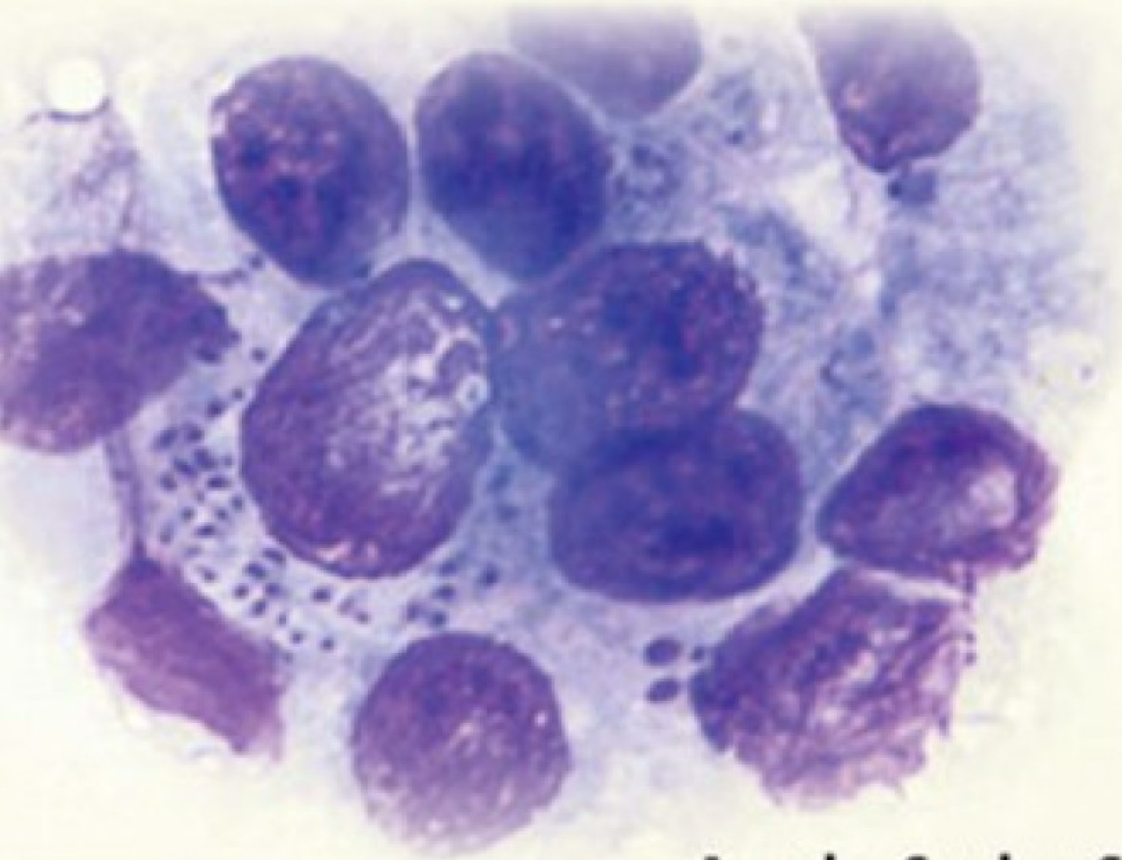
JAYPEE

Essentials of MEDICAL PARASITOLOGY



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Section 1

Introduction

Chapter 1 General Introduction: Parasitology

1 General Introduction: Parasitology

Chapter Outline

- Taxonomy of parasites
- Parasite
- Host
- Host-parasite relationship
- Transmission of parasites
- Life cycle of the parasites
- Pathogenesis of parasitic diseases
- Immunology of parasitic diseases
- Laboratory diagnosis of parasitic diseases
- Treatment of parasitic diseases
- Expected questions

Medical Parasitology deals with the study of animal parasites, which infect and produce diseases in human beings.

TAXONOMY OF PARASITES

According to the binomial nomenclature as suggested by Linnaeus, each parasite has two names: a genus and a species name.

These names are either derived from: names of their discoverers, Greek or Latin words of the geographical area where they are found, habitat of the parasite, or hosts in which parasites are found and its size and shape.

All parasites are classified under the following taxonomic units—the kingdom, subkingdom, phylum, subphylum, super class, class, subclass, order, suborder, super family, family, genus and species.

The generic name of the parasite always begins with an initial capital letter and species name with an initial small letter, e.g., *Entamoeba histolytica*.

PARASITE

Parasite is a living organism, which lives in or upon another organism (host) and derives nutrients directly from it, without giving any benefit to the host.

Protozoa and helminths (animal parasites) are studied in Medical Parasitology.

Parasites may be classified as:

- **Ectoparasite:** They inhabit the surface of the body of the host without penetrating into the tissues. They are important vectors transmitting the pathogenic microbes. The infection by these parasites is called as **infestation**, e.g., fleas or ticks
- **Endoparasite:** They live within the body of the host (e.g., *Leishmania*). Invasion by the endoparasite is called as **infection.**

The endoparasites are of following types:

- **Obligate parasite:** They cannot exist without a parasitic life in the host (e.g., *Plasmodium* species)
- **Facultative parasite:** They can live a parasitic life or free-living life, when the opportunity arises (e.g., *Acanthamoeba*)
- **Accidental parasite:** They infect an unusual host (e.g., *Echinococcus granulosus* infect humans accidentally)
- **Aberrant parasite or wandering parasite:** They infect a host where they cannot live or develop further (e.g., *Toxocara* in humans).

HOST

Host is defined as an organism, which harbors the parasite and provides nourishment and shelter.

Hosts may be of the following types:

- **Definitive host:** The host in which the adult parasites replicate sexually (e.g., anopheles species), is called as definitive host. The definitive hosts may be human or nonhuman living things
- **Intermediate host:** The host in which the parasite undergoes asexual multiplication is called as intermediate host. (e.g., in malaria parasite life cycle, humans are the intermediate hosts)
  - Intermediate hosts are essential for the completion of the life cycle for some parasites
  - Some parasites require two intermediate hosts to complete their different larval stages. These are known as the first and second intermediate hosts respectively (e.g., Amphibian snails are the first intermediate host and aquatic plants are the second intermediate host for *Fasciola hepatica*)

Hosts can also be:

- **Reservoir host:** It is a host, which harbours the parasites and serves as an important source of infection to other susceptible hosts. (e.g., dog is the reservoir host for cystic echinococcosis)
- **Paratenic host:** It is the host, in which the parasite lives but it cannot develop further and not essential for its life cycle is known as paratenic host (e.g., fresh water prawn for *Angiostrongylus cantonensis,* big suitable fish for plerocercoid larva of *Diphyllobothrium latum* and freshwater fishes for *Gnathostoma spinigerum*). It functions as a transport or carrier host
- **Amplifier host:** It is the host, in which the parasite lives and multiplies exponentially.

HOST-PARASITE RELATIONSHIP

The relationship between the parasite and the host, may be divided into the following types:

- **Symbiosis:** It is the close association between the host and the parasite. Both are interdependent upon each other that one cannot live without the help of the other. None of them suffer any harm from each other
- **Commensalism:** It is an association in which the parasite only derives the benefit without causing any injury to the host. A commensal is capable of living an independent life
- **Parasitism:** It is an association in which the parasite derives benefit from the host and always causes some injury to the host. The host gets no benefit in return.

Disease: The disease is the clinical manifestation of the infection, which shows the active presence, and replication of the parasite causing damage to the host. It may be mild, severe and fulminant and in some cases may even cause death of the host.

Carrier: The person who is infected with the parasite without any clinical or sub clinical disease is referred to as a **carrier**. He can transmit the parasites to others.

TRANSMISSION OF PARASITES

It depends upon:
- Source or reservoir of infection
- Mode of transmission.

Sources of Infection

- **Man:** Man is the source or reservoir for a majority of parasitic infections (e.g., amoebiasis, enterobiasis, etc.) The infection transmitted from one infected man to another man is called as **anthroponoses**
- **Animal:** The infection which is transmitted from infected animals to humans is called as **zoonoses**. The infection can be transmitted to humans either directly or indirectly via vectors. (e.g., cystic echinococcosis from dogs and toxoplasmosis from cats)
- **Vectors:** Vector is an agent, usually an arthropod that transmits the infection from one infected human being to another. Vector can be biological or mechanical. An infected blood sucking insect can transmit the parasite directly into the blood during its blood meal.

Note: Vectors have been dealt in detail in Medical Entomology (Chapter 16).

- **Contaminated soil and water:** Soil polluted with human excreta containing eggs of the parasites can act as an important source of infection, e.g., hookworm, *Ascaris* species, *Strongyloides* species and *Trichuris* species.

  Water contaminated with human excreta containing cysts of *E. histolytica* or *Giardia lamblia*, can act as source of infection
- **Raw or under cooked meat:** Raw beef containing the larvae of *Cysticercus bovis* and pork containing *Cysticercus cellulosae* are some of the examples where undercooked meat acts as source of infection
- **Other sources of infection:** Fish, crab or aquatic plants, etc.

Modes of Transmission

The infective stages of various parasites may be transmitted from one host to another in the following ways:

- **Oral or feco-oral route:** It is the most common mode of transmission of the parasites. Infection is transmitted orally by ingestion of food, water or vegetables contaminated with feces containing the infective stages of the parasite. (e.g., cysts of *E. histolytica*, and ova of *Ascaris lumbricoides*)
- **Penetration of the skin and mucous membranes:** Infection is transmitted by the penetration of the larval forms of the parasite through unbroken skin (e.g., filariform larva of *Strongyloides stercoralis* and hookworm can penetrate through the skin of an individual walking barefooted over fecally contaminated soil), or by introduction of the parasites through blood-sucking insect vectors. (e.g., *Plasmodium* species, *Leishmania* species and *Wuchereria bancrofti*)
- **Sexual contact:** *Trichomonas vaginalis* is the most frequent parasite to be transmitted by sexual contact. However, *Entamoeba, Giardia* and *Enterobius* are also transmitted rarely by sexual contact among homosexuals
- **Bite of vectors:** Many parasitic diseases are transmitted by insect bite (Table 16.2 in Chapter 16) such as: malaria (female anopheles mosquito), filariasis (Culex), leishmaniasis (sandfly), Chagas' disease (reduviid bug) and African sleeping sickness (tsetse fly)
- **Vertical transmission:** Mother to fetus transmission is important for few parasitic infections like *Toxoplasma gondii*, *Plasmodium* spp. and *Trypanosoma cruzi*.
- **Blood transfusion:** Certain parasites like *Plasmodium* species, *Babesia* species, *Toxoplasma* species, *Leishmania* species and *Trypanosoma* species can be transmitted through transfusion of blood or blood products
- **Autoinfection:** Few intestinal parasites may be transmitted to the same person by contaminated hand (external auto-infection) or by reverse peristalsis (inter-

nal autoinfection). It is observed in *Cryptosporidium parvum, Taenia solium, Enterobius vermicularis, Strongyloides stercoralis* and *Hymenolepis nana.*

LIFE CYCLE OF THE PARASITES

The life cycle of the parasite may be direct (simple) or indirect (complex).

- **Direct/simple life cycle:** When a parasite requires only one host to complete its development, it is referred as direct/simple life cycle (Table 1.1)
- **Indirect/complex life cycle:** When a parasite requires two hosts (one definitive host and another intermediate host) to complete its development, it is referred as indirect/complex life cycle (Table 1.2). Some of the helminths require three hosts (one definitive host and two intermediate hosts) (Table 1.3).

Table 1.1: Direct/simple life cycle—parasites that need only one host (man)

Protozoa	***Helminths***
• *Entamoeba histolytica* • *Giardia lamblia* • *Trichomonas vaginalis* • *Balantidium coli* • *Cryptosporidium parvum* • *Cyclospora cayetanensis* • *Isospora belli* • *Microsporidia*	***Cestodes*** • *Hymenolepis nana* ***Nematodes*** • *Ascaris lumbricoides* • Hookworm • *Enterobius* spp. • *Trichuris trichiura* • *Strongyloides* spp.

PATHOGENESIS OF PARASITIC DISEASES

The parasites can cause damage to humans in various ways.

- **Mechanical trauma:**
  - **Eggs:** Trematode eggs being large in size, can be deposited inside the

Table 1.2: Indirect/complex life cycle: parasites requiring one definitive host and one intermediate host

Man acts as definitive host		
Parasites	***Definitive host (man)***	***Intermediate host***
Leishmania spp.	Man	Sandfly
Trypanosoma cruzi	Man	Reduviid bugs
Trypanosoma brucei	Man	Tsetse fly
Taenia solium (intestinal taeniasis)	Man	Pig
Taenia saginata	Man	Cattle
Hymenolepis diminuta	Man	Rat flea
Schistosoma spp.	Man	Snail
Trichinella spiralis	Man	Pig
Filarial worms	Man	Mosquito (culex, aedes, anopheles) and flies (blackflies and deerflies)
Dracunculus medinensis	Man	Cyclops
Man acts as intermediate host		
Parasites	***Definitive host***	***Intermediate host***
Plasmodium spp.	Female anopheles mosquito	Man
Babesia spp.	Tick	Man
Sarcocystis lindemanni	Cat and dog	Man
Toxoplasma gondii	Cat	Man
Echinococcus granulosus	Dog	Man
Taenia solium (Cysticercosis)	Man	Man

Table 1.3: Indirect/complex life cycle: parasites requiring one definitive host and two intermediate hosts

Parasites	*Definitive host*	*First intermediate host*	*Second intermediate host*
Diphyllobothrium spp.	Man	Cyclops	Fish
Fasciola hepatica	Man	Snail	Aquatic plant
Fasciolopsis buski	Man	Snail	Aquatic plant
Paragonimus spp.	Man	Snail	Crab and fish
Clonorchis spp.	Man	Snail	Fish
Opisthorchis spp.	Man	Snail	Fish
Gnathostoma spinigerum	Cat, dog and man	Cyclops	Fish

intestinal mucosa (*Schistosoma mansoni*), bladder (*Schistosoma haematobium*), lungs (*Paragonimus*), liver (*Fasciola hepatica*) and can cause mechanical irritation
  - **Larvae:** Migration of several helminthic larvae (hookworms, *Strongyloides* or *Ascaris*) in the lungs produce traumatic damage of the pulmonary capillaries leading to pneumonitis
  - **Adult worms:** Adult worms of hookworm, *Strongyloides*, *Ascaris* or *Taenia* get adhere to the intestinal wall and cause mechanical trauma
- **Space occupying lesions:** Certain parasites produce characteristic cystic lesion that may compress the surrounding tissues or organs, e.g., hydatid cysts and neurocysticercosis
- **Inflammatory reactions:** Most of the parasites induce cellular proliferation and infiltration at the site of their multiplication, e.g., *E. histolytica* provokes inflammation of the large intestine leading to the formation of amoebic granuloma. Adult worm of *W. bancrofti* causes mechanical blockage and chronic inflammation of the lymphatics and lymph vessels. Trematode eggs can induce inflammatory changes (granuloma formation) surrounding the area of egg deposition
- **Enzyme production and lytic necrosis:** Obligate intracellular parasites of man (*Plasmodium, Leishmania* and *Trypanosoma*), produce several enzymes, which cause digestion and necrosis of host cells. *E. histolytica* produces various enzymes like cysteine proteinases, hydrolytic enzymes and amoebic pore forming protein that lead to destruction of the target tissue
- **Toxins:** Some of the parasites produce toxins, which may be responsible for pathogenesis of the disease, e.g., *E. histolytica*. However, in contrast to bacterial toxin, parasitic toxins have minimal role in pathogenesis
- **Allergic manifestations:** Many metabolic and excretory products of the parasites get absorbed in the circulation and produce a variety of allergic manifestations in the sensitized hosts

  Examples include schistosomes causing cercarial dermatitis, rupture of hydatid cyst producing anaphylactic reactions and occult filariasis (tropical pulmonary eosinophilia)
- **Neoplasia:** Some of the parasitic infections can contribute to the development of neoplasia (e.g., *S. haematobium* causes bladder carcinoma, *Clonorchis* and *Opisthorchis* cause cholangiocarcinoma)
- **Secondary bacterial infections:** Seen in some helminthic diseases (schistosomiasis and strongyloidiasis).

IMMUNOLOGY OF PARASITIC DISEASES

The immune response against the parasitic infections depends on two factors:

- **Host factors:** Immune status, age, underlying disease, nutritional status, genetic constitution and various defense mechanisms of the host
- **Parasitic factors:** Size, route of entry, frequency of infection, parasitic load and various immune evasion mechanisms of the parasites.

Broadly, the host immunity against the parasitic diseases may be of two types:

1. Protective immune response
   i. Innate immunity
   ii. Adaptive/acquired immunity
2. Unwanted or harmful immune response (hypersensitive reactions).

Protective Immune Response

Both innate and acquired immunity play an important role in protecting the hosts against parasites. Some of the parasitic infections can be eliminated completely by the host immune responses (complete immunity) while few are difficult to eliminate. In some infections, the immune defense of the host is sufficient to resist further infection but insufficient to destroy the parasite. Immunity lasts till the original infection remains active and prevents further infection. This is called as *infection immunity* or *premunition* or *concomitant immunity* or *incomplete immunity*. This is observed in malaria, schistosomiasis, trichinellosis, toxoplasmosis and Chagas' disease.

(i) *Innate Immunity*

Innate immunity is the resistance which an individual possesses by birth, due to genetic and constitutional make-up.

Factors influencing innate immunity

- **Age of the host:** Both the extremes of age are more vulnerable to parasitic infections. Certain diseases are common in children like giardiasis and enterobiasis while certain infections occur more commonly in adults like hookworm infection. Congenital infection occurs commonly with *Toxoplasma gondii*; whereas newborns are protected from falciparum malaria because of high concentration of fetal hemoglobin
- **Sex:** Certain diseases are more common in males like amoebiasis where as females are more vulnerable to develop anemia due to hookworm infection
- **Nutritional status:** Both humoral and cellular mediated immunity are lowered and neutrophil activity is reduced in malnutrition
- **Genetic constitution of the individuals:** People with hemoglobin S (sickle cell disease), fetal hemoglobin and thalassemia hemoglobin are resistant to falciparum malaria where as Duffy blood group negative red blood cells (RBCs) are resistant to vivax malaria.

Components of innate immunity

- **Anatomic barriers (skin and mucosa):** Skin is an important barrier for the parasites that enter by cutaneous routes like *Schistosomes*, hookworm and *Strongyloides*
- **Physiologic barriers:** It includes temperature, pH, and various soluble molecules like lysozyme, interferon and complement. Gastric acidity acts as a physiologic barrier to *Giardia* and *Dracunculus*
- **Phagocytosis:** Phagocytes like macrophages and microphages (neutrophils, basophils and eosinophils) act as first line of defense against the parasites
- **Complements:** They play an important role for killing the extracellular parasites by forming membrane attack complexes; that leads to the formation of holes in the parasite membrane
- **Natural killer cells:** Natural killer cells (NKs) are another important mediator of innate immunity. They play a central role in killing few of the helminthic parasites.

(ii) *Acquired/Adaptive Immunity*

This is the resistance acquired by an individual during life following exposure to an agent. It is mediated by antibody produced by B lymphocytes (humoral immune response) or by T cells (cell mediated immune response).

Cell mediated immune response

- When a parasite enters, the parasitic antigens are processed by the antigen presenting cells, (e.g., macrophages) which present the antigenic peptides to T helper cells. The antigen presenting cells also secrete interleukin-1 (IL-1) that activates the resting T helper cells. Activated T helper cells differentiate into Th-1 and Th-2 cells
- T helper cell-1 secrete interleukin-2 (IL-2) and interferon gamma (IFN-γ)
  - Interleukin-2 activates the cytotoxic T cells and NKs, which are cytotoxic to the target parasitic cells. They produce perforin and granazyme that form pores and lyse the target cells
  - IFN-γ activates the resting macrophages which in turn become more phagocytic and release free radicals like reactive oxygen intermediate (ROI) and nitric oxide (NO) that kill the intracellular parasites
- T helper cells-2 release IL-4, IL-5, IL-6 and IL-10 which are involved in activation of B cells to produce antibodies [immunoglobin E (IgE) by IL-4]. IL-5 also acts as chemoattractant for the eosinophils. Eosinophilia is common finding in various helminthic infections.

Humoral immune response

Th-2 response activates the B cells to produce antibodies which in turn have various roles against the parasitic infections. They are:

- **Neutralization** of parasitic toxins (mediated by IgA and IgG)
- **Preventing** attachment to the gastrointestinal tract (GIT) mucosa (mediated by secretory IgA)
- **Agglutinating** the parasitic antigens thus preventing invasion (mediated by IgM)
- **Complement activation (by IgM and IgG):** Complements bind to the Fc portion of the antibody coated to the parasitic cells. Activation of the complements leads to membrane damage and cell lysis
- **Antibody dependent cell-mediated cytotoxicity (ADCC)** is important for killing of the helminths. NKs bind to the Fc portion of the IgG antibody coated to the helminths. Activation of NKs leads to release of perforin and granazyme that in turn cause membrane damage and cell lysis
- **Mast cell degranulation:** IgE antibodies coated on mast cells when get bound to parasitic antigens, the mast cells become activated and release a number of mediators like serotonin and histamine.

The Unwanted or Harmful Immune Responses

Sometimes immune responses may be exaggerated or inappropriate in the sensitized individuals on re-exposure to the same antigen. Such type of immunopathologic reactions are called as hypersensitivity reactions that may be harmful to the hosts causing tissue damage. These are of four types (Table 1.4).

Parasitic Factors that Evade the Host Immune Response

Sometimes the hosts find it difficult to contain the parasitic infections mainly because of the following reasons:

- Large size of the parasites
- Complicated life cycles
- Antigenic complexicity.

There are a number of mechanisms by which the parasites evade the host immune responses (Table 1.5).

LABORATORY DIAGNOSIS OF PARASITIC DISEASES

It plays an important role in establishing

Table 1.4: Hypersensitivity reactions seen in parasitic diseases

Hypersensitive reactions	*Parasitic diseases*
Type I hypersensitivity reactions These are allergic or anaphylactic reactions, occurring within minutes of exposure to parasitic antigens due to IgE mediated degranulation of mast cells	• Cercarial dermatitis (Swimmer's Itch) in schistosomiasis • Loeffler's syndrome in ascariasis • Ground itch (Hookworm infection) • Anaphylaxis due to leakage of hydatid fluid (*Echinococcus granulosus*) • Casoni's test (done in the diagnosis of hydatid disease). • Tropical pulmonary eosinophilia (occult filariasis)
Type II hypersensitivity reactions These are mediated by IgG or rarely IgM antibodies produced against the antigens on surfaces of the parasitic cells causing antibody mediated destruction of the cells by i) the complement activation or ii) by the NK cell activation (ADCC -antibody dependent cell mediated cytotoxicity)	• Anemia in malaria • Black water fever in malaria following quinine therapy • Myocarditis in Chagas' disease • Killing of the helminths by NK cells
Type III hypersensitivity reactions Immune complexes are formed by the combination of parasitic antigens with the circulating antibodies (IgG) which get deposited in various tissues	• Nephrotic syndrome in *Plasmodium malariae* • Katayama fever in schistosomiasis • African trypanosomiasis • Onchocerciasis
Type IV hypersensitivity reactions This is T-cell mediated delayed type of hypersensitivity reaction. Previously sensitized T helper cells secrete a variety of cytokines, on subsequent exposure to the parasitic antigens. Usually, the pathogen is cleared rapidly with little tissue damage. However, in some cases, it may be destructive to the host resulting in granulomatous reaction	• Elephantiasis (in filariasis) • Granulomatous disease in schistosomiasis and other helminthic infections • Leishmaniasis

Abbreviations: IgE, immunoglobulin E; IgG, immunoglobulin G; IgM, immunoglobulin M; NKs, natural killer cells.

the specific diagnosis of various parasitic infections. Following techniques are used in diagnosis of parasitic infections (has been discussed in detail in Chapter 15):

- Parasitic diagnosis—either microscopically or macroscopically
- Culture methods
- Immunodiagnostic methods (antigen and antibody detection)
- Intradermal skin tests
- Molecular methods
- Xenodiagnostic techniques
- Animal inoculation
- Imaging techniques.

TREATMENT OF PARASITIC DISEASES

Treatment of parasitic disease is primarily based on chemotherapy and in some cases by surgery.

Antiparasitic Drugs

Various chemotherapeutic agents are used for the treatment and prophylaxis of parasitic infections (Table 1.6).

Surgical Management

For management of parasitic diseases like cystic echinococcosis and neurocysticercosis surgery is indicated.

Table 1.5: Immune evasion mechanisms of the parasites

Immune evasion mechanisms	***Parasites involved***
By intracellular location	*Plasmodium* spp, *Babesia* spp., *Trypanosoma* spp., *Toxoplasma* spp., *Leishmania* spp. and *Microsporidia*
Enters an immunologically protected site soon after infection	*Plasmodium* spp. entering into hepatocytes
Leave the site where the immune response is already established	*Ascaris* undergoes intestinal phase and migratory lung phase during its life cycle
Survives in macrophages by preventing phago-lysosome fusion	*Leishmania, Trypanosoma* and *Toxoplasma*
Antigenic shedding (*capping*): Surface membrane antigens of the parasites bound to the antibodies undergo redistribution so that the parasite is covered by a folded membrane that later extrude as a cap containing most of the antibodies that were originally bound to the membrane	*Entamoeba histolytica, Trypanosoma brucei* and *Acylostoma caninum*
Antigenic variation: By change of antigenic composition, the parasites can be protected from the antibodies which are formed against the original antigens	*P. falciparum* (pf-EMP protein), *Giardia* and *Trypanosoma brucei*
Antigenic mimicry: The adult flukes of *Schistosoma* get coated with the host red cell antigens and histocompatibility antigens, so that they are not recognized as foreign and live free from host attack	*Schistosoma* spp.
Inhibit antibody binding	*Schistosoma mansoni*
Lymphocyte suppression	*Schistosoma mansoni*
Polyclonal stimulation of lymphocytes	*P. falciparum, Trypanosoma brucei, Babesia, Trichinella* and *E. histolytica.*
Suppression of immune system	*Trypanosoma, Plasmodium* and *Lesimania*

Table 1.6: Common antiparasitic drugs, their mechanism of action and clinical indications

Antiparasitic Drugs		
Drugs for amoebiasis	***Mechanism of action***	***Clinical indications***
Metronidazole, tinidazole and ornidazole (nitroimidazole compound)	Bioactivated to form reduced cytotoxic products which damage DNA	DOC for the amoebic colitis, amoebic liver abscess, and other extraintestinal amoebiasis
Dehydroemetine	Inhibits protein synthesis	Parenterally used for severe hepatic amoebiasis.
Chloroquine	Probably by concentrating in parasite food vacuoles.	Used for extra intestinal amoebiasis.
Paromomycin (Aminoglycoside)	Inhibits protein synthesis by binding to 16S ribosomal RNA	Effective luminal agent
Diloxanide furoate (Acetanilide compound)	Unknown; it is thought to interfere with protein synthesis	Effective luminal agent
Iodoquinol (8-hydroxyquinoline compound)	Unknown	Luminal agent
Amphotericin B	Complex and multifaceted.	DOC for *Naegleria fowleri*

Contd...

Contd...

Drugs for flagellates	*Mechanism of action*	*Clinical indications*
Intestinal/Genital Flagellates		
Giardiasis		
Metronidazole and tinidazole	Bioactivated to form reduced cytotoxic products which damage DNA.	DOC for Giardiasis
Nitazoxanide	Interference with the PFOR enzyme dependent electron transfer reaction which is essential for anaerobic energy metabolism.	
Furazolidone	Cross linking of DNA	Given to children
Paromomycin	Protein synthesis inhibitor in non-resistant cells by binding to 16S ribosomal RNA.	Can be given in pregnancy
Trichomoniasis		
Metronidazole or tinidazole	Bioactivated to form reduced cytotoxic products having nitro groups which damage DNA.	DOC for trichomoniasis, given to both the partners
Hemoflagellates		
Trypanosomiasis		
Chagas' disease		
Nifurtimox	Forms nitro-anion radical metabolite, which reacts with the nucleic acids of the parasite, causing a significant breakage in the DNA	Chagas' disease
Benznidazole	Production of free radicals, to which *Trypanosoma cruzi* is particularly sensitive	Effective in the treatment of reactivated *T. cruzi* infections caused by immunosuppression (AIDS patients or patients of organ transplants)
Sleeping sickness		
Pentamidine	Accumulates to micromolar concentrations within the parasite to kill it by inhibiting enzymes and interacting with DNA	DOC for East African sleeping sickness
Suramin	Trypanocidal activity; inhibits enzymes involved with the oxidation of reduced NADH	DOC for West African sleeping sickness
Leishmaniasis		
Sodium stibogluconate Meglumine antimoniate	Inhibition of the parasite's glycolytic and fatty acid oxidative activity resulting in decreased reducing equivalents for antioxidant defense and decreased synthesis of ATP	Leishmaniasis
Amphotericin B	Complex and multifaceted	Leishmaniasis
Paromomycin	Protein synthesis inhibitor in non-resistant cells by binding to 16S	Leishmaniasis

Contd...

Contd...

Miltefosine	Can trigger programmed cell death (apoptosis)	Leishmaniasis
Drugs for malaria	***Mechanism of action***	***Clinical indications***
Chloroquine	Probably, concentrating in parasite food vacuoles, preventing the polymerization of the hemoglobin into the toxic product hemozoin	DOC for uncomplicated benign malaria
Artemisinin derivative (Artemisinin or artemether or arte-ether)	Generate highly active free radicals that damage parasite membrane	DOC for complicated or falciparum malaria
Quinine	Probably similar to chloroquine; still not clear	DOC for complicated or falciparum malaria
Mefloquine	Same as chloroquine	DOC for complicated or falciparum malaria
Primaquine	Generating reactive oxygen species	DOC for relapse of vivax malaria
Sulfadoxine-pyrimethamine	Inhibits the production of enzymes involved in the synthesis of folic acid within the parasites	DOC for complicated or falciparum malaria
Lumefantrine	Accumulation of heme and free radicals	Complicated or falciparum malaria
Drugs for babesiosis	***Mechanism of action***	***Clinical indication***
Clindamycin plus quinine		DOC for severe babesiosis
Atovaquone plus azithromycin		DOC for mild babesiosis
Drugs for toxoplasmosis	***Mechanism of action***	***Clinical indications***
Cotrimoxazole (Trimethoprim-sulfamethoxazole)	Inhibiting folate synthesis from PABA (para aminobenzoic acid), thus inhibiting purine metabolism	DOC for prophylaxis in HIV infected people
Spiramycin	Inhibition of protein synthesis in the cell during translocation	DOC in pregnancy
Drugs for Cryptosporidium	***Mechanism of action***	***Clinical indications***
Nitazoxanide	Interferes with the PFOR enzyme-dependent electron-transfer reaction, which is essential to anaerobic metabolism in protozoan and bacterial species	DOC for *Cryptosporidium* infection
Drugs for Isospora and Cyclospora	***Mechanism of action***	***Clinical indications***
Cotrimoxazole (Trimethoprim-sulfamethoxazole)	Inhibiting folate synthesis from PABA (Para aminobenzoic acid), thus inhibiting purine metabolism	DOC for *Isospora* and *Cyclospora* infection
Drugs for cestodes	***Mechanism of action***	***Clinical indication***
Praziquantel	Increases the permeability of the membranes of parasite cells toward calcium ions which induces contraction of the parasites, resulting in paralysis in the contracted state	DOC for all cestode infections

Contd...

Contd...

Niclosamide	Niclosamide uncouples oxidative phosphorylation	Alternative drug for cestode infections
Albendazole	Causes loss of the cytoplasmic micro tubules leading to impaired uptake of glucose by the larval and adult stages of the susceptible parasites, and depleting their glycogen stores	Given for cysticercosis and hydatid disease
Drugs for trematodes	***Mechanism of action***	***Clinical indication***
Praziquantel	Increases the permeability of the membranes of parasite cells toward calcium ions which induces contraction of the parasites, resulting in paralysis in the contracted state	DOC for most of the trematode infections
Triclabendazole	Binds to beta-tubulin and prevent the polymerization of the microtubules	DOC for *Fasciola hepatica* and *F. gigantica*
Drugs for nematodes	***Mechanism of action***	***Clinical indication***
Intestinal nematodes		
Mebendazole or albendazole	Causes loss of the cytoplasmic microtubules leading to impaired uptake of glucose by the larval and the adult stages of the susceptible parasites, and depleting their glycogen stores	DOC for most of the intestinal nematodes
Pyrantel pamoate	Acts as a depolarizing neuromuscular blocking agent, thereby causing sudden contraction, followed by spastic paralysis of the helminths	Alternative drug for intestinal nematodes
Ivermectin	Kills by interfering with nervous system and muscle function, in particular by enhancing inhibitory neurotransmission resulting in flaccid paralysis	More affective for disseminated strongyloidiasis. Alternative drug for *Trichuris* infections
Filarial nematodes		
Diethylcarbamazine (DEC)	An inhibitor of arachidonic acid metabolism in microfilaria. This makes the microfilaria more susceptible to phagocytosis	DOC for lymphatic filariasis, *Loa loa* and *Mansonella* infections
Albendazole	Causes loss of the cytoplasmic microtubules leading to impaired uptake of glucose by the larval and the adult stages of the susceptible parasites, and depleting their glycogen stores	Alternative drug for lymphatic filariasis, *Loa loa* and *Mansonella* infections

Contd...

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Ivermectin	Kills by interfering with nervous system and muscle function, in particular by enhancing inhibitory neurotransmission	Used for lymphatic filariasis in Africa DOC for onchocerciasis Alternative drug for *Loa loa* and *Mansonella* infections
Doxycycline	Targets the intracellular *Wolbachia* present inside the Microfilaria	Alternative drug for lymphatic filariasis

Abbreviations: DNA, deoxyribonucleic acid; DOC, drug of choice; RNA, ribonucleic acid; PFOR, pyruvate ferredoxin oxidoreductase enzyme; ATP, adenosine triphosphate; NADH, nicotinamide adenine dinucleotide.

EXPECTED QUESTIONS

I. Write short notes on:
- (a) Paratenic host
- (b) Reservoir host
- (c) Indirect/complex life cycle
- (d) Immune evasion mechanisms of the parasites
- (e) Antiparasitic drugs

II. Differentiate between:
- (a) Definitive host and intermediate host
- (b) Direct and indirect life cycle

III. Multiple choice questions (MCQs):

1. A host harboring adult or sexual stage of a parasite is called:
- (a) Definitive host
- (b) Intermediate host
- (c) Reservoir host
- (d) None of the above

2. Parasite which may be transmitted by sexual contact is:
- (a) *Trypanosoma cruzi*
- (b) *Trichomonas vaginalis*
- (c) *Trypanosoma brucei*
- (d) *Ascaris*

3. Cholangiocarcinoma is associated with chronic infection of:
- (a) *Paragonimus westermani*
- (b) *Fasciola hepatica*
- (c) *Clonorchis sinensis*
- (d) *Schistosoma haematobium*

4. Which of the following parasite is transmitted by dog:
- (a) *Taenia saginata*
- (b) *Hymenolepis nana*
- (c) *Echinococcus granulosus*
- (d) *Diphyllobothrium latum*

5. Blood-sucking vector may transmit:
- (a) *Ascaris lumbricoides*
- (b) *Ancylostoma duodenale*
- (c) *Strongyloides stercoralis*
- (d) *Plasmodium*

Answers

1. (a) 2. (b) 3. (c) 4. (c) 5. (d)

Section 2

Protozoology

Chapter 2 Introduction to Protozoa

Chapter 3 Amoeba

Chapter 4 Flagellates—I (Intestinal and Genital)

Chapter 5 Flagellates—II (Hemoflagellates)

Chapter 6 Sporozoa—I (Malaria Parasite and Babesia)

Chapter 7 Sporozoa—II (Opportunistic Coccidian Parasites)

Chapter 8 Miscellaneous Protozoa

2 Introduction to Protozoa

Chapter Outline

- General features of protozoa
- Classification of protozoa
- Expected questions

GENERAL FEATURES OF PROTOZOA

The protozoa are unicellular eukaryotic cells that perform all the physiological function.

- More than two lakhs protozoa are named but only about 70 species belonging to nearly 30 genera infect human beings
- Many of these protozoa are relatively harmless but few may cause some of the important diseases of tropical countries like malaria, kala azar, sleeping sickness and Chaga's disease, etc which together threaten one quarter of the population of the World
- With the advent of HIV/AIDS, some of them are increasingly being recognized as opportunistic pathogens like toxoplasmosis, cryptosporidiosis, etc
- In general, protozoa are placed between prokaryotes and higher eukaryotes
  - Like bacteria, they are small, single celled, 1–150 μm size, short generation time, higher reproduction rates and have a tendency to induce immunity to reinfection in those who survive
  - On other hand, the protozoa are undoubtedly lower eukaryotes as they possess cellular organelles and have similar metabolic pathways.

CLASSIFICATION OF PROTOZOA

The Traditional 1980s Classification

Based on the recommendation of the committee on Systematics and Evolution of the Society of Protozoologists conducted by Levine et al (1980), the protozoan parasites were classified (Table 2.1)

Though it satisfied the requirements of the protozoologists but couldn't meet some of the requirements of medical parasitologists.

Corliss's Interim User Friendly Classification (1994)

Corliss proposed a user-friendly classification trying to meet the requirements of both protozoologists and medical parasitologists.

He divided the living creatures into six kingdoms. Unicellular parasites (generally accepted as protozoa) are categorized into two phylum—Archezoa and Protozoa.

Table 2.1: The traditional 1980s classification of protozoa (Levine et al., 1980)

Phylum	*Subphylum*	*Super class*	*Class*	*Subclass*	*Order*	*Suborder*	*Genus*
Sarcomastigophora (Amoeba and flagellates)	Mastigophora		Zoomastigophorea		Kinetoplastida	Trypanosomatina	*Leishmania* *Trypanosoma*
					Retortamonadida		*Retortamonas* *Chilomastix*
					Diplomonadida	Diplomonadina	*Giardia*
						Enteromonadina	*Enteromonas*
					Trichomonadida		*Trichomonas* *Pentatrichomonas* *Dientamoeba*
	Sarcodina	Rhizopoda	Lobosea	Gymnamoebia	Amoebida	Tubulina	*Entamoeba* *Endolimax* *Iodamoeba*
						Acanthopodina	*Acanthamoeba*
					Schizopyrenida		*Naegleria*
Apicomplexa (sporozoans)			Sporozoea	Coccidia	Eucoccidiida	Eimeriina	*Eimeria* *Toxoplasma* *Cryptosporidium* *Cyclospora* *Isospora* *Sarcocystis*
						Haemosporina	*Plasmodium*
				Piroplasmia	Piroplasmida		*Babesia*
Microspora			Microsporea		Microsporida	Apansporoblastina	*Enterocytozoon* *Encephalitozoon* *Pleistophora* *Trachipleistophora* *Brachiola* *Nosema* *Vittaforma* *Microsporum*
Ciliophora			Kinetofragmino-phorea	Vestibuliferia	Trichostomatida	Trichostomatina	*Balantidium*

Adapted from: Topley and Wilson's Microbiology and Microbial Infections (Parasitology volume), 10th edition.

Molecular Classification (2000)

The hierarchical system can be accurately represented by the ribonucleic acid (RNA) and protein sequences of the organisms. With advance of molecular techniques, the ribosomal RNA and protein sequences are studied, and a new classification has been devised.

- Cavalier and Smith's six kingdoms classification—molecular classification is based on the six kingdom theory proposed by Cavalier and Smith (1998). They are bacteria, protozoa, animalia, fungi, plantae and chromista
- The unicellular protozoan parasites constitute thirteen phyla of which the human parasites belong to seven phyla which are distributed in three kingdoms—Protozoa, Fungi and Chromista (Table 2.2)
- The description in this book will be according to this classification.

Kingdom Protozoa

Unicellular eukaryotic, phagotrophic, non-photosynthetic organism without a cell wall.

Subkingdom Archezoa

Unicellular eukaryotic organisms exhibiting various prokaryotic features in ribosomes and transfer ribonucleic acid (tRNA) and lacking mitochondria and other organelles.

- **Phylum Metamonada:** Unicellular intestinal flagellates (2–8 numbers)
- **Phylum Parabasalia:** Unicellular flagellates with one or more nuclei and numerous flagella and parabasal body.

Subkingdom Neozoa

Unicellular eukaryotic organisms typically possessing mitochondria and other organelles.

- **Phylum Amoebozoa:** Unicellular eukaryotic organisms with pseudopodia used for locomotion and feeding
  - **Class Amoebaea:** free living amoeba with and mitochondria
  - **Class Entamoebidea:** Obligate amoeba with secondary loss of mitochondria
- **Phylum Percolozoa:** Unicellular organisms having 1–4 temporary flagella and mitochondria but lacking Golgi bodies
- **Phylum Euglenozoa:** Unicellular organisms having 1–4 flagella, mitochondria and Golgi bodies
- **Phylum Sporozoa:** Unicellular eukaryotic organisms possessing apical complex made up of polar rings, rhoptries, micronemes and conoid
- **Phylum Ciliophora:** Unicellular organisms having cilia as loco motor organ and two nuclei of different size and ploidy—(1) macronucleus and (2) micronucleus.

Kingdom Fungi

Eukaryotic heterotrophic organisms lacking plastids but possessing cell wall containing chitin and β-glucan.

Kingdom Chromista

Unicellular eukaryotic, photosynthetic filamentous or colonial, organisms (in part "algae"); some with secondary loss of plastids.

Table 2.2: Molecular classification (2000)

Kingdom	*Subkingdom*	*Phylum*	*Class*	*Order*	*Genus*
Protozoa	Archezoa	Metamonada	Trepomonadea	Diplomonadida	*Giardia*
				Enteromonadida	*Enteromonas*
			Retortamonadea	Retortamonadida	*Retortamonas* *Chilomastix*
		Parabasalia	Trichomonadea	Trichomonadida	*Trichomonas* *Pentatrichomonas* *Dientamoeba*
	Neozoa	Amoebozoa	Entamoebidea	Euamoebida	*Entamoeba* *Endolimax* *Iodamoeba*
			Amoebaea	Acanthopodida	*Acanthamoeba*
		Percolozoa	Heterolobosea (flagellated amoeba)	Schizopyrenida	*Naegleria*
		Euglenozoa	Kinetoplastea	Trypanosomatida	*Leishmania* *Trypanosoma*
		Sporozoa	Coccidea	Eimeriida	*Eimeria* *Toxoplasma* *Cryptosporidium* *Cyclospora* *Isospora* *Sarcocystis*
				Haemosporida	*Plasmodium*
				Piroplasmida	*Babesia*
		Ciliophora	Litostomatea	Vestibuliferida	*Balantidium*
Fungi		Microspora	Microsporea	Microsporida	*Enterocytozoon* *Encephalitozoon* *Pleistophora* *Trachipleistophora* *Brachiola* *Nosema* *Vittaforma* *Microsporum*
Chromista	Chromobiota	Bigyra	Blastocystea		*Blastocystis*

Adapted from: Topley and Wilson's Microbiology and Microbial Infections (Parasitology volume), 10th edition.

EXPECTED QUESTIONS

I. Write short notes on:

(a) The traditional 1980s classification of parasites

(b) Molecular classification (2000) of parasites

(c) Subkingdom Neozoa

II. Multiple choice questions (MCQs):

1. Which of the following protozoa belongs to phylum Euglenozoa?

(a) *Leishmania* species

(b) *Entamoeba* species

(c) *Cryptosporidium* species

(d) *Plasmodium* species

2. Which of the following protozoa belongs to kingdom Chromista?

(a) *Isospora* species

(b) *Babesia* species

(c) *Giardia* species

(d) *Blastocystis* species

3. Which of the following protozoa belongs to order Schizopyrenida?

(a) *Plasmodium* species

(b) *Naegleria* species

(c) *Acanthamoeba* species

(d) *Entamoeba* species

4. Which of the following protozoa belongs to phylum Sporozoa?

(a) *Giardia* species

(b) *Toxoplasma* species

(c) *Plasmodium* species

(d) *Entamoeba* species

Answers

1. (a) 2. (d) 3. (b) 4. (c)

3 Amoeba

Chapter Outline

- Classification of amoeba
- Intestinal amoeba
  - Pathogenic intestinal amoeba
  - Nonpathogenic intestinal amoeba
- Free-living amoeba
- Expected questions

CLASSIFICATION OF AMOEBA

Amoeba is a single-celled protozoa that constantly changes its shape. The word **"amoeba"** is derived from the Greek word **"amoibe"** meaning **"change"**. They constantly change their shape due to presence of an organ of locomotion called as " pseudopodium"

Classification Based On Habitat

Amoebae are classified as intestinal amoebae and free living amoebae.

- **Intestinal amoebae:** They inhabitat in the large intestine of humans and animals. *Entamoeba histolytica* is the only pathogenic species. Others are nonpathogenic such as—*E. dispar, E. moshkovskii, E. coli, E. polecki, E. hartmanni, E. gingivalis, Endolimax nana,* and *Iodamoeba butschlii*
- **Free-living amoebae:** They are small free living and opportunistic pathogens. Examples are *Acanthamoeba* species, *Naegleria fowleri, Balamuthia mandrillaris* and *Sappinia diploidea*

Taxonomical Classification

According to the traditional 1980s classification—amoeba belongs to the Phylum Sarcomastigophora, Subphylum Sarcodina, Superclass Rhizopoda, Class Lobosea, Subclass Gymnamoebia, Order Amoebida and Family Endamoebidae.

However, in last 30 years, with the advent of molecular technique, the taxonomy is changed and currently the new molecular classification is followed (Table 3.1).

PATHOGENIC INTESTINAL AMOEBA

Entamoeba histolytica

Introduction

E. histolytica is worldwide in distribution but more common in tropical and subtropical countries.

- *E. histolytica* has three subspecies—*E. histolytica* subspecies *histolytica*, *dispar* and *moshkovskii*

Table 3.1: Taxonomy of Amoeba

Kingdom	*Subkingdom*	*Phylum*	*Class*	*Order*	*Genus*
Protozoa	Neozoa	Amoebozoa	Entamoebidea	Euamoebida	*Entamoeba* *Endolimax* *Iodamoeba*
			Amoebaea	Acanthopodida	*Acanthamoeba*
		Percolozoa	Heterolobosea (flagellated amoeba)	Schizopyrenida	*Naegleria*

- Cysts and trophozoites of all the three subspecies are morphologically indistinguishable
- However, on the basis of extensive genetic, immunological, and biochemical analysis, currently all the three subspecies are formally accepted as different yet closely related species
- *E. histolytica* is the pathogenic species causing amoebic dysentery and a wide range of other invasive diseases, including amoebic liver abscess, where as other two are considered as nonpathogens that colonize the large intestine.

History

E. histolytica was first described by Fedor Losch (1875) from Russia.

- The species name was first coined by Fritz Schaudinn in 1903
- Brumpt had described and designated the nonpathogenic form of *E. histolytica* as *E. dispar* in 1993.

Epidemiology

Amoebiasis is a major health problem worldwide.

- The largest burden of the disease occurs in tropics of China, Central and South America, and Indian subcontinents affecting 10% of the world's population. (500 million)
- It is the third most common parasitic cause of death in the world (after malaria and schistosomiasis). Approximately 50 million cases and 110, 000 deaths are reported annually by WHO (World health Oranization)
- In India, the prevalence rate is around 15% (ranges from 3.6% to 47.4%) with a higher prevalene reported from Maharashtra, Tamilnadu and Chandigarh.

Morphology

E. histolytica has three stages—(1) trophozoite, (2) precyst and (3) cyst (immature and mature).

Trophozoite

It is the invasive form as well as the feeding and replicating form of the parasite found in the feces of patients with active disease.

- It measures 12–60 μm (average 15–20 μm) in diameter
- Cytoplasm of trophozoite is divided into a clear ectoplasm and a granular endoplasm
- Granular endoplasm looks as ground glass appearance and contains red blood cells (RBCs), white blood cells (WBCs) and food vacuoles containing tissue debris and bacteria. RBCs are found only in the stage of invasion
- **Pseudopodia:** Ectoplasm has long finger like projections called as pseudopodia (organ of locomotion); which exhibits active, unidirectional rapid progressive and purposeful movement
- Nucleus is single, spherical, 4–6 μm size, contains central dot like compact karyosome surrounded by a clear halo. Nuclear membrane is thin and delicate and is lined by a layer of fine chromatin granules. The number of chromosomes varies between 30 and 50

- The space between the karyosome and the nuclear membrane is traversed by spoke like radial arrangement of achromatic fibrils (cart wheel appearance)
- Amoebic trophozoites are anaerobic parasites. They lack mitochondria, endoplasmic reticulum and Golgi apparatus. (Fig. 3.1A).

Precyst

It is the intermediate stage between trophozoite and cyst.

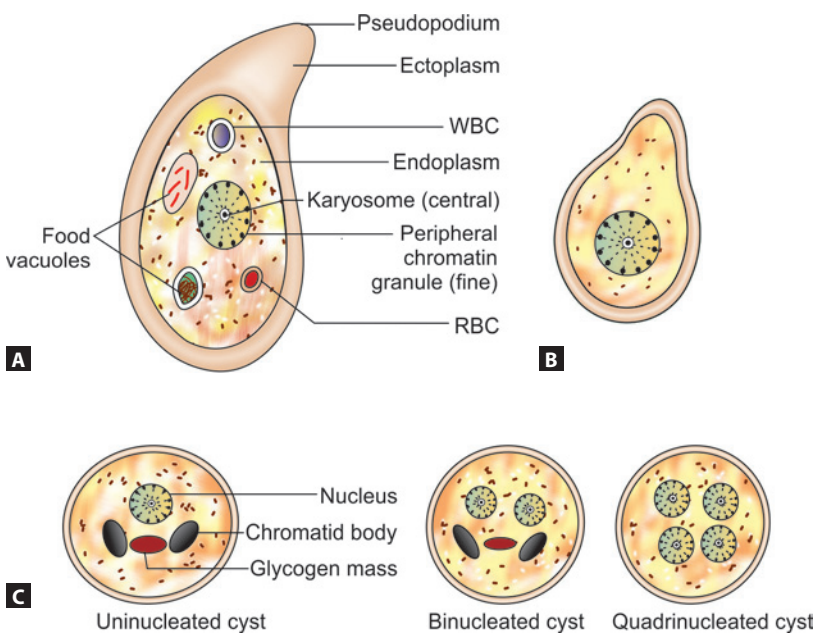
- It is smaller to trophozoite but larger to cyst (10–20 μm)
- It is oval with a blunt pseudopodia. Food vacuoles and RBCs disappear. Nuclear structures are same as that of trophozoite (Fig. 3.1B).

Cyst

It is the infective form as well as the diagnostic form of the parasite found in the feces of carriers as well as patients with active disease.

- It measures 10–20 μm (average 12–15 μm) in diameter (Fig. 3.1C)
- Nuclear structures are same as in trophozoites. First, the cyst is uninucleated; later the nucleus divides to form binucleated and finally becomes quadrinucleated cyst
- Cytoplasm of uninucleated cyst contains 1–4 numbers refractile bars with rounded ends called as **chromatoid bodies** (aggregation of ribosome**)** and a large **glycogen mass** (stains brown with iodine)
- Both chromatoid body and glycogen mass gradually disappear, and they are not found in mature quadrinucleated cyst
- Cysts are present only in the gut lumen; they never invade the intestinal wall.

"Minuta" form of *Entamoeba histolytica:* They are the commensal phase of *E. histolytica*, living in the lumen of gut. They are usually smaller in size (trophozoite 12–14 μm and cyst < 10 μm) and often mistaken as *E. hartmanni.*



Figs 3.1A to C: *Entamoeba histolytica* (schematic diagram) (A) trophozoite; (B) precyst; (C) cysts

Life Cycle (Fig. 3.2)

Host: *E. histolytica* completes its life cycle in single host, i.e. man.

Infective form: Mature quadrinucleated cyst is the infective form. It can resist chlorination, gastric acidity and desiccation and can survive in a moist environment for several weeks.

Note: Trophozoites and immature cysts can be passed in stool of amoebic patients, but they can't serve as infective form as they are disintegrated in the environment or by gastric juice when ingested.

Mode of transmission:

- **Feco-oral route (most common):** By ingestion of contaminated food or water with mature quadrinucleated cysts
- **Sexual contact:** Rare, either by anogenital or orogenital contact. (especially in developed countries among homosexual males)
- **Vector:** Very rarely, flies and cockroaches may mechanically transmit the cysts from feces, and contaminate food and water.

Development in man (small intestine)

- **Excystation:** In small intestine, the cyst wall gets lysed by trypsin and a single tetranucleated trophozoite (metacyst) is liberated which eventually undergoes a

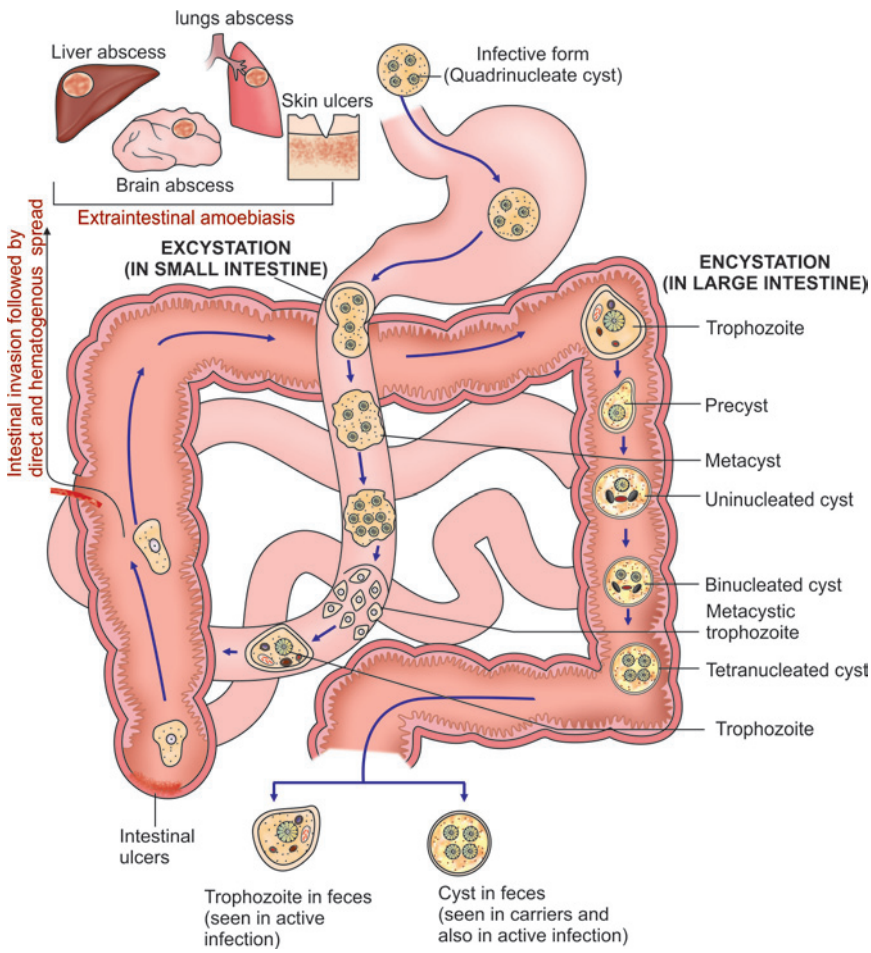


Fig. 3.2: Life cycle of *Entamoeba histolytica*

series of nuclear and cytoplasmic divisions to produce eight small **metacystic trophozoites**
- Metacystic trophozoites are carried by the peristalsis to ileocecal region of large intestine and multiply by binary fission, and then colonize on the mucosal surfaces and crypts of the large intestine
- After colonization, trophozoites show different courses depending on various factors like host susceptibility, age, sex, nutritional status, host immunity, intestinal motility, transit time and intestinal flora
- **Asymptomatic cyst passers:** In majority of individuals, trophozoites don't cause any lesion, transform into cysts and are excreted in feces
- **Amoebic dysentery:** Trophozoites of *E. histolytica* secrete proteolytic enzymes that cause destruction and necrosis of tissue, and produces flask shaped ulcers on the intestinal mucosa. At this stage, large numbers of trophozoites are liberated along with blood and mucus in stool producing amoebic dysentery. Trophozoites usually degenerate within minutes
- **Amoebic liver abscess:** In few cases, erosion and necrosis of small intestine are so extensive that the trophozoites gain entrance into the radicals of portal veins and are carried away to the liver where they multiply causing amoebic liver abscess.

Development in man (large intestine)

- **Encystation:** After some days, when the intestinal lesion starts healing and patient improves, the trophozoites transform into precysts then into quadrinucleated cysts which are liberated in feces
- Encystation occurs only in the large gut. Cysts are never formed once the trophozoites are excreted in stool
- Factors that induce cyst formation include food deprivation, overcrowding, desiccation, accumulation of waste products, and cold temperatures
- Mature quadrinucleated cysts released in feces can survive in the enviornment and become the infective form. Immature cysts and trophozoites are some times excreted, but get disintegrated in the environement. (Fig. 3.2).

Pathogenesis

Trophozoite of *E. histolytica* is the major invasive form and possesses many virulence factors that play role in the pathogenesis of intestinal as well as extraintestinal amoebiasis (Table 3.2).

Pathogenesis of Intestinal Amoebiasis

Trophozoites invade the colonic mucosa producing characteristic ulcerative lesions and profuse bloody diarrhea (amoebic dysentery). Males and females are affected equally with a ratio of 1:1.

Amoebic ulcer

The classical ulcer is **flask-shaped** (broad base with a narrow neck).

- It may be localized to ileocecal region (most common site) or sigmoidorectal region or may be generalized involving the whole length of the large intestine
- Ulcers are usually scattered with intervening normal mucosa
- It may be superficial (confined to muscularis mucosa and heal without scar) or deep ulcer (beyond muscularis mucosa and heals with scar formation)
- Size ranging from pin head to inches
- Shape round to oval
- Margin ragged and undermined
- Base is formed on muscle coat.

Complications of intestinal amoebiasis (Fig. 3.3)

There are following types of complications:
- **Fulminant amoebic colitis:** Resulting from generalized necrotic involvement of entire large intestine, occurs more commonly

Table 3.2: Virulence factors of *Entamoeba histolytica*

Amoebic lectin antigen
• It is a 260 kDa galactose and N-acetylgalactosamine inhabitable surface protein (Gal/NAG lectin) • It has two subunits—heavy (170 kDa) and—light (35 kDa) subunits linked by disulfide bridge. The 170 kDa subunit has a cytoplasmic and toxic membrane domain • Lectin antigen is the principle virulence factor, present on the surface of trophozoites of pathogenic *E. histolytica* but not on nonpathogenic *E. dispar*. Its various pathogenic mechanisms are: – *Adhesion*: By binding to glycoprotein receptors on intestinal and hepatic surfaces – *Cytotoxicity*: By contact dependent cytolysis of the target cells by increasing the calcium level – *Complement resistance*: 170kDa subunit resembles CD59 (a human complement blocker) that prevent C5b-9 complex formation
Amoebapore
(5 kDa Amoebic pore forming protein): It inserts ion channels in the target cell membrane causing leakage of ions. Its equivalent found in *E. dispar* is called as dispar pores
Cysteine proteases
They degrade extracellular matrix, responsible for invasion, secreted only by trophozoites of pathogenic *E. histolytica*. Examples include histolysin, amoebapain and cathepsin B like proteases
Hydrolytic enzymes
Such as RNAse, neutral protease and phosphatases—help in the destruction of the target tissue
Neuraminidase and metallocollagenase
Help in invasion

in immunocompromised patients and in pregnancy

- **Amoebic appendicitis:** Results when the infection involves appendix
- **Intestinal perforation and amoebic peritonitis:** Occurs when the ulcer progresses beyond the serosa
- **Toxic megacolon and intussusception** (segment of intestine invaginates into the adjoining intestinal lumen, causing bowel obstruction)
- **Perianal skin ulcers:** By direct extension of ulcers to perianal skin
- **Amoeboma (amoebic granuloma):** A diffuse pseudotumor like mass of granulomatous tissue found in rectosigmoid region

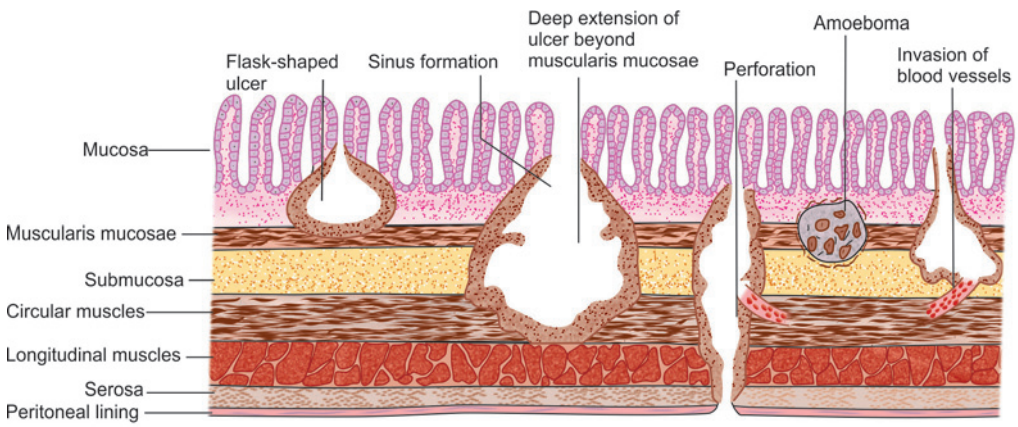


Fig. 3.3: Complications of intestinal amoebiasis (cross section of intestinal wall)

- **Chronic amoebiasis:** It is characterized by thickening, fibrosis, stricture formation with scarring and amoeboma formation.

Pathogenesis of Extraintestinal Amoebiasis

Following 1–3 months of intestinal amoebiasis, about 5–10% of patients develop extraintestinal amoebiasis. Liver is the most common site (because of the carriage of trophozoites through the portal vein) followed by lungs, brain, genitourinary tract and spleen.

Amoebic liver abscess

The most common group affected: Adult males (male and female ratio is 9:1).

The most common affected site is the posterior-superior surface of the right lobe of liver. Abscess is usually single or rarely multiple (Fig. 3.4).

- Amoebic trophozoites occlude the hepatic venules; which leads to anoxic necrosis of the hepatocytes. Inflammatory response surrounding the hepatocytes leads to the formation of abscesses
- Microscopically the abscess wall is comprised of:
  - Inner central zone of necrotic hepatocytes without amoeba
  - Middle zone of degenerative hepatocytes, RBC, few leucocytes and occasionally amoebic trophozoites
  - Outer zone: comprised of healthy hepatocytes invaded with amoebic trophozoites
- **Anchovy sauce pus:** Liver abscess pus is thick chocolate brown in color. The fluid is acidic and pH 5.2–6.7 and is comprised of necrotic hepatocytes without any pus cells and occasional amoebic trophozoites (mainly found in last few drops of pus as amoebae multiply in the wall of abscess).



Fig. 3.4: Cross section of liver showing amoebic liver abscess (right side)
Courtesy: HOD, Department of Pathology, Meenakshi medical college, Chennai

Complications of amoebic liver abscess

With continuous hepatic necrosis, abscess may grow in various direction of liver discharging the contents into the neighboring organs (Fig. 3.4).

- Right sided liver abscess may rupture externally to skin causing **granuloma cutis** or rupture into lungs (pulmonary amoebiasis with trophozoites in sputum) or into the right pleura (**amoebic pleuritis**)
- Rupture of liver abscess below the diaphragm leads to subphrenic abscess and generalized peritonitis
- Left sided liver abscess may rupture into stomach or left pleura or pericardial cavity (**amoebic pericarditis**)
- Hematogenous spread can occur from liver affecting brain, lungs, spleen and genitourinary organs.

Clinical Manifestations of Amoebiasis

Asymptomatic amoebiasis

About 90% of infected persons are asymptomatic carriers and excrete cysts in their feces. Now it is confirmed that many of these carriers harbor *E. dispar.*

The remaining 10% of people (who are truly infected by pathogenic *E. histolytica*) produces a spectrum of diseases varying from intestinal amoebiasis to amoebic liver abscess.

Intestinal amoebiasis

Incubation period varies from one to four weeks. Intestinal amoebiasis is characterized

by four clinical forms:

1. **Amoebic dysentery:** Symptoms include bloody diarrhea with mucus and pus cells, colicky abdominal pain, fever, prostration, and weight loss. Amoebic dysentery should be differentiated from bacillary dysentery (Table 3.3)
2. **Amoebic appendicitis:** Presented with acute right lower abdominal pain
3. **Amoeboma:** It present as palpable abdominal mass
4. **Fulminant colitis:** Presents as intense colicky pain, rectal tenesmus, more than 20 motions/day, fever, nausea, anorexia and hypotension.

Laboratory Diagnosis Intestinal amoebiasis

- Stool microscopy by wet mount, permanent stains, etc—detects cysts and trophozoites
- Stool culture
  - Polyxenic and axenic culture
- Stool antigen detection (copro-antigen)—CIEP, ELISA, ICT
- Serology
  - Amoebic antigen—ELISA
  - Amoeboic antibody—IHA, ELISA and IFA
- Isoenzyme (zymodene) analysis
- Molecular diagnosis
  - Nested multiplex PCR and real time PCR

Table 3.3: Differences in stool characters between amoebic dysentery and bacillary dysentery

Character	***Amoebic dysentery***	***Bacillary dysentery***
Pathology		
Ulcer	Deep	Shallow
Margin	Ragged and undermined	Uniform
Intervening mucosa	Normal	Inflamed
Necrosis type	Pyknosis (pyknotic bodies)	Karyolysis (ghost cells)
Cellular response	Mononuclear cells	Polymorphonuclear cells
Stool macroscopic feature		
Number of motion	6–8/day	> 10/day
Amount	Copious amount	Small quantity
Color	Dark red	Bright red
Odor	Offensive	Odorless
Reaction	Acidic	Alkaline
Consistency	Not adherent to the container	Adherent to the container
Stool microscopic feature		
Red blood cells (RBCs)	In clumps	Discrete or in rouleaux
Pus cells	Few	Numerous
Macrophages	Few	Numerous, may contain RBCs, so can be mistaken as *E. histolytica*
Eosinophils	Present	Absent or rare
Charcot Leyden crystal	Present	Absent
Pyknotic body (nuclear remains of tissue cells and leukocytes)	Present	Absent
Ghost cell	Absent	Present
Organism detected	*E. histolytica* cyst or trophozoite	Bacteria (*Shigella*)

Amoebic liver abscess

Presents with tender hepatomegaly, fever with weight loss, sweating and weakness, rarely jaundice, and cough.

Laboratory Diagnosis of Intestinal Amoebiasis

Sample collection

Stool is the specimen of choice. Minimum of three stool samples should be collected on consecutive days as amoebae are shed intermittently.

- Other samples include rectal exudates and rectal ulcer tissues collected by colonoscopy
- Stool specimen should be collected in wide mouthed clean container before administration of interfering substances like kaolin, bismuth, barium sulfate, antiamoebic drugs
- It should be examined immediately within 1–2 hours of collection or can be preserved in polyvinyl alcohol or merthiolate iodine or formalin. However, refrigeration is not recommended.

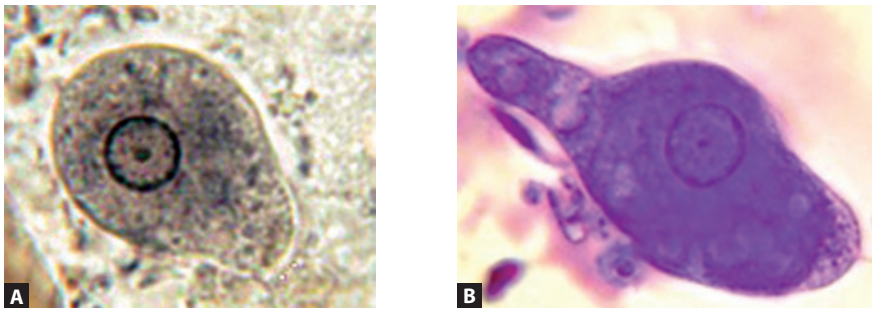
Stool microscopy

Direct examination of stool by saline and iodine mount is done to demonstrate:

- Trophozoites (Fig. 3.5)
- Quadrinucleated cysts (Fig. 3.6)

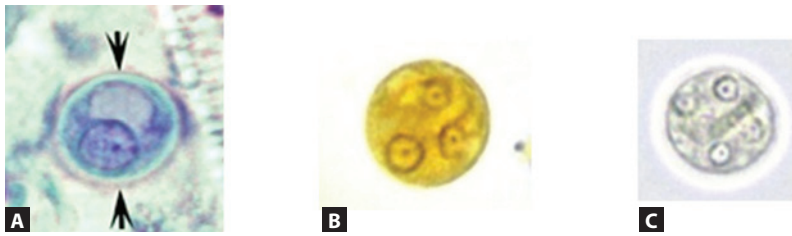
With saline mount, motility of the trophozoites can be appreciated while iodine mount clearly demonstrates the internal structures of the cyst

- Microscopy is poorly sensitive (25–60% with single sample) but the sensitivity increases to 85–95% when three stool samples are examined
- When the amoeba load in stool is less (as in chronic amoebiasis or convalescent



Figs 3.5 A and B: Trophozoite of *Entamoeba histolytica* shows finger like psuedopodia and visible fine chromatin granule lining the nuclear membrane (A) hematoxylin stain; (B) trichrome stain

Source: Giovanni Swierczynski, Bruno Milanesi."Atlas of human intestinal protozoa Microscopic diagnosis" (*with permission*)



Figs 3.6 A to C: Cyst of *Entamoeba histolytica* (A) Giemsa stain shows uninucleated cyst (with glycogen vacuole); (B) iodine mount shows immature cyst (three nuclei); (C) saline mount shows mature cyst (with four nuclei)

Source: Giovanni Swierczynski, Bruno Milanesi. "Atlas of human intestinal protozoa Microscopic diagnosis" (*with permission*)

stage), stool samples can be examined after concentration by formalin ether sedimentation method
- Stool or colonoscopy guided biopsy samples can also be examined by staining with permanent stains like trichrome, periodic acid Schiff (PAS), and hematoxylin and eosin (H & E) stains. Internal structures of cysts and trophozoites are well demonstrated by permanent stains (Figs 3.5 and 3.6 A)
- Cyst and trophozoites of *E. histolytica* are indistinguishable from that of *E. dispar* or *E. moshkovskii* except the presence of RBCs in trophozoites of *E. histolytica* (which might not be there after dysentery episode is over). So, the report should always be sent as "cyst or trophozoite of *E. histolytica/dispar/moshkovskii* found in the stool microscopy."

Stool culture

Culture methods are not routinely used for diagnosis. They are useful in research and teaching purpose. Culture methods are discussed in detail in Chapter 15.

Polyxenic culture: Culture media contains bacterial supplement, starch and serum providing nourishment to amoeba.
- Polyxenic medias are used for cultivation of amoeba from stool samples of chronic and asymptomatic carriers passing less number of cysts
- Stool culture shows 50–70% sensitivity and 100% specificity (gold standard)
- Various culture medias used are:
  - National Institute of Health (NIH) media
  - Boeck and Drbohlav egg serum medium containing Locke's solution
  - Balamuth's medium
  - Nelson's medium
  - Robinson's medium.

Axenic culture: It lacks bacterial supplement, e.g., diamond's medium. Axenic culture is useful when the bacterial flora interferes with the test results such as:
- Studying pathogenicity of amoeba
- Testing antiamoebic drug susceptibility
- Preparation of amoebic antigen in mass for serological tests
- For harvesting the parasite to determine the zymodeme pattern.

***Stool antigen detection* (*coproantigen*)**

Various tests used to demonstrate amoebic coproantigen in stool are:
- Counter current immune electrophoresis (CIEP)
- Enzyme-linked immunosorbent assay (ELISA)
- Immunochromatographic test (ICT)

Since, the antigens get denatured by stool preservatives, only fresh or frozen stool sample should be used.
- **ELISA detecting 170 kDa of lectin antigen** in stool shows more than 95% sensitivity and specificity. It can also differentiate pathogenic *E. histolytica* (lectin antigen positive) and nonpathogenic *E. dispar* (lectin antigen negative)
- **Immunochromatographic test** is available for the simultaneous detection of antigens specific for *Giardia lamblia*, *E. histolytica/E. dispar*, and *Cryptosporidium parvum* from stool, (commercially available as the triage parasite panel). It shows sensitivity (83–96%) and specificity (99–100%).

Serology

Amoebic antigen: Amoebic antigen in serum is found only in patients with active infection and disappears after clinical cure, so its presence in serum indicates recent and active infection.
- ELISA is done using monoclonal antibody specific for lectin antigen—usually positive in early stage of the disease (sensitivity of 65%)
- ELISA is also available using monoclonal antibody specific for various other antigens like—serine rich *E. histolytica* protein (SREHP), lysine rich surface antigen and lipophosphoglycan (LPG)

- Other older methods for antigen detection include:
  - CIEP
  - Coagglutination test
  - Slide agglutination test.

Amoebic antibody: Serum antibodies appear only in the later stages of intestinal amoebiasis

- Various tests include:
  - ELISA
  - Indirect fluorescent antibody (IFA) test
  - Indirect hemagglutination (IHA) test
- IHA using crude antigens shows 10% sensitivity in asymptomatic cysts passers and 50–60% sensitivity in acute infection
- ELISA detecting antibody against lectin antigen shows 90% sensitivity in convalescent stage and 75–85% in early stage.

Isoenzyme (zymodeme) analysis

E. histolytica possesses several isoenzymes like malic enzyme, hexokinase, isomerase, and phosphoglucomutase.

- When these isoenzymes are subjected to electrophoresis, based on the electrophoretic pattern (zymodeme pattern) and mobility of the isoenzymes, *Entamoeba* can be speciated
- It can also be used to differentiate *E. histolytica* and *E. dispar*
- However, zymodeme analysis has a number of disadvantages such as: difficulty of performing the test, time-consuming and difficulty in preparing the antigens by culture.

Molecular diagnosis

Nested multiplex polymerase chain reaction (PCR) is available targeting small subunit ribosomal ribonucleic acid (rRNA) genes that can differentiate *E.histolytica, E.dispar* and *E.moshkovskii* with a sensitivity of nearing 90% and specificity of 90–100 % (Table 3.4)

Real-time PCR can be used as alternate to the conventional PCR. It is more sensitive, quantitates the parasite load and takes less time with less contamination rates.

Table 3.4: Sensitivity and specificity of various tests in the diagnosis of amoebiasis

Test	*Specimen*	*Sensitivity*		*Specificity*
		Amoebic dysentery	*Amoebic liver abscess*	
Microscopy (wet mount/permanent stain)	Stool	25–60%	< 10%	10–50%
	Liver abscess fluid	NA	< 25%	100%
Antigen detection (ELISA)	Stool	> 95%	Negative	> 95%
	Serum	65% (Early stage)	75% (late), 100% (early before treatment)	> 90%
	ALA fluid	Negative	100% (early before treatment)	90–100%
Antibody detection (ELISA)	Serum (acute infection)	75–85%	70–100%	> 85%
	Serum (convalescent infection)	> 90%	> 90%	> 85%
Culture and Isoenzyme analysis		50–70% (problems in case of mixed infections)	< 25%	Gold standard
PCR-based assays	Stool/ALA fluid	Stool > 90%	ALA fluid 100%	90–100%

Abbreviations: NA, not applicable; ELISA, enzyme linked immunosorbent assay; ALA, amoebic liver abscess; PCR, polymerase chain reaction

Other nonspecific findings

- Charcot Leyden crystals in stool
- Moderate leucocytosis in blood

Laboratory Diagnosis — Amoebic liver abscess

- Microscopy—detects trophozoites
- Stool culture
- Antigen detection—ELISA
- Antibody detection—IHA, IFA, ELISA, CIEP, CFT, SAT, CIA, etc
- Histopathology—PAS stain
- Molecular diagnosis—PCR
- Ultrasonography

Laboratory Diagnosis of Amoebic Liver Abscess

Microscopy

Microscopy of liver pus can detect trophozoites (but never cyst) with less than 25% sensitivity. However, it confirms the diagnosis. Stool microscopy is not useful.

Stool culture

Though it is considered as the gold standard test, the sensitivity is low (<25%).

Antigen detection

Lectin antigen is usually absent in stool but can be demonstrated in serum (70% sensitive in late stage, 100% sensitive when tested before treatment), liver pus (100% sensitive when tested before treatment) and saliva (70% sensitive).

Antibody detection

Antibody detection methods like IHA, IFA, ELISA, CIEP, complement fixation test (CFT), staphylococcal adherence test (SAT) and carbon immune assay (CIA) are used in establishing the diagnosis of amoebic liver abscess

- However, antibody persists even after the cure, so it cannot differentiate recent and old infection
- IHA (using crude antigenic extract) and IFA with titer of 1:256 and 1:200 respectively are considered as significant
- ELISA is now replacing IHA and has reported sensitivity of 90% and specificity of 85%.

Histopathology

The trophozoites in pus aspirate can be demonstrated by histopathological stains. In PAS stain, organism appears bright pink with green blue background.

Molecular diagnosis

PCR done on amoebic liver pus approaches sensitivity of 100% and specificity of 90–100%.

Ultrasonography

Ultrasonography (USG) of liver shows the site of the abscess and its extension.

Treatment — Amoebiasis

- Metronidazole or tinidazole is the drug of choice for intestinal amoebiasis and amoebic liver abscess (Table 3.5)
- Other measures include fluid and electrolyte replacement and symptomatic treatment.

Prevention

Preventive measures are as follows:

- Avoidance of the ingestion of food and water contaminated with human feces
- Treatment of asymptomatic persons who pass *E. histolytica* cysts in the stool may help to reduce opportunities for disease transmission.

Vaccination

Till now, there is no effective vaccine licensed for human use. However, colonization blocking vaccines are under trial targeting three *E. histolytica* specific antigens such as: SREHP 170 kDa subunit of lectin antigen and 29kDa cysteine rich protein.

NONPATHOGENIC INTESTINAL AMOEBA

Entamoeba dispar

E. dispar is morphologically indistinguishable (both cyst and trophozoite) from *E. histolytica*,

Table 3.5 Antiamoebic drugs and their recommended therapeutic dosages

Drug	*Dosage*
Luminal amoebicide	
(that acts on amoeba present in gut lumen) • Paromomycin	30 mg/kg in three divided doses for 5–10 days
• Diiodohydroxyquin	–
• Diloxanide furoate	–
• Iodoquinol	650 mg orally thrice a day for 20 days
Tissue amoebicide	
(effective on intestinal wall, liver and other tissue) • Emetine and dehydroemetine	–
Hepatic amoebicide	
• Chloroquine	–
Luminal and hepatic amoebicide	
• Metronidazole	750 mg thrice a day for 5–10 days
• Tinidazole	2 g/day with food for 3 days
• Benzimidazole	–

so it may be considered as a subspecies of *E. histolytica*.

- It can be distinguished from *E. histolytica* by:
  - Zymodeme study (hexokinase isoenzyme pattern)
  - Molecular methods, PCR amplifying small subunit rRNA gene)
  - Detection of lectin antigen in stool
  - RBC inside trophozoites—present only in *E. histolytica*.
- It was described by Brumpt in 1993
- It is nonpathogenic, usually colonizes in the large intestine (10 times more than *E. histolytica*) but doesn't invade intestinal mucosa
- It grows well in polyxenic media, however, poorly grows on axenic media
- *E. dispar* doesn't induce antibody production.

Entamoeba moshkovskii

E. moshkovskii is also morphologically indistinguishable from *E. histolytica* and *E. dispar* (may be the third subspecies of *E. histolytica)*.

- This species was first described from Moscow sewage by Tshalaia in 1941 and was thereafter reported to occur in many different countries including India
- It can be distinguished from *E. histolytica* by isoenzyme analysis, molecular methods and detection of lectin antigen
- Though it is a nonpathogen harboring in intestine but recent studies from Bangladesh and India have reported *E. moshkovskii* as a sole potential pathogen in patients presenting with gastrointestinal symptoms and/or dysentery, highlighting the need for further study to investigate the pathogenic potential of this organism.

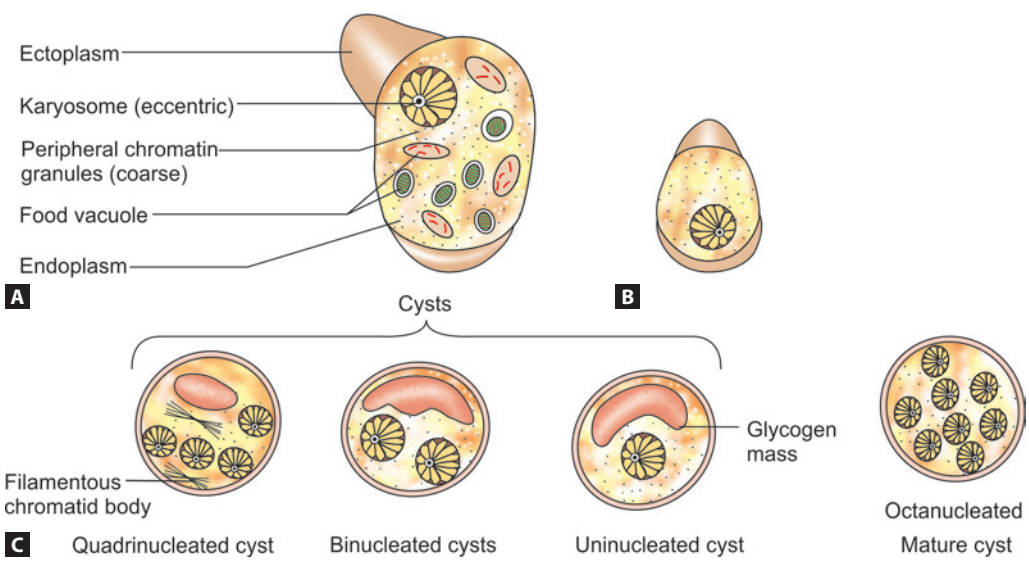
Entamoeba coli

E. coli is a nonpathogenic amoeba that colonizes the large intestine.

- The life cycle is similar to *E. histolytica*
- It has also three forms—trophozoites, precyst and cyst (Table 3.6, Figs 3.7, 3.8 and 3.9)
- It is frequently found in the stool samples of healthy individuals and should be differentiated from that of *E. histolytica* (Table 3.6).

Table 3.6: Differences between *Entamoeba histolytica* and *Entamoeba coli*

	Entamoeba histolytica	*Entamoeba coli*
Trophozoite		
Size	15–20 μm	20–25 μm
Motility	• Very active and unidirectional purposeful motility • Pseudopodia with finger like projection	• Sluggish, nonpurposeful and aimless motility in any direction • Blunt pseudopodia
Cytoplasm	Clearly differentiated to ectoplasm and endoplasm	Not differentiated
Cytoplasmic inclusions	RBC, leucocytes, tissue debris and bacteria	Same except it doesn't contain RBC
Nucleus	• Karyosome is small and central • Nuclear membrane is thin and lined by fine chromatin granules	• Karyosome is large and eccentric • Nuclear membrane is thick and lined by coarse chromatin granules
Precyst		
	10–20 μm size, oval with blunt pseudopodium, no food vacuoles and nucleus same as trophozoite	Same as *E. histolytica* except size is 20 μm
Cyst		
Size	12–15 μm	15–25 μm
Nucleus	Same as trophozoite	Same as trophozoite
Number of nuclei	1–4	1–8
Chromatoid body	Thick bars with rounded ends	Filamentous and thread like ends



Figs 3.7A to C: *Entamoeba coli* (schematic diagram) (A) trophozoite; (B) precyst; (C) cyst

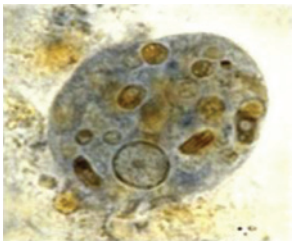


Fig. 3.8: Trophozoite of *Entamoeba coli* (Iron hematoxylin stain) shows nucleus with coarse peripheral chromatin and abundant food vacuoles in the cytoplasm containing fecal debris

Source: Giovanni Swierczynski, Bruno Milanesi. "Atlas of human intestinal protozoa Microscopic diagnosis" (*with permission*)

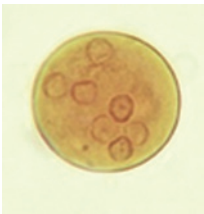


Fig. 3.9: Cyst of *Entamoeba coli* (Iodine mount) shows seven nuclei

Source: Giovanni Swierczynski, Bruno Milanesi. "Atlas of human intestinal protozoa Microscopic diagnosis" (*with permission*)

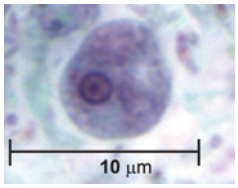


Fig. 3.10: *Entamoeba hartmanni* (smear showing trophozoite)

Source: DPDx Image Library, Centre for Disease Control and prevention (CDC), Atlanta (*with permission*)

Entamoeba hartmanni

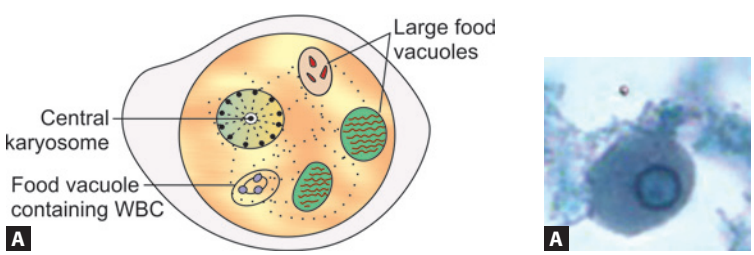
It is also known as small race variant of *E. histolytica,* i.e. morphologically it is similar to *E. histolytica* but of smaller size (trophozoite is 8–10 µm and cyst is 6–8 µm (Fig. 3.10).

- It is nonpathogenic and colonizes the large intestine
- Its life cycle is similar to *E. histolytica*.

Entamoeba gingivalis

It is the first parasitic amoeba of humans to be described; recovered from the soft tarter between the teeth.

- It is unusual in two respects:
  1. It inhabits in the mouth rather than in the large intestine
  2. Only trophozoite stage exists; no cystic stage
- Trophozoite is similar to that of *E. histolytica* trophozoite except (Fig. 3.11 A and B):
  - Smaller in size (10–15 µm)
  - Larger food vacuoles containing WBCs (only *Entamoeba* species that contains WBCs)
  - Nucleus similar to that of *E. histolytica*
- It is recovered from:
  - Vaginal secretions of women using intrauterine devices
  - Oral cavities of patients on radiation therapy and human immunodeficiency virus (HIV) infection
  - Patients with pyorrhea alveolaris
- Though it is considered nonpathogen, but still needs further study to determine its pathogenicity.



Figs 3.11 A and B: Trophozoite of *Entamoeba gingivalis* (A) schematic diagram; (B) trichrome stain

Source: B- DPDx Image Library, Centre for Disease Control and prevention (CDC), Atlanta (*with permission*)

Entamoeba polecki

It is a nonpathogenic amoeba usually found in the intestine of pigs and monkeys.

- However, human infection is rare, mainly restricted to Papua New Guinea where it is the most common intestinal amoeba in humans
- The trophozoites measure 10–12 μm size, motility nonprogressive and sluggish (like *E. coli*) and contains one nucleus having central karyosome and fine peripheral chromatin (like *E. histolytica*) (Fig. 3.12)
- Cyst is of 5–11 μm size and has one nucleus with features similar to that of trophozoite. It has many chromatoid bodies with thread-like ends (like *E. coli*) and cytoplasm has a large nonglycogen inclusion mass (Fig. 3.12).

Endolimax nana

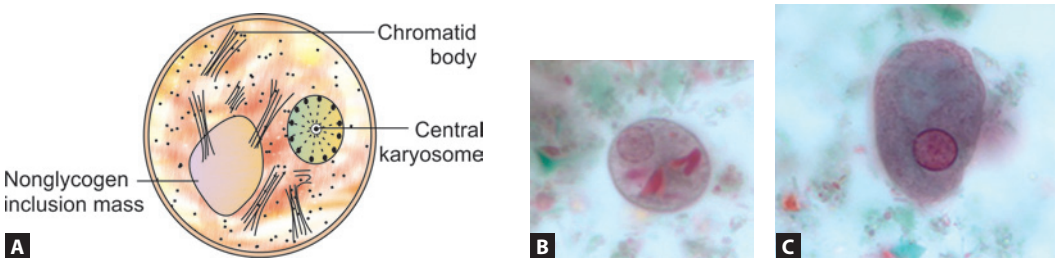
It is a small (*nana* means small) nonpathogenic amoeba.

- It is worldwide in distribution, frequently resides in the large intestine of humans and other animals
- Trophozoite measures 8–10 μm in size and shows sluggish motility
- Cyst is 6–8 μm in size and contains one to four nuclei. Cytoplasm doesn't have chromatoid body or glycogen vacuole
- Nucleus (both trophozoite and cyst)—Karyosome is eccentric and irregular; from which several achromatic strands extend to the nuclear membrane. There is no peripheral chromatin on nuclear membrane (Fig. 3.13).

Iodamoeba butschlii

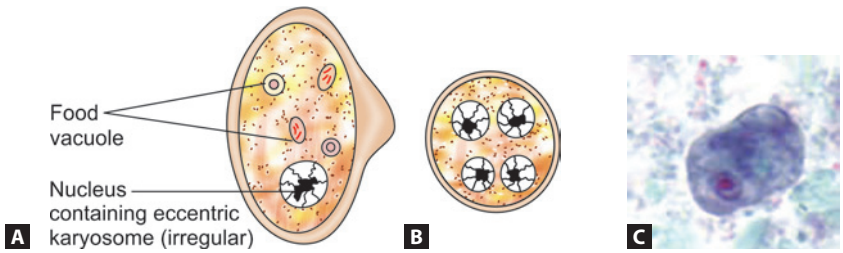
It is also worldwide in distribution though less common than *E. coli* and *E. nana*

- Trophozoite is 12–15 μm in size. The ectoplasm and endoplasm are not differentiated. Cytoplasm is more vacuolated. Nucleus is similar to that of the cyst



Figs 3.12 A to C: *Entamoeba polecki* (A) cyst (schematic diagram); (B) cyst (trichrome stain); (C) trophozoite (trichrome stain)

Source: B- and C- DPDx Image Library, Centre for Disease Control and prevention (CDC), Atlanta (*with permission*)



Figs 3.13 A to C: *Endolimax nana* (A and B) trophozoite and cyst (schematic diagram); (C) trophozoite (trichrome stain)

Source: C- DPDx Image Library, Center for Disease Control and Prevention (CDC), Atlanta (*with permission*)

- Cyst measures 10–12 μm in size, round to oval and mostly is uninucleated
  - Nucleus has central karyosome surrounded by refractile chromatin granules **(bull's eye appearance or basket nucleus)**. On permanent smear, the nucleus may appear to have a halo surrounding the karyosome
  - Cytoplasm of the cyst contains large iodine stained glycogen mass or iodophilic body (hence named as *Iodamoeba*) and no chromatoid body (Fig. 3.14).

FREE-LIVING AMOEBA

These amoebae are small, freely living, widely distributed in soil and water and can cause opportunistic infections in humans.

- Among the many genera of free-living amoebae that exist in nature, only four genera have an association with human disease. They are:
  1. *Naegleria fowleri* is a causative agent of primary amoebic meningoencephalitis (PAM)
  2. *Acanthamoeba* species causes granulomatous amoebic encephalitis (GAE) and amoebic keratitis in contact lens wearers
  3. *Balamuthia mandrillaris* causes GAE
  4. *Sappinia diploidea*
- They differ from intestinal amoeba by:
  - Naturally found freely outside the host in the environment (soil and water)
  - Possesses plenty of mitochondria (intestinal amoeba lack mitochondria)
  - Nuclear membrane is distinct, not lined by peripheral chromatin granules and nucleolus is large, deep stained. (Intestinal amoeba has a delicate nuclear membrane, small pale stained nucleolus)
  - Cause opportunistic infection affecting central nervous system (CNS).

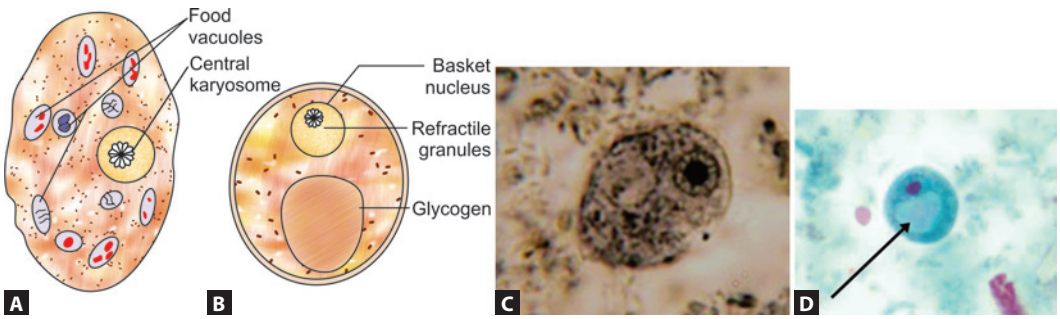
Naegleria fowleri

Naegleria is a free-living amoeba, typically found in warm fresh water, such as ponds, lakes, rivers and hot springs. It is also found in soil, near warm-water discharges of industrial plants and swimming pools.

- Only one species, *N. fowleri*, is known to cause infection, although two other species, *N. australiensis* and *N. italica*, can cause infection in mice
- *N. fowleri* (also known as "the brain-eating amoeba") is first described by physicians M. Fowler (hence named as *fowleri*) and R. F. Carter in Australia in 1965.

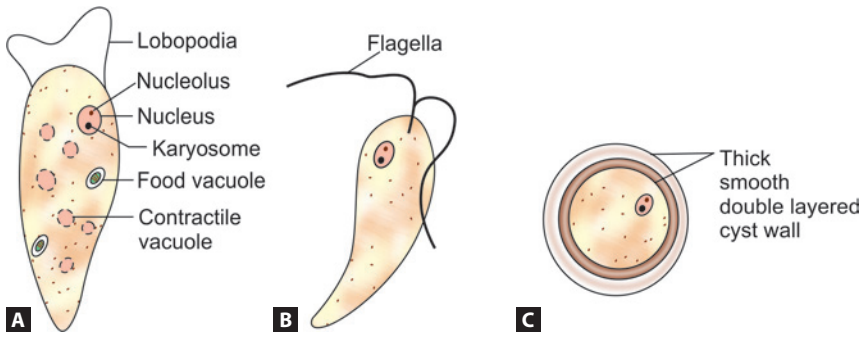
Morphology

Naegleria fowleri exists in nature as cyst and trophozoite forms (Fig. 3.15).



Figs 3.14 A to D: *Iodamoeba butschlii* (A and B) trophozoite and cyst (schematic diagram); (C) trophozoite (Iron hematoxylin stain) shows basket nucleus and glycogen vacuole; (D) cyst (stained)

Source: C- Giovanni Swierczynski, Bruno Milanesi. "Atlas of human intestinal protozoa microscopic diagnosis" (*with permission*); D- DPDx Image Library, Centre for Disease Control and prevention (CDC), Atlanta (*with permission*)



Figs 3.15 A to C: *Naegleria fowleri* trophozoite stage (schematic diagram) (A) amoeboid form; (B) flagellated form; (C) cyst stage

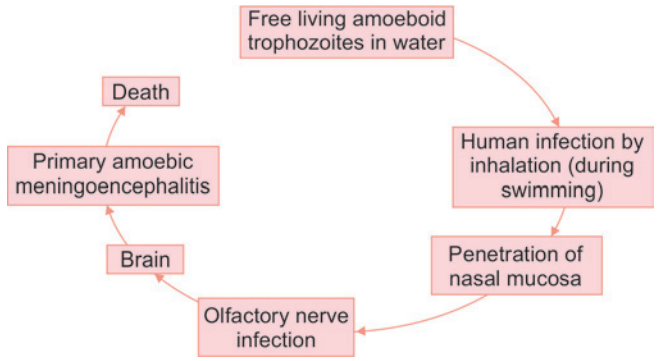


Fig. 3.16: Life cycle of *Naegleria fowleri*

Trophozoite Stage

The trophozoites occur in two forms, amoeboid and flagellated form. Both measure 8–15 μm.

- **Amoeboid form:** It is the only recognizable form in humans. It possesses lobate pseudopodia (called as **lobopodia**). Cytoplasm is granular with food vacuoles; nucleus shows central karyosome and no peripheral chromatin. It is the only replicating form and it divides by binary fission (Fig. 3.15 A)
- **Flagellated form:** When the amoeboid forms are exposed to a change in ionic concentration such as placement in distilled water at 27–37°C, they transform to pear shaped flagellated form that possess two flagella at the broader end. This change occurs very quickly within a few hours. They show typical jerky or spinning motility. When the flagella are lost, they revert back to amoeboid form (Fig. 3.15 B).

Cyst Stage

Cysts measure 7–15 μm in size and is surrounded by a thick, smooth double wall. Nucleus is identical to that found in the trophozoite. Cysts are not found in tissue (humans) but can be grown in culture. (Fig. 3.15 C).

Life Cycle and Pathogenicity (Fig. 3.16)

Infective form: Amoeboid form is the invasive form and also the usual infective form of the parasite.

Mode of transmission: Man acquires infection by nasal contamination during swimming in fresh hot water bodies like ponds, river, swimming pools or lakes. Rarely, if the flagellated or cyst form enters, soon they revert back to amoeboid form.

- **CNS invasion:** The amoeboid form invades the nasal mucosa, cribriform plate and travels along the olfactory nerve to reach brain. The penetration initially results in significant necrosis and hemorrhages in the nasal mucosa and olfactory bulbs
- The two main mechanisms of pathogenesis are:
  1. Direct ingestion of the brain tissue by producing food cups or **amoebostome** into which the cytopathic enzymes are liberated
  2. Contact dependent cytolysis mediated by hemolytic proteins, cytolysins and phospholipase enzymes
- Gradually, it produces an acute suppurative meningoencephalitis, which becomes hemorrhagic and necrotic later
- Only amoeboid trophozoites are found in cerebrospinal fluid (CSF) and in brain tissue; but not other forms.

Clinical Features (Primary Amoebic Meningoencephalitis)

N. fowleri causes acute suppurative fulminant infection of CNS known as primary amoebic meningoencephalitis (PAM).

- It is so named because to distinguish it from the secondary invasions of CNS caused by *E. histolytica*
- PAM usually occurs in healthy children or young adults with recent history of swimming in fresh hot water
- **Incubation period:** 1–2 days to 2 weeks after exposure. Clinical course is acute and fulminant
- The initial symptoms include changes in the taste and smell (due to olfactory nerve involvement) followed by headache, anorexia, nausea, vomiting, high fever, and signs of meningeal involvement like stiff neck and a positive Kernig's sign
- Secondary symptoms include confusion, hallucinations, lack of attention, ataxia, and seizures
- The mortality rate is nearly 98%. Death occurs within 7–14 days after exposure.

Epidemiology

- The first case was reported from water and soil from Australia and from sewage sludge of India
- Till now more than 200 cases of PAM have been reported mainly from USA (> 90 cases) and also from other parts of the world like Czechoslovakia, Australia, New Zealand and Brazil
- In India, it is reported from Mangalore, Kolkata and Rajasthan (> 20 cases reported so far).

Laboratory Diagnosis — Naegleria fowleri

- Cerebrospinal fluid analysis
- Microscopy
- Culture on nonnutrient agar
- Isoenzyme analysis
- Molecular methods—PCR
- Imaging methods—CT and MRI

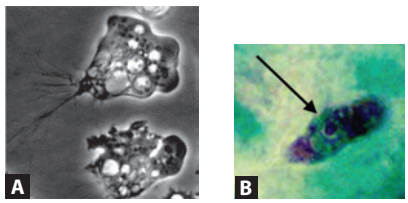
Laboratory Diagnosis

Cerebrospinal fluid analysis

CSF is thick purulent, with polymorpho-nuclear cells more than 20,000/µl, elevated protein and reduced sugar level (mimic bacterial meningitis).

Microscopy

- **Direct microscopy:** Motile amoeboid trophozoites can be demonstrated in wet mount preparation of CSF made with cover slip (counting chamber is not used as trophozites in CSF mimic leucocytes) Other forms are not seen in CSF
- Care should be taken to differentiate the trophozoites from leukocytes. Motile trophozoite containing a spherical nucleus with large karyosome is the clue for identification
- **Phase contrast microscope** yields better result than light microscope. (Fig. 3.17A)
- **Histopathological staining** (Wright's or Giemsa) of CSF or brain biopsied tissue may demonstrate trophozoites with sky blue cytoplasm with a pink nucleus (Fig. 3.17B)



Figs 3.17A and B: *Naegleria fowleri* (A) saline mount (phase contrast microscopy) top—flagellated trophozoite, below—amoeboid trophozoite; (B) arrow shows amoeboid trophozoite (hematoxylin and eosin stain)

Source: DPDx Image Library, Centre for Disease Control and prevention (CDC), Atlanta (*with permission*)

- Refrigeration is not recommended if there is a delay in examining the CSF
- If the parasite load is low then CSF can be centrifuged at low speed (150 rpm for 5 minutes). Trophozoites are not damaged, they only lose their pseudopodia
- Trophozoites can also be demonstrated by **direct fluorescence antibody** staining of centrifuged CSF using monoclonal antibody.

Culture

CSF sample can be cultivated on **nonnutrient agar (Page's saline and 1.5% agar)**, lawn cultured with bacterial supplement like *E. coli. Naegleria* feeds on bacteria and crawls over the lawn culture of *E. coli* to produce trails (**Trail sign**).

Enflagellation test

When the scrapping of the nonnutrient agar is transferred to sterile tubes containing distilled water, *N. fowleri* undergoes transformation to a pear shaped flagellate form.

Isoenzyme Analysis

Isoenzyme analysis has been developed for the specific identification of *N. fowleri* cultured from the CSF and brain specimens of the patients as well as from the environment samples.

Molecular Methods

Both conventional PCR and nested PCR assays have been described for the identification of *N. fowleri* targeting specific 5.8s rRNA genes and internal transcribed spacer genes

Imaging Methods

Computed tomography (CT) scan and magnetic resonance imaging (MRI) show obliteration of cisterns, and diffuse enhancement around midbrain, subarachnoid space and over cerebrum.

Treatment	Naegleria fowleri
• No effective treatment is available for PAM • Amphotericin B has considerable anti *Naegleria* effect. Four cases were treated successfully with amphotericin B. • Other drugs like rifampicin, azithromycin and antifungals like miconazole and voriconazole are also found to be effective.	

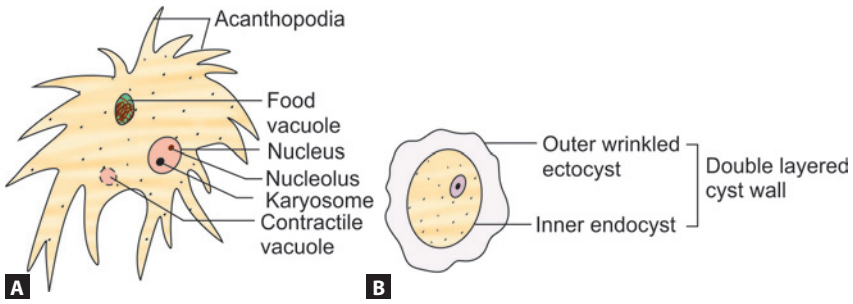
Acanthamoeba

Acanthamoeba species is ubiquitous and present worldwide. They have been isolated from soil, fresh and brackish waters

- Griffin and Sawyer proposed the name in 1975. It is so named because of the spine like pseudopodia present in trophozoite (called as acanthopodia).
- More than 24 species have been identified. Important ones that cause human infection include *Acenthamoeba astronyxis*, *A. castellanii, A. culbertsoni* and *A. polyphaga*.
- It principally affects CNS and eye.
- ***Reservoir for bacteria:*** Approximately 20–24% of clinical and environmental isolates of *Acanthamoeba* harbor bacterial pathogens such as *Legionella* species *Mycobacterium avium* and *Listeria,* and may serve as a potential reservoir and act as **Trojan horse** of the microbial world.

Morphology

Acanthamoeba species exists in nature as cyst and trophozoite forms. There is no flagellated form. (Fig. 3.18 A and B).



Figs 3.18 A and B: *Acanthamoeba* species (schematic diagram) (A) amoeboid form; (B) cyst

Trophozoite

It is larger than *Naegleria* measuring 15–25 μm size

- It bears spine or thorn like pseudopodia **(acanthopodia)**
- **Nucleus:** Single with central karyosome and no peripheral chromatin.

Cyst

It is double walled (outer wrinkled ectocyst and inner endocyst), nuclear characteristics are same as trophozoite.

Life Cycle and Pathogenesis (Fig. 3.19)

- **Mode of transmission:** Man acquires infection by inhalational route by aerosol contaminated with cyst or trophozoite, or rarely by direct spread through broken skin or infected eye
- From lungs, trophozoites reach CNS by hematogenous route

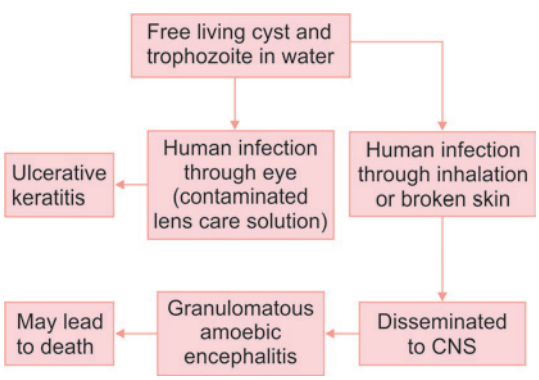


Fig. 3.19: Life cycle of *Acanthamoeba* species

- It causes **GAE (granulomatous amoebic encephalitis)** in immunocompromised patients like HIV positive patients and keratitis in healthy individuals.
- **GAE** is characterized by:
  - **Insidious onset:** Incubation period varies from several weeks to months
  - **Chronic course:** Lasts for months to years
  - History of immunosupression or underlying disease or trauma
  - **Pathology:** Focal granulomatous lesions in brain
  - Lymphocytosis of CSF
  - **Symptoms:** Confusion, dizziness, nausea, headache, stiff neck and sometimes seizure and hemiplegia
  - More than 200 cases of GAE due to *Acanthamoeba* have been reported so far, half of those from USA. From India, few cases are reported from Vellore and other places.
- **Amoebic keratitis** is characterized by:
  - Corneal spread with *Acanthamoeba* occurs following:
    - trauma (onset is rapid)
    - contact lens use; especially present in the lens cleaning solution (onset is slow)
    - contaminated water (onset is slow)
  - **Mechanism of adhesion:** Mannose binding protein on *Acanthamoeba* adheres to glycoprotein receptors on corneal epithelium

- Characterized by-corneal infiltration and ulcerations, iritis, scleritis, hypopyon (pus in anterior chamber), severe pain, and loss of vision
- In India, it is commonly associated with contact lens users (75–93% of cases) However, a recent South Indian study done at Arvind eye hospital, Madurai had shown an increase trend of *Acanthamoeba* keratitis in noncontact lens wearers.

- In **HIV patients**, it produces:
  - GAE
  - Nasal ulcers
  - Cutaneous ulcers and abscess
  - Musculoskeletal abscesses.

Laboratory Diagnosis Acanthamoeba

- Direct microscopy
  - Wet mount examination of CSF or conrneal scrapping
  - Permanant staining of brain biopsy
- Culture on nonnutrient agar
- Indirect fluorescent antibody technique
- Molecular methods—PCR
- CSF examination—lymphocytosis

Laboratory Diagnosis

Direct microscopy

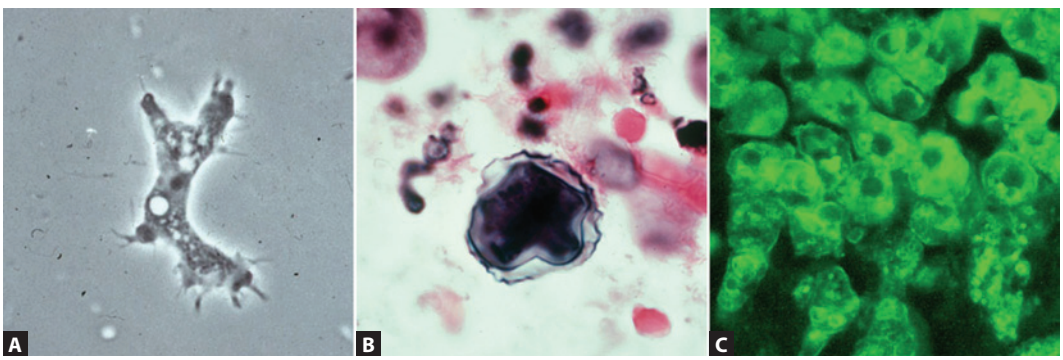
- **Wet mount examination:** CSF or corneal scrapping is done to demonstrate both cyst and trophozoites (Fig. 3.20A). Phase contrast microscope gives better results:
  - Trophozoite is characterized by acanthopodia
  - Cyst has two layers with an outer wrinkled ectocyst.
- **Permanent staining:** It is done for CSF and brain biopsy. Hematoxylin and eosin stain, trichrome stain are used to visualize characteristic morphology of trophozoite such as prominent nucleolus, contractile vacuole and cytoplasmic vacuole (Fig. 3.20B)
- **Calcofluor stain:** It is recommended to visualize the double walled cyst.

Indirect fluorescent antibody technique

Indirect fluorescent antibody technique (IFAT) with specific antisera can be used for speciation of *Acanthamoeba*. *A. culbertsoni* and *A. castellani* are the most frequently identified species in CSF; where as *A. polyphaga* and *A. castellanii* from corneal scrapping (Fig. 3.20C).

Culture

- Various culture media used are nonnutrient agar with bacterial supplement, tryptic soy agar with horse blood, buffer charcoal yeast extract (BCYE) incubated at 30°C



Figs 3.20 A to C: *Acanthamoeba* species (A) trophozoite in CSF saline mount; (B) cyst in brain tissue (hematoxylin and eosin stain); (C) indirect fluorescent antibody technique of *Acanthamoeba* species viewed under UV microscopy

Source: DPDx Image Library, Centre for Disease Control and prevention (CDC), Atlanta (*with permission*)

- However, unlike *Naegleria, Acanthamoeba* is not readily isolated from culture (Table 3.7)
- Compared to centrifuation of CSF, Parasite detection rate is higher when CSF sample is cultured following filtration through celluose membrane.

Treatment	Acanthamoeba

Granulomatous amoebic encephalitis

- Unfortunately, there are no therapies with proven efficacy against this disease. Only three cases are survived so far
- They were treated with multidrug combinations that included cotrimoxazole, ketoconazole, pentamidine, flucytosine and rifampin. Surgery was done in one of those patients.

Amoebic keratitis

- Topical antiseptic agents such as a biguanide or chlorhexidine are used
- In severe cases of vision impairment may need penetrating keratoplasty.

Balamuthia mandrillaris

Balamuthia mandrillaris is a free-living, heterotrophic amoeba that also causes GAE.

History

The name goes in the honor of the late Professor William Balamuth and it was first discovered in a pregnant mandrill (an old world monkey) at San Diego.

Epidemiology

It is distributed in the temperate regions of the world. Till now more than 150 cases are reported, half of them being form USA and South America. There are no reports from India yet.

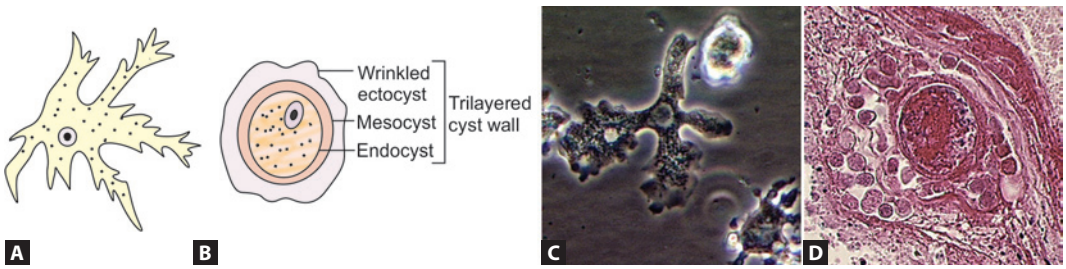
Life Cycle

It is similar to *Acanthamoeba*. It has trophozoite and cyst form (no flagellated form) (Fig. 3.21).

Table 3.7: Differences in the characteristics of *Naegleria* and *Acanthamoeba*

Character	*Naegleria fowleri*	*Acanthamoeba*
Disease	Primary amoebic meningoencephalitis	Granulomatous amoebic encephalitis • Ulcerative keratitis
Risk factor	Swimming in contaminated water	Immunodeficiency
Clinical course	Acute	Sub-acute to chronic
Pathology	Diffuse suppurative changes	Focal granulomatous inflammation
Trophozoites	• Two forms, amoeboid and flagellated form • Lobated and blunt pseudopodium (lobopodia) • 8–15 μm size	• One form, no flagellated form • Thorn like pseudopodium (acanthopodia) • 15–25 μm size
Cyst	• Not present in tissue or CSF • Small (7–15 μm), thick and smooth double wall	• Can be found in tissue or CSF • Larger (12–20 μm), thin wrinkled double wall
Spread	Direct neural spread	Hematogenous spread
CSF Leukocytes	Neutrophils	Lymphocytes
Culture	• Require bacterial supplement • Don't grow with > 0.4% NaCl	• May grow without bacterial supplement • Not affected by NaCl
CT scan	Unremarkable (such as basal arachnoiditis), no specific feature	Space occupying lesion is seen

Abbreviations: CSF, cerebrospinal fluid; NaCl, sodium chloride; CT, computed tomography



Figs 3.21A to D: *Balamuthia mandrillaris* (A and B) trophozoite and cyst (schematic diagram); (C) trophozoite (saline mount); (D) cyst (hematoxylin and eosin stain)

Source: C- and D- DPDx Image Library, Centre for Disease Control and prevention (CDC), Atlanta (*with permission*)

- The trophozoite is approximately 30 μm, irregular with finger like pseudopodia
- The cyst measures 6–30 μm, surrounded by a three-layered cell wall (outer wrinkled ectocyst, middle mesocyst and inner thin endocyst), and an abnormally large, vesicular nucleus.

Clinical Features

It may enter the body through the respiratory tract or through open wounds. In CNS, it causes GAE. It also can cause skin lesion.

Diagnosis

Microscopy

CSF microscopy reveals trophozoites and cysts.

Culture

It can be cultured on monkey kidney cell line, HEp2, Vero and diploid macrophage cell line. It doesn't grow on agar plate culture coated with bacteria.

It can be differentiated from *Acanthamoeba* species by:

- Microscopy: Nucleus contains more than one nucleoli and cyst wall is tri-layered
- IFAT using specific antisera
- Culture on cell lines but not in agar plate
- PCR targeting mitochondrial small subunit rRNA gene.

Sappinia diploidea

- It is newly recognized pathogenic free-living amoeba found in soil and water
- The characteristic feature is both trophozoite and cyst stages are binucleated
- The trophozoite is oval, measures 40–70 μm. The mature cyst is round and measures 15–30 μm
- *S. diploidea* can be cultivated on nonnutrient agar plate coated with bacteria.
- Till now, only one case of amoebic encephalitis has been reported.

EXPECTED QUESTIONS

I. Write essay on:

(a) Describe the life cycle, pathogenesis and laboratory diagnosis of *Entamoeba histolytica*?

(b) List the free-living amoeba. Write in detail about their life cycle, pathogenesis and laboratory diagnosis?

II. Write short notes on:

(a) Amoebic liver abscess

(b) *Entamoeba dispar*

III. Differentiate between:

(a) Features of *Entamoeba dispar* and *Entamoeba histolytica*

(b) Amoebic dysentery and bacillary dysentery

(c) Features of *Entamoeba coli* and *Entamoeba histolytica*

(d) Features of *Naegleria* and *Acanthamoeba*

(e) Features of *Acanthamoeba* and *Balamuthia*

Contd...

Contd...

IV. Multiple choice questions (MCQs):

1. **What would be the most likely manifestation of extraintestinal amoebiasis?**
   (a) High periodic fever
   (b) Draining skin lesion
   (c) Enlarged painful spleen
   (d) Tender, enlarged liver
2. **A 30-year-old female having habit of keeping her contact lenses in tap water. She noticed deterioration of vision and visited an ophthalmologist who diagnosed her with severe retinitis. Culture of the water as well as vitreous fluid would most likely reveal:**
   (a) *Naegleria*
   (b) *Entamoeba histolytica*
   (c) *Acanthamoeba*
   (d) *Entamoeba coli*
3. **Mature cyst of *Entamoeba histolytica* differs from *Entamoeba coli* by:**
   (a) Larger and uninucleated
   (b) Smaller and binucleated
   (c) Smaller and quadrinucleated
   (d) Larger and quadrinucleated
4. **All nonpathogenic amoebae live in the lumen of large intestine except:**
   (a) *Entamoeba dispar*
   (b) *Entamoeba moshkovskii*
   (c) *Entamoeba gingivalis*
   (d) *Endolimax nana*
5. **Lobopodia are seen in trophozoites of:**
   (a) *Naegleria fowleri*
   (b) *Acanthamoeba* species
   (c) *Balamuthia mandrillaris*
   (d) *Sappinia diploidea*
6. ***Balamuthia* causes:**
   (a) Primary amoebic meningoencephalitis
   (b) Granulomatous amoebic encephalitis
   (c) Keratitis
   (d) Liver abscess
7. **Infection with *Naegleria* can be acquired by:**
   (a) Swimming in lakes, ponds or pools containing infective forms
   (b) Parenteral route
   (c) Orogenital contact
   (d) Feco-oral route
8. **Which is not a feature of CSF in primary amoebic meningoencephalitis?**
   (a) Purulent
   (b) Lymphocytic leucocytosis
   (c) High protein
   (d) Low glucose content
9. **Cysts of *Entamoeba histolytica* are formed in:**
   (a) Lumen of the large intestine
   (b) Tissues
   (c) Lumen of the small intestine
   (d) Epithelium of large intestine
10. **Infective form and invasive form of *Entamoeba histolytica* are:**
    (a) Trophozoite and quadrinucleated cyst
    (b) Quadrinucleated cyst and trophozoite
    (c) Both trophozoite
    (d) Both quadrinucleated cyst

Answers

1. (d) 2. (c) 3. (c) 4. (c) 5. (a) 6. (b) 7. (a) 8. (b) 9. (a) 10. (b)

4 Flagellates—I (Intestinal and Genital)

Chapter Outline

- Classification of flagellates
- Giardia lamblia
- Trichomonas vaginalis
- Other intestinal flagellates of minor importance
- Expected questions

CLASSIFICATION OF FLAGELLATES

This group of parasites bear flagella as the organ of locomotion.

- Flagella are slender, long and thread-like extension of cytoplasm. Its intracellular portion is called as **axostyle** or **axoneme**. Flagella arise from kinetoplast (made up of copies of mitochondrial DNA) which in turn consists of:
  - Blepharoplast or basal body or kinetosome from which flagellum arises
  - Parabasal body, through which it passes as axostyle
- In most of the flagellates, the flagella are external except in *Dientamoeba fragilis* which bears internal flagellum.

Taxonomic Classification

Different flagellates belong to three different phyla (Table 4.1).

Table 4.1: Taxonomic classification of flagellates

Kingdom	*Subkingdom*	*Phylum*	*Class*	*Order*	*Genus*
Protozoa	Archezoa	Metamonada	Trepomonadea	Diplomonadida	*Giardia*
				Enteromonadida	*Enteromonas*
			Retortamonadea	Retortamonadida	*Retortamonas* *Chilomastix*
		Parabasalia	Trichomonadea	Trichomonadida	*Trichomonas* *Pentatrichomonas* *Dientamoeba*
	Neozoa	Euglenozoa	Kinetoplastea	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	Ileocecal region
Trichomonas vaginalis	Vagina and urethra
Blood and somatic flagellates	***Habitat***
Leishmania	Blood and tissue
Trypanosoma	Blood and tissue

Classification Based on Habitat

They are grouped into intestinal, genital and blood flagellates (Table 4.2).

GIARDIA LAMBLIA

History

Giardia lamblia was first observed by A.V. Leeuwenhoek in 1681 while examining his own stool.

The parasite was named after Dr F. Lambl of Prague and Prof. A.Giard of Paris in 1859. They have described the morphology of the parasite in the human intestine.

Classification

Giardia can be differentiated to various species based on the origin of the host.

- *G. lamblia* infects humans and other mammals, *G.muris* in mice, *G.agilis* in amphibians, *G.psittaci* in birds and *G. microti* in voles
- *G. lamblia* can further be differentiated into seven genotypes from A to G, out of which genotype A and B usually infect humans.

Epidemiology

G. lamblia is worldwide in distribution, it is considered as one of the most common parasitic diseases, causing both endemic and epidemic intestinal disease and diarrhea.

Geographical area: More common in warm climate of tropics and subtropics.

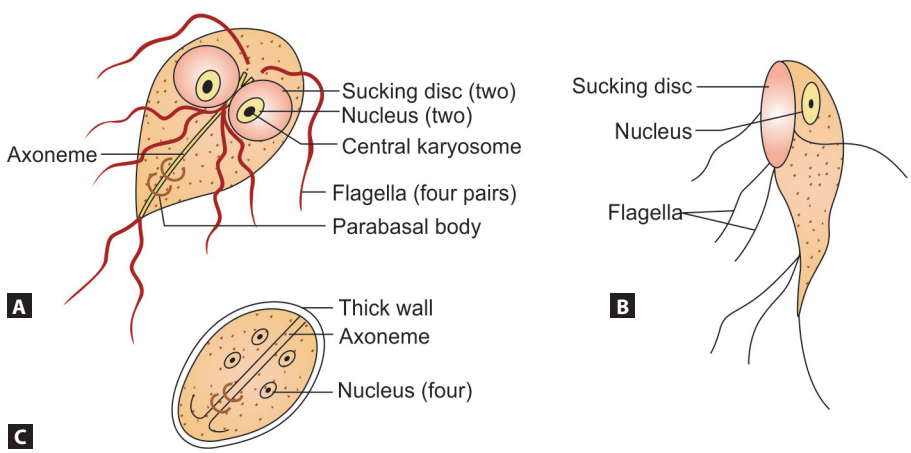
In India: Prevalence of giardiasis in children ranges from 2.67 to 32%. Majority of infections are caused by genotype B as reported by a South Indian study.

Habitat

Duodenum and upper part of jejunum.

Morphology

It occurs in two forms—(1) trophozoite and (2) cyst (Fig. 4.1).



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

Trophozoite

The trophozoite has a falling leaf-like motility, usually measures 10–20 μm in length and 5–15 μm in width.

- Shape:
  - In front view, it is pear shaped (or tear drop or tennis racket shaped) with rounded anterior end and pointed posterior end
  - Laterally, it appears as a curved portion of a spoon (sickle shaped)
- It is convex dorsally while the ventral surface has a concavity bearing a bilobed adhesive disc. Hence, it appears as sickle shaped in lateral view
- Trophozoite is bilaterally symmetrical; on each side from the midline it bears (Figs 4.1A and B):
  - One pair of nuclei
  - Pair of median bodies
  - Four pairs of basal bodies or blepharoplast (from which the axoneme arises)
  - Four pairs of flagella—two lateral, one ventral and one caudal pair of flagella
  - Pair of parabasal bodies (connected to basal bodies through which the axoneme passes)
  - Pair of axoneme or axostyle (the intracellular portion of the flagella).

Cyst

Giardia cyst is oval shaped, measures 11–14 μm in length and 7–10 μm in width.

- It contains four nuclei and remnants of axonemes, basal bodies and parabasal bodies (Fig. 4.1C)
- It is the infective form as well as the diagnostic form of the parasite.

Life Cycle (Fig. 4.2)

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

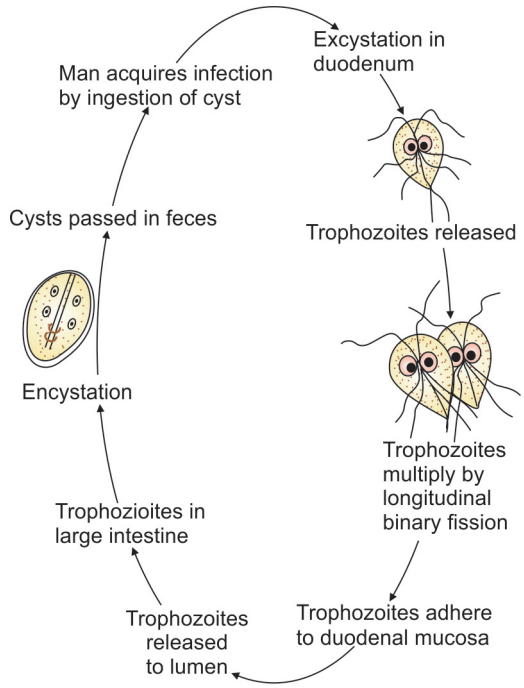


Fig. 4.2: Life cycle of *Giardia lamblia*

Development in Man

- **Excystation:** Two trophozoites are released from each cyst in the duodenum within 30 minutes of entry
- **Multiplication:** Trophozoites multiply by longitudinal binary fission in the duodenum.
- **Adhesion:** Trophozoites adhere to the duodenal mucosa by the bilobed adhesive ventral disc

  This is achieved by the microtubules of median bodies, contractile proteins and lectins present on the surface of adhesive disc that bind to the intestinal receptors (sugar molecules)
- In active stage of the disease, sometimes the trophozoites are excreted in diarrhea stool
- **Encystation:** Gradually when the trophozoites pass down to large intestine, encystation begins
  - Promoting factors for encystation are the conjugated bile salts, alkaline pH and cholesterol starvation

- **Encystation specific vesicles (ESV)** appear in the cytoplasm that helps in processing and transportation of the cyst wall protein antigens to the exterior of the plasma membrane to synthesize the cyst wall
- Encystation begins with retraction of the flagella followed by condensation of the cytoplasm and finally formation of the cyst wall
- On maturation, nuclei divide to become four. The mature cysts excreted in feces can survive better in the environment and are infective to man.

Pathogenicity

- **Infective dose:** As few as 10–25 cysts can initiate the infection
- **Risk factors:** Children are commonly affected. Other high-risk groups are elderly debilitated persons and patients with cystic fibrosis, poor hygiene, and immunodeficiency syndromes such as common variable hypoglobulinemia. However, association with acquired immunodeficiency syndrome (AIDS) patient is not been confirmed yet
- **Several pathogenic mechanisms** have been postulated that include:
  - Trophozoites adhere to the duodenal mucosa and cause disruption of the intestinal epithelial brush border that leads to increase permeability and malabsorption
  - Very rarely, elaboration of enterotoxin such as cystein rich surface protein 136 (CRP-136)
- **Malabsorption:** There could be various types which include:
  - Malabsorption of fat (steatorrhea)—leads to foul smelling profuse frothy diarrhea
  - Disaccharidase deficiencies (lactate, xylose)—leading to lactose intolerance
  - Malabsorption of vitamin B12 and folic acid
  - Protein loosing enteropathy
- **Antigenic variation:**
  - *Giardia* undergoes frequent antigenic variation due to a cysteine rich protein on its surface called **variant surface protein (VSP)**
  - This helps the parasite in evasion of the host immune system and resistant to intestinal proteases which inturn leads to persistence of infection resulting into chronic and recurrent illness.

Clinical Features

Clinical course of giardiasis can be divided into three stages:

1. **Asymptomatic carriers:** Most infected persons are asymptomatic, harboring the cysts and spreading the infection
2. **Acute giardiasis:**
   - Incubation period varies from 1 week to 3 weeks (average 12–20 days). Symptoms may develop suddenly or gradually
   - Common symptoms include diarrhea, abdominal pain, bloating, belching, flatus and vomiting
   - Diarrhea is often foul smelling with fat and mucus but no blood
   - The acute stage lasts for 1 week but usually resolves spontaneously. Very rarely, in some children may last for months
3. **Chronic giardiasis:**
   - It may present with or without a previous acute symptomatic episode
   - Symptoms are intermittent and recurring
   - Common symptoms include recurrent episodes of foul smelling diarrhea, foul flatus, sulfurous belching with rotten egg taste, and profound weight loss leading to growth retardation
   - Uncommon symptoms such as—fever, presence of blood and/or mucus in the stools, and other signs and symptoms of colitis
   - Extraintestinal manifestations have been described, such as urticaria,

anterior uveitis, salt and pepper retinal changes and arthritis.

Laboratory Diagnosis Giardia lamblia

- Stool examination—detects cysts and trophozoites
- Entero-test
- Antigen detection in stool (copro-antigen)—ELISA, ICT
- Antibody detection in serum—ELISA, IFA
- Culture
- Molecular method—PCR
- Radiological findings—barium meal, X-ray

Laboratory Diagnosis

Stool Examination

Giardia cysts can be demonstrated by iodine and saline wet mount preparations but they cannot differentiate active disease from carriers (Fig. 4.3).

Demonstration of the trophozoites with falling leaf like motility by saline mount indicates active stage of the disease (Fig. 4.4).

- *Giardia* adheres firmly to the duodenal mucosa by adhesive disc leading to intermittent shedding. Hence, repeated stool examination (at least three consecutive samples) should be done
- Sensitivity varies from 60% to 80% with one stool and more than 90% after three stools examination
- **Concentration techniques** like zinc sulfate floatation or formalin ether sedimentation methods are employed to increase the chance of detection
- **Duodenal sampling:** If stool examination is negative, then direct duodenal samples like aspirates (obtained by entero-test) or biopsy (done by endoscopy) should be processed.

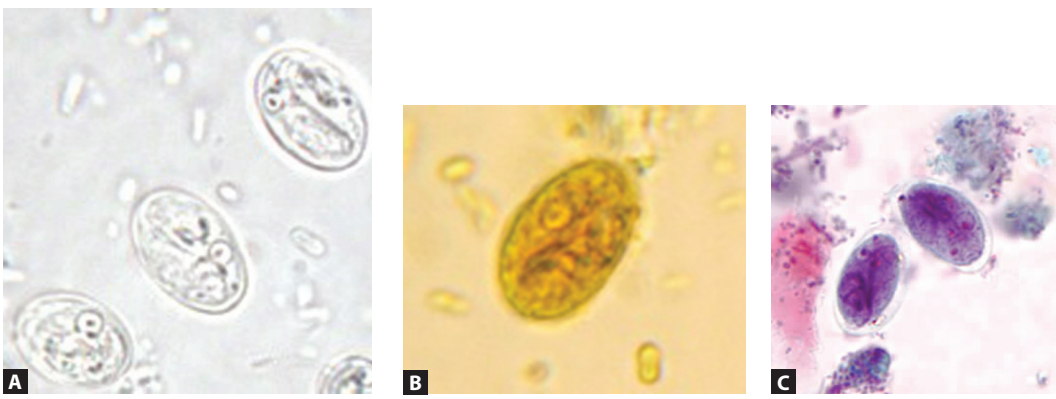
Entero-test

It uses a gelatin capsule attached to a thread.

- One end of the thread is attached to the inner aspect of the patient's cheek, and then, the capsule is swallowed
- Capsule gets dissolved in the intestine releasing the thread which is kept there for 4–6 hours to take the duodenal fluid
- Later, the thread is withdrawn and shaken in saline to release trophozoites which can be detected microscopically
- The entero-test is also useful in the search for other upper intestinal parasites such as *Strongyloides stercoralis* (Fig. 4.5).

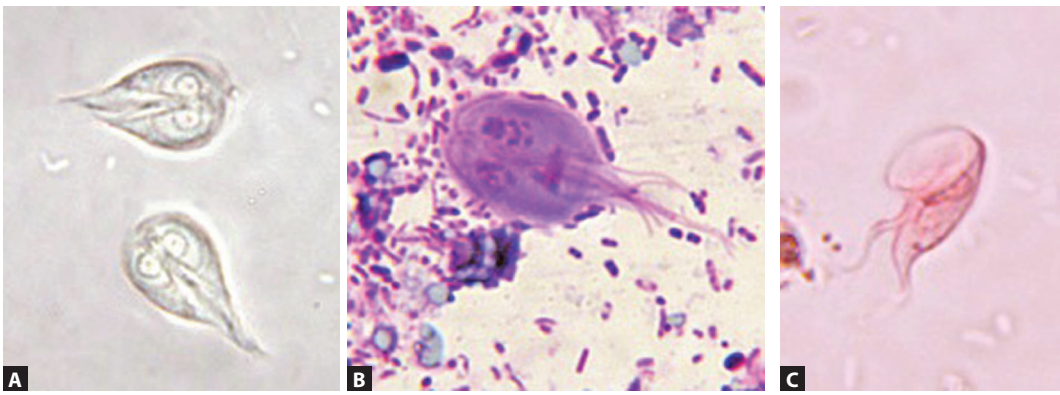
Antigen Detection in Stool (Copro-antigen)

The enzyme linked inmunosorbent assay (ELISA) and direct fluorescent antibody



Figs 4.3A and B: Cysts of *Giardia lamblia* (A) saline mount (B) iodine mount (C) trichrome stain

Source: A- and B- Giovanni Swierczynski, Bruno Milanesi "Atlas of human intestinal protozoa Microscopic diagnosis" (*with permission*); C- DPDx Image Library, Centre for Disease Control and prevention (CDC), Atlanta (*with permission*)



Figs 4.4A to C: Trophozoites of *Giardia lamblia* (A) saline mount front view; (B) Giemsa stain front view; (C) merthiolate iodine formalin (MIF) stain lateral view (spoon shaped)

Source: Giovanni Swierczynski, Bruno Milanesi. "Atlas of human intestinal protozoa Microscopic diagnosis" (*with permission*)

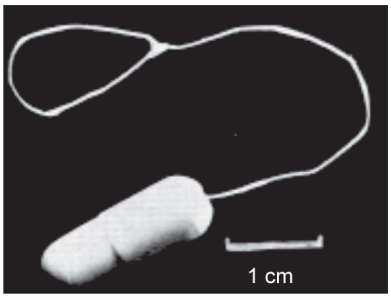


Fig. 4.5: Entero-test equipment showing duodenal capsule attached with thread at other end

tests are available using labeled monoclonal antibodies against cyst wall protein antigens. Both the tests are highly sensitive (90–100%) and specific (99–100%). They are very useful in microscopy negative samples and also in outbreak situations.

Rapid **immunochromatographic test** (commercial name **triage parasite panel**) has been developed that simultaneously detect antigens of *Giardia, Entamoeba histolytica* and *Cryptosporidium* with comparable sensitivity and specificity like ELISA. It is simple, easy to perform, doesn't require any costly instruments and can be done at peripheral laboratory.

Antibody Detection

Both indirect fluorescent antibody (IFA) and ELISA formats are developed to detect antibodies in serum.

- But unlike microscopy and antigen detection, presence of antibody cannot differentiate recent and past infection
- Hence, serology is only helpful for epidemiological purpose for estimating the prevalence of infection.

Culture

Giardia can be cultivated in axenic media like Diamond's media used for *E. histolytica.*

- Culture is done for research purpose and to prepare the antigens
- It is not routinely used because of the difficulty in isolating *Giardia* from patient samples.

Molecular Methods

Detection of *Giardia* nucleic acid by polymerase chain reaction (PCR) or by gene probes is highly sensitive and specific

- It is used to detect the parasites in water samples or to genotype the isolates from various mammalian hosts
- However, its use in routine laboratory diagnostics is limited.

Radiological Finding

X-ray after barium meal is generally nonspecific and may be positive in 20% of cases

- It shows an increased secretion and irregular thickening of small bowel folds
- Barium meal may also interfere with the stool examination. So, stool samples should be collected before the barium meal.

Treatment	Giardia lamblia
• Metronidazole (250 mg thrice daily for 5 days) is usually affective in more than 90% of cases of giardiasis • Tinidazole (2 g once orally) is more effective than metronidazole • Nitazoxanide (500 mg twice daily for 3 days) is an alternative agent for treatment of giardiasis • Furazolidone is given to children and 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.	

Prevention

Giardiasis can be prevented by:
- Improved food and personal hygiene
- Boiling or filtering of potentially contaminated water
- Treatment of asymptomatic carriers
- No vaccine is currently available.

TRICHOMONAS

Trichomonas differ from other flagellates as they lack the cyst stage. They exist as only trophozoites.
- Trichomonas belongs to:
  - Class: Trichomonadea
  - Order: Trichomonadida
  - Family: Trichomonadidae
- Three species of *Trichomonas* infect humans. They are:
  1. *Trichomonas vaginalis* is the only pathogen. It resides in the genital tract
  2. *Pentatrichomonas hominis:* Non-pathogen, resides in large intestine
  3. *Trichomonas tenax:* Nonpathogen, resides in mouth (teeth and gum).

TRICHOMONAS VAGINALIS

It is the most common parasitic cause of sexually transmitted diseases (STDs).
- Females are commonly affected than males
- It was first observed by Donne in 1836 from the purulent genital discharge of a female
- Though it is an eukaryote, its metbolism is similar to a primitive anaerobic bacteria
- Carbohydrate is utilized fermentatively. It is unable to synthesize fatty acid, sterols, purines and pyrimidines and hence depends on exogenous sources.

Morphology

Trophozoites are the only stage, there is no cystic stage.

Trophozoites

It is pear (pyriform) shaped, measures 7–23 μm and 5–15 μm wide (Fig. 4.6), resides in vagina and urethra of women and urethra, seminal vesicle and prostate of men.
- It shows characteristic jerky or twitchy motility in saline mount preparation
- It bears five flagella—four anterior flagella and one lateral flagellum called as **recurrent flagellum** as it curves back on the surface of the parasite and traverses as undulating membrane and stops halfway down the side

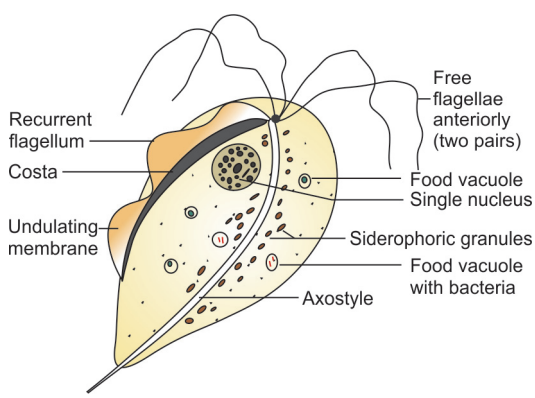


Fig. 4.6: Trophozoite of *Trichomonas vaginalis* (schematic diagram)

of the trophozoite. It doesn't come out free posteriorly
- The undulating membrane is supported on to the surface of the parasite by a rod like structure called as **costa**
- The axostyle runs down the middle of the trophozoite and ends in the pointed end of the posterior pole
- It has a single nucleus containing central karyosome with evenly distributed nuclear chromatin and the cytoplasm contains a number of siderophore granules along the axostyle
- The respiratory organelle is called as **hydrogenosome**.

Life Cycle

Trophozoites are the infective stage as well as the diagnostic stage.
- Asymptomatic females are the reservoir of infection and transmit the disease by sexual route
- Trophozoites divide by longitudinal binary fission giving rise to a number of daughter trophozoites in the urogenital tract which can infect other individuals.

Pathogenicity and Clinical Features

Trichomoniasis is the most common parasitic cause of STDs.
- It is worldwide in distribution and accounts for 10% of cases of vulvovaginitis
- Incubation period is variable (4–28 days)
- **Predisposing factors:**
  - Binding to the vaginal epithelium by various metabolic enzymes secreted by the trophozoites like adhesins, proteolytic enzymes, iron regulated proteins, erythrocyte binding proteins, etc
  - Vaginal pH of more than 4.5 facilitates infection
  - Hormonal levels
  - Coexisting vaginal flora
  - Strain and relative concentration of the organisms present in the vagina
- **Asymptomatic infection:** 25–50% of individuals are asymptomatic, harboring the trophozoites and can transmit the infection
- **Acute infection (vulvovaginitis):**
  - Females are commonly affected and are presented as vulvovaginitis, characterized by profuse foul smelling purulent vaginal discharge. Discharge may be frothy (10% of cases) and yellowish green color mixed with a number of polymorphonuclear leukocytes
  - Strawberry appearance of vaginal mucosa **(Colpitis macularis)** is observed 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:** In chronic stage, the disease is mild with pruritus and pain during coitus. Vaginal discharge is scanty, mixed with mucus
- **Complications:**
  - Rarely it is associated with complications like pyosalpinx, endometritis, infertility, low birth weight and cervical erosions

Laboratory Diagnosis Trichomonas vaginalis

- Direct microscopy
  - Wet saline mounting
  - Permanent stain
  - Acridine orange fluorescent stain
  - Direct fluorescent antibody test
- Culture— gold standard method
- Antigen detection in vaginal secretion—ELISA, ICT, etc
- Antibody detection—ELISA
- Molecular method—PCR
- Other supportive test
  - Raised vaginal pH
  - Positive whiff test

- There is also an association of increased HIV transmission and cervical dysplasia
- Respiratory distress may be seen in few cases.

Laboratory Diagnosis

Direct Microscopy

- **Samples:** Vaginal, urethral discharge, urine sediment and prostatic secretions can be examined
- **Wet (saline) mounting** of fresh samples (within 10–20 minutes of collection) should be done to demonstrate the jerky motile trophozoites and pus cells. Its sensitivity is variable (40–80%)
- **Permanent stain:** Giemsa stain and Papanicolaou stain are routinely performed to demonstrate the morphology trophozoites (Fig. 4.7)
- **Acridine orange fluorescent stain** can be used. It is rapid and sensitive; comparable to wet mount
- **Direct fluorescent antibody test (DFA):** Trophozoites are detected by staining with fluorescent labeled monoclonal antibodies. DAF test is more sensitive (70–90%) than wet-mount examination.

Culture

Culture is the gold standard method for diagnosis. It is highly sensitive 95% and specific (100%). It is positive even in microscopy negative samples.

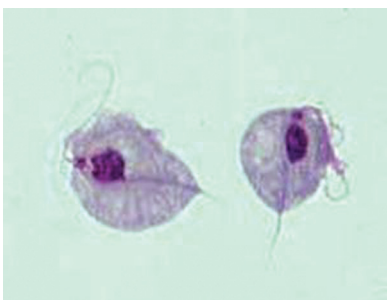


Fig. 4.7: *Trichomonas vaginalis* trophozoite (Giemsa stain)
Source: DPDx Image Library, Centre for Disease Control and prevention (CDC), Atlanta (*with permission*)

- Specimen should be collected properly and processed immediately (preferably bedside)
- Cultures should be incubated for 3–7 days or longer, followed by mounting of the culture to demonstrate the trophozoites
- If facilities are available, special container like **"InPouch TV"** can be used. It contains a specimen transport container, growth chamber for incubation and a slide for mounting
- Various culture medias can be used like:
  - Lash's cysteine hydrolysate serum media
  - Diamond's trypticase yeast maltose media
  - Cysteine peptone liver maltose media
  - Cell lines like McCoy cell line highly sensitive, can detect as low as three trophozoites/mL.

Antigen Detection in Vaginal Secretion

Antigen detection methods are more sensitive than microscopy, easy to perform and indicates recent infection.

- A rapid immunochromatographic test (ICT) (dipstick) is available which shows result within 10 minutes, requires no sophisticated instruments. Compared to culture, it is 83% sensitive and 99% specific
- ELISA using monoclonal antibodies has been developed; which shows sensitivity of 89% and specificity of 97%.

Antibody Detection

ELISA is available using whole cell antigen preparation and aqueous antigenic extract to detect antitrichomonial antibodies in serum and vaginal secretion of the patients.

However, antibodies persist for longer time, hence cannot differentiate between current infection and past infection. More over, its sensitivity is variable with variable antibody response.

Molecular Methods

- PCR detecting *T. vaginalis* specific beta tubulin genes are available with sensitivity and specificity comparable to culture

- PCR based ELISA format has been developed for urine samples (sensitivity 90% and specificity 93%)
- Recently, transcription-mediated amplification test has been developed for urine and genital specimens from men and women.

Other Supportive Tests

- **Raised vaginal pH (> 4.5):** It is not specific as the vaginal pH is also raised in bacterial vaginosis. However, in vaginal candidiasis, the pH is not raised
- **Positive whiff test:**
  - Fishy odor is accentuated when a drop of 10% KOH is added to vaginal discharge due to production of amine
  - It is positive in more than 75% of cases
  - It is also positive in bacterial vaginosis
- Excess of polymorphonuclear neutrophils on wet mount (seen in more than 75% of cases).

Treatment	Trichomonas vaginalis

Metronidazole or tinidazole

- Drug of choice, 2g, 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

Prevention

Trichomoniasis can be prevented by:

- Treatment of both the partners
- Safe sex practices like use of condoms
- Avoidance of sex with infected person
- **Vaccine:** There is no effective vaccine licensed so far. However, trials are going on targeting potential immunogenic antigens like 100 kDa protein.

OTHER INTESTINAL FLAGELLATES OF MINOR IMPORTANCE

Pentatrichomonas hominis

It is worldwide in distribution found both in warm and temperate climates.

- It is a harmless commensal present in large intestine
- Trophozoite is pyriform shaped, measures 5–15 μm long and 7–10 μm wide, similar to that of *T. vaginalis*, except that the undulating membrane is extended throughout the body.

Trichomonas tenax

T. tenax is a harmless commensal in the mouth (gum and tartar of the teeth).

- However, few cases of respiratory infection and thoracic abscesses are reported from Western Europe particularly in patients with cancer or other underlying lung disease
- The trophozoite is similar to *P. hominis* (undulating membrane is extended throughout the body); but it is smaller (5–12 μm long and 5–10 μm wide) and more slender
- Prevalence may vary from 0% to 0.25% depending on the oral hygiene.

Chilomastix mesnili

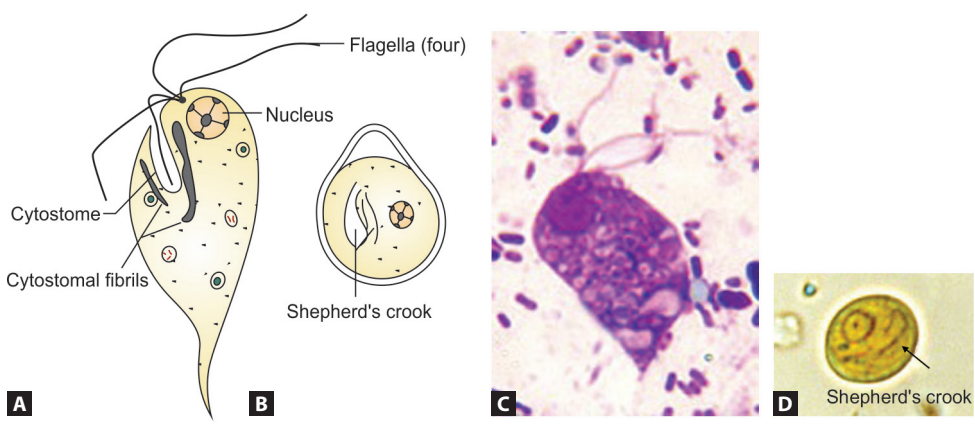
Chilomastix mesnili is a harmless commensal of cecum and colon in man.

- It is worldwide in distribution, found more frequently in warm climate
- It has two stages—trophozoites and cyst stages (Fig. 4.8):
  1. **Trophozoite:**
     - It is pear-shaped, measuring 10–15 μm in length and 4–6 μm in width
     - At the anterior end, there is a single nucleus and a distinct groove present near the nucleus called as cytostome
     - It has four flagella—three anterior and one in cytostome
     - It shows stiff, rotary movement
     - Cytostome is supported by two cytostomal fibrils right one is prominent and curved, left one is straight and less conspicuous (Figs 4.8A and C)
  2. **Cyst:**
     - It is the infective stage
     - It is lemon shaped with a narrow anterior end, surrounded by a cyst wall
     - It measures 6–10 μm in length and 4–6 μm in width
     - Bears a single nucleus; cytoplasm is densely granular, separated from the cyst wall at the anterior end
     - Remnant of the curved cytostomal fibrils can be seen, called as shepherd's crook (Figs 4.8B and D)
- Both the forms can be demonstrated by permanent staining of the stool samples
- Since it's a commensal so no treatment is required
- Prevention depends on improved personal hygiene.

Enteromonas hominis

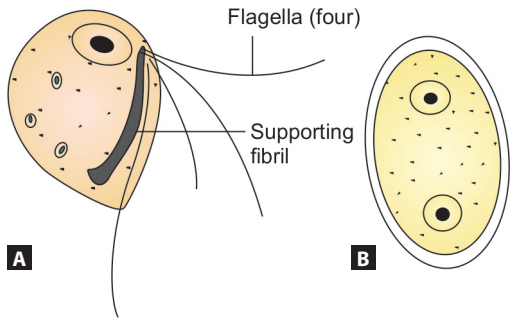
Enteromonas hominis is considered as a non-pathogenic commensal that is rarely encountered in man's in the large intestine (cecum).

- It is reported from both tropical (warm) and temperate (cold) climate
- It exists in two forms—(1) trophozoite and (2) cyst
  1. **Trophozoite:**
     - Oval to pear shaped, smaller in size, measuring 8–9 μm long and 5–6 μm wide
     - It possesses four flagella—three anterior and one recurrent. The recurrent flagellum extends free posteriorly and supported by a darkly stained fibril
     - It shows jerky forward movement
     - Nucleus is placed anteriorly and there is no cytosome
     - Cytoplasm is vacuolated and contains numerous bacteria (Fig. 4.9A)



Figs 4.8A to D: *Chilomastix mesnili* (A and B) trophozoite and cyst (schematic diagram); (C) trophozoite (Giemsa stain); (D) cyst (iodine stain)

Source: C- and D- Giovanni Swierczynski, Bruno Milanesi "Atlas of human intestinal protozoa Microscopic diagnosis" (*with permission*)



Figs 4.9A and B: *Enteromonas hominis* (schematic diagram) (A) trophozoite; (B) cyst

2. **Cyst:**
   - It is the infective stage
   - It is oval, measuring 6–8 μm long and 4–6 μm wide
   - Possesses one to four nuclei, binucleated being most common (two nuclei lie at opposite poles) (Fig. 4.9B)
   - It resembles like the cyst of *Endolimax nana*

- Infection is transmitted by ingestion of contaminated cyst
- Both the forms can be demonstrated by permanent staining of the stool samples
- Since it is a commensal, no treatment is required
- Prevention depends on improved personal hygiene.

Retortamonas intestinalis

Retortamonas intestinalis is a harmless commensal found less commonly in human's large intestine.

- It is reported from both tropical (warm) and temperate (cold) climate
- It exists in two forms—trophozoite and cyst

1. **Trophozoite:**
   - Elongated pyriform or oval shaped, smaller in size, measuring 6–7 μm long and 3-4 μm wide
   - It bears two flagella-one anterior and one posterior. It shows jerky movement
   - It has a cytostomal groove anteriorly with cystostomal fibrils and a single nucleus (Fig. 4.10A)

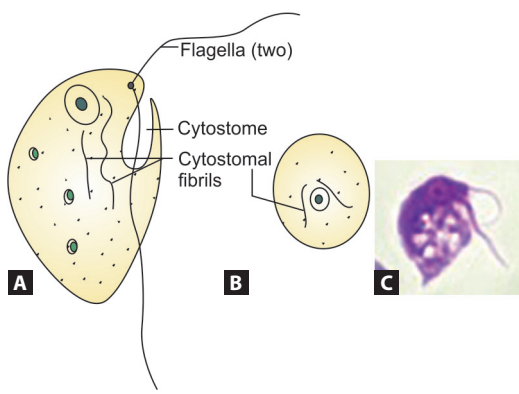
2. **Cyst:**
   - It is the infective stage
   - It is pear shaped, measuring 4–7 μm long and 5 μm wide
   - It resembles like the cyst of *Chilomastix* having singe nucleus and cytostome with supporting fibrils extends above nucleus (Fig. 4.10B)

- Infection is transmitted by ingestion of contaminated cyst
- Both the forms can be demonstrated by permanent staining of the stool samples
- Since it is a commensal, there is no therapy indicated
- Prevention depends on improved personal hygiene.

Dientamoeba fragilis

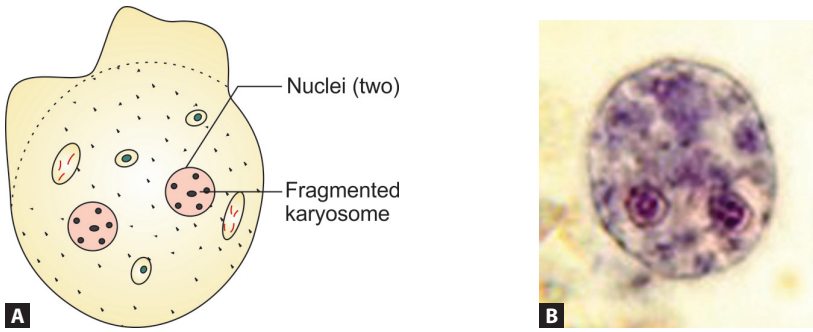
Dientamoeba fragilis is a common commensal that lives in the lumen of the cecum and upper colon of humans.

- It was initially thought to be an amoeba as it bears no external flagella but recently, by electron microscopic studies; it is reclassified as an amoeboflagellate as the



Figs 4.10A and B: *Retortamonas intestinalis* (A and B) trophozoite and cyst (schematic diagram); (C) trophozoite (Giemsa stain)

Source: C- Giovanni Swierczynski, Bruno Milanesi" Atlas of human intestinal protozoa Microscopic diagnosis" (*with permission*)



Figs 4.11A and B: Trophozoite *of Dientamoeba fragilis* (A) schematic diagram; (B) iron hematoxylin stain showing two nuclei with fragmented karyosome

Source: B- Giovanni Swierczynski, Bruno Milanesi "Atlas of human intestinal protozoa microscopic diagnosis" (*with permission*)

flagellum is internal. It closely resembles *Histomonas*, a parasite infecting turkeys.

- It is cosmopolitan in distribution with incidence rate varies from 1.4% to 19%.
- Higher incidence is reported in children.

Morphology and Life Cycle

Trophozoite is the only stage. Cyst stage is not been confirmed till date.

Trophozoite

- It is irregular in shape (amoeboid), relatively small, varying from 9 μm to 12 μm
- **Nucleus:** one to four in number (commonly two nuclei in 60–80% of cases, hence named as *Dientamoeba*) (Figs 4.11A and B)
- The nuclear chromatin is usually fragmented into three to five granules (hence named as **fragilis**), no peripheral chromatin on the nuclear membrane
- The cytoplasm is usually vacuolated and may contain ingested debris as well as some large uniform granules

The life cycle is not fully understood. Trophozoites are the infective forms; transmitted by feco-oral route. They multiply in the large intestine and excreted in feces.

Pathogenesis

This is controversial; the pathogenic status in not well defined.

- Some authors believe that there may be two distinct genotypes one of which may be pathogenic
- The organism has been reported in association with mucous diarrhea, abdominal pain and tenderness, nausea, vomiting, and low-grade fever.

Laboratory Diagnosis — Diantamoeba fragilis

- Stool examination
- Antigen detection in stool
- Antibody detection in serum—IFA

Laboratory Diagnosis

Stool examination

Trophozoite doesn't survive for longer time hence the fresh direct wet preparations should be examined immediately or stained by permanent stains.

- The recommended stains are Fields', giemra and iron hematoxylin stain
- Trophozoites are destroyed in a formol-ether concentration technique.

Antigen Detection in Stool

Both immunofluoresence and enzyme immunoassays are commercially available.

Antibody detection in serum

It can be detected by IFA technique.

Treatment	Diantamoeba fragilis
Tetracycline or metronidazole is effective. However, antimicrobial drugs are not preferred as its pathogenicity is not confirmed. Symptomatic treatment is sufficient.	

EXPECTED QUESTIONS

I. Write short notes on:
(a) Giardiasis
(b) Trichomoniasis
(c) *Dientamoeba fragilis*
(d) *Chilomastix mesnili*

II. Multiple choice questions (MCQs):

1. How many pairs of flagella are present in the trophozoite of *Giardia lamblia*?
(a) One
(b) Two
(c) Four
(d) Eight

2. *Giardia lamblia* resides in:
(a) Sigmoid colon
(b) Colon
(c) Duodenum
(d) Vagina

3. Which is the most common cause of steatorrhea:
(a) *Ascaris lumbricoides*
(b) *Giardia lamblia*
(c) *Entamoeba histolytica*
(d) *Enteromonas hominis*

4. Trophozoites of which of the following bear two nuclei with fragmented karyosome?
(a) *Dientamoeba fragilis*
(b) *Chilomastix mesnili*
(c) *Retortamonas intestinalis*
(d) *Enteromonas hominis*

5. Diagnosis of which of the following parasite uses Entero-Test?
(a) *Cyclospora* species
(b) *Entamoeba histolytica*
(c) *Giardia lamblia*
(d) *Dientamoeba fragilis*

6. Which of the following parasite doesn't have a cyst stage?
(a) *Enteromonas hominis*
(b) *Dientamoeba fragilis*
(c) *Giardia lamblia*
(d) *Chilomastix mesnili*

Answers

1. (c) 2. (c) 3. (b) 4. (a) 5. (c) 6. (b)

5 Flagellates—II (Hemoflagellates)

Chapter Outline

- Introduction
- Morphology of hemoflagellates
- Leishmania
  - Old world leishmaniasis
  - New world leishmaniasis
- Trypanosoma
  - Trypanosoma cruzi
  - Tryponasoma brucei complex
- Expected questions

INTRODUCTION

Hemoflagellates are the flagellated protozoa that are found in peripheral blood circulation. They complete their life cycle in two hosts, i.e. vertebrate host and insect vector; therefore, called as **digenetic** or **heteroxenous parasites.** Hemoflagellates of medical importance belongs to:

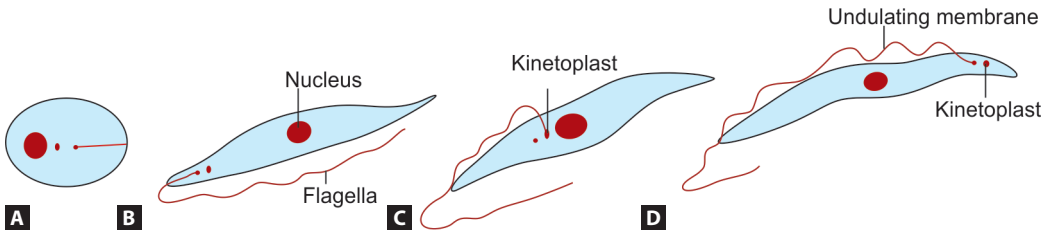
- Phylum: Euglenozoa
- Class: Kinetoplastea
- Order: Trypanosomatida
- Family: Trypanosomatidae
- Genera: *Leishmania* and *Trypanosoma.*

MORPHOLOGY OF HEMOFLAGELLATES

Hemoflagellates have an oval to elongated body, nucleus, and a single flagellum arising from kinetoplast.

- **Kinetoplast:** It consists of blepharoplast and parabasal body connected by a delicate fibril (cytoskeleton). It lies tangentially or at right angle to the nucleus. It represents multiple copies of mitochondrial DNA
- **Axoneme (or axostyle):** It extends from blepharoplast to the cell wall. It represents the intracellular portion (root) of flagellum
- Based upon arrangement of flagellum, they exist in four morphological stages—(1) amastigote, (2) promastigote, (3) epimastigote and (4) trypomastigote. Names are ended with a suffix **"mastigote"** (Greek word Mastix means whip) (Fig. 5.1)

1. **Amastigote form:** Round to oval, lacks flagellum, found in reticuloendothelial cells of man infected with *Leishmania* and *Trypanohsoma cruzi*
2. **Promastigote form:** Lanceolate shaped; kinetoplast is anterior to nucleus (antenuclear kinetoplast). Flagellum arises from the anterior end. It is found in the mid gut of insect vector. This is the infective stage of *Leishmania* to man
3. **Epimastigote form:** Elongated, kinetoplast is placed close to the nucleus (juxtanuclear kinetoplast). Flagellum arises from the



Figs 5.1A to D: Various morphological forms of flagellates (schematic diagrams) (A) amastigote; (B) promastigote; (C) epimastigote; (D) trypomastigote

lateral side and traverses the body as a short undulating membrane and comes out from the anterior end. This form is seen for *Trypanosoma* in insect vector

4. **Trypomastigote form:** Elongated and spindle shaped with central nucleus. Kinetoplast lies near the posterior end. Flagellum arises posteriorly and runs as long undulating membrane. It is the infective stage of *Trypanosoma* found in insect vector and peripheral blood of humans.

LEISHMANIA

Leishmaniasis is caused by the obligatory intracellular protozoa of the genus *Leishmania*. Primarily it affects the reticulo-endothelial system of the host.

- *Leishmania* species produce widely varying group of clinical syndromes ranging from self-healing cutaneous ulcers to fatal visceral disease
- Leishmaniasis is mainly a zoonotic disease affecting dogs, foxes, jackals and rodents. Animal reservoir plays a major role for transmission; except in Indian subcontinent where it is anthropophilic affecting only humans
- The parasite is transmitted by bite of the female sandfly vector (Fig. 16.5).

Classification of Leishmaniasis

Leishmania has two subgenera *L. Leishmania* and *L. Viannia*.

- The main difference between the two subgenera is that promastigotes of the subgenus *Viannia* develop in the midgut and hindgut of sandfly where as that of subgenus *Leishmania* develop in the anterior portion of the alimentary tract of sandfly
- Both of the subgenera comprise of nearly 20 species. (Table 5.1)
  - **Old world leishmaniasis:** Affects Asia, Africa and Europe and transmitted by sandfly (Genus *Phlebotomus*)
  - **New World Leishmaniasis:** Affects Central and South America and transmitted by sandfly (Genus *Lutzomyia*)
- Clinical syndromes of leishmaniasis include:
  - Visceral leishmaniasis (VL)
  - Post–kala-azar dermal leishmaniasis (PKDL)
  - Cutaneous leishmaniasis (CL)
  - Diffuse cutaneous leishmaniasis (DCL)
  - Leishmaniasis recidivans (LR)
  - Mucocutaneous leishmaniasis (MCL)

OLD WORLD LEISHMANIASIS

Leishmania donovani

History

Leishmania donovani causes VL or kala azar (a hindi term meaning "black fever")

- It was named after two scientists who discovered the parasite in the same year 1903
  1. Sir William Boog Leishman in London observed the amastigotes form of the

Table 5.1: Classification of *Leishmania*

Species	*Geographical distribution*	*Clinical syndrome*	*Vector (sandfly)*	*Reservoir*	*Transmission*
Old World Leishmaniasis					
Leishmania Leishmania (L. L.) donovani Complex					
L. L. donovani	South Asia (Indian subcontinent)	VL (Kala-azar) PKDL	*Phlebotomus argentipes*	Humans	Anthroponotic
	Sudan, Ethiopia, Kenya and Uganda	VL, PKDL	*P. orientalis, P. martini*	Humans/ rodents	Anthroponotic/ Zoonotic
	Middle East, Africa and China	VL	*P. perniciosus*		Zoonotic
L. L. infantum	Mediterranean, Middle East, Central Asia and China	VL, CL	*P. perniciosus*	Dogs, foxes, jackals, etc	Zoonotic
L. L. tropica Complex					
L. L. tropica (Delhi boil)	Western India, North Africa, Mediterranean littoral, Middle East	CL, LR	*P. sergenti*	Humans	Anthroponotic
L. L. aethiopica	Ethiopia, Uganda, and Kenya	CL, DCL	*P. longipes*	Hyraxes	Zoonotic
L. L major	Middle East, India, China Africa, central and western Asia	CL	*P. papatasi*	Rodents	Zoonotic
New World Leishmaniasis					
L. L. chagasi (new world variant of *L.L. infantum*)	Central and South America	*VL, CL*	*Lutzomyia* spp.	Dogs, foxes, etc	Zoonotic
L. L. Mexicana Complex	Central America and northern parts of South America	CL, DCL	*Lutzomyia* spp.	Forest rodents	Zoonotic
L. Viannia braziliensis Complex	South and Central America	CL, MCL	*Lutzomyia* spp.	Forest rodents	Zoonotic

Abbreviations: VL, Visceral leishmaniasis; PKDL, post-kala-azar dermal leishmaniasis; CL, cutaneous leishmaniasis; LR, leishmaniasis recidivans DCL, diffuse cutaneous leishmaniasis; MCL, mucocutaneous leishmaniasis

parasite in the liver of a British soldier died at Dumdum, Kolkata. (Hence also known as Dum-Dum fever)

2. Sir Donovan who found the amastigotes in the splenic smear from a patient from Chennai

- Charles Nicolle, a 1928 Nobel laureate, at the Pasteur Institute of Tunis, characterized the new world VL and cultivated the etiologic agent.

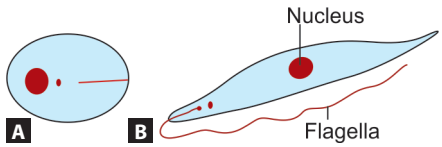
Morphology

Leishmania occurs in two forms:

Amastigote form

It is an obligate intracellular form and the infective stage to vector, sandfly.

- Found in reticuloendothelial cells like macrophages, neutrophils, endothelial cells of liver, spleen, bone marrow, etc. of



Figs 5.2A and B: *Leishmania* species (schematic diagram) (A) amastigote form; (B) promastigote form

the vertebrate hosts like humans, dogs and rodents

- Round to oval, 3-5 μm in size
- **Nucleus***:* It measures less than 1 μm, oval to round, located in center or side of the cell
- **Kinetoplast:** Consists of copies of mitochondrial DNA. It is made up blepharoplast and parabasal body connected by a delicate fibril (cytoskeleton). It lies at right angle to the nucleus
- **Axoneme:** It extends from blepharoplast to the cell wall. It represents the intracellular portion (root) of flagellum
- There is no external flagellum and it is nonmotile
- **Vacuole:** It is a clear space, lies adjacent to axoneme (Fig. 5.2A).

Promastigote form

This is an extracellular form, infective stage to humans.

- It is mainly found in sandfly and in culture
- It is motile and contains single anterior flagellum
- Pear shaped, 8–15 μm length
- Nucleus is situated centrally and kinetoplast is placed near the anterior end transversely
- **Axoneme:** Represents the intracellular portion of flagellum (Fig. 5.2B).

Life Cycle (Fig. 5.3)

Host: *Leishmania* completes its life cycle in two hosts:

1. **Vertebrate host** (man, dog, rodents, etc.)
2. **Insect vector (female sandfly):** *Phlebotomus argentipes*

Infective form: Promastigote forms present in the midgut (majority) or foregut (small proportion) of female sandfly

Mode of transmission: By bite of an infected sandfly mainly during the late evening or the night time. Minimum 10–1,000 promastigotes per infective bite are required to initiate the infection

In vertebrate hosts, including humans:

- Promastigotes are regurgitated from the midgut rarely or directly discharged from foregut (proboscis) of the female sandfly into the skin of the vertebrate host
- Promastigotes are phagocytosed by the skin macrophages and transform into amastigote forms within 12–24 hours
- The amastigote forms inside the macrophages multiply further causing cell rupture and release into the circulation
- Amastigotes are carried out in the circulation to various organs like liver, spleen and bone marrow and invade the reticuloendothelial cells like macrophages, endothelial cells, etc.

In sandfly:

- During the blood meal taken up by the sandfly, the amastigotes are ingested and transformed into promastigote forms in the insect midgut
- Promastigotes multiply by longitudinal fission and pass through various stages such as:
  - **Amastigote → procyclic promastigote → nectomonad promastigote → haptomonad promastigote → leptomonad promastigote → metacyclic promastigote**
  - The metacyclic promastigotes multiply in the midgut of vector by binary fission and a small proportion migrates to the foregut (proboscis). They infect a new host during another blood meal
- The duration of the life cycle in sandfly varies from 4 to 18 days depending on the species.

Pathogenicity

Various factors contribute to the pathogenesis such as:

- The phagocytosis of the promastigotes is facilitated by binding of the promastigote surface antigens such as 63 kDa glycoprotein (gp-63)

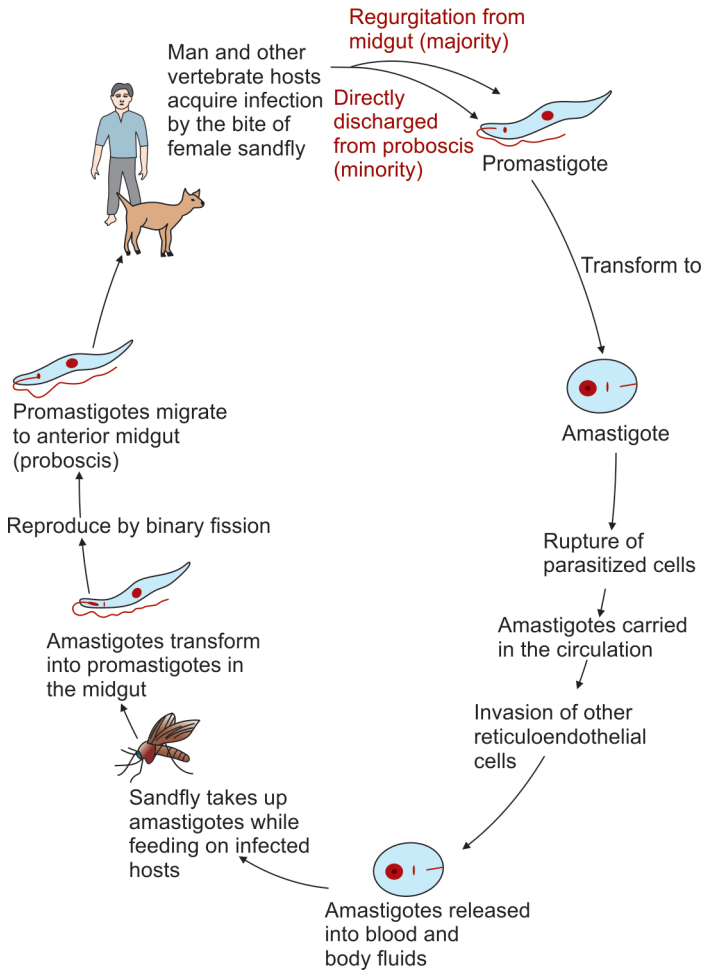


Fig. 5.3: Life cycle of *Leishmania donovani*

and lipophosphoglycan (LPG) to complement receptors (CR3 and Cq1) on macrophages.

- The gp-63 antigen also gives protection from proteolytic enzymes secreted from the phagolysosome
- LPG is the principle virulence factor, exhibits variety of functions. It prevents phagosome maturation and protects the parasite against hydrolytic enzymes secreted from the phagolysosome
- The amastigotes multiply within acidic parasitophorous vacuoles
- **Glycosyl phosphatidyl inositols (GPIs)** is a major surface protein on amastigotes, helps in protecting from phagolysosomal attack inside the macrophage.

Host Immune Response

Depending on the host immune response, the amastigotes are either killed or allowed to multiply inside the macrophages.

- Like leprosy, the immunology of leishmaniasis is complex and bipolar. It has two extreme poles, each which is characterized by one of the two type of T helper subset responses, i.e. T helper 1 or T helper 2 responses.

T helper 1 response

Th-1 response is induced by interleukin -12 (IL-12) which leads to increase production of interferon γ (IFN-γ) and IL-2.

- At the cellular level, IFN-γ activates macrophages which in turn kill amastigotes by induction of nitric oxide synthase and oxidative killing mechanisms
- Th1 response is observed in:
  - The majority of individuals who mount a successful immune response and control the infection
  - Cutaneous leishmaniasis
  - Patients after recovering/treated for VL
  - Leishmaniasis recidivans
- These individuals exhibit a delayed-type hypersensitivity (DTH) to leishmanial antigens (positive leishmanin skin test).

T helper 2 response

Stimulation of Th-2 cells results in increase production of IL-10 and IL-4.

- It is observed in patients developing active VL and in diffuse CL
- IL-10 inhibits macrophages to kill amastigote by downregulating the production of (TNF-α)and nitric oxide. That helps in enhanced survival and growth of the parasite
- Patients do not show positive leishmanin skin test
- The parasite uses the macrophage much like a Trojan horse
- Amastigotes are released periodically by rupture of the macrophages
- They disseminate through the regional lymphatics and the vascular system to infect the reticuloendothelial cells of various organs
- This results in remarkable enlargement of the spleen, liver and bone marrow dysfunction.

Clinical Features

Visceral leishmaniasis

Visceral leishmaniasis is mainly caused by the *L. donovani* and sometimes by *L. infantum,* (designated as *L. chagasi* in the New World) together known as *L. donovani* complex. (Table 5.2).

Table 5.2: Various forms of visceral leishmaniasis

Characters		***Old World VL***		***New World VL***
	Indian VL (kala-azar)	***Infantile VL***	***African VL***	***Mediterranean VL***
Agent	*Leishmania donovani*	*L. infantum*	*L. donovani*	*L. chagasi*
Vector	*Phlebotomus argentipes*	*P. perniciosus*	*P. orientalis, P. martini*	*Lutzomyia longipalpis*
Epidemiology	India	Middle East, Central Asia, China and Mediterranean basin	Sudan, Ethiopia, Kenya and Uganda	Central and South America
Age affected	Young adults	Infants and children < 5 years of age	Adults	Children
Reservoir	Anthroponotic (human)	Zoonotic (canine)	Anthroponotic, Rarely- Zoonotic (rodents)	Zoonotic (canine)
PKDL	Common	Less common	Common	Less common
Lymphnode invovlement	Less common	More common, Aggravated by poor nutrition	Less common	Less common

Abbreviations: VL, visceral leishamaniasis; PKDL, post-kala-azar dermal leishmaniasis

- Incubation period ranges from 2–6 months.
- The hallmark of VL is a triad of fever, hepatosplenomegaly and pancytopenia
- **Fever:** The most common symptom of VL is an abrupt onset of moderate to high grade fever associated with rigor and chills. Typically it is described as double rise of fever in 24 hours
- **Splenomegaly:** It is the most consistent sign. The spleen may become hugely enlarged and palpable below the umbilicus. (Fig. 5.4A)
- Hepatomegaly (usually moderate in degree) soon follows splenomegaly
- **Lymphadenopathy:** Common in most of the African endemic regions (rare in Indian subcontinent)
- **Hyperpigmentation:** Mostly seen in brown-skinned individuals from Indian subcontinent
- **Pedal edema and ascites:** Occur due to hypoalbuminemia, may be seen in advanced illness
- Mucosal lesions in mouth and nasopharynx-Seen in Sudan, rare in India
- **Hematological abnormalities** (bone marrow dysfunction):
  - Anemia (normocytic and normochromic): Appears early and may become severe enough to cause congestive heart failure
  - Leucopenia
  - Thrombocytopenia: Can lead to epistaxis, retinal hemorrhages, and gastrointestinal bleeding
  - Hypergammaglobulinemia (due to polyclonal B cell activation)
- **Leishmanoma:** Nodular skin lesions seen in African cases only
- Weight loss (cachexia)
- **Secondary infections:** Such as measles, pneumonia, tuberculosis, bacillary or amoebic dysentery and gastroenteritis are common.

Post-kala azar dermal leishmaniasis

It is a nonulcerative lesion of skin occurs in 2–50% of patients of VL following the completion of treatment.

- Mainly seen in India and East African countries (Table 5.3)
- It develops as hypopigmented macule (most common feature) near mouth which later on spreads to face and then to arms and trunk (extensor surfaces) and finally becomes nodules resembling leprosy (Figs 5.4B to D)
- Ocular lesions like conjunctivitis and



Figs 5.4A to D: Real images showing clinical features (A) splenomegaly seen in visceral leishmaniasis; (B) hypopigmented skin changes in early PKDL; (C and D) extensive facial nodular lesions in late PKDL

Source: World Health Organization "Manual on visceral leishmaniasis control" Slide1/Desjeux; Slide 4 and 5/ El Hassan; Slide 6/ Bryceson (*with permission*)

Table 5.3: Post-kala azar dermal leishmaniasis from Indian Subcontinent and East Africa

Feature	*Indian subcontinent*	*East Africa*
Most affected country	Bangladesh followed by India	Sudan
Incidence among patients with VL	2–20%	~50% (rare in other part of Africa)
Interval between VL and PKDL	2–10 years	Can occur during VL to 6 months
Age affected	Any age	Mainly children
History of prior kala-azar	Not necessarily	Yes
PKDL persists for	Long period (20 years)	Few months
Treatment with antimonial	2–4 months	2 months
Course	Resolve slowly (noncompliance)	Spontaneous cure usually occurs

Abbreviations: VL, visceral leishamaniasis; PKDL, post-kala-azar dermal leishmaniasis

uveitis are associated in some patients.
- Sometimes, PKDL occurs in subclinical patients without a history of VL.
- **The diagnosis is based on:**
  - Amastigote can be detected in the skin in more than 80% of cases in the Sudan. It is more easily detected from nodular lesions than other lesions
  - Serological tests: Direct agglutination test (DAT) and antibodies to rK39 antigen are positive in most of the cases.

Treatment	PKDL
• Extended course of antimonial is given for a period of 2–4 months • They respond poor treatment and often serve as reservoir of infection during inter-endemic cycles.	

Leishmaniasis with HIV co-infection

Co-infection of HIV with VL has been reported from more than 35 countries.
- Mainly it is reported from Southern Europe (France, Italy, Spain and Portugal) where 50–75% of adult cases of VL are HIV positive and 7–17% of HIV infected people with fever have amastigotes
- Also reported from other places like sub-Saharan African and Indian subcontinent
- In India, it is reported from Bihar, sub-Himalayan region and other North Indian states. Various studies reported the co-infection prevalence around 2–6%
- Both HIV and *Leishmania* affect each other's pathogenesis

  Effect on HIV:
  - *Leishmania* appears to cause activation of latent HIV
  - It expresses high level of chemokine receptor (CCR5)receptors on macrophages

  Effect on *Leishmania*:
  - HIV causes activation of T helper 2 cells response leading to disease progression
  - *Leishmania* uptake is enhanced by the HIV infected macrophages
  - Associated with more relapses
- HIV co-infected patients don't show the classic signs of VL like hepatosplenomegaly
- But they present with atypical features due to loss of immunity with presence of more gastrointestinal tract (GIT)and pulmonary symptoms
- CD4 T cell count often fall below 50/μL (almost always < 200/μL)
- There is consideration to include leishmaniasis in Center for Disease Control and Prevention (CDC) clinical category C for the definition of AIDS as an opportunistic pathogen
- **Diagnosis:** Serodiagnostic tests are usually negative. Amastigote are demonstrated

from unusual sites such as bronchoalveolar lavage fluid and buffy coat region of blood

Treatment	HIV/VL coinfection
Liposomal amphotericin B is the drug of choice for HIV/VL co-infection. But response is poor with frequent relapses.	

Epidemiology

Leishmaniasis occurs in 98 countries; most of them developing countries of tropical and temperate regions.

- More than 350 million people are at risk, with an overall prevalence of 12 million
- Two million cases occur annually, of which 1–1.5 million are CL (and its variations) and 500,000 are VL
- Four largest foci of VL (90%): India and neighboring countries like Nepal, Bangladesh, Sudan and Brazil
- In Indian subcontinents, VL is anthroponotic, while zoonotic VL is reported from Middle East, Pakistan and other countries from Western Asia to China.
- **India:** India is the worst affected country. Bihar is affected the most followed by Jharkhand, West Bengal and Uttar Pradesh. 48 districts with more than 165 millions of people are at risk. In 2012, more than 20,000 cases are reported from India with 23 deaths.

Laboratory Diagnosis — Leishmania donovani

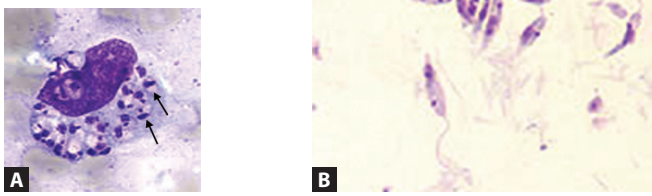
- Microscopy (detects LD bodies)
  - Splenic aspiration: Most sensitive
  - Bone marrow aspiration: Most commonly preferred
  - Lymph node aspirates (in African patients)
  - Liver biopsy
  - Peripheral blood smear(in HIV infected people)
  - Biopsy of various organs (in HIV infected people)
- Culture (detects promastigotes)
  - NNN medium
  - Schneider's liquid medium
- Antidbody detection in serum
  - CFT using WKK antigen of tubercle bacilli
  - ELISA
  - Direct agglutination test
  - ICT using rk39 antigen
- Nonspecific tests to detect hypergammaglobulinemia
  - Napier's aldehyde test
  - Chopra antimony test
- Molecular method—PCR
- Leishmanin test (montenegro test)
- Animal inoculation—golden hamster
- Pancytopenia.

Laboratory Diagnosis

Microscopy

Demonstration of amastigotes inside the macrophages (also known as **Leishman Donovan bodies** or **LD bodies**) is the gold standard method for the diagnosis of VL (Fig. 5.5A). Smears should be stained with Leishman, Giemsa or Wright stains. The various samples include:

- **Splenic aspiration:** The sensitivity of splenic smear examination is excellent (> 95%) but splenic puncture is associated with risk of hemorrhage. Grading of LD bodies from splenic smear is useful in determining the parasitic load and monitoring the response to treatment
- **Bone marrow aspiration:** Iliac crest aspirate is the most commonly preferred sample though the sensitivity is around 60–85%. If bone marrow findings are negative but the clinical suspicion is strong, then the splenic aspiration is indicated
- **Lymph node aspiration:** It is useful only in African cases of kala-azar and sensitivity is low (50%)
- **Liver biopsy:** Less sensitive and carries the risk of hemorrhage
- **Peripheral blood smear:** Amastigotes within mononuclear cells and neutrophils can be seen in a stained blood smear.



Figs 5.5A and B: (A) Amastigote form [arrows shows inside a macrophage (Giemsa stain)]; (B) smear shows promastigote form (Giemsa stain) from culture
Source: DPDx Image Library, Centre for Disease Control and prevention (CDC), Atlanta (*with permission*)



Fig. 5.6: NNN medium
Source: World Health Organization, "Manual on visceral leishmaniasis control" (Slide22/Alvar) (*with permission*)

Sensitivity increases by making thick smears, using centrifuged blood and making smears from buffy coat particularly in HIV patients

- **Biopsy specimens of various organs:** Like oropharynx, stomach, or intestine. This is particularly important in patients with AIDS.

Culture

- **Sample:** Aspirations from spleen, bone marrow or other tissue and also buffy coat.
- **Medium:**
  - **NNN medium** (described by Novy, McNeal–1903 and Nicolle-1908): Novy-MacNeal-Nicolle (NNN) medium is a biphasic medium, composed of two parts salt agar and one part defibrinated rabbit blood (Fig. 5.6)
  - ***Schneider's Liquid medium:*** It contains Schneider's *Drosophila* insect medium supplemented with 30% fetal calf serum. It is found to be more sensitive than NNN media
  - Semisynthetic fetal calf serum free medium
  - Microculture method using microcapillary tubes
- Inoculated specimens are incubated at ambient temperature (22–26°C) upto 4 weeks
- Amastigotes transform into promastigotes in the culture fluid which are detected by staining with Giemsa stain (Fig. 5.5B)
- Culture fluid is examined for twice a week for first 2 weeks and once a week thereafter for up to 4 weeks; before they are reported as negative
- Culture is found to be positive in 75% of cases.

Antibody detection in serum

In general, the serological tests are sensitive, but less specific. False-positive results may occur due to cross-reacting antibodies in patients with leprosy, Chagas' disease, CL, and other infections. Antibodies cannot differentiate current and past infection. More so, antibodies may be absent or present in low titer in patients with AIDS

- **Complement fixation test (CFT):** Cross reacting antibodies are detected in patient's blood by using nonspecific antigens like human tubercle bacilli antigens called as WKK antigen (as described by Witebsky, Klingenstein, Kuhn). It is not in use nowadays
- Enzyme-linked immunosorbent assay **(ELISA)** and indirect fluorescent antibody **(IFA)** test, (Fig. 5.7A) are the newer tests found to be more sensitive and have replaced CFT
- **Direct agglutination test:** Patient serum is incubated with extract of axenic amastigote antigens in a microtitre plate. Mat formation indicates a positive result where as a negative result is reported when button is formed. It is found to be 100% sensitive and specific. It is simple, rapid and doesn't need any instrument. However, antibodies persist up to 5 years after the treatment. (Fig. 5.7B)
- **Immunochromatographic test***:* Immunochromatographic test (ICT) detects leishmanial antibody by using *L. infantum* recombinant kinesin antigen (rk39). It claims 98% sensitivity and 90% specificity, however, the sensitivity is low in East Africa and in HIV patients. Like DAT, it is also simple, rapid and doesn't need any instrument (useful in field studies).

Nonspecific tests to detect hypergammaglobulinemia

- **Napier's Aldehyde test:** Patient's serum is added with a drop of 40% formalin in a test tube. Positive test is indicated by jellification of the serum forming milk white opacity like that of white of a boiled egg within 20 minutes

  Disadvantages include:
  - It is negative in the first three months
  - False positive results are seen with *Schistosoma japonicum, Trypanosoma cruzi,* multiple myeloma and cirrhosis
  - It is negative in CL cases
- **Chopra's antimony test:** Positive test is indicated by formation of profuse flocculation when patient's serum is mixed with 4% urea stibamine solution.

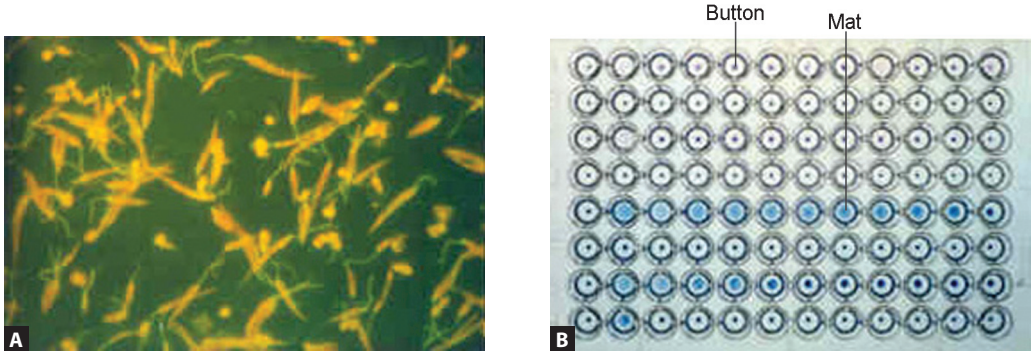
Molecular methods

Qualitative detection by polymerase chain reaction (PCR) and quantitative detection by real-time PCR are available targeting *Leishmania* specific kinetoplast (mitochondrial) DNA. It is mostly confined to the reference laboratories with sensitivity varying from 70% to 93%.

Leishmanin test (Montenegro test)

Introduced by Sir Montenegro in South America.

- It is a delayed hypersensitivity skin test to



Figs 5.7A and B: (A) Indirect fluorescent antibody test; (B) direct agglutination test

Source: "Manual on visceral leishmaniasis control", World Health Organization A- Slide31/Evans, B- (Slide32/Alvar) (*with permission*)

a suspension of killed *L. donovani* promastigote injected intradermally
- A positive test is indicated by induration of more than or equal to 5 mm in 72 hours
- Positive test indicates prior exposure to *Leishmania* antigens
- It is positive in people with good cell-mediated immunity (CMI):
  - Asymptomatic individuals: It is used for epidemiological survey to estimate the burden of the disease
  - Cutaneous leishmaniasis
  - 6-8 weeks after recovery from VL
  - Leishmaniasis recidivans
- However, this test is negative in [when CMI is low]:
  - Active visceral leishmaniasis
  - Diffuse cutaneous leishmaniasis.

Animal inoculation

Intranasal inoculation of specimens to golden hamsters yields amastigotes after several months. It is not in use nowadays.

Nonspecific tests

- Complete blood count—to detect pancytopenia
- Elevated liver enzymes
- Reversal of albumin globulin ratio (reflects hypergammaglobulinemia)

Leishmania tropica Complex

It includes three species—*L. tropica, L. aethiopica* and *L. major.* They cause old world Cutaneous Leishmaniasis (Table 5.4).
- *L. tropica* is reported from Western India (mainly Rajasthan), Middle East and Mediterranean coast. It mainly affects urban area hence known as agent of urban anthroponotic CL
- *L. aethiopica* infects people from Ethiopia, Uganda and Kenya
- *L. major* is reported from Middle East, India, China, Africa, and central and western Asia. It mainly affects rural area hence known as agent of rural zoonotic CL.

Life Cycle

The life cycle of the *L. tropica* complex is same as *L. donovani* except:
- The species of vector sandfly are different-
  - *L. tropica*—vector is *P. sergenti*
  - *L. aethiopica*—vector is *P. longipes*
  - *L. major*—vector is *P. papatasi*
- Reservoir of infection:
  - *L. tropica*—is man (anthroponotic)
  - *L. aethiopica*—is *Hyraxes* (Zoonotic)
  - *L. major*—is rodents (zoonotic)
- In humans, the amastigote forms reside in reticuloendothelial cells of skin (they do not migrate to viscera).

Clinical Features

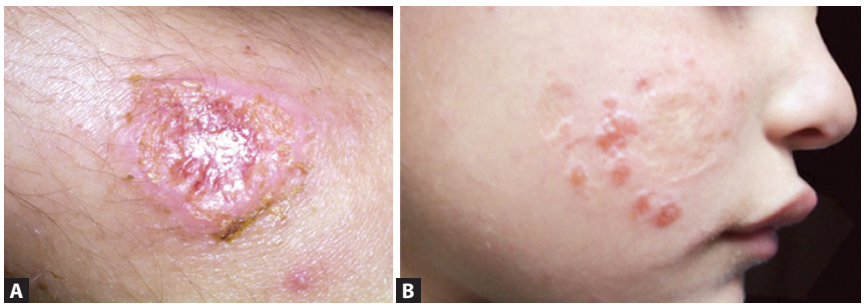
Cutaneous leishmaniasis

It is caused by *L. tropica* complex. This condition is also known as **"Oriental sore"**,

Table 5.4: Various agents of cutaneous leishmaniasis

Species	*Geographical distribution*	*Clinical syndrome*	*Vector (Sandfly)*	*Reservoir*	*Transmission*
Leismania Leishamania tropica (Oriental sore)	Western India, North Africa, and Middle East	CL, LR	*Phelobotomus sergenti*	Humans	Anthroponotic
L. L. aethiopica	Ethiopia, Uganda, and Kenya	CL, DCL	*P. longipes*	Hyraxes	Zoonotic
L. L. major	Middle East, India, China Africa, Central and Western Asia	CL	*P. papatasi*	Rodents	Zoonotic

Abbreviations: CL, cutaneous leishmaniasis; LR, leishmania recidivans; DCL, diffuse cutaneous leishmaniasis



Figs 5.8A and B: Real images showing clinical features of (A) cutaneous leishmaniasis; (B) leishmaniasis recidivans

Source: A- World Health Organization, "Manual on visceral leishmaniasis control" (*with permission*); B- Global Skin Atlas/Image Number 2268/Nameer Al-Sudany (*with permission*)

Delhi Boil, Aleppo Boil and Baghdad Button, etc (Fig. 5.8A).

- Oriental sore usually occurs on face and hands
- It begins as papule, becomes nodular and finally it ulcerates
- The margins of the ulcers are raised, painless and indurated
- Lesions may be single or multiple and vary in size from 0.5 cm to more than 3 cm
- Mostly, it heals spontaneously leaving behind a scar
- There may be satellite lesions, especially in *L. major* and *L.tropica* infections.

Leishmaniasis recidivans

It is a granulomatous response occurs years after healing of primary sore due to *L. tropica*.

- Characterized by new lesions formed on the face, usually scaly, erythematous papules and nodules develop in the center or periphery of a previously healed sore
- CMI is intact and skin test is positive
- Very few parasites can be demonstrated in the smears from the lesions (Fig. 5.8B).

Diffuse cutaneous leishmaniasis

It is a rare form of leishmaniasis, caused by *L. amazonensis* and *L. mexicana* in South and Central America (New World) and by *L. aethiopica* in Ethiopia and Kenya (old World).

- Characterized by the lack of a CMI response to the parasite
- Low CMI leads to widespread cutaneous disease—symmetric or asymmetric distribution of various lesions like papules, nodules, plaques, and areas of diffuse infiltration, non ulcerative lesions with heavy load of parasites
- The delayed type hypersenstivity (DTH) response is negative, so skin test, i.e. Montenegro test is negative.

Laboratory Diagnosis — Leishmania tropica complex

- Microscopy—detects amastigotes
- Culture—NNN medium
- Montenegro test—positive except in diffuse CL

Laboratory Diagnosis

Microscopy

Amastigotes can be demonstrated from indurated edge of the lesions or biopsy from the margin of the ulcer.

Culture

Aspiration from the ulcers can be cultured in NNN medium for the isolation of promastigote forms.

Montenegro test

Positive leishmanin skin test indicates delayed hypersensitivity reaction to the parasite. However, it is negative in diffuse CL.

Treatment Old World Leishmaniasis

Supportive therapy

Correction of pancytopenia should be done by blood transfusion. Similarly, other associated conditions should be managed promptly.

Specific antileishmanial drugs

Pentavalent antimonial

- It is the drug of choice in most endemic regions of the world, except in Bihar (due to emergence of drug resistance)
- Two pentavalent antimonial (SbV) preparations are available:
  - Sodium stibogluconate (100 mg of SbV/mL)
  - Meglumine antimoniate (85 mg of SbV/mL)
- **WHO recommendations 1995**
  - For VL, the daily dose is 20 mg/kg by rapid intra venous (IV) infusion or intramuscular (IM) injection, and therapy continues for 28–30 days till smear microscopy is negative
  - For CL, 1–3 ml of antimonial preparations should be infiltrated at the base of the lesions for two to three times at interval of 1–2days
- **Resistance to antimonials**
  - Increased resistance has been reported to *L. tropica, L. major* and *L. mexicana* in comparison to *L. donovani*
  - ***L. donovani*:** Resistance is only reported from North Bihar. Mishandling of antileishmanial drugs is the single most important contributor to the development of drug resistance
  - **Mechanisms:** Occurs due to failure of reduction of SbV (prodrug) to its active form SbIII inside the resistant *L. donovani* amastigotes.

Amphotericin B

- It is currently used as a first-line drug in Bihar for the treatment of VL. In other parts of the world, it is used when initial antimonial treatment fails
- It is also the drug of choice for the New world mucocutaneous leishmaniasis (MCL)
- Conventional amphotericin B deoxycholate is administered in doses of 0.75–1.0 mg/kg on alternate days for a total of 15 infusions
- Alternatively the lipid formulations of AmB are used which have lower side effects

Paromomycin

It is an aminoglycoside antibiotic with antileishmanial activity. It is given IM at a dose of 11 mg of base/kg daily for 21 days

Miltefosine

It is the first oral compound approved for the treatment of leishmaniasis. It is given as daily dose of 50 mg once or twice for 28 days.

Prevention

Vaccine Trials

Currently no vaccine is available for the prevention of leishmaniasis. However, several trials are going on.

- Both killed and live-attenuated vaccine trials are on going targeting antigens derived from killed promastigotes
- Trials for recombinant and synthetic vaccines are also on going using gp-63 antigen.

Control Measures

Vector control measures to eradicate sandfly:

- Personal prophylaxis by using insect repellents or bed nets
- Control of canine or rodent reservoir
- *Phlebotomus* doesn't fly high above the ground level and it is nocturnal in habitat. So, sleeping at top floors also can prevent transmission
- Early treatment of all cases (mainly anthroponotic VL and PKDL cases).

NEW WORLD LEISHMANIASIS

It is mainly caused by:

- *Leishmania Viannia* (*L.V.*) *braziliensis* complex

- *Leishmania Leishmania* (*L.L.*) *mexicana* complex
- *L.L. chagasi* (new world variant of *L.L. infantum*)

The main difference between the two subgenera is promastigotes of the subgenus *Viannia* develop in the midgut and hindgut of sandfly where as that of subgenus *Leishmania* develop in the anterior portion of the alimentary tract of sandfly.

The morphology and life cycle of new world *Leishmania* species are identical to that of *L. donovani* except:

- Geographical distribution-restricted to central and south America
- **Vector:** *Lutzomyia* species
- **Reservoir of infection:** Dogs, foxes (zoonotic)
- The amastigote forms in humans reside in reticuloendothelial cells of skin and mucus membrane (don't invade viscera).

Clinical Features of New World Leishmaniasis

Leishmania Mexicana Complex

L. mexicana complex infected people develop CL similar to those seen with old world cutaneous disease (Table 5.5).

- *L. mexicana* causes a specific form of CL called as **chiclero ulcer** (or bay sore) characterized by persistent ulcerations in pinna seen in Central America among workers living in forests harvesting chicle plants to collect chewing gum latex. 30% of people are infected during the first year of exposure (Fig. 5.9)
- *L. mexicana* and *L. amazonensis* produce DCL similar to that is described earlier for *L. aethiopica.*

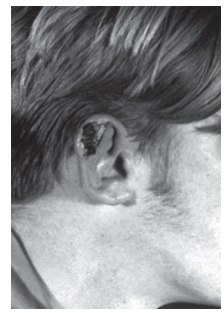


Fig. 5.9: Real image showing chiclero ulcer in pinna

Source: Public Health Image Library, ID#15062/David O. Madorsky: Capt. Loren Quigg, Centre for Disease Control and Prevention (CDC), Atlanta (*with permission*)

Leishmania Viannia braziliensis Complex

They cause MCL and also CL similar to oriental sore but they are more severe (Table 5.6).

Espundia (*mucocutaneous leishmaniasis*)

L. braziliensis infects mucous membrane of the nose, oral cavity, pharynx or larynx months to years after the CL.

- It is seen in 1–3% of patients infected with *L. braziliensis,* more in males of age 10–30 years

Table 5.5: *Leishmania Leishmania mexicana* complex

Species	*Geographical distribution*	*Clinical syndrome*	*Vector*	*Reservoir*	*Transmission*
Leishmania Leishmania mexicana	Central America and Northern parts of South America	CL (chiclero ulcer), DCL,MCL	*Lutzomyia* spp.	Forest rodents Marsupial and Humans	Zoonotic
L. L. amazonensis		CL, DCL and MCL			
L. L. venezuelensis		CL			
L. L. pifanoi		CL, DCL			
L. L. garnhami		CL			

Abbreviations: CL, cutaneous leishmaniasis; DCL, diffuse cutaneous leishmaniasis; MCL, mucocutaneous leishmaniasis



Fig. 5.10: Real image showing mucocutaneous leishmaniasis or espundia
Source: Calvopina et al. BMC Infectious Diseases 2006 (*with permission*)

- The initial symptoms are often nasal stuffiness, erythema and mucopurulent discharge.
- It may eventually involve the upper lip, buccal, pharyngeal, or laryngeal mucosa (Fig. 5.10)
- Ulcerative lesions are formed with erosion of the soft tissue and the cartilages leading to loss of lips, soft part of nose and soft palate
- Gradually, the nasal septum may be destroyed, resulting in nasal collapse with hypertrophy of upper lip and nose leading to development of **"tapir nose"**

Forest yaws and uta: The cutaneous lesions of *L. V. guyanensis* and *L. V. peruviana* are known as forest yaws (pain bois) and uta respectively.

Leishmania Leishmania chagasi

L.L. chagasi is the new world variant of *L. L. infantum.*

- Causes mediterranean VL and CL
- Occurs in Central and South American region
- It is zoonotic (canine reservoir)
- Vector: *Lutzomyia* species
- Age: Children are affected commonly

Laboratory Diagnosis New World leishmaniasis

- Microscopy—detects amastigotes
- Culture—NNN medium
- Montenegro test—Positive (except in DCL and active VL)
- Antibody detection—Poorly sensitive in DCL and MCL

Laboratory Diagnosis of New World Leishmaniasis

Microscopy

Amastigote forms within the macrophages are found abundant in the lesions of DCL followed by CL and VL when stained with Giemsa or Leishman stain. However, in lesions of MCL fewer parasites are found.

Culture

Skin and mucosal biopsy specimens are first minced to release the organisms and then

Table 5.6: *Leishmania Viannia braziliensis* complex

Species	*Geographical distribution*	*Clinical syndrome*	*Vector*	*Reservoir*	*Transmission*
Leishmaina Viannia braziliensis	Brazil	CL and MCL (espundia)	*Lutzomyia* spp.	Dogs, foxes, forest rodents and humans	Zoonotic
L. V. panamensis	Panama and Colombia	CL and MCL			
L. V. guyanensis	Guyana	CL (forest yaws) and MCL			
L. V. peruviana	Peru	CL (Uta) and MCL			

Abbreviations: CL, cutaneous leishmaniasis; MCL, mucocutaneous leishmaniasis

inoculated in NNN media and Schneider's *Drosophila* medium.

Montenegro Test

Positive Leishmanin skin test indicates delayed hypersensitivity reaction to the parasites. However, it is negative in diffuse CL and active VL (*L. chagasi*).

Antibody Detection

Antibodies are detected in patients with active VL by various formats described before. However, they show variable sensitivity in CL and poor response in MCL and DCL.

Treatment	New world leishmaniasis

- In contrast to Old World CL, systemic therapy is recommended for New World CL as the lesions are more chronic, multiple and shows tendency for mucosal involvement
- Pentavalent antimonial is the drug of choice, administered as a dose of 20 mg/kg for 30 days
- In case of relapse, liposomal amphotericin B (2–3 mg/kg for 20 days) or miltefosine (2.5 mg/kg for 28 days) are given.

TRYPANOSOMA

Trypanosomes are hemoflagellates that reside in peripheral blood and tissues of their host. They can be classified as:

Human Trypanosomes

They show strict geographical distribution:

- ***Trypanosoma cruzi:*** It is the causative agent of South American trypanosomiasis (also called as **Chagas' disease**) in man and transmitted by insect vector reduviid bug
- ***Trypanosoma brucei:*** It causes African trypanosomiasis, transmitted by tsetse fly. It has three important subspecies out of which only two of them infect humans
  - ***Trypanosma brucei rhodesiense:*** It is the causative agent of East African sleeping sickness
  - ***Trypanosma brucei gambiense:*** It is the causative agent of West African sleeping sickness
- ***Trypanosoma rangeli:*** It is a nonpathogenic species that rarely infects humans in South America.

Animal Trypanosomes

- ***Trypanosoma brucei brucei:*** It causes **"nagana"**, a disease affecting cattle in Africa
- *T. congolense* and *T.vivax* cause disease similar to that of *T. brucei brucei*
- ***Trypanosma evansi:*** It causes **"Surra"** in horses and other animals. It is transmitted by flies (tabanidae and stomoxys). Many animal cases are reported in India
- ***Trypanosma lewisi:*** It causes a harmless infection affecting rodents
- ***Trypanosma equiperdum:*** It causes **"Stallion's disease"** in horses. It is transmitted by sexual route (not by insect vector).

TRYPANOSOMA CRUZI

It is the causative agent of South American trypanosomiasis or Chagas' disease.

- It was first discovered by Brazilian scientist **Carlos Chagas,** isolated from reduviid bug (triatomine bugs) and blood of infected monkeys. Later on he found it causing human infection also
- Hence the condition is named as Chagas' disease. He named the parasite as *T. cruzi* after his guide Oswaldo Cruz.

Habitat

In humans, *T. cruzi* exists in two forms: (1) amastigote and (2) trypomastigote form.

- Amastigotes are intracellular parasite found in reticuloendothelial cells of spleen, liver, lymph node, bone marrow, and myocardium. They are also found in cells of epidermis and striated muscles
- Trypomastigotes are extracellular and found in peripheral blood.

Epidemiology

Chagas' disease is mainly restricted to South and Central American countries like Brazil, Argentina, Venezuela, etc.

- Currently, it is estimated that 8 million people are chronically infected with *T. cruzi* and 14,000 deaths occur due to the illness every year
- It is a zoonotic disease, having many animal reservoirs like dogs, cats, opossums and rodents.

Morphology

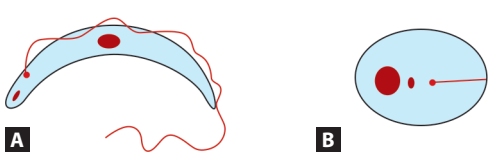
- In vertebrate host, it exists mainly in two forms—(1) trypomastigote form and (2) amastigote form
- In insect vector (reduviid bug), it exists as all four forms, i.e. (1) trypomastigote, (2) amastigote, (3) promastigote and (4) epimastigote form.

Trypomastigote Form

- It is spindle shaped; measures around 20 μm and appears as C or U shaped
- It is seen in the peripheral blood of the infected patients in two forms—(1) long slender form and (2) short stubby form
- It consists of a central nucleus and large kinetoplast situated posteriorly from which flagellum originates and traverses the whole body as undulating membrane and comes out from the anterior end as free flagellum
- It doesn't multiply (Fig. 5.11 A).

Amastigote Form

- It is found inside cells of striated muscle (skeletal and cardiac), nervous tissue and reticuloendothelial cells



Figs 5.11A and B: *Trypanosoma cruzi* (schematic diagram) (A) trypomastigote form (B) amastigote form

- When fully developed, a large number of amastigote may be found in a cyst like cavity
- This is indistinguishable from those found in *Leishmania* infection
- It is round to oval, 26 μm in size having a large nucleus, rod shaped kinetoplast and axoneme but no flagella
- It is the multiplying form of the parasite (Fig. 5.11 B).

Life Cycle (Fig. 5.12)

Host: *T. cruzi* passes its life cycle in two hosts—(1) humans and (2) vector reduviid bugs or kissing bugs or triatomine bugs (*Triatoma infestans, Rhodnius prolixus* and *Panstrongylus megistus*) (Fig. 5.13).

Infective form: Metacyclic trypomastigote form is the infective forms, found in feces of reduviid bugs.

Mode of transmission: Reduviid bugs are nocturnal in habitat and humans get infection when abraded skin, mucous membranes, or conjunctivae become contaminated with reduvid bug's feces containing infective form of the parasite.

T. cruzi can also be transmitted by the blood transfusion, organ transplantation from mother to fetus or very rarely by ingestion of contaminated food or drink, and most importantly by laboratory accidents.

Development in Man

The parasite invades the reticuloendothelial cells and other tissues like muscle (cardiac, skeletal and GIT muscles) and nervous tissue and transforms into amastigote form.

- In these tissues, the amastigotes multiply by binary fission forming a cyst like mass of growth known as pseudocyst
- Many amastigotes within the pseudocyst are transformed into motile C shaped nonmultiplying trypomastigote forms.
- On rupture of the pseudocyst, the trypomastogotes are liberated to blood. They are of two types:

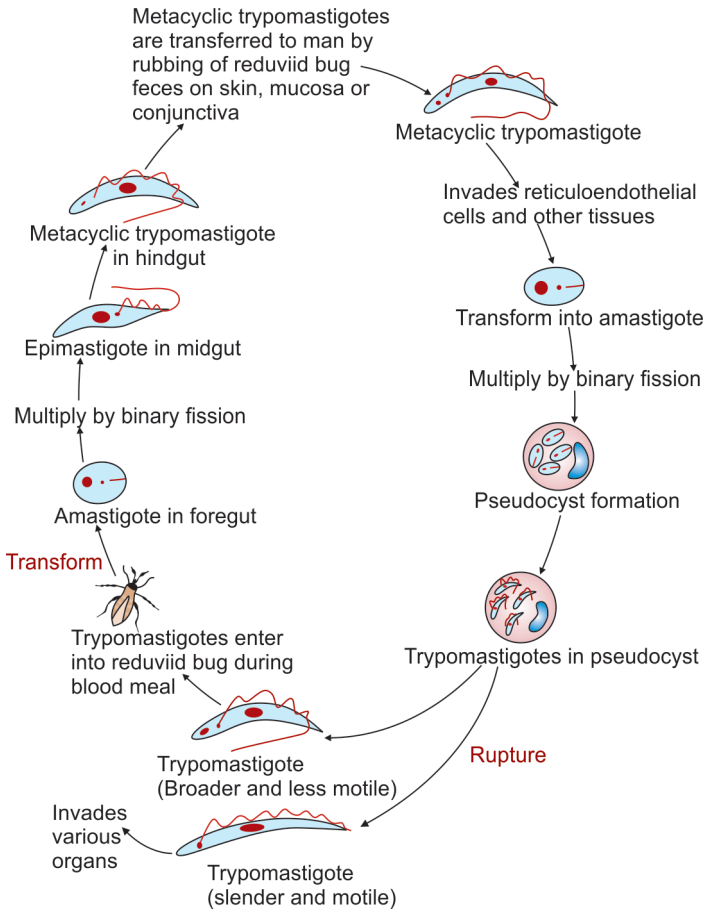


Fig. 5.12: Life cycle of *Trypanosoma cruzi*



Fig. 5.13: Reduviid bug (real image)

Source: DPDx Image Library, Centre for Disease Control and prevention (CDC), Atlanta (*with permission*)

- **Slender highly motile forms:** Have an elongated nucleus, sub terminal kinetoplast and a short free flagellum. They are the invasive forms, eventually migrate to many organs, penetrate the cells and continue the life cycle
- **Broader less motile forms:** Have an oval nucleus, terminal kinetoplast and a long free flagellum. They persist in the blood to be taken up by the insect vector during a blood meal.

Development in Reduviid Bugs

Broader less motile trypomastigote forms are transmitted to reduviid bugs during the blood meal.

- They transform into amastigote forms in the foregut
- In the midgut, the amastigote forms multiply by binary fission and divide to form epimastigote forms
- They finally transform into metacyclic

trypomastigote forms in the hindgut and are excreted in the bug's feces
- The insect cycle takes about 10–15 days (extrinsic incubation period). There is no transovarian transmission seen in the bugs and once infected, they retain the infection throughout the life by the molting cycles.

Pathogenesis and Clinical Feature

Average incubation period is around 1 week. *T. cruzi* causes American trypanosomiasis (also called as Chagas' disease) which can be acute or chronic type.

Acute Chagas' Disease

It is characterized by:
- **Chagoma:** An erythematous subcutaneous nodule is formed at the site of deposition of bug's feces. It is painful, commonly occurs on face and may take 2–3months to resolve
- **Romana's sign:** When the parasites enter through conjunctiva, there occurs an unilateral painless edema of the eye lid and conjunctivitis (Fig. 5.14)
- Generalized lymphadenopathy and hepatosplenomegaly may also appear
- Severe myocarditis and neurologic signs like meningoencephalitis occur occasionally, especially in children
- Usually within 4–8 weeks, patient either recovers spontaneously or develops chronic *T. cruzi* infection.



Fig. 5.14: Real image showing romana's sign in the eyelid

Source: Public Health-image library, ID # 2617/ Dr. Mac Melvin, Centre for Disease Control and Prevention (CDC), Atlanta (*with permission*)

Chronic Chagas' Disease

Chronic Chagas' disease manifests years or even decades after the initial infection. It occurs due to multiplication of the parasites in the muscles (skeletal, cardiac and GIT) and nervous tissue.

Autoimmune hypothesis: Though not fully proved, an autoimmune mechanism has been suggested. *T. cruzi* antigens cross react with mammalian antigens (molecular mimicry) and many autoreactive antibodies have been detected in infected patients
- **Asymptomatic (indeterminate form):** Most frequent form, typically observed in the beginning of the chronic phase. Many individuals live in this stage throughout the life
- **Cardiac form:** Occurs in 30% of the patients. Patient develops dilated cardiomyopathy, rhythm disturbances like right bundle-branch block, and thromboembolism
- **Gastrointestinal form:** Involvement of muscles of GIT leads to megaesophagus (manifested as dysphagia, chest pain, and regurgitation) and megacolon (manifested as abdominal pain and chronic constipation)
- **Pulmonary form:** Repeated episodes of aspiration pneumonitis are common (especially during sleep) in patients with severe esophageal dysfunction
- Mixed forms are observed in 10% of the patients.

Congenital Trypanosomiasis

Rarely, *T. cruzi* can be transmitted transplacentally both in acute and chronic stage of the disease. It is manifested as low birth weight, still birth, rarely myocarditis and neurological alterations.

In HIV Infected People

HIV infected people are at a greater risk of reactivation of underlying *T. cruzi* infection and are more prone to develop meningoencephalitis.

Immune Response

Both cell-mediated and humoral immunity are involved against the parasite. IgM antibodies appear early during acute infection. Then the class switch over occurs and IgG and IgA antibodies predominate in the chronic stage of the disease. Antigenic variation which is the characteristic feature of African trypanosomiasis, is rarely observed in *T. cruzi*. CMI is mainly involved in tissue destruction in the chronic stage such as cardiomyopathy and megacolon.

Laboratory Diagnosis Trypanosoma cruzi

- Peripheral blood microscopy by wet mount, thick or thin smear—detects trypomastigotes
- Culture—NNN medium or Yager's liver infusion tryptose medium
- Antibody detection in serum—ELISA, IFA, CFT, RIPA
- Antigen detection from serum, urine—by CLIA
- Molecular methods—PCR
- Animal inoculation—Mice
- Xenodiagnosis—nymph of reduviid bugs

Laboratory Diagnosis

Peripheral Blood Microscopy

In acute Chagas' disease, the trypomastigotes (Fig. 5.15A) are frequently found in peripheral blood which can be detected by:

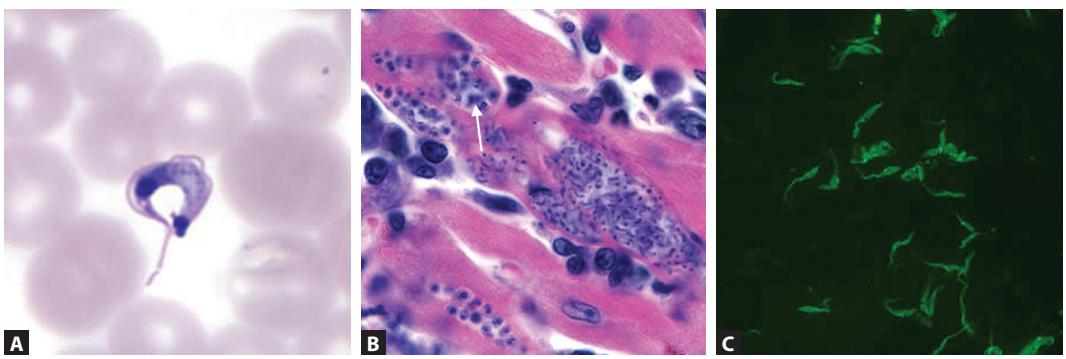
- Wet mount preparation of anticoagulated blood or buffy coat can be done to see the rapid movements of trypomastigotes
- **Thick and thin smear:** (Stained by Giemsa stain) thick smear is more sensitive in detecting the parasite whereas the thin smear helps in differentiating *T. cruzi* with morphologically similar looking *T. rangeli* (Table 5.7)
- Blood concentration techniques like micro-hematocrit method and Strout method of buffy coat preparation may be employed if the parasite count is low
- Amastigotes can be demonstrated in heart tissue obtained at autopsy stained by histopathological stain (Fig. 5.15B).

Culture

Blood is inoculated in NNN medium or Yager's liver infusion tryptose medium, incubated at 25°C and observed for the epimastigote forms for up to 30 days before they are considered negative. Culture is more sensitive than smear microscopy.

Antibody Detection

Chronic Chagas' disease is diagnosed by the detection of specific IgG antibodies against *T. cruzi* antigens. IgM antibodies are diagnostic for congenital infection.



Figs 5.15A to C: *Trypanosoma cruzi* (A) trypomastigote form (thin blood smear stained with Giemsa) (B) amastigote forms in heart tissue stained by hematoxylin and eosin (C) indirect fluorescent antibody test showing trypomastigote forms

Source: DPDx Image Library, Centre for Disease Control and prevention (CDC), Atlanta (*with permission*)

Table 5.7: Differences between trypomastigote form of *Trypanosoma cruzi* and *Trypanosoma rangeli*

	Trypanosoma cruzi	*Trypanosoma rangeli*
Pathogenicity	Pathogen, causes Chagas' disease	Nonpathogenic
Size of trypomastigote	Average 20 μm	Average 30 μm
Shape	Often 'C' shaped	Rarely 'C' shaped
Kinetoplast	Large and terminal	Small and sub-terminal
Posterior end	Short blunt	Long pointed
Location in reduviid bug	• Hindgut (feces) • Transmitted by rubbing of reduvid bug's feces on abraded skin	• Usually found in salivary gland • Transmitted by bite of bugs

- Several methods are employed like CFT (Guerreiro Machado test), ELISA, IFA (Fig. 5.15C), IHA (indirect hemagglutination test) and Western blot
- However, false positive reactions may occur in patients with *T. rangeli* infection, leishmaniasis, syphilis, etc
- Radioimmunoprecipitation assay (Chagas' RIPA) is a highly sensitive and specific (confirmatory) method for detecting antibodies to *T. cruzi.*

Antigen Detection

T. cruzi specific antigens from serum and urine of the infected patients are detected which are very useful for diagnosing acute infection and congenital transmission. Recently, a chemiluminescence immunoassay (CLIA) has been developed for blood bank screening and for the monitoring the response to treatment.

Molecular Methods

PCR is available that detects *T. cruzi* specific kinetoplast or nuclear DNA in blood. It is more sensitive than microscopy and serology for the diagnosis of chronic disease. It can detect as low as one trypomastigote per 20 mL of blood. It is also useful in monitoring the response to treatment and for the diagnosis of congenital infection.

Animal Inoculation

Blood or CSF of the patients is inoculated intraperitoneally into mice. Trypomastigotes can be demonstrated from the blood of mice within 10 days of inoculation.

Xenodiagnosis

The infected patients are exposed to 20 numbers of laboratory maintained nymphs of reduviid bugs daily for 3 days and the dropping of the insects are examined monthly for 3 months for the presence of the epimastigote forms. This is more sensitive to detect light chronic infection.

Treatment Trypanosoma cruzi

- Therapy for Chagas' disease is still unsatisfactory. Only two drugs—(1) nifurtimox and (2) benznidazole have been available
- **In acute disease:**
  - Benznidazole is considered as the drug of choice in Latin America. The recommended oral dosage is 5 mg/kg per day for adults and 5–10 mg/kg per day for children for 60 days
  - Nifurtimox is given 8–10 mg/kg for adults and 15–20 mg/kg for children in four divided doses for 90–120 days
- **In chronic disease:** These drugs lack efficacy and may cause many side effects.

Prophylaxis

Prevention of the disease in endemic countries depends on control of vector. This includes residual insecticides, health education and housing improvement.

TRYPONASOMA BRUCEI COMPLEX

T. brucei was first demonstrated by Sir Bruce in 1895 from horses suffering from "nagana". Forde in 1902 had demonstrated the parasite in man whereas Kleine had demonstrated the parasite in the vector tsetse fly. The name "*Trypanosoma*" was coined by Dutton in 1902. *T. brucei* complex consists of three subspecies:

1. ***T. brucei gambiense:*** Agent of West African sleeping sickness
2. ***T. brucei rhodesiense:*** Agent of East African sleeping sickness
3. ***T. brucei brucei:*** Causes "nagana", a disease affecting cattle in Africa. It doesn't infect humans.

Life Cycle (Fig. 5.16)

Host: *T. brucei* passes its life cycle in two hosts.

1. The **vertebrate host** is man and other animals
2. **Invertebrate host** is the **tsetse fly** (genus *Glossina)* (Fig. 16.6.) Both male and female flies bite man and serve as vectors. *Glossina palpalis* group serves as the vector for *T. brucei gambiense* whereas *Glossina morsitans* group is the vector for *T. brucei rhodesiense*.

Infective form: The metacyclic trypomastigote forms are found in salivary gland of tsetse fly

Mode of transmission: By the bite of tsetse fly, trypomastigote forms are transmitted to the punctured wound from the saliva of the tsetse fly (Fig. 5.16).

Development in Man

- At the site of inoculation, they transform into long slender trypomastigote forms which multiply by binary fission
- They transform into an intermediate stage and then into nondividing short stumpy form without free flagellum
- Subsequently the parasites invade the blood

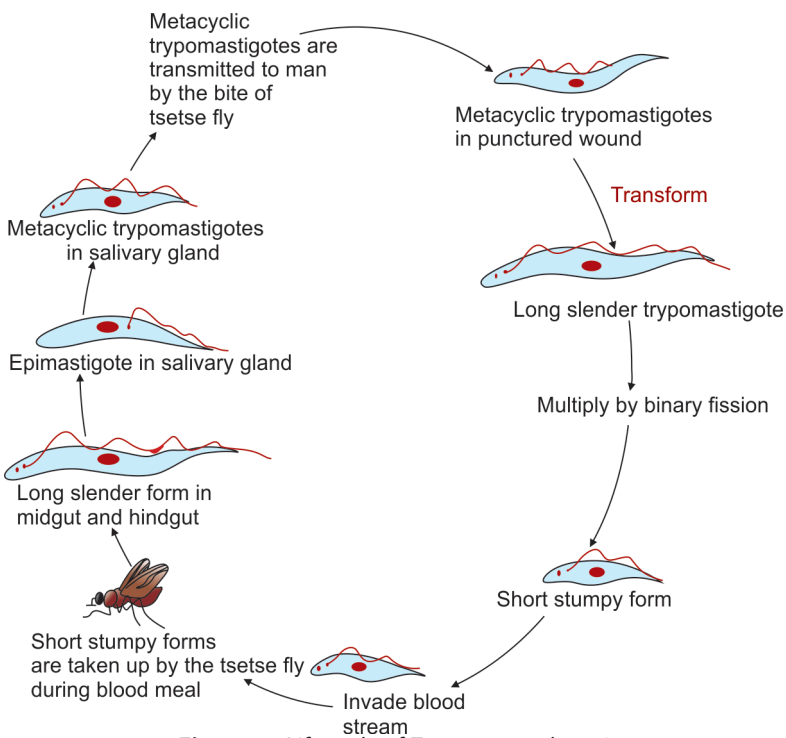


Fig. 5.16: Life cycle of *Trypanosoma brucei*

stream resulting in parasitemia and migrate to various organs including CNS
- The short stumpy forms are the infective form to the tsetse fly, hence the transformation of long slender trypomastigotes into short stumpy forms is critical for the transmission of the parasite.

Development in Tsetse Fly

- The short stumpy trypomastigote forms are taken up by the tsetse fly along with the blood meal
- They become long slender forms in the midgut and hindgut of the insect where they multiply and finally reach the salivary gland
- They attach to the epithelial cells of salivary ducts and transform into broad epimastigote forms
- Finally, the epimastigotes develop into metacyclic trypomastigote forms which are the infective forms to man
- It takes around 3 weeks from the time of blood meal till the fly becomes infective (extrinsic incubation period), then the fly remains infected throughout the life.

Antigenic Variation

Trypomastigotes undergo periodic antigenic variation leading to frequent change of antigenic nature of variable surface glycoprotein (VSG) antigens present on their surface. This serves as the key mechanism of evading host immune response.

- Genome of *Tryponasoma brucei* contains thousands of genes that undergo gene switching (like mutations, deletions, additions or recombinations) leading to formation of new **variant antigenic types (VATs)**.
- Every 5–10 days, a new wave of genes evolves, that code for a new batch of VATs. Host immunity is strain specific hence, is not able to eliminate the new waves of parasitemia.

Pathogenesis and Clinical Feature

In general, *T. brucei gambiense* develops a chronic course with slow progression whereas *T. brucei rhodesiense* runs an acute course with rapid progression and early death (Table 5.8).

Table 5.8: Comparison between *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense*

	Trypanosoma brucei gambiense	***Trypanosoma brucei rhodesiense***
Disease	West African sleeping sickness	East African sleeping sickness
Vectors	Tsetse flies (*Glossina palpalis* group)	Tsetse flies (*Glossina morsitans* group)
Primary reservoir	Humans	Animals (Antelope and cattle)
Human illness	Chronic central nervous system (CNS) disease	Acute (early CNS disease) up to 9 months
Duration of illness	Months to years	< 9 months (before that the death occurs)
Lymphadenopathy	Frequent, cervical lymphadenopathy (winter bottom sign)	Minimal (Axially And inguinal)
Parasitemia	Low	High
Virulence	Less	More
Rodent inoculation	Not useful	Diagnostic
Epidemiology	Rural populations	Workers in wild areas, rural populations, and tourists in game parks
Respond to drugs	Less resistant	More resistant

Trypanosomal Chancre

A self-limited inflammatory lesion may appear a week after the bite of an infected tsetse fly.

Stage I Disease

It is characterized by a systemic febrile illness that occurs due to dissemination of the parasite through the lymphatics and bloodstream.

- Lymphadenopathy is prominent in West African trypanosomiasis. The posterior cervical nodes are commonly involved and become soft, rubbery and nontender called as **winterbottom's sign**
- Pruritus, maculopapular rashes and transient edema are common
- Delayed sensation to pain is noted (Kerandel's sign)
- Hepatosplenomegaly may be seen in few cases
- Hematologic manifestations include moderate leukocytosis, thrombocytopenia, anemia and production high levels of polyclonal IgM.

Stage II Disease (Sleeping Sickness)

It involves invasion of the CNS. The presence of trypanosomes in perivascular areas of CNS is accompanied by intense infiltration of mononuclear cells.

- Patient develops characteristic progressive daytime somnolence (hence called as "**sleeping sickness**"), with restlessness and insomnia at night
- Other features include listless gaze, loss of spontaneity, and abnormal speech with few extrapyramidal signs like choreiform movements, tremors and fasciculations
- CSF findings include increased pressure, elevated total protein and pleocytosis, with frequently demonstration of trypanosomes.

Laboratory Diagnosis — Trypanosoma brucei

- Direct microscopy—detects trypomastigotes
  - Serial blood sample examination
  - CSF examination
  - Lymphonode aspirate
- Antibodies from serum and CSF—card agglutination test, ELISA, IFA
- Antigen from serum and CSF—ELISA
- Molecular method—PCR
- Culture—inoculated into KIVI
- Animal inoculation in mice

Laboratory Diagnosis

Direct Microscopy

- **Specimen:** Useful samples are multiple blood samples (due to periodic release of trypomastigotes in blood), chancre fluid, CSF, lymphnode aspirate and bone marrow aspirate.

Serial blood sample examination

- **Wet mounting:** It is done to demonstrate highly motile trypomastigotes
- **Thin and thick films:** Smear is fixed and stained with Giemsa stain to visualize the trypomastigote forms. Detection limit of thick smear is 1parasite/200 high power field and that of thin smear is 2000 parasite/mL (or 100/mL following centrifugation) (Fig. 5.17)
- If the parasitemia is low, then blood concentration methods are followed such as: **Microhematocrit centrifugation** using QBC capillary tube coated with Acridine orange. It claims sensitivity of 55–90%

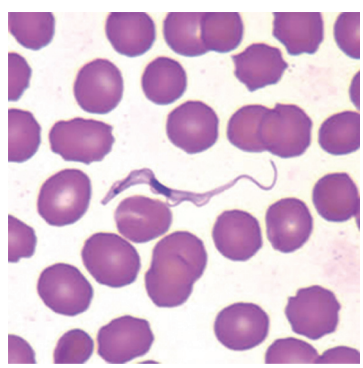


Fig. 5.17: Trypomastigote form in peripheral blood smear examination (Giemsa stain)

Source: DPDx Image Library, Centre for Disease Control and prevention (CDC), Atlanta (*with permission*)

- **Mini anion exchange centrifugation:** Involves separation of trypomastogotes from blood using anion exchange chromatography followed by low speed centrifugation. It can detect as low as less than 100 organisms/mL.

Cerebrospinal fluid examination

Centrifuged deposit of the CSF is examined for the presence of **Mott cells** (also called as Morula or Mulberry cells). Mott cells are abnormal plasma cells containing large eosinophilic inclusions of IgM immunoglobulins. Other CSF findings include increased protein level, lymphocytosis, increased pressure and occasional presence of trypomastigotes.

Lymphnode aspirate

It is useful for *T. brucei gambiense* and shows variable sensitivity of 40–80%.

Antibodies from Serum and CSF

- Card agglutination test for trypanosomes (CATT) for *T. brucei gambiense* has been developed for field use and mass screening. This is highly sensitive (96%) but less specific
- Recently, newer methods like ELISA (using VSG antigen) and indirect fluorescent antibody (IFA) test are available to aid in the diagnosis but they have variable sensitivity and specificity
- Detection of serum and CSF IgM is of great diagnostic value.

Antigens from Serum and CSF

Antigen detection by ELISA is useful for clinical staging of disease to determine CNS infection and for monitoring the response to treatment (antigens are rapidly cleared following improvement).

Molecular Methods

PCR assays have been developed which show a detection limit of 5 parasites/mL. Other molecular methods available are branched DNA assay, real time PCR, LAMP (loop mediated amplification technique) and FISH (fluorescent in situ hybridization).

Culture

Samples can be inoculated into KIVI (kit for *in vitro* isolation) and trypomastigotes are recovered in 7–10 days. However, culture is not routinely performed and only used as a standard for the evaluation of other diagnostic tests.

Animal Inoculation in Mice

It is highly sensitive for the isolation of *T. brucei rhodesiense* but not useful for *T. brucei gambiense.*

Treatment	Trypanosoma brucei complex
The drugs used for treatment of African sleeping sickness are suramin and pentamidine. Alternate drugs are eflornithine, and the organic arsenical melarsoprol. Treatment is based on type of disease (West or East African) and presence or absence of CNS invasion (Table 5.9).	

Prophylaxis

Vector control strategies like destruction of the insect's habitats, elimination of reservoir

Table 5.9: Treatment of african trypanosomiasis

Causative organism	*Stage I (normal cerebrospinal fluid)*	*Stage II (abnormal cerebrospinal fluid)*
Trypanosoma brucei gambiense (West African)	Pentamidine Alternative: Suramin	Eflornithine Alternative: Melarsoprol
Trypanosoma brucei rhodesiense (East African)	Suramin	Melarsoprol

sources, etc can be done to control African trypanosomiasis. Vaccines are not available as the parasite undergoes frequent antigenic variations.

EXPECTED QUESTIONS

I. Write essay on:
- (a) Classify hemoflagellates. Describe the life cycle, pathogenesis and laboratory diagnosis of *Leishmania donovani*?
- (b) Describe the life cycle, clinical feature and laboratory diagnosis of *Trypanosoma cruzi*?

II. Write short notes on:
- (a) Post kala azar dermal leishmaniasis
- (b) Cutaneous leishmaniasis
- (c) African sleeping sickness
- (d) Diffuse cutaneous leishmaniasis (DCL)
- (e) New world Leishmaniasis
- (f) Chagas' disease

III. Differentiate between:
- (a) West African sleeping sickness and East African sleeping sickness
- (b) Indian and East African PKDL
- (c) *Trypanosoma cruzi* and *Trypanosoma rangeli*

IV. Multiple choice questions (MCQs):

1. Vector for leishmaniasis:
- (a) Sandfly
- (b) Reduviid bugs
- (c) Tsetse fly
- (d) *Anopheles* mosquito

2. Old world leishmaniasis is caused by:
- (a) *Leishmania donovani*
- (b) *Leishmania tropica*
- (c) *Leishmania infantum*
- (d) All of the above

3. New World leishmaniasis is caused by:
- (a) *Leishmania donovani*
- (b) *Leishmania braziliensis* complex
- (c) *Leishmania tropica*
- (d) *Leishmania major*

4. Amastigote form of *Leishmania donovani* resides in the:
- (a) Gastrointestinal tract of insect vector
- (b) Salivary gland of mosquito
- (c) Cells of reticuloendothelial system
- (d) NNN culture media

5. Oriental sore is caused by:
- (a) *Leishmania mexicana* complex
- (b) *Leishmania braziliensis* complex
- (c) *Leishmania tropica*
- (d) *Leishmania chagasi*

6. *Leishmania donovani* can be cultivated in:
- (a) Blood agar
- (b) NNN medium
- (c) Diamond's medium
- (d) RPMI 1640 medium

7. Chiclero's ulcer is caused by:
- (a) *Leishmania mexicana* complex
- (b) *Leishmania braziliensis* complex
- (c) *Leishmania peruviana*
- (d) *Leishmania chagasi*

8. Espundia is caused by:
- (a) *Leishmania mexicana* complex
- (b) *Leishmania braziliensis* complex
- (c) *Leishmania peruviana*
- (d) *Leishmania chagasi*

9. Best animal model used for inoculation of *Leishmania donovani*?
- (a) Hamster
- (b) Guinea pig
- (c) Rabbit
- (d) Mouse

10. American trypanosomiasis (Chagas' disease) is caused by:
- (a) *Trypanosoma brucei gambiense*
- (b) *Trypanosoma rangeli*
- (c) *Trypanosoma brucei rhodesiense*
- (d) *Trypanosoma cruzi*

Answers

1. (a) 2. (d) 3. (b) 4. (c) 5. (c) 6. (b) 7. (a) 8. (b) 9. (a) 10. (d)