Ministry of Higher Education

&

Scientific Research

University of MOSUL

College of Nursing

Practical Medical Microbiology2

(Part I Protozoa)

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2nd stage
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Lab: 1

1. Protozoa

- **General characteristic feature of protozoa:**

The protozoa are **unicellular eukaryotic** organisms, most of which are **microscopic**. They have a number of specialized **organelles** that are responsible for **life functions** and that allow further **division** of the group into classes. Most protozoa multiply by **binary fission** and are ubiquitous worldwide.

- **Classification of Clinically Important Protozoa:**

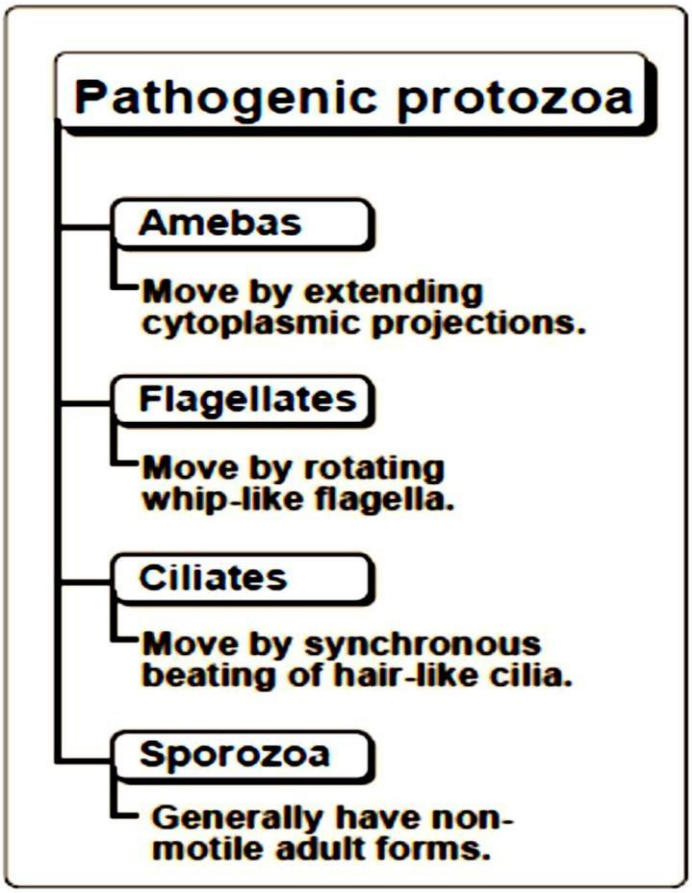


Figure 1.1: Clinically relevant protozoa, classified according to mode of locomotion

1. Amoeba: Classification:

Kingdom : Protista
Subkingdom : Protozoa
Phylum : Sacromastigophora
Subphylum : Sarcodina
Superclass : Rhizopoda
Class : Lobosea
Order : Amoebida

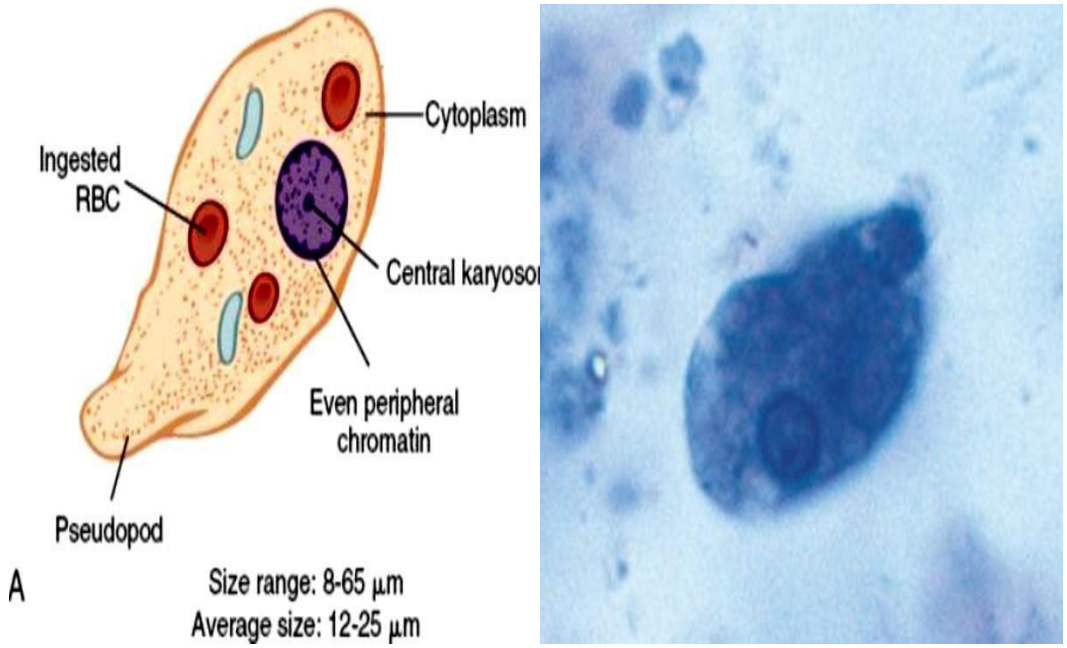
Protozoa Amoebae genus	Natural habitat
Entamoeba histolytica	Large intestine
Entamoeba dispar	Large intestine
Entamoeba coli	Large intestine
Entamoeba gingivali	Oral cavity

1. *Entamoeba histolytica:*

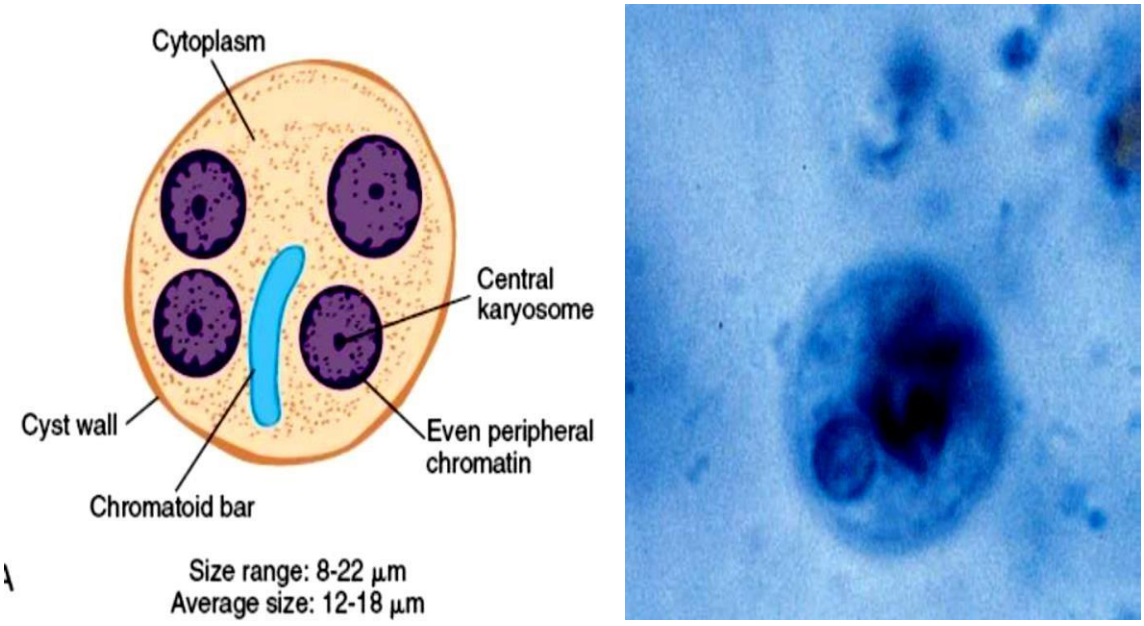
Sites of infection : Intestinal site(Large intestine) and Extra intestinal site (liver , lung , , spleen, skin, and brain.

- **Disease :** Amebiasis , Amebic dysentery , hepatic abscesses ,lung abscesses , Skin amoebiasis
- **Stages :** It has four stages: **Trophozoite, precyst, cyst, metacyst .**
- **Infective stage** : **Tetra nucleated cyst (having 4 nuclei)**
- **Diagnostic stage** : Motile trophozoite , cyst
- Mode of transmission: Ingestion of mature cyst through contaminated food or water.
- **Morphology:**

Characteries	Trophozoite	Cyst
Size range	8-86um	8-22um
Shape	Arregular shape	spherical to round
Motility	Progressiv,finger-like hyaline pseudopods.	Non- motility
Number of nuclei	One	1-4
Karyosome	Small and central	Small and central
Periphral chromatin	Fine and evenl distributed	Fine and evenl distributed
Cytoplasm	Finely granular	Finely granular
Cytoplasminclusion	Ingested RBC	chromatoid bars, diffuse glycogen mass in younger cysts

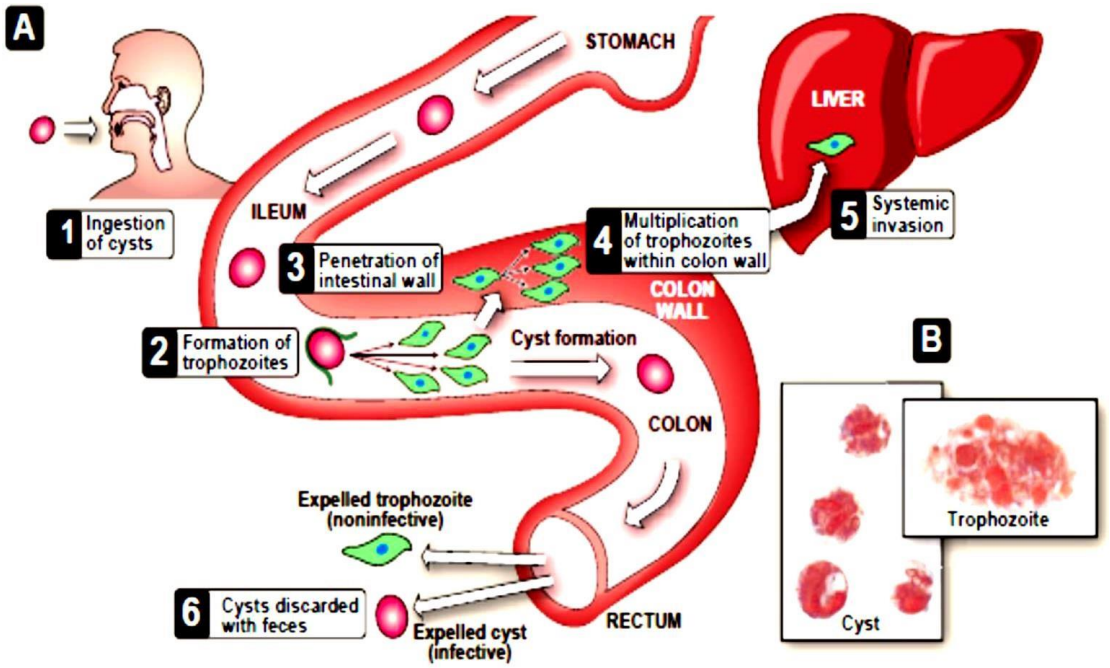


Entamoeba histolytica **trophozoite**



Entamoeba histolytica **cyst.**

Life cycle: direct life cycle



- **Laboratory Diagnosis:**
- **Intestinal amoebiasis** :
- Direct microscopic examination of the stool to **recover motile trophozoite (**containing red cells are diagnostic of amoebic dysentery).
- **In extra intestinal amoebiasis** :
- laboratory tests, including immunologically based procedures, may be used. Methods currently available include antigen tests, enzyme-linked immunosorbent assay (ELISA), indirect hemagglutination (IHA), gel diffusion precipitin (GDP), and indirect immunofluorescence (IIF).

2. Entamoeba coli:

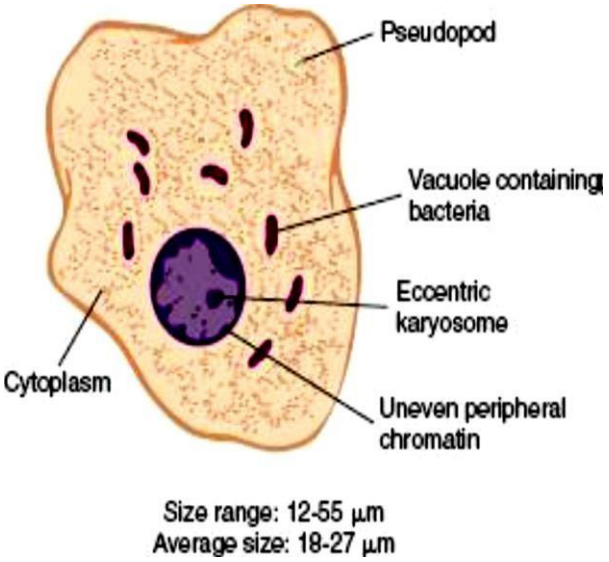
- Habitat : large intestine
- Disease **:**.(non pathogen)
- Stages : ***Trophozoite , cyst.***
- Infective stage : **Cyst**
- Diagnostic stage : Trophozoites and cysts in stool .

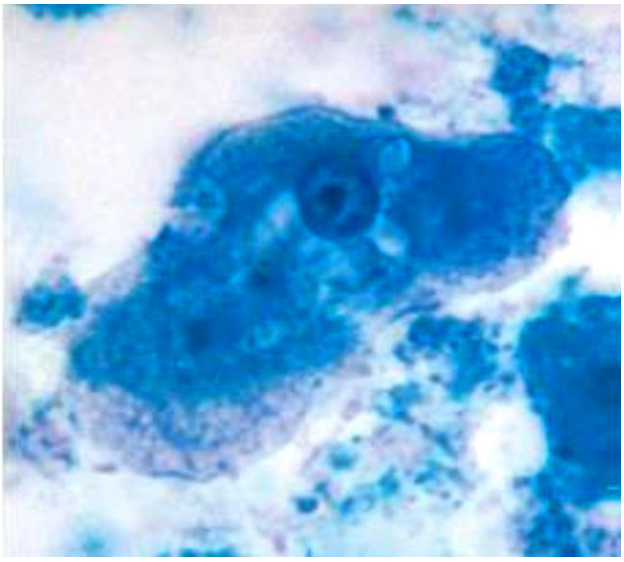
Morphology:

Characteries	Trophozoite	Cyst
Size range	5-15 µm	8-35µm
Shape	Arregular shape	Round to spherical
Motility	Non Progressiv, blunt pseudopods.	Non- motility
Number of nuclei	One	1-8
Karyosome	Large, irregular shape, eccentric	Large, irregular shape, eccentric
Periphral chromatin		Unevenly distributed
Cytoplasm	Coarse and granulated	Coarse and granulated
Cytoplasminclusion	Vacuoles containing bacteria often visible	diffuse glycogen mass in younger cysts ,Thin chromatoid bars with pointed to splintered ends in young cysts

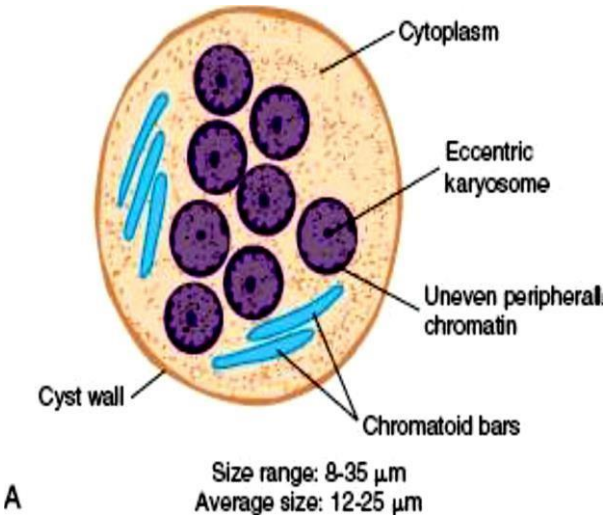
- Laboratory Diagnosis:

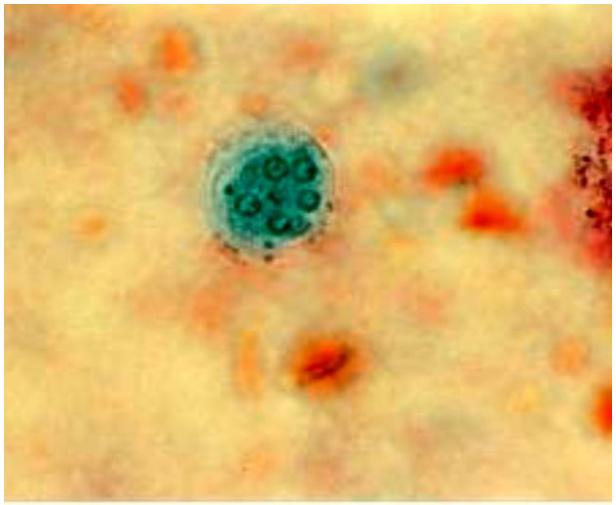
Stool examination is the method of choice for the recovery of *E. coli* **trophozoites and cysts**. Although not considered as being pathogenic, the presence of *E. coli* suggests ingestion of contaminated food or drink.





***Entamoeba coli* Trophozoite stage**





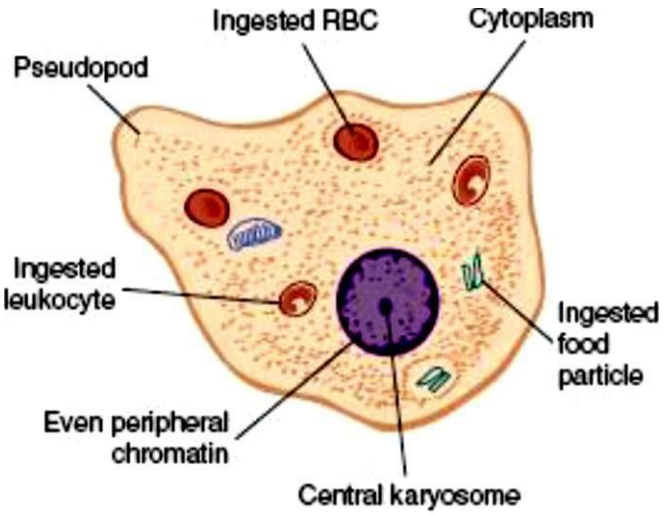
Entamoeba coli Cyst stage

3. *Entamoeba gingivalis:*

- Habitat : Oral cavity , vaginal and cervical areas.
- Disease **:** It is nonpathogenic but **opportunistic**
- Stages **:** Only **Trophozoite (No cyst stage)**
- Infective stage **:** Trophozoite
- Diagnostic stage Trophozoite
- Reproduction **:** Asexually reproduction by Binary fission

Morphology:

Characteries	Trophozoite
Size range	8-20 µm
Motility	Active, varying pseudopod appearance
Number of nuclei	One
Karyosome	Centrally located
Peripheral chromatin	Fine and evenly distributed
Cytoplasm	Finely granular



Size range: 8-20 µm

- Laboratory Diagnosis:
- An accurate diagnosis of *E. gingivalis* trophozoites may best be made by examining **mouth scrapings** particularly from the **gingival area**.
- Material from the **tonsillar crypts** and **pulmonary abscess**, as well as **sputum**, may also be examined.

- **Vaginal and cervical material** may be examined to diagnose *E. gingivalis* in the vaginal and cervical areas.

Lab: 3

- **Ciliates:** are a group of protozoans characterized by the presence of hair-like organelles on the surface of their membranes called **cilia** that are used for **locomotion**.

Kingdom : Animalia
Phylum : Protozoa
Subphylum : Ciliophora
Class : Ciliates
Genus : ***Balantidium coli***

- ***Balantidium coli*** **(Intestinal species):**

Balantidium coli considered as the **large protozoan parasite** known to humans. It is causes the disease **Balantidiasis** . It is the only member of the ciliate phylum known to be pathogenic parasitize to humans.

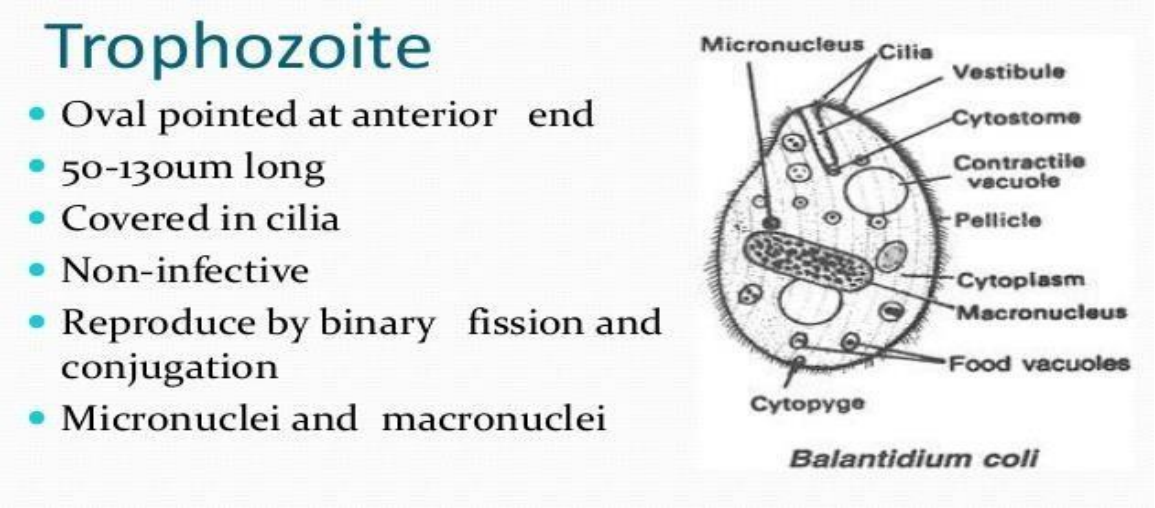
- **Habitat** : large intestine (cecum).
- **Life cycle** : Direct.
- **Disease :** Zoonotic intestinal disease Balantidiasis
- **Stages**: Trophozoite, cyst .
- **Definitive host**: human, **swine,** horses, ...
- **Locomotion**: cilia
- **Infective stage** : cyst
- **Diagnostic stage** : Both cyst and trophozoite
- **mechanism of transmission**: by contaminated water (most important) and food.
- The life cycle of this parasite consist of **two stages:**

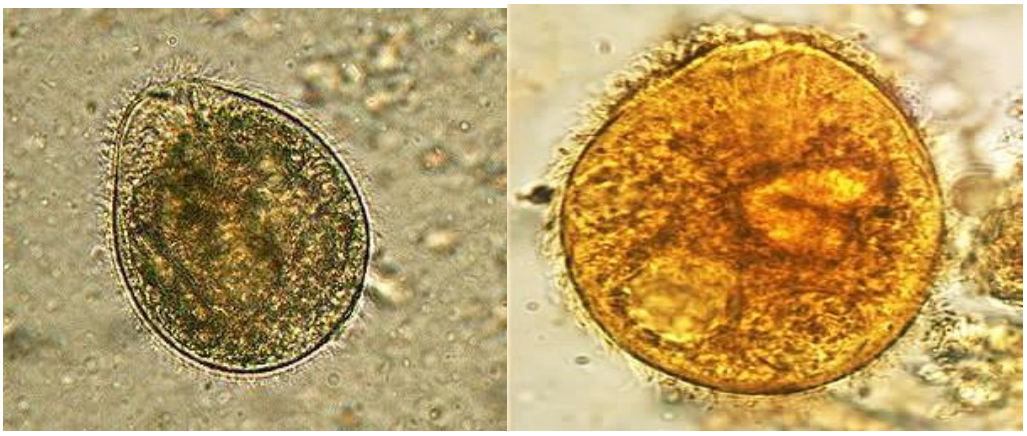
A- **Trophozoite stage**: The typical characteristics of this stage are:
1- **Ovoid to sac-shaped** tapers at the anterior end.
2- Cytoplasm is **granular** and have **two nuclei**:

  • Micronucleus (small spherical-shaped)
  • Macronucleus (kidney-shaped)

3- Having one to two contractile vacuoles in the granular cytoplasm and food vacuoles (ingested microbes " bacteria ").
4- There is **cytosome** in the anterior end (mouth like structure).
5- A large of cilia surrounded the organism and one protective wall surrounded the organism.
6- • The B. coli trophozoite is often referred to as resembling a sac in its shape.

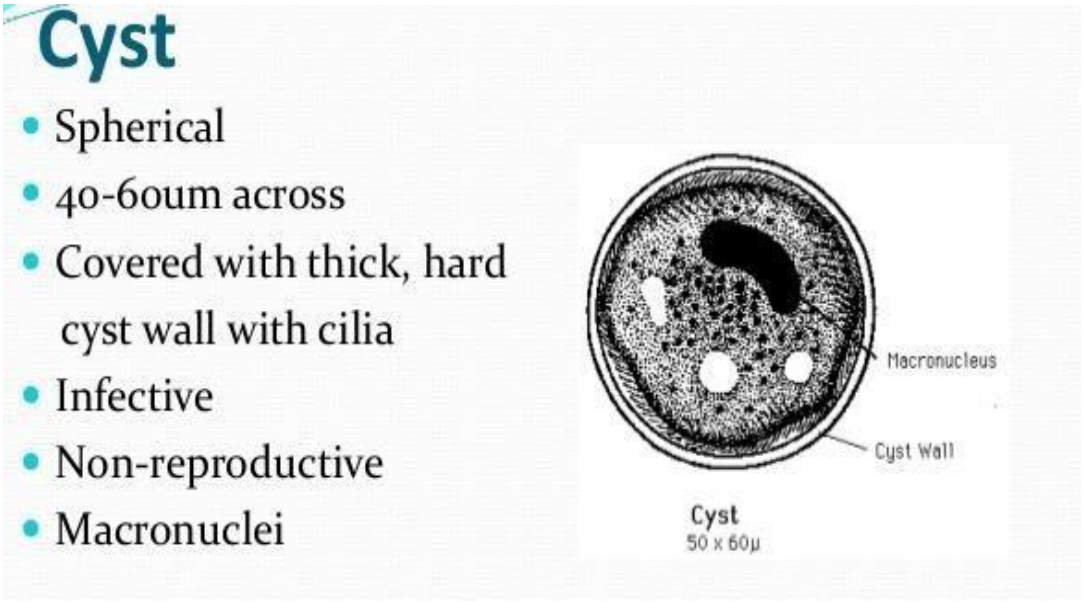
As of this shape, the organism was named *Balantidium*, which means " **little bag** ".

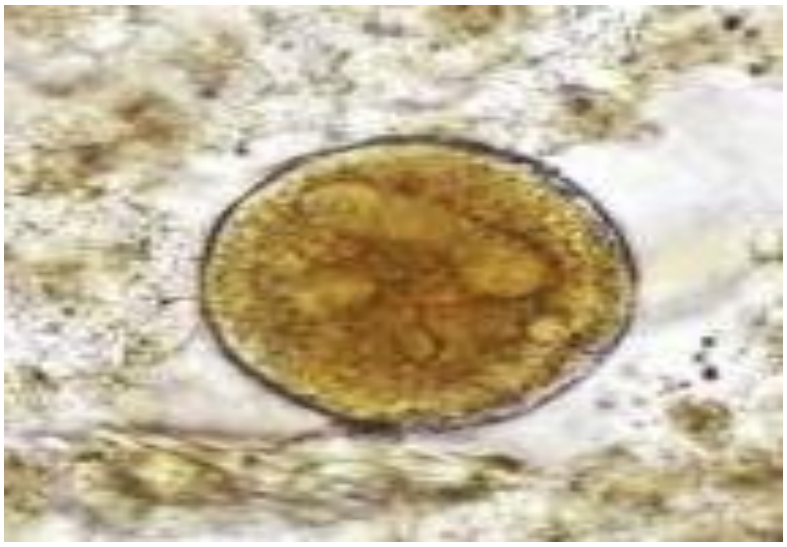




B- **Cyst stage** : The typical characteristics of this stage are :

1- Sub-spherical to oval-shaped .
2- Cytoplasm is granular and have two nuclei :
   - Micronucleus (small spherical-shaped)
   - Macronucleus (kidney-shaped)
3- Having one to two contractile vacuoles in young cyst but does not have food vacuoles .
4- Doesn't have cytosome .
5- Double – protective cyst wall surrounded the organism and arrow of cilia visible in between the two cyst wall layers of cyst .





Diagnosis:

1- Clinical symptoms

Blantidiasis may be resemble amebic dysentery . Acute infections are characterized by up to **15 liquid stools per day containing pus** , **mucus and blood** . Patients who suffer from chronic infection may develop a tender colon , anemia , chachexia and occasional diarrhea .

2- Laboratory Diagnosis

By examining stool samples for the presence of trophozoites and cyst

Lab: 3

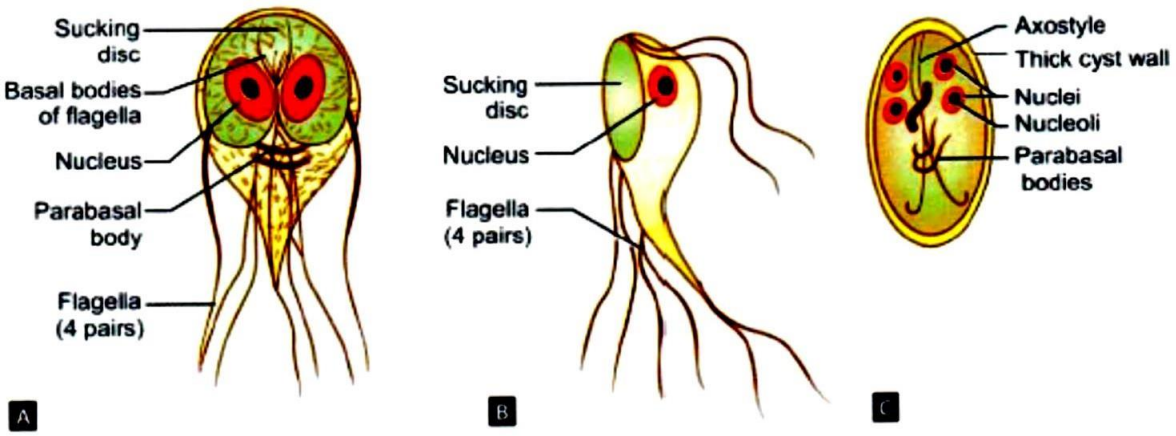
Kingdom : Protista
Phylum : Sarcomastigophora
Class : Zoomastigophora
Geneus : 1. *Giardia intestinalis* (*Giardia lamblia*)
: 2. *Chilomastix mesnili*
: 3. *Trichomonas spp.*

1. ***Giardia lamblia:***

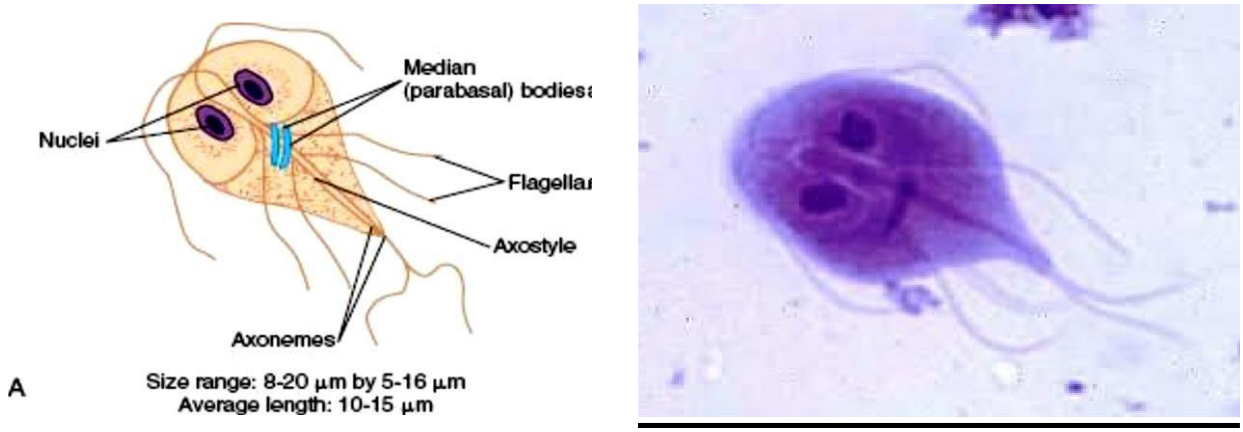
- **Habitat** : Small intestine (duodenum and jejunum)
- **Disease** : **Giardiasis**
- **Stages** : Trophozoite, cyst**.**
- **Infective stage** : Cyst
- **Diagnostic stage** : Motile trophozoite , cyst stage
- **Mode of transmission**: by ingestion of **cysts** in contaminated water and food

Morphology:

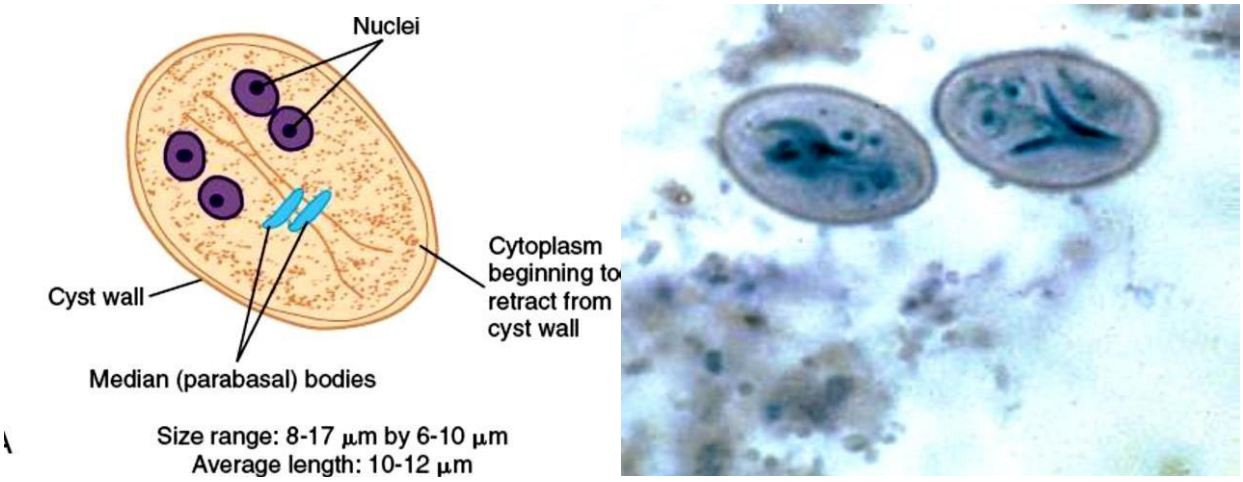
Characteries	**Trophozoite**	**Cyst**
Size range	8-20 µm long , 5-16 µm wide	8-17 µm long , 6-10 µm wide
Shape	Pear-shaped, teardrop Bilaterally symmetrical	Ovoid
Motility	Falling leaf	Non- motility
Number of nuclei	Two ovoid-shaped,	Immature cyst, two Mature cyst, four
Karyosome	large karyosome	Central karyosomes
Periphral chromatin	Absent	Absent
Flagella	Four pairs, origination of each: One pair, anterior end	Absent
Other structures	One pair, posterior end ,Two pair, central, extending laterally Two median bodies Two axonemes Sucking disk	Median bodies:two in immature cyst or four in fully mature cyst Interior flagellar structures*



(A) Trophozoite. (B) Ventral view; Lateral view: and (C) Quadrinucleate cyst



***Giardia lamblia* trophozoite**



***Giardia lamblia* cyst**

Laboratory Diagnosis:

1-Stool Examination: Giardiasis can be diagnosed by identification of **cysts** of *Giardia lamblia* in the formed **stools** and the **trophozoites and cysts** of the parasite in **diarrheal stools** by **saline** and **iodine** wet preparations..

• On macroscopic examination, fecal specimens containing G. *lamblia* may have an **offensive odor, are pale colored and fatty, and float in water.**
2-Enterotest (String Test) .

3- Examination **of Duodenal contents** by **aspiration and Biopsies from upper small intestine**.

4- **Fecal antigen detection** by enzyme immunoassays(EIA) and enzyme-linked immunosorbent assay(ELISA)

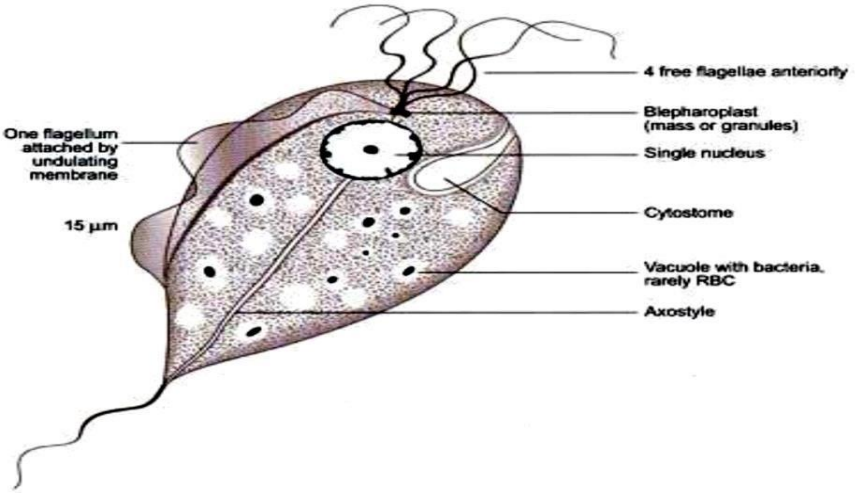
5-Molecular Method: by using **PCR.** Technique.

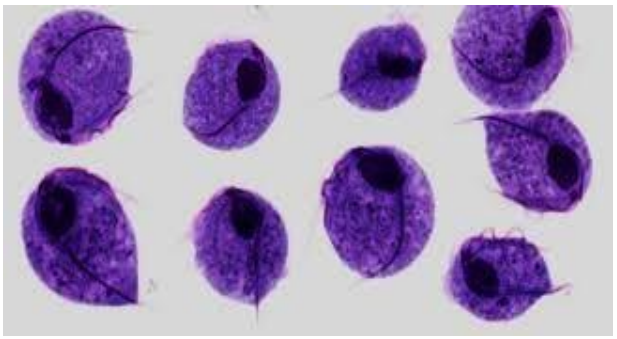
2. Trichomonas vaginalis

- **Disease**: Vaginitis
- **Habitat** : vagina , urethra , prostate
- **Infective stage**: Trophozoit**e**
- **Mode of transmission** : sexual intercourse& contaminated clothes
- **Trophozoit:** pear or oval shape , one nucleus , 4 free anterior flagellates , fifth backward with undulating membrane , Axostyle
- **Definitive host**: Human

- **Diagnosis:**

1- Direct smear in general urine examination
2- vaginal discharge examination.
3- urethra and seminal fluid examination.





Trichomonas vaginalis –Trophozoite

- ***Trichomonas hominis:***
- **Disease**: Non- pathogenic
- **Habitat** : Large intestine may seen in small intestine
- **Infective stage**: Trophozoite
- **Mode of transmission**: ingestion of contaminated food or water
- **Trophozoit:** pear shape , one nucleus , 3-5 flagellates , one flagellum united with the parasite body forming undulating membrane , Axostyle
- **Definitive host**: Human
- **Diagnosis**: Direct smear of stool examination

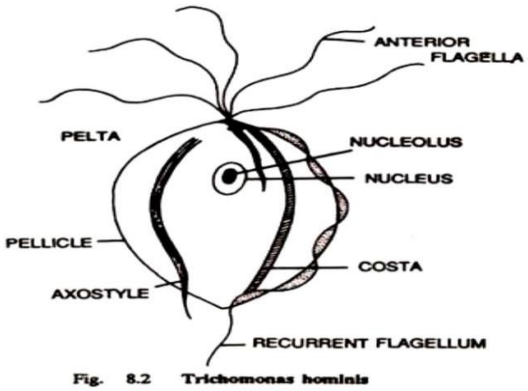


Fig. 8.2 Trichomonas hominis

- ***Trichomonas vaginalis* –Trophozoite**
-

- ***Trichomonas tenax***
- **Disease**: cause disease of gum
- **Habitat** : mouth
- **Infective stage**: Trophozoit
- **Mode of transmission** : contamination of dental equipments
- **Trophozoit:** pear or oval shape , one nucleus , 4 free anterior flagellates , fifth backward with undulating membrane , Axostyle
- **Definitive host**: Human
- **Diagnosis**: Swab from mouth.

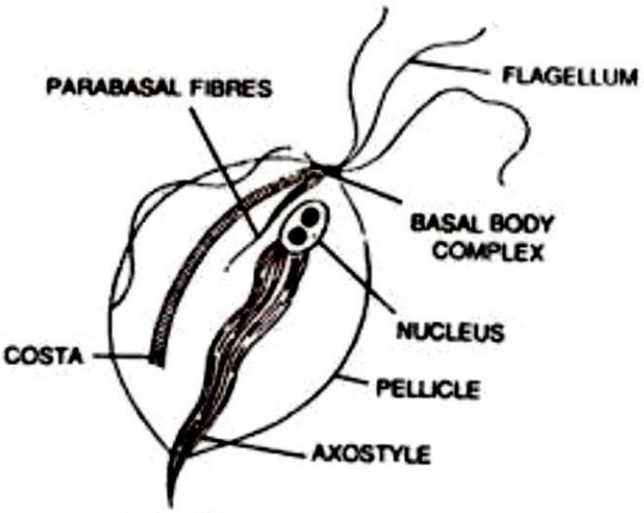
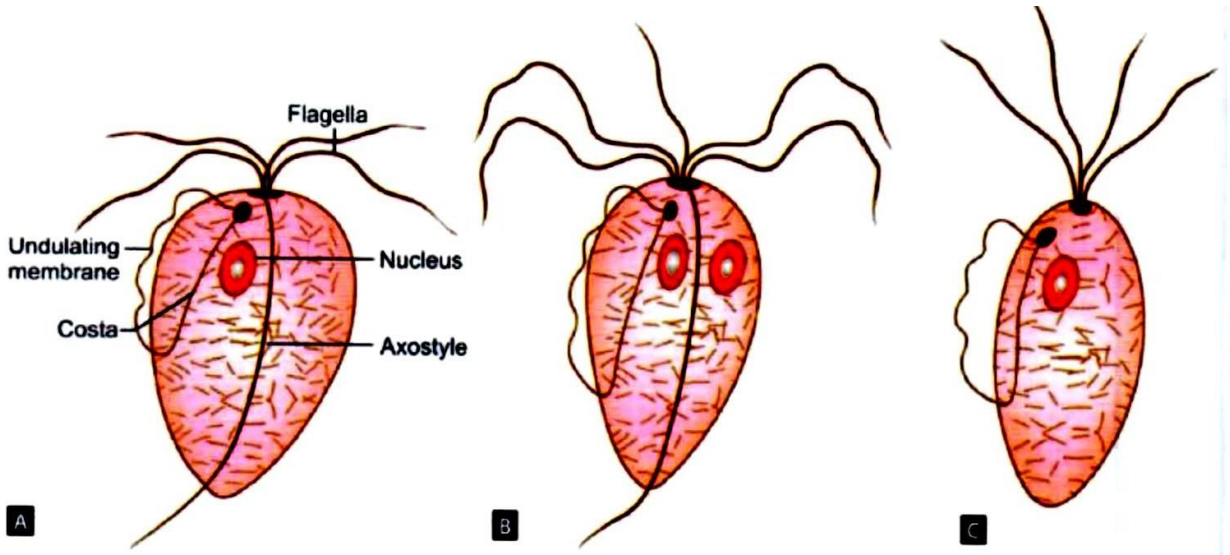


Fig. 8.3 Trichomonas tenax



Trichomonas* species. (A) *T. vaginalis;* (B) *T. hominis;* and (C) *T. tenax

Lab: 4

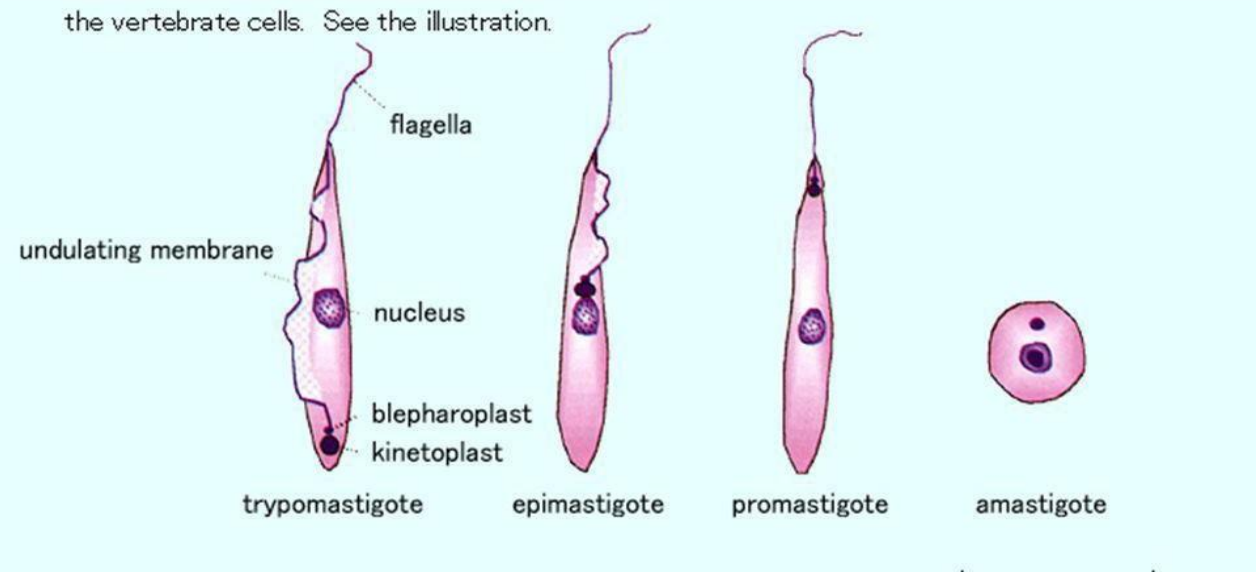
Hemoflagellates (blood and tissue flagellates protozoa)

It is called **Hemoflagellates** because they have a **flagellum** and require **blood medium** to culture them. It is included ***Trypanosoma spp.*** *and* ***Leishmania spp***.

Life cycle**: Indirect life cycle** need two hosts:
Definitive Host - Humans and their domestics .
Intermediate Host - Insect vectors .

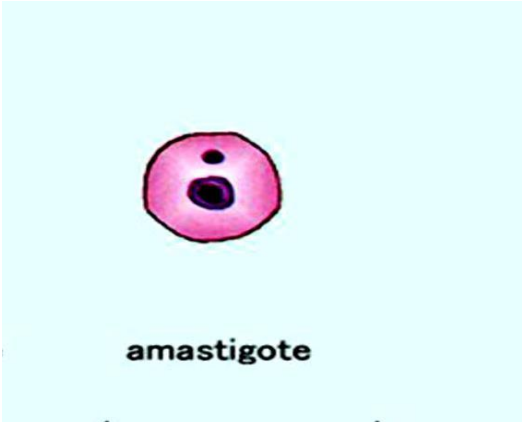
***Morphological forms of hemoflagellates:**
1-Amastigote Form
2-Promastigote Form
3-Epimasigote Form
4-Trypomastigote Form.

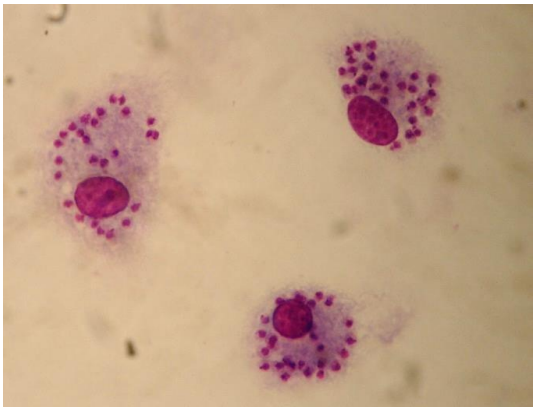
Different stages of Haemoflagellates



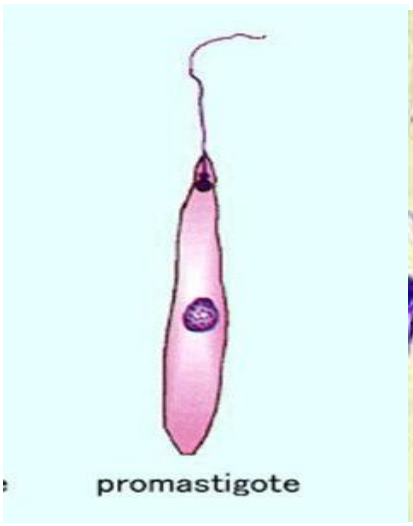
Amastigote (Leishmania) form:

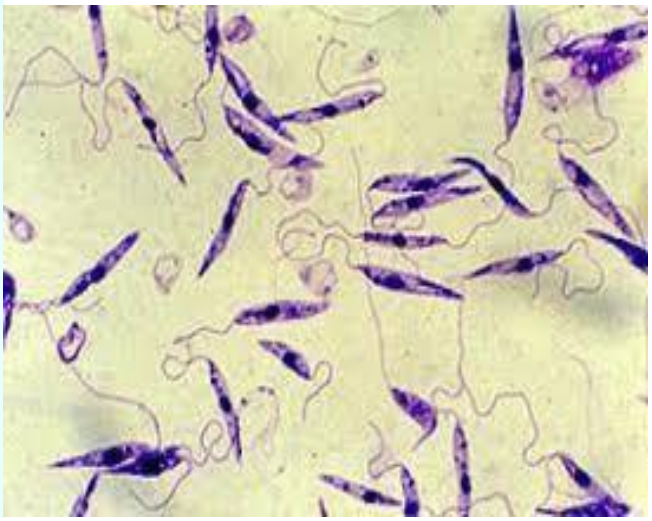
Round or oval in shape, 2-5 microns in diameter, no free flagellum, No undulating membrane, The only intracellular forms of all Leishmania species and *Trypanosome cruzi*



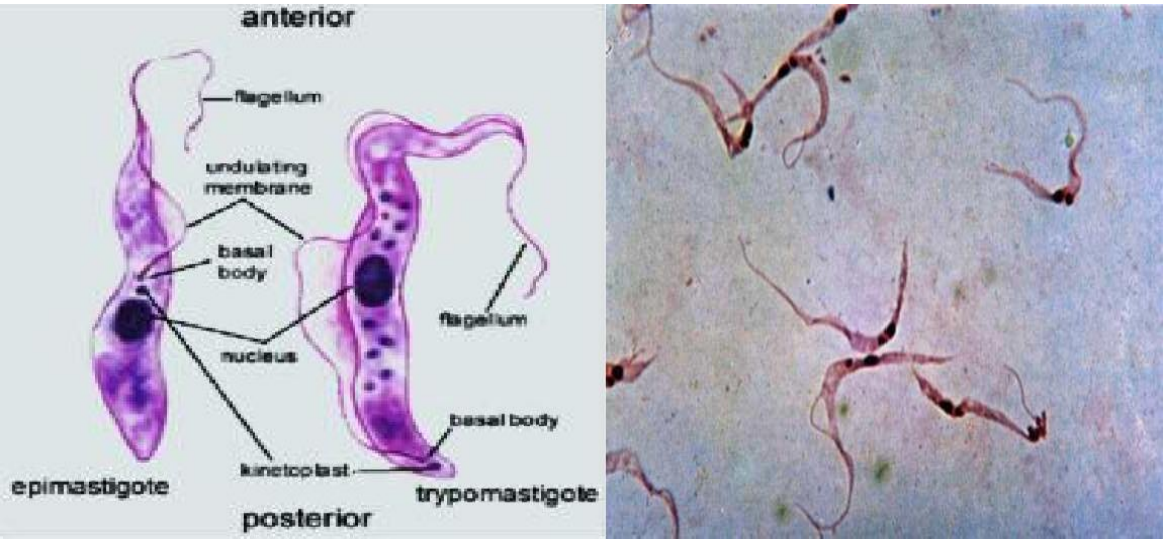


1-Promastigote (leptomonad) form:
Elongated (spindle in shape), have centrally located nucleus and the kinetoplast situated at the anterior end. From blepharoplast, **single free flagellum** projects from the anterior end, **equal or longer** than the body length. This form has **no undulating membrane.**



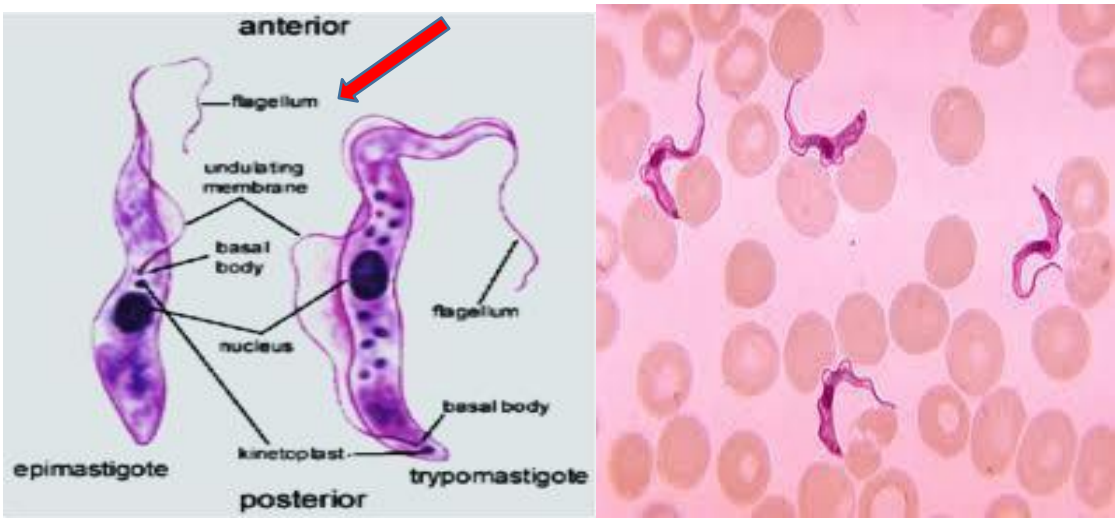


2-Epimastigote (crithidia) form:
Elongated form, slightly wider than promastigote, single free flagellum, single nucleus , undulating membrane, the kinetoplast is just anterior to the nucleus. It is found in the invertebrate host and in culture media (of Trypanosome species).



3- Trypomastigote (Trypanosome) form:

Elongated form with highly polymorphism from rather short and stumpy to a long slender from. In stained blood film, *Trypanosoma cruzi* appears as C or U shape, single free flagellum, single Nucleus , presence of undulating membrane . The kinetoplast and axonemes are found at the posterior end relative to the nucleus. This form is found in the peripheral blood of vertebrates and is the diagnostic stage of *Trypanosoma* species.



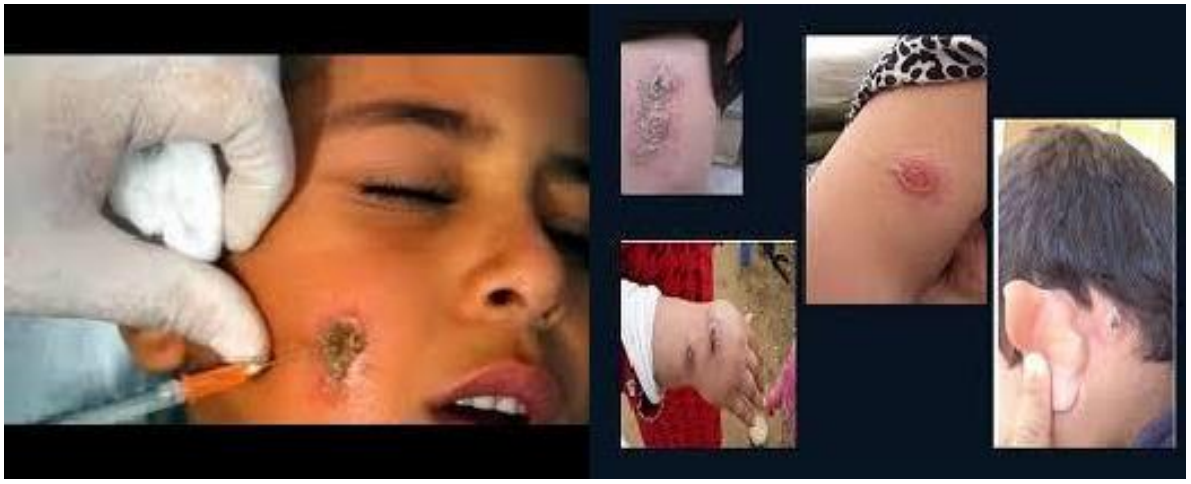
- ***Leishmania spp:***

There are three clinical types of leishmaniasis:
1- Cutaneous
2- Mucocutaneous
3- Visceral

1- Leishmania tropica:

Disease: Cutaneous leishmaniasis , oriental sore , Baghdad boil, Delhi boil
Habitat: skin
Morphology: Amastigote , promastigote
Infective stage: Promastigote
Vector (intermediate host) : Sand fly
Mode of transmission: by biting of sand fly
Diagnosis:

- Smear : Giemsa stain- microscopy examination for (amastigote) from infected tissue samples taken from the edges of lesions or lymph node aspirates.
- Biobsy: microscopy for (promastigote).



2- Leishmania donovani:

Disease: Viseral leishmaniasis , Kala-azar disease.
Habitat: liver , spleen , bone marrow
Morphology: Amastigote , promastigote
Infective stage: Promastigote
Vector: Sand fly
Mode of transmission: by biting of sand fly
Diagnosis: Microscopy , Culture in **NNN** medium or Schneider's Drosophila medium

- Serodiagnosis (ELISA).

3- Leishmania braziliensis:

Disease: Mucocutaneous leishmaniasis , Espundia
Habitat: Nasopharyngeal, mucosa membrane
Morphology: Amastigote , promastigote
Infective stage: Promastigote
Vector: Sand fly
Mode of transmission: by biting of sand fly
Diagnosis: Microscopy (finding the organism in a histological section of the lesion provides definitive diagnosis of Mucocutaneous leishmaniasis.

Lab 5

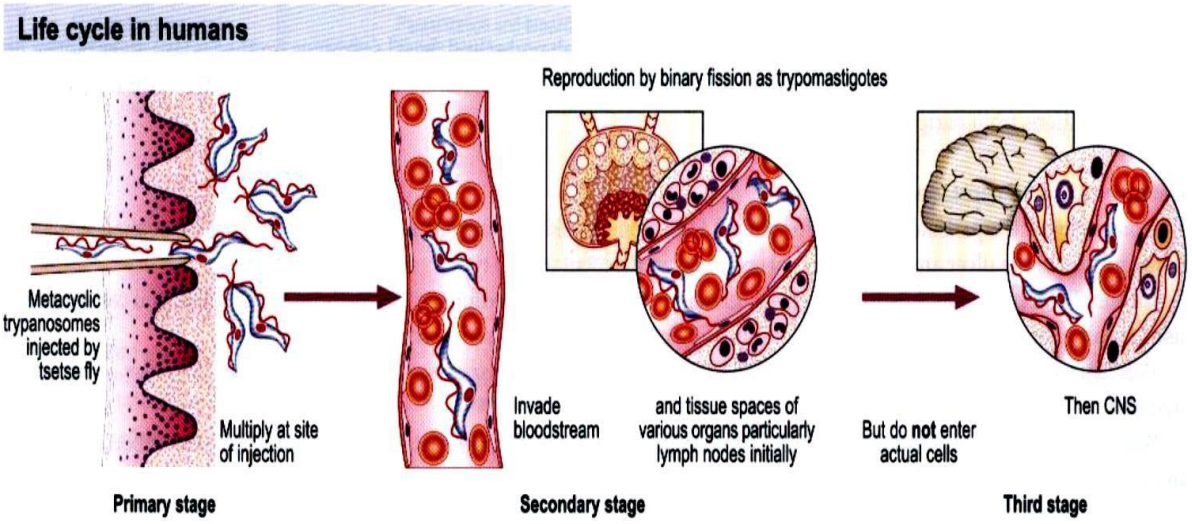
- **Trypanosoma:**

1. **Trypanosoma *bruci*** involves two species:
   - **Trypanosoma gambiense.**
   - **Trypanosoma rhodesiense**

2. **Trypanosoma *cruzi***

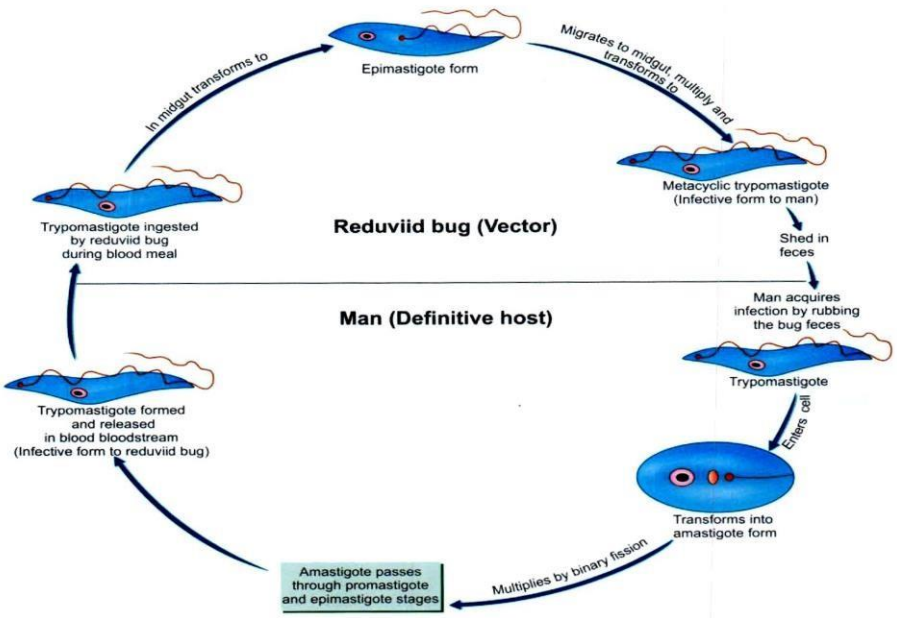
2- Trypanosoma *bruci*

Disease: sleeping sickness African trypanosomiasis
Habitat: liver , spleen , CNS
Morphology: Amastigote , promastigote , epimastigote , Metacyclic trypomastigotes
Infective stage: Metacyclic trypomastigotes
Vector: Tsetse fly
Mode of transmission: by biting of Tsetse fly
Diagnosis: Blood film ,wet mount stained smear of C.S.F., PCR



2- Trypanosoma *cruzi:*

Disease: Chagas disease ,south American trypanosomiasis
Habitat: blood , RBC , heart , muscle
Morphology: Amastigote , promastigote , epimastigote , metacyclic trypomastigotes
Infective stage: metacyclic trypomastigotes
Vector: Reduviid bug(***Triatoma infestans***)
Mode of transmission: During or following the blood meal, the infected bugs discharge the feces containing the infected metacyclic trypomastigotes which is enter the host through the bite wound or intact mucous membranes (conjunctiva, mouth), so this method called contaminative method.
Diagnosis: Blood film, PCR,.



***T. cruzi* causes cutaneous stage (chagoma)**

Ocular lesion (Roman's sign)

Lab 6

Sporozoa

Phylum :

Apicomplexa.

Class : Coccidia.

Order : Eucoccidia.

Genus :

Plasmodium.

1- Plasmodium vivax

General Properties :

- **Habitat :** Young and immature Red blood corpuscles (R.B.Cs)
- **Appearance of infected RBCs :** Enlarged, distorted
- **Disease :** Benign tertian malaria, vivax malaria.
- **Vector :** Female of Anopheles Mosquito .
- **Human Stages :**
- Ring form , Developing trophozoite Immature schizont , Mature schizont Merozoites , Microgametocyte Macrogametocyte
- **Mosquito Stages :** Zygotes, Ookinete ,Oocyst , Sporozotes**.**
- **Infective stage :** Sporozotes .
- **Diagnostic stage :** all human stage .

- ***Morphology***

Morphologic Form	Typical Characteristics
Ring form	**Delicate cytoplasmic ring measuring one third of RBC diameter , Single chromatin dot Ring surrounds a vacuole ,**
Developing trophozoite	**Irregular ameboid appearance , Ring remnants common , Brown pigment becomes apparent, increases in number and visibility as parasites mature**
Immature schizont	**Multiple chromatin bodies ,Often contains clumps of brown pigment**
Mature schizont	**12 to 24 merozoites occupy most of infected red blood cell , Merozoites surrounded by cytoplasmic material , Brown pigment may be present**
Microgametocyte	**Large pink to purple chromatin mass surrounded by colorless to pale halo Brown pigment common**
Macrogametocyte	**Round to oval cytoplasm , Eccentric chromatin mass , Delicate light-brown pigment may be visible throughout cell**

2.Plasmodium ovale

General Properties :

- **Habitat :** Only young and immature cells .
- **Appearance of infected RBCs :** Oval and enlarged, distorted with ragged cell walls.
- **Disease :** Benign tertian malaria, ovale malaria.
- **Vector :** Female of Anopheles Mosquito .
- **Human Stages :** Ring form

Developing trophozoite Immature

schizont Mature schizont Merozoites

Microgametocyte Macrogametocyte

- **Mosquito Stages :** Zygotes, Ookinete ,Oocyst , Sporozotes**.**
- **Infective stage :** Sporozotes .
- **Diagnostic stage :** all human stage .

- **Morphology**

Morphologic Form	Typical Characteristics
Ring form	**Resembles that of P. vivax Ring larger in size than P. vivax Ring thick and often somewhat ameboid in appearance**
Developing trophozoite	**Ring appearance usually maintained until late in development Ameboid tendencies not as evident as in P. vivax .**
Immature schizont	**Progressive dividing chromatin surrounded by cytoplasmic material—often maintains circular shape early in development**
Mature schizont	**Parasites occupy 75% of RBCs. Rosette arrangement of merozoites (average of eight merozoites typically present)**
Microgametocyte	**Large pink to purple chromatin mass surrounded by colorless to pale halo Brown pigment common smaller in size *P. vivax*,**
Macrogametocyte	**Round to oval cytoplasm , Eccentric chromatin mass , Delicate light-brown pigment may be visible throughout cell smaller in size than *P. vivax*,**

3. Plasmodium malariae

General Properties :

- **Habitat :** Only mature cells
- **Appearance of infected RBCs :** Typical Characteristics (Based on Giemsa Stain)
- **Disease :** Quartan malaria, malarial malaria.
- **Vector :** Female of Anopheles Mosquito.
- **Human Stages :** Ring form
- Developing trophozoite Immature schizont
  Mature schizont Merozoites
  Microgametocyte Macrogametocyte
- **Mosquito Stages :** Zygotes, Ookinete ,Oocyst , Sporozotes**.**
- **Infective stage :** Sporozotes .
- **Diagnostic stage :** all human stage .
- **Morphology**

Morphologic Form	Typical Characteristics
Ring form	**Smaller than P. vivax , Occupies one sixth of the RBC**
Developing trophozoite	**Nonameboid solid cytoplasm that may assume roundish, oval, band, or bar shape Cytoplasm contains coarse dark brown pigment; may mask chromatin material Vacuoles absent in mature stages**
Immature schizont	**Similar to that of P. vivax, only smaller; may contain large and dark peripheral or central granules .**
Mature schizont	**Typically contains 6 to 12 merozoites arranged in rosettes or irregular clusters Central arrangement of brown-green pigment may be visible Infected RBC may not be seen because developing parasites often fill the cell completely.**
Microgametocyte & Macrogametocyte	**Similar to *P. vivax*, only smaller in size; pigment usually darker and coarser Older forms assume an oval shape.**

4- Plasmodium falciparum

- **Mosquito Stages :** Zygotes, Ookinete ,Oocyst , Sporozotes**.**
- **Infective stage :** Sporozotes .
- **Diagnostic stage :** all human stage .

General Properties :

- **Habitat :** May infect cells of all ages
- Appearance of infected RBCs : Normal size, no distortion
- **Disease** : Black water fever, malignant tertian malaria, falciparum malaria.
- Vector : Female of Anopheles Mosquito .
- **Human Stages** : Ring form

  Developing trophozoite , Immature schizont , Mature schizont Merozoites , Microgametocyte , Macrogametocyte

- **Morpholog**

Morphologic Form	Typical Characteristics
Ring form	**Circle configuration (one chromatin dot) or headphone configuration (two chromatin dots)** **Scanty cytoplasm , Small vacuole usually visible** **Multiple rings common , Accolé forms possible**
Developing trophozoite	**Heavy rings common** **Fine pigment granules Mature forms only seen in severe infections**
Immature schizont	**Multiple chromatin bodies surrounded by cytoplasm Only detected in severe infections**
Mature schizont	**Typically consists of 8-36 ,merozoites (average, 24) in cluster arrangement Only detected in severe infections**
Microgametocyte	**Sausage- or crescent-shaped Dispersed central chromatin with nearby black pigment usually visible**
Macrogametocyte	**Sausage- or crescent-shaped Compact chromatin Black pigment surrounding chromatin may be visible**

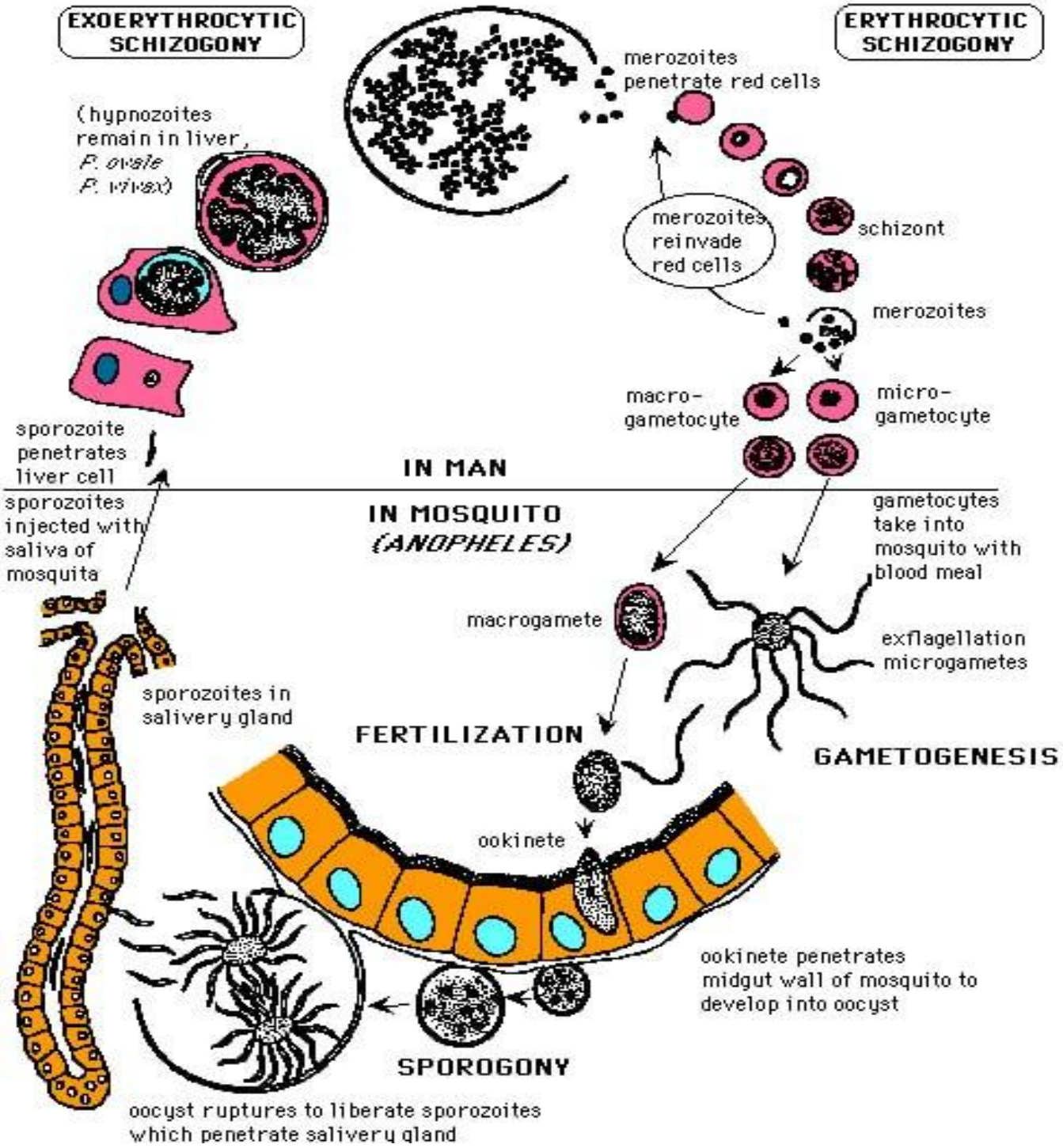
- **Laboratory Diagnosis**

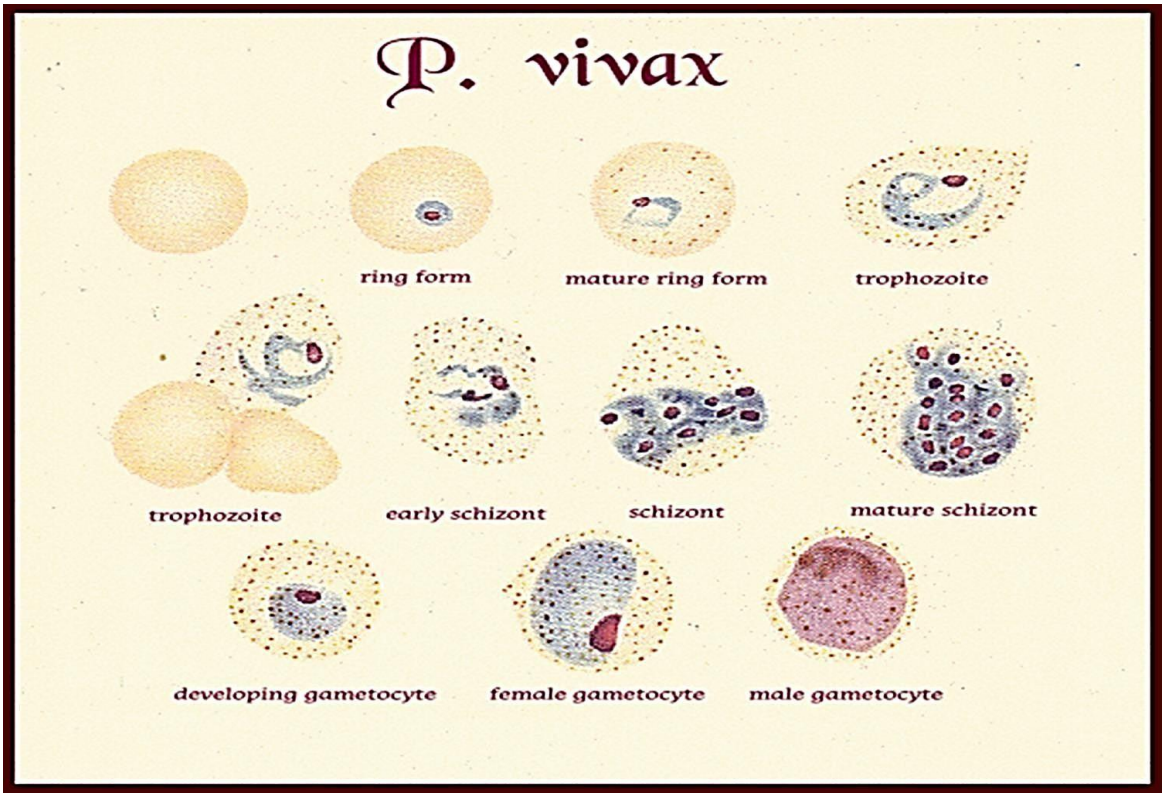
1- **Thick and thin blood films :** All developmental stages of parasites may be seen in blood film stained with **Giemsa s**tain.

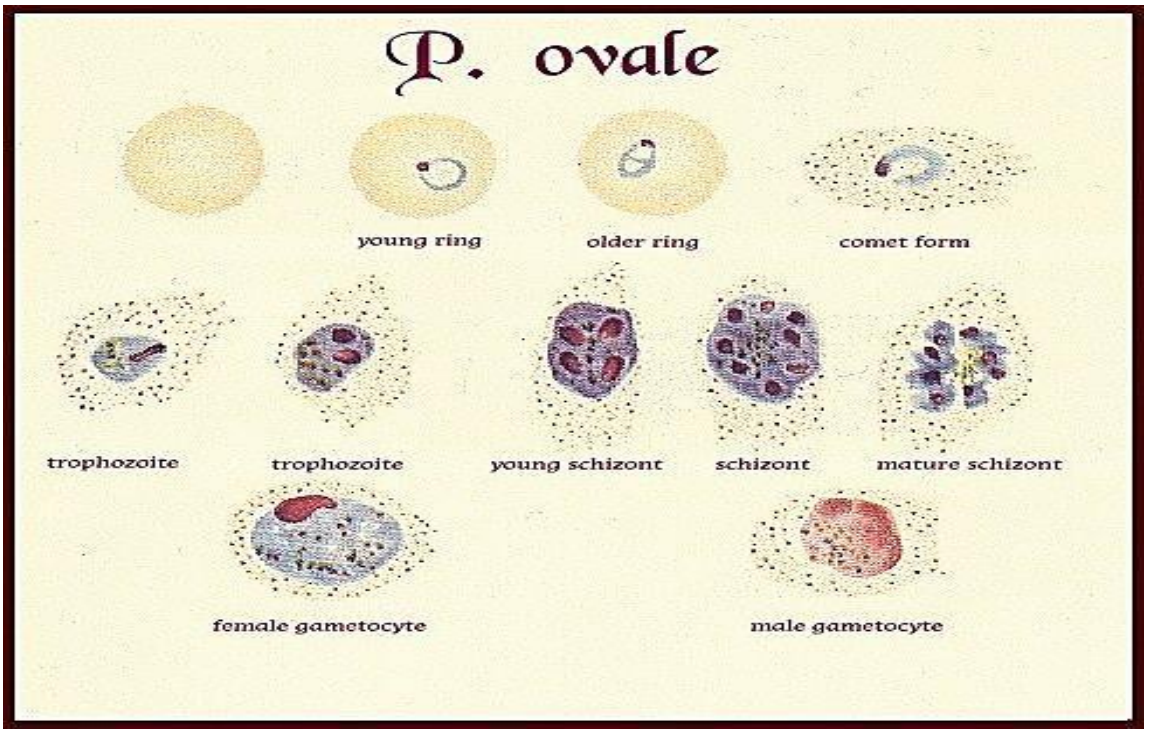
2- **Serological tests** : Among the tests used are :

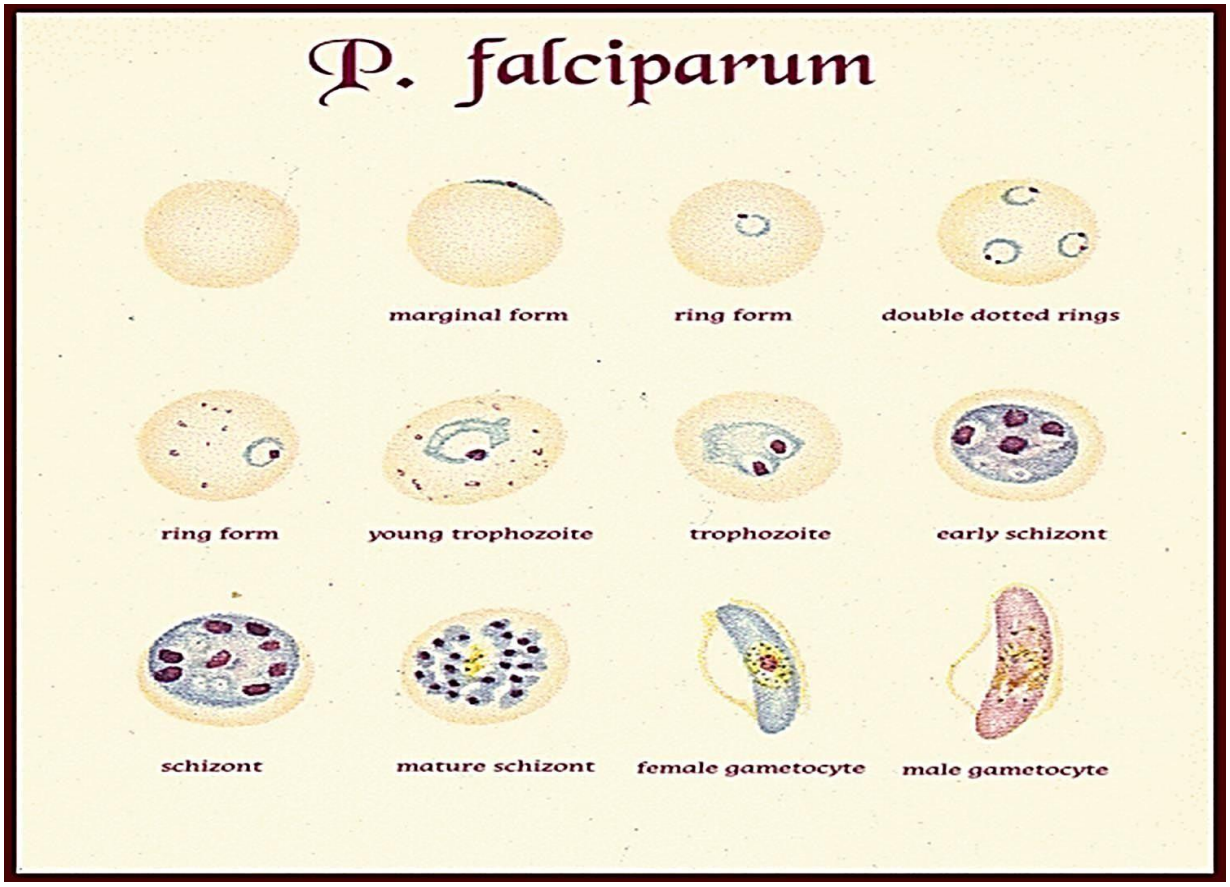
a) Indirect fluorescent antibody test (IFAT),
b) Indirect hemagglutination antibody (IHA) test,
c) Enzyme-linked immunosorbent assay (ELISA).

The life-cycle of *Plasmodium vivax* in man & the mosquito. (after Vickerman and Cox, 1967)




P. vivax
ring form
mature ring form
trophozoite
trophozoite
early schizont
schizont
mature schizont
developing gametocyte
female gametocyte
male gametocyte


P. ovale
young ring
older ring
comet form
trophozoite
trophozoite
young schizont
schizont
mature schizont
female gametocyte
male gametocyte



-Toxoplasma gondii

Disease: Toxoplasmosis.

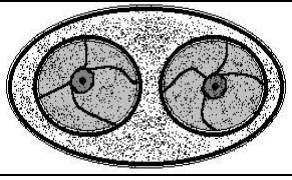
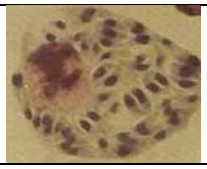
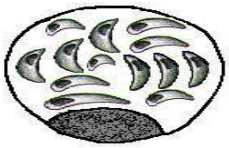
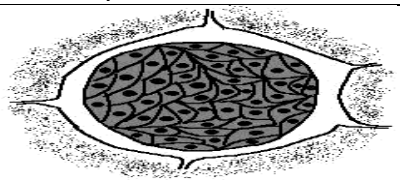
Habitat: Epithelial cells of small intestine or other tissue of the host.

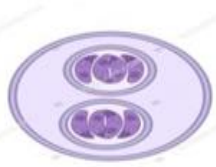
Definitive host :Cats

intermediate host: Human, Mammals, Birds

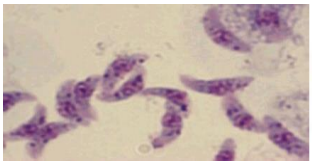
Infective stage : Oocyst, Bradyzoite. **Diagnostic stage :** Tachyzoite , Bradyzoite. **Mode of infection :**

- Consuming undercooked meat of animals that had tissue cysts
- Consuming food or water contaminated with infected cat feces
- Infection of a fetus transplacentally from the mother .
- A less likely method of infection is receiving a blood transfusion or organ transplant from individuals harboring tissue cyst.

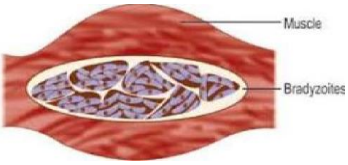
Oocyst	
Size range	25 to 35 µm long , 10 to 15 µm wide
Appearance	Transparent
Shape	Oval
Other features	**Mature oocyst** contains two sporocysts, each containing four sporozoites.
Figure	 
Tachyzoite (Trophozoite)	
General comment	Actively multiplying morphologic form
Size	3 to 7 µm by 2 to 4 µm
Shape	Crescent shaped , often more rounded one end
Number of nucleui	One
Other features	Contains a variety of organelles that are not readily visible
Figure	 
Bradyzoite (Cyst)	
General comment	Slow - growing morphologic form
Size	Smaller than tachyzoites
Physical appearance	Similar to that of the tachyzoites
Number of nucleui	One
Other features	Hundreds to thousands of bradyzoites enclose themselves to form a cyst .
Figure	



Oocyst



Tachyzoite



Bradyzoite

Ministry of Higher Education

&

Scientific Research

University of MOSUL

College of Nursing

Practical Medical Microbiology2

(Part II Helminthes)

Dr. Duaa Mohammed
Lecturer Amera Ali

2nd stage
2024-2025

Helminthes

Helminthes are classified into :

A- **Platyhelminthes Phylum (flat worms)** divided into two classes :

1- Cestodes class

2- Trematodes class

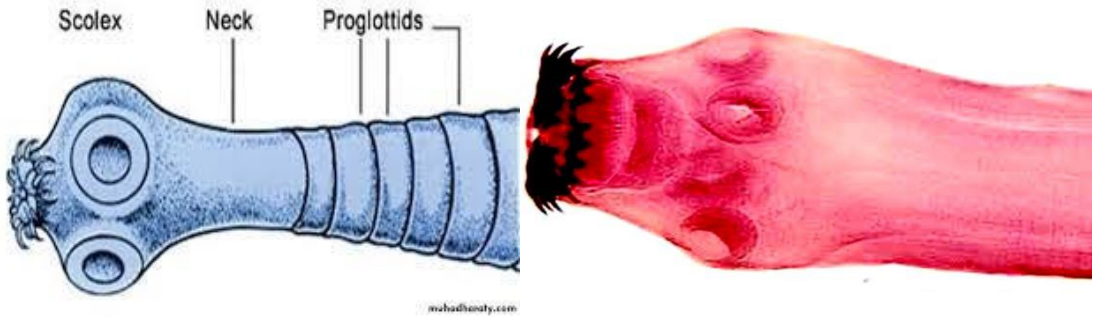
B- **Nemathelminthes Phylum (**Class **Nematoda or round worm)**

➢ Platyhelminthes Phylum (flat worms)

➢ Cestode Morphology:

The body of cestoda called strobilla consist of 3 part :

1. Scolex(head): - All cestoda has scolex but different in shape for each worm and help attachment in host tissues and used in diagnosis.



2. Neck: It is narrow and unsegmented , the segmentation or strobillization ocurrs in neck.
3. Proglottid (segments) – There are three types of segments in strobilla :

- Immature segments: located near from the neck and the sex organs are immature.
- Mature segment: It is containing both male and female organs and sex organs are fully mature.(presence numbers of ovaries and testis)
- Gravid segments: it is look like a sac filled with fertilized eggs. The gravid segment is separated from the strobilla and appear with stool.

Phylum : Platyhelminthes .

Class : Cestodes

Genus : ***1) Taenia***

2) Echinococcus

3) Hymenolepis

1-Taenia:

A. *Taenia saginata*

B. *Taenia Solium*

General Properties *:*

- Common name: **Beef tapeworm (** ***Taenia saginata)*****.**

Pork tapeworm (***Taenia Solium)*** **.**

- Final host **:** man
- Intermediate host: **cow (** ***Taenia saginata*****).**

: pig **(** ***Taenia Solium)***

- Disease : **Taeniasis , beef tapeworm infection.**

Taeniasis(adult), cysticercosis (Larva) .

- Habitat : **Wall of small intestine**
- Body region :
    1. **Scolex (Head): The hold fast organ**
    2. **Neck: Posterior to the scolex .**
    3. **Stobilla(trunk) : made up of proglottid.**
- Stages : **Adult worm , Egg , Larva .**
- Infective stage : *T. saginata* **(Larva:** ***cysticercus bovis*** **)**

T. solium **(Larva:** ***cysticercus cellulosae*****)**

- Diagnostic stages : proglottid or egg
- Reproduction : **Tape worms are Hermaphrodites .**

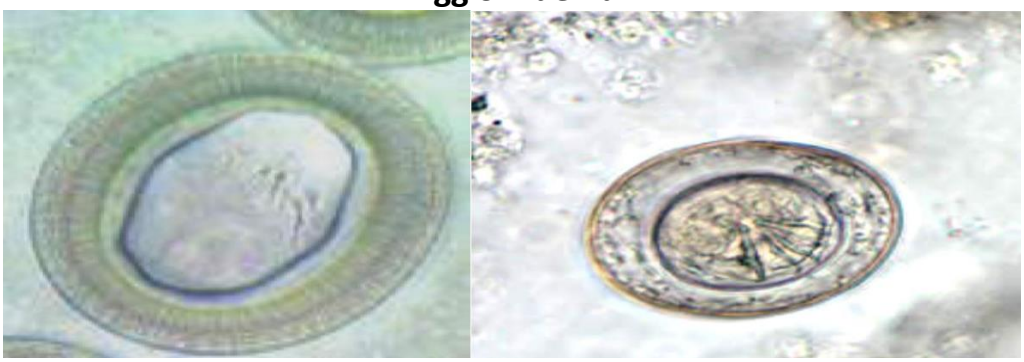
- **Morphology:**

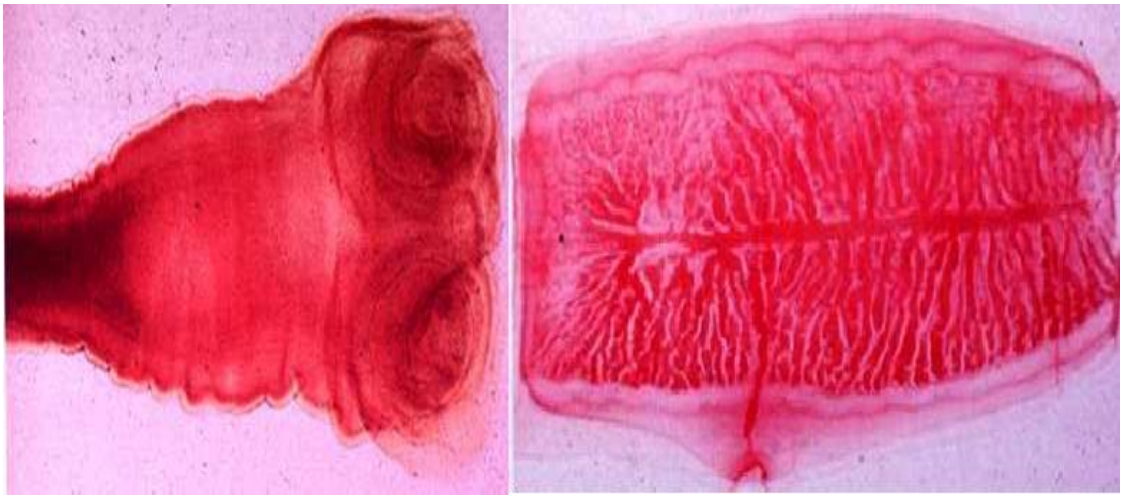
Adult Worm		
Characteries	***Taenia saginata***	***Taenia Solium***
Length	5-10 meters	3 meters
Scolex shape	pyriform scolex has 4 suckers but no rostellum.	globular scolex has rostellum with 2 rows of hooklets.
Uterus branches	15-30 branches	7-15 branches
Proglottids number	1000 -2000 proglottid.	fewer than 1000 proglottid
Eggs number	100.000 egg	30.000-50.000 egg
Infective stage	***Cysticercus bovis***	***Cysticercus cellulosae***
Egg		
Size range	28-40 µm by 18-30 µm	
Hooklets	Three pairs; hexacanth embryo	
Other features	Radial striations on yellow brown in color	

- **Laboratory Diagnosis:**

1. Demonstration of proglottid or egg in faeces.
2. Serodiagnosis: ElISA.
3. Adhesive tape technique.

Egg of *Taenia*

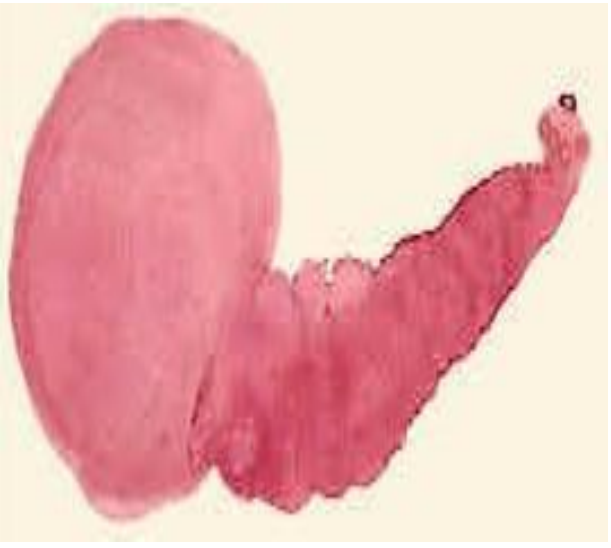




Scolex **proglottid**

Taenia saginata

Taenia solium (Adult) ***Cysticercus***



Echinococcus granulosus

General Properties :

Common name : **Dog tape worm, hydatid tape worm.**

Disease : **Echinococcosis, hyatid cyst, hyatid disease, hyatidosis.**

Habitat : **1. liver and lungs (Man) ⟹ larval form**

1. small intestine (dog and other canines) ⟹ Adult worm

Intermediate host : **Sheep and other herbivores.**

Accidental intermediate host : **Human .**

Definitive host : **Dogs or wild canine .**

Body region :

1. **Scolex (Head): The hold fast organ**
2. **Neck: Posterior to the scolex .**
3. **Stobilla : (short)The main bulk, made up of proglottids.**

Stages : **Adult worm , Egg , Larva (Hydatid Cysts larval stages) .**

Infective stage : **Embronated egg .**

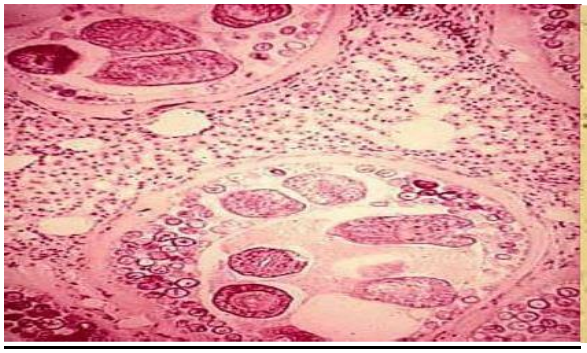
Diagnostic stages : **scolices, daughter cysts, brood capsules, or hydatid sand.**

- **Morphology:**

Adult Worm	
Characteries	*Echinococcus granulosus*
Length , Body shape	up to 6 mm long, ribbon-like
Scolex shape	pyriform in shape rostellum with 28 to 50hooks in 2 rows 4 suckers.
Strobila	Short with neck ,one immature ,one mature and one or two gravid proglottids
Mature segment:	with male and female genital organ, male with 45- 65 testes.
Gravid segment	measures more than half the total length of the whole tape worm with sac like uterus.

Egg	
Shape & Size range	spherical , 31-40 –m in diameter
Other feature	Outer shell surround ---- with radially striated embryophore (inner shell) Hexacanth embryo

Larval stage (Hydatid cyst)	
Protective coverings	Cyst wall; multiple laminated germinal tissue layers
Basic cyst makeup	Fluid-filled bladder
Structures that arise from inner germinal layer	Daughter cysts
	Brood capsules
Other possible structures present	Hydatid sand



Laboratory Diagnosis:

- **Imagery**

Hydatids are found during X-radiography, ultrasonography.

- **Immunodiagnostic techniques**

Generally less sensitive then imagery

- **Microscopy**

Fluid aspirated from hydatid cyst will show many protoscolices

Hymenolepis nana

*** Hymenolepiasis is an infection by adult and larval stage of *H. nana*. It is found worldwide, primarily limited to children in war climate.**

Common name : **Dwarf tape worm .**

Disease : **Hymenolepiasis nana, Dwarf tape worm infection .**

Habitat : **small intestine**

Intermediate host : **fleas, beetles, rats, and house mice.**

Definitive host : **humans and rodents**

Body region :

1. **Scolex (Head): The hold fast organ**
2. **Strobila , 3. Mature proglottids , 4. Gravid proglot .**

Stages : **Adult worm , Egg , Larva .**
Infective stage : **Embronated egg .**
Diagnostic stages : **Embronated egg**
Morphology:

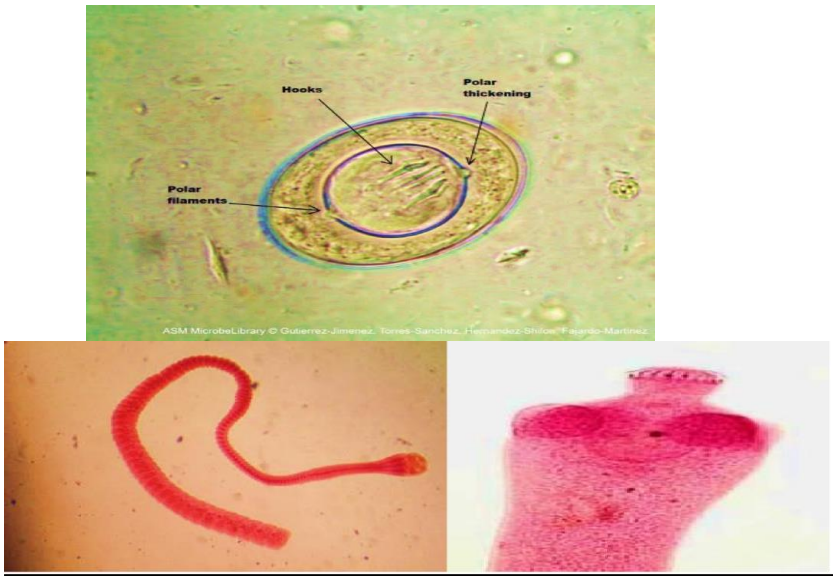
It is a small species, The scolex bears a retractable rostellum armed with a **single** circle of 20 to 30 **hooks**. The scolex also has **four suckers**. The neck is long and slender, and the segments are wider than long.

- The oncosphere is covered with a thin, hyaline, outer membrane and an inner, thick membrane with polar thickenings that **bear several filaments.**
- The neck is long and slender, the region of growth.

The strobila starts with short, narrow proglottids, followed with mature ones.

- **Laboratory Diagnosis:**

Laboratory diagnosis of *H. nana* is accomplished by examining stool samples for the characteristic eggs.



Class: Trematodes

Blood Flukes Schistosomes:

- ***Schistosoma Species:***

  - **General Properties:**

Schistosoma SP	***Schistosoma mansoni***	***Schistosoma japonicum***	***Schistosoma haematobium***
Common name	Manson's blood fluke	Blood fluke	Bladder fluke
Disease	Schistosomiasis mansoni	Schistosomiasis japonicum	urinary schistosomiasis
Habitat	intestine , in intra hepatic portal veins ,vesical venules , pulmonary pulmonary	veins that surround the intestinal tract, as well as in the blood passages of the liver.	vesical venules
Final host	vertebrate	vertebrate	vertebrate
Intermediate host	Snail	Snail	Snail
Stages	Adult worm , Egg , cercaria	Adult worm , Egg , cercaria	Adult worm , Egg , cercaria
Infective stage	cercaria	cercaria	cercaria
Diagnostic stages	Egg	Egg	Egg

Morphology:

Morphology	*S. mansoni*	*S. japonicum*	*S.haematobium*
Male	6.4-9.9 mm in length has 6-9 grape like cluster testes	12-20 mm by 0,5 mm. has 7 testes	10-15 mm in length &1 mm in diameter. has 4-5 testes
Female	7.2-14 mm with	15-30 mm in length &width 0.1-0.3 mm	20mm in length &0.25 mm in width
Egg	large ,rounded at both ends & has lateral spine near one pole .	Round ,has Small; lateral	rounded at one pole & has a terminal spine at the other

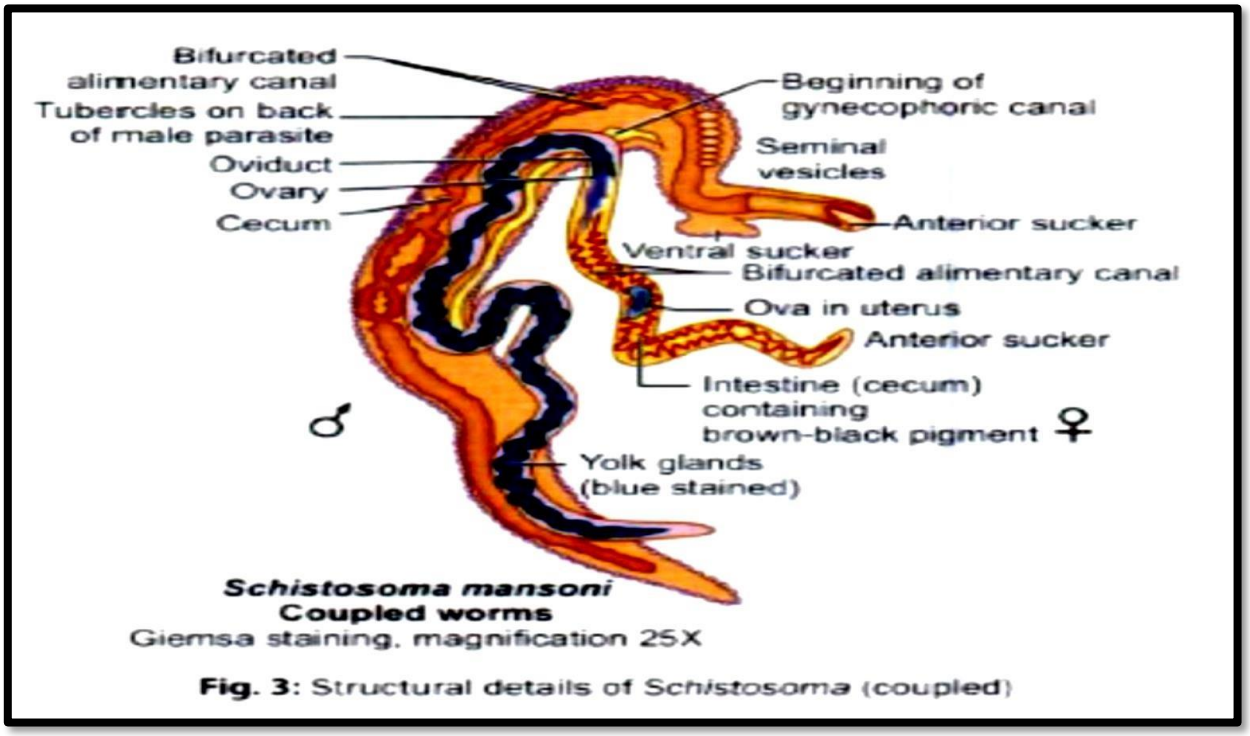
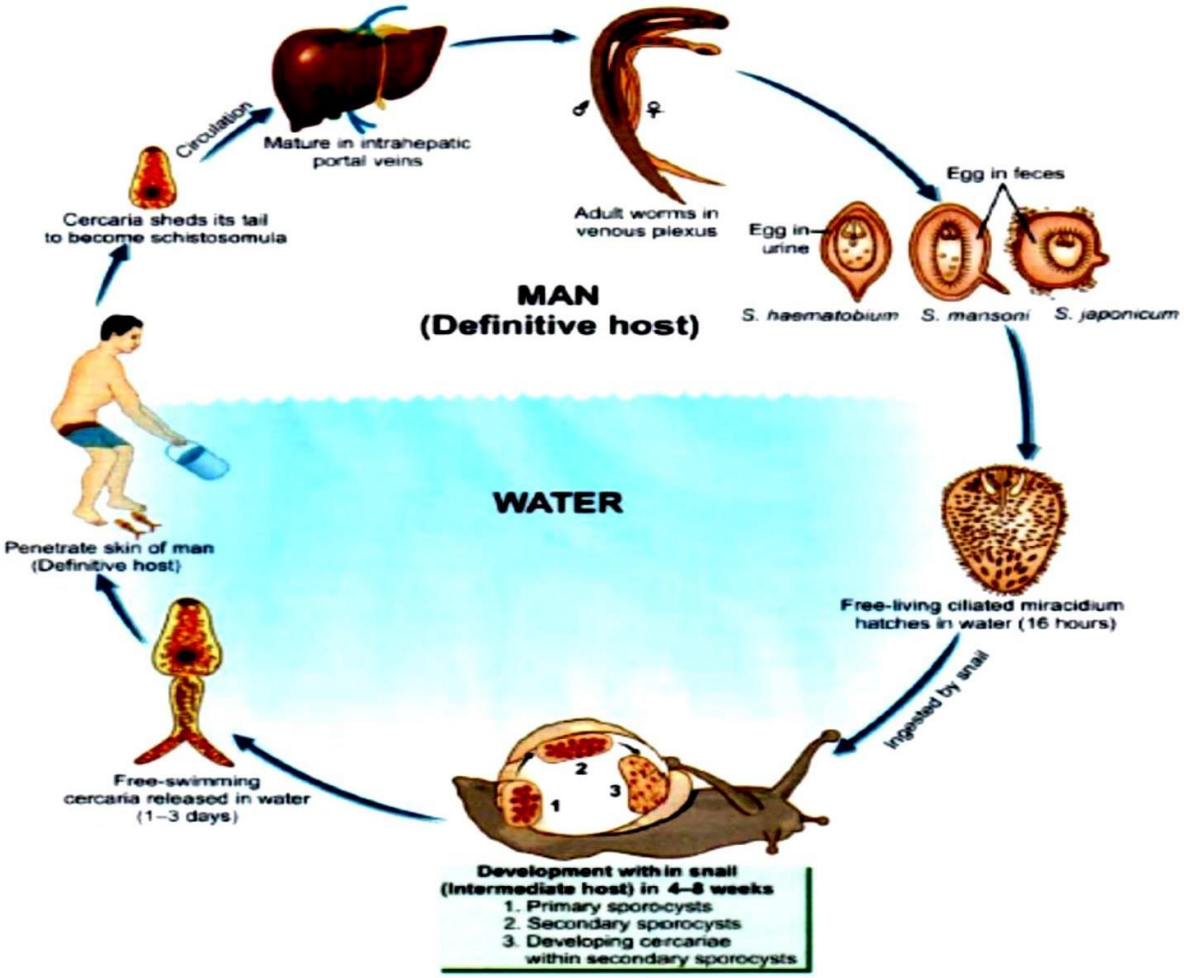
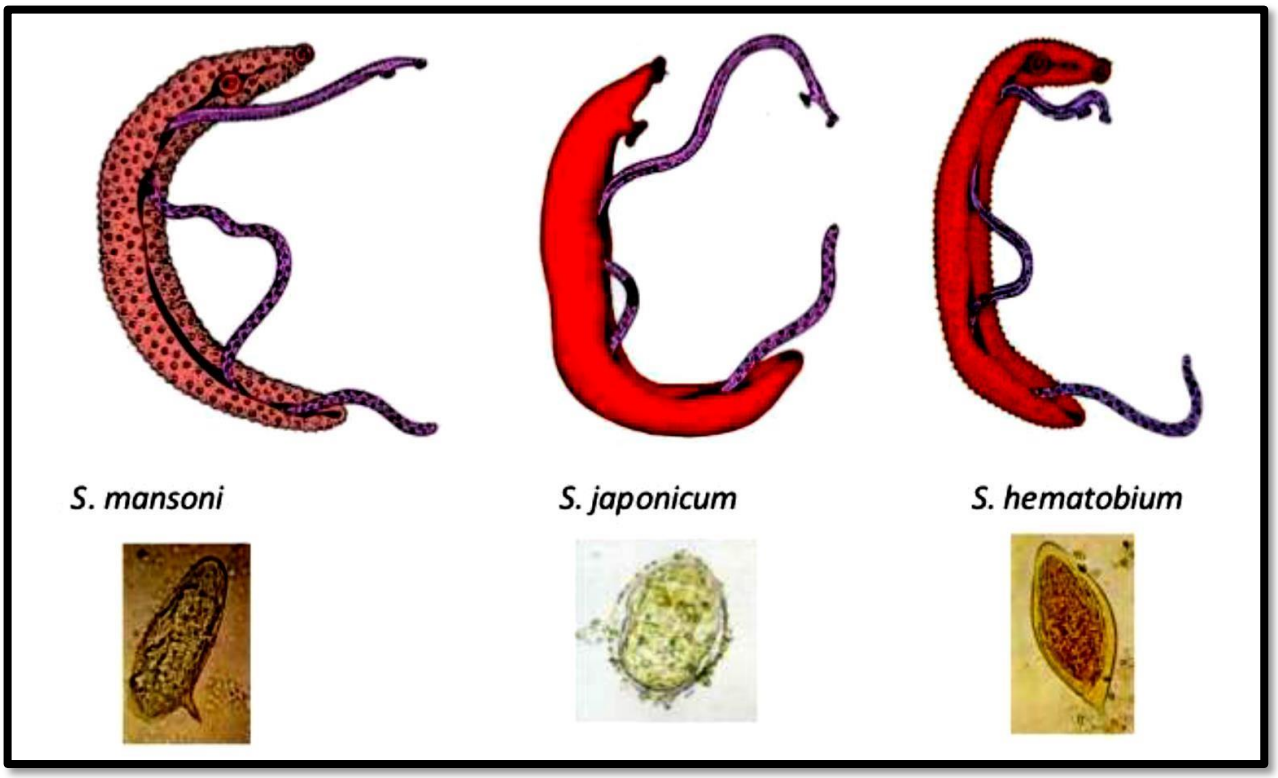


Fig. 3: Structural details of *Schistosoma* (coupled)



Laboratory Diagnosis

- Laboratory diagnosis of ***S. mansoni*** **and** ***S. japonicum*** is accomplished by recovery of the eggs in **stool** or rectal biopsy specimens.
- The specimen of choice for the recovery of ***S. haematobium*** eggs is a concentrated **urine** specimen.
- Immunodiagnostic techniques, including ELISA, , PCR. are also available.


S. mansoni
S. japonicum
S. hematobium


S. mansoni
Stool
S. haematobium
Urine
S. japonicum
Stool

Fasciola hepatica (Sheep liver fluke)*:*

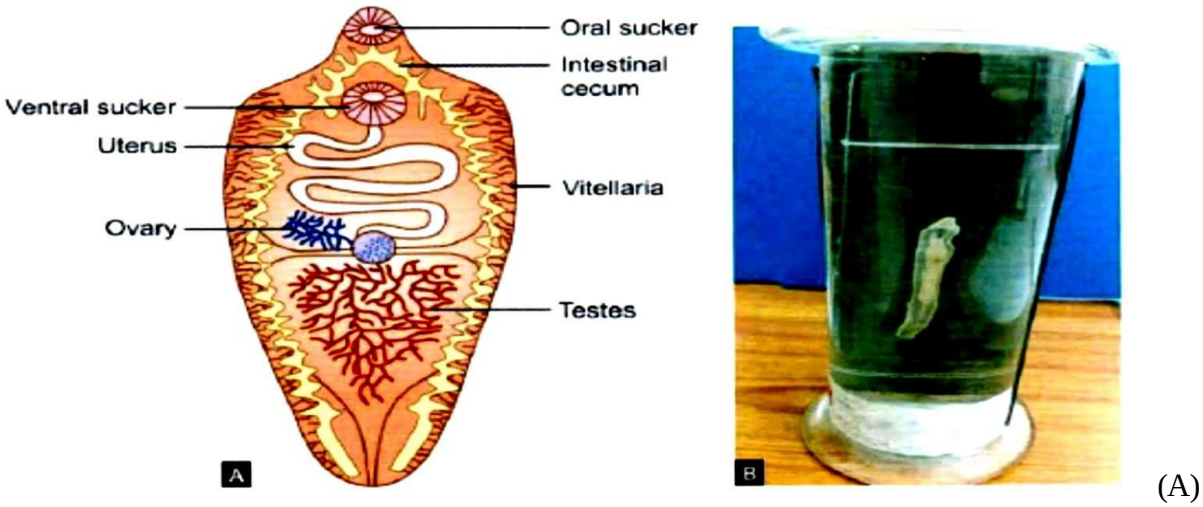
General Properties*:*

- Common name: **sheep liver fluke.**
- Diseases: **fascioliasis, sheep liver rot.**
- Habitat: **Large bile ducts & gall bladder.**
- Final Host: **sheep, goats & occasionally man.**
- Intermediate Host: **Snail.**
- Stages: **Adult worm, Egg, miracidium, sporocyst, cercariae, Metacercariae.**
- Infective stage: **Metacercaria**
- Diagnostic stages: **Embronated egg**

Morphology:

Adult worm It is large in size, flat leaf-shaped fluke measuring 30 mm long and 15 mm broad, gray or brown in color. It has a conical projection anteriorly containing an oral sucker and is rounded posteriorly.

The adult worm lives in the biliary tract of the definitive host for many years about 5 years in sheep and 10 years in humans.



(A) *Fasciola hepatica;* (B) Specimen showing *Fasciola hepatica*

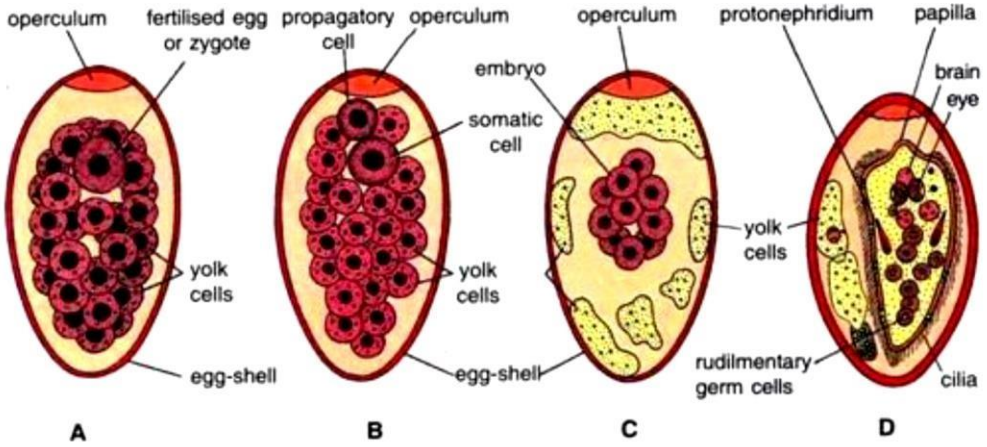


Fig. 41.14. *Fasciola hepatica*. Early stages of development. A—Fertilised egg; B—Two cell stage; C—Many cell stage; D—Miracidium in capsules.

- **Laboratory Diagnosis:**

1-Stool microscopy: Demonstration of eggs in feces or aspirated bile from duodenum is the best method of diagnosis. Eggs of *F. hepatica* and *F. buski* are indistinguishable.
2-Blood picture: It reveals eosinophilia.
3-Serodiagnosis: Serological tests such as immunofluorescence, ELISA, immunoelectrophoresis and complement fixation are helpful in lightly infected individuals for detection of specific antibody. ELISA becomes positive within 2 weeks of infection and is negative after treatment. In chronic fascioliasis, Fasciola coproantigen may be detected in stool.
4-Imaging: Ultrasonography, computed tomography **(CT)** scan, endoscopic retrograde cholangiopancreatography **(ERCP)** and percutaneous cholangiography may be helpful in diagnosis.

Adult of *F. hepatica*

Egg of *F. hepatica*

B- **Nemathelminthes Phylum (**Class **Nematoda or round worm)**

A- Ascaris Iumbricoides (Round worm)

- **General Properties :**
  - Common name : **large intestinal roundworm ,round worm of man.**
  - Diseases : **ascariasis , round worm infection .**
  - Habitat : **large intestine**
  - Stages : **Adult worm , Egg , Larva .**
  - Infective stage : **Embronated egg and larva .**
  - Diagnostic stages : **Embronated egg**
- **Morphology:**

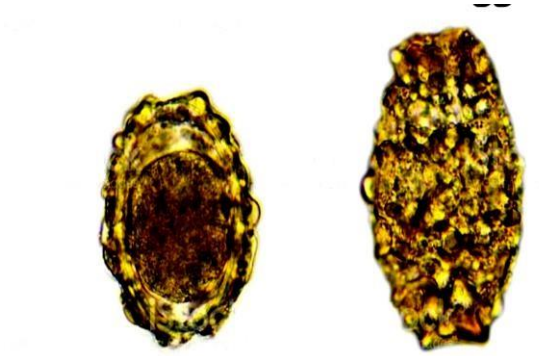
Ascaris iumbricoides Egg		
Parameter	**Unfertilized egg**	**fertilized egg**
Size	85-95um by 38-45um	40-75umby 30-50um
Shape	subsperical	Rounder than nonfertilized version
Embryo	Unembryonated; amorphous mass of protoplasm	Undeveloped unicellular embro
Shell	Thin ,usually corticated	Thick chitin may be corticated or decorticated

Ascaris iumbricoides Adult		
Parameter	**Adult Female**	**Adult Male**
Size	22-35cm	Up to 30 cm
color	Creamy white pink tint	Creamy white pink tint
Posterior end	Pencil lead thickness	Promint incurved tail

- **<u>Laboratory Diagnosis</u>:**

1- General stool examination to see eggs , adult worm may be recovered in several specimen types .

2- Enzyme linked immunosorbent assay (ELISA) is also available .



Fertilised egg Unfertilised egg

B. Entrobius vermicularis (Pin Worm)

- **General Properties :**
  - Common name : **Pinworm , seat worm .**
  - Diseases : **enterobiasis , pinworm infection .**
  - Habitat : **small intestine**
  - Stages : **Adult worm , Egg , Larva .**
  - Infective stage : **Embronated egg .**
  - Diagnostic stages : **Embronated egg**
- **Morphology**

Enterobius vermicularis Egg	
Parameter	Description
Size	48-60 um long , 20-35 um wide
Shape	Oval , one side flattened
Embryo	Stage of development varies ; my be unemdryonated , embryonated ,mature
Shell	Double-layered ,thick ,colorless

Enterobius vermicularis Adult		
Parameter	**Adult Female**	**Adult Male**
Size	7-14 mm	2-4mm
Width	Up to 0.5mm	≤o.3mm
Color	Yellowish -White	Yellowish -White
Tail	Pointed resemble pinhead	Strongly curved and the lateral view of the worm forms an inverted question mark

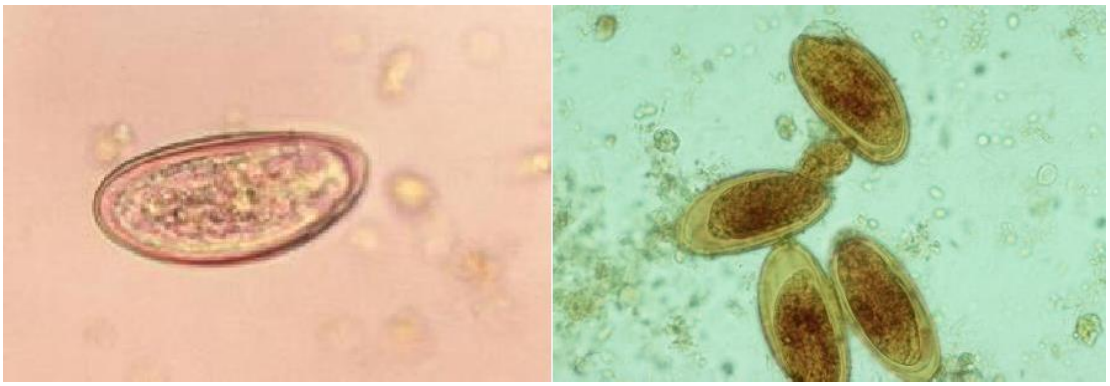
Laboratory Diagnosis:

1) The eggs are recovered from perianal skin by using cellophane tape technique and examined microscopically.

2) The technique preferably done at night or in the early morning before bathing.

Adult Male

Adult Female



Egg

C- *Trichuris trichiura* (The Whipworm)

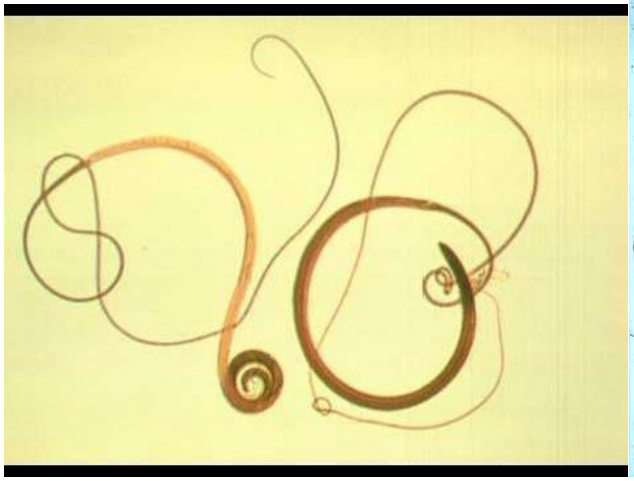
- **General Properties :**
  - Common name : **Whipworm .**
  - Diseases : **Trichuriasis , Whipworm infection .**
  - Habitat : **small intestine**
  - Stages : **Adult worm , Egg , Larva .**
  - Infective stage : **Embronated egg .**
  - Diagnostic stages : **Embronated egg**
- **Morphology**

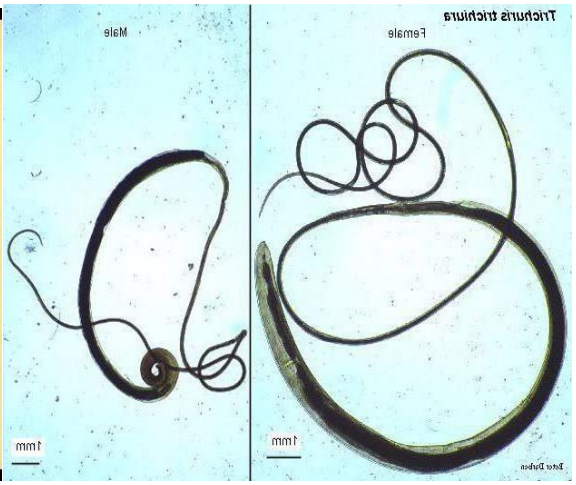
Trichuris trichiura Egg	
Parameter	**Description**
Size	50-55 by 25 um
Shape	Barrel ,football ,hyaline polar plug at each end
Embryo	Unicellular ; undeveloped
Shell	Smooth ; yellow –brown color because of bile contact

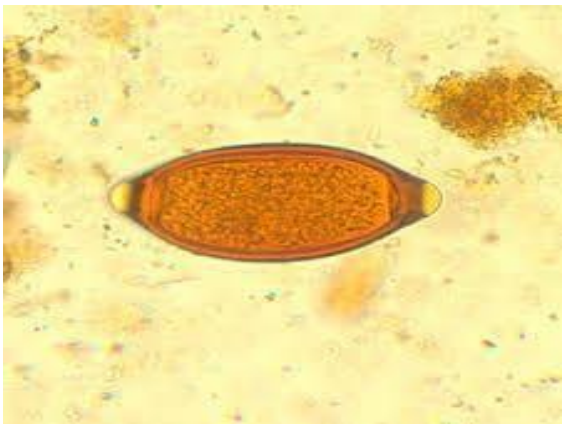
Trichuris trichiura Adult		
Parameter	**Adult Female**	**Adult Male**
Size	2.5 cm long	Males usually smaller than females
Anterior end	Colorless ; resembles whip handle; contains a slender esophagus	Colorless ; resembles whip handle; contains a slender esophagus
Posterior end	Pinkish –gray ; resembles whip itself ; contains digestive and reproductive systems ,club shape end	Possess prominent curled tail

Laboratory Diagnosis:

1. General stool examination to see the characteristic eggs .
2. Diarrheal or dysenteric stools contain eosinophils and charcot –leyden crystals.
3. Adult or immature worm my be seen attached to the prolapsed rectum or at sigmoidoscopy .







D- *Ancylomstoma duodenale*

General Properties :

- Common name : **large intestinal roundworm ,round worm of man .**
- Diseases : **, hook worm infection , *Ancylostomiasis* ,*necatoriasis*.**
- Habitat : **small intestine**
- Stages : **Adult worm , Egg , Larva (rhabditiform larva ,filariform larva).**
- Infective stage : **filariform larva .**
- Diagnostic stages : **Embronated egg.**

- **Morphology:**

Ancylostoma duodenale Egg	
Parameter	**Description**
Size	Length 55-60 um Width 35-40 um
Embryo	Two –four or eight –cell stage
Shell	Smooth ,coloeless

Ancylostoma duodenale Adult		
Parameter	**Adult Female**	**Adult Male**
Size	9-12mm long 0.25 -0.5mm wide	5-10mm long 0.2-0.4 mm wide
Posterior end	Tapered at the posterior end	Prominent posterior copula tory bursa
mouth	Contains actual teeth	

Ancylostoma duodenale larva		
Parameter	**Rhabditiform larva**	**Filariform larva**
Size	Newly hatched 270 by 15 um 5day old , 540-700 um long	Short ,more slender
Other feature	Long buccal cavity ; small genetal primordium	It has closed mouth ,elongated esophagus and pointed tail

- **<u>Laboratory Diagnosis :</u>**

General stool examination for eggs demonstration, larvae may mature and hatch from the eggs