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African sleeping sickness in

The Democratic Republic of Congo

- On the edge of an outbreak?

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1. Abstract

1.1 Abstract in English

Background: African sleeping sickness is one of the World Health Organizations (WHO) defined neglected tropical diseases. It is endemic in 36 sub-Saharan Africa countries. Even though the incidence has decreased in all these countries the last decade, the incidence of infected travelers is increasing due to increased tourism. The disease is challenging to diagnose, and is regarded as fatal if left untreated.

Material and methods: This is a literature review based on systematic search in PubMed. In addition cross-section surveys were carried out in Zanzibar (Tanzania), a former HAT-endemic island (n=50) and in Kasongo (the Democratic Republic of Congo, DRC), a current HAT-infected area (n=50). Clinical experience was obtained in different health care centers and hospitals in Kinshasa (DRC) and Kasongo (DRC). We interviewed people on all levels working with HAT. E.g the health minister of Maniema, the leader of the Belgium partner (BTC-CTB) and mobile team workers in Kinshasa, and remote Kasongo.

Results and principal findings: Human African trypanosomiasis (HAT) still poses a major problem in the remote and poorest parts of Africa. The lack of vaccine and prophylaxis against HAT makes it even more important with sufficient prevention and control programs. The disease is caused by the parasite of the species *Trypanosoma brucei*. Its vector is the tsetse fly. The disease is divided into two stages, separated by whether trypanosomes are present in the central nervous system or not. The first stage presents a fever illness. The second stage presents several of neurological symptoms, such as sleeping disorder. The clinical feature alone is insufficient to set the diagnosis, and the diagnostic tools presented today do not show enough accuracy. The treatment of late stage HAT has severe side effects. The last 60 years has only seen one new drug for HAT. We found the awareness of HAT in Zanzibar to be limited to 30%. In Kasongo 78% of the population were found to have knowledge of HAT. Among those who had knowledge, only 32% knew that the tsetse fly was the transmitter.

Conclusion: As the incidence of HAT is decreasing, so is the attention. HAT may well go from being a neglected disease to an ignored disease. Thus we cannot rule out the possibility of a new outbreak. To prevent an increase of HAT, or even a new epidemic, it is of great importance to maintain the prevention and control programs. The development of more specific diagnosis and staging tools is urgent. The Holy Grail will be the development of a vaccine, though there is still a long way to go.

1.2 Abstract in Norwegian

Bakgrunn: Afrikansk sovesyke er av som WHO definert blant neglisjerte tropisk sykdommer. Sykdommen er endemisk i 36 land sør for Sahara. Selv om sykdommen har vært i tilbakegang i alle disse landene det siste 10-året, er forekomsten økende blant reisende grunnet økt turisme. Sykdommen er utfordrende å diagnostisere, og er ansett som fatal dersom den ikke behandles.

Material og metode: Dette er en litteraturstudie basert på systematisk søk i PubMed. I tillegg ble det utført en tverrsnittstudie i Zanzibar (Tanzania), en tidligere endemisk øy (n=50), og i Kasongo (DRC), et nåværende endemisk området (n=50). Klinisk erfaring ble oppnådd ved ulike helsesentre og sykehus i Kinshasa (DRC) og Kasongo (DRC). Vi intervjuet personer på ulike nivåer, som arbeider med afrikansk sovesyke. Dette inkluderer helseministeren i proviensen Maniema (DRC), lederen av de belgiske partnerne (BTC-CTB) og lederen for de mobile enhetene i Kinshasa og Kasongo.

Resultater: Afrikansk sovesyke utgjør fortsatt et stort problem i de avsidesliggende og fattigste delene av Afrika. Mangelen på vaksine og profylakse mot sovesyken, gjør det spesielt viktig med tilstrekkelige prevensjon- og kontrollprogrammer. Sykdommen er forårsaket av parasitter i arten *Trypanosoma brucei*. Dens vektor er tsetsefluen. Sykdommen deles inn i to stadier, avhengig av om parasitter er til stede i sentralnervesystemet eller ikke. Det første stadiet presenterer seg som uspesifikk febersykdom. Det andre stadiet er karakterisert ved et utall av neurologiske utfall og symptomer, som forstyrrelse i søvnmønsteret. Den kliniske presentasjon er alene ikke nok til å stille diagnosen. Samtidig viser ikke dagens diagnostiske tester tilstrekkelige testegenskaper. Behandlingen av stadium to har alvorlige bivirkninger. De siste 60 årene har det kun kommet et nytt medikament for behandling av afrikansk sovesyke. Vi fant at 30 % på Zanzibar hadde hørt om sykdommen. I Kasongo (DRC), var 78 % klar over sykdommen. Av disse viste 32 % at tsetsefluen var vektor for sykdommen.

Konklusjon: Ettersom forekomsten av Afrikansk sovesyke reduseres, vil også oppmerksomheten rundt sykdommen reduseres. Sovesyken er i fare for å gå fra å være en neglisjert til å bli en ignorert sykdom. Vi kan derfor ikke utelukke mulighet for et nytt utbrudd. For å forhindre økende insidens, eller til og med en ny epidemi, er det svært viktig å opprettholde dagens prevensjons- og kontrollprogrammer. Utviklingen av bedre diagnostiske verktøy er av avgjørende betydning. Den hellige gral er utviklingen av en vaksine, dog er veien mot dette fortsatt lang.

2. Abbreviations

AAT	Animal african trypanosomiasis
CNS	Central nervous system
CSF	Cerebrospinal fluid
DDT	Dichloro-diphenyl-trichloroethane
DRC	Democratic Republic of Congo
GIS	Geographical information system
GPS	Global position system
HAT	Human African trypanosomiasis
HCB	Hexa-chlorobenzene
INRB	Institut National de Recherche Biomédicale
NECT	Nifurtimox-eflornithine combination therapy
NGO	Non-governmental organization
NSSCP	National sleeping sickness control programmes
NTD	Neglected Tropical Diseases
PATTEC	Pan African tsetse and trypanosomiasis eradiction campaign
PM	Peritrophic matrix
ROS	Reactive oxygen species
S1	Stage 1
S2	Stage 2
SAT	Sequential aerial techniques
SIT	Sterile insect technique
sqm	Square metre
T&T	Tsetse and trypanosomiasis
TB	Trypanosoma brucei
TBB	Trypanosoma brucei brucei
TBG	Trypanosoma brucei gambiense
TBR	Trypanosoma brucei rhodesiense
WHO	World health organisation

3. Introduction

According to the World Health Organization (WHO), human African trypanosomiasis (HAT) is the deadliest disease found on the earth (1). No vaccine is developed (2), and there is no commercial interest in developing new drugs. Three of four main drugs used in treatment were registered before 1950. The most recent drug was initially developed as an anti-cancer drug, only by coincidence found to be effective against HAT. HAT is a disease which the poorest of the poor are suffering from (3).

This devastating disease is caused by a parasite, the trypanosome. Its vector is the tsetse fly. The disease is divided into two different forms, based on the geographical and on the clinical picture. The West African sleeping sickness, the chronic form, is found in central and west Africa and caused by *Trypanosoma brucei gambiense* (TBG). The east African, the acute form, is found in the east and the southern parts of Africa, and caused by *Trypanosoma brucei rhodesiense* (TBR). TBG is today responsible for more than 95% of all HAT cases. 36 sub-Saharan countries are today regarded as endemic for HAT (4).

The disease is divided into two stages, ultimately divided by whether parasites are present in the central nervous system (CNS) or not. The two different form of sleeping sickness generally present with the same symptoms, but at a different progression. Both forms are regarded as fatal if left untreated. East African sleeping sickness has a median survival time of 6-12 months untreated, whereas untreated west African sleeping sickness has a median survival time of three years (5, 6).

4. Material and method

4.1 Literature search strategy

This paper was put together largely based on systematic search in PubMed (figure 1) done in March/April 2012. In addition, an active outreaching approach was carried out, seeking information in several of books and different Internet sites of interest. At all time with particular focus on DRC.

4.2 DRC

DRC has 80% of the world's HAT cases (12). To get a complete understanding why this is, a voyage to DRC was necessary. The first week was spent in Kinshasa, the second in Kindu and Kasongo, villages belonging to the Manemia province. During the two weeks in DRC a medical student, Paul Tunda, was our friend, collaborator and translator. Provincial parliament member Honorable Chef Tunda arranged the practical part with permissions, meetings and transport. 92% ($ 13 000) of the costs of this study are self-financed. The remaining part is contributed from the University of Tromsø.

4.2.1 Institut National de Recherche Biomédicale (INRB), Kinshasa

We spent valuable time at the INRB by the help of Dr. Dieudonné Mumba Ngoyi, from whom we got the latest epidemiological numbers of DRC. We were demonstrated the screening test for west African sleeping sickness, CATT, and got the opportunity to observe living trypanosomes in the microscopy, originating from infected mice. Furthermore we gained knowledge on the research done by the INRB concerning African trypanosomiasis.

4.2.2 Hospitals and meetings

At Kinshasa University Hospital we examined stage 2 HAT patients. At Kasongo hospital we examined stage 1 HAT patients. Both in Kinshasa and Manemia we interviewed people dealing with HAT. Professors, doctors, nurses, leaders of mobile teams, provincial health minister, The Belgian Development Agency (BTC-CTB) representative, were our objects. The field work gave us clinical experience.

The meetings gave us valuable information. Some of it used in this paper as personal communication.

4.2.2 Surveys

At Zanzibar and in Kasongo we made cross-section surveys (n=50 x 2). We did not distinguish between sexes, while age was registered. Despite the selection is too small to conclude with a statistical accuracy, one get an idea of the awareness of HAT.

4.2.2.1. Zanzibar

Zanzibar is an earlier endemic HAT island. The spoken language is Swahili and the English knowledge is limited. The survey (figure 34) was sustained in Jambiani, a village east on the island. Inclusion criteria were knowledge of English and belonging to Zanzibar. The survey was sustained orally. If the word sleeping sickness was unknown, the Swahilian word *malale*, meaning sleeping sickness, was used**.**

4.2.2.2. Kasongo

Kasongo is an HAT endemic village in Manemia. Manemia is one of eleven provinces east in DRC. The survey (figure 6 and 7) was sustained orally. Paul Tunda translated the questions to Swahili, the spoken language in Kasongo. Inclusion criteria were belonging to Kasongo.

4.3 Other information

WHOs long time HAT expert prof. dr. Perrez Simarro has aided us with accuracy in the result part together with Prof. Reto Brun, Ph.D, Head of Parasite Chemotherapy, Swiss Tropical and Public Health Institute.

5. Results

5.1 Epidemiology

Where tsetse fly can be found, chances are HAT is also present. The distribution of HAT coincides with the occurrence of the sub-Saharan tsetse fly. Co-existence between humans and trypanosomiasis is well known, and demonstrated by the fact that humans are resistant against all the trypanosomes, with exception of TBR and TBG, whereas none of the arboreal primates are resistant (13).

5.1.1 Historical epidemiology

5.1.1.1 The pre-colonial period

The written records of the pre-colonial period are limited (14). The first written account of HAT was made by Yaqut in the 11th century. The 11th century saw extensive trading between Arabic peoples and the Wangarian people in West Africa. It was in this period the Arabian geographer Abu Abdallah Yaqut (1179-1229) made a description from a village in the region: *The people and even their dogs where just skin and asleep* (15). The description suggests that HAT had a huge impact even before the start of the western colonization of Africa. However no serious epidemics, such as the ones seen in the 19th and 20th centuries, are known prior to the colonization (14).

5.1.1.2 The colonial period

Huge sleeping sickness epidemics occurred in both west and east Africa subsequent to colonization by the European powers in the 19th and the 20th centuries (14). There were 3 major epidemics of sleeping sickness: 1) 1896-1906, 2) 1920-1940 and 3) 1970-1990 (13). During the colonial period it is estimated that several millions died as a consequence of African sleeping sickness, but official records of the exact number of the disease in that period are lacking. To understand the negative influence of this disease we can compare the sleeping sickness epidemics with the HIV/AIDS epidemic today. The main reason for this enormous devastating epidemics is thought to be caused by socio-economic changes seen during the colonization (14). Even though the first epidemic made the colonial authorities start an enormous investigation into the disease, it ended

up as a fatal epidemic, e.g. causing the death of 2/3 of the population of Uganda. This first epidemic forced the colonial government to search for the cause of the disease and a treatment for it. In 1903 they found the parasite in a human, and further on in 1905 the first man was cured of HAT (16).

The first and second epidemics

The first epidemic mainly affected Uganda and Congo Free State, now known as DRC. Estimates have been made saying that between 300 000 and 500 000 died. Since they had discovered the microbiologic agent and they knew that the tsetse was the vector, a major preventive program was started. In British Uganda this was carried out hard-handedly. Because the vegetation around the lake provided typical habitat for the tsetse, the inhabitants around Lake Victoria were forcibly removed from their home villages (16).

Further on in this part of the article we will have a look at new cases of the disease in the period 1920 to 1997 (figure 2).

The WHO describes a second epidemic that started in the 1920s, which affected a number of African countries. It lasted until 1940. The epidemic was controlled thanks to mobile teams organizing the screening of millions of people at risk. By the mid-1960s, the disease had almost disappeared (18). As we can see from figure 2 the incidences of HAT in 1960 was historically low. But three decades later an incidence of the same scale as the one in the 1920s was reported.

5.1.1.3 The post-colonial period

Third epidemic and the new era against HAT

The current situation in 1996: 40 000 cases of HAT were reported, but estimates were that 400 000 cases were undiagnosed and therefore untreated (19). The main reason for this new HAT epidemic was that in the late 1960s African countries started breaking free from the European colonial powers. The newly established countries did not focus on the rare illness that HAT had become (17).

In 2001 Dr. Gro Harlem Brundtland, WHO Director-General said: *We can now look forward to halting the spread of sleeping sickness* (17). This seems odd compared to

the devastating increase seen in figure 2, and the incidence of HAT in 1996. What had happen and what happened?

1997: WHO passed a resolution which strongly advised access to diagnosis and treatment and the reinforcement of surveillance and control activities (17).

2000: The Pan African Tsetse and Trypanosomiasis Eradiction Campaign (PATTEC) was initiated by the African Union summit in Lomé. PATTECs aim was to make Africa a tsetse and trypanosomiasis free zone (20).

2001: WHO formed a private partnership with the two pharmaceutical companies Sanofi-Aventis, which produce pentamidine®, melarsoprol® and eflornithine®, and Bayer AG which produce suramin® and Nifurtimox® (21). The partnership resulted in distribution of HAT drugs needed to treat patients in all endemic countries, free of charge (21, 22).

2003: World Health Assembly (the decision-making body of WHO) gathered the member states to support the effort to eliminate the disease as a public health problem. This led the WHO program to intensify its coordination efforts, bringing together non-governmental organizations (NGOs), national control programs and research institutions, as well as private and public contributors (17).

2006: 20 of the 36 endemic countries achieved the target of reporting no new cases (19).

2009: After continued control efforts, the number of cases reported has dropped below 10 000 (9878) for first time in 50 years (19).

2010**:** The Democratic Republic of Congo (DRC) alone declared over 5000 new cases per year (23). 7139 new cases of HAT were reported, but WHO estimates that the actual number are 30 000 (19).

Many factors need to be taken into consideration in order to get the correct understanding of how the HAT problem, as we have seen, has decreased. But all over it is important to have the political will to eliminate HAT. It is also important to remember that the first decade of the 21st century saw fewer civil wars and better social conditions than 1980-2000 all over Africa (24), facilitating access to diagnosis and treatment easier.

WHO reports that 36 countries are endemic to HAT (19), even though some of them have no reported cases from the last decade (17) (table 2 and 3). The meaning of the word endemic is *steady state of a disease*. $E = R_0 + S = 2$, where R_0: is Basic reproduction number. S: susceptible individuals. In 2006, 20 countries did not report any new cases of HAT. WHO still reports them as being endemic, despite that R_0 is 0. In an epidemiology approach it seems that HAT is eliminated, but the statistics are built on reported cases, and WHO highlights that we overall have to multiply the reported HAT cases with 10 to get the true number of incidences.

5.1.2 Current epidemiology

Since TBG and TBR have different etiology, epidemiology and distribution, distinguishing the two forms of HAT is important. Today TBR are found in east Africa and TBG are endemic in western and central Africa(3). Roughly, the two forms are separated by the great rift valley (25) (figure 3). Uganda is the only country where both forms of HAT are found. Even though they currently are separated in Uganda, a spread of TBR towards the northwest is seen, which means that we may see an overlap of the two forms in the future (4).

5.1.2.1 The true burden of TBG today

Table 2 and table 3 show a decrease in new reports of HAT. Of all HAT cases 95% off them are caused by TBG, the West African sleeping sickness. Even though many endemic countries have not detected any new HAT cases the last 10 years, HAT still is a major problem in DRC, Angola and Sudan (19). DRC alone reported 5018 new cases in 2011 and 80% of all HAT cases are found in DRC (12) (figure 4).

TBG in DRC

DRC is Africa's second largest country (26) with a landmass of 2.3 million km^2 (27). This could to some degree explain all the HAT cases (figure 5). From 2011, the new cases are not equally dispersed across the county. DRC is divided into 281 health zones, where 26 of them report over 200 new cases, illustrating that some zones are more heavily affected, compared to others, where no cases are reported.

Agriculture and infrastructure

WHO states that "the people most exposed to the tsetse fly and therefore the disease are in rural populations dependent on agriculture, fishing, animal husbandry or hunting" (19). 37,5% of the labor force in DRC are working in the agriculture sector, meaning that 30 million people are under risk from being exposed. The infrastructure in DRC is poorly developed. DRC has an area on the order of 8 to 1 compared to Norway. Still, DRC has only 2,794 km of paved roads compared to Norway's 93,509 km (28). The lack of appropriate roads makes it difficult to access HAT affected villages (29).

Economics

The health system in DRC is financed by the DRC government and international partners (30). 5% of the Health Departments budget is earmarked to the fight against sleeping sickness (8). However many people dealing with HAT at a high level, never see this money (12, 29).

A study from Buma, a remote village outside Kinshasa, saw that the cost of each household following a diagnosis of HAT was equivalent to 5 months income for that household. Despite the fact that almost all HAT control programs subsidize the cost of drugs and hospitalization, there are still some indirect costs related to getting the HAT diagnosis. Patients are not able to work during the hospitalization period, where median time is 10 days (range 7-45). After hospitalization there is an enforced rest of 90 days (range 30-270). The authors of the study suggested that this cost may have contributed to patients frequently refusing to seek treatment, even though treatment is provided for free (31).

Knowledge

Knowledge among the DRC-inhabitants, of HAT, is illustrated in a cross section study in Kasongo, village in endemic province of Manemia. Figure 6 and figure 7 illustrates that 28% of the population have no knowledge of HAT. Among those who have knowledge, 32% knew that the tsetse fly was the transmitter.

5.1.2.2 The true burden of TBR today

TRB is responsible for the acute form of HAT and is found in the eastern and southern parts of Africa. In endemic countries it counts for less than 5% of the reported HAT cases (19), and between 1997 and 2006 it counted only 3% of the total reported cases of HAT (17). However, we should bear in mind the under-reporting of TBR. A study from Uganda emphasized that TBR was under-reported on the order of 12 to 1 in 2005 (32). The true burden of TBR in Uganda taking into consideration (Table 3), means an incidence at 3348 in 2005.

5.1.2.3 Sleeping sickness in Caucasian travelers

"Global village" is a modern term used to describe how the world is connected through the internet. However, the world is also connected by the possibility to travel. Many people, especially from the western world, use the world as global village, e.g. by travelling to the African landmass. Consequently an increase in incidents of imported tropical diseases is seen, especially in the last decade, including HAT (18) (figure 8), while at the same time HAT incidences in the endemic countries have decreased (17).

Contrary to the endemic countries, TBR HAT dominates, responsible for 78% of the imported HAT cases. Safari trips to e.g. Tanzania (18), and the seldomness of western travelers in areas highly endemic to TBG (4) are the main reasons for these adverse statistics.

5.2 Etiology

5.2.1 The Vector

5.2.1.1 Introduction

Tsetse fly is the vector of the trypanosomes causing HAT and animal African trypanosomiasis (AAT). The trypanosome is a protozoan (33). In general protozoans have two different strategies of transferring them self from one mammal to another: Fecal-Oral, like toxoplasmosis, and vector transmission like HAT. There are several vector transmitted protozoans (figure 9).

The tsetse fly is not only a transmitter. It works also as an important host for the trypanosomes, which develop from the procyclic trypomastigote stage to the metacyclic trypomastigote stage inside the tsetse (35). The transmission of HAT occurs when the tsetse penetrates the skin of a mammal and thereby provides access to the mammals circulatory system (36) (figure 10). The word tsetse is derived from Tswana, a language spoken in Southern Africa. The meaning of the word is "fly destructive to cattle" (34).

5.2.1.2 Tsetse classification

Tsetses are two winged insects, which places them in the order *diptera* along with other flies and mosquitoes (37) (figure 11).

There are 31 species and subspecies of tsetse flies, which are divided into three ecologically separate groups (9): The palpalis group, the morsitans group, and the fusca group (figure 12). Their only morphological difference lies in the structure of their genitalia, but they can be distinguish by the ecological habitat they inhabit (39).

Palpalis group

The tsetses in this group are the main contributors to TBG HAT (9), and are responsible for more than 90% of HAT cases (2). Five of the nine species found in this group are potential vectors of HAT and AAT. Four of the five species that are potential vectors of HAT and AAT are found in DRC (39). The species in this group

are found along rivers and lakes, which are often in close vicinity to human habitation (34, 36), hence their second name, riverine tsetse (9).

Morsitans group

There are seven species and sub-species in the morsitans group, all of which are potential vectors of HAT. The morsitans group is the major contributor to AAT (9). Their typical habitat is Savannah (9). Even though the morsitans are found in endemic areas for both TBR and TBG, it is mainly associated with transmission of TBR (34).

Fusca group

The habitat of the Fusca group is forests (9). None of the species in this group are potential vectors for HAT. However two of them are potential vectors AAT (39).

5.2.1.3 Tsetse, the etiology

Unlike other *dipterians*, e.g mosquitos, both male and female tsetse feed on blood, which means that both of them function as vectors for trypanosomes. However despite that a tsetse has fed on a trypanosome infected host, it is not given that the tsetse will be a HAT transmitter. Several resistance mechanism has been discovered as explanations for this (40), and tsetse males are found more infectious compared to females (40). Under ideal conditions in laboratories, 40% or more of the flies will cure themselves (41). The most important factors prohibiting the tsetse from spreading the trypanosomes are the intrinsic factors like protease and reactive oxygen species (ROS). Other factors stopping the transmission are ecological factors and host factors (39). The trypanosome life cycle within the tsetse is complex, and overall the tsetse resistance mechanisms are poorly understood. During the last decade some of the intrinsic factors have been demonstrated. The most researched trypanosome is *Trypanosomia brucei brucei* (TBB), which is the cause of AAT. This is due to lack of risk to infect the HAT investigators (40).

Intrinsic tsetse factors

Protease

When a tsetse lacerates the skin of a HAT infected mammal, the mammal is infected with the trypanosome in its trypomastigotes part of its cycle. The infected

blood first enters the crop after which the parasite continues to the gut, which is filled with highly lethal digestive proteases. The majority of trypanosomes die here (42)(figure 13). Some of the trypanosomes are survivors that get the chance to proliferate to a non-mammalian infective (43) procyclic form (12), which literally means a trypanosome with a coat of procyclin. Advantage of procyclin is the nature of resistants against proteases. The tsetse uses proline as its energy source, which the procyclic form of the trypanosome is able to utilize. The trypanosome is switching from the sugar it uses as energy source in its trypomastigote form, to the tsetses proline as energy source in its procyclic form (42).

ROS

Another antimicrobial defense system discovered is ROS (35). The epithelium found in the midgut of the tsetse is, in addition to protease, also producing ROS, thought to be quite apoptotic to the trypanosomes. This has been proven in a study where anti-oxidants were added to TBG infected blood fed to tsetses. Consequently they saw that the infection rate increased from 15% in the control group (n=88) to 44% (n=91) (44). ROS and proteases work together to clear the majority of the trypanosomes at an early stage of the infection route (35)

Physical barrier

The midgut of the tsetse is surrounded by a glycosaminoglycan rich layer strengthened with chitin. The layer is called the peritrophic matrix (PM), and is a strong layer protecting the underlying epithelium. The PM and epithelium is called the ectoperitropic space. Some of the survivors from the protease and ROS inferno manage to pass into that space 3-5 days after the tsetse has fed on the blood. This mechanism is not fully understood. Further on the trypanosomes in the ectoperitropic space move anterior towards the proventriculus (cardia), where they cross the PM and continue along the esophagus canal to the salivary gland (42).

Transferrin

Several barriers are thought to exist along the trypanosomes migration route towards the salivary gland. One of them is transferrin, known as an acute infective protein in humans. An increase in transferrin levels is observed during infection by trypanosomes. Knockdown of transferrin RNA increased the trypanosomes

chances of survival in the tsetse, proving that transferrin helps the tsetse fly to fight back the trypanosomes (41). During the migration from the midgut to the salivary gland the trypanosome transforms to the mammalian infective metacyclic form (43).

5.2.2 The parasite

5.2.2.1 Introduction

The causative agent of human African sleeping sickness belongs to the species *Trypanosoma brucei* (TB), which traditionally is divided into three sub-species based on the clinical and geographical picture. One of the sub-species, *Trypanosoma brucei brucei* (TBB), is contributing to disease in wild and domestic animals, nagana, alongside other non-human pathogen trypanosomes (45). The two other sub-species, *Trypanosoma brucei gambiense* (TBG) and *Trypanosoma brucei rhodensiense* (TBR), have got the ability to infect and cause disease in humans. Whereas infection by TBR present an acute and highly progressive disease in the eastern and southern parts of Africa, infection by TBG shows a more chronic character and is present in the western and central parts of Africa. The common story is that both infection by TBG and TBR are regarded as fatal if left untreated (13).

5.2.2.2 Taxonomy and morphology

The species of TB are eukaryote unicellular flagellated organisms. They are elongated cells varying in length from 15 to 35 μm (4). The flagellum makes them highly moveable and active when seen in microscope (attached film clip). The sub-species of TB are morphological indistinguishable (46), thus analysis of DNA are needed to separate them. TB is classified within the order of *Kinetoplastida*, referring to the organization of its mitochondrial DNA into a kinetoplast (figure 14). The characteristic kinetoplast is a huge network of circular DNA situated near the basal bodies of the flagellum, and has even been claimed to be the structurally most complex mitochondrial DNA in nature(47). TB is further defined within the family of *Trypanosomatidae* alongside *Leishmania*. Finally it shares its genus, *Trypanosoma*, with *Trypanosoma cruzei*, the parasite responsible for American trypanosomiasis, also known as chagas disease(48, 49).

5.2.2.3 Further classification of the parasite

TBG and TBR can be divided into subgroups based on the clinical picture, though only TBG present two genetic distinct groups.

TBR shows two separate presentations of disease, which differ in clinical feature, progression and immune response. One found in the north, and the other found mainly seen in the south. In addition they present different types of serum resistance-associated (SRA) gene, coding the protein that makes TBR resistant to trypanolytic factors (TLFs) in human serum. The northern form, recognized by SRA type 1, is characterized by acute onset with fast progression to stage 2 and has been described in Uganda, Tanzania and Kenya. The southern form of TBR, acknowledged by SRA type 2, shows a much more chronic profile and has been described in Zambia, Ethiopia, Malawi and Tanzania. Thus Tanzania is the only country known to present both forms(51).

Though it is easy to suspect that the type of SRA dictates the severity and progression of disease, so is not necessary the truth. Difference in cytokine response is likely to largely contribute to the difference in the clinical feature. Whereas TNF-α dominates the cytokine response in the north, the southern type shows an immune response predominated by TGF-β. Surely there has been shown a clear association between TNF-α levels and severity of disease. TNF-α has even been suggested to play a vital role in the dysfunction of the blood-brain-barrier (BBB), leading to stage 2 of disease. On the other hand, TGF-β in high concentration is regarded as an anti-inflammatory mediator suppressing macrophages and natural killer cells production of major pro-inflammatory mediator of HAT such as INF-γ and TNF-α. The difference in cytokine response observed is more likely to be a result of polymorphism in several of to date unknown virulence genes, than a direct result of difference in SRA type. Another hypothesis is that the geographical separated presentation could be explained by various degree of genetic resistance against trypanosomes in different host populations (52, 53).

As stated, TBG can be separated into two genetic distinct groups. The first, TBG group 1, is the most common agent of HAT and present the classic chronic picture. The second, TBG group 2, has been described as TBR-like in its clinical presentation. Although more virulent, the latter group shows variable resistant to trypanolytic factors (54).

Recent evaluation of the relationship between TBG group 1, TBG group 2, TBB and TBR certainly confirm TBG group 1 as a distinct genetic group. TBG group 2 was even shown to be more similar to TBB than to TBG group 1. The relationship between TBR and TBB is very close, frequently more similar to each other than within its own group (figure 15). With this in mind it has been suggested to consider TBR as a phenotypic expression, or being sub-species of TBB, only differing in the expression of SRA. Certainly, recent research supports the thesis that SRA can be transmitted from TBR to TBB making the original TBB human-pathogen. This close genetic relationship is of clinical interest as it increases the genetic pool available for the parasite. Increased genetic pool improves the parasites chances of developing drug resistance, in response to continuous drug pressure (55).

5.2.2.4 Life cycle

The life cycle (figure 16) of the trypanosomes starts off with a tsetse fly of any sex enjoying a blood meal of an infected host. TBR, only occasionally TBG, can infect animals. Though the exposed animals will not fall sick, instead they play a vital epidemiologic role as reservoir of the parasite. Cattle is an important reservoir for TBR, though most wild animals in game parks can certainly carry the parasite. Whereas TBR is zoonotic, TBG is mainly anthroponotic, as humans largely serve as reservoir (13). In infected individuals there will be two forms of trypomastigotes in the blood: a long slender capable of diving, and thereby causing disease, and a short stumpy, presumably non-diving, but with the ability to transmit to and infect the tsetse fly. The latter one enters the fly's midgut where it differentiates into longer slender procyclic trypomastigotes, with the capability to divide. After numerous multiplications the procyclic trypomastigotes migrate to the salivary

glands where it transforms into epimastigotes. In the salivary glands the epimastigotes keeps multiplying until the succeeding blood meal. As it is inoculated during the upcoming meal it differentiates into infectious metacyclic trypomastigotes, without the ability to divide. Following the local inflammation at site of inclusion the metacyclic trypomastigotes migrate to extracellular spaces in the body, such as the lymph and vascular system, where it completes its life cycles by the transformation into blood stream trypomastigotes (56).
The cycle is complex, and is not always completed. Only about 0,1% of all tsetse flies carries mature trypanosomes with the ability to infect (4).

A primary lesion at site of tsetse bite, known as chancre, will occur in some cases, characterized by accumulation of macrophages, granulocytes and lymphocytes(57).
The trypanosomes will rapidly spread to the blood and lymph system. In both systems the parasite can cross the capillaries to reach and cause inflammation in tissue throughout the body.

During the infection there will be waves of parasitemia as the immune system tries to eliminate the parasites. Throughout the descending phase the long slender trypomastigotes will be replaced by non-dividing short stumpy trypomastigotes, which can only continue its life in a tsetse fly. The substance that promotes this differentiation is to date not known. Interestingly the stumpy trypomastigotes produce prostaglandin D_2, which at a critical level induces apoptosis (figure 17) of itself. It has been hypnotized that the concomitant decrease in parasitemia is an important survival mechanism for the trypanosomes as it prevents too rapid proliferation of the parasite, which potentially could threaten the life of the host and thereby decreasing the parasites chances of transmitting further (58).

At a later stage of disease the trypanosomes will reach the brain and cerebrospinal fluid by crossing the blood-brain-barrier (BBB) supposedly through the choroid plexus. In both stages the long slender trypomastigotes will multiply with a doubling time of 5-10 hours (59).

5.2.2.5 Immunopathogenesis

As the African trypanosomes solely live extracellular in its host (59) it persistently experience pressure in a hostile environment, thus it needs to find a way around the immune system.

How the parasite evades the immune system

Being a parasite the first strategy of survival in a host should always be to avoid detection by the immune system. The human-pathogen African trypanosomes manage this by several mechanisms.

The human serum contains trypanolytic factor (TLFs), which destroys all non-human-pathogen trypanosomes. Two different TLFs have been described, TLF-1 and TLF-2(60). They are closely related as both compose the two components, apolipoprotein L1 (APOL1) and hapotglobin-related protein (Hpr). When the two components are united they constitute an active trypanolytic HDL-lipoprotein (4). APOL-1 acts as an ion channel forming protein in the lysosome of the trypanosomes. Certainly, a reported case of human infection by the non-human pathogen *Trypanosoma evasi* could be due to the lack of APOL1 in this patient (4). Hpr binds to free hemoglobin (Hb), the hemoglobin probably being released from the erythrocytes early on in the infection. The Hpr-Hb complex has been suggested to execute two functions. First, it may function as a ligand binding to Hpr-Hb-receptor (HpHbR) at the trypanosomes. Secondly, it may take part in the killing of the parasite by catalyzing the peroxidation of lysosomal membrane lipids. The combination of Hpr-Hb complex and apol-1 leads to osmotic lysis of the parasites(60).

TBG and TBR have developed resistance against these factors through different means. TBR has evolved the serum resistant (SRA) protein. Regarded the hallmark protein of TBR, the intracellular SRA protein interacts and prevent lysis of the TBR either by neutralizing APOL-1 or by redirecting it away from the lysosomes. For TBG, which lacks the SRA protein, the mechanism is still largely unknown. Although not explaining the resistant satisfactory TBG group 1 shows some alternation in the presentation of HpHbR which are likely to contribute to some

extent. First, it expresses few HpHb-receptors. Secondly, the gene coding the HpHb-receptors in TBG group 1 present numerous of point mutations(60).

Interestingly there has been proven an associating between non-diabetic nephropaty and two variants of APOL1 (G1 and G2), which are overrepresented in African americans. The allels G1 and G2 of APOL1, has in *in vitro* trials showed to kill TBR, but not TBG. Thus these variants are likely to have been selected for in evolution, as it contributes to killing the trypanosomes. This recent discovery highlights that trypanosomes are likely to have been a major problem in the history of Africa, as the mentioned alleles are found throughout Africa. Still needing further infestation this discovery could lead to the development of new treatment for HAT, as well as contributing to assessment of risk for developing non-diabetic nephropaty in patients with these alleles (61).

Whilst the trypanosomes have slipped through the innate immune system, they still have to escape the adaptive immune response. It does so by the means of its 12-15 nm thick surface coat (59). The trypanosomes are covered by 10 millions (62) identical variant surface glycoproteins (VSG), which also protects the trypanosomes from lytic factors in plasma. The immune system will recognize a certain VSG expressed, and start to produce IgM- and IgG-antibodies. These antibodies will destroy most of the trypanosomes, but there will always be a few trypanosomes that have changed their surface coat and thereby are able to escape and multiply. With only one specific variant expressed at a given time and the ability to express 1000-2000 different variants of VSG, the adaptive immune system is surely playing a losing game, never being able to eradicate the parasite completely. This ongoing cycle with waves of parasitemia corresponds to the recurrent episodes of fever patients with HAT typically suffer from. The fact that 10 % of the trypanosomes genome is dedicated to the variety of its surface coat truly highlights the evolutionary importance of the VSG in the co-exiting with host (4, 63).

How the parasite modulates the immune response in its favor

For the trypanosomes it is not all about hiding for the immune system. It also confronts it in a rather exceptional way by manipulating the immune response

favoring parasite survival rather than death. Huge modifications have been observed in both the innate and the adaptive immune system during infection by human-pathogen trypanosomes, which include disorders in the complement system, antigen presentation as well as defects concerning T- and B-cells(59).

A stunning example of this is alternative macrophage activation carried out by the parasite. The trypanosomes facilitate an immune response dominated by T helper cells 2 (Th2-cells) rather T helper cells 1 (Th1-cells). All of the following processes being described here happen in the macrophage influence by Th1- and Th2-cells. A Th2-response will induce host arginase, converting L-arginin to L-ornithine. Where L-ornithine is an essential growth factor for the trypanosomes, L-arginin is substrate for NO-synthase. Thus reduction in the concentration of L-arginin will lead to less generation of trypanotoxic NO. With this in mind we may look at three different scenarios (figure 18). A) The parasite does not manage to reroute the immune response from Th1 to Th2. Domination by the Th1-cells will stimulate the formation of the trypanocidal factors NO and TNF-α by the macrophages. At the same time inhibition of Th2 will leave plenty of arginine available as substrate for the making of NO-synthase. In addition, low Th2 activity will favor the formation trypanocidal IgM. B) In contrast a Th2 dominated immune response will favor the creation of IgG1 leading to chronic infection. Regulatory T-cells, in low concentration, together with Th2-cells inhibit the formation of NO as well as increasing the activity of arginase – both favoring survival of the parasite. C) As the Th1 and Th2 response exacerbate with simultaneous development of resistant against NO, excessive amount of inflammatory mediator are being creating leading to host, and thereby parasite death (13).

Even though the massive antigen variation shown, still is regarded the most important escape mechanism, it is clear that the parasite has evolved very sophisticated methods for survival in the host. Through natural selection in a persistent hostile environment it seems like the parasite has managed to modify a potential lethal immune response, making it possible for the parasite to survive in the human body just long enough for its transmission to a tsetse. This peculiar

parasite-host relationship makes HAT one of the most intricate modeling tools for research on regulation of host immune response(59).

How and why the trypanosomes reaches the brain

The process of how the trypanosomes cross the blood-brain-barrier (BBB) (figure 19) and reach the brain is complex and poorly understood. Another question is why the trypanosomes would want to reach the brain. Seeing how introduction of trypanosomes to the brain eventually leads to host death, it is reasonable to question if invasion of the brain is an evolutionary dead end. It has still been suggested that the presence of trypanosomes in the central nervous system (CNS) potentially could suit as a reservoir contributing relapsing parasitemia, as bidirectional transmigration of trypanosomes across the BBB has been proven in *in vitro* trials(64).

The site of crossing the BBB most commonly recognized is the highly vascular choroid plexus, though it also occurs in other regions (59, 63). Only a few post-mortem examinations of human beings have been carried out, thus trials carried out on animals contribute most of knowledge concerning the neuropathogensis of HAT today. From experiments on infected animals three phases of invasion of the CNS have been described. In the first phase inflammation of the meninges appears. The second phase is characterized by perivascular affection, following a final phase where trypanosomes reach the brain parenchyma leading to encephalitis(65).

Though there are still more questions than answers regarding the neuropathogensis in HAT, parasite cysteine proteases seem to play an important role. *In vitro* models suggest that the parasite crosses the BBB partly through activation of pathways depending on Ca^{2+} in brain microvascular endothelial cells (BMEC), thought to be facilitated by parasite cysteine proteases. This has been testified by the observation of temporary changes in the intracellular concentration of calcium in BMEC in the environment of trypanosomes or trypanosome-like medium. In this manner the cysteine protease brucipain shows particular interest in research(51).

In addition to cysteine proteases, pro-inflammatory cytokines seems to play a vital role in the accumulation and crossing of parasites and T-cells across the BBB, in a likely to be multi-step process. White blood cells are able to cross the BBB through the tight junctions and by transcytosis near the tight junctions of the BMEC. Dysfunction of the BBB, facilitating the migration of trypanosomes across the BBB, seems to be partly facilitated by IFN-γ and IFN-γ induced chemokines such as CXCL10. CXCL10, in particular, has been in the attention of researchers as a potential staging tool, as it has been observed in S2 but is absent in S1. Other chemokines seen in S2, which are regarded as attractive candidates as staging molecules are CXCL8, CCL2 and CCL3, which alongside IFN-γ have been proven to be associated with the severity of disease(63).

Furthermore, a broad range of chemical mediators, such as host TNF-α, IFN-γ and host and parasite-derived PGD_2, influences sleeping disorder and affection of the circadian rhythms seen in HAT(63).

In conclusion the neuropathogensis of HAT is still poorly understood and needs further investigation. It is of particular interest to understand the neuropathogensis as it may contribute to better staging tools as well as treatment of S2-subjects. It seems as both parasites and host-derived factors play a vital role in the migration of parasites across the BBB (51, 63, 65). Understanding how the trypanosomes disturb the sleep cycle, may contribute to a better of understanding of other diseases where sleep is affected such as narcolepsy, certain neuropsychiatric disorders as well as alternation in sleeping pattern during normal aging (63).

5.3 Clinical feature

5.3.1 Introduction

The clinical symptoms and signs in HAT are unspecific and show a high degree of variety, both on an inter-individual and inter-regional level. Notably travellers in general present with different symptoms than people living in endemic regions (6). As there is no symptom or sign regarded as pathognomonic for HAT mapping the risk of exposure is of great anamnestic value. Groups at risk of developing TBR

HAT include fishermen, hunters and game wardens. Thus travellers visting game parks in the eastern parts of Africa will always be at risk of infection by TBR. Infection by TBG is seen in any epidemic areas were humans are in contact with water (66).

5.3.2 Sleeping sickness in endemic regions

In general the symptom and findings concerning infection by TBG and TBR are very much the same. The difference is rather in the onset and progression of the disease. Whereas infection by TBR has an acute onset and present a highly progressive disease, TBG shows a more chronic and protracted character with typical symptoms often presented months or even years after exposure (13).

HAT is separated into two stages, ultimately divided by whether trypanosomes are present in the central nervous system or not. The average time to reach stage 2 of the disease is estimated to be just over one year and three weeks for TBG and TBR, respectively (6).

Stage 1 – The Haematolymphatic stage

The first stage is characterized by the presence of trypanosomes in extracellular tissue throughout the body, notably the blood and lymphatic system, hence the name, yet absence of trypanosomes in the CNS.

The most common onset of disease is fever illness with headache accompanied by muscular and joint pain (figure 20). The headache has been reported to be the most common symptom (67) and is being described as severe and persistent. The muscular and joint pains are experienced alongside fever seizures, which last for 1-3 days being linked to recurrent parasitemia. The episodes with fever are particularly severe and frequent in TBR-patients. In the case of infection by TBG the symptoms are often of such mild nature, that medical contact is not necessary being carried out (10). Other leading signs and symptoms of stage I include lymphadenopathy, pruritus, and to a lesser extent the presence of trypanosomal chancres and hepato-splenomegaly (4).

Whereas infection by TBR often shows a general lymphadenopathy, TBG typically present with enlarged posterior cervical lymph node (Winterbottom´s sign). Pruritus is frequent, though not always complained about. In such cases the manifestation of scratch marks are useful pointers (10). A primary lesion on site of inclusion, called chancre (figure 21), is seen in 19 % of patients infected by TBR (4), but is rarely seen in infection by TBG. It occurs after 2-3 days as a tender and painful elevated edematous papule, and resolves after two-three weeks. Along side local edema, erythema and lymphadenopathy is seen (66). As many HAT-patients, especially those infected by TBG, does not seek medical advice within three weeks, the actual share suffering of a chancre is thought to be considerable higher then reported (68). Though the presence of trypanosomal-like chancre surely is a good lead, it is not always easy to differ from other insect bites or skin infections, such as cutaneous anthrax (66).
Internal organs that are most commonly affected are the heart and kidney, though failure of these organs is less common (70). Cardiovascular pathology includes dysrhythmia, heart murmurs and low blood pressure. A thorax x-ray could visualize enlargement of the heart. This is due to pericardial effusion and dilatation of the heart. Anemia is a common feature and is especially prominent in infection by TBR. In severe cases, mainly late stage 2, it may even lead to heart failure (10). Myocarditis is another complication most frequently seen in infection by TBR (66).

On the list of other signs and symptoms of stage I (figure 22) intercurrent infections are noteworthy, especially pneumonia, which in some cases is the cause of death(10).

Stadium 2 – The meningoencephalitic stage

The symptoms and signs described in stage I will persist through stage II, often enhancing. As the name predicts symptoms of the second stage are of neurological origin and include sleeping disorder, mental change as well as a variety of sensory and motoric disorders (figure 23).

The hormonal circadian rhythms are being disturbed as the trypanosomes cross the blood-brain-barrier, thereby causing sleeping disorder as well as the name of the disease. In the past a characteristic inverse sleeping pattern has been

described, though recent research points to a more fragmented sleeping pattern with ongoing sleep-wake cycles lasting for only hours both day and night (13). As the disease progresses the sleeping disorder will too. Indeed polysomnography has been suggested as a non-invasive staging tool suitable to monitor disease and effect of treatment in children (71).

Various mental changes have been described as the disease progresses into the second stage. This includes confusion, disorientation as well as a variety of psychiatric disorders such as personality disorder, psychotic reactions and change in mood (euphoria and depression) and behavior (apathy and aggression) (10). Psychiatric symptoms may well dominate the picture in the second stage. There have even been reported cases in Europe where misdiagnosed patients wrongly have been admitted to psychiatric clinics, delaying the diagnosis and crucial treatment for several years (72).

Multiple motoric disorders may occur such as tremor, choreoathetosis, hypertonia and coordination's disorder. The observed hypertonia is believed to be of extra-pyramidal origin. Hypotonia has also been described, and could either be of cerebellar origin or be caused by sensory disorders. Other sensory changes seen in second stage HAT-patients include paresthesia, hyperaesthesia (Keradel´s sign) and loss of sense of position. In addition abnormal tendon-, skin- (Babinski sign) and primitive reflexes may occur, accompanying other neurological disorders of stage II (figure 22) (10).

If treatment is insufficient the patient will finally reach the terminal phase characterized by demyelization and atrophy of brain tissue, leading to changes of consciousness, dementia and coma. Finally in a state of cachexia the patient dies, with or because of opportunistic infections(13).

5.3.3 Sleeping Sickness in Travelers

The clinical picture displayed by HAT in travelers from non-endemic regions, differs in significant compared to that of natives in endemic countries suffering from the same disease.

First of all, HAT will show an acute onset in travelers independent of causative species. Whereas the incubation time of TBG in natives is estimated to be 18 months, the disease will in 75 % of the TBG-infected travelers debut within one month. Secondly, two of the symptoms regarded as hallmarks of the diseases in natives, lymphadenopathy and sleeping disorder, appears much less frequent in travelers. Third, skin manifestation is much more common in travelers. Chancres have got a reported incident in travelers of 55,6% and 87,9 % in stage I, TBG and TBR respectively(18). The trypanosomal rash is another skin manifestation barely seen in natives that develops in travellers(73). The fleeting non-itching skin rash appears as ring-formed rashes (figure 24), which melts into polycyclic structures with a diameter ranging from one to ten cm(10). The rash is typically located on the trunk and the proximal limbs(74). Finally, affection of gastrointestinal organs are more common in travelers. Even though hepato- and splenomegaly also are seen in natives, others gastrointestinal manifestation such as diarrhea and icterus are close to only observed in travelers(18).

As for immigrants from endemic countries, living in non-endemic countries, they show a clinical presentation similar to that of natives in endemic regions (18). For this reason HAT should be considered in immigrants even years after return from endemic areas.

5.4 Differential diagnosis

Correct diagnostic requires knowledge of the other local diseases.

Differential diagnosis of stage 1 mainly covers other fever illnesses such as malaria, the relapsing fever, typhoid fever, as well as brucellosis and arboviral infection. HAT stage 1 should also be considered in patients presenting with myocarditis, who has been at risk of being exposed (66).

Differential diagnosis of stage 2 includes a variety of inflammatory processes in CNS. This includes bacterial meningitis, cerebral malaria and HIV-AIDS related infection, such as cryptococcal meningitis. Psychiatric diseases and personality disorder may dominate the clinical feature. Many cases of patients being

misdiagnosed and admitted to psychiatric clinics has been described, thereby delaying much needed treatment. In addition neuromuscular disorder, such as Parkinson and any expensive process in the brain, such as tumor and hematomas, are differential diagnosis of stage 2 (66).

5.5 Diagnosis

5.5.1 Introduction

It is crucial both to provide the diagnosis as early as possible, as well as getting the staging right. Whereas lack of treatment will lead to certain death, the current treatment of S2-subjects has known severe side effects, notably encephalopathy (75, 76). In this setting it is clear that precise diagnostic will benefit of lower costs of control programs, fewer follow-ups of presumably healthy individuals who are misdiagnosed with HAT (false positive), as well as avoiding severe side effects of incorrect staged individuals - which ultimately could be lethal.

As described the clinical feature shows a broad spectrum of symptoms and signs, which of none is significant to set the diagnosis. For this reason laboratory tests are necessary.

5.5.2 Diagnosis of TBG HAT-patients

In order to diagnose and threat TBG in an adequate manner a three-step approach is being used in the field. First screening, secondly diagnostic confirmation and third staging(4).

5.5.2.1 Screening

The card agglutination test for trypanosomiasis (CATT) was introduced in 1978 (77), and is still the only low cost screening method available for use in the field (figure 25). Besides being cheap, it certainly has practical benefits of being simple and fast, making it possible to screen hundreds of individuals on a daily basis. In addition both capillary blood, serum, as well as blood samples collected on filter papers can be used for analysis (78).

CATT has a reported sensitivity and specificity of 87-98% and 93-95 %. Still as it is usually being used in population with prevalence of HAT below 5 %, the positive predicting value is too low to confirm the diagnosis(4).

5.5.2.2 Diagnostic confirmation

The ones who get a positive CATT (figure 26) test result (CATT-seropositive) are further investigated by microscopic examinations either of lymph node aspirate, blood, or both for parasitological confirmation. Examination should be carried out as soon as possible to prevent the parasites for lysis. Trypansomes are vulnerable once they are brought out of their natural environment. They are rapid killed if they are expose to direct sunlight, though they may survive for a few hours if kept in a cold and dark place (79). When present, aspiration of cervical lymph nodes are performed. It has a reported sensitivity ranging from 40 to 80 % highly depending on the specific parasite strain, current stage (higher sensitivity for S1) and the local distribution of other diseases that could cause lymphadenopathy.

As for examination of thin or thick blood film, the sensitivity is generally too low concerning detection of TBG. For this reason concentration methods such as microhaematocrit centrifugation technique (mHCT), Quantitative buffy coat (QBC) and mini-Anion Exchange Centrifugation Technique (mAECT) (figure 27) are recommended (78). If parasites eventually are detected the individuals are staged in order to get the right treatment.

In the case of CATT-seropositive individual who are not confirmed by microscopic assay a variety of regimes and algorithms are being used (figure 28). There are no standards and the numbers of methods available at a certain healthcare center will to some extent limit the algorithms. Surely a recent study on five different algorithms used by Médecins sans frontiers (MSF) highlights the common feature in the lack of evidence. The authors conclude that new diagnostic tools, specific and simple enough to be used in the field, should replace all present algorithms (80). A huge challenge in the making of algorithms is that most diagnostic tools show different sensitivity and specificity in different populations (79).

Although being highly depending on the present algorithm, it is common to start off with another CATT in the case of CATT-seropositive individuals. The CATT is this time preformed on ¼-diluted sera instead of whole blood, and additionally 1/8-diluted sera if the first one is positive. If the mentioned dilutions are negative the WHO recommends two years follow-up. The follow-ups may be challenging to achieve, as the typical regions struggling with TBG are rural areas, often with poor healthcare infrastructure. Depending on the algorithms being used in a certain healthcare center, individuals who test positive for CATT diluted sera could either be sent to staging, re-examined by microscopic procedures or simply be sent home(7). In the democratic republic of Congo it is common to retest CATT-seropositive on a monthly basis. If the area is considered to have a prevalence of disease above 1 % the HAT- suspective individuals are treated if CATT remains positive on the first control one month after the initial CATT (12). Whether CATT-seropositive subjects, who are not confirmed by microscopic detection should be treated or not, is still controversial. These individuals could potentially constitute a human reservoir, and close follow-ups have certainly been suggested (81).

5.5.2.3 Staging

As earlier stated the importance of proper staging cannot be ignored. Whilst S2-threathment surely is associated with adverse effects, inadequate treatment will lead to inevitable death.

WHO defines S2 as the presence of either of the following in the cerebrospinal fluid: detection of trypanosomes, increased white blood cell count or increased concentration of proteins (10). All mentioned are accessible and requires lumbar puncture. Increased amount of proteins (*> 370 mg/L)* are nevertheless no longer recommended as a staging tool for HAT. If parasites are present, all other parameters are irrelevant and the patient is categorized as S2. As parasites are not always detectable in the CSF, the numbers of white blood cell counts are most frequently used. The cut-off value normally used is 5 cells/µl, though the faction between 5 and 20 cells/µl are widely discussed. The latter group surely contains individuals both with and without symptoms of neural affection. In addition the effects of S2-medications in this group is variant (4). In some countries, such as Angola and Côte d'Ivoire, 20 cells/µl is being used as the cut-off value (79).

5.5.3 Diagnosis of TBR HAT-patients

The diagnostic approach of TBR differs from TBG in several ways. First, there is no screening method available. Thus clinical features, such as presence of chancre, play a greater role. In addition the concentration of trypanosomes is much higher with TBR than the case is for TBG. Hence microscopic examination is easier, and in most cases the only diagnostic tool needed. Finally, affection of other unspecific biological markers, such as anemia and thrombocytopenia, is common and more severe in infection by TBR (4, 79). As for staging the methodology concerning TBR and TBG is the same.

5.6 Treatment

There are four main drugs for treating HAT available (figure 29). The first one came on the marked in 1922, the last in 1981. The two others were introduced before 1950 (82). There are no commercial interests connected to developing new drugs for the treatment of HAT (3). All of them are administered parentally and adverse drug reactions are seen in all of them (3). As described in the diagnosis chapter, what kind of stage the HAT patient is in is of great importance because drugs used to treat stage 2 patients can cause severe drug reactions. Adverse reactions and difficulties with the administration of the drug in stage 2 patients, makes hospitalization during treatment necessary.

Pentamidine® is the first line treatment of TBR HAT stage 1 patients. It is the only first line treatment of HAT that can be administered intramuscularly. The dosage is 4mg/kg/day in one week (4). The intramuscular injection often causes local pain at the site of injection. Other Adverse effects, like gastrointestinal problem and hypoglycemia are normal and seen in 5-40% of treated patients (4). Relapse is seen in 7% of treated patients (3) (figure 30).
Suramin® is the first line treatment of TBR HAT stage 1 patients. Its administered as intravenous injections at 20 mg/kg. Suramin is administrated 5 times, with one week interval. Relapse is seen in under 4% of treated patients (3).

Melarsoprol® is used in the second stage of both TBR and TBG patients. It's the most commonly used drug in treating patients with second stage TBG (4). It is

administered as injections in three series. There is no agreement on what the most appropriate regimen is and different regimens are found (75). WHO regimen from 1998 consists of daily injections for 3 day**s** followed by no injections in 7 days**.** (3). Drugs used to treat second stage HAT have to cross the blood brain barrier (BBB) since the trypanosomes are found in the Central Nerve System (CNS). Melarsoprol® is liposoluble, making the crossing of the BBB possible. A variety of adverse reactions and a high relapse rate is seen when using this drug. This has caused some hospitals, like Kinshasa Hospital, to abandon the use of the drug. The most severe adverse reaction is encephalopatic syndrome with an incidence rate of between 1,5% to 28% (75), of which 10-50% die (3). When using Melarsoprol® it is normal to combine it with Prednisolone, even though no studies have been able to show any decrease in adverse reactions as a result of this combination (75). In DRC relapse rates of up to 50% are found (3).

Eflornithine® is the first line treatment of TBG stage 2 HAT patients because of the reduced mortality rate compared to Melarsoprol®, and can also be used against TBG stage 1. It is not used on TBR HAT patients (4). Eflornithine® is administered through infusion. The newest recommendations are 400 mg/kg divided on four infusion per day for 14 days (3). It was developed in the 1970s as an anti-cancer drug (75), and the most common adverse reaction to the drug is bone marrow suppression, which is normal in anti cancer treatments (4). The relapse rates are found to be 7.8% (3). Eflornithine® is the latest developed first line drug

Combination therapies have the last decade been given an increased interest. Where the mentioned drugs are combined with each other, or other drugs are tried (3). The Nifurtimox®-Eflornithine® Combination Therapy (NECT) is found to be significantly better than monotherapy and other combination therapies, taking into consideration the incidence of adverse effects and mortality (75). WHO now recommends NECT as first line treatment for TBG stage 2 patients in hospitals where it can be applied. Nifurtimox® as monotherapy can be used against both stages of TBG where other therapies have failed. The advantage of Nifuritmox® is that the administration of it is oral (83).

Follow up after treatment is mandatory. Threated patients should be followed up for two years, with control visits at three and six month and at six-month intervals thereafter. At this visits the doctor should evaluate the patients clinical condition, together with examination of the blood and the cerebrospinal fluid (CSF). A patient is considered cured only when during a two year follow-up periode no trypanosomes can be detected and when the CSF stayed or returned to normal (3, 84)

5.7 Complications and Sequels

Complications could both be caused by treatment and by the disease. Encephalopathy is a feared complication associated with the stage 2 drug melarsoprol® (75, 76). For patients suffering and recovering from stage 2, neurological and psychiatric sequels (figure 31) are common (13). Any damage caused in the meningoencefalic stage could ultimately be irreversible (1). In addition, several reproductive disorders including infertility, high abortion rates and occasionally congenital infection, have been described (66, 85).

5.8 Prognosis

Although there have been described certain cases of individuals who seem to be resistant to infections by TBG, known as trypanotolerance (86, 87), HAT is regarded as fatal if left untreated. The prognostic outcome will highly depend on stage and duration of the disease at the time of diagnosis and start of treatment. If treatment starts in S1 the survival rate is generally high. On the other hand, there is a marked increase in the risk of sequels and decrease of survival as the disease progress into the second stage. If left untreated the time from inclusion of trypanosomes to death in TBG and TBR have been estimated to three years and 6-12 months, respectively (5, 6).

5.9 Control and Prevention

Trypanosome causes both human african trypanomiasis (HAT) and animal african trypanosomiasis (AAT) - also known as nagana. This paper focuses on the human disease. The vector of both diseases is the tsetse fly.

Animal african trypanosomiasis (AAT) and the poverty seen in Sub-Saharan countries, have a connection. WHO states that "Of Africa's 37 tsetse-infested countries, 20 rank among the world's poorest 25 nations" (figure 32). AAT is a threat to livestock, and livestock is a major income source in many Sub-Saharan countries. Susceptible livestock infected with AAT has a reduced calving rate of 11-20 % (32). This together with an agricultural production loss of $ 5 billions headlights what a major problem AAT is (88). The fight against tsetse and trypanosomiasis (T&T) is therefore also a fight against poverty (32) (figure 33).

Human African trypanosomiasis is there no presently vaccine against (2), and prophylaxis is not recommended (4). This leaves control and prevention as the two main strategies available for reducing or eliminating HAT. Control means to cure the already HAT infected patients, and prevention is to control the tsetse, i.e. to cut the circle of transmission.

5.9.1 Control

Control of HAT patients is to cure HAT patients. It is based on active and passive surveillance.

Active surveillance is patients who by them self-search treatment of HAT. Passive surveillance is a method for identifying HAT patients who for different reasons have not sought treatment. Passive surveillance is used in endemic areas where TBG is found, since TBG HAT patients have a long incubation time and can be infected without signs of disease. As long as these patients remain untreated, they function as a reservoir of TBG. Consequently, active and passive surveillance are essential in order to defeat TBG HAT (89), while passive surveillance has no place in the fight against TBR HAT (32). TBR patients have a short incubation time and becomes seriously ill and die relatively fast without treatment (89).

When an area, zone or village is found to be endemic for TBG HAT a mobile team starts passive surveillance, meaning that a team will carry out a screening, using CATT-tests and further treat the infected patients in the population. A typical

mobile team consists of 6-10 people with different tasks. Some are lab technicians who perform the CATT-tests (4), others work on educating people about the disease, while some work as drivers and guards (32). According to the provincial leader of mobile teams in Kinshasa, Dr. Erick Mwamba, his mobile teams have the capacity to screen 300 persons a day. They will normally stay in one location for 20 days. Most HAT experts see mobile teams as the most effective response to control TBG HAT (32).

5.9.2. Prevention

5.9.2.1. Prevention among travelers

Prevention of HAT means preventing the possibility of being bit by the tsetse. Travelers could choose to avoid HAT endemic areas or, if traveling in endemic areas do their best to prevent being bit by the tsetse fly. For instance by keeping car windows closed while driving and by wearing clothes that cover wrists and ankles (4).

5.9.2.2. Prevention in endemic areas

Also in endemic areas prevention of HAT means preventing the possibility of being bit by the tsetse, consequently the only way to prevent HAT is vector regulation. I.e. regulation of tsetse fly populations (2, 4). This matter of fact has sparked much investigation into the tsetse biology, ecology and behavior (90). Unlike most insects, tsetses are k-strategists meaning that the fertilized female tsetse only gives birth to one larva approximately every 10th day (39). Since TBR is zoonotic, controlling the vector is essential in order to defeat the east African sleeping sickness (89). Despite control (active and passive surveillance) are needed a to defeat TBG, vector regulation also play a central role to defeat the TBG HAT.

To control and eliminate the tsetse fly, its distribution must be known. Geographical information system (GIS), known as the best tool to determine the tsetse distribution, and global position system (GPS) are therefore used (90). GIS uses old maps of the tsetse distribution together with new registered predictor variables such as vegetation, temperature and moisture. Combined with data from hospitals, that register new cases of HAT, this provides a good instrument to mapping the tsetse's distribution (91). In the case of a positive indication, one of

the tsetse control techniques that are discussed in the following chapter can be applied.

Previously used tsetse control techniques

Slaughter of wild animals and land clearing was the start of HAT and AAT prevention in the late 19th century and early 20th century. Slaughter of wild animals was a method used to prevent TBR since it is zoonotic, and wild animals function as reservoirs (92). Land clearing was a technique that destroyed tsetse habitat, which meant it was a highly efficient prevention method both for TBG and for TBR (90). However, due to the ecological consequences WHO cannot recommend these two methods anymore (92).

Ground spraying with Dichloro-diphenyl-trichloroethane (DDT) and hexa-chlorobenzene (HCB) were earlier used for prevention of both HAT and AAT by spraying the compound on the vegetation, the tsetse flies habitat. It is not used today due to concerns about side effects, like accumulation in the food chain and possible carcinogenetic effects (92).

Currently recommended tsetse control techniques

Sequential aerial techniques (SAT)

Tsetse flies are susceptible to modern insecticides (92). The aim of SAT is to eliminate the tsetse by using aircraft, which sprays low dosages of biodegradable insecticides over an area. SAT has been used successfully to eradicate tsetse in Botswana (90). The general cost of SAT is US$ 590 per square metre (sq m) (93).

Sterile insect technique (SIT)

Tsetse females reproduce only once during their lifespan. It is this reproduction strategy that makes SIT possible. Where sterile males are released into a population the overall fertility is reduced (93). SIT has thus effectively been used to eradicate tsetse flies from the Tanzanian Island Zanzibar in 1997 (4), after the release of 8.5 million sterile males between 1994 and 1996 (94). The awareness of HAT on Zanzibar today is limited, where only 30% know about the disease (figure 34). SIT is the most expensive control technique with a cost between US$ 1000 US and US$ 13000 per sq m (93).

Trapping

The first successful tsetse trap was used to eradicate *Glossina palpalis* from an island of the west coast of Africa, Principe, in 1914. The trap consisted of a black textile coated with lime, carried on the back of the plantation workers (92). Traps, as we know them today, were first made to collect tsetse flies in the start of the 20th century. Not until the 80's were traps to control tsetse flies, like the biconical trap made at a large scale (95) (figure 35). There has been a huge development within the tsetse traps the last three decades. Today several traps are used to control the tsetse. Different traps are developed in order to defeat different groups of tsetse in different countries (37). The cost of eliminating tsetse flies from an area by traps are between US$ 500 and US$ 950 per sq m (93).

Trap color, odor and shape

The trap only has a chance at catching the flies if it has the correct color (95). Tsetse flies are attracted to the colours blue and black. This has been proved by several behavioral studies. One study explains the tsetses attraction to blue by suggesting it is mistaking the blue for a shadow when it seeks shadow to rest. Shadow is perceived as blue to a tsetse, due to the reflection of the sky. The optimal blue is found to be royal blue cotton (96). From the 1970's different odors have been added to the traps to make them more efficient (37). E.g. buffalo urine increases the catches 10 times (92). In general different odors are components from the hosts breath, urine and skin secretion. The odors are quite attractive to flies in the *Glossina morsitans* group, whereas no increase in efficiency is seen with regard to the other two groups of tsetse (37). Shape is the main difference between the traps. The biconical is the most common shape (92) (figure 35).

The best tsetse control techniques

Overall traps are the most effective method in the fight against tsetse since it prevents reinvasion. The majority of traps found today are made to eliminate flies from the *Morsitans* group, the group of tsetse which is the main vector of AAT (92). The reason it has not been used widely against *G. palpalis*, the main vector for HAT (2), is that tsetse flies in this group disperse less than flies in the *G. morsitans* group. This implicates that the density of traps have to be higher. Consequently the

method becomes too expensive (92) compared to the most cost effective insecticide method (9). The problem with insecticides methods like SAT is that it can't prevent reinvasion (93). SIT is only highly effective in isolated areas likes islands (94).

Ecology

PAATEC has the aim to eradicate tsetse and trypanosomais from the African landsmass (97). Consequently 8.7 million km^2 on the African continent, which today is uninhabitable because of the tsetse, will be inhabitable. However,as well as possible positive impacts there are also a risks: A tsetse free Sub-Saharan Africa may cause irreversible damaged from over grazing and cultivation in less than 10 years from eradication. If, a dramatic loss of biodiversity, soil erosion and destruction of wild life, will be seen (88). HAT and AAT are major problems for inhabitants of endemic areas, but it is important to be aware of the consequences of eradicating tsetse, due to the ecological crisis it is likely to cause.

6. Discussion

6.1 HAT - one of twenty neglected tropical diseases

Why should one be aware of HAT, and why should we pay attention to a disease with under 10 000 reported cases worldwide? (19) Even when considering estimates made by WHO, who multiplies the number of reported cases with a factor of 10, the estimated burden of HAT will be an annularly incidence of 100 000 (22). Furthermore this number is thought to be to be a major overestimation, a more probable multiplication factor being 4 (12). This together with the ecological crisis, which is predicated as a consequence of a tsetse and HAT free Sub-Saharan (88), one could say paying attention to HAT is meaningless.

The start of the 20th century saw an enormous effort to control and prevent HAT. Resulting in an elimination of reported HAT cases in the start of the 60s (figure 2). After the African break-free from the colonial powers an increase of HAT were reported, ending into the third epidemic with a peak at the 90s. Today WHO has concluded that HAT will be eradicated in the future (98), and PAATEC has the aim to eradicate tsetse and trypanosomiasis from the African landsmass (97). However, HAT is considered as one of twenty neglected tropical diseases (NTD), as defined by WHO (22). HAT has developed from being a Sub-Saharan issue in the 90s to now being a issue mainly in Angola, Sudan DRC (19). DRC alone reported 5018 new cases in 2011 (8) and 80% of all HAT cases are found in DRC (12). To defeat HAT, sufficient prevention and control strategies have to be in focus.

6.2 Prevention.

The use of tsetse traps is currently the most cost-effective prevention technique found (92). To reach PATTECs aim of total tsetse eradication, other prevention techniques should be given attention. This includes vaccine development and increasing knowledge of inhabitants in HAT endemic areas.

6.2.1 Vaccine

The development of a vaccine would obviously make a hug impact in the prevention of HAT. However, the high degree of antigen variation shown by TB has made most researchers doubt that the development of a vaccine ever will occur (4,

13), though others believe it could be possible. The latter emphasizes the reports of individuals in endemic areas apparently resistant to infection, known as trypanotolerance, which could provide clues in the development of a possible vaccine. In addition, it has been claimed that it will be virtually impossible to completely eradicate the reservoir of the parasite in endemic regions. Therefore development of a vaccine should be the ultimate goal (11).

6.2.2 Knowlegde

One other important preventive strategy is to increase knowledge of HAT, in endemic countries. Due to the risk of re-invasion of the tsetse, it is also important to sustain and increase the knowledge, in former HAT endemic countries. Awareness of HAT will make people search treatment faster if they are bit by the tsetse, or if they have the symptoms. Late diagnosed patient function as a reservoir for the parasite, which increases the HAT transmission (89). Figure 6 and 7 show the results of a cross section study in Kasongo, 2012. Figure 34 illustrates that 28% of the population have no knowledge of HAT. Among those who knew HAT, only 32% knew the tsetse as the causative agent. At Zanzibar, an HAT endemic island until 1997 (94), only 29% had knowledge about the disease (figure 34). No one in the age between 14 to 22 years in Kasongo knew about tsetse as the cause of HAT. One approach to strengthen the prevention, could be to implement basic facts about HAT into primary and secondary school (30).

6.3 Controll

Control of HAT patients is to cure HAT patients with drugs. It is based on active and passive surveillance.

Active surveillance is patients who by them self-search treatment of HAT. Passive surveillance is a method for identifying HAT patients who for different reasons have not sought treatment. 50% of HAT patients are found by passive surveillance carried out by mobile teams. Diagnostic tools are needed in both passive and active surveillance.

6.3.1 Diagnosis

6.3.1.1 Introduction

The key to any successful HAT control program is accurate diagnostic tools. This is particularly true in the regions close to eradication of the disease. In such areas precise diagnostic and screening methods are decisive to pinpoint the last few cases in humans, as well as in animals, contributing the reservoir (99). The steps in the three-step diagnostic pathway each have its limitations in inadequate specificity and sensitivity.

6.3.1.2 Screening

Even though CATT must be considered a success story, largely contributing in the fight against HAT, this widely used screening method has got some issues that should be stated.

First CATT is based on anti-parasite antibodies, thus it recognizes certain antibodies made by the adaptive immune system, and not the parasite, nor parasite-antigens. These antibodies are known to cross-react with other non-human pathogenic trypanosomes (77). In addition other diseases which present with non-specific B-cell proliferation such as malaria, HIV-AIDS and tuberculosis, could interfere the outcome of a CATT-test (7). In this manner there is a risk of false-positive, and thereby needless treatment and excessive follow-ups.

A second issue concerning CATT is that it is based on solely one strain of TBG (LiTat 1.3). Even though this strains VSG-variant is thought to be expressed at least once during an infections by TBG, it certainly has been proven not always to be the case (100, 101). However, it should be noted that there in recent years has been developed a latex agglutination test (LATEX/TBG) which combines the VSG variants of LiTat 1.3, 1.5 and 1.6 (102). Though it is true that the LATEX/TBG could be suitable in certain regions (103, 104), conventional CATT is still preferred as the test of choice in mass screening in most areas of concern (105, 106).

Apart from the problem with false negative and positive, the CATT is not suitable for use in follow-up, as the antibodies remain in the blood up to three years after

treatment. Another problem is how to deal with the cases of aparasitaemic CATT-seropostive. To date there is still uncertainties to what degree follow-up and treatment, should be carried out on such individuals. Furthermore CATT is not available in single units, thus it is only suitable for mass screening.

As opposed to the lateral flow rapid diagnostic test (RDT), which is being used in the diagnosis of malaria (*Plasmodium falciparum)*, CATT in addition requires trained personnel (107). The RDT applies a dipstick format, similar to that diabetic patients use. This screening method detects parasite antigen, another feature which still is lacking in the screening of HAT (7). Development of a similar test for TBR has even been claimed to be the only obstacle to wipe out TBR HAT (99).

6.3.1.3 Diagnostic confirmation

Detection of the parasite in blood in mainly a problem in the case of TBG where the concentration can vary from less then 100 trypanosomes/ml, to easily detected levels above 10 000 trypanosomes/ml (107). Infection by TBR generally presents with high degree of parasitemia, and is usually easily detected by microscopic examination.

The detection limit of plain wet and thick blood films show low sensitivity, with a detection limit of 10 000 and 5000 trypanosomes/ml, respectively (table 4). They are still being used in some health care centers, as they are simple and inexpensive (79).

Due to the lack in sensitivity in wet and think blood smear, concentration methods such as mHCT, QBC and mAECT are usually preformed. mHCT is often the test of choice, due to its relative simplicity combined with low cost. mAECT is the better choice in the sense of sensitivity. Although mAECT is considerable more expensive than mHCT (€ 2-3 per sample), it has been proven to be cost-efficient (112).

The HAT-patients with a concentration of trypanosomes below 100 trypanosomes/ml still remains a concern. Estimation suggests that 30 % off all TBG-infected patient are below this limited. Thus they go undetected by the conventional methods, though a new improved mAECT have got a detection limit

as low as 50 trypanosomes/ml (4). Concerning detection limit, tests aimed to detect nucleic acids of the parasite seems to be superior compared to all conventional methods. Still clinical validation of possible target sequences remains. Three nucleic acids-based techniques are undergoing investigation: PCR, Loop-Mediated Isothermal Amplification (LAMP) and, Nucleic acid Sequence Based Amplification (NASBA).

PCR has been shown to possess a detection limit of 1 trypanosomes/ml for detection of TBG and 1000 trypanosomes/ml for detection of conventional TBR SRA. A recent systematic review comparing 11 studies on PCR showed an overall sensitivity and specificity of 99 % and 97,7 % respectively, for detection of trypanosomes in blood. Most studies included analyzed common sequences of TBR and TBG. There was not enough material available to draw any conclusion on PCR as a staging tool. Neither was there enough material to draw conclusions on NASBA and LAMP as diagnostic or staging tool. In conclusion it was stated PCR is specific enough to replace microscopic examination for stage 1 disease, but further studies needs to confirm this in a representative population (108). A major obstacle with PCR is that it requires a well-equipped laboratory, with trained personnel, and a cooling system for the reagents (99). Thus the utility value is low in endemic regions, where such equipment rarely is available. Alongside immunofluorescence and ELISA-based serological tests, PCR is mainly being used in non-endemic countries, and in research (107).

Due to cost-related issues concerning PCR, LAMP and NASBA shows particular interest in a cost-efficient point of view. The latter two has the advantage of running on isothermal amplification techniques. Thus it is not depending on the expensive thermocycler being used for PCR. Running under isothermal conditions, both techniques can be done on simple water bath or by using a heating block (99).

NASBA has been proven to be significantly better then microscope alone. Recently NASBA was coupled with an oligochromatographic dipstick format (NASBA-OC), which could provide bedside diagnosis within 5-10 min. It was proven to be better then microscope of blood sample, as well as blood samples treated with mHCT and

mAECTH in diagnosing infection by TBG. In addition NASBA-OC for TBG showed higher sensitive and specificity then PCR-OC of the same gene. It should be noted that investigation of CSF were not carried out, thus no conclusions can be made on whether NASBA-OC may be suitable as a staging tool. In addition the test showed rather low sensitivity for TBR, and were not compared to TBR PCR (113). The major obstacle preventing NASBA from being used in the field is that it demands procedures for purification and preservation of the fragile RNA, similar to PCR (99).

LAMP is a promising new diagnostic tool with obvious practical benefits. As already stated it demands no advanced aperture, as is the case with PCR. Isothermal condition on 60-65 degree Celsius for 30-40 min is enough (99). The interpretation is simple, and can even be spotted by the naked eye, or by spectrophotometry, where PCR requires gel electrophoresis. A primer developed for detection of TBG has shown a detection limit as low as 1 femtogram DNA, corresponding to 0.01 trypanosomes/ml. It should though be noted that this has only been proven by using purified DNA. Further investigation remains to see if this holds the truth also in fields conditions (7). Another sequence for the detection of the subgenus *Trypanozoon* has been shown to detect 0.001 trypansomes/ml. In addition a template used to detect the SRA-gene of TBR has been proven to detect 10 trypanosomes/ml blood. Combination of the two latter for the detection of TBR was further superior compared to PCR. A prototype LAMP kit has recently (july 2011) been introduce, that currently is being evaluated at several of health care centers (99). LAMP still requires electricity and a cooling system for its reagents (107). Time will show if LAMP will prove to be the promising tool many believes, or if it will end up being just another tool in the hands of the researchers.

Finally its should be noted that there is currently being developed a nanbody-based test for detection of parasite antigen (107).

6.3.1.4 Staging

Correct staging is crucial seeing how lack of treatment will lead to death, whereas stage 2 drugs have severe adverse effects. The ultimate goal is to find staging tools, which do not include lumbar puncture, as lumbar puncture is a major obstacle for

seeking medical advice as well as maintaining adequate follow-up. To date no promising substance for staging, which would exclude lumbar puncture has been proposed. In addition to rummage through the blood for potential markers, it would be of high value to identify candidates at sites not demanding invasive procedures, such as salvia, urine and tear. Indeed it has been detected trypanosome antibodies in salvia (99).

On the list of promising marker, which could improve the accuracy of staging is IgM in CSF. A latex IgM test for staging has been developed (114). Although being promising it still requires further clinical validation. The INF-gamma-dervied chemokine CXCL10 (IP-10) shows particular interest. In combination with CXCL8 (Interleucin-8) and H-FABP (heart fatty binding protein) it showed a sensitivity of 97 % and specificity of 100 % in staging HAT. The weakness of these immune-dervied molecules is that they surely could be elevated due to other causes of neural inflammation, such as cerebral malaria and active tuberculosis. Other possible staging markers are beta-2-microglobulin, matrix metalloprotease 9, intercellular adhension molecule 1 and neopterin. All mentioned still requires lumbar puncture (99).

6.3.1.5 Follow-up

The lack of follow-up due to the unpopular lumbar puncture as well as problem with infrastructure, distance and population movement is a challenge both on an individual level as well as regional in the fight against HAT. Active follow-up is being carried out to some extent, but is certainly not an easy or low-cost solution. Apart from finding alternative tools for detection of trypanosomes in the CNS, any algorithm which could reduce the follow-up time will be essential.

Combination criteria taking into consideration the level of IgM and white blood cell counts in CSF has been suggested to reduce the follow-up to a maximum of 12 months. This algorithm should though be validated further in a larger scale, before implantation (115). In addition IL-10 and protein concentration has been evaluated to possibly reducing the follow-up time (99).

Whereas PCR is not suitable for follow-up, as DNA is detectable up to two years post-treatment, NASBA could possibly constitute to reduction in the follow-up time. This is due to the fact that RNA is less stabile then DNA, and thus dissolves in less time. In addition it has been suggested that LAMP may be used to reduce the follow-up time, though no studies concerning this has currently been brought out yet (99).

6.3.2 Mobile teams

Control consists of active and passive surveillance. When the mobile teams are performing passive surveillance in Kasongo, a endemic HAT area in the eastern DRC, they are dealing with road problems on their way to the villages. The road surface, consisting of sand, gets so slippery during rain that maximum speed will be 5 km/h. Together with the lack of bridges, heavy rain thus makes crossing the rivers impossible (figure 36).

Their main problem however, is lack of financial means. Because of the financial situation working is only possible three out of twelve months this year, despite that there may be villages in need of screening (29).

The Belgian Development Agency (BTC-CTB) has for decades been strongly involved in the fight against sleeping sickness in DRC. The general conclusion of The Declaration of Paris from march 2005 was to implement existing partners work into governmental health systems, which is seen as a sustainable use of resources (116). Consequently the BTC –CTB will end their support to the mobile teams, passive surveillance, as of 2013 (30). HAT control will be converted into the primary health care, meaning that HAT control in the future will consist of only the active surveillance. In DRC 50 % of HAT cases are detected by passive surveillance by mobile teams (117), and the primary health care facilities are often insufficient or non-existing, meaning that the diagnostics and treatment of HAT will be missing (98).

Mobile teams also work with HAT prevention, e.g. by educating village inhabitants on the disease where there have been one or more detected HAT cases. At the

same time will mobile team place tsetse traps in the area (29). If the control of HAT in DRC in the future only will consist of active surveillance, it is important that people have knowledge of the disease. Patients diagnosed at a late stage poses a problem, both because the patients then function as a reservoir for the parasite and because of the risk of irreversible damages and deaths among the patients.

6.3.3 Drugs

Drugs are essential to control HAT. The private partnership WHO and two pharmaceuticals companies made in 2001 resulted in distribution of HAT drugs needed to treat patients in all endemic countries, free of charge (21, 22).

Despite this, Honorable Dauda Saleh Sendo, the Minister of Health in Manemia, emphasizes their difficulties with financing the high costs of the drugs. He also claims the lack of availability, especially pentamidine®, which makes it difficult to treat TBG stage 1 patients (118).

Sanofi-Aventis distributes and funds together with WHO (21) the delivery of pentamidine® to Kinshasa. Where the drugs are given to national sleeping sickness control programs (NSSCPs). Sanofi-Aventis highlights there is no shortages in delivering HAT-drugs (119). The NSSCP distribution into DRC provinces is financed by WHO annually with US$ 63 000, which is fair enough (21). The governmental lack of information regarding WHOs drugs support of HAT, illustrates HAT as a neglected disease.

6.4 To be or not to be

The Ministry of Health in Manemia, have limited resources, which also have to cover the fight against several other major health problems such as malaria, anemia due to malnutrition and diarrhea among children (29). Consequently, the governmental resources and interest to defeat HAT in DRC is lacking (8, 12, 21). The effort and money dedicated by the BTC-CTB is seen as one of the most important resource for the fight against sleeping sickness in DRC (98). When BTC-CTB terminates their HAT support in Kasongo and the rest of DRC, the mobile teams will have no alternative way to sustain their work (30). Due to Paris declaration the future will see implementation of existing HAT partners work into

governmental health systems (116). If this, together with the end of the passive surveillance by mobile teams happens, HAT will go from being a neglected to an ignored disease (98). The setting is similar to that of the 60s where the newly established African countries did not focus on HAT control and prevention (17), and the third epidemic became a fact (figure 2).

If this, WHO will most likely, together with private partners and local health authorities, start increasing their effort against HAT. And the future may see a T&T free DRC. A T&T free DRC will open huge areas which today is uninhabitable, and irreversible damaged from overgrazing and cultivation are possible results (88). This implicates that the ecological part also should been taken into consideration when dealing with HAT

7. Conclusion

As the incidence of HAT is decreasing(19), so is the attention. HAT may go from being a neglected disease to an ignored disease (98). Historically and today passive surveillance of mobile teams has played an important role in the fight against HAT. DRC reports 80% of all HAT cases (3), and the funding of these teams, in DRC, are planned to come to a closer in 2013 (30), due to Paris declaration (116). The governmental interests and resources are limited to continue the former partners effort against HAT (8, 12, 30). Consequently the situation seen in the 80s and 90s is likely to occur.

In general further research is needed to develop better low-cost solutions for bedside diagnosis and staging. In addition it is important to obtain better understanding of the neuropathogensis of HAT, as it may lead to better treatment. The Holy Grail is the development of a vaccine, though there is still a long way to go.

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10. Appendix

Tables

Table 1. Fact table HAT (made by authors).

	Trypanosoma brucei gambiense	*Trypanosoma brucei rhodesiense*
Distribution (7)	West and central parts of Africa	East and southern parts of Africa
Epidemiology (8)	7600 reported cases (2011)	400 reported cases (2011)
Vector (9)	Mainly tsetse from palpalis group	Mainly tsetse from the morsitans group
Reservoir (7)	Anthropologic (humans)	Zoonotic (animals)
Symptoms and signs (10)	Mild onset. Low grad grade fever, headache, personality disorders and sleeping disorder. Rarely skin manifestation.	Acute onset with high fever and persistent severe headache. Affection of visceral organs. More common with skin manifestation. Fast progression to stage 2.
Diagnosis (7)	Screening: available Diagnosis: Microscopy, concentration methods are usually preformed. Staging: Lumbar puncture	Screening: Not available Diagnosis: Microscopy, concentration methods are usually preformed. Staging: Lumbar puncture
Vaccine (11)	Non-existing.	Non-existing.
Treatment (3)	Stage 1: Pentamidine Stage 2:Melarsoprol + Eflornithine	Stage 1: Suramine Stage 2: Melarsoprol
Prognosis (5, 6)	Median survival time (untreated): three years	Median survival time (untreated): 6-12 months

Table 2. TBG HAT patients. Annularly incidence between 1997 and 2006

Countries	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006
More than 1,000 new cases per year										
Angola	8,275	6,610	5,351	4,546	4,577	3,621	3,115	2,280	1,727	1,105
DRC	25,094	26,318	18,684	16,975	17,322	13,853	11,481	10,369	10,269	8,023
Sudan	737	1,726	1,312	1,609	1,804	3,163	3,076	1,766	1,869	809
More than 100 but less than 1,000 new cases per year										
Chad	122	134	187	153	138	715	222	483	190	276
CAR	730	1068	869	988	717	570	538	737	666	460
Congo	142	201	91	111	894	1005	682	859	398	300
Uganda	1123	971	1036	1141	424	562	501	354	304	270
Less than 100 new cases per year										
Cameroon	10	54	32	27	13	32	33	17	3	15
Côte d'Ivoire	185	121	104	169	84	92	51	72	40	29
Equatorial Guinea	67	62	28	16	17	32	23	22	17	13
Gabon	11	6	38	45	30	25	26	48	53	31
Guinea	88	99	68	52	72	124	116	84	94	48
Nigeria	0	0	27	14	14	26	31	10	21	3
No new cases with control activities present										
Benin	0	0	20	72	83	8	3	0	0	0
Burkina Faso	1	15	15	8	8	2	3	2	0	0
Ghana	0	0	0	1	0	0	0	0	0	0
Mali	0	0	0	18	3	2	0	0	0	0
Togo	0	0	0	0	0	0	0	0	0	0
No new cases and no control activities										
Gambia	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Guinea Bissau	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Liberia	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Niger	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Senegal	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Sierra Leone	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Total	36,585	37,385	27,862	25,945	26,200	23,832	19,901	17,103	15,651	11,382

CAR, Central African Republic; DRC, Democratic Republic of the Congo; nd, no data reported

Table 2: DRC, Angola and Sudan are the only countries reporting more than 1000 cases annually. WHO regards 24 countries as endemic for TBG, despite 11 have not reported any new cases from 2004 (17)

Table 3. TBR HAT patients. Annularly incidence between 1997 and 2006

Countries	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006
More than 100 but less than 1,000 new cases per year										
Tanzania	354	299	288	347	258	226	111	157	183	125
Uganda	217	283	283	266	426	328	321	318	479	245
Less than 100 new cases per year										
Malawi	7	10	11	35	38	43	70	47	41	58
Zambia	nd	nd	15	9	6	17	7	35	20	57
Sporadic new cases										
Kenya	5	14	22	12	14	13	0	0	0	1
Mozambique	nd	nd	nd	nd	nd	1	nd	1	nd	nd
Rwanda	nd	nd	nd	nd	8	27	5	22	nd	nd
Zimbabwe	9	nd	nd	nd	nd	nd	nd	nd	4	nd
No new cases										
Botswana	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Burundi	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Ethiopia	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Namibia	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Swaziland	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Total	592	606	619	669	750	655	514	580	727	486

nd, no data reported

Table 3: 5% of all HAT cases are caused by TBR. Only Uganda and Tanzania report more than 100 new cases annually (17).

Table 4 – Strengths and weaknesses of different diagnostic tools for HAT.

	Detection limit (trypanosomes/ml)	Advantages	Disadvantages
Wet blood film	10 000 (7, 79, 107)	- Simple and low-cost.	- Low sensitivity.
Think blood film	5 000 (7, 79, 107)	- Simple and low-cost. - Enhanced sensitivity, compared to wet blood film.	- Low sensitivity. - Time-consuming, compared to wet blood film (10-20 min per sample).
mHCT	500 (79, 99)	- Relatively simple. - Fitted for mass screening.	- Moderate time-consuming.
QBC	450 (79)	- Simple and less time-consuming than think blood smear. - Fitted for mass screening. - Is not being affected by co-infection by malaria.	- The fragile material required for this procedure complicates daily transportation by the mobile teams. - Requires a darkroom for interpretation.
mAECT	50-100 (79, 107-109)	- Most sensitive test being used in the field to date. - Proven to be cost-efficient.	- Time-consuming and expensive (€ 2-3 per test).
PCR	1-1000 (110, 111)	- Superior sensitivity to all tests being used in the field.	- Demands well-equipped laboratory. - Needs further validation.
NASBA	10 (107)	- Runs on isothermal conditions, which makes it less expensive than PCR.	- Requires procedures for purification and preservation, an obstacle for use in the field. - Needs further validation.
LAMP	0.01-10 (7, 99)	- Does not require the elaborate purification methods that PCR and NASBA require. - Easy to interpret.	- Demands electricity and a cooling system for its reagents. - Needs further evaluation.

Table 4 sums up the current, and possible diagnostic tools in the future. The detection limit for PCR, nucleic acid sequence based amplification (NASBA) and loop-mediated isothermal amplification (LAMP), should be read with some reservation, as current studies are insufficient (made by authors).

Figures

Figure 1. Systematic search in PubMed illustrated in flow chart (made by authors).

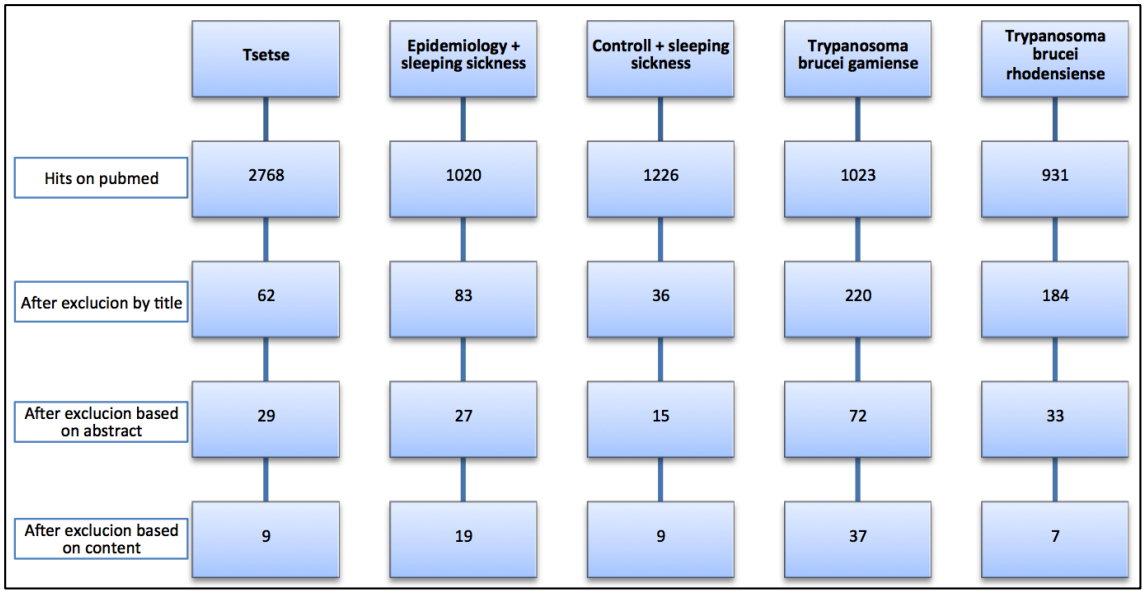
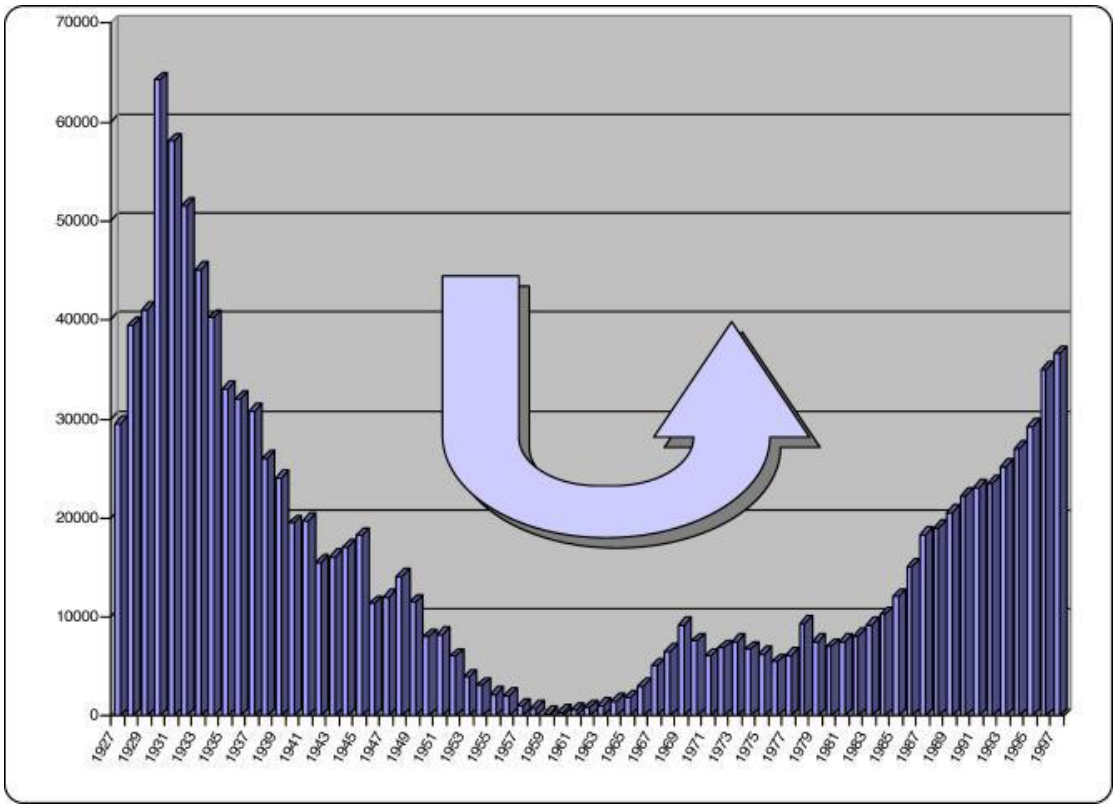


Figure 2. Reported HAT cases all over Africa in the 20th century



Explanation to figure 2: Reported annularly incidence of HAT all over Africa from 1927 to 1997. The incidence seen in the 20s illustrates the second HAT epidemics. The incidence in the 90s illustrates the third HAT epidemic (17).

Figure 3. Map of Africa illustrating the HAT epidemiological status

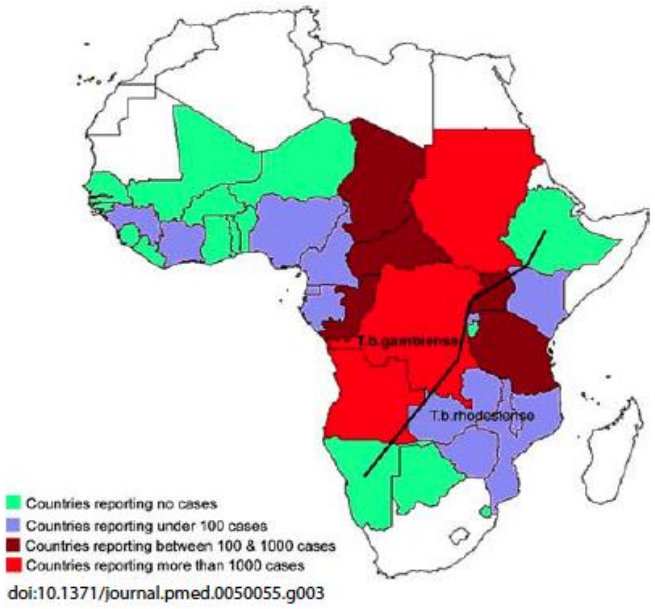


Figure 3: TBR and TBG are roughly separated of the Great rift valley. Uganda is the only country where both of them are present (17).

Figure 4. TBG HAT patients reported DRC between 2000 and 2011

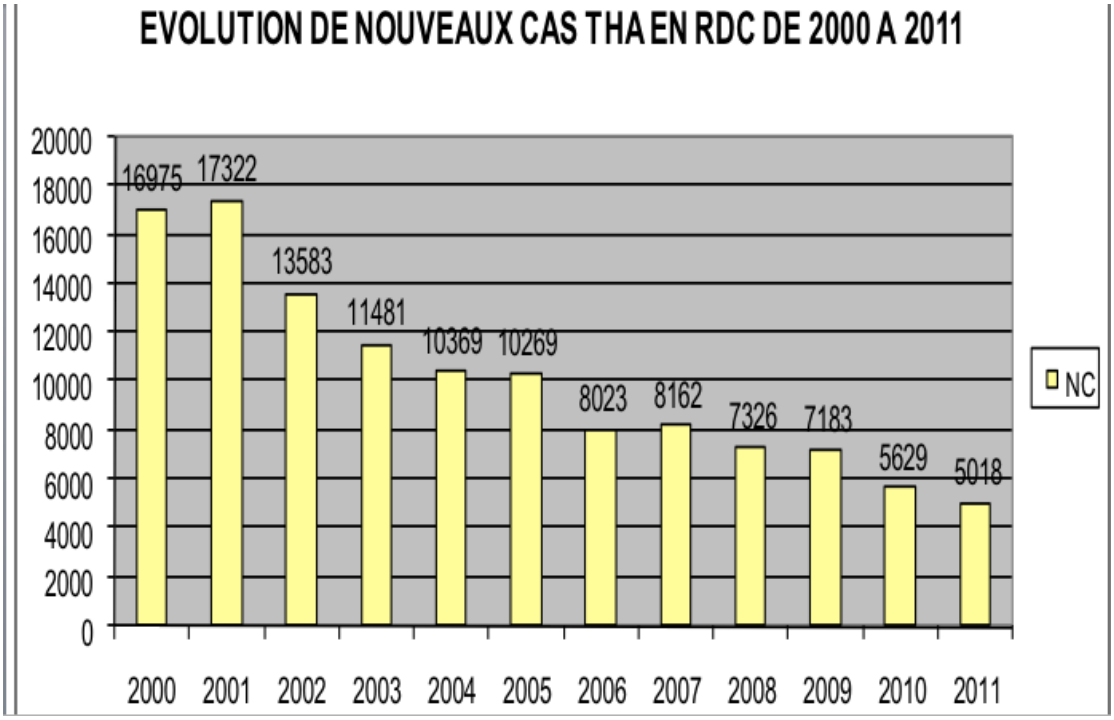
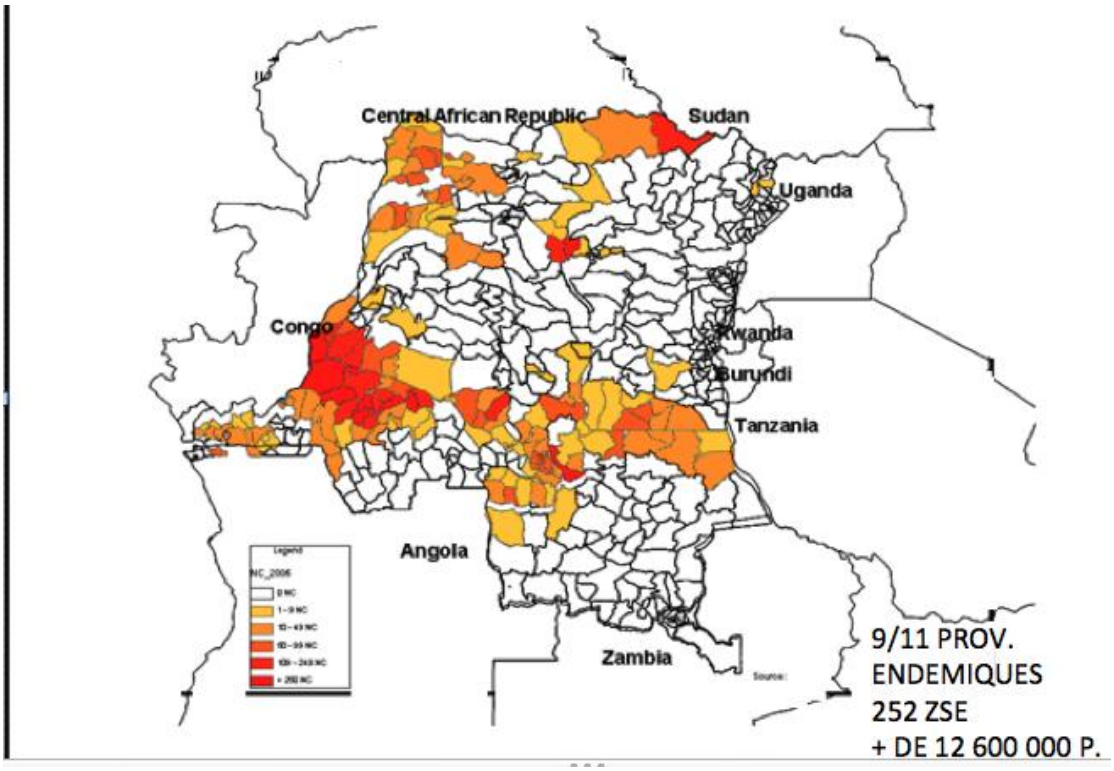


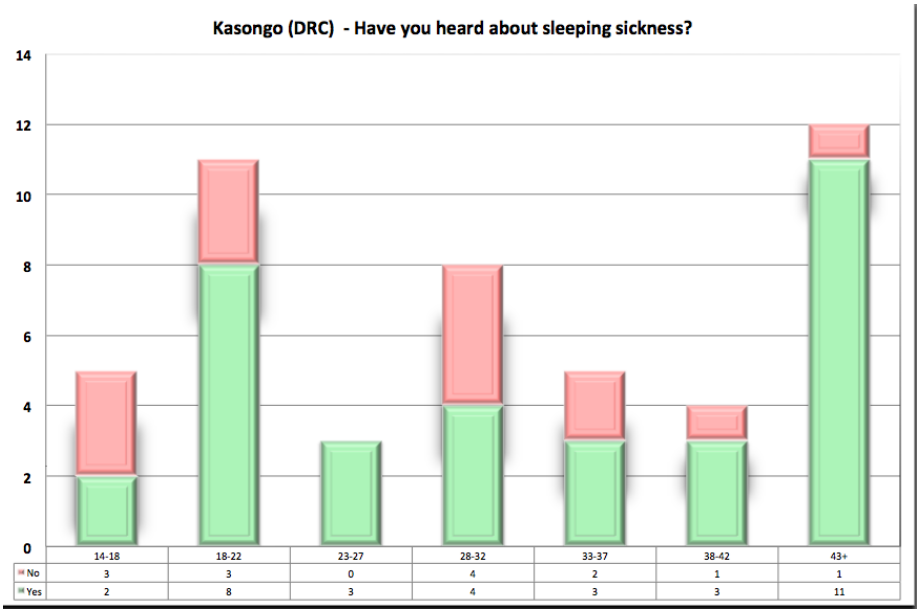
Figure 4: The incidence of HAT in DRC is decreasing. Even though they have 80% of all the reported HAT patients (8).

Figure 5. Illustrating DRCs 281 health zones, with epidemiological status of HAT.



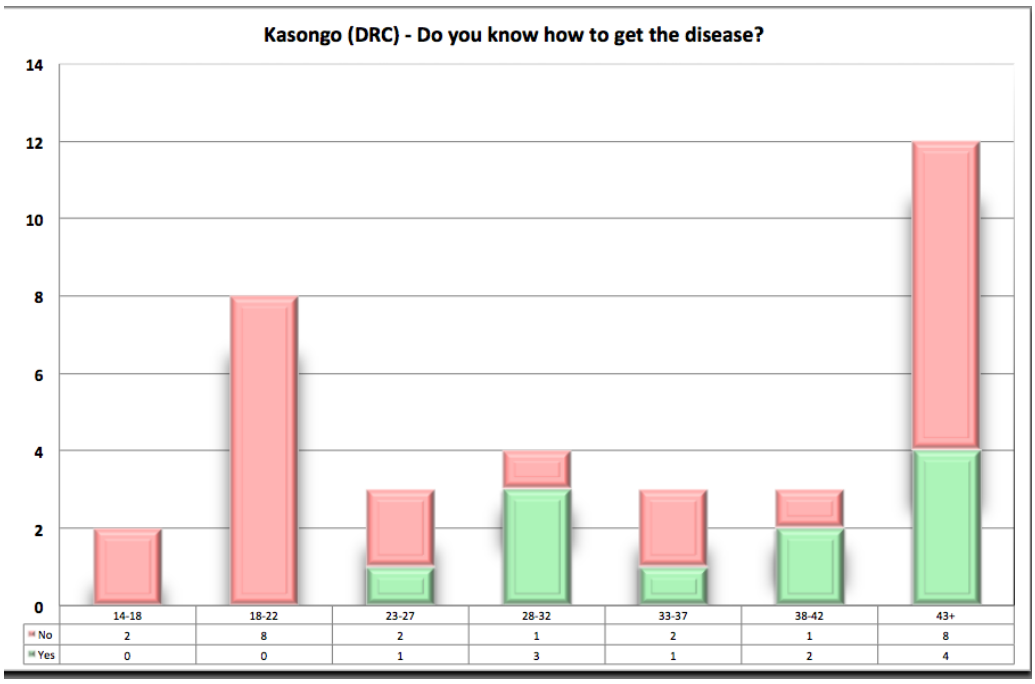
Explanation figure 5: In 2011 the majority of DRCs health zones reports zero new cases (white). 26 health zones reports over 200 TBG HAT cases (red) (8).

Figure 6. Cross section survey, Kasongo, DRC, 2012 (made by authors)



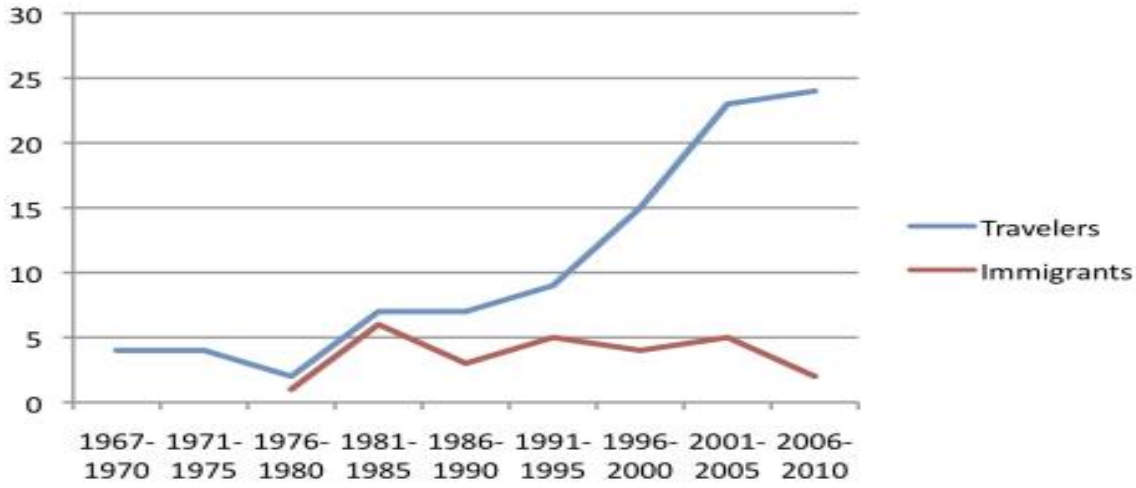
Explanation figure 6: Knowledge of HAT in seen in all groups.

Figure 7. Cross section survey, Kasongo, DRC, 2012



Explanation figure 7: Knowledge of tsetse as the transmitter of HAT is limited, and none existing in the groups 14-17 and 18 - 22 (made by authors).

Figure 8. Sleeping sickness reported cases over years in travellers



Explanation to figure 8: The last two decades show an increase of HAT reported cases in travelers, despite the decrease of HAT cases in endemic areas (18).

Figure 9. Vector transmitted protozoa

Vector Transmitted Protozoa

Protozoa		Vectors	
Parasite	**Disease**	**Common Name**	**Genera**
Trypanosoma gambiense, T. brucei	African sleeping sickness	tse-tse	*Glossina*
Trypanosoma cruzi	Chagas' disease	kissing bugs, etc.	*Triatoma, Rhodnius*
Leishmania	leishmaniasis	sand fly	*Phlebotomus, Lutzomyia*
Plasmodium	malaria	mosquito	*Anopheles*
Babesia	babesiosis	tick	*Ixodes*

Explanation figure 9: Tsetse is a potential vector and host of the protozoan trypanosomes, the cause of HAT (34).

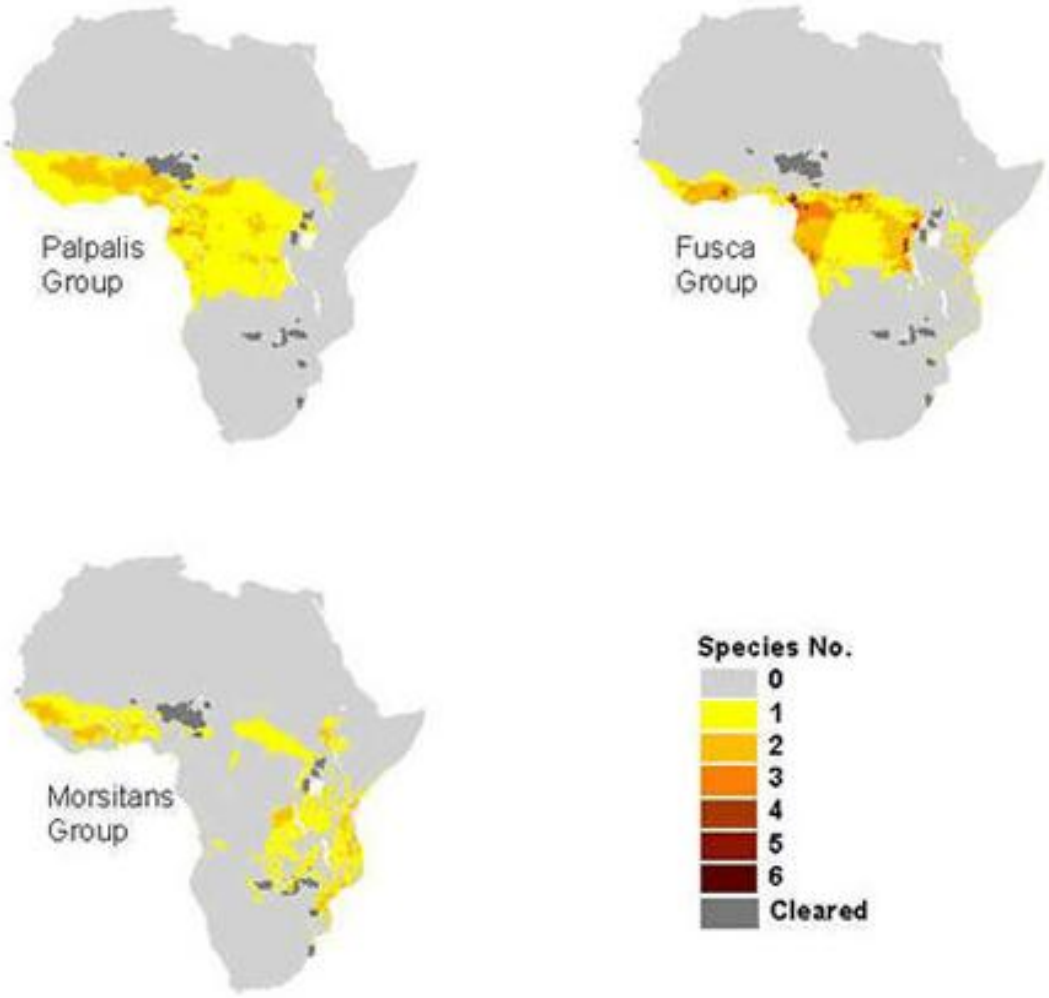
Figure 10. The tsetse fly (picture taken by authors).

Explanation figure 10: An adult tsetse is yellow, brown, or black, and 6-14 mm long. The characteristic used to distinguish it, from house flies, is the rest of wings at the flies abdomen.

Figure 11. Tsetse classification.

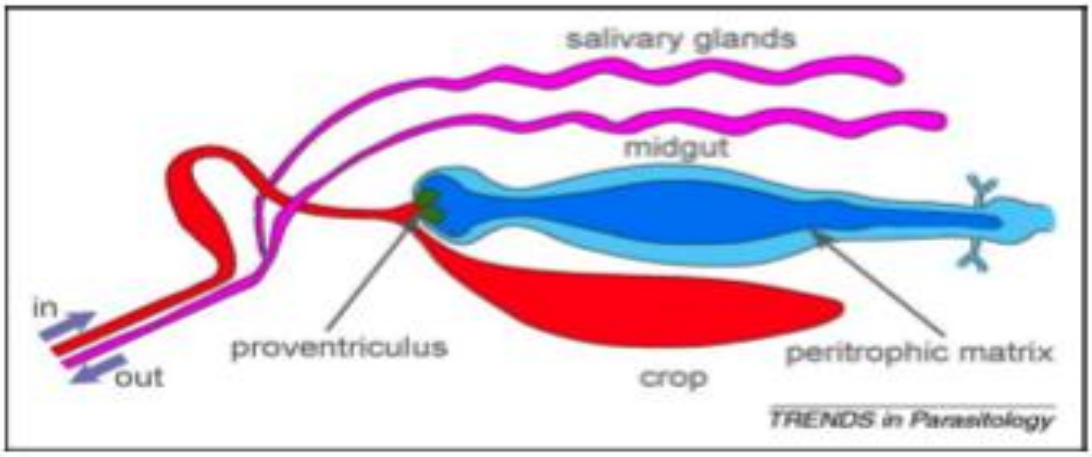
Explanation figure 11: Tsetse is classified into the order *diptera*, and further into the family *glossinidae* (38).

Figure 12. Distribution of tsetse groups.



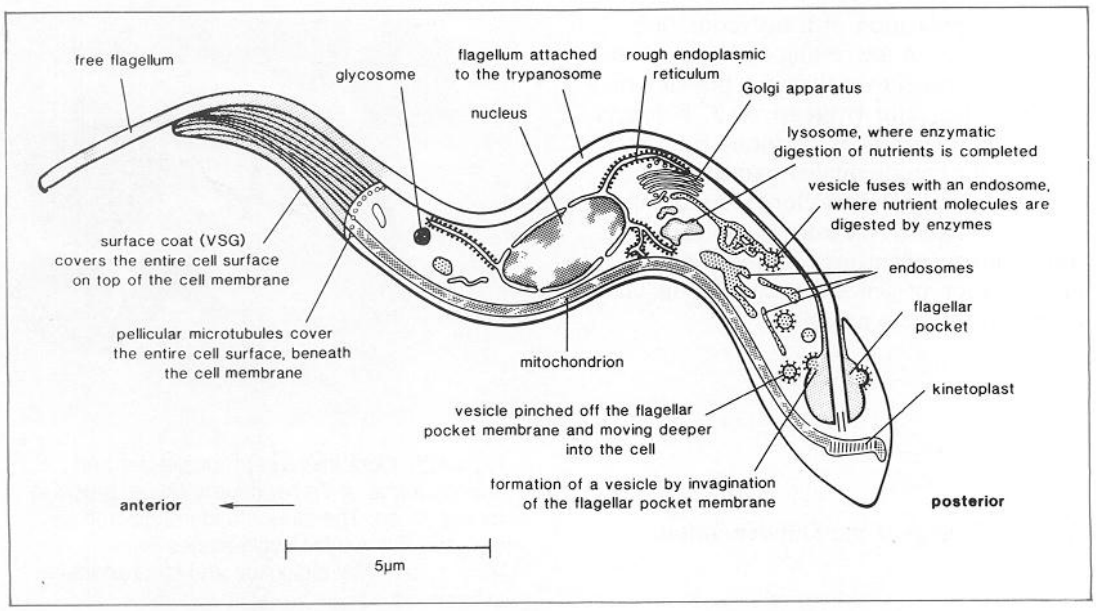
Explanation figure 12: Distribution of the three groups of tsetse. Palpalis group found in west and central Africa, morsitans in east Africa. Respectively main transmitter of TBG and TBR (39).

Figure 13. Schematic illustration of tsetse viscera.



Explanation figure 13: The trypanosomes enter the tsetse midgut after a blood meal. Further they migrate to the ectotropic space. From here, there is an anterior movement to the proventriculus (cardia) In the end they have a differentiation into the mammal-infective metacyclic form, in the salivary glands (35).

Figure 14. Major organelles of the intermediate bloodstream form of *Trypanosoma brucei*.



Basal bodies are not visualized in figure 14, but are situated near the kinetoplast and the flaggelar pocket (50).

Figure 15. Comparison of genetic variation and structure within different isolates of *Trypanosoma brucei.*

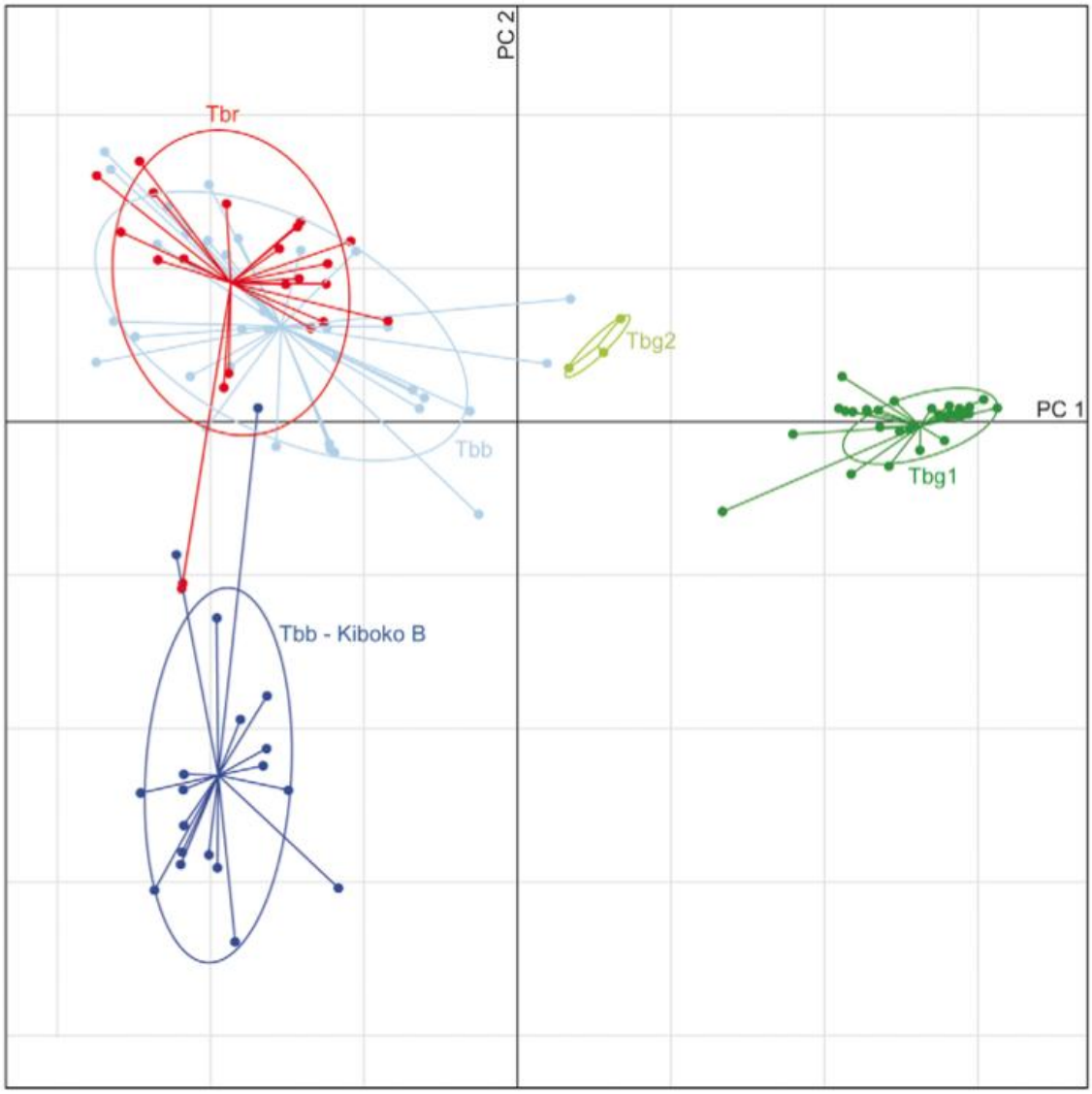


Figure 15 illustrates the genetic between different isolates of Trypanosoma brucei. The dots represent individual genotypes of different strain within different groups of TB. The ellipsis enclosing the groups represents the 95-percentile. The group Tbb - Kikboko B (dark blue) was sampled in Tanzania in 1970-71. It is not known why these samples differ from the other Tbb samples. Possible the Kikboko B-group represents the truly restrictive animal trypanosomiasis form of Tbb, not being able to obtain SRA and therby becoming human-pathogen (55).

Figure 16. Life cycle of the human-pathogen trypanosomes

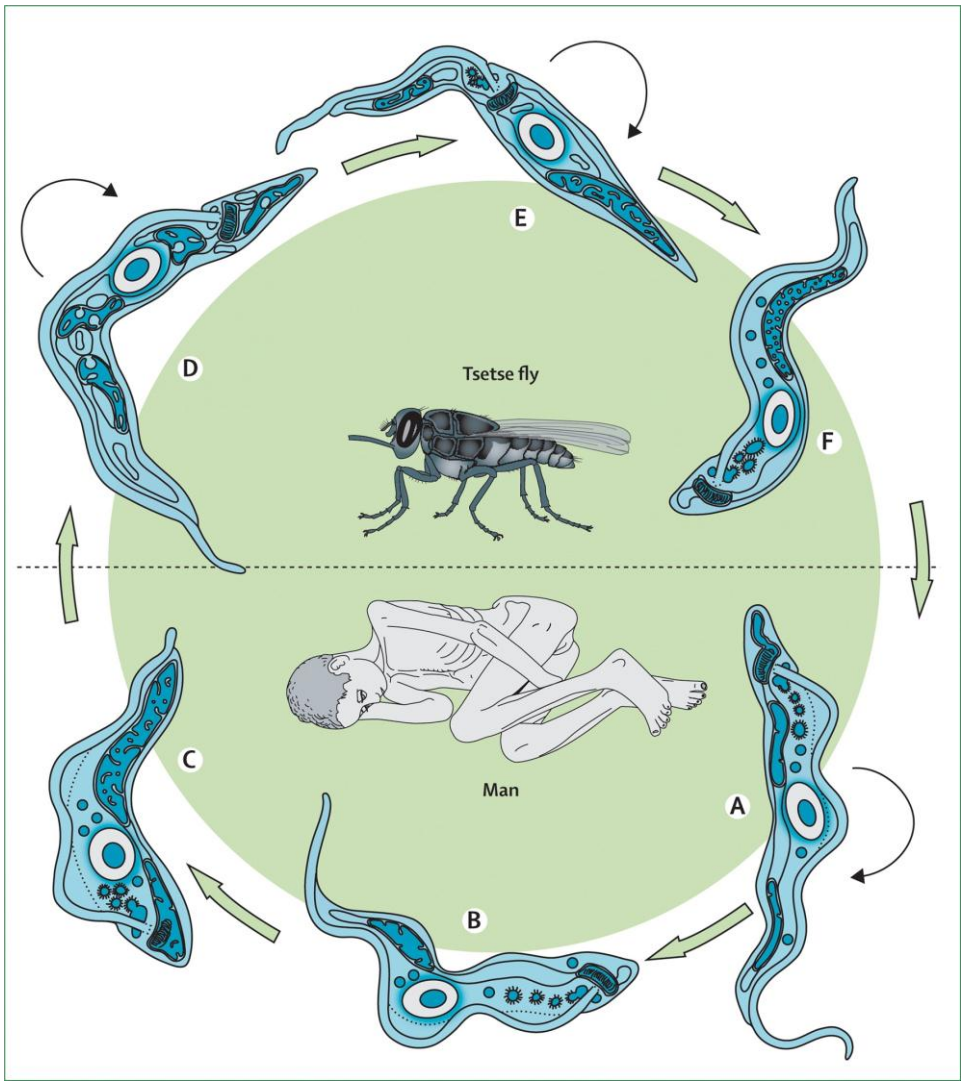


Figure 16 highlights the polymorphism shown by trypanosomes in and tsetse. In man: a) Diseases-causing, dividing long slender trypomastigotes, b) Intermediate trypomastigotes, c) Infective, short stumpy trypomastigotes. In Tsetse: d) dividing, procyclic trypomastigotes in the midgut, e) migrating epimastigotes keep proliferation in the salivary glands and f) Infective non-dividing metacyclic trypomastigotes (4).

Figure 17. Differensation from longer slender to short stump trypomastigotes.

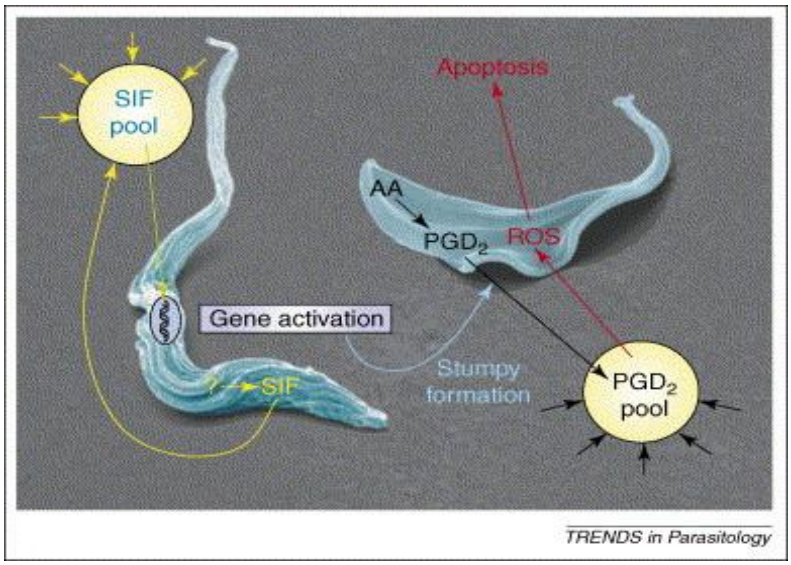


Figure 17 shows a hypothesis on how differensiation of trypanosomes happens in the human body. An unknown differensation factor (SIF) induces differensiation of the long slender trypomatigotes to the stumpy form at a given treshold. The stumpy form uses arachidonic acid (AA) to produce prostaglandin D2 (PGD2). At a given treshold the PGD2 induce apoptosis through mechanisms involving increase intracelluar concentraion of reactive oxygen species (ROS) (58).

Figure 18. Host immune response in HAT.

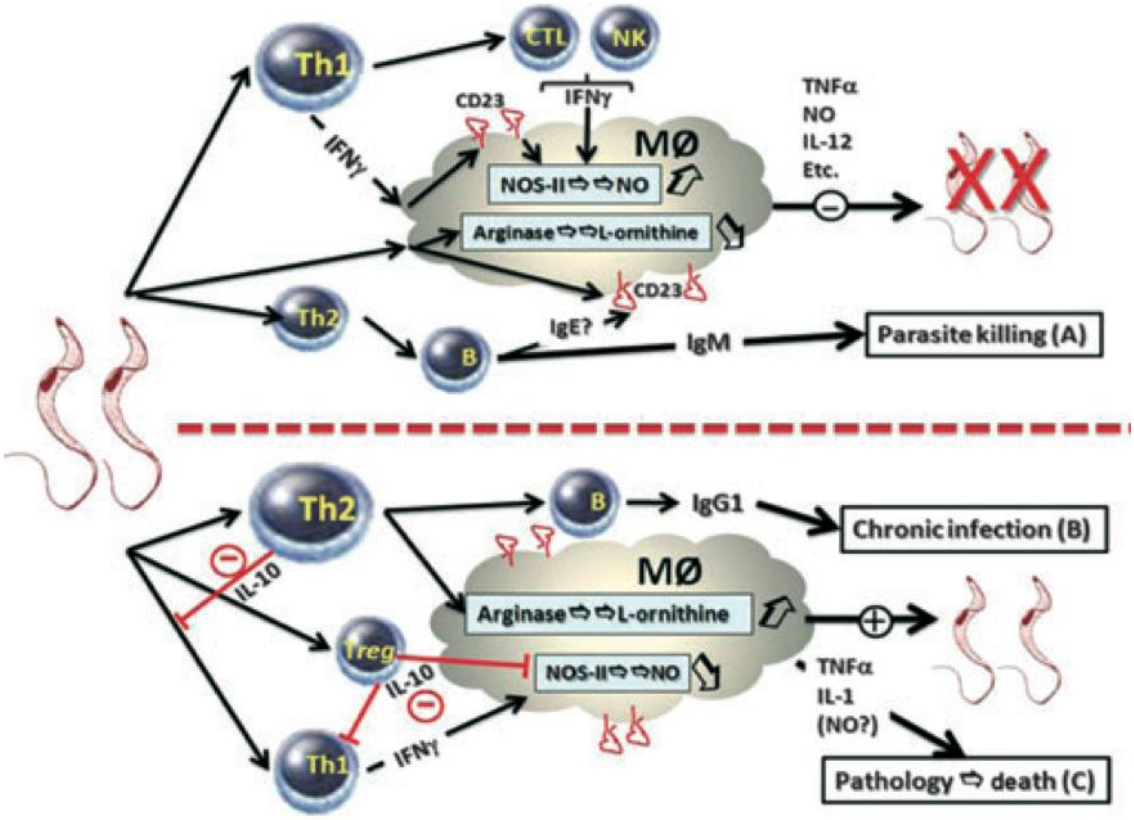


Figure 18 illustrates different outcomes of infection by trypanosomes based on to what degree T-helper 1 cells (Th1) and T-helper 2 cells (Th2) dominate the immune response. Trypanosomes will modulate the immune response to a Th2-response, favoring parasite survival. A Th2-response will make the macrophages (MØ) produce the essential growth factor L-ornithine, while the production of trypanotoxic NO is suppressed. In addition IgG1 will be created during a Th2-response, instead of trypanocidal IgM seen in Th1-responses, favoring chronic infection. Regulatory T-cells (Treg) in low concentration will also inhibit the production of NO. During a Th1-response, the Th1-cells will stimulate cytotoxic T-cells (CTL) and natural killer cells (NK-cells), which will stimulate the MØ to produce NO. In addition L-arginase will be used as a substrate for NO-synthase, leading to more trypanotoxic NO and less of the essential L-ornithine. As the Th1- and Th2-response exacerbate and NO resistance is developed, excessive amount of pro-inflammatory cytokine will lead to host death (13).

Figure 19. Crossing of trypanosomes across the blood-brain-barrier.

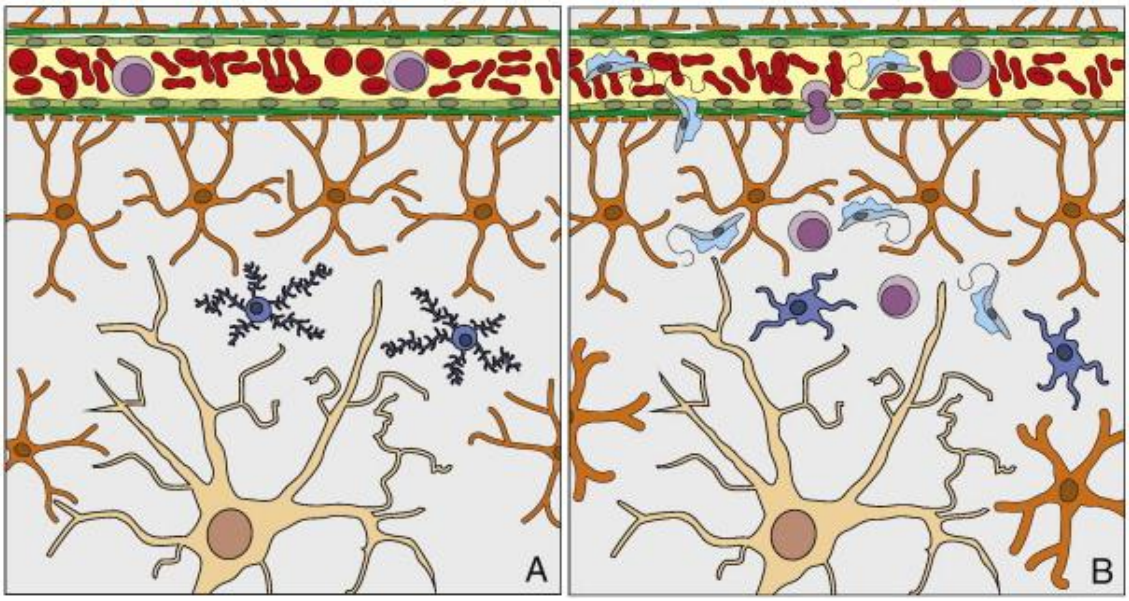


Figure 19 shows: a) normal brain parenchyma and b) *Trypanosoma brucei* entering the brain in late stage. After crossing the endothelium the trypanosomes may get caught temporary between the two basal membranes constituting the BBB. This can be observed histologically as perivascular cuffing (not illustrated here). Local activation of astrocytes and glia cells is likely to occur and plays part in the neuropathogensis of late HAT (65).

Figure 20. Educative poster used by the mobile teams in Kasongo, DRC (picture taken by authors).

Figure 21. Trypanosomal chancre.

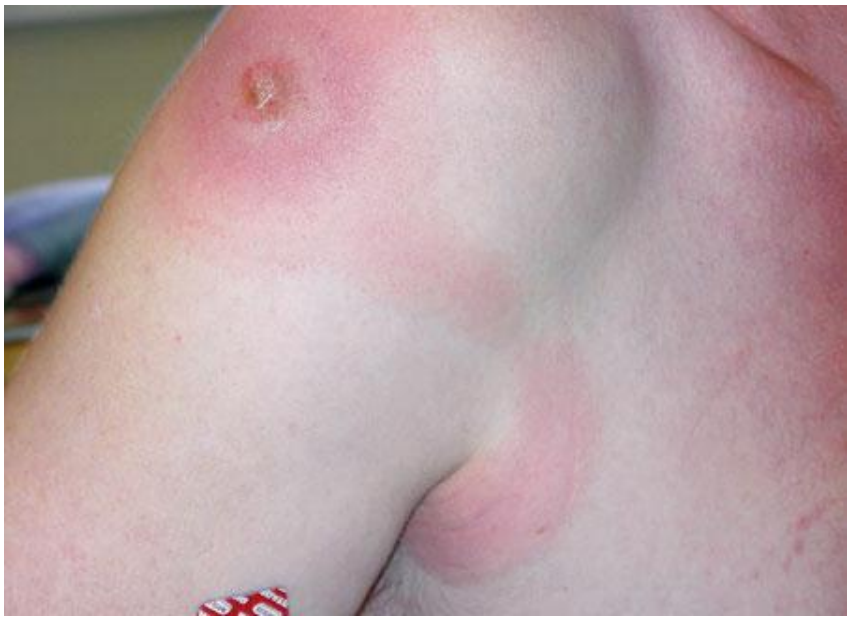


Figure 21 shows a trypanosomal chancre, and subsequent lymphangitis towards the axilla highlighting the affection of the lymphatic system *(69)*.

Figure 22. Major sign and symptoms in HAT.

First (haematolymphatic stage)	Second (meningoencephalitic) stage
Chancre	Sleep disturbances
Lymphadenopathy	Alteration of mental state
Fever	temporospatial disorientation
Headache	personality disorders
Pruritus (scratch marks)	behavioural changes
Skin rash	alteration of mood
Hepatomegaly	Abnormal reflexes
Splenomegaly	osteotendinous reflexes
Musculoskeletal pains	clonus
Anaemia	abnormal cutaneous reflexes
pallor of the conjunctivae	Tone disorders
Oedema	hypertonia
arms	hypotonia
legs	Abnormal movements
Ascites	tremor
Cardiovascular disorders	choreoathetosis
dysrhythmia	Sensory disorders
heart murmurs	paraesthesia
hypotension	deep hyperaesthesia
cardiac dilatation	Coordination disorders
Endocrinological disorders	ataxia
moon face	abnormal gait
amenorrhoea	Other neurological disorders
abortion	convulsions
impotence	hemiplegia
Renal involvement	neurovegetative disorders
albuminuria	archaic reflexes
Intercurrent infections	deterioration of consciousness
lung infections	coma

Figure 22 shows main sign and symptoms put together by a WHO Expert Committee in 1998 (10).

Figure 23. Severe case of african trypanosomiasis at the University Hospital in Kinshasa, DRC (by authors).

Figure 24. Trypanosomal rash in a French travellers pectoral area (74).

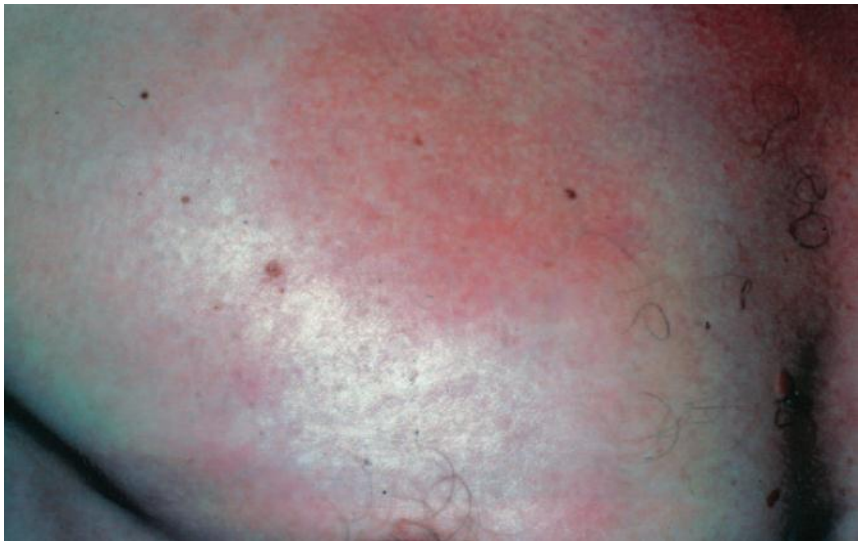


Figure 25. The card agglutination test for trypanosomiasis (picture taken by authors).

Figure 26. Interpretation of the CATT at the INRB (picture taken by authors).

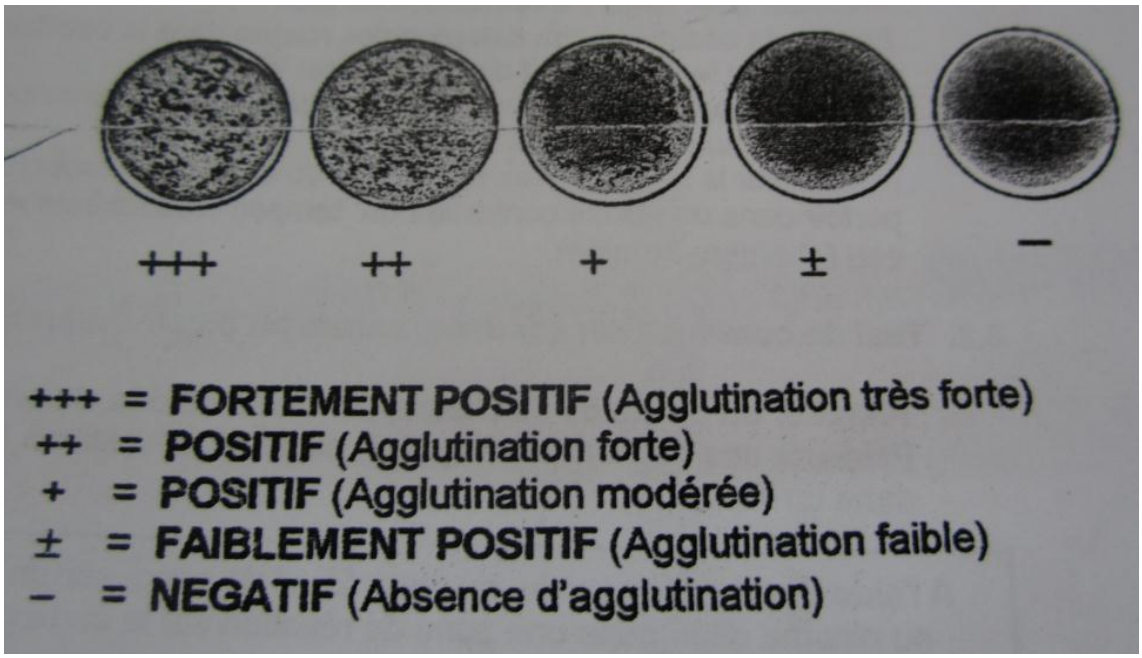


Figure 27. mAECT at *Institut National de Recherche Biomédicale* (INRB) in Kinshasa, DRC (picture taken by authors).

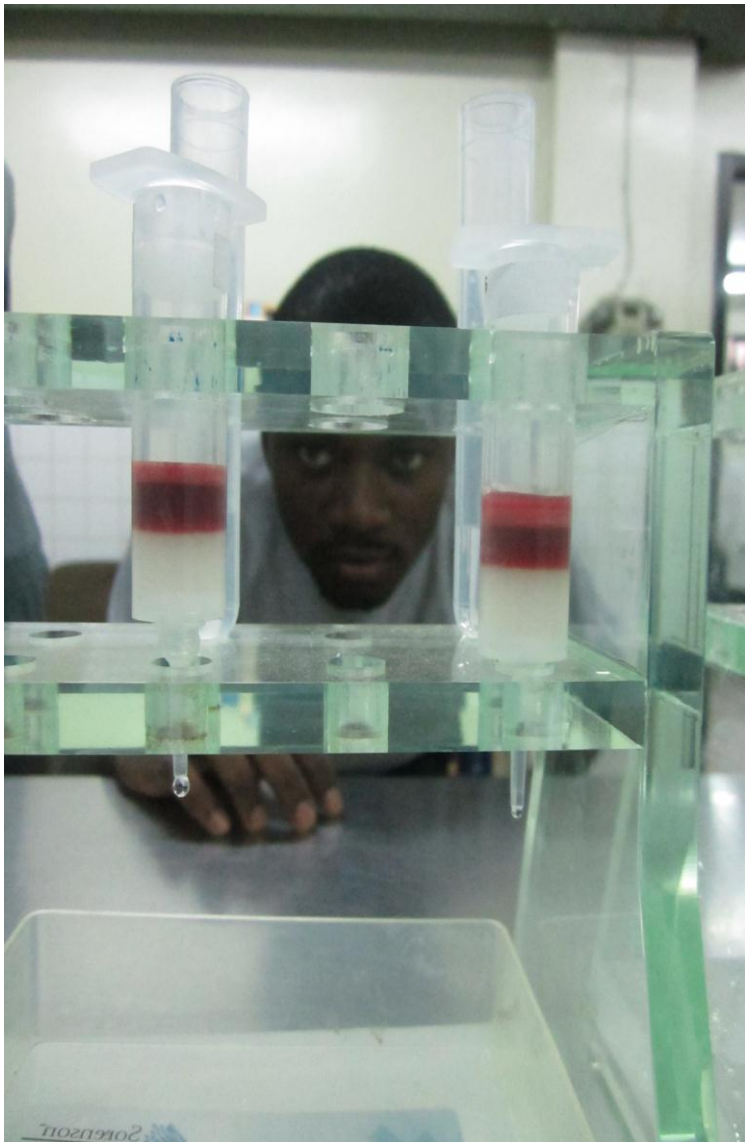


Figure 28. Algorithm being used in the Republic of Congo by the MSF (80).

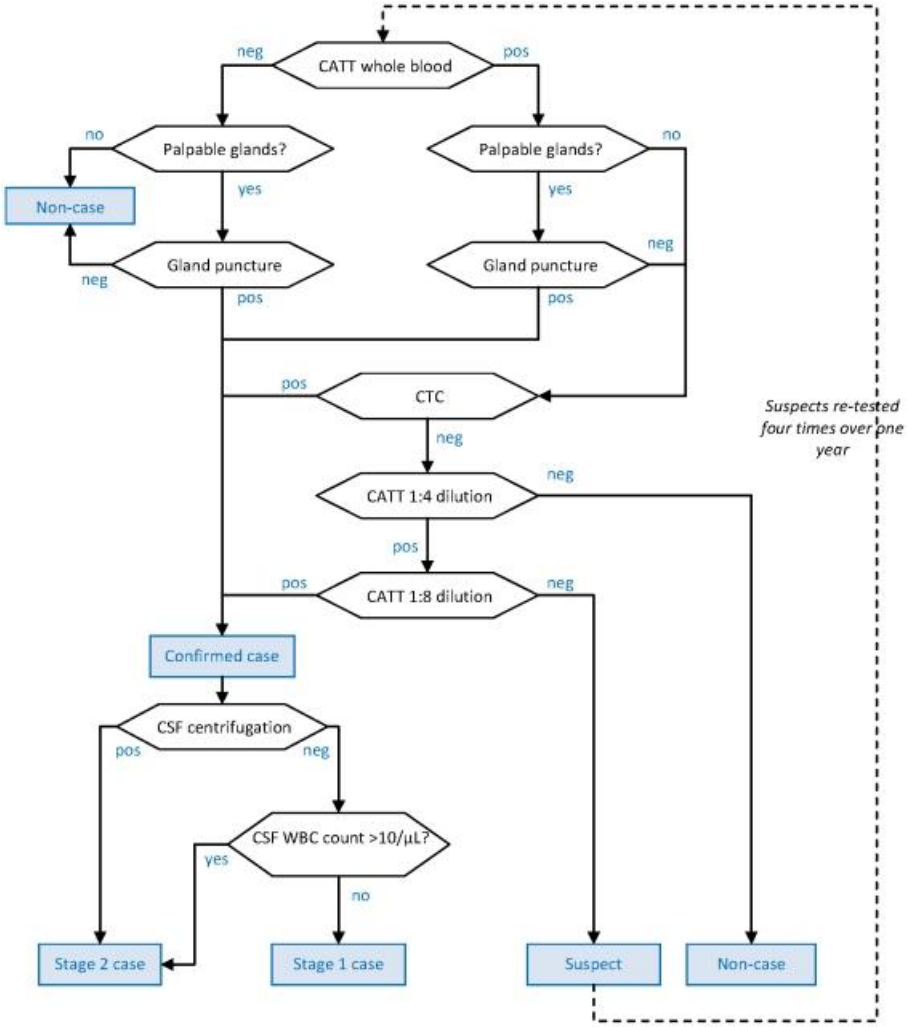


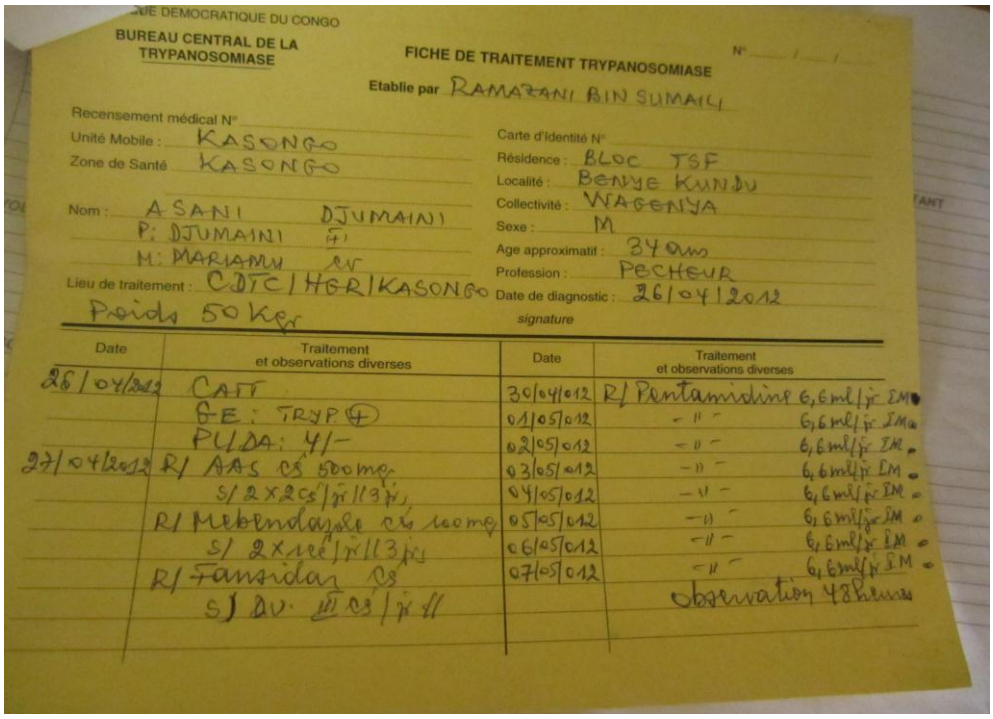
Figure 29. Standard treatments for HAT, and main adverse reactions (4).

	Stage	Route of application	Dosing	Main adverse drug reactions
Trypanosoma brucei gambiense				
Pentamidine*	First	Intramuscular	4 mg/kg bodyweight at 24 h intervals for 7 days	Hypoglycaemia, injection site pain, diarrhoea, nausea, vomiting
Eflornithine	Second	Intravenous Infusion of >30 min	100 mg/kg bodyweight at 6 h intervals for 14 days	Diarrhoea, nausea, vomiting, convulsions; anaemia, leucopenia, and thrombocytopenia
Melarsoprol†	Second	Intravenous	2·2 mg/kg bodyweight at 24 h intervals for 10 days	Encephalopathic syndromes, skin reactions (pruritus, maculopapular eruptions), peripheral motoric (palsy) or sensorial (paraesthesia) neuropathies, thrombophlebitis
Trypanosoma brucei rhodesiense				
Suramin*	First	Intravenous	Test dose of 4–5 mg/kg bodyweight at day 1, then five injections of 20 mg/kg bodyweight every 7 days (eg, day 3, 10, 17, 24, 31); maximum dose per injection 1 g	Hypersensitivity reactions (acute, late); albuminuria, cylinduria, haematuria, peripheral neuropathy
Melarsoprol*	Second	Intravenous	Three series of 3·6, 3·6, 3·6 mg/kg bodyweight, the series spaced by intervals of 7 days; maximum dose per day 180 mg	Encephalopathic syndromes, skin reactions (pruritus, maculopapular eruptions), peripheral motoric (palsy) or sensorial (paraesthesia) neuropathies, thrombophlebitis

*Endemic countries: according to national legislature or guidelines. †Only where eflornithine is not available or where melarsoprol is first-line treatment according to national guidelines.

Table: **Standard treatments for human African trypanosomiasis and main adverse reactions**

Figure 30. Treatment card used at TBG stage I patient in Kasongo (picture taken by authors).



E DEMOCRATIQUE DU CONGO

BUREAU CENTRAL DE LA TRYPANOSOMIASE

FICHE DE TRAITEMENT TRYPANOSOMIASE

N°

Etablie par RAMAZANI BIN SUMAILI

Recensement médical N°

Unité Mobile : KASONGO

Zone de Santé KASONGO

Carte d'identité N°

Résidence : BLOC TSF

Localité : BENYE KUNDU

Collectivité : WAGENYA

Nom : ASANI DJUMAINI

P: DJUMAINI

M: MARIAMU

Sexe : M

Age approximatif : 34 ans

Profession : PECHEUR

Lieu de traitement : CDTC/HGR/KASONGO

Date de diagnostic : 26/04/2012

Poids 50 Kgs

signature

Date	Traitement et observations diverses	Date	Traitement et observations diverses
26/04/2012	CATT	30/04/012	R/ Pentamidine 6,6ml/jr IM
	G-E : TRYP (+)	01/05/012	- " - 6,6ml/jr IM
	PUDA : 4/-	02/05/012	- " - 6,6ml/jr IM
27/04/2012	R/ AAS cs 500mg	03/05/012	- " - 6,6ml/jr IM
	S/ 2x2cs/jr/13jrs	04/05/012	- " - 6,6ml/jr IM
	R/ Mebendazole cs 100mg	05/05/012	- " - 6,6ml/jr IM
	S/ 2x1cs/jr/13jrs	06/05/012	- " - 6,6ml/jr IM
	R/ Fansidar cs	07/05/012	- " - 6,6ml/jr IM
	S/ DU. III cs/jr/1		Observation 48heures

Figure 31. Patient (in the middle) at the University Hospital in Kinshasa (DRC), with aphasia and motoric squeal due to delay in diagnosis and treatment and diagnosis. One of the authors at the right (picture taken by authors).

Figure 32. Tsetse distribution and income per capita in Africa (32).

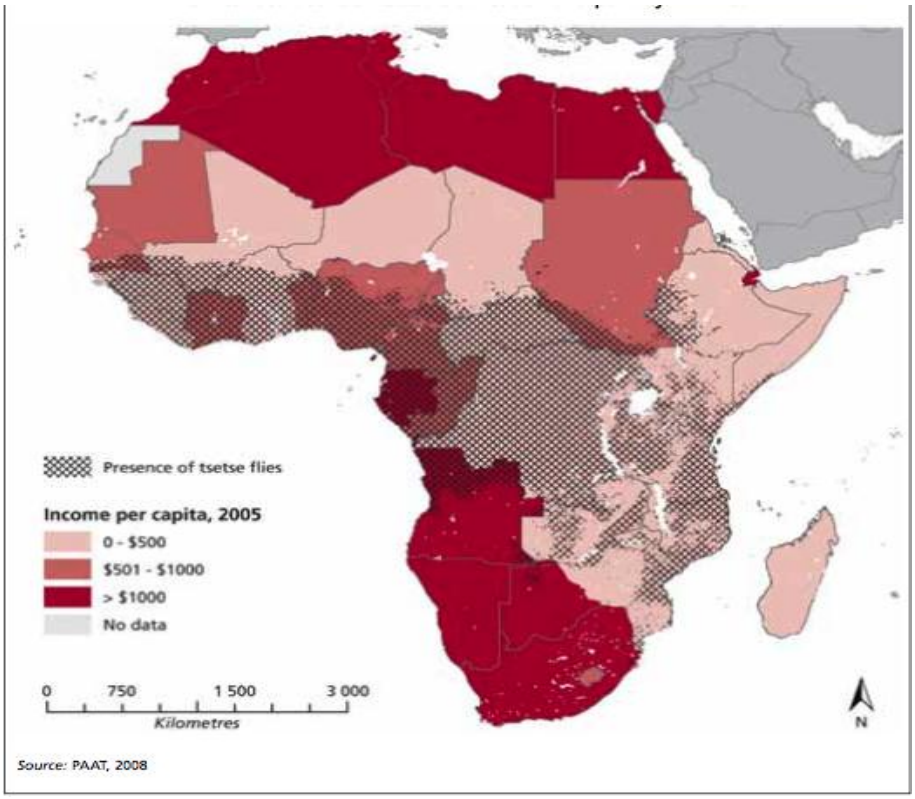
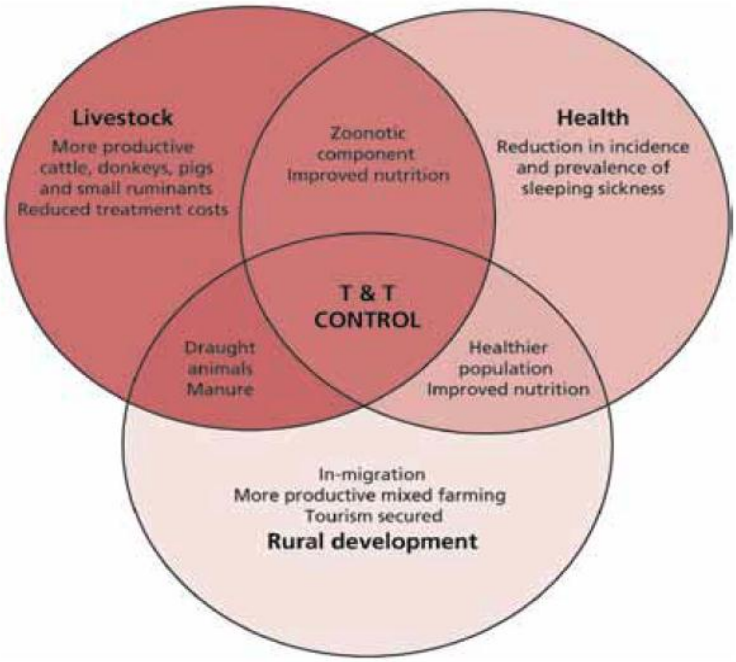
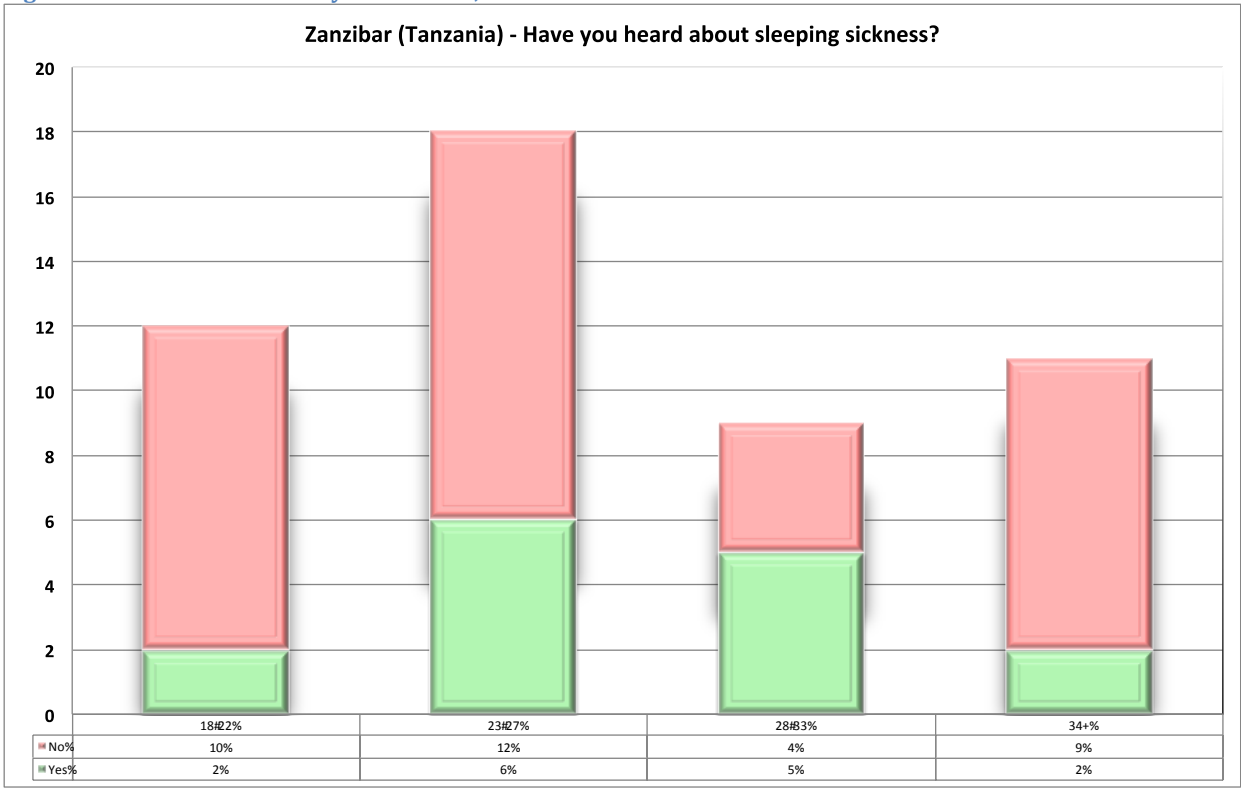


Figure 33. Benefits arising of T&T control.



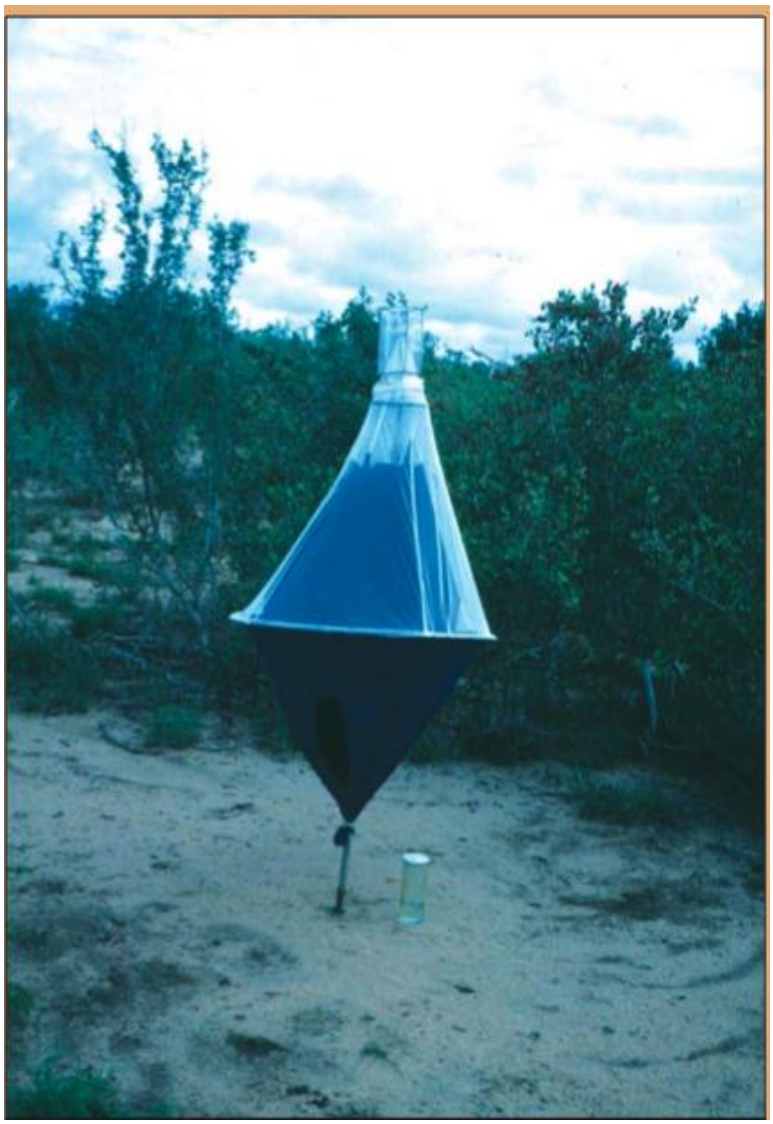
Explanation figure 33: The intersectoral nature of the benefits arising from programs designed to control tsetse-transmitted trypanosomiasis (32).

Figure 34. Cross section survey at Zanzibar, Tanzania 2012.



Explanation figure 34: Zanzibar is an earlier HAT endemic island. Study carried out without distinguishing between sexes. Age is registered.

Figure 35. Biconical Trap



Explanation figure 35: The most common used tsetse trap, the biconical trap. Used both to collect tsetse and to prevent HAT. The Biconical trap consists of two cones each 80 cm wide, an upper cone 73 cm high and a lower cone 60 cm high, joined at their widest point (92).

Figure 36. Car stop due to heavy rain and a bad road (picture taken by authors).

Explanation to figure 36: The authors experienced the roads in the Manemia province in the eastern DRC. The main road from Kindu to Kasongo, N31, is 250 km long. Expected travel time can be all from 6 hours to 20 hours, entirely depending on the weather