Acanthamoeba Species

A. culbertsoni is the species most often responsible for human infection but other species like **A. polyphagia**, **A. castalleni**, and **A. astromyx** have also been reported.

Distribution This is an opportiunistic protozoan pathogen found worldwide in the environment in water and soil. 2

Morphology

Acanthamoeba exists as active **trophozoite** form and a resistant **cystic** form.

The trophozoite is large, 20–50 µm in size and characterized by spine-like pseudopodia (acanthopodia).'

It differs from Naegleria in not having a flagellate stage and in forming cysts in tissues .

Cyst The double-walled cysts are highly resistant.

The cysts are present in all types of environment, all over the world.

Acanthamoeba cysts

Life Cycle

Both **trophozoites and cysts are infective**. Human beings acquire by inhalation of cyst or trophozoite , ingestion of cysts, or through traumatized skin or eyes. After inhalation of aerosol or dust containing trophozoites and cysts, the trophozoites reach the lungs and from there, they invade the central nervous system through the blood stream, producing **granulomatous amoebic encephalitis** (GAE).

Clinical Disease

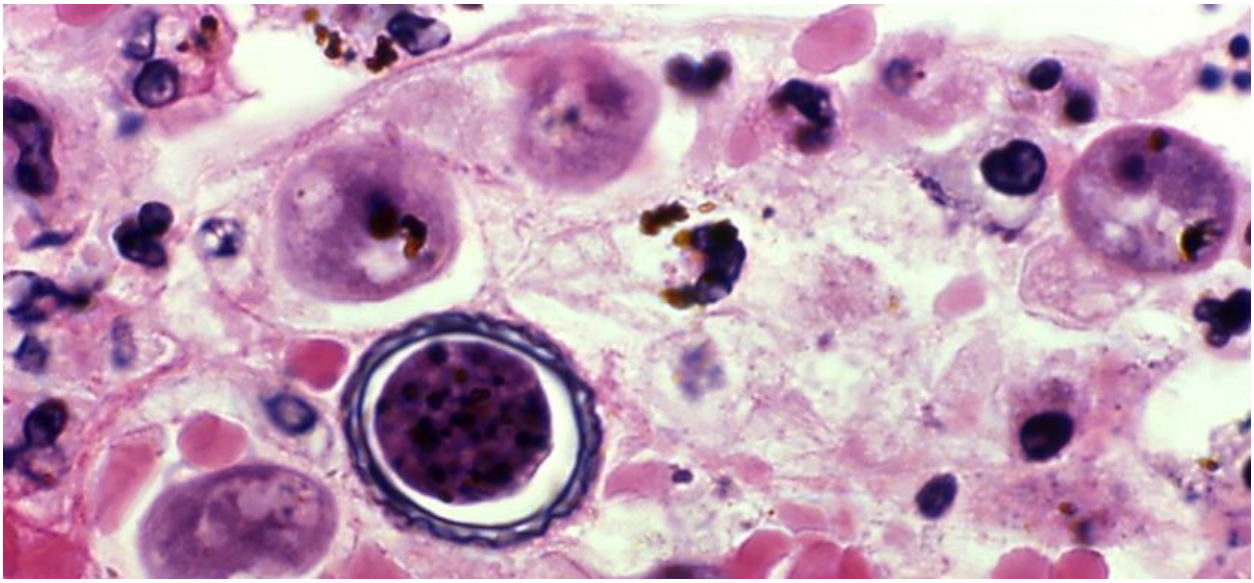
It presents chiefly as 2 chronic conditions—keratitis and encephalitis.

Acanthamoeba keratitis: An infection of the eye that typically occurs in healthy persons and develops from the entry of the amoebic cyst through abrasions on the cornea.

Majority of such cases have been associated with the use of **contact lenses**. **Unilateral photophobia, excessive tearing, redness** and **foreign body sensation are the earliest signs**.

Granulomatous amoebic encephalitis(GAE):

It is a serious infection of the brain and spinal cord that typically occurs in persons with a compromised immune system. GAE is believed to follow inhalation of the dried cysts. Clinical picture is that of intracranial space-occupying lesions with seizures, pareses, and mental deterioration.



Laboratory Diagnosis

Diagnosis of amoebic keratitis is made by demonstration of the **cyst** in corneal scrapings by **wet mount**, **histology** and **culture**. Growth can be obtained from corneal scarpings inoculated on nutrient agar and incubated at 30°C.

Diagnosis of GAE is made by demonstration of trophozoites and cysts in brain biopsy, culture, and immnofluroscence microscopy using monoclonal antibodies. CSF shows lymphocytic pleocytosis, slightly elevated protein levels, and normal or slightly decreased glucose levels. **CT scan** of brain provides inconclusive findings.

Many different assays have been developed for the detection of

antibodies,

- **Indirect hemagglutination (IHA).**
- **Latex agglutination.**
- **Immuno electrophoresis.**
- **Counter immune electrophoresis (CIE).**
- **Amebic gel diffusion test.**
- **Immunodiffusion.**
- **Complement fixation.**
- **Indirect immunofluorescence assay (IFA).**
- **Enzyme-linked immunosorbent assay (ELISA).**

Diphyllobothrium Latum: Common name: Fish tape worm/Broad tape worm

Habitat: The adult worm is found in the small intestine

Life cycle: indirect

Definitive host : man

First intermediate host: Fresh water copepod, mainly of genera Cyclops

Second intermediate host: Fresh water fish

Infective form to Fresh : procercoid in Fresh water copepod

Infective form to human: Third stage plerocercoid larva Fresh

Morphology

Adult worm very long, measuring up to 10 meters or more. It is the largest tape worm inhabiting the small intestine of man.

As in all cestodes, the adult worm has 3 parts: scolex, neck, and strobila.

Scolex (head) is spoon-shaped. It carries 2 slit-like longitudinal sucking grooves (bothria), one dorsal and the other ventral. The scolex lacks suckers and hooks

Neck is thin, unsegmented and is much more longer than the head.

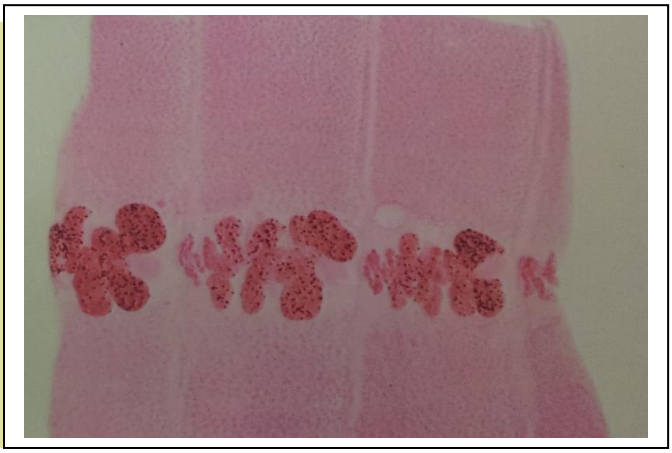
Strobila consists of 3,000–4,000 proglottids, consisting of immature, mature, and gravid segments. The mature proglottid is broader than long, and is practically filled with male and female reproductive organs

The testes are represented by numerous minute follicles situated laterally in the dorsal plane.The female reproductive organs are arranged along the midline, lying ventrally. The ovary is bilobed. The large rosette-like uterus lies in the center.

Three genital openings are present ventrally along the midline—the openings of the vas deferens, vagina, and uterus.

The fertilized ova develop in the uterus and are discharged periodically through the uterine pore.

Scolex Diphyllobothrium Latum



Diphyllobothrium latum proglottid

Egg

A single worm may pass about a million eggs in a day. Egg is broadly ovoid, about 65 µm by 45 µm, with a thick, light brown shell . It has an operculum at one end and often a small knob at the other.The freshly passed egg contains an immature embryo The embryo with 6 hooklets inside the egg is called the oncosphere. The egg does not float in saturated salt solution and is bile stained. They are not infective to humans.

Diphyllobothrium latum Egg

Clinical Features

The pathogenic effects of diphyllobothriasis depend on the mass of the worm, absorption of its byproducts by the host, and deprivation of the host's essential metabolic.

In some persons, infection may be entirely asymptomatic, while in others there may be an evidence of mechanical obstruction.

abdominal discomfort, diarrhea, nausea, weakness, weightloss, and anemia. Patients may be by noticing the strands of proglottids passed in their feces.

A kind of pernicious anemia, sometimes caused by the infection,

The anemia develops because the tape worm absorbs large quantity of vit B 12 and interferes with its ileal absorption, leading to vit B 12 deficiency.

In severe cases, patients may exhibit neurologic sequelae of vit B 12 deficiency.

Laboratory Diagnosis : Stool Microscopy

Eggs are passed in very large number in feces, and therefore, their demonstration in feces offers an easy method of diagnosis. The Proglottids passed in feces can also be identified by their morphology.

Serodiagnosis: coproantigen detection test is available to diagnose diphyllobothriasis.

Dipylidium caninum, also called the flea tapeworm, double-pored tapeworm, or cucumber tapeworm

Final host: children , dogs

Intermediate host : flea or louse will harbor the **infective** cysticercoid

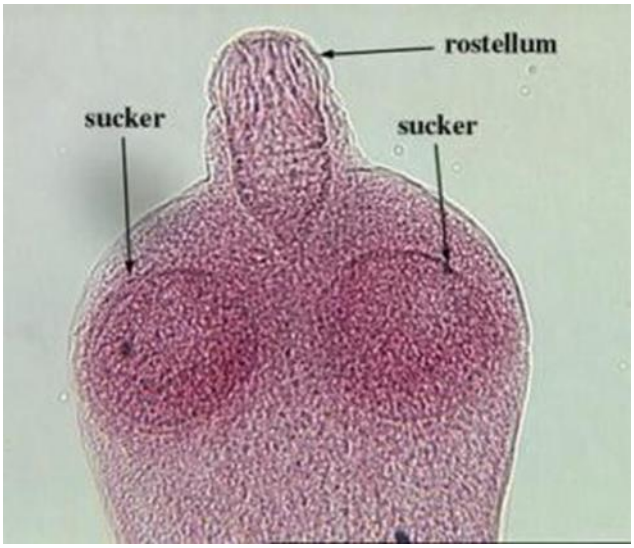
Site of infection: small intestine

Morphology

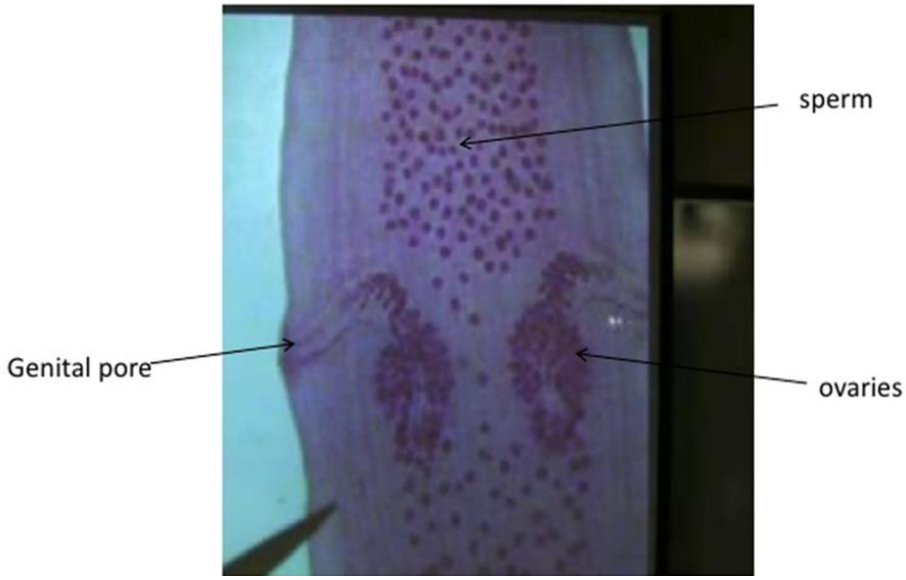
in reference to the shape of its cucumber-seed-like proglottids, though these also resemble grains of rice or sesame seeds), 10-70 cm in length

Scolex:
- conical-shaped and has four suckers. There is a rostellum armed with several rings of small hooks.

-provided with of hooks.



Segments(stroblia) 200segments,diagnostic feature(pumpkin-seed shape).they contain two sets of male and female reproductive organs. They therefore demonstrate two genital pores that lead to their name as the “double-pored and gravid segments :contain egg capsule, The number of eggs can range from 5 to 30



Eggs Dipylidium caninum eggs are round to oval and contain an oncosphere that has 6 hooklets. And present as egg capsule contain 5 to 30 or more eggs . segments Dipylidium caninum segments have two genital pores, one in the middle of each lateral margin. They are pumpkin seed-shaped.

Clinical signs

Most infections with Dipylidium caninum are asymptomatic. may relieve anal pruritis. The most striking feature in animals and children consists of the passage of proglottids. These can be found in the perianal region, in the feces, on diapers, The proglottids are motile when freshly passed and may be mistaken for maggots or fly larvae

Diagnosis

Microscapy .Observe gravid proglottids and Eggs in stool, or near anus White Shaped like cucumber seed\ Usually intact

Protozoa: General Characteristics

Protozoa are single-celled eukaryotic microorganisms belonging to kingdom Protista. The body wall is covered by cell membrane. Its cytoplasm is made up of ectoplasm and endoplasm. The nucleus is usually single but may be double or multiple. Reproduction can be asexual (e.g. binary fission, schizogony, endodyogeny) or sexual (e.g. gametogony). Protozoa can be divided into the following groups:

1. **amoebae** (Has pseudopodia as a mean of locomotion) Amoebae of medical importance:
   - Amoeba in the large intestine: Entamoeba histolytica
   - Free-living amoebae in CNS and eye: Naegleria, Acanthamoeba
2. **Flagellates** (Has flagella as organ of locomotion) Flagellates of medical importance:
   - Hemoflagellates: Trypanosoma, Leishmania
   - Gastrointestinal: Giardia lamblia
   - Urogenital: Trichomonas vaginalis
3. Apicomplexa (Has a structure called apical complex which serves as the organ of attachment to host cells. They have an alternating sexual and asexual life cycle) Apicomplexa of medical importance:
   - Blood: Plasmodium, Babesia
   - Tissue: Toxoplasma gondii, Sarcocystis
   - Gastrointestinal: Cryptosporidium, Cystoisospora, Cyclospora
4. Ciliate (Has cilia for locomotion) Ciliate of medical importance:
   - Gastrointestinal: Balantidium coli

Entamoeba histolytica

Direct live cycle

Infective stage . cyst

Site of infection . colon and liver

infecting humans and other primates Mammals such as dogs and cats can become infected transiently

Morphology

***Entamoeba histolytica* occurs in 3 forms.**

1. Trophozoite

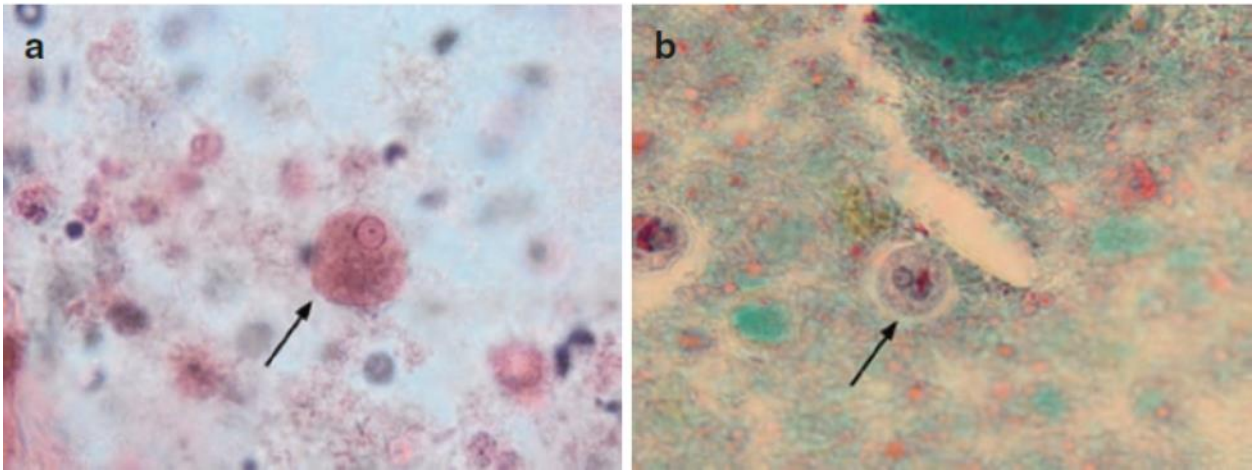
2. Precyst

3. Cyst

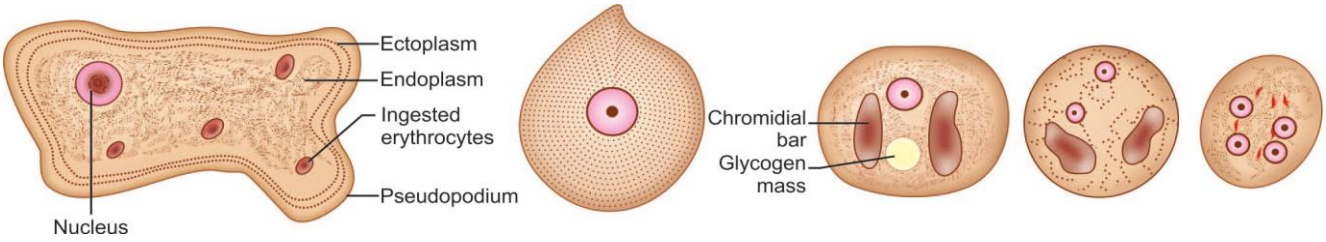
Trophozoite is the vegetative form of the parasite and the only form present in tissues. It is irregular in shape and varies in size from. Contains nucleus, food vacuoles and phagocytosed erythrocytes. **Pseudopodia** are finger-like projections formed by movements of ectoplasm in one direction. Its nucleus is spherical. reproduces by binary fission.

Precystic stage the trophozoites undergo encystment in the intestinal lumen. Before encystment, the trophozoite extrudes its food vacuoles and rounds up to form a precystic stage. It secretes a cyst wall to become cyst.

The cyst is spherical in shape. **Immature cyst contains a single nucleus**, a glycogen vacuole and chromatoid bars which are cigar shaped with rounded ends. The chromatoid bars are visible in saline. With iron haematoxylin stain, nuclear chromatin and chromatoid bodies appear deep blue or black. When stained with iodine, the glycogen mass appears golden brown while the nuclear chromatin and karyosome bright yellow. **Mature cyst contains 4 nuclei**. The glycogen mass and chromatoid bars disappear in mature cyst. Conditions.



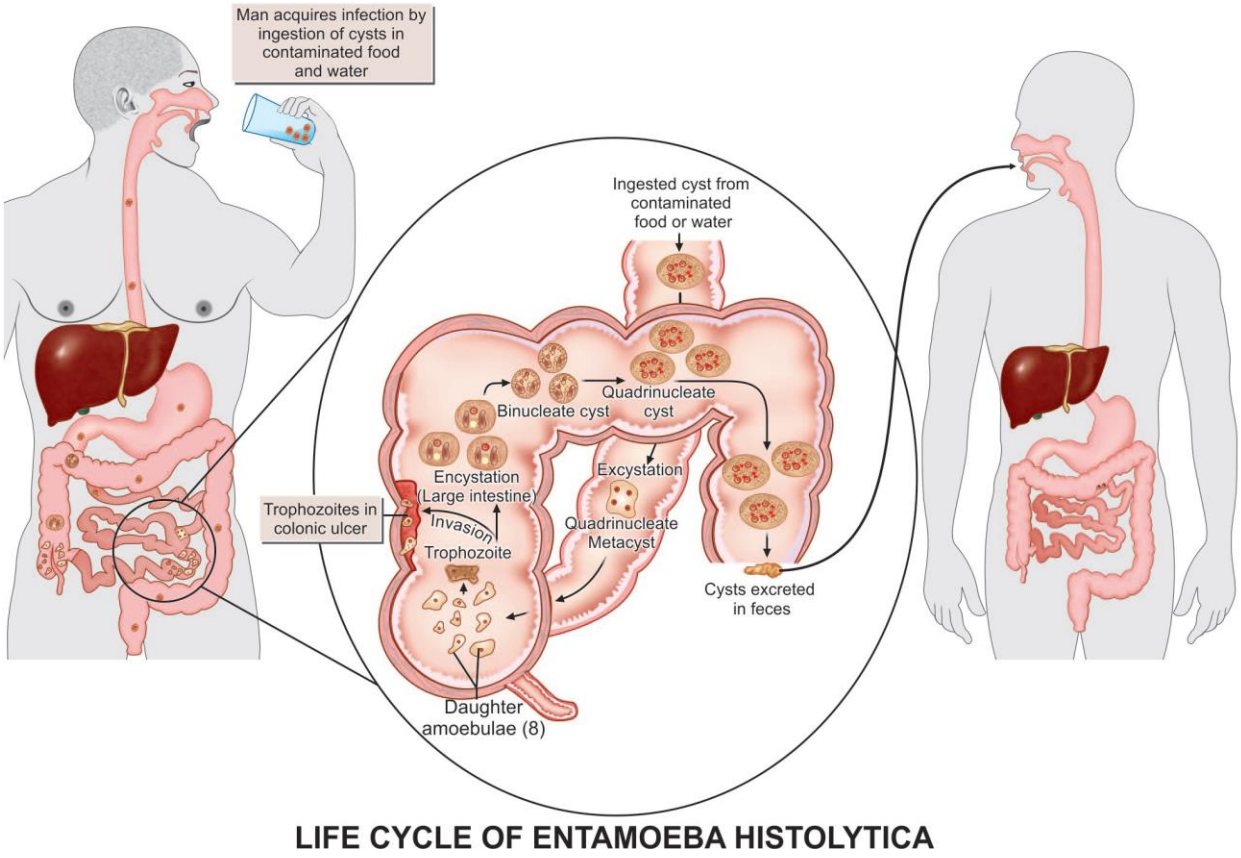
Entamoeba histolytica. (a) Trophozoite, (b) Cyst



Entamoeba histolytica. A. Trophozoite; B. Precystic stage; C. Uninucleate cyst; D. Binucleate cyst; E. Mature quadrinucleate cyst

Life Cycle

The cysts (usually found in formed stools) and trophozoites are passed out in faeces of infected human. Cysts are ingested via contaminated food or water. In the intestine, the cysts undergo excystation and form trophozoites. As the trophozoite passes down the intestine, it undergoes encystation and is excreted in the faeces. *Entamoeba histolytica* completes its life cycle in human host. In the majority of cases, *E. histolytica* remains as a commensal in the large intestine. They are carriers or asymptomatic cyst passers and are responsible for maintenance and transmission of infection in the community.



LIFE CYCLE OF ENTAMOEBA HISTOLYTICA

Symptoms

1-can include fulminating **dysentery, bloody diarrhea**,

2- weight loss, fatigue, abdominal pain,

3- **ulcerations :** 'ulcer' in the tissue, typically described as flask-shaped.

3. The amoeba can actually 'bore' into the intestinal wall, causing lesions and intestinal symptoms, and it may reach the blood stream. From there, it can reach different vital organs of the human body, usually the liver, but sometimes the lungs, brain, spleen, etc. A common outcome of this invasion of tissues is a **liver abscess**, which can be fatal if untreated. Ingested red blood cells are sometimes seen in the amoeba cell cytoplasm.

Pathology

majority of cases, infection is asymptomatic and the carrier is unaware they are infected. However, in an estimated 10% of cases E. histolytica causes disease. Once the trophozoites are excysted they colonize the large bowel, remaining on the surface of the mucus layer and feeding on bacteria and food particles. Occasionally, and in response to unknown stimuli, trophozoites move through the mucus layer where they come in contact with the epithelial cell layer and start the pathological process. E. histolytica has a lectin that binds to galactose and N-acetylgalactosamine sugars on the surface of the epithelial cells, The lectin normally is used to bind bacteria for ingestion. The parasite has several enzymes such as pore forming proteins, lipases, and cysteine proteases, which are normally used to digest bacteria in food vacuoles but which can cause lysis of the epithelial cells by inducing cellular necrosis and apoptosis when the trophozoite comes in contact with them and binds via the lectin. Enzymes released allow penetration into intestinal wall and blood vessels, sometimes on to liver and other organs.[20] The trophozoites will then ingest these dead cells. This damage to the epithelial cell layer attracts human immune cells and these in turn can be lysed by the trophozoite, which releases the immune cell's own lytic enzymes into the surrounding tissue, creating a type of chain reaction and leading to tissue destruction. This destruction manifests itself in the form of an 'ulcer' in the tissue, typically described as flask-shaped because of its appearance in transverse section. This tissue destruction can also involve blood vessels leading to bloody diarrhea, amebic dysentery. Occasionally, trophozoites enter the bloodstream where they are transported typically to the liver via the portal system. In the liver a similar pathological sequence ensues, leading to amebic liver abscesses. The trophozoites can also end up in other organs, sometimes via the bloodstream, sometimes via liver abscess rupture or fistulas. In all locations, similar pathology can occur.

Diagnosis

1. Diagnosis of intestinal amoebiasis

1- microscopic examination for cysts or trophozoites,. Iodine-stained preparation is commonly for this purpose. The trophozoite of E. histolytica stains yellow to light brown..Trichrome stain is useful to demonstrate intracellular features of both trophozoites and cysts. Direct wet mount and iron haematoxylin staining to demonstrate trophozoites.
2- Stool Culture, Stool culture is a sensitive method in diagnosing chronic and asymptomatic intestinal amoebiasis.
3- Serological test.
4- PCR are available for diagnosis, and are more sensitive and specific than microscop

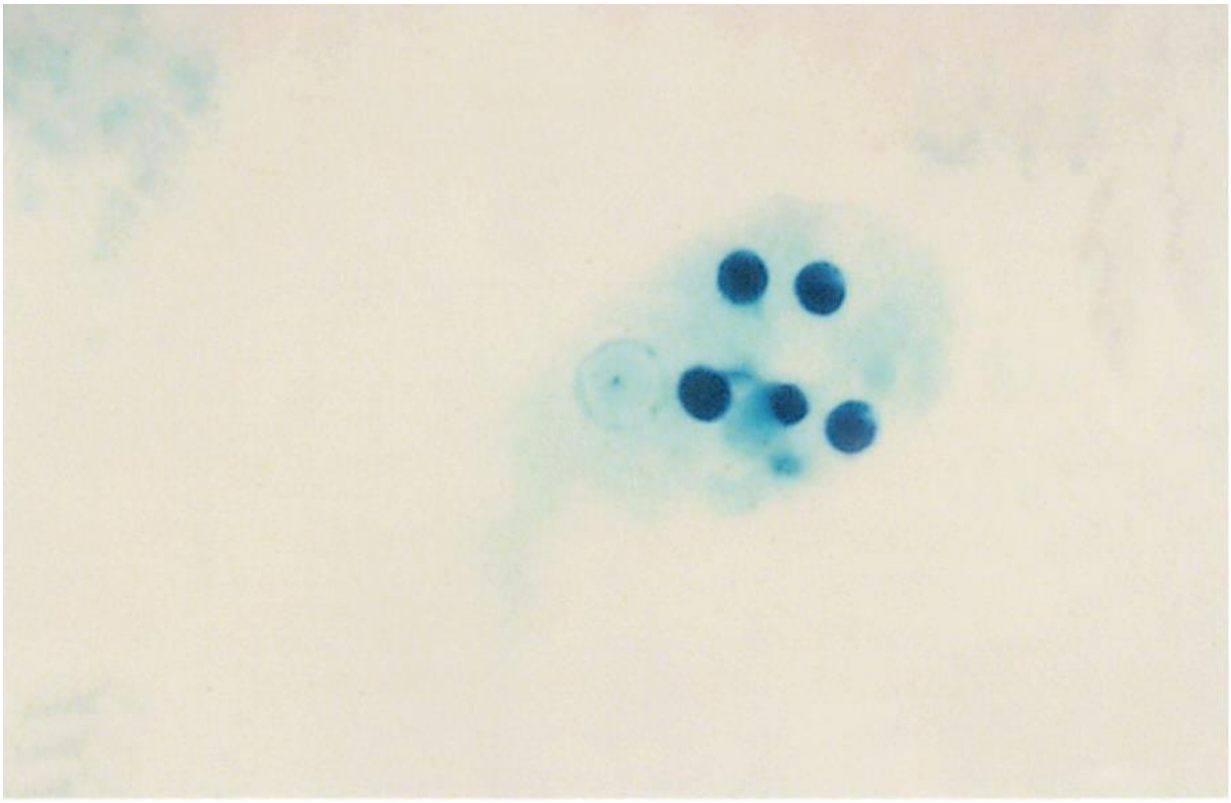
Entamoeba histolytica (cyst)

2. Diagnosis of extraintestinal amoebiasis

(a) Microscopic examination Demonstration of trophozoites in pus aspirated from the wall of liver abscess. smears of aspirates or scrapings obtained by proctoscopy, and aspirates of abscesses or other tissue specimen. The pus obtained from the centre of the abscess may not contain amoebae as they are confined to the wall of the abscess. Cysts are not found in extraintestinal lesions. Stool examination rarely can detect E. histolytica cyst.

(b) Molecular diagnosis PCR of pus aspirated from ALA

(c) Serodiagnosis



Entamoeba histolytica (1,000). This trophozoite in feces contains five .

Entamoeba histolytica (1,000) showing four nuclei. Iodine stain

Entamoeba Coli

E. coli presence in healthy persons.

It is worldwide in distribution and a nonpathogenic commensal intestinal amoeba.

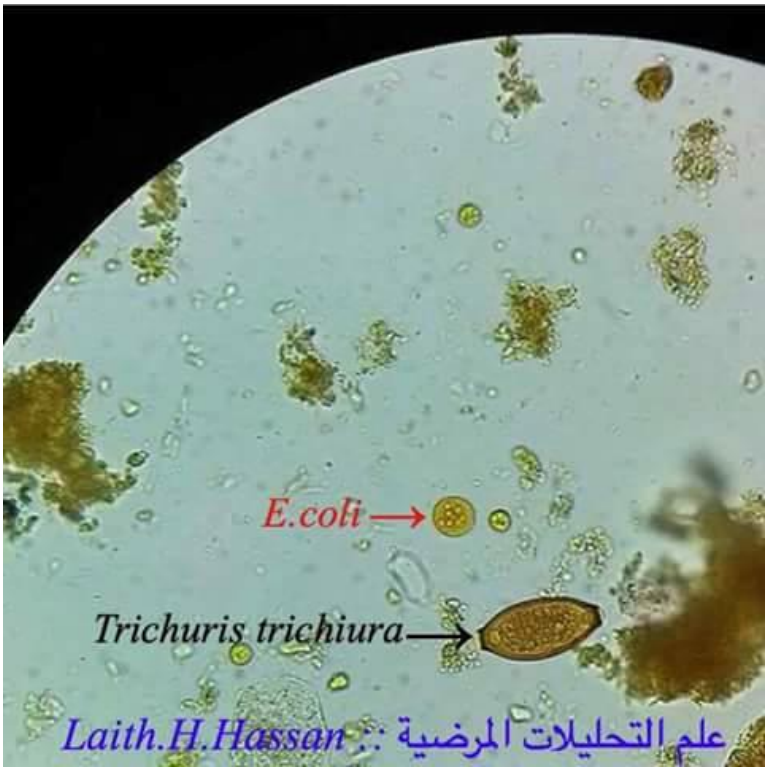
It is larger than E. histolytica

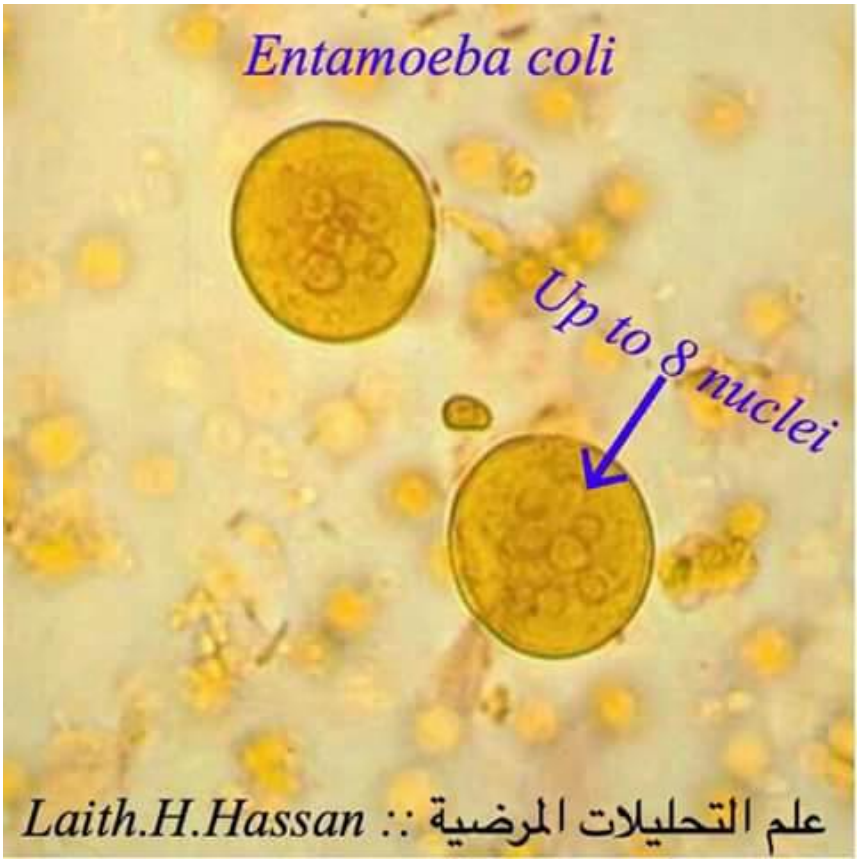
The nucleus is clearly visible in unstained films

Cysts are large with a prominent glycogen mass in the early stage.

The mature cyst has 8 nuclei

The life cycle is the same as in E.histolytica except that it remains a luminal commensal without tissue invasion and is nonpathogenic.


E.coli
Trichuris trichiura
Laith.H.Hassan :: علم التحليلات المرضية


Entamoeba coli
Up to 8 nuclei
Laith.H.Hassan :: علم التحليلات المرضية

ENTAMOEBA DISPAR

Entamoeba dispar that can result in either a harmless of the intestine or may progress to an invasion of the colon wall and damage to other host tissues such as the liver, lung, and brain (amoebiasis). Often a clinical diagnosis of amoebiasis by Entamoeba can be confirmed and usually depends on the visualization of parasites using light microscopy for either a wet smear or a stained smear. E. coli, E.

dispar and E. histolytica, may be differentiated by comparative sizes and minor morphological differences.

Morphology Same as E. histolytica.

TABLE 3-4 Comparison of *E. histolytica* with Common Nonpathogenic Amoebae

E. HISTOLYTICA	*E. COLI*	*E. HARTMANNI*	*E. DISPAR*
Trophozoites	**Trophozoites**	**Trophozoites**	**Trophozoites**
20–40 µm	20–25 µm	8–10 µm	15–20 µm
Motility increased when warm	Sluggish and nondirectional movement	Less progressive motility than *E. dispar*	Progressive movement
Extended pseudopodia	Broad, blunt pseudopodia		Extended pseudopodia
Cysts	**Cysts**	**Cysts**	**Cysts**
8–20 µm	20–25 µm	6–8 µm	12–15 µm
1 to 4 nuclei (4 in mature form)	8 nuclei	4 nuclei	4 nuclei
Squat, oval chromatoid bodies	Pointed chromatoid bodies	Blunt chromatoid bodies	Blunt chromatoid bodies

Echinococcus granulosus **Common name: Dog tape worm**

Type of life cycle. Indirect life cycle

final host: carnivores (dogs).

site infection: small intestine

intermediate host: humans and herbivorus animals (sheep, goat, cattle and horse**)**

Infective stage: Hydatid cyst

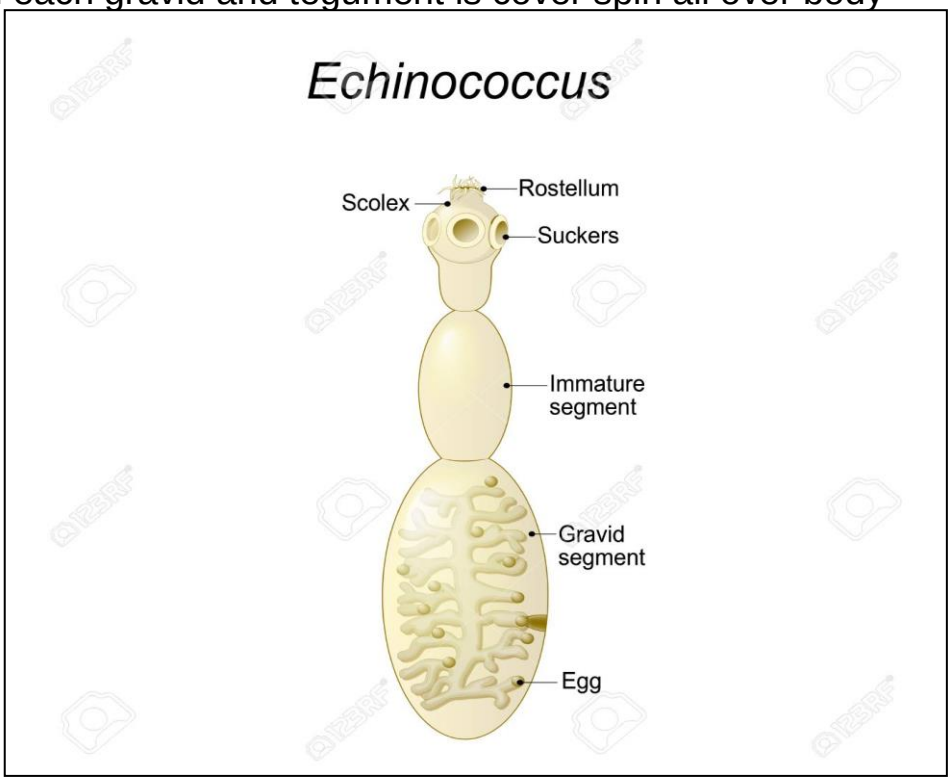
Morphological of the adult worm :

scolex globular or pea shape have rostullum retractable, double row of hooks 30-36 & four Sucker

adult parasite length 3-6 mm, wide 1-1.5mm ,The strobila is composed of only 3 proglottids, the anterior immature, the middle mature, and the posterior gravid segment,

Mature segment have testes distributed in middle (15-65), genital pore posterior middlehave cirrus sac , vitelline gland

gravid segment have lateral dilatation sac like uterus (diverticulum) and full 500 – 1500 eggs in each gravid and tegument is cover spin all over body



Egg

The eggs of Echinococcus are indistinguishable from those of Taenia species. It is ovoid in shape and brown in color. It contains an embryo with 3 pairs of hooklets.

Morphology

- **Egg:**
  Spherical
  30 – 40 mic. In diameter.
  With an outer brownish, radially striated embryophore which surrounds a hexacanth embryo or onchosphere.

Larval Form

The larval form is found within the hydatid cyst developing inside various organs of the intermediate host. It represents the structure of the scolex of adult worm and remains invaginated within a vesicular body. After entering the definitive host, the scolex with suckers and rostellar hooklets, becomes exvaginated and develops into adult worm.

Clinical Features

Most of infection is asymptomatic and accidentally discovered. Clinical disease develops only when the hydatid cyst has grown big enough to cause symptoms. Disease results mainly from pressure effects caused by the enlarging cysts.

In about half the cases, the primary hydatid cyst occurs **in liver (63%)**, Hepatomegaly, pain, and jaundice.

The next common site is the **lung (25%)** . Cough, chest pain, pneumothorax, and dyspnea

In the kidney (2%), hydatid cyst causes pain and hematuria.

Other sites affected include spleen (1%), brain (1%), pelvic organs, orbit, and bones (3%).

hypersensitivity to the echinococcal antigen. If minute amounts of hydatid fluid seeping through the capsule. Hypersensitivity may cause **urticaria.** But if a hydatid cyst ruptures spontaneously or during surgical interference, massive release of hydatid fluid may cause severe or even fatal anaphylaxis.

Diagnosis in intermediate host : Human

1-symptoms : Rash , redness , weakness , fever ,diarrhea, vomiting , (Other symptoms according to location of the cyst) .

2-Serological test: IHAT , IFAT , ELISA , Immune electrophoresis test

. 3- PCR.

4- X- Ray or ultrasonography , computerised tomography , magnetic resonanc

Protozoa: General Characteristics

Protozoa are single-celled eukaryotic microorganisms belonging to kingdom Protista. The body wall is covered by cell membrane. Its cytoplasm is made up of ectoplasm and endoplasm. The nucleus is usually single but may be double or multiple. Reproduction can be asexual (e.g. binary fission, schizogony, endodyogeny) or sexual (e.g. gametogony). Protozoa can be divided into the following groups:

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Entamoeba histolytica

Direct live cycle

Infective stage . cyst

Site of infection . colon and liver

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Morphology

***Entamoeba histolytica* occurs in 3 forms.**

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2. Precyst

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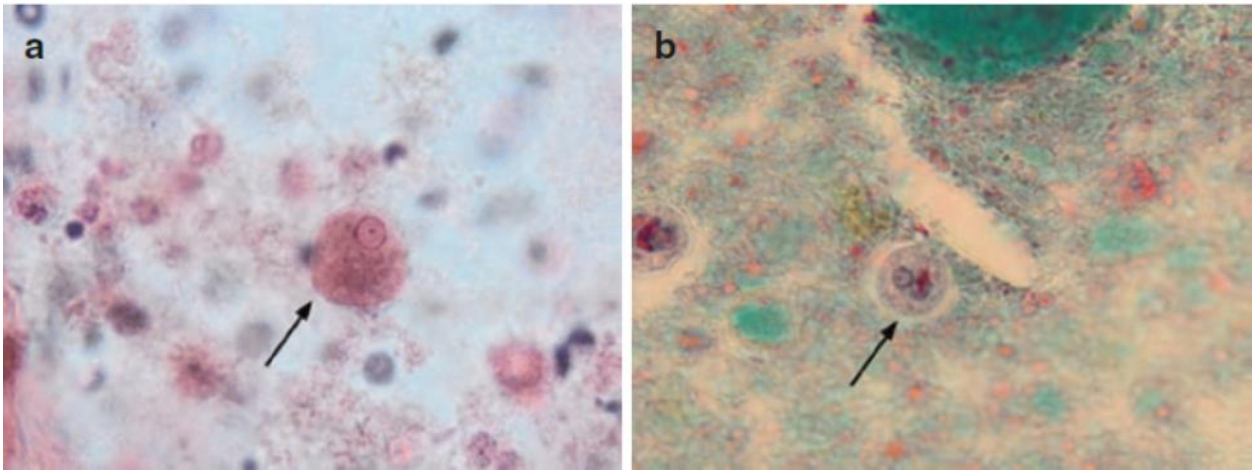

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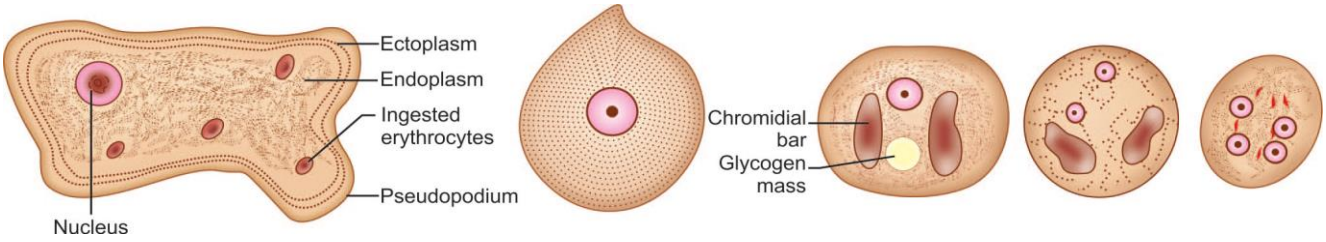

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Life Cycle

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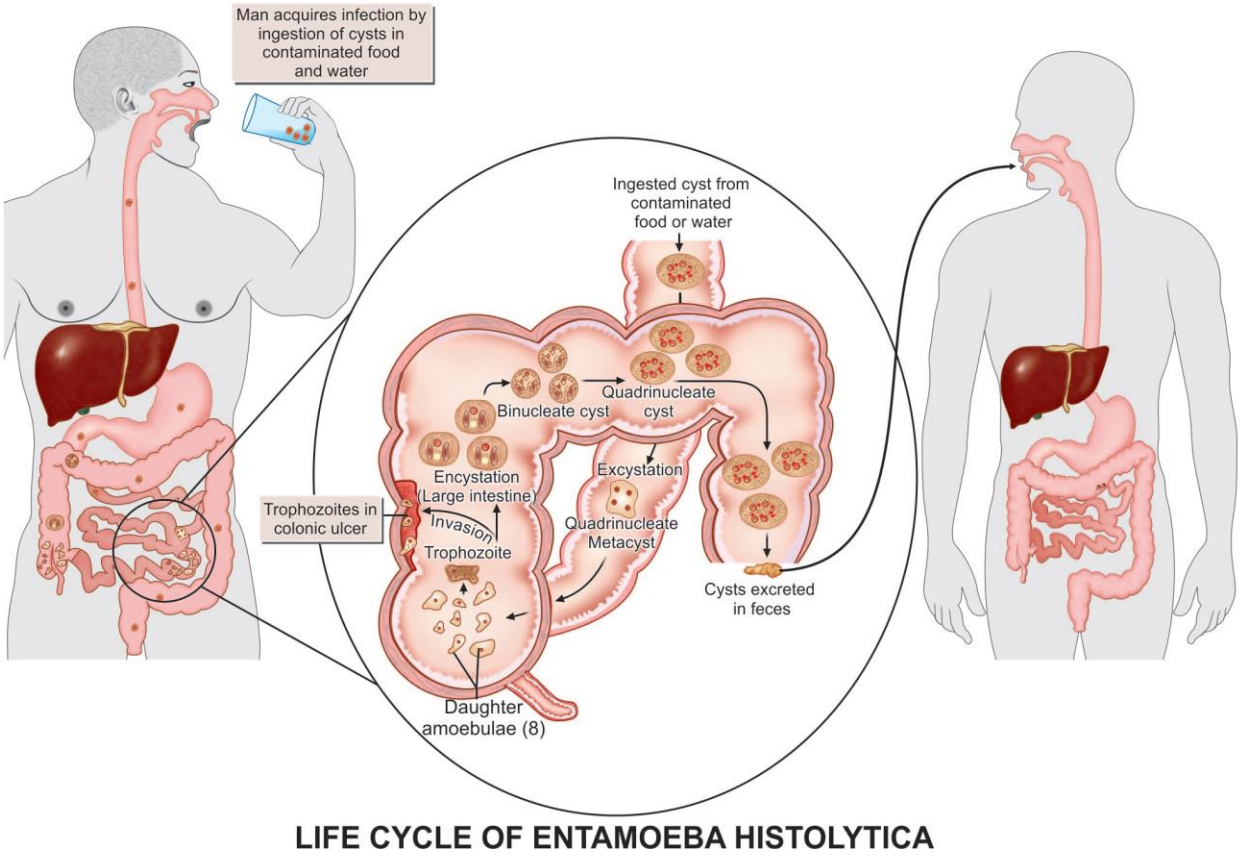

LIFE CYCLE OF ENTAMOEBA HISTOLYTICA

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3. The amoeba can actually 'bore' into the intestinal wall, causing lesions and intestinal symptoms, and it may reach the blood stream. From there, it can reach different vital organs of the human body, usually the liver, but sometimes the lungs, brain, spleen, etc. A common outcome of this invasion of tissues is a **liver abscess**, which can be fatal if untreated. Ingested red blood cells are sometimes seen in the amoeba cell cytoplasm.

Pathology

majority of cases, infection is asymptomatic and the carrier is unaware they are infected. However, in an estimated 10% of cases E. histolytica causes disease. Once the trophozoites are excysted they colonize the large bowel, remaining on the surface of the mucus layer and feeding on bacteria and food particles. Occasionally, and in response to unknown stimuli, trophozoites move through the mucus layer where they come in contact with the epithelial cell layer and start the pathological process. E. histolytica has a lectin that binds to galactose and N-acetylgalactosamine sugars on the surface of the epithelial cells, The lectin normally is used to bind bacteria for ingestion. The parasite has several enzymes such as pore forming proteins, lipases, and cysteine proteases, which are normally used to digest bacteria in food vacuoles but which can cause lysis of the epithelial cells by inducing cellular necrosis and apoptosis when the trophozoite comes in contact with them and binds via the lectin. Enzymes released allow penetration into intestinal wall and blood vessels, sometimes on to liver and other organs.[20] The trophozoites will then ingest these dead cells. This damage to the epithelial cell layer attracts human immune cells and these in turn can be lysed by the trophozoite, which releases the immune cell's own lytic enzymes into the surrounding tissue, creating a type of chain reaction and leading to tissue destruction. This destruction manifests itself in the form of an 'ulcer' in the tissue, typically described as flask-shaped because of its appearance in transverse section. This tissue destruction can also involve blood vessels leading to bloody diarrhea, amebic dysentery. Occasionally, trophozoites enter the bloodstream where they are transported typically to the liver via the portal system. In the liver a similar pathological sequence ensues, leading to amebic liver abscesses. The trophozoites can also end up in other organs, sometimes via the bloodstream, sometimes via liver abscess rupture or fistulas. In all locations, similar pathology can occur.

Diagnosis

1. Diagnosis of intestinal amoebiasis

1- microscopic examination for cysts or trophozoites,. Iodine-stained preparation is commonly for this purpose. The trophozoite of E. histolytica stains yellow to light brown..Trichrome stain is useful to demonstrate intracellular features of both trophozoites and cysts. Direct wet mount and iron haematoxylin staining to demonstrate trophozoites.
2- Stool Culture, Stool culture is a sensitive method in diagnosing chronic and asymptomatic intestinal amoebiasis.
3- Serological test.
4- PCR are available for diagnosis, and are more sensitive and specific than microscop

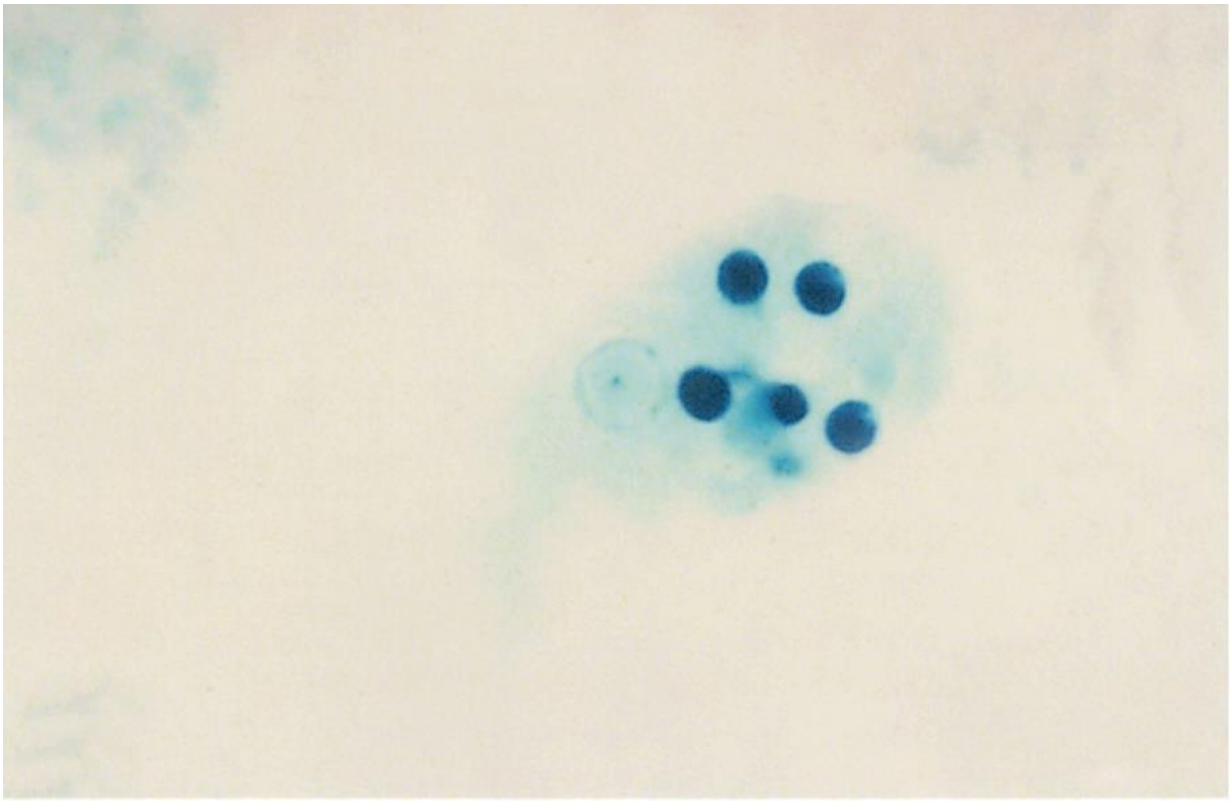
Entamoeba histolytica (cyst)

2. Diagnosis of extraintestinal amoebiasis

(a) Microscopic examination Demonstration of trophozoites in pus aspirated from the wall of liver abscess. smears of aspirates or scrapings obtained by proctoscopy, and aspirates of abscesses or other tissue specimen. The pus obtained from the centre of the abscess may not contain amoebae as they are confined to the wall of the abscess. Cysts are not found in extraintestinal lesions. Stool examination rarely can detect E. histolytica cyst.

(b) Molecular diagnosis PCR of pus aspirated from ALA

(c) Serodiagnosis



Entamoeba histolytica (1,000). This trophozoite in feces contains five .

Entamoeba histolytica (1,000) showing four nuclei. Iodine stain

Entamoeba Coli

E. coli presence in healthy persons.

It is worldwide in distribution and a nonpathogenic commensal intestinal amoeba.

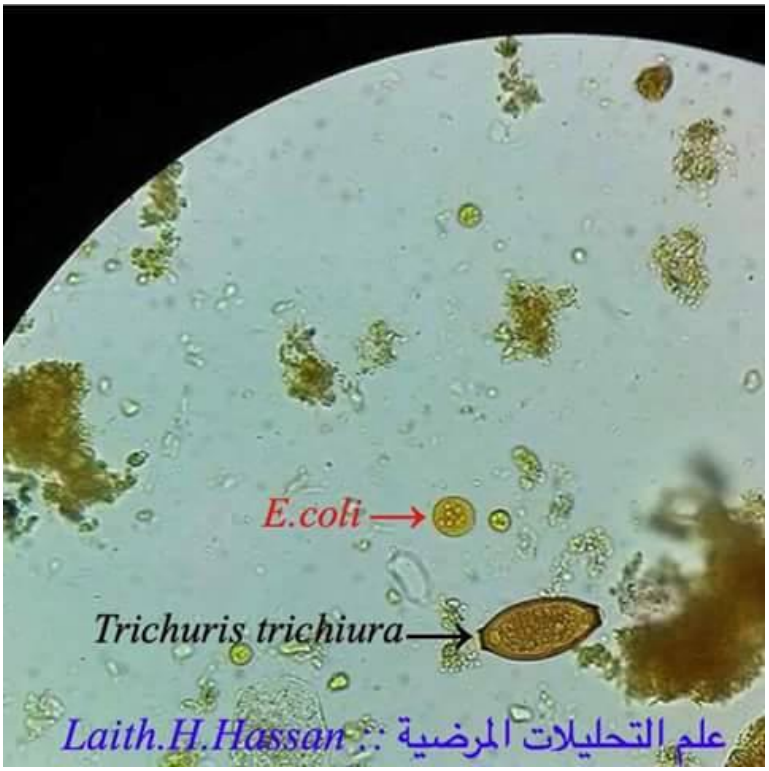
It is larger than E. histolytica

The nucleus is clearly visible in unstained films

Cysts are large with a prominent glycogen mass in the early stage.

The mature cyst has 8 nuclei

The life cycle is the same as in E.histolytica except that it remains a luminal commensal without tissue invasion and is nonpathogenic.


E.coli
Trichuris trichiura
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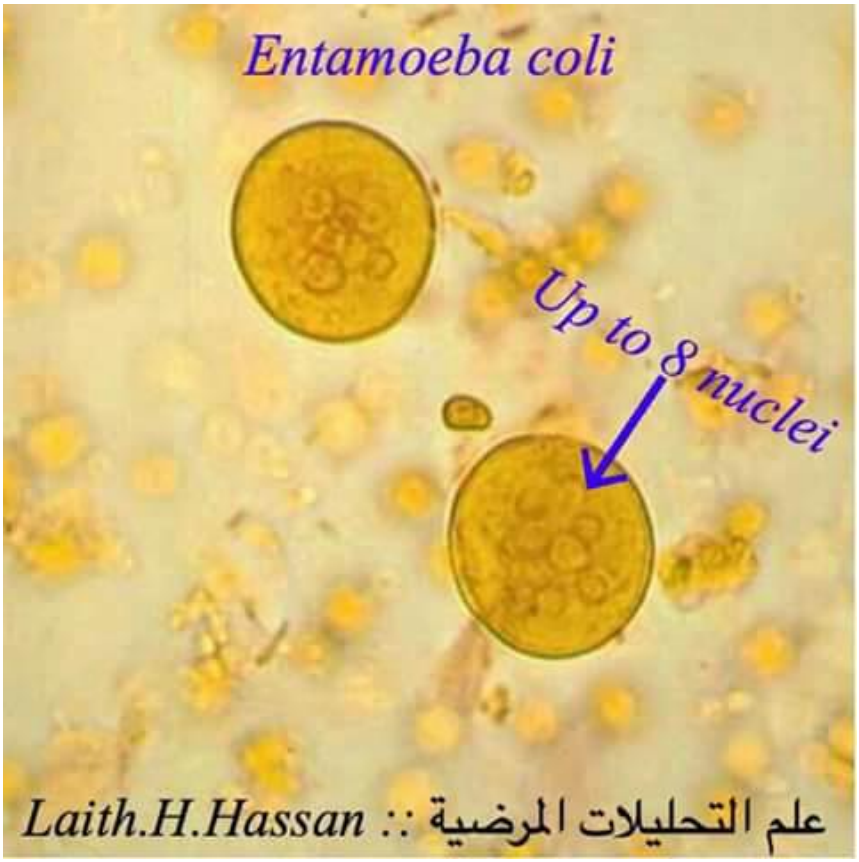
Entamoeba coli
Up to 8 nuclei
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ENTAMOEBA DISPAR

Entamoeba dispar that can result in either a harmless of the intestine or may progress to an invasion of the colon wall and damage to other host tissues such as the liver, lung, and brain (amoebiasis). Often a clinical diagnosis of amoebiasis by Entamoeba can be confirmed and usually depends on the visualization of parasites using light microscopy for either a wet smear or a stained smear. E. coli, E.

dispar and E. histolytica, may be differentiated by comparative sizes and minor morphological differences.

Morphology Same as E. histolytica.

TABLE 3-4 Comparison of *E. histolytica* with Common Nonpathogenic Amoebae

E. HISTOLYTICA	*E. COLI*	*E. HARTMANNI*	*E. DISPAR*
Trophozoites	**Trophozoites**	**Trophozoites**	**Trophozoites**
20–40 µm	20–25 µm	8–10 µm	15–20 µm
Motility increased when warm	Sluggish and nondirectional movement	Less progressive motility than *E. dispar*	Progressive movement
Extended pseudopodia	Broad, blunt pseudopodia		Extended pseudopodia
Cysts	**Cysts**	**Cysts**	**Cysts**
8–20 µm	20–25 µm	6–8 µm	12–15 µm
1 to 4 nuclei (4 in mature form)	8 nuclei	4 nuclei	4 nuclei
Squat, oval chromatoid bodies	Pointed chromatoid bodies	Blunt chromatoid bodies	Blunt chromatoid bodies

3-Enterobius Vermicularis : Common name: Pinworm

Habitat: Adult worms are found in the caecum, appendix, and adjacent portion of ascending colon.

Life Cycle: E. vermicularis is monoxenous, passing its entire life cycle in the human host. It has no intermediate host

Natural host: Man

Infective form: Embryonated eggs

Morphology

Adult Worm: The adults are short, white, with pointed ends, looking like bits of white thread. The mouth is surrounded by (cervical alae]. The esophagus has a double-bulb structure

Female Worm

The female is 8–13 mm long and 0.3–0.5 mm thick. Its posterior third is drawn into a thin pointed pin-like tail. The vulva is located just in front of the middle third of the body and opens into the single vagina

Male Worm

The male worm is 2–5 mm long and 0.1–0.2 mm thick. Its posterior end is tightly curved ventrally, sharply truncted and carries a prominent copulatory spicule

Egg

The egg is colorless. It floats in saturated salt solution. It has a characteristic shape, being elongated ovoid, flattened on one side, and convex on the other.

Clinical Features

Enterobiasis occurs mostly in children.The worm produces intense irritation and pruritus of the perianal and perineal area (pruritis ani), when it crawls out of the anus to lay eggs. This leads to scratching and excoriation of the skin around the anus. As the worm migrates out at night, it disturbs sleep.

Laboratory Diagnosis

1- **Demonstration of Eggs**

Eggs are present in the feces only in a small proportion of patients and so feces examination is not useful in diagnosis. They are deposited in large numbers on the perianal and perineal skin at night and can be demonstrated in swabs collected from the sites early morning. Swabs from perianal folds are most often positive. a

2- **NIH Swab Method** The NIH swab (named after National Institutes of Health, USA) has been widely used for collection of specimens.
3- **Scotch Tape Method** Another method for collection of specimens is with scotch tape (adhesive transparent cellophane tape)
4- **Demonstration of Adult Worm**

The adult worms may sometimes be noticed on the surface of stools. They may occasionally be found crawling out of the anus while the children are asleep

4-Ascaris Lumbricoides : Common name: Roundworm

Life cycle of Ascaris involves only 1 host.

Natural host: Man. There is no intermediate host.

Infective form: Embryonated eggs

Habitat: Adult worms live in the small intestines (85% in jejunum and 15% in ileum).

morphology

Adult Worm They are large cylindrical worms, the anterior end being more pointed than the posterior. They are pale pink colored when freshly passed in stools, but become white outside the body. The mouth at the anterior end has 3 finely lips, 1 dorsal and 2 ventrolateral

Male Worm The adult male worm is little smaller than female. It measures 15–30 cm in length and 2–4 mm in thickness Its posterior end is curved ventrally to form a hook and carries 2 copulatory spicules.

Female Worm The female is larger than male, measuring 20–40 cm in length and 3–6 mm in thickness. Its posterior extremity is straight and conical. The vulva is situated mid-ventrally, near the junction of the anterior and middle thirds of the body.

Egg: Two types of eggs are passed by the worms; fertilized and unfertilized.

1-The fertilized eggs,

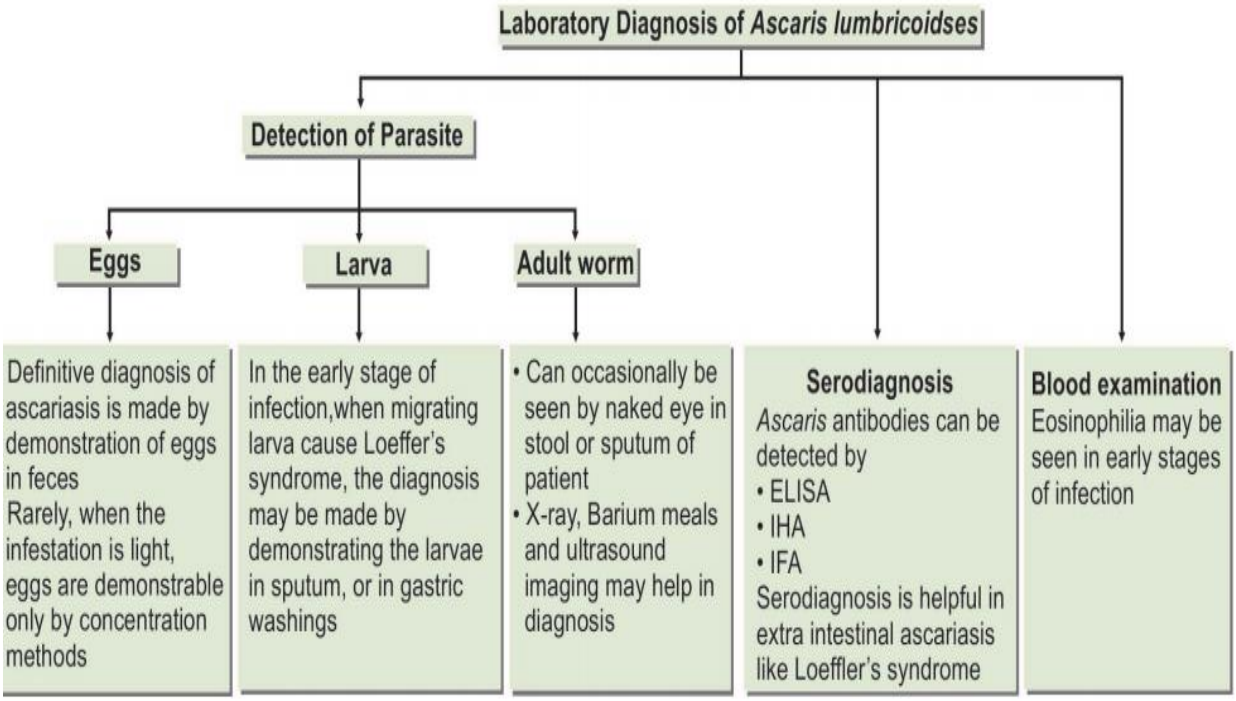
Round or oval in shape

- Size 75 µm × 405 µm
- Golden brown in color
- Surrounded by thick smooth translucent shell
- In the middle of the egg is a large unsegmented ovum.
- Floats in saturated solution of common salt

2-The unfertilized eggs,

Ellipitical in shape

- Narrower and longer
- 80 µm × 55 µm in size
- Has a thinner shell with an irregular coating of albumin
- Does not float in salt solution


Laboratory Diagnosis of Ascaris lumbricoidses
Detection of Parasite
Eggs
Larva
Adult worm
Definitive diagnosis of ascariasis is made by demonstration of eggs in feces
Rarely, when the infestation is light, eggs are demonstrable only by concentration methods
In the early stage of infection,when migrating larva cause Loeffer's syndrome, the diagnosis may be made by demonstrating the larvae in sputum, or in gastric washings
• Can occasionally be seen by naked eye in stool or sputum of patient
• X-ray, Barium meals and ultrasound imaging may help in diagnosis
Serodiagnosis
Ascaris antibodies can be detected by
• ELISA
• IHA
• IFA
Serodiagnosis is helpful in extra intestinal ascariasis like Loeffler's syndrome
Blood examination
Eosinophilia may be seen in early stages of infection

Examination of Stool

Collection of Fresh Stool Specimen

Normally passed stool are preferable, although samples obtained after purgative (sodium sulphate) or high saline enema may also be used.

Writ data on each sample.

All stool specimens should be collected in a suitable, clean, wide mouthed container like a plastic container with a light fitting lid, waxed cardboard box.

All fresh specimens should be handled carefully because each specimen represents a potential source of infectious material.

The specimen should not be contaminated with water, urine, or disinfectants.

Grossly examination Stool should be examined for its consistency, color, odor, and presence of blood or mucus. In some instances, parasites may be seen on gross inspection, as in the case of roundworm, pinworm, or tapeworm proglottids.

Examination of fresh specimens is necessary for observing motility of protozoan parasites

Microscopic Examination

For detection of parasites, use different methods for different purposes. The methods include examination of: (i) wet mounts, (ii) thick smears and, (iii) permanent stained preparations.

Various concentration methods can be used to increase the sensitivity of microscopic examination.

If there is a delay in examination, use of preservatives like formalin, sodium acetate, and polyvinyl alcohol is recommended.

Wet Mounts

Unstained wet film: The unstained wet film is made by emulsifying a small quantity of stool in a drop of normal saline placed on a slide and applying a coverslip on top, avoiding air bubbles. The entire field under coverslip should be systematically examined with low power objective (10X) under low light intensity. Any suspicious object may then be examined with the high power objective.

Wet saline mounts: Wet saline mounts are particularly useful for detecting live motile trophozoites of E. histolytica, Balantidium coli, and Giardia lamblia. Eggs of helminths are also readily seen. Rhabditiform larvae of Strongyloides stercoralis are detected in freshly passed stool.

Eosin staining: Eosin 1% aqueous solution, can be used for staining wet films. Eosin stains Trophozoites and cysts of protozoa, as well as helminth larvae and thin-walled eggs stand out as pearly-white objects against a pink background and can be easily detected. Eosin also indicates the viability of cysts; live cysts are unstained and dead ones are stained pink.

Iodine staning: Iodine staining of wet mounts is another standard method of examination Lugol's iodine diluted (5 g iodine, 10 g potasium iodide and 100 mL of distilled water) used. Iodine helps to confirm the identity of cysts, as it prominently stains the nuclei. Protozoan cyst stained with iodine show yellow gold cytoplasmnd pale refractile nuclei.

Thick Smears these are not useful for routine examination, but are valuable in surveys for intestinal helminth eggs. The method described by Kato and Miura in 1954 is known as the Kato thick smear technique.

About 50 mg stool is taken on a slide and covered with a special wet table cellophane coverslip soaked in glycerine containing aqueous malachite green.

The preparation is left for about an hour at room temperature, during which the glycerine clears the stool, enabling the helminth eggs to be seen distinctly under

low power magnification. This method is however not useful for diagnosis of protozoa or helminth larvae.

Permanent Stained Smears

Permanent stained smears are examined normally under oil immersion (100X) objective.

Confirmation of the intestinal protozoan, both trophozoites and cysts, is the primary purpose of this technique.

Helminthic eggs and larvae take up too much stain and usually cannot be identified.

Permanent smear can be prepared with both fresh and polyvinyl alcohol preserved stool specimen.

methods commonly used are the iron-hematoxylin stain and Wheatley's trichrome stain. The iron hematoxylin is the older method, but is more difficult.

Iron-hematoxylin stain

Procedure

Fecal smear on a slide is fixed in Schaudinn's solution for 15 minutes and is immersed successively for 2–5 minutes in 70% alcohol, 70% alcohol containing a trace of iodine, and then 50% alcohol for 2–5 minutes.

 It is washed in water for 5–10 minutes

immersed in 2% aqueous ferric ammonium sulphate solution for 5–15 minutes

It is again washed in water for 3–5 minutes

stained with 0.5% aqueous hematoxylin for 5–15 minutes

 It is washed for 2–5 minutes and differentiated in saturated aqueous solution of picric acid for 10–15 minutes

It is then washed for 10–15 minutes and dehydrated by passing through increasing strengths of alcohol, cleared in toluene or xylol and mounted.

Trichrome stain (Wheatley's method)

The trichrome technique of Wheatley for stool specimens is a modification of Gomori's original staining procedure for tissue.

It is a quicker and simpler method, which produces uniformly well-stained smears of the intestinal protozoa, human cells, yeast cells, and artefact material in about 45 minutes or less.

Procedure The smear is fixed in Schaudinn's solution and taken successively through alcohol, as above.

Trichrome stain (chromotrope 2R, light green SF, phosphotungstic acid in glacial acetic acid, and distilled water) is then applied for 5–10 minutes, differentiated in acid-alcohol dehydrated, cleared, and mounted.

Reagents of Trichrome stain

Chromotrope 2R 0.6 g

Light green SF 9.3 g

Phosphotungstic acid 0.7 g

Acetic acid (glacial) 1.0 mL

Distilled water 100 mL

Modified Ziehl-Neelsen (acid-fast) stain

Kinyon's acid-fast stain

Cryptosporidium and Isospora have been recognized as causes of severe diarrhea in immunocompromised hosts but can also cause diarrhea in immunocompetent hosts.

Method of preparing **Modified Ziehl-Neelsen (acid-fast) stain**

- **ethanol (add 50 mL of absolute ethanol and 50 mL of distilled water)**
- **Kinyoun's carbol fuchsin.**

Solution A: Dissolve 4 g of basic fuchsin in 20 mL of 95% ethanol.

Solution B: Dissolve 8 g of phenol crystals in 100 mL of distilled water.

Mix solution A and B, and store at room temperature

- **acidic alcohol (ethanol 97 ml + 3 m HCL 95 %)**
- **Alkaline methylene blue.**
- **Dissolve 0.3 g of methylene blue in 30 mL of 95% ethanol, and add 100 mL of distil water.**

Procedure

Smear 1–2 drops of specimen on the slide and allow it to air dry.

Fix with absolute methanol for 1 minute.

Flood the slide with Kinyoun's carbol fuchsin and stain it for 5 minutes.

Rinse the slide thoroughly with water.

Decolorize by using acidic alcohol for 30 second

Rinse slid with wter and drain.

 Counterstain with methylene blue for 1- 2 minute.

 Rinse the slide with water and air dry.

 Examine with high dry objective.

To see internal morphology, use the oil objective (100X).

Concentration Methods

When the parasites are scanty in stools, routine microscopic examination may fail to detect them. It is then necessary to selectively concentrate the protozoan cysts and helminth eggs and larvae. Concentration may be done using fresh or preserved feces. Several concentration techniques have been described. They can be classified as the **floatation** or **sedimentation** methods.

In floatation method, the feces is suspended in a solution of high specific gravity, so that parasitic eggs and cysts float up and get concentrated at the surface.

In sedimentation method, the feces is suspended in a solution with low specific gravity, so that the eggs and cysts get sedimented at the bottom, either spontaneously or by centrifugation.

Floatation Methods

Saturated salt solution technique Procedure

 A simple and popular method is salt-floatation using a saturated solution of sodium chloride, having a specific gravity of 1.2. About 2 mL of the salt solution is taken in tube and 1 g of feces is emulsified in it. The container is then filled completely to the brim with the salt solution. A cover slide is placed on the container, so that it is in contact with the surface of the solution without any intervening air bubbles. After standing for 20– 30 minutes, the slide is removed, without jerking, reversed to bring the wet surface on top, and examined under the microscope. A coverslip need not to be applied if examination is done immediately. Any delay in examination may cause salt crystals to develop,

interfering with clarity of vision. This simple method is quite useful for detecting the eggs of the common nematodes such as roundworm, hookworms, and whipworm, but is not applicable for eggs of tapeworms, unfertilized egg of Ascaris lumbricoides, eggs of trematodes, and protozoan cysts.

Zinc sulphate centrifugal floatation

Procedure

 Make a fine suspension of about 1 g of feces in 10 mL of water and strain through gauze to remove coarse particles.

Collect the liquid in a small test tube and centrifuge for 1 minute at 2,500 revolutions per minute. Pour off the supernatant, add water, resuspend, and centrifuge in the same manner, repeating the process, till the supernatant is clear. Pour off the clear supernatant, add a small quantity of zinc sulphate solution (specific gravity 1.18–1.2), and resuspend the sediment well.

Add zinc sulphate solution to a little below the brim and centrifuge at 2,500 revolution per minute for 1 minute.

Take samples carefully from the surface, using a wire loop, transfer to slide, and examine under the microscope. A drop of dilute iodine helps to bring out the protozoan cysts in a better way.

This technique is useful for protozoan cysts and eggs of nematodes and small tapeworms, but it does not detect unfertilized roundworm eggs, nematode larvae, and eggs of most trematodes and large tapeworms.

Sugar floatation technique Sheather's sugar floatation technique is recommended for the detection of cryptosporidia infection.

Prepared by dissolving 500 gm in 320 ml distil water with adding 6.5 gm phenol

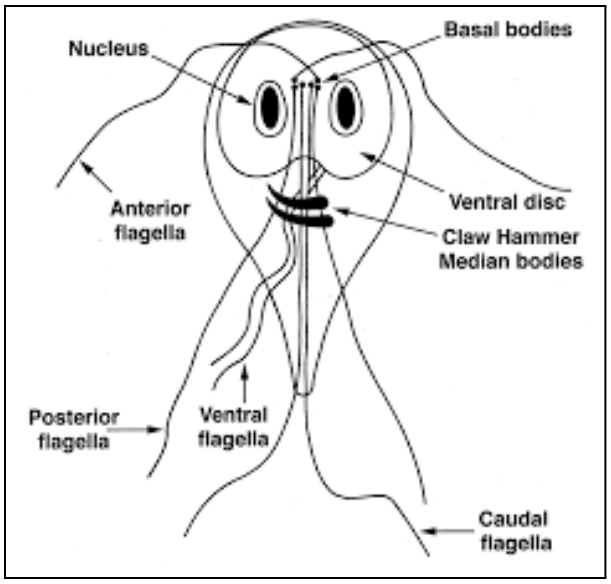
Sedimentation Methods

Formol-ether sedimentation technique

Formol-ether concentration method has been the most widely used sedimentation method.

Procedure Emulsify 1–2 g feces in 10 mL of water and let large particles sediment. Take the supernatant and spin at 2,500 revolutions per minute for 2–3 minutes. Discard the supernatant. Add 10% normal-saline, mix well, and let it stand for 10 minutes.

Add 3 mL ether and shake well. Spin at 2,500 revolutions per minute for 2–3 minutes. Four layers will form—a top layer of ether, a plug of debris at the interface, the formalin-saline layer, and the sediment at the bottom. Carefully detach the debris from the sides of the tube and discard the top 3 layers. Suspend the sediment in a few drops of fluid and examine wet mount and iodine preparation. Ethyl acetate can be conveniently used in its place, with equally good results. The method is useful for all helminth eggs and protozoan cysts.



Giardia lamblia

Direct life cycle

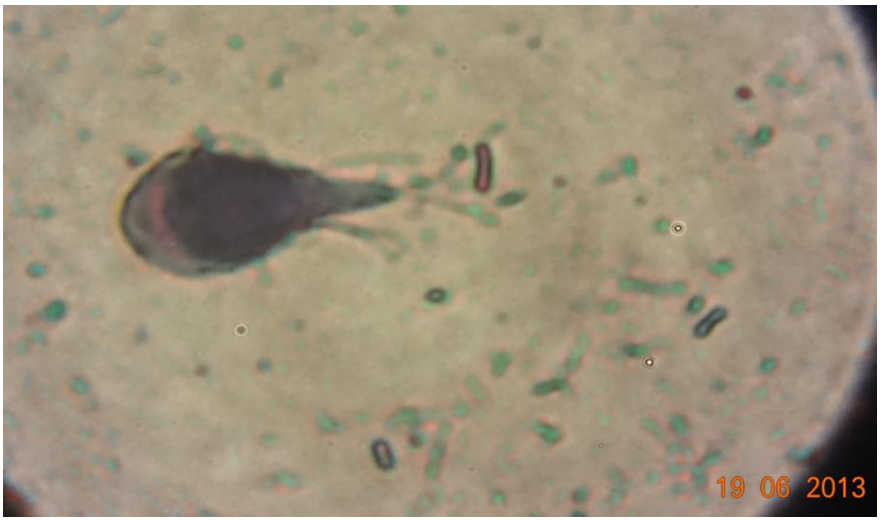
Infective stage. Cyst

Habitat and Site of infection . small intestine

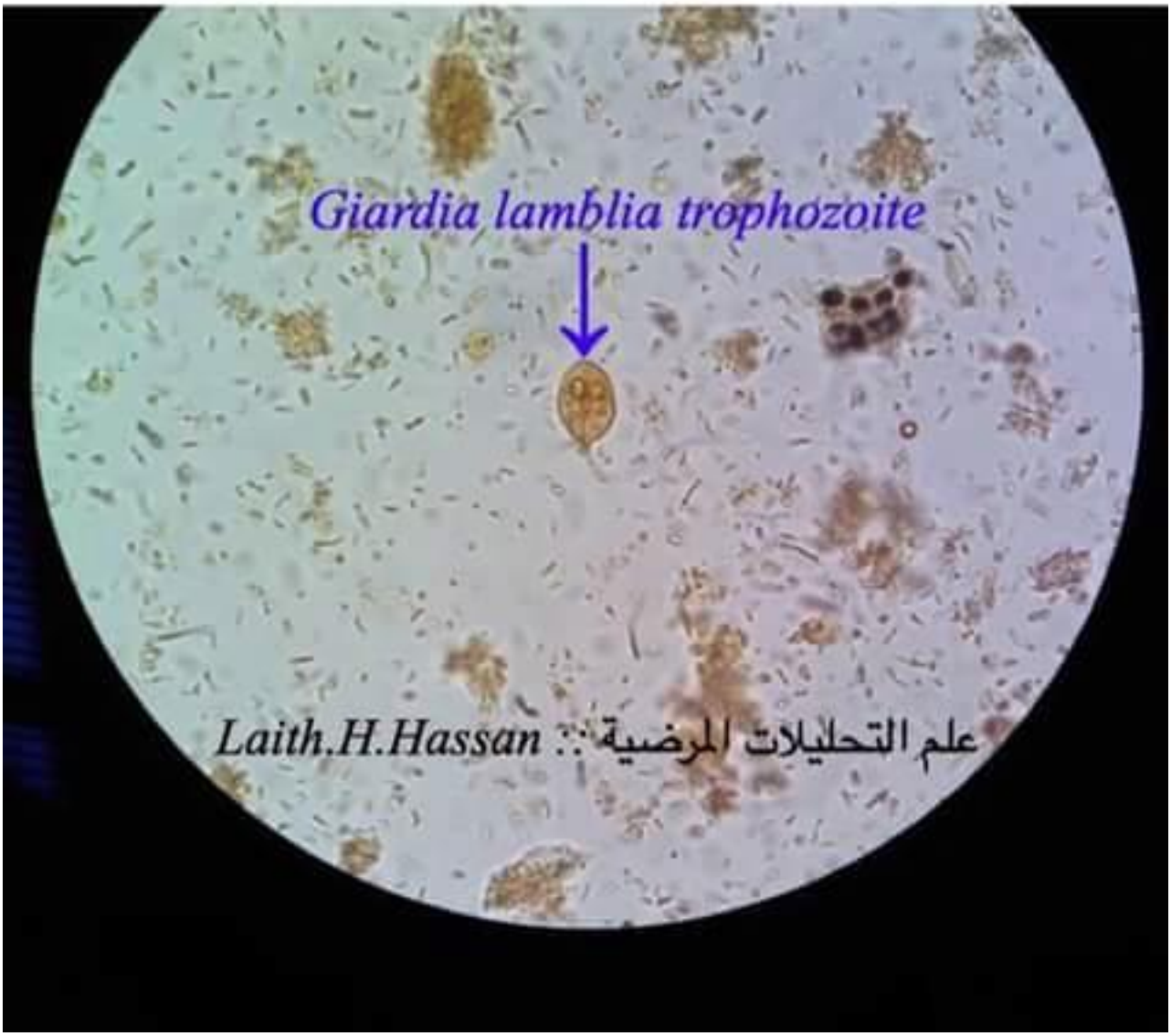
in the duodenum and upper jejunum.

Morphology It exists in 2 forms:

1. **Trophozoite**
2. The trophozoite is in the shape of (heart shaped or pyriform shaped) and is rounded anteriorly and pointed posteriorly. Dorsally, it is convex and ventrally, it has a concave sucking disc, which helps in its attachment to the intestinal mucosa. It is bilaterally symmetrical and possesses.
3. 1 pair of nuclei and 4 pairs of flagella
4. Blepharoplast, from which the fl agella arise (4 pairs)
5. 1 pair of axostyles, running along the midline



***Giardia* trophozoite Giemsa stain**


Giardia lamblia trophozoite
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Giardia lamblia trophozoite
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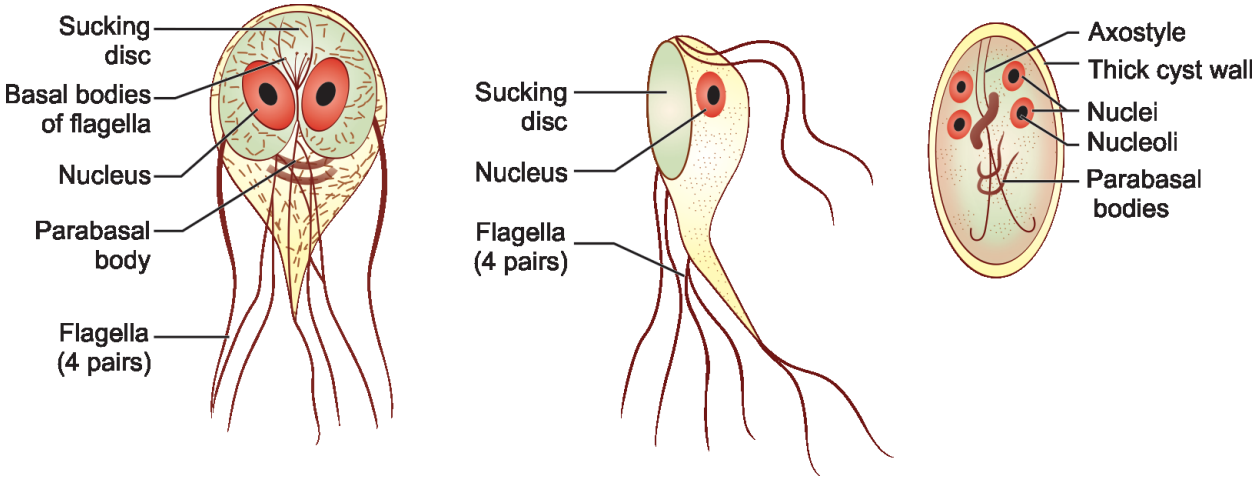
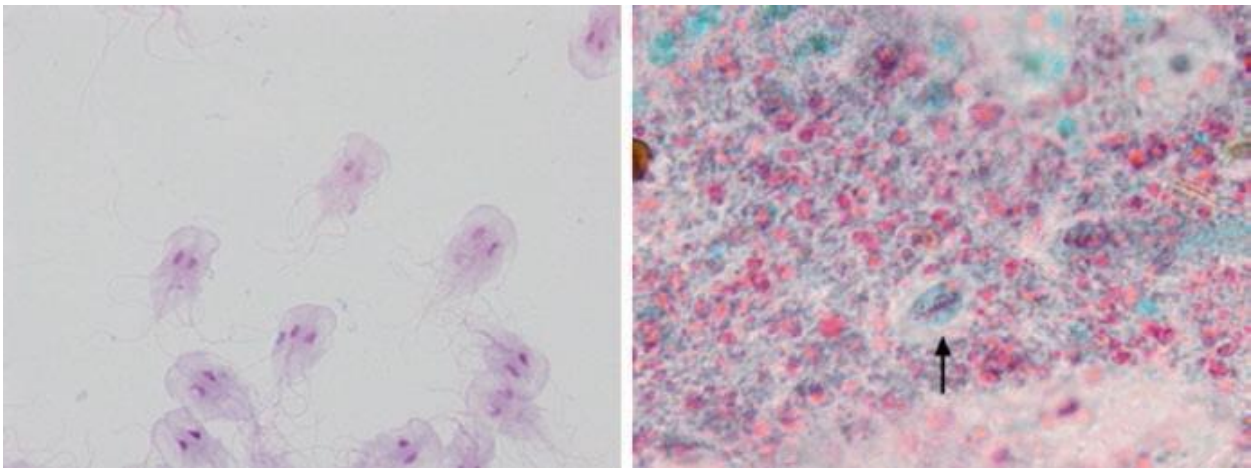


Trophozoite. A. Ventral view; B. Lateral view; C. Quadrinucleate Cyst

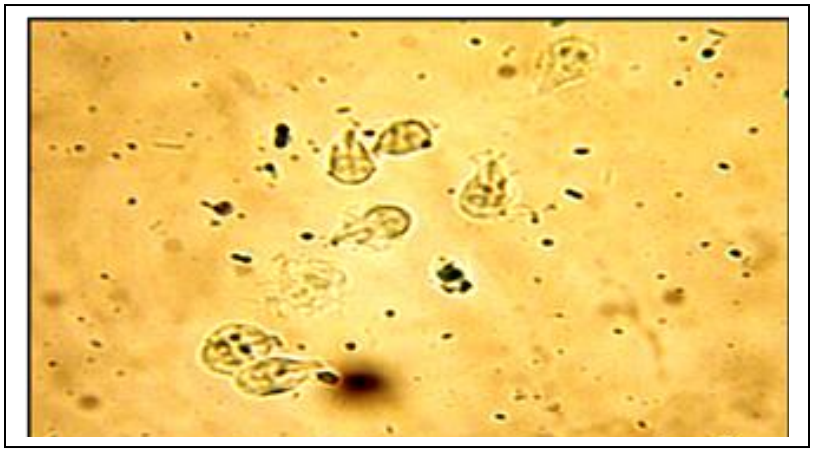
2. Cyst

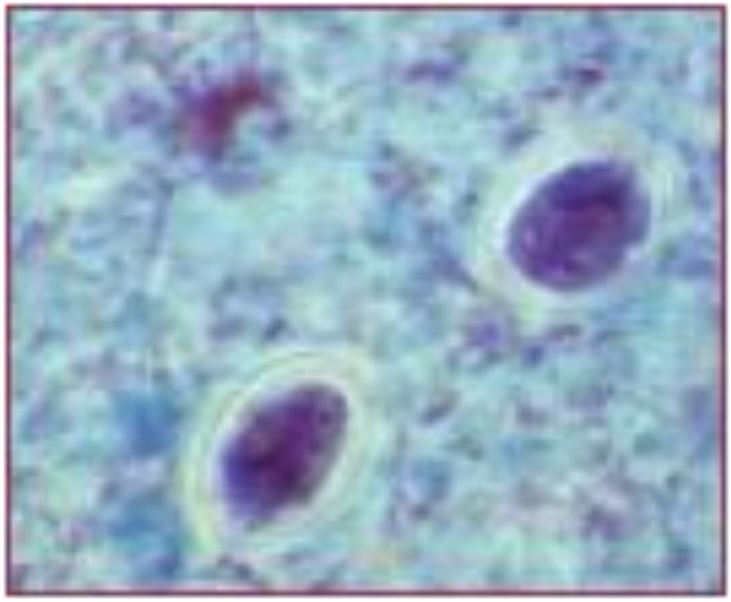
Cyst is the infective form of the parasite. The cyst is oval. A young cyst contains 2 nuclei. A mature cyst contains 4 nuclei. The axostyle lies diagonally.



Giardia lamblia. (a) Trophozoites, (b) Cyst

: Giardia trophozoite &cyst





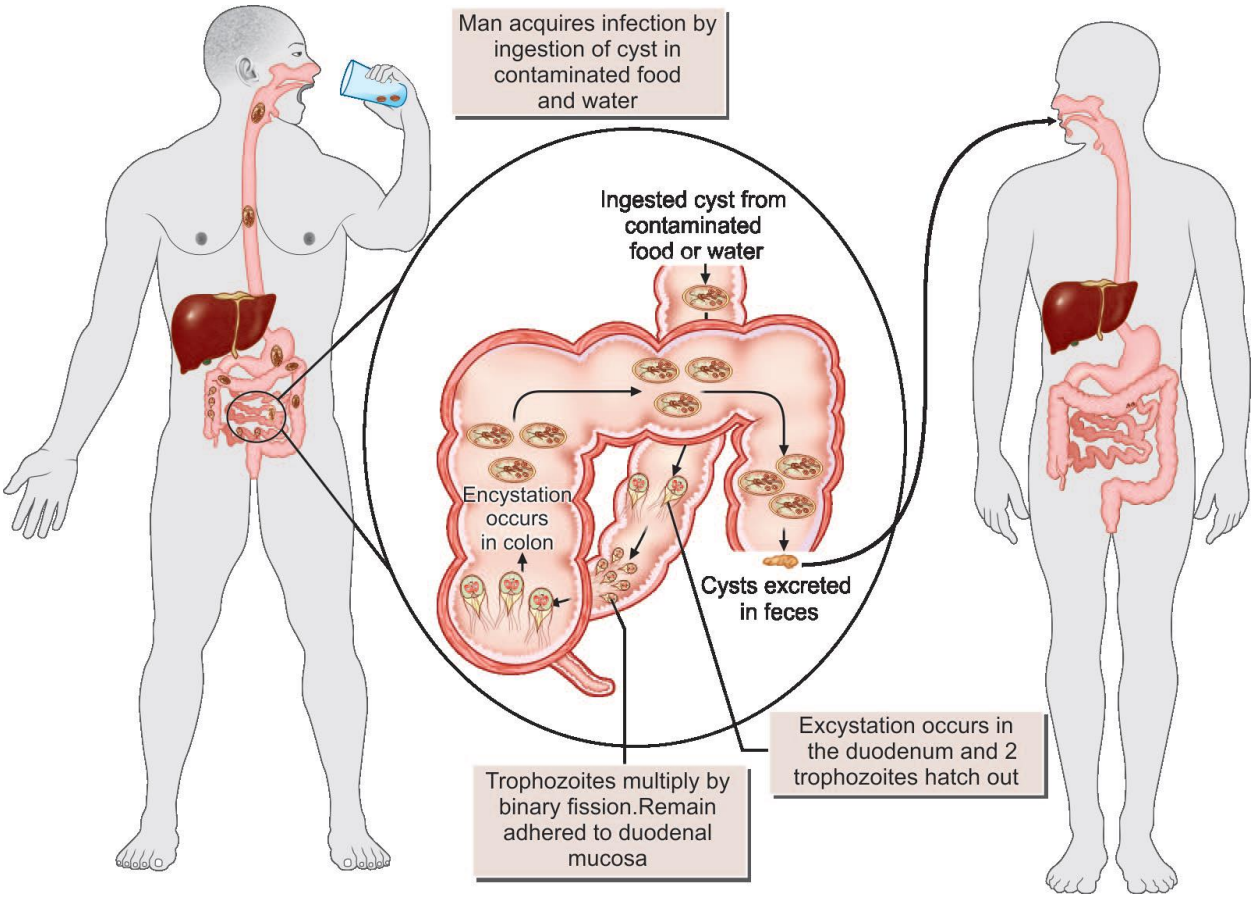
Pathogenesis and Clinical Features

Trophozoite does not invade the tissue, but remains adhered to intestinal epithelium by means of the sucking disc causing stunting and shortening of the villi. Patients are usually asymptomatic, but in some cases, giardiasis may cause diarrhoea**, fat malabsorption (steatorrhea**), dull epigastric pain and flatulence. The **stool contains excess mucus and fat**. Children may develop chronic diarrhoea, malabsorption of fat and vitamin A and weight loss. Incubation period is about 2 weeks.

Life Cycle.

Cysts are passed out in stool of an infected human. Infective cysts are ingested. The cyst excysts to release trophozoite in the small intestine. The trophozoites

multiply by binary fission. The trophozoite encysts to become cyst which is passed out in the stool. Trophozoites are passed in loose stools. Giardia completes its life cycle in 1 host. Infective stage is the mature cyst. Human acquires infection by ingestion of cysts in contaminated water and food. Direct person to person transmission may also occur in children.

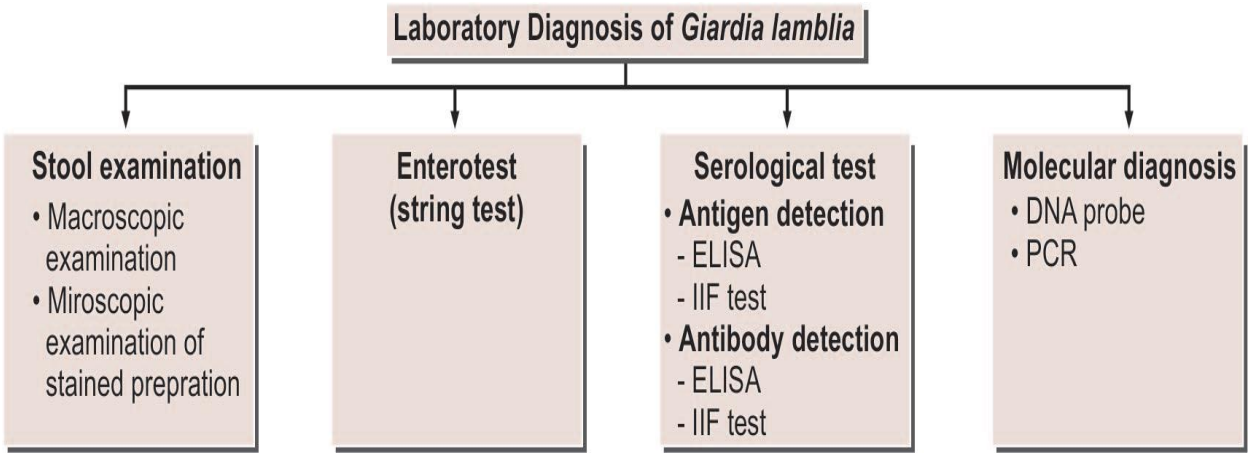


1. Microscopic examination

Detection of cysts and trophozoites in stools by direct saline, iodine wet preparations and use of concentration technique like formal ether. Often, multiple stool specimens need to be examined. In asymptomatic carriers, only the cysts are seen. Fixed stool smear can be stained with trichrome to identify cysts and trophozoites.

2- serological test such as ELISA

3. Molecular diagnosis PCR on stool specimen.



Hymenolepis nana

Common name: Dwarf tape worm

Only tapeworm In human has direct life cycle. And in rodents in direct life cycle

Infective stage . cysticercoid larva

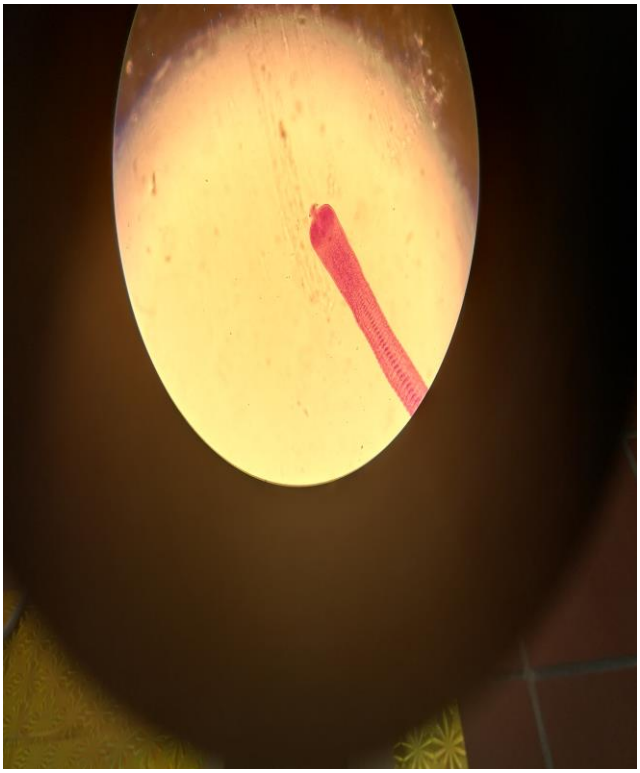
Site of infection small intestine

Morphology of H.nana Adult worm are only 10-45 mm long and 0.5- 1 mm wide They have 100-200 segments that are wider then they are long

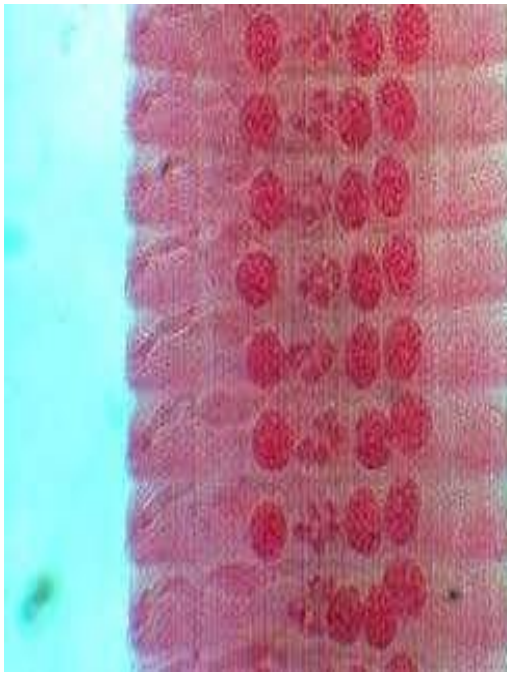
Scolex is small, globular (rounded), cup-like has four suckers and retractile rostellum with a single row of 20–30 hooks.

Neck is long and slender. Segment wider than long. Genital pores are unilateral (side of the segment), Each segment contains 3 testes and a single ovary. Hymenolepis nana hermaphroditic. Uterus persist as sac lik

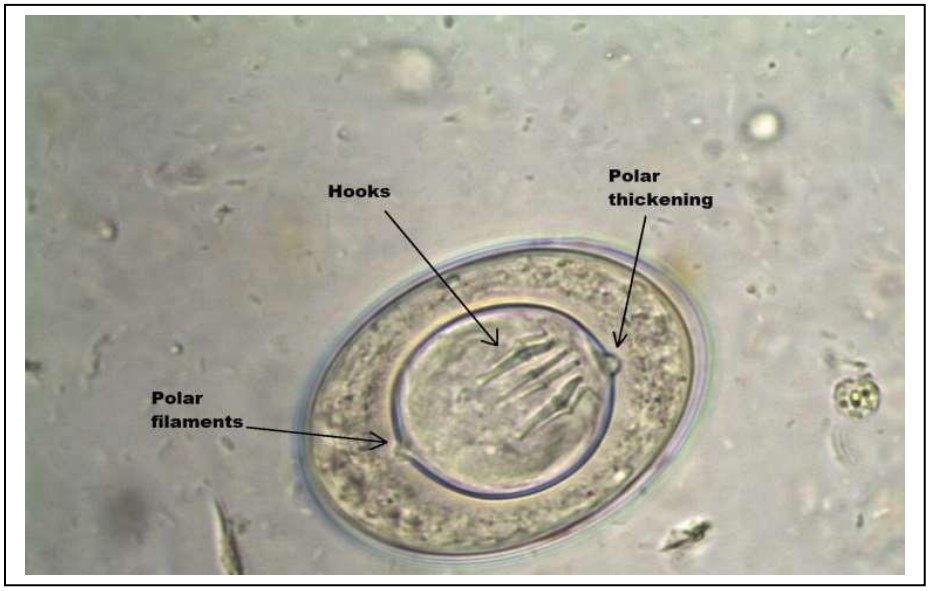
Scolex of H.nana



Mature segment



H. nana egg is colourless, almost transparent, round to oval , Shell consists of two distinct membranes between it polar filaments. they are embryonated and have a 6-hooked oncospheres inside the shells.. On inner membrane there are two small "knobs" or poles from which 4–8 filaments arise and spread out between the two membranes.



Symptoms are:

-Those who have symptoms may experience nausea, weakness, loss of appetite, diarrhea, and abdominal pain. headache, or have difficulty sleeping.

Diagnosis

1- Microscopic examination of stool the diagnosis is based on the observation of eggs in the stool
2- Serology ,indirect hemagglutination, ELISA

3- polymerase chain reaction (PCR) tests

Leishmania:

Final host : humans and animals (especially dogs)

Site of infection macrophage , bone marrow , spleen

Indirect life cycle

Intermediate host Vector sand flay

Infective stage promistogoit

The genus Leishmania belongs to a family Trypanosomatidae (order Kinetoplastida). Leishmaniasis is a vector-borne zoonotic disease caused by obligate intracellular parasitic protozoa of the genus Leishmania.

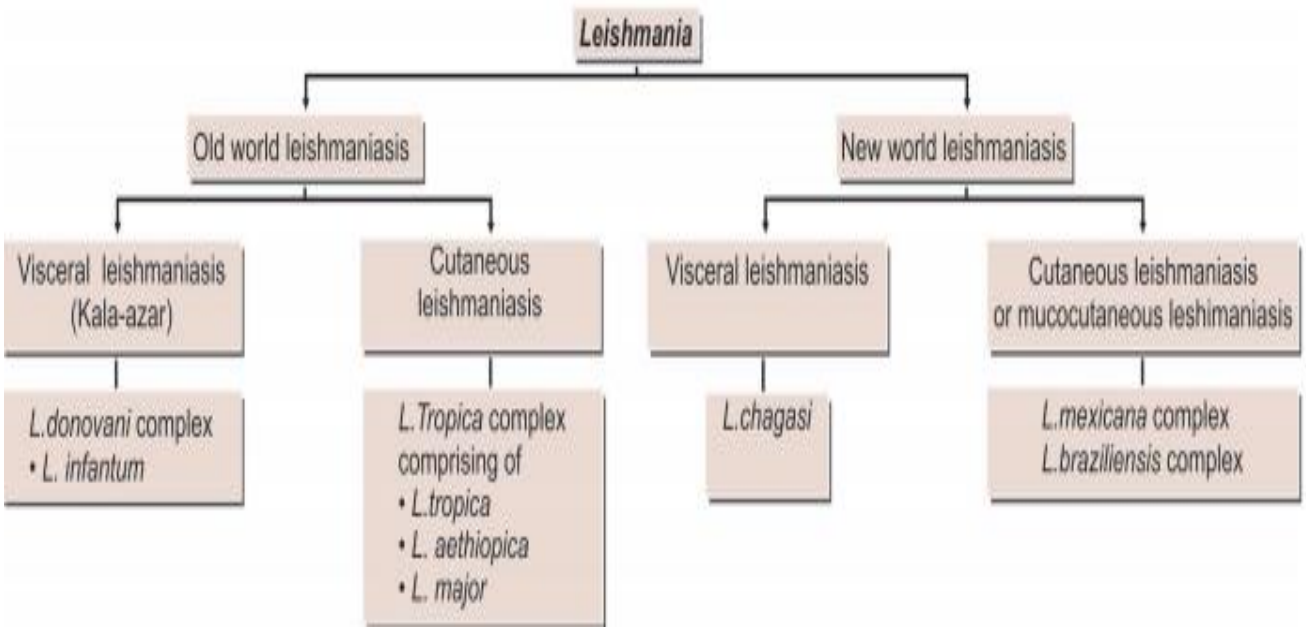
Leishmania infection is transmitted to humans and to other mammals by the bite of an infected sand Fly vector. The infection can rarely be transmitted by other means such as blood transfusions, needle sharing, or from mother to child during pregnancy.

Leishmaniasis is divided in two form according distribution

1- Old word Leishmaniasis 2- New word Leishmaniasis

Leishmaniasis is divided in three form according lesion

1- cutaneous, 2- muco-cutaneous 3- visceral leishmaniasis.



The causative agent:

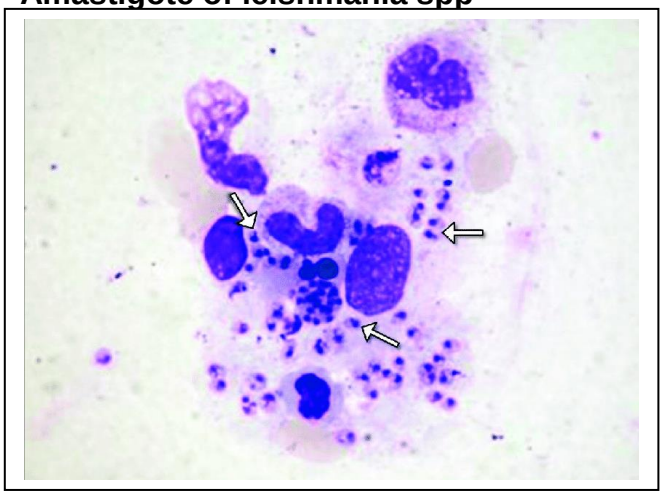
Leishmania species	Disease form in humans	Geographical distribution	Vectors
Leishmania aethiopica@	Localised cutaneous leishmaniasis,	Ethiopia, Kenya	*Phlebotomus*
L. major@	Localised cutaneous leishmaniasis	North Africa, Middle East and Central Asia, Sub- Saharan Africa and Sahel belt,Sudan, North India, Pakistan	*Phlebotomus*
L. mexicana*	Localised cutaneous leishmaniasis	Central America	*Lutzomyia*
*L.amazonensis**	Localised cutaneous leishmaniasis	South America, north of the Amazon	*Lutzomyia*
*L.braziliensis**	Localised cutaneous leishmaniasis and Mucocutaneous leishmaniasis	South America, and Central America and Mexico	*Psychodopygus* and *Lutzomyia spp.*
L. infantum@	Visceral leishmaniasis; Localised cutaneous leishmaniasis	Mediterranean basin; Middle East and Central Asia to Pakistan; China; Central and South America, southern Europe, northwest Africa	*Phlebotomus*
L. donovani@	Visceral leishmaniasis	Ethiopia, Sudan, Kenya, India, China, Bangladesh, Burma	*Phlebotomus*
@Old world species *New world species			

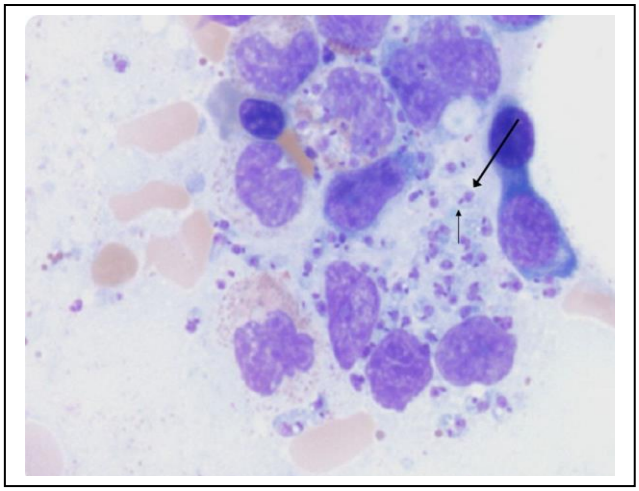
Morphology of the parasite

Leishmania are **two forms**: **Amastigote form**: Amastigotes are ovoid and **non flagellated** form of Leishmania, measuring 3-5 µm in length. On simple light microscopy, a central round or oval nucleus and adjacent but smaller round or rod shaped **kinetoplast** can be discovered. An in folding of the surface membrane creates an internal space, termed as 'flagellar pocket'. **The flagellum is not functional** in amastigotes and does not extend beyond the cell body. Immediately below the origin of the flagellum lies a dense mass of mitochondrial DNA known as kinetoplast.

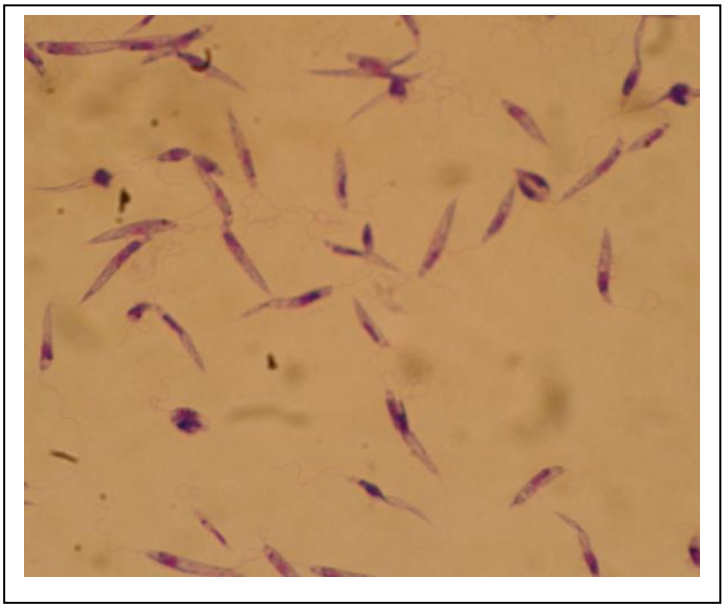
Promastigote form: In the sand fly host the parasite is found in the promastigote form. The mature metacyclic promastigotes are accumulated in the midgut and foregut. The main difference from amastigotes is that the cell body is elongated, in the range of 8-15 µm, the flagellum emerges from the cell body, and is functional, making these cells motile.

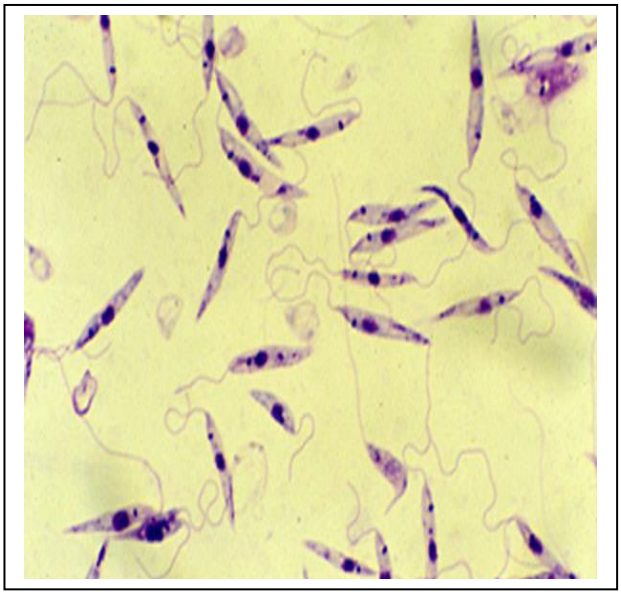
Amastigote of leishmania spp





Promastigote of leishmania spp..





The disease has three main forms, depending on the parasite species:

Cutaneous leishmaniasis

produces skin lesions mainly on the face, arms and legs

this form is often self-healing, it can create serious disability and permanent scars. After recovery cutaneous leishmaniasis induces immunity to re-infection by the species of Leishmania that caused the disease.

The disease is caused mainly by **L. major, L. tropica and L. athtiubica .** The main vectors for cutaneous leishmaniasis are **Phlebotomus sergenti** and **Ph. papatasi**.

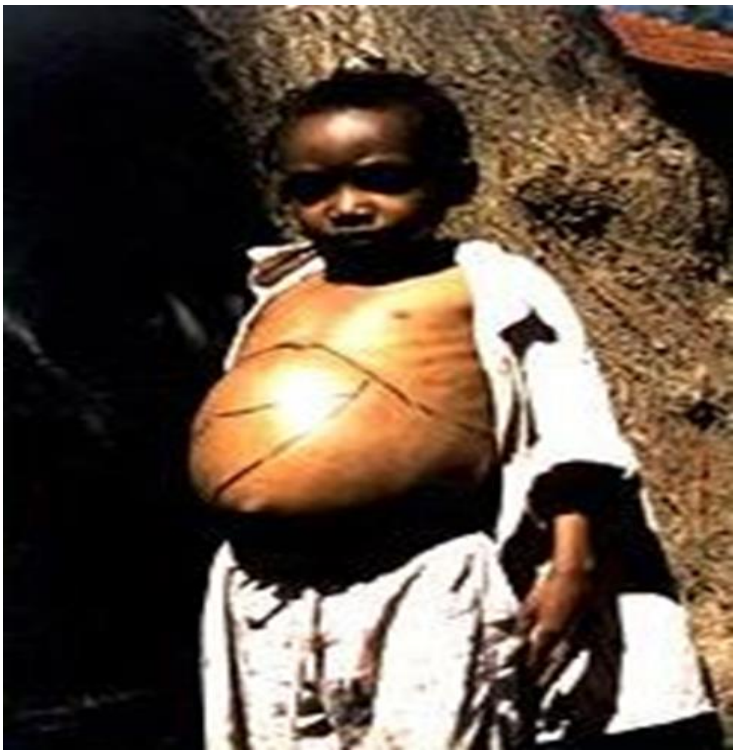
Mucocutaneous leishmaniasis, also called **'espundia'** in South America, causes disfiguring lesions to the face; it destroys the mucous membranes of the nose, mouth and throat. Reconstructive surgery of deformities is an important part of therapy.

Visceral leishmaniasis, **also known as 'kala azar', or Dumdum fever**

is characterized by irregular fever, weight loss, swelling of the liver and spleen and anaemia. It is the most severe form of Leishmaniasis, and is usually fatal if left untreated. The incubation period can be months or years and, unlike the cutaneous forms of leishmaniasis, it involves the internal organs. It is highly endemic in the **Indian and East Africa**. It is mainly caused by **L. infantum** in Europe, where cases have been reported by countries in the southern and western parts, the Balkan region, central Asia and southern Caucasus.

cutaneous leishmaniasis

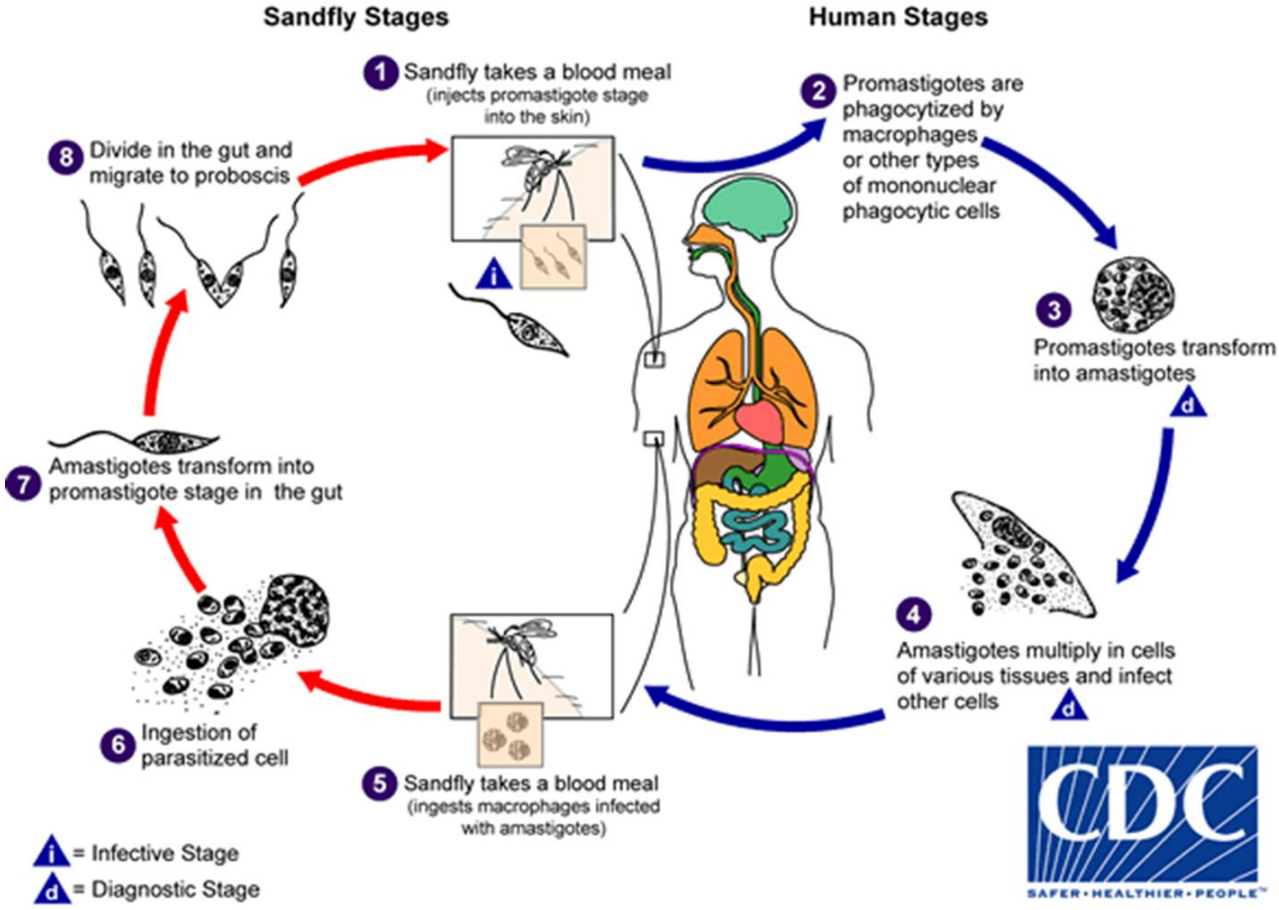
Mucocutaneous Leishmaniasis



Visceral Leishmaniasis

Life cycle:

Leishmaniasis is transmitted by the bite of infected female phlebotomine sand flies. The sand flies inject the infective stage (i.e., promastigotes) from their proboscis during blood meals. (1)Promastigotes that reach the puncture wound are phagocytized by macrophages (2). and other types of mononuclear phagocytic cells. Promastigotes transform in these cells into the tissue stage of the parasite (i.e., amastigotes)(3). which multiply by simple division and proceed to infect other mononuclear phagocytic cells (4). Parasite, host, and other factors affect whether the infection becomes symptomatic and whether cutaneous or visceral leishmaniasis results. Sand flies become infected by ingesting infected cells during blood meals (5) and (6). In sand flies, amastigotes transform into promastigotes, develop in the gut (7). (in the hindgut for leishmanial organisms in the Viannia subgenus; in the midgut for organisms in the Leishmania subgenus), and migrate to the proboscis (8).



DiagnosisAmastigotes are demonstrated in smears taken from lesions of skin and mucous membrane. L mexicana amastigotes are larger than those of L braziliensis and their kinetoplast is more centrally placed.

For microscopic demonstration of the parasite, the materials collected are:

€ Peripheral blood

€ Bone marrow

€ Splenic aspirate

€ Enlarged lymph node.

- The smears are stained by Leishman, Giemsa, or Wright's stains and examined under oil immersion objective.
- Amastigote parasite can be seen within the macrophages, often in large numbers. A few extracellular forms can also be seen.

€ **- Examination of a thick blood film.**

€ - Examine buffy coat smear,

€ - Bone marrow aspirate: Bone marrow aspirate is the most common diagnostic specimen collected. the sternal marrow is aspirated by puncturing the sternum at the level of the 2nd or 3rd intercostal space, using a sternal puncture needle.

€ This consists of a short stout needle with a stylet. It has a movable guard, which is fixed at 1–2 cm from the tip, depending on the thickness of the chest wall over the sternum. After disinfecting and anesthetizing the skin, the needle is introduced into the sternal marrow and about 0.5 mL of marrow fluid is aspirated using a syringe. The puncture wound is sealed with celloidin or tincture benzoin. Bone marrow samples can also be obtained by puncturing the iliac crest.

Splenic aspirates:

€ Splenic aspirates are richer in parasites and therefore, are more valuable for diagnosis. But, the procedure can sometimes cause dangerous bleeding and therefore, should be done carefully and only when a marrow examination is inconclusive.

Lymphnode aspirates:

€ Lymphnode apsirates are not useful in the diagnosis of Indian Kala-azar, although it is employed in visceral leishmaniasis in some other countries.

Culture

Culturing material obtained from ulcers in NNN medium demonstrates promastigote. Different tissue materials or blood are cultured on 3 NNN (Novy, Neal and Nicolle) medium. This is a rabbit blood agar slope consisting of 2 parts of salt agar and 1 part of defibrinated rabbit blood. The material is inoculated into the water of condensation and culture is incubated at 22°–24°C for 1–4 weeks. At the end of each week, a drop of culture fluid is examined for promastigotes under high power objective or phase contrast illumination (Fig. 5.12). Other biphasic medium, like Schneider's drosophila tissue culture medium with added fetal calf serum can also be used.

Animal inoculation

Animal inoculation is not used for routine diagnosis.

The material is inoculated intraperitoneally

The inoculated animals are kept at 23°–26°C. In positive cases, the amastigote can be demonstrated in smears taken from ulcers or nodules developing at the sites of inoculation or from the spleen

Animal inoculation is a very sensitive method, but takes several weeks to become positive.

Serodiagnosis

Detection of antigen: The concentration of antigen in the serum or other body fluids is very low. ELISA developed for detection of leishmanial antigen.

Detection of antibodies: CFT was the first serological test used to detect serum antibodies in visceral leishmaniasis. The antigen originally used, was prepared from human tubercle bacillus by Witebsky, Kliengenstein, and Kühn (hence, called WKK antigen).

CFT using WKK antigen becomes positive early in the disease, within weeks of infection. Specific leishmanial antigens prepared from cultures

have been used in a number of tests to demonstrate

specific antibodies. These tests include:

ˆ Indirect immunofluroscent antibody test (IFAT)

ˆ Counter immunoelectrophoresis (CIEP)

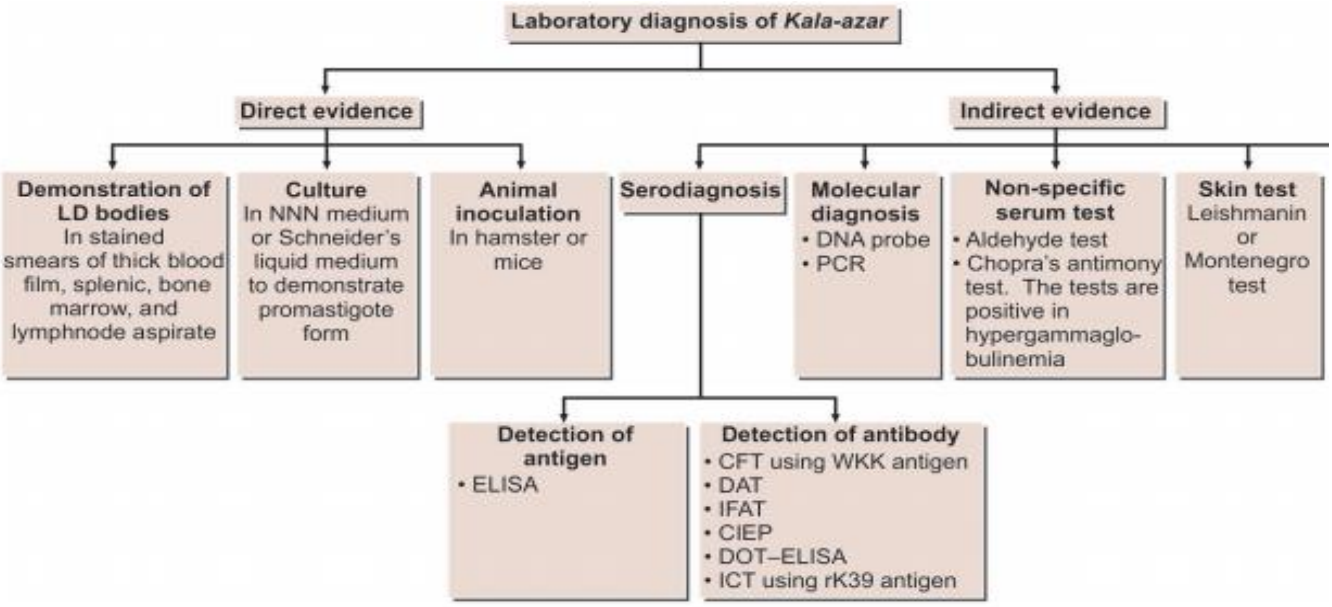
ˆ ELISA and DOT-ELISA

Leishmanin skin test (Montenegro test):

€ It is delayed hypersensitivity test. This was first discovered by Montenegro in South America and hence, named after him. € 0.1 mL of killed promastigote suspension (106 washed

promastigotes/mL) is injected intradermally on the dorsoventral aspect of forearm. Positive result is indicated by an induration and erythema of 5 mm or more after 48–72 hours. Positive result indicates prior exposure to leishmanial parasite. In active Kala-azar, this test is negative and becomes positive usually 6–8 weeks after cure from the disease.

Molecular diagnoss: PCR



Naegleria Fowleri

It is the only species of genus Naegleria, which infects man. N. fowleri causes the disease **primary amoebic meningi encephalitis** (PAM), a brain infection that leads to destruction of brain tissue.

History and Distribution

N. fowleri is a heat-loving (thermophilic) amoeba that thrives in warm water at low oxygen tension and is commonly found in warm freshwater (e.g. lakes, rivers, and springs) and soil.

Morphology

N .fowleri occurs in 3 forms:

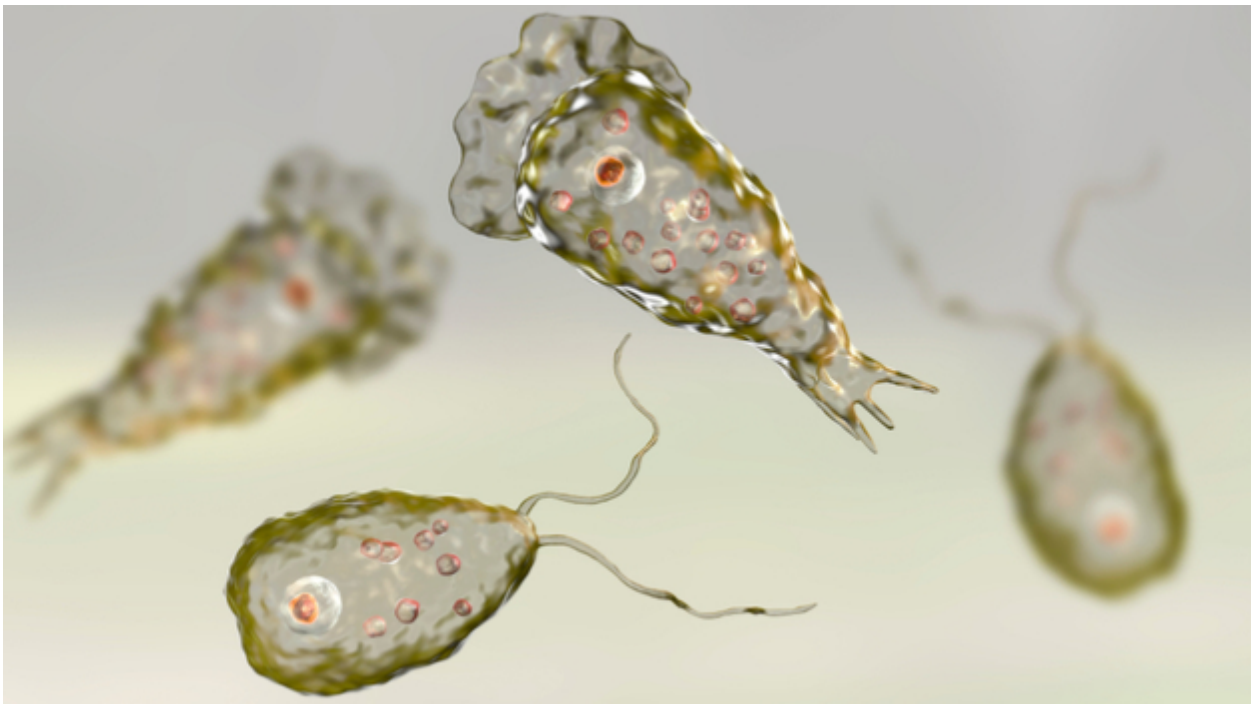
Cyst , **Amoeboid trophozoite form** and **Flagellate trophozoite form**.

Amoeboid form

The amoeboid form is about 10–20 µm, showing rounded pseudopodia, a spherical nucleus and pulsating vacuoles. . Amoeboid form is the feeding, growing, and replicating form of the parasite, seen on the surface of vegetation, mud, and water. It is the **invasive stage** of the parasite and the **infective form** of the parasite.

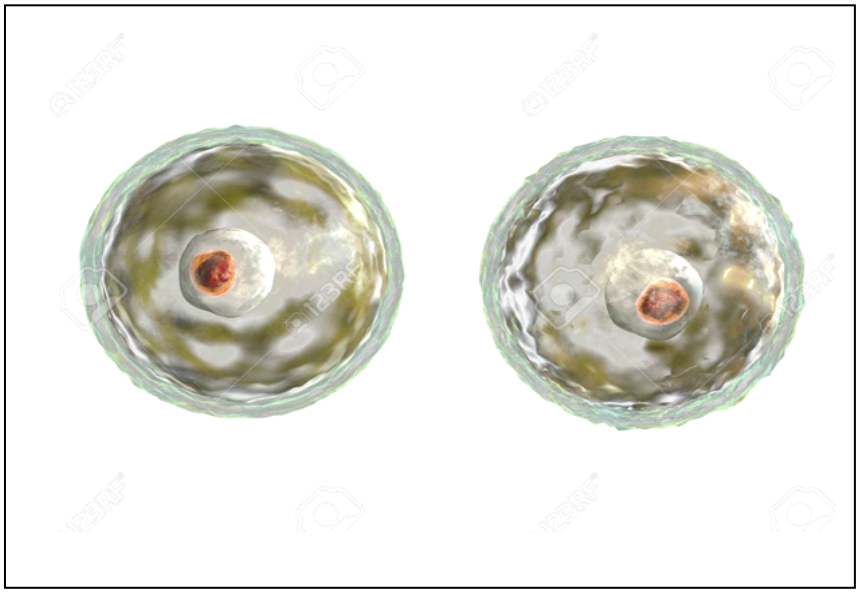
Flagellate form

The biflagellate form occurs when trophozoites are transferred to distilled water. This transformation of trophozoites to biflagellate pear-shaped form occurs within a minute. The flagellate can revert to the amoeboid form, hence N. fowleri is classified as amoeboflagellate.



Cyst Stage Trophozoites encyst due to unfavorable conditions like food deprivation, desiccation, cold temperature, etc.

The cyst is 7–10 µm in diameter and has a smooth double wall. They are the resting or the dormant form and can resist unfavorable conditions. Cysts and flagellate forms of N. fowleri have never been found in tissues of cerebrospinal fluid (CSF).



Life Cycle

- Typically, infection occurs when people go swimming in warm freshwater river or ponds and poorly- maintained swimming pools or nasal irrigation using contaminated tap water
- The life cycle of N. fowleri is completed in the external environment.
- The amoeboid form of trophozoite multiplies by binary fission.
- Under unfavorable conditions, it forms a cyst and which undergoes excystation in favorable conditions.
- Flagellate form of trophozoite helps in the spread of N. fowleri to new water. Since the amoeboid form is the invasive stage, hence, the flagellate forms revert to amoeboid forms to become infective to man.

Clinical Features

The amoebae invade the nasal mucosa and pass through the olfactory nerve branches into the meninges, and brain to initiate an acute purulent meningitis and encephalitis, called as **primary amoebic meningoencephalitis** (PAM) .

The incubation period varies from 2 days to 2 weeks.

In the incubation period, the patient experiences **anosmia**. The disease advances rapidly, causing fever, headache, vomiting, **stiff neck**, ataxia, seizure, and coma. Cranial nerve palsies, especially of the third, fourth, and sixth nerves have also been documented. The disease almost always ends fatally within a week (average 5 days).

Laboratory Diagnosis

- The diagnosis of PAM is based on the finding of motile Naegleria trophozoites in wet mounts of freshly-obtained CSF.
- Wet film examination of CSF may show trophozoites.
- Cysts are not found in CSF or brain.
- At autopsy, trophozoites can be demonstrated in brain histologically by immunofluroscent staining.
- Culture N. fowleri can be grown in several kinds of liquid axenic media or non-nutrient agar plates. Both trophozoites and cysts occur in culture.
- Molecular Diagnosis Newer tests based on polymerase chain reaction (PCR) technology are being developed.

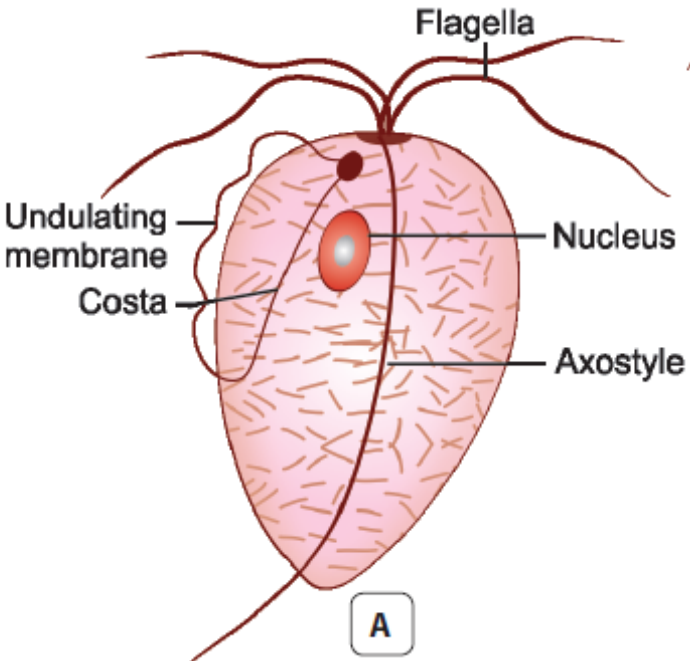
Trichomonas vaginalis

Distribution

It is distributed worldwide.

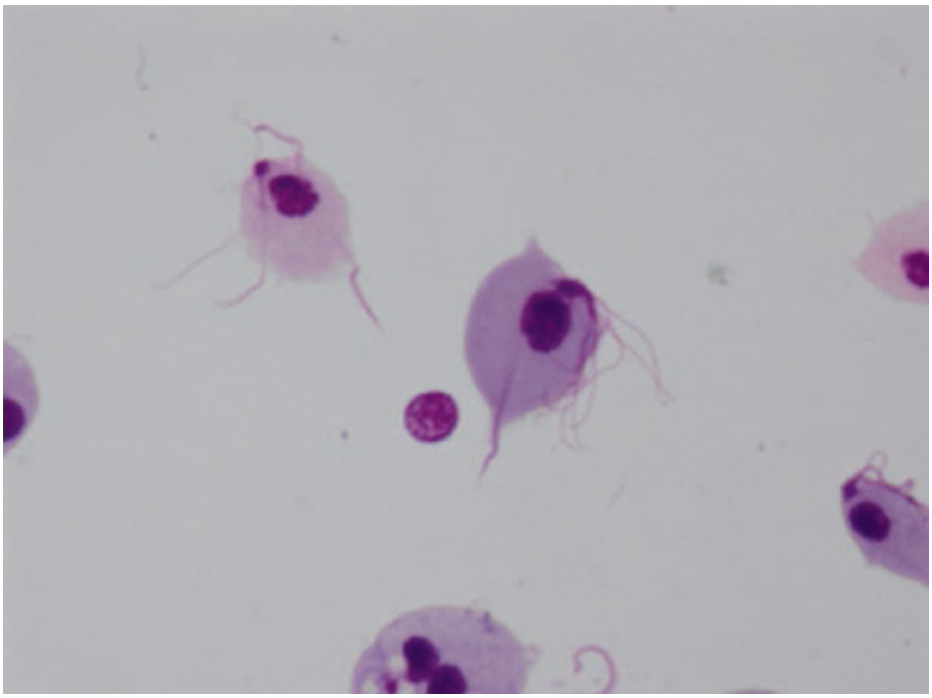
Morphology

It only exists in the trophozoite stage. It is pear-shaped or ovoid. with a short undulating membrane reaching up to the middle of the body. it has four anterior flagella and fifth running along the outer margin of the undulating membrane, A prominent axostyle runs throughout the length of the body and projects posteriorly like a tail. It is motile with a rapid jerky or twitching type movement.



Habitat

In females, it lives in vagina and cevix and may also be found in Bartholin's glands, urethra, and urinary bladder. In males, it occurs mainly in the anterior urethra, but may also be found in the prostate and preputial sac.



Trichomonas vaginalis trophozoites

Life Cycle

Trophozoites live in the vagina and cervix and may also be found in Bartholin's glands, urethra and urinary bladder in females. In males, it occurs mainly in the anterior urethra, but may also be found in the prostate. Trophozoites multiply by longitudinal binary fission. Trophozoites in vagina or orifice of urethra can be found in the vaginal and prostatic secretions and urine Life cycle of T. vaginalis is completed in a human host. There is no cystic stage. The trophozoite is transmitted directly from person to person. Sexual transmission is the usual mode of infection. Trichomoniasis often coexists with other sexually transmitted diseases; like candidiasis, gonorrhoea, syphillis, or human immunodeficiency virus (HIV). Babies may acquire infection during birth from infected mothers. Fomites such as towels have been implicated in transmission.

Pathogenesis and Clinical Features

Trichomonas vaginalis infects the vagina and secretes **cysteine, proteases, lactic acid and acetic acid**,

which disrupt the glycogen levels and lower the pH of the vaginal fluid. Trophozoite does not invade the vaginal mucosa. The infection can range from mild irritation to severe inflammation.

Infection is often asymptomatic, particularly in males, although sometime may develop urethritis, epididymitis and prostatitis.In

females, it may produce severe itching in the genital area with **foul smelling** ,yellowish green frothy discharge, dysuria, burning sensation with urination . Cervical erosion is common. The incubation period is 4 days to 4 weeks.

Diagnosis

1. Microscopic examination

Vaginal or urethral discharge is examined microscopically in saline wet mount preparation for the motility of the trophozoite. In males, trophozoites may be found in urine or prostatic secretions. Fixed smears can be stained with Giemsa stains. Direct fluorescent antibody (DFA) is another method of parasite detection.

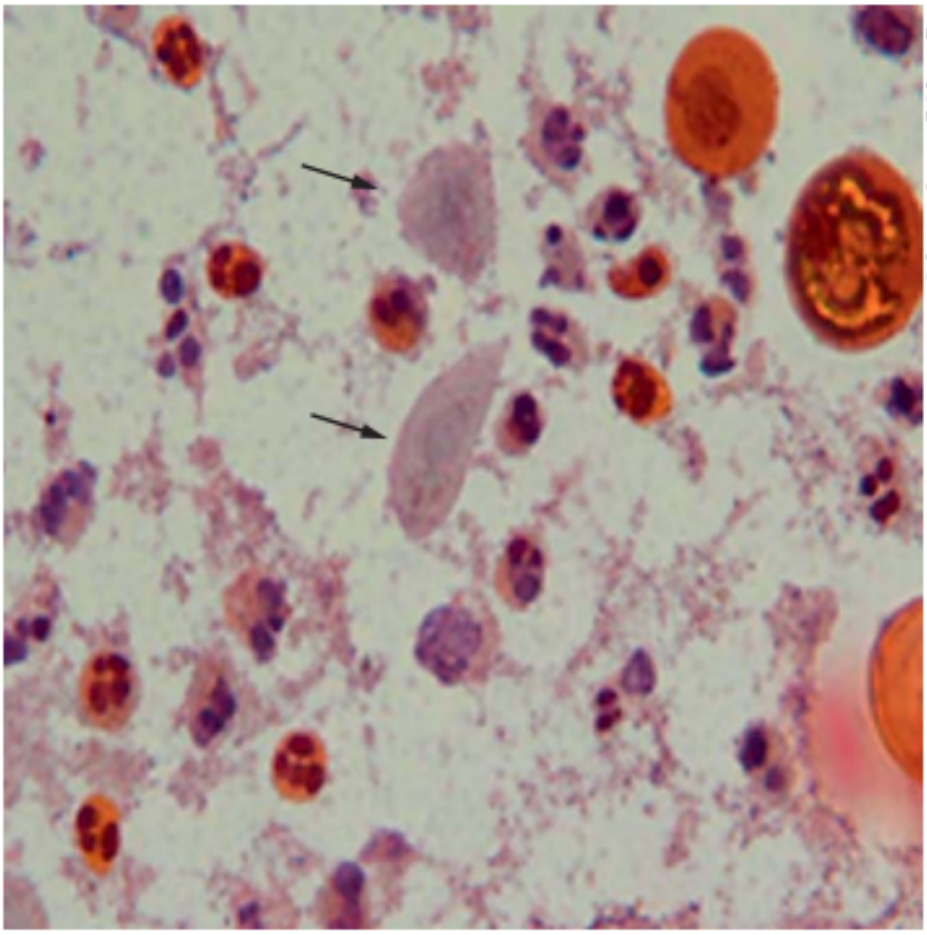
2. Culture

Culture is recommended when direct microscopy is negative and is considered as a 'gold standard' as well as the most sensitive (95%) method for the diagnosis of T. vaginalis infection. It grows best at 35°–37°C under anaerobic conditions. The optimal pH for growth is 5.5–6.0. It can be grown in a variety of solid or liquid media, tissue culture, and eggs. Cysteine-peptone-liver-maltose (CPLM) medium and plastic envelope medium (PEM) are often used.

Papanicolaou method (pap)

Each specimen was smeared on a clean slide and fixed in ether-alcohol for 30 minutes.

- The specimens were then stained by the Papanicolaou method as follows:
- Harris's haematoxylin without acetic acid for 5 minutes,
- rinsed in tap water
- differentiated in 1% acid alcohol for 30 seconds and water for 2 minutes.
- Smears were taken to 95%alcohol
- stained in Orange 6 for 2 minutes,
- rinsed in 95% alcohol
- stained in Eosin 35 for 2minutes.
- Smears were then taken to two changes of absolute alcohol, xylene and mounted in DPX.
- The stained smears were examined under the light microscope at low and high power objectives for the presence of T.vaginalis. The stained slides had been examined using the microscope (×10 objective), for a general view, and then with ×40 objective for morphological details identification.



Cervical smear of T. vaginalis (arrows) . Pap stain. ×400.

Trichomonas Tenax

T. tenax, also known as T. buccalis, is a harmless commensal which lives in mouth-in the periodontal pockets, carious tooth cavities, and less often in tonsillar crypts.

It is smaller (5–10 μm) than T. vaginalis.

It is transmitted by kissing, through salivary droplets, and fomites. There are sporadic reports of its involvement in respiratory infections and thoracic abcesses.

Better oral hygine rapidly eliminates the infection and no therapy is indicated.

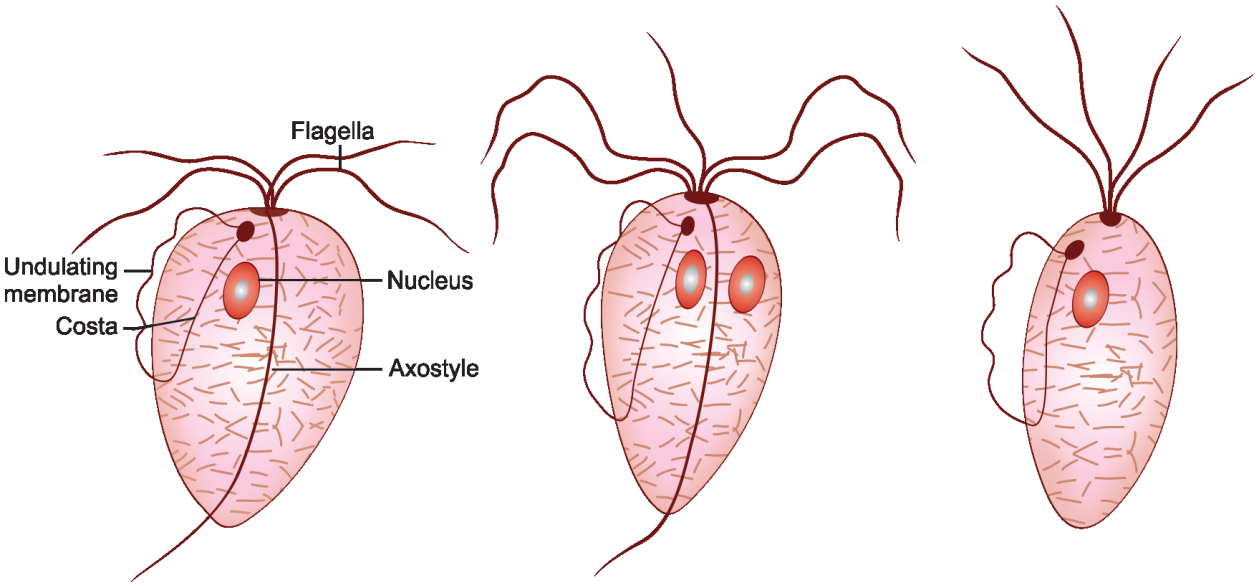
Trichomonas Hominis

T. hominis measures 8–12 μm, pyriform-shaped, and carries 5 anterior fl agella and an undulating membrane that extends the full length of the body.

It is a very harmless commensal of the caecum

Microscopic examination of stool will reveal motile trophozoite of T. hominis.

Transmission occurs in trophic form by fecal-oral route



Trichomonas species. A. T. vaginalis; B. T. hominis; C. T. tenax

Balantidium Coli

 It is the **only ciliate protozoan** parasite of humans

 It is the **largest protozoan** parasite of humans.

Habitat B. coli resides in the **large intestine** of man, pigs, and monkeys.

cyst is the infective stage

Morphology

B. Coli occurs in 2 stages – **trophozoite and cyst**

Trophozoite

 The trophozoite is actively motile and is invasive stage of the parasite

  large ovoid cell measuring up to 200 µm are sometimes seen.

 anterior end is narrow and posterior end is broad. At the anterior end, there is a groove (peristome) leading to the mouth (cytostome), and a short funnel shaped gullet (cytopharynx).

 Posteriorly, there is a small anal pore (cytopyge).

 The cilia around the mouth are larger (adoral cilia).

 The motility of trophozoite is due to the presence of short delicate cilia over the surface of the body.

  has 2 nuclei—a large kidney-shaped macronucleus and lying in its concavity a small micro nucleus.

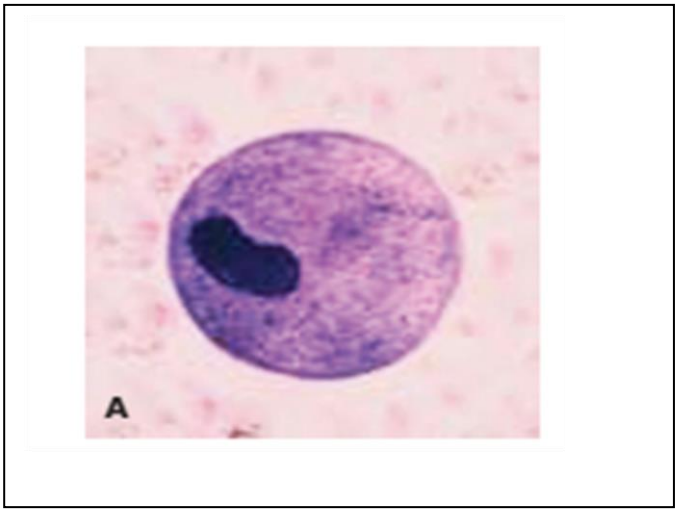
 The cytoplasm has 1 or 2 contractile vacuoles and several food vacuoles.

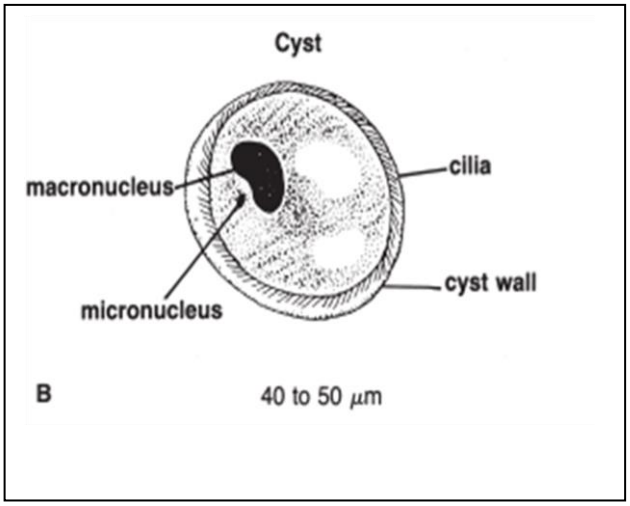
Cyst

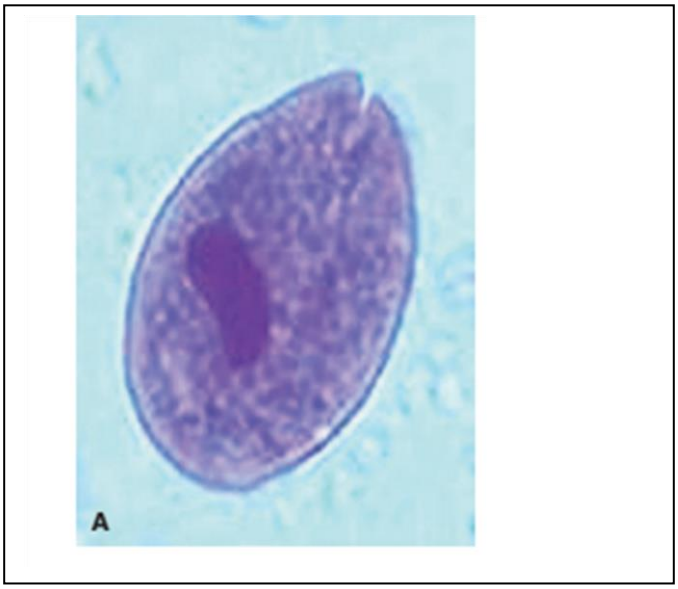
The cyst is spherical in shape and measures 40–60 µm in diameter.

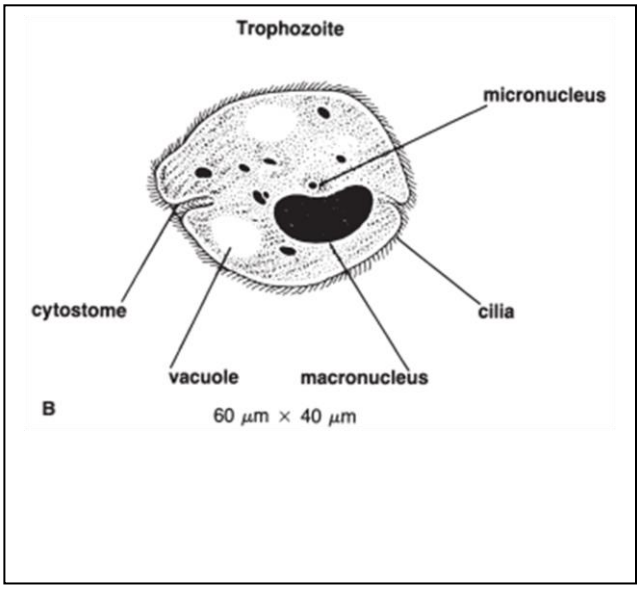
 It is surrounded by a thick and transparent double layered wall.

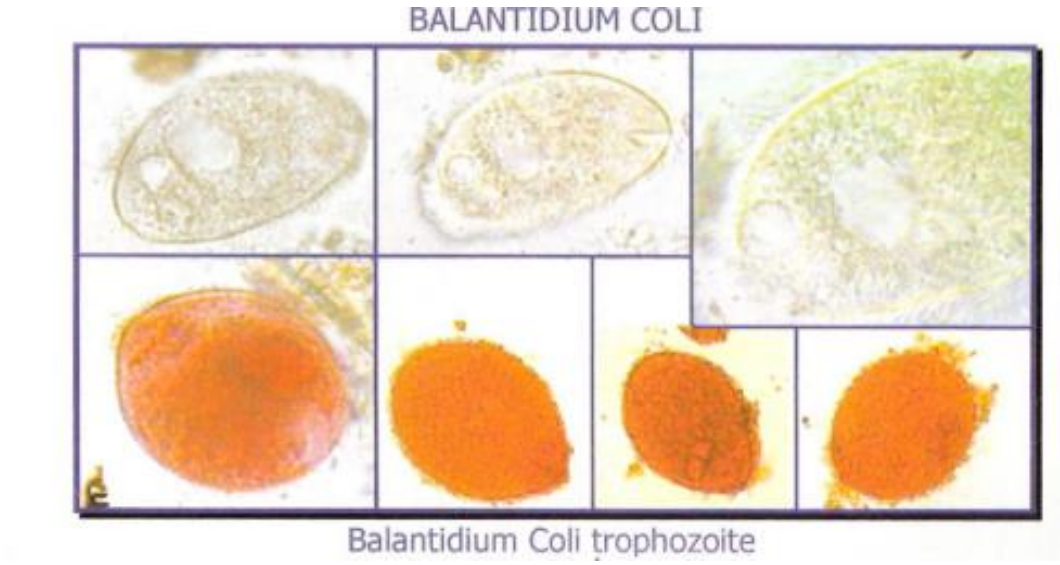
 The cytoplasm is granular. Macronucleus, micro nucleus, and vacuoles are also present in the cyst.



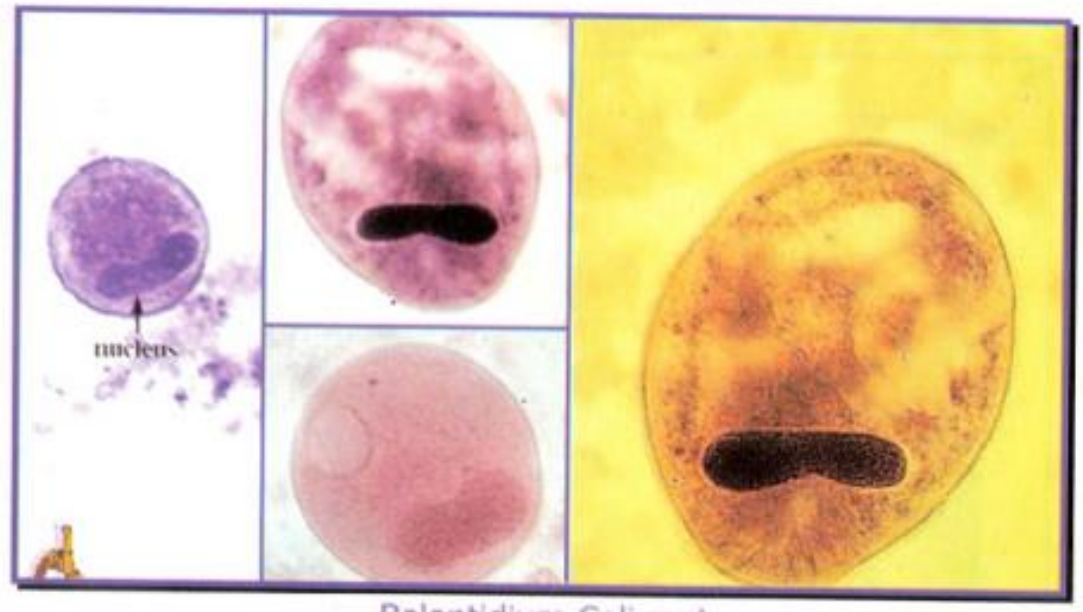








Balantidium Coli trophozoite



Balantidium Coli cyst

Life Cycle

B. coli passes its life cycle in one host only (**direct life cycle**).

Natural host: Pig.

Accidental host: Man.

Infective form: Cyst.

Mode of transmission: Balantidiasis is a zoonosis. Human beings acquire

infection by ingestion of food and water contaminated with feces containing the cysts of B. coli.

Clinical Features

Most infections are asymptomatic.

 Symptomatic disease or balantidiasis resembles amoebiasis causing diarrhea or dysentery with abdominal colic, tenesmus, nausea, and vomiting.

Laboratory Diagnosis of Stool Examination

Diagnosis of B. coli infection is established by demonstration of trophozoites and cysts in feces.

 Motile trophozoites occur in diarrheic feces and cysts are found in formed stools.

Biopsy

When stool examination is negative, biopsy specimens and scrapings from intestinal can be examined for presence of trophozoites and cysts.

Culture

B. coli can also be cultured in vitro in **Locke's egg albumin** medium or **NIH polyxenic medium** like Entamoeba histolytica,

ISOSPORA BELLI: completes its life cycle in one host**. (direct)**

Host: human

Infective stage the **sporulated oocyst**

Site of infection: small intestine

Mode of transmission: Man gets infection by ingestion of food and water contaminated with sporulated oocyst.

Morphology

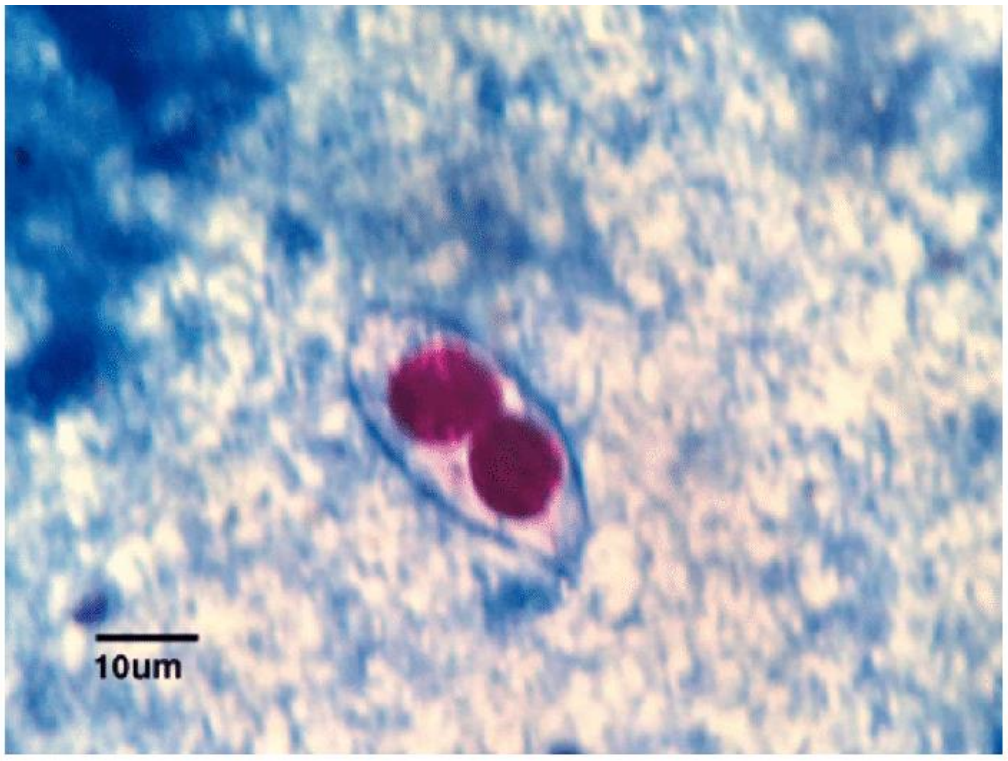
Oocysts of I. belli are elongated-ovoid and measure 25 µm × 15 µm.

Each oocyst is surrounded by a thin smooth 2 layered cyst wall

Immature oocyst seen in the feces of patients contain **two sporoblasts**.

The oocysts mature outside the body.

On maturation, the sporoblast convert into **sporocysts**. Each sporocyst contain **4 crescent-shaped sporozoites**



Clinical Features Infection is usually asymptomatic.

Clinical illness includes abdominal discomfort, mild fever, diarrohea, and malabsorption. **The diarrohea is usually watery** and does not contain blood or pus and is self-limiting.

Laboratory Diagnosis of Stool Examination

It may be difficult to demonstrate the transparent oocyst in saline preparation of stool.

Stool concentration techniques**(flotation and sedimentation)** may be required when direct wet mount of stools are negative.

The staining technique used are **Modifi ed Ziehl-Neelsen** stain or Kinyoun acid fast staining of stool smear. **In these methods, pink** colored acid fast large oocyst (>25 µm) can be demonstrated.

The stool smear can also be stained by auramine rhodamine and Giemsa stains.

Malaria

Malaria is the most important tropical disease known to man. It remains a significant problem in many tropical areas, especially in sub-Saharan Africa.

Malaria is caused by the **Genus: Plasmodium**.
There are **four species** which infect both **humans** and **animals**:
Causative agents of human malaria:

1. Plasmodium vivax

2. Plasmodium falciparum

3. Plasmodium malariae

4. Plasmodium ovale

Habitat

In human, the parasites are found in the **erythrocytes and hepatocytes**.

- ❖ They **live intracellularly**, at least during part of their life cycle.
- ❖ At some stage in their life cycle, they possess a structure called the apical complex, by means of which they attach to and penetrate host cells.
- ❖ These protozoa are therefore grouped under the Phylum Apicomplexa.

- **Genus: Plasmodium. Three different species of malaria parasite infecting man,**
  P. vivax, P. malariae, and P. falciparum

Vectors malaria is transmitted by over 60 species of **female Anopheles mosquito**.

Malaria life cycle:

Life Cycle

Malaria parasite passes its life cycle in 2 hosts. **(in direct)**

Definitive host: Female Anopheles mosquito.

Intermediate host: Man.

The life cycle of malarial parasite comprises of 2 stages— an asexual phase occurring in humans, which act as the intermediate host

sexual phase occurring in mosquito, which serves as a definitive host for the parasite .

infective forms: sporozoites

Clinical presentation or Clinical Features

- **Benign Malaria**
- consists of periodic bouts of fever with rigor, followed by anemia and splenomegaly. Severe headache, nausea, and vomiting are common.
- The 3 successive stages—
- **cold stage, hot stage, and sweating stage.**
- In the cold stage, that lasts for 15–60 minutes, the patient experiences intense cold and uncontrollable
- shivering. This is followed by the hot stage, lasting for 2–6 hours, when the patient feels intensely hot. The temperature mounts to 41°C or higher.
- Afterwards comes the sweating stage, when the patient is drenched in profuse sweat.

Malignant Tertian Malaria

- The most serious and fatal type of malaria is malignant tertian malaria caused by P. falciparum. When not treated promptly and adequately, dangerous complications develop.

.

Plasmodium vivax:

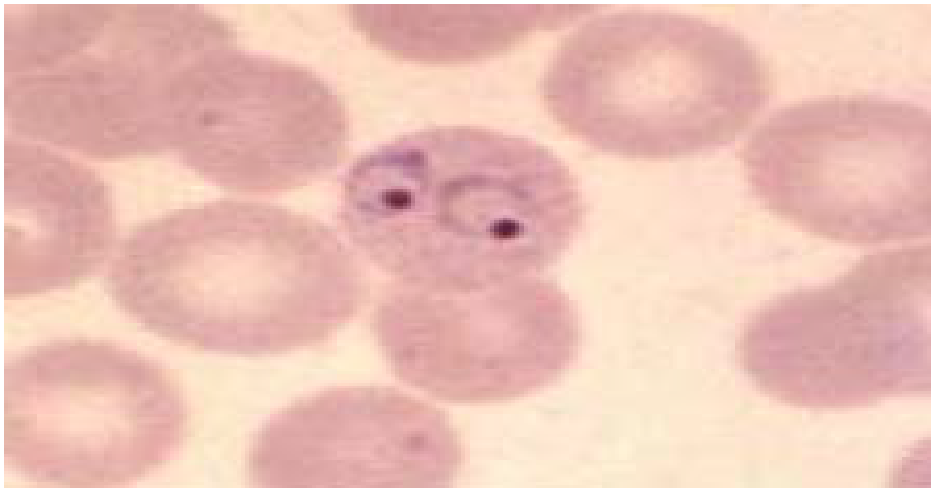


Figure 2: Plasmodium vivax Trophozoite.

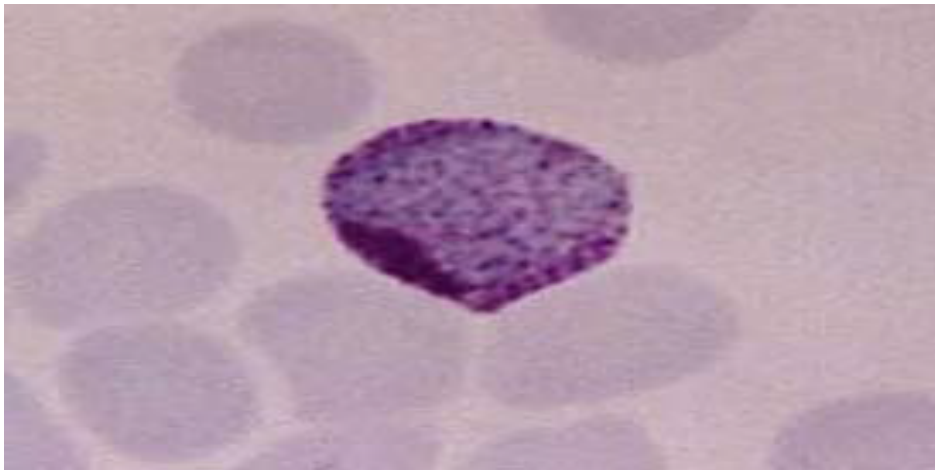


Figure 3: Plasmodium vivax Mature female gametocyte.

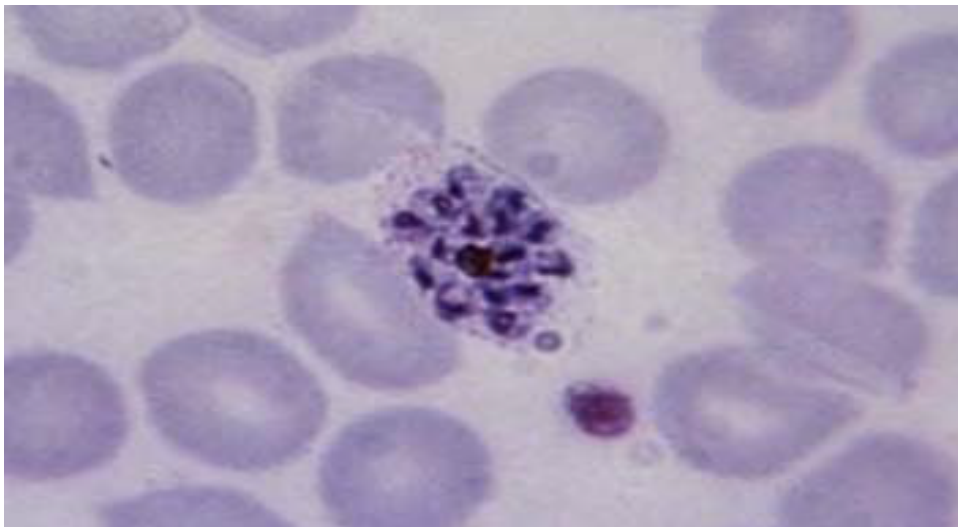


Figure 4: Plasmodium vivax schizont.

Plasmodium falciparum:

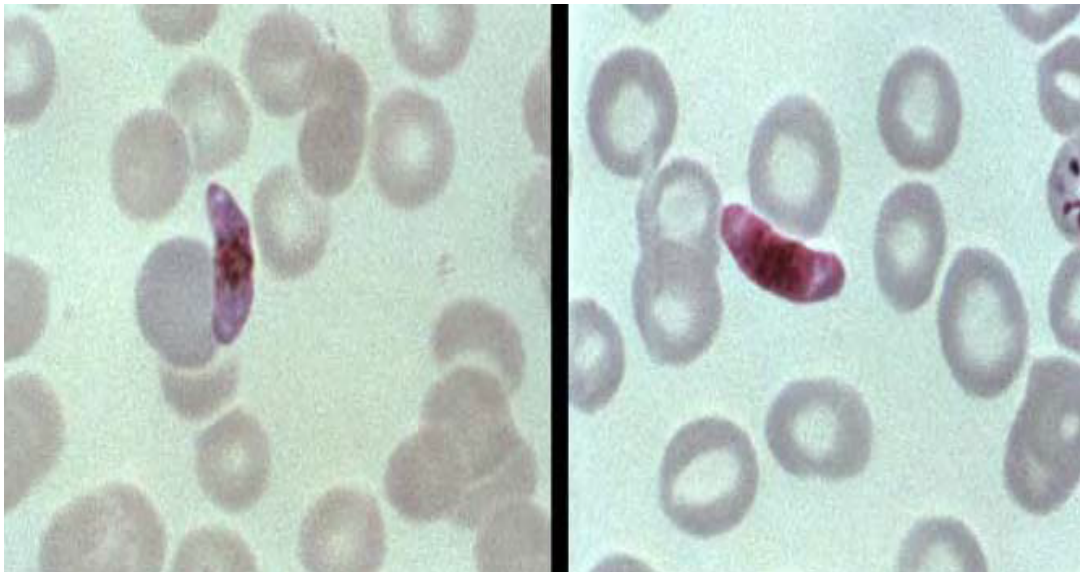


Figure 5: Plasmodium falciparum gametocytes.

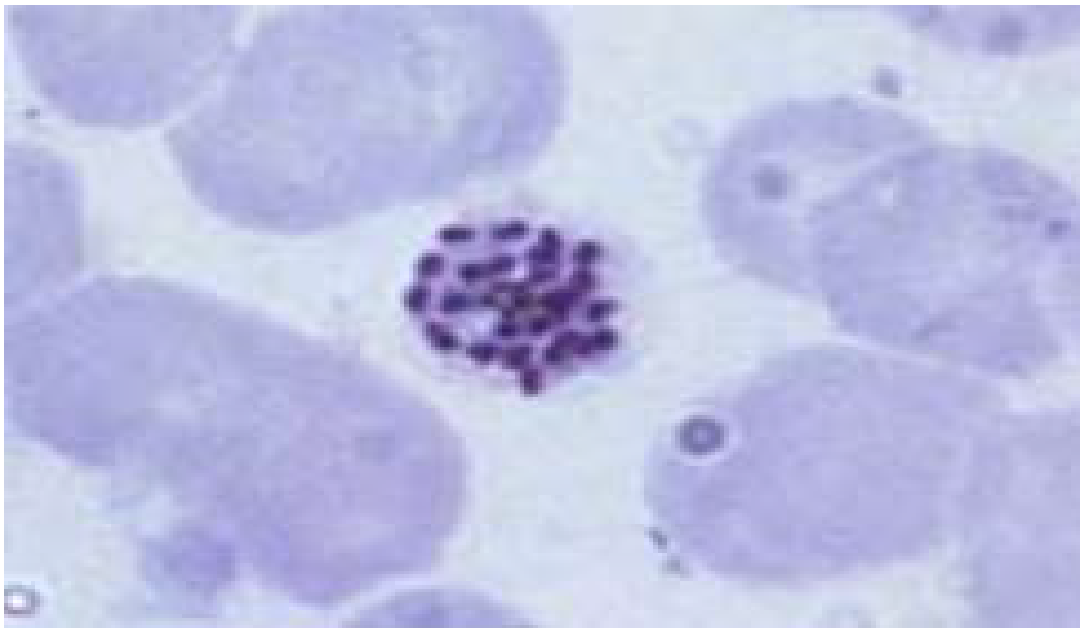


Figure 6:Plasmodium falciparum schizont.

Laboratory Diagnosis

Diagnosis of malaria can be made by demonstration of malarial parasite in the blood. Two types of smears are prepared from the peripheral blood. One is called **thin smear** and the other is **called thick smear**.

Thin smears: They are prepared from capillary blood of fingertip and spread over a good quality slide by a second slide held at an angle of 30°–45°. Thins smears are air dried rapidly, fixed in alcohol and stained by one of stains such as Leishman, Giemesa, Thins smears are used for detecting the parasites and determining the species.

Thick smears: drops of blood are spread over a small area (about 10 mm). The thick film is dried and It is not fixed in methanol. Thick film is stained similar to thin film. The stained film is examined under the oil immersion microscope. The thick film is more sensitive, when examined by an experienced person. Thick film is more suitable for rapid detection of malarial parasite, **particularly when they are few (**as low as 20 parasites/µL).

serodiagnosis

indirect hemagglutination (IHA),

indirect fluroscent antibody test (IFA), and

enzyme-linked immunosorbent assay (ELISA).

Molecular Diagnosis

Taeniasis

Taeniasis: is the parasitic infection of humans caused by adult tapeworm species; i.e. *Taenia saginata* **(beef tapeworm)**, *Taenia solium* **(pork tapeworm)** or *Taenia asiatica* (Asian tapeworm).

T. solium and *T. saginata* have a worldwide distribution but incidence is higher in developing countries where as *Taenia asiatica* is limited to Asia.

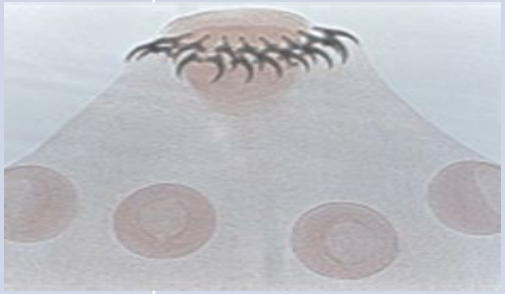
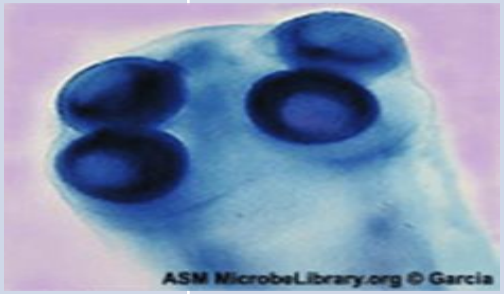
T. solium tapeworm infections can lead to human cysticercosis.

The major differences between *T. solium* and *T. saginata* is summarized in this table:

Taenia saginata	*Taenia solium*	Properties
		
Human	Human	Definitive host
Cow/Cattle	Pig	Intermediate Host

Taenia saginata	*Taenia solium*	Properties
Taeniasis only	Taeniasis and Cysticercosis	Disease
ileum	Duodenum	Adult
cystisercus bovis	*cysticercus cellulosae*	common larval name
in cardiac and .skeletal muscle	in cardiac and skeletal muscle, brain, eye, .subcutaneous tissue	localization of infective stage

Taenia saginata	*Taenia solium*	Properties
Size of adult worm: 5 m or less (sometime up to (25 m	Size of adult worm: 2-7 m	Size
• The scolex (“head”) is quadrate in outline and has four circular suckers. • Rostellum and hooklets are absent (i.e. unarmed scolex)	• The scolex head is globular in outline and has four circular suckers. • The head is provided with the rostellum armed with double row of alternating large and small hooklets (22-32). The rostellar hooklets are shaped like daggers or Arbian poniards (armed scolex)	Scolex

Taenia saginata	*Taenia solium*	Properties
		
• The number of proglottids varies from 1000- 2000. • The gravid uterus consists of a central longitudinal stem with 15-30 lateral branches on each side; these in turn sub-branch leaving practically no space in between. • The gravid segments are expelled singly • *T. saginata* may produce up to 100,000 eggs per proglottid	• The total number of proglottids (segments) is an average of 700-1000. • The gravid uterus consists of a median longitudinal stem with 5-13 compound lateral branches on each side • The gravid segments are expelled passively , in chains of 5-6 at a time, and not singly. • *T. solium* may produce 50,000 eggs per proglottid.	**Proglottids**

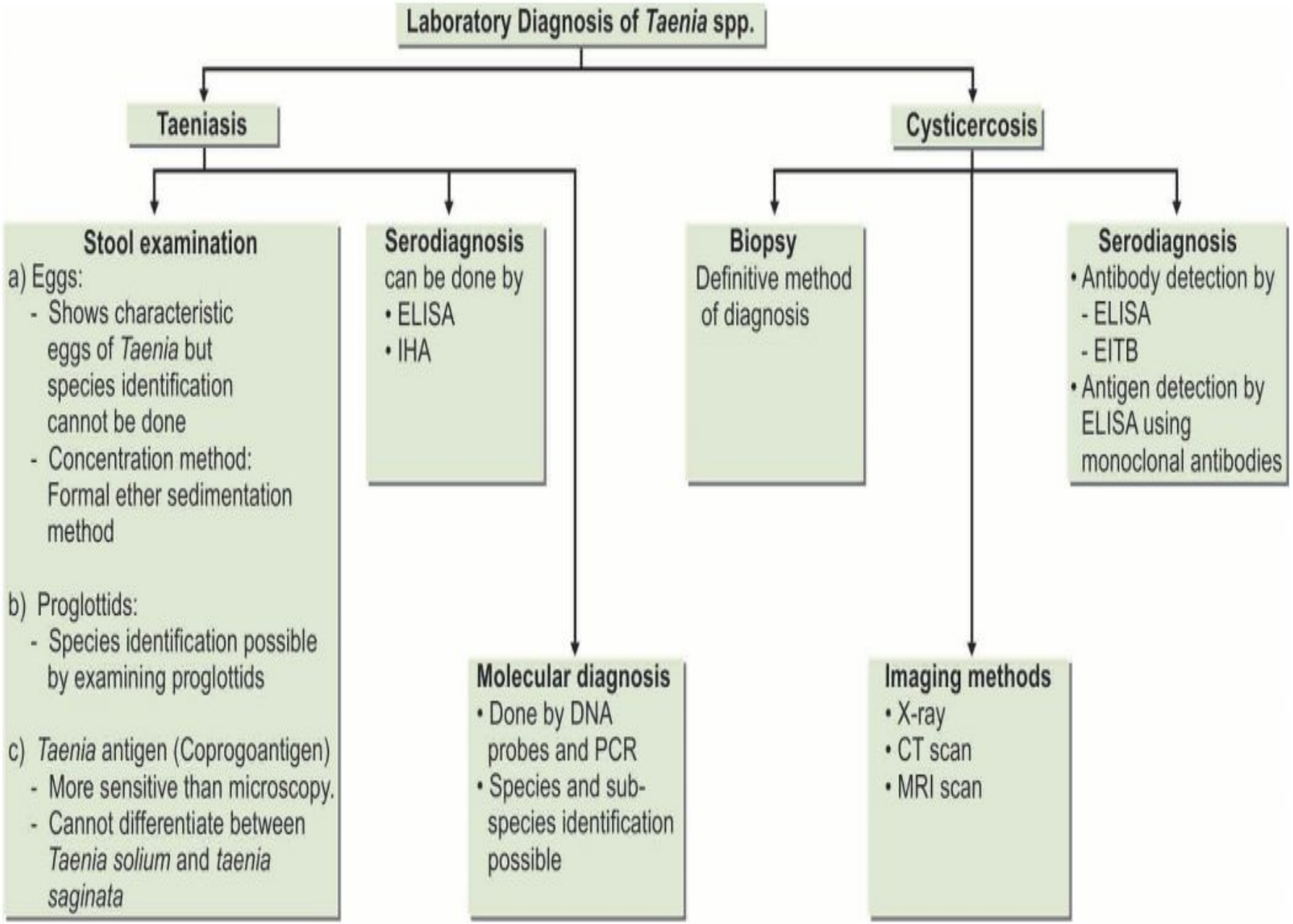
Taenia saginata	*Taenia solium*	Properties
		
800-1200	375-575	Testicles (quantity)
2	3	Ovaries (number lobes)
present	Absent	vaginal sphincter
single or grouped (actively and spontaneously)	single or grouped (passively)	mode of leaving the host

Taenia solium	*Taenia solium*	Properties
• Eggs of Taenia solium and Taenia saginata are morphologically indistinguishable	• The eggs are about 30-35 micrometers in diameter and are bile stained. • The internal oncosphere contains six refractile hooks. • The eggs are not floated in the saturated solution of NaCl. • Eggs of *Taenia solium* and Taenia saginata are morphologically indistinguishable.	Eggs
	 	

Taenia saginata	*Taenia solium*	Properties
Eggs of *Taenia saginata* are not infectious. Human who ingest T. saginata eggs do not develop cysticercosis.	Infectious to humans (Human who ingested food contaminated with egg of *Taenia solium* may develop cysticercosis)	Eggs (Infectious (nature
Human beings are infected through the eating of undercooked beef containing the cysticerci (“measly” beef)	Infection is common among those eating raw or insufficiently cooked measly pork containing the cysticerci.	Transmission

Cysticercosis

- *T. soliume* ggs can also infect humans and cause cysticercosis (larval cysts in lung, liver, eye and brain) resulting in blindness and neurological disorders.
- cysticercosis ocular involvement occurs and muscular involvement is high


Laboratory Diagnosis of *Taenia* spp.
Taeniasis
Cysticercosis
Stool examination
a) Eggs:
- Shows characteristic eggs of *Taenia* but species identification cannot be done
- Concentration method: Formal ether sedimentation method
b) Proglottids:
- Species identification possible by examining proglottids
c) *Taenia* antigen (Coprogoantigen)
- More sensitive than microscopy.
- Cannot differentiate between *Taenia solium* and *taenia saginata*
Serodiagnosis
can be done by
• ELISA
• IHA
Molecular diagnosis
• Done by DNA probes and PCR
• Species and sub-species identification possible
Biopsy
Definitive method of diagnosis
Imaging methods
• X-ray
• CT scan
• MRI scan
Serodiagnosis
• Antibody detection by
- ELISA
- EITB
• Antigen detection by ELISA using monoclonal antibodies

1-Schistosoma Haematobium

Definitive host: Humans are the only natural definitive hosts. No animal reservoir is known.

Intermediate host: Fresh water snails. Bulinus species

Infective form: Cercaria larva :

Habitat

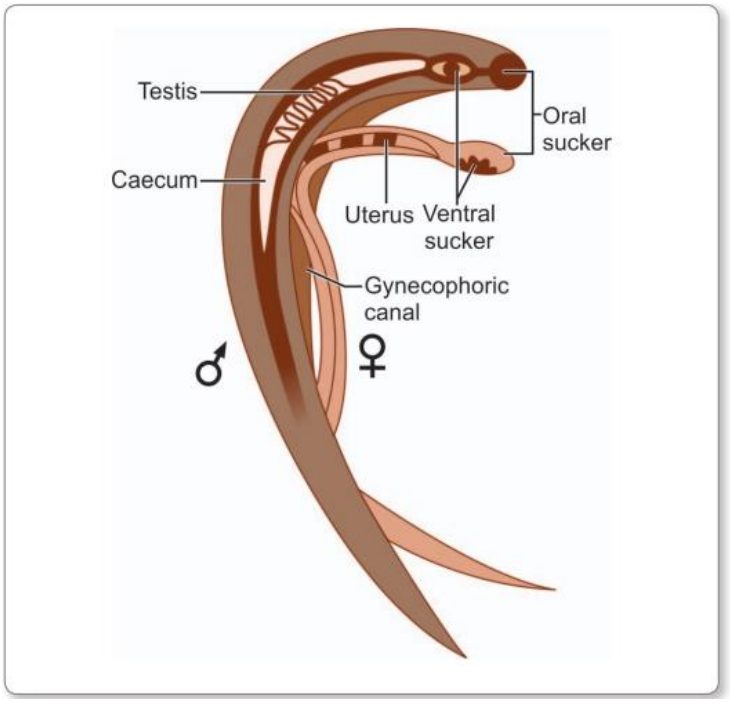
The adult worms live in the vesical and pelvic plexuses of veins.

Morphology

Adult worm

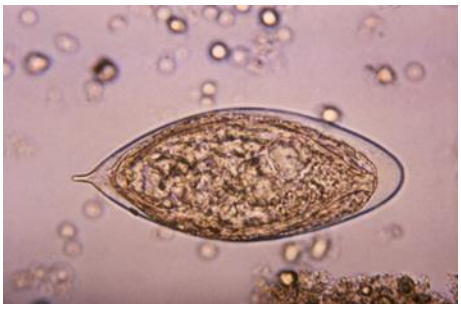
The male is 10–15 mm long by 1 mm thick and covered by a finely tuberculated cuticle. It has 2 muscular suckers, the oral sucker being small and the ventral sucker large and prominent. Beginning immediately behind the ventral sucker and extending to the caudal end is the canal, in which the female worm is held .

The adult female is long and slender, 20 mm by 0.25 mm with the cuticular tubercles confined to the two ends. The gravid worm contains 20–30 eggs in its uterus at one time and may pass up to 300 eggs a day.

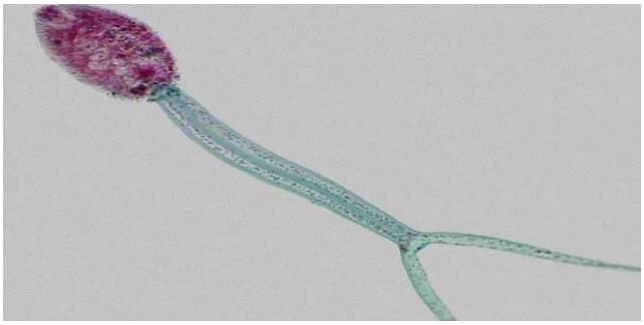


Egg

The eggs are elongated , about 150 µm by 50 µm, nonoperculated, with a brownish yellow transparent shell carrying a terminal spine at one pole; the terminal spine being characteristic of the species



Cercaria larva : The cercaria has an elongated ovoid body and forked tail



Pathogenicity and Clinical Features

-Skin penetration, The clinical features during the incubation period may be local cercarial dermatitis or general anaphylactic or toxic symptoms. Cercarial dermatitis consists of itching and petechial lesions at the site of entry of the cercariae (swimmer's itch).

Clinical features during oviposition hematuria (endemic hematuria)€ Hematuria is initially microscopic, but becomes gross, if infection is heavy.

Laboratory Diagnosis Urine Microscopy

The eggs with characteristic terminal spines can be demonstrated by microscopic examination of centrifuged deposits of urine or by filtration of a known volume of urine through nucleopore filters. Eggs can also be seen in the seminal fluid in males.

Histopathology

Schistosome infection may also be diagnosed by demonstrating its eggs in bladder mucosal biopsy. squamous cell carcinoma of the bladder

Detection of Antigen

detection of specific schistosome antigens in serum or urine. Two glycoprotein antigens associated with the gut of adult schistosomes: circulating anodic antigen (CAA) and circulating cathodic antigens (CCA) can be demonstrated by ELISA . The test is very sensitive and

specific, but is available only in specialized laboratories

Detection of Antibody

Several serological tests have been described for detection of specific antibody, but are not very useful as they cannot differentiate between present and past infection. These include complement fixation test (CFT), bentonite flocculation test, indirect haemagglutination (IHA), immunofluorescence, and gel diffusion tests.

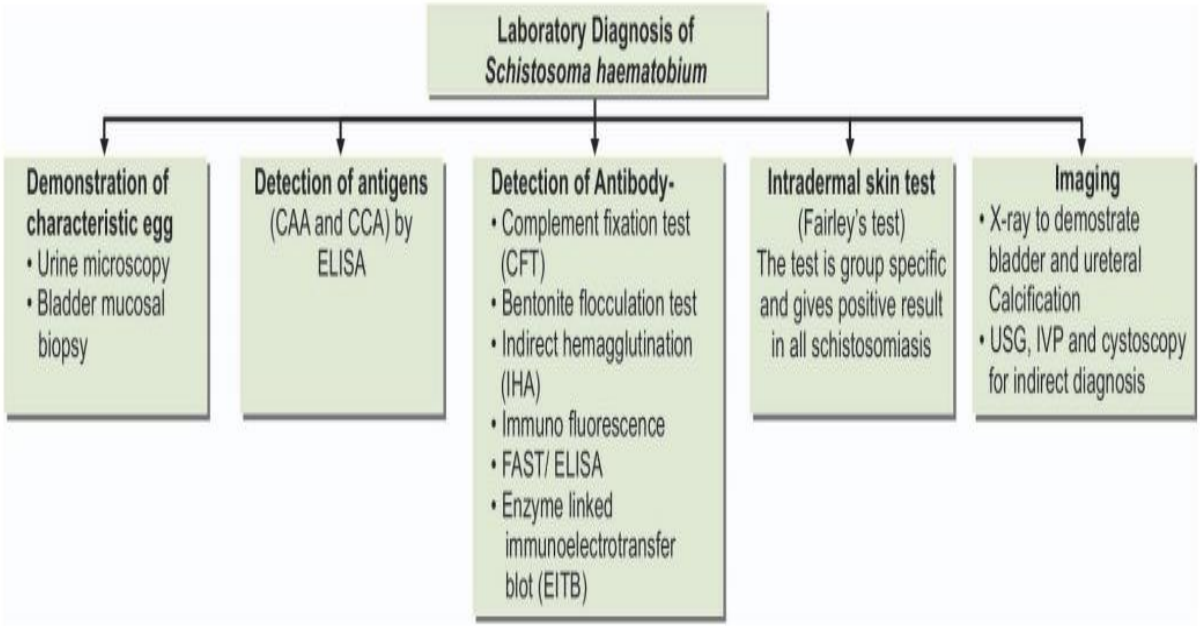
Two serological tests available at Centers For Disease Control and Prevention (CDC) are-the falcon assay screening test (FAST)/ELISA and the confirmatory enzyme-linked immunoelectrotransfer blot (EITB). Both these tests are highly sensitive and specific (95% sensitive and 99% specific). In (FAST) ELISA test, y (Flowchart).

Intradermal Skin Test (Fairley's test)

Skin tests are group-specific and give positive results in all schistosomiases. The intradermal allergic test uses antigen from infected snails, cercariae, eggs, and adult schistosomes from experimentally-infected laboratory animals.

Imaging

X-ray of the abdomen may show bladder and ureteral calcification. Ultrasonography (USG) is also useful in diagnosing S. haematobium infection. USG may show hydroureter and hydronephrosis. Intravenous pyelogram (IVP) and cystoscopy are also useful in indirect diagnosis of the disease



2-Schistosoma Mansoni

Definitive host: Humans are the only natural definitive hosts, though in endemic areas monkeys and baboons have also been found infected.

Intermediate host: Planorbid fresh-water snails **.**

Infective form: Fork-tailed cercaria.

Habitat: Adult worm lives in the inferior mesenteric vein. In humans, the schistosomulae mature in the liver and the adult worms move against the blood stream into the venules of the inferior mesenteric group in the sigmoidorectal area. Eggs penetrate the gut wall, reach the colonic lumen, and are shed in feces.

Morphology

S. mansoni resembles S. haematobium in morphology and life cycle, except— The adult worms are smaller and their integuments studded with prominent coarse tubercles. In the gravid female, the uterus contains very few eggs, usually 1–3 only.

The egg has a characteristic lateral spine , more near to the rounded posterior end. The eggs are non operculated and yellowish brown.

Laboratory Diagnosis

Stool Microscopy Eggs with lateral spines may be demonstrated microscopically in stools. **Kato-katz thick smear** or other concentration methods may be required when infection is light. Kato-katz thick smear provides quantitative data on the intensity of infection, which is of value in assessing the degree of tissue damage and monitoring the effect of chemotherapy.

Rectal Biopsy

Proctoscopic biopsy of rectal mucosa may reveal eggs when examined as fresh squash preparation between 2 slides.

Serological Diagnosis

Serological diagnosis by detecting schistomal antigen and antibody is similar to that of S.haematobium.

Imaging

Ultrasonography (USG) is useful to detect hepatosplenomegaly and periportal fibrosis.

Blood Examination

Blood examination may reveal eosionophilia, and increased levels of alkaline phosphatase.

3-Schistosoma Japonicum

Common name: Oriental blood fluke

Definitive host: Man is the definitive host but in endemic areas, natural infection occurs widely in several domestic animals and rodents, which act as reservoirs of infection.

Intermediate host: Amphibian snails

Infective form for humans: Fork tailed cercaria.

Habitat

The adult worms are seen typically in the venules of the superior mesenteric vein draining the ileocecal region. They are also seen in the intrahepatic portal venules and hemorrhoidal plexus of veins.

Morphology

Morphologically, they are similar to the schistosomes described above except— The adult male is comparatively slender (0.5 mm thick) and does not have cuticular tuberculations. In the gravid female, the uterus contains as many as 100 eggs at one time and up to 3,500 eggs may be passed daily by a single worm.

Egg. The eggs are smaller and more spherical than those of S. haematobium and S. mansoni. The egg has no spine, but shows a lateral small rudimentary knob.

Differentiating features between the 3 species of Schistosoma are illustrated in Table

	Schistosoma haematobium	*Schistosoma mansoni*	*Schistosoma japonicum*
Habitat	Veins of the vesical and pelvic plexuses, less commonly in portal vein and its mesenteric branches	Inferior mesenteric vein and its branches	Superior mesenteric vein and its branches
Morphology			
Size: *Male*	1.5 cm x 1 mm	1 cm x 1 mm	1.2–2 cm x 0.5 mm
Female	2 cm x 0.22 mm	1.4 cm x 0.25 mm	2.6 cm x 0.3 mm
Integument	Finely tuberculated	Grossly tuberculated	Non-tubercular
Number of testes	4–5 in groups	8–9 in a zigzag row	6–7 in a single file
Ovary	In the posterior one-third of the body	In the anterior half of the body	In the middle of the body
Uterus	Contains 20-30 eggs	1–3 eggs	50 or more eggs
Egg	Elongated with terminal spine	Elongated with lateral spine	Round with small lateral knob
Cephalic glands in Cercariae	2 pairs oxyphilic and 3 pairs basophilic	2 pairs oxyphilic and 4 pairs basophilic	5 pairs oxyphilic, no basophilic
Distribution	Africa, Near East, Middle East, India	Africa and south America	China, Japan, far east (oriental)
Definitive host	Man	Man	Man (mainly) domestic animals & rodents (which act as reservoir of infection
Intermediate host	Snail of Genus *Bulinus*	Snail of Genus *Biomphalaria*	Amphibian snail of Genus *Oncomelania*

Toxoplasma Gondii

In direct… life cycle **Final host … cat**
Intermediate … host human **Infective stage… oocyst**
Site of infection .. tissue

The trophozoite and tissue cyst represent stages in asexual multiplication (schizogony), while the the oocyst is formed by sexual reproduction (gametogony or sporogony).

All 3 forms occur in domestic cats and other felines, which are the definitive hosts and support both schizogony and gametogony.

Morphology … T. gondii occurs in 3 forms

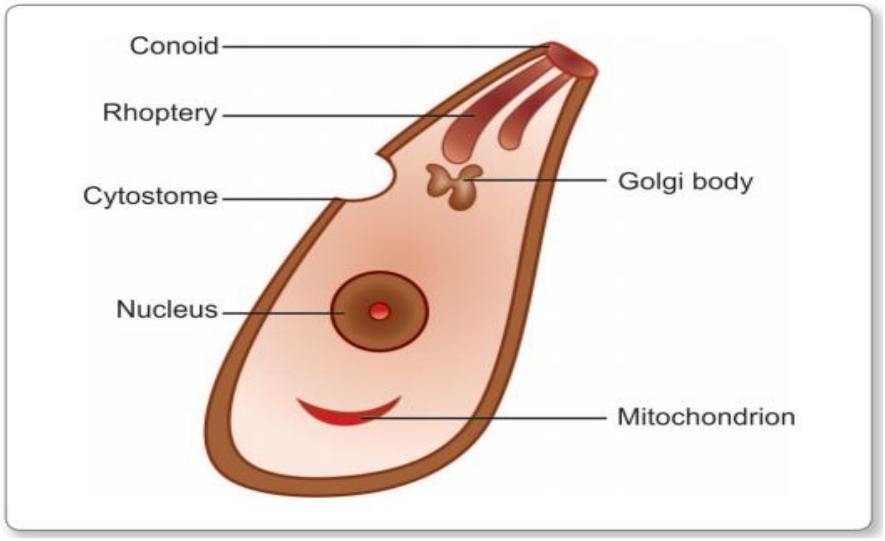
Trophozoites (Tachyzoites)€

Tissue cyst (bradyzoites).

€ Oocyst.

All the 3 forms are infectious to man.

1-Trophozoites (Tachyzoites) The trophozoite is crescent-shaped, with one end pointed and the other end rounded. and It measures 3–7 µm in length. The nucleus is ovoid and is situated at the blunt end of the parasite. Electron microscopy reveals an apical complex at the pointed end



Toxoplasma gondii. Trophozoite (tachyzoite), fine structure seen by electron microscopy

2-Tissue cyst (bradyzoites).

Tissue cysts are the resting form of the parasite. They are found during chronic stage of the infection and can be found in **the brain (most common site),** skeletal muscles, and various other organs.

The cyst is round or oval, 10–20 µm in size and contains numerous bradyzoites. Cysts remain viable in tissue for several years. They reach various tissues and organs through blood and lymphatic dissemination.

3- Oocyst

Oocysts develop only in definitive hosts – in the intestine of cats and other felines but not in humans. It is oval in shape and measures 10–12 µm in diameter. Each oocyst is surrounded by a thick resistant wall. The oocysts is formed by sexual reproduction

(gametogony). undergo sporulation in the soil with formation of 2 sporocysts, each containing 4 sporozoites. The sporulated oocyst is infective. Oocyst is very resistant to environmental conditons and can remain infective in soil for about an year.

Clinical sign

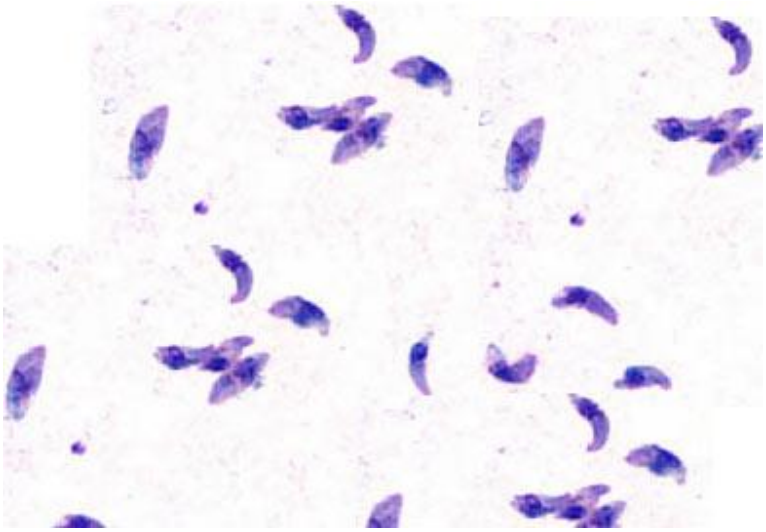
toxoplasmosis is often asymptomatic in healthy adults. People with weakened immune systems are headache, confusion, poor coordination, seizures, lung problems that may resemble tuberculosis, pneumonia, or **blurred vision caused by severe inflammation of the retina (ocular toxoplasmosis).** This can cause damage to the brain (encephalitis) or the eyes **(necrotizing retinochoroiditis)**. **during pregenancy or Congenital toxoplasmosis is associated with fetal death and miscarriage and abortion**, Infants infected via placental transmission **hydrocephaly**, **hypocephaly** or **nasal malformations**. The toxoplasmic trophozoites causing acute toxoplasmosis are referred to as tachyzoites, and are typically found in bodily fluids

Laboratory Diagnosis

1-Microscopy

Tachyzoites and tissue cysts can be detected in various specimens like blood, sputum, bone marrow aspirate, cerebrospinal fluid (CSF), aminiotic fluid, and biopsy material from lymphnode, spleen, and brain. Smear made from above specimens is stained by Giemsa,or Gomori methanamine silver (GMS) stain.Tachyzoites appear as crescent-shaped structures with blue cytoplasm and dark nucleus.

Tachyzoites or cyst can also be demonstrated effectively by fluroscent conjugated antibody technique in tissue biopsy or impression smear.



Toxoplasma gondii

2-Animal Inoculation

Toxoplasma can be isolated by inoculating body fluids, blood, or tissue specimes by intraperitoneal inoculation in mice or in tissue culture. Mice should be examined for Toxoplasma in their peritoneal exudate after 7–10 days of infection

3-Serology

a--Antibody detection Tests for detecting IgG antibody include:

€ **Enzyme-linked immunosorbent assay (ELISA)**

€ Indirect fluroscent antibody test (IFAT)

€ Latex agglutination test

€ Sabin-Feldman dye test.

Positive IgG titer (>1:10) can be detected as early as 2–3 weeks after infection. Peak level of antibody is observed in blood 4–8 weeks after infection.

b--Antigen detection

Detection of antigen by ELISA indicates recent Toxoplasma infection.

In AIDS and other immonocompromised patients, antigen detection is very useful.

Detection of antigen in amniotic fluid is helpful to diagnose congenital toxoplasmosis.

Molecular Methods

polymerase chain reaction (PCR) are used to detect Toxoplasma from different tissues and body fluids..

Imaging

Magnetic resonance imaging (MRI) and computed tomography (CT) scan are used to diagnose toxoplasmosis with central nervous system involvement.

Ultrasonography (USG) of the fetus in utero at 20–24 weeks of pregnancy is useful for diagnosis of congenital toxoplasmosis.

1--Trichuris Trichiura Common name: Whipworm

Life Cycle: direct

Natural host: Man. No intermediate host is required

Infective form: Embryonated eggs contaning Rhabditiform larva.

Habitat

T. trichiura lives in the large intestine. The adult worms are found attached to the wall of the caecum and less commonly to appendix, colon, and anal canal.

Morphology

Adult Worm

The male worm is 30–45 mm long, while the female is slightly larger, about 40–50 mm. The worm resembles a whip, with the anterior thin and thread-like and the posterior thick and fleshy, appearing like the handle of a whip. The attenuated anterior portion, which contains the capillary esophagus, is embedded in the mucosa. The posterior part contains the intestines and reproductive organs. The posterior end of the male is coiled ventrally, while the hind end of the female is straight, blunt, and rounded .The worm has a lifespan of 5–10 years.

Egg

The egg has a characteristic appearance. It is brown in color being bile-stained. It has a triple shell. It is barrel-shaped and about 50 µm long and 25 µm wide in the middle, with a projecting mucus plug at each pole containing an unsegmented ovum . The plugs are colorless. The egg floats in saturated salt solution. When freshly passed, the egg contains an unsegmented ovum. At this stage, it is not infective for humans. The fertilized female lays about 5,000 eggs per day.

Laboratory Diagnosis

Stool Examination

The characteristic barrel-shaped eggs are found in stools. The degree of infection can be assessed by egg counts. Less than 10 eggs per smear in direct stool preparation is considered light infection and more than 50 per smear as heavy infection. Light infection is not considered to cause clinical disease.

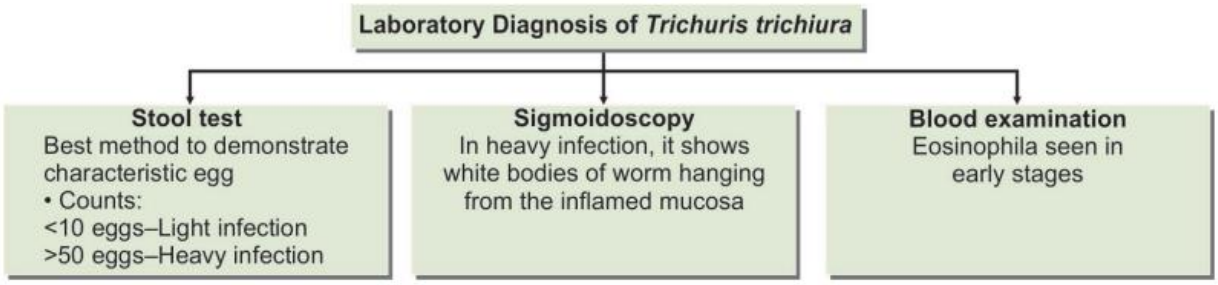
Sigmoidoscopy

Sigmoidoscopy is useful as worms are found in the rectal mucosa in whipworm diarrhea and dysentery. In heavy infection, sigmoidoscopy may show white

bodies of worm hanging from the in amed mucosa, the so called coconut cake rectum.

Blood Examination

Differential leukocyte count (DLC) may show upto 25% eosinophila in the early stage of the disease



2-Ancylostoma Duodenale ……. Hookworm

Habitat

The adult worms live in the small intestines of infected persons, mostly in the jejunum

Life Cycle: Life cycle of ancylostoma is completed in a single host

Definitive host: Humans are the only natural host. No intermediate host is required like other helminths.

Infective form: Third stage larva.

Morphology

Adult Worm

They are relatively stout cylindroidal worms. The body is curved with the dorsal aspect concave and the ventral aspect convex. The mouth is dorsally. The prominent buccal capsule, with a hard chitin-like substance carries 6 teeth; 4 hook-like teeth ventrally, and 2 knob-like with a median cleft dorsally.

Male Worm

The male worm is smaller than female worm 8-11 mm in length and 0.4 mm thick. The posterior end of the male is expanded into a copulatory bursa which consists of 3 lobes; 1 dorsal and 2 lateral. Each lobe is supported by 13 chitinous rays . There are 2 long retractile bristle-like copulatory spicules.

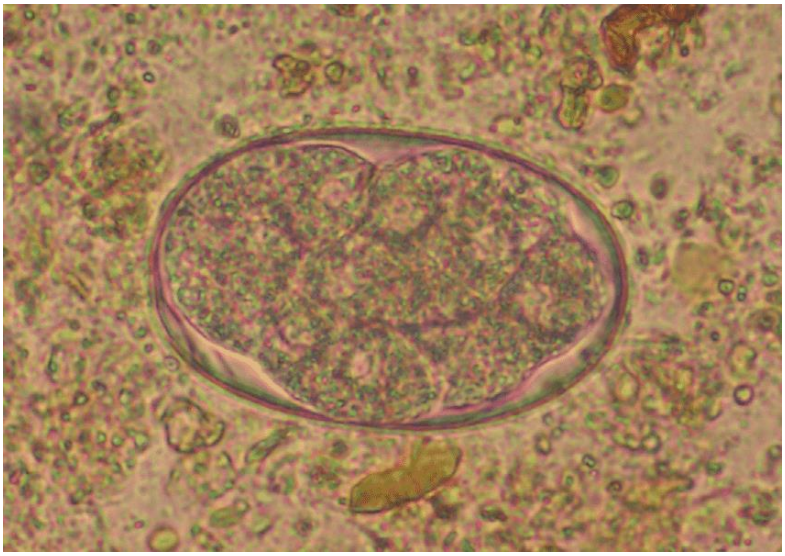
Ancylostoma duodenale MALE 10x tail

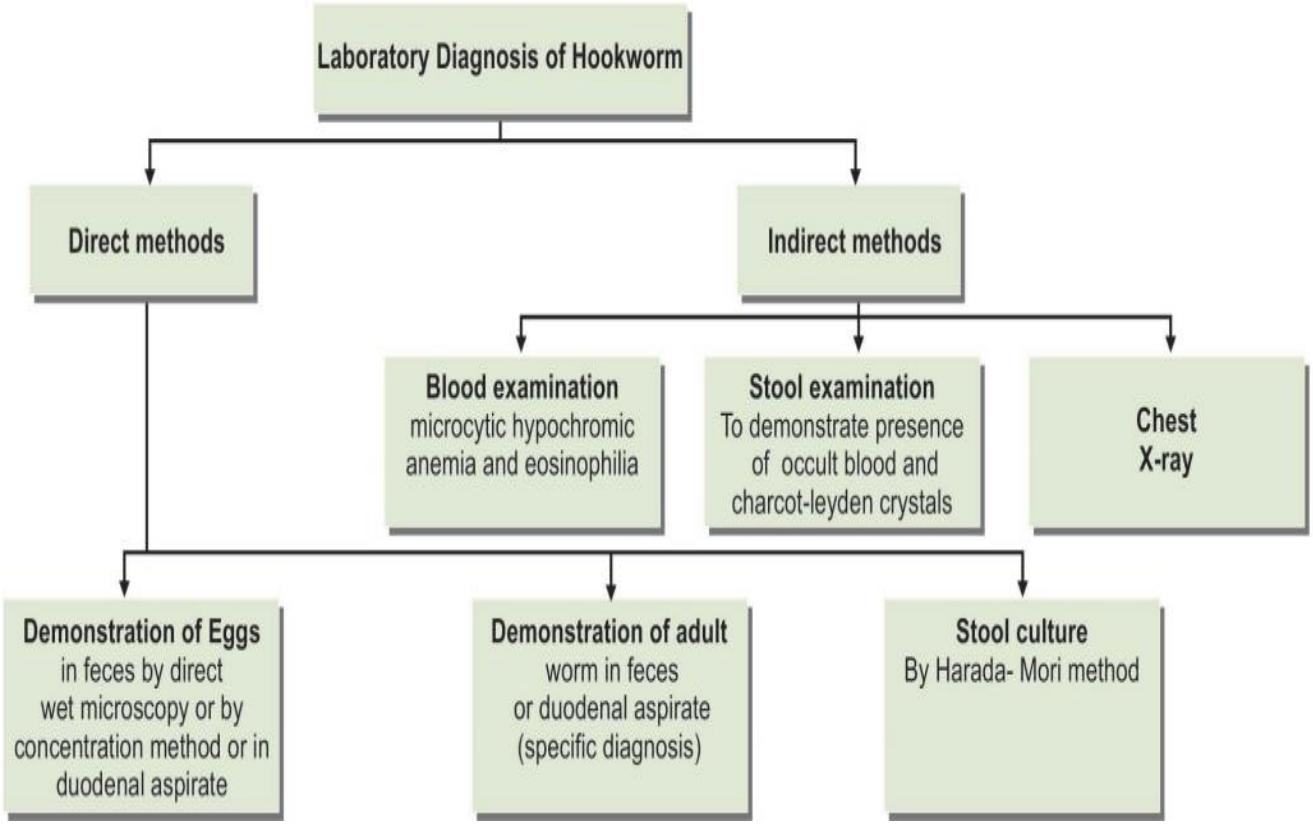
Female Worm

The female worm is larger, 10 to 13 mm long and 0.6 mm, Its hind end is conoid, with a subterminal anus situated ventrally. The vulva opens ventrally at the junction of the middle and posterior thirds of the body.

Egg

The egg of hookworm is: Oval or elliptical, measuring 60 µm by 40 µm. Colorless. Surrounded by a thin transparent hyaline shell membrane. Floats in saturated salt solution. When released by the worm in the intestine, the egg contains an unsegmented ovum. During its passage down the intestine, the ovum develops. When passed in feces, the egg contains a segmented ovum, usually with 4 or 8 blastomeres.





Trichinella Spiralis Common name: Trichina worm

Habitat Adult worms live deeply buried in the mucosa of small intestine (duodenum or jejunum) of pig, bear, rat, or man. The encysted larvae are present in the striated muscles of these hosts. There are no free-living stages.

Life Cycle: has a direct life cycle, which means it completes all stages of development in one host.

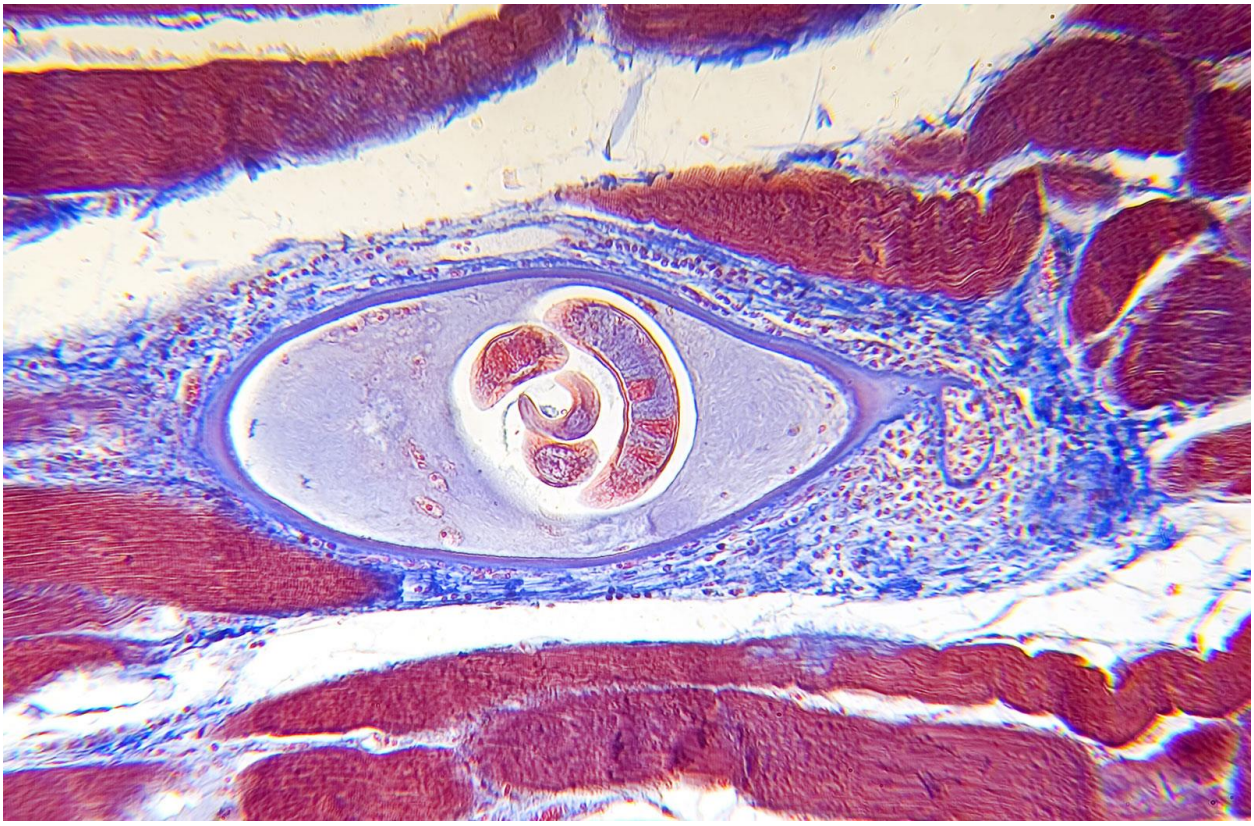
host: Pig. Man.

Infective form: Encysted larva found in the muscles of pigs and other animals

Morphology of The adult T. spiralis, a small white worm just visible to the naked eye, is one of the smallest nematodes infecting humans. The male measures about 1.5 mm by 0.04 mm and the female about 3 mm by 0.06 mm (twice the

length of male).The anterior half of the body is thin and pointed, well adapted for burrowing into the mucosal epithelium

Trichinella Spiralis adult worm



Encysted larvae are present in the striated muscles of these hosts

Diagnosis

Direct Methods

i. Detection of spiral larvae in muscle tissue by performing muscle biopsy. biceps, gastrocnemius, or pectoralis muscles are usually selected for biopsy

ii. Detection of adult worms and larvae in the stool during the diarrheic stage

iii. Xenodiagnosis: For xenodiagnosis, biopsy bits are fed to laboratory rats, which are killed a month or so, later. The larvae can be demonstrated more easily in the muscles of such infected rats

Indirect Methods

ii. Blood examination: It shows eosinophilia (20–95%).

iii. Serology: There is massive hypergammaglobulinemia with elevated serum IgE.

T. spiralis antibody can be detected by enzymelinked immunosorbent assay (ELISA)

Radiological examination: Calci- ed cysts may be demonstrated on X-ray examination.

vi. **Molecular methods** like multiplex polymerase chain reaction