

Subject: Zoology

Production of Courseware

-Content for Post Graduate Courses



Paper No. : 08 Biology of Parasitism

Module : 06 Morphology, Life cycle, Mode of infection of *Trypanosoma*



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1. Learning Outcomes

After studying this module, you shall be able to:

- ✓ Know the Human African Trypanosomiasis disease.
- ✓ Learn different forms of Human African Trypanosomiasis.
- ✓ Know the distribution and major epidemics of the disease.
- ✓ Understand the life cycle of *Trypanosoma gambiense*.
- ✓ Learn the mode of infection, symptoms, diagnosis and prevention of the disease.

2. Introduction

Human African trypanosomiasis, also known as sleeping sickness, is a vector-borne parasitic disease caused by protozoa which belongs to the genus *Trypanosoma*. The parasite is classified into three subspecies: *Trypanosoma brucei gambiense*, *T. brucei rhodesiense*, and *T. brucei brucei*. The later subspecies is not pathogenic to humans. These subspecies cannot be differentiated morphologically.

T. b. gambiense is distributed in western and central Africa which causes chronic disease whereas, *T. b. rhodesiense* found in eastern and southern Africa and responsible for acute severe disease (figure 1).

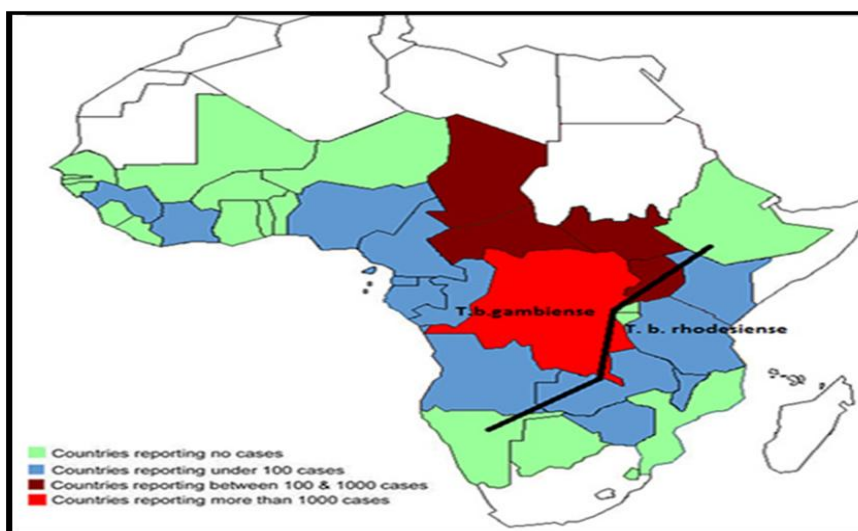


Figure.1: Distribution of *T. b. gambiense* and *T. b. rhodesiense* cases in different parts of Africa.

The epidemiology of these parasite species also differs and depends on the distribution of their vectors, *Glossina palpalis* and *Glossina morsitans* respectively. The *G. palpalis* is found in the areas of vegetation near rivers or cultivated fields. The *G. morsitans* mainly depends on wild animals in savannah which are far away from human settlements. The disease is transmitted to humans by tsetse fly which gets their infection from humans or from wild animals harboring the parasites.

Some species of Trypanosomes are non pathogenic and does not cause any harm to the humans while other species such as *Trypanosoma gambiense* are pathogenic and causes severe disease in human and animals. In this module we will discuss about morphology, life cycle and pathogenesis of *Trypanosoma gambiense*. We will also take a glance at diagnosis and treatment of the disease.

3. Forms of Human African Trypanosomiasis

Human African Trypanosomiasis takes different forms, depending on the parasite involved:

- *Trypanosoma brucei gambiense* is found in west and central Africa and currently accounts for around 95% of sleeping sickness cases and causes a chronic infection. In this form the individual infected with the parasite can live without any symptoms of disease for several months or years. When a symptom first arises, the patient is already in an advanced state of disease where central nervous system is affected.
- *Trypanosoma brucei rhodesiense* is present in eastern and southern Africa. This form represents 5% cases and causes acute infection. The symptoms of the disease appear within few months or weeks after infection. The disease spreads rapidly and invades the central nervous system.
- *Trypanosoma cruzi* occurs mainly in about twenty one Latin American countries. It is called American trypanosomiasis or Chagas disease. The causal organism is a different species from those causing the African form of the disease.
- *T. rangeli* is a non pathogenic trypanosome found in the blood of man in Venezuela and Colombia.

4. Animal Trypanosomiasis

Other species of the *Trypanosoma* genus are pathogenic to animals which cause animal trypanosomiasis in wild and domestic animals. In cattle, the disease is known as *Nagana*, a Zulu word, which means "to be depressed".

Animals can also become host for human pathogen parasites, mainly *T. b. rhodesiense* and play important role as reservoir of the parasites. Animals can also be infected by *T. b. gambiense* and act as a reservoir. However the exact epidemiological role of this reservoir is not yet well known. The disease in domestic animals especially cattle are major barrier to the economic development of affected rural areas.

5. Distribution and Major Epidemics of the Disease

The disease, sleeping sickness is endemic and highly prevalent in some regions of sub-Saharan Africa (figure 2), covering near 36 countries having more than 60 million people. Many of the affected populations live in remote areas having limited access to adequate health services which obstructs the surveillance and hence diagnosis and treatment of cases. Despite its sporadic occurrence among travelers, *T. b. rhodesiense* has been found more common in European and American tourists than *T. b. gambiense* because *T. b. rhodesiense* is present in areas not visited by emigrants. According to recent study, total African population at risk of sleeping sickness is 69.3 million, with one third population being at a 'very high' to 'moderate' risk and rest of the population at a 'low' to 'very low' risk. About 48,000 people died of sleeping sickness in 2008.

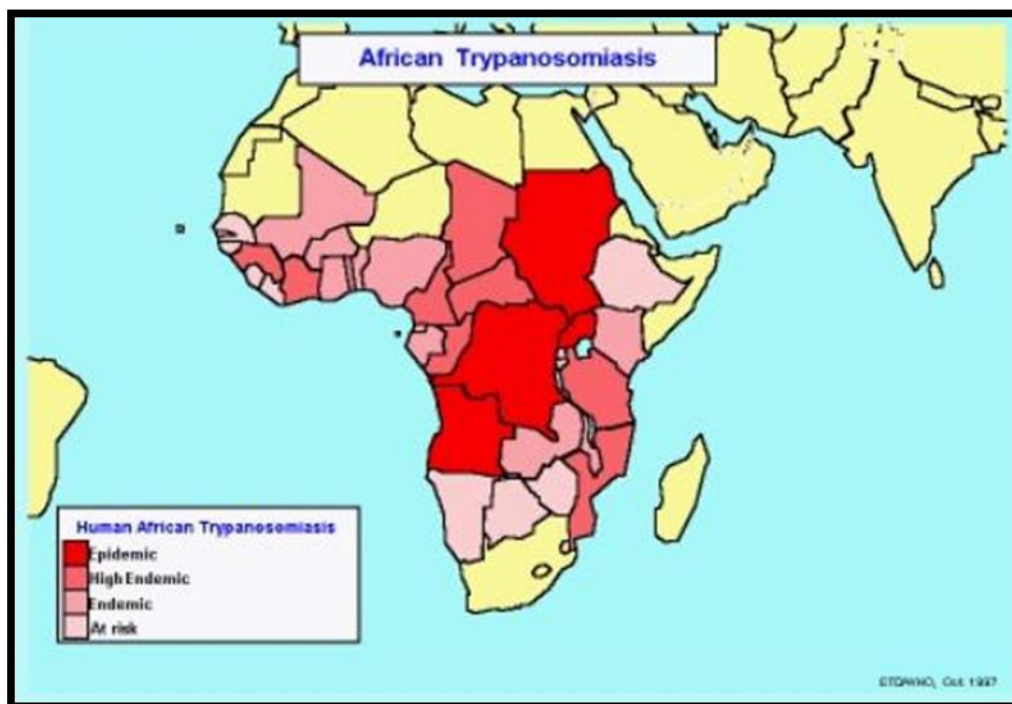


Figure.2: Status of African Trypanosomiasis in Africa

Four major epidemics were recorded in recent history of Africa:

- ✓ First endemic occurred mostly in Uganda and Congo Basin between the year 1896 and 1906.
- ✓ Two epidemics in 1920 in most of the African countries.
- ✓ Third epidemics occurred in 1970 in several African countries
- ✓ Fourth epidemics occurred in 2008 in the Uganda.

WHO (2000 and 2001) established public-private partnerships with Aventis Pharma and Bayer Health Care, which support endemic countries in controlling disease and supply drugs without any cost for the treatment of patients. The efforts of WHO, bilateral cooperation, national control programmes and nongovernmental organizations during the 1990's and in the early 21st century has stopped and reversed the upward trend of new cases of the disease.

6. *Trypanosoma gambiense*

T. gambiense as a human parasite (figure 3) was first discovered by Forde in 1901. Sir David Bruce in 1895 first found that the tsetse fly (figure 4) which is responsible for transmitting the

disease sleeping sickness. *T. gambiense* is confined mainly to Central and West Africa, Particularly in Nigeria and Congo. Areas near the rivers and lakes have the greatest incidence of infection, since the insect vector, the tsetse fly lives there in low and thick vegetation.



Figure.3: Trypanosomes in human blood smear.

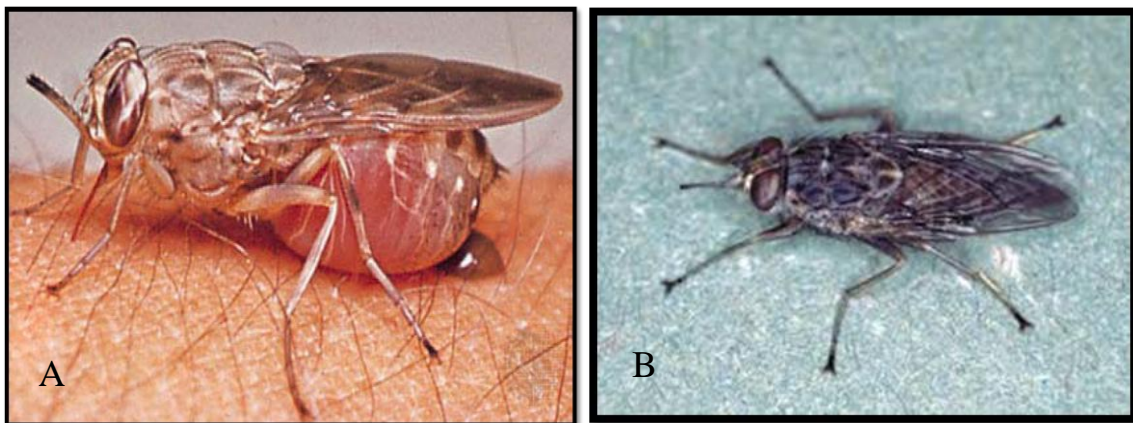


Figure.4: Tsetse fly (A) fly fed with blood (B) fly unfed.

6.1: Morphology:

T. gambiense are microscopic, elongate, flattened and have fusiform body pointed at both ends and covered by a membranous pellicle which maintains the form of body (figure 5). It measures about 10 μm to 40 μm in length and 2.5 μm to 10 μm in width. A single flagellum arises from a basal body situated near the posterior end and curves in a spiral form round the

body forming undulating membrane, thrown into 3 or 4 folds depending upon the length of the parasite. The undulating membrane is believed to be an adaptation for locomotion in the blood. The flagellum is free at the anterior end. The nucleus is large and oval, situated in the centre of the body and the cytoplasm contains numerous greenish refractile granules called volutin granules. These granules store food particles mainly glycogen and phosphate. At the base of the flagellum is located the basal granule or blepharoplast close to which is another granule, the parabasal body.

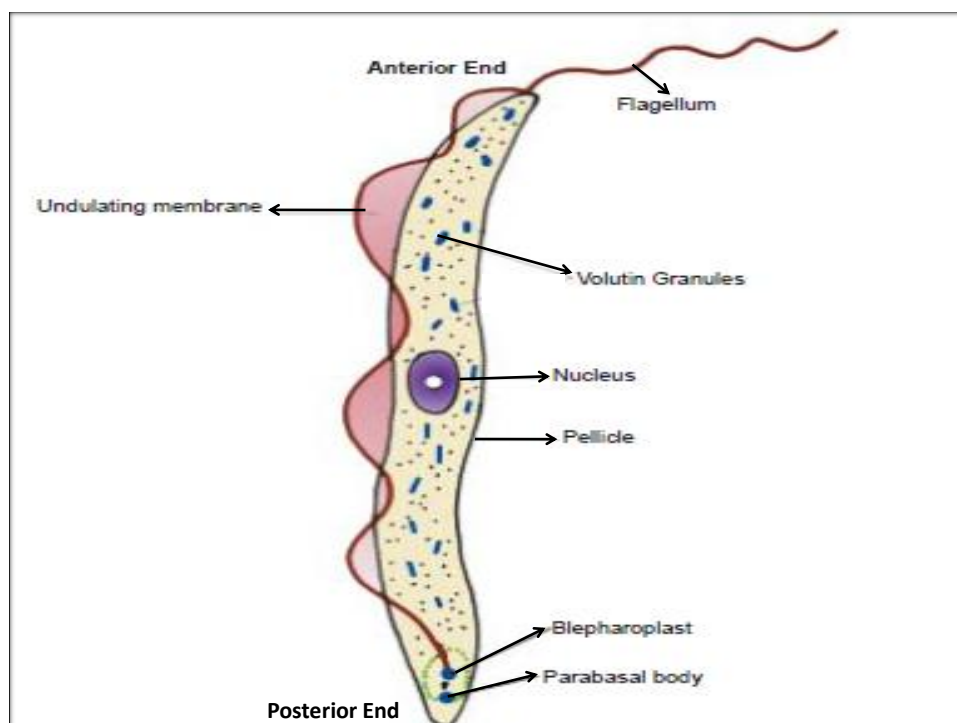


Figure.5: Morphology of *Trypanosoma gambiense*.

6.2: Life cycle:

T. gambiense is a digenetic parasite which requires two hosts for completing the life cycle (figure 6). The primary host is humans and the intermediate host is blood sucking insect Tsetse fly of the genus *Glossina*. The mammals like pig, antelopes and buffaloes often act as reservoir host harbouring the parasite. When Tsetse fly sucks the blood from infected individual or wild mammal, it carries Trypanosomes to its mid gut where they divide asexually by longitudinal binary fission. Here the parasite changes their morphology and give rise to metacyclic forms which are short and stumpy. At this stage, the fly is said to be infective. When the infected tsetse fly bites a healthy human host, it releases these metacyclic

trypanosomes in the blood stream of host and repeats the life cycle. Sexual reproduction is unknown in *T. gambiense*.

It is essentially a parasite of connective tissue in human where it multiplies readily. It consumes large amount of glucose and invades the regional lymph nodes through the lymphatic systems and also invades the blood system causing parasitaemia. It finally localizes in the brain. It is to be noted that African sleeping sickness is a disease which affects the central nervous system.

6.2.1: Life cycle in human:

Infection: The infection by parasite is initiated when tsetse fly harboring the infective metacyclic form, bite the healthy individual. When the fly bites, it releases trypanosomes into blood stream which develop into long slender form and multiply asexually by binary longitudinal fission at the site of inoculation. These become 'stumpy' via 'intermediate' forms. Consequently the parasites invade the blood stream and causes parasitaemia. The trypomastigote forms, mainly the short stumpy forms are taken up by the tsetse fly along with its blood meal and undergo a series of complex biological development inside the insect host before becoming infective to man.

Multiplication: All stages of parasites in humans are extracellular as they are present in the blood cells. In human blood, the metacyclic forms which are devoid of free flagellum become transformed into long slender forms equipped with long flagella. These stages can freely swim by beating of their free flagellum along with the vibratile movements of the undulating membrane. They multiply asexually by longitudinal binary fission and obtain energy by anaerobic process of glycolysis.

Metamorphosis: When absorption of glucose ceases due to antibodies which are produced in blood, is hampered glycolysis. As a result, the trypanosomes stop dividing and shrink to short stumpy forms, which are lacking free flagellum. These stumpy forms do not feed and ultimately die if they are not sucked up by tsetse fly along with the blood meal from infected human.

Relapse of infection: It has also been reported that some of the long and slender forms of trypanosomes do not undergo any transformation, but change their antigen in blood to which the host has produced the antibodies. These unaltered slender forms continue to survive and multiply in blood leading to future relapses of the infection.

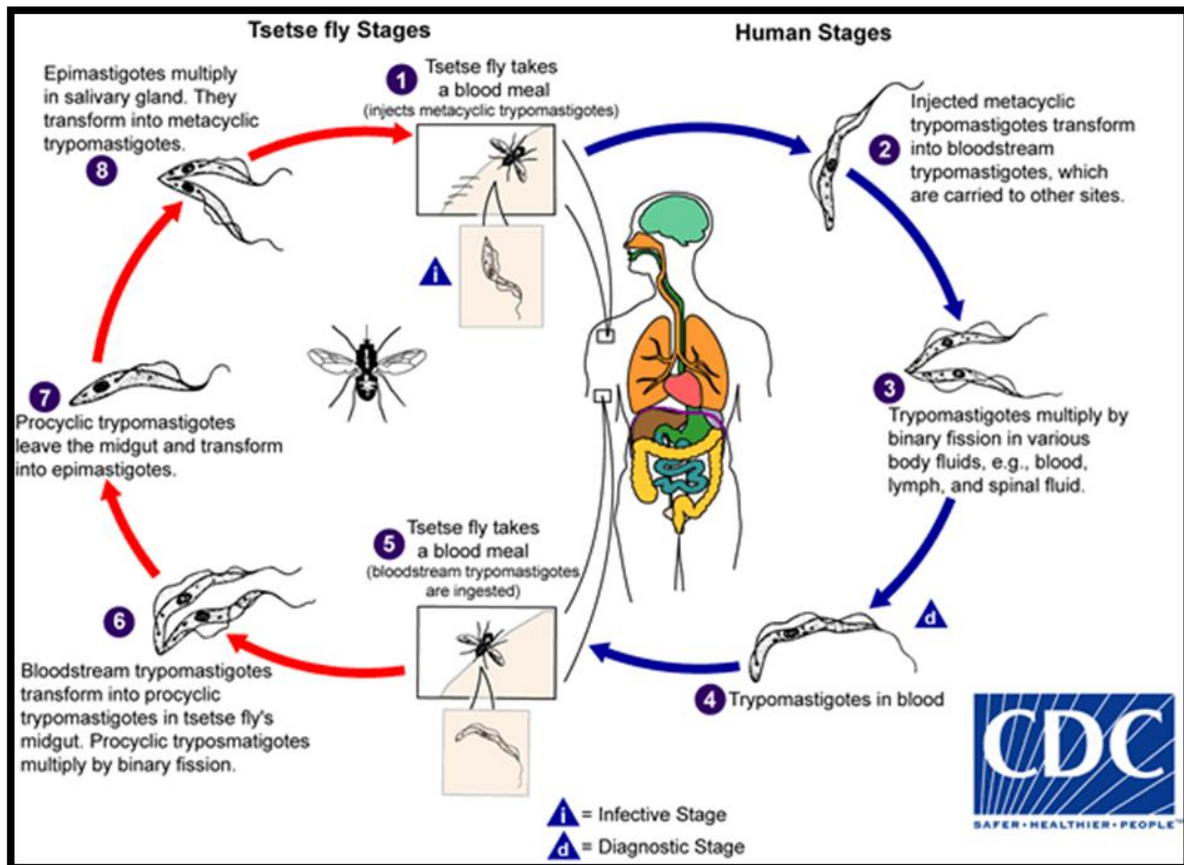


Figure.6: Life cycle of *Trypanosoma gambiense* in human and tsetse fly.

6.2.2: Life cycle in tsetse fly:

Transfer to tsetse fly: When tsetse fly feed on the blood of an infected person, it also sucks short stumpy forms of parasite along with the blood. It is the stumpy forms which continue development in the vector.

Development in mid gut: Further developments of stumpy forms proceeds in the insect mid gut within peritrophic membrane. In the mid gut parasite transforms into long slender form and multiply asexually by longitudinal binary fission. The kinetoplast moves farther from the

posterior end of body. The energy yielding process is related to mitochondrial oxidation of pyruvic acid.

Development in salivary gland: After sometime, the long slender forms migrate into salivary glands via oesophagus and mouthparts of insects. Here, they metamorphose into the crithidial forms with shortened body, reduced free flagellum and the kinetoplast in front of the nucleus. The mitochondria develop an extensive network of cristae and parasite respire more economically as blood glucose gradually declines. The crithidial forms multiply in the lumen of salivary glands and transform into slender metacyclic forms. When the tsetse fly bites a healthy person, it transfers the metacyclic stage into his blood where they initiate another infection.

Sleeping sickness: *T. gambiense* causes the disease of West African sleeping sickness. It is different from American sleeping sickness or Encephalitis which is caused by filterable virus.

7. Pathogenesis

7.1: Mode of infection:

Inoculative method: by the bite of the infective tsetse fly, *Glossina*: Both male and female suck the blood and can transmit the infection. They bite by daylight, usually in the early morning and evening.

The metacyclic stage is introduced by the tsetse fly with the saliva into the subcutaneous pool of blood on which it feeds. Some of the parasites may enter the blood stream directly and majority of them entangled in the tissue space. The initial growth of trypomastigotes occurs in the tissue space which form a favourable nidus or possibly here the organisms can escape the action of antibodies which might be developed. It is to be noted that while the trypomastigotes are multiplying in the subcutaneous tissue, the organisms are either absent or present in small numbers only in the peripheral blood.

It has been suggested that although unlikely yet the connective tissue damage caused by the trypomastigotes may be due to an exaggerated immune response (autoimmune reaction or massive release of kinin) rather than to any direct effect (mechanical damage due to motility)

of this relatively non toxic organism. The presence of trypomastigotes in the subcutaneous connective tissue excites host's immune response in two ways.

- a) By producing large amount of non specific immunoglobulins which are however not capable of sensitizing the antigen. Antibodies are produced in response to the secretion of an exo-antigen of the trypomastigotes.
- b) By heavily infiltrating the site of infection with macrophages, the cells competent to deal with the invaders. The neutrophils take peculiarly little interest in the defense and are therefore not much in evidence.

Thus it will be seen that there is no lack of mobilization of the hosts defensive mechanism but it is the cellular defense which plays the dominant role. The macrophages could be seen to remove the living trypomastigotes in the tissue space. The release of kinins may help to attract macrophages, it also increases the capillary permeability of tissues and may explain the oedematous swollen subcutaneous tissue at the site of infection.

Furthermore, trypanosomes are surrounded by a coat that is composed of variant surface glycoproteins (VSG). These proteins act to protect the parasite from any lytic factors that are present in human plasma. The host's immune system recognizes the glycoproteins present on the coat of the parasite leading to the production of different antibodies (IgM and IgG). These antibodies will then act to destroy the parasites that circulate around the blood. However, from the several parasites present in the plasma, a small number of them will experience changes in their surface coats, resulting in the formation of new VSGs. Thus, the antibodies produced by the immune system will no longer recognize the parasite leading to proliferation until new antibodies are created to combat the novel VSGs. Eventually the immune system will no longer be able to fight off the parasite due to the constant changes in VSGs and infection will arise.

7.2: Clinical features:

Bite of tsetse fly causes local irritation which subsides after few days. A trypanosomal chancre may develop at the site of inoculation of trypomastigotes introduced by the bite of the infected tsetse fly. It is a hard painful nodule and fluid withdrawn from it contains actively dividing trypomastigotes. It subsides in a week or two without suppurating. The

symptom can appear after several months or a year in Gambian form but symptoms may appear after two weeks in case of Rhodesian form.

It is characterized by the infection of blood stream, involvement and enlargement of lymph nodes and eventually invasion of the central nervous system. The early symptoms are fever, loss of nocturnal sleep, severe headache, and feeling of oppression. A fleeting circulate erythematous rash may appear on the chest and shoulder.

Lymph node enlargement, particularly of the posterior triangle of the neck is a feature of Gambian disease whereas invasion of CNS is very rapid in case of 'rhodesian' form. As the CNS is involved, the symptoms of meningo-encephalitis develop resulting in classical sleeping sickness (figure 7). In due course, the patient fall asleep, first at regular interval and then lies prostrate in coma. Finally, the patient becomes thin and exhausted, accompanied by signs of malnutrition. Disruption of the sleep cycle is an important symptom of this stage that gave the disease the name 'sleeping sickness'.



Figure.7: A child suffering from sleeping sickness.

The person infected from disease experience unsystematic and uneven 24-hour rhythm of the sleep-wake cycle. The patient sleeps in daytime and at night time shows periods of wakefulness. Other neurological symptoms of the disease include tremor, confusion,

paralysis, general muscle weakness, hemiparesis and paralysis of a limb. Parkinson like movements may also arise due to non-specific movement and speech disorders. The person infected from sleeping sickness may also exhibit psychiatric signs like aggressive behaviour, irritability, psychotic reactions or apathy which can sometimes dominate the clinical diagnosis. If the disease is not treated, it can invariably become fatal, with progressive mental deterioration that leads to coma, systemic organ failure and finally death. In case of *T. b. rhodesiense*, an untreated infection will lead to death within few months; however, infection with *T. b. gambiense* will lead to death of the patient after several years when left untreated. Tryptophol is a chemical compound which stimulates sleep in humans. It is the chemical that is produced by the trypanosomal parasite in sleeping sickness. The major mode of transmitting the disease is the bite of an infected tsetse fly but there are several other ways through which people are infected with sleeping sickness.

- ✓ The infection can be spread from pregnant mother to her child because the trypanosomes are able to cross the placenta and cause the disease to the fetus.
- ✓ The mechanical transmission is also possible through other blood sucking insects. However, assessment of epidemiological impact of transmission is very difficult.
- ✓ Accidental infections may also be possible in the laboratories due to pricks from contaminated needles.

7.3: Disease management:

Disease management can be done in three major steps.

- ✓ The first step is the screening for potential infection which can be done by serological tests (only available for *T. b. gambiense*) and confirms major symptoms such as swollen cervical glands.
- ✓ The second stage is to diagnose the presence of the parasite.
- ✓ The last step is staging, which is done to find out the state of disease progression. This involves collection and examination of cerebro-spinal fluid from lumbar region which helps in determining the course of treatment.

7.4: Treatment:

The Gambian or African trypanosomiasis or sleeping sickness can be treated during early stage but once the parasites enter into the cerebrospinal fluid of central nervous system, it is

very difficult to control the disease. There are a number of drugs that are used to control the disease. Drugs such as suramin sodium, Bayer 205, Atoxyl and Tryparsamide have been useful in early stages of infection. Other drugs such as Parsenophenyl butyric acids, germanin and pentamidine are also effective in treatment of early cases. Orsanine is quite effective in cases where the Central nervous system is involved. Melarsen oxide is rapid in action and is less toxic.

Suramin (discovered in 1921 and used to treat *T.b. rhodesiense* sleeping sickness) and pentamidine (discovered in 1941 and used to treat *T.b. gambiense* sleeping sickness) are considered to be the drugs of choice for early acute infection. These drugs cannot pass the blood brain barrier, so they are not of any value when the CNS is involved in which case arsenical is needed. The arsenical includes melarsoprol (discovered in 1949) which is used in both forms of infection but show undesirable side effects in the patients. Other effective drug is Eflornithine (registered in 1990) which is less toxic than melarsoprol and effective against *T.b. gambiense*.

For Rhodesian infection IV suramin in a dose of 1gm for 1st, 3rd, 7th 14th, 21st should be given after a test dose of 100 mg- 200mg.

For Gambian infection IM pentamidine in a dose of 4mg/kg/day for 10 days is recommended. In case of neurological involvement of both Rhodesian and Gambian infection melarsoprol in a dose of 2-3.6mg/kg/day by IV route for 3 days followed by 2nd course of 3 days at an interval of 7-10 days is prescribed. The dose of the drug in second course is given 3-6 mg/kg/day for 3 days. 3rd course can be repeated 15-21 days later.

Eflornithine in a dose of 400 mg/kg/day in four divided doses for 14 days also give encouraging result in cases of Gambian infection with CNS involvement.

7.5: Prophylaxis (Prevention):

Prevention depends upon the eradication of vector i.e., the tsetse fly. It can be eradicated by destruction of the habitat of the vector. It is supplemented by the use of insecticides such as spraying of DDT over bushy areas in the vicinity of villages.

Game destruction program to eliminate the blood meal of the fly and isolation of the human population from areas known to harbor infective animals can be done to prevent the spreading of the disease. This is much more important in Gambian disease.

Chemoprophylaxis: Pentamidine is effective against Gambian disease. A single intramuscular injection of 4 mg/kg will provide a preventive effect for at least six months.

Further protection is obtained by injecting one gram of Suramin (Antrypol) every two or three months.

8. Summary

Human African trypanosomiasis is a vector-borne parasitic disease caused by the protozoa which belongs to the genus *Trypanosoma*. The disease sleeping sickness is endemic and highly prevalent in some regions of sub-Saharan Africa, covering near 36 countries having more than 60 million people. Four major epidemics occurred in recent history in Africa.

T. gambiense as a human parasite was first discovered by Forde in 1901 and is confined mainly to Central and West Africa Particularly in Nigeria and Congo. The parasite initially lives in the blood stream and lymph glands of the patient and later invades the cerebrospinal fluid of the CNS. The parasite is transmitted by vector, *Glossina palpalis*.

T. gambiense are microscopic and covered by a membranous pellicle. It also bears a single flagellum which curves in a spiral form round the body forming undulating membrane adapted for locomotion in the blood.

T. gambiense is a digenetic parasite which requires two hosts human and tsetse fly for completing the life cycle. When Tsetse fly sucks the blood from infected individual, the parasite divide asexually in the mid gut of the insects by longitudinal binary fission and forms metacyclic trypanosomes which are infective and ready to infect new host. When the fly bites, it invades the regional lymph nodes through the lymphatic systems and also invades the blood system, finally localizes in the brain and affect CNS

Lymph node enlargement, particularly of the posterior triangle of the neck is a feature of Gambian disease whereas invasion of CNS is very rapid in case of 'rhodesian' form. The symptoms of meningo-encephalitis develop, resulting in classical sleeping sickness in which the patient falls asleep in initial stage at regular interval and then lies prostrate in coma. Disruption of the sleep cycle is an important symptom of this stage that gave the disease the name 'sleeping sickness'.

There are a number of drugs that are used to control the disease. Drugs such as suramin sodium, Bayer 205, Atoxyl and Tryparsamide have been useful in early stages of infection. Other drugs such as Parsenophenyl butyric acids, germanin and pentamidine are also effective in treatment of early cases. Orsanine is quite effective in cases where the Central nervous system is involved. Melarsen oxide is rapid in action and less toxic.