

جامعة الانبار

كلية: الصيدلة

قسم: العلوم المختبرية السريرية

اسم المادة باللغة العربية: احياء مجهرية طبية II

اسم المادة باللغة الإنكليزية: **Medical Microbiology II**

المرحلة: الثانية

التدريسي: م. د. رواء علي حسين

عنوان المحاضرة باللغة العربية: السوطيات

عنوان المحاضرة باللغة الإنكليزية: **Flagellates Protozoa**

---

## **Flagellates Protozoa**

**Sub-phylum: Mastigiphora**

**Class: Zoomastigophora**

**Suborder 2. Diplomonadina**

## **INTRODUCTION**

Flagellates are unicellular microorganisms. Their locomotion is by lashing a tail-like appendage called a flagellum or flagella and reproduction is by simple binary fission. **There are three groups of flagellates:**

**a-Flagellates of digestive tract:( Lumen dwelling flagellates)**

-*Giardia lamblia* ,

-*Chilomastix mesnili* ,

**b-Flagellates of genital organs: (Lumen dwelling flagellates)**

-*Trichomonas vaginalis*.

### **c-Hemoflagellates(Flagellates of blood and tissue.)**

-Trypanosoma species.

-Leishmania species.

- -The flagellates protozoa are distinguished by having in their trophozoite stage one to several thread-like extensions of the ectoplasm(flagella) ; arising from a complex system of axonemes extending along the midline which arising from a basal body .

### ***Giardia lamblia***

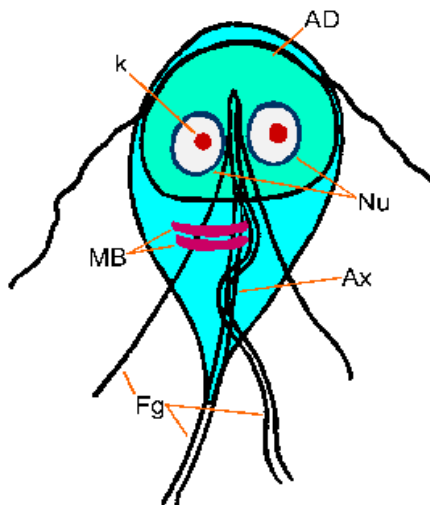
*Giardia lamblia* is a flagellate of world-wide distribution. It is more common in warm climates than temporal climates . It is the most common flagellate of the intestinal tract, causing Giardiasis. Humans are the only important reservoir of the infection.. *Giardia* inhabits the crypts of the duodenum and upper jejunum. Giardiasis is an infection of the upper small bowel, which may cause diarrhea.

Disease: **Giardiasis, Lambliasis, steatorrhea.**

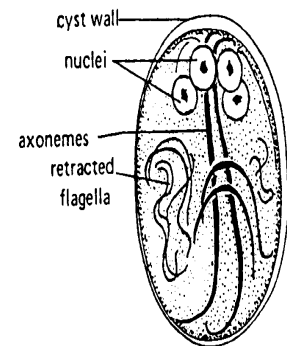
### **Morphology**

The parasite has both a trophic and cystic stage. The trophozoites of *G. lamblia* is pear shaped, with a broad anterior and much attenuated posterior . It is 10 -12µm long and 5-7 µm wide, bilaterally symmetrical, and has two nuclei with central karyosomes . It is also relatively flattened with a large sucking disk on the anterior ventral side, which serves as the parasites

method attachment to the mucosa of the host. The trophozoite also has two median bodies the function of the median bodies is not known, but most believe they are somehow involved with the adhesive disk and its formation, and four pairs of flagella. The fibrils are called axonemes (Ax) or (axostyles). They attach themselves to the surface of the jejunal or duodenal mucosa by their disc-like suckers which are found on their ventral surface. They multiply in the gut by binary fission.



Trophozoite of *G. lamblia*



cyst of *G. lamblia*

### Morphology of Cysts

It is ovoid in shape; 8-12  $\mu\text{m}$  long x 7-10  $\mu\text{m}$  wide and have thin cyst wall. Four nuclei present, often concentrated at one end. Flagella shorten and are

retracted within cyst, axonemes provide internal support and parabasal bodies may also seen.

## **MODE OF TRANSMISSION**

- ✓ Infection is occurred by ingestion of cyst in contaminated food & water.
- ✓ Direct transmission from person to person may occur in children, , mentally ill persons.

## **CLINICAL FEATURES**

**1-Silent cases without any symptoms.**

**2-Intestinal :which include the following:-**

1. Malabsorption syndrome (Steatorrhoea)

It has been suggested that the coating of large surface areas of upper small bowel by giardia may act as a barrier to fat absorption and thus cause the steatorrhea (fatty or oil diarrhea)

2. Mucus diarrhea

3. Dull epigastric pain

4. Flatulence

5. Chronic enteritis

6. Acute enterocolitis

**Incubation period : about 2 weeks**

**3. General :**

1. Fever

2. Anaemia

3. Weight loss

4. Allergic manifestations.

4. Chronic cholecystopathy

- ❖ Note: Stool containing a large amount of mucus and fat and no blood.
- ❖ However 50% of *G. lamblia* infections are symptomless, although severe infections may develop in immunocompromised hosts..

## **Laboratory Diagnosis**

### **1-Stool Examination**

- ❖ Identification of cysts in formed stool and trophozoites & cysts in diarrhoeic stool or after a purgative.
- ❖ In asymptomatic carriers only cysts are seen.
- ❖ Macroscopy : offensive odour, pale coloured & fatty stool.
- ❖ Microscopy : saline & iodine wet preparations.
- ❖ Multiple specimens need to be examined.
- ❖ Concentration techniques like formal ether or zinc acetate are used.

### **2- Enterotest (STRING TEST)**

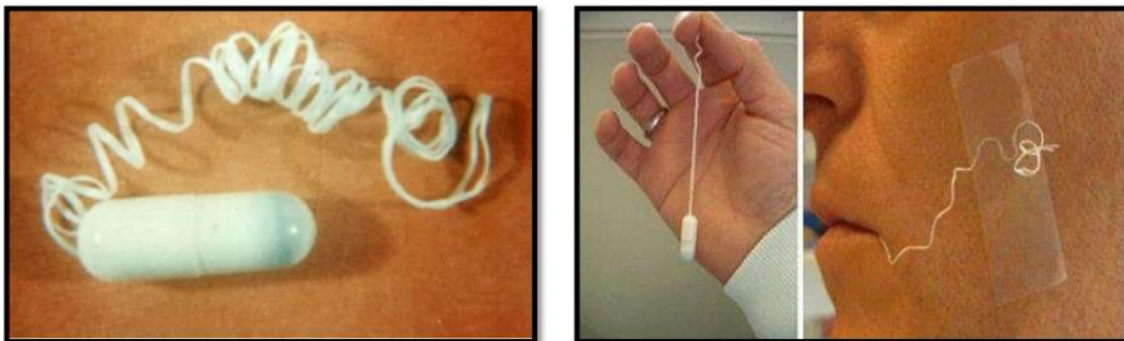
Method for obtaining duodenal specimen (upper part of small intestine)

Procedure :

- A coiled string with a small weighted gelatin capsule is swallowed by the patient & the free end of the string is attached to the side of the patient's face.
- The capsule dissolves in the stomach & the string which is weighted at its distal end, passes into the duodenum.
- After 2-4 hrs the string is withdrawn & placed in a saline with mechanical shaking.
- The centrifuged deposit of saline is examined by wet mount technique to detect the presence of motility of the organism or specific morphological forms of trophozoites of Giardia (and larvae of Strongyloides stercoralis).

**When the test should performed ???**

- Entero-test is performed when a physician suspects a parasitic infection, but no parasites were found in stool sample.
- As its sensitivity is comparable to duodenal aspirate, it eliminates the need of duodenal intubation.



**Enterotest (String test)**

**3- Serological test**

**Serodiagnosis**

**A-Antigen detection in feces –**

- 1- ELISA

2- IIF (Indirect immunofluorescent tests)

3- Immuno-chromatographic strip test

-Antigen present – active infection.

-Giardia specific Ag 65 (GSA 65) detection by ELISA kits.

-Sensitivity - 95% Specificity – 100% compared to microscopy.

-Tests are not for routine purpose.

-It is for epidemiological & control purposes

### **B-Antibody detection –**

1- IIF

2- ELISA

-Tests can't differentiate between recent & past infection.

- Lack sensitivity & specificity.

-Antibody detection (anti Giardia IgG Ab) is useful for epidemiological & pathophysiological studies.

-The presence of anti Giardia secretory IgA Ab in breast milk protects breast fed infants from giardiasis

### **4-Molecular method**

DNA based techniques are available now.

They are used to demonstrate the genome of the parasite.

- PCR

- DNA probe

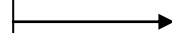
### **Treatment**

-Metronidazole – 250mg x 3 times daily x 5 days. (Cure rate -95%)

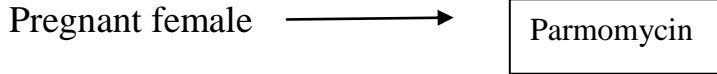
-Tinidazole – 2 g single dose. (More effective)

-Furazolidone

-Nitazoxamide



Children (less adverse effects)



### *Chilomastix mesnili*

It is thought to be non-pathogenic although the trophozoite has been associated with diarrheic stool. This is the largest flagellate found in man. The natural habitat of *Chilomastix mesnili* is the colon.

#### **Morphology of trophozoite:**

The parasite has both a trophic and cystic stage. The trophozoite of *C. mesnili* is pear shaped and measure 6-20µm in length. It is unsymmetrical rounded anteriorly and spirally twisted posteriorly. It has one large nucleus with a small karyosome and three flagella that extend from the nucleus at the anterior end of the parasite. A distinct oral groove or cytosome can be seen near the nucleus with its sides being supported by two filaments. They are known to move in a directional manner.

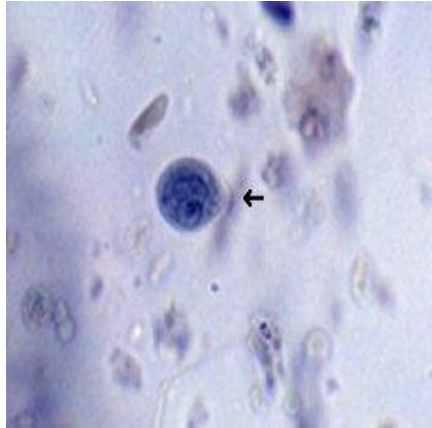


**Trophozoite of *C. mesnili***



## Morphology of cysts

The cyst is 6-9 $\mu$ m; it has a large single nucleus with a large karyosome. is lemon shaped , it has a thick hyaline wall and having the characteristic internal features of the trophozoite.



**The cyst of *C. mesnili***

## Laboratory Diagnosis

The characteristic lemon shaped cysts can be seen in a formol-ether concentrate. Motile organisms can be seen in a wet preparation of a fresh stool .

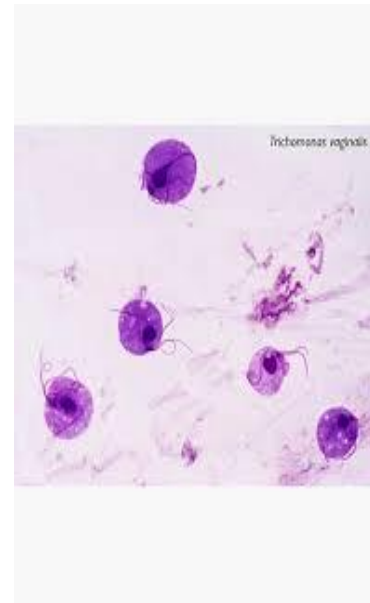
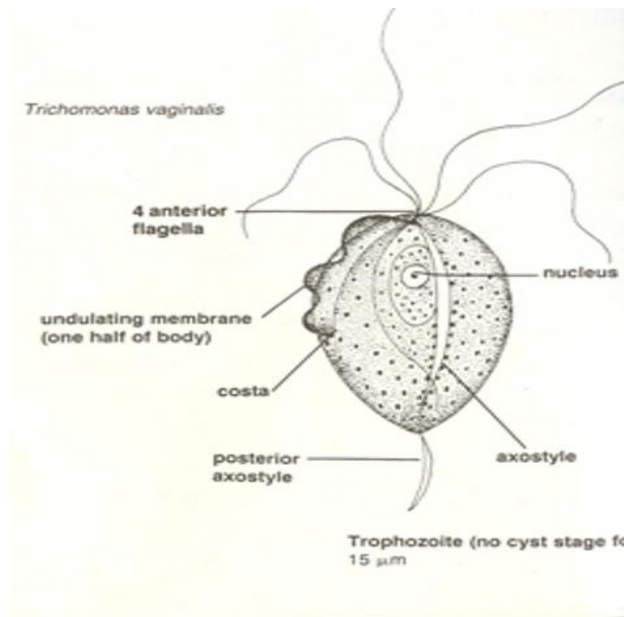
## *Trichomonas vaginalis*

This protozoan parasitizes in the vagina, urethra and prostate and causes trichomonas vaginitis, urethritis and prostatitis. Trichomoniasis vaginalis is prevalent all over the world.

## Morphology

Only the trophozoite stage is found in its life cycle. The trophozoite is ovoid or pear-shaped, 10~30 $\times$ 5~15 $\mu$ m in size. It has 4 anterior flagella and one

posterior flagellum which turns back and is attached to the to the body by an undulating membrane. The undulating membrane of *T. vaginalis* is very short, only one-half of its body length. There is nucleus and the axostyle project posteriorly out of the body. The motility is jerky and non-directional.



**Trophozoite of *Trichomonas vaginalis***

**Trophozoite shape under microscope**

### **Mode of transmission**

- Trophozoite cannot survive outside and so infection has to be transmitted directly from person to person.
- Sexual transmission is the usual mode of transmission.
- Trichomoniasis often coexists with other sexually transmitted diseases; like candidiasis, gonorrhea, syphilis, or human immunodeficiency virus (HIV).
- Babies may get infected during birth.
- Fomites such as towels have been implicated in transmission.

- **Trophozoite divides by binary fission.**
- **Incubation period is roughly 10days**

### **Clinical manifestation**

#### **❖ WOMEN ( SYMPTOMATIC)**

-Vulvo vaginitis (Trichomonal vaginitis)

-Urethritis

#### **❖ IN MEN (ASYMPTOMATIC)**

Urethritis, epididymis, prostatitis, and superficial ,penile ulcerations.

Irritation inside the penis, mild discharge, discharge may be purulent to mucoid or slight burning after urination or ejaculation.

### **COMPLICATIONS**

#### **❖ (WOMEN)**

-PID

-Premature birth

-Low birth weight

-Increased risk of transmission of HIV

-Increased chance of cervical cancer

**May also cause Pneumonia, bronchitis, and oral leisons.**

#### **❖ In men:**

- Prostatitis
- Epididymitis
- Urethral stricture
- Infertility.

## **SPECIMENS**

**IN WOMEN :** vaginal discharge, endocervical specimens.

**IN MEN:** Prostatic fluid, less commonly semen.

common specimens urethral swab, early morning first voided urine sediment

## **LAB DIAGNOSIS.**

### **1. MICROSCOPY**

**Trichomonas in the vaginal discharge can be demonstrated by;**

- Wet mount
- Acridine orange staining
- Papanicolau stain (PAP smear)
- Direct fluorescent antibody (DFA)staining

Vaginal or urethral discharge is examined

microscopically in saline wet mount preparation for characteristic, jerky and twitching motility and shape.

**In males trophozoites may be found in urine or prostatic secretions.**

- DFA is more sensitive.

### **2. Culture**

- Consider as gold standard for the diagnosis.
- Is recommended when direct microscopy is negative
- most sensitive (95%)

- Grows best at 35-37C under anaerobic condition
- Can be grown in a variety of solid or liquid media , tissue culture, and eggs. Cystein-peptone-liver maltose (**CPLM**) medium and plastic envelope medium (**PEM**) are often used.

### **3. Antigen detection in vaginal smears**

ELISA is used for demonstration of Trichomonas antigen in vaginal specimens.

### **4. Molecular diagnosis**

- DNA probes
- PCR(highly sensitive (97%) and specific (98%) test

#### **TREATMENT**

- Metronidazole 2g orally as a single dose or 250mg three times daily for 7 days.
- Metronidazole is contraindicate in pregnancy due to is mutagenecity, so topical therapy with clotrimazole is applied

### **The Ciliates**

The ciliates belong to the family Ciliophora. They possess simple cilia or compound ciliary organelles, two types of nuclei and a large contractile vacuole. The only member of the ciliate family to cause human disease is *Balantidium coli*

#### ***Balantidium coli***

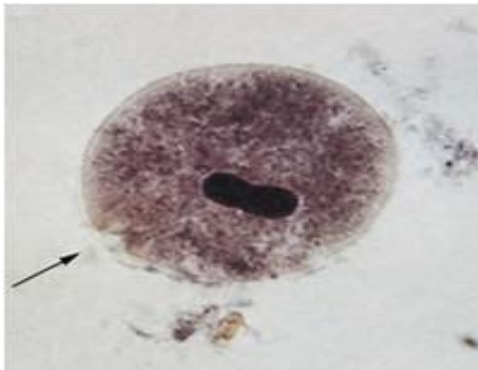
The organisms inhabit the large intestine, cecum and terminal ileum where they feed on bacteria. The most common hosts being humans, pigs and rodents. Human infection is usually from pigs and is rare.

Disease: Balantidiasis

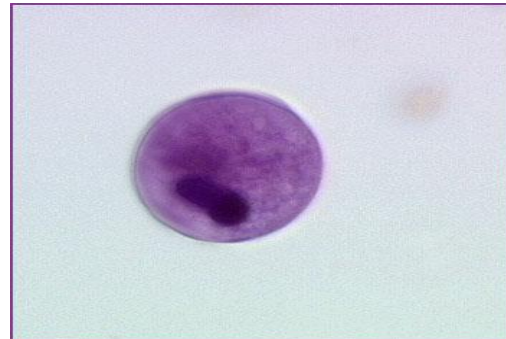
### **Morphology of the Trophozoite**

Trophozoites of *B. coli* measure approximately 30-150 $\mu$ m in length x 25-120 $\mu$ m in width but have been known to attain lengths of up to 200 $\mu$ m. They are oval in shape and covered in short cilia. A funnel shaped cytosome can be seen near the anterior end. the two nuclei are visible. The macronucleus is

long and sausage-shaped, and the spherical micronucleus is nested next to it, often hidden by the macronucleus .Multiplication is by binary fission in the trophozoite stage.



Trophozoite of *B. coli*



Cyst of *B. coli*

### **Morphology of the Cyst**

The cyst is spherical or ellipsoid and measures from 30-200 $\mu$ m by 20-120 $\mu$ m. It contains 1 macro and 1 micronucleus. The cilia are present in young cysts and may be seen slowly rotating, but after prolonged encystment, the cilia disappear. The cyst, ingested by a fresh host, excysts to liberate the trophozoite.

### **Clinical Disease**

Severe *B. coli* infections may resemble amebiasis. Symptoms include diarrhea, nausea, vomiting, and anorexia. The diarrhea may persist for long periods of time resulting in acute fluid loss. *Balantidium coli* also has the potential to penetrate the mucosa resulting in ulceration just as those of *E. Entamoeba histolytica*.

### **Laboratory Diagnosis**

Wet preparations of fresh and concentrated stool samples reveal the characteristic cysts and motile trophozoites. They are easier to identify in direct-smear saline preparations than permanently stained fecal smears.