MEDICAL PROTOZOOLOGY

Protozoa: These are unicellular organisms that occur singly or in colony formation. Each protozoan is a complete unit capable of performing all functions.

Morphology: Protozoa have wide range of size (1-150µ). The structure of protozoan cell is formed of a cytoplasmic body and a nucleus.

1. Cytoplasm:

a. Ectoplasm: The outer hyaline layer that is responsible for ingestion of food, excretion, respiration, protection and sensation.

Some structures develop from ectoplasm as:

- Organs of locomotion; pseudopodia, flagella and cilia.

- Organs for food intake or excretion; peristome, cytostome and cytopyge.

b. Endoplasm: The inner granular part of cytoplasm that is responsible for nutrition and reproduction. The endoplasm contains number of structures as: food vacuoles, foreign bodies, contractile vacuoles and chromatoid bodies.

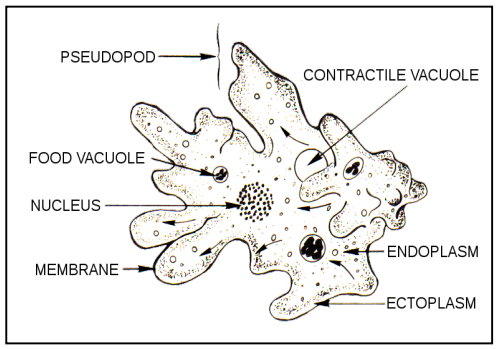
2. Nucleus: It is the most important structure, as it regulates the various functions and reproduction. It is formed of:

a. Nuclear membrane.

b. Nuclear sap (nucleoplasm).

c. Chromatin granules.

d. Karyosome (nucleolus): It is a DNA containing body, situated centrally or peripherally within the nucleus.



General morphology of protozoa.

Biology of protozoa:

1. Movement: Protozoa may move by pseudopodia, cilia and flagella with or without undulating membrane.

2. Respiration: It may be by direct taking of oxygen or by using oxygen liberated from metabolic processes.

3. Nutrition: It is through:

a. Absorption of liquid food.

b. Ingestion of solid material through the ectoplasm by pseudopodia or the cytostome and become surrounded by food vacuoles.

- Digestive enzymes assimilate the food and the undigested particles are extruded through the surface of the body or through a specialized opening (cytopyge).

4. Excretion: It is performed by osmotic pressure, contractile vacuoles, diffusion or cytopyge.

5. Secretion: Protozoan cell can secrete cyst wall, digestive enzymes, pigments, proteolytic enzymes, haemolysins, cytolysins, toxic and antigenic substances.

6. Reproduction:

The parasite multiplies only in the trophozoite stage. The methods of reproduction are of the following types:

I. Asexual reproduction:

1. Simple binary fission: It is either longitudinal or transverse into two organisms.

2. Multiple fission (schizogony, merogony or sporogony): In this process the nucleus undergoes several successive divisions followed by division of cytoplasm into small parts to produce large number of small merozoites or sporozoites within the schizont, e.g. *Plasmodium*.

3. Budding:

a. Internal budding:

- Endodyogeny: Division into two organisms inside the mother cell. It occurs in human intestine and cyst or pseudocyst, e.g. *Toxoplasma gondii*.

- Endopolygeny: Division into several organisms at once by internal budding. It takes place in the intestine of cat in *Toxoplasma gondii*.

b. External budding (Ectomerogony): Simultaneous division into several organisms inside the cyst or pseudocyst, e.g. *Toxoplasma gondii*.

II. Sexual reproduction:

1. Gametogony or Syngamy: It means fusion of two cells one is female (macrogamete) and the other is the male cell (microgamete), e.g. *Plasmodium*.

2. Conjugation: It means fusion of the nuclei from two organisms. Conjugation may be rejuvenation process in some protozoa and reproductive process in others, e.g. *Blantidium coli*.

- Reproduction usually occurs asexually in protozoa; although, sexual reproduction occurs in ciliates and sporozoa.

Life cycle:

1. Simple life cycle: Intestinal and luminal protozoa require only one host, within which they multiply asexually, and transfer from one host to another directly.

2. Complex life cycle: Most blood and tissue parasites pass alternatively in a vertebrate and an invertebrate host, this is called alternation of generation (i.e. transmission is indirect). The sexual multiplication occurs in one host and the asexual multiplication in another host.

Classification of Protozoa

1. Phylum: Sarcomastigophora (Amoebae and Flagellates):

a. Sub-phylum: Sarcodina (Amoebae):

i. Parasitic Amoeba.

ii. Free-living Amoeba.

b. Sub-phylum: Mastigophora (Flagellates):

i. Intestinal and uro-genital flagellates e.g. *Giardia intestinalis, Dientamoeba fragilis* (Amoeba-like flagellate), and *Trichomonas vaginalis*.

ii. Blood and tissue (haemo-somatic) flagellates: *Leishmania* and *Trypanosoma* species.

2. Phylum: Ciliophora, e.g. *Balantidium coli*.

3. Phylum: Apicomplexa (Sporozoa or Coccidia), e.g. *Plasmodium*, *Toxoplasma gondii*, *Cryptosporidium parvum*, *Cystoisospora belli* and *Cyclospora cayetanensis*.

SARCOMASTIGOPHORA

Sarcodina (Amoebae)

Amoebae may be parasitic or free-living, pathogenic or non-pathogenic.

Parasitic amoebae

General characters:

1. Parasitic amoebae have both trophozoite and cyst stage (infective form).
2. Trophozoites have pseudopodia for locomotion.
3. Nutrition occurs through pseudopodia.

4. They multiply by simple binary fission.
5. They inhabit the large intestine.
6. Species:
    a. Potentially pathogenic: ***Entamoeba histolytica***.
    b. Non-pathogenic (Commensal):
        1. ***Entamoeba coli***
        2. ***Entamoeba hartmanni***
        3. ***Entamoeba dispar***
        4. ***Iodamoeba butschlii***

Entamoeba histolytica

Geographical distribution: Worldwide distribution especially in tropical areas and poor communities.

Morphology:

Entamoeba histolytica has 3 stages:

1. Trophozoite (Vegetative or growing stage):

- Size: 10-60 μ (average 20 μ).

- Shape: Irregular outline with finger like pseudopodia and active movement.

- Cytoplasm: It is formed of outer clear hyaline, refractile ectoplasm and inner granular endoplasm containing nucleus, food vacuoles, erythrocytes (RBCs), occasionally bacteria, and tissue debris.

- Nucleus: It has **centrally located fine karyosome and peripheral chromatin dots** arranged regularly at the inner side of the nuclear membrane.

2. Precyst:

- Smaller than the trophozoite but larger than cyst (10-20 μ).

- Rounded or oval with blunt pseudopodia and sluggish movement.

- No food vacuoles or RBCs.

- It contains a single nucleus similar to that of the trophozoite.

3. Cyst:

- It is rounded, 10-15 μ in diameter.

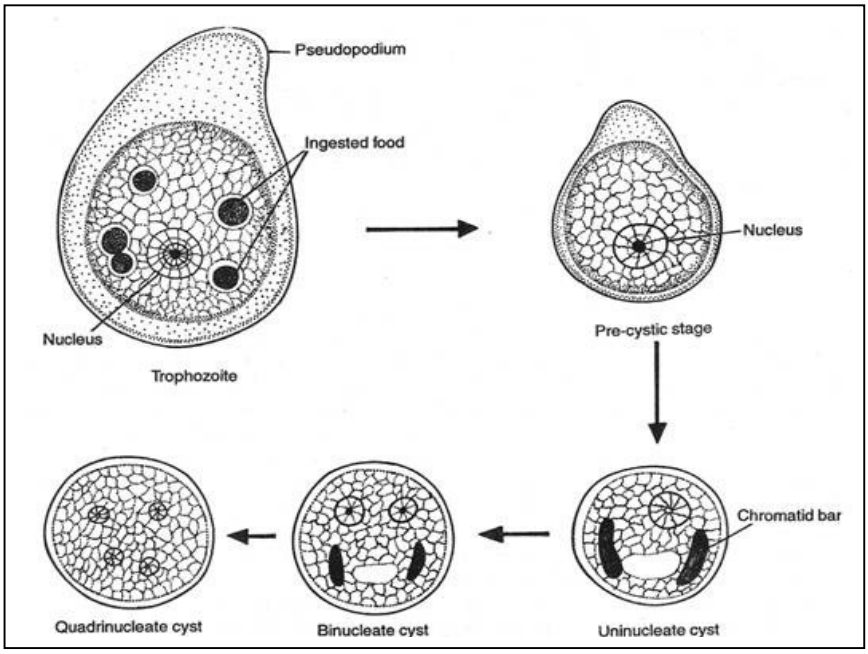
- Has smooth refractile cyst wall.

- The early cyst contains glycogen vacuoles and **1-4 chromatoid bodies** which are sausage-shaped with rounded ends. They are formed of RNA & DNA, and represent stored proteins which are consumed with repeated nuclear division.

- Immature cysts may be mono- or bi-nucleated.

- Mature cysts contain 4 nuclei formed by mitotic division.

- Nuclei are similar to that of the vegetative form.



Three stages of *Entamoeba histolytica*.

Life cycle:

- Habitat:

a. Trophozoite: Inhabits the **wall and lumen of the large intestine**, with extra-intestinal metastases (liver, lung and brain, etc.).
b. Cyst: Inhabits the **lumen of the large intestine**.

- Definitive host: Man.

- Intermediate host: No.

- Reservoir hosts: Dogs, rats and monkeys.

- Infective stage: Mature quadrinucleated cyst.

Mode of infection:

1. Ingestion of **mature quadrinucleated *E. histolytica* cysts** in contaminated food or drink, or through infected food handlers.
2. Mechanical transmission by flies and cockroaches.
3. Autoinfection: feco-oral route (hand to mouth contact).

- On ingestion, the trophozoites disintegrate in the stomach, while only the mature cysts resist the stomach acidity and pass to the small intestine.

- The cyst wall is digested by action of **trypsin** and **excystation occurs in the proximal small intestine**, where metacystic stage escapes and **divides into 8 small amoebae**.

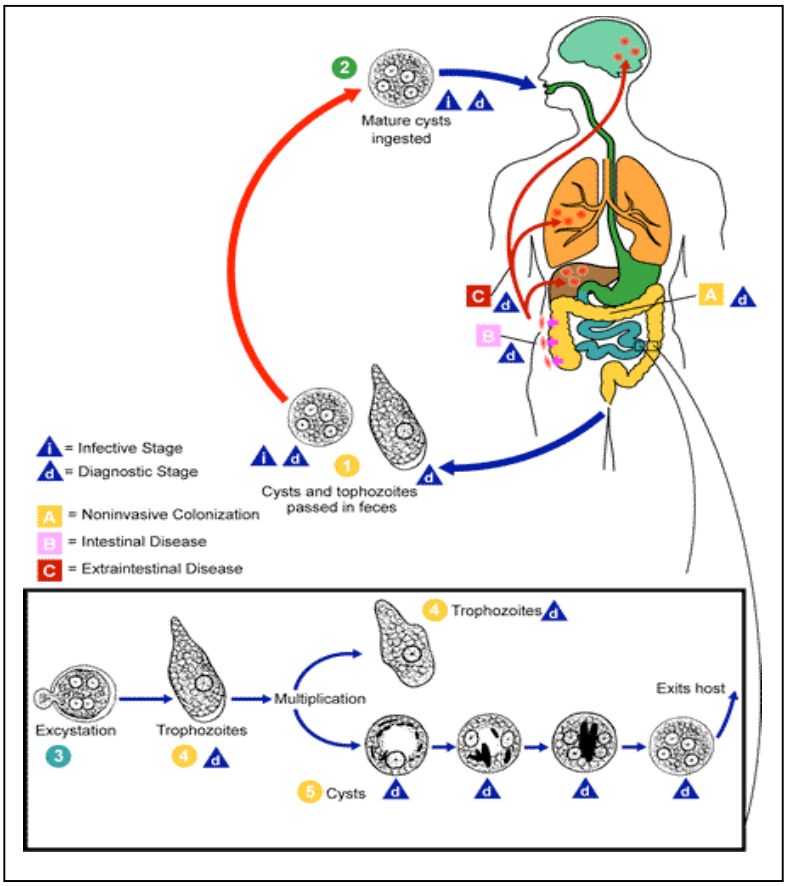
- These trophozoites move down to the **ilio-caecal region**, multiply by binary fission, and then pass to the **lumen of colon,** where they may remain, feeding on starch or mucus and pass in liquid stool, or may undergo **encystation** and **cysts pass with formed stool**.

- Also, trophozoite may invade the wall of large intestine by their **lytic secretion** to invade the host tissues through blood vessels (**extra-intestinal invasion**).

Pathogenesis:

E. histolytica causes **intestinal and extra-intestinal amoebiasis**.

- E. *histolytica* lives in large intestine usually as a commensal without producing any clinical manifestation, but sometimes they become pathogenic and attack the mucosa (10% of cases).



Life cycle of *Entamoeba histolytica*.

The pathogenic activities of *E. histolytica* depend upon:

1- The resistance of the host, state of nutrition, associated infectious or debilitated diseases.

2- Virulence and invasiveness of amoebic strain and number of amoebae.

3- Local conditions of the intestinal tract: Invasion is facilitated by carbohydrate diet, injury of mucosa, bacterial flora and food stasis, e.g. constipation.

- *Entamoeba* trophozoites attach themselves to the surface epithelium aided by an enzyme called ***E. histolytica*** **lectin** and start crawling over the mucosa.

-Trophozoites secrete cytolytic enzymes; **haemolysins and pore-forming enzymes** (amoeba pore), which lead to necrosis of epithelial cells with pore formation. Amoebae absorb nourishment from the dissolved tissues and ingest RBCs and tissue fragments through pseudopodia encirclement.

-Tophozoites enter to the submucosa through the hole formed in the epithelial layer and continue the process of cytolysis downwards and laterally.

- Early lesion is a tiny area of necrosis in the superficial mucosa or small nodular elevation with minute opening that leads to flask shaped cavity containing cytolysed cells, mucus and **amoeba trophozoites**, while **amoeba cysts never found in tissues**. This ulcer is called **flask shaped** or **crater like ulcer**.

- The lesions vary from small ulcers distributed over the mucosa, to large irregular ulcers, each with undermined edge and necrotic base with yellow purulent membrane covering its base. **Ulcers are more common in the ileo-caecal region followed by the sigmoid-rectal region**.

- With progress of lesions→ sloughing of large mucosal parts exposing large necrotic areas.

- Ulcer expansion can penetrate the intestinal wall →intestinal perforation with hemorrhage and peritonitis.

- Repeated inflammation and healing →deposition of fibrous tissue and granuloma formation around the ulcer with thickening of the intestinal wall that may be mistaken as a tumour or tuberculous granuloma and is called **amoeboma**. It is composed of collagen, fibrous tissue and chronic inflammatory cells.

- Invasion of blood vessels may lead to spread of amoebae causing **extra intestinal amoebiasis:**

1. Amoebic liver abscess: It usually occurs due to direct transport of trophozoites from the large intestine via the portal vein.

- It may be single or multiple, located in the **upper right lobe of the liver**.

-The lesion starts as small necrotic foci which tend to coalesce into a single abscess and continues to enlarge as the trophozoites destroy and ingest liver cells.
-The abscess contains lysed hepatocytes, erythrocytes, bile and fat, giving its content a colour from yellowish to reddish (**Anchovy- sauce**).

2. Pulmonary amoebiasis:

- It usually results from direct extension from the liver across the diaphragm but may be also haematogenous.

- Lung abscess may be single or multiple, in the **lower lobe of right lung**.

3. Cerebral amoebiasis:

- Haematogenous spread from amoebic liver abscess or pulmonary amoebiasis usually causes single brain abscess.

- It results in **secondary amoebic meningoencephalitis**, with severe destruction of brain tissue.

Clinical picture:

The clinical picture of amoebiasis may be:

I. Intestinal amoebiasis:

1. Asymptomatic infections:

- These account for the majority of cases (80-90 %).

- There is vague abdominal discomfort, malaise, constipation alternating with mild diarrhea.

- These patients are cyst passers and they are called **healthy carriers**.

2. Symptomatic infections:

a. Acute intestinal amoebiasis (Amoebic dysentery):

- Incubation period from 1-4 weeks but may range from few days to months or years.

- There is **severe dysentery** (colic + tenesmus + frequency of defecation + blood + mucus and shreds of necrotic mucosa in stool) and abdominal tenderness.

- The patient is usually afebrile and non-toxic.

b. Chronic amoebic colitis (Non-dysenteric colitis):

- Chronic intermittent diarrhea.

- Abdominal pain and distension (**Uncomfortable belly** or **growling abdomen)**.

- Weight loss and weakness.

c. Complications of symptomatic intestinal amoebiasis:

1. Fulminant amoebic colitis. The patient is febrile and toxic.
2. Amoeboma. It is palpable, firm, painful, movable, chronic nodular lesion occurring mainly in the caecum, sigmoid colon or rectum.
3. Thick **mega-colon** and colonic stricture associated with obstructive symptoms.
4. Appendicitis, intestinal perforation and peritonitis.
5. Haemorrhage due to erosion of intestinal blood vessels.
6. Peri-anal ulceration.

Differences between amoebic and bacillary dysentery.

Characteristics	**Amoebic dysentery**	**Bacillary dysentery**
1. Clinical picture: Incubation period	Long	Short (< 7 days)
Onset	Slow	Acute
Abdominal tenderness	Localized	Generalized
Tenesmus	Moderate	Severe
Fever	-	+
2. Stool: Nature	Faeces mixed with blood and mucus	Blood and mucus with little or no faeces
Odour	Offensive	Nil
Consistency	Not adherent	Adherent to container
Frequency	6-8 times/day	> 10 times/day
Reaction	Acidic	Alkaline
3. Microscopy: Pus cells	Few	Numerous
RBCs	In clumps	Scattered or in rouleaux
Macrophages	Few	Numerous
Amoeba trophozoites	+	-
Bacteria	-	+
Charcot-Leyden Crystals	+	-

II. Extra intestinal amoebiasis:

1. Hepatic amoebiasis:

a. Diffuse amoebic hepatitis:

- It is a non- specific reaction of liver to the necrotic debris and toxic materials.

- The liver is enlarged and tender with pain in the right hypochondrium.

- Temperature is usually elevated.

b. Amoebic liver abscess:

- The liver is enlarged and tender with pain in the right hypochondrium.

- Elevation of the right diaphragm with severe pain referred to the right shoulder.

- Fever, chills, toxemia, anorexia with leukocytosis.

- Jaundice occurs with multiple lesions or affection of biliary tract.

-The abscess may extend through the diaphragm to the lung, pericardium, peritoneal cavity or rupture through the abdominal wall.

2. Pulmonary amoebiasis:

- It is characterized by chest pain, cough, dyspnea, chills, fever and leukocytosis.

- Hepatobronchial fistula is usually associated with expectoration of **chocolate-brown sputum**.

3. Amoebic brain abscess: It acts as a brain tumor (**Space-occupying lesion**).

4. Cutaneous amoebiasis:

- It results from fistula formation (intestinal, hepatic, or perineal).

- Lesions can be highly destructive, simulating epithelioma.

5. Genitourinary amoebiasis:

- In females, vulva, vagina or cervix can be affected by spread from perineum or fistula formation.

-The destructive lesions resemble carcinoma.

Diagnosis:

- Clinical diagnosis:

1. History of travel to or residence in an endemic area.

2. Signs and symptoms on physical examination.

- Laboratory diagnosis:

I. Diagnosis of intestinal amoebiasis:

1. Stool examination:

a. Macroscopy.

b. Microscopy:

-Proper collection, preservation and examination of stool samples using saline, iodine or eosin smears, or permanent stained smears with trichrome or iron-haematoxylin.

-Repeated stool examination and concentration methods by zinc sulphate floatation, may be required especially in chronic cases.

c. Stool culture: Using Robinson's medium. It is a sensitive method for diagnosing chronic and asymptomatic intestinal amoebiasis.

d. Detection of amoebic copro-antigens: By enzyme-linked immunosorbent assay (ELISA).

e. Molecular diagnosis.

2. Sigmoidoscopic examination: For detection of trophozoites and associated pathology.

3. Serodiagnosis: Antibodies to *E. histolytica* can be detected by indirect haemagglutination (IHA) test, immunofluorescence assay (IFA) test, and ELISA in invasive intestinal amoebiasis.

II. Diagnosis of extra-intestinal amoebiasis:

1. Microscopic examination:

- For detection of trophozoites in:

a. Aspirated pus or biopsy from amoebic liver or lung abscess.

b. Sputum in pulmonary amoebiasis.

c. CSF in cerebral amoebiasis.

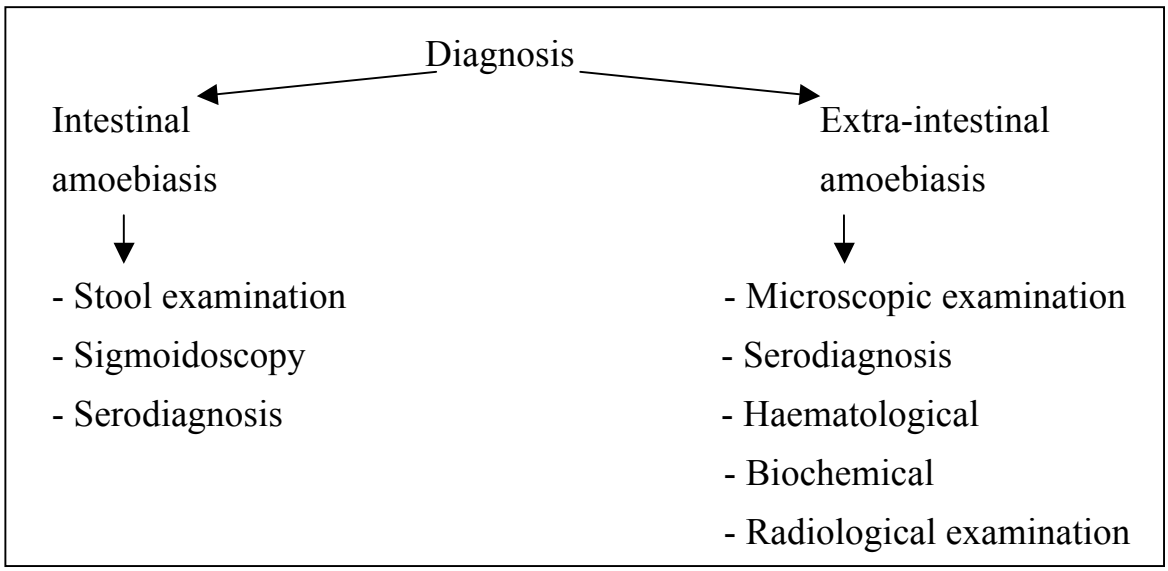
- Stool samples are not of much value as cyst can be detected in less than 15% of hepatic ameobiasis.

2. Serodiagnosis: The circulating amoebic antigens or antibodies can detected by IHA, IFA or ELISA.

3. Haematological diagnosis: Leukocytosis is noted in amoebic liver abscess.

4. Biochemical diagnosis: Raised alkaline phosphatase and serum glutamic oxaloacetic transaminase (SGOT) level in amoebic liver abscess.

5. Radiological examination: Amoebic liver, lung or brain abscesses can be diagnosed by ultra-sonography (US), computed axial tomography (CT) or magnetic resonance imaging (MRI).



Laboratory diagnosis of amoebiasis.

Treatment:

1. Luminal amoebicides: They act in the intestinal lumen.

-Diloxanide fluorate (Furamide).
- Metronidazole (Flagyl).
- Tinidazole (Fasigen).
- Paromomycin.
- Iodoquinol.

2. Tissue amoebicides: They act against the tissue invasive form.

a. Amoebicides acting on all types of tissues:

- Metronidazole.
-Tinidazole.
- Emetine hydrochloride.

b. Amoebicides acting only on liver tissue:

- Chloroquine phosphate.

c. Amoebicides acting only on the intestinal wall:

- Tetracycline.

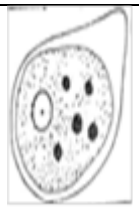
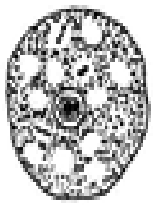
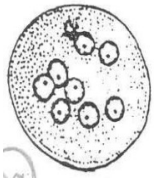
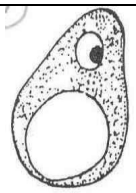
3. Amoebic liver abscess: Aspiration of pus + amoebicides.

- Although metronidazole and tinidazole act as both luminal and tissue amoebicides, but none of them reach high level in the intestinal lumen. Therefore, patients with amoebic colitis or amoebic liver abscess should also take another luminal amoebicides (paromomycin) to ensure eradication of infection.

Prevention and control:

1. Environmental sanitation as: Anti-fly measures, proper sewage disposal, safe water supply and avoid using excreta as fertilizer.
2. Health education for: Washing green vegetables, fruits and hands before eating.
3. Treatment of cases, especially carriers.

Differences between *E. histolytica*, *E. coli* and *Iodamoeba butschlii*.

Characteristics	***Entamoeba histolytica***	***Entamoeba coli***	***Iodamoeba butschlii***
1. Trophozoite: Size	10-60µ	10-30µ	8-16µ
Motility	Active	Sluggish	Sluggish
Pseudopodia	Finger-shaped	Blunt	Blunt
Cytoplasm	Clearly differentiated	-	-
RBCs	+	-	-
Bacteria	-	+	+
Vacuoles	Few	Multiple	Multiple
Karyosome	Small, central	Large, eccentric	Large, eccentric
Chromatin Granules	Fine, regular	Coarse, irregular	-
			
2. Cyst: Size	10-15µ	10-30µ	7-14µ
Shape	Rounded	Rounded	Rounded, oval
Nuclei	1-4	1-8	Single
Chromatoid Bodies	Rod-shaped	Splinters	-
Glycogen Mass	Diffuse	Diffuse	Big circumscribed vacuole
			
3. Pathogenicity	Potentially pathogenic	-	-

***Entamoeba hartmani*:** It is non-pathogenic and morphologically similar to *E. histolytica* but differs in:

1. Trophozoite: It is small about 4-12μ, and ingests bacteria only. Nucleus contains large eccentric karyosome and coarse peripheral chromatin granules.

2. Cyst: 5-10μ and chromatoid bodies are in the form of rice-grain shaped.

***Entamoeba dispar*:** It is non-pathogenic and morphologically similar to *E. histolytica*, but the trophozoite of *E. dispar* does not contain RBCs.

Case study:

A 13-year-old male complained of frequent diarrhea, fever, malaise and abdominal colic. Stool culture for pathogenic bacteria was negative. Parasitological examination of stool revealed mono-nucleated, irregularly-shaped organisms about 20 μ, containing RBCs in the cytoplasm.

Questions:

1. What is the parasitic cause of this patient's infection?
2. How is this infection transmitted? What is the infective stage?
3. Mention the complications of this parasitic infection.
4. Develop diagnostic procedures to confirm your diagnosis.
5. Propose a treatment regimen and control measures for this infection.

Free-living amoebae

Free-living amoebae are found in moist soil, decaying vegetations and all types of water, especially water containing bacteria. They are **amphizoic** parasites, as they can multiply both in the host (**endozoic**) and in free-living (**exozoic**) conditions.

Three types of free-living amoebae are pathogenic to man:

1. *Naeglaria fowleri* (an amoeboflagellate).
2. *Acanthamoeba castellani*.
3. *Balamuthia mandrillaris*.

Naeglaria fowleri
(Brain-eating amoeba)

Geographical distribution: Cosmopolitan.

Morphology:

1. Trophozoite:

a. Amoeboid form (Vegetative and growing form):

- Size: 10-20μ.

- Shape: Elongate with broad anterior end and tapering posterior end.

- Cytoplasmic inclusion: Food vacuoles, contractile vacuole and phagocytic vacuoles known as **amoebostomes**.

- Nucleus: Has a **large central karyosome**.

- Motility: Actively motile with broad rounded pseudopodia (**lobopodia**).

- It inhabits CNS tissues and multiplies by simple binary fission.

- Trophozoite takes the amoebic form in tissues and CSF.

b. Flagellate form:

- Shape: Pear-shaped or oval.

- Flagella: Two long equal flagellate.

- Cytoplasmic inclusion: Single posterior contractile vacuole.

- Nucleus: As trophozoite.

- Amoeba changes to flagellated form when comes in contact with warm water and occasionally in CSF.

- It never presents in tissues.

2. Cyst:

- Size: 7-10µ.

- Shape: Rounded.

- Wall: **Smooth double wall**.

- Nucleus: Mono-nucleated.

- Cytoplasmic inclusions: Contractile and food vacuoles.

- It presents only in soil, never in tissues or CSF.

Life cycle:

- Habitat: Soil and warm fresh water. In man it attacks the CNS.

- Infective stage: Amoeboid trophozoite.

Mode of infection: Through the nasal route.

1. Swimming or sniffing in contaminated water.

2. Inhalation of contaminated air.

- Amoeboid trophozoites in contaminated water enter the nose, migrate through the nasal mucosa → cribriform plate → olfactory nerve → olfactory pulp → base of the brain → disseminate to the brain tissue.

- The amoeboid trophozoite feeds and divides by binary fission. It transforms transiently into the flagellate trophozoite, which is none feeding and can't divide, but can reverts back to amoeboid form.

- The amoeboid trophozoite transforms into cyst stage.

- Exystation of the cyst releases trophozoite.

Pathogenesis:

Naegleria fowleri causes **primary amoebic meningo-encephalitis (PAM or PAME)**.

- Amoeboid trophozoite is specifically **neurotropic**, penetrates the cribriform plate and multiplies along the base of the brain, where it feeds on nerve tissue, by means of an **amoebostome**, resulting in significant necrosis and bleeding; causing acute meningoencephalitis.

- In the subarachnoid space, an inflammatory exudate of neutrophils and monocytes is seen.

- In the grey matter, there is haemorrhage and extension of the inflammatory exudates, rounded amoebae and necrosis of the tissues are also seen.

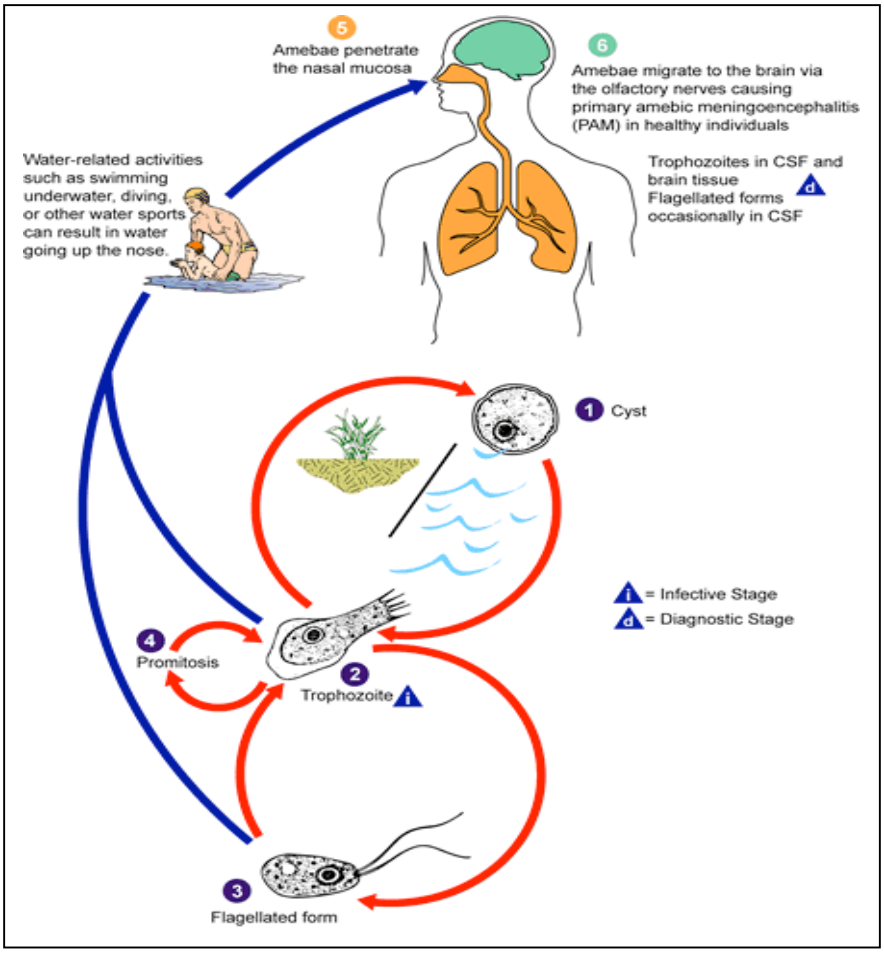
- In the white matter of the brain and spinal cord, there is demyelination, although amoebae and cellular exudate are absent there. Demyelination may be due to the production of **phospholytic enzyme** or **enzyme-like substance** by the growing amoebae in the adjacent grey matter.

Clinical picture:

The signs and symptoms of *Naegleria fowleri* infection are similar to bacterial meningitis, which lowers the chances of initially diagnosing PAM. The clinical course of PAM is dramatic and death usually occurs within a week, so that diagnosis is usually made after the person has died.

1. Stage I: Nausea, vomiting, severe frontal headache, fever, blocked nose with alteration of smell or taste (acute onset of upper respiratory tract infection).

2. Stage II: Signs of meningeal irritation as stiffness of neck **(Kernig's sign**), photophobia, seizures, altered mental status, and coma.



Life cycle of *Naeglaria fowleri*.

Diagnosis:

- Clinical diagnosis:

History of swimming or diving in lakes, ponds or bath spa, 2-6 days prior to onset of meningeal irritation manifestations may suggest the possibility of PAM.

- Laboratory diagnosis:

a. Microscopic examination: Wet mounts of fresh, uncentrifuged CSF revealing trophozoites are clues to a potential diagnosis of PAM. CSF is purulent but with no bacteria, marked raised cell count; mainly polymorph-nuclear leucocytes, elevated protein (> 1gm / L) and low glucose (< 5gm / L). This is in contrast to the viral meningitis where cells are mainly mononuclear cells with low protein.

- CSF smears can be stained with: Haematoxylin and eosin (H&E), periodic acid-Schiff (PAS), Giemsa, or Wright stains.

- At autopsy: Amoeboid trophozoites can be detected in brain tissue by immunofluorescent staining.

b. Culture: Using 1.5% non- nutrient agar seeded with *Escherichia coli*. Both trophozoites and cysts can be seen.

c. Molecular diagnosis.

d. Mice inoculation.

e. Blood sample: It reveals polymorph nuclear leukocytosis that may reach up to 25.000 with preponderance of neutrophils.

Treatment:

1. The patient must be hospitalized and given palliative treatment.
2. Amphotericin-B is administered intravenous and intrathecal.
3. Miconazole or rifampin may be given to potentiate the action of amphotericin-B.

Prevention and control:

1. Adequate chlorination of water of swimming pools and public water supplies.
2. Avoid immersing the head in water during swimming.

Acanthamoeba castellani and *Balamuthia mandrillaris*

Geographical distribution: Worldwide.

Morphology:

1. ***Acanthamoeba castellani***

a. Trophozoite: 20-40 μ, characterized by multiple small spiky pseudopodia (**acanthopodia**) with sluggish motility. Nucleus has large central karyosome.

b. Cyst: Spherical, 15-20 μ, mononucleated and has **polygonal double wall** with many pores (**osteoles**).

-There is no flagellate form.

2. ***Balamuthia mandrillaris***: It is like *Acanthamoeba*, but the trophozoite is pleomorphic, large (12-60 μ), and actively motile by broad or finger-like pseudopodia. Cyst is 6-30 μ, more or less spherical, has **three-layered cyst wall**.

Life cycle:

- Habitat:

-Both trophozoite and cyst stages may exist in the environment and in tissues.

- In the environment: Brackish and fresh water, soil and dust.
- In man: CNS, eye, skin and lungs.

- Infective stage: Trophozoite and cyst.

- Source of infection: Dust, water and contact lens fluid.

Mode of infection:

1. Inhalation of air, aerosol or dust contaminated with trophozoite or cyst.
2. Direct invasion through skin and mucosal ulcers.
3. Through the use of contaminated contact lenses.

- After inhalation, the trophozoites reach lungs, and then invade the CNS through the blood stream.

- Life cycle is simple between the active trophozoite and the resistant cyst stage, where trophozoites multiply by simple binary fission.

Pathogenesis:

1. They are **opportunistic parasites** causing severe disease in immuno-compromised persons; with infected tissues contain both trophozoites and cysts.

2. Tissue invasion is slow producing **chronic granulomatous amoebic encephalitis (GAE)**.

3. Parasitic granuloma of skin & lungs and disseminated infection.

4. In addition, *Acanthamoeba* causes keratitis.

Clinical picture:

1. Granulomatous amoebic encephalitis (GAE):

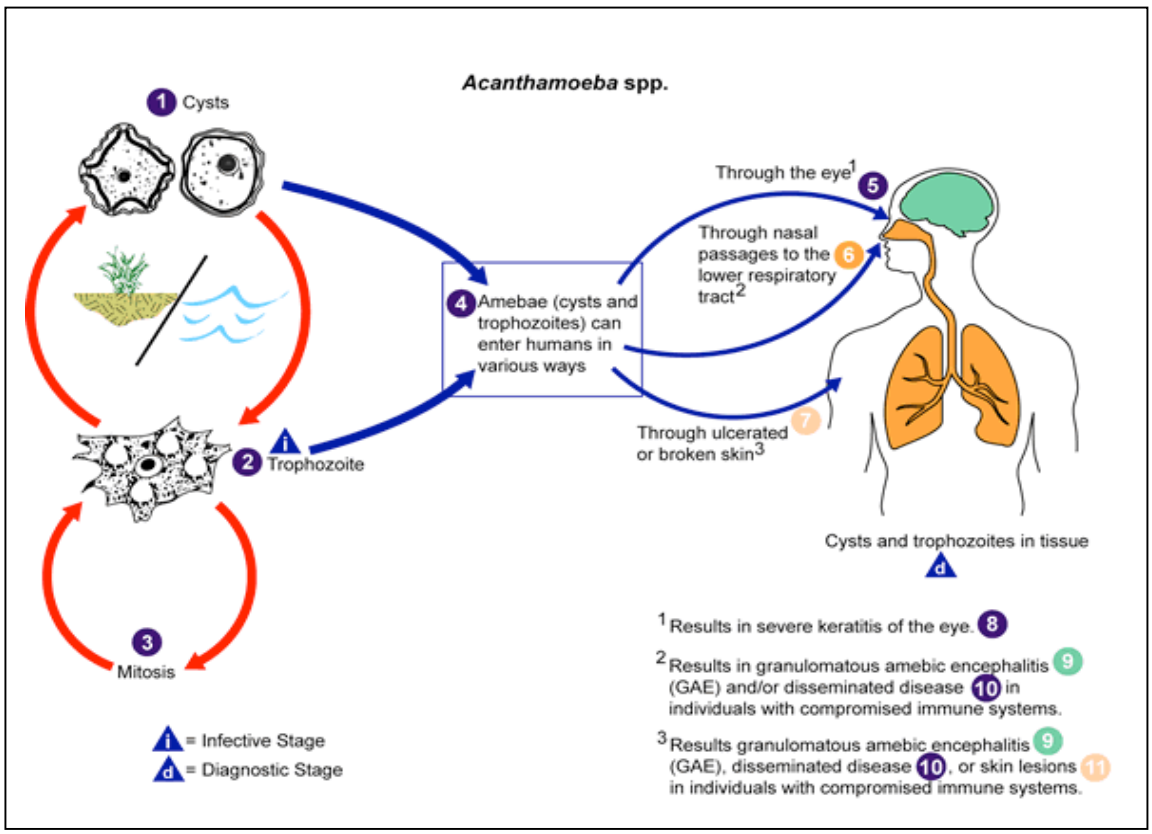
- The course is usually subacute or chronic, lasting from weeks to even years.

- Clinical picture is that of intracranial space-occupying lesions with headache, seizures, mental deterioration, paresis, nausea and vomiting may also occur.

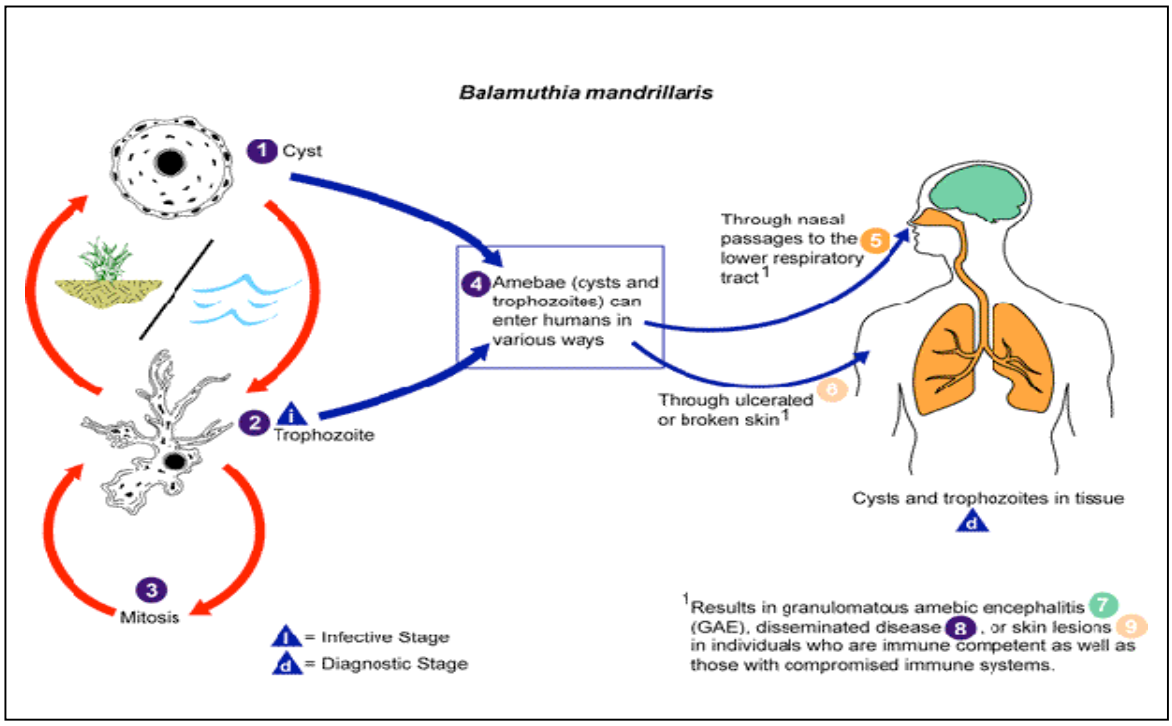
2. Amoebic Keratitis:

-The disease is a chronic progressive ulcerative keratitis caused by ***Acanthamoeba***, characterized by severe unilateral ocular pain, photophobia, annular corneal infiltration, congested conjunctiva and loss of vision or even eye perforation may occur.

3. Chronic granulomatous skin lesions.



Life cycle of *Acanthamoeba castellani*.



Life cycle of *Balamuthia mandrillaris*.

Diagnosis:

- Clinical diagnosis: Full history taking and clinical examination.

- Laboratory diagnosis:

1. GAE:

a. Identification of amoebic trophozoites and/or cysts in CSF or brain tissue biopsy by wet mount or after staining with H&E, PAS, Giemsa, or immunofluorescent technique.

b. Culture on non-nutrient agar seeded with *Escherichia coli*.

c. CT scan of brain.

2. Amoebic keratitis: Corneal scrapings or histologic sections for detection of the organism by direct microscopy or after staining and culture.

Treatment:

1. No effective treatment is available for GAE, but sulfadiazine, pentamidine and rifampicin are being used.
2. Keratitis is treated with antibiotics and topical miconazole ointment. In severe case, keratoplasty can be done.

Prevention and control:

1. Health education.
2. Avoid swimming in stagnant water.
3. The use of proper contact lens fluid.
4. Avoid wearing contact lenses whenever possible.

Case study:

A 12-year-old boy was admitted to hospital with symptoms of photosensitivity, altered mental status, and a sudden frontal headache starting two days prior. A cerebrospinal fluid (CSF) sample taken the day after the patient was admitted revealed motile amoeba. That same day the patient was treated with amphotericin B, however; the patient died two days after admittance to hospital.

Questions:

1. What is the possible parasitic cause?
2. Explain the mode of infection in this case.
3. Analyze the cause of demyelination in this infection.
4. Develop a control plan for this parasitic infection.

Differences between free living amoebae.

Characteristics	***Naegleria fowleri***	***Acanthamoeba Castellani***	***Balamuthia mandrillaris***
I. Morphological stages: **1. Amoeboid trophozoite:** a. Size	Small (10-20 μ)	Medium (20-40μ)	Large (12-60 μ)
b. Pseudopodia	Single, rounded and broad	Multiple, spine-like	Multiple, broad or finger-like
c. Motility	Active	Sluggish	Active
2. Flagellate trophozoites	+	-	-
3. Cyst: a. Size	7-10 μ	15-20 μ	6-30 μ
b. Cyst wall	Smooth double wall	Polygonal double wall	Three-layered cyst wall
c. Encystation in tissues	-	+	+
II. Route of entry	Nasal	Nasal, corneal or cutaneous	Nasal, corneal or cutaneous
III. Pathogenesis: 1. Disease	PAE	GAE, keratitis, skin granuloma, and disseminated disease	GAE, skin granuloma and disseminated disease
2. Clinical course	Acute	Subacute or chronic	Subacute or chronic
3. Opportunistic infection	-	+	+

MASTIGOPHORA (Flagellates)
Intestinal and urogenital flagellates

General characters:

1- Infection occurs in the intestine or the uro-genital system.

2- The infective stage may be either the trophozoite or the cyst form.

3- Transmission of infection is a direct one.

Intestinal flagellates
Giardia intestinalis (*Giardia lamblia*)

Geographical distribution: World-wide. It is considered the main cause of diarrheal out breaks from contaminated water supplies.

Morphology:

1. Trophozoite:

- It is pear-shaped, bilaterally symmetrical, measuring 12x6 µ. It has:
  - **Two sucking discs**, each contains vesicular nuclei.
  - **Four pairs of flagella**, each arises from a **blepharoplast** and has free end.
  - The intracytoplasmic parts of the caudal pair of flagella run along the midline as **axostyles**.
  - **Two curved median parabasal bodies**, lie posterior to the sucking discs.

2. Cyst:

- It is oval, 10x5 µ, and has double-colourless wall.
- Contains **four nuclei** usually gathered at one pole.
- Remnants of flagella and median bodies and axostyles are clearly seen.

Life cycle:

- Habitat:

a. Trophozoite: Inhabits the upper part of the **small intestine**, sticks closely to the mucosa and may penetrate down into the crypts of the mucosa. It may also be found in the **gall bladder** and **biliary drainage**.

b. Cyst: Inhabits the **lumen of the intestine**.

- Definitive host: Man.

- Reservoir hosts: Many animals (dogs, rodents, monkeys…etc). *Giardia* is considered one of the most known zoonotic diseases.

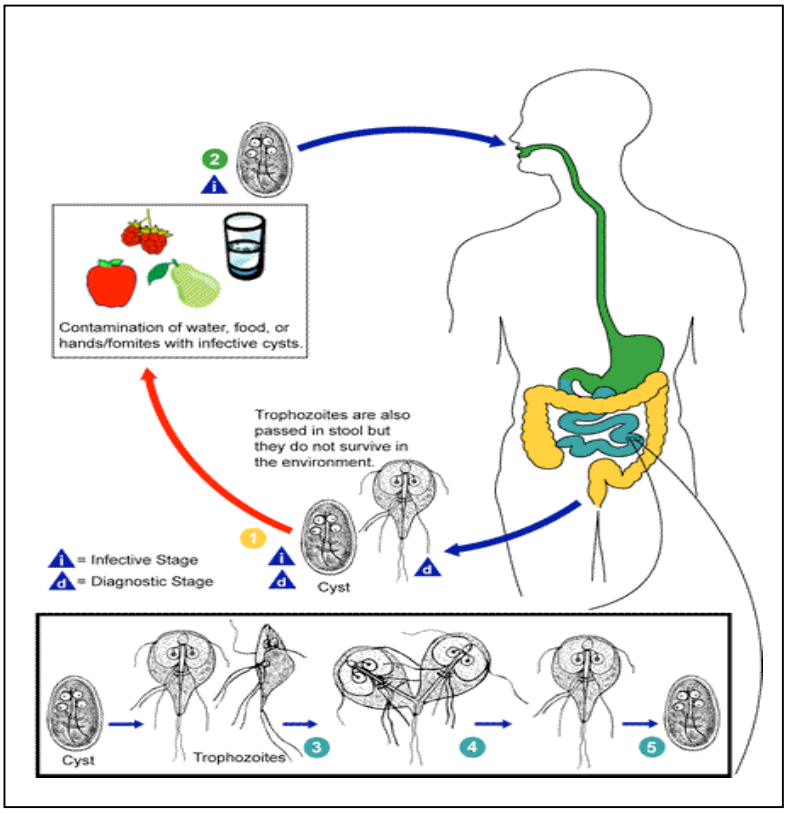
- Infective stage: Mature quadrinucleated cyst.

Mode of infection:

1. Cysts may be ingested with food, drinks, contaminated water or transmitted by house flies, cockroaches ….etc.

2. Person to person transmission occurs especially in nurseries, male homosexuals, mentally ill persons and among school children. *Giardia* is considered one of the **nosocomial (hospitally-transmitted) infections**.

3. Autoinfection by hand to mouth transmission also occurs.



Life cycle of *Giardia intestinalis*.

- Within half an hour of ingestion, the cyst excysts in the small intestine → two trophozoites, which multiply successively by longitudinal binary fission and colonize in the duodenum, feeding by **pinocytosis**.

- During unfavourable conditions, encystment occurs usually in the colon.

- Cysts are passed in stool and remain viable in soil and water for several weeks.

Pathogenesis:

- Trophozoites of *Giardia* live closely to the intestinal mucosa by their sucking discs. They may produce considerable mechanical irritation to the tissues.

- Attachment is facilitated by a **lectin** produced by the parasite and activated by duodenal secretions, leading to derangement of normal villous architecture.

- Giardiasis causes shortening, blunting, and even total atrophy of the villi, with inflammatory foci in the crypts and lamina propria.

- Malabsorption of fat and carbohydrates and fatty diarrhea **(steatorrhea)** among children are the most important sequelae of giardiasis. Occasionally, *Giardia* may colonize the gall bladder causing cholangitis and cholecystitis.

Pathogenic mechanisms postulated for malabsorption and steatorrhea:

1. Inflammation and mechanical blockage of intestinal mucosa by large numbers of trophozoites.
2. Shortening and atrophy of intestinal villi with altered jejunal motility.
3. Reduced secretion of intestinal enzymes.
4. Bacterial jejunal colonization potentiates the damage done by *Giardia*.
5. De-conjugation of bile salts.
6. Secretion of entero-toxins.
7. Competition for essential nutrients.
8. Achlorohydria, hypogammaglobulinaemia and deficiency of secretory IgA.

Resistance to giardiasis:

- Resistance to giardiasis and host defense is indicated by spontaneous cure of the disease which may occur after about 40 days.
- Lymphocytes, macrophages and secretory IgA may have a role.
- Human milk is able to kill *Giardia* trophozoites due to the presence of lipase and secretory IgA. So, it can afford protection to breast fed babies.

Clinical picture:

- The prepatent period is usually 2 weeks.

- **Giardiasis** may be asymptomatic in a good proportion of cases.

- Symptoms may be in the form of:

1. Mucus diarrhea, fat malabsorption (steatorrhea), flatulence, dull epigastric pain, crampy abdominal pain, and anorexia.

2- Severe symptoms: Occur in immunocompromized patients as persistent diarrhea (steatorrhea), hypoproteinaemia, fat soluble vitamin deficiency, lactose intolerance, weight loss, biliary colic and jaundice may occur.

Diagnosis:

- **Clinical diagnosis:** Clinical history and presentation of the disease.

- Laboratory diagnosis:

1. Stool examination:

a. Macroscopy: Faecal specimens containing *G. lamblia* may have an offensive odour, are pale in colour and fatty.

b. Microscopy:

- Stool examination for trophozoites and/or cysts by direct smear, eosin and iodine smears, and by concentration methods.

- Repeated stool examination for three times as the parasite is intermittently shed.

c. Detection of *Giardia* copro-antigens: By ELISA, immunochromatographic strip tests and indirect immunofluroscent tests (IIF).

d. Molecular diagnosis.

2. Examination of duodenal contents for trophozoites:

a. Entero-test (String test).

b. Duodenal aspiration and duodenal biopsy.

3. Serodiagnosis: Antibodies to *Giardia* are detected by IFA and ELISA.

Treatment:

1. Metronidazole (Flagyl).
2. Tinidazole (Fasigen) is more effective than metronidazole.
3. Albendazole.
4. Parmomycin, an oral aminoglycosides can be given to pregnant females.

Prevention and control:

- As amoebiasis.

Case study:

A 9-year old girl complained of epigastric pain, diarrhea and flatulence. Her stool was offensive, pale and greasy. Microscopic examination revealed motile protozoa.

Questions:

1. What is your suggestive diagnosis?
2. Define the habitat of the parasite and the probable sources of infection.
3. Demonstrate the infective stage.
4. Mention the possible complications of this parasitic infection if the patient is immunocompromized.
5. Create a proper treatment plan for this infection.

Dientamoeba fragilis

It was considered as an amoeba, but recently its flagellated nature was discovered by electron microscope. So, it is now reclassified as an **amoeba-flagellate** with antigenic similarity to *Trichomonas*.

Geographical distribution: Worldwide.

Morphology:

Trophozoite: 7-12 µ, characterized by the presence of **two nuclei**, their karyosomes are composed of even granules 4-8 in number. It is actively motile by leaf-like pseudopodia, with cytoplasm contains food vacuoles and bacteria.

- **It has no cyst stage**.

Life cycle:

- **Habitat:** Mucosal crypts of large intestine. It may ingest RBCs but never invades the tissues.

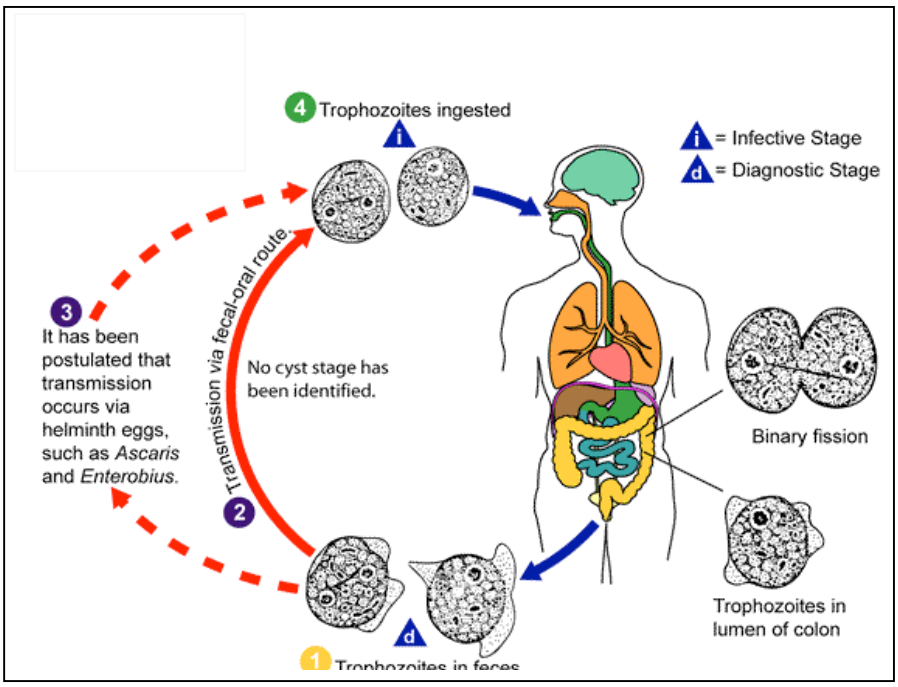
- **Definitive host:** Man.

- **Infective stage:** Trophozoite.

Mode of transmission:

1. Via faecal-oral route.
2. By the eggs of *Enterobius vermicularis* or *Ascaris*.

Multiplication: By longitudinal binary fission.



Life cycle of *Dientamoeba fragilis*.

Clinical picture: The clinical manifestations of **dientamoebiasis** are variable, as intermittent diarrhea, abdominal pain, flatulence, nausea, anorexia, and fatigue.

Diagnosis: Careful examination of fresh and/or iron-haematoxylin-stained smears for detection of trophozoites. At least 3 stool specimens should be collected over a period of a week.

Treatment:
1. Metronidazole (Flagyl).
2. Iodoquinol.
3. Paromomycin.
4. Tetracycline.

Prevention and control: Appropriate hygienic and sanitary measures.

Urogenital flagellates
Trichomonas vaginalis

Geographical distribution: Worldwide.

Morphology:
Trophozoite:
- It is pear-shaped, 17X10 µ, with a rapid **jerky** movement.

- It has an antero-lateral **cytostome**.

- The cytoplasm is granular with a single anterior nucleus.

- It has 4 flagella anteriorly, another flagellum attached to the body by undulating membrane, presents at the anterior 1/3 of body with no free end. The 6^{th} flagellum passes through the body as **axostyle** which projects out of the body.

- A thick marked rod called the **parabasal body** is present between the axostyle and the undulating membrane.

- **It has no cyst stage**.

Life cycle:
- Habitat:
   a. **Female**s: Posterior fornix of the vagina, cervix, and urethra.
   b. **Males**: Urethra, epididymis, seminal vesicles and prostate.

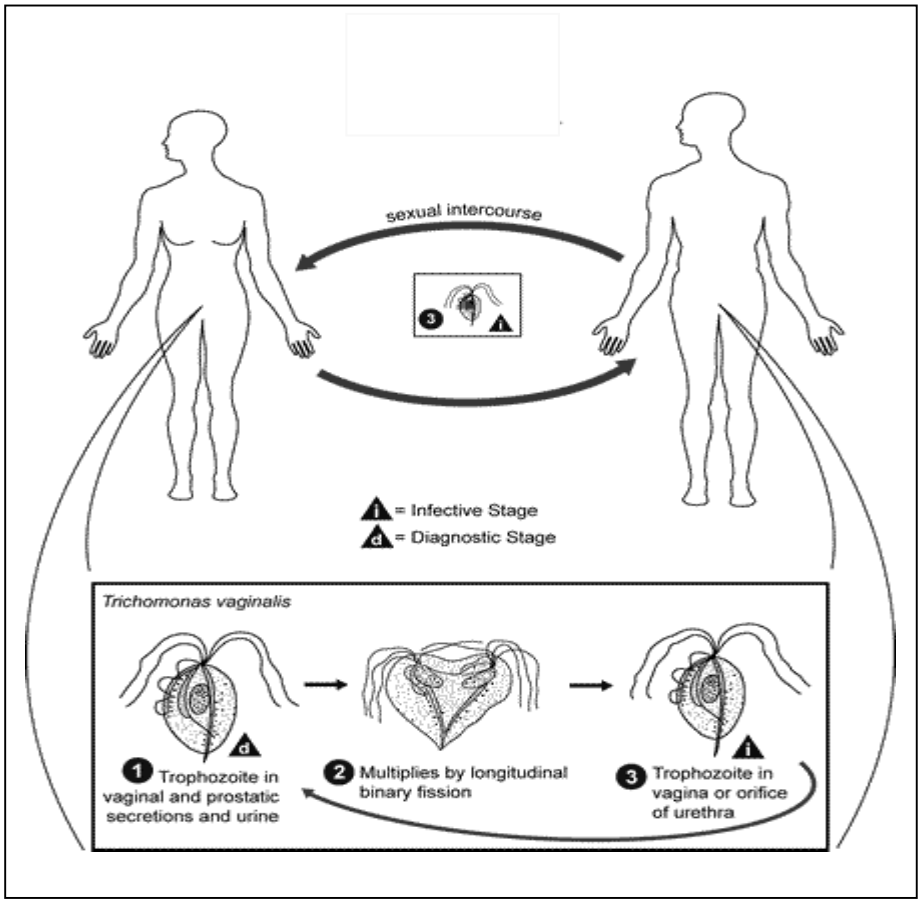
- Definitive host: Man.

- Infective stage: Trophozoite.

Mode of infection:

1. Sexual transmission or by contaminated toilet seats and towels.
2. From infected mothers to babies during birth.

- Multiplication: By longitudinal binary fission.



Life cycle of *Trichomonas vaginalis*.

Pathogenesis:

- *Trichomonas vaginalis* causes trichomoniasis, trichomonad vaginitis, urethritis, epididymitis, vesiculitis and prostatitis.

- The parasite is able to kill target cells by direct contact without phagocytosis (**dependent cytopathic effect**).

- Additionally, *T. vaginalis* produces a **cell detaching factor**, its amount correlates with the severity of the clinical infection.

- **In female**, the vaginal wall is red, showing oedma, petechial hemorrhages (**strawberry mucosa**), mucosal erosion and necrosis. The mucosa is infiltrated

with lymphocytes, plasma cells and polymorphonuclear leucocytes. A relationship between trichomoniasis and cervical carcinoma is suggested.

- **In male**, urethritis, vesiculitis, epididymitis and prostatitis may occur.

Predisposing factors for pathogenicity:

1. Change of the normal vaginal bacterial flora and pH.
2. Decrease in the secretory IgA.

Clinical picture: Trichomoniais may be asymptomatic in infected males (95%) and females (50%).

- Symptoms may be in the form of:

1. Females:

- Vaginal itching and burning with an offensive, frothy, profuse leucorrheic discharge forming a pool in the posterior fornix.

- Dyspareunia (painful sexual intercourse), frequency of micturition and dysuria, also cystitis may occur.

2. Males:

- Dysuria, and prostate may be enlarged and tender.

3. New born: *Trichomonas* respiratory tract infection and conjunctivitis may affect infants during vaginal delivery of an infected mother.

Diagnosis:

- **Clinical diagnosis**.
- **Laboratory diagnosis:**

1. Microscopy:

- **In females:**

- Specimens are obtained through a vaginal speculum by using cotton –tiped applicator stick, and then the applicator is placed in glucose–saline before examination of the precipitate for the organisms.

a. Direct wet smear examination for characteristic jerky motility and shape of trichomonad trophozoites.

b. Fixed smears may be stained with Giemsa, Leishman and Papanicolaou stain.

- **In males:** Examination of prostatic fluid.
- **In both sexes**: Urine examination may beneficial.

2. Culture: On modified Diamond's medium.

3. Immunodiagnosis: For detection of *T.vaginalis* antigens.

a. Direct fluorescent antibody test using labeled monoclonal antibodies.

b. ELISA.

4. Molecular diagnosis.

Treatment:

1. Both partners must be treated at the same time.
2. Metronidazole is the most effective drug.
3. Restoration of normal vaginal acidity by vaginal douching with lactic acid or vinegar seems beneficial in mild vaginal trichomoniasis**.**

Prevention and control:

1. Good personal hygiene.
2. Avoidance of sexual contact with infected partners and use of condoms.
3. Treatment of diagnosed cases, and simultaneous treatment of sexual partners.

Case study:

A pregnant female complained of vaginal itching and burning sensation with profuse and offensive discharge. Gynecological examination revealed redness, oedma, and strawberry-like vaginal mucosa.

Questions:

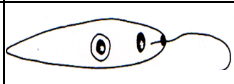
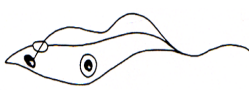
1. What is your suggestive diagnosis?
2. How is this infection transmitted?
3. Demonstrate the infective stage.
4. Mention the possible complications of this parasitic infection to her newborn.
5. Develop diagnostic procedures to confirm your diagnosis.
6. Propose a suitable treatment regimen for this case.

Blood or body fluid and tissue flagellates
(Haemo-somatic flagellates)

General characters:

1. They live in blood & tissues of man and vertebrate hosts and in gut of vectors.
2. Two genera are pathogenic to man: ***Leishmania*** and ***Trypanosoma***.
3. They acquire two or more of the following morphological stages: amastigote, promastigote, epimastigote and trypomastigote.
4. They have a single nucleus, kinetoplast and a flagellum.
5. Kinetoplast consists of a deeply stained **parabasal body** and an adjacent dot-like **blepharoplast**.
6. Flagellum is thin, originating from the blepharoplast. The intra-cytoplasmic portion of the flagellum is called **axoneme**. In some forms, the free flagellum is attached to the surface of the parasite as an **undulating membrane**.
7. All members require an insect vector as an intermediate host.
8. All stages multiply by longitudinal binary fission.
9. For smears of body fluids, Leishman, Giemsa, and Romanowsky´s Wrights stains are used for identifying the parasite, while H& E stain is used for tissues.
10. They can be cultured on Nicoll, Novy and Mac Neal (NNN) medium.

Differences between various stages of haemo-somatic flagellates.

Differences	**Amastigote**	**Promastigote**	**Epimastigote**	**Trypomastigote**
1. Size	3-4 µ	20 x 3 µ	10-20 x 4 µ	15-30 x 5 µ
2. Shape	Rounded	Elongated, lanceolate	Elongated	Elongated, spindle
3. Position of kinetoplast	Beside the nucleus	At the anterior end	In front of the nucleus	At the posterior end
3. Undulating Membrane	-	-	+ (short)	+ (long)
4. Free Flagellum	-	+	+	+
5. Habitat	***Leishmania*** & ***Trypanosoma cruzi*** as intra-cellular form in vertebrates	***Leishmania*** a. In vector b. In culture	***Trypanosoma*** a. In vector b. In culture	***Trypanosoma*** a. In vector b. In culture c. In blood, lymph & CSF of vertebrates
6. Schematic diagram				

Leishmania species

General characters:

1. *Leishmania* species occur as intracellular **amastigote** form in vertebrate hosts and as **promastigote** form in insect and culture.

2. *Leishmania* species and subspecies infecting man, have the same morphology and life cycle in insect, but differ in geographical distribution, host specificity, vector, clinical picture, antigenic structure, etc... They cause 3 different diseases:

-Visceral leishmaniasis caused by ***Leishmania donovani* complex**.

-Cutaneous leishmaniasis caused by ***Leishmania tropica* complex** and ***Leishmania mexicana* complex**.

-Mucocutaneous leishmaniasis caused by ***Leishmania* braziliensis complex**.

Leishmania donovani complex

Geographical distribution: All species of ***Leishmania donovani* complex** cause **visceral leishmaniasis** and are distributed as:

A. In old world:

- *L. donovani*: India, Pakistan, Indonesia, Thailand, Central Africa and Sudan.
- *L. infantum:* Mediterranean area, Middle East and China.

B. In new world:

- *L. chagasi:* America (Central and South America).

Morphology:

1. Amastigote form (Leishman Donovan body): In reticuloendothelial cells (RECs) all over the **human body** and **reservoir host** (**vertebrate hosts**), typically intracellular in macrophages.

2. Promastigote form: In **insect vector** (**invertebrate host**) and **culture**.

Life cycle:

- Habitat: RECs of viscera, especially spleen, liver, bone marrow, intestinal mucosa and mesenteric lymph nodes.

- Definitive host: Man.

- Reservoir host: Dogs, rodents, wild and domestic animals.

- Insect vector: Female sand flies of the genus *Phelebotomus* in the old world, and *Lutzomyia* in the new world.

- Infective stage: Promastigotes.

Mode of infection:

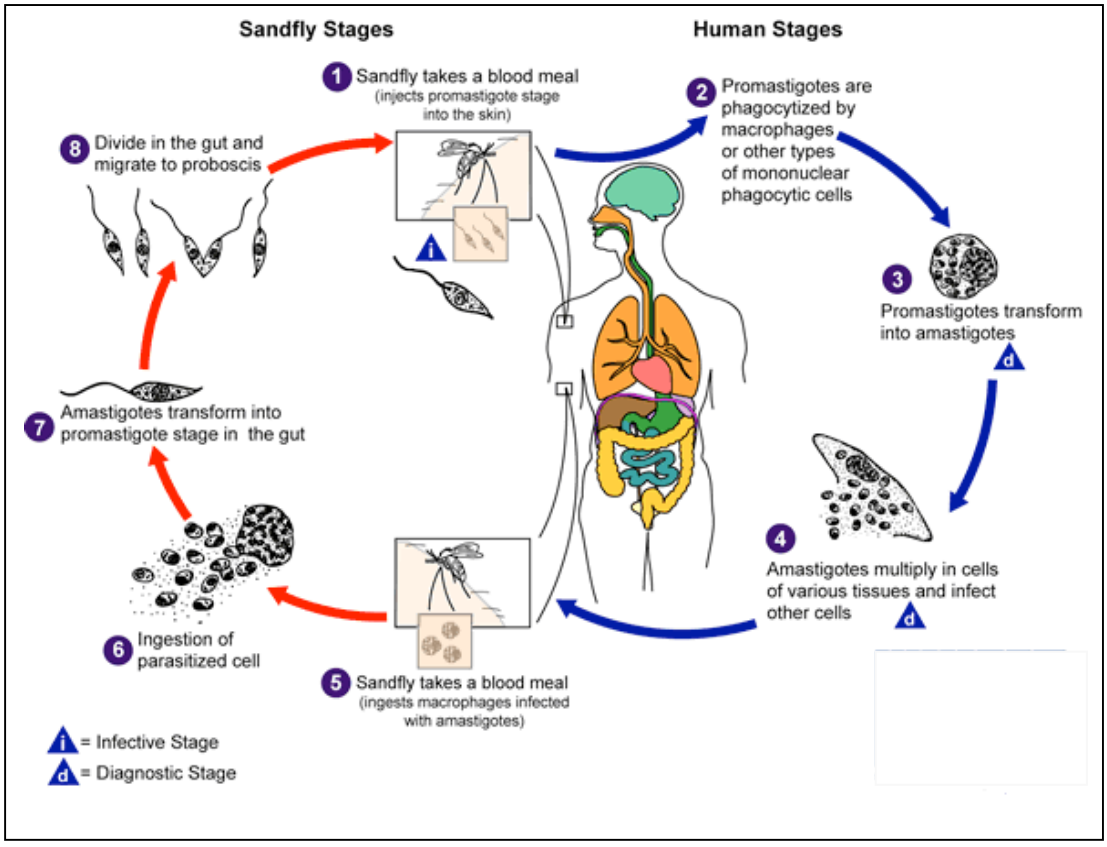
1. Bite of infected sand fly.
2. Blood transfusion.
3. Direct from man to man in epidemics by nasal secretions.
4. Congenital (vertical transmission from mother to fetus).
5. Accidental infection in the laboratory.

- Man acquires the infection when the infected female sand fly attempts a blood meal, where some of the **promastigotes** in the buccal cavity are regurgitated, and introduced into the skin bite by their motility.

- Promastigotes are phagocytosed by skin macrophages, where they metamorphose into **amastigotes** that reproduce by binary fission.

- Ruptured parasitized cells release large number of amastigotes into circulation.

- Blood monocytes phagocytose the free amastigotes and carry them to the viscera, where they produce generalized infection of the RECs.



Life cycle of *Leishmania donovani*.

- Amastigotes in blood are taken by the female sand fly during blood meal.

- In the mid-gut of the sand fly, the amastigotes are metamorphosed into promastigotes and multiplied by binary fission (**Cyclo-propagative development**), until the lumen of the mid-gut is completely blocked.

- After 6-9 days, the promastigotes migrate to the pharynx which becomes blocked by the parasites, then to buccal cavity and proboscis.

- When **blocked sand fly** attempts subsequent blood meal, some of promastigotes are regurgitated, and introduced into the skin bite and the cycle is repeated.

Pathogenicity:

- ***L. donovani*** causes **visceral leishmaniasis, kala-azar, black fever** or **dumdum fever**, an **opportunistic disease**. Immunocompromised status and diets lacking protein, iron, vitamin A and zinc increase the risk of severe form.

- The parasitized macrophages are present in small numbers in the blood, but are numerous in the RECs mainly of kupffer cells of liver, littoral cells of spleen, peyer's patches of intestine, bone marrow and lymph nodes.

-The amastigotes multiply enormously in the fixed macrophages, causing blockade of the reticuloendothelial system. This leads to **marked hyperplasia** and **destruction of reticuloendothelial tissue** in these organs.

- Multiplication of amastigotes in the RECs of liver, spleen and lymph nodes results in **hepatosplenomegaly** and **lymphadenopathy**, respectively.

- The bone marrow is heavily infiltrated with parasitized macrophages, which replace the hematopoietic tissues resulting in **pancytopenia**.

- Lymphoid macrophage cells in intestinal submucosa are packed with parasites causing **ulceration** and **dysenteric symptoms**, with leishmanial bodies in faeces.

- Urinary tract: kidneys, pelvis and bladder wall may be infiltrated with parasitized macrophage cells causing break down of mucosa with viable leishmanial bodies escape in urine.

- Naso-pharyngeal affection results in mucopurulent discharge containing leishmanial bodies.

Clinical picture:

1. The incubation period is usually 2-6 months.

2. The onset can be acute or chronic.

3. A primary skin lesion at the site of infection (**Leishmanioma**) preceding visceral disease has been described in Sudan.

4. In Mediterranean area, kala-azar is common in infants and young children**.**

5. The clinical illness begins with fever. It may be continuous, remittent with a **twice-daily rise**, or irregular.

Causes of fever in Kala-azar: It is due to release of pyrogens by the invaded macrophages due to:

- Phagocytosis of amastigotes.
- Uptake of cellular debris from ruptured parasitized macrophages.

6. Hepatosplenomegaly with progressive and massive enlargement of the spleen.

7. Normocytic normochromic anemia is a significant feature of kala-azar with hemoglobin levels of 5-10 g/dl.

Types and causes and of anemia in Kala-azar:

a. Normocytic normochromic anemia:

- Increased sequestration and destruction of RBCs due to hyperspleenism.
- Decreased erythropoiesis due infiltration of bone marrow by parasitized macrophages.
- Autoantibodies to red cells may cause hemolysis.
- Hemorrhage.
- Alterations in RBCs membrane permeability.
- Production of haemolysin by the parasites.

b. Macrocytic anemia: Due to reticuloendothelial hyperplasia and fatty infiltration of the liver leading to deficient storage of vitamin B12.

c. Microcytic anemia: Due to lack of iron absorption from intestine.

8. Lymphadenopathy in African patients.

9. Diarrhea and/or dysentery.

10. Epistaxis and bleeding from gums.

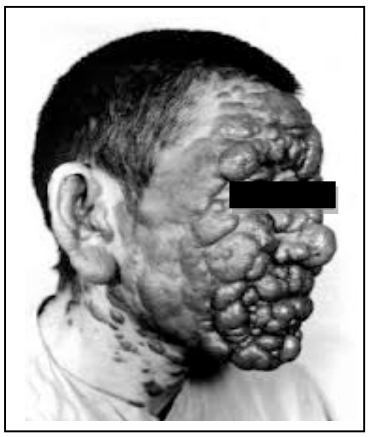
11. Weakness, weight loss and emaciation.

12. Skin becomes dry, thin, rough, and darkly pigmented (hence the name **kala-azar**, or **black fever**). A **butterfly distribution** over the nose is common.

13. **Post kala-azar dermal leishmaniasis (PKDL):**

- It appears after **spontaneous** or **drug cure** (Pentostam) of kala-azar (6 months - 5 years).
- It is common in the Indian and African type of kala-azar.

- It is due to migration of the parasites from viscera to the skin.
- The skin lesions are **chronic**, **progressive** and **painless hypopigmented macules**, **erythematous patches**, or **yellowish pink non-ulcerative granulomatous nodules**.
- It is localized in the outer surface of the body mostly the face especially on nose, chin, cheeks, lips, forehead and ears, resembling Lepromatous leprosy or disseminated cutaneous leishmaniasis.



Post kala-azar dermal leishmaniasis.

14. Untreated severe cases of visceral leishmaniasis are fatal, either directly from the disease or concurrent diseases as pneumonia, tuberculosis, and dysentery.

Diagnosis:

- Clinical diagnosis: In endemic areas, Kala-azar may be suspected in patients specially children with persistent, irregular or remittent fever, often with a double daily peak, hepatosplenomegaly, anemia, leucopenia and emaciation.

- Laboratory diagnosis:

I. Direct:

1. Microscopy:

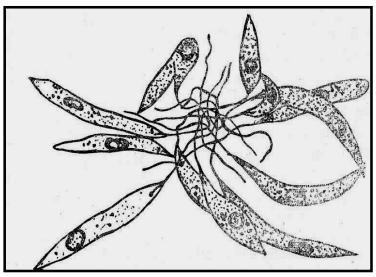
- Detection of **amastigotes** in smears made from the material collected from:
  - Peripheral blood by **thick film** or **buffy coat smears,** which show a **diurnal periodicity**.
  - Bone marrow puncture (sternal or iliac crest).
  - Splenic puncture (spleen pulp).
  - Enlarged lymph node aspirate or puncture.
  - Liver puncture.
  - Nasopharyngeal secretions, stool and urine as the parasite may also be found in atypical sites.

- Nodular lesions in PKDL.

- The smears of body fluids are stained with Leishman, Giemsa or Wrights stain while H& E stain is used for tissue sections.

- Amastigotes can be seen inside the macrophages in large numbers and little extracellular form can also be seen.

2. Culture: Materials are cultured on NNN medium. **Promastigotes** in the form of **rosette grouping of parasites** can be detected 1-4 weeks after cultivation.



Culture form of *Leishmania donovani*.

3. Animal inoculation: Intra-peritoneal inoculation of hamster by aspirated specimens. In positive cases, the **amastigotes** can be seen in smears taken from ulcers or nodules at site of inoculation or from the spleen, weeks post infection.

II. Indirect:

1. Immunological diagnosis:

a. Serological tests: Specific leishmanial antigens prepared from cultures are used to detect anti-leishmanial antibodies using some tests as: IFA, IHA, ELISA, complement fixation test (CFT), direct agglutination test (DAT), and a specific rapid immunchromatographic dipstick (ICT).

b. Leishmanin skin test (Montenegro test):

- It is a delayed hypersensitivity skin test.
- 0.1ml of killed promastigotes of *L. donovani* is injected intradermal.
- Positive result is indicated by an induration and erythema of 5 mm or more after 48-72 hours.
- Positive test indicates past infection with *Leishmania* parasites as it becomes positive 6-8 weeks after cure.
- The test is negative in active infection due to marked depression of cellular immune response and in PKDL.

2. Molecular diagnosis: It helps in species identification of *Leishmania*.

3. Blood picture:

- Complete blood count shows normocytic normochromic anemia, leucopenia and thrombocytopenia (**Aplastic anemia**).

- Serum shows hypergammaglobulinemia and low albumin level.

Treatment:

1. **Supportive treatment:**

- Diet rich in vitamins, iron and liver therapy.
- Treatment with appropriate antibiotics for secondary bacterial infection.
- Blood transfusion, necessary for patients with severe anemia or bleeding.

2. Specific treatment:

a. Systemic therapy (parenteral)

- Pentavalant antimony compounds: Pentostam (Sodium stibogluconate).
- Amphotericin B.
- Interferon gamma, combined with pentostam, has recently been reported to be effective when relapse of the disease occur.

b. Systemic therapy (oral)

- Miltefosine: It is the first oral drug approved for treatment of leishmaniasis.

Prevention and control:

1. Control of sand flies by destruction of their breeding grounds near human habitations and by the use of residual chlorinated hydrocarbon.
2. Control of reservoir hosts will reduce the sources of infection.
3. Personal prophylaxis by using bed nets, window mesh screen, insect repellents and spraying of insecticides.
4. Treatment of infected persons.

Case study:

A 10- year- old boy from the Mediterranean area was complaining of fever and diarrhea for 4 weeks. Clinical examination revealed hepatosplenomegaly. His blood picture showed anaemia and leucopenia. Bone marrow specimens revealed intracellular and extracellular rounded parasites, about 3-4 μ, with central nucleus and an axoneme.

Questions:

1. What parasitic cause do you suspect?
2. Illustrate the mode of infection in such case.
3. What is the prognosis of this disease?
4. Propose other diagnostic procedures to confirm your diagnosis.
5. Predict two other complications that can occur with this infection.

Leishmania tropica complex

Geographical distribution: All species of ***L. tropica* complex** cause **old world cutaneous leishmaniasis** and are distributed as:

a. ***Leishmania tropica***: Middle and Far-East, Mediterranean area.
b. ***Leishmania major***: Central Asia, Middle-East and Africa. **It is recorded in Egypt (Sinai, Sohag and Minia)**.
c. ***Leishmania aethiopica***: Ethiopia and Kenya.

Morphology: *L. tropica* complex morphology is indistinguishable from that of *L. donovani*.

Life cycle:

- Habitat: The amastigote form inhabits the RECs of skin.

- Definitive host: Man.

- Reservoir host:

- Dogs for ***L. tropica***.
- Desert gerbils and rodents for ***L. major***.
- Wild rabbits and rodents for ***L. aethiopica***.

- Insect vector: Female sand fly of the genus *Phelebotomus.*

- Infective stage: Promastigotes.

Mode of infection:

1. Bite of infected sand fly.
2. Direct contact.
3. The stable fly (***Stomoxys calcitrans***) may transmit the organisms mechanically from an open ulcer or through unbroken skin.

- The life cycle is similar to that of *L. donovani* in sand fly. In man, after inoculation of promastigotes, the **amastigotes reside and multiply in the RECs of the skin, without invasion of blood or internal organs**.

Pathogenicity: Cutaneous leishmaniasis is characterized by:

1. Multiplication of amastigotes in the skin macrophages leading to formation of papule, nodule and ulcer.
2. The ulcer may be single or multiple, that heals over months to years, leaving scar.
3. Recovery from cutaneous leishmaniasis gives a life-long immunity against the same *Leishmania* species.

Clinical picture: The manifestations of cutaneous leishmaniasis are variable according to the causative species.

1. *Leishmania tropica*: It causes **dry oriental sore**, **Delhi boil** or **urban cutaneous leishmaniasis**.

- The incubation period is long up to six months.

- The lesion develops on the exposed parts of the body, particularly on the face and hands, as single or multiple lesions.

- It appears as a localized nodule, with granulomatous reaction around.

- The nodule ulcerates after several months and the ulcer appears with sharp cut edges, raised indurated margin and scanty exudates.

- The dry ulcers usually heal spontaneously within a year.

2. *Leishmania major*: It causes **wet sore**, **moist sore** or **rural cutaneous leishmaniasis**.

- The incubation period is short, few days or weeks.

- The lesion usually affects the lower limbs.

- It starts as small itchy papules, at first dry, then becomes moist, thick and brown, forming crusts which fall leaving shallow oozing ulcers with raised margin, granulation tissue at the base and seropurulent exudates.

- Ulceration occurs very early and heals more rapidly than ***L. tropica***.

- Secondary bacterial infection usually occurs.

3. *Leishmania aethiopic*a: It causes **diffuse cutaneous leishmaniasis**.

- The disease is presented by chronic diffuse cutaneous lesions.

- It starts as a single lesion, then spread slowly due to proliferation of the parasites, till the whole body is covered with nodules, but don't ulcerate.

- It is characterized by **low humoral** and **cell-mediated immunity**.

- It is difficult to treat.

Diagnosis:

- **Clinical diagnosis:** The type of lesion is a helpful feature.
- **Laboratory diagnosis:**

I. Direct:

1. Microscopy: For detection of **amastigotes** in**:**

- Smears aspirated or scraped from the **edge of the lesion** and stained with Leishman, Giemsa or Wrights stain.

- Biopsy of skin lesion stained with H & E.

2. Culture: Materials are cultured on NNN media to see **promastigotes**.

3. Animal inoculation.

II. Indirect:

1. Leishmanin skin test (Montenegro test):

- It is helpful, becomes positive few days after infection.

- The test is negative in diffuse cutaneous leishmaniasis.

2. Serological tests: These are of limited value in the diagnosis of cutaneous leishmaniasis as the patient has no detectable level of circulating antibodies.

Treatment:

1. Local measures:

- Surgical excision especially in single lesions.
- Scraping (curettage).
- Plastic surgery for scars or disfiguring nodules.
- Local heating of lesion by infra-red rays or freezing therapy by carbon dioxide.
- Local injection of 10% atebrine solution.
- I.D. injection of interferon gamma around lesions promotes healing of ulcers.
- Cleanliness to prevent secondary bacterial infection.
- Secondary infection needs local or systemic antibiotic.

2. Systemic treatment:

a. Systemic therapy (parenteral)

- **Pentostam** is the drug of choice. Two or three courses may be needed.
- If the sores are 1-3 in number, treatment may be facilitated by local infiltration of the drug into the edges of the ulcers.
- Antimony-resistant cases or diffuse cutaneous leishmaniasis can be treated with pentamidine.

b. Systemic therapy (oral)

- Miltefosine.

Prevention and control:

- Due to sylvatic and rural nature of the disease, it is difficult to control the source of infection.
- Preventive and control measures are similar to those of visceral leishmaniasis.

Case study:

A young man arriving from Jordan after working there for several years. He has a chronic ulcer on his cheek with clean cut edge that resists treatment by antibiotics.

Questions:

1. What is your diagnosis?
2. Illustrate the mode of infection by this disease.
3. How can you investigate such case?
4. Propose a therapeutic scheme for this patient.
5. Develop a control plan for this parasitic infection.

Leishmania mexicana complex and *Leishmania braziliensis* complex

Geographical distribution: They cause **new world cutaneous** and **mucocutaneous leishmaniasis**, respectively, in Central and South America.

Morphology: It is the same as that of other species of *Leishmania*.

Life cycle:

- The life cycle is similar to that of *L. tropica complex* except:
  - **Reservoir host:** Forest rodents, cats and dogs.
  - **Insect vector:** *Lutzomyia* species.
  - **Mode of infection:**
    1. Bite of infected sand fly.
    2. Direct contact.

Pathogenicity and clinical picture:

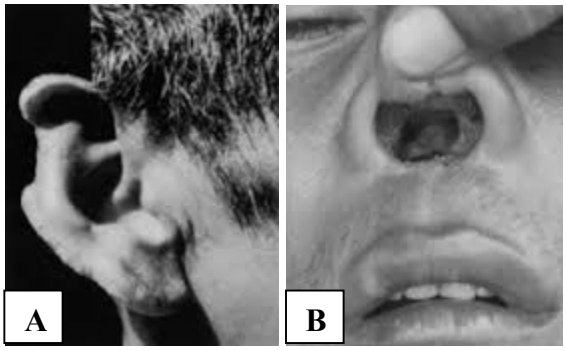
1. *L. mexicana* complex:

- It causes **cutaneous leishmaniasis**.
- Lesion is usually single, causing destruction of ear cartilage (**Chiclero ulcer**).
- It occurs in the forest workers who collect the chicle gum.

2. *L. braziliensis* complex:

- It causes **muco-cutaneous leishmaniasis**.
- Lesion is small painless nodule as with oriental sore, which ulcerates.
- Lymphatic spread occurs.
- Muco-cutaneous lesions in the face generally develop, several years after the cutaneous one and commonly become painful, with erosion of the nasal septum, palate, or larynx which is accompanied by loss of voice.

- Oedema, tissue destruction, scarring of ulcerated lesions and secondary bacterial infection can combine, producing mutilation of the face (**Espundia**).
- Death may develop from aspiration pneumonia, or septicemia.



A. Chiclero ulcer; B. Espundia.

Diagnosis and treatment: As described for cutaneous leishmaniasis.

- In *L. braziliensis*, amastigotes can also be demonstrated in smears taken from lesions of mucous membrane.

Prevention and control: As mentioned for visceral leishmaniasis.

***Leishmania* species and sub-species involved in human diseases**.

Species and sub-species	**Disease**
1. *L. donovani* complex: a. *L. donovani donovani*	- Old World visceral leishmaniasis. - Post kala-azar dermal leishmaniasis
b. *L. donovani infantum*	- Old World visceral leishmaniasis - Infantile kala-azar
c. *L. donovani chagasi*	- New World visceral leishmaniasis
2. *L. tropica* complex: a. *L. tropica*	- Old World cutaneous leishmaniasis - Dry oriental sore or Delhi boil
b. *L. major*	- Old World cutaneous leishmaniasis - Wet oriental sore
c. *L. aethiopica*	- Old World cutaneous leishmaniasis - Diffuse cutaneous leishmaniasis
3. *L. mexicana* complex	- New World cutaneous leishmaniasis - Chiclero ulcer
4. *L. braziliensis* complex	- New world muco-cutaneous leishmaniasis - Espundia

Trypanosoma species

Trypanosomes infecting humans are classified according to the method of development in the insect vector into 2 groups:

1. Salivarian trypanosomes (anterior station development): Trypanosomes in the gut of insect vector migrate anteriorly to the mouth and salivary glands, so the infection is transmitted through saliva.

- Species: *Trypanosoma brucei* complex, causing African trypanosomiasis, sub-species are: ***a. Trypanosoma brucei gambiense***.

b. Trypanosoma brucei rhodesiense.

2. Stercorarian trypanosomes (posterior station development): Trypanosomes migrate to the hind-gut of insect vector and are passed in faeces.

- Species: ***Trypanosoma cruzi***, causing American trypanosomiasis.

Trypanosoma brucei gambiense

Geographical distribution: Central and West Africa.

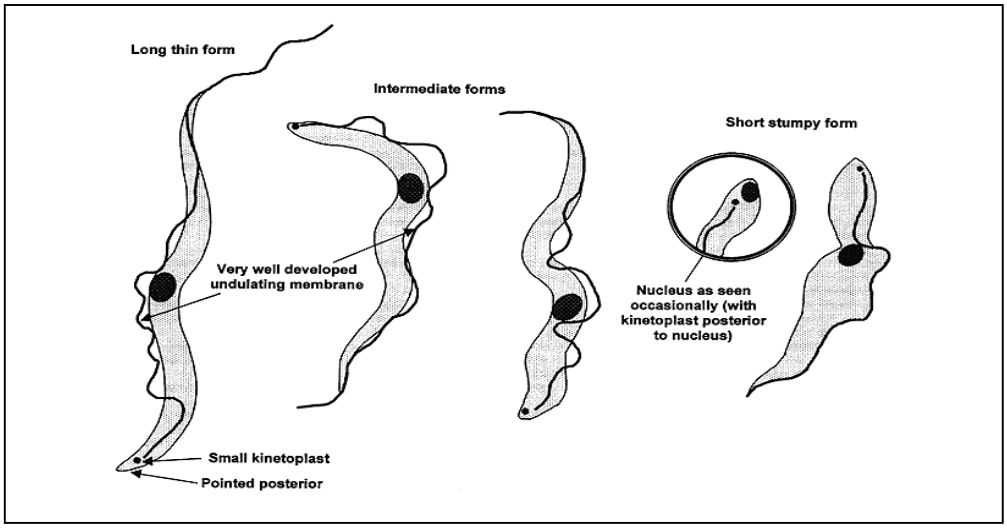
Morphology:

1. In the vertebrate hosts: *T. brucei gambiense* exists as **trypomastigotes**, which have a small subterminal kinetoplast at the posterior end of the parasites, and are **polymorphic**, appearing in 3 forms:

a. **Long slender form:** 30μ, with a long free flagellum and actively motile.

b. **Intermediate form:** 25μ, with a short free flagellum.

c. **Short stumpy form:** 20μ, without a free flagellum and sluggish.



Trypomastigote forms of *Trypanosoma brucei* in vertebrate host.

2. In the insect vector:

a. **Long slender trypomastigotes**.
b. **Epimastigotes**.
c. **Metacyclic trypomastigotes** (short stumpy trypomastigotes).

Life cycle: It passes in 2 hosts.

- Habitat: Blood, lymphatics, lymph nodes, CNS and CSF.

- Vertebrate hosts: Mainly man, although domestic animals as pigs, goats and dogs can act as chronic asymptomatic carriers of the parasite.

- Invertebrate hosts: Both sexes of *Glossina palpalis* and *Glossina tachinoides*.

- Infective stage: Metacyclic trypomastigotes.

Mode of infection:

1. Biological transmission by the bite of infected *Glossina*.
2. Blood transfusion.
3. Congenital.
4. Mechanical transmission of infection by blood-suckling flies.

- Man acquires the infection by the bite of ***Glossina***, where the infective metacyclic trypomastigotes are inoculated in the skin during a blood meal.

- The metacyclic trypanosomes multiply by binary fission at site of inoculation, causing local swelling, called **trypanosomal chancre**.

- The metacyclic forms are transformed into trypomastigotes which spread, via the blood stream and lymphatics, throughout the body, and continue replication by binary fission as **extracellular stages**.

- The tsetse fly becomes infected with trypomastigotes when taking a blood meal from an infected vertebrate host.

- In the midgut, the short stumpy trypomastigotes are transformed into long ribbon forms (**procyclic trypomastigotes**), multiply by binary fission, leave the midgut, and transform into epimastigotes, which reach the salivary glands, and continue multiplication by binary fission, then transform to the **non-dividing metacyclic trypomastigotes** (infective form) in vector saliva.

- The life cycle in vector is a **cyclo-propagative development** of about 3 weeks.

- Tsetse fly harbouring the infective metacyclic forms is infective to man and the cycle is repeated.

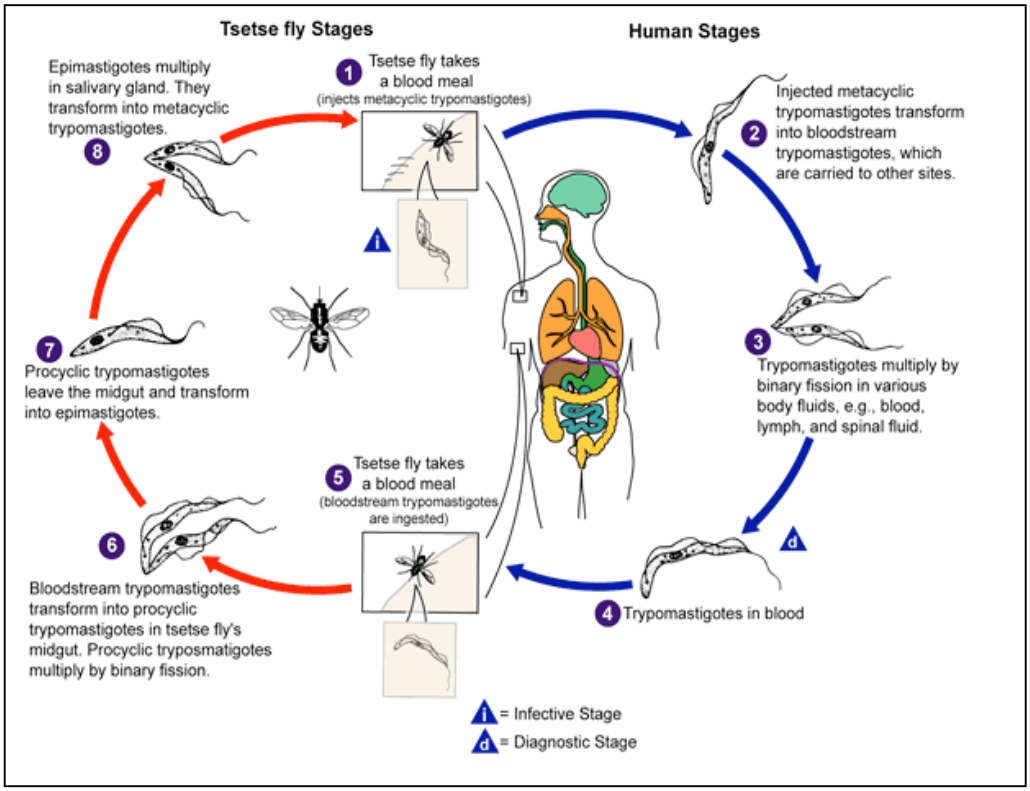
Pathogenicity:

- *T. brucei gambiense* causes **West African trypanosomiasis** (**West African sleeping sickness**). This Gambian form is characterized by:

1. **Trypanosomal chancre**: It is a local inflammatory response at the site of tsetse bite, with intense cellular infiltration, oedema and divided trypomastigotes.

2. Systemic spread of trypomastigotes via tissue fluid, mainly leads to **lymphadenopathy**. The lymph nodes show congestion, haemorrhage and marked macrophages infiltrate, then undergo degenerative changes with excess fibrosis.

3. Spread of trypomastigotes to CNS leads to **chronic meningoencephalitis**. Increases in glial cells occur throughout CNS, and perivascular infiltration with mononuclear cells, leading to ischemic softening of tissues and petechial haemorrhage. There is also neuronal degeneration, and heavy infiltration of meninges with lymphocytes, plasma cells, and **morula cells of Mott**.



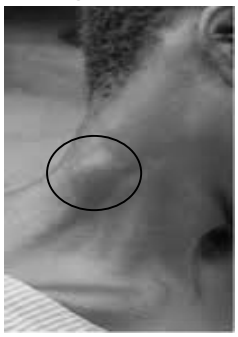
Life cycle of *Trypanosoma brucei*.

Clinical picture:

1. A trypanosomal chancre appears within few days at the site of bite. It is an indurated painful swelling, which lasts for 1-2 weeks.

2. Stage I disease: Characterized by haematogenous and lymphatic dissemination of the parasites.

a. Parasitaemia with irregular headache, fever, rash, joint and muscle pain and anaemia.
b. Enlargement of cervical lymph nodes especially of the posterior cervical region (**Winterbottom's sign**), or generalized lymphadenopathy.



Winterbottom's sign.

c. Hepatosplenomegaly.

3. Stage II disease: It involves invasion of CNS, which occurs after several months, and **sleeping sickness** starts. It is manifested by behavioral and personality changes, such as a mental apathy, slow speech, tremors, involuntary movements and convulsions, abnormalities in the sleep patterns as nocturnal insomnia with sleepiness during the day, hypersomnia and finally coma followed by death from the disease, or concurrent infection.

Diagnosis:

- **Clinical diagnosis:** History of traveling or residence in areas of Africa where the disease occurs.
- **Laboratory diagnosis:**

I. Direct:

Demonstration of trypanosomes in samples from chancre aspirate, blood (thick blood or buffy coat smears), lymph node aspirate, bone marrow, and CSF by:

1. Microscopy:

a. Wet mount smears examination to detect motility of trypanosomes.

b. Examination of Giemsa-stained smears to detect the morphological characteristics of the **polymorphic trypomastigotes**.

2. Culture: The organisms are difficult to grow on NNN medium; hence culture is not routinely used for primary isolation of the parasites.

3. Animal inoculation: Intraperitoneal inoculation of specimens into guinea pigs, rats or mice. The animal is killed after one week and examined for the trypomastigotes and characteristic pathological lesions.

- Posterior nuclear shift phenomenon: A blood sample of a patient with sleeping sickness is injected into a laboratory animal. Examination of animal's blood sample, one week post infection, shows shift of the nucleus of some trypanosomes to the posterior end of the parasites. This phenomenon disappears after repeated sub-passage in the experimental animals.

II. Indirect:

1. Serodiagnosis:

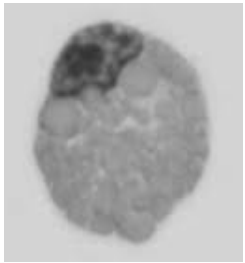
a. Antibody detection: IHA, IFA, CFT, ELISA, and card agglutination test for trypanosomiasis (CATT) are used to detect very high levels of the specific antibodies (**IgM**) in the serum 2-3 weeks after infection. Also, specific antibodies are detected in CSF by IFA and ELISA.

b. Antigen detection: Antigens from serum and CSF can be detected by ELISA.

2. CSF examination:

- There is raised pressure, cell count (mainly lymphocytes) and proteins in CSF.

- Morula cells of Mott, which are atypical plasma cells with unilateral nucleus and many cytoplasm vacuoles, representing stored immunoglobulins. They are pathognomonic of sleeping sickness.



Morula cell of Mott in CSF.

3. Haematological diagnosis:

- Anaemia and thrombocytopenia.
- Moderate leukocytosis.
- High levels of immunogobulins, mainly IgM.

4. Molecular diagnosis.

5. Imaging: CT scan of the brain shows cerebral oedema and MRI shows white matter enhancement in patients with late stage CNS involvement.

Treatment:

1. In stage I, when CNS is not involved:

- Suramin.
- Pentamidine.

2. In stage II, when CNS is involved:

- Melarsoprol (Mel-B): is the drug of choice, as it can pass the blood brain barrier. This drug shouldn't be administered to pregnant women.

3. In both early & late stages of the disease:

- Eflornithine or DFMO (Ornithyl).

Prevention and control:

- Mass treatment of patients.
- Combat of tsetse flies.

Case study:

An African patient presented with enlarged cervical lymph nodes, hepatosplenomegaly, fever and generalized weakness. He had a history of having indurated painful swelling on his face before his complaints.

Questions:

1. What is your possible diagnosis and the causative parasite?
2. If blood film is negative, what other specimens may reveal the organism?
3. Illustrate the mode of infection in such case.
4. What change in the serum proteins is highly suggestive of active infection?
5. Predict the complication that can occur if this infection is untreated.

Trypanosoma brucei rhodesiense

Geographical distribution: East and Central Africa.

Morphology, habitat and life cycle: They are similar to *T. brucei gambiense*, but the disease is actually a **zoonosis**, with the reservoir hosts are wild animals as antelope, and domestic animals as cattle, and the vector is *Glossina morsitans*.

Pathogenicity and clinical picture:

- ***T. brucei rhodesiense*** causes **East African trypanosomiasis** (**East African sleeping sickness**). The disease is similar to Gambian form with some variations:
- Short I.P. with more rapid and fatal course.

- CNS is involved early and patients usually die before reaching the sleeping sickness stage.

- Fever and rigors are more frequent and severe.
- Trypanosomes appear in blood early with plentiful numbers.
- Myocarditis and emaciation are prominent.
- Lymphadenopathy is less prominent.

Diagnosis: As in *T. brucei gambiense*, but trypanosomes are plentiful in blood and show more **posterior nuclear shift** after animal inoculation with blood.

Treatment:
- Must be early and suramin is the drug of choice.
- In case of neurological involvement, melarsoprol can be given.

Prevention and control:
- Treatment of patients.
- Control of vectors.

Differences between West African and East African trypanosomiasis.

Characteristics	**West African**	**East African**
1. Organism	*Trypanosoma brucei gambiense*	*Trypanosoma brucei rhodesiense*
2. Distribution	West and Central Africa	East and Central Africa
3. Insect vectors	*Glossina palpalis* & *Glossina tachinoides*	*Glossina moristans*
4. Reservoirs	Mainly humans	Mainly animals as antelopes, pigs, goats, dogs and cattle
5.Course of the disease	Chronic	Acute
6. Lymphadenopathy	More common	Less common
7. Mortality rate	Low	High
8. Trypanosomes in the peripheral blood	Few	Numerous and appear early
9. Virulence to laboratory animals	Less virulent	More virulent
10. Posterior nuclear shift phenomenon	Rare	Common

Trypanosma cruzi

Geographical distribution: South and Central America.

Morphology:

1. In the vertebrate hosts: *Trypanosoma cruzi* exists in both amastigote and trypomastigote forms.

a. **Amastigotes:** They are multiplying intracellular parasites.

b. **Trypomastigotes**: They are non-multiplying extracellular forms, **monomorphic**, C- or S-shaped, with large kinetoplast and wedge-shaped posterior end.

2. In the insect vector: Trypomastigotes, epimastigotes and metacyclic trypomastigotes.

Life cycle:

- Habitat:

a. Amastigotes in the cells of striated muscles (heart and skeletal), neurological cells of the nervous tissues and inside the cells of the reticuloendothelial system.

b. Trypomastigotes in the peripheral blood.

- Definitive host: Man.

- Insect vector: *Triatoma megista* (winged bug or of reduviid bug).

- Reservoir host: Wild animals as armadillos &opossums, and domestic animals.

- Infective stage: Metacyclic trypomastigotes.

Mode of infection:

1. Contamination of skin wounds, conjunctiva and mucous membranes by bug's faeces, containing the infective metacyclic trypomastigotes.
2. Blood transfusion.
3. Organ transplantation.
4. Accidental laboratory-acquired infection.
5. Transplacental.

- Man acquires the infection when the infective metacyclic trypanosomes in faeces of **night-biting *Triatoma*** are deposited during blood meal, and rubbed into the bite puncture, skin abrasion or mucous membranes as conjunctiva.

- Metacyclic trypanosomes are engulfed by local histiocytes, transform to amastigotes and multiply by binary fission, producing skin swelling (**Chagoma**).

- Infected cells rupture, liberating amastigotes which transform into trypomastigotes, invading circulation with dissemination to a variety of tissues.

- The bloodstream trypomastigotes do not replicate (different from the African trypanosomes). Replication resumes only when the parasites enter another cell.

- Kissing bug is infected by feeding on host blood contains trypomastigotes.

- In the insect vector there is a **cyclo-propagative development**, as the ingested trypomastigotes transform into epimastigotes in the midgut, where they multiply.

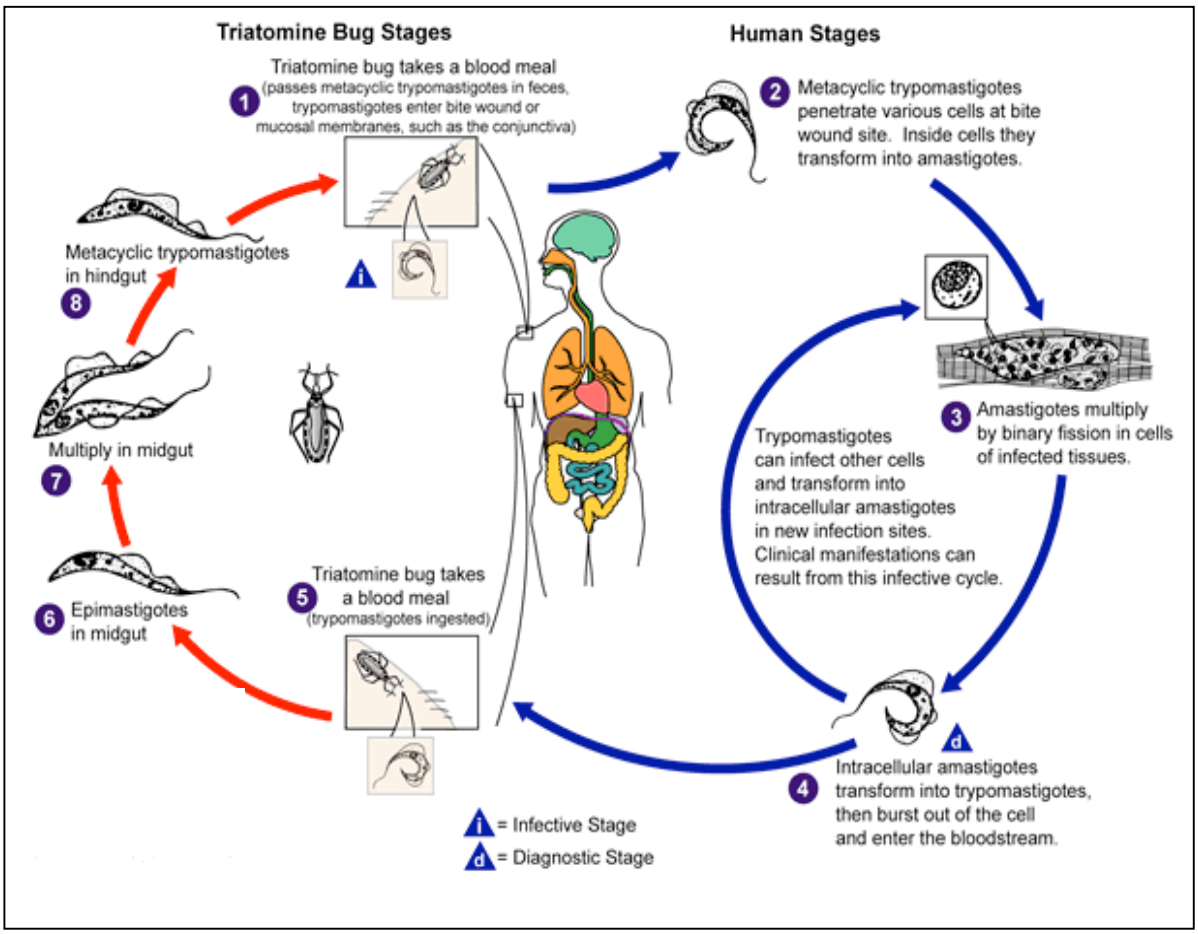
- After 10 days, the epimastigotes differentiate into **non-dividing metacyclic trypomastigotes** (infective form) in the hindgut, that pass out with faeces during blood meal and the cycle is repeated.

Pathogenicity and clinical picture:

- ***T. cruzi*** causes **Chagas' disease** or **South American trypanosomiasis**. Pathogenicity depends on the affected parasitized cells and stage of the disease.

1. Acute stage: It occurs 1-2 weeks after infection and may last for 1- 4 months.

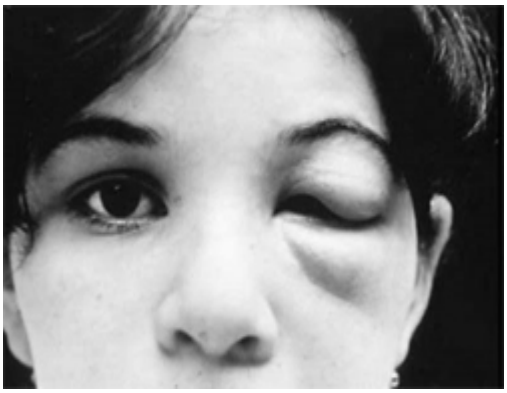
- It is often seen in children and infants.



Life cycle of *Trypanosma cruzi*.

a. Chagoma: A localized oedematous swelling with erythema at the site of inoculation accompanied with local lymphadenopathy. It contains multiplying amastigotes in histiocytes.

b. Romana's sign: Inoculation of the parasite in conjunctiva causes unilateral facial oedema of cheek, upper and lower eyelids, usually with conjunctivitis and enlargement of ipsilateral pre-auricular lymph nodes.



Romana's sign.

c. In few patients, there may be **fever**, **generalized lymphadenopathy**, **hepatosplenomegaly**, toxic depression of bone marrow and **anaemia**.

- In severe infections, the patients may die of meningoencephalitis and acute myocarditis and acute congestive heart failure.

- Usually within 1-2 months, acute manifestations resolve and patients enter the asymptomatic or intermediate phase of chronic *T. cruzi* infection.

2. Chronic stage:

-It is found in adults and adolescents and becomes manifested years or even decades after the initial infection.

-Multiplication of *T. cruzi* inside the tissue cells causes inflammatory response, irreversible cellular destruction and fibrosis of muscles and nerves that control the tone of hollow organs, this is manifested by:

- **Cardiomegaly**, cardiac arrhythmias and congestive heart failure.

- **Megaoesophagus**, due to destruction of intramural plexus; manifested by dysphagia and aspiration pneumonia.

- **Megacolon**, due to destruction of mesenteric plexus; manifested by intractable constipation and abdominal distention.

- **Other megaviscera**, as the small intestine, urinary bladder and uterus.

- **Thyroiditis** and thyroid insufficiency.

- Less commonly, peripheral nervous involvement causing **spastic paralysis**.

- Immunosuppression results in exacerbation of infection.

Diagnosis:

- **Clinical diagnosis:** History of traveling or residence in areas of South and Central America where the disease occurs.

- **Laboratory diagnosis:**

I. Direct:

Diagnosis is made by demonstration of *T. cruzi* in peripheral blood or tissue biopsy of involved lymph node or muscle (calf and deltoid), liver, spleen and bone marrow by:

1. Microscopy:

a. Examination of wet mount of peripheral blood reveals motile trypomastigotes. Thin and thick blood smears, stained with Giemsa, shows **monomorphic trypanosomal forms in C** or **S shape**. **However, a blood smear works well only in the acute stage when parasites are seen circulating in blood.**

b. H & E stained tissue specimens show **amastigotes**.

2. Culture: It is more sensitive than smear microscope. **Epimastigotes** and **trypomastigotes** are found on the NNN medium, 1-6 weeks after incubation.

3. Animal inoculation: Intra-peritoneal inoculation of specimens into guinea pig or mice. After 10 days, blood is collected and examined for the **trypomastigotes**.

4. Xenodiagnosis: This is the method of choice in suspected Chagas' disease, if other methods failed to **detect very low parasitaemia, especially during the early phase of the disease**. It depends on feeding a clean bred triatomine bugs on the suspected patient blood, 2-4 weeks later, dissection of the *Triatoma* gut reveals **epimastigotes** and **metacyclic trypomastigote** forms.

II. Indirect:

1. Immunological diagnosis:

a. Serological tests: They are the methods of choice for diagnosis of **chronic Chagas' disease**. Diagnosis is made by testing with at least two different serologic tests.

- Antigen detection: *T. cruzi* antigens can be detected in urine and sera using ELISA.
- Antibody detection: Antibodies **(IgG)** against *T. cruzi* can be detected by:

IHA, IFA, ELISA, CFT, DAT, and Chagas´ Stat-Pak rapid immunochromatographic test. False positive results are common with other disease as **Leishmaniasis**.

b. Cruzin test: It is an intradermal test used for the diagnosis of Chagas' disease. The antigen (cruzin) used is prepared from cultured trypanosomes. It gives a delayed hypersensitivity reaction in positive cases.

2. Molecular diagnosis: Can be used for diagnosis of **chronic Chagas' disease**.

3. Endoscope: It is valuable for diagnosis of megaviscera.

4. Barium dye meal and barium dye enema: They help in visualization of megaoesophagus and megacolon, respectively.

5. X-ray chest and **electrocardiography (ECG):** They are useful for diagnosis and prognosis of cardiomyopathy seen in **chronic Chagas' disease**.

Treatment:

1. Nifurtimox (lampit): It inhibits intracellular development of *T.cruzi*. It is the drug of choice in treatment of acute and early chronic cases.
2. Benznidazole (Radanil).
3. Diuretics: for treatment of congestive heart failure.
4. Treatment of megacolon: if possible, is dependent on removal of the aganglionic undilated segment and the redundant portion of the dilated segment.

Prevention and control:

1. Control of winged bugs.
2. Treatment of cases.
3. Personal protection by using repellants and bed net.
4. Control of reservoir hosts.
5. Serological screening of blood donors for ***T. cruzi***.

Case study:

A South American patient with a past history of unilateral oedema of eyelids and face, is complaining now of irregular heartbeats and severe constipation. On examination, ventricular arrhythmia was observed. X ray after barium dye meal showed marked enlargement of colon.

Questions:

1. Mention the possible parasitic cause and the mode of infection.
2. Propose other diagnostic procedures for this case.
3. Develop a control plan for this parasitic infection.

CILIOPHORA

Balantidium coli

Geographical distribution: Worldwide, especially among people in close contact with pigs.

Morphology:

Balantidium coli occur in 2 stages:

1. Trophozoite:

- It is the largest protozoan parasite of humans, average size 150 x 50 μ.
- Oval in shape, body covered with cilia as organ of locomotion.
- The anterior end is provided with a prominent V-shaped **cytostome** (mouth), and the posterior end has a prominent **cytopyge** (anus).
- The parasite has 2 nuclei, a large kidney-shaped **macronucleus** situated in the middle of the body and concerned with **vegetative functions** (nutrition and movement), and a small rounded **micronucleus** lies in the depression of the macronucleus and concerned with **generative functions** (multiplication).
- The cytoplasm also has food vacuoles, and 2 contractile vacuoles for excretion.

2. Cyst:

- Spherical, measures 50 μ.
- Has a thick and transparent double- layered wall.
- The cytoplasm has macronucleus, micronucleus, and contractile vacuoles.
- Cilia may be present in young cysts.

Life cycle:

- **Habitat:** Large intestine (especially the caecum).
- **Definitive host:** Pigs (natural host) and man (accidental host).
- **Reservoir hosts:** Pigs, monkeys and rats.
- **Infective stage:** Cyst.

Mode of infection: Through ingestion of cysts with food or drink contaminated with pigs and other animal reservoirs or human excreta.

- Following ingestion, excystation occurs in the small intestine, and each cyst gives a single trophozoite which migrates to large intestine.
- The trophozoites reside in the lumen of large intestine, where they replicate **asexually by transverse binary fission** and **sexually by conjugation**, with exchange of nuclear material, followed by separation of the two organisms.

- After a period of multiplication and growth, trophozoites undergo encystation and cysts are passed with faeces and the cycle is repeated.

Pathogenicity:

- In healthy individuals, *B. coli* lives in the lumen of large intestine without invasion of the intestinal wall.

- *B. coli* causes **balantidial dysentery** when the resistance of host is lowered.

- The disease occurs when trophozoites invade colonic mucosa and submucosa, which is facilitated by the **cytolytic enzymes** (hyaluronidase) produced by the parasite and the **boring action of its cilia** (tissue invader in man).

- Balantidial invasion may be followed by 2ry bacterial infection and formation of flask-shaped ulcers, with wider mouth than in *E. histolytica*, but with **no invasion of blood vessels** and **no extra-intestinal metastasis**, while perforation and peritonitis are more common.

Clinical picture:

1. Most infections are asymptomatic.
2. Symptomatic disease:
   - **a.** Acute balantidial dysentery: It resembles amoebiasis causing dysentery with abdominal colic, nausea, vomiting and marked loss of weight.
   - **b.** Fulminating infection may occur with **intestinal perforation** and **peritonitis** which may progress rapidly to death.
   - **c.** Chronic balantidiasis with intermittent diarrhea.

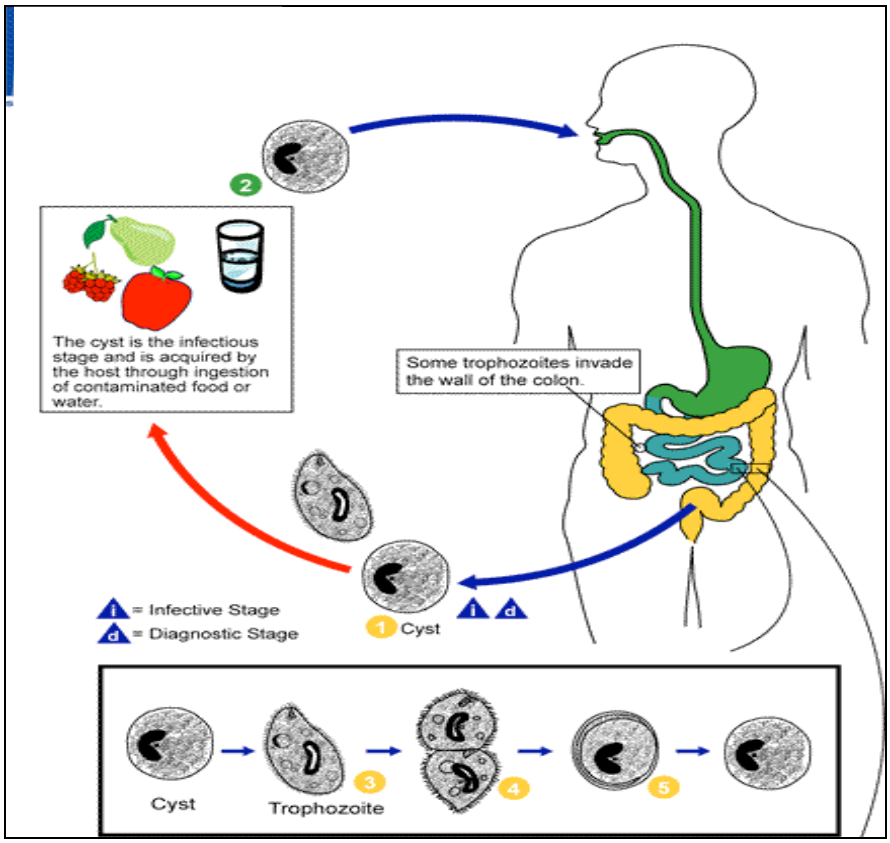
Diagnosis:

- Clinical diagnosis: History of close contacts with swine and manifestations of dysentery.

- Laboratory diagnosis:

1. Microscopy: Stool examination for **cysts in formed stool and/or trophozoites in diarrheic stool**. *B. coli* are excreted intermittently and once outside the colon are rapidly destroyed. Thus, stool specimens should be collected repeatedly, and immediately examined or preserved to enhance detection of the parasite.

2. Endoscopy: When stool examination is negative, for visualization of lesion. Also, biopsy specimens can be examined for presence of trophozoites.



Life cycle of *Balantidium coli*.

Treatment:

1. Tetracycline is the drug of choice.
2. Oxytetracycline.
3. Metronidazole (Flagyl).

Prevention and control:

1. Treatment of infected patients.
2. Sanitary disposal of stool.
3. Avoid contact with pigs.
4. Good personal hygiene.

Case study:

A 30-year-old male with a history of rearing pigs, complained of dysentery with diarrhea. Stool examination revealed mucus, RBCs, and active motile protozoa.

Questions:

1. What is your suggestive diagnosis?
2. What type of host is the pig?
3. Demonstrate the infective stage.
4. What are the possible complications and treatment of this infection?

APICOMPLEXA

Sporozoa or Coccidia

General characters:

1. The coccidian are unicellular protozoa belonging to the phylum Apicomplexa.
2. They live **intracellular**, at least during a part of their life cycle.
3. They do not possess any organs of locomotion, but at some stages (**sporozoites, merozoites** and **ookinete**) in their life cycle, possess a structure called **apical complex**, by which they can attach to and penetrate host cells.
4. All coccidian have **sexual gametogony phase** and **asexual schizogony phase**.
5. Many of them show an alternation of hosts; a definitive and intermediate host.
6. They include:
   a. **Haemosporina**: *Plasmodium* and *Babesia*.
   b. **Eimeriorina**: *Toxoplasma gondii*, *Cryptosporidium parvum*, *Cystoisospora belli* and *Cyclospora cayetanensis*.

Haemosporina

Plasmodium

Species:

Four species of *Plasmodium* cause human malaria:

1. *Plasmodium vivax*: Benign tertian malaria.
2. *Plasmodium ovale*: Benign tertian malaria.
3. *Plasmodium malariae*: Benign quartan malaria.
4. *Plasmodium falciparum*: Tertian or subtertian malignant malaria.

Geographical distribution:

- *P. vivax*: The most widely distributed species found in tropical, subtropical and temperate areas.
- *P. ovale*: West Africa.
- *P. malariae*: Tropical Africa and Far East.
- *P. falciparum*: Africa and Far East.

Life cycle:

- Definitive host: **Female *Anopheles* mosquito**.
- Intermediate host: **Man**.
- Reservoir host: **No**. **However, in *P. malariae*, chimpanzee can be affected and act as a reservoir of infection**.
- **Habitat:**

  In mosquito: Gut, salivary glands.

In man: Intracellular inside the liver cells and RBCs.

- Infective stage:

a. Sporozoites (in **mosquito-borne** malaria).

b. Merozoites and/or trophozoites (in **blood-borne** malaria).

Mode of infection:

1. Bite of infected female ***Anopheles***.
2. Blood-borne transmission:
   a. Blood transfusion (whole blood and packed RBCs).
   b. Shared syringes among drug addicts.
   c. Transplacental transmission.
   d. Organ transplantation.

- The life cycle of malaria parasites is **heteroxenous** (alternation of generations between two hosts), where an **asexual cycle** occurs in man (**intermediate host**), and **sexual cycle** occurs in female *Anopheles* (**definitive host**).

I. Human cycle (Asexual cycle):

- In this cycle the malaria parasites multiply **asexually** by division; **schizogony**, which occurs in 2 sites, in the liver cells **(exoerythrocytic schizogony)** and in the RBCs **(erythrocytic schizogony)**.

1. Exoerythrocytic schizogony or merogony (Tissue phase):

a. Initial tissue phase:

-During blood meal, a malaria-infected female *Anopheles* inoculates **sporozoites** with saliva into human host, which are carried within 30 minutes, by blood stream to the liver, and form **parasitophorous vacuoles** in hepatocytes.

-The spindle-shaped **sporozoites** become rounded and transform into **trophozoites**, which multiply by schizogony, resulting in formation of thousands of pear-shaped **merozoites** with enlarged infected liver cells.

-The mature schizont and the infected liver cells rupture in 6-15 days, releasing thousands of merozoites into the blood stream, with **no clinical symptoms**.

-The interval between the inoculation of sporozoites into the human host and the first appearance of malaria parasite in blood is called the **pre-patent period**.

b. Latent tissue phase:

- In ***P. vivax*** and ***P. ovale***, some sporozoites remain dormant in liver cells as **hypnozoites**. Months or years later, some hypnozoites are activated, start exo-erythrocytic schizogony, and release merozoites invading RBCs causing **relapse**.

Comparison of exo-erythrocytic phases of human malaria parasites.

Differences	*P. vivax*	*P. ovale*	*P. malariae*	*P. falciparum*
1. Duration in days	8	9	15	6
2. Hypnozoites	+	+	-	-
3. No. of merozoites	10.000	15.000	2.000	30.000

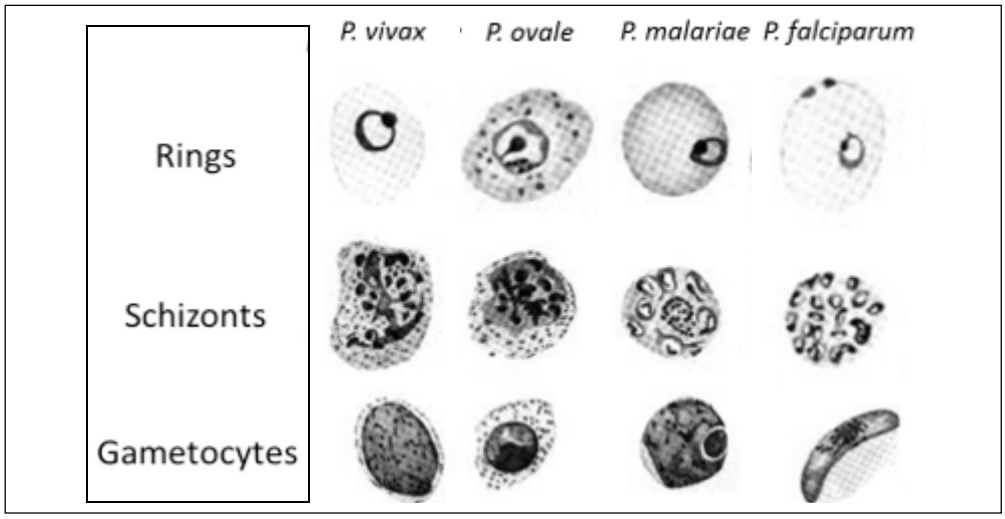
2. Erythrocytic schizogony or merogony:

- The merozoites released by exo-erythrocytic schizogony attach to the RBCs by their **apical complex**, and then lie within an intra-erythrocytic **parasitophorous vacuoles** formed by red cell membrane, by a process of invagination.
- In the infected RBC, the merozoite appears rounded with vacuolated cytoplasm and the nucleus at one pole. This parasite is called the **ring stage** or **young trophozoites**.
- Young trophozoite feeds on the haemoglobin of RBC. The degradation products of haemoglobin appears as residual pigment granules inside the cytoplasm of the parasite, called **malaria pigment** or **haemozoin pigment**. Also, **stippling** occurs in the cytoplasm of **infected RBCs**.
- As the ring stage develops, it enlarges in size and becomes irregular in shape. This is called the **old trophozoite**.
- The nucleus of old trophozoite divides by mitosis followed by division of cytoplasm to become mature **schizonts** within 2-3 days.
- The mature schizont contains **8-32 merozoites** and **haemozoin**. The cytoplasm not sharing in the formation of merozoites is called the **residual body**.
- The mature schizont ruptures releasing the merozoites, haemozoin and residual body into the circulation. Therefore, the typical **malarial paroxysms** occur by the 3rd or the 4th day, and malaria is described as tertian or quartan.
- Erythrocytic merozoites can re-invade new RBCs and repeat the erythrocytic cycle destroying each erythrocyte they infect, but never re-invade liver cells.

3. Gametogony:

- After few erythrocytic cycles, some merozoites invade new RBCs and instead of developing into trophozoites and schizonts, develop into sexually differentiated forms, **gametocytes**, where maturation is completed in 4 days.
- The mature gametocytes are round-shaped (***P. vivax, P. ovale*** and ***P. malariae***) or crescent-shaped (***P. falciparum***), with prominent pigment granules.

- The female gametocyte is large (**macrogametocyte**) with compact eccentric nucleus and pale blue cytoplasm, while the male gametocyte is small (**microgametocyte**) with large central nucleus and pale blue cytoplasm.
- Gametocytes do not cause any febrile illness in the host and individual who harbours gametocytes is a carrier. They are produced for propagation of species.
- Gametogony starts inside RBCs of intermediate host and is completed in the mosquito, the definitive host.



Stages of erythrocytic schizogony of human malaria parasites.

II. Mosquito cycle (Sexual cycle or Sporogony):

- When female *Anopheles* ingests parasitized RBCs during a blood meal, all parasitic stages are digested in the stomach, except micro- and macro-gametocytes, which start a complex cycle of **cyclo-propagative development**.
- They escape from their RBCs envelope, and from one **microgametocyte,** 4-8 microgametes are developed by process of division called **exflagellation**. While, **macrogametocyte** matures by a process of **nuclear reduction** division giving rise to only one macrogamete.

Exflagellation: It is a process in which the male microgametocyte in the stomach of female *Anopheles*, undergoes division of its chromatin into 6-8 nuclei that migrate to the periphery of the parasite with part of the cytoplasm. They form several whip-like actively motile filaments (uninuclear microgametes), which then detach from the parent cell forming the individual microgametes.

- After half to two hours of the blood meal, one of the male gametes fertilizes the female gamete forming a rounded **zygote**.

- The **zygote** elongates and develops into a **motile ookinete** with an apical complex.

- **Ookinete** penetrates the gut wall, comes beneath the basement membrane, secretes a thin wall and develops into a spherical **oocyst**.

- The **oocyst** undergoes asexual division by **sporogony**, and thousands of **sporozoites** are formed. Rupture of oocyst will release sporozoites into the body cavity of female *Anopheles*, where some find their way to salivary glands.

- Sporogony is completed in about 1-4 weeks.

- When female *Anopheles* takes a blood meal from another human, the sporozoites are injected with the mosquito's saliva and the cycle is repeated.

Pathogenicity of malaria:

The major clinical manifestations of malaria are due to the products of erythrocytic schizogony and host´s reaction to them.

I. Destruction of parasitized RBCs:

-Rupture of infected RBCs at the end of a schizogony cycle results in:

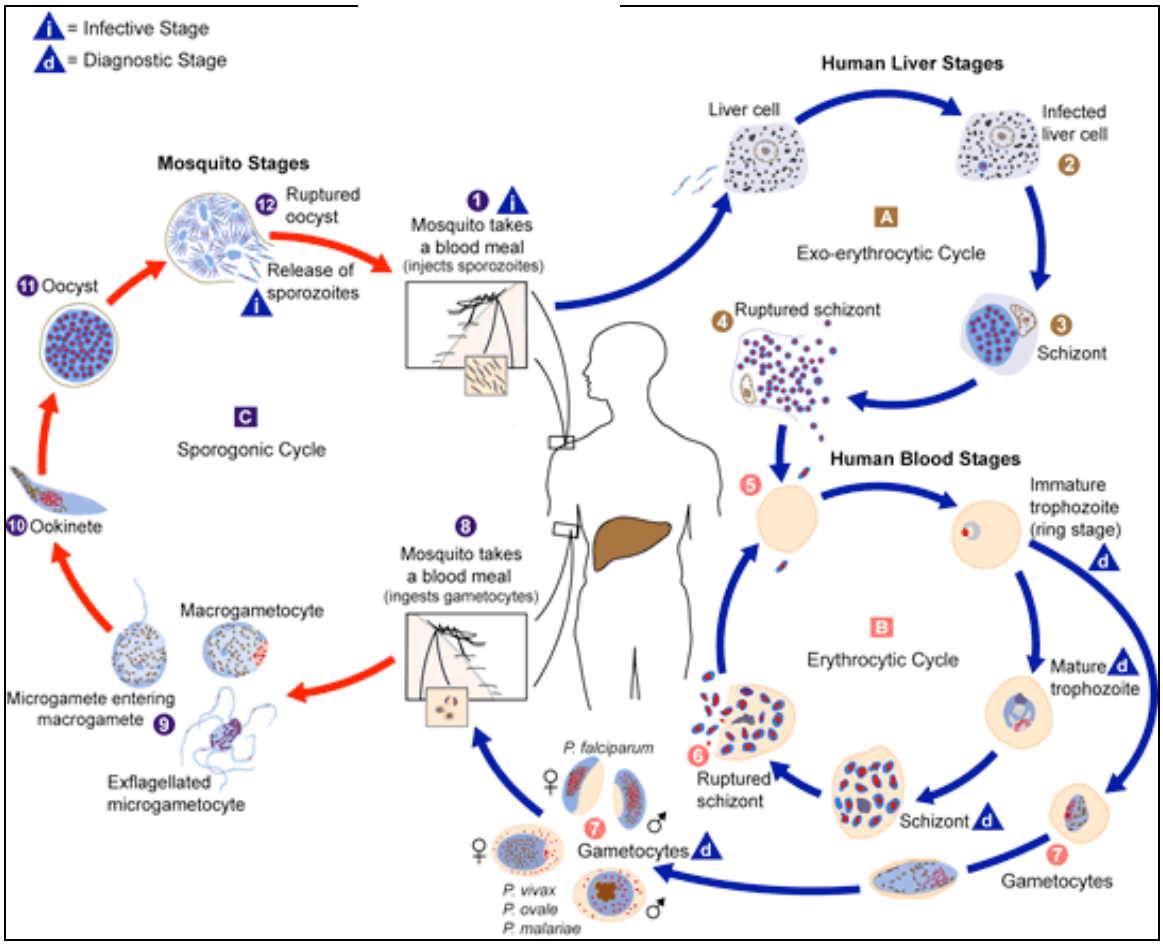
a. **Tissue hypoxia** because of reduction of blood flow by parasitized RBCs and subsequent **fatty degeneration of liver and spleen**.

b. **Release of haemozoin and parasite metabolites** in blood stream resulting in **hepatosplenomegaly**. The soft, large spleen becomes susceptible to spontaneous rupture and in chronic infection it becomes firm and fibrotic. Kidneys are also enlarged and congested.

c. **Haemolytic anaemia** and **jaundice**.

Causes of anaemia in malaria:

1. Obligatory destruction of RBCs at merogony.
2. Destruction of large number of RBCs by complement-mediated and autoimmune hemolysis.
3. Increased clearance of both parasitized and non-parasitized RBCs by the spleen.
4. Decrease erythropoiesis in bone marrow due to increased tumour necrosis factor.
5. Shortened red cell survival.
6. Failure of the host to recycle the iron bound in haemozoin pigments.



Life cycle of malaria parasites.

II. Host inflammatory response:

Occurs as an immune response of the host to the liberated parasite metabolites and malaria pigments.

a. **Fever** coincides with rupture of erythrocytic schizont with release of merozoites, parasitic pigments, and residual body in the blood stream. These materials activate tissue macrophages, which in turn produce interleukin-1, tumour necrosis factor and pyrogens which cause fever.

b. **Activation of complement and immune complexes formation** as a result of the antigen excess situation in chronic quartan malaria may lead to the deposition of these circulating antigen-antibody complexes within renal glomeruli leading to **nephrotic syndrome**.

III. Additional pathology associated with *P. falciparum*:

a. In *P. falciparum* infection, erythrocytic schizogony takes place in capillaries of internal organs as brain, kidney, spleen, bone marrow, and intestine. Knobs formation on the surface of RBCs infected with late stages of parasites (**during the second half of the 48 hour life cycle**) and the resulting increase in rigidity, lead to their adherence to receptors on the

endothelium of internal capillaries, a phenomenon termed **cytoadherence**. Also, infected RBCs adhere to uninfected RBCs, resulting in **rosetting**. These lead to **sequestration** of RBCs which ultimately block blood flow, with subsequent infarctions and haemorrhage, mainly in brain and large intestine. All these factors contributing to the development of severe disease (**malignant malaria** or **pernicious syndrome**).

b. **Acute renal failure**, tubular necrosis from tissue anoxia.
c. **Black water fever** (malarial haemogloblinuria) due to massive intra-vascular haemolysis caused by anti-erythrocyte antibodies, leading to massive absorption of haemoglobin by renal tubules with its passage in urine causing haemoglobinuria (red urine). Sometimes, the haemoglobin is transformed into met-haemoglobin in the renal tubules, causing black-coloured urine; **black water**.
d. **Adrenal and retinal haemorrhage**.
e. **Pulmonary oedema** due to disseminated intravascular clotting.
f. **Cardiac oedema**, blocked capillaries and degenerated foci.
g. **Spontaneous abortion**.

Clinical picture:

1. **Incubation period:**

- It is the interval between the inoculation of the sporozoites into the human host and appearance of the earliest manifestation of the disease (1st paroxysm).
- It represents the duration of exo-erythrocytic cycle.
- Patient may feel malaise, muscle pain, headache, loss of appetite and fever.

2. **Malarial paroxysms:**

The typical picture of malaria consists of series of **febrile paroxysm**, followed by **anaemia** and **splenomegaly**.

The febrile paroxysm occurs in 3 successive stages; cold, hot and sweating.

a. **Cold stage:** Intense cold and uncontrollable shivering for 15-60 minutes.
b. **Hot stage:** Intense heat, flushing, nausea, vomiting and severe headache, lasting for 2-6 hours.
c. **Sweating stage:** Decreased temperature and profuse sweating, lasting for 2- 3 hours.

- The paroxysm usually begins in the **early afternoon** and lasts for 8-12 hours.

- It synchronizes with the erythrocytic schizogony cycle. With a 48-hour cycle, the **fever recurs every third day; tertian malaria**, and with 72-hour cycle, the **fever recurs every fourth day; quartan malaria**.

3. Anaemia of microcytic or a normocytic hypochromic type and **jaundice**.

4. Splenomegaly and **hepatomegaly**.

5. Tropical splenomegaly syndrome (**TSS**) or **hyper-reactive malarial syndrome (HMS):** A chronic benign condition occurs with any type of plasmodia, seen in some adults in endemic areas. This results from abnormal immunological response to malaria and is characterized by:

- Hypersplenism and hepatomegaly.
- High titers of circulating anti-malarial antibodies.
- Hypergammaglobulinemia (**IgM**).
- Presence of circulating immune complex.
- Absence of malaria parasites in peripheral blood smears.
- Normocytic normochromic anaemia which does not respond to haematinics or antihelmentics.
- Differs from other types of splenomegaly in its response to anti-malarial drugs.

6. Nephrotic syndrome (oedema, proteinuria and hypo-albuminaemia) in ***P. malariae*** infection.

7. Pernicious malaria (acute falciparum malaria): It is a series of phenomena occurring in ***P. falciparum*** infection, which if not treated, threatens patient´s life.

Clinical types:

a. **Cerebral malaria**: Manifested by headache, hyperpyrexia, coma and paralysis.

b. **Black water fever**: It is seen in patients with repeated ***P. falciparum*** infection and inadequate treatment with quinine. Clinical manifestations include vomiting, prostration with passage of dark red or black urine. This condition may be complicated with acute renal failure and circulatory collapse.

c. **Algid malaria**: Characterized by peripheral circulatory failure, rapid pulse, low blood pressure, cold wet skin and profound shock. There may be severe abdominal pain, vomiting (gastric type), watery diarrhea (choleric type), or passage of blood in feces (dysenteric type).

d. **Septicaemic malaria**: It is characterized by high continuous fever with dissemination of parasite to various organs, causing multiorgan failure.

8. Recurrence of malarial attack:

a. Relapse: It is the recurrence of clinical manifestations of malaria and the re-appearance of peripheral parasitaemia months or years after subsidence of a previous attack, in the absence of a new mosquito bite.

- **Species:** Relapse occurs in ***P. vivax*** and ***P. ovale*** (infections last up to 4 years).

- **Cause:** It is due to activation of the dormant **hypnozoites** initiating exo-erythrocytic schizogony, with the production of erythrotropic merozoites.
- Can be prevented by giving primaquine to eradicate hypnozoites.

b. Recrudescence: It is a recurrence of clinical attack of malaria, few weeks or many years after apparent clinical cure, without re-infection.

- **Species:** Recrudescence can occur in **all *Plasmodium* species**, but it is more common in ***P. falciparum*** (up to 2 years) and ***P. malariae*** (up to 40 years).
- **Causes:** It results from the persistence of some **erythrocytic parasites** at a sub-clinical level, which start to multiply to reach significant numbers. It may be due to:

a. Incomplete anti-malarial therapy.
b. Anti-malarial drug resistance.
c. Changes of the surface antigens (**antigenic variation**) of the parasites resulting in evasion of the host immune response.
d. Splenectomy or immunosuppression.

- Can be prevented by adequate drug therapy or use of new antimalarial drugs in case of drug resistance.

Malaria is classified as severe when any of the following criteria is present:

1. Decreased consciousness.
2. Significant weakness.
3. Two or more convulsions.
4. Low blood pressure (< 70 mmHg in adults and 50 mmHg in children).
5. Breathing problems.
6. Circulatory shock.
7. Kidney failure or haemoglobinuria.
8. Bleeding problems, or hemoglobin less than 50 g/L.
9. Pulmonary oedema.
10. Blood glucose less than 40 mg/dL.
11. Acidosis or lactate levels of greater than 5 mmol/L.
12. A parasite level in the blood of greater than 100,000/µl in low-intensity transmission areas, or 250,000/µl in high-intensity transmission areas.
13. Parasite count greater than 2% in non-immune patient.

Diagnosis:

- **Clinical diagnosis:** In endemic areas, malaria must be suspected in all cases of typical malarial paroxysm or fever.

- Laboratory diagnosis:

1. Parasitic diagnosis: Examination of **thin** and/or **thick Leishman** or **Geimsa-stained blood smears**. All erythrocytic stages can be detected in peripheral blood **except in *P. falciparum***, **only ring form alone or with gametocytes** can be detected.

- **Provocative tests** are indicated in chronic infection, when no parasites are seen in peripheral blood. This may be done by subcutaneous injection of 0.5 ml adrenaline **(Ascoli's test)**, injection of TAB vaccine, milk or cold shower. So, the spleen contracts and squeezes its blood content to the peripheral circulation.

2. Therapeutic diagnosis: The non-subsidence of the febrile paroxysms after the administration of anti-malarial drug for 3 days, means that the case is not malaria.

3. Serodiagnosis:

a. Circulating antibodies can be detected by IHA, IFA and ELISA.

b. Circulating antigens can be detected by ELISA.

c. **Rapid immunochromatographic test** for detection of malaria antigens by using a dipstick impregnated with specific monoclonal antibodies.

4. Molecular diagnosis.

5. Haematological diagnosis: Anaemia and reticulocytosis.

6. Biochemical diagnosis:

- Hypergammaglobulinemia and low albumin level.
- Hyperglycemia or hypoglycemia.
- Hyperkalemia.

Malarial survey:

Estimation of malarial endemicity in a locality is important for preventive and control measures. In the life cycle of malaria parasites, there is alternation of hosts, man (intermediate host) and female *Anopheles* (definitive host). So, malarial survey includes both human and mosquito factors.

1. Human factors:

A representative population sample of a certain locality is examined for:

a. **Parasitic index:** For parasitic stages in the peripheral blood.
b. **Haemoglobin index:** For haemolytic anaemia.
c. **Splenic index:** For splenomegaly.

2. Mosquito factors:

Anopheline mosquitoes are collected and examined for the percentage distribution of males and females. The predomination of females indicates high endemicity. A sample of females are dissected and examined for:

a. **Oocyst index:** For prevalence of oocysts in the wall of the stomach.
b. **Sporozoite index:** For prevalence of sporozoites in the salivary glands.

Treatment:

I. General and supportive measures: Given to treat symptoms and complications, e.g. antipyretics, fluids and electrolytes replacement and blood transfusion.

II. Antimalarial drugs: They are used with various objectives as:

- Therapeutic: To eradicate the erythrocytic cycle and produce clinical cure.

- Radical cure: To eradicate the exoerythrocytic cycle to prevent relapse.

- Gametocidal: To destroy gametes to prevent transmission of infection to mosquito.

- Chemoprophylaxis: To prevent infection in non-immune person visiting endemic areas.

A. Treatment of uncomplicated malaria:

1. Suppressive treatment (Erythrocytic schizonticides): These drugs act on the erythrocytic stages, e.g. 4-aminoquinoline as chloroquine, quinine, and atebrine.

2. Prophylactic treatment (Tissue schizonticides): These drugs act on the exoerythrocytic stages, e.g. 8-aminoquinolines as primaquine.

- In blood-borne malaria (no exoerythrocytic stages), one of the anti-exoerythrocytic drugs should be given because it has a gametocidal effect.

3. Radical treatment: Two drugs are given to eradicate plasmodia, one acting on the erythrocytic stages, to improve the symptoms (chloroquine), and another one acting on the exoerythrocytic stages to prevent relapse (primaquine).

B. Treatment of complicated falciparum malaria:

1. Chloroquine-sensitive falciparum malaria: Treated with chloroquine along with primaquine (gametocidal).

2. Chloroquine-resistant falciparum malaria: Artemisinin combined therapy (ACT) should be used.

- ACT consists of an **artemisinin** or its derivatives combined with long-acting antimalarial drug as amodiaquine, mefloquine or sulfadoxine-pyrimethamine.

Differences between mosquito-borne and blood-borne malaria.

Differences	Mosquito-borne malaria	Blood-borne malaria
1. Infective stage	Sporozoite	Merozoite and/or trophozoites
2. Incubation period	Long	Short
3. Exo-erythrocytic schizogony	Present	Absent
4. Hypnozoites	May be present	Absent
5. Relapse	May occur	Does not occur
6. Schizonticidal drugs	No radical cure	Can be radically cured
7. Radical treatment	Required	Not required

Prevention and control:

1. Mass treatment of infected cases.
2. Mosquito control.
3. Chemoprophylaxis: It is used to prevent erythrocytic infections by giving one of the tissue schizonticides. Primaquine is given for healthy individuals, one day before visiting a malaria-endemic area and continued for 4 weeks after the last exposure.
4. Vaccination.

Case study:

A 30-year-old Sudanese male was referred to a hospital in coma. Clinical examination revealed high-grade fever (41°C), low pulse rate (58/min), soft palpable spleen, hepatomegaly, jaundice and signs of cardiac oedema. His wife reported that during the last two weeks he suffered from recurrent attacks of fever, preceded by shivering and ended by profuse sweating.

Questions:

1. What is your possible diagnosis and the causative parasite?
2. Illustrate the mode of infection in such case.
3. How to confirm your diagnosis?
4. Predict two other phenomena that can occur if this infection is untreated.
5. Propose a therapeutic plan for this case.

Comparison of erythrocytic phases of human malaria parasites.

Differences	*P. vivax*	*P. ovale*	*P. malariae*	*P. falciparum*
1. Duration	48 hours	48 hours	72 hours	48 hours or less
2. Parasitized RBCs: a. Type	Reticulocytes	Reticulocytes	Old RBCs	All types
b. Size and shape	Enlarged, pale	Oval, slightly enlarged	Normal size	Normal size, knobbed
c. Parasite pigments	Yellowish brown & fine	Darker and coarser than *P. vivax*	Dark brown & coarse	Dark brown or black & coarse
d. RBCs stippling (pigment granules)	Schuffner's fine dots	Earlier & prominent Schuffner's	Ziemann's fine dots	Maurer's coarse dots
e. No. of affected cells	Moderate	Moderate	Low	Very high
3. Ring form: a. Size	1/3 RBC	1/3 RBC	1/3 RBC	1/6 RBC
b. Number	Single	Single	Single	Multiple
c. Shape	Thin cytoplasmic rim & fine chromatin dot	Thin rim & large chromatin	Thin cytoplasmic rim & fine chromatin	Accole' or aplique'form & may be double chromatin
4. Trophozoite	Amoeboid	Compact	Band form	Compact
5. Schizont: a. Size	Large	Medium	Medium	Small
b. No. of merozoites	16, rosette form	8	8, rosette form	16
6. Gametocytes	Rounded	Rounded	Rounded	Crescent
7. Stages in blood film	All stages	All stages	All stages	Ring & Gametocytes

Eimeriorina

General characters:

1. These coccidian parasites are characterized by a thick-walled oocyst stage that is typically excreted with the faeces of the definitive host.

2. Some coccidians (***Toxoplasma***) have a complicated life cycle, involving **tissue cysts** and **multiple hosts** (i.e., heteroxenous).

3. Other coccidians (***Cryptosporidium***, ***Cystoisospora***, and ***Cyclospora***) carry out their entire life cycle **within the intestinal epithelial cells of the host**.

4. They are generally considered **opportunistic pathogens**.

Toxoplasma gondii

Geographical distribution: Worldwide.

Morphology:

Toxplasma gondii occurs in 4 forms:

1. Trophozoite (Tachyzoite):

- It is crescent, 3X6 µ, with pointed anterior end and rounded posterior end.
- It has an ovoid posterior nucleus and anterior paranuclear granules.
- It is the active multiplying stage, seen intracellular in various tissues.
- It multiplies by **endodyogeny** within cytoplasmic vacuoles of any nucleated cell.
- It is found in the **acute stage** of infection.

2. Pseudocyst:

- It is full of rapidly multiplying **tachyzoites**.
- It has **no cyst wall**.
- It is localized inside the RECs.
- The tachyzoites multiply by **endodyogeny** and **ectomerogeny**.
- It is found in the **acute stage** of infection.

3. True tissue cyst:

- The cyst is round or oval, 5-50 µ and contains slowly multiplying **bradyzoites**.
- It has **cyst wall**.
- It is found in the brain (most common site), skeletal and cardiac muscles and various organs.
- The bradyzoites multiply by **endodyogeny** and **ectomerogeny**.
- It is found in the **chronic stage** of infection.
- It remains viable for years, and immunosuppression causes reactivation of cysts.

4. Oocyst:

- This stage is only present in **cats and other felines**.

- It is oval, 10X12 µ and surrounded by a thick resistant wall.

- Non-infectious when excreted in unsporulated or immature stage in cat´s faeces.

- It sporulates, by **sporogony**, within 1-5 days and becomes infectious.

- The mature or sporulated oocyst contains 2 sporocysts, each containing 4 sporozoites (**disporocystic tetrazoic oocyst)**.

- It may remain viable in moist shaded soil for a year or more.

Life cycle:

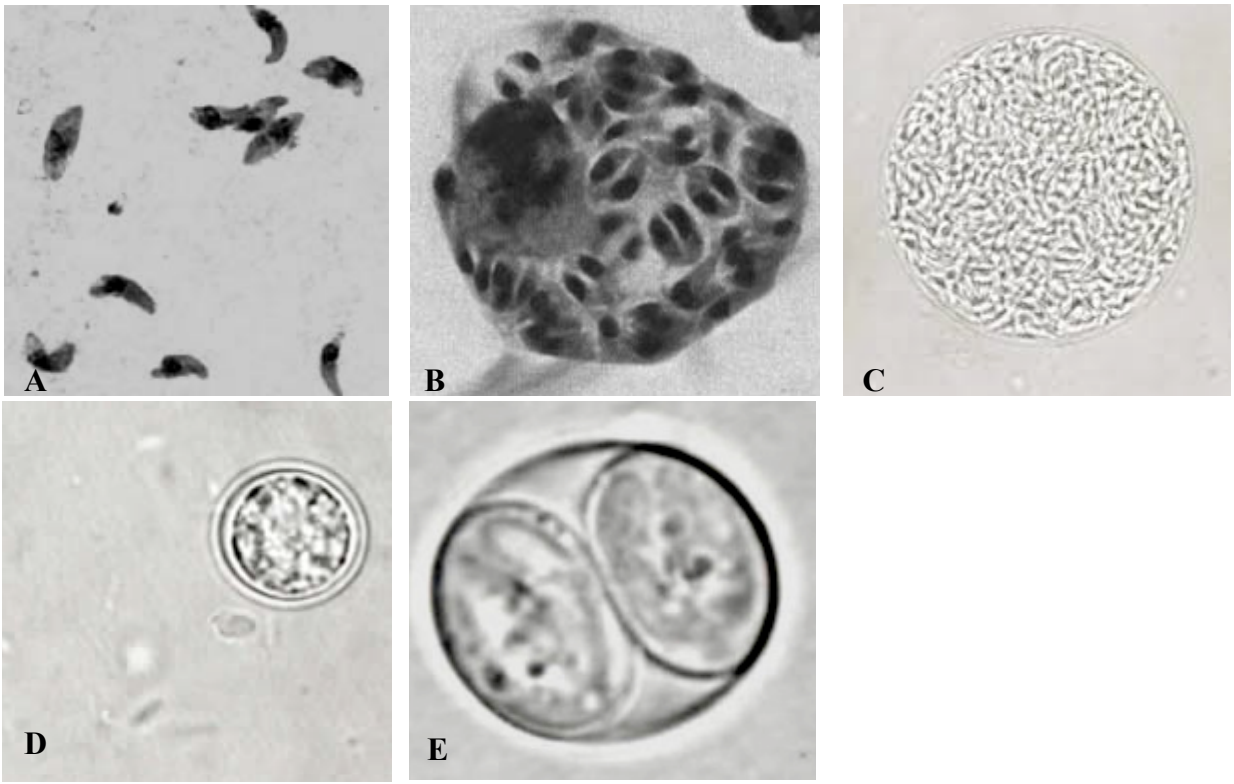
- Habitat:

T. gondii is an obligate intracellular parasite, which is found inside the RECs, brain, skeletal and cardiac muscles, and any other nucleated cells.

- Definitive host: Cats and other felines.

- Intermediate host: Man and other mammals (mice, rabbits, goat, sheep, cattle, and pigs), reptiles and birds.

- Infective stage: All stages are infectious to humans; tachyzoites, pseudocysts, true tissue cysts and sporulated oocysts.



***Toxoplasma gondii*. A. Trophozoites; B. Pseudocyst; C. True cyst; D. Unsporulated oocyst; E. Sporulated oocyst.**

Mode of infection:

1. Oral route via ingestion of:

- Mature oocysts in food and drinks contaminated with cat's faeces.
- Pseudocysts or true cysts in raw or undercooked contaminated meat.
- Tachyzoites in unpasteurized goat's and cow´s milk.

2. Inhalation of mature oocysts.

3. Blood transfusion.

4. Organ transplantation.

5. Contamination of mucous membrane, skin abrasions during handling and preparation of fresh infected meat, or laboratory workers who handle infected blood can also acquire infection through accidental inoculation.

6. Transplacental route, where the tachyzoites can be transmitted from infected pregnant woman to the fetus via the blood stream (placenta).

7. Sexually transmitted or by artificial insemination with semen from infected male.

- The life cycle of *T. gondii* is **heteroxenous** (alternation of generations between two hosts), where an **asexual cycle** occurs in the **intermediate host**, and **sexual cycle** occurs in the **definitive host**.

I. Exoenteric cycle (Asexual cycle):

- It occurs in intermediate hosts (man, and other mammals, reptiles and birds).

- Sporozoites from sporulated oocysts and trophozoites from tissue cysts, enter the epithelial cells of intestinal mucosa, and proliferate as **tachyzoites** by **endodyogeny**.

- Tachyzoites continue to multiply and may spread locally to invade new cells.

- Some tachyzoites also spread to distant extra-intestinal organs (e.g. brain, heart, skeletal muscles, eye, liver, spleen and placenta) by invading lymphatic and blood, forming **tissue cysts**.

- Tachyzoites invade and multiply inside the RECs by either **endodyogeny** or **ectomerogeny** to form **pseudocysts**.

- When the pseudocyst ruptures, the tachyzoites will invade either new macrophages or any other cells, multiply by **endodyogeny** or **ectomerogeny**, forming **true tissue cysts** full of **bradyzoites**.

- The dormant bradyzoites inside the cyst can be reactivated in **immunosuppression** causing renewed infection in the host.

- The released trophozoites can enter the blood and the cycle is repeated.

- **Man acts as blind or final host or dead end for *T. gondii***.

II. Enteric cycle (Sexual cycle):

- It occurs in cats and other felines, definitive hosts.

- Both **sexual reproduction** (**gametogony**) and **asexual reproduction** (**schizogony**) occur within the mucosal epithelial cells of the small intestine of cat.

- Cats acquire infection by their predatory habit of feeding on muscles, brain and other tissues of infected mice, harboring the tissue cysts, or by eating raw infected meat of domestic animals, or by ingestion of mature oocysts passed in their faeces.

- The sporozoites and trophozoites are released in the small intestine, penetrate its mucosal epithelial cells and multiply inside by several **asexual cycles** of **schizogony** (**endopolygony**), leading to formation of **merozoites**.

- Some **merozoites** (from rupture of schizont) may enter extraintestinal tissues resulting in formation of **tissue cysts** in other organs of the body as in man.

- Other **merozoites** invade the intestinal mucosa again and transform into **micro- and macrogametocytes**, and **sexual cycle** (**gametogony**) begins, with formation of **microgamete** and **macrogamete**.

- A **macrogamete** is fertilized by **microgamete** with production of zygote which develops into an oocyst.

- Unsporulated oocysts are shed in the cat's faeces for 1-2 weeks, and take 1-5 days to sporulate in the environment and become infective.

- Man and other intermediate hosts acquire infection by ingesting the sporulating oocysts and the cycle is repeated.

Pathogenicity:

- In **toxoplasmosis,** proliferation of tachyzoites in the host cells (intestinal and extra-intestinal), causes cellular death with focal necrosis and surrounding inflammatory cells.

1. In acute infection, the outcome of the disease depends on host immune status.

a. **In immunocompetent individuals**, tachyzoites disappear from various organs.

b. **In immunocompromised patients**, there is dissemination of the parasites through the blood stream to various organs as brain, eyes, lungs, heart, liver, spleen, kidneys, lymph nodes, bone marrow and skeletal muscles, where they form pseudocysts causing severe necrotizing lesions and disease progression.

2. In chronic infection, true tissue cysts remain viable in tissues for years in resting form. In immunodeficient status, their reactivation cause clinical disease.

Clinical picture:

1. Congenital toxoplasmosis:

- This occurs when the mother get infected for the first time during pregnancy. But, in some woman with chronic infection, reactivation of tissue cysts leads to liberation of trophozoites, which may infect the fetus.

- The risk of fetal infection rises with progress of pregnancy. In contrast, the severity of fetal damage is high, when infection is transmitted in early pregnancy.

- Clinical manifestations of congenital infections may be:

a. Early manifestations: Still birth, abortion, hydrocephalus, microcephaly and microphthalmia. The most common sequelae are **retinochoroiditis** that affects vision and results in blindness, **cerebral calcification**, **convulsions** (**clinical triad**). In some cases, fever, lymphadenopathy, hepatosplenomegaly, anaemia, thrombocytopenia, petechial rash, jaundice, and myocarditis may present at birth.

b. Late manifestations: Mental retardation, visual affection and psychomotor disturbance in adolescence and adulthood.

2. Acquired toxoplasmosis:

- It is asymptomatic in 80-90% of healthy hosts.

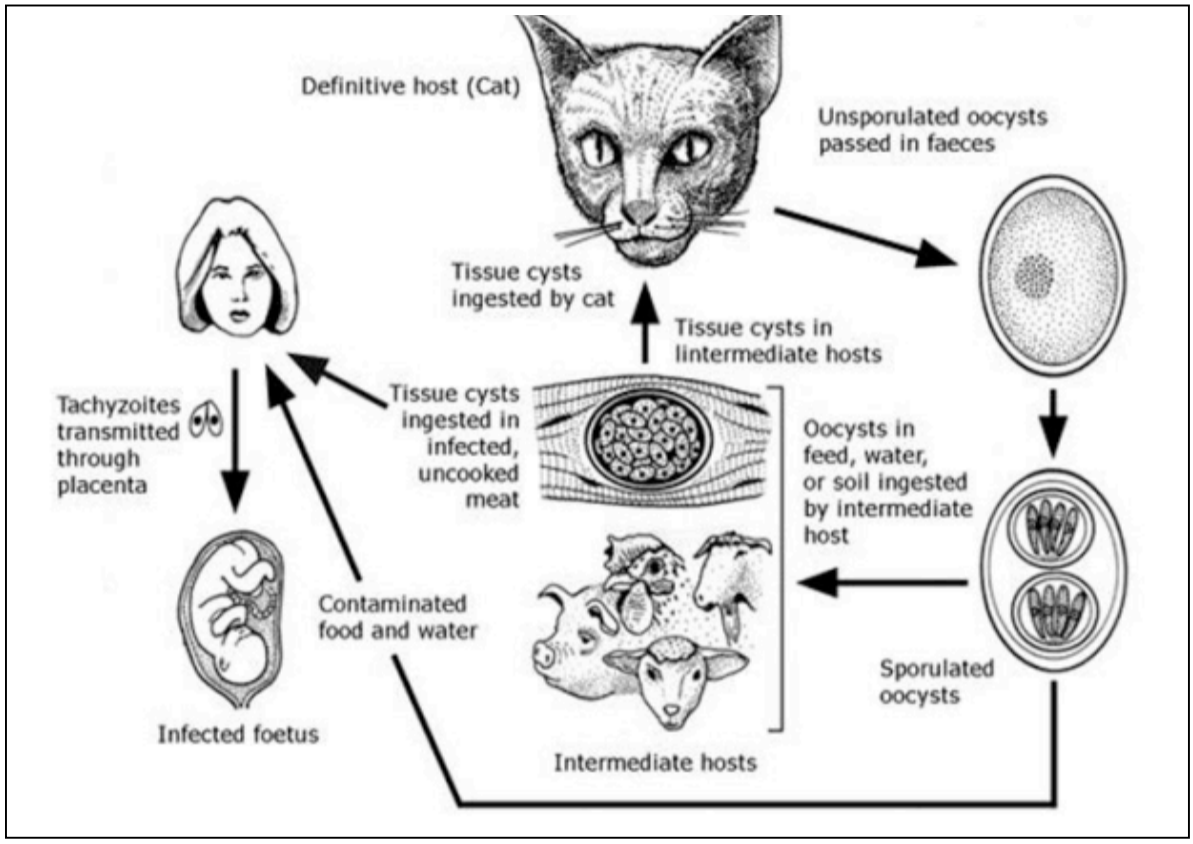
-The classical clinical sign of acute acquired toxoplasmosis is **lymphadenopathy** and the deep cervical lymph nodes are the most commonly affected. The infected lymph nodes are discrete and non-tender.

-Mild fever, headache, myalgia (**Flu-like syndrome**), and hepatosplenomegaly are often present.

3. Toxoplasmosis in immunocompromised patients:

- In these patients affection of brain is more common, with meningoencephalitis, and neuropsychiatric manifestations.

- Pneumonia, myocarditis, chorioretinitis and hepatosplenomegaly may occur.



Life cycle of *Toxoplasma gondii*.

Diagnosis:

- Clinical diagnosis:

- A combination of signs as hydrocephalus or microcephaly, chorioretinitis and signs of intracerebral calcification make diagnosis of congenital toxoplasmosis probable.

- Acquired toxoplasmosis is diagnosed by exclusion from other diseases of the reticuloendothelial and lymphatic systems.

- Laboratory diagnosis:

I. Direct:

1. Microscopy:

- Detection of trophozoites and tissue cysts in lymph node, bone marrow, spleen, placenta, blood, CSF, and amniotic fluid smears stained by Giemsa, PAS, or Gomori methanamine silver (GMS) stain.

2. Animal inoculation: *Toxoplasma* can be detected by intraperitoneal inoculation of infective material in mice. After 7-10 days, peritoneal fluid is examined for trophozoites. Mice are sacrificed after 3 weeks and examined for tissue cysts.

II. Indirect:

1. Serodiagnosis:

a. **Sabin-Feldman dye test:** It detects a circulating cytoplasm modifying antibody. Patient's serum is mixed with a *Toxoplasma* trophozoites suspension and methylene blue is added. **If the parasite fails to take the stain, the test is considered positive**. The test gives false positive results in *Sarcocytis* and *Trichomonas vaginalis* infections.

b. **Antibody detection:**

- Tests for detecting IgG antibody include: ELISA, IFA, and IHA.

- The serum IgM can be measured by ELISA.

- Detection of specific **IgM** antibodies indicates acute infection, while positive **IgG** titer indicates latent infection.

- IgM detected in babies' blood is fetal in origin as **maternal IgM doesn't cross the placenta**.

- IgA-ELISA test is also used for detecting congenital infection in newborns.

c. **Antigen detection:**

- Detection of antigen by ELISA indicates recent *Toxoplasma* infection.

- It is useful in immunocompromised patients.

- Detection of antigen in amniotic fluid is helpful to diagnose congenital toxoplasmosis.

2. Molecular diagnosis:

- Can be used for diagnosis of *T. gondii* DNA in blood, CSF, urine, and different tissues.
- It is valuable especially in immunocompromised patients in whom antibody titers are low or absent.
- Also, it can be used on amniotic fluid in case of congenital infection.

3. Imaging:

- MRI and CT scan are used to diagnose CNS involvement.
- US of fetus at 20-24 week of pregnancy is useful to diagnose congenital toxoplasmosis.

Treatment:

1. Congenital toxoplasmosis:

- Neonates with congenital infection are treated with **pyrimethamine and sulfadiazine** with folinic acid for one year.
-Systemic corticosteroids may be given to alleviate chorioretinitis.

2. Immunocompetent individuals:

- Most healthy people recover from toxoplasmosis without treatment.
- Persons who develop persistent, severe symptoms can be treated with a combination of drugs such as **pyrimethamine and sulfadiazine or clindamycin**, plus folinic acid.

3. Immunocompromised individuals:

- Immunosuppressed patients who are positive for *T. gondii* and have a **$CD4^+$ T-lymphocyte count less than 100/µl** should receive prophylactic measures against *Toxoplasma* encephalitis. **Trimethoprim-sulfamethoxazole** is the drug of choice, or dapsone- pyrimethamine.
- Prophylaxis against *Toxoplasma* encephalitis should be discontinued in patients whose $CD4^+$ T-lymphocyte becomes more than 200/µl for 3 months after successful treatment.

4. Pregnant women:

- **Spiramycin** (Rovamycin) should be taken for 4 weeks, to treat the infected pregnant women to reduce the risk of transplacental transmission.

Prevention and control:

1. Women who are or may become pregnant should avoid contacts with cats or cleaning the litter box, and undergo routine serological screening.
2. Individuals at risk, mainly children and immunocompromised individuals should avoid contacts with cats and their faeces.
3. Proper washing of hands, vegetables and fruits before eating.
4. Proper washing of hands and utensils after handling raw meat.
5. Proper freezing and cooking of meat before eating.
6. Never fed raw meat to cats; only dry, cooked or canned meat should be fed.
7. Cats should be kept indoors and litter boxes changed daily. Cats' faeces should be flushed down the toilet or burned. Litter pans should be cleaned by immersing them in boiling water.
8. Screening for *T. gondii* antibody should be done in all blood banks.

Case study:

A young lady works in medical laboratory. She had been married 3 years ago, and she got pregnant and aborted twice.

Questions:

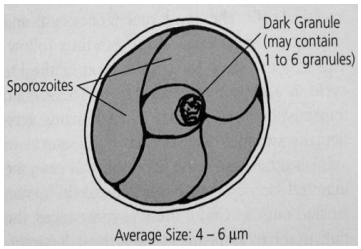
1. What is the possible parasitic cause?
2. What is the probable method of infection in such case?
3. Propose a good therapeutic plan for this case.
4. Develop a control plan for this parasitic infection.

Cryptosporidium parvum

Geographical distribution: Worldwide.

Morphology:

- Oocyst is spherical or oval, 4-6 µ in diameter.
- It contains 4 naked curved sporozoites (**no sporocyst**) with a residuum in between, which is formed of numerous granules and a spherical globule.



- There are 2 types of oocysts, thick- walled (80%) and thin- walled (20%).

Life cycle:

- **Habitat:** Beneath the brush boarder of the small intestinal mucosa (mainly jejunum), within the host cell membrane, but not within the cell cytoplasm (**intracellular but extra cytoplasmic position**).
- **Definitive host:** Man.
- **Intermediate host:** No.
- **Reservoir hosts:** Cattle, dogs and cats.
- **Infective stage:** Oocysts.

Mode of infection:

1. Faecal-oral route by ingestion of thick-walled oocysts in contaminated food or drinks.
2. Internal autoinfection by thin walled oocysts, which release sporozoites in situ.
3. Zoonotic transmission from the reservoir animals.
4. Air-borne infection.
5. Sexual transmission among homosexual individuals (oral-anal contact).

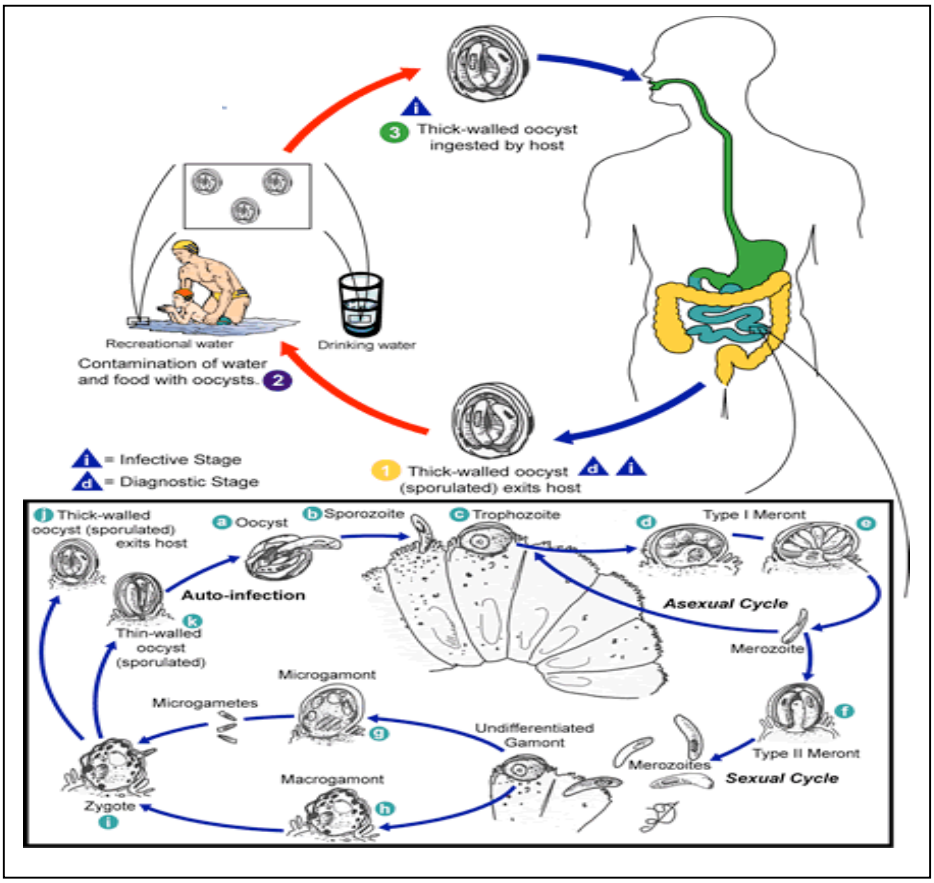
- The parasite completes its life cycle, asexual (**schizogony**) and sexual (**gametogony**) phases in a single host (**monoxenous**).
- Ingested oocysts release sporozoites in the upper gastro-intestinal tract, which invade epithelial cells of small intestine to be restricted to the apical surface of the cells; intracellular but extra cytoplasmic, within a **parasitophorous vacuole**.
- There, they undergo asexual multiplication (**schizogony**) and then sexual multiplication (**gametogony**) with formation of **macro-** and **micro-gamonts**.
- After fertilization, zygotes change to oocysts that sporulate in the infected host.

- Sporozoites released from the **thin-walled oocysts** infect the same host by **internal autoinfection**, while **thick-walled oocysts** are infective upon **excretion in faeces**, thus permitting direct and immediate faecal-oral transmission.
- Oocysts can remain viable in the environment for long period.

Pathogenicity:

- *C. parvum* causes **cryptosporidiosis**, a significant cause of water-borne outbreaks, travellers, house hold, nosocomial, and day care unit's diarrhea.

- The pathogenesis of *Cryptosporidium* is **restricted to the apical surface of the epithelial cells of small intestine** without affection of the host cell cytoplasm.
- Inflammatory changes occur as a result of infection, with crypts hyperplasia and villous atrophy of the affected area of small intestine, causing profuse watery diarrhea.



Life cycle of *Cryptosporidium parvum*.

Causes of diarrhea in cryptosporidiosis:

1. Villous atrophy.
2. Reduction in the intestinal mucosal surface.
3. Decrease in the absorbing capacity of the small intestine.
4. Decrease in the digestive enzymes of the intestinal mucosal.

- Malabsorption and bacterial fermentation of unabsorbed sugar and fatty acids cause offensive diarrhea.

- In immunocompromised patients, dissemination of infection to lungs, oesophagus, colon, biliary tract, pancreas, and urinary bladder may occur.

Clinical picture:

1. In immunocompetent persons:

- Most cases are asymptomatic.
-There may be a short-term enteropathy with self-limited diarrhea lasting for 1-2 weeks. The most frequent symptoms are watery and offensive diarrhea, nausea, vomiting, abdominal cramps, anorexia, loss of weight and low-grade fever.

2. In immunocompromised patients:

- Chronic, severe, profuse, watery, green, frothy and offensive diarrhea, as frequent as 5-10 or may reach over 25 motions / day, causing significant fluid and electrolyte depletion, weight loss, emaciation and abdominal pain.
- Malabsorption may also lead to dehydration, weight loss, and death if it is not controlled.
- Extraintestinal infection of respiratory system (**respiratory cryptosporidiosis**), hepatitis, cholecystitis, cholangitis and pancreatitis have been reported.

Diagnosis:

- Clinical diagnosis: Clinical history and presentation of the disease.

- Laboratory diagnosis:

I. Direct:

1. Stool examination:

a. Microscopic examination of direct smear preferably after concentration by **floatation techniques** as: **Sheather's sugar**, **sodium chloride**, **zinc sulphate**, or **formalin-ether flotation**.

b. Acid fast staining techniques for detection of *Cryptosporidium* oocysts as: **Modified Ziehl-Neelsen** stain where oocysts appear deep red with blue granules against pale green background, or by **kinyoun acid-fast** or **safranin stain**.

c. Fluorescent staining with **auramine-phenol**.

2. Examination of duodenal aspirates obtained by entero-test.

3. Examination of jejunal biopsy.

4. Sputum examination in respiratory cryptosporidiosis.

II. Indirect:

1. Serodiagnosis:

a. **Antibody detection:** IFA and ELISA.

b. **Antigen detection:** ELISA for detection of *Cryptosporidium* antigens in stool.

2. Molecular diagnosis: For detection of *Cryptosporidium* DNA in stool and biopsy material.

Treatment:

1. **In immunocompetent patients**: Cryptosporidiosis is self-limited.
2. **In immunocompromised patients**:

- Nitazoxanide or paromomycin.
- Supportive treatment: Fluid, electrolyte and nutrient replacement.

Prevention and control:

1. Treatment of infected patients.

2. Environmental sanitation as: Anti-fly measures, proper sewage disposal, safe water supply and avoid using excreta as fertilizer.

3. Washing green vegetables and fruits before eating.

4. Avoid contamination of food and drinks with faecal oocysts.

5. Strict personal hygiene as washing of hands after defecation and before eating.

6. Avoidance of zoonotic infection.

7. *Cryptosporidium* oocysts are very tough, resist most disinfectants. Only prolonged exposure to a chlorine concentration of 80 ppm for 2 hours, 10% formalin, or temperatures > 60 °C, or less than -20°C can kill them.

8. Proper filtration through a ≤ 1 μ filter or smaller or boiling of drinking water for 1 minute is essential particularly for immunocompromized patients.

9. In hospitals, contaminated instruments and equipments should be autoclaved to 65°C for 20-30 minutes to avoid nosocomial infection.

10. Contact with infected materials must be avoided by using gloves, gowns and hand washing.

Cystoisospora belli

(Formerly known as *Isospora belli*)

Geographical distribution: More common in tropics and sub-tropics.

Morphology:

1. Unsporulated oocyst:

- It is oval, 30 X 12 μ, and surrounded by a translucent double-layered cyst wall.

- It contains a spherical mass of protoplasm, which divides into 2 sporoblasts before sporulation.

- Since sporulation requires 3-4 days, unsporulated oocyst is the form usually seen in faeces.

2. Mature sporulated oocyst:

- It contains two sporocysts.
- Has 4 curved, sausage-shaped nucleated sporozoites (**disporocystic tetrazoic**).

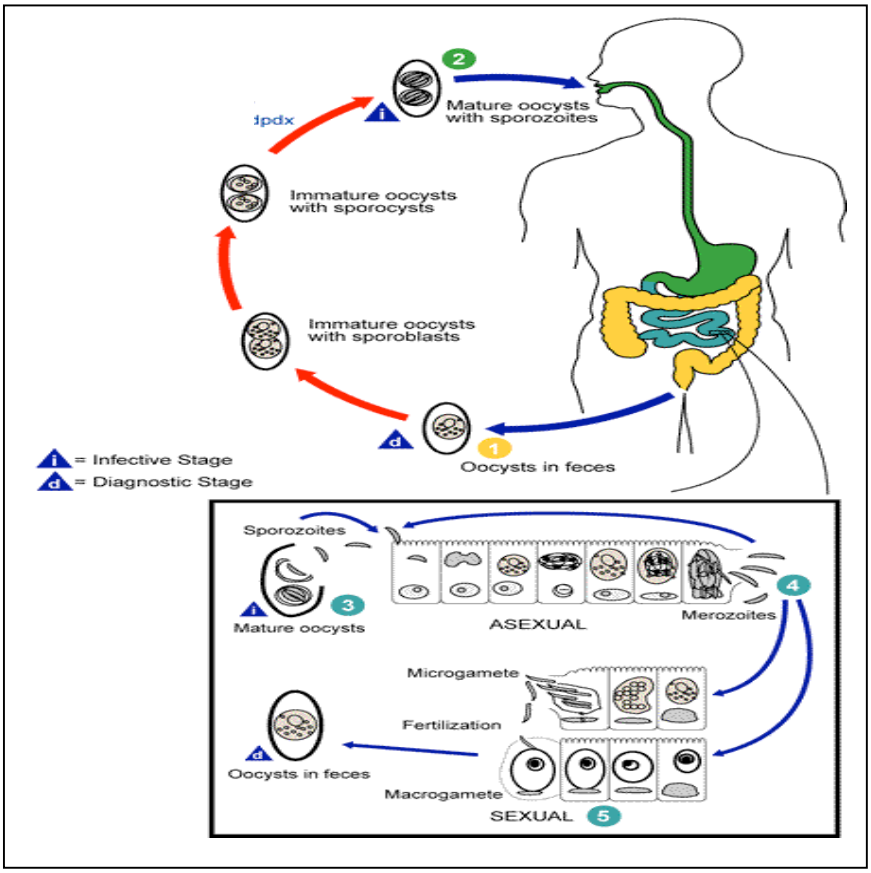
***Cystoisospora belli*. A. Immature oocyst;** **B. Mature oocyst**.

Life cycle:

- **Habitat:** Epithelial lining of the small intestine (duodenum and jejunum).
- **Definitive host:** Man is the only recognized source of *Cystoisospora belli* infection, unlike cryptosporidiosis, cystoisosporiasis is not a zoonotic disease.
- **Infective stage:** Mature sporulated oocyst.
- **Mode of infection:** By ingestion of food or drink contaminated with sporulated oocysts.

- *Cystoisospora belli* is a **host-specific parasite**, completing its life cycle (asexual and sexual phases**) in man**.

- After ingestion of sporulated oocyst, sporocysts excyst in the small intestine and release their sporozoites, which invade the epithelial cells and initiate asexual multiplication (**schizogony)** within a **parasitophorous vacuole**.
- Upon rupture of the schizonts, the merozoites are released, invade new epithelial cells, and continue the cycle of asexual multiplication.
- Some merozoites begin the sexual cycle (**gametogony**), with the development of gametocytes. Fertilization results in the development of oocysts.
- Immature unsporulated oocysts are released in the lumen of the bowel, while sporulation occurs in the environment within 3-4 day.
- If the sporulated oocysts are ingested by man, the cycle is repeated.
- Sporulated oocysts remain viable for months in the environment.



Life cycle of *Cystoisospora belli*.

Pathogenicity:

- *Cystoisospora belli* causes **cystoisosporiasis**, a cause of **traveller's diarrhea,** with more severe form of the disease in infants and young children than adults.

-The pathogenesis of *Cystoisospora belli* is similar to that of *Cryptosporidium*, except that *Cystoisospora belli* invades the host cell cytoplasm.

- Inflammatory changes develop in the affected epithelium after invasion of enterocytes by the parasites, resulting in flattened mucosa with villous atrophy and crypts hyperplasia, and infiltration of lamina propria with eosinophils, lymphocytes and plasma cells.

- Peripheral eosinophilia.

- In immunocompromised patients, the infection is disseminated to liver, bile duct, large intestine and spleen.

Clinical picture:

1. In immunocompetent individuals:

- Most cases are asymptomatic.
- There may be a short-term enteropathy with self-limited diarrhea (few days to a

week), steatorrhea, abdominal colic and fever. Bowel movements usually from 6-10/ day, watery to soft foamy, and offensive, associated with weight loss.

2. In immunocompromised patients:

- It often presents with chronic, profuse, watery diarrhea, anorexia, weakness and weight loss associated with malabsorption syndrome. Dehydration can develop and be life- threatening.
- Extraintestinal infection of liver, spleen and bile duct has been observed.

Diagnosis:

- Clinical diagnosis.

- Laboratory diagnosis:

I. Direct:

1. Stool examination:

a. Microscopic examination of direct smear preferably after concentration by floatation techniques.

b. Acid fast staining techniques for detection of *Cystoisospora belli* oocysts as: Modified Ziehl-Neelsen, which shows pink-staining oocysts that contain bright red sporoblasts (protoplasmic mass), the cyst wall doesn't stain, and it is usually outlined by a stain precipitate, or by kinyoun acid-fast or safranin stain.

c. Fluorescent staining with **auramine-phenol**.

2. Examination of duodenal aspirates obtained by entero-test.

3. Examination of intestinal biopsy.

II. Indirect:

1. **Charcot Leyden crystals** can be detected in stool.

2. Eosinophilia, which is generally not seen in other enteric protozoal infections, can be detected in cystoisosporiasis.

Treatment:

1. **In immunocompetent patients**: No treatment in self-limiting infection.
2. **In immunocompromised patients**:

- Oral cotrimoxazole, a combination of trimethoprim and sulfamethoxazole (Bactrim, Septra, and Cotrim).
- For patients intolerant to sulfonamides, pyreimethamine can be used.
- It may be necessary to continue a maintenance dose with cotrimoxazole to prevent relapses.
- Fluid and electrolyte replacement.

Prevention and Control:

1. Treatment of infected patients.
2. Control of food and water born infection.
3. Utilize approaches used for inactivation of *Cryptosporidium parvum* oocysts.

Cyclocospora cayetanensis

Geographical distribution: More common in tropical and sub-tropical regions.

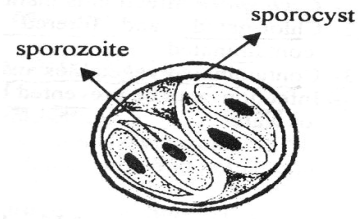
Morphology:

1. Unsporulated oocyst:

- It is spherical, 8-10μ, with central morula, containing 6-9 retractile granules.
- Since sporulation requires 5-10 days, unsporulated oocyst is the form usually seen in faeces.

2. Mature sporulated oocyst:

- It contains 2 sporocysts; each contains 2 crescent-shaped sporozoites (**disporocystic dizoic**).



Life cycle:

- **Habitat:** Jujenal enterocytes.
- **Definitive host:** Man
- **Infective stage:** Mature sporulated oocysts.
- **Mode of infection:** By ingestion of food or drink contaminated with sporulated oocysts.

- *Cyclocospora cayetanensis* is a **host-specific parasite**, completing its life cycle phases in **man**.

- After ingestion of the sporulated oocysts, they excyst in the small intestine, and the released sporozoites invade the jujenal enterocytes, living within a **parasitophorous vacuole**, where they initiate asexual cycle (**schizogony)**.

- Then, sexual cycle (**gametogony**) takes place resulting in the formation of unsporulated oocysts which are excreted in patients' faeces.

- An obligatory phase of maturation of oocysts in the environment occurs in 5-10 days with the development of sporulated oocysts.

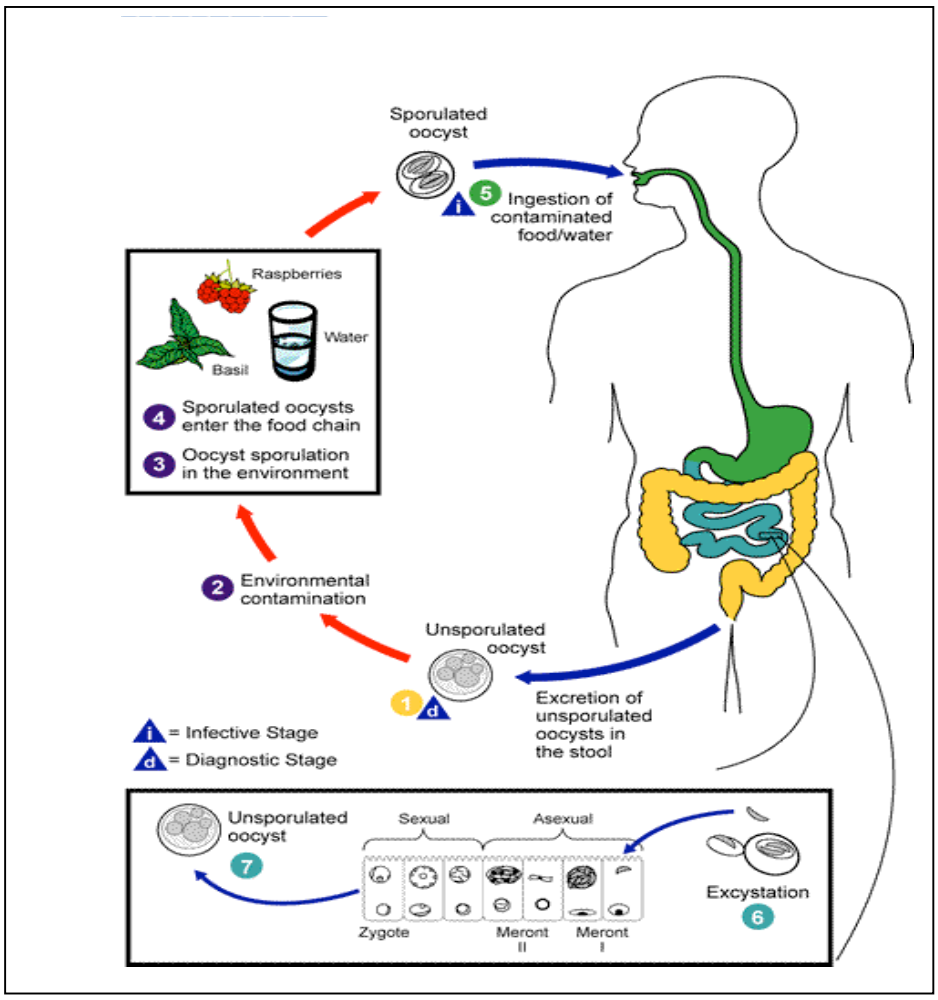
- If man ingests the sporulated oocysts, the cycle is repeated.

Pathogenicity:

- *Cyclocospora cayetanensis* causes cyclosporiasis. The disease is the cause of **unexplained summer diarrhea** and similar illness following travel to tropics.

- The pathogenesis of *Cycloclospora* is of similar to that *Cryptosporidium* **except** that ***Cycloclospora* invades the host cell cytoplasm**, while *cryptosporidium* is restricted to the apical surface of the cells.

- The parasitic invasion of the enterocytes, leads to damage and death of the cells due to parasite multiplication and inflammation mediated by T-cells or mast cells, resulting in **villous atrophy and crypts hyperplasia**.



Life cycle of *Cycloclospora cayetanensis*.

Clinical picture:

1. In immunocompetent patients: Diarrhea is self-limited within 3-4days. There is abrupt onset of watery diarrhoea that tends to relapse. It is accompanied by nausea, vomiting, flatulence and abdominal cramps.

- Infection may cause anorexia, fatigue, loss of weight and low grade fever.

2. In immunocompromized patients: Diarrhea is severe, prolonged for 3 weeks or longer and tends to recur, with colicky abdominal pain, malaise, vomiting, dehydration, substantial weight loss and muscle pain. **Biliary affection** may also develop.

Diagnosis:
- Clinical diagnosis.
- Laboratory diagnosis:
1. Stool examination:
a. Microscopic examination of direct smear preferably after concentration by floatation techniques.

- The key for diagnosis is concentration of the oocysts from faecal samples without the use of formalin because of low number of oocysts. **Sheather's sucrose flotation** is the best procedure. Also, the addition of **2.5-5% potassium dichromate** allows the sporocysts to sporulate at room temperature, and become more visible.

b. Acid fast staining techniques for detection of oocysts as: **Modified Ziehl-Neelsen**, or **safranin stain**.

c. Examination by UV Fluorescence microscope: Oocysts exhibit blue auto fluorescence.

2. Examination of duodenal aspirates obtained by entero-test.

3. Examination of jejunal biopsy.

Treatment:
- Cotrimoxazole, a combined treatment with trimethoprim and sulfamethoxazole is effective.
Prevention and Control:
1. Treatment of infected patients.
2. *Cyclospora* oocysts resist chlorine; infection can be prevented by boiling of water or disinfection by Ozone.
3. Proper washing of vegetables and fruits.

Case study:

A 40-year-old homosexual man with severe diarrhea was admitted to a University Medical Center. The patient had up to 10 episodes of diarrhea per day, and lost 9 kg in the period of 1 week. Stool specimens were tested for a wide panel of enteric pathogens (bacteria, viruses, helminths, and protozoa). The parasitologic examination of stools showed oocysts in the range of 4-5µ. No other pathogen was found in the specimens. The patient was treated with paromomycin. On the second day of treatment, the diarrhea promptly resolved, decreasing from 10 to 2 attacks per day.

Questions:

1. What is the possible parasitic cause?
2. What is the probable method of infection in such case?
3. Propose other procedures to confirm the diagnosis.
4. Predict two complications that can occur if this infection is untreated.
5. Develop a control plan for this parasitic infection.