

Medical Laboratory Techniques Department Lecture 4 : Genital, Intestinal, Oral flagella Dr.Fatima H.Abbas



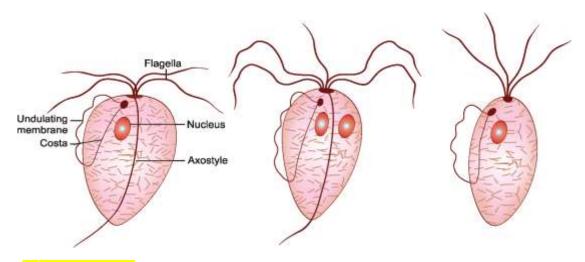
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Genital, Intestinal, Oral flagella

Trichomonas

Trichomonas differs from other flagellates, as they exist only in trophozoite stage. Cystic stage is not seen.

*Genus *Trichomonas* has 3 species, which occur in humans.



- 1- T. vaginalis
- 2- T. hominis
- 3- T. tenax

1. T.vaginalis (Genital flagella)

Morphology

It is **pear-shaped** or **ovoid** and measures 10–30 μm in length and 5–10 μm in breadth 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, which is supported at its base by a flexible rod, **costa**.
- * A prominent **axostyle** runs throughout the length of the body and projects posteriorly like a tail.
- *The cytoplasm shows prominent siderophillic اليفة للحديد granules, which are most numerous alongside the axostyle and costa.
- *It is motile with a rapid jerky ارتعاشية or twitching متشنجة type movement.

Habitat

In females, it lives in vagina and cervix 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.

Life cycle

Life cycle of *T. vaginalis* is completed in a single host either male or female.

Mode of transmission:

The trophozoite cannot survive outside and so infection has to be transmitted directly from person to person. Sexual transmission is the usual mode of infection.

- Trichomoniasis often coexists with other sexually transmitted diseases; like candidiasis, gonorrhea السيلان, syphillis, or human immunodeficiency virus (HIV).
- Babies may get infected during birth.
- Fomites الأدوات such as towels الفوط have been implicated in transmission.
- Trophozoites divide by binary fission.
- As cysts are not formed, the **trophozite** itself is the **Infective form**.
- Incubation period is roughly 10 days.

Protozoan's transmitted by sexual contact

- Trichomonas vaginalis
- Giardia lamblia
- Entamoeba histolytica

Pathogenesis

T. vaginalis particularly infects squamous epithelium and not columnar epithelium. It secretes cystine proteases, lactic acid, and acetic acid, which disrupt the glycogen levels and lower the pH of the vaginal fluid.

- It is an **obligate parasite** and cannot live without close association with the vaginal, urethral, or prostatic tissues.
- Parasite causes petechial hemorrhage نزف حبري (strawberry فراولي mucosa), metaplastic changes, and desquamation of the vaginal epithelium.
- Intracellular edema and so called **chicken-like epithelium**, is the most characteristic feature of trichomoniasis.

Clinical Features

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

- In females, it may produce severe pruritic vaginitis with an offensive, yellowish green, often frothy discharge افرازات رغوية, dysuria عسر البول, and dyspareunia عسر الجماع. Cervical erosion تاكل
- Endometritis and pyosalpingitis are infrequent complications.
- Rarely, neonatal pneumonia and conjunctivitis have been reported in infants born to infected mothers.
- The incubation period of trichomoniasis is 4 days to 4 weeks.

Laboratory Diagnosis

1. Microscopic examination

- 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.

- Fixed smears may be stained with acridine orange, papanicolaou, and Giemsa stains.
- Direct fluroscent antibody (DFA) is another method of detection of parasite and is more sensitive than the wet mount.

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.

3. Serology

- ELISA is used for demonstration of *T. vaginalis* antigen in vaginal smear using a monoclonal antibody for 65-KDA surface polypeptide of *T.vaginalis*.

4. Molecular method

- DNA hybridization and PCR are also highly sensitive (97%) and specific (98%) tests for the diagnosis of trichomoniasis

1. T.hominis (Intestinal flagella)

Non-pathogenic .The natural habitat is the lumen of the cecum. Trophozoite is pear shaped(1nucleus at anterior end," 3-5flagella 3anti.,1 post", central karyosome, axostyle in the middle and undulating membrane is the whole length). There is no cyst stage. Laboratory diagnosis by stool examination. Transmission occurs by ingesting trophozoites.

2. T.tenax (Oral flagella)

Non-pathogenic .The natural habitat is the mouth particularly gums and teeth. Life cycle consist of trophozoite {5 flagella "4 to anterior end and 1 to post.", containing undulating membrane extend 2/3 body length and gives the jerky motion(wave-like)} and there is no cyst stage. Laboratory diagnosis by mouth scrapings.

Hemoflagellates

The blood and tissue flagellates belong to the family <u>Trypanosomatidae</u>. The family consists of 6 genera, of which 2 genera *Trypanosoma* and *Leishmania* are pathogenic to humans.

General Characteristics

- They live in the blood and tissues of man and other vertebrate hosts and in the gut of the insect vectors.
- Members of this family have a single nucleus, a kinetoplast, and a single flagellum.
- **Nucleus** is round or oval and is situated in the central part of the body.

- **Kinetoplast** consists of a deeply staining **parabasal body** and adjacent dot like **blepharoplast**. The body and blepharoplast are connected by one or more thin fibrils.
- **Flagellum** is a thin, hair like structure, which originates from the blepharoplast. The portion of the flagellum, which is inside the body of the parasite and extends from the blepharoplast to surface of the body, is known as **axoneme**. A free flagellum at the anterior end traverses on the surface of the parasite as a narrow **undulating membrane**.
- **Hemoflagellates** exist in two or more of four morphological stages. These forms **amastigote**, **promastigote**, **epimastigote** and **trypomastigote**.
- Parabasal body and blepharoplast together constitute the Kinetoplast.

Differences between Various Morphological Stages of Hemoflagellates

	Amastigote	Promastigote	Epimastigote	Trypomastigote
Morphological characteristics	Rounded or ovoid, without any external flagellum. The nucleus, kinetoplast, and axial filaments can be seen. The axoneme extends upto the anterior end of the cell	Lanceolate in shape. Kinetoplast is anterior to the nucleus (antenuclear kineloplast) near the anterior end of the cell, from which flagellum emerges. There is no undulating membrane	Elongated, with the kinetoplast placed more posteriorly, though close to and in front of the nucleus (juxtanuclear kinetoplast). The flagellum runs alongside the body as a short undulating membrane, before emerging from the anterior end	This stage is elongated, spindle- shaped with a central nucleus. The kinetoplast is posterior to the nucleus (postnuclear kinetoplast) and situated at the posterior end of the body. The flagellum runs alongside the entire length of the cell to form a long undulating membrane before emerging as a free flagellum from the anterior end
Seen in	Trypanosoma cruzi and Leishmania as intracellular form in vertebrate host	It is the infective stage of Leishmania, found in the insect vector as well as in cultures in-vitro	It is the form in which Trypanosoma brucei occur in salivary gland of the vector tsetse fly and Trypanosoma cruzi in the midgut of the vector reduviid bug. Note: This stage is lacking in Leishmania.	This is the infective stage of trypanosomes found in arthropod vector and in the blood of infected vertebrate. Note: This stage is lacking in Leishmania
Schematic illustration	N PBA	NO B	NO BOTTO	ENU

N = Nucleus; P = Parabasal body; B = Blepharoplast; A = Axoneme; U = Undulating membrane; F = Flagellum.

- All members of the family have similar life cycles. They all require an insect vector as an intermediate host.
- Multiplication in both the vertebrate and invertebrate host is by **binary fission**. **No sexual cycle is known**.

Leishmania

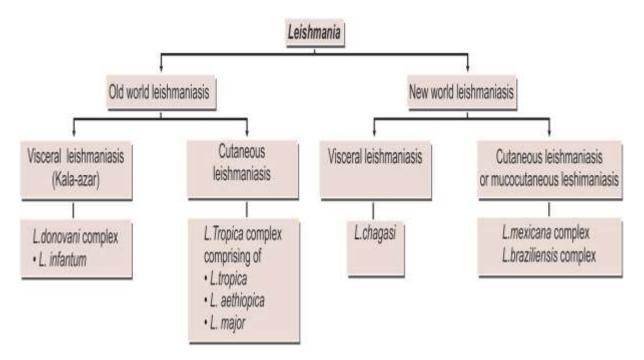
General Characteristics

The genus *Leishmania* is named after Sir William Leishman, who discovered the flagellate protozoa causing Kala azar, the Indian visceral leishmaniasis.

- All members of the genus *Leishmania* are **obligate intracellular parasites** that pass their life cycle in **2hosts**: the mammalian host and the insect vector, female sand fly.
- In humans and other mammalian hosts, they multiply within macrophages, in which they occur exclusively in the amastigote form, having an ovoid body containing a nucleus and kinetoplast.
- Spindle shaped body and a single flagellum arising from anterior end.
- Leishmaniasis has an immense geographical distribution in the tropics and subtropics of the world, extending through most of the Central and South America, part of North America, central and Southeast Asia, India, China, the Mediterranean region, and Africa.
- The disease affects the low socioeconomic group of people. Overcrowding, poor ventilation, and collection of organic material inside house facilitate its transmission.

Across the tropics, 3 different diseases are caused by various species of genus *Leishmania*. These are:

- **Visceral leishmaniasis:** The species *L. donovani* complex infecting internal organs (liver, spleen, and bone marrow) of human is the causative parasite.
- Cutaneous leishmaniasis: The species *L. tropica* complex, *L. aethiopica*, *L. major* and *L. Mexicana complex* are the causative parasite.
- Mucocutaneous leishmaniasis: It is caused by the L. braziliensis complex.



Distribution and disease caused by *Leishmania* spp.

Old World Leishmaniasis

<mark>Leishmania Donovani</mark>

L. donovani causes visceral leishmaniasis or Kala-azar. It also causes the condition, Post Kala-azar Dermal Leishmaniasis (PKDL).

History and Distribution

Sir William Leishman in 1900, observed the parasite in spleen smears of a soldier who died of '**Dumdum fever**' or Kalaazar contracted at Dum Dum, Calcutta. Leishman reported this finding from London in 1903. In the same year, Donovan also reported the same parasite in spleen smears of patients from Madras. The name *Leishmania donovani* was, therefore given to this parasite. The amastigote forms of the parasite as seen in smears from patients are called **Leishman Donovan** (LD) bodies.

Habitat

The amastigote (LD body) of *L. donovani* is found in the reticuloendothelial system. They are found mostly within the macrophages in the spleen, liver, bone marrow and less often in other locations such as skin, intestinal mucosa, and mesenteric lymph nodes.

Morphology

The parasite exists in 2 forms mammals.

- **Promastigote form:** in the sand fly and in artificial culture.
- Amastigote
- The amastigote form (LD body) is an ovoid or rounded cell, about 2–4 μm in size.
- It is typically intracellular, being found inside macrophages, monocytes, neutrophils, or endothelial cells.
- They are also known as **LD bodies**.
- Smears stained with Leishman, Giemsa, or Wright's stain

Life Cycle

L. donovani completes its life cycle in 2 hosts. **Definitive host:** Man, dog, and other mammals.

Vector: Female sand fly (*Phlebotomus* species).

Infective form: Promastigote form present in midgut of female sand fly.

Mode of transmission:

- Humans acquire by bite of an infected female sand fly.
- It can also be transmitted vertically from mother to fetus, by blood transfusion, and accidental inoculation in the laboratory.
- Incubation period: Usually 2–6 months, occasionally it may be as short as 10 days or as long as 2 years.

