NATIONAL OPEN UNIVERSITY OF NIGERIA

SCHOOL OF SCIENCE AND TECHNOLOGY

COURSE CODE: BIO 310

COURSE TITLE: PROTOZOOLOGY

Course Code & Course Title:	**BIO 310: Protozoology**
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GENERAL INTRODUCTION

A parasite is an organism that obtains food and shelter from another organism and derives all benefits from this association. The parasite is termed obligate when it can live only in a host; it is classified as facultative when it can live both in a host as well as in free form. Parasites that live inside the body are termed endo-parasites whereas those that exist on the body surface are called ecto-parasites. Parasites that cause harm to the host are pathogenic parasites while those that benefit from the host without causing it any harm are known as commensals.

2.0 Objectives

By the end of this lecture unit, students should:

-have a clear knowledge of what parasites are and the different types of parasites

-have a clear understanding of the phylum apicomplexa, its characteristics and replication

-be clearly introduced to protozoans, its structure and functions.

3.0 Definition of terms:

1. A host:- The organism that harbors the parasite and suffers a loss caused by the parasite

2. Definitive host:- This is the host in which the parasite lives its adult and sexual stage.

3. Intermediate host:- This is the host in which a parasite lives as the larval and asexual stage.

4. Reservoir hosts: - These are hosts that harbor the parasite and thus ensure continuity of the parasite's life cycle and act as additional sources of human infection.

5. Vector:- This is an organism (usually an insect) that is responsible for transmitting the parasitic infection.

While animals and plants are more visible to the naked eye, protozoa (unicellular organisms) constitute the majority of Eukaryotic life. Studying the biology of a model parasitic protozoan can therefore provide insight into the basic biology of eukaryotes (whose cells contain a nucleus, as distinct from bacteria and archaebacteria). Studying these organisms also provides insight into host-parasite interactions and mechanisms of pathogenesis.

4.0 The Phylum Apicomplexa

The Phylum Apicomplexa encompasses over 5,000 species of unicellular parasitic protozoa, many of which cause serious disease in humans and animals. For example, Plasmodium parasites are transmitted by mosquitoes, infect red blood cells and cause malaria, a disease with devastating impact on children in sub-Saharan Africa, Southeast Asia and South America. Other Apicomplexan parasites of medical importance include Cryptosporidium and Toxoplasma, which infect a large percentage of the population worldwide, and can cause debilitating and potentially fatal illness in immunocompromised individuals. Toxoplasma is a leading cause of congenital neurological birth defects, transmitted by consumption of undercooked meat or ingestion of material contaminated with cat feces (this is why pregnant women are advised not to empty the kitty litter box!).

4.1 General characteristics of Apicomplexa

Although they cause very different diseases, all apicomplexan parasites originated from a common ancestor and consequently share many characteristics:

1. All are obligate intracellular parasites … they must invade and replicate within host cells in order to survive.

2. They traverse a complex life cycle, differentiating (developing) through various forms, often in different host species/tissues in the course of infection. In particular, sexual and asexual reproduction often occurs in different species (such as mosquitoes and humans for *Plasmodium*).

3. From a cell biological perspective, apicomplexan parasites contain all of the organelles one might expect in a eukaryote, including the nucleus, endoplasmic reticulum, Golgi apparatus, and mitochondrion.

Apicomplexan parasites also possess distinctive subcellular organelles, including:

Specialized secretory organelles, termed rhoptries and micronemes, that deploy their cargo in a coordinated fashion during parasite attachment to the host cell, invasion, establishment of the intracellular "parasitophorous vacuole" within which they reside and replicate, and modulation of the host cell.

A plastid organelle, known as the "apicoplast" (apicomplexan plastid), acquired through "secondary endosymbiosis", in which an ancestral parasite ate a eukaryotic alga, and retained the algal plastid.

4.2 **How apicomplexan parasites replicate.**

Once the parasite has invaded a host cell, its survival is critically linked to replication. The replicative process occurs entirely within a specialized intracellular vacuole termed the parasitophorous vacuole. As the parasite divides, the host cell swells and eventually bursts (a lytic infection), causing disease through direct tissue damage. Similarly, in malaria, *Plasmodium* parasites lyse infected red blood cells, causing anemia (other factors may also exascerbate anemia in severe disease). Toxoplasma damages multiple organs

systems, but is particularly noted for its ability to quickly destroy brain and fetal tissues.

Apicomplexan parasites replicate via an unusual process in which daughter parasites are assembled within a single mother cell. In the asexual "tachyzoite" stages of *Toxoplasma* infection, two daughter cells emerge from each mother (endodyogeny). In *Plasmodium* "merozoites", up to 16 or more daughters form within a single mother cell ("schizogony"). Assembly of daughter cells within the mother offers several advantages, such as the ability to eliminate indigestible waste products (such as the toxic heme left over after digestion of hemoglobin by Plasmodium) by simply leaving them behind. These parasites don't need lysosomes! Assembly of the cytoskeleton from the top (apical end) down, also ensures that polarity is preserved, as required for invasion of the host cell. Replication by endodyogeny or schizogony also poses challenges: a complex process is required to ensure that each daughter inherits a complete set of organelles. Note that unlike division in the typical 'textbook' eukaryotic cell, "M" phase (including organellar replication) is a lengthy process, completely encompassing the DNA replication hallmark of "S" phase. This suggests that unique checkpoints are likely to operate in the apicomplexan cell cycle.

5.0 STRUCTURE AND FUNCTION OF PROTOZOA

Protozoa, like other eukaryotic cells, have a nucleus, an endoplasmic reticulum, mitochondria and a Golgi body and lysosomes. In addition, because they lead an independent existence they possess a variety of

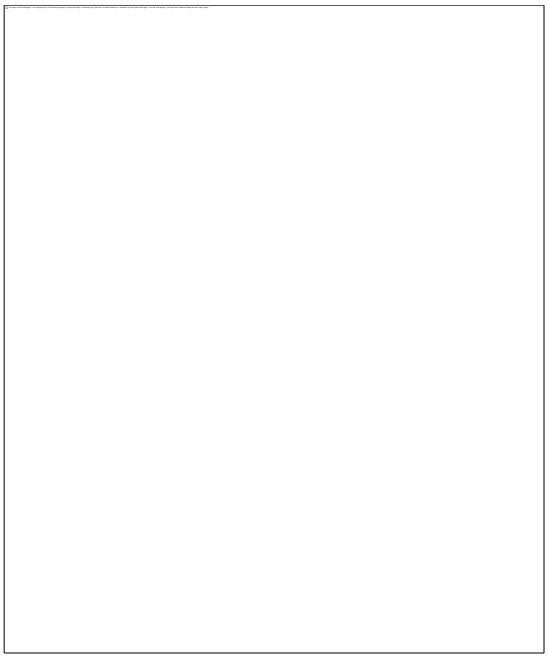

Fig. 5.1a.*Trypanosoma brucei* showing the flagellum and undulating membrane

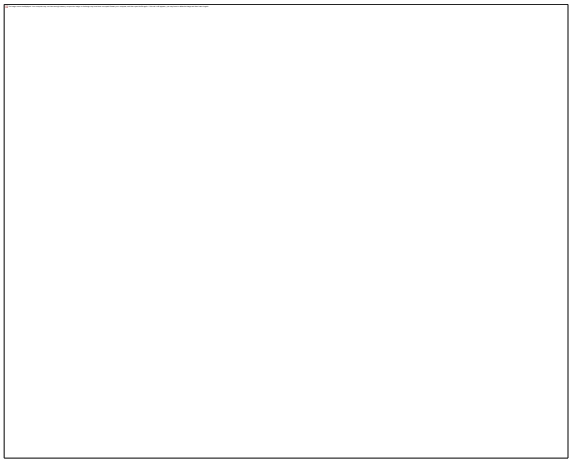

Fig. 5.1b. The morphology of the intestinal protozoan *Balantidium.*

other subcellular structures or organelles with distinct organisational features and functions.

Thus locomotion, in, for example, the genus Trypanosoma (Fig. 5.1a.) is facilitated by a single flagellum, and in some other protozoa by several flagella. A flagellum is a contractile fibre, arising from a structure called a basal body, and in some species is attached to the body of the protozoan along its length, so that when the flagellum beats, the cell membrane (pellicle) is pulled up to form an undulating membrane. Someetimes, also, it projects beyond the protozoan body as a free flagellum. During movement the shape of these organisms is maintained bymicrotubules in the pellicle.

Other protozoa, such as Balantidium (Fig. 5.1b), move by means of cilia which are fine, short hairs, each arising from a basal body; these cover much of the body surface and beat in unison to effect movement. In such species a mouth or cytostome is present and the ciliary movement is also used to waft food towards this openmg.

A third means of locomotion, used by protozoa such as Entamoeba (Fig. 5.1c.) are pseudopodia, which are prolongations of cytoplasm. Movement occurs as the rest of the cytoplasm flows into this prolongation.

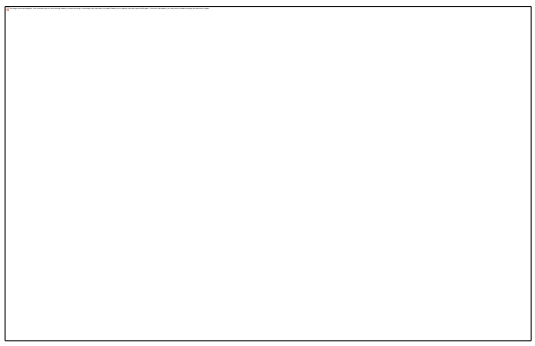

Fig. 5.1c.*Enatamoeba histolytica* has an amoeboid trophozoite stage and a non-motile cystic stage with nuclei.

The pseudopodium also possesses a phagocytic capacity and can function as a cup, which closes, enveloping particulate food material in a vacuole.

Finally, some protozoa, such as the extracellular stages of the *Eimeria*, have no obvious means of locomotion, but are nevertheless capable of gliding movements.

The nutrition of parasitic protozoa usually occurs by pinocytosis or phagocytosis, depending on whether tiny droplets of fluid or small objects of macromolecular dimension are taken into the

cell, In both cases, the process is the same, the cell membrane gradually enveloping the droplet or object which has become adherent to its outer surface, When, this is complete, the particle is carried into the cell where fusion with lysosomes effects digestion, Finally, undigested material is extruded from the cell. As noted above, some ciliated protozoa and also some stages of the organisms causing malaria obtain food through a cytostome, At the base of the cytostome the food enters a vacuole for digestion within the cell. Metabolic products are excreted by diffusion through the cell membrane.

The infective stage of some protozoa is called a sporozoite, while the term trophozoite is applied to that stage of the protozoa in the host, which feeds and grows, until division commences, In most protozoa, reproduction is asexual and is accomplished by binary fission or, in the case of within *Babesia* erythrocytes, by budding, Another form of asexual reproduction, which occurs in the subphylum Sporozoa is merogony (schizogony), In the latter process, the trophozoite grows to a large size while the nucleus divides repeatedly. This structure is called a meront (schizont) and, when mature, each nucleus has acquired a porrtion of the cytoplasm so that the schizont is filled with a large number of elongated separate organisms called merozoites. The meront eventually ruptures, liberating the individual merozoites.

Protozoa that only divide asexually generally have a short generation time, and since they cannot exchange genetic material, rely on mutants to provide the variants necessary for natural selection. However, most Sporozoa at certain stages in their life cycle also have a sexual phase of reproduction, called gametogony, which may be followed by a free-living maturation phase, or sporogony. Sometimes, as in *Eimeria*, both asexual and sexual phases occur in vertebrate host while in others, such as *Plasmodium*, the asexual phase occurs in the vertebrate host and the sexual phase in the arthropod vector.

6.0 CONCLUSION

The Protozoa contains unicellular organisms, which belong to the Kingdom, Protista. Protozoa are more primitive than animals, and no matter how complex their bodies may be, all the different structures are conntained in a single cell.

Protozoans, like most organisms, are eukaryotic, in that their genetic information is stored in chromosomes contained in a nuclear envelope. In this way they difffer from bacteria which do not have a nucleus and whose single chromosome is coiled like a skein of wool in the cytoplasm. This primitive arrangement, found only in bacteria, rickettsia and certain algae, is called prokaryotic and such organisms may be regarded as neither animal nor plant, but as a separate kingdom of prokaryotic organisms, the Monera.

Tutor- marked assignment (TMA)

1. Differentiate between a trophozoite and a sporozoite

2. Enumerate the various mode of movement of protozoans giving examples

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MODULE 1: BLOOD AND TISSUE PROTOZOA

1. Introduction

Blood protozoa of major biological/medical significance include members of genera

i). Trypanosoma (*T. brucei* and *T. cruzi*)

ii). Leishmania (*L. donovani*, *L. tropica* and *L. braziliensis*)

3. Plasmodium (*P. falciparum*, *P. ovale*, *P. malariae* and *P. vivax*)

4. *Toxoplasma gondii*; and

5. Babesia (*B. microti*).

Objectives

At the end of this lecture unit, the student should:

-have a good understanding of blood and tissue protozoans of major biological significance

-be able to differentiate between the various protozoans using their morphological and life cycle characteristics

-be able to differentiate between the various species within the genera.

Unit 1: TRYPANOSOME

The haemoflagellates all belong to the family Trypanosomatidae, and include the trypanosomes and leishmanias.

MORPHOLOGY

Trypanosomes have a leaf-like or rounded body conntaining a vesicular nucleus, and a varying number of subpellicular microtubules lying beneath the outer membrane. There is a single

flagellum arising from a kinetosome or basal granule. An undulating membrane is present in some genera and the flagellum lies on its outer border. Posterior to the kinetosome is a rod-shaped or spherical kinetoplast containing DNA. Members of this family were originally parasites of the intestinal tract of insects, and many are still found in insects. Others are heteroxenous, spending part of their life cycle in a vertebrate host and part in an invertebrate host.

Members of the genus *Trypanosoma* are heteroxenous and pass through amastigote, promastigote, epimastigote and tryptomastigote stages in their life cycle. In some species only tryptomastigote forms are found in the verteorate host; in others, presumably more primitive species, both amastigote and tryptomastigote forms are present.

In the tryptomastigote form, the kinetoplast and kinetosome are near the posterior end and the

Flagellum forms the border of an undulating membrane that extends along the side of the body to the anterior end.

In the **epimastigote** form, the kinetoplast and kinetosome are just posterior to the nucleus and the undulating membrane forward from there.

In the **promastigote** form, the kinetoplast and the kinetosome are still further anterior in the body and there is no undulating membrane.

In the **amastigote** form, the body is rounded and the flagellum emerges from the body through a wide, funnel-shaped reservoir.

Epidemiology

Trypanosoma brucei gambiense is predominant in the western and central regions of Africa, whereas *Trypanosoma brucei rhodesiense* is restricted to the eastern third of the continent. 6,000 to 10,000 human cases are documented annually. 35 million people and 25 million cattle are at risk. Regional epidemics of the disease are cause of major health and economic disasters.

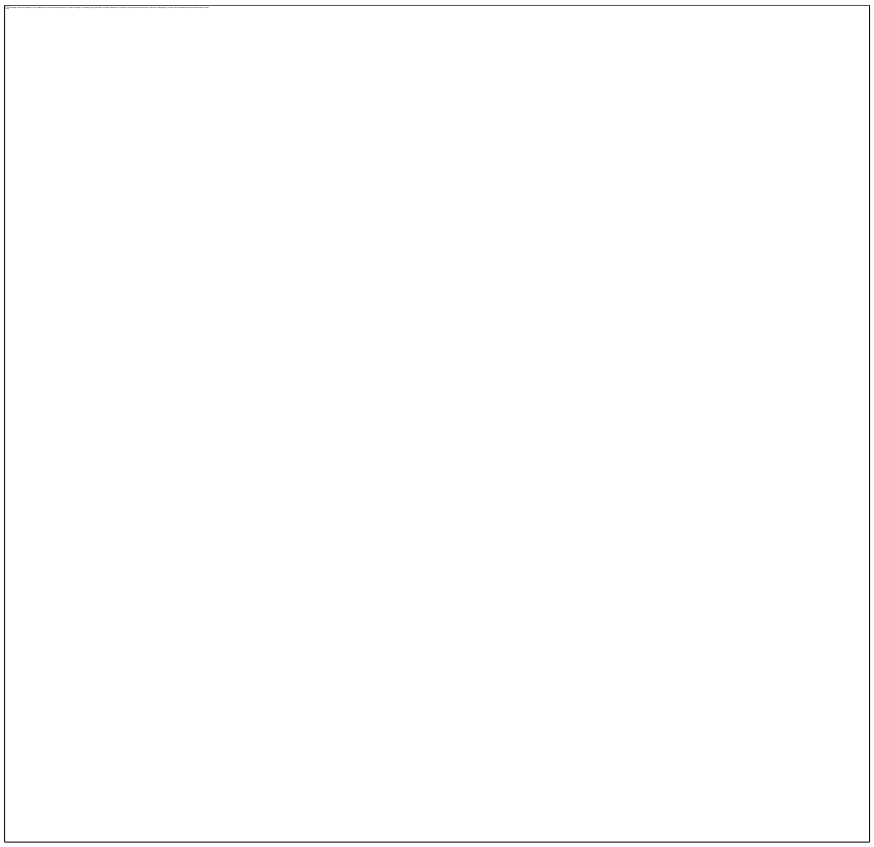

Fig. 2.1.1: Distribution of West African or Gambian Sleeping Sickness and East African or Rhodesian Sleeping Sickness

Morphology

T. b. gambiense and *T. b. rhodesiense* are similar in appearance: The organism measures 10 - 30 micrometers x 1-3 micrometers. It has a single central nucleus and a single flagellum originating at the kinetoplast and joined to the body by an undulating membrane. The outer surface of the organism is densely coated with a layer of glycoprotein, the variable surface glycoprotein (VSG).

Life cycle

The infective, metacyclic form of the trypanosome is injected into the primary host during a bite by the vector, the tsetse fly. The organism transforms into a dividing trypanosomal (trypomastigote) blood form as it enters the draining lymphatic and blood stream. The trypanosomal form enters the vector during the blood meal and travels through the alimentary canal to the salivary gland where it proliferates as the crithidial form (epimastigote) and matures to infectious metacyclic forms. Trypomastigotes can traverse the walls of blood and lymph capillaries into the connective tissues and, at

a later stage, cross the choroid plexus into the brain and cerebrospinal fluid. The organism can be transmitted through blood transfusion

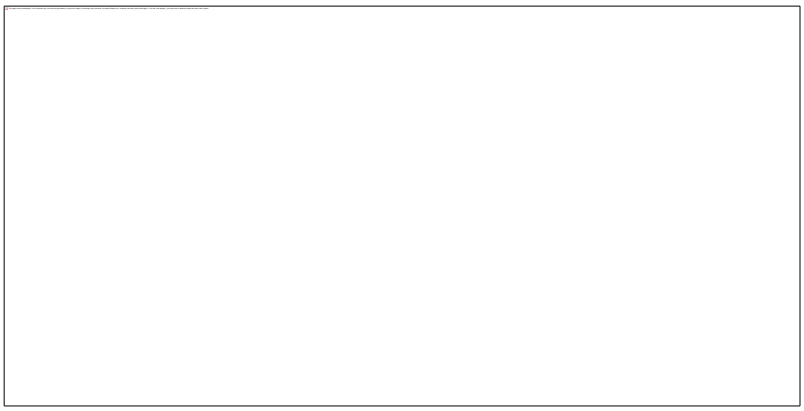

Fig.2.1.2: Glossina (Tsetse fly) vector of Trypanosome

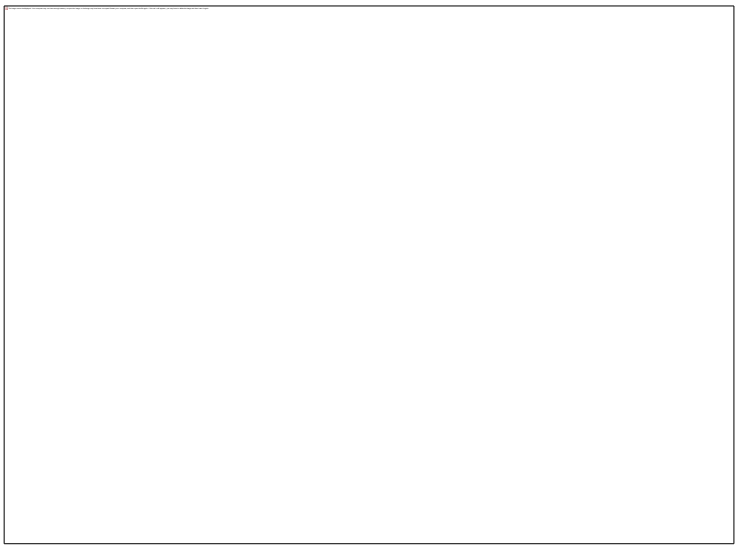

Figure 2.1.3: Forms of *Trypansoma brucei* obsreved in the tstese fly and in the human blood stream

Trypanosomiasis is the disease caused by trypanosomes.

African trypanosomiasis (Sleeping sickness)

Etiology

There are two clinical forms of African trypanosomiasis:

1) A slowly developing disease caused by *Trypanosoma brucei gambiense* and

2) A rapidly progressing disease caused by *T. brucei rhodesiense*.

Symptoms

The clinical features of Gambian and Rhodesian disease are the same, however they vary in severity and duration. Rhodesian disease progresses more rapidly and the symptoms are often more pronounced. The symptoms of the two diseases are also more pronounced in Caucasians than in the local African population. Classically, the progression of African trypanosomiasis can be divided into three stages: the bite reaction (chancre), parasitemia (blood and lymphoid tissues), and central nervous system (CNS) stage.

Bite reaction: A non-pustular, painful, itchy chancre forms 1-3 weeks after the bite and lasts 1-2 weeks. It leaves no scar.

Parasitemia: Parasitemia and lymph node invasion is marked by attacks of fever which starts 2-3 weeks after the bite and is accompanied by malaise, lassitude, insomnia headache and lymphadenopathy and edema. Painful sensitivity of palms and ulnar region to pressure (Kerandel's sign) may develop in some Caucasians. Very characteristic of Gambian disease is visible enlargement of the glands of the posterior cervical region (Winterbottom's sign). Febrile episodes may last few months as in Rhodesian disease or several years as in Gambian disease. Parasitemia is more prominent during the acute stage than during the recurrence episodes.

CNS Stage: The late or CNS stage is marked by changes in character and personality. They include lack of interest and disinclination to work, avoidance of acquaintances, morose and melancholic attitude alternating with exaltation, mental retardation and lethargy, low and tremulous speech, tremors of tongue and limbs, slow and shuffling gait, altered reflexes, etc. Males become impotent. There is a slow progressive involvement of cardiac tissue. The later stages are characterized by drowsiness and uncontrollable urge to sleep. The terminal stage is marked by wasting and emaciation. Death results from coma, intercurrent infection or cardiac failure.

The clinical features of Rhodesian disease are similar but briefer and more acute. The acuteness and severity of disease do not allow typical sleeping sickness. Death is due to cardiac failure within 6-9 months.

Pathology and Immunology

An exact pathogenesis of sleeping sickness is not known, although immune complexes and inflammation have been suspected to be the mechanism of damage to tissues. The immune response against the organism does help to eliminate the parasite but it is not protective, since the parasite has a unique ability of altering its antigens, the VSG. Consequently, there is a cyclic fluctuation in the number of parasites in blood and lymphatic fluids and each wave of parasite represents a different antigenic variant. The parasite causes polyclonal expansion of B lymphocytes and plasma cells and an increase in total IgM concentration. It stimulates the reticuloendothelial function. It also causes severe depression of cell mediated and humoral immunity to other antigens.

Diagnosis

Detection of parasite in the bloodstream, lymph secretions and enlarged lymph node aspirate provides a definitive diagnosis in early (acute) stages. The parasite in blood can be concentrated by centrifugation or by the use of anionic support media. Cerebrospinal fluid must always be examined for organisms. Immuno-serology (enzyme-linked immune assay, immunofluorescence) may be indicative but does not provide definite diagnosis.

Treatment and control

The blood stage of African trypanosomiasis can be treated with reasonable success with Pentamidine isethionate or Suramin. These drugs have been reported also to be effective in prophylaxis although they may mask early infection and thus increase the risk of CNS disease. Cases with CNS involvement should be treated with Melarsoprol, an organic arsenic compound.

The most effective means of prevention is to avoid contact with tsetse flies. Vector eradication is impractical due to the vast area involved. Immunization has not been effective due to antigenic variation.

American trypanosomiasis (Chagas' disease)

Etiology

Chagas' disease is caused by the protozoan hemoflagellate, *Trypanosoma cruzi*.

Epidemiology

American trypanosomiasis, also known as Chagas' disease, is scattered irregularly in Central and South America, stretching from parts of Mexico to Argentina. Rare cases have been reported in Texas, California and Maryland. It is estimated that 16-18 million people are infected by the parasite and 50 million are at risk. About 50,000 people die each year from the disease

Morphology

Depending on its host environment, the organism occurs in three different forms. The trypanosomal (trypomastigote) form, found in mammalian blood, is 15 to 20 microns long and morphologically similar to African trypanosomes. The crithidial (epimastigote) form is found in the insect intestine. The leishmanial (amastigote) form, found intracellularly or in pseudocysts in mammalian viscera (particularly in myocardium and brain), is round or oval in shape, measures 2-4 microns and lacks a prominent flagellum.

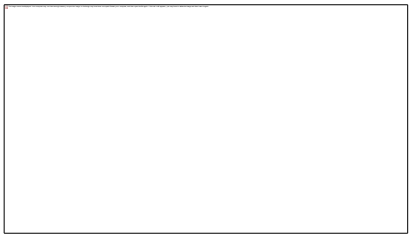

Fig.2.1.4*: Trypanosoma cruzi,* from experimentally infected mice.

Figure 2.1.5: *Trypanosoma cruzi*. As seen under the microscope X10

Life cycle

The organism is transmitted to mammalian host by many species of kissing (riduvid) bug, most prominently by *Triatoma infestans*, *T. sordida*, *Panstrongylus megistus* and *Rhodnius prolixus*. Transmission takes place during the feeding of the bug which normally bites in the facial area (hence the name, kissing bug) and has the habit of defecating during feeding. The metacyclic trypamastigotes, contained in the fecal material, gain access to the mammalian tissue through the wound which is often rubbed by the individual that is bitten. Subsequently, they enter various cells, including macrophages, where they differentiate into amastigotes and multiply by binary fission. The amastigotes differentiate into non-replicating trypomastigotes and the cells rupture to release them into the bloodstream.

Additional host cells, of a variety of types, can become infected and the trypomastigotes once again form amastigotes inside these cells.

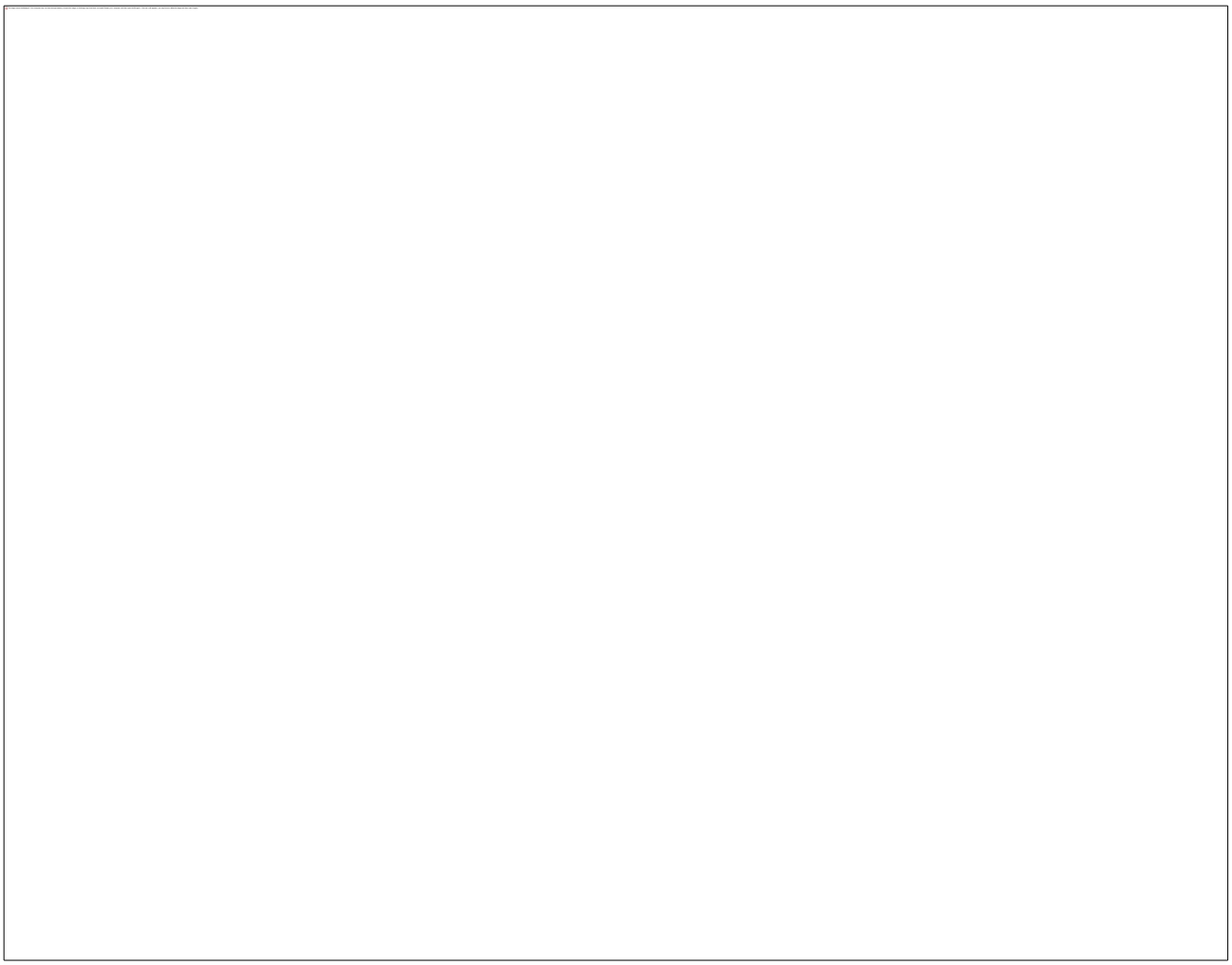

Fig.2.1.6: Life cycle of *Trypanosoma cruzi*. In man and in the triatomid bug *Triatoma infestans.*

Other arthropods, bed bugs, ticks and keds can also act as vectors

Uninfected insect vectors acquire the organism when they feed on infected animals or people containing trypomastigotes circulating in their blood. Inside the alimentary tract of the insect vector, the trypomastigotes differentiate to form epimastigotes and divide longitudinally in the mid and hindgut of the insect where they develop into infective metacyclic trypomastigotes. Transmission may also occur from man to man by blood transfusion and by the transplacental route.

More than one hundred mammalian species of wild and domestic animals including cattle, pigs, cats, dogs, rats, armadillo, raccoon and opossum are naturally infected by *T. cruzi* and serve as a reservoir.

Symptoms

Chagas' disease can be divided into three stages: the primary lesion, the acute stage, and the chronic stage. The primary lesion, chagoma, appearing at the site of infection, within a few hours of a bite, consists of a slightly raised, flat non-purulent erythematous plaque surrounded by a variable area of hard edema. It is usually found on the face, eyelids, cheek, lips or the conjunctiva, but may occur on the abdomen or limbs. When the primary chagoma is on the face, there is an enlargement of the pre- and post- auricular and the submaxillary glands on the side of the bite. Infection in the eyelid, resulting in a unilateral conjunctivitis and orbital edema (Ramana's sign), is the commonest finding.

Acute Stage: The acute stage appears 7-14 days after infection. It is characterized by restlessness, sleeplessness, malaise, increasing exhaustion, chills, fever and bone and muscle pains. Other manifestations of the acute phase are cervical, axillary and iliac adenitis, hepatomegaly, erythematous rash and acute myocarditis. There is a general edematous reaction associated with lymphadenopathy. Diffuse myocarditis, sometimes accompanied by serious pericarditis and endocarditis, is very frequent during the initial stage of the disease. In children, Chagas' disease may cause meningo-encephalitis and coma. Death occurs in 5-10 percent of infants. Hematologic examination reveals lymphocytosis and parasitemia.

Chronic Stage: The acute stage is usually not recognized and often resolves with little or no immediate damage and the infected host remains an asymptomatic carrier. An unknown proportion (guessed at 10-20%) of victims develop a chronic disease. They alternate between asymptomatic remission periods and relapses characterized by symptoms seen in the acute phase. Cardiac arrhythmia is common. The chronic disease results in an abnormal function of the hollow organs, particularly the heart, esophagus and colon. The cardiac changes include myocardial insufficiency, cardiomegaly, disturbances of atrio-ventricular conduction and the Adams-Stoke syndrome. Disturbances of peristalsis lead to megaesophagus and megacolon.

Pathology and Immunology

The pathological effects of acute phase Chagas' disease largely result from direct damage to infected cells. In later stages, the destruction of the autonomic nerve ganglions may be of significance. Immune mechanisms, both cell mediated and humoral, involving reaction to the organism and to autologous tissues have been implicated in pathogenesis.

T. cruzi stimulates both humoral and cell mediated immune responses. Antibody has been shown to lyze the organism, but rarely causes eradication of the organism, perhaps due to its intracellular localization. Cell mediated immunity may be of significant value. While normal macrophages are targeted by the organism for growth, activated macrophages can kill the organism. Unlike *T. brucei*, *T. cruzi* does not alter its antigenic coat. Antibodies directed against heart and muscle cells have also been detected in infected patients leading to the supposition that there is an element of autoimmune reaction in the pathogenesis of Chagas' disease. The infection causes severe depression of both cell mediated and humoral immune responses. Immunosuppression may be due to induction of suppressor T-cells and/or overstimulation of macrophages.

Diagnosis

Clinical diagnosis is usually easy among children in endemic areas. Cardiac dilation, megacolon and megaesophagus in individuals from endemic areas indicate present or former infection. Definitive diagnosis requires the demonstration of trypanosomes by microscopy or biological tests (in the insect or mice). Antibodies are often detectable by complement fixation or immunofluorescence and provide presumptive diagnosis.

Treatment and Control

There is no curative therapy available. Most drugs are either ineffective or highly toxic. Recently two experimental drugs, Benznidazol and Nifurtimox have been used with promising results in the acute stage of the disease, however their side effects limit their prolonged use in chronic cases.

Control measures are limited to those that reduce contact between the vectors and man. Attempts to develop a vaccine have not been very successful, although they may be feasible.

Unit 2: LEISHMANIA

Leishmania are ovoid organisms within the macrophage and possess a rod-shaped kinetoplast associated with a rudimentary flagellum, which, however, does not extend beyond the cell margin. The parasites are found in the amastigote stage in cells of the vertebrate host and in the promastigote stage in the intestine of the sandfly,

In the vertebrate host Leishmania is found in the macrophages and other cells of the reticuloendothelium system in the skin, spleen, liver, bone marrow, lymph nodes and mucosa. It may also be found in leucocytes in the blood.

This leishmanial, or amastigote form, after ingestion by a sandfly, transforms into a promastigote form in the insect gut in which the kinetoplast is situated at the posterior end of the body. These divide repeatedly by binary fission, migrate to the proboscis, and when the insect subsequently feeds, are inoculated into a new host. Once within a macrophage the pro"mastigote reverts to the amastigote form and again starts to divide.

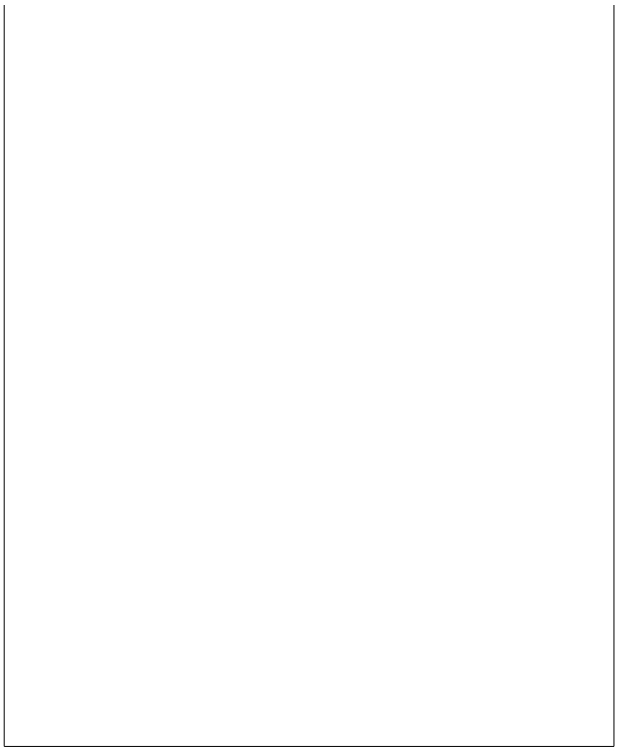

Fig. 2.2.1.*Leishmania.*(a) Promastigote form. (b) Amastigote form

Leishmania occur primarily in mammals, although ten species have been described in Old World lizards. They cause disease in man, dogs and various rodents. Leishmania have a heteroxenous life cycle, are transsmitted by sandflies of the genus Phlebotomus in the Old World and Lutzomyia in the New World.

Hypopylaria are primitive species found in old world lizards, which become infected following ingesstion of sandflies. Development occurs in the sandfly hindgut.

Peripylaria develop in both the hindgut and foregut of sandflies and infect both lizards and mammals. Transmission in mammals is by bite of sandflies.

Figure2.2.2 : Picture of a sand fly biting a human arm.

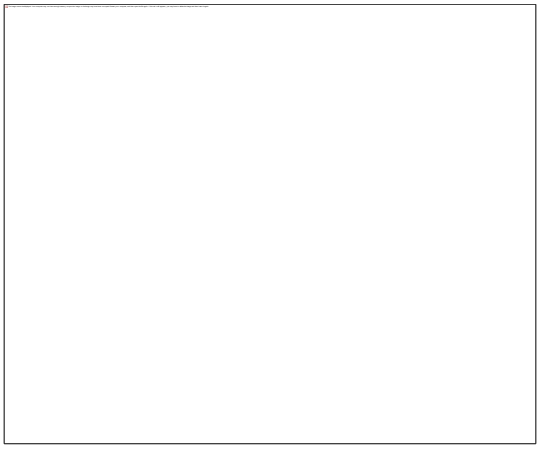

Figure 2.2.3: metacyclic promastigotes of Leishmania parasite

Epidemiology

Leishmaniasis is prevalent world wide: ranging from south east Asia, Indo-Pakistan, Mediterranean, north and central Africa, and south and central America.

Morphology

In man, the parasites occur in the amastigote form only, but in the insect vector the promastigote form is assumed. The amastigote forms are small, ovoid or round bodies (often called Leishman-Donovan bodies) about 2-5µm in diameter by 1 – 3 µm whereas the leptomonad measures 14 - 20 microns by 1.5 - 4 microns, a similar size to trypanosomes. Under light microscopy, the cells are seen to contain a central nucleus, a rod-shaped kinetoplast and (sometimes) a basal body (from which the flagellum arises.

Life cycle

The organism is transmitted by the bite of several species of blood-feeding sand flies (*Phlebotomus*) which carry the promastigote in the anterior gut and pharynx. The parasites gain access to mononuclear phagocytes where they transform into amastigotes and divide until the infected cell ruptures. The released organisms infect other cells. The sandfly acquires the organisms during the blood meal; the amastigotes transform into flagellate promastigotes and multiply in the gut until the anterior gut and pharynx are packed. Dogs and rodents are common reservoirs figure 2.2.4.

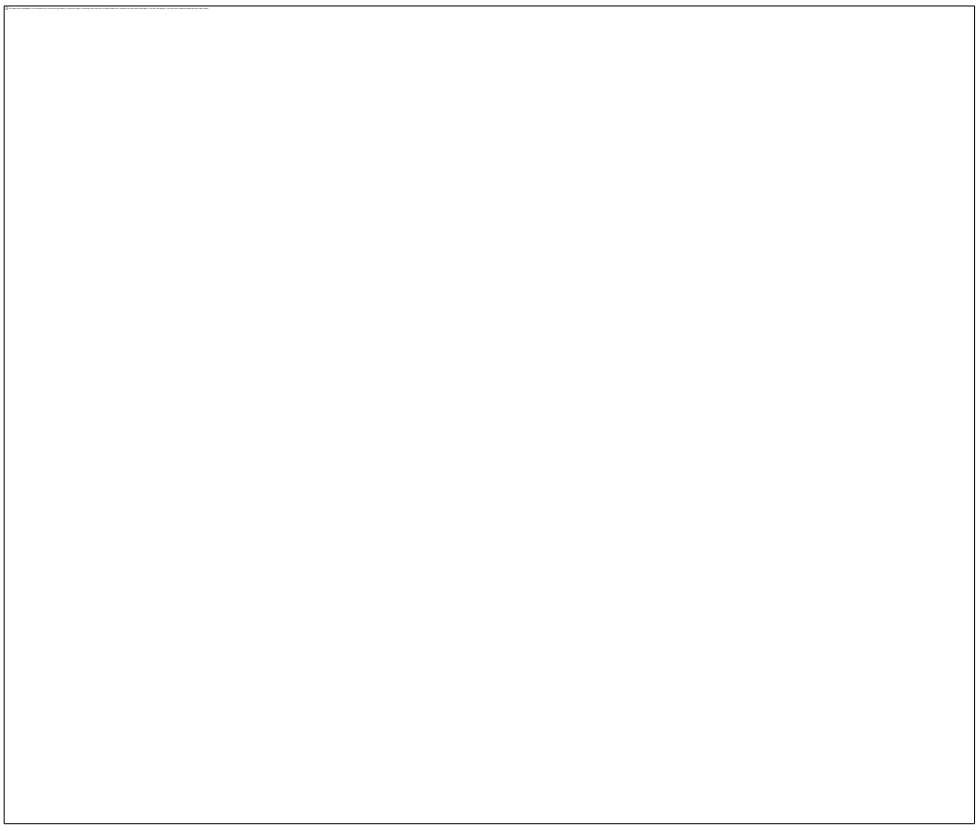

Fig. 2.2.4: Life cycle of *Leishmania donovani* and *L tropica* in man and vector, *Phlebotomus argentipes*

Leishmaniasis is the disease caused by Leishmania

Etiology

Several species of Leishmania are pathogenic for man: *L. donovani* causes visceral leishmaniasis (Kala-azar, black disease, dumdum fever); *L. tropica* (*L. t. major, L. t. minor* and *L. ethiopica*) cause cutaneous leishmaniasis (oriental sore, Delhi ulcer, Aleppo, Delhi or Baghdad boil); and *L. braziliensis* (also, *L. mexicana* and *L. peruviana*) are etiologic agents of mucocutaneous leishmaniasis (espundia, Uta, chiclero ulcer).

Symptoms

Visceral leishmaniasis (kala-azar, dumdum fever):*L. donovani* organisms in visceral leishmaniasis are rapidly eliminated from the site of infection, hence there is rarely a local lesion, although minute papules have been described in children. They are localized and multiply in the mononuclear phagocytic cells of spleen, liver, lymph nodes, bone marrow, intestinal mucosa and other organs. One to four months after infection, there is occurrence of fever, with a daily rise to 102-104 degrees F, accompanied by chills and sweating. The spleen and liver progressively become enlarged. With progression of the diseases, skin develops hyperpigmented granulomatous areas (kala-azar means black disease). Chronic disease renders patients susceptible to other infections. Untreated disease results in death.

Cutaneous leishmaniasis (Oriental sore, Delhi ulcer, Baghdad boil): In cutaneous leishmaniasis, the organism (*L. tropica*) multiplies locally, producing of a papule, 1-2 weeks (or as long as 1-2 months) after the bite. The papule gradually grows to form a relatively painless ulcer. The center of the ulcer encrusts while satellite papules develop at the periphery. The ulcer heals in 2-10 months, even if untreated but leaves a disfiguring scar figure 2.2.5. The disease may disseminate in the case of depressed immune function.

Fig.2.2.5 : Cutenous Leishmaniasis

Mucocutaneous leishmaniasis (espundia, Uta, chiclero): The initial symptoms of mucocutaneous leishmaniasis are the same as those of cutaneous leishmaniasis, except that in this disease the organism can metastasize and the lesions spread to mucoid (oral, pharyngeal and nasal) tissues and lead to their destruction and hence sever deformity figure 2.2.6. The organisms responsible are *L. braziliensis*, *L. mexicana* and *L. peruviana*.

Fig.2.2.6 :mucocutenous Leishmaniasis

Pathology

Pathogenesis of leishmaniasis is due to an immune reaction to the organism, particularly cell mediated immunity. Laboratory examination reveals a marked leukopenia with relative monocytosis and lymphocytosis, anemia and thrombocytopenia. IgM and IgG levels are extremely elevated due to both specific antibodies and polyclonal activation.

Diagnosis

Diagnosis is based on a history of exposure to sandfies, symptoms and isolation of the organisms from the lesion aspirate or biopsy, by direct examination or culture. A skin test (delayed hypersensitivity: Montenegro test) and detection of anti-leishmanial antibodies by immuno-fluorescence are indicative of exposure.

Treatment and Control

Sodium stibogluconate (Pentostam) is the drug of choice. Pentamidine isethionate is used as an alternative. Control measures involve vector control and avoidance. Immunization has not been effective.

Unit 3: PLASMODIUM

Parasites of the genus Plasmodium are responsible for the disease 'malaria' in both animals and man. Although the species attacking man have been most extensively studied, considerable use has been made of species in laboratory animals, especially *P. knowlesi* and *P. cynomolgi* in monkeys, *P. relictum*, *P. cathemerium* and *P. gallinaceum* in birds, and *P. berghei*, *P. yoelii* and *P. chabaudi* in rodents. Species also occur in other mammmals such as squirrels and bats and in amphibians and reptiles, but these have not been much studied. Although it has been known for many years that some species of monkey malaria (e.g. *P. cynomolgi*) are transsmissible to man, it is only comparatively recently that it has been found that human malaria can be transmitted to several species of monkeys. The Colombian night monkey, *Aotus trivirgatus*, has proved to be an especially valuable laboratory model for malarial research.

The genus Plasmodium contains the 'true' malarial parasites (in contrast to the haemoproteids); the recognition that profound differences exist between the various species has led to the further subdivision into seven subgenera, which are as follows:

Plasmodium: Parasitic in primates, with exo-erythrocytic schizogony in parenchymal cells of liver; erythrocytic schizonts large; gametocytes round.

Vinckeia: Parasitic in non-primate mammals with exo-erythrocytic schizogony in parenchymal cells of liver; erythrocytic schizonts usually small; gametocytes round.

Laverania: Parasitic in primates, with exo-erythrocytic schizogony in parenchymal cells of liver; gametocytes crescentic with perinuclear pigment.

Haemamoeba: Parasitic in birds; exo-erythrocytic schizogony in reticulo-endothelial cells; erythrocytic schizonts large; gametocytes round.

Giovannolaia: Parasitic in birds, exo-erythrocytic schizogony in reticulo-endothelial cells; erythrocytic schizonts large; gametocytes elongate.

Novyella: Parasitic in birds; exo-erythrocytic schizoogony in reticulo-endothelial cells; erythrocytic schizonts small; gametocytes elongate.

Huffia: Parasitic in birds; exo-erythrocytic schizonts in the haemopoietic (i.e. blood-forming) system; gametoocytes elongate.

Using the subgeneric designations, the correct zoologiical nomenclature for the four species infecting man is *Plasmodium (Plasmodium) vivax, P. (P.) ovale, P. (P.) malariae* and *P. (Laverania) falciparum*. However, as is common practice in the literature, the subgeneric name, have been omitted in this write up.

Epidemiology

There are an estimated 200 million global cases of malaria leading a mortality of more than one million people per year. *P. falciparum* (malignant tertian malaria) and *P. malariae* (quartan malaria) are the most common species of malarial parasite and are found in Asia and Africa. *P. vivax* (benign tertian malaria) predominates in Latin America, India and Pakistan, whereas, *P. ovale* (ovale tertian malaria) is almost exclusively found in Africa.

Morphology

Malarial parasite trophozoites are generally ring shaped, 1-2 microns in size, although other forms (ameboid and band) may also exist. The sexual forms of the parasite (gametocytes) are much larger and 7-14 microns in size. *P. falciparum* is the largest and is banana shaped while others are smaller and round. *P. vivax* causes stippling of infected red cells.

Life cycle

Malarial parasites are transmitted by the infected female anopheline mosquito which injects sporozoites present in the saliva of the insect. Sporozoites infect the liver parenchymal cells where they may remain dormant (hypnozoites) or undergo stages of schizogony to produce schizonts and merogony to produce merozoites (meronts). When parenchymal cells rupture, thousands of meronts are released into blood and infect the red cells. *P. ovale* and *P. vivax* infect immature red blood cells whereas *P. malariae* infects mature red cells. *P. falciparum* infects both. In red cells, the parasites mature into trophozoites. These trophozoites undergo schizogony and merogony in red cells which ultimately burst and release daughter merozoites. Some of the merozoites transform into male and female gametocytes while others enter red cells to continue the erythrocytic cycle. The gametocytes are ingested by the female mosquito, the female gametocyte transforms into ookinete, is fertilized, and forms an oocyst in the gut. The oocyte produces sporozoites (sporogony) which migrate to the salivary gland and are ready to infect another host. The liver (extraerythrocytic) cycle takes 5-15 days whereas the erythrocytic cycle takes 48 hours or 72 hours (*P. malariae*). Malaria can be transmitted by transfusion and transplacentally figure 2.3.1.

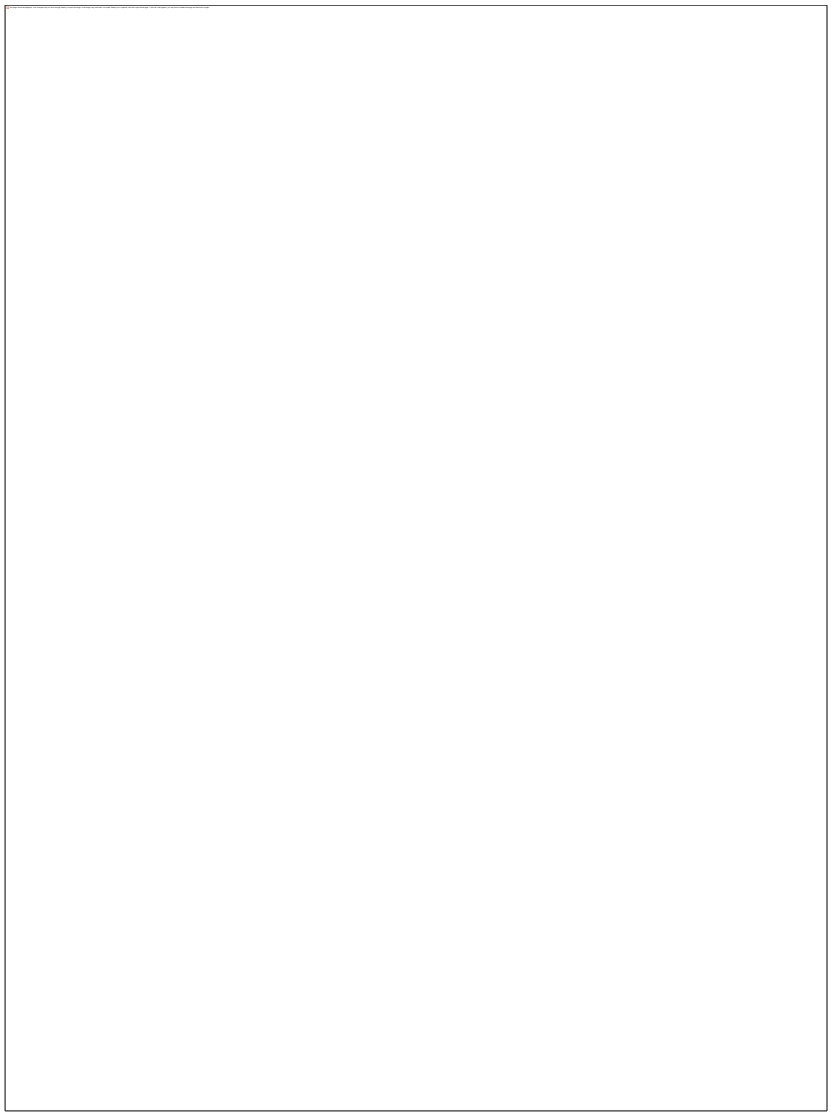

Figure 2.3.1: life cycle of *Plasmodium parasite*

Malaria is the disease caused by *Plasmodim* parasite.

Human malaria :It is not always realised that malaria is one of the world's greatest killers. It remains endemic in some 102 countries with more than half the world's population at risk. There are probably more than 100 million cases of the disease throughout the world, of which perhaps a million are fatal. In spite of control programmes in many countries, the situation has shown little improvement partly due to world economic and/or political problems, there have been downtrends in some countries, notably India and China. The problem has grown worse in some rural areas which have undergone intensive economic development, especially parts of Asia and in the Amazon region of Latin America. It should also be emphasised that non-endemic countries

are not immune to infection as cases of imported malaria were being reported. A major research effort has been directed towards developing a vaccine against the disease, In spite early optimism, success in this has so far eluded workers and remains a major unachieved (and perhaps unachievable) goal.

Etiology

Four *Plasmodium* species are responsible for human malaria These are *P. falciparum, P. vivax, P. ovale* and *P. malariae*.

There are four species of Plasmodium that infect man and result in four kinds of malarial fever.

P. vivax: benign, simple or tertian malaria.

P. falciparum: aestivo-autumnal, malignant tertian, pernicious quotidian, subtertian or tropical malaria. *P. malariae*: quartan ague, or quartan malaria.
P. ovale: ovale tertian malaria.

Of the above species, *P. vivax* shows the widest distribution, being prevalent throughout the tropics and many temperate regions. Vivax malaria is characterised by relapses: reappearances of symptoms after a latent of about up to 5 years, as is infection with P. ovale, which occurs chiefly in tropical Africa. Such relapses are due to the sudden activation of hypnozoites (sleeping merozoites) in liver cells. *P. falciparum* is most common in tropical and subtropical areas and causes the most dangerous, malignant form of malaria, which fortunately does not have relapses *P. malariae* is widely distributed but is much less common than *P. vivax* or *P. falciparum*. Although falciparum malaria or malariae malaria do not show relapses, they are subjected to 'recrudescences': repeated manifestations of infection after a relatively short latent period between 3 months and 1 year.
Human malaria can develop in certain monkey species. The pattern of the life cycles of these species follow the general life cycle already given (Fig.2.3.1) but there are important physiological differences which are often reflected in the nature of the diseases produced.

Many of these characteristics are sufficiently definite to be used as criteria for identification in blood films but species differentiation may not be possible in the earliest stages. In *P. vivax* and *P. ovale* infections, erythrocytes become enlarged and paler in colour when the parasites have grown beyond the ring stage. In stained preparations, infected corpuscles show characteristic dots called Schuffner's dots (*P. vivax* and *P. ovale*) Ziemann's dots (*P. malariae*) or Maurer's dot (*P. falciparum*).The pigment granules vary in size and shape but their appearance and colour depend on number of factors such as intensity of light, type of filter used, etc.

Characteristics of species

P. falciparum. The most important malarial parasite, the disease it produces runs an acute course and often terminating fatally. It is a significant cause of aborrtion or stillbirth and even death of non-immune pregnant women. It is responsible for some 50 per cent of all the malarial cases throughout the world. Its distribution is restricted to warm and tropical countries.

The special features of its life cycle may be summarised as follows:

(a) It attacks erythrocytes of all ages indiscriminately so that a high density of parasites may be rapidly reached. In extreme cases up to 48 per cent of the red cells may be parasitised.

(b) Multiple infections (polyparasitism) resulting in several ring forms in a corpuscle are not uncommon

(c) The later stages in the asexual cycle, that is, the growth to schizonts, do not occur in the peripheral blood as in other forms of malaria, except in severe cases, so that only rings and crescents are found in blood films. After twenty-four hours, the ring forms and older trophozoites show a tendency to clump together and adhere to the visceral capillary wall and become caught up in the vessels of the heart, intestine, brain or bone marrow in which the later asexual stages are completed. This behaviour, together with the fact that the subtertian malaria is more toxic, are the principal reasons why this type is so dangerous.

(d) Sporulation is not so well synchronised as in other species so that fever paroxysms may be longer drawn out.

(e) EE forms do not persist in the tissues and hence relapses do not occur.

P. vivax.Causes the benign tertian form of malaria which is responsible for about 43 percent of all cases in the world and has the widest geographical distribution. Although generally not life-threatening, it can cause severe, acute illness. Several points in its life cycle may be noted:

(a) The degree of infection is low, for only the young immature corpuscles (reticulocytes) are attacked; about 2 per cent of erythrocytes are parasitised.

(b) The periodicity of the asexual cycle is closely synchronised.

(c) Hypnozoites develop in the liver, so that relapses may occur (Fig. 2.3.1 above).

The morphological features of a sub-species *bastianellii* of the simian species *P. cynomolgi* bear a striking resemblance to those of *P. vivax*; differentiation between these two is diffficult.

P. malariae.A relatively rare parasite producing quartan malaria which is responsible for about seven percent of the malaria in the world. Particular points of interest are:

(a) Infected erythrocytes are not larger than uninfected ones and sometimes even smaller.

(b) Mature erythrocytes are attacked and rarely reticulocytes, so that the density of parasites is very low; about 0.2 per cent of erythrocytes are parasitised.

(c) It is often difficult to distinguish between a large trophozoite and an immature gametocyte.

P. ovale. This is a species rarely encountered; it is confined essentially to the tropics and subtropics although reported from many continents. The type of fever it produces (ovale tertian) is milder than the benign tertian of *P. vivax*. Special points of interest are:

(a) It morphologically resembles *P. malariae* in most of its stages.

(b) The changes produced in the erythrocytes in general are similar to those produced by *P. vivax*, but Shuffer's dots appear considerably earlier in the ring stage.

(c) In the oocyst the pigment granules are (usually) characteristically arranged in two rows crossing each other at right angles.

(d) Hypnozoites develp in the liver so that relapses may occur.

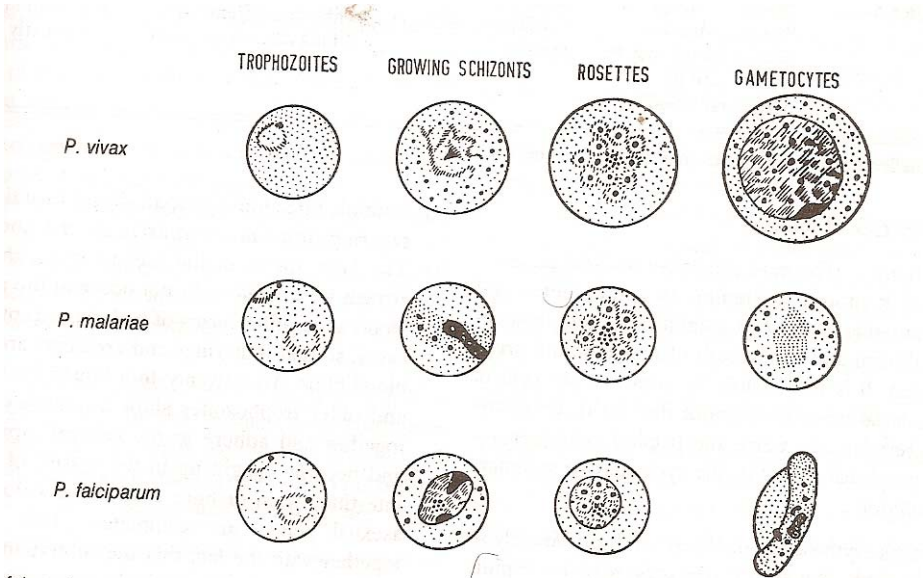

Fig. 2.3.2: Comparism of various stages of the three common species of Plasmodium infecting man

Vectors of human malaria.

As far as is known, the plasmodia of man will develop only in female mosquitoes of the genus *Anopheles* and not in any other arthropod. Almost any species can be infected with plasmodia in the laboratory, but many species are poor vectors and not natural ones. For a mosquito to be efficient vector, it must present certain characteristics: (a) it must be susceptible to infection and present physico-chemical and nutritional characteristics suitable for the development of plasmodia; (b) it must bite man in preference to animals; (c) it must not be shy of human habitation; (d) its span of life must be sufficiently long to permit sexual development of the plasmodium.

Symptoms

The symptomatology of malaria depends on the parasitemia, the presence of the organism in different organs and the parasite burden. The incubation period varies generally between 10-30 days. As the parasite load becomes significant, the patient develops headache, lassitude, vague pains in the bones and joints, chilly sensations and fever. As the disease progresses, the chills and fever become more prominent. The chill and fever follow a cyclic pattern (paroxysm) with the symptomatic period lasting 8-12 hours. In between the symptomatic periods, there is a period of relative normalcy, the duration of which depends upon the species of the infecting parasite. This interval is about 34-36 hours in the case of *P. vivax* and *P. ovale* (tertian malaria), and 58-60 hours in the case of *P. malariae* (quartan malaria). Classical tertian paroxysm is rarely seen in *P. falciparum* and persistent spiking or a daily paroxysm is more usual.

The malarial paroxysm is most dramatic and frightening. It begins with a chilly sensation that progresses to teeth chattering, overtly shaking chill and peripheral vasoconstriction resulting in cyanotic lips and nails (cold stage). This lasts for about an hour. At the end of this period, the body temperature begins to climb and reaches 103-106 degrees F (39- 41degrees C). Fever is associated with severe headache, nausea (vomiting) and convulsions. The patient experiences euphoria, and profuse perspiration and the temperature begins to drop. Within a few hours the patient feels exhausted but symptom-less and remains symptomatic until the next paroxysm. Each paroxysm is due to the rupture of infected erythrocytes and release of parasites.

Without treatment, all species of human malaria may ultimately result in spontaneous cure except with *P. falciparum* which becomes more severe progressively and results in death. This organism causes sequestration of capillary vasculature in the brain, gastrointestinal and renal tissues. Chronic malaria results in splenomegaly, hepatomegaly and nephritic syndromes.

Pathology and immunology

Symptoms of malaria are due to the release of massive number of merozoites into the circulation. Infection results in the production of antibodies which are effective in containing the parasite load. These antibodies are against merozoites and schizonts. The infection also results in the activation of the reticuloendothelial system (phagocytes). The activated macrophages help in the destruction of infected (modified) erythrocytes and antibody-coated merozoites. Cell mediated immunity also may develop and help in the elimination of infected erythrocytes. Malarial infection is associated with immunosuppression.

Diagnosis

Diagnosis is based on symptoms and detection of parasite in Giemsa stained blood smears. There are also antibody tests.

Treatment and Control

Treatment is effective with various quinine derivatives (quinine sulphate, chloroquine, meflaquine and primaquine, etc.). Drug resistance, particularly in *P. falciparum* and to some extent in *P. vivax* is a major problem. Control measures are eradication of infected anopheline mosquitos. Vaccines are being developed and tried but none is available yet for routine use.

Unit 4: BABESIA

Morphology

The trophozoite is very similar to the ring form of the *Plasmodium* species.

Life cycle

The organism (sporozoite) is transmitted by a tick and enters the red cell where it undergoes mitosis and the organisms (merozoite) are released to infect other red cells. Ticks acquire the organism during feeding on an infected individual. In the tick, the organism divides sexually in the gut and migrates into the salivary gland.

Babesiosis is the disease caused by Babesia parasite.

Etiology

Babesia microti is the only member of the genus that infects man.

Symptoms
Babesiosis is associated with hemolytic anemia, jaundice, fever and hepatomegaly, usually 1-2 weeks after infection.

Diagnosis
Diagnosis is based on symptoms, patient history and detection of intraerythrocytic parasite in the patient or transfer of blood in normal hamsters which can be heavily parasitized.

Treatment and control

Drugs of choice are clindamycin combined with quinine. The patient may recover spontaneously. One should avoid tick exposure and, if bitten, remove the tick from the skin immediately.

Unit 5: TOXOPLASMA

This remarkable species was known as a potential parasite of man for many years but its true nature as a coccidian was only discovered not too long ago. It was known to occur in all warm-blooded animals - mammals and birds - but its nature and means of transmission remained a mystery.

The two stages known were the trophozoite and the cyst, also known as a pseudocyst (see below). The trophozoites (figure 2.5.1) were intracellular parasites which could invade almost any nucleated cell within the host.

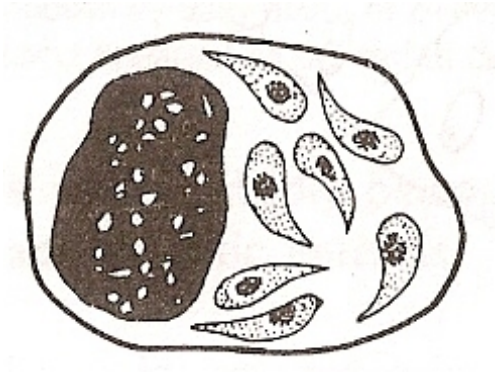

Fig.2.5.1 :*Toxoplasma gondii*, trophozoites in macrophage

The cysts had well-formed walls and contained organisms which multiplied within the cysts, as many as 1000-3000 organisms being found within a single cyst. The trophozoite and the cystic form appeared to be almost identical in morphology, but they exhibited a striking physiological difference in that the trophozoites were rapidly killed in acid pepsin whereas the cystic forms could survive 1 h of acid pepsin digestion. This physiological difference should have provided a clue to the life cycle as it suggested that there was a further development of an intestinal phase in the life cycle. This, in fact, proved to be the case when workers in a number of different countries, almost simultaneously, pieced together the puzzle and showed that *T gondii* developed a sexual stage in the intestine of a cat and was, in fact, a coccidian closely related to (but not identical to) an Isospora species. This discovery revolutionized this area of protozoology and opened the door to the solution of the taxonomy of several other

Epidemiology

Toxoplasma has worldwide distribution and 20%-75% of the population is seropositive without any symptomatic episode. However, the infection poses a serious threat in immunosuppressed individuals and pregnant females.

Morphology

The intracellular parasites (tachyzoite) are 3x6 microns, pear-shaped organisms that are enclosed in a parasite membrane to form a cyst measuring 10-100 microns in size. Cysts in cat feces (oocysts) are 10-13 microns in diameter.

Life cycle

The natural life cycle of *T. gondii* occurs in cats and small rodents, although the parasite can grow in the organs (brain, eye, skeletal muscle, etc.) of any mammal or birds. Cats gets infected by ingestion of cysts in flesh. Decystation occurs in the small intestine, and the organisms penetrate the submucosal epithelial cells where they undergo several generations of mitosis, finally resulting in the development of micro- (male) and macro- (female) gametocytes. Fertilized macro-gametocytes develop into oocysts that are discharged into the gut lumen and excreted. Oocysts sporulate in the warm environment and are infectious to a variety of animals including rodents and man. Sporozoites released from the oocyst in the small intestine penetrate the intestinal mucosa and find their way into macrophages where they divide very rapidly (hence the name tachyzoites) and form a cyst which may occupy the whole cell. The infected cells ultimately burst and release the tachyzoites to enter other cells, including muscle and nerve cells, where they are protected from the host immune system and multiply slowly (bradyzoites). These cysts are infectious to carnivores (including man). Unless man is eaten by a cat, it is a dead-end host figure 2.5.2.

Fig.2.5.2: Life cycle of *Toxoplasma gondii*

Toxoplasmosis is the disease caused by Toxoplasma parasite.

Etiology

Toxoplasma gondii is the organism responsible for toxoplasmosis

Symptoms

Although Toxoplasma infection is common, it rarely produces symptoms in normal individuals. Its serious consequences are limited to pregnant women and immunodeficient hosts. Congenital infections occur in about 1-5 per 1000 pregnancies of which 5-10% result in miscarriage and 8-10% result in serious brain and eye damage to the fetus. 10-13% of the babies will have visual handicaps. Although 58-70% of infected women will give birth to a normal offspring, a small proportion of babies will develop active retino-chorditis or mental retardation in childhood or young adulthood. In immunocompetent adults, toxoplasmosis, may produce flu-like symptoms, sometimes associated with lymphadenopathy. In immunocompromised individuals, infection results in generalized parasitemia involvement of brain, liver lung and other organs, and often death.

Immunology

Both humoral and cell mediated immune responses are stimulated in normal individuals. Cell-mediated immunity is protective and humoral response is of diagnostic value.

Diagnosis

Suspected toxoplasmosis can be confirmed by isolation of the organism from tonsil or lymph gland biopsy.

Treatment

Acute infections benefit from pyrimethamine or sulphadiazine. Spiramycin is a successful alternative. Pregnant women are advised to avoid cat litter and to handle uncooked and undercooked meat carefully.

CONCLUSION

Biologically/medically significant blood and tissue protozoans includes members of the genera Trypanosoma (*T. brucei* and *T. cruzi*), Leishmania (*L. donovani*, *L. tropica* and *L. braziliensis*) Plasmodium (*P. falciparum*, *P. ovale*, *P. malariae* and *P. vivax*), *Toxoplasma gondii*; and Babesia (*B. microti*).They cause different disease conditions in their hosts such as sleeping sickness, malaria, leishmaniasis etc. They can be found in different parts of the world including Africa.

Tutor- marked assignment (TMA)

1. List the six major blood protozoans of biological/medical importance and explain one
2. Give the life cycle of plasmodium parasite.
3. Write short notes on: (i) African sleeping sickness. (ii) Leishmania.

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MODULE 2: INTESTINAL AND UROGENITAL PROTOZOA

Introduction

Intestinal and luminal protozoa significant to human health include

Entamoeba histolytica (Amebae)

Balantidium coli (Ciliates)

Giardia lamblia and *Trichomonas vaginalis* (Flagellates)

Cryptosporidium parvum and *Isospora belli* (Sporozoa)

3.1 Objectives

At the end of this lecture nit, students should:

-be acquinted with genera and species of protozoans that constitutes intestinal and urogenital protozoans.

-Know their epidemiology, morphological characteristics and life cycle.

Unit 1: AMEBAE

Species of Entamoeba have been found in a number of invertebrate and vertebrate hosts. Five species of the genus Entamoeba infect man: *E. histolytica*, a species which is sometimes pathogenic in the caecum and colon; *E. hartmanni*, a harmless species closely related to *E. histolytica* and not regarded as a separate species by some workers; *E. coli*, a harmless species; *E. gingivalis*, in the mouth; and *E. polecki*, a species rare in man and probably normally occurring in pigs. The relationship between these species is complicated by the discovery of numerous 'strains' of some species.

Infections of *Entamoeba histolytica* commonly result in amoebiasis, the term 'histolytica' literally meaning 'tissue-dissolving', referring to the potential carnivorous habits of this organism. The qualification potentially is stressed, for a high percentage of individuals infected with

entamoebae show no symptoms of disease. Where clinical symptoms result, the disease is referred to as invasive amoebiasis; the non-invasive infection is sometimes called lumenal amoebiasis.

In the developing world, amoebiasis causes some 450 million infections per annum, about 50 million incidents and about 100000 deaths. Invasive amoebiasis is prevalent in certain areas of the world including West and South East Africa, China, the whole of SE Asia, Mexico and the western portion of South America, and the Indian subcontinent. Possibly only about 10 per cent of infections result in invasive amoebiasis; the remainder of those infected appear to remain asymptomatic carriers who pass cysts in their stools.

Epidemiology

0.5 to 50% of the population world wide harbors *E. histolytica* parasites with the higher rates of infection being in underdeveloped countries. 1 to 3% of the population of the USA are infected. Infection is associated with poor hygiene. Humans are the principal host, although dogs, cats and rodents may be infected.

Morphology

Trophozoite: This form has an ameboid appearance and is usually 15-30 micrometers in diameter, although more invasive strains tend to be larger. The organism has a single nucleus with a distinctive small central karyosome. The fine granular endoplasm may contain ingested erythrocytes. The nuclear chromatin is evenly distributed along the periphery of the nucleus figure 3.3.1

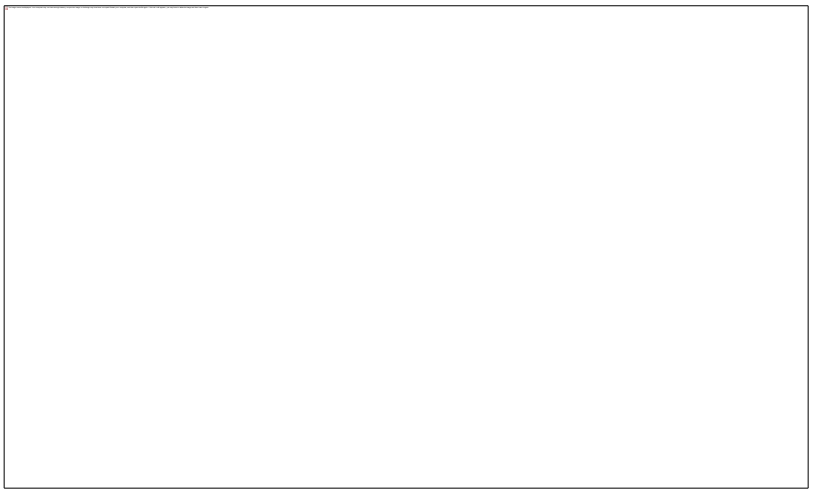

Fig. 3.3.1: Trophozoite *E. histolytica*

Cyst:*Entameba histolytica* cysts are spherical, with a refractile wall; the cytoplasm contains dark staining chromatoidal bodies and 1 to 4 nuclei with a central karyosome and evenly distributed peripheral chromatin.

Lifecycle

Infection occurs by ingestion of cysts on fecally contaminated food or hands. The cyst is resistant to the gastric environment and passes into small intestine where it decysts. The metacyst divides into four and then eight amoebae which move to the large intestine. The majority of the organisms are passed out of the body with the feces but, with larger bolus of infection, some amebae attach to and invade the mucosal tissue forming "flask-shaped" lesions (bomb craters). The organisms encyst for mitosis and are passed through with feces. There are no intermediate or reservoir hosts figure 3.3.2.

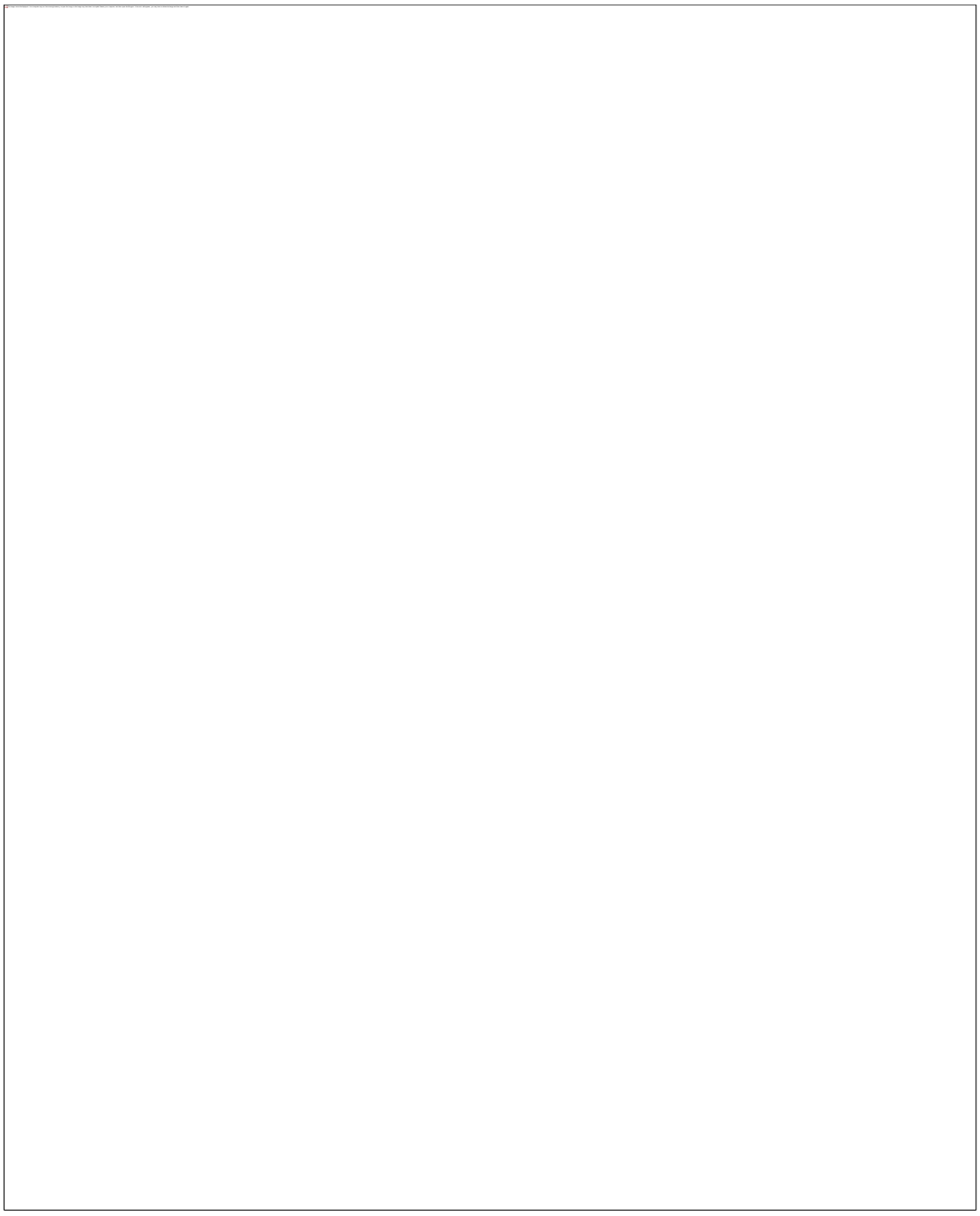

Figure 3.3.2: life cycle of *E. histolytica*

AMEBIASIS (amebic dysentery, amebic hepatitis) is the disease caused by Entamoeba parasite

Etiology

E. histolytica is the major cause of amebic dysentery.

Symptoms

Acute: Frequent dysentery with necrotic mucosa and abdominal pain.

Chronic: Recurrent episodes of dysentery with blood and mucus in the feces. There are intervening gastrointestinal disturbances and constipation. Cysts are found in the stool. The organism may invade the liver, lung and brain where it produces abscesses that result in liver dysfunction, pneumonitis, and encephalitis.

Pathology
Intestinal ulcers (craters/flasks) are due to enzymatic degradation of tissue. The infection may result in appendicitis, perforation, stricture granuloma, pseudo-polyps, liver abscess; sometimes brain, lung and spleen abscesses can also occur. Strictures and pseudo-polyps result from the host inflammatory response.

Immunology
There is an antibody response after invasive infection (liver abscess or colitis) but it is of questionable significance in immunity, as there is recurrence of enteric episodes in these patients.

Diagnosis
Symptoms, history and epidemiology are the keys to diagnosis. In the laboratory, the infection is confirmed by finding cysts in the stool. *E. histolytica* infection is distinguished from bacillary dysentery by the lack of high fever and absence PMN leukocytosis.

Distinction must be made from other non-pathogenic intestinal protozoa (*e.g., Entamoeba coli, Entamoeba hartmanni, Dientamoeba fragilis, Endolimax nana, Iodamoeba buetschlii, etc.*).

Treatment
Iodoquinol is used to treat asymptomatic infections and metronidazole is used for symptomatic and chronic amebiasis, including extra-intestinal disease.

Other species of Entamoeba:

1. *Entamoeba coli.*

This is a non-pathogenic species whose distribution is world-wide, occurring in some 30 per cent of the world's population. It is found in the large intestine and distinguished from *E. histolytica* by a number of features, chief of which are: (a) it has slower movement; (b) its pseudopodium is mainly coarser and not clear like that of *E. histolytica*; (c) its nucleus is coarser and with an eccentric karyosome (d) it has a larger number of food vacuoles.

The precystic stages are difficult to distinguish from those of *E. histolytica*, critical observation of the nucleus being necessary. The cysts, however, are easily differentiated from those of *E. histolytica* by having eight nuclei and the chromatoid bodies, which, when present, have splintered, but never rounded ends. *E. coli* is entirely a scavenger, feeding on bacteria and

detritus in the large intestine. It is not capable of eroding the intestinal mucosa, but, as an indiscriminate feeder, may phagocytose blood cells if these are available.

b) *Entamoeba gingivalis* .

A common parasite of the human mouth; related species occur in the mouths of dogs, horses and donkeys. The spaces between the teeth and the soft pits of the gums offer ideal surfaces for amoebae, because in these sites bacteria and detritus abound. *E. gingivalis* resembles *E. histolytica*; it has a crystal-clear ectoplasm and moves, actively. Its food vacuoles are usually numerous and contain bacteria, leucocytes and occasionally red cells. The pathogenicity of this species has long been a matter of dispute and has never been established.

c) *Entamoeba polecki*

This is a species which normally occurs in pigs. It forms uninucleate cysts and shows some morphological differences from the cyst of *E. histolytica*. It is generally considered to be a rare parasite of man but may be more common than supposed.

d) *Entamoeba moshkovskii*

This species is not an animal parasite, but it may conveniently be discussed with the other forms. It is a free-living species, which was first discovered in a sewage:-disposal plant in Moscow and has since been shown be of world-wide occurrence. A large number of strains have been identified. Morphologically it closely resembles *E. histolytica* throughout life cycle, including encystment and metacystic development, differing from it only in details. Yet all experiments to establish this species in rats or amphibians, animals which might act as hosts in sewage beds, have failed, and there seems no doubt that it is a e free-living form.

e) *Entamoeba invadens*

This species is increasingly being used as an experimental model for the study of amoebiasis. This is partly on account of its resemblance to *E. histolytica* and partly because it causes invasive amoebiasis in reptiles. It is probably relatively harmless in turtles, which may have been the original hosts,' but is highly pathogenic to snakes, which may die within two weeks

f) *Entamoeba ranarnm*

The morphology of this species is indistinguishable from that of *E. histolytica*. It is commoner in tadpoles than adult frogs.

Unit 2: GIARDIA

A flagellate of this genus is quite unlike any of the other species in shape or habits. It has been described in front view as looking like a 'tennis racquet without a handle' and it has a comical, face-like appearance. The body is a 'tear-drop shaped' with a convex dorsal surface and a concave ventral one. The latter possesses two depressions, sometimes termed *adhesive discs* (suckers) which make contact with the intestinal cells of the host. A single or double *median body*, unique to this genus, is found just below the adhesive discs. Giardia exhibits perfect bilateral symmetry, and there are double sets of nuclei, flagella and kinetosomes. Each nucleus has been shown to contain a haploid number of chromosomes.

Ultrastructural studies, show that the so-called adhesive disc is in fact a structure composed of supportive, rather than contractile, elements which remain a fixed shape; the term 'striated' disc has been suggested. The median body superficially resembles the axostyle of trichomonads, but the organisation of the microtubules which compose it, and the fact that the body is not always present, make it a distinctive structure. No structures identifiable as mitochondria, smooth endoplasmic reticulum or Golgi complex have been identified in Giardia. The central flagella are 'ribbon-like', and thus morphologically adapted to their suggested pumping function. Studies with ferritin have demonstrated that particulate material can be ingested by pinocytosis.

Species of Giardia are confined in their distribution to the small intestine, particularly the duodenum, occasionally invading the bile ducts. In severe infections they may carpet large areas of the mucosa. Nutritionally the duodenum is the richest habitat in the alimentary canal and, as mentioned above, the organism makes very close contact with the mucosa.

Epidemiology

Giardia has worldwide distribution and is not uncommon in South Carolina. It is the most frequent protozoan intestinal disease in the US and the most common identified cause of water-borne disease associated with breakdown of water purification systems, drinking from contaminated streams, travel to endemic areas (Russia, India, Rocky Mountains, etc.) and day care centers.

Morphology

Trophozoite: *Giardia* is a 12 to 15 micrometer, half pear-shaped organism with 8 flagella and 2 axostyles arranged in a bilateral symmetry. There are two anteriorly located large suction discs. The cytoplasm contains two nuclei and two parabasal bodies.

Cyst: *Giardia* cysts are 9 to 12 micrometer ellipsoidal cells with a smooth well-defined wall. The cytoplasm contains four nuclei and many of the structures seen in the trophozoite figure 3.2.1.

Fig. 3.2.1: trophozoite of *Giardia lamblia*

Life cycle

Infection occurs by ingestion of cysts, usually in contaminated water. Decystation occurs in the duodenum and trophozoites (trophs) colonize the upper small intestine where they may swim freely or attach to the sub-mucosal epithelium via the ventral suction disc. The free trophozoites encyst as they move down stream and mitosis takes place during the encystment. The cysts are passed in the stool. Man is the primary host although beavers, pigs and monkeys are also infected and serve as reservoirs figure 3.2.2.

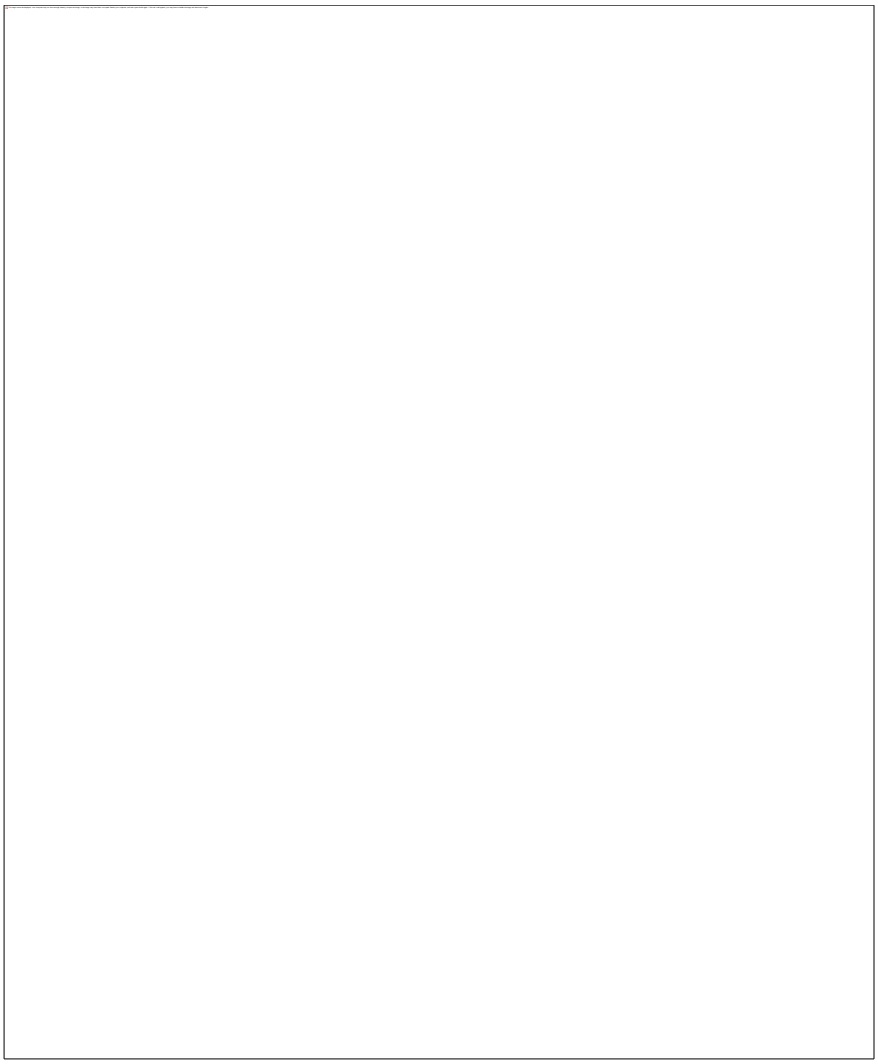

Figure 3.2.2: life cycle of *Giardia lamblia*

Giardiasis (lambliasis) is the disease caused by Giardi parasite.

Etiology
Giardia lamblia (a flagellate)

Symptoms
Early symptoms include flatulence, abdominal distension, nausea and foul-smelling bulky, explosive, often watery, diarrhea. The stool contains excessive lipids but very rarely any blood or necrotic tissue. The more chronic stage is associated with vitamin B_{12} malabsorption, disaccharidase deficiency and lactose intolerance.

Pathology
Covering of the intestinal epithelium by the trophozoite and flattening of the mucosal surface results in malabsorption of nutrients.

Immunology
There is some role for IgA and IgM and there is increased incidence of infection in immunodeficient patients (*e.g.* AIDS).

Diagnosis
Symptoms, history, epidemiology are used in diagnosis. *Giardia* caused dysentery is distinct from other dysenteries due to lack of mucus and blood in the stool, lack of increased PMN leukocytes in the stool and lack of high fever. Cysts in the stool and trophs in the duodenum can be identified microscopically after content has been obtained using a string device (Enterotest®). Trophs must be distinguished from the non-pathogenic flagellate *Trichomona hominis*, which is an asymmetrical flagellate with an undulating membrane.

Treatment
Metronidazole is the drug of choice.

CONCLUSION

The medically/biologically significant intestinal and urogenital protozoans significant to human health includes *Entamoeba histolytica* (Amebae), *Balantidium coli* (Ciliates), *Giardia lamblia* and *Trichomonas vaginalis* (Flagellates) and*Cryptosporidium parvum* and *Isospora belli* (Sporozoa). These have been incriminated in various health challenges of the lumen and the urogenital tract.

Tutor- marked assignment (TMA).

1a. write short note on the morphology and life cycle of *Entamoeba histolytica*
1b. list the other species of Entamoeba.
1. of what significance/importance is Entamoeba to man?
2. Sketch/illustrate the trophozoite of *Giardia lamblia.*
3. Illustrate the life cycle of *Giardia lamblia*

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MODULE 3: OTHER INTESTINAL PROTOZOA

Introduction

Balantidium coli and *Cryptosporidium (parvum)* are both zoonotic protozoan intestinal infections with some health significance. *Isospora belli* is an opportunistic human parasite.

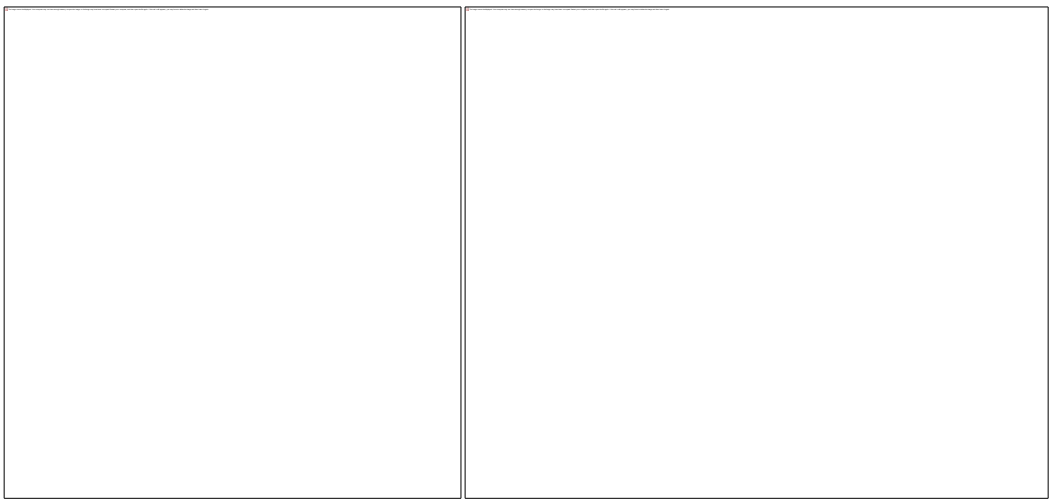
Objectives:

At the end of this lecture, students should:

-have a good knowledge of protozoans that are considered to be primarily of importance in animals but could also infect man (zoonotic importance).

Unit 1: *Balantidium coli*

This is a parasite primarily of cows, pigs and horses. The organism is a large (100 x 60 micrometer) ciliate with a macro- and a micro-nucleus. The infection occurs mostly in farm workers and other rural dwellers by ingestion of cysts in fecal material of farm animals. Man-to-man transmission is rare but possible. Symptoms and pathogenesis of balantidiasis are similar to those seen in entamebiasis, including intestinal epithelial erosion. However, liver, lung and brain abscesses are not seen. Metronidazole and iodoquinol are effective.



A B

Figure 4.1A and B *Balantidium coli* trophozoites. These are characterized by: their large size (40 µm to more than 70 µm) the presence of cilia on the cell surface - particularly visible in (B)

a cytostome (arrows) a bean shaped macronucleus which is often visible - see (A), and a smaller, less conspicuous micronucleus

Unit 2: ***Cryptosporidium parvum***

Cryptosporidium parvum (C. parvum) is a small round parasite measuring 3 to 5 micrometers which is found in the gastrointestinal tract of many animals and causes epidemics of diarrhea in humans via contaminated food and water. Humans are infected by ingestion of *C. parvum* oocysts containing many sporozoites. The sporozoites are released in the upper GI tract and attach to the gut mucosal cells where they divide to produce merozoites. The merozoites invade other mucosal cells and further multiply asexually. Some of the merozoites differentiate into male and female gametocytes and form an oocyst in which they multiply and differentiate into sporozoites. The mature oocyst is excreted with fecal material and infects other individuals.

When a large number of humans in a community have diarrhea, the most likely cause is *C. parvum*. A small bolus of infection may cause mild diarrhea, whereas a larger intake of organisms may cause more pronounced symptoms including copious watery diarrhea, cramping abdominal pain, flatulence and weight loss. Severity and duration of symptoms are related to immuno-competence. In AIDS patients, the organism may cause prolonged, severe diarrhea and the organisms may invade the gallbladder, biliary tract and the lung epithelium. There is no approved effective treatment for cryptosporidiasis, although paromycin is used as an investigational drug.

There are a variety of antibody tests for detection but many of these detect other species of *Cryptosporidium* than *C. parvum.* Sensitive polymerase chain reaction tests are available for *C. parvum* detection in environmental and animal samples.

Unit 3: ***Isospora belli***

Isospora belli is a rare infection of normal humans, although it is being seen in increasing numbers in AIDS patients. The infection occurs via the oro-fecal route. The infective stage of the organism is an oval oocyst which, upon ingestion, follows the same course as *C. parvum*. The disease produces symptoms similar to those of giardiasis. In normal individuals, mild infections resolve themselves with rest and mild diet and heavier infections can be treated with sulpha drugs. The treatment may have to be carried on for a prolonged period in AIDS patients.

CONCLUSION.

While*Balantidium coli* and *Cryptosporidium (parvum)* are both zoonotic (i.e causing infection in both animals and man) protozoans causing intestinal infections with some health significance,*Isospora belli* is an opportunistic human parasite.

Tutor- marked assignment (TMA).

Write short notes on the following:

1. *Balantidium coli* and

2. *Cryptosporidium (parvum)*

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MODULE 4: LUMINAL PROTOZOA

Objectives:

By the time the students would have gone through this lecture unit, they should:

-know protozoans parasitizing other organs aside from blood and intestines

-understand their morphology and life cycle

Unit 1: TRICHOMONAS

Trichomonad' is a general term used for members of the Order Trichomonadida, which includes a large number of species parasitising the tubular organs of a wide range of hosts. Species readily obtained for laboratory study are *Trichomonas muris* in laboratory mice and rats and *Trichomonas gallinae* from the crop of pigeons.

Epidemiology
Trichomonas vaginalis has a world-wide distribution; incidence is as low as 5% in normal females and as high as 70% among prostitutes and prison inmates.

Morphology
The trophozoite form is 15 to 18 micrometers in diameter and is half pear shaped with a single nucleus, four anterior flagella and a lateral flagellum attached by an undulating membrane. Two axostyles are arranged asymmetrically. The organism does not encyst.

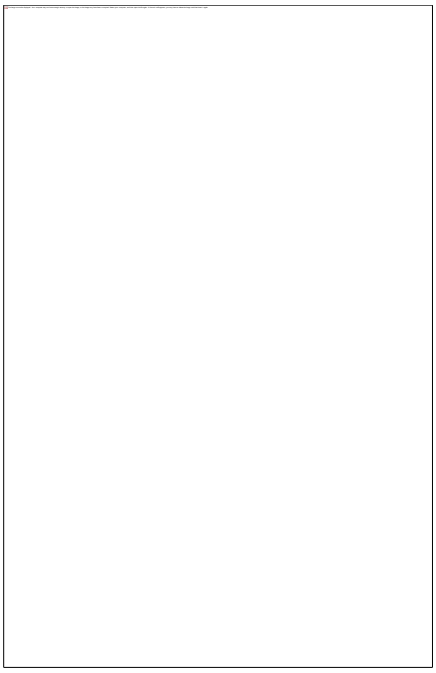

Figure 5.1: *Trichomonas vaginalis* from man.

Lifecycle
T. vaginalis colonizes the vagina of women and the urethra (sometimes prostate) of men. Infection occurs primarily via sexual contact, although non-venereal infections are possible. The organism does not encyst and divides by binary fission which is favored by low acidity (pH > 5.9; the normal pH is 3.5 to 4.5). There is no non-human reservoir.

Trichomoniasis is the disease caused by this organism

Etiology
Trichomonas vaginalis (a flagellate)

Symptoms
T. vaginalis infection is rarely symptomatic in men, although it may cause mild urethritis or occasionally prostatitis. In women, it is often asymptomatic, but heavy infections in a high pH environment may cause mild to severe vaginitis with copious foul-smelling yellowish, sometimes frothy discharge.

Pathology
The organism causes contact-dependent damage to the epithelium of the infected organ.

Diagnosis
Clinical suspicion may be confirmed by finding the organism in Giemsa-stained smears of vaginal discharge or, in difficult cases, by cultivation of a swab sample in Diamond's medium. Trophozoites must be distinguished from the non-pathogenic flagellate *Trichomona hominis*.

Treatment
Metronidazole (although teratogenic) is effective in both males and females. Vinegar douche may be useful. Personal hygiene and the use of condoms are helpful.

Tutor- marked assignment (TMA).

Write short notes on the morphology and life cycle of

Trichomonas vaginalis

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