



Flagellates—I

(Intestinal and Genital)

The background of the slide features a close-up, slightly blurred image of an open book. The book has a vibrant red cover and a white page. The text on the page is faint and illegible. The overall lighting is warm, with a gradient from orange to red at the top, transitioning to a lighter, more neutral tone towards the bottom.

Learning objectives

At the end of the session, the students will know

Classification of flagellates

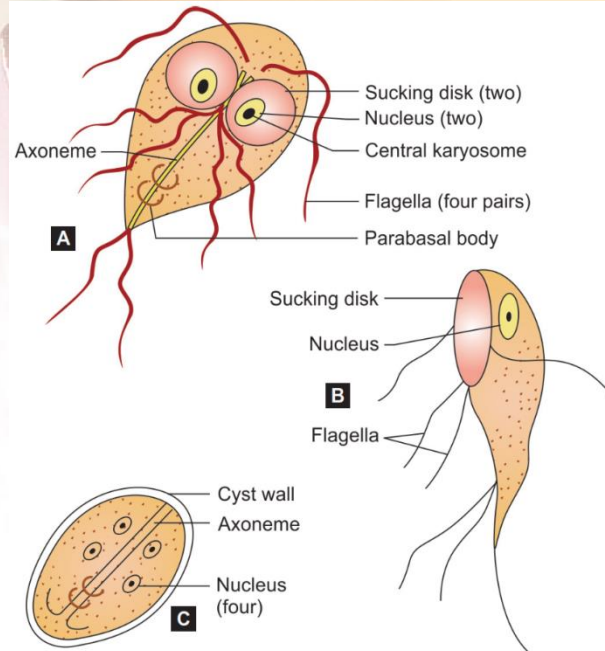
Table 4.1: Taxonomic classification of flagellates

<i>Kingdom</i>	<i>Subkingdom</i>	<i>Phylum</i>	<i>Class</i>	<i>Order</i>	<i>Genus</i>
Protozoa	Archezoa	Metamonada	Trepomonadea	Diplomonadida	<i>Giardia</i> <i>Enteromonas</i>
			Retortamonadea	Retortamonadida	<i>Retortamonas</i> <i>Chilomastix</i>
			Trichomonadea	Trichomonadida	<i>Trichomonas</i> <i>Pentatrichomonas</i> <i>Dientamoeba</i>
	Neozoa	Euglenozoa	Kinetoplastidea	Trypanosomatida	<i>Leishmania</i> <i>Trypanosoma</i>

Table 4.2: Classification of flagellates based on habitat

<i>Intestinal/genital flagellates</i>	<i>Habitat</i>
<i>Giardia lamblia</i>	Duodenum and jejunum
<i>Enteromonas hominis</i>	Large intestine
<i>Retortamonas intestinalis</i>	Large intestine
<i>Chilomastix mesnili</i>	Cecum
<i>Dientamoeba fragilis</i>	Cecum and colon
<i>Trichomonas tenax</i>	Mouth (teeth and gum)
<i>Pentatrichomonas hominis</i>	Ileocecal region
<i>Trichomonas vaginalis</i>	Vagina and urethra
<i>Blood and somatic flagellates</i>	<i>Habitat</i>
<i>Leishmania</i>	Blood and tissue
<i>Trypanosoma</i>	Blood and tissue

Morphology



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

Life Cycle

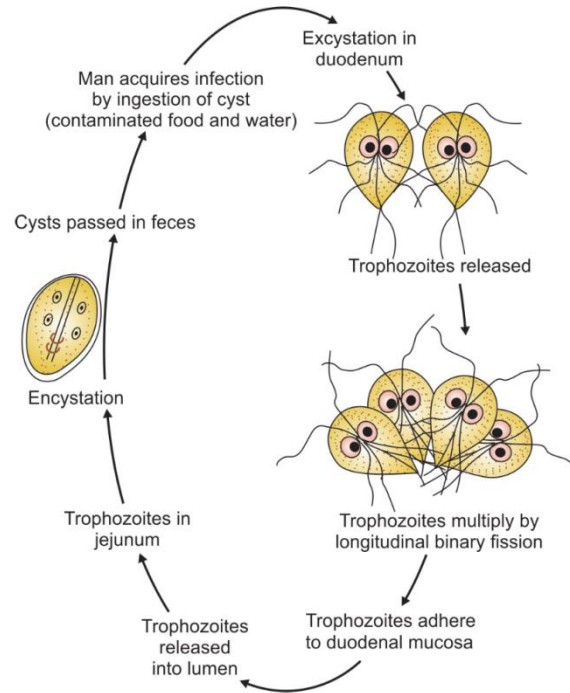


Fig. 4.2: Life cycle of *Giardia lamblia*

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

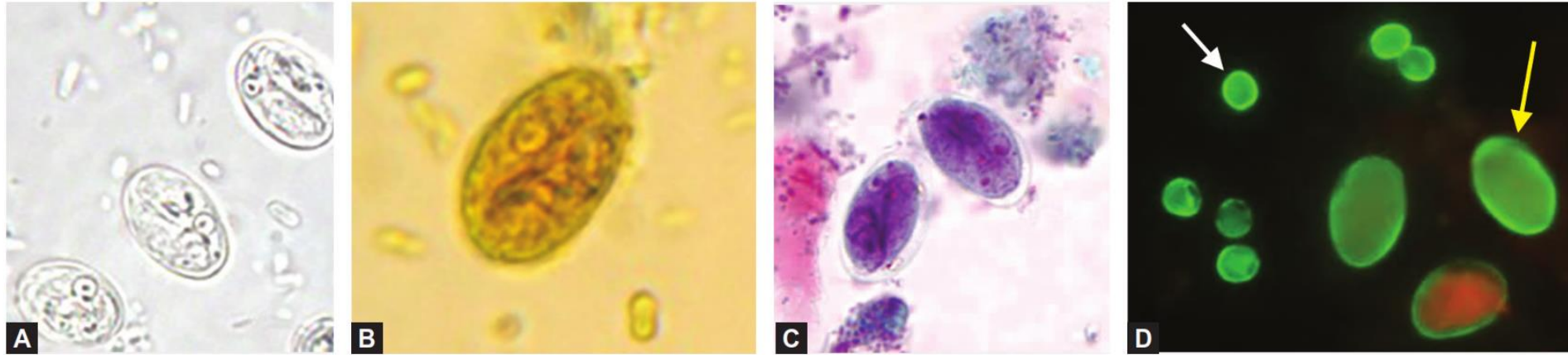


Laboratory Diagnosis

Giardia lamblia

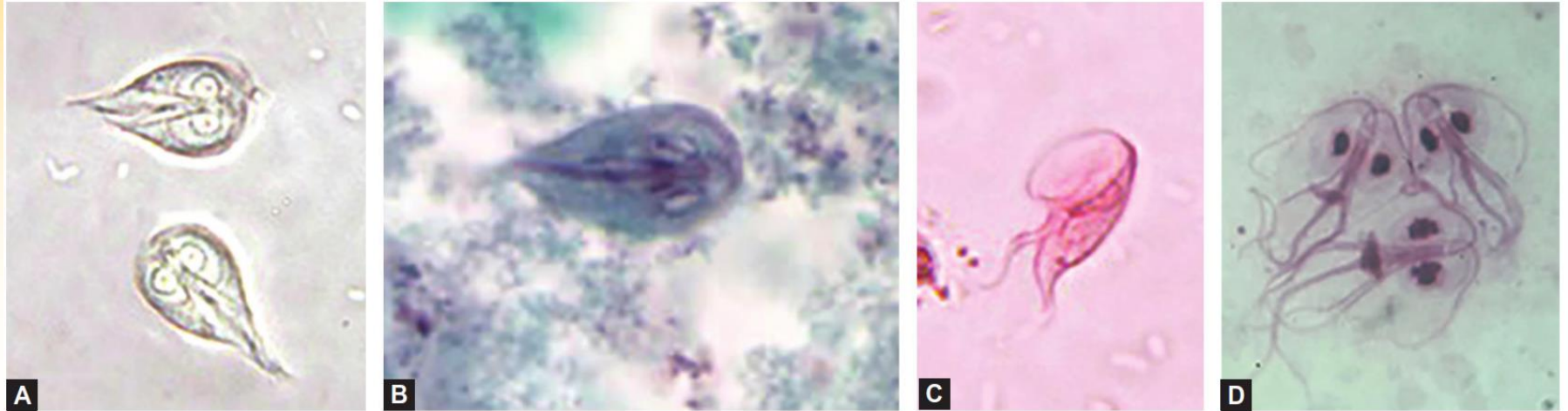
- ❑ **Stool examination** (saline mount)—detects
 - Cysts (oval, 4 nuclei)—indicates carrier/active stage
 - Trophozoites (pear shaped, falling leaf motility)—indicates active infection
 - Other methods—iodine mount, trichrome stain, DFA
- ❑ **Entero-test**—duodenal sampling, with the help of gelatin 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.

Cysts



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

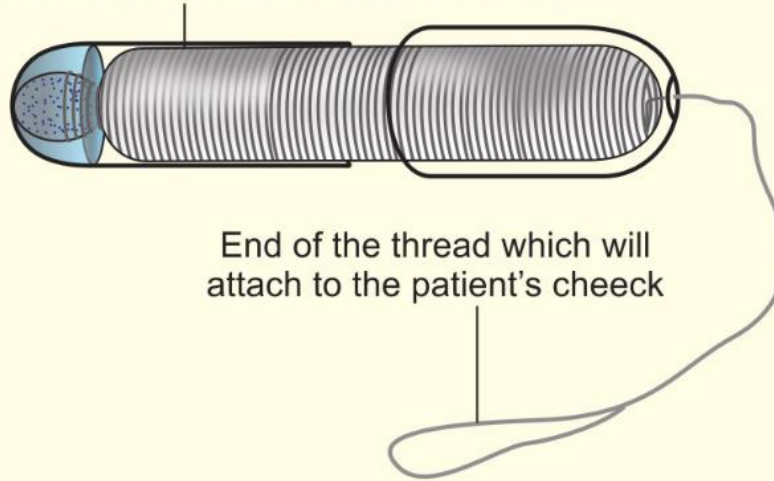
Trophozoites



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)

Entero-test

Gelatin capsule containing the thread
with a weight attached at the other end



End of the thread which will
attach to the patient's cheek

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

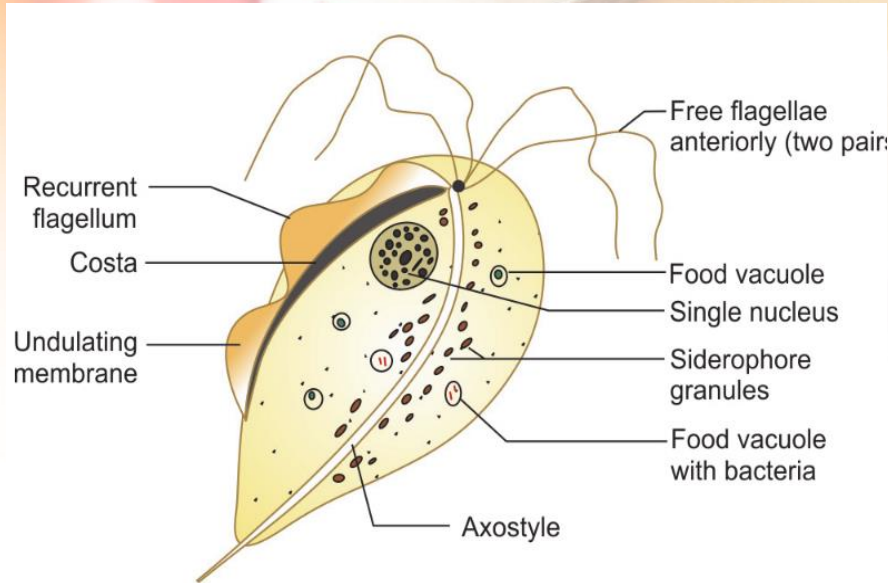


Fig. 4.6: Trophozoite (flagellated) of *Trichomonas vaginalis* (schematic diagram)

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

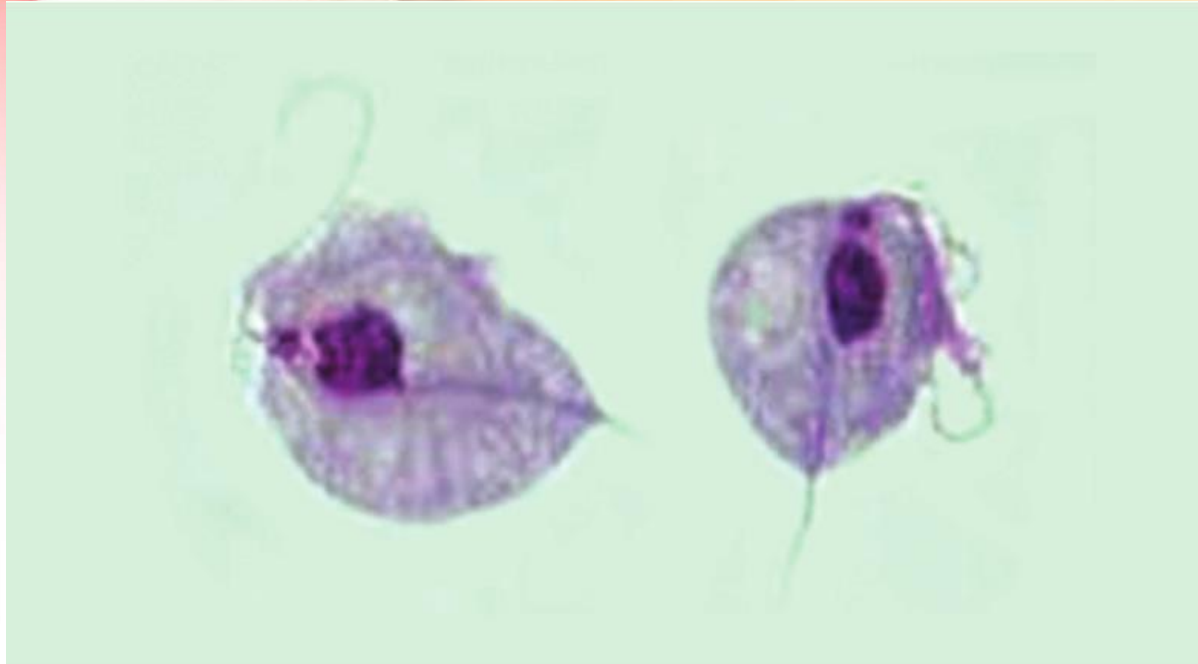


Fig. 4.7: *Trichomonas vaginalis* trophozoite (Giemsa stain)

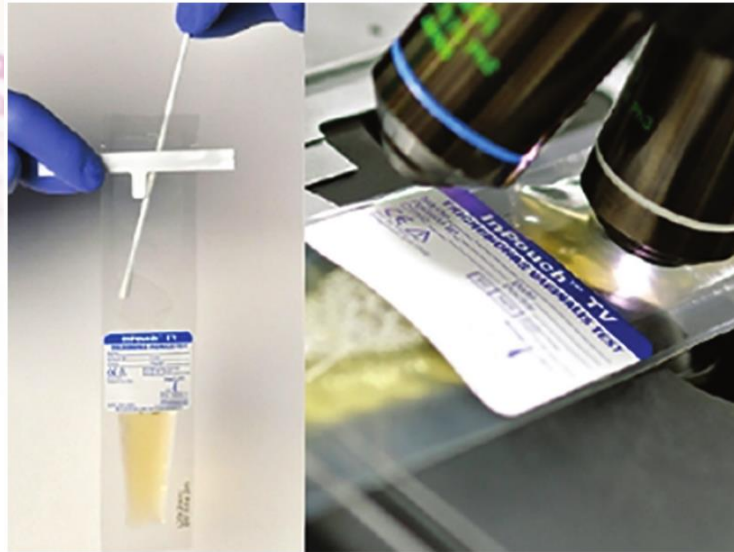


Fig. 4.8: InPouch TV diagnostic system for culturing *T. vaginalis*. The swab containing the specimen is inoculated into the highly selective liquid medium present within the plastic pouch; incubated for 4–7 days at 37°C and then examined under microscope for presence of trophozoites

Treatment

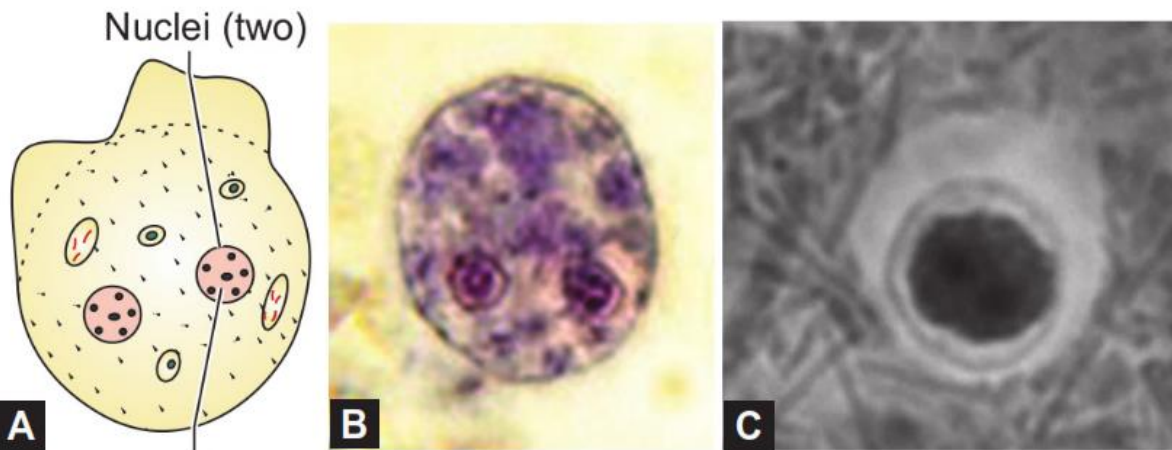
Trichomonas vaginalis

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 g) 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. Iodoquinol, paromomycin are the other useful agents.