# **Flagellates—I** (Intestinal and Genital)

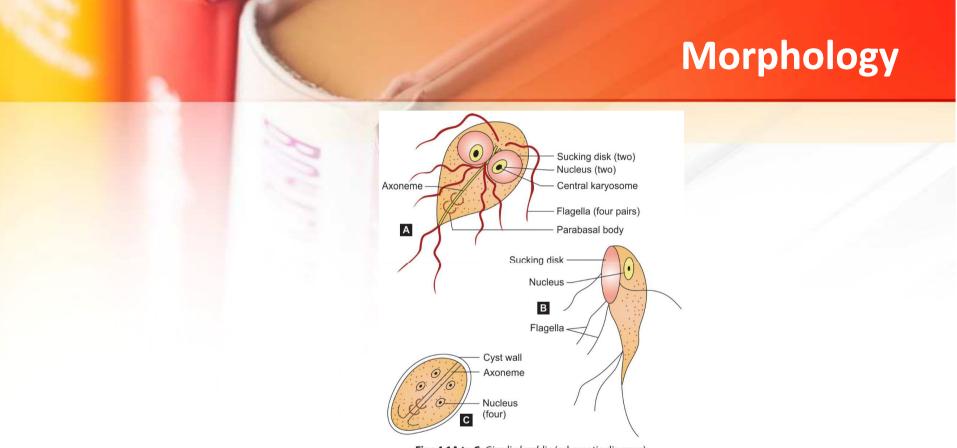
## Learning objectives

At the end of the session, the students will know

# **Classification of flagellates**

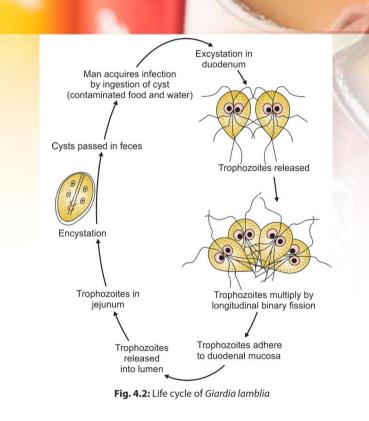
Table 4.1: Taxonomic classification of flagellates					
Kingdom	Subkingdom	Phylum	Class	Order	Genus
Protozoa	Archezoa	Metamonada	Trepomonadea	Diplomonadida	Giardia Enteromonas
			Retortamonadea	Retortamonadida	Retortamonas Chilomastix
			Trichomonadea	Trichomonadida	Trichomonas Pentatrichomonas Dientamoeba
	Neozoa	Euglenozoa	Kinetoplastidea	Trypanosomatida	Leishmania Trypanosoma

Table 4.2: Classification of flagellates based on habitat				
Intestinal/genital flagellates	Habitat			
Giardia lamblia	Duodenum and jejunum			
Enteromonas hominis	Large intestine			
Retortamonas intestinalis	Large intestine			
Chilomastix mesnili	Cecum			
Dientamoeba fragilis	Cecum and colon			
Trichomonas tenax	Mouth (teeth and gum)			
Pentatrichomonas hominis	lleocecal region			
Trichomonas vaginalis	Vagina and urethra			
Blood and somatic flagellates	Habitat			
Leishmania	Blood and tissue			
Trypanosoma	Blood and tissue			



**Figs 4.1A to C:** *Giardia lamblia* (schematic diagram): (A) Trophozoite front view; (B) Trophozoite lateral view; (C) cyst

# Life Cycle



## Life Cycle

- **Host:** Giardia completes its life cycle in one host.
- Infective form: Cysts are the infective form.
- Mode of transmission: Man acquires infection by ingestion of food and water contaminated with mature cysts or rarely by sexual route (mainly in homosexuals).

## Pathogenicity

- Infective dose: 10-25 cysts can initiate the Infection
- Malabsorption: This could be of various types such as:
  - Malabsorption of fat (steatorrhea)- leads to foul smelling profuse frothy diarrhea
  - Disaccharides deficiencies (lactose, xylose)- leading to lactose intolerance
  - Malabsorption of vitamin A, B12 and iron
  - Protein loosing enteropathy.
- Antigenic variation: Giardia undergoes frequent antigenic variations due to a cysteine rich protein on its surface called variant surface protein (VSP)

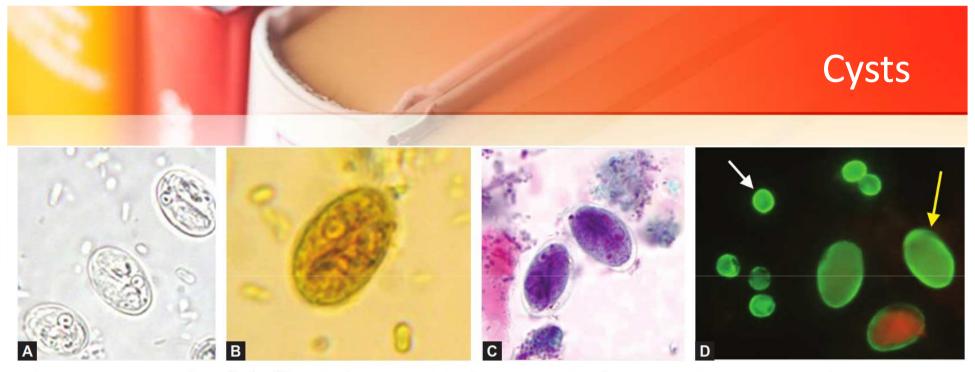
## **Clinical features**

- Asymptomatic carriers
- Acute giardiasis
  - Incubation period varies from 1 week to 3 weeks
  - Common symptoms diarrhea, abdominal pain, bloating, belching
  - Diarrhea is often foul smelling with fat, and mucus but no blood
- Chronic giardiasis:
  - Recurrent episodes of foul smelling diarrhea
  - Extraintestinal manifestations such as urticaria, anterior uveitis, salt and pepper retinal changes and arthritis

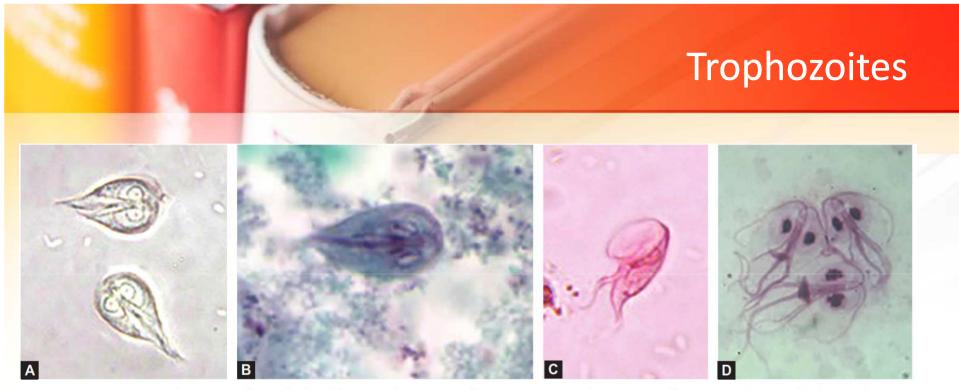
### Laboratory Diagnosis

### Giardia lamblia

- Stool examination (saline mount)—detects
  - Cysts (oval, 4 nuclei)—indicates carrier/active stage
  - Trophozoites (pear shaped, falling leaf motility)—indicates active infection
  - > Other methods—iodine mount, trichrome stain, DFA
- Entero-test—duodenal sampling, with the help of gelatin capsule attached to a thread
- Antigen detection in stool (coproantigen)—ELISA, ICT (Triage Parasite Panel)
- Antibody detection in serum—ELISA, IFA
- **Culture**—for research purpose, not for diagnostics
- Molecular method—PCR, BioFire FilmArray, genotyping
- **Radiological findings**—barium meal X-ray.



Figs 4.3A to D: Cysts of *Giardia lamblia*: (A) Saline mount; (B) Iodine mount; (C) Trichrome stain; (D) Direct immunofluorescence assay (vellow arrow indicates *Giardia* cyst and white arrow indicate oocyst of *Cryptosporidium*)



Figs 4.4A to D: Trophozoites of *Giardia lamblia* (A) Saline mount front view; (B) Trichrome stain front view; (C) Merthiolate iodine formalin (MIF) stain lateral view (spoon shaped); (D) Giemsa stained mucosal imprint (front view)

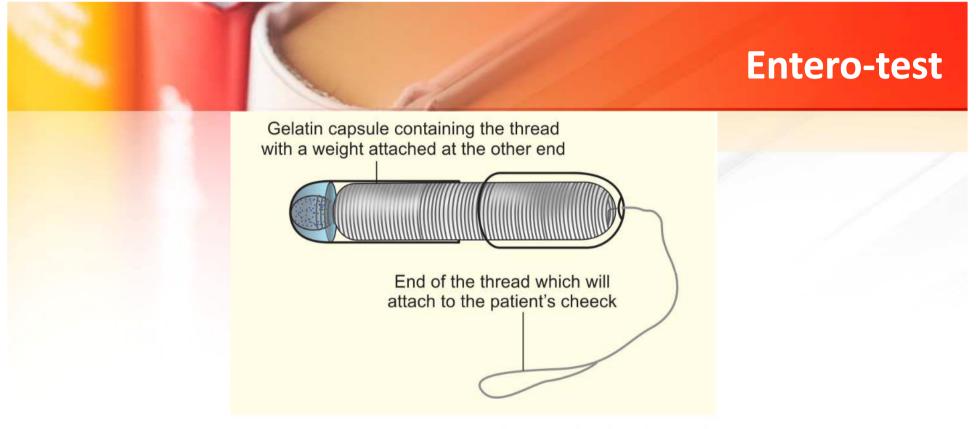


Fig. 4.5: Entero-test equipment showing duodenal capsule attached with thread at other end

### Treatment

### Giardia lamblia

- Metronidazole (250 mg thrice daily for 5 days) is usually effective in more than 90% of cases of giardiasis
- Tinidazole (2 g once orally) is more effective than metronidazole; considered as the drug of choice
- Nitazoxanide (500 mg twice daily for 3 days) is an alternative agent for treatment of giardiasis
- Furazolidone is given to children and auranofin, paromomycin can be given in pregnancy
- In patients with AIDS and hypogammaglobulinemia, giardiasis is often refractory to treatment. Prolonged therapy with metronidazole (750 mg thrice daily for 21 days) has been successful
- Wheat germ agglutinin supplemented diet, albendazole and auranofin can be used for treatment of giardiasis in future; needs further research
- Metronidazole resistance has been reported in *Giardia*; linked to pyruvate ferredoxin oxidoreductase (PFOR) gene. Auranofin is shown to be effective against metronidazole-resistant strains of *Giardia*. Cysts are more resistant to metronidazole than trophozoites.

## **TRICHOMONAS**

- Trichomonas belongs to:
  - Class: Trichomonadea
  - Order: Trichomonadida
  - Family: Trichomonadidae.
- Species
  - T. vaginalis is the only pathogen. It resides in the genital tract
  - Pentatrichomonas hominis: Nonpathogen, large intestine
  - T. tenax: Nonpathogen, resides in mouth (teeth and gum)

# Morphology

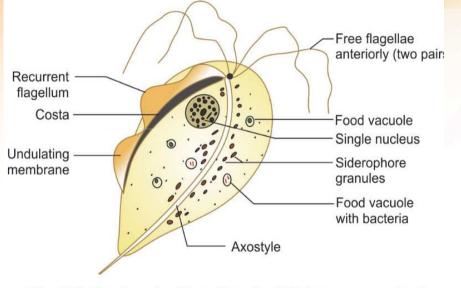


Fig. 4.6: Trophozoite (flagellated) of *Trichomonas vaginalis* (schematic diagram)

## **Exists** as

- Trophozoite (flagellate and ameboid form)
- Pseudocystic stages.
- There is no cystic stage

# Life Cycle

- Flagellated trophozoites are the infective stage as well as the diagnostic stage.
- Transmission:
  - Asymptomatic females transmit the disease by sexual route.
  - Though rare, but evidence of non-sexual transmission via fomites
- On exposure to oxygen, the anaerobic flagellated trophozoites undergo cytoskeletal rearrangement and transform into tissue-feeding and actively dividing ameboid trophozoites
- The amoeboid trophozoites divide by longitudinal binary fission and infect urogenital tract
- They again transform back to flagellated trophozoites, which infect other individuals.

## **Clinical feature**

- Asymptomatic infection: 25-30%
- Acute infection (vulvovaginitis)
  - Females are commonly affected and are presented as vulvovaginitis (thin profuse foul smelling purulent discharge)
  - Strawberry appearance of vaginal mucosa (Colpitis macularis) -in 2% of patients. It is characterized by small punctate hemorrhagic spots on vaginal and cervical mucosa
  - Other features include dysuria and lower abdominal pain
  - In males, the common features are nongonococcal urethritis and rarely epididymitis, prostatitis and penile ulcerations
- Chronic infection
- Complications

## Laboratory Diagnosis

### Trichomonas vaginalis

- Direct microscopy—detects trophozoites (pears shaped, jerky motility)
  - Wet saline mount
  - Permanent stain
  - Acridine orange fluorescent stain
  - Direct fluorescent antibody test
- □ Culture (e.g. InPouch TV)— gold standard method
- □ Antigen detection in vaginal secretion—ELISA, ICT, etc.
- □ Antibody detection—ELISA using whole cell antigen
- Molecular method—PCR detecting beta tubulin genes
- □ Other supportive test: Raised vaginal pH, Whiff test

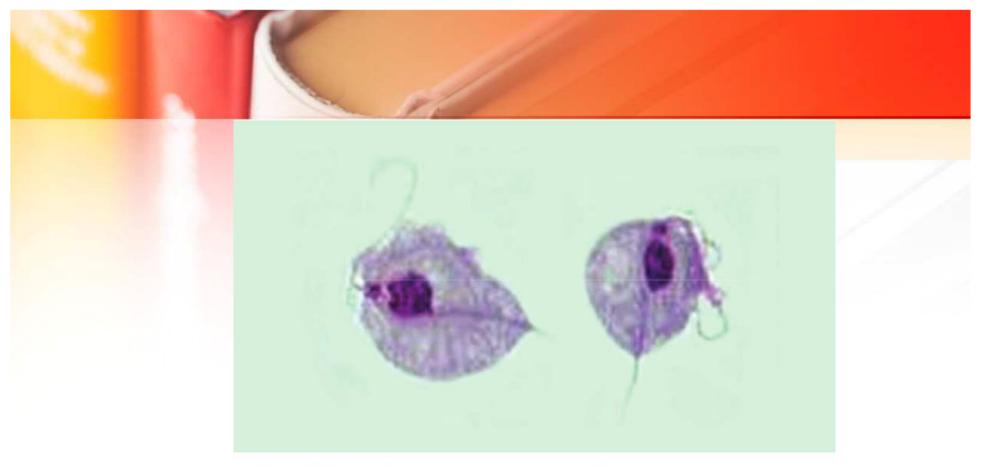


Fig. 4.7: Trichomonas vaginalis trophozoite (Giemsa stain)



**Fig. 4.8:** InPouch TV diagnostic system for culturing *T. vaginalis*. The swab containing the specimen is inoculated into the highly selective liquid medium present within the plastic pouch; incubated for 4–7 days at 37°C and then examined under microscope for presence of trophozoites

#### Treatment

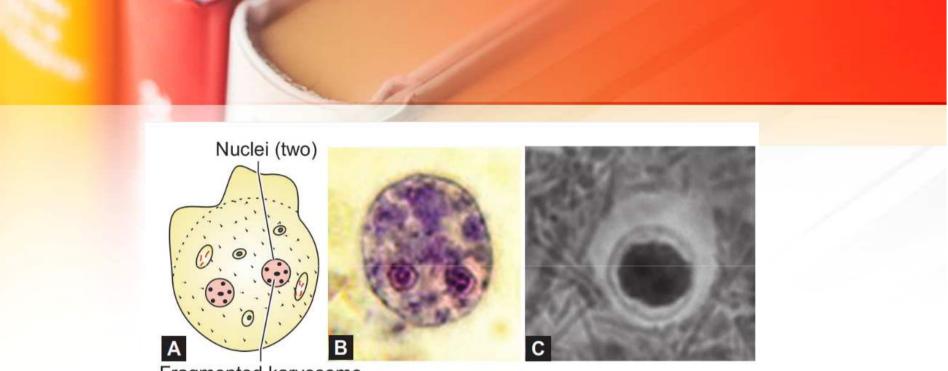
#### Trichomonas vaginalis

#### Metronidazole or tinidazole

- **Standard therapy:** 2 g, single dose is usually effective
- □ Both the sexual partners must be treated simultaneously to prevent reinfection, especially asymptomatic males
- Resistance to metronidazole:
  - > Resistance is rare but has been reported:
    - ♦ 2.5–10% to metronidazole
    - Less than 1% to tinidazole
  - > The mechanism of development of resistance to metronidazole is controlled by hydrogenosome
  - Metronidazole requires hydrogen as an electron acceptor which is provided by hydrogenosome present in *T. vaginalis*
  - In metronidazole-resistant *T. vaginalis*, the expression levels of the hydrogenosomal enzymes like ferredoxin are reduced dramatically, which probably eliminates the ability of the parasite to activate metronidazole
  - Resistance is relative and can be overcome with higher doses of oral metronidazole
- □ **If standard therapy fails:** a second dose of metronidazole (2 q) is given
- Refractory cases (i.e. failure after two doses of standard therapy)—Here, treatment with metronidazole 2 g for 5 days is recommended
- □ For hypersensitivity to metronidazole: As there is no other therapy available, desensitization to metronidazole is the only option.

## **Dientamoeba fragilis**

- Morphology- It exists in three forms: trophozoite, precyst and cyst
- Life Cycle- Cysts are the infective forms; transmitted by feco-oral route.
  - Cysts transform to trophozoites which multiply in the large intestine and excreted in feces.
  - True cysts are rarely seen in feces although precystic forms may be seen rarely (5%).



Fragmented karyosome

**Figs 4.12A to C:** *Dientamoeba fragilis*: (A) Schematic diagram of trophozoite; (B) Iron hematoxylin stain showing trophozoite having two nuclei with fragmented karyosome; (C) Cyst (saline mount)

## Laboratory Diagnosis

- Stool examination- trophozoites
- Culture-Loefflers slope medium
- Antigen detection in stool-immunofluorescence and enzyme immuno assays
- Antibody detection in serum-IFA technique and immunoblot
- Molecular methods- Multiplex real-time PCR, EasyScreen PCR

## Treatment

Dientamoeba fragilis

Tetracycline or metronidazole is effective. lodoquinol, paromomycin are the other useful agents.