East African Regional External Quality Assessment Scheme (EA-REQAS)

Learning Sheet Number Four

AFRICAN TRYPANOSOMIASIS

INTRODUCTION

Human African Trypanosomiasis, commonly known as *Sleeping Sickness,* is an infectious disease caused by protozoa of the genus *Trypanosoma.* There are two main morphologically identical species: *Trypanosoma brucei rhodesiense* and *Trypanosoma brucei gambiense.* Trypanosomes are transmitted to humans by bites of tsetse flies (*Glossina* genus) that have acquired the parasite from infected humans or animals. Trypanosomes have a complex life cycle, with part of their development in the digestive tract of tsetse flies. Both sexes of tsetse fly feed on blood.

Trypanosomes remain a constant threat to the lives of humans, cattle, and other domesticated animals throughout sub-Saharan Africa. Rural populations living in areas where transmission occurs, who depend on agriculture, fishing, animal husbandry and hunting, are the most exposed to tsetse fly bites. Displaced populations, war and poverty are important factors leading to increased transmission. *T. brucei gambiense* is more common in West and Central Africa and represents more than 90% of reported cases. *T. b. gambiense* causes a chronic infection and a person can be infected for months or years without major signs or symptoms of disease. On presentation, patients are often in an advanced stage of the disease with central nervous system involvement. *T. brucei rhodesiense* is more common in eastern and southern Africa and represents less than 10% of reported cases. *T. b. rhodesiense* causes an acute infection, with first signs and symptoms occurring a few months or weeks after infection. This disease develops rapidly to involve the central nervous system (CNS). Trypanosome infections are invariably fatal if left untreated, taking weeks or years depending on the species and virulence of individual strains.

There are no vaccines or modern drugs to treat trypanosomiasis. The few drugs available have their origins in the first half of the 20th century and are all very toxic. The limiting factor for drug development is the lack of resources and motivation for manufacturing new drugs for exotic diseases in distant countries that lack the ability to pay for them. Trypanosomiasis is regarded as one of the world's neglected tropical diseases.

American Trypanosomiasis or Chagas' disease occurs in Central and South America. The causal organism, *Trypanosoma cruzi,* is transmitted by reduvid bugs and is a different species of trypanosome from those causing Human African Trypanosomiasis. Chagas' disease causes heart disease. Chagas' disease does not occur in Africa.

CLINICAL FEATURES

Trypanosomes multiply in the subcutaneous tissues at the site of the tsetse fly bite, and then spread to lymph and blood – the first stage (haemo-lymphatic phase). In time, the parasites cross the blood-brain barrier and infect the central nervous system (CNS) – the second stage (meningo-encephalitic phase). Other routes of infection include:

- Mother-to-child infection: trypanosomes can cross the placenta and infect the foetus.
- Accidental infections have occurred in laboratories due to pricks from contaminated needles.

During the first stage of the disease, patients complain of fever, headaches, joint pains and itching. During the second stage, patients develop confusion, sensory disturbances and poor coordination. Disturbances of the sleep cycle, which give the disease its name, are an important feature of the second stage.

Since the clinical features are non- specific, the diagnosis must be confirmed by laboratory testing. Diseases such as malaria, enteric fever, tuberculosis, meningitis and HIV infection may mimic or even coexist with trypanosomiasis. Staging is a key step that classifies the patient into the first or second stage of the disease. The diagnosis and staging of the disease must be highly accurate as melarsoprol, the most widely used treatment for second stage trypanosomiasis, is highly toxic.

LABORATORY DIAGNOSIS OF TRYPANOSOMIASIS

Parasitological diagnosis

Microscopic examination of chancre fluid aspirate, lymph node aspirate, blood or CSF provides direct evidence of trypanosome infection. Parasites are examined in wet (motile parasites) or Romanowsky-stained (fixed parasites) preparations. Parasite numbers in blood in *T. b. gambiense* infection may be below the microscopic detection limit; therefore failure to demonstrate parasites does not exclude infection. Serial examination of blood on consecutive days increases test sensitivity. When possible, concentration methods should be used to improve sensitivity of parasite detection. It is essential to keep time between sampling and examination as short as possible as immobilisation and lysis of trypanosomes occur in a few hours. Trypanosomes are rapidly killed by direct sunlight but can survive for a few hours if the sample is kept in a cool, dark place.

***1. Chancre aspirate*:** Trypanosomes can be detected in chancre fluid a few days earlier than in blood. This method is seldom applied in the field because most infections are detected much later, when the chancre has resolved.

***2. Lymph node aspirate*:** Detection of enlarged lymph glands is performed systematically in all patients with suspected trypanosomiasis. Sensitivity varies between 40 – 80% depending on the parasite strain and stage of disease (sensitivity is higher during the early stages).

***3. Blood films*:** Trypanosomes can be seen moving in wet blood film preparations – the movement of the surrounding erythrocytes attracts attention. Examination of 20µl of blood as a stained thick blood film improves sensitivity and is the technique of choice for blood examination when no centrifuge is available. Apart from trypanosomes, other parasites such as microfilariae and *Plasmodium* spp. may also be detected.

4. Microhematocrit centrifugation: Trypanosomes are concentrated in the white blood cell layer (buffy coat) between the plasma and the erythrocytes. Capillary tubes, mounted in a special holder, are directly examined at low magnification for motile parasites. The sensitivity of the technique increases with the number of tubes examined; usually 6 – 8 tubes are prepared for each patient. The presence of microfilariae in blood can make visualisation of the much smaller trypanosomes difficult.

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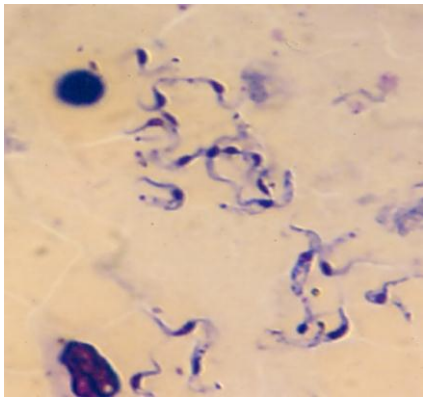
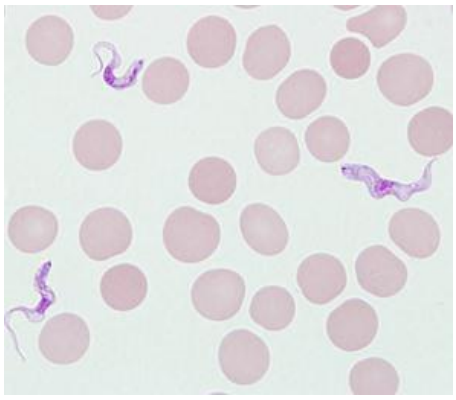
5. Cerebrospinal Fluid (CSF) Examination: Staging of patients relies on examination of CSF. CNS involvement is defined by the presence of one or more of the following in CSF: (i) raised white blood cell count (>5 cells/µl), (ii) trypanosomes (iii) increased protein content (>370 mg/litre).



Trypanosoma spp in a thick blood film



Trypanosoma spp in a thin blood film

Serological testing

Serological tests are used for screening patients and establishing suspicion of infection. Current serological tests detect antibodies in blood 3 – 4 weeks after infection. Seropositivity must be interpreted with caution in previously treated patients as antibodies may persist for up to 3 years after cure. Serological tests may also be useful for epidemiological surveys.

1. CATT/T. b. gambiense: the Card Agglutination Test for Trypanosomes (CATT) is a fast and simple agglutination assay for detecting *T. b. gambiense*-specific antibodies in blood, plasma, or serum. There is still no equivalent to the CATT widely available screening for *T. b. rhodesiense* infection. The *LATEX/T. b. gambiense* has been developed as an alternative to the CATT. A new lateral flow rapid diagnostic test (RDT) for *T. b. gambiense* has been developed that is of low cost and stable at a temperature of 40°C for up to 2 years and is currently under evaluation.

2. Antibody detection in CSF: CSF in second stage patients contains high levels of immunoglobulins, especially IgM, which can be used as a strong marker for second stage disease. Unfortunately, IgM detection in CSF is not readily carried out in the field due to lack of simple and robust tests. However, a latex agglutination test for IgM in CSF (LATEX/IgM) has recently been developed for field use.

3. Other serological tests: immunofluorescent antibody (IFA) and enzyme-linked immunosorbent assay (ELISA) tests can be performed with serum, filter paper eluates and CSF. The sophisticated equipment required for IFA and ELISA methods limits their use to reference laboratories for remote testing of samples collected during surveys.

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TREATMENT

First stage treatment

- **Suramin:** Suramin is the drug of choice for both species of infection in first stage disease, and is given as 1 g intravenously on spaced days over a 3 week period, following a test dose of 200 mg. Suramin must be given at least 24 hours before lumbar puncture to prevent contamination of the CSF with trypanosomes. Suramin may cause allergic reactions.
- **Pentamidine**: A daily 200 mg intramuscular injection of pentamidine is given for 7 – 10 days for treating first stage *T. b. gambiense* infection. Despite a few undesirable effects, it is well tolerated.

Second stage treatment

- **Melarsoprol**: Intravenous injections of 3.6 mg/kg per day are given in courses of 3 days duration with 7 day rest periods in-between. The drug is derived from arsenic and is highly toxic; the safety margin is low. The most severe side effect is a reactive encephalopathy which can be fatal (3 – 10% of cases).
- **Eflornithine**: Intravenous infusions of 200 mg are given daily for 14 days. The drug is only effective against *T.b. gambiense,* but is an alternative to melarsoprol treatment. The regimen is strict and difficult to apply.

CONTROL OF TRYPANOSOMIASIS

Trypanosomiasis control programmes are based on the elimination of the parasite human reservoir by mass screening of the population and treatment of all infected persons. Therefore the availability of accurate, practical, and cheap screening and confirmatory tests is vital. Control programmes undertake the following activities:

1. Reduction of human-fly contact; cutting trees around water contact points 20 metres each side of the banks and for 200 metres up and downstream of crossing points.
2. Active case finding and treatment.
3. Use of tsetse fly traps impregnated with insecticide and placed at high human-fly contact points.
4. Elimination of adult tsetse flies using insecticides. The most effective method is aerial spraying with insecticide.

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