

Medical Laboratory Techniques Department Lecture 3: Mastigophora Dr.Fatima H.Abbas



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Mastigophora

General Classification

Depending on their habitat, they can be considered under:

- Lumen-dwelling flagellates: Flagellates found in the alimentary tract and urogenital tract.
- Hemoflagellates: Flagellates found in blood and tissues.
- Most luminal flagellates are nonpathogenic commensals. Two of them cause clinical diseases-*Giardia lamblia*, which can cause diarrhea and *Trichomonas vaginalis*, which can produce vaginitis and urethritis.

Group	Parasites	Habitat
Lumen-dwelling flagellates	Giardia lamblia Trichomonas vaginalis Trichomonas tenax Trichomonas hominis Chilomastix mesnili Enteromonas hominis Retortamonas intestinalis Dientamoeba fragilis	Duodenum and jejunum Vagina and urethra Mouth Large intestine (caecum) Large intestine (caecum) Large intestine (colon) Large intestine (colon) Large intestine (colon)
Hemoflagellates	Leishmania spp. Trypanosoma brucei Trypanosoma cruzi	Reticuloendothelial cells Connective tissue and blood Reticuloendothelial cells and blood

Giardia Lamblia

Habitat

G. lamblia lives in the duodenum and upper jejunum and is the only protozoan parasite found in the lumen of the human small intestine.

Morphology: It exists in 2 forms: Trophozoite (or vegetative form) and Cyst (or cystic form).

Trophozoite The trophozoite is in the shape of a tennis racket (heart-shaped or pyriform shaped) and is rounded anteriorly and pointed posteriorly.

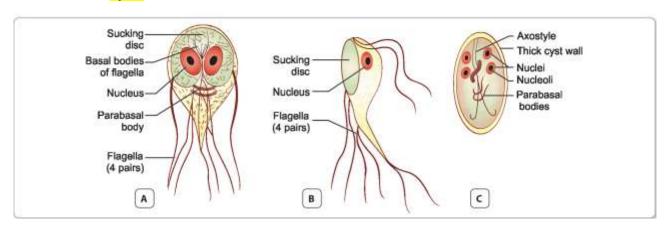
- It measures 15 μm x 9 μm wide and 4 μm thick.
- Dorsally, it is convex and ventrally, it has a concave sucking disc, which helps in its attachment to the intestinal mucosa.
- It is bilaterally symmetrical and possesses.
 - 1 pair of nuclei
 - 4 pairs of flagella
 - Blepharoplast, from which the flagella arise (4 pairs)

- 1 pair of axostyles, running along the midline
- Two sausage shaped parabasal or median bodies, lying transversely posterior to the sucking disc.
- The trophozoite is motile, with a slow oscillation about its long axis, often resembling falling leaf.

Cyst

It is the infective form of the parasite.

- The cyst is small and oval, measuring 12 μm x 8 μm and is surrounded by a hyaline cyst wall.
- Its internal structure includes 2 pairs of nuclei grouped at one end. A young cyst contains 1 pair of nuclei.
- The axostyle lies diagonally, forming a dividing line within cyst wall.
- Remnants of the flagella and the sucking disc may be seen in the young cyst.



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

Life Cycle

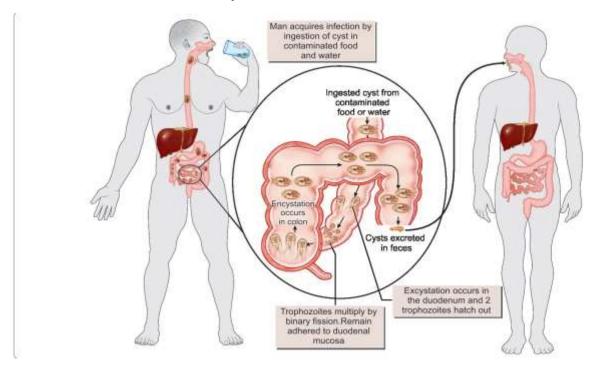
Giardia passes its life cycle in 1 host.

Infective form: Mature cyst.

Mode of transmission:

- Man acquires infection by ingestion of cysts in contaminated water and food.
- Direct person-to-person transmission may also occur in children, male homosexuals, and mentally-ill persons المرضى عقليا.
- Enhanced susceptibility to giardiasis is associated with blood group A, achlorhydria مخدر, chronic use of cannabis, use of cannabis مخدر, chronic pancreatitis, malnutrition, and immune defects such as 19A deficiency and hypogamma-globulinemia.

- Within half an hour of ingestion, the cyst hatches out into two trophozoites, which multiply successively by binary fission and colonize in the duodenum.
- The trophozoites live in the duodenum and upper part of jejunum, feeding by pinocytosis.
- During unfavorable conditions, encystment occurs usually in colon.
- Cysts are passed in stool and remain viable in soil and water for several weeks.
- There may be 200,000 cysts passed per gram of feces.
- Infective dose is 10-100 cysts.

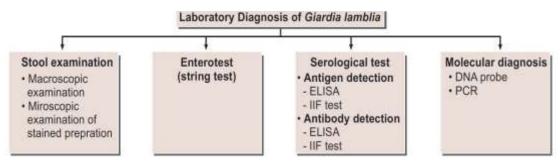


Pathogenicity and Clinical Features

G. lamblia is typically seen within the crypts of duodenal and jejunal mucosa. It does not invade the tissue, but remains tightly adhered to intestinal epithelium by means of the sucking disc.

- They may cause abnormalities of villous architecture التركيب الزغابي by cell apoptosis and increased lymphatic infiltration تسرب الليمفاوي of lamina propria.
- Variant specific surface proteins (VSSP) of giardia play an important role in virulence and infectivity of the parasite.
- Often they are asymptomatic, but in some cases, Giardia may lead to mucus diarrhea, fat malabsorption (steatorrhea), dull ثقيل epigastric pain, and flatulence انتفاخ البطن. The stool contains excess mucus and fat but no blood.

- Children may develop chronic diarrhea, malabsorption of fat, vitamin A, protein, sugars like xylose disaccharides, weight loss.
- Occassionally, Giardia may colonize the gall bladder, causing biliary colic and jaundice.
- Incubation period is variable, but is usually about 2 weeks.



Laboratory Diagnosis

❖ Stool Examination

- Giardiasis can be diagnosed by identification of cysts of *Giardia lamblia* in the formed stools and the trophozoites and cysts of the parasite in diarrheal stools.
- On macroscopic examination fecal specimens containing G. lamblia may have an offensive odor, are pale colored and fatty, and float in water.
- On microscopic examination cysts and trophozoites can be found in diarrheal stools by saline and iodine wet preparations.
- Often multiple specimens need to be examined and concentration techniques like formal ether or zinc acetate are used. In asymptomatic carriers, only the cysts are seen.

Enterotest (String test)

A useful method for obtaining duodenal specimen is entrotest . A coiled thread خيط ملفوفا inside a small weighted gelatin capsule is swallowed by the patient, after attaching the free end of the thread in check . The capsule passes through the stomach to the duodenum. After 2 hours, the thread is withdrawn, placed in saline and is mechanically shaken. The centrifuged deposit of the saline is examined for Giardia. The use of entrotest is not recommended because of the very high cost of the test.

Serodiagnosis

a. Antigen detection

Enzyme-linked immunosorbent assay (ELISA), immuno-chromatographic strip tests and indirect immunofluroscent (IIF) tests using monoclonal antibodies have been developed for detection of Giardia antigens in feces.

- The presence of antigen indicates active infection.
- Commercially available ELISA kits (ProSpec T/Giardia kit) detects *Giardia*-specific antigen 65 (GAS 65).

- The sensitivity of the test is 95% and specificity is 100%, when compared to conventional microscopy.
- The test may be used for quantification of cysts and in epidemiological and control studies, but not for routine use.

b. Antibody detection

IIF test and ELISA are used to detect antibodies against Giardia.

- Demonstration of antibodies is useful in the epidemio-logical and pathophysiological studies.
- These tests cannot differentiates between recent and past infection and lack sensitivity and specificity.

* Molecular Method

•DNA probes and polymerase chain reaction (PCR) have been used to demonstrate parasitic genome in the stool specimen.

Chilomastix mesnili

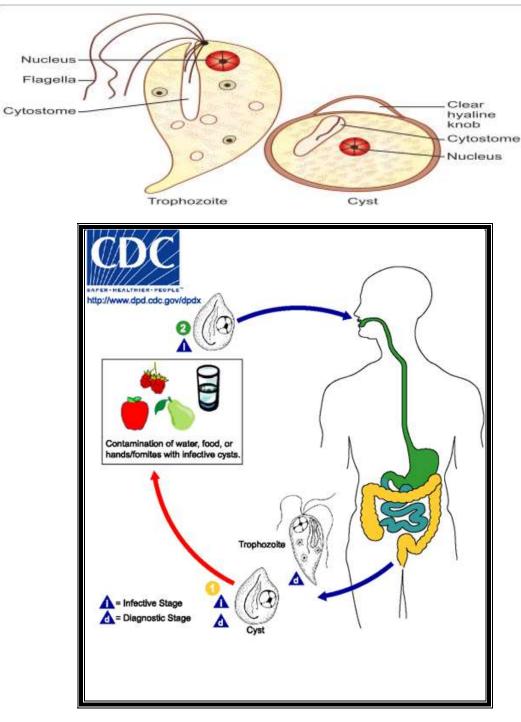
It is nonpathogenic. The natural habitat in the colon. The transmission occurs where infective stage (cyst) are ingested through hand to mouth contamination or via contaminated food or drink.

Trophozoite

Pear shaped .The broad anterior end tapers toward the posterior end of the organism The single nucleus is located in the anterior end of the trophozoite. Karyosome may be found located either centrally or eccentrically. Peripheral chromatin is absent. Trophozoites characteristically have four flagella. Three extend out of the anterior end and the fourth is shorter and extends posterior form the cytosome .The cytosome is located to one side of the nucleus. Trophozoite also contain spiral groove results in curved posture at the posterior end.

Cyst

Are usually lemon shaped and possess a clear anterior hyaline knob. A large single nucleus consisting of a large central karyosome and no peripheral chromatin, is usually located toward the anterior end of the cyst. The well-defined cytosome may be found to one side of the nucleus.



Life cycle of C. mesinili

Laboratory diagnosis

Examination of freshly passed liquid stools typically reveal only trophozoite. Formed stool samples usually reveal only cysts. Samples of semiformed consistency may contain both trophzoites and cysts.

<mark>Dientamoeba Fragilis</mark>

- It is unique as it has only trophozoite stage but no cyst stage.

- The name *Dientamoeba fragilis* is derived from the binucleate nature of trophozoite (Dientamoeba) and the fragmented appearance (fragilis) of its nuclear chromatin.
- It lives in colonic mucosal crypts, feeding on bacteria. It does not invade tissues, but may rarely ingest RBCs.
- In the absence of cyst stage, its mode of transmission is not clear. Possibly, it is transmitted from person-to-person by the fecal-oral route or by the eggs of *Enterobius vermicularis* and other nematodes, which may serve as a vector.
- Laboratory diagnosis is made by demonstration of trophozoites in stool.