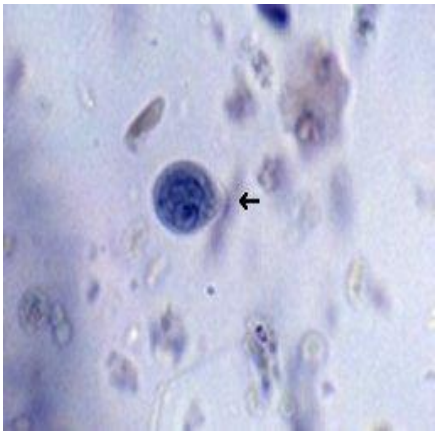
with a small karyosome and three flagella that extend from the nucleus at the anterior end of the parasite. A distinct oral groove or cytosome can be seen near the nucleus with its sides being supported by two filaments. They are known to move in a directional manner.



Trophozoite of *C. mesnili*

Morphology of cysts

The cyst is 6-9μm; it has a large single nucleus with a large karyosome. is lemon shaped , it has a thick hyaline wall and having the characteristic internal features of the trophozoite.



The cyst of *C. mesnili*

Laboratory Diagnosis

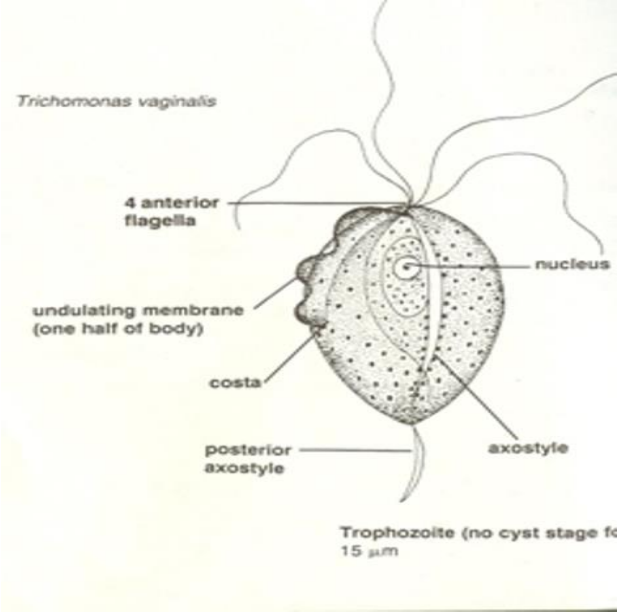
The characteristic lemon shaped cysts can be seen in a formol-ether concentrate. Motile organisms can be seen in a wet preparation of a fresh stool .

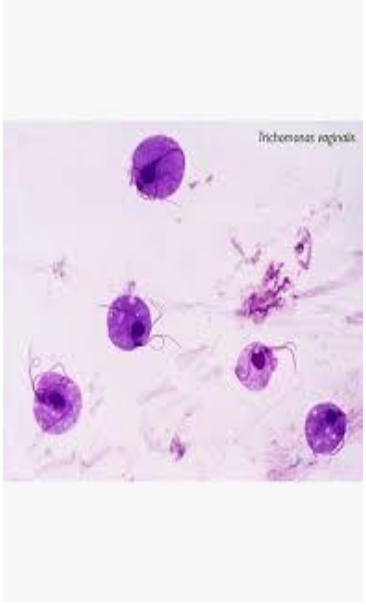
Trichomonas vaginalis

This protozoan parsitizes in the vagina, urethra and prostate and causes trichomonas vaginitis, urethritis and prostatitis. Trichomoniasis vaginalis is prevalent all over the world.

Morphology

Only the trophozoite stage is found in its life cycle. The trophozoite is ovoid or pear-shaped, 10~30×5~15μm in size. It has 4 anterior flagella and one posterior flagellum which turns back and is attached to the to the body by an undulating membrane. The undulating membrane of *T. vaginalis* is very short, only one-half of its body length. There is nucleus and the axostyle project posteriorly out of the body. The motility is jerky and non-directional.





Trophozoite of *Trichomonas vaginalis* **Trophozoite shape under microscope**

Mode of transmission

-Trophozoite cannot survive outside and so infection has to be transmitted directly from person to person.

- Sexual transmission is the usual mode of transmission.

- Trichomoniasis often coexists with other sexually transmitted diseases; like candidiasis, gonorrhea, syphillis, or human immunodeficiency virus (HIV).

-Babies may get infected during birth.

- Fomites such as towels have been implicated in transmission.

- **Trophozoite divides by binary fission.**
- **Incubation period is roughly 10days**

Clinical manifestation

- **WOMEN (SYMPTOMATIC)**

-Vulvo vaginitis (Trichomonal vaginitis)

-Urethritis

- **IN MEN (ASYMPTOMATIC)**

Urethritis, epididymis, prostatitis, and superficial ,penile ulcerations.

Irritation inside the penis, mild discharge, discharge may be purulent to mucoid or slight burning after urination or ejaculation.

COMPLICATIONS

- **(WOMEN)**

-PID

-Premature birth

-Low birth weight

-Increased risk of transmission of HIV

-Increased chance of cervical cancer

May also cause Pneumonia, bronchitis, and oral leisons.

- **In men:**

– Prostatitis

– Epididymitis

– Urethral stricture

– Infertility.

SPECIMENS

IN WOMEN : vaginal discharge, endocervical specimens.

IN MEN: Prostatic fluid, less commonly semen**.**

common specimens urethral swab, early morning first voided urine sediment

LAB DIAGNOSIS.

1. **MICROSCOPY**

Trichomonas in the vaginal discharge can be demonstrated by;

-Wet mount

- Acridine orange staining

-Papanicolau stain (PAP smear)

-Direct fluorescent antibody (DFA)staining

Vaginal or urethtral discharge is examined

microscopically in saline wet mount preparation for characteristic, jerky and twitching motility and shape.

In males trophozoites may be found in urine or prostatic secretions.

• DFA is more sensitive.

2. Culture

•Consider as gold standard for the diagnosis.

- Is recommended when direct microscopy is negative
- most sensitive (95%)
- Grows best at 35-37C under anaerobic condition
- Can be grown in a variety of solid or liquid media , tissue culture, and eggs. Cystein-peptone-liver maltose (**CPLM**) medium and plastic envelope medium (**PEM**) are often used.

3. Antigen detection in vaginal smears

ELISA is used for demonstration of Trichomonas antigen in vaginal specimens.

4. Molecular diagnosis

• DNA probes

• PCR(highly sensitive (97%) and specific (98%) test

TREATMENT

- Metronidazole 2g orally as a single dose or 250mg three times daily for 7 days.
- Metronidazole is contraindicate in pregnancy due to is mutagenecity, so topical therapy with clotrimazole is applied

The Ciliates

The ciliates belong to the family Ciliophora. They possess simple cilia or compound ciliary organelles, two types of nuclei and a large contractile vacuole. The only member of the ciliate family to cause human disease is *Balantidium coli*

Balantidium coli

The organisms inhabit the large intestine, cecum and terminal ileum where they feed on bacteria. The most common hosts being humans, pigs and rodents. Human infection is usually from pigs and is rare.

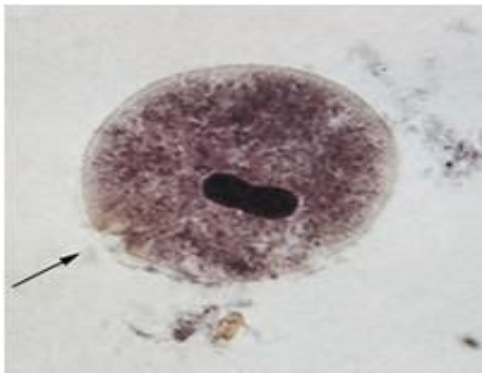
Disease: Balantidiasis

Morphology of the Trophozoite

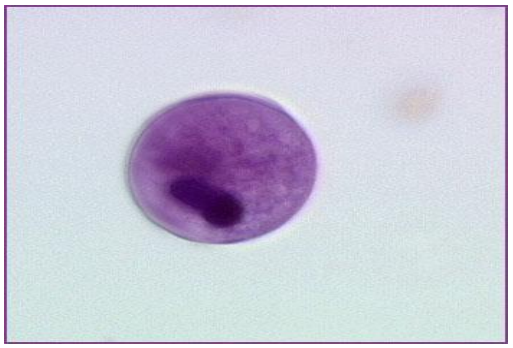
Trophozoites of *B. coli* measure approximately 30-150μm in length x 25-120μm in width but have been known to attain lengths of up to 200μm. They

are oval in shape and covered in short cilia. A funnel shaped cytosome can be seen near the anterior end. the two nuclei are visible. The macronucleus is

long and sausage-shaped, and the spherical micronucleus is nested next to it, often hidden by the macronucleus .Multiplication is by binary fission in the trophozoite stage.



Trophozoite of *B. coli*



Cyst of *B. coli*

Morphology of the Cyst

The cyst is spherical or ellipsoid and measures from 30-200µm by 20-120µm. It contains 1 macro and 1 micronucleus. The cilia are present in young cysts and may be seen slowly rotating, but after prolonged encystment, the cilia disappear. The cyst, ingested by a fresh host, excysts to liberate the trophozoite.

Clinical Disease

Severe *B. coli* infections may resemble amebiasis. Symptoms include diarrhea, nausea, vomiting, and anorexia. The diarrhea may persist for long periods of time resulting in acute fluid loss. *Balantidium coli* also has the potential to penetrate the mucosa resulting in ulceration just as those of E *Entamoeba histolytica*.

Laboratory Diagnosis

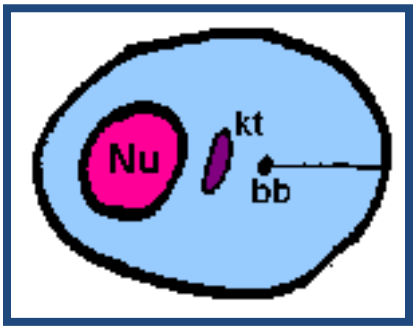
Wet preparations of fresh and concentrated stool samples reveal the characteristic cysts and motile trophozoites. They are easier to identify in direct-smear saline preparations than permanently stained fecal smears.

Haemoflagellates

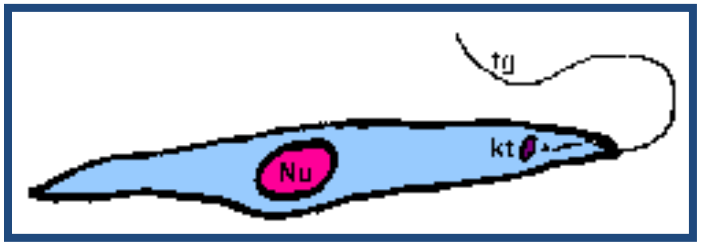
Medically important haemoflagellates require two hosts to complete their life cycle; some are called (digenetic or heteroxenous). They live in the blood and tissue of human and other vertebrate hosts and also in the gut of insect vectors. Haemoflagellates infecting human belong to two genera, in the family trypanosomatidae (Trypanosome and Leishmania).

Haemoflagellates exist in two or more of four morphological stages, these are:

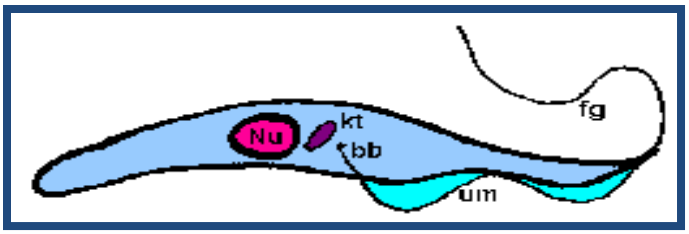
1- **Amastigote (leishmanial form):**
This stage is rounded or oval shaped without flagellum. This stage found intracellular in vertebrate hosts.



2- **Promastigote (leptomonal form):**
This stage is lanceolate where kinetoplast is anterior to the nucleus, from which arises the short flagellum. There is no undulating membrane this is the **infective stage of leishmania found in the mid-gut of insect.**

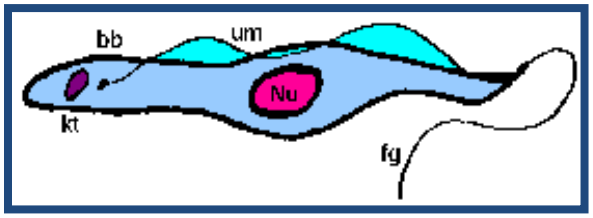


3. Epimastigote (crithidial form)
This stage is elongated with the kinetoplast placed more posteriorly and in front the nucleus. The flagellum extends alongside the body as a short undulating membrane.



4.Trypomastigote (trypanosomal form):

This stage is more elongated, spindle-shaped with central nucleus and the kinetoplast posterior to the nucleus, situated at the posterior end of the body. The flagellum extends alongside the entire length of the cell to form long undulating membrane before protruding from anterior end. This stage lacking in Leishmania



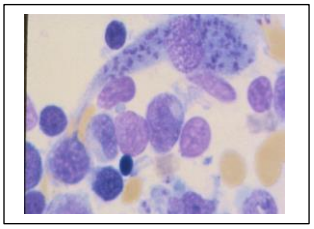
Leishmania

Leishmaniasis is one of the most important vector-borne diseases of humans. This parasitic disease can be caused by many species of *Leishmania*, most of which are zoonotic. In humans, different species of the parasite are associated with different forms of the disease. Many *Leishmania* spp. cause skin ulcers and nodules. A few of these organisms can also affect the mucous membranes, and may cause disfiguring lesions of the nose. Other species damage the internal organs and cause human visceral leishmaniasis, a life-threatening condition. Two forms of leishmaniasis, cutaneous and visceral, are seen in humans. The form of the disease and the usual clinical signs vary with the species of *Leishmania*. Some infections remain asymptomatic.

- ***Leishmania donovani* (Visceral leishmaniasis (VL))** (Liver, spleen and bone marrow)
- ***Leishmania tropica*** **(Cutaneous leishmaniasis (CL)** (Skin and mucous membranes)

Leishmania has two morphological stages in the life cycle:

1- Insect stage (Promastigote, motile, mid gut)
2- Mammalian stage (Amastigote, non-motile, intracellular).
Amastigotes measure 2-3 micrometers, with a large nucleus and



Kinetoplast. Amastigotes mainly live within cells of the RE system, but have been found in nearly every tissue and fluid of the body.

Transmission of Leishmaniasis

1. Bite of sand flies Phlebotomine Sandflies
2. artificial transmission of leishmania via the sharing of contaminated syringes and needles, from one intravenous drug user to another.
3. Rarely, Leishmaniasis is spread from a pregnant woman to her baby (Materno-fetal transplacental transmission). Blood transfusion also can spread Leishmaniasis.

Diagnosis

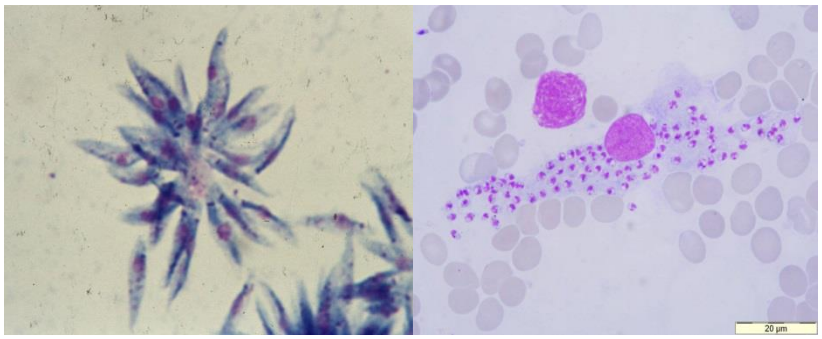
1- Clinical diagnosis: signs and symptoms (patient's history, travel and vectors).
2- Laboratory diagnosis:

Cutaneous leishmaniasis :

- Tissue sample (scraping, aspirate or punch biopsy) for smear (Giemsa stain) and culture (N.N.N medium)

Visceral leishmaniasis :

- Bone marrow biopsy or splenic aspirate for smear (Giemsa stain) and culture (N.N.N medium)
- Serology (ELISA) (IFAT).
- PCR
- Skin test
- Inoculate serum of infected person in lab. animals.



Promastigotes in rosettes in a culture of an orient sore on N.N.N. medium (Giemsa stain)

Ovoid small intracellular parasites in a bone marrow aspirate (Giemsa stain).

Trypanosomes

All members of the genus trypanosome exist at some time in their life cycle, as the trypanomastigote (trypanosomal stage) with an elongated spindle-shaped body, a

central nucleus, a posterior kinetoplast and a long undulating membrane. A blood sucking insect constitutes the intermediate host and vector, the vector becomes infective to the vertebrate host only after an extrinsic inoculation period during which the parasite undergoes development and multiplication. In the vector, the trypanosomes follow one of two modes of development, salivaria and stercoraria.

Classification of trypanosomiasis:

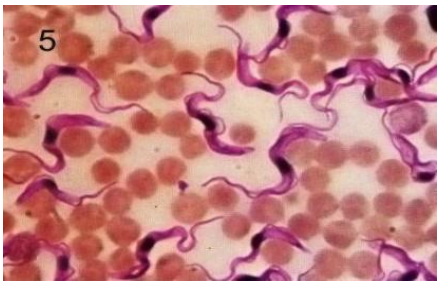
The trypanosomes infecting man are classified into the following groups:
1. *T. brucei* subspecies "(human strains) causing African Trypanosomiasis or sleeping sickness.

- *T. brucei gambiemse* that cause West African Trypanosomiasis transmitted by *Glossina* (tsetse fly)
- *T. brucei rhodesiense* that cause East African Trypanosomiasis transmitted by Glossina (tsetse fly).

2. *T. cruzi*, causing South American trypanosomiasis or chaga's disease transmitted by *Triatoma* (winged bug).

3. *T. rangeli*, non-pathogenic trypanosomes causing harmless human infection in South America.

East African Trypanosomiasis (Rhodesian sleeping sickness) caused by Trypanosoma brucei rhodesiense transmitted by tsetse fly (Glossina)

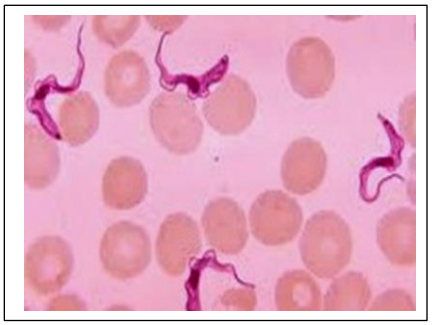


East Africa *T.brucei*

Diagnosis

Diagnosis is established by the determination of the trypanosomes in the peripheral blood, bone marrow. Lymph nodes or Cerebrospinal fluid. The methods available are:

1- Clinical picture (signs and symptoms)
2- Microscopic examination of unstained or stained blood film

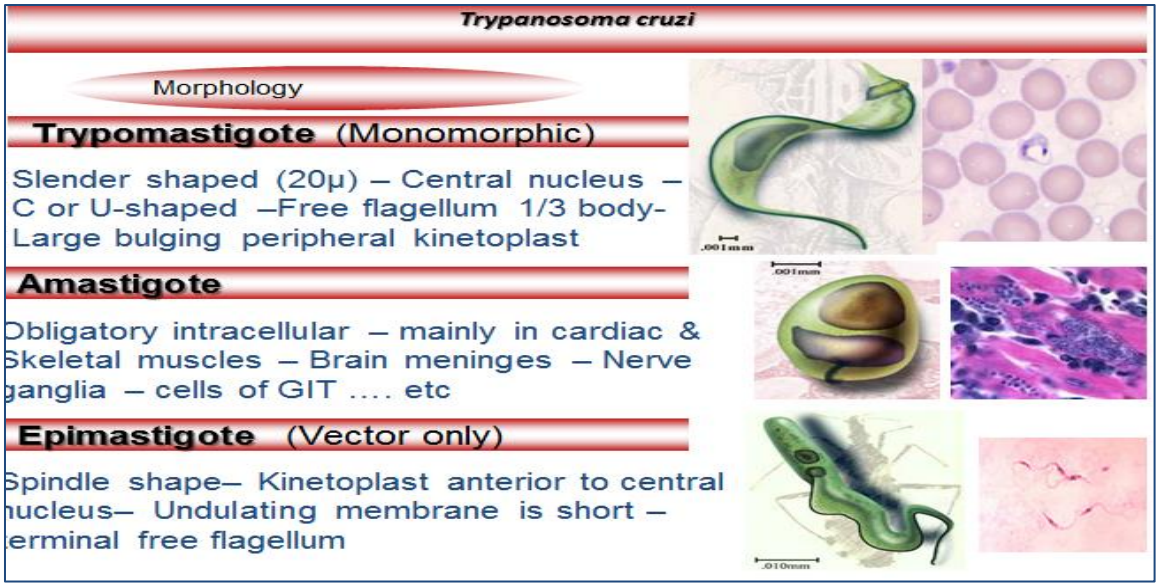


3- Culture on suitable media such as NNN medium or or Weinmann's media to detect Epimastigote)
4- Serological methods (DAT, IHT, Gel precipitation immunofluorescence and ELISA).
5- Molecular technique Polymerase chain reaction (PCR).
6- Animal's inoculation.

South American trypanosomiasis (chaga's disease)

The causative agent of South American trypanosomiasis is *T. cruzi*. This parasite passes its life cycle in two hosts, vertebrate host including humans and the insect vector, the reduviid bug, winged bug, kissing bug (triatomate).

The feces of infected bugs contain the **metacyclic trypomastigotes which are the infective forms**. Infection is acquired when they are rubbed into the bite wound or enter through mucosal surfeces, particularly the conjunctiva, being transferred there by the person's fingers.

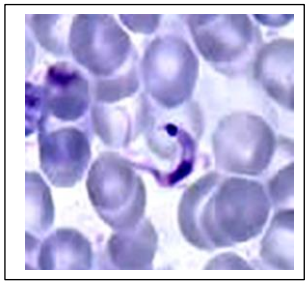


Mode of infection

1- Mainly by contamination of skin or abrasion by winged bug feces.
2- Rarely by blood transfusion, through placenta or mothers milk to here baby.

Diagnosis:

- Diagnosis is by demonstration of *T. cruzi* in blood or biopsy, in stained peripheral blood smears, the **trypomastigote often appears in C-shaped form**. While, in Biopsy from lymph node, liver or spleen **(Amastigotes) is found**.



- culture on N.N.N. medium or its modifications (Epimastigotes)
- animal inoculation
- xenodoiagnosis, may be attempted by allowing the parasite-free reduviid bug to bite the patient and by demonstrating the parasite in its intestinal contents.
- Polymerase chain reaction (PCR).

Xenodiagnoses

Highly efficient method demonstrate low level of parasite in blood. In this method the laboratory bred winged bug is starved for 2 weeks then fed on suspected patient's blood – 30 days later, it feces & gut examined for trypanosomes.

Malaria

Malaria is a disease that is transmitted by the bite of female *Anopheles* mosquito. Malaria is caused by protozoa of the *Plasmodium* species. There are four species which infect both humans and animals:

1-***Plasmodium vivax*** (benign tertian malaria) .The developmental cycle in the blood lasts approximately 48 h.

2- ***Plasmodium ovale*** (ovale tertian malaria). The developmental cycle in the blood lasts approximately 48 h.

3- ***Plasmodium malariae*** (quartian malaria). The developmental cycle in the blood lasts approximately72 h.

4- ***Plasmodium falciparum*** (malignant tertian malaria or falciparum malaria or black water fever) .The developmental cycle in the blood lasts approximately 48 h.

Plasmodium requires two hosts, the definitive invertebrate host (**vector**), and intermediate host vertebrate host **(mammals, birds and lizards**.)

Infective stage: **sporozoite**

Morphology of *Plasmodium* species

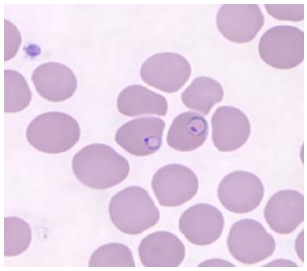
*1- **Plasmodium vivax**:-*

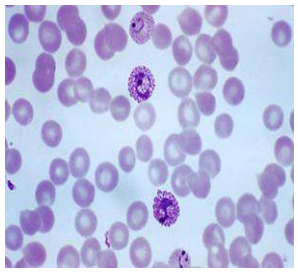
- Infected RBC enlarged, Small Trophozoites **(Ring form**) has large chromatin dots and develop to form trophozoite stage or amoeboid stage which has irregular shape.

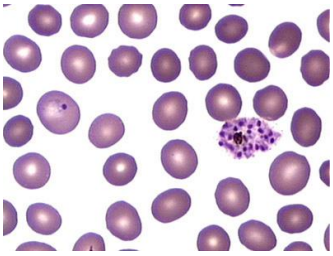
- *P. vivax* **trophozoites** show amoeboid cytoplasm, large chromatin dots,. Schüffner's dots (reddish granules) may appear more fine in comparison to those seen in *P. ovale*.

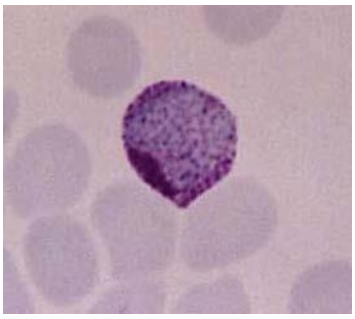
- Chromatin divided forming **schizont** within 48 hr and produce 12-24 merozoites (usually16).may cause rapture of RBC.

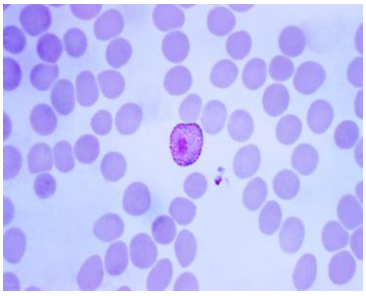
- **Gametocytes** are Round to oval shape fill the RBC. Microgametocyte or male gametocyte has distributed chromatin granules ,whereas the macrogametocyte or female gametocyte has compact chromatin usually peripheral in distribution .


Ring stage


Amoeboid stage


schizont


Macrogametocytes

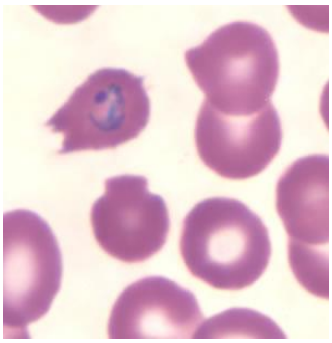

Microgametocyte

2-*Plasmodium ovale*:-

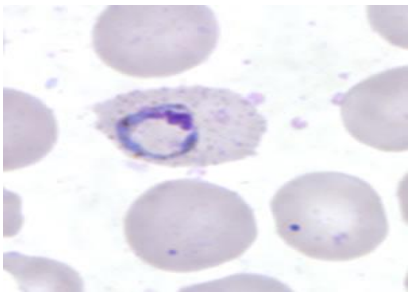
Similar to *P. vivax* , red blood cells (rbcs) can be normal or slightly enlarged, may be round to oval and contain ring form.

Trophozoites of *P. ovale* have sturdy cytoplasm, large chromatin dots, and can be compact to slightly irregular, show Schüffner's dots and fimbrinated appearance of edges of infected RBCs.

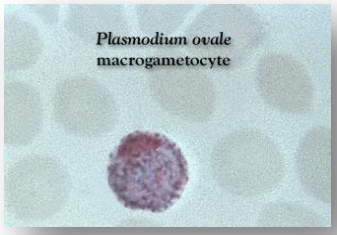
Schizonts have 6 to 12 merozoites, usually 8 with large nuclei clustered around a mass of dark-brown pigment.



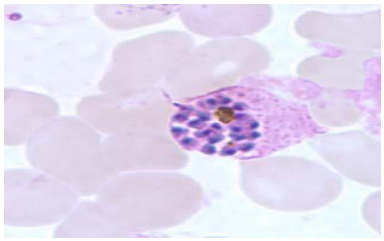
Ring form



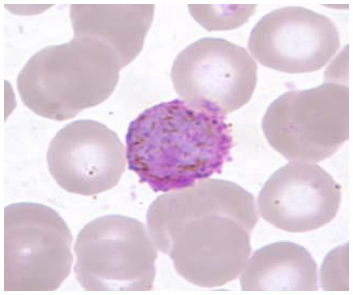
Trophozoite



Macrogametocytes



Schizonts



Microgametocyte

<u>Gametocytes</u> are round to oval and may almost fill the red blood cells.

3- ***Plasmodium malariae*:-**

- Early trophozoites / **<u>ring forms</u>**, compact rings containing one mass of chromatin.
- **<u>The trophozoite</u>** growing and forms band shape across the RBC.
- Through 72 hours growing to schizont, which has a rosette with 8-10 oval merozoites.

- **<u>The gametocytes</u>** are resembled to *P. vivax* but smaller.

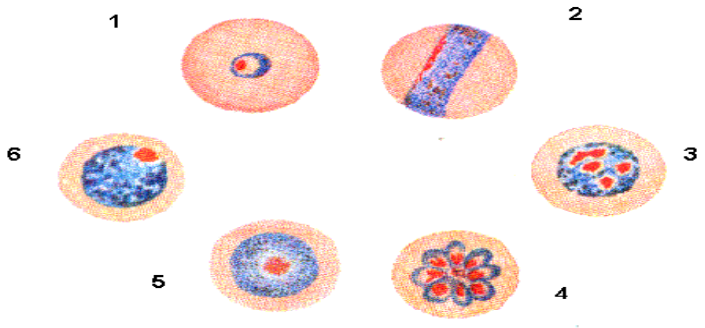


Illustration 9-9. Diagrammatic illustration of the morphology of the different stages of the *Plasmodium malariae* life cycle in thin blood films. **1)** Early trophozoites / ring forms, compact rings containing one mass of chromatin. **2)** Developing trophozoites, small and compact (often band forms) with an inconspicuous vacuole. **3)** Immature schizonts, compact and almost fill the red blood cell which contains scattered pigment. **4)** Mature schizonts, almost fill the red blood cell. **5)** Micro-gametocytes, low numbers appear after 7–14 days. **6)** Macrogametocytes, low numbers appear after 7-14 days.

4- ***Plasmodium falciparum***:-

- We can see only early (**<u>ring-form</u>**) and gametocytes in the peripheral blood.
- More than one ring form in RBcs or double chromatin dots.
- **<u>Schizont</u>** seldom found in the blood and it resemble to *P. vivax* and have 8-24 merozoite.
- **<u>The Gametocytes</u>** have banana shape, so called crescent.
- The female form, or macrogametocyte, is usually more slender and somewhat longer than the male. The nucleus is small and compact, The male form, or microgametocyte, is broader than the female sausage shaped. and the nucleus, which less compact than in the female,
- Presence of Maurer's dot In RBC , which are often seen in older ring

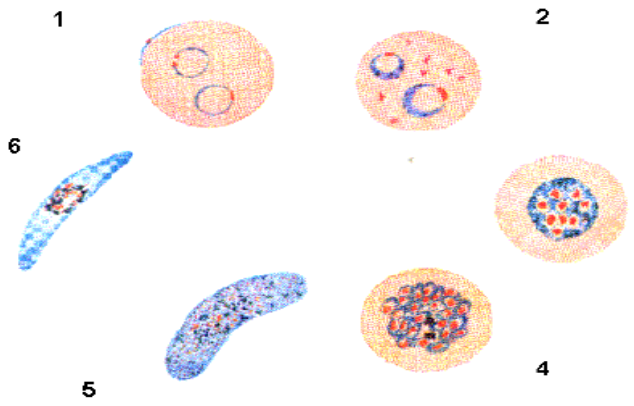


Illustration 9-6. Diagrammatic illustration of the morphology of the different stages of the *Plasmodium falciparum* life cycle in thin blood films. **1)** *P. falciparum* early trophozoites / ring forms. **2)** Developing trophozoites (rarely seen in peripheral blood). **3)** Immature schizonts (rarely seen in peripheral blood). **4)** Mature schizonts, almost fill the red blood cell. **5)** Microgametocytes, large numbers appear after 7–12 days. **6)** Macrogametocytes, large numbers appear after 7-12 days.

Transmission of malaria

- Female Anopheline mosquito
- Blood transfusion .
- Mother to child .

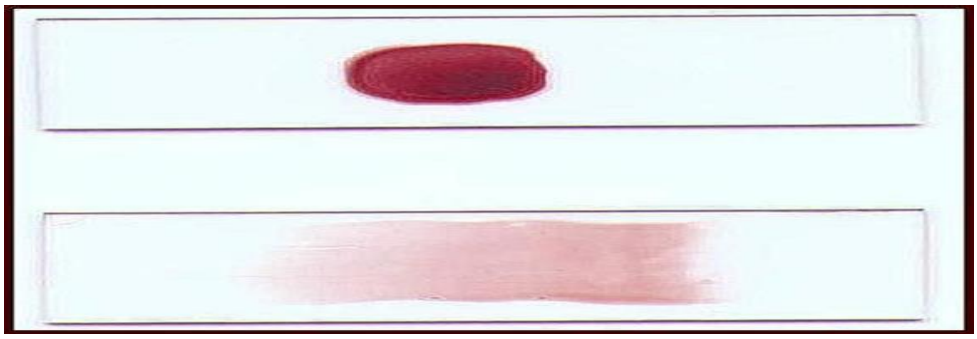
Clinical Manifestations

The pathology and clinical manifestations associated with malaria are almost exclusively due to the asexual erythrocytic stage parasites. Plasmodium infection causes an acute febrile illness which is most notable for its periodic fever paroxysms occuring at either 48 or 72 hour intervals.

The malarial paroxysm will usually last 4-8 hours and begins with a sudden onset of chills in which the patient experiences an intense feeling of cold despite having an elevated temperature. This is often referred to as the cold stage, or rigor, and is characterized by a vigorous shivering. Immediately following this cold stage is the hot stage. The patient feels an intense heat accompanied by severe headache. Fatigue, dizziness, anorexia, myalgia, and nausea will often be associated with the hot stage. Next a period of profuse sweating will ensue and the fever will start to decline. The patient is exhausted and weak and will usually fall a sleep. Followed by splenomegaly and anemia

Laboratory Diagnosis

The definitive diagnosis of malaria infection is still based on finding malaria parasites in blood films. In thin films the red blood cells are fixed so the morphology of the parasitized cells can be seen. However, malaria parasites may be missed on a thin blood film when there is a low parasitemia. Therefore, examination of a thick blood film is recommended.



Class: coccidia,
Family: Sarcocystidae

Toxoplasma gondii

It is the causative organism of toxoplasmosis, Congenital Toxoplasmosis it can cause severe damage and even death of the fetus. It occurs in three forms; trophozoite, tissue cyst and oocyst. The trophoziote and tissue cyst represent stages in asexual multiplication(Schizogony). While the oocyst is formed by sexual reproduction (gametogony or Sporogony).

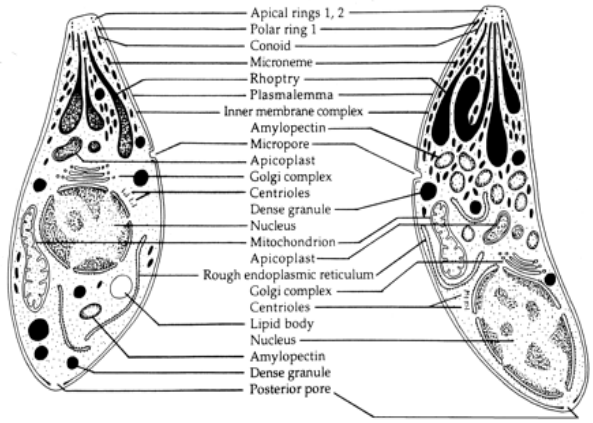
1-**Trophoziotes:-**

The trophozoite is crescent – shaped, with one end pointed and the other end rounded. It is measured approximately 2-3 μm by 4-7 μm. The nucleus is ovoid and located near the blunt end of the parasite, there is no cilia, flagella or pseudopodes. Trophoziotes proliferate by endodygony or internal budding ,they found in tissues during acute stage of infection and invade any nucleated cell of all mammalian cells accept non nucleated erythrocytes. The

rapidly proliferating trophozoites in acute infection in any cell of the intermediate host are called **tachyzoites**.

2- **Tissue cysts *:***

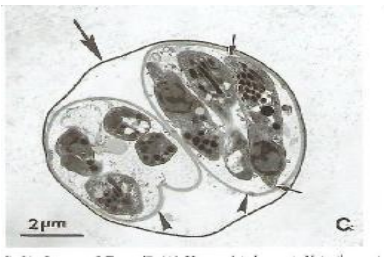
They are formed during the chronic phase of the infection and can be found in the muscle and various other tissues and organs , especially brain, skeletal and cardiac muscles and can persist, inactivated, in the body for a very long time. It range from 10-20 μm may contain several thousands of organisms. The slowly multiplying parasite within the cyst during chronic infection are called **bradyzoites**. They have nucleus situated toward the posterior end whereas the nucleus in tachyzoite is more centrally located.



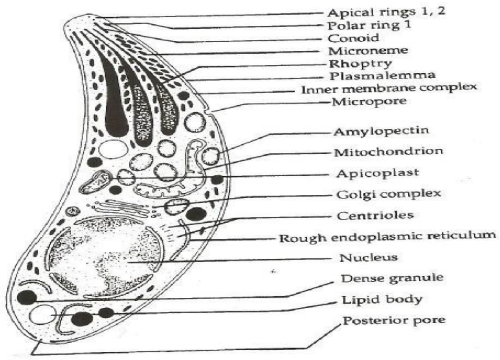
The tachyziote (left) and a bradyziote (right) of *T.gondii*

3-Oocysts:

These develop only in definitive hosts of (cats and other felines).It is sepherical or ovoid, about 10 to 12 μm in size. Cat shed millions of oocysts per day in faeces for about two weeks during the primary infection. It becomes infections only after development in soil or in water for a few days to form mature. Oocyst which contains two sporocysts, each one consist of four sporozoite is the infective form.



The Oocyst of *T.gondii*



The sporozoite of *T.gondii*

Definitive host is cats (any members of the feline family), in which sexual reproduction (gametogony or Sporogony), is occur to form oocyst. .
Intermediate hosts is humans , mouse and others, in which asexual multiplication (Schizogony) is occur to form the trophoziote and tissue cyst.

So the infective stage of *Toxoplasma gondii* are:

1-Oocyst with contaminated food and drunk.

2-Tissue cyst within uncooked meat.

Toxoplasmosis appears to be transmitted by:-

1-consuming raw or undercooked meat containing *T. gondii* tissue cysts.

2-ingesting water, soil, vegetables, or anything contaminated with oocysts shed in the feces of an infected animal

3-blood transfusion or organ transplant

4-transplacental transmission from mother to fetus,

Laboratory Diagnosis*:-*

1- Serological Tests:
-Direct agglutination test,

-Complement fixation test (CFT***).***

-Enzyme linked immunosorbant assay (ELISA)

-Indirect fluorescent assay (IFA).

2-Demonstration of trophozoite in brain biopsy, bone marrow aspiration, CSF and amniotic fluid.

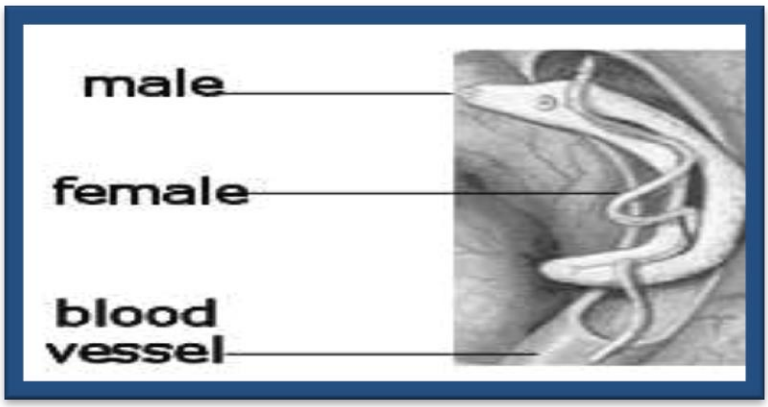
3-Demonstration of cysts in placenta and tissues of newborn.

4-Histopathology of lymph nodes.

5-Polymerase Chain Reaction (PCR).

Schistosomes (Blood flukes)

The Schistosomes are blood trematodes belonging to the Phylum Platyhelmintha. They differ from other trematodes in that they have separate sexes (dioecious trematodes) and are located in blood vessels of the definitive host. .The male is broader than females and its lateral borders are rolled ventrally into cylindrical shape producing along groove called the gynaecophoric canal, in which the female is hold during copulation.the name schistosome (Greek shisto-split and soma body). Schistosome were formerly called Bilharzia after Theoder bilharz who first observed.



They require definitive (human) and intermediate hosts (snail) to complete their life cycle. Infective stage **cercariae**

There are five species of Schistosomes responsible for human disease; **S. mansoni, S. haematobium** and **S. japonicum** with **S. mekongi** and **S. intercalatum** being less common. Schistosomiasis (bilharziasis) is a water borne disease constituting an important public health.

Schistosoma haematobium (Vesical Blood fluke , Bilharzia haematobium)

It causes **urinary schistosomiasis**. It occurs in Africa, India and the Middle East. The intermediate host is the **Bulinus** snail and definitive host are human.

Morophology

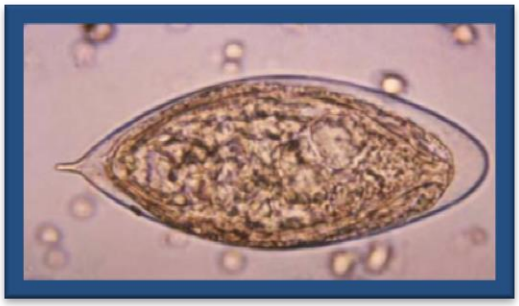
Adults are found in veins of urinary bladder and the females deposit their eggs in the walls of the bladder and finally making their way into the urine

The male is 10-15mm long by1 mm thick and **covered by a finely tuberculated cutical**. It has two suckers ,the oral sucker being small and ventral sucker large and prominent. behind the ventral sucker and extending to caudal end is the gynaecophoric canal, in which the female is hold**. 4-5 large testes** which discharge through a genital pore posterior to the ventral sucker. **The two ceca are united in second half of the body**.

The female is delicately cylindrical , 20 mm in length and diameter of 0.25 mm. The genital organs composed of ovary which is located in **second half of the body** from which extend ovi duct open in ootype . long uterus contains a**bout 20-100 eggs**, and vitellaria (yolk glands) **which located in posterior half of body.** The adult worms are longer than those of *S. mansoni*

Schistosoma haematobium (male)

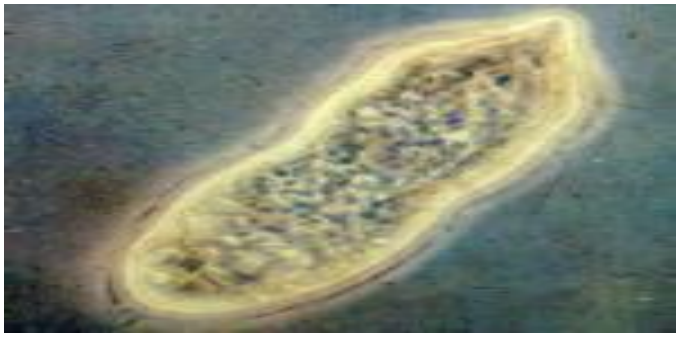
The eggs are ovoid , about 150 µm by 50 µm with brownish yellow transparent shell carrying a terminal spine at one pole.



- Miracidium

Hatches from the egg in slightly alkaline clean water with a temp. between 25 0C to 310C.

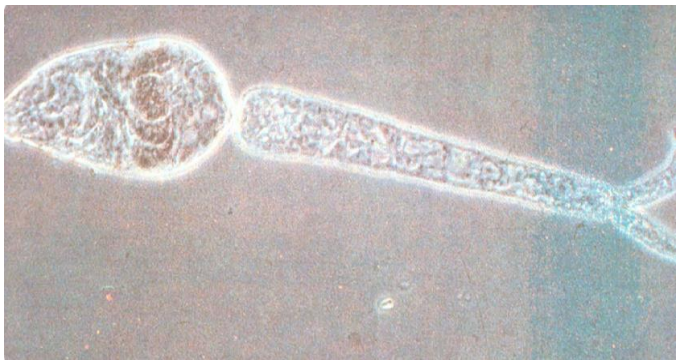
Free swimming cilated embryo liberated from the egg and infect snails..



Miracidium

Cercaria

- The cercaria has elongated ovoid body and forked tail.
- Emerges from daughter sporocysts
- Escapes from the snail
- Infects man by skin penetration



Cercaria

Pathology of Schistosomiasis

Clinical Disease caused by schistomes can be classified depending on the following stages:-

1. Skin penetration and incubation period
2. Egg deposition and extrusion
3. Tissue proliferation and repair.

- ✓ Cercarial dermatitis **(Swimmer's Itch)** following skin penetration, results in a maculopapular rash and can last 36 hours or more.

- ✓ After mating, the eggs are laid in the venules of the bladder and many penetrate through the mucosa, enter the lumen of the bladder and are excreted in the urine accompanied by blood. Thus hematuria, burning micturition and proteinuria are characteristic (urinary schistosomiasis)
- ✓ In chronic disease, eggs become trapped in the bladder wall resulting in the formation of granulomata, Fibrosis and Lung and CNS involvement
- ✓ Chronic urinary schistosomiasis is associated with squamous cell bladder cancer

Laboratory Diagnosis

- The definitive diagnosis of urinary schistosomiasis is made by finding the characteristic ova of *S. haematobium* in urine

- Terminal urine should be collected as the terminal drops contain a large proportion of the eggs. The urine can either be centrifuged and the deposit examined microscopically for ova.
- Eggs can sometimes be found in seminal fluid in males.,
- A bladder biopsy may be used in some cases

***Schistosoma mansoni* (Manson's blood fluke)**

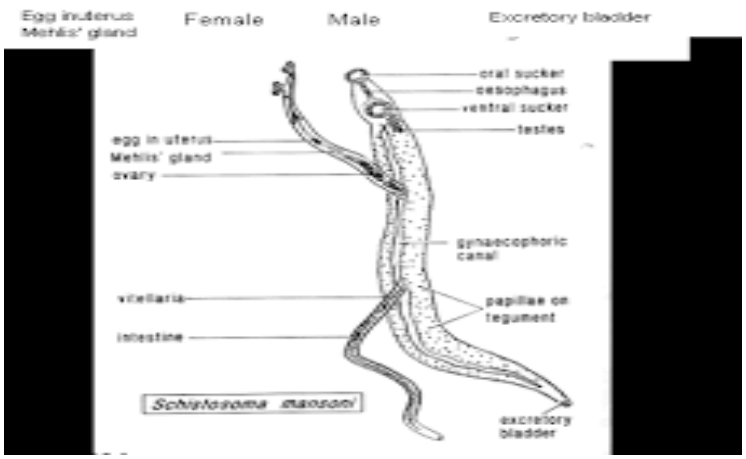
The adults are smaller than those of the other species. *S. mansoni* occurs in West and Central Africa, Egypt, Malagasy, the Arabian Peninsula, Brazil, Venezuela and the West Indies The intermediate host is an aquatic snail of the genus ***Biomphalaria*.** Man is the most common definitive host.

Morphology.

The adult worms live in smaller branches of the inferior mesenteric vein in the lower colon.

Male: The male ranges in size from 1-1.4 cm in length and the body is covered by coarse tubercles, **two ceca united in the first half** of the body so it has long united cecum. Oral and ventral suckers are present, with the ventral one being larger serving to hold the worms in place, preventing them being carried away by the circulatory current The male surrounding female with his gynaecophoric canal. **It has 6-9 testes.**

Female: The female is 1.5-2.0 cm in length. The genital organs ovary is situated **in the first half of body**. uterus short contain **a few eggs 3-5 eggs** .vitellaria (yolk glands) **extend between ootype to posterior end** The female parasite is darker, and it looks gray. The darker color is due to the presence of a pigment (hemozoin) in its digestive tube. This pigment is derived from the digestion of blood



The ova of *S. mansoni* are 114-175µm long by 45-68µm wide. They are light yellowish brown, elongate and possess a lateral spine

Clinical Disease

1. Cercarial dermatitis (swimmers itch)
2. Causes intestinal schistosomiasis

Dysentery blood and mucus in stool, hepatomegaly ,splenomegaly

Papillomata in intestine ,periportal fibrosis,hematemesis, lung and CNS involvement.

Laboratory Diagnosis

Microscopy

Laboratory confirmation of *S. mansoni* infection can be made by finding the eggs in the feces after an iodine stained, formol-ether concentration method. When eggs cannot be found in the feces, a rectal biopsy can be examined.

Serology

Serological tests are of value in the diagnosis of schistosomiasis when eggs cannot be found. An enzyme linked immunosorbent assay (ELISA) using soluble egg antigen

Schistosoma japonicum (Oriental blood fluke)

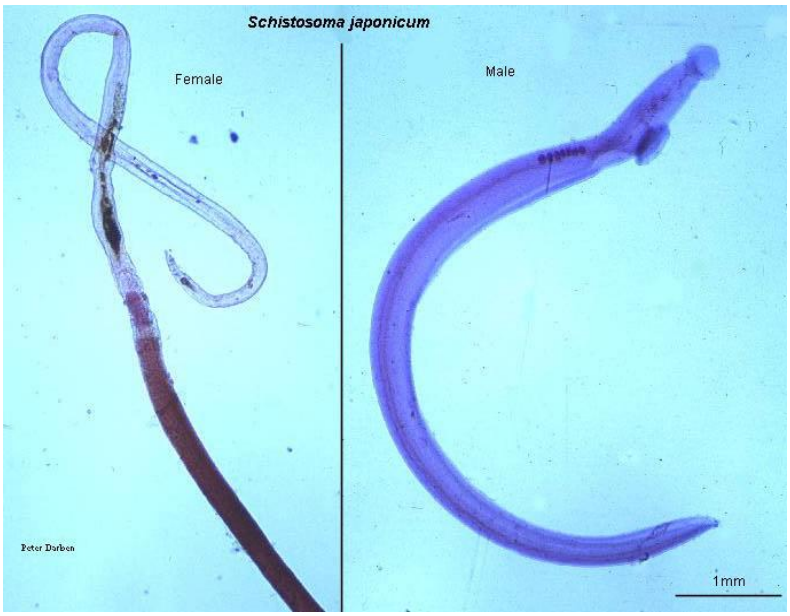
Schistosoma japonicum is found in China, Japan, the Philippines, and Indonesia. It causes disease of the bowel with the eggs being passed out in the feces. It differs form *S. mansoni* and *S. haematobium* in that it is a zoonosis in which a large number of mammals serve as reservoir hosts; cats, dogs and cattle playing major roles in the transmission of the disease. The life cycle is not very different from that of *S. mansoni*, the intermediate hosts are from the subspecies *Oncomelania hupensis*. Sexual maturity is reached in about four weeks and eggs may be seen in the feces as quickly as five weeks

Morphology.

The worms live coupled together in the superior, mesenteric veins and deposit 1500–3500 eggs per day in the vessels of the intestinal wall.

The males of this species are slightly larger than the other Schistosomes and they measure ~ 1.2 cm by 0.5 mm.,has two suckers oral and ventral suckers,two ceca united in posterior part of the body to form short united ceca, The adult worms are longer and narrower than the *S. mansoni* worms..There are 6-7 testes located near ventral sucker ,the tegument is nontuberculated(smooth)

The females measure 2 cm by 0.4 mm.The location of ovary in the middle of the body and the number of eggs in uterus 50-300. The ova are about 55-85µm by 40-60µm, oval with a minute lateral spine or knob



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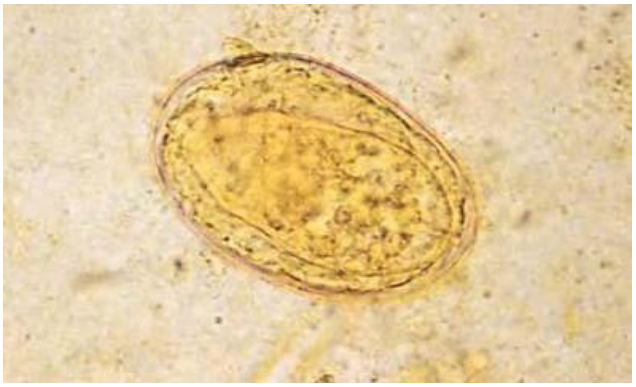
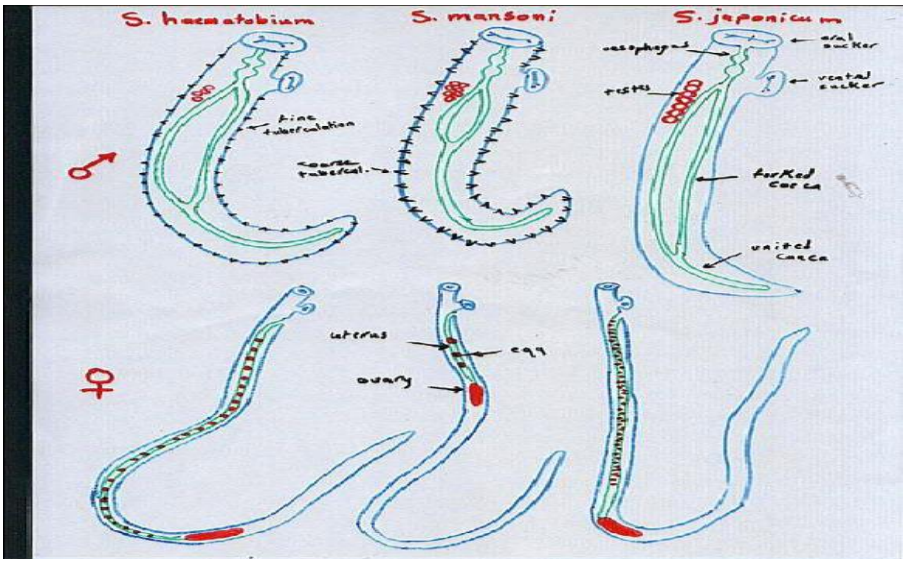


Eggs of S. ***japonicum***



Morphology of schistosoma spp

Clinical Disease

Cause **oriental schistosomiasis** or **Katayama fever** is associated with heavy primary infection and egg production. Clinical features include high fever, hepatosplenomegaly, lymphadenopathy, eosinophilia and dysentery. This syndrome occurs a few weeks after primary infection.Katayama fever is more commonly seen in people with their first infection such as migrants and tourists. However it is seen in native residents of China infected with *S. japonicum*

- The eggs which are sequesters in the intestine mucosa or submucosa initiate granulomatous reactions, resulting in the formation of pseudotubercles.

- Due to the number of eggs released by the females the infection is more severe than one with *S. mansoni*.

Laboratory Diagnosis

Laboratory confirmation of *S. japonicum i*nfection can be made by finding the eggs in the feces after an iodine stained, formol-ether concentration method. When eggs cannot be found in the feces, a rectal biopsy can be examined

Differentiating Features of Schistosomes:-

Feature	*S. haematobium*	*S. mansoni*	*S. japonicum*
Testes (male)	4-5 large testes	6-9 testes	6-7 testes
Ovary (Female)	located in second half of the body contain 20-100 ova	is situated in the first half of body contain a few eggs 3-5 eggs	The location of ovary in the middle of the body contain 50-300
Intestine	long	longest	short
Egg	Terminal spine	Lateral spine	Lateral knob
Intermediate Host	Bulinus	Biomphalaria	Oncomelania
Definitive Host	Human	Human	Human and domestic animals