Module Objectives

At the end of this module, students will be able to:

1. Identify different classes of protozoans
2. List those of parasitic importance
3. Describe the life cycle of at least 3 protozoans.

<table>
<thead>
<tr>
<th>PROTOZOA</th>
<th>HELMINTHS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unicellular</td>
<td>Multicellular</td>
</tr>
<tr>
<td>Single cell for all functions</td>
<td>Specialized cells</td>
</tr>
</tbody>
</table>

1: Amoebae: move by pseudopodia.
2: Flagellates: move by flagella.
3: Ciliates: move by cilia
4: Apicomplexa(Sporozoa) tissue parasites

Round worms (Nematodes):
- elongated, cylindrical, unsegmented.

Flat worms:
- Trematodes: leaf-like, unsegmented.
- Cestodes: tape-like, segmented.

PROTOZOA

The term protozoa implies ‘first animals’ as (proto) ‘animals' (zoa). Although molecular phylogenetic studies indicate that protozoa are among the earliest branching eukaryotes (see phylogenetic tree), such a definition does not provide much descriptive information. Protozoans are not easily defined because they are diverse and are often only distantly related to each other. As the primary hunters of the microbial world, protozoa help in continuing the equilibrium of bacterial, algal and other microbial life forms.

Protozoa also means ‘little animal’. They are named so because many species act like small animals. They search for and collect other microbes as food. Previously, protozoa were specified
as unicellular protists possessing animal-like characteristics such as the capability to move in water. Protists are a class of eukaryotic microorganisms which are a part of the kingdom Protista. Protozoa possess typical eukaryotic organelles and in general exhibit the typical features of other eukaryotic cells. For example, a membrane bound nucleus containing the chromosomes is found in all protozoan species. However, in many protozoan species some of the organelles may be absent, or are morphologically or functionally different from those found in other eukaryotes. In addition, many of the protozoa have organelles that are unique to a particular group of protozoa.

The term ‘protozoan’ has become debatable. Modern science has shown that protozoans refer to a very complex group of organisms that do not form a clade or monophylum. This has led scientists to give up the term protozoa. Hence, the sub-kingdom Protozoa is not used today. Currently, protozoa are defined as single-celled, heterotrophic, or colonial eukaryotes possessing non-filamentous structures.

**Characteristics of Protozoa**

Protozoa do not have a cell wall and therefore can have a variety of shapes. Nevertheless, some of the protozoans have a pliant layer, a pellicle, or a stiff shell outside the cell membrane.
Protozoa exhibit a wide variety of morphologies. There is no one shape or morphology which would include a majority of the protozoa. Shapes range from the amorphous and ever-changing forms of ameba to relatively rigid forms dictated in part by highly ordered cytoskeletons or secreted walls or shells. Their sizes range from 10 to 55 micrometers, but they can be as large as 1 mm. The largest protozoa are called xenophyophores, which can measure up to 20 centimeters in diameter. Several protozoan species express photosynthetic or other pigments and thus are colored. Many protozoan species exhibit complex life cycles with multiple stages. Sometimes the different life cycle stages are so dissimilar that they have been mistaken for completely different species.

Protozoa are found in moist environments virtually everywhere. As a group, the protozoa are extremely adaptable. Individual species, though, generally have specific niches. Like all other organisms, protozoa must be able to acquire and metabolize nutrients from their environment (i.e., heterotrophic). Many protozoa simply absorb solutes (i.e., osmotrophy) from their media, while some are scavengers that ingest solid material (i.e., phagotrophy). Predatory protozoa either actively hunt down or passively ambush other organisms (typically bacteria or other protozoa). Some protozoa are photosynthetic and can capture the energy of the sun and convert it to usable chemical energy (i.e., autotrophic or phototrophic). Many protozoa are not restricted to a single feeding mechanism and can utilize combinations of the above (i.e., mixotrophic).

Protozoa can also be viewed as free-living or symbiotic. Generally free-living organisms are found in the soil or aqueous environments, whereas symbionts live in close association with another organism. Symbiosis implies a physiological dependency of one organism on another organism and not just a close physical association between two organisms. Generally this dependency is in the form of nutrition. Different forms of symbiosis can be distinguished which reflect the nature of the association between the two organisms (Box).

<table>
<thead>
<tr>
<th>Symbiotic Associations</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Commensalism</strong></td>
</tr>
<tr>
<td><strong>Mutualism</strong></td>
</tr>
<tr>
<td><strong>Parasitism</strong></td>
</tr>
</tbody>
</table>
Protozoa prefer living in moist and aquatic habitats. Their cysts can be found in the bleakest parts of the ecosphere.

Protozoa are found drifting in the oceans, seas, and freshwater. They are at the base of food chains.

**Motility and the Cytoskeleton**

The earliest observations of protozoa noted their motility. This motility resulted in their classification as 'animals', which were distinguished from the non-motile 'plants'. However, motility is not a universal feature of protozoa and different protozoa utilize different mechanisms for their movement (Table). In fact, protozoa were initially classified based in part on their mechanism of motility.

Cilia and flagella are subcellular structures which propel protozoa through a fluid medium. Flagella are long whip-like structures which propel the organism as a result of wave-like beat which is propagated through their length. Flagellated protozoa typically have one or a few flagella per organism. In contrast, ciliated protozoa are usually covered with rows of numerous cilia. The beats of these cilia are coordinated and function like oars to propel the organism. Cilia and flagella can also assist in the procurement of food, reproduction and other functions. Cilia and flagella are made up of the same protein components and are actually equivalent structures. Both are membrane bound filamentous projections from the cell. The filament, known as an axoneme, is composed of a series of parallel microtubules. Movement is produced when the microtubules slide past each other. The force which mediates this sliding motion is generated by a protein called dynein. Dyneins are 'motor proteins' which convert the chemical energy released by ATP hydrolysis into a mechanical energy. Microtubules are cytoskeletal elements which also play important roles in cell shape and are a major component of the mitotic spindle.

In contrast to the swimming exhibited by flagellates and ciliates, ameba are protozoa that crawl along a solid substratum in a fashion known as 'ameboid movement'. The ameba projects out a pseudopodium, or false foot, from the cell body. The pseudopodium then attaches to the substratum and then pulls the rest of the cell body forward. The force involved in this movement is generated by another cytoskeletal system, which is comprised of actin and myosin. Actin forms long filaments, also known as microfilaments, and myosin is a motor protein which moves along the microfilaments in an ATP dependent manner. Muscle contraction is another example of the force generation via actin-myosin cytoskeletal elements. In a mechanistic sense,
Phagocytosis is a form of ameboid movement also involving microfilaments. In this case the pseudopodia are extended to surround the particle being ingested. Fusion of the pseudopodia with the cell body results in the internalization of the particle within a vacuole.

Apicomplexa also crawl along a substratum, but by a different mechanism than the ameba. The mechanism of this so-called 'gliding motility' is just beginning to be understood and probably involves both microfilament and microtubule based cytoskeletal systems. Apicomplexa also exhibit intracellular forms and invasion of the host cell also involves this gliding motility.

**Reproduction**

Protozoa, like all other organisms, reproduce. The most common form of reproduction in protozoa is asexual binary fission. In other words, a single organism will divide into two equal organisms. A slight modification of this binary fission, called budding, is when one of the newly formed cells is smaller than the other. Typically the larger cell is called the mother and the smaller is the daughter. Some protozoa will form an intracellular bud and essentially give birth. Another variation of binary fission is a multiple fission or segmentation. In this situation, several rounds of nuclear replication occur without cytokinesis. This multinucleated cell will then form multiple progeny simultaneously.

Many protozoa exhibit sexual reproduction in addition to the asexual forms of reproduction. This sexual reproduction can involve the production and fusion of gametes in processes similar to higher organisms. The Ciliophora undergo a conjugation in which opposite mating types will pair and directly exchange genetic material (i.e., DNA). Sometimes sexual reproduction is an obligatory step in the life cycle, whereas in other cases the organism can reproduce asexually with an occasional round of sexual reproduction.

The life cycle of protozoa changes between proliferative stages and dormant cysts.

When in the cystic stage, protozoa can live in utmost temperatures or harsh chemicals, or without nutrients, water, or oxygen for a long time. Being a cyst enables parasitic species to dwell on the host externally. This lets them transmit from one host to another. In the form of trophozoites, protozoa feed actively. The transition of a trophozoite to a cyst is called encystation and the transition back to a trophozoite is called excystation.

The mode of nutrition of protozoa is heterotrophic, and most species obtain food by phagocytosis. Phagocytosis is the process where the cell changes shape by sending out pseudopodia to make contact with food particles.
Protozoa take food into the cell at a point called the cytostome. The food is ingested by them and lysosomal enzymes digest the food. There are also certain types of protozoa that take in food by their cell membranes. Some others such as the amoeba, surround food and absorb it. Others have mouth pores into which they pull in food.

Protozoans digest their food in spaces called vacuoles. Contractile vacuoles that are found in protozoa thriving in freshwater, excrete water that penetrates into the cells by osmosis. While chewing down the food, protozoans produce and release nitrogen.

Classification

The protozoa group comprises more than 65,000 species. All the protozoan species belong to the kingdom Protista. Many kinds of protozoa are symbionts. Some of the protozoan species are parasites and some are predators of bacteria and algae. Some examples of protozoans are dinoflagellates, amoebas, paramecia, and plasmodium.

Classification of Protozoa:

The phylum protozoa has been divided into four classes. The classification is based principally on their mode of locomotion.

Class I:

Rhizopoda (Rhiza= root; podus = foot). Protozoa having peculiar temporary organelles for locomotion, called pseudopodia or false feet. Examples—Amoeba proteus; Entamoeba histolytica (causing dysentery).

Class II:

Mastigophora (Mastix =whip; phoros=bearer) or Flagellata (flagellum = whip). Protozoa that move by the lashing of whip-like organelles called flagella. Example—Euglena viridis; Trypanosoma gambiensis (causing African sleeping-sickness); Leishmania donovani (causing kala-azar).

Class III:

Sporozoa (Spora—seed; animal). Parasitic protozoa possessing no locomotor organelles and reproducing by means of spores. Example—Monocystis gregarina; Plasmodium vivax (causing malaria).

Class IV:

Ciliophora (Cilium— eyelash). Protozoa that move by hair-like cilia. Example—Paramoecium caudatum (the slipper- animalcule); Vorticella campanula (the bell-animalcule).

Based on the mode of locomotion, protozoa have been divided into four types.
1. Amoeboids

An amoeboid (ameba or amoeba) is a type of cell or organism that is capable of changing its shape, mainly by extending and retracting pseudopods. They are normally found in the soil and in aquatic habitats. They move by using pseudopods. They typically ingest their food by phagocytosis. They extend their pseudopods to engulf a prey. They do not possess a mouth or cytostome.

There is no specific place on the cell where phagocytosis takes place. The food sources of amoebae differ. Some of them feed on bacteria and other protists. Some others feed on dead organic material. Some also feed by absorbing dissolved nutrients through vesicles. The examples of amoeboids are *Amoeba proteus*, *Chaos carolinense* (the giant amoeba), *Naegleria fowleri* (the brain-eating amoeba), *Entamoeba histolytica* (the intestinal parasite of commensals and humans), and *Dictyostelium discoideum* (the multicellular social amoeba).

2. Flagellates

Euglena
Flagellates are organisms which have one or more whip-like organelles called flagella. They may be solitary, colonial, free-living or parasitic. Parasitic forms live in the intestine or bloodstream of the host. An example of a parasitic flagellate is Trypanosoma, which has an interesting life cycle as it uses two hosts; humans and tsetse fly. Many other flagellates like dinoflagellates live as plankton in the oceans and fresh water. Some flagellates are autotrophic while others are heterotrophs.

Flagellates are divided into two classes:

**Phytomastigophorea:** The Phytomastigophorea includes protozoans that contain chlorophyll. They can produce their food photosynthetically, like plants. Examples include Euglena and Dinoflagellates. Euglena is regarded as both an alga and a protozoan.

**Zoomastigophorea:** It is the phylum commonly called zooflagellates. Zooflagellates include protozoans which are colorless. They ingest organic substances by osmotrophy (uptake of dissolved organic compounds through plasma membrane) or phagotrophy (engulfing prey in food vacuoles). They may be free-living, symbiotic, commensal, or parasitic. Examples include hypermastigids, holomastigotoides, and trichomonads.

3. Ciliates

Paramecium

The ciliates are a group of protozoans which possesses hair-like organelles called cilia. Cilia are used in swimming, crawling, attachment, feeding, and sensation. Most ciliates are heterotrophs. They eat organisms such as bacteria and algae. They sweep the food by their modified oral cilia into their oral groove (mouth). The food is moved with the help of cilia through the mouth pore into the gullet, which forms food vacuoles.
Some ciliates do not have a mouth and they feed by absorption (osmotrophy), and some others are predatory and feed on other protozoa, especially ciliates. Some ciliates also parasitize animals. Examples of ciliates include free-living forms like *Paramecium caudatum, Stentor polymorpha, Vorticella campanula* and parasitic forms like *Balantidium coli*.

There are three types of ciliated protozoa. They are free-swimming ciliates, crawling ciliates, and stalked ciliates. All of them use cilia for locomotion and capturing food. Examples of free-swimming ciliates include *Litonotus* and *Paramecium*. Examples of crawling ciliates are *Aspidisca* and *Euplotes*.

### 4. Sporozoans

![Polar Rings, Microtubules, Mitochondria, Pellicular Cisterna, Nucleus, Ribosomes, Plasma Membrane, Apical End](image)

**Plasmodium**

Sporozoans are non-motile, unicellular protists, usually parasites. These protozoans are also called intracellular parasites. An example is *Plasmodium vivax*, causing malaria in humans. The earlier stage sporozoan forms show some movement. They do not possess locomotion organelles in their later stage.

Four main groups of sporozoans (based on spore structure) include:

**Apicomplexa:** The Apicomplexa, also called Apicomplexia, are a large phylum of parasitic protists. They are spore-forming unicellular parasites. Most of them have a unique organelle that is made up of a type of plastid called an apicoplast, and an apical complex structure. The organelle is used by the organism for penetrating into a host cell. Flagella or pseudopods are found only in certain gamete stages. This group includes organisms like *coccidia, gregarines, piroplasms, haemogregarines*, and *plasmodium*. All organisms of this phylum have an infectious stage, the sporozoite. All the species of this group, except *Nephromyces*, a symbiont in marine animals, are endoparasites of animals.

**Microsporidia:** The microsporidia constitute a group of spore-forming unicellular parasites. They were at a time known to be protists, but are now known to be fungi. They have a polar tube or polar filament in the spore with which they infiltrate host cells. Microsporidia do not have mitochondria, and instead possess mitosomes. They also do not have flagella. Most organisms in
this group infect animals and insects and a few infect humans. Microsporidia can also infect hosts which are themselves parasites.

**Ascestosporea:** They are a group of protists that are parasites of animals, especially marine invertebrates. Two groups which come under this are the haplosporids and paramyxids. Haplosporid spores have a single nucleus and an opening at one end, covered with an internal diaphragm. After emerging, it develops within the cells of its host, usually a marine invertebrate. However, some infect other groups or freshwater species. Paramyxids grow within the digestive system of marine invertebrates, and produce multicellular spores.

**Myxosporidia:** The Myxosporea are a class of microscopic parasites, belonging to the Myxozoa (group of parasitic animals of aquatic environment). They have a life cycle which comprises vegetative forms in two hosts, an aquatic invertebrate, usually an annelid, and an ectothermic vertebrate, usually a fish.

**As a phylum, protozoa are divided into three subphyla.**

1. **Subphylum Sarcomastigophora**

   The subphylum Sarcomastigophora belongs to the kingdom Protista and includes many unicellular or colonial, autotrophic, or heterotrophic organisms. It is divided into three superclasses, the Mastigophora, the Sarcodina and the Opalinata.

   **Superclass Mastigophora:** This group of protozoa is also flagellates. They move with the help of flagella. They feed on bacteria, algae, and other protozoa.

   **Superclass Sarcodina:** This group includes amoeba, heliozoa, radiozoa, and foraminifera. Amoeba have pseudopods that are used for locomotion and feeding. In amoeba, the flagellae are lobe-like protrusions that extend from the cell membrane. In heliozoa, radiozoa, and foraminifera, the pseudopods are like needles jutting out from the cells.

   **Superclass Opalinata:** The opalines are a small group of protists, which belong to the family Opalinidae. The microscopic organisms of this group are opalescent (having or emitting an iridescence like that of an opal) in appearance when they come under full sunlight. Most opalines live as endocommensals (a commensal living within the body of its host) in the large intestine and cloaca of frogs and toads. They are sometimes found in fish, reptiles, molluscs, and insects.

2. **Subphylum Sporozoa**

   Sporozoa include organisms that are also called sporozoans or intracellular parasites. In the early stages, they show some movement. They do not possess locomotor organelles in their later stage. All forms of sporozoa are parasites. They include plasmodium, the malarial parasite.

3. **Subphylum Ciliophora**

   This group of organisms is of ciliates. Their locomotion is with the help of cilia. The cilia enable them to move quickly, stop suddenly, and turn sharply while following their prey. The types include free-living forms like paramecium and parasitic forms like balantidium coli. Many ciliates eat bacteria, fungi, and other protozoa.
Based on the mode of nutrition, protozoa are divided into the following two types.

1. Free-living protozoa

Euglena

The free-living protozoa are those which do not infect or live on hosts for their survival. They may produce their food photosynthetically, or eat bacteria, yeast and algae. Example: Euglena

2. Parasitic protozoa

They depend on their hosts for survival. They take in fluids from the body of their hosts. Example: Plasmodium

Based on the mode of respiration, protozoa are classified into two groups.

1. Aerobic Protozoa

Amoeba Proteus

Most species of free-living protozoa are aerobic. They cannot live without oxygen. Aerobic protozoa are tiny and so are capable of getting oxygen from the liquid medium by diffusion. Example: Amoeba proteus
2. Anaerobic Protozoa

Giardia

They can survive in the absence of oxygen and are not commonly found amidst eukaryotic organisms. Normally, anaerobic eukaryotes are either parasites or symbionts of multicellular organisms that have originated from aerobic ancestors. Examples: *Giardia* and *Trichomonads*
KINETOPLASTIDS

Kinetoplastids Causing Human Disease

- African trypanosomes
  (African sleeping sickness)
- Trypanosoma cruzi
  (Chagas’ disease)
- Leishmania species
  (leishmaniasis)

The kinetoplastids are a widespread group of flagellated protozoa. Members of this group parasitize virtually all animal groups as well as plants and insects. There are also free-living kinetoplastids which feed on bacteria in aquatic, marine and terrestrial environments. Three distinct kinetoplastids cause human disease: African trypanosomes (African sleeping sickness), Trypanosoma cruzi (Chagas’ disease), and Leishmania species (leishmaniasis). All three are parasites of the blood and/or tissues of the human host and are transmitted by arthropod vectors.

The major distinguishing feature of this group is a subcellular structure known as the kinetoplast. The kinetoplast is a dark Giemsa-staining structure which is distinct from the nucleus. The size of the kinetoplast will vary according to species. The kinetoplast is found near the basal body which is located at the base of the flagellum (Figure). Because of this location near the flagellum, it was previously believed that the kinetoplast was somehow associated with cell movement—hence the name. However, the kinetoplast is actually a distinct region of the mitochondria and is not involved in motility. The staining of the kinetoplast is due to mitochondrial DNA. In fact, the existence of extranuclear (i.e., organellar) DNA was first demonstrated in the kinetoplastids.

Typically, the kinetoplastids are depicted as long slender organisms. However, the kinetoplastids exhibit several morphological forms which are defined by the position of the kinetoplast in relation to the nucleus and the length of the undulating membrane. Cellular features of the kinetoplastids include:

- A single flagellum present in many of the morphological forms. A paraxial rods runs along beside the axoneme. The flagellum is sometimes attached to cell body to form undulating membrane.
- The flagellum emerges from a flagellar pocket. Endo- and exocytosis is limited to this flagellar pocket.
- A single and often branched mitochondria with discoid (rarely flattened or tubular) cristae characterized by the ktDNA discussed above.
- The presence of a peroxisome-like organelle called the glycosome in which glycolysis occurs.
A cytoskeleton composed of subpellicular (also called cortical) microtubules which run the length of the organism.

Morphological Forms

Several different morphological forms of kinetoplastids are observed. These various morphological forms are associated with different life cycle stages in the various species. The different forms are distinguished by the position of the kinetoplast in relation to the nucleus and the presence or absence of an undulating membrane. The four major morphological forms of kinetoplastids which cause human disease are:

**trypomastigote**

The kinetoplast (kt) is located on the posterior end of the parasite. The flagellum emerges from the posterior end and folds back along the parasite's body. This attachment of the flagellum to the body forms an undulating membrane (um) that spans the entire length of the parasite and the free flagellum emerges from the anterior end. This is considered the anterior end since the flagellum pulls the organism and the end with the free flagellum is the front in reference to the direction of movement. The undulating membrane functions like a fin and increases the motility of the organism.

**epimastigote**

The kinetoplast (kt) is more centrally located, usually just anterior to nucleus (Nu). The flagellum (fg) emerges from the middle of the parasite and forms a shorter undulating membrane (um) than observed in trypomastigotes. Epimastigotes are less motile than trypomastigotes.

**promastigote**

The kinetoplast (kt) is towards the anterior end and a free flagellum (fg) with no undulating membrane emerges from the anterior end. The free flagellum emerges from in all three motile forms is designated as the anterior end because they swim in that direction. In other words, the flagellum pulls the organism.
amastigote

The parasite is more spherical in shape and has no free flagellum. A basal body (bb) and the base of the flagellum are present. The kinetoplast (kt) is usually detectable as a darkly staining body near the nucleus (Nu). This form is an intracellular stage.

AFRICAN TRYPANOSOMIASIS

African trypanosomiasis, also known as African sleeping sickness, exhibits a patchy distribution in equatorial Africa depending upon specific topographical features and the presence of the vector. The control of African trypanosomiasis is complicated by poverty, political instability, and civil wars often found in areas endemic for the parasite and vector. An estimated 60 million people in 36 nations are at risk of infection. The incidence of the disease had been increasing from the mid-1960s to the end of the 20th century with an estimated 300,000-500,000 cases occurring annually in 1998. However, increased awareness and programs initiated by the WHO have led to a decreasing incidence and in 2009 there were less than 10,000 cases (Simerro et al., 2011)

The parasites responsible for causing African sleeping sickness belong to a group of closely related trypanosomes in the Trypanosoma brucei species complex. Three morphologically indistinguishable species are recognized:

- **T. brucei** infects game animals/livestock (causes nagana)
- **T. rhodesiense** causes E. African trypanosomiasis
- **T. gambiense** causes W. and Central African sleeping sickness

(Some authors consider these as subspecies: T. brucei brucei, T. b. rhodesiense, T. b. gambiense.)

*T. brucei* is a natural parasite of wild game in Africa and are non-infective to humans. This inability to infect humans is due to a trypanosome lytic factor found in human sera. *T. brucei* and two morphologically distinct trypanosomes, *T. vivax* and *T. conglolense*, are major pathogens for wild and domestic animals and have far reaching effects on raising livestock. In fact, although trypanosomiasis can be a devastating human disease, the greatest impact of trypanosomiasis on human health is at the agricultural level. Large areas of Africa are unsuitable for raising cattle and other livestock due to the presence of the tsetse vector and the transmission of trypanosomes. This contributes to protein deficient diets among the indigenous population.

As the names imply, *T. gambiense* and *T. rhodesiense* are distinguished by their geographical distributions. *T. rhodesiense* is found in East Africa and *T. gambiense* is found in West and Central Africa. (Rhodesia is the former name for Zimbabwe.) The restricted distributions of the African trypanosomes are determined by the vectors. African trypanosomes are transmitted by
several species within the genus *Glossina*, commonly known as the tsetse. In addition to being transmitted by different vectors, *T. gambiense* and *T. rhodesiense* are distinguished by animal reservoirs, epidemiology, and disease virulence.

<table>
<thead>
<tr>
<th>Attribute</th>
<th><em>T. rhodesiense</em></th>
<th><em>T. gambiense</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>tsetse vector</td>
<td><em>G. morsitans</em> group</td>
<td><em>G. palpalis</em> group</td>
</tr>
<tr>
<td>ecology</td>
<td>dry bush, woodland</td>
<td>rainforest, riverine, lakes</td>
</tr>
<tr>
<td>transmission cycle</td>
<td>ungulate-fly-human</td>
<td>human-fly-human</td>
</tr>
<tr>
<td>non-human reservoir</td>
<td>wild animals</td>
<td>domestic animals</td>
</tr>
<tr>
<td>epidemiology</td>
<td>sporadic, safaris</td>
<td>endemic, some epidemics</td>
</tr>
<tr>
<td>disease progression</td>
<td>rapid, often fatal</td>
<td>slow (~1 yr) acute ⇒ chronic</td>
</tr>
<tr>
<td>parasitemia</td>
<td>high</td>
<td>low</td>
</tr>
<tr>
<td>asymptomatic carriers</td>
<td>rare</td>
<td>common</td>
</tr>
</tbody>
</table>

The tsetse vector of *T. rhodesiense* is found in woodland or dry bush environments. Its natural vertebrate host is the antelope and other wild ungulates. Disease transmission depends upon coming in contact with infected vectors and is often associated with hunters or safaris. *T. rhodesiense* is primarily a *zoonosis* and little human-fly-human transmission occurs. The disease typically progresses rapidly and is often fatal without treatment. Although variants of *T. rhodesiense* which cause a slowly progressing chronic disease have been identified (MacLean *et al*, 2004). Molecular data suggest that *T. rhodesiense* may be a host-range variant of *T. brucei*.

*T. gambiense* is transmitted by vectors found along rivers and lakes which are often in close proximity to human habitation. Consequently, the disease tends to be endemic and domestic animals may serve as reservoirs. The disease is much less virulent and is characterized by a slow progression from an acute disease to a chronic disease. It is believed that *T. gambiense* has been associated with humans for much longer than *T. rhodesisense* and thus possibly accounts for the lower virulence of *T. gambiense*.
Life Cycle

**Metacyclic trypomastigotes** (MT) in the saliva of the tsetse are transferred to the bloodstream of the mammalian host as the tsetse feeds. The parasite exhibits a trypomastigote morphology in the bloodstream and is extracellular. These extracellular forms undergo an antigenic variation to evade the host immune system. Within the bloodstream the trypanosome undergoes asexual replication by longitudinal binary fission. These replicating forms are generally long slender (LS) parasites. In addition to the long slender forms, intermediate and short stumpy (SS) forms are also found within the bloodstream of the mammalian host. The short stumpy forms are thought to be preadapted for the tsetse. However, *in vitro* experiments suggest that all bloodstream forms are infective for the tsetse.

The tse-tse can ingest trypanosomes with its blood meal. The bloodstream trypomastigote differentiates into a **procyclic trypomastigote** (PT) within the gut of the tsetse. Accompanying this differentiation is a loss of the VSG surface coat and changes in the mitochondria and metabolism. The environment within the gut of fly is quite different than that of the mammalian bloodstream. The mammalian bloodstream is rich in glucose and parasite exhibits a high rate of glycolysis which is carried out in a special organelle known as the glycosome. Because of this abundance of glucose the parasite does not carry out oxidative phosphorylation within the mitochondria and consequently the mitochondria are acristate and have minimal electron transport activity. Within the vector, though, mitochondrial functions associated with aerobic metabolism return and cristae develop within the mitochondria. The procyclic trypomastigotes undergo multiple rounds of asexual replication within the midgut of the tsetse. The procyclic stage can also be cultured in vitro.

The focus in medical parasitology courses tends to be on the complex interactions between the parasite and the human host which result in pathology. However, parasites also interact with and undergo complex developmental processes in the vector. Vectors are more than 'flying syringes'. (Although *T. evansi*, a trypanosome infecting horses and camels, is transmitted *mechanically* by horseflies.) One problem for the trypanosome is that it must move from the gut to the salivary glands of the tsetse. The exact mechanism by which the parasite migrates from the tsetse gut to the saliva glands is not known. Two routes have been proposed: 1) the classical route in which the parasite 'backtracks' through the digestive system and migrates up the salivary duct, or 2) the direct route in which the parasite penetrates the peritrophic membrane and gut epithelium to gain access to the hemolymph.

After reaching the salivary glands the procyclic trypomastigotes transform into **epimastigotes** (E) and attach to epithelial cells via their flagella. The epimastigotes probably undergo further
replication within the salivary gland. The epimastigotes are non-infective for the mammalian host and they must first mature into **metacyclic trypomastigotes** (MT). During this maturation the surface coat is reformed, the mitochondria lose their cristae and the parasite detaches. These trypomastigotes are free within the lumen of the salivary gland waiting to be transferred to a vertebrate host when the tsetse feeds again, thus completing the life cycle.

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**Sex in African trypanosomes?**

The long-standing dogma has been that trypanosomes exhibit no sexual stages. However, molecular studies (first reported in 1986) indicate that genetic recombination occurs in the African trypanosomes within the tsetse vector. Although genetic recombination clearly occurs, it is not obligatory and it is rare. It is not yet known exactly when this recombination occurs within the life cycle. Experimental evidence indicates that hybrids are only found in salivary glands and not in procyclic stages found in the gut. Most likely the fusion is between epimastigotes and not metacyclic trypomastigotes. No haploid gamete stages have been observed the biparental inheritance of the kDNA suggests that mitochondria exchange DNA.

A genetic cross between *T. brucei* and *T. rhodesiense* has been successfully carried out in the laboratory. In regards to the human serum resistance phenotype (its mode of action), both parental phenotypes (i.e., sensitive and resistant), as well as intermediate forms, were detected in the progeny.

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**Disease Course and Symptoms**

Infection with African trypanosomes can result in disease manifestations ranging from asymptomatic or mild to a severe fulminating disease. *T. rhodesiense* is more likely to cause a rapidly progressing and fulminating disease than *T. gambiense*. *T. gambiense* tends to cause a slow progressing disease which may either be self-limiting or develop into a chronic disease involving the lymphatics and the central nervous system (CNS). The infection is almost always fatal with few documented cases of individuals clearing the parasites and surviving.

The infection is initiated when metacyclic trypomastigotes are introduced from the salivary glands of the tsetse into the bite wound. Generally there is an asymptomatic incubation period of 1-2 weeks in which the trypomastigotes are replicating within the tissue near the site of the bite. Occasionally, a local inflammatory nodule known as a 'trypanosomal chancre' is observed during this period. Chancres are usually tender and painful and ulceration may occur.

The trypomastigotes will then invade the capillaries and enter the circulatory system during this incubation period and continue to replicate within the blood of the human host. The establishment of this acute blood stage infection is characterized by irregular episodes of fever and headache. In the case of *T. gambiense* the number of parasites in the blood tends to be very low and often the infected person exhibits no symptoms, whereas most persons infected with *T.*
*rhodesiense* will exhibit much higher parasitemias and a more pronounced fever sometimes associated with rigor.

Disease progression is often characterized by invasion of the lymphatics in *T. gambiense* infections. Symptoms during the lymphatic stage include enlarged lymph nodes (particularly post-cervical group), weight loss, weakness, rash, itching, and edema as well as the continued intermittent febrile attacks. Higher parasitemias are often associated with the symptomatic periods. The infection can spontaneously resolve during either the blood stage or the lymphatic stage. There is usually little evidence of lymphatic involvement in *T. rhodesiense* infections. In general, the symptoms during the earlier stages of the infection tend to be non-specific (fever, malaise, headache, weakness) and may infect multiple organs.

- A hallmark feature of African trypanosomiasis is the invasion of the CNS and nervous system impairment.
- Trypanosomes crossing the blood-brain barrier result in a generalized meningoencephalitis characterized by progressively worsening symptoms.
- Indications of nervous impairment include: apathy, fatigue, confusion, somnolence, and motor changes (such as tics, slurred speech, and incoordination). The changes in sleep patterns are often characterized by extreme fatigue during the day and extreme agitation at night.
- Generally it is 6-12 months (or even years) after the infection before the neurological symptoms start to become apparent in the case of *T. gambiense*.
- Neurological manifestations can occur within weeks after *T. rhodesiense* infections. If untreated, the CNS stage of the disease will almost always progress to include convulsions or coma followed by death in both *T. gambiense* and *T. rhodesiense* infections.

<table>
<thead>
<tr>
<th>Disease Progression</th>
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<tbody>
<tr>
<td>- 1-3 week incubation period (± trypanosomal chancre)</td>
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<tr>
<td>- acute blood stage infection characterized by intermittent fever and headache</td>
</tr>
<tr>
<td>- invasion of lymphatics characterized by wasting and other symptoms</td>
</tr>
<tr>
<td>- CNS involvement and nervous impairment</td>
</tr>
<tr>
<td>- convulsions or coma leading to death</td>
</tr>
</tbody>
</table>
Life Cycle of Trapanosomiasis

During a blood meal on the mammalian host, an infected tsetse fly (genus *Glossina*) injects metacyclic trypomastigotes into skin tissue. The parasites enter the lymphatic system and pass into the bloodstream. Inside the host, they transform into bloodstream trypomastigotes, are carried to other sites throughout the body, reach other body fluids (e.g., lymph, spinal fluid), and continue the replication by binary fission. The entire life cycle of African trypanosomes is represented by extracellular stages. The tsetse fly becomes infected with bloodstream trypomastigotes when taking a blood meal on an infected mammalian host. In the fly’s midgut, the parasites transform into procyclic trypomastigotes, multiply by binary fission, leave the midgut, and transform into epimastigotes. The epimastigotes reach the fly’s salivary glands and continue multiplication by binary fission. The cycle in the fly takes approximately 3 weeks. Rarely, *T. b. gambiense* may be acquired congenitally if the mother is infected during pregnancy.
Diagnosis

Confirmed diagnosis depends upon the detection of trypanosomes in the blood, lymph node aspirations, or spinal fluid. Typically few trypanosomes are detected in the blood or other bodily fluids during the *T. gambiense* infections. The trypanosomes are more likely to be detected during symptomatic periods (eg. during febrile episodes).

One important issue in diagnosis of African trypanosomiasis is to distinguish the late encephalitic stage of the disease from the early stage. This is important since the treatment is different depending on whether there is CNS involvement (see below). Criteria for CNS involvement include detection of parasites in the cerebral spinal fluid (CSF) or elevated white
blood cells (>5/microliter) in the CSF. There is some controversy in regards to the exact level of white blood cells in the CSF should constitute a classification of CNS involvement.

**Treatment**

Suramin and pentamidine are the recommend drugs during the acute stage without CNS involvement, whereas melarsoprol or eflornithine are recommend if the CNS is involved (Table below). Pentamidine is less toxic than suramin. However, it is not effective against *T. rhodesiense*. All four of these drugs are provided free of charge by the World Health Organization through public and private partnerships with pharmaceutical firms. The prognosis is generally excellent if treatment starts during the acute stage.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Use</th>
<th>Drawbacks</th>
</tr>
</thead>
</table>
| **Pentamidine** | Effective against early-stage *gambiense* disease | • Adverse side effects  
• Non-oral route |
| **Suramin**   | Effective against early-stage *gambiense* and *rhodesiense* disease | • Adverse side effects  
• Non-oral route |
| **Melarsoprol** | First line drug for late-stage *gambiense* and *rhodesiense* disease involving CNS | • Adverse side effects, especially encephalopathy  
• Fatal in 1-5% of cases  
• Parasite resistance  
• Non-oral route |
| **Eflornithine** | Effective against late-stage *gambiense* disease involving CNS | • High cost  
• Not effective against *T. rhodesiense*  
• Non-oral route - has to be given intravenously (needs hospitalization for 14 days) |

**Prevention and Control**

Wearing protective clothing or using insect repellents are the recommended prophylactic measures against African trypanosomiasis. Other measures to lessen contact with the day-biting tsetse, such as avoidance of streams and water holes during the warm dry season, can also be...
taken. Prophylactic drugs are contraindicated since they may mask latent CNS infections and promote drug resistance. In addition, the available drugs are somewhat toxic.

Control activities are primarily focused on *T. gambiense* and involve reducing the number of infected humans as well as reducing the vector. Systematic surveillance and treatment of infected persons is an effective control measure since humans are the primary reservoir and the disease in the early stages is often asymptomatic or mild and slowly progressing. Control of the riverine tsetse includes destruction of their habitats and breeding places by clearing stream banks of trees and shrubs. Widespread application of insecticides can also be used to reduce the number of tsetse.

The use of traps and targets are another potential means to reduce the number of tsetse in a localized area. Traps and targets function by attracting the tsetse to a contraption that collects orReview on African Trypanosomiasis:

- Reviews on African Trypanosomiasis: