Protozoan Parasites

2

The protozoan parasites are known to parasitize both human beings and domesticated animals, thus causing immense loss to human society. These parasites cause different important diseases like malaria, Chagas disease, sleeping sickness, kala-azar, leishmaniasis, etc. those are major diseases of tropical countries bringing death to human beings and their domesticated animals.

In this chapter the flagellates like *Trichomonas vaginalis*, *Giardia intestinalis*, *Trichomonas hominis*, *Trypanosoma cruzi*, *Trypanosoma gambiense*, *Leishmania donovani* are discussed about their history of discovery, habit and habitat, morphology, modes of infection, life cycle, pathogenesis, energy production, diagnosis, epidemiology of the parasites and immune response of the host.

Parasitic amoebae like *Entamoeba histolytica*, *Acanthamoeba*, *Naegleria fowleri*, *Entamoeba coli*, *Dientamoeba fragilis*, *Iodamoeba butschlii* are described concerning their same factors as the parasitic flagellates.

Coccidia like *Cryptosporidium parvum*, four species of malarial parasites, *Toxoplasma gondii*, *Babesia* and an intestinal *ciliophoran*, *Balantidium coli* causing diseases like dysentery are also discussed under the same subheadings.

Systematics of all these protozoan parasites are written for the benefit of the students who are interested in the classification of these parasites to know their exact position in the animal kingdom.

Protozoa is the subkingdom under Kingdom Animalia. This scheme of classification is introduced by N.D. Levine in the year 1980. Before that Protozoa was regarded as a phylum in the scheme of classification by T.J. Parker and W.A. Haswell in 1940. In the year 1942 A.J. Marshall and W.D. Williams edited a book where Parker and Haswell's classification was accepted and described.

In the year 1675 Antony Van Leewenhoek found for the first time the microscopic, single celled organisms and described them. In the year 1818 Scientist Goldfuss for the first time coined the term Protozoa for this group of organisms. After the discovery of cell by Robert Hooke in the year 1839, Van Siebold in 1845

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understanding the characteristics of Protista and on the basis of this discovery he for the first time applied the name Protozoa for the microscopic unicellular organisms.

N.D. Levine in the year 1980 divided the subkingdom Protozoa into seven phyla. Out of these seven phyla the phylum Sarcomastigophora, phylum Apicomplexa, phylum Microspora, phylum Ascetospora, phylum Ciliophora contain parasitic organisms. Examples are in phylum Sarcomastigophora organisms like Giardia, Trypanosoma, Amoeba, etc. are parasites. In phylum Apicomplexa organisms like Gregarina, Monocystis, Plasmodium, Toxoplasma, etc. are the parasitic animals. In the phylum Microspora all the members of the phylum are endoparasites. The members of the phylum Ascetospora are parasites. In phylum ciliophora very few are parasitic organisms.

The Protozoa are unicellular organisms and the single cell performs all the vital functions of life. As protozoans are very very small they can be studied only under microscope. The main structure of any member of the parasitic Protozoa consists of protoplasm enclosed in a cell membrane, plasmalemma. The protoplasm is divided into cytoplasm and nucleus. Though parasites some have the power of movement. The movement is performed by either pseudopodia or cilia or flagella, etc.

Some of the protozoan parasites are:

Entamoeba histolytica	*Plasmodium falciparum*
Giardia intestinalis	*Toxoplasma gondii*
Trichomonas vaginalis	*Cryptosporidium parvum*
Balantidium coli	*Babesia bigemina*
Trypanosoma cruzi	
Trypanosoma brucei	
Leishmania donovani	
Plasmodium vivax	
All are infective to human beings	

The Phylum Protozoa though it is now called kingdom Protozoa as per the classification of N.D. Levine (1980) have more than 45,000 species of which 10,000 are parasitic in invertebrates and in all species of vertebrates. Human and their domesticated animals serve as the hosts of the protozoan parasites like malaria, Chagas disease, sleeping sickness, Leishmaniasis, etc. those are considered major diseases of the third world tropical countries. In poultry, dairy and domesticated animals theileriosis and coccidiosis present a continuous threat to the persons who are in these business and trying to produce food for human beings.

In the living world kingdom Protozoa have three Phyla under which there are a number of parasites those attack human beings and domesticated animals. They are:

Kingdom Protozoa
Phylum
Sarcomastigophora
Sub Phylum
Sarcodina
Class
Lobosea
Free living species
1) Acanthamoeba sp.
2) Naggleria sp.
Intestinal species
1) Entamoeba histolytica
2) Entamoeba coli
3) Dientamoeba fragilis
4) Iodoamoeba butschlii
Mastigophora
Class
Zoomastigophora
Intestinal species
1) Giardia intestinalis
2) Trichomonas hominis
Vaginal species
1) Trichomonas vaginalis
Blood and tissue species
1) Leishmania donovani
2) Trypanosoma gambiensae
3) Trypanosoma cruzi
Cilliophora
Class
Kinetofragminiphora
Intestinal species
1) Balantidium coli
Apicomplexa
Class
Sporozoa
Blood species
1) Plasmodium vivax
2) Plasmodium falciparam
3) Plasmodium malariae
4) Plasmodium ovale
Tissue species
1) Toxoplasma gondii
Intestinal species
1) Cryptosporidium parvum

2.1 Flagellates

2.1.1 *Trichomonas vaginalis*

Trichomonas vaginalis is largest in size among the Trichomonads in human beings.

2.1.1.1 History of Discovery

This parasite was first discovered by Donne in 1836 in vaginal secretion. Next year he called this parasite *Trichomonas vaginalis*. He created the genus Trichomonas.

Geographical distribution: It is a common vaginal flagellate protozoan parasite distributed throughout the world. It is prevalent in Negro women twice as high as white women. *T. vaginalis* is also found in man occasionally though it is a parasite of women particularly. The disease caused by it is known as vaginitis. *Trichomonas vaginalis* is also found in man in the urine or in prostate secretion after prostatic massage.

2.1.1.2 Morphology

It is pear shaped, length varies from 10 to 30 μ but in average 20 μ long and 5–10 μ in breadth. This protozoan flagellate exists in trophozoite stage only, like other flagellates they do not form cysts. The trophozoites have five flagella (Fig. 2.1). Four of them are anterior flagella and one posterior. The posterior one remains along the margin of the undulating membrane which does not extend beyond the middle of

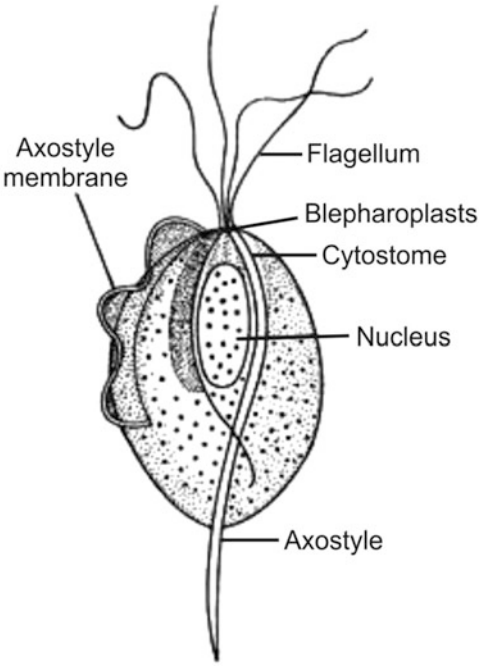


Fig. 2.1 *Trichomonas vaginalis*

the body. The body is supported by a stiff axostyle that originates from the anterior end and projects posterior beyond the body like a tail spine. The undulating membrane is attached to the body by a flexible rod known as costa. The projected axostyle is at posterior end and the organism seen to anchor themselves to the debris by this structure. The nucleus is oval in shape and is formed of scanty scattered chromatin granules. In the cytoplasm of the body some deep staining granules are seen and they are also found in rows along the costa known as metachromatic granules. The cytostome or cell mouth is not very distinct and the cell contains few food vacuoles. Beside the nucleus there is a structure called parabasal apparatus which is sausage shaped and light stained body. There is also a deep staining fibril that extends up to the middle of the body is the parabasal fibril.

The fresh active trophozoites exhibit jerky movements.

2.1.1.3 Habitat

T. vaginalis is primarily a parasite of vagina of women, it also invades the Skene's gland in urethra. In males they are found in urethra and prostate. *T. vaginalis* found in abundance in the upper part of the vagina up to the cervix of the uterus but do not enter into the uterus.

Scientists have found that *T. vaginalis* is associated with a low acidic creamy white frothy discharge.

2.1.1.4 Modes of Infection

T. vaginalis infection is accompanied by low acidity of the vagina, thin epithelium and less glycogen content in the cells. Normally the pH of the vaginal passage is highly acidic which prevents the infection. It is found that in children the vaginal passage is not highly acidic. The trophozoites of *T. vaginalis* are resistant to environmental changes. They may survive in urine and damp towel for a considerable period. These help in the spread of infection. Living trophozoites are seen on wet under cloths even after 24 h. *T. vaginalis* may infect the foetus during passing through the birth canal of the infected mother.

The trophozoites multiply by binary fission. The parasite is transmitted by sexual contact and also by sharing of towels or undergarments.

2.1.1.5 Life Cycle

The trophozoites are infective forms. It resides in vaginal passage, bartholin gland and the urethra of women and urethra, prostrate, seminal vesicles and epididymis in man. The multiplication of the trophozoites takes place by binary fission. The parasite is transmitted by sexual contact and also by sharing of towels or undergarments.

They cause vaginitis after an incubation period of about a month.

2.1.1.6 Pathogenicity

They cause vaginitis after an incubation period of about 1 month. The symptoms of vaginitis by *T. vaginalis* are a yellow frothy bad odorous discharge. The infection is also associated with leucorrhoeic condition. The vulva region becomes very much

red due to congestion of capillaries, feeling of itching, painful micturition, tendency of frequent micturition and painful coitus also occur. In males the infection is asymptomatic but spreads the infection to female sexual partner during coitus.

2.1.1.7 Energy Production

Trichomonads are aerobic organisms. They acquire energy from incomplete degradation of simple sugars producing lactic acids and acetic acids. (see *Trichomonas hominis*).

In in vitro culture, it is found that these organisms grow most successfully in glucose and maltose.

ATP is formed in the cytoplasm by substrate level phosphorylation which is used by the organisms and energy is liberated from ATPs.

2.1.1.8 Diagnosis

In females *T. vaginalis* is found in sedimental urine, vaginal secretion swab but in males in the urine or in prostatic secretion after prostatic massage.

2.1.1.9 Prophylaxis

Male and female sexual partners are treated simultaneously. Its control and prevention needs time and patience.

2.2 Intestinal Flagellates

2.2.1 *Giardia intestinalis*

2.2.1.1 History of Discovery

The flagellate parasite was first seen by Leeuwenhoek in 1681 while examining his own stool. Author (Das) separated for the first time the *Giardia* in Indian Goats and Lions (Das and Jha 1967; Jha et al. 1968).

2.2.1.2 Geographical Distribution

These flagellate parasites are cosmopolitan in distribution.

2.2.1.3 Habit and Habitat

Giardia is a parasite of human beings and found in the duodenum and other parts of small intestine. Sometimes they are found in the colon and bile duct.

2.2.1.4 Structure

Giardia occurs in two different forms: Trophozoite and cyst.

Trophozoite

The body is bilaterally symmetrical and measures 10–18 μ in length. The shape of the body is like 'tear drop'. The dorsal surface is convex and ventral surface is concave. The concave ventral surface has two rigid bilobed concave adhesive discs.

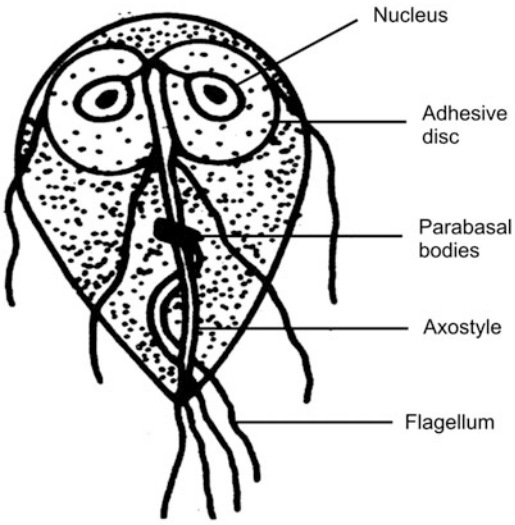


Fig. 2.2 Trophozoite of *Giardia intestinalis* (*Advanced Parasitology*, Das)

These two ventral discs occupy most of the anterior part on the ventral surface of the trophozoites. These adhesive discs are organelle of attachment which make contact and attach the parasite with intestinal wall of the host (Fig. 2.2). The attachment is also achieved by a hydrodynamic force generated by a pair of ventral flagella and a mechanical force developed by contractile protein like giardin present in the ventral disc (Karyakarta and Damle 2003).

Just below the adhesive discs there is a single sometimes double median bodies which stain black in iron-alum-haematoxylene. There are four pairs of flagella: anterior, posterior, ventral and caudal pair.

There are two nuclei placed at the broader end of the body containing haploid number of chromosomes. A single or double axostyle (Kinetosome) is seen in the mid ventral line of the body.

Giardia is unable to synthesize phospholipids and sterols which are necessary for its growth and metabolism. They use phospholipids and sterols found in the intestine of the host. It is proved that bile salts present in the intestine facilitate the entry of these organic chemicals within the trophozoites.

Cysts

The fully formed cyst is oval in shape and measures 12 μm × 7 μm in dimension. The cyst wall is thin and cytoplasm does not fill the entire cyst. There are four nuclei which remain clustered at one end or lie in pairs at opposite poles. The remains of disintegrated flagella are seen as streak in iodine preparation. The axostyle lies diagonally.

2.2.1.5 Life Cycle

Mature cysts are the infective forms, the normal dose is 10–100 cysts. The transmission route is faecal–oral. Excystation happens in small intestine of the host. The

process is initiated due to the presence of pancreatic enzymes present in the small intestine.

Giardia undergoes multiplication by binary fission in the upper part of the intestine while it stays in trophozoite stage. In unfavourable condition in the small intestine encystment of the parasite takes place. Usually encystment takes place in large intestine. An acidic environment causes the *Giardia* to form cysts.

A tough resistant cyst wall is formed around the organism. Within the cyst parasite undergoes binary division and two cells are formed. The cytoplasm of the cyst does not fill entirely the cyst a gap around the inner side of cyst wall remains.

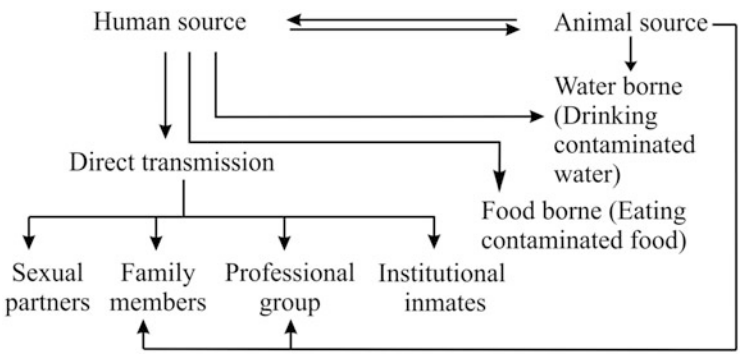
Excystation occurs in upper part of the small intestine of the new host. Two individual trophozoites come out from the cyst. The trophozoites attach themselves to the mucosa coat of the intestinal wall with the help of ventral sucker of the parasite. They used to multiply by longitudinal binary fission every 6–10 h interval and population increases tremendously within the intestine. The trophozoites feed through the processes of pinocytosis.

The parasite completes its life cycle in a single host. They are monogenic parasite.

2.2.1.6 Transmission

Trophozoites after encystation come out from its host along with the faeces of the host. They remain viable in soil and water for 2 weeks. Then the cysts transmit to the new healthy host with contaminated food and drink with cysts of *Giardia*.

2.2.1.7 Possible Pathway



2.2.1.8 Pathogenicity

Most of the *Giardia* infection is symptomless. When the infection is symptomatic the incubation period is 1–3 weeks. Early symptoms included diarrhoea, abdominal pain, nausea, vomiting. Acute infection subsides within 2 weeks. In subacute infection to chronic one diarrhoea, abdominal irritation with excess of mucus production take place. Sometime inflammation of gall bladder occurs. If the

population of parasite is large then malabsorption of fat may cause. Patient may complain of looseness of bowel and greasy stools with excess of fat.

2.2.1.9 Pathways Associated with Energy Production

Giardia is capable of incorporating certain monosaccharides into glycogen. Though the parasites have no mitochondria yet it can use oxygen if available. The organisms depend upon flavin-dependent substrate level phosphorylation for the requirement of energy. The end-products are ethanol, carbon dioxide and acetate.

2.2.1.10 Immunological Response

Giardia infection in human beings produces anti giardial antibodies and IgA-dependent host defence eradicate trophozoites. IgA binds to the surface of the trophozoites of *Giardia* which inhibits them to attach intestinal epithelium and expelled them from the body of the host.

2.2.1.11 Diagnosis

Diagnosis includes microscopical examination of freshly passed stool for trophozoites and cysts of Giardia.

Trophozoites of *Giardia* may be recovered from aspirated liquid from the duodenum or from the bile duct.

Control: Gastrointestinal parasites like *Giardia* may be controlled by strict method of assimilation and by encouraging hygienic habits in the community.

2.2.2 *Trichomonas hominis*

2.2.2.1 History

It was discovered by Davaine in the year 1860 and he called it *Cercomonas hominis.* As they possess five anterior flagella they are included in the genus Pentatrichomonas.

It is a non-pathogenic intestinal flagellate parasite. They are living as harmless commensals in the ileo-caecal region of human intestine. They may be seen in diarrohoeic stool. They exist only in trophozoite stage.

2.2.2.2 Morphology

The size ranges from 8 to 12 μm in length, the body is pear shaped. There is single ovoid nucleus with small central karyosome located at the round anterior end. They possess three to five anterior and one posterior flagellum. The undulating membrane is supported at the base by a rod like structure, the costa. The axostyle runs through the middle of the body and ends in a pointed extremity. The cytostome is conical.

The movement seen in freshly evacuated stool is jerky. They divide by binary fission. Infection is generally asymptomatic.

2.2.2.3 Habitat

T. hominis resides in the large intestine of human beings. Here they divide by binary fission and form a large colony. They consume bacteria and debris as food. *T. hominis* have been found from liver abscess. They have been found to withstand the acidic condition of the stomach.

2.2.2.4 Life Cycle

Transmission occurs through contamination. Flies also may act as mechanical vector. No cysts are formed and trophozoite is the infective form. The infection rate is high where sanitation is poor. It is observed that they cannot form colony in the mouth or urinogenital tract.

2.2.2.5 Diagnosis

The diagnosis is made from routine examination of diarrhoeic stool.

2.2.2.6 Pathogenesis

They are harmless commensal.

2.2.2.7 Pathways Associated with Energy Production

Trichomonads are anaerobic. They degrade carbohydrates to short chain organic acids, lactic and acetic acids and as usual carbon dioxide. Trychomonads in the absence of oxygen produce molecular hydrogen.

Trichomonads have organelles called hydrogenosomes because of their action, Hydrogenosomes are like mitochondria those are absent in the Trichomonads. In the hydrogenosomes DNA and cardiolipin are absent but these are present in the membranes of mitochondria. Two closely opposed membranes of 6 nm thick surround the hydrogenosomes (Schimidt and Roberts 2013).

By the process of glycolysis pyruvic acid is formed in the cytoplasm of Trichomonads and a portion of this pyruvic acid is reduced to lactic acid by the metabolic enzyme lactic dehydrogenase which is excreted but rest portion enters into hydrogenosomes. This is oxidatively decarboxylated. The electrons are accepted by ferredoxin. This is an iron sulphur protein that serves as electron acceptor in metabolic reactions. These electrons are changed to protons under anaerobic condition by the metabolic enzyme hydrogenase present in the hydrogenosomes to form molecular hydrogen. Oxidation of pyruvic acid to acetic acid generate ATP, i.e. generation of energy in the cell (Schimidt and Roberts 2013).

2.3 Parasitic Amoebae

2.3.1 *Entamoeba histolytica*

2.3.1.1 History

Entamoeba histolytica, the protozoan intestinal parasite was first discovered by Losch in 1873 in St. Petersburg, Russia. It was discovered from the faeces of a young peasant suffering from blood dysentery. Large number of parasites were found from his watery, bloody stool. Losch also observed that these parasitic amoebae contained RBCs in their food vacuoles.

Schaudin after 40 years in 1903 observed that intestinal amoebae can cause disease so they are pathogenic to man. This amoebic parasites are sometimes become tissue invader causing serious fatal disease. *Entamoeba histolytica* during its life time may become invasive form and secrete an enzyme which has lytic effect on tissues of the host and so they are called histolytica. But these parasitic organisms normally reside in the upper part of the large intestine as a commensal but sometimes invade the intestinal mucosa and cause amoebiasis.

2.3.1.2 Geographical Distribution

Entamoeba histolytica is cosmopolitan in distribution. But its prevalence is greater in tropical and sub-tropical countries and gangetic plain of India than temperate countries. The incidence is considerably higher in rural and densely populated urban areas than in the clean cities.

2.3.1.3 Habit and Habitat

E. histolytica is a unicellular, protozoa, microscopic endoparasite of human beings. They live as commensal but sometimes invade the mucosa and such invasion of the mucosa coat of the intestine of the hosts cause serious type of amoebiasis. In chronic cases, they enter into the blood circulation and migrate to the organs like spleen, liver, lungs and brain also, where they create ulcer and abscess causing death of the host. *E. histolytica* in its life cycle have trophozoite, cyst and pre-cystic stages. Cystic stage is the infective form and infection takes place by cyst from infected person to healthy persons. The harmful stage is the trophozoite.

2.3.1.4 Morphology

E. histolytica have two distinct forms: Trophozoite and cyst. But some are of opinion that they also have another form called pre-cystic forms but they remain in that form only for a very short time.

Trophozoite

Trophozoites of *E. histolytica* are very much active, motile and feeding forms. They sometime transfer into pathogenic forms to human beings.

The size of the trophozoite is 18–30 μ in diameter. The covering of the body, the plasma membrane is thin, elastic and semi-permeable in nature. The enclosed cytoplasm is differentiated into outer ectoplasm and inner endoplasm. The ectoplasm

is clear and the endoplasm is granular. The body surface contains pseudopodia which are small, round or elongated projection formed from the ectoplasm.

The granular endoplasm contains a nucleus which is visible in a living specimen and can be studied from a fixed and stained one. Nucleus is enclosed by a thin and delicate membrane whose inner surface is beaded in appearance due to presence of chromatin granules arranged in a peripheral ring. In the nucleus there is a small dot like endosome surrounded by a clear space. The fluid filled space between the nuclear membrane and endosome is marked by spoke like striations of nucleoplasm giving the nucleus a cart wheel appearance. The size of the nucleus is 4–6 μ in diameter.

In the endoplasm there are food vacuoles with ingested RBCs, WBCs, fragments of epithelial cells and bacteria. But no contractile vacuoles are present, because osmotic concentration of protoplasm of *E. histolotica* is equal to that of the intestinal fluid of the host and so no water enters into the organism by osmosis. So there is no need for osmoregulation.

E. histolytica during movement produces broad, blunt pseudopodium. The **nutrition** is holozoic and it feeds by phagocytosis.

In this connection it may be mentioned that some scientists opined that there are two races: Magna and minuta. The magnas are 20–40 μ in diameter and are tissue invaders. The minutas are 13–15 μ in diameter and remain in the lumen of the intestine feeding on bacteria and organic materials.

Pre-cystic Phase

This is a phase between trophozoite and cyst. Before the formation of cyst trophozoites eliminate all food vacuoles, then round up and shrink by condensation of cytoplasm. It now measures 10–20 μ in diameter. The nuclear morphology remains same with that of trophozoite.

Cystic Phase

The cystic form is small, spherical, non-motile and non-feeding form. A delicate refractile cyst wall develops around the parasite. The cysts measure 12–15 μ in diameter. In case of minute forms cysts measure 6–9 μ in diameter.

The nuclear morphology is same with that of trophozoite. But appears large because of the condensation of the cytoplasm of trophozoites.

At the very onset, the cyst contains single nucleus, but soon it divides by binary fission and forms two nuclei and the cyst is called binucleate. Then again by another fission the nuclei become four in number. The cyst is called quadrinucleate. The cytoplasm of the cyst becomes clear and more or less transparent.

The measurement of nuclei is 2 μ in diameter each. In immature cyst there are broad rod shaped 1–4 bodies with blunt round ends called chromatoid bodies. These bodies stain deeply with iron-alum haematoxylene. These bodies are ribo-nucleo protein (RNP) in nature. These bodies disappear in the mature cyst. Disappearance is due to the dispersion of the RNP. The young cyst contains glycogen mass which also disappears gradually.

2.3.1.5 Life History

E. histolytica requires only one host to complete its life cycle and therefore *Entamoeba* is called monogenetic parasite.

Binary Fission

The trophozoites multiply by binary fission in the lumen of the large intestine. The scientists are of opinion that some trophozoites take mucus, blood corpuscles, bacteria, etc. from the wall of the intestine and forms ulcer there. Here in the mucosal folds they divide by binary fission. Some mature trophozoites due to peristaltic movement of the intestine dislodge from the mucus layer and become inactive instantly. These inactive parasites transform into pre-cystic stage. Some trophozoites when passed in faeces, die on exposure to air, gastric acid also destroy them. So trophozoites cannot transmit infection (Fig. 2.3).

During binary fission in the mucus wall of large intestine the nucleus of the trophozoite undergoes mitosis without the disappearance of the nuclear membrane. The mitosis of nucleus is followed by cytokinesis. Two daughter organisms are formed. They grow rapidly in size by taking food from the host and continue to form invasive forms.

For transmission of infection and continuation of species the pre-cystic forms again transform into cyst.

Encystation

In the process of encystation the trophozoite becomes non-motile, round and devoid of any food vacuole. This stage is called pre-cystic stage. The precysts are smaller than trophozoites and larger than cysts. They now secrete a highly refractile cyst wall around themselves and transform into cysts. Cysts are formed only in the lumen of the intestine and not in the tissues. The cyst at this stage is uninucleate and measures 8–22 μ in diameter and is spherical.

The immature cysts have a single nucleus of 5 μ in size, diffused glycogen and rod shaped bodies formed of RNP. These are called chromatoid bodies and stain black in iron-alum haematoxylene. The glycogen may reserve as food and chromatoid bodies contain ribonucleic protein.

The cysts mature after two mitotic divisions. The single nucleus of the cysts first become double and called binucleate cysts. Then after second mitotic division four nuclei are formed and the cysts are called quadrinucleate cysts. The nuclear size is successively reduced from 5 to 2 μ in diameter. The whole process is completed within a few hours. Simultaneously the glycogen mass and chromatoid bodies gradually disappear.

The tetranucleate cysts are infective forms. They are transmitted to new hosts. These tetranucleate cysts do not develop further and pass out of the body through faeces from the hosts. They are highly resistance to desiccation and survive about 10 days in moist outside environment. They withstand the gastric acid present in the stomach of the new healthy host. The strength of chlorination used in the municipal water supply cannot kill the cysts of *E. histolytica* only boiling and filtration can kill the cysts and prevent infection.

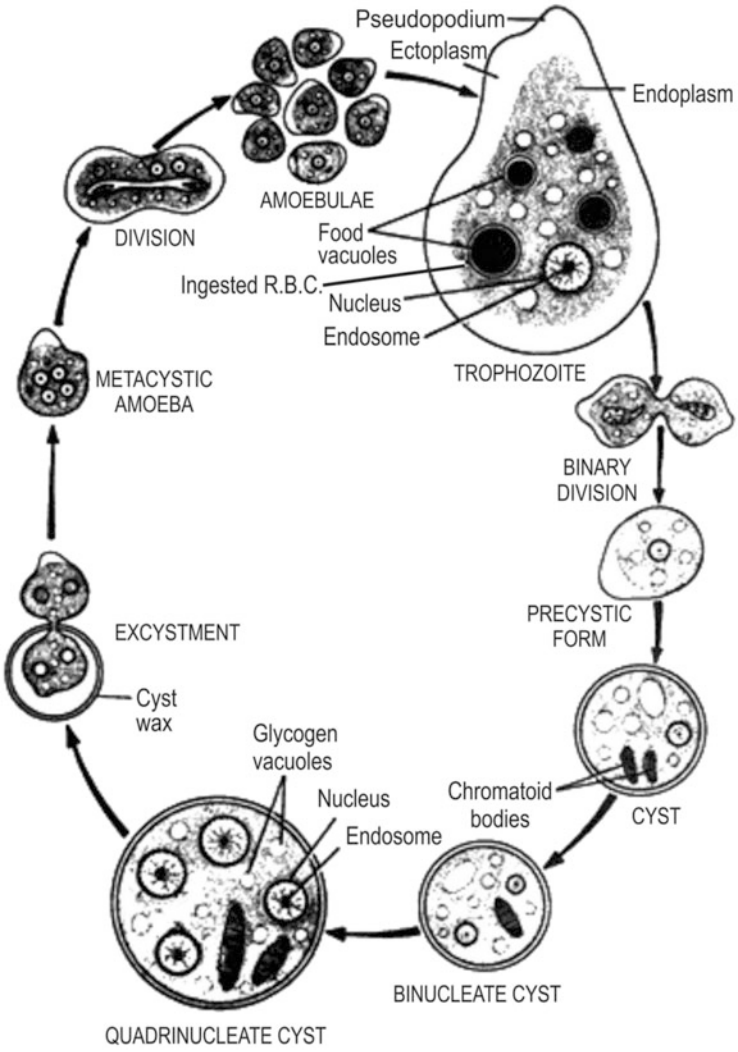


Fig. 2.3 Life cycle of *Entamoeba histolytica* (*Advanced Parasitology*, Das)

2.3.1.6 Pathways Associated with Energy Production

E. histolytica grows best in an oxygen free environment and for that reason it was once considered an obligate anaerobe. But it is proved that the organism can use oxygen in very low concentration. In vitro this parasitic amoebae cannot tolerate an oxygen concentration of 10% or higher and this is lethal to them. But carbon dioxide is required for their growth.

Glucose and galactose are the major carbohydrates, those are used by the *E. histolytica* from which they produce ethanol, acetate and carbon dioxide

obviously the energy also. In the presence of sublethal concentrations of oxygen the end-products remain same but the proportions are different. If oxygen is present in the environment they store glycogen through E.M pathways also fructose phosphate is phosphorylated for energy.

2.3.1.7 Transmission of Infection

Infection takes place through ingestion of mature cysts in food and drink. The route of infection is called faecal–oral. The contamination takes place by flies, food handlers like cooks, food sellers, hawkers when they deal with unwashed hands. The unhygienic condition in the rural areas of some countries is a common source of infection. Cross-connection between sewage and waterlines may lead to water borne epidemics of amoebiasis.

After infection within the new host the cysts pass up to the ileo-caecal region, then alkaline environment and trypsin act on the cyst wall lead to the excystation in this region of the G.I. tract of the host.

A quadrinucleate metacyst emerges from each cyst. Each metacyst immediately divides by binary fission, its nuclei divide to produce eight small uninucleate metacystic trophozoites called amoebuli. These amoebuli pass into the large intestine and grow into mature trophozoites.

The mature trophozoites live in the lumen of the intestine without invading the intestinal mucosa in about 90% of infected persons. In such persons the trophozoites after sometime encyst themselves in certain condition like overpopulation of the parasite, pH change, changes in availability of food and oxygen. The cysts then pass outside along with the faeces of the host. These persons are called cyst passers they are actually the carrier and responsible for the spread of the disease.

In the remaining 10% of the infected persons the *Entamoeba histolytica* invade the host's tissue and cause tissue amoebiasis leading to liver abscess, brain abscess, etc. resulting in the death of the patient.

2.3.1.8 Advanced Idea About *E. histolytica*

They cause major calcium ion influx to the cells of the wall of large intestine resulting in cell death and ulcer formation.

2.3.1.9 Analysis of the Epithelial Damage Produced by *E. histolytica* Infection

Infection is initiated by interaction of the pathogen with intestinal epithelial cells. The interaction leads to disruption of intercellular structures such as tight junctions (TJ). TJ ensures sealing of the epithelial layer to separate host tissue from gut lumen. Recent studies provide evidence that disruption of TJ by the parasitic protein is prerequisite for *E. histolytica* invasion. Thus the analysis of molecular mechanisms involved in TJ disassembly during *E. histolytica* invasion is of paramount importance to improve our understanding of amoebic pathogenesis.

A comprehensive study has been made on the life style of *E. histolytica* on the whole genome level allowing identification of new virulent gene and signalling pathways and process relevant to amoebic biology.

The genome of *E. histolytica* is predicted to the 24 Mb with 14 chromosomes and is functionally tetraploid.

Much progress has been made in transcriptional profile of *E. histolytica* to study diverse aspects of amoebic biology including genes involved in amoebic pathogenesis assessing differences between virulent and nonvirulent amoebic strains.

In the last year multiple studies have reported a large prevalence of amoebic seroconversion of invasive amoebiasis and amoebic liver abscess among HIV positive individuals compared with HIV negative individuals.

Immune response by host: Acute and chronic *Entamoeba histolytica* infection in colon of human beings can initiate post-inflammatory response. In acute amoebiasis, it is seen an increase in the Th2 response and this is indicated by an increase production of IL-4 cytokine. In chronic condition patient exhibit little or no change in their CD4+/CD8+ ratio. It is also proved beyond doubt that Th1 and Th2 responses remain unchanged in these patients. But in the asymptomatic patients, it is observed that the level of IFN-y increases.

2.3.1.10 Pathogenesis

Trophozoites normally multiply within the large intestine in the crypts of mucus membrane. They feed on bacteria present in the gut, some available starches and mucus secretions. The invasive forms hydrolyse the mucus membrane. At this stage they do not need bacteria as their food. It is observed that oxidation–reduction potential initiate invasiveness of the parasites along with pH of the intestine.

The invasive forms produce ulcers on the intestinal wall and reach blood vessels. Now they swim in the blood circulation and reach lung, liver and brain also. The interesting phenomenon is these invasive forms reach their dead-end because they cannot come out of the host to infect others.

The cytoplasmic extension called filopodia are seen in electron micrographs attach to host cell and secrete cytotoxic substance for cytolysis of the host cells.

Intestinal ulcer is produced in the caecum, appendix, and throughout the length of the colon. Death may occur from perforation of colon. Sometime due to tissue response to chronic ulcer where active trophozoites may remain inflammated along with a granulomatus mass may form on the inner side of the intestinal wall called amoeboma which creates intestinal obstruction. This type of abnormality happens in central south America.

The chronic amoebic infection is so dangerous that secondary lesions are found in most of the organs of the body. Out of all organs liver is highly susceptible. Whatever may be the case the infection starts from intestinal abscess. The active trophozoites are carried by the bloodstream to the veinules of the mesentery and go to the liver via hepatic portal system. Here in the liver the parasites form abscess and the disease is now called hepatic amoebiasis.

The next vulnerable organ is the lung. Most of the time the infection comes from hepatic amoebiasis due to rupture of liver abscess. It is also observed that lung may affect independently. Other organs of the body may affect, but that is very rare, like brain, penis, and/or very rare site is the skin. The other very rare sites may be

kidneys, glands like adrenal, spleen, male and female genital organs. But it is seen that all these abscesses in these organs remain bacteriologically free.

2.3.1.11 Diagnosis

The examination of stool smear preparation then fixed and stained preparation will help to observe the cysts. But repeated examination is essential as per the experience of laboratory technician. Cysts can be found in faeces during the convalescent stage of amoebic colitis. Cysts can be seen in saline preparation and in iodine preparation.

A special culture medium TYIS-33 is used to culture *E. histolytica*. DNA probes are used to distinguish between tissue invasive and non-invasive forms of *E. histolytica*.

Serological test is required to detect antiamoebic antibodies which will help to diagnose invasive forms of *E. histolytica*.

Another method of diagnosis in case of extra intestinal amoebiasis is the immunological test ELISA method application for detection of amoebic lectin antigens.

2.3.2 *Acanthamoeba*

This facultative parasite is under Phylum Sarcomastigophora, subphylum Sarcodina class Lobosa. Genus *Acanthamoeba* have five species. They are found in the tissues of human beings sometimes as facultative parasites.

These facultative parasites attack skin and/or central nervous system and cause infection in immunocompromised persons. The disease they cause is the corneal ulcer and keratitis, i.e. corneal inflammation and opacity of the cornea. It is also found that immunocompetent persons also sometimes suffer.

In 1990 it was estimated that so many cases of meningoencephalitis were detected which is due to *Acanthamoeba* infection.

The movement of the organism is very slow and has small spiky lobopodia. The five species have different invasive potential. The pathogenic organisms have ability to attach with host cells. Most of these organisms are found on beaches and multiply in saline water ranging from 0 to 3% salinity.

It was also discovered the persons with contact lenses are prone to *Acanthamoeba* infection due to washing of contact lenses with homemade saline water contaminated with *Acanthamoeba*. It is also discovered that public swimming pools are sometime source of infection. The parasites most of the time remain in fresh water and soil as free living organisms. Studies have demonstrated that if there is bacterial contamination in contact lens cleaning solution then these facultative parasites rapidly multiply increasing the rate of infection in the eyes.

Surprisingly it is also observed that some bacterial species like *Pseudomonas aeruginose* secretes toxin which is lethal to *Acanthamoeba*.

Acanthamoeba is a parasite of opportunistic infection in immune compromised persons with AIDS.

2.3.3 *Nagleria fowleri*

2.3.3.1 History

Nagleria fowleri is a free living amoeba belongs to excavate forms of Protista under the group Percolozoa or Heterolobosa. Excavates are a major assemblage of unicellular eukaryotes. Excavate contains a variety of free living and symbiotic forms of which some are important parasites of human beings.

N. fowleri though a free living amoeba but if found opportunity becomes pathogen causing the disease called primary amoebic meningo encephalitis or PAM.

The disease was first reported by Fowler and Carter in 1965.

Geographical distribution: The disease is reported from Australia, Czechoslovakia, the USA, Britain and New Zealand. Only two cases have been reported from India by Pan and Ghosh in the year 1971.

2.3.3.2 Morphology

N. fowleri is a soil amoeba. They are found in three forms: trophozoite, cyst and flagellate.

The trophozoites are elongated and measure 10–22 μ × 7 μ. They are actively motile with one broad and one pointed extremity. They move more than two body lengths per minute. The cytoplasm has a pulsating vacuole and a large nucleus. The nucleus is seen only in the fixed and stained specimen. The nucleus is single, large, 2 μm in diameter and has a large nucleolus and fine nuclear membrane. It has a large central karyosome and a perinuclear halo.

They convert from amoeboid trophozoites to biflagellate forms. The flagellated forms are 6–10 μm in diameter. They are more or less ovoid in shape having two flagella at the anterior side and a vacuole at the posterior side. The size and shape of the nucleus are same with that of the trophozoite.

2.3.3.3 Infection and Transmission

This parasite was first isolated by Carter in the year 1972. *Nagleria* is also known as brain eating amoeba. They are usually found in mud and warm fresh water from 25 to 35 °C in amoeboid or flagellated form. The amoeboid trophozoite converts from motile trophozoite to biflagellate form when it comes in contact with water. The non-feeding and non-dividing biflagellate helps the spread of *Nagleria* to fresh pools when it rains. The biflagellate forms do not multiply for multiplication they have to convert to trophozoite forms (Fig. 2.4).

The infective forms trophozoites enter through olfactory neuroepithellium of human beings during swimming in water heavily infested with these organisms. Sometimes infection also takes place by inhalation of particles of decaying animal manure which supports the growth of these amoebae.

They attack especially children and young healthy adults.

Besides trophozoite and biflagellate forms they may remain in the form of cyst. The cysts are uninucleate and possess double cyst wall. The structure of nucleus is same with that of the trophozoites.

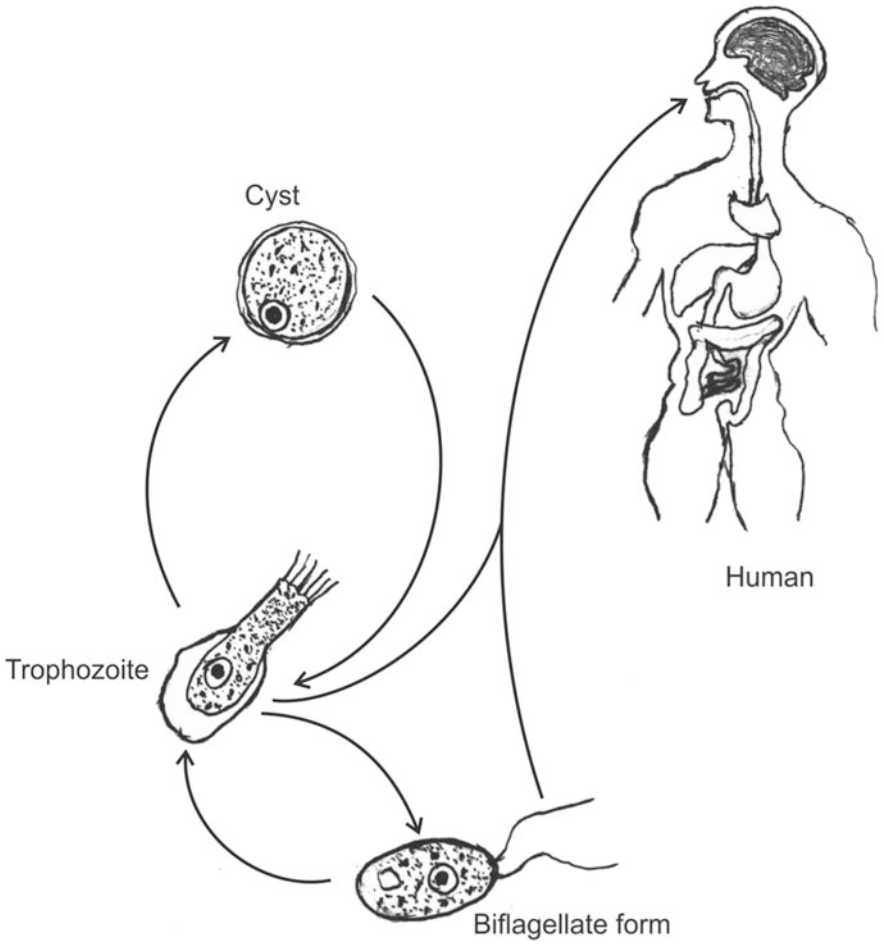


Fig. 2.4 Transmission of *N. fowleri* in human beings

2.3.3.4 Pathogenesis

The trophozoites and cysts are found in C5F and brain tissue, respectively, and flagellate form in CSF in the infected persons.

They invade and attack central nervous system via nose rather olfactory mucosa. As the amoebae penetrate the olfactory mucosa necrosis and haemorrhage start in the olfactory bulbs. Now amoebae climb along the olfactory nerve through floor of the cranium into the brain. The amoebae reaching the brain begin to consume the nervous tissue of the brain by means of sucker like structure extended from the cell surface. *Nagleria fowleri* in this condition is pathogenic to human beings causing primary amoebic meningitis or PAM disease.

The disease is known as PAM to distinguish it from the disease Amoebic encephalitis or Amoebic meningitis which is caused by *E. histolytica* in which lesions and abscess are formed in the brain.

The incubation period of PAM ranges from 2 to 15 days. The amoeboid trophozoites multiply in the grey matter of the brain.

The patient first feels the change in the perception of taste and smell followed by vomiting, severe headache, nausea, fever and ultimately rapid onset of coma followed by death in 2 weeks.

2.3.4 *Entamoeba coli*

2.3.4.1 History

This nonpathogenic amoeba was first discovered by Grassi in the year 1879. Mackinon and Dible found *E. coli* in Chimpanzee and other monkeys in the year 1938. This is a very common commensal amoeba living in the intestine of man, but they are also found in other primates. The author (Das) with his mentor Late Dr. H.N. Ray observed *E. coli* in the faeces of *Macaca speciosa*, *Papio porcarius, Cynocephalus mormon* and *Cercopithecus mona* (Thesis submitted for Ph.D in the year, 1970).

2.3.4.2 Geographical Distribution

It is distributed worldwide.

2.3.4.3 Morphology

The *Entamoeba coli* are found in two stages, Trophozoite and cyst with a transitional stage known as precyst are also found.

2.3.4.4 Trophozoite

It is said to be one of the largest amoeba residing in the colon. The more or less round or oval trophozoite varies in size from 15 to 50 μm in diameter. The movement of trophozoite is sluggish. The cytoplasm is not so clear. The endoplasm is granular and opaque. Within endoplasm there are a number of food vacuoles containing bacteria but no RBCs have ever found.

The nucleus of the trophozoite sometimes may be seen in unstained preparations. The karyosome is eccentric, the coarse chromatin granules remain arranged on the thick nuclear membrane.

The trophozoites of *E. coli* observed by the author (Das) in the faeces of stump tailed monkey were more or less similar to those recovered from man (Markel and Voge 1958; Noble and Noble 1961). Dobell demonstrated that *E. coli* of man may be experimentally transmitted to macaques.

2.3.4.5 Cyst

The round cyst varies from 15 to 20 μm in diameter. There are 1–8 karyosome placed eccentrically. Glycogen mass is visible in binucleate stage. The chromatoid bodies

are square or with pointed ends. In the mature cysts glycogen mass or chromatoid bodies are not found. The size of the cysts varies from 10 to 33 μm in diameter. Author (Das) worked with these amoebae of primates. The investigation recorded the size ranging from 16.8 to 36.0 μm in diameter of the cysts of *E. coli* instead 10–33 μm as recorded in different literature (Levine 1961).

The chromatoid bars with broken, pointed or square ends varied in size and shape were very much similar with those of *E. coli* of man.

Ectocystic envelop quite similar to *E. histolytica* was found over the cysts of *E. coli* recovered from Stump tailed monkey and Mandrill. In nature it is strongly positive and also resisted saliva digestion (Das and Ray 1967). The function might be protective to prevent destruction when it comes in contact with soil. A PAS positive cystic wall and innumerable PAS positive granules are seen scattered within the cytoplasm.

The endosome is dot shaped and surrounded by loosely arranged fine granules of chromatin often the presence of a thin membrane around the endosome was also noticed. The peripheral chromatin was usually arranged in a beaded manner. Periendosomal granules appeared as delicate fibrils or sometime completely absent. The absence of periendosomal granules was mostly found in the cysts recovered from Stump tailed monkey. The nuclear structure of *E. coli* cysts of man was similar to those found in the cysts recovered from monkeys.

The trophozoites of *E. coli* seen in the faeces of Stump tailed monkey were more or less similar to those recovered from man (Markel and Voge 1958; Noble and Noble 1961).

Dobell demonstrated that *E. coli* of man can be experimentally transmitted to macaques.

Regarding pathogenesis, *E. coli* infection is nonpathogenic and shows no symptoms of infection in monkeys.

2.3.5 *Dienmtaoeba fragilis*

2.3.5.1 History

This harmless commensal was first discovered by Jepps and Dobell in the year 1918. About 60 years ago Dobell was of opinion that *D. fragilis* was very closely linked to the genus *Histomonas*. But Camp and his co-workers on the basis of immunological evidence positioned *Dientamoeba* within a sub family of Monocercomonadidae. In the genus the species *Dientamoeba fragilis* infects about 4% of human beings.

2.3.5.2 Geographical Distribution

It is cosmopolitan in distribution.

2.3.5.3 Morphology

Trophozoites are only observed and cysts are still not known of this type of amoebae. It is also a harmless commensal of human beings and a number of primates.

The trophozoites are sensitive and decompose very quickly in faeces or water. The size of the trophozoite is 6–12 μm in diameter and the rarest intestinal amoebae of human beings. The cytoplasm is differentiated into ecto and endoplasm. Single pseudopodium is present. The cytoplasm contains food vacuoles containing bacteria, cellular debris, starch granules, etc., but never RBCs.

The nuclear character shows the large chromatin granules are usually 6 in number which forms a star shaped cluster.

The salient feature is two nuclei connected by a filament which may be seen under light microscope and so the name is given *Dientamoeba*. The endosome is present eccentrically.

2.3.5.4 Habitat

D. fragilis resides in the caecum of human beings. As they are harmless commensals they devour cellular debris and bacteria. Some study carried in some parts of Canada showed some people remain infected with *D. fragilis*.

2.3.5.5 Symptoms

The symptoms of the disease found in the study of Canada were diarrhoea, abdominal pain, liquid stool, etc. scientists are of opinion that *D. fragilis* infection often occurs with other parasitic amoebae, so it cannot be ascertained that all the symptoms are caused by *D. fragilis*.

The mode of infection cannot be described as there is no cysts present and trophozoites can survive in the upper digestive tract.

The binucleate trophozoites of *D. fragilis* were encountered in the faeces of *Papio hamadryas, Papio porcariaus, Cynocephalus mormon* and *Cercopithecus nictitans* in our study.

The study done by one of the authors (N. Das) recorded primates as new hosts because no such hosts of *D. fragilis* are recorded by any earlier investigators. But presence of *D. fragilis* in the sheep of California was recorded by Noble and Noble.

The trophozoites found in our study were unusually oblong or ovoid measured 7.2–14.4 μm in length and 4.8–10.8 μm in breadth. *D. fragilis* from human sources are usually 9.0–18.0 μm in diameter. The two nuclei were small and connected by a delicate filament. This structure degenerates quickly with the death of the amoeba.

The endosome varied a great deal in morphology. It may be compact or loosely arranged, composed of six to eight chromatin granules placed centrally or it may be represented by chromatin plaques near the margin of the nuclei. Sometimes the endosome was very large, filling up almost the entire nuclear space. Peripheral chromatin was never observed. Periendosomal granules were present in a fibrillar form connecting the endosome with the nuclear membrane.

2.3.5.6 Pathogenesis

It was thought to be nonpathogenic. There were reports of mucus diarrhoea, sometimes virulent with these amoebae only.

D. fragilis is generally regarded as a rare species, but we found in 5 out of 12 hosts examined.

2.3.6 *Iodoamoeba butschlii*

2.3.6.1 History

The amoebae were first discovered by Prowazek in 1912 and it was first described by Dobell in 1919.

2.3.6.2 Habitat

They reside as harmless commensals in the colon of man and other primates like *Macaca speciosa, Papio hamadryas, Papio porcarius, Semnopithecus entellus, Cercopithecus nictitans* and *Pan satyrus*.

The only species of *Iodoamoeba* known as *butschlii* is capable of infecting human beings and other primates and mammal like pigs. The main or original host of this species of amoeba is the pig. The propensity of this parasite in human beings is 4–8%.

2.3.6.3 Geographical Distribution

It is cosmopolitan in distribution.

2.3.6.4 Morphology

The parasite exists in two forms trophozoite and cyst.

2.3.6.5 Trophozoite

It measures 9–14 μm in length and 4–10 μm in breadth. They have blunt pseudopodia and crawl slowly with the help of pseudopodia. The trophozoites have clear cytoplasm and granular endoplasm. The nucleus is vesicular and has a large endosome. The midway between nucleus and nuclear membrane there are lightly stained granules between the nuclear membrane and the endosome, there are some strands which are achromatic.

The cytoplasm of trophozoites has food vacuoles which are full of bacteria and yeasts.

The *Iodoamoeba butschlii* have pre-cystic stage. The shape is ovoid that contains no undigested food. The precyst secretes a hard cyst wall and the shape becomes ovoid. They measure 6–15 μm in length and 4–10 μm in breadth.

2.3.6.6 Cyst

The nucleus of the cyst is 9–12 μm having a glycogen vacuole which stains with iodine. So the name is iodoamoeba. In the matured cyst the nucleus is pushed towards the nuclear membrane. The karyosome is placed more or less near the nuclear membrane. This parasite is nonpathogenic to human beings.

2.3.6.7 Biology

I. butschlii resides in the large intestine especially near the caecum. As the route of infection is faecal–oral so infection spreads through food and drink contaminated with cysts. It is observed that infection may be due to faeces of infected human beings and pig.

During our study (Das and Ray) this common intestinal nonpathogenic parasite was observed in the *Macaca speciosa, Papio hamadryas, Papio porcarius, Semnopithecus entellus, Cercopithecus nictitans* and *Pan satyrus.*

The trophozoites recovered from different primates in our (Das and Ray) investigation ranged from 8.4 to 21.6 μm in length and 7.2–18 μm in breadth. But earlier the measurement recorded was 9.00–14 μm (Wenrich 1937) or from 4.00 to 20.00 μm (Levine 1961). Simitch et al. (1959) isolated trophozoites of *I. butschlii* from 156 pigs were morphologically similar with that of *I. butschlii* of man. But between two hosts there were differences in character and so they named them *I. suis*.

The nucleus measured 4.8–8.2 μm in diameter containing a single round endosome The earlier record was 2–6 μm in diameter (Wenrich 1937). The peripheral chromatin was present in trophozoites in the form of a thick ring. But its presence in the trophic form was not mentioned earlier (Wenrich 1937; Levine 1961). The periendosomal granules was not observed always but when present, it was in the form of fibrils extending from the endosome to the nuclear membrane (Das and Ray 1970).

The size of the ovoid cysts of *I. butschlii* was 8.4–26.6 μm long and 8.4–26.6 μm long and 8.4–15.6 μm broad. The measurement recorded by Hoare (1959), Markel and Voge (1958), Wenrich (1937) and others did not agree with our observation.

The cyst wall of *I. butschlii* from *Pan satyrus* was alcian blue positive suggesting acid mucopolysaccharide in nature.

Granular or alveolar cytoplasm of the cyst of *I. butschlii* contained PAS positive glycogen vacuole which sometime seemed to have pushed the nucleus to one side of the cyst.

The chromatoid bars, very slender, small and curved were seen in the cysts recovered from white nosed monkey only. Levine (1961) was of opinion that there were no chromatoid bars in the cysts but contained deeply stained volutin like granules.

Single large round nucleus measuring 3.0–7.2 μm in diameter showed a delicate, alcian blue positive nuclear membrane. This feature was not noticed by earlier workers.

The peripheral chromatin of the nuclear membrane of *I. butschlii* was seen to occur in the cysts from Langur, white nosed monkey and Chimpanzee. The presence of the chromatin granules in the *I. butschlii* cysts was not reported earlier.

Regarding pathogenicity, no pathogenic symptoms have been seen in the primates harbouring *I. butschlii*. There was an unique instance of fatal infection by *I. butschlii* in a Japanese soldier captured in New guinea.

2.4 Haemoflagellates

2.4.1 *Trypanosoma cruzi*

Trypanosoma cruzi is a haemoflagellate parasitic protozoa, the causative agent of the disease called Chagas disease or American trypanosomiasis in central and South America.

Trypanosoma cruzi is transmitted by the insect vector *Triatoma infestans, Panstrongylus megistus* and *Rhodinus prolixyus*, the blood sucking bugs of family Reduviidae.

The disease may also spread by blood transfusion, organ transplantation, ingestion of food contaminated with parasite and from mother to her foetus.

2.4.1.1 History

The disease was named after the Brazilian Physician, Carlos Chagas, who first reported the disease in 1909. But the disease was not recognized as a major disease in human beings until 1960. Chagas discovered that in the intestine of Triatoma, a reduviid bug, a flagellate protozoan parasite of genus *Trypanosoma* resides. He experimentally proved that the disease can be transmitted to marmoset monkeys taking the help of infected reduviid bugs. Chagas for the first time named the species *Trypanosoma cruzi* after the name of famous Brazilian Physician Oswald Cruz. Oswald Cruz successfully eradicated the epidemic of yellow fever, small pox and bubonic plague in Rio de Janeréo and he is famous for his such work.

2.4.1.2 Symptoms

The symptoms of Chagas disease vary in course of infection local swelling at the site of bite of the bug is seen. With the progress of the disease chronic symptoms of heart disease, malformation and malfunction of the intestine of the host appear. If untreated the disease becomes fatal.

In human beings the disease manifests in two stages: an acute stage appears just after initial infection and chronic stage that develops over a period of many years.

The acute stage continues for the first few weeks or months after infection. The symptoms manifested are fever, fatigue, body aches, headache, rash on the skin, loss of appetite, diarrhoea, nausea and vomiting. On physical and clinical examination mild enlargement of liver and spleen, swollen glands and local swelling at the site of infection are found. Most important symptom is swelling of the eyelid on the side of the face near the bite of the bug. If untreated patients die from severe inflammation of the heart muscles or brain. Some patients develop neurological disorders.

2.4.1.3 Morphology of the Parasite

The causative agent of the disease is a parasitic haemoflagellate, *Trypanosoma*. This parasitic haemoflagellate lives in the blood of man and other animals causing disease. The *T. cruzi* is a curved, stumpy trypanosome measures about 20 μ in length with a pointed posterior end and an elongated nucleus at the centre of the body. A large egg shaped kinetoplast is present at the posterior end. A narrow slightly wavy

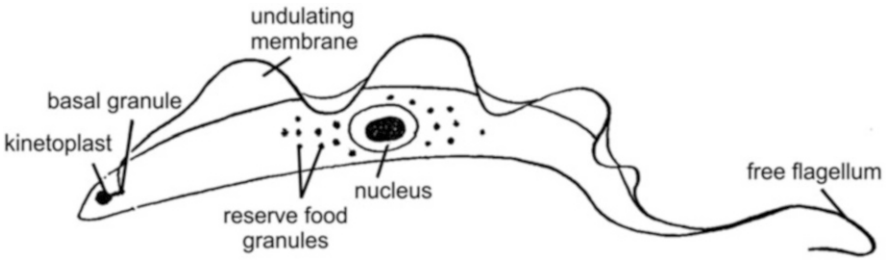


Fig. 2.5 Morphology of *Trypanosoma cruzi* (*Advanced Parasitology*, Das)

undulating membrane and a long free flagellum are the salient features of the said *Trypanosoma* (Fig. 2.5).

The morphology changes during their development. They remain as trypomastigote form in the peripheral blood of vertebrate host in acute stage of the disease and do not multiply within the blood of human beings.

When they enter into the bloodstream of the vertebrate host, trypomastigotes are phagocytosed by different cells of the immunity system including macrophages and quickly transform into amastigote form. The change is caused by the low pH of the lysosomal contents during phagocytosis. The resulting amastigote avoids the lysosomal action by entering into the cytoplasm of the infected cell. Amastigote forms live in muscles of heart, skeletal system, nerve cells and cells of R.E. system which is formed from metacyclic trypomastigote form. Amastigote forms are spherical and measure 2–4 μm in diameter and are the multiplying forms.

They complete their life cycle in two hosts: man and Reduviid bugs. Within the insect host they change from amastigote to promastigote, promastigote to epimastigote, epimastigote to metacyclic form of trypomastigote. Multiplication can take place in any of these developmental stages.

Horizontal transmission takes place from one vertebrate host to another by blood sucking bug *Triatoma*. After an infective feeding development takes place within the invertebrate host, reduviid bug. So certain time is required before it can infect another healthy individual. Infectiveness of the bugs develops only after the development of metacyclic form of trypomastigote within the bugs which requires some time.

Ingested trypomastigote in the sucked blood develops in the intestine of the insect vector from where they go backwards to the hind gut of the infected insect. Transmission happens mostly by rubbing the faecal matter of the insect contaminated with metacyclic trypomastigotes into the wound caused by the insect bite.

2.4.1.4 Developmental Stages

Amastigote form: Represented by round forms without any external flagellum and have a nucleus and a kinetoplast.

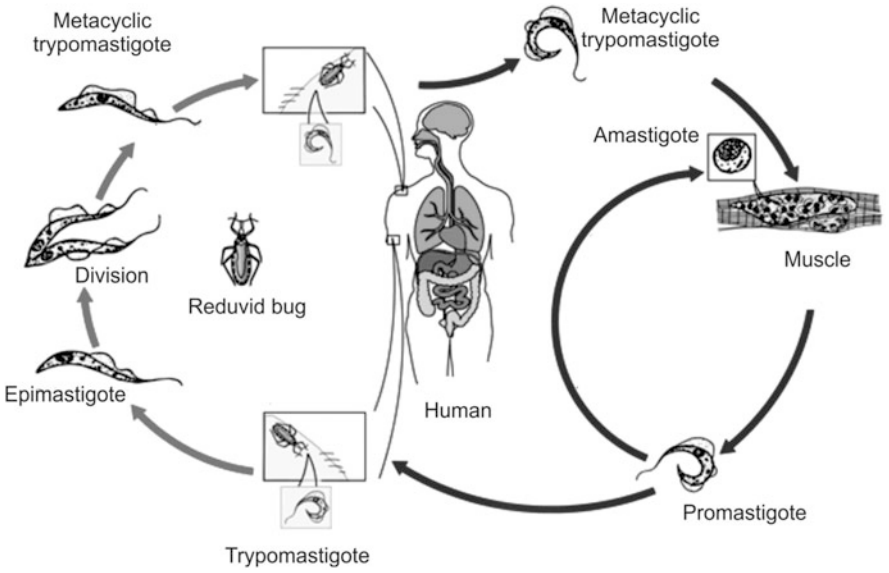


Fig. 2.6 Life cycle of *T. cruzi*

Promastigote form: Represented by forms with the kinotoplast present anterior to the nucleus, a single long slender flagellum which arises from a basal granule very near to the kinetoplast.

Epimastigote form: Represented by forms with kinetoplast located anterior and close to the nucleus the flagellum arises from the basal granule very near to the kinetoplast and is connected with the body up to the anterior end by an undulating membrane.

Trypomastigote form: Represented by forms with post nucleus kinetoplast located at the posterior end of the body and flagellum is attached to the body for most of its length with an undulating membrane.

2.4.1.5 Life Cycle

Trypanosoma cruzi requires two hosts, definitive and intermediate, to complete its life cycle. Human beings are the definitive hosts and reduviid bugs like *Triatoma infestans*, *Panstrongylus megistus* and *Rhodinis prolixus* are the intermediate hosts.

T. cruzi does not multiply in the blood of vertebrate host though it is a haemoparasite but divides rapidly in the tissue of human beings and the animals. The parasites multiply in the tissues of the heart and voluntary muscles, central nervous system and in different glands of the body.

The infective form of the *T. cruzi* is called metacyclic trypomastigote. These forms are liberated by the infected intermediate host in their excreta (Fig. 2.6). The intermediate hosts, the reduviid bugs of genus Triatoma and others bite human beings and suck blood from the face specially the eyelids. The bugs are known as kissing bugs for their special choice of the face of the vertebrate host for bitting and

sucking blood. The human beings are infected because of the rubbing of the eye at the site of bite where they defaeciate during bite. The human beings rub the site as they feel irritation due to bite of bug and sucking of blood the infective forms are liberated along with the faeces due to squeezing out of slapping. Animals are infected by eating the bugs or licking the site of the bite.

During the day, the bugs hide in crevices of the walls and roof of the house. The bugs come down during night when the victims are sleeping. After the bite and sucking of blood from an infected person the bugs take some period of time and then bite and defaeciate on the face of another person and same thing happens.

T. cruzi may also be transmitted through blood transfusion, organ transplantation and breast milk. Chagas disease may also spread congenitally through placenta and accounts for about 13% of still born deaths in some regions of Brazil.

The trypomastigote forms which the bugs suck along with the blood meal reach in their intestine and within 24 h the trypomastigote forms transform into epimastigote and multiply profusely. The epimastigote forms after division pass to the rectum where they give rise to metacyclic trypomastigotes which are same in shapes, size and structure with the trypomastigote forms found in the blood of vertebrate hosts and are the infective forms. They are found in the larval bugs in about 6th day and 10th to 15th day in adults after the sucking of blood. As many as 3500 of metacyclic trypomastigote forms are found in 1 cubic millimetre of the excreta of the insect.

Within the human beings the metacyclic trypomastigotes enter into different tissues of definitive host where they divide quickly. The organs most vulnerable are the spleen, liver, lymph glands and all types of muscle cells. Besides nervous and reproductive systems, intestine and bone marrow are sometimes invaded. During the stage of rapid intracellular division the parasites transform into amastigote form then change into promastigote, epimastigote and trypomastigote form. The tissues or cells loaded with the parasites rupture liberating the parasites which either infect another tissue or disperse to other parts of the body through blood or lymph. In the early acute stage the parasites can live in the blood but in chronic stage when antibody reaction started in the vertebrate host the blood forms are not seen but can be found in large number in the tissues.

The ultimate trypomastigote forms in the blood are metacyclic trypomastigotes.

2.4.1.6 Pathways Associated with Energy Production

American *Trypanosoma cruzi* differs from African *Trypanosoma gambiensae* in their physiological function. The parasite is a 'partial aerobic fermenter'. The parasites exhibit Krebs cycle and classical cytochrome system in their bloodstream forms. In all the stages of life cycle of *T. cruzi* the well-developed mitochondrial cristae suggest that oxygen metabolism does not differ at all. It is proved that oxygen consumption is same in the intracellular stages and bloodstream trypomastigote and the stages that are found in insect also.

The mitochondria of Trypanosomes change in function and structure in each stage of their life cycle. Within the insect vector the parasites enter into the salivary glands. Here the mitochondria develop branches with large numbers of cristae and

fully functional TCA cycle. It is able to oxidise proline and other amino acids present in the haemolymph of the insect vector.

A complete glycolytic pathway remains in all the stages with glucose as the major carbohydrate. Some portions of the consumed glucose are degenerated entirely into carbon dioxide while the rest portion is incompletely degraded to organic acids like succinic acid and acetic acid.

2.4.1.7 Pathogenicity

Chagas disease affects 16–18 million people in the year 2008 and 100 million people are at risk. It is estimated that the disease kills about 20,000 people annually.

The disease is present in 18 countries in the American continent ranging from Southern USA to Southern Argentina.

In the South America the wild reservoir of *T. cruzi* are opossums, raccoons, armadillos, squirrels and mice. Opossums are interestingly important as reservoir host because the *Trypanosoma* can complete its life cycle in the anal glands of the animal without the help of insect vector.

The first entry of the metacyclic trypomastigotes within the human body induce a local inflammation and swelling at the site of bite of the vector.

Acute causes of the disease are found in infants or young children. The first sign is oedematous swelling of the eyelid and sometime other parts of the face usually only on one side of the face where the bug bites for blood sucking. The first sign of swelling due to inflammation is called 'Chagoma' because the parasites after entry into blood go to the local lymph nodes where they are colonizing in tissues mainly in subcutaneous fat and begin to multiply in the phagocytic cells of the immune system. In later stages other chagoma develops in different parts of the body. In early infection patients complain severe headache with continuous fever and extreme exhaustion.

After sometime the acute stage disappears and chronic stage starts which according to many physicians persists for life. There is a long latent period with a few or no symptoms. Actually at that time the disease is progressing. In the chronic stage the symptoms are oedema, inflamed lymph glands and enlargement of liver and spleen in the patients. The patients who are suffering for a long period with the infection of *T. cruzi* anaemia develops sometimes nervous disturbance also as the parasite colonize in the muscle cells and nerve cells. In severe cases death may occur within 2 or 3 weeks.

Diseased condition of the heart is the common symptom in the patient because the parasites choose heart muscle and attack them. Heart block and other abnormalities are seen in electrocardiogram.

When acute phase progresses, i.e. when eyelid and other parts of eye are parasitized then oedema and swelling take place. Then the symptom is known as 'Roman's sign'.

2.4.1.8 Host Defence Mechanism

For all the stages of life cycle of *Trypanosoma cruzi* causing Chagas disease, cell mediated immunity is very much important. The investigation shows that membrane

glycoproteins of parasite activate host cytokine production as a result increases macrophage killing capacity linked to NO production.

The response of the infected host is found in the preparation of specific antibodies and the activation of phagocytes stimulated by IFN-γ. In the early stages of acute infection increase the production of IL-2 and NK cell activity. During acute phase of infection intracellular amastigote secretes glutathione *S*-transferase in the bloodstream of host. T cell response to the infection decreases with decreased secretion of IL-2 and IFN-γ by which the parasites can avoid close observation of immune system.

Postacute or chronic infection is influenced by humoral response. It is seen that circulating IgG acts as protective immunoglobulin. Investigation on this has proved that nonprotective antibodies are also produced and they remain in bloodstream even after the patient is cured.

2.4.1.9 Diagnosis

Diagnosis is direct by examining the thick blood film under microscope. Besides blood film the parasites may be found from cerebrospinal fluid, fixed tissues and lymph. During fever of the patients in peripheral blood a large number of trypomastigotes may be found.

It is very difficult to find the parasites in the patients suffering in the chronic stage of the disease.

There is another method of diagnosis, the method is called xenodiagnosis. In this method the bug, *Triatoma* which are reared in laboratory are subjected to feed on the suspected patient and then after 10–15 days the intestine of bugs are taken out and a smear is prepared of the gut contents, if trypanosomes are present they may be seen under microscope.

In chronic cases complement fixation test or other immunologistic tests are performed to be sure of *Trypanosoma cruzi* infection.

2.4.1.10 Prophylaxis

Parasites should be controlled by treating the disease with specific drugs.

Destruction of vectors by spraying insecticides like DDT, or Dieldrin or BMC is very effective.

Personal guard is very important by using mosquito net.

2.4.2 *Tryponosoma gambiense*

2.4.2.1 History

Joseph Everett Dutton identified the parasite and in 1902 he proposed the name *Trypanosoma gambiense. The Trypanosoma* causing sleeping sickness in man was first discovered in Gambia by Dutta and Forde in 1902.

2.4.2.2 Geographical Distribution

Trypanosoma gambiense is found in Western Africa from Senegal to London. They are prevalent along the river side of Cairo and Nigeria and extended to Lake Tanganyika and south Sudan and *T. brucei* is prevalent in East Africa. *T. brucei* is found in many African wild animals and domestic animals like horses, camels and is infective for almost all types of mammals except baboons and man.

T. gambiense and *T. brucei* both are similar in all respects except their geographical distribution and some clinical manifestations.

2.4.2.3 Habitat

T. gambiense is the causative agent of the disease African sleeping sickness in man. The parasites reside in the bloodstream and in the lymph glands of human beings and are called haemoflagellates. It is also seen that in late stage they may invade the CSF.

They are mainly found in the lymph glands like spleen and cause enlargement of spleen. After 3 months they are found in CSF even in the brain tissue and spinal cord.

2.4.2.4 Morphology

The African polymorphic Tryponosomes vary in their measurement from 15 to 30 μ with exceptionally long or short forms.

The haemoflagellate *Trypanosoma* have polymorphic forms, all these are developmental stages. The different forms are:

1. Trypanosoma or Trypomastigote form: This form measures 12–30 μm in length and 2–4 μm in breadth. Nucleus is placed at the centre and kinetoplast is located at the posterior end of the cell. Undulating membrane nearly spread almost the whole length of the cell with a free flagellum.
2. Epimastogote or crithidial form: Cell body elongates with kinetoplast lying anterior and close to the nucleus and the flagellum arises from the basal granules very near to kinetoplast and is connected with the cell body up to the anterior end by an undulating membrane.
3. Promastigote or Leptomonad form: Represented by forms with kinetoplast anterior to the nucleus, a single slender long flagellum arises from a basal granule very near to kinetoplast.
4. Amastigote or Leishmanial form: Represented by round forms without any external flagellum and contains a nucleus and a kinetoplast.

The *Trypanosoma gambiense* when resides in vertebrate host like man it remains in trypomastigote form in the bloodstream and lymph glands. It is slender, elongated having a blunt posterior end and a finely pointed anterior end. It is 10–30 μm in length and 2.5–10 μm in breadth. The end from where the flagellum arises is the anterior end which is pointed (Fig. 2.7).

The large, oval, conspicuous nucleus located centrally. The kinetoplast which is situated at the posterior end is very small. The flagellum arises from a basal granule which is very near to the kinotoplast. The undulating membrane runs along the

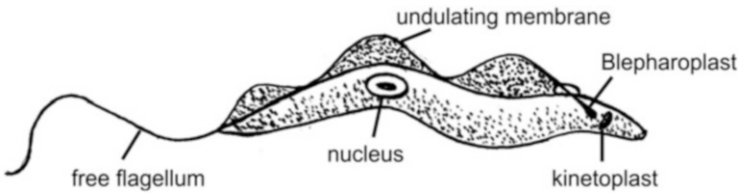


Fig. 2.7 Morphology of *Trypanosoma gambiense* (*Advanced Parasitology*, Das)

almost entire length of the body and extends beyond the anterior end as a free flagellum.

The *Trypanosoma gambiense* exists in various forms during their development, i.e. they are polymorphic.

2.4.2.5 Life Cycle

Trypanosoma gambiense requires two different hosts vertebrate and invertebrate, man and insect to complete its life cycle. The definitive host is the human beings or other mammals. The intermediate host is the insect vector tsetse fly, *Glossina palpalis*. When the infected insect vector bites healthy human beings the infective forms of trypanosome the metacyclic trypomastigotes are introduced into the blood of human beings which are stored in the salivary gland of the tsetse fly. The action is just like female anopheles mosquitoes perform in case of malarial infection.

Trypanosoma is an extracellular parasite resides in the bloodstream. The metacyclic forms when enter into the blood of human beings remain without free flagellum. They develop into flagellated long slender form, the trypomastigote form. Within the bloodstream they divide longitudinally by longitudinal binary fission. The division starts from kinetoplast which divides into two first. The nucleus divides by the process of mitotic karyokinesis. The cytokinesis starts from anterior end. Ultimately it is divided into two. After a number of divisions the long, slender forms become short stumpy forms with flagellum.

These stumpy forms enter into the gut of the vector during sucking of blood. These forms when reach the mid-gut become transformed into long, slender trypomastigote forms. These long, slender trypomastigotes multiply in the blood meal of mid-gut and ultimately reach salivary glands of the vector where they transform into epimastigote forms. Epimastigotes soon multiply and create metacyclic trypomastigotes (Fig. 2.8).

The metacyclic trypomastigotes waiting in the salivary gland of the vector reach human beings with the bite of the insect vector *Glossina palpalis*. The cycle is completed.

According to the investigators the trypanosomes stop multiplying when the glycolysis process is disturbed. The long slender forms become short stumpy forms due to loss of energy and want of food. The stumpy forms ultimately die if they are not sucked by the tsetse fly.

When the insect vector sucks the blood from the infected person the stumpy forms reach mid-gut along with the sucked blood and development within the insect vector

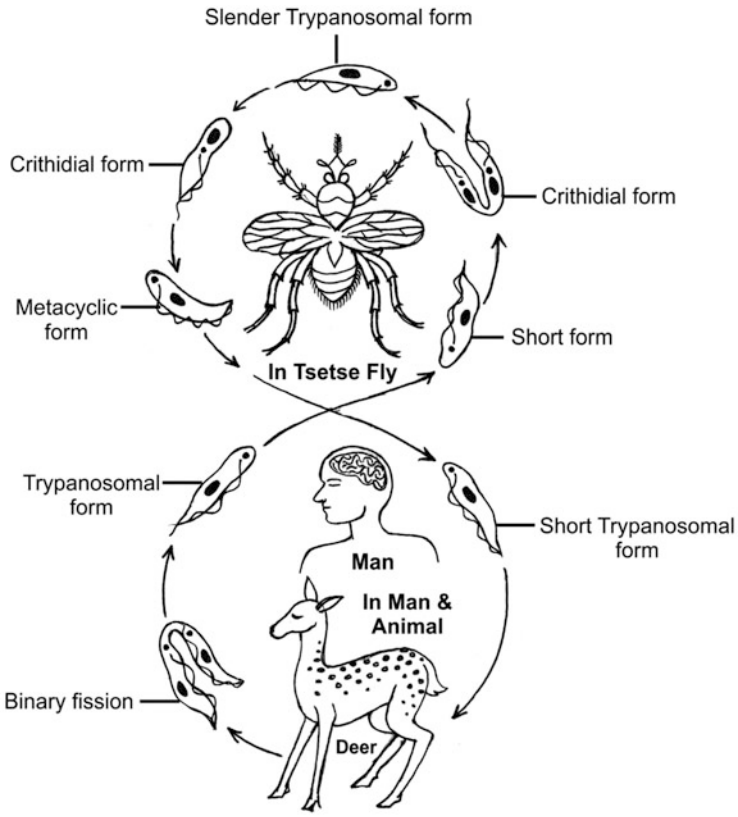


Fig. 2.8 Life cycle of *T. gambiense*

starts. It is found that in case of trypanosome the stumpy forms develop within the cavity of the peritrophic membrane of the insect. The stumpy forms transform into long, slender forms.

These long, slender forms go to the extra peritrophic cavity, i.e. the space between the peritrophic membrane and the body wall here they multiply for some days. By the 15th day they liberate from this space and enter into the proventriculus.

These long, slender forms from the proventriculus go straight to the salivary glands. Here in the salivary glands they multiply again and transform into first epimastogotes and then into metacyclic trypomastigotes which are the infective forms. The time taken by the parasite for development within the vector is about 20 days.

The vector now becomes fully ready to infect another healthy person to start new another healthy person to start new infection.

2.4.2.6 Pathways Associated with Energy Production

The long slender trypomastigote obtains energy rich compounds by substrate level phosphorylation through glycolysis which occurs in glycosomes. The reduced NAD produced in glycolysis is oxidised in the glycosomes.

The epimastigote with its well-developed mitochondria utilizes oxidative phosphorylation for ATP synthesis. Energy is procured from glycolysis. Epimastigotes in the lumen of insect gut evolve an efficient system for synthesis of energy rich compounds. The different situation arises when trypanosomes remain in the blood clot in the intestine of vector. The parasites then produce degraded glucose by glycolysis in TCA cycle.

A single mitochondrion is present in the long slender trypomastigote extending anteriorly from the kinetoplst. Their cristae are few in number, short and tubular in size. But in the parasites when remain in the intestine of vector mitochondria is well developed and extends both posterior and anterior from kinetoplast also cristae are innumerable and plate like in form and size. The infective forms for the tsetse flies are the short stumpy forms. But the transitional forms are long slender, noninfective forms. Whereas mitochondria present in the metacyclic trypomastigote form is more or less similar to those of forms present in the bloodstream. The stages in the salivary gland with tubular cristae in their mitochondria indicate less electron transfer system.

2.4.2.7 Pathogenicity

The *Trypanosoma gambiense* is the causative agent of African sleeping sickness, the human disease. It is the agent of chronic form of sleeping sickness. These flagellate parasitic protozoa enter the central nervous system and produce the chronic sleeping sickness.

A red inflamed area called trypanosome chencre develops after 1 week of the bite of tsetse fly. This chancre or wound heals but in the meantime the parasites (metacyclic trypanosomes) invade blood and lymph channels produce parasitemia. The large number of parasite invades all the organs of the body of hosts. The infected lymph nodes are congested and become swollen. Especially the nodes of neck, groin and legs are swollen. These swollen nodes are termed Winterbothom's sign. When the Trypanosomes in the circulating blood increases in number is in the early stage of infection fever starts. Then the symptoms are generalized pain, persistent headache, weakness and cramps in the body.

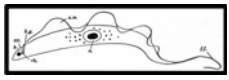
Parasitemia is seen until the specific antibodies are produced. Most of the trypanosomes degenerate due to specific antibodies produced in the host.

When the parasites enter central nervous system the symptoms are tremor of the tongue and trunk of the patient and then paralysis follows. This condition arises when the patient feels sleepiness and found falling asleep even while standing or eating. Ultimately dies due to either malnutrition or pneumonia or myocarditis with severe congestive heart failure.

2.4.2.8 Immune Response by the Host

It is a very peculiar phenomenon the parasites can change their outer variant surface glycoprotein (VSG) coat. Each VSG is immunogenic but different from the

Table 2.1 The differences of features between African and American *Trypanosoma*

Features	African	American
Distribution	Western and Central Africa	Central and South America
Disease causing organism	*Trypanosoma gambiense*	*Trypanosoma cruzi*
Vector	*Glossina palpalis* (Tsetse fly)	*Triatoma infestans* (Reduviid bug)
Reservoir	Human	Cats and dogs
Multiplication	Divides in blood	Does not divide in blood
Infective forms	Metacyclic trypomastigote	Metacyclic trypomastigote
Route of infection	Biting of insect vector	Biting of insect vector
Polymorphic form	Amastigote forms are not seen	All forms are seen
Depiction		

preceding VSG. They change their VSG every 8–10 days to hide themselves from immune response of the host. Each time the new VSG variant produces repeated parasitemia. This goes on for months. Then the parasites invade central nervous system.

As the number of parasites increases the VSG of the parasites creates IgM response by the host. The complement produces lysis effect on the parasites which results decrease in the number of parasitic population.

It is found that less than 1% of the surviving parasites, those who possess a variant VSG, again proliferate until they produce host response. In this way a cycle of remission alternating with parasitaemia occurs until the death of the host.

2.4.2.9 Comparative Account of African and American Sleeping Sickness

African sleeping sickness is somewhat different from American sleeping sickness which is called 'Chagas disease'. Though, both are caused by haemoflagellate of genus *Trypanosoma* (Table 2.1).

2.4.3 *Leishmania donovani*

2.4.3.1 History of Discovery

Leishmania donovani was discovered by Leishman and Donovan, both of them reported the presence of this organism simultaneously, Leishman from London in May1903 and Donovan from Madras in July1903.

Leishmania donovani is generally of two types: visceral Leishmaniasis known as Kala-Azar and cutaneous leishmaniasis produce lesion or local sores on the skin.

2.4.3.2 Geographical Distribution

It is found in many places in India, China, Africa, Southern Europe, South America and Russia.

In India it was once very common infection in Assam and Bengal along the coasts of the Ganges and Brahmaputra. It was also endemic in Bihar, Jharkhand, Orissa, Madras and the eastern parts of Uttar Pradesh.

2.4.3.3 Habitat

It is a parasite of blood and tissues of human beings and is intracellular living within WBC or hepatocytes, spleen, bone marrow, lymph glands, etc. They are found both inside and outside the cells and some are present in bloodstream and inside monocytes. The parasite is the causative agent of the disease Kala-azar.

A number of canaines wild and domestic are reservoir hosts and young children are mostly affected.

The vector of the disease is *Phlebotomus argentipes*.

2.4.3.4 Different Strains of *Leishmania*

The amastigote forms of the parasite infect adults and adolescent of human beings and there is no reservoir host of the strain found in India. But a more virulent, clinically similar strain is transmitted by *Phlebotomus* species in east Africa and wild rodents serve as their reservoir hosts.

A third strain is prevalent in Central and South America whose vector is *Phlebotomus longiceps* and their reservoir hosts are wild and pet dogs.

Leishmania donovani causing the visceral leishmaniasis showing symptoms of fever and enlargement of liver and spleen, cutaneous leishmaniasis caused by *Leishmania tropica* showing sores on the skin, lesions on mucus membrane of the nose, mouth, etc.

Leishmania is an important pathogenic zooflagellate. Actually there are two species *Leishmania donovani* and *L. tropica*. *L. donovani* is responsible for various types of visceral leishmaniasis.

In the year 1953 Biagi described all types of visceral Leishmaniasis which are known as Indian kala-azar, Chinese kala-azar, Mediterranean kala-azar, African kala-azar, Russian kala-azar and American kala-azar. The Indian kala-azar is transmitted by sandfly, *Phlebotomus argentipes*.

2.4.3.5 Disease

The symptoms of the disease are very misleading and same with that of typhoid, malaria or dysentery but no symptoms appear until the body resistance is lowered. The incubation period is generally 8 or 9 months.

At the initial stage there is an irregular fever along with hepatomegaly and spleenomegaly, anaemia, rheumatic fever and weakness. Cirrhosis of liver and hypertension may also result. The number of WBC population decreases. If the patient is not treated properly usually die within months. Sometimes after treatment and recovery post kala-azar dermal leishmanial nodules in the skin appear.

In Sudan a number of cases of extensive mouth lesions have been seen in which parasites were found in abundance though not in liver or spleen.

2.4.3.6 Morphology

The parasite exists in two forms: amastigote and promastigote.

Amastigote forms are found in human bodies as L.D. bodies and promastogote forms are found in insect vector sandfly.

The amastigote of *L. donovani* are small, ovoid and 2–4 μ in diameter. The L.D. bodies divide by binary fission and are mainly found in macrophages. The infected macrophages are found in quite considerable number in liver and spleen.

The promastigotes are found in the insect vector *Phlebotomus argentipes*. Promastigotes are small, oval and 15–20 μm × 1–2 μm in size.

Amastigote form: At this stage parasites are intracellular and reside within R.E system of man and other vertebrates.

It is round or oval bodies. Nucleus is located more or less at the centre or periphery. Absence of flagellum, kinetoplast present at right angle to the nucleus. There is a delicate filament present from kinetoplast to the margin of the cell called axonema. Sometimes there is a clear vacuole located along side the axonema. Axonema is the root of the flagellum.

Promastigote form: This stage is found only in the stomach tissue of sandfly, *Phlebotomus argentipes*.

This stage is represented by long, slender, spindle shaped body. A mature promastigote is 15–20 μm in length and 1–2 μm in breadth.

Nucleus is large, round and present either in the centre or at periphery. Kinotoplast is distinct and present posterior to the basal granule from where the flagellum arises. Flagellum is long either measures the same length of the body or more and present at the anterior end.

2.4.3.7 Life Cycle

Leishmania is a zooflagellate which requires two different hosts to complete its life cycle.

The main or definitive host is man or other vertebrates in which the parasite reproduces asexually (Fig. 2.9).

In the life cycle of *Leishmania*, when they reside within the mammalian host the amastigote infects macrophages, the cells of immune response, it is quite paradoxical. The parasites after entering the macrophages establishes itself in an intracytoplasmic vacuole called parasitophorous vacuole. Lysosomes fuse with this vacuole. The amastigotes defend themselves from the enzymatic actions of lysosomes and reside and reproduce within the parasitophorous vacuole. It is an interesting observation.

A number of mammals act as natural reservoir hosts of *Leishmania*. The most common of them are wild and domestic dogs and rodents. Leishmaniasis is therefore a zoonosis for human beings.

The intermediate host an insect vector known as sandfly, *Phlebotomus argentipes* is a blood sucking insect.

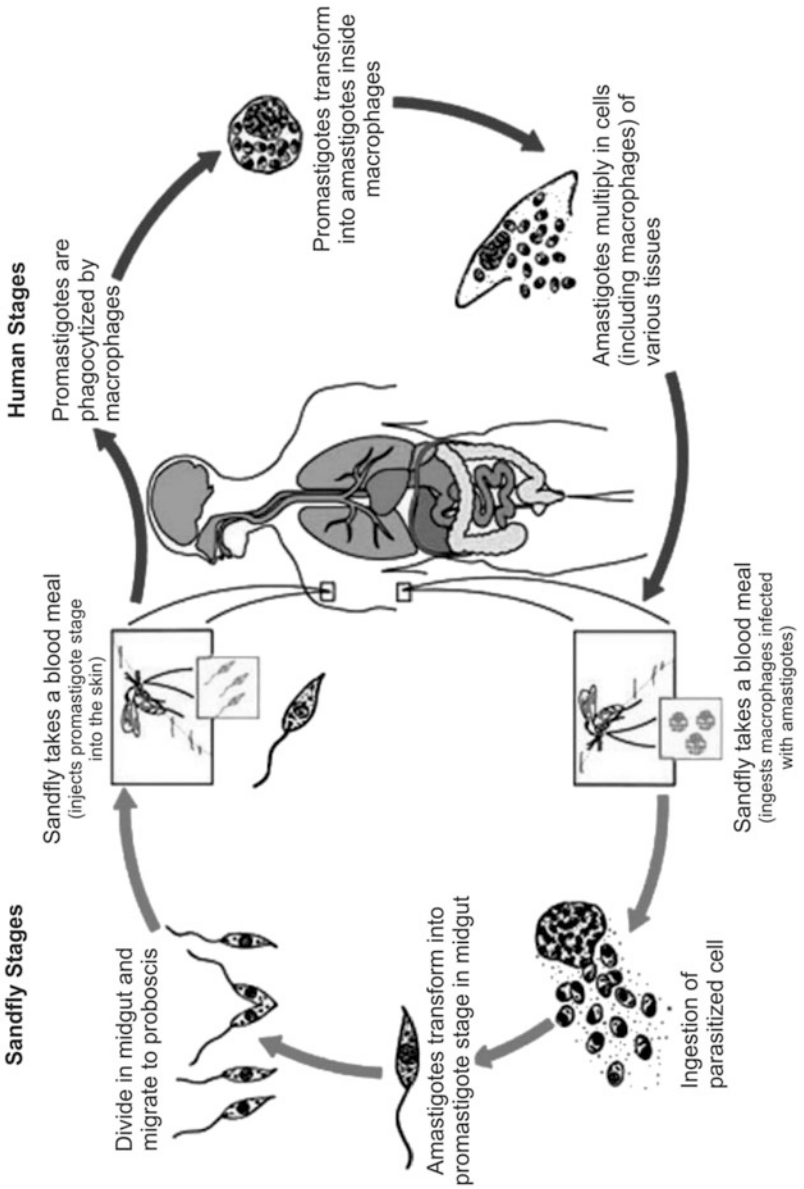


Fig. 2.9 Life cycle of *Leishmania donovani* (*Advanced Parasitology*, Das)

The reservoir hosts are dogs, squirrels, jackals, etc. in which the parasites do not undergo any change in morphology but help in spreading the disease from them to human beings. In the Mediterranean countries transmission from dog to man is more frequent than from man to man.

The infective forms of man are the promastigotes which are developed in the stomach wall of the insect vector. During bite of the sandfly the saliva causes vasodilation of the blood vessels where they bite human beings. The parasites are injected into the human beings. The parasites straight enter into the macrophages. Within the macrophages the promastigotes lose their flagella and develop into amastigote form. These amastigote forms undergo asexual binary fission to increase their population.

The macrophages have receptor on their surface, the parasites become attached to the macrophages and macrophages by their process of phagocytosis take the parasites within them and the amastigotes reproduce in large number.

The enormous population is formed within the macrophages make the cell to rupture. To increase their population the librated amastigotes again enter into fresh macrophages and in this way the cycle goes on. Some amastigotes swim free in the bloodstream, some are present in the neutrophils and monocytes.

At this stage a sandfly bites the infected person and sucks blood for her blood meal which is required by the female insect for the maturation of her ova. During sucking of blood the amastigote forms present in the bloodstream and tissue fluid of human beings are sucked in into the stomach of the sandfly. The amastigotes reach the mid-gut of the fly and transform here into promastigote forms.

The promastigote forms in the mid-gut tissue reproduce by longitudinal binary fission. The promastigote form with free flagellum migrates to the anterior part of the fly in the pharynx and buccal cavity. The pharynx becomes loaded with the promastigotes between 6 and 9 days after the sucking of blood meal from the infected person. In the case of sandfly salivary gland is not affected. Now when the sandfly again bites and sucks blood from a healthy person horizontal transmission takes place by introducing promastigotes into the blood of the vertebrates including man. The cycle is completed.

2.4.3.8 Pathogenicity

The incubation period of kala-azar of Indian origin is 3–6 months. But many times it remains asymptomatic. The fever like rheumatic fever sometimes is the early symptom. Then the hepatomegaly and spleenomegaly occur. Anaemia starts due to destruction of blood corpuscles in enormous number. The skin of the patient becomes pigmented, dry, rough and harsh. In case of some patients alopecia starts.

If not treated the infected person dies within 2 years.

Leishmaniasis affects mostly children in between 1 and 4 years in Mediterranean, south-west Asia, China. In East Africa and in India the children suffer from Leishmaniasis at the age of 5–9 years. The usual symptoms are fever, weight loss, diarrhoea, anorexia along with spleenomegaly, anaemia and pigmentation in the skin.

Post kala-azar symptoms sometimes involve lesions in the mouth cavity, nasal cavity and around mouth. Face may be mutilated so badly that people may hate themselves and outcast.

2.4.3.9 Enzyme Activities and Pathways Associated with Energy Production

In the genus *Leishmania* carbohydrate metabolism takes place in kinetoplast, mitochondria and glycosomes of the amastigote and promastigote forms.

In the amastigote forms carbohydrate metabolism takes place partially by anaerobic metabolism as there is lack of cytochrome system. The process occurs in glycosomes and cytoplasm produce acetic acid and lactic acids as end-products. ATP is formed by substrate level phosphorylation. When the amastigote forms are ingested by the insect vector they (amastigote forms) change into promastigote forms. With the change, the mitochondria grow, increase and become functionally and morphologically well developed with an active cytochrome system and functional TCA cycle. In this atmosphere, the cell utilizes aerobic metabolism producing ATP by oxidative phosphorylation.

The mitochondrial proliferation is directly linked to the kinetoplastic DNA. The chemical components of the pellicle of amastigote forms protect the cell from the hydrolytic action of the macrophage lysosomal enzymes.

2.4.3.10 Immune Response by the Host

Leishmaniasis according to WHO is an AIDS related opportunistic disease. *Leishmania* infection is controlled by the infected macrophages which induce cell mediated immunity by stimulating Th1 cells to produce IFN-y. Leishmaniasis and HIV simultaneous infection change the Th1/Th2 cell balance. For example, this type of double infection depresses Th1 cell activity and increases Th2 cell population. Inhibition of Th1 cell activity decreases the possibility of cure, while increased activity of the Th2 cell (IL-10) tends to depress macrophage activation. So here in this case the *Leishmania* infection becomes more virulent. On the other hand stimulates HIV infection by stimulating the secretion of IFN-y factor which stimulates HIV replication.

HIV infection suppresses cell mediated immunity due to T cells inhibition and promotes *Leishmania* multiplication. So this co-action resulting out of HIV and *Leishmania* infection simultaneously becomes fatal for the patients.

2.4.3.11 Diagnosis

Direct evidence: By microscopical examination of the stained blood film taken from the patient. It is often successful because of the presence of amastigote forms of the parasite in the peripheral blood.

But because of the less number of parasites present in the blood this may not produce positive result.

For positive result preparation of thick blood film and/or by centrifuging citrated blood or by making a blood culture taking blood from the patients is done. But it takes long time to prepare blood culture.

2.4.3.12 Indirect Evidence

By blood count, i.e. TC, DC, serological test and complement fixation test,

2.4.3.13 Prophylaxis

Eradication of vector Sandfly, the infective agent, control of reservoir hosts and the use of mosquito net during sleeping are the best procedure.

2.5 Coccidia

2.5.1 *Cryptosporidium parvum*

2.5.1.1 History

Tyzzar first observed *Cryptosporidium* in the year 1907 in the crypts of the gastric mucus layer. It was discovered in the laboratory mice. It was reported from human beings in the year 1976. In 1980 it is found in HIV patient.

Cryptosporidium is a genus of subkingdom Protozoa. It is an apicomplexa and contains 24 species still known today. Out of these 24 species only *C. parvum, C. homini, C. canis, C. felis, C. meleagridis* and *C. muris* have the ability to infect human beings. The disease caused by the protozoan parasites known as cryptosporidiosis which is a type of gastrointestinal illness with diarrhoea.

It is also found in HIV positive patients having symptoms of copious diarrhoea.

2.5.1.2 Geographical Distribution

It is cosmopolitan in distribution.

It is not only a parasite of human beings it is found in birds, cats, turkeys, cattle, sheep and goats, etc. The infection can spread from one host species to some other animals

2.5.1.3 Characteristics and Life Cycle

Though *P. vivax*, an apicomplexan needs mosquito as vector for the spread of the diseases. *Cryptosporidia* do not need an insect vector for completing its life cycle. It completes its life cycle within a single host so they are called monogenetic parasite.

Cryptosporidium infection happens by ingesting the oocysts through contaminated food and/or drinks. Sometimes through food sources like chicken salad.

The infective form is the oocyst. The oocyst is 2–5 μm in diameter. There are two types of oocysts: one thick walled and other thin walled oocysts. The oocysts are refractile and have some prominent granules within them and remain clustered near the margin of the cell.

The thin walled oocysts reinfect the same host but thick walled oocysts come out from the host through faecal matters and possibly respiratory secretions in the outside environment (Fig. 2.10).

Cryptosporidiosis is typically an acute short-term disease.

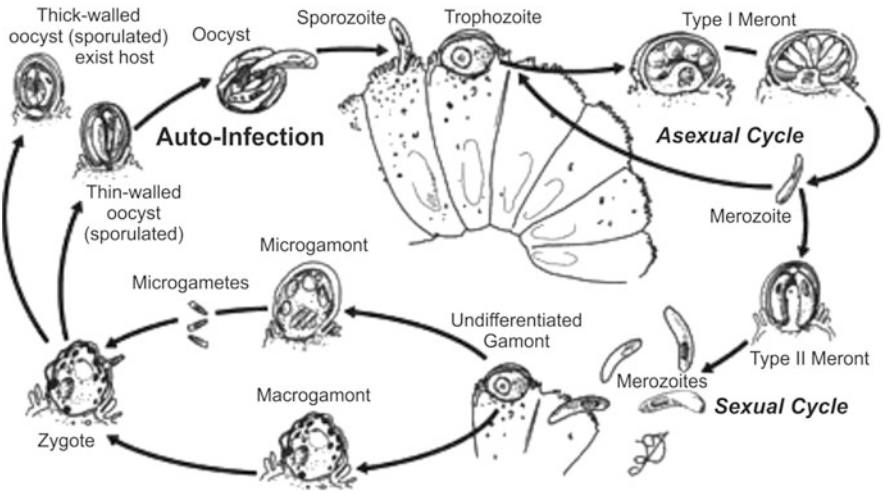


Fig. 2.10 Life cycle of *Cryptosporidium parvum* (*Advanced Parasitology*, Das)

The disease sometimes becomes severe in children and immunocompromised persons like AIDS patients. It is reported that there were many out breaks of cryptosporidiosis in water parks, community swimming pools and day care centres. Zoonotic and anthroponotic transmission of *C. parvum* happens through intimate contact with infected pet animals and vector contaminated with the faeces of contaminated animals.

The parasites remain in lower intestine and may remain there for 5 weeks.

The transmission of the disease takes place through thick walled hard oocysts.

Both the thin walled and thick walled oocysts contain within them four sporozoites.

The four sporozoites are liberated in the intestine of the host. The sporozoites enter through the outer membrane of the gastrointestinal brush bordered epithelial cells and remain there.

Here they develop into trophozoites. These trophozoites are 3–6 μm in size. These trophozoites here undergo asexual multiplication either schizogony or merogony. The result of the schizogony produces merozoites those infect new cell of the intestinal wall repeatedly, i.e. autoinfection takes place. The merozoites are 7 μm in size and form 8 banana shaped meronts.

The trophozoites multiply by schizogony and like *Plasmodium* also produce micro- and macrogametocytes. From microgametocytes microgamets are formed and fertilize macrogamets. Microgamets form non flagellated rod shaped 16 microgamets those are 1.5–2 μm in size. The fertilization produces oocysts both thick and thin walled. Oocysts are resistant to most disinfectants.

The thick walled oocysts escape from the host along with the faeces and other routes of the host after 5 days of infection and seek opportunity to infect new healthy individuals.

2.5.1.4 Pathogenicity

In developing countries where sanitation is poor *Cryptosporidia* infection is common in infants.

In developed countries the infection may take place in children. Cryptosporidiosis is typically an acute, short-term infection, but may become severe in immunocompromised persons.

C. parvum produces voluminous watery diarrhoea and the patient suffers for several months. The incubation period is 2 weeks. The severity of infection depends upon the immunity status of the patient.

In an immunocompromised person the symptoms that accompany diarrhoea are abdominal pain, weight loss, flatulence, nausea and vomiting.

The risks of infections in certain group of people by cryptosporiodosis are:

1. The people who swim regularly in different pools where sanitation is not so perfect. The faeces with oocysts deposited on the soil may mix with the water of the pool during rainy season.
2. Parents of infected children may be infected with the disease due to intimate contact and handling of the children.
3. Nurses or people who take care of the people infected with cryptosporidiosis.
4. People handling infected cattle.

Host defence mechanism: It appears that at the initial stage of infection, IL-15 secretion occurs for initial clearance of the parasite. Human memory response takes place by CD4+ cells and IFN-γ. Very little is known about the activity of B cells and CD8+ cells. In autoinflammatory and healing process TGF-β cells and IL-10 secretion are involved. Reinfection is prevented by the production of IgA.

2.5.1.5 Epidemiology

Reservoirs of *Cryptosporidium* infection are a number of animals. The route of infection is faecal–oral. Current and his co-workers in the year 2000 infected puppies, kittens, goats, etc. experimentally with oocysts taking from an immunodeficient person. They discovered that 12 immunocompetent persons were infected with *C. parvum* who were handling calves. So cryptosporidiosis is a zoonotic disease.

The zoonotic capability of *C. parvum* is discovered by surveys on cattle. It was found that 10,000 cattle, 65% of the diaries have infected animals. Cryptosporidiosis is an opportunistic infection in persons with AIDS. Parasites are distributed in the immune competent and asymptomatic population. It may be considered a usual causative agent of chronic diarrhoea in human population.

2.5.1.6 Diagnosis

Oocysts can be seen in faecal smears after staining with Jenner-Giemsa stain. Oocysts are seen as blue spherical structure under microscope.

Instead of Giemsa stain fluorescent staining with auramine phenol is also applied.

Indirect immunofluorescence using specific antibody may be used as a special technique.

In acute diarrhoea, the presence of oocysts is large in number but if their number is less then concentration technique may be applied with formal ether solution.

ELISA technique may be applied to detect antibodies.

ELISA technique in stool using monoclonal antibody is highly specific.

Diagnosis is done by observing oocysts in faeces. The oocysts may be induced to sporulate in 5% potassium dichromate solution. Concentration of oocysts with the help of centrifugation technique is also useful.

2.6 Malarial Parasites

2.6.1 *Plasmodium vivax*

Plasmodium is a protozoan parasite, a causative agent of the notorious disease malaria. The malaria recognized as the disease which played an important role in the progress and development of a country. The Japanese soldiers retreat in World War II due to wide spread infection of their soldiers with malaria and the defeat of Japan is due to the sufferings of large number of soldiers with malaria. Malaria caused more than five times casualties as did combat. A country cannot prosper without sound public health. But malaria caused disaster in the several Asian and African countries. Malaria is responsible for unemployment, communism and war opposed to peace, prosperity and contentment (Chandler and Read 1961).

2.6.1.1 History

Malaria means mala aria that is bad air. People at that time believed that bad air coming at evening through window is the cause of the disease until Laveran discovered the parasites in the blood of a patient in the year 1880. In the year 1891 Romanowsky for the first time stained the parasite and in 1898 Sir Ronald Ross discovered the mosquito cycle of the bird malaria working at P.G. Hospital in Calcutta and for this work he was awarded with the noble prize in 1902. But according to the Encyclopaedia Britannica Sir Ronald Ross discovered that the anopheles mosquito as the malaria vector on 20th August, 1897 in Secunderabad. Ross made this landmark discovery while dissecting the stomach tissue of female anopheles mosquito blood fed 4 days previously on a malarial patient.

2.6.1.2 Geographical Distribution

It is cosmopolitan in distribution especially in tropical countries.

2.6.1.3 Habitat

The malarial parasites reside in liver and red blood corpuscles of human beings. It is an intracellular parasite and mainly found within the RBCs where they develop through different stages and destroy the R.B.Cs in large number during their

development. The parasites consume haemoglobin and produce malarial pigment due to metabolism of the parasite.

The malaria parasite completes their life cycle in two hosts: vertebrate and invertebrate hosts. Sexual reproduction (sporogony) of the parasites takes place within the stomach of female mosquito but asexual reproduction (schizogony) takes place in the blood of human beings exactly RBC and liver parenchyma.

2.6.1.4 Different Species of *Plasmodium*

The disease malaria in man is caused by the protozoan parasite of the genus *Plasmodium* and there are four different species of genus *Plasmodium*.

Plasmodium vivax: The disease is called Benign tertian malaria. Here the paroxysm of chill and fever is felt at the interval of 48 h.

Plasmodium falciparum: Malignant tertian malaria is caused by the parasite *Plasmodium falciparum*. Here also the paroxysm of chill and fever is felt every 48 h. But the disease is dangerous with high mortality rate.

Plasmodium malariae: Quartan malaria is caused by *P. malariae.* The feeling of chill and fever occur every 72 h interval, i.e. on every 4th day.

Plasmodium ovale: Mild tertian malaria is caused by *P. ovale*. Here fever occurs every 48 h interval.

2.6.1.5 Life Cycle

To complete the life cycle the malaria parasite requires two different hosts vertebrate and invertebrate, man and female Anopheles mosquito.

The parasite reproduces asexually in the liver cells and RBCs of man and is called asexual generation. The process of asexual reproduction is known as schizogony.

Most of the parasites infect human beings or vertebrate animals, the sexual reproduction takes place in the vertebrate host and they are called definitive host. But in case of malaria parasite the sexual reproduction or gametogony takes place in the invertebrate vector, female anopheles mosquito.

The life history of Plasmodium is divided into four stages:

1. Pre-erythrocytic stage: The development and reproduction within the liver cells before actual infection of RBC.
2. Erythrocytic stage: The growth and reproduction within the red blood corpuscles, RBCs.
3. Exoerythrocytic stage: The development and reproduction within the liver cell after the infection in RBCs the effect of which is relapse.
4. Sexual cycle: The fertilization of anisogamates and consequent growth and reproduction of the parasites in the stomach of the female Anopheles mosquito.

1. Pre-erythrocytic stage: Human beings are infected when the infected female anopheles mosquito bites a healthy person and sucks blood by the help of sucking mouth parts, proboscis. During sucking of blood to prevent coagulation in the proboscis mosquitoes inject saliva in which there is an anticoagulant. With

the saliva the infective forms of malarial parasites enter into the human blood as the infective form, sporozoites remain stored in the salivary glands of mosquitoes. The sporozoites after entering into the bloodstream swim in the blood for 30 min or so and hide themselves in the parenchyma cells of liver of the vertebrate host to protect from immune reaction of the host.

Sporozoites stay here for nearly 8 days growth and reproduction of the parasites take place here.

The infective forms sporozoites measure 12–14 μ in length and 1 μ in breadth. They are very slender and curved structure. The nucleus is situated at the middle. There is a complex structure called apicomplex present at the anterior terminal end or at apex of the sporozoites. The apical complex helps the parasites to enter within the liver parenchyma cells.

Then the sporozoites transform into schizont within liver cells. The schizonts are round or oval. The nucleus remains at one side called growing schizont. The growing schizont then matures and becomes 42 μ in diameter in average. Each mature schizont asexually divides, the process is known as schizogony into 12,000 small merozoites.

According to Huff (1946) there occur two successive generations of merozoites in the liver cells of man. The first generation is called cryptozoites. Cryptozoites then again enter into other liver cells and second generations of merozoites are formed called metacryptozoites. The metacryptozoites then invade the bloodstream and enter within RBCs.

In some species the pre-erythrocytic metacryptozoites continue to multiply indefinitely within the liver cells and they are the source of exoerythrocytic cycle.

The pre-erythrocytic merozoites or metacryptozoites are 1.5 μm in length and 0.5 μm in breadth. They are oval in shape and nucleus is present at the centre. The time taken by the parasites to develop and reproduce within the liver cells is called incubation period and no symptoms are witnessed at that time. It is found experimentally that if quinine is administered the parasites are not destroyed at this time.

2. Erythrocytic stages: The metacryptozoites now enter into the bloodstream in innumerable number like shower. They invade RBCs by making an invagination of the plasma membrane of the RBCs. It is found experimentally that the invasion of parasites into the RBCs takes place in three consecutive stages and takes about 10–20 s.

Now after entering within RBCs the metacryptozoites start consuming haemoglobin and develop into trophozoites.

Trophozoites measure 2.5–3 μm in diameter. At the initial stage it looks like a ring so they are called signet ring stage. It appears like a little signet ring as the centre of the parasite is occupied by a vacuole surrounded by a delicate ring of cytoplasm which stains blue and small nucleus at one side which stains red with usual Giemsa's stain. The ring of *P. vivax* is 1/3rd of the diameter of RBCs. Haematin pigment appears in the cytoplasm at this time. Haematin or haemozoin pigment is produced when haemoglobin is digested by the parasites and it is the insoluble polymer of haeme. The trophozoites now grow and become double in

size. The RBCs now become pale in colour and some granules appear at the margin of the RBCs called Schuffner's dots stain red. After 48 h the trophozoites mature and transform into schizonts. Each and every erythrocytic schizont by the process of schizogony creates 12–14 merozoites.

The erythrocytic merozoites formed by the division of nucleus of the trophozoites into two, then four and ultimately 12–14. The nuclei take up peripheral position in the schizont and small portion of cytoplasm concentrates around each fragment of chromatin material. The matured merozoites then rupture the RBC and come out of the corpuscles. These erythrocytic merozoites then attack new young RBCs and the erythrocytic cycle is repeated again and again.

From the ruptured RBCs toxin, the pigment and other waste products left behind by the parasites also come out of the RBCs and mix with the bloodstream. Just at the time of rupture of thousands of RBCs, the paroxisms of chill and fever is felt by the patients. The pigments and waste products that left behind when the merozoites are liberated and deposited in the spleen or under the skin cause pale yellow colour characteristic of malaria patient.

Some of the erythrocytic merozoites instead of forming schizonts again and again develop into single nucleated organisms, gamonts. There are two types of gamonts: microgametocytes or male gametocytes and macrogametocytes or female gametocytes.

The female gametocyte is 10–12 μ in diameter. Presence of pigments in the cytoplasm and the nucleus remains at the margin of the gametocyte.

The male gametocyte is 9–10 μ in diameter. Granule in the cytoplasm is less in number and the nucleus is present at the centre.

3. Exocrythrocytic stage: The pre-erythrocytic merozoites and erythrocytic merozoites sometimes again attack the liver cells to continue the liver cycle. The product of the exocrythrocytic merozoites again attacks RBCs if the erythrocytic cycle is destroyed by using medicine and this is called relapse. The exocrythrocytic products are known as hypnozoites or phenerozoites.
4. Sexual cycle: Both the gametocytes do not develop further in the human body. They wait for the mosquitoes to suck themselves up in their (vectors') stomach. The difference in temperature initiates formation of active gamets from the gametocytes. When a female anopheles mosquito for their maturation of ovum sucks warm blood from the infected human beings, the gametocytes come out from the RBCs and transform into gamets. When removed from the warm blood gametocytes only develop further. But other asexual forms perish very soon in the stomach of the mosquito.

   At first the male gametocytes through a complex process transform into eight flagellated male gametes after coming out from the RBCs. From the gametocytes eight flagella like structure develop, the nucleus divides thrice to form eight nuclear fragments, around each nuclear fragment cytoplasm concentrates and ultimately break free. The process of formation of flagellated male gamets from male gametocyte is called exflagellation.

The female gametocytes in the meantime just come out from the RBCs. Now with the help of meiosis and creation of polar body the gamets mature and form female gamets.

According to Garnham the male and female gamets present in the blood meal of mosquito fuse and fertilization occurs resulting in the formation of zygote. After sometime the zygotes become active and motile. The motile zygote is called ookinete. These motile ookinete then touch the wall of the stomach of the mosquito and form oocyst by invading the stomach wall and forming a cyst around it at the outerside of the stomach wall.

But Howard (1960) was of opinion that round inactive zygotes are formed on the periphery of blood meal lodged in the fold of the stomach wall formed due to digestion of the blood meal and contraction of the stomach wall which was distended due to sucking of blood. But zygotes which are formed in the centre of the blood meal do not have the chance to come in touch with the stomach wall and perish. They are unfortunate zygotes. They become extremely flattened and are called ookinetes. But ookinetes, the unfortunate zygotes are voided with the faeces of the mosquito.

But according to Garnham et al. the ookinetes actively move and ultimately come in touch with the mosquito's stomach wall and further development takes place.

The oocysts now develop on the outside of the stomach wall of the mosquito, they grow in size from 6 to 60 μ in diameter. There may be as many as 50 oocysts in a single mosquito. Within the oocysts cell division takes place the first is meiosis followed by mitosis. Within the oocysts gradually develop sporoblasts. Within sporoblasts sporozoites are formed by the process of sporogony. In each oocyst there may be 10,000 sporozoites, very slender, curved and crescent shaped. When the sporozoites mature they rupture the oocysts and are liberated in the body cavity of the mosquito. Then the thousands of sporozoites migrate towards salivary gland and stay there. During the bite of the mosquito the sporozoites are injected into a healthy person along with the saliva. The cycle is completed (Fig. 2.11).

2.6.1.6 Enzyme Activities and Pathways Associated with Energy Production

Glucose is the main carbohydrate from which the parasites acquire energy through glycolysis. In the erythrocytic stages when the parasites remain within the RBCs, they are considered as facultative anaerobes.

The end-products of carbohydrate metabolism are lactic acid, formic acid and acetic acid. The parasites fix carbon dioxide at this stage and the enzymes that help the process are vulnerable to quinine and chloroquinine.

2.6.1.7 Pathogenicity

As the malaria parasites draw nutrition from the haemoglobin and RBC ruptures every 48 h the large number of RBCs are destroyed leading to anaemia of the patient. Haemolytic jaundice also results in the malaria disease.

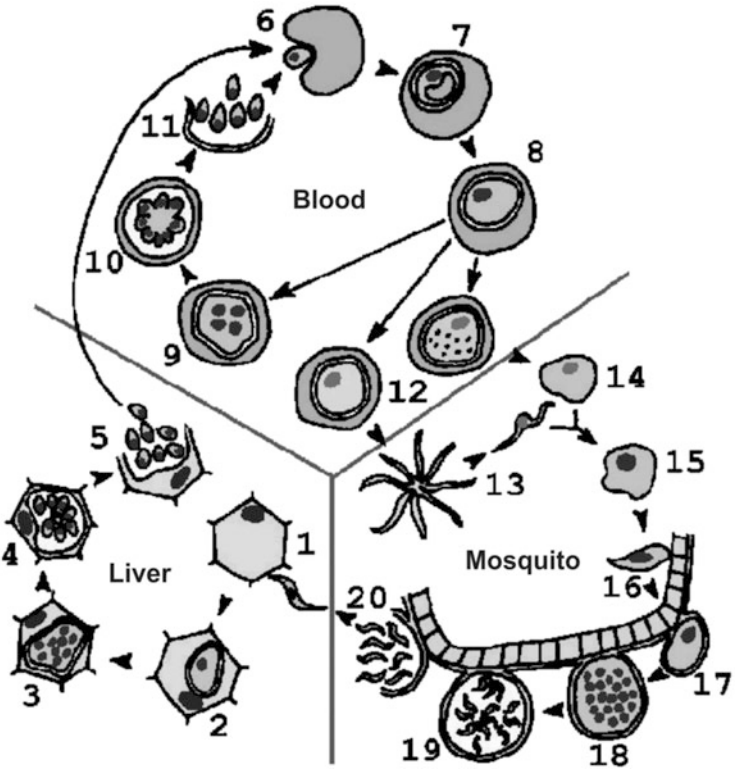


Fig. 2.11 Asexual and sexual cycle of *Plasmodium vivax.* 1. Sporozoite entering hepatocyte, 2. Trophozoite within hepatocyte, 3. Growing schizont in hepatocyte, 4. Merozoites in hepatocytes, 5. Merozoites liberating from hepatocyte, 6. Merozoite entering RBC, 7. Ring stage within RBC, 8. Schizont in RBC, 9. Merozoite in RBC, 10. Rosette formation, 11. Merozoites liberating from RBC, 12. Micro and Macro-gametocyte, 13. Exflagellation, 14. Microgametocyte, 15. Zygote, 16. Ookinete, 17. Oocyst, 18. Sporoblast, 19. Sporozoites within sporoblast, 20. Sporozoites liberating

The malaria patient may feel malaise, loss of appetite, headache and slight fever before the first paroxysm. Then patient suffers from shivering, nausea and vomiting.

Due to destruction of large number of RBCs and liberation of toxin the enlargement of liver and spleen of the patient is observed. The degenerated and ruptured RBCs may clump and create obstruction in the normal flow of blood. Due to excessive haemolysis bilious vomiting may occur. In chronic cases hepatomegaly and spleenomegaly are observed in the patients.

2.6.1.8 Host Defence Mechanism

The immune response by the host is quite different in two stages: Pre-erythrocytic stage within liver and erythrocytic stage within RBCs.

It is believed that CD8+ T cells are the main components that play a part in pre-erythrocytic immunity. On the other hand CD4+ T cells control the immunity in

erythrocytic stages. Protection from malaria infection is that the number of parasites are held in check by the immunity system of the host. Therefore host remains asymptomatic. It is found that when a person completely cured then again he or she may be susceptible. It is also found that infants in endemic areas are protected by the antibodies formed within the mother.

In case of severe infection balance between the Th1 (IFN-γ) and Th2 (IL-4) mediated immune response affects seriously. IL-4 secretion increases along with the severity of infection.

2.6.1.9 Diagnosis

Malarial parasites can be detected by microscopical examination of blood film. In most of the cases parasites can be seen under microscope by preparing thick and thin blood film. But before the examination of blood film antimalarial drugs should not be administered.

In this film parasites can be detected and at the same time can be identified which of the species is infected.

Other tests for detection are serological test, blood count, complement fixation test, etc.

2.6.1.10 Prophylaxis

(a) Use of mosquito net at night to prevent mosquito bite.
(b) Use of antimalarial drugs.
(c) Destruction of adult mosquitoes by spraying DDT or gammaxine.
(d) Destruction of larval stages by using larvicides.
(e) Elimination of breeding places of mosquitoes.

2.6.2 *Plasmodium falciparum*

2.6.2.1 History

The name of the disease malaria was given in the year 1753. At that time people believed that the disease is related to the bad air. In the year 1880 Laveran for the first time discovered the parasite in the blood film preparation. In 1886 Golgi demonstrated the erythrocytic schizogony of benign tertian malaria and differentiated between benign and quartan malaria. In 1949 Short and others demonstrated the pre-erythrocytic schizogony of *P. falciparum*. In 1897 Willium H. Welch gave the name *Plasmodium falciparum* which ICZN formally adopted the name in 1954. But it is proved that a German physician Johann Freidrich Meckel was the first to observe *P. falciparum*, at that time he did not know what it is.

2.6.2.2 Geographical Distribution

Malarial parasites are found in all the countries of the world extending from 40° south to 60° north. The endemic areas are the tropical zone.

The *Plasmodium falciparum* is the causative agent of malignant malaria or cerebral malaria causes clinical illness, often very severe, affects 100 million and

over a million people die from malaria. Malaria threatens 2200 million persons, about 40% of the world population undermining health and welfare of the families, endangering the survival of children, debilitating the active population and straining both countries and people's scare resources. The vector in our country is *Anopheles stephensi*.

Like *Plasmodium vivax*, *P. falciparum* has pre-erythrocytic, erythrocytic stage and sexual cycle but exoerythrocytic stage is absent in case of *P. falciparum*.

2.6.2.3 Pre-erythrocytic Stage

Malaria sporozoites are released into the blood with the bite of an infected female Anopheles mosquito and within minutes attach to and invade liver cells by binding to the hepatocyte receptor located on the basolateral surface of liver cells. Within the liver cells malaria parasites multiply rapidly, so as many as 40,000 merozoites are released when the hepatocytes rupture. They attack new and old RBCs. It is found that a type of glycoprotein, namely glycophorin A present on the membrane of the RBCs acts as receptor of *P. falciparum*. Within the RBCs the parasites multiply in a membrane bound digestive vacuole hydrolysing haemoglobin by secreting enzymes that include haem polymerase.

As the parasites mature within RBCs they change morphology from ring to schizont form. The ring stage is small in shape 1.2 μ in diameter. It consists of a fine and thread like uniform cytoplasmic ring with a distinct nucleus often projecting beyond the ring or lying outside the ring. The parasites after attaching itself to the margins or the edge of the host cell the nucleus and a small part of the cytoplasm remaining almost outside. There may be two nuclei in a ring. There may be more than one ring stage in a single RBC. The infected RBCs remain unchanged in shape and size. The colour of the cell is reddish violet in Leishman stain. Schuffner's dots are not seen but instead 6–12 Maurer's dots staining brick red are seen. The ring stage and mature trophozoites disappear within 24 h and are sequestrated in the internal organs like brain, heart, spleen, bone marrow, etc. because the outer surface membrane of RBCs produce proteins and form knob like structure. For this protein the infected RBCs bind together with the post capillary venular epithelium. This binding causes sequestration of the infected RBCs in the venules. But surprisingly the RBCs those are with gametocytes have no knob like structure on their surface and so they do not attach with the venular endothelium and pass freely within the venules. So only early ring stages and gametocytes are seen in the peripheral blood of patients infected with *P. falciparum* 8–32 merozoites usually 16 merozoites are formed within RBCs.

In falciparum infection only the ring stages are seen in the slide preparation because all other stages are sequestrated in the internal organs of the patient. Mature schizont occupied 2/3rd of the RBC. Within the schizont 8–32 (generally 8–18) merozoites are formed. The merozoites come out by bursting the RBCs and attack new RBCs. If the rate of infection is high there may be 500,000 parasites in 1 mL of blood.

Some of the merozoites as usual form gametocytes. The gametocytes are seen in the peripheral blood though they develop within internal organs. They are elongated

and crescent shaped. So the species is called *falciparum*. With the growth of the gametocytes, the Red Blood Cells are gradually used up only their membrane remains in the form of a covering enclosing the gametocytes.

The size of the mature gametocytes are about one and half times large then the RBCs harbouring them, so the RBCs are stretched beyond recognition and can only be detected by the concave side of the parasites projecting outward in the form of arched rim.

The first appearance of the gametocytes takes place after 21 days of inoculation of sporozoites by the mosquito. The female gametocyte is generally more slender and elongated than the male counterpart. The cytoplasm of the female gametocyte stains deep blue, small nucleus is placed at the centre. It measures 10–12 μ by 2–3 pigments are distributed around nucleus.

The male gametocyte measuring 8–10 μ by 2–3 μ is sausage shaped, cytoplasm stains faint blue, nucleus is deep pink and pigments are scatteredly distributed.

Sometimes the number of gametocytes may be as high as 50,000–150,000 per μL of blood. The gametocytes remain viable for 30–60 days sometimes 120 days in peripheral blood. The mosquito cycle can start only if the infection rate is moderate, i.e. in the slide preparation there is 1 parasite in every 20 WBC count.

In case of *P. falciparum* relapse does not occur as there is no exoerythrocytic cycle. But recrudescence may occur due to late bursting of merozoites. In the laboratory it is found that during production of parasites para-aminobenzoic acid PABA is necessary. The research workers are now trying to develop medicine which will disturb the PABA formation or restrict use of PABA by the parasites.

2.6.2.4 Sexual Cycle

The sexual cycle of *P. falciparum* is same like that of *P. vivax.*

2.6.2.5 Pathogenicity

As the malarial parasites draw nutrition from the haemoglobin so RBC ruptures every 48 h. The large numbers of RBCs are destroyed leading to anaemia. Haemolytic jaundice also occurs in the *falciparum* malaria.

In the *falciparum* malaria sometimes severe complications may arise. Death occurs due to severe anaemia and blockage of capillaries and small blood vessels in the brain. A breakdown product of haemoglobin called bilirubin cause jaundice in the patient. In the falciparum malaria a common symptom is hypoglycaemia which may leads to coma. In recent years it is discovered that release of TNF and IFN-γ cause serious changes in metabolic process. It is found that the meninges, the covering of the brain, becomes red due to excess load of parasites in the capillary network. The circulation is choked resulting in decrease in the oxygen supply to the brain tissues. The lack of oxygen starts breakdown of glycogen into lactic acid and necrosis develop.

The temperature of the patient increases because of the presence of malarial toxin in large quantity. The WBCs become excited and liberate pyrogen. The pyrogen dissolves in plasma and reacts on the hypothalamus of brain. Constriction of vasomotor starts and pyroxysm of chill and fever is felt by the patients. The

Table 2.2 Comparison of different species of malarial parasites (Erythrocytic forms)

Features	*P. falciparum*	*P. malariae*	*P. ovale*
Ring	More than one in one corpuscle. Presence of two nuclei frequent, 1/6th to 1/5th of the diameter of corpuscle	Same as *P. vivax*	Same as *P. vivax*, Schuffner's dots present even at this stage
Corpuscles infected with schizonts	Not found in peripheral circulation, size normal, presence of Maurer's dots instead of Schuffner's dots	Size and colour normal, absence of Schuffner's dots	Oval in shape, often fabricated, corpuscle not much enlarged, presence of Schuffner's dots
Growing schizont	Usually compact, round, pigment coarse and black, not seen in peripheral circulation	More compact and round, pigment coarse and black	Usually round, pigment brownish, coarse and scattered
Merozoites	Occupy 2/3rd to 3/4th of corpuscle, number of merozoites from 8 to 32, not seen in peripheral circulation	Nearly fill normal size corpuscle, 6–12 merozoites arranged in petals of rose	Occupy 3/4th of oval corpuscle, 8–10 merozoites arranged like a bunch of grapes
Microgametocytes	Microgametocyte small with pale blue cytoplasm, nucleus pink in colour, pigment granules scattered. Crescent shaped	Same as *P. vivax*	Same as *P. vivax*
Macrogametocytes	Macrogametocytes longer and more slender, deep blue cytoplasm, small red nucleus, pigments more concentrated near centre. Crescent shaped	Same as *P. vivax*	Same as *P. vivax*
Interval between cycle	48 h	72 h	48 h

symptoms of cerebral malaria are also hyperpyrexia and coma. Acute *P. falciparum* infection produces high temperature, severe anaemia, cerebral symptoms, renal failure, pulmonary oedema and death (Table 2.2).

2.6.2.6 Resistance and Resurgence

Very recently it is seen that several parasitic diseases which had been considered quite under control or eradicated have been found to infect with stronger pathogenicity. This upsurge in malaria is due to the emergence of new strain of malarial parasites which are now resistant to available drugs as well as insecticide resistant vectors. The crowded condition of human inhabitation coming from resettlement of refuges due to political disturbance, famine, etc. hasten the progress of transmission.

Recrudescence and relapse are for lowering of antibody titre. Large family of genes encode variant antigens in *P. falciparum*. Tolerance of the parasites is due to loss of reactivity to TNF.

2.6.2.7 Host Immune Response

Plasmodium has two cycles in human beings—one pre-erythrocytic phase which starts when sporozoites infect hepatocytes and second cycle is an erythrocytic cycle when merozoites infect RBCs.

Suppression of CD4+ T helper cells function, decrease CD8+ cytotoxic cell differentiation and release of IFN-γ. The above mentioned two processes are important for immunity against cerebral malaria for extra erythrocytic stages.

It is found that the antigens from asexual erythrocytic stages of the parasites stimulate the immune system of the patient to produce specific antibodies which are present in IgG and IgM of the serum gamma globulin. The cell mediated and humoral defence mechanisms of the patient are effective only against the asexual erythrocytic stages of the parasites. They are not effective on the gametocytes. Decrease activity of cytotoxic cell functions prevents degeneration of sporozoites infected hepatocytes. While suppression of IFN-γ prevents stimulation of macrophages resulting release of uncontrolled number of merozoites during extra erythrocytic phase. One of the consequences of these changes is that AIDS patient exhibits increase incidence of cerebral malaria.

2.6.2.8 Energy Metabolism

Though the oxygen is available parasites acquire energy from the degradation of glucose to lactic acid. It is found that *Plasmodium* species of birds have mitochondria with cristae. But asexual stages of mammalian *Plasmodium* parasites have no cristae in their mitochondria but asexual stages within the stomach wall of mosquitoes have mitochondria with prominent cristae.

Erythrocytic stages of the parasites are facultative anaerobes. They consume oxygen when it is available. They use oxygen for synthesis of nucleic acids. But a classical cytochrome system is not found in the malarial parasites. The parasites cannot synthesize coenzyme A and it is obtained from the host. The Plasmodial parasites of mammals fix carbon dioxide into phosphoenol pyruvate. The antimalarial drugs actually inhibit the enzymes, carboxykinase or phosphoenol pyruvate carboxylase.

It is quite interesting that 25 different proteins have been found in different species of *Plasmodium*. These enzymes are very important for maturation and release of merozoites from infected RBCs and haemoglobin digestion within RBCs. It is found that ferriprotoporphyrin (FP) is formed from the digestion of haemoglobin by the parasites this FP is believed to inhibit secretion of plasmodial proteins.

A membraneous structure noticed under EM in Apicomplexa. They are called Apicoplasts which have four membranes. Parasitologists have suggested that apicoplasts have a number of functions one of which is survival and transmission of *P. falciparum*.

2.6.2.9 Diagnosis

Microscopical examination of blood film taken during pyrexial interval of falciparum malaria will show the presence of parasites. In acute falciparum malaria blood picture is normal or shows less number of leucocytes.

Passive haemagglutination test: If the infection is recent it shows positive result. Here infected erythrocytes are agglutinised. This test gives result in *P. falciparum* infection because a number of infected erythrocytes are present in this case.

2.6.3 *Plasmodium malariae*

2.6.3.1 History

Like other *Plasmodium*, *P. malariae* also cause the disease malaria in human beings. The disease caused by this parasite is called Quartan malaria. Golgi in the year 1885 gave an accurate description of *P. malariae*.

2.6.3.2 Geographical Distribution

It is cosmopolitan in distribution. It is mainly found in India, tropical Africa, Sri Lanka, Malaya, Java, New guinea and some parts of Europe.

It is the only human malaria species that is regularly found in wild animals also.

2.6.3.3 Life Cycle

Like all other species of malaria they have also pre-erythrocytic, erythrocytic, exoerythrocytic and sexual cycle.

Pre-erythrocytic Cycle

The pre-erythrocytic schizogony has not always seen in man but Parasitologists believe that this tissue cycle is present in man.

Probably the duration of tissue phase of this parasite is nearly 15 days. Garnham noticed that the tissue phase of this parasite is like *P. inui* (a parasite of monkeys). Bray in 1960 by an experiment showed that pre-erythrocytic schizont of *P. malariae* in the liver of Chimpanzee by inoculating sporozoites taking from salivary glands of infected mosquito.

The pre-erythrocytic schizont in early stage is 5.5 μm in diameter with 5 nuclei while the mature schizont is about 22 μm and merozoites are formed which are over 2000 in number.

Erythrocytic schizogony takes 13–16 days to complete within RBCs of man. The young ring form, i.e. trophozoite has the same appearance like the signet ring stage of *P. vivax*. It is less amoeboid and cytoplasm is somewhat thicker than that of *P. vivax*. The trophozoite of *P. malariae* often stretches across the RBCs and takes a band like appearance. Now they begin to collect pigment when they are 6–8 h old. In the cytoplasm of the parasites dark brown or black colour or course pigment granules appear. The infected RBCs are not enlarged in size.

Schizont is round in shape and measures 6.5–7 μm in diameter. After 45–54 h the nucleus divides resulting in 6–12 merozoites. They arrange themselves around the

pigment at centre the parasites look like daisy head. Merozoites measure 2–2.5 μm in diameter.

Gametocytes are round and measure 7–7.5 μm in diameter. The female gametocytes are somewhat large than male gametocytes. Gametocytes mature within 6 days. The gametocytes are not found in peripheral blood they develop within internal organs.

It is found that the gametocytes when become matured come out in the peripheral blood and remain there for a long time.

Exoerythrocytic Schizogony

It is found that after the disappearance of erythrocytic stages of *P. malariae* relapse may occur even up to 32 or 55 years (Garnham 1966).

2.6.4 *Plasmodium ovale*

It is very rare and because of its oval structure they are called *Plasmodium ovale*. The parasites are the causative agent of the ovale tertian malaria.

2.6.4.1 Geographical Distribution

They are distributed in tropical countries but are found also in Europe and the USA. They are mostly found in the west coast of Africa.

2.6.4.2 Life Cycle

Pre-erythrocytic Schizogony

Their morphology is more or less same with *P. vivax* so the diagnosis is very difficult. The time taken to complete pre-erythrocytic cycle is more or less 9 days. Some schizonts have been found on 5th and 9th day in the tissue of liver.

A mature schizont measures about 70–80 μm in length and 40–50 μm in breadth. Merozoites are about 15,000 within a schizont. They are spherical about 1.8 μm in length and with a nucleus on one side.

Erythrocytic Schizogony

The schizogony completes in about 48 h. The ring stage in its initial phase has a round nucleus and the ring stage measures 2–2.5 μm in diameter. The pigments at this stage are coarse and dark brown in colour. In these parasites some granules appear and called James's dots.

Mature schizonts are round or oval and measure 6.2 μm in diameter. Four to 16 merozoites are formed but usually eight nuclei are produced due to asexual division.

The merozoites measure 2–2.5 μm in diameter. The shape of the nucleus is crescent.

2.6.4.3 Gametocyte

Gametocytes develop from the erythrocytic merozoites. The gametocytes of *P. ovale* are oval in shape and in the infected RBCs James's dots are seen. The infected RBCs are irregular in outline. Gametocytes appear in the peripheral blood after a long period. They appear in large number after 3 weeks of infection and at that time mosquitoes get infected from the infected persons.

2.6.4.4 Sexual Cycle

Same as *P. vivax*.

2.6.4.5 Exoerythrocytic Cycle

The exoerthrocytic forms of *P. ovale* have been demonstrated in the liver tissue of Chimpanzee after 18–40 days of sporozoites infection (Bray 1957).

2.7 Piroplasm

2.7.1 *Babesia* sp.

Babesia is a genus of protozoan haemoparasite (blood parasite) residing within the RBCs of human and nonhuman mammals. It is the causative agent of the disease babesiosis. The disease was called earlier as Piroplasmosis. More than 100 species of *Babesia* are known still today but only a few are responsible for the disease in human beings. Human infection is accidental and rare.

The distribution is very much restricted, in the USA, *Babesia microti* and in the Europe *Babesia divergens* are found. The most well-known species is *Babesia bigemina* which can cause mild to severe sometimes fatal disease and have world-wide distribution.

The disease babesiosis is also called Red water fever as the patients excrete red urine due to presence of haemoglobin in the urine known as haemoglobinourea.

At the onset of the disease, the babesiosis in human beings is shown symptoms like malaria. So most of the time the disease is misdiagnosed.

The symptoms of Babesiosis are irregular fever, chill, headache, general lethargy, pain and bodily discomfort. In severe cases cause haemolytic anaemia, jaundice, laboured breathing and haemoglobinourea. Haemoglobinourea occurs due to lytic effect of parasites on RBCs.

Resistant persons with healthy spleen most of the time recover without any treatment. But in splenectomised persons the infection is severe and becomes fatal within 5–10 days of infection.

Splenectomised persons suffer from severe haemolytic anaemia with hepatomegaly and spleenomegaly.

In *B. microti* infection which takes place mainly in the USA, sometimes complications arise like acute respiratory failure, congestive heart failure and renal failure. Among the hospitalized patients with babesiosis 5–10% becomes fatal. There is always a higher risk of death among the elderly persons infected with

severe babesiosis. Babesiosis is characterized by destruction of RBCs and excretion of haemoglobin with urine. Babesiosis is also called Red water fever.

In *B. divergens* infection restricted in Europe mortality rate is about 42% and always have severe symptoms. Here also infected persons suffer from haemoglobinourea, jaundice, persistent high fever, chill and sweat. In untreated individuals develop pulmonary oedema and ultimately renal failure occurs.

2.7.1.1 History

The species *Babesia* was first discovered by Romanian Scientist Victor Babes in 1888. He discovered severe haemolytic anaemia in cattle and sheep due to babesiosis. But he identified the causative agent as bacteria and named it *Haematococcus bovis*.

In 1893 Theobald Smith and Fred Kilborne identified the parasite as the cause of Texas Cattle fever which is later known as babesiosis.

Smith and Kilborne are the first scientists to discover that the disease is transmitted by a tick of the *Boophelus* group called *Rhipicephalus*. This discovery for the first time recognized the role of arthropod as vector of the disease in the field of medical science.

In 1957 human infection by *Babesia* was discovered. The very first patient was a splenectomised person. In nonsplenectomised person the infection was first observed in 1969 and it proved that the protozoan parasite *Babesia* is the cause of the babesiosis in human beings.

2.7.1.2 Morphology

Babesia represents itself in three forms: Sporozoites, trophozoites and merozoites. Sporozoites are the infective stage of the parasite. *Babesia* enters into the RBCs at sporozoite stage. Within the RBCs the sporozoites transform into trophozoites. Sporozoite becomes round and forms a ring. The trophozoites undergo asexual division schizogony within the RBC and convert into merozoites. The RBCs when become filled up with merozoites rupture and large number of infected RBCs burst resulting haemolytic anaemia.

In the meantime the parasite assumes tetrad structure. The tetrad morphology of the parasite present in the RBCs makes the diagnosis very easy and differentiate *Babesia* from *Plasmodium*.

Trophozoites appear as ring forms. They are 2–4 μm in diameter and have a small chromatin dot with scanty cytoplasm. Trophozoites start feeding haemoglobulin but no pigment is produced. The tetrad morphology is best seen in Giemsa's stain in thin blood smear. They multiply by budding process and form merozoites. The merozoites coming out from the infected RBCs again attack new and uninfected RBCs to repeat the cycle.

B. bigemina is the causative agent of the disease red water fever or Texas fever in cattle throughout the world.

Infection of *Babesia* in human beings is very rare but infection takes place when the tick bites for blood meal. It is seen that if the biting tick is not removed from the

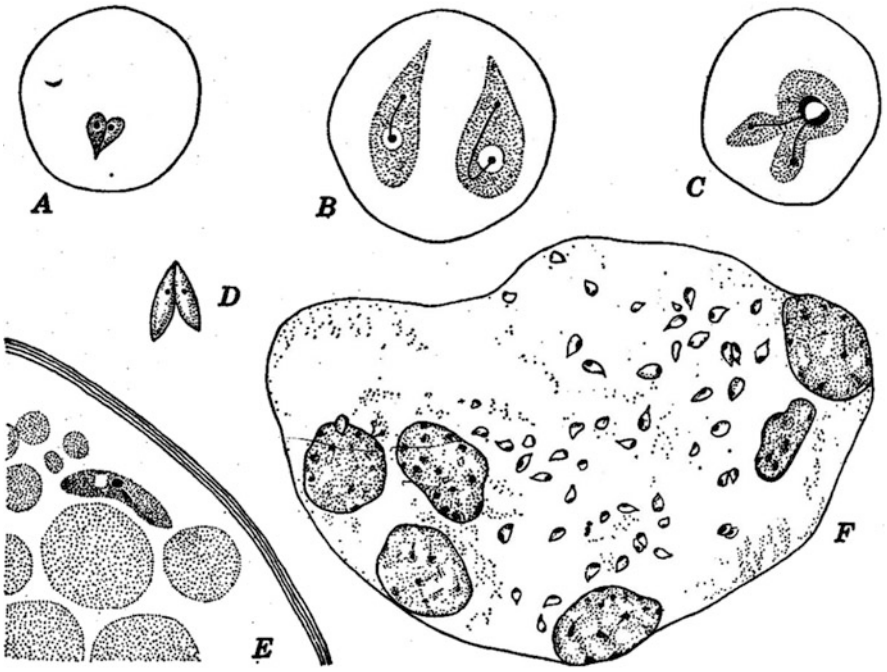


Fig. 2.12 Life history of *Babesia bigemina.* A. Trophozoite in RBC during binary fission, B. Pyriform in RBC, C. Division in RBC, D. Associated isogametes in the gut of tick, E. Ookinete in section of ovum of tick, F. Sporozoites in salivary gland of tick

skin remain there for 3–10 days. The longer period of biting is associated with higher probability of acquiring the parasite from the tick.

2.7.1.3 Life Cycle

Life cycle of *B. microti* requires a biological vector like rodent or tick. The transmission of infection occurs by arthropodan host tick of genus *Boophilus*. The ticks of genus *Boophilus* or Rhipicephilus are parasite on rat or rodents, when they suck blood meal from the rat the infected tick introduces sporozoite into the blood of the rodent. The sporozoites within a short time directly enter into the RBCs and undergo development via trophozoites and merozoites formed within the infected RBCs. Some of the merozoites like *Plasmodium* develop into gametocytes (Fig. 2.12).

The tick, the definitive host, as the sexual reproduction of *Babesia* takes place within ticks, during their blood meal from infected rat or rodent all the stages of *Babesia* like sporozoites, merozoites, gametocytes are sucked into the alimentary canal of the ticks. But except the gametocytes all other stages degenerate. Only the gametocytes develop into gamets and fertilization takes place in the hind gut of the tick and then through gametogony, the sexual reproduction, the parasites invade the reproductive organ of tick. They multiply extensively and migrate to all the tissue

of the developing tick embryo through ovary. So the transmission is called transovarial transmission. Some of the parasites enter the salivary glands and from there they are transmitted to new vertebrate host by the young ticks. Adult ticks do not transmit the disease (Dennis 1932). Besides at the nymphal stage of the tick if they bite infected human beings the parasites enter into the g.i. tract of tick through blood meal remain within the tick and multiply within the tick all through its developmental stages. Now all the stages of tick during development become infective. *Babesia* can be passed on from one generation to another only once via transovarial transmission as Ticks strictly maintain single host feeding cycle on cattle. But *B. microti* are not transmitted transovarially in human beings.

In the USA and Latin America Ixodes is the most common tick. This hard tick is usually known as deer tick transmits various tick bearing diseases of wild animals like deer.

In 2003 Centre for disease control (CDC), U.S.A. reported that more than 40 cases of Babesiosis transmitted due to transfusion of packed red blood cells in patients. After that the Government of the USA made a strict law to screen the blood used for transfusion for *Babesia* antibodies.

On the wall of the hind gut of tick fertilized female gametes form oocytes. Within the oocytes sporogony takes place and sporozoites are formed ultimately. The sporozoites by rupturing the oocytes released into the coelomic cavity of the body and migrate to the reproductive organ and to the eggs. In the embryo the parasites increase in almost all tissues including salivary gland.

In some cases the infected seed tick bite human beings to suck blood. In this process the sporozoites of *Babesia* are introduced into the blood of the human beings along with the salivary secretion of the ticks. Human beings act as intermediate host as the asexual reproduction of the parasite takes place in the RBCs of human beings. The adult tick is not responsible for the transmission of the infection.

2.7.1.4 Pathogenicity

The symptoms are irregular fever, chill, sweating, muscle pain, fatigue, mild enlargement of spleen and mild haemolytic anaemia. The illness continues for weeks and months. The disease is severe in adult cattle than in calves. The incubation period of the disease is 8–15 days after infection.

In severe cases 75% of the RBCs may be degenerated, no severe anaemia happens. Due to overloading of breakdown products of haemoglobin jaundice occurs and due to clearance of haemoglobin through kidney the colour of the urine becomes red.

The disease may be controlled through eradication of ticks.

2.7.1.5 Host Defence Mechanism

The infection is sometime fatal for cattle and human beings. The parasites multiply within RBCs causing destruction of RBCs. But the disease is long lasting. The recovered animals become resistant to reinfection for acquired immunity. It is found that immune response of sporozoites is antibody response. There is some proof of

cell mediated killing involving macrophages. As the antibodies have been found so immunization against Babesiosis in cattle and dogs is successful.

2.8 Ciliophora

2.8.1 *Balantidium coli*

2.8.1.1 History

In the year 1857 Malmsten was first to discover the organism in two human beings with dysentery. He identified it as a species of *Paramoecium* and called it *Paramoecium coli*. Leuckart described morphologically similar species from the intestine of pig in 1861.

A parasitic Ciliophoran intestinal protozoa is the *Balantidium coli*. It is the largest protozoan ciliate parasitic of human beings. The parasite causes dysentery in man. The disease is called balantidiasis.

It resides in large intestine of human beings, monkeys and pigs.

2.8.1.2 Geographical Distribution

It is cosmopolitan in distribution.

2.8.1.3 Morphology

The parasite has two forms: trophozoite and cyst. The trophozoite of the species is large in size measuring about 28–152, 7.22–12.3 μm in dimension. The shape is almost round or ovoid. At the anterior end there is a depression called peristome. The entire body is covered with a delicate pellicle having longitudinal rows of fine cilia. Cilia are the organs of locomotion.

At the bottom of the peristome there is a cytostome. The cytostome is the cell mouth from here the food is taken within the organism. The food vacuoles circulate within the endoplasm. There are two nuclei present in the cell body: macro and micro nuclei. The macronucleus is large slightly curved and concave at both sides. It is about 2/5th of the length of the body. The micro nucleus is very small and situated at the concavity of the macronucleus. At the anterior end from the peristome large cilia come out they are called adoral cilia which help during ingestion (Fig. 2.13).

The cysts those are commonly found in faeces are ovoid and about 45–55 μm in diameter. The cyst wall is tough and made of one or two layers. The nuclear shape and structure is same with that of trophozoites. The cysts have a very small contractile vacuole and one or two refractile body. No multiplication occurs within the cyst. The cysts are infective forms and are found in the faeces of human beings. Transmission of infection takes place through cysts. Surviving trophozoites and cysts are greenish or yellow in colour seen under microscope.

2.8.1.4 Life Cycle

Balantidium coli resides in the lumen of the large intestine specially caecum and colon of man and other animals transform into cysts and they escape through the

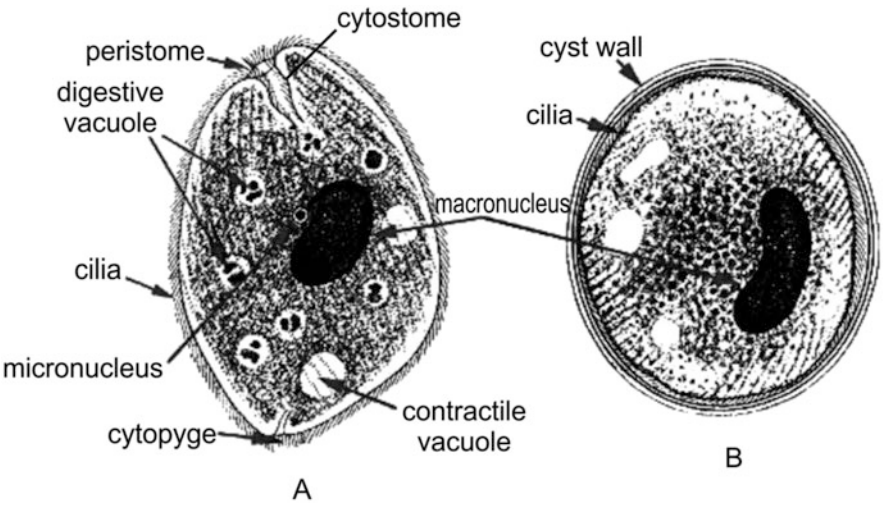


Fig. 2.13 (**a**) Trophozoite of *Balantidium coli*, (**b**) cyst

faeces of pig and are ingested by children. Because of the question of adaptation in new environment of intestinal symbiotic flora in a new host it cannot be transmitted easily. But when they become adapted to the new intestinal environment of human beings it becomes a pathogen which can create havoc. *B. coli* is seen that it cannot produce lesions within the intestine of animals except Primates. But they may infect secondarily in the lesions produced otherwise.

Trophozoites divide by transverse binary fission within the lumen of the intestine. During binary fission the peristome and cytostome remain present at the anterior end and they are formed at the anterior end of the newly formed posterior daughter. Some reported that conjugation is also seen sometimes especially in culture media.

The transmission route is faecal–oral. The *Balantidium* infection in man only occurs when cysts from animal sources are ingested through contaminated food and drink in large numbers.

Infection takes place when a host ingests cysts during the consumption of food and drink contaminated with cysts. Once the cysts are ingested they pass through the G.I. tract of the hosts. The cysts receive some protection from degradation of acidic environment of the stomach. The tough hard cyst wall gives protection from the acid of the stomach. It is proved that the cysts remain healthy at a pH greater than 5 but at less than 5 the cyst wall is damaged and destroyed. Infection occurs in malnourished individuals who have less acidic environment in their stomach.

Once the cysts reach the small intestine excystation occur and trophozoites are produced within the intestine. The trophozoites colonized in the large intestine where they live in the lumen and feed on the intestinal flora.

Some trophozoites undergo encystation after a period of growth and multiplication. Some trophozoites invade the wall of the colon of the host using proteolytic enzymes and reproduce there and some of them return in the lumen again. In the

lumen of the intestine of the host the trophozoites undergo encystation. Encystation takes place due to dehydration of the intestinal contents and occurs in the distal part of the large intestine and the process may also occur in the outside environment in faeces.

2.8.1.5 Pathogenicity

Balatidium coli lives in the caecum and colon of the hosts like human beings, pigs, rats and other mammals. The trophozoites devour like other ciliates through cytostome. It is found that sometimes the organism, i.e. trophozoites of *B. coli* produce proteolytic enzymes which act on cracked or damaged intestinal epithelium of the host. At the same time hyaluronidase is also secreted and this enzyme helps in the enlargement of the ulcer in the intestinal wall. The ulcers are like the amoebic ulcers usually flask shaped with a narrow neck proceeding into a sac like depression in the submucosa layer of the intestine. Here the parasites divide and an ulceration is formed. This may lead to necrosis and perforation of the large intestine may occur. In extreme condition lung or liver may be infected. Besides urinogenital organ, vaginal, uterine and bladder infections may be seen. The parasites do not transmit readily from one species to another because of their food and adaptation in the new hosts. It requires a period of time to adjust to the symbiotic flora of the intestine of new host. Once it is adapted the parasite may become a serious pathogen to human beings.

Balantidiasis infection may take place in immunocompetent persons but the infection never becomes serious.

Usually in the patients with symptoms diarrhoea occurs. The abscess in the liver or in other organs is very very rare. Infection not likely occurs in the malnourished individuals due to low acidity in the stomach or in the immunocompromised individuals.

2.8.1.6 Diagnosis

Saline preparation of the diarrhoeal faeces shows trophozoites in the microscope, if it is present. Cysts can be found also in saline preparation of formed stool.

2.8.1.7 Epidemiology

Balantidium in human beings is found in the people of Philippines though it is cosmopolitan in distribution. The parasites are found among people who are associated with swine. The infection in human beings is rare and only 1%. But it is found that infection rate is very high in pigs and it is seen that in slaughter house in Japan the infection rate was 100%. In middle east countries it is seen that the prevalence of Balantidiasis is connected with the increased population of wild boar.

The ability of the trophozoites to encyst when they come out increases the rate of infection from reservoir host. These cysts remain viable for a long period in the faeces of pig. It is proved that the pigs are common source of infection in human beings. It is seen that infection disappears in healthy person. But they may be carriers of infection.

Some are of opinion that cysts found in pigs cannot infect human beings. The parasites found in pigs are *Balantidium suis*, completely a separate species.

2.9 *Toxoplasma*

2.9.1 *Toxoplasma gondii*

Toxoplasma gondii is a parasitic protozoa, a causative agent of the disease Toxoplasmosis of man and animals.

2.9.1.1 History

In the year 1908 Charles Nicolle and Louis Manceaux discovered a protozoan parasite in a rodent called Gundii, *Ctenodactylus gundi* at the Pasteur Institute in Tunis. They named the parasite *Toxoplasma gundi*.

In the same year Alfanso Splender found the same organism in a rabbit in Brazil. It is like *Plasmodium* an obligate intracellular parasite. The disease is extremely common and asymptomatic but sometimes becomes very dangerous.

But it was for the first time found in human beings in an infant girl delivered full term by caesarean section on May 23, 1938, at Babies' Hospital in New York City.

It was believed by the scientists that transmission of *T. gondii* may be due to consumption of undercooked meat (Weinman and Chandler 1954). To prove this way of transmission in 1960 the cyst wall of oocysts was shown to dissolve in the proteolytic enzymes present in the stomach and as a result infectious bradyzoites are released into the stomach and then pass into the intestine. This type of transmission in human beings was tested in an orphanage in Paris in 1965.

In Bombay in the year 1959 *Toxoplasma gondii* was found in strict vegetarians also. This led to believe that there must be another major route of infection besides meat consumption and in 1970 oocysts were discovered in cat faeces. The faecal–oral route of infection was demonstrated with the help of oocysts experimentally in laboratory.

For 10 long years from 1970 to 1980 quite a number of experiments were done to test the ability of the animals to shed oocysts of *T. gondii*. Seventeen different species of Felids (cat family) have been found to shed oocysts but never the non-felids as the sexual reproduction of *T. gondii* and subsequent formation of oocysts never occur in non-felids.

The disease is asymptomatic although mild flu like symptoms may occur during the first few weeks of infection.

But in immunocompromised persons, AIDS patients and infants the disease may be serious and occasionally fatal.

2.9.1.2 Geographical Distribution

The parasite is cosmopolitan in distribution and can infect all warm blooded animals including human beings. It is believed to be one of the most common parasite and is estimated that one-third of the world population harbour this parasite within them.

2.9.1.3 Morphology

The parasites are crescent shaped or oval measuring 6–12 μm in length and have a distinct central nucleus. They are presented in pairs or groups of pairs.

Electron microscopical picture shows the presence of an organelle at the anterior end which may be a cytostome or a holdfast and fibrils extending two-thirds of the body length which is responsible for gliding movement of the parasite.

The parasites are found swimming in the bloodstream, within tissues and within cells of R.E. system, W.B.C. and epithelial cells.

Toxoplasma has a number of phases: Oocysts or tissue cysts, Tachyzoites, Merozoites, Bradyzoites and Sporozoites.

Oocysts

Oocysts are product of sexual reproduction and are formed only in the intestine of cats, the definitive host. They are spherical in shape measuring $10 \times 15\,\mu m \times 18–12\,\mu m$ in size.

Tissue Cysts

Same as oocysts but are product of asexual reproduction in tissues. They are 12–100 μm in size and contain 50 to several thousand bradyzoites.

Sporozoites

Sporozoites are formed within mature oocysts. Mature oocysts contain two sporocysts each with four sporozoites. These are very small in size.

Tachyzoites

They are motile, multiply rapidly and are responsible for increasing population of the parasites within the host. Tachyzoites are crescent shaped, measuring 3–7 μm × 2–4-μm in size and have one end more round than the other.

Merozoites

They also divide quickly and increase population of the parasite within the cat's intestine before sexual reproduction. They are also very small.

Bradyzoites

These are slowly dividing phase of the parasites. When an infected host consumes oocyst, bradyzoites come out from the cyst, infect epithelial cells of intestine of the host and slowly convert into the rapidly dividing stage tachyzoites. After sometimes tachyzoites convert back to bradyzoites. They reproduce inside host cells and form oocysts or tissue cysts in the new host, cysts usually range between 5 and 50 μm in diameters.

2.9.1.4 Life Cycle

Life cycle of *Toxoplasma* is very much complex and interesting.

The cat is the definitive host where sexual cycle is completed. All other warm blooded animals (non-feline) are intermediate host.

There is only one species of *Toxoplasma*, *T. gondii* known still today. The *T. gondii* has no choice about its host, it can infect all kinds of warm blooded animals like birds and mammals. Experimentally it is shown that it can survive even

in lizards and turtles. As indicated by antibody reaction, the parasite is known to occur in 59% of dogs, 34% of cats, 48% of goats, 30% of pigs, 20% of rats, 10% in pigeons. But in human beings in the USA it is 17–35% and 0–68% in Eskimos at Tahiti (Chandler and Read 1961).

The human infection is found to be highest in warm, moist areas, but less in warm, dry areas and very few in very cold areas of the globe.

About transmission it is observed that the closest friends of human beings are dogs and cats and they are the reservoirs of the disease. The rats are also very important animals acting as reservoirs of the disease.

The transmission of the disease from person to person is very rare. Laarman in the year 1956 reported mechanical transmission by Stomoxys (Stable fly) and fleas after feeding them with *Toxoplasma* infected animals. These flies and fleas have been reported to harbour *Toxoplasma* for 60 days after experimental feeding of an infected animal (Fig. 2.14).

Life cycle of *Toxoplasma* has been divided into three components for the convenience of study:

1. In definitive host—Feline cycle.
2. In definitive host and intermediate host—feline and non-feline cycle.
3. In two intermediate hosts—non-feline to non-feline cycle.

Definitive Host Cycle

Toxoplasma multiplies through sexual reproduction only in cats or animals of feline groups.

The cycle starts by consuming rat heavily infected with *Toxoplasma*. The product of sexual reproduction is oocyst which is formed in the intestine of cats.

Then the oocysts escape from the body of the definitive host through faeces of the host. The oocysts reach the external environment on soil. The oocysts then mature within 2 days. During maturation the oocysts contain two sporocysts and each sporocyst contains four sporozoites.

Some sporozoites in case of cat only enter into the epithelial cells of gut and produce enteroepithelial cycle. Other sporozoites enter into the mucus membrane and start development process in the lamina propria, lymph nodes, WBC and other organs.

Within the cats the result of the cycle in gut epithelium are the tachyzoites. The dividing stages in acute infection, tachyzoites 8–32 in number, accrued within parasitophorus vacuole in the host cell and ultimately ruptures escaping the parasites which will again infect other cells.

After some such cycle of asexual multiplication gametogony starts: macro and microgamets are produced. They are formed within 3–15 days of infection. Gametocytes are formed all along the small intestine, especially in the ileum. Very small percentage like 2–4% gametocytes are male gametocytes. The microgametocytes develop 12 microgamets from each microgametocyte. The microgamets fertilize the macrogamets and form zygotes. Zygote converts into an

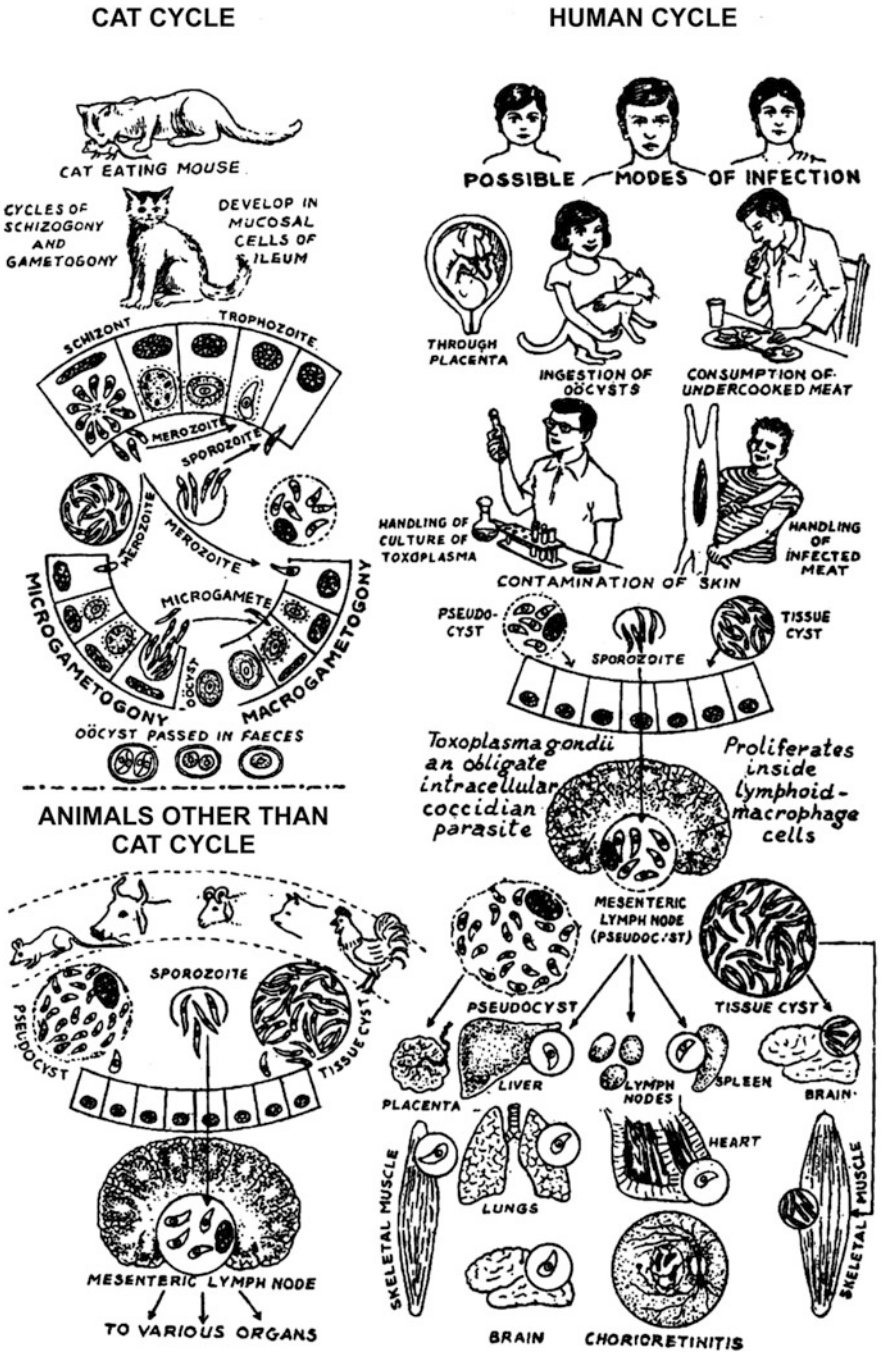


Fig. 2.14 Life cycle of *Toxoplasma gondii* (in different animals)

oocyst. Oocysts then come out in the faeces of cat after 3–5 days of infection by cysts.

After some such cycle of asexual multiplication gametogony starts: macro and microgametocytes are produced. They form micro and macrogamets and are fertilized to form zygote and zygote converts into an oocyst. Oocysts then come out in the faeces of cat after 3–5 days of infection by cysts. Oocysts then deposited on soil, in water, food as anything having contact with the faeces. The cysts may survive and remain infective for a number of months in cold moist climate.

Definitive Host to Intermediate Hosts

Ingestion of oocysts by human beings or other warm blooded animals starts the feline–non-feline cycle.

The common route of infection is faecal–oral route. Human beings consume unwashed vegetables in the form of salad or unknowingly drink oocysts contaminated water or by handling the faeces of a domesticated or pet infected cat.

Though cats are also infected by ingesting oocysts the intermediate hosts are infected by ingesting oocysts.

When oocysts or tissue cysts are ingested by human beings or non-feline animals through contaminated food and drink the cyst wall dissolves by the action of proteolytic enzymes present in the stomach and small intestine. The infective forms of parasites are released from the oocysts and invade epithelial cell of the intestine of the intermediate hosts. The parasites inside the intestinal epithelium convert into tachyzoites, the motile and rapidly multiplying cellular stage of the parasites, which infect the other cells and organs of the host.

Inside the intestinal epithelial cells the tachyzoites multiply inside a vacuole formed within the cell known as parasitophorous vacuole created during the entry of the parasites inside the cells. Now the parasites remain as intracellular but extra cytoplasmic one.

The tachyzoites multiply inside the parasitophorous vacuole of the infected cell until the cell dies and ruptures. Then the tachyzoites are released which find their way into the bloodstream and ultimately reach organs and tissues of the body including brain of the host where they form tissue cysts.

With the spread of the tachyzoites throughout the body of the host the immune system of the host react and causes *T. gondii* tachyzoites to transform into bradyzoites. The bradyzoites form clusters and are now known as tissue cysts because the cyst wall is produced from the membrane of the parasitophorous vacuole. The bradyzoites containing tissue cysts can form in any organ and found mainly in brain, eyes and striated muscles including heart muscles. It is seen that the choice of organ by the bradyzoites depends upon the hosts like majority of the parasites form tissue cysts in muscle tissues in case of pig while majority of cysts are found in brain in case of mice.

The cysts within the tissues are formed to avoid the immunity reactions of the hosts.

The transmission in case of human beings takes place through ingestion of undercooked meat, unwashed vegetables and contaminated drink. Transplacental transmission also occurs from mother to foetus.

Intermediate (Non-feline) to Intermediate (Non-feline) Cycle

Bradyzoites in the tissues cysts of non-feline animals, i.e. intermediate hosts undergo only asexual multiplication. Bradyzoites infect the intestinal cells of the intermediate host and starts endodyogeny, i.e. two daughter cells develop inside a parent cell. The parent cell then rupture and two daughter cells are released. The individual parasite then again undergoes the same process forming four parasites. These tachyzoites infect new cells and can invade all cells except RBCs.

T. gondii theoretically may be passed to intermediate hosts through consumption of tissue cysts in meat. But the life cycle of parasite begins and completes only when the parasite enters into a feline host (from rat to cat) where the parasite undergoes sexual reproduction.

2.9.1.5 Transmission

1. Consumption of raw or undercooked meat.
2. Drinking of unpasteurized goat milk.
3. Contact with contaminated soil.
4. Consumption of unwashed and unboiled raw vegetables or fruits.
5. Handling of infected cat and dogs.
6. Blood transfusion or organ transplant.
7. Transplacental transmission when mother is infected during pregnancy.

2.9.1.6 Precaution

As suggested by United States Centre for disease control and prevention.

From food:

1. Peeling and washing of consumable fruits and vegetables thoroughly.
2. Freezing meat for several days at subzero temperature before consumption.
3. Avoid eating undercooked meat.
4. Washing of hands with hot soapy water for the dog or cat handlers.
5. To drink only pasteurized milk.

From environment:

1. Changing and disposing of cat and dog litter daily.
2. Wearing of gloves during gardening.
3. Drinking of only filtered water.

2.9.1.7 Pathogenicity

In immunocompetent patients most of the time the Toxoplasmosis is overlooked as it is asymptomatic.

Toxoplasmosis if symptomatic is mild and remains only for a few days.

In acute Toxoplasmosis the symptoms are cervical lymphadenopathy, headache, illness and fever. Then the symptoms subside and the patients become carrier for the rest of their life as the tissue cysts remain in their organs.

Immunocompromised patients may be patients with AIDS or the patients on which immunosuppressive drugs are regimented develop severe illness. The common symptoms in AIDS patient if infected with *T. gondii* is encephalitis. This condition arises when the immunity developing WBC, CD4 cell count decrease below 100 cells/cubic millimetre. If the patients in this condition remain untreated the disease may be fatal.

It is found that transplacental transmission in pregnant mother does not occur if the infection took place more than 6 months before conception. The risk of infection of the foetus by transplacental transmission during pregnancy depends upon the trimester. The risk of infection by *T. gondii* is lowest in the first trimester and highest during third trimester.

The typical symptoms noticeable in an infected offspring are hydrocephaly, microcephaly, intra-cerebral calcifications, convulsions, chorioretinitis, optic nerve atrophy, hepato-spleenomegaly and jaundice.

In acute infection, the infection takes place in intestine at the onset.

In subacute infection, the tachyzoites start destroying cells of the liver, lung, heart, brain and eyes. It is found that CNS is damaged more than other organs.

Chronic infection takes place when the patients develop immunity. In this condition tachyzoites formation is suppressed. Now bradyzoites are formed from tachyzoites. The bradyzoites are also called zoitocysts. These bradyzoites remain intact for years but the disease is now asymptomatic. It is seen if the infection reach retinal cells of the eyes then it cause blindness. In chronic infection also myocarditis, heart damage, pneumonia may develop. Spontaneous abortions like serious phenomenon may occur.

2.9.1.8 Diagnosis

Clinical diagnosis can be done from the symptoms like cerebral calcification, chorioretinitis, hydrocephalus, symptoms like pneumonia, myocarditis, spleenomegaly, hepatomegaly, etc.

Direct methods may be applied like presence of parasites in the blood smears, bone marrow puncture, centrifuged deposit of CSF by staining with Giemsa and examined under microscope.

By serological tests like Sabin–Feldman dye test, indirect immunofluorescence, indirect haemagglutination, complement fixation test and ELISA.

2.9.1.9 Epidemiology

The toxoplasmosis, a disease is distributed worldwide. The disease is associated with cats mainly. Though birds and other mammals may be infected. The complete natural cycle remains within cats and mice. The mice devour materials contaminated with faeces of cats where there may be oocysts of *Toxoplasma*. If the cats consume such infected mice, they are infected.

Human toxoplasmosis is a zoonotic disease. Human infection takes place through food and drink contaminated by mature oocysts of *Toxoplasma gondii* and/or undercooked meat having tissue cysts. Over and above cockroaches and flies act as mechanical vector.

Infection may be transmitted through blood or WBC transfusion. The incubation period of Toxoplasmosis is ordinarily 1–3 weeks.

The result of infection depends upon the immunity status of the persons concerned. The important of all is the incident of congenital toxoplasmosis. Out of all it is such a disease that the stage of a parasite known as tachyzoites have been found in the nasal, vaginal and eye secretions. Not only that breast milk, saliva, urine, seminal fluid may also contain the infective forms of the parasite.

2.9.1.10 Host Defence Mechanism

Toxoplasmosis is an opportunistic disease. The disease manifests in two states: an acute stage when early spread of tachyzoites take place by the lymphocytes to the tissues another chronic stage when cysts are formed.

During the acute stage IFN-γ is produced by the NK cells which are induced by IL-12 coming from macrophage along with TNF-α.

IFN-γ and TNF-α activate anti-Toxoplasma action in macrophages. During chronic stage Th2 type response occurs. Here CD8+ cells and IFN-γ play major roles.

2.10 Systematic of the Studied Protozoan Parasites

2.10.1 *Entamoeba histolytica*

Kingdom	Protista
Subkingdom	Protozoa
Phylum	Sarcomastigophora
Subphylum	Sarcodina
Class	Lobosea
Order	Amoebida
Family	Entamoebidae
Genus	*Entamoeba*
Species	*histolytica*

2.10.1.1 General Characters of Kingdom Protista

1. They are mostly unicellular organisms.
2. These eukaryotes have a nucleus and organelles.
3. They are mostly heterotrophic organisms.
4. Symbiosis or parasitism is observed in the member of Protista.
5. Locomotory organs are cilia or flagella or pseudopodia.

2.10.1.2 Subkingdom Protozoa

1. All are microscopical.
2. Body composed of single cell.
3. Single cell performs all the biological functions.
4. Body is naked or covered by pellicle.
5. Single cell usually contains single nucleus.
6. Presence of contractile vacuole in free living forms.
7. Free living, symbiotic or parasitic.
8. Reproduction asexual or sexual.

2.10.1.3 Phylum Sarcomastigophora

1. Locomotion with the help of pseudopodia or flagella.
2. Reproduction by binary fusion or syngamy.

2.10.1.4 Subphylum Sarcodina

1. Body is naked.
2. Mostly pseudopodia are present.
3. Presence of a single nucleus.
4. Most of the members are free living, some are parasitic.

2.10.1.5 Class Lobosea

1. Pseudopodia are lobose type.
2. Presence of single or more nucleus.

2.10.1.6 Order Amoebida

1. Presence of a cell membrane which encloses the cytoplasm and cell organelles.
2. As there is no cell wall, so cellular structure is not definite.
3. Presence of a single nucleus.

2.10.1.7 Family Entamoebidae

1. Cells are small.
2. Presence of a simple life cycle.
3. Trophozoites feeds on bacteria.

2.10.1.8 Genus *Entamoeba*

1. It is found as internal parasite of G.I. tract of human beings or may be commensal.

2.10.1.9 Species *histolytica*

1. An anaerobic parasitic protozoa.
2. Infects digestive tract of human beings and other primates.
3. Trophozoetes ingest RBC.
4. Trophozoetes transform into cysts.
5. Occasionally penetrate the intestinal wall by a type of secretion and enters into blood vessels and form abscess in different organs.

2.10.2 *Giardia intestinalis*

Kingdom	Protista
Subkingdom	Protozoa
Phylum	Sarcomastigophora
Subphylum	Mastigophora
Class	Zoomastigophora
Order	Diplomonidida
Family	Hexamitidae
Genus	*Giardia*
Species	*intestinalis*

2.10.2.1 Kingdom Protista
Same as Entamoeba.

2.10.2.2 Subkingdom Protozoa
Same as Entamoeba.

2.10.2.3 Phylum Sarcomastigophora
Same as Entamoeba.

2.10.2.4 Subphylum Mastogophora
1. Presence of single or more than one flagella.
2. Asexual reproduction by binary fission.

2.10.2.5 Class Zoomastigophora
1. Absence of chloroplast.
2. Sexual reproduction may be seen in some cases.

2.10.2.6 Order Diplomonidida
1. Presence of two nuclei in the body.
2. Presence of four flagella.
3. They are zooflagellates.
4. Live as parasites or commensals in the digestive system of host.

2.10.2.7 Family Hexamitidae
1. Intestinal parasites of invertebrate and vertebrate hosts.
2. Bilaterally symmetrical.
3. Oval in shape with two nuclei.

2.10.2.8 Genus *Giardia*
1. Anaerobic flagellated protozoan parasite.
2. Colonize and reproduce in the small intestine of the hosts.

2.10.2.9 Species *intestinalis*

1. A flagellated parasitic microorganism.
2. Colonize and reproduce in small intestine of man and other vertebrates.

2.10.3 *Trichomonas vaginalis*

Kingdom	Protista
Subkingdom	Protozoa
Phylum	Sarcomastigophora
Subphylum	Mastigophora
Class	Zoomastigophora
Order	Trichomonadida
Family	Trichomonadidae
Genus	*Trichomonas*
Species	*vaginalis*

Kingdom, Subkingdom, Phylum, Subphylum and Class same as *Giardia*.

2.10.3.1 Order Trichomonadida

1. They are anaerobic protista.
2. They are zooflagellate.
3. Presence of three to six flagella.
4. One trails or borders an undulating membrane.
5. Resides within digestive system of vertebrates.
6. They may be uninucleate or multinucleate.

2.10.3.2 Genus *Trichomonas*

1. They are anaerobic excavate parasites of vertebrates.
2. Presence of four flagella.
3. One recurrent, along the outer margin of the undulating membrane.
4. Costa originate in the kinetosomal complex at the anterior of the parasite.
5. Presence of axostyle.

2.10.3.3 Species *vaginalis*

1. Causes sexually transmitted infection.
2. It is an anaerobic flagellated protozoan parasite.
3. Usually resides in vagina and urethra of female human beings.

2.10.4 *Trichomonas hominis*

Up to Genus same as *T. vaginalis*.

2.10.4.1 Species *hominis*

1. Flagellate parasitic protozoa.
2. They are nonpathogenic.
3. Sometimes causes diarrhoeic stools.
4. Resides in large intestine of the host.

2.10.5 *Nagleria fowleri*

Up to Subphylum same as *Entamoeba histolytica*.

Class	Heterolobosea
Order	Schizopyrenida
Family	Vahlkamppfidae
Genus	*Nagleria*
Species	*fowleri*

2.10.5.1 Class Heterolobosea

1. Free living heterotrophs.
2. Many are amoeboflagellates.
3. Some of these can reversibly transform into flagellates.

2.10.5.2 Order Schizopyrenida

1. They are small amoebas.
2. Many of them can transform into flagellates.

2.10.5.3 Family Vahlkamppfidae

1. They are uninucleate.
2. Can transform from amoeba to flagellate.
3. Forms polar masses in mitosis.

2.10.5.4 Genus *Nagleria*

1. Presence of DNA in the nucleus.
2. Presence of membrane bound organelles.

2.10.5.5 Species *fowleri*

1. Presence of two flagella in their flagellate stage.
2. May be parasite and causes primary amoebic meningitis.

2.10.6 *Trypanosoma cruzi*

Kingdom	Protista
Subkingdom	Protozoa
Phylum	Sarcomastigophora
Subphylum	Mastigophora
Class	Zoomastigophora
Order	Kinetoplastida
Family	Trypanosomatidae
Genus	*Trypanosoma*
Species	*cruzi*

Up to Class same as *T. vaginalis.*

2.10.6.1 Order Kinetoplastida

1. Parasitic life style.
2. Cell body is elongated.
3. Locomotion by flagellum.
4. Presence of kinetoplast containing DNA.
5. Kinetoplast is a special compartment of mitochondria.

2.10.6.2 Family Trypanosomatidae

1. A type of protozoan parasite.
2. They are dixenous.
3. Infection through insect vector.

2.10.6.3 Genus *Trypanosoma*

1. A monophylectic group of unicellular organisms.
2. Parasitic flagellate protozoa.
3. Locomotion like corkscrew.

2.10.6.4 Species *cruzi*

1. Parasitic to human beings,
2. Metacyclic trypomastigotes and amastigotes stages found in the life cycle of human hosts.

2.10.7 *Trypanosoma gambiense*

Kingdom	Protista
Subkingdom	Protozoa
Phylum	Sarcomastigophora
Subphylum	Mastigophora
Class	Zoomastigophora

(continued)

Order	Kinetoplastida
Family	Trypanosomatidae
Genus	*Trypanosoma*
Species	*gambiense*, *rhodesiense*, *brucei*

Up to Genus same as *T. cruzi.*

2.10.8 *Leishmania donovani*

Kingdom	Protista
Subkingdom	Protozoa
Phylum	Sarcomastigophora
Subphylum	Mastigophora
Class	Zoomastigophora
Order	Kinetoplastida
Family	Trypanosomatidae
Genus	*Leishmania*
Species	*donovani*

Up to Family same as *T. cruzi.*

2.10.8.1 Genus *Leishmania*

1. Presence a well-defined nucleus and other cell organelles.
2. Presence of kinetoplasts and flagella.
3. Presence of amastigote form in the mononuclear phagocytes and circulatory systems of human beings.

2.10.8.2 Species *donovani*

1. Causes the disease leishmaniasis.
2. Responsible for visceral leishmaniasis or kala-azar.
3. Infection is transmitted by sandfly.

2.10.9 *Plasmodium vivax*

Kingdom	Protista
Subkingdom	Protozoa
Phylum	Apicomplexa
Class	Sporozoa
Order	Haemosporida
Family	Haemosporidae
Genus	*Plasmodium*
Species	*vivax*, *falciparum*, *malariae*, *ovale*

Up to Subkingdom same as *Entamoeba.*

2.10.9.1 Phylum Apicomplexa

1. Presence of apical complex, only seen under E.M.
2. Presence of one or more polar ring.
3. Locomotory organ is microfibril.
4. Flagella may be present in gonad.
5. All members are intracellular parasite.
6. Sporozoites are formed within spore.

2.10.9.2 Class sporozoa

1. Colloid forms complete ring.
2. Sporozoites are formed from oocytes.
3. Sexual and asexual reproduction both are seen.

2.10.9.3 Order Haemosporida

1. They are blood parasites.
2. The zygote is capable of movement called ookinite.
3. Sporozoites are free not enclosed by sporocysts.

2.10.9.4 Family Haemosporidia

1. Parasite produces pigment.
2. Asexual cycle takes place in peripheral blood.

2.10.9.5 Genus *Plasmodium*

1. Parasites are responsible for the disease malaria.

There are four species vivax, falciparum, malariae, ovale.

2.10.10 *Cryptosporidium parvum*

Kingdom	Protista
Subkingdom	Protozoa
Phylum	Apicomplexa
Subphylum	Sporozoa
Class	Sporozoasida
Subclass	Coccidiasina
Order	Eucoccidiida
Suborder	Eumerii
Family	Cryptosporidiidae
Genus	*Cryptosporidium*
Species	*parvum*

Up to Phylum same as *Plasmodium*.

2.10.10.1 Subphylum Sporozoa

1. Absence of any special organ for locomotion.
2. Reproduce asexually by fission followed by sexual union or syngamy.
3. Presence of alternation of generations.

2.10.10.2 Class Sporozoasida

Reproduce by sexual and asexual process oocysts are produced.

2.10.10.3 Subclass Coccidiasina

Life cycle usually involves mesogony, gametogony or sporogony. Gamonts are small.

2.10.10.4 Order Eucoccidiida

Mesogony or schizogony present.

2.10.10.5 Suborder Eimeriine

Micro and macro gamonts produce independently. Zygote is non-motile.

2.10.10.6 Family Cryptosporidiidae

1. Oocysts contain four naked sporozoites.
2. Absence of sporocysts.
3. Endogenous stages with attachment organelle.
4. Life cycle monoxenous.

2.10.10.7 Genus *Cryptosporidium*

1. Important gastrointestinal agents.
2. Presence of two types of oocysts, a thin walled and a thick walled.
3. Both contain four sporozoites.
4. Thin walled oocyst reinfects the same host.
5. Thick walled oocysts are exit forms.

2.10.10.8 Species *parvum*

1. Life cycle in a single host.
2. Sporozoites 4 in number are liberated in the intestine of host.
3. Infection remains restricted in the brush border cells of intestine.
4. Oocysts are resistant to most disinfectants.

2.10.11 *Babesia bigemina*

Kingdom	Protista
Subkingdom	Protozoa
Phyllum	Apicomplexa
Class	Sporozo

(continued)

Order	Haemosporida
Family	Piroplasmida
Genus	*Babesia*
Species	*bigemina*

Up to Order same as *Plasmodium*.

2.10.11.1 Family Piroplasmida

1. Piroplasm parasitize RBCs of the host.
2. Presence of apical complex with polar ring.
3. They lack flagella.
4. Do not form oocysts or spores.
5. Sporogony occurs within invertebrate vector.

2.10.11.2 Genus *Babesia*

1. Pear shaped appearance in blood cells.
2. Sometimes may be spherical or amoeboid in shape.
3. Ticks are vector.

2.10.11.3 Species *bigemina*

1. Presence of intraerythrocytic merogony.
2. Sporogony forms sporonts which produce sporoblasts.

2.10.12 *Balantidium coli*

Kingdom	Protista
Subkingdom	Protozoa
Phylum	Ciliophora
Subphylum	Mastigophora
Class	Zoomastigophora
Order	Spirotrichida
Family	Heterotrichina
Genus	*Balantidium*
Species	*coli*

Up to Class Zoomastigophora is same as *T. vaginalis.*

2.10.12.1 Order Spirotrichida

1. They are a group of ciliate protozoa.
2. They have prominent oral cilia.
3. The body of cilia is fused to form cirri in some.

2.10.12.2 Family Heterotrichina

1. Presence of a distinct mouth, cytostome and a cytopyge (anal pore).
2. The cilia cover the entire body arranged in longitudinal or obliquely spiral parallel row.

2.10.12.3 Genus *Balantidium*

1. Presence of two nuclei macro and micro.
2. Macronucleus large, slightly curved and vegetative in function.
3. Micro-nucleus, vesicular type, acts as generative nucleus.

2.10.12.4 Species *coli*

1. Parasite is able to form cyst.
2. All are parasitic to vertebrate and invertebrate hosts.
3. Only species of medical importance.

2.10.13 *Toxoplasma gondii*

Up to Order same as Cryptosporidium.

2.10.13.1 Family Sarcocystidae

1. Parasites of the carnivorous hosts.
2. They are characterized by an obligatory two hosts life cycle.
3. Infection takes place through predator relationship.
4. Cysts are not found in raccoon dogs.

2.10.13.2 Genus *Toxoplasma*

1. Occurs in three morphological form—Trophozoite.
2. Produces cysts in the muscles of intermediate hosts.
3. The cysts contain numerous bradyzoites.

2.10.13.3 Species *gondii*

1. Tissue cysts occur in muscles and other tissue.
2. Parasites multiply slowly within the host cell and develop a cyst wall.

References

Bray RS (1957) Studies on the exo-crythocytic cycle in the genus Plasmodium. Mem Lon Sch Hyg Trop Med 12:192

Chandler AC, Read CP (1961) Introduction to parasitology. Wiley and Toppan Co Ltd, Tokyo

Das N, Jha D (1967) On a new flagellate, *Giardia panthera* n sp, from the gut of a lion (*Panthera leo*). Ind J Anim Health VI(II):305–307

Das N, Ray HN (1967) PA Schiff positive ectocystic envelop in *Entamoeba coli* type cyst from Mandrill (*Cynocephalus mormon*). J Protozool USA 14(Suppl):29 Art 131

Das N, Ray HN (1970) Cytomorphological studies of some parasitic protozoa. Thesis submitted for Ph. D. degree of N.Das at University of Calcutta

Dennis EW (1932) The life cycle of *Babesia bigemina* of Texas cattle fever in the tick *Margaropus annulatus*. Univ Calif Publ Zool 36:263–298

Garnham PCC (1966) Malarial parasites and other Haemosporidia. Blackwell Publication, Oxford

Hoare CA (1959) Amoebic infection in animals. Vet Rev Annot 5:91

Howard L (1960) Studies on the mechanism of infection of mosquitoes by malarial parasites. DPH thesis, John Hopkins University

Huff CG (1946) The relation and acquired immunity of various avian hosts to the cryptozpites and metacryptozoites of *Plasmodium gallineaceum* and *Plasmodium relictum*. J Inf Dis 78:99–117

Jha D, Das N, Ray HN (1968) Giardial infection in Indian goats. Ind J Anim Health VII(I):39–42

Karyakarta R, Damle A (2003) Medical parasitoloogy. Book Allied, Kolkata

Levine ND (1961) Protozoan parasites of domestic animals and man. Burges Publishing, Minneapolis, p 129

Levine ND (1980) Journal of protozoology. Society of Protozologists, Athens

Markel EK, Voge H (1958) Diagnostic medical parasitology. WB Saunders, Philadelphia

Noble ER, Noble GA (1961) Parasitology: the biology of animal parasites. Lea & Febiger, Philadelphia

Schimidt GD, Roberts LS (2013) Foundations of parasitology. McGraw-Hill International, New York

Simitch T et al (1959) Contribution à la connaissance de la fauna des protozooire intertinaux de pore de yogoslavia. Ard Inst Pasteur, D'Algeric 37:401

Weinman D, Chandler AH (1954) Toxoplasmosis in swine and rodents: reciprocal oral infection and potential human hazard. Proc Soc Exp Biol 87:211–216

Wenrich DH (1937) Studies on Iodoamoeba butschlii (Protozoa) with special reference to nuclear structure. Proc Am Philosop Soc 77:183