In the 1960s, it appeared that human African trypanosomiasis (HAT) could be effectively controlled, but by the beginning of the twenty-first century several decades of neglect had led to alarming numbers of reported new cases, with an estimated 300 000 people infected. The World Health Organization (WHO) responded with a series of initiatives aimed at bringing HAT under control again. Since 2001, the pharmaceutical companies that produce drugs for HAT have committed themselves to providing them free of charge to WHO for distribution for the treatment of patients. In addition, funds have been provided to WHO to support national sleeping sickness control programmes to boost control and surveillance of the disease. That, coupled with bilateral cooperation and the work of nongovernmental organizations, helped reverse the upward trend in HAT prevalence. By 2012, the number of reported cases was fewer than 8000. This success in bringing HAT under control led to its inclusion in the WHO Roadmap for eradication, elimination and control of neglected tropical diseases, with a target set to eliminate the disease as a public health problem by 2020. A further target has been set, by countries in which HAT is endemic, to eliminate gambiense HAT by reducing the incidence of infection to zero in a defined geographical area.

This report provides information about new diagnostic approaches, new therapeutic regimens and better understanding of the distribution of the disease with high-quality mapping. The roles of human and animal reservoirs and the tsetse fly vectors that transmit the parasites are emphasized. The new information has formed the basis for an integrated strategy with which it is hoped that elimination of gambiense HAT will be achieved. The report also contains recommendations on the approaches that will lead to elimination of the disease.

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WHO Expert Committee on human African trypanosomiasis

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Control and surveillance of human African trypanosomiasis

Report of a WHO Expert Committee

World Health Organization

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Abbreviations and acronyms

CATT	card agglutination test for trypanosomiasis
CATT-P	CATT performed on plasma (or serum) dilutions
CATT-WB	CATT performed on undiluted whole blood
CNS	central nervous system
CSF	cerebrospinal fluid
EDTA	ethylene diamine tetraacetic acid
ELISA	enzyme-linked immunosorbent assay
FAO	Food and Agriculture Organization of the United Nations
GPS	global positioning system
HAT	human African trypanosomiasis
HPLC	high-performance liquid chromatography
Ig	immunoglobulin
IMPAMEL	programme for improved application of melarsoprol
LAMP	loop-mediated isothermal amplification
LED	light-emitting diode
mAECT	mini-anion exchange centrifugation technique
mAECT-BC	mAECT on buffy coat
mHCT	micro-haematocrit centrifugation technique
NECT	nifurtimox–eflornithine combination therapy
PCR	polymerase chain reaction
R_0	basic reproduction number
SRA	serum resistance-associated
TDR	UNDP/World Bank /WHO Special Programme for Research and Training in Tropical Diseases
TGSGP	*Trypanosoma brucei gambiense*-specific glycoprotein
VSG	variant surface glycoprotein
WBC	white blood cell
WHO	World Health Organization

WHO Expert Committee on control and surveillance of human African trypanosomiasis

Geneva, 22–26 April 2013

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1. Introduction

A World Health Organization (WHO) Expert Committee on the control and surveillance of human African trypanosomiasis (HAT) met in Geneva, Switzerland, from 22 to 26 April 2013. Dr H. Nakatani, Assistant Director-General for HIV/AIDS, Tuberculosis, Malaria and Neglected Tropical Diseases, opened the meeting on behalf of Dr M. Chan, Director-General of WHO.

HAT is a disease that afflicts populations in rural Africa, where the tsetse fly vector that transmits the causative trypanosome parasites thrives. There are two forms of HAT: one, known as gambiense HAT, is endemic in West and Central Africa and causes over 95% of current cases; the other, known as rhodesiense HAT, is endemic in East and southern Africa and accounts for the remainder of cases. The presence of parasites in the brain leads to progressive neurological breakdown. Changes to sleep–wake patterns are among the symptoms that characterize the disease, also known as "sleeping sickness". Eventually, patients fall into a coma and die if not treated. Different treatments are available against parasites present in the haemo-lymphatic system (first-stage) and those that have entered the brain (second-stage). Currently, lumbar puncture is required to select the appropriate drug.

Despite significant progress, the current drugs are unsatisfactory because of the complexity of their administration, the need for hospitalization and the toxicity of the drugs. In the 1960s, it appeared that HAT could be effectively controlled, but, by the beginning of the twenty-first century, several decades of neglect had led to alarming numbers of reported new cases, with an estimated 300 000 people infected. The resurgence of the disease was considered a public health calamity. WHO responded with a series of initiatives aimed at bringing HAT under control again. Since 2001, the pharmaceutical companies that produce drugs for HAT have committed themselves to provide them free of charge to WHO for distribution for the treatment of patients. In addition, funds have been provided to WHO to support national sleeping sickness control programmes to boost control and surveillance of the disease.

That, coupled with bilateral cooperation and the work of nongovernmental organizations, helped reverse the upward trend in HAT prevalence. By 2012, the number of reported cases was fewer than 8000, although the actual number was estimated to be about 20 000 because of incomplete surveillance.

This success in bringing HAT under control led to its inclusion in the WHO "roadmap for eradication, elimination and control of neglected tropical diseases", with a target set to eliminate the disease as a public health problem by 2020, when fewer than one new case per 10 000 inhabitants in at least 90% of endemic foci is expected. A further target has been set by countries in which HAT

is endemic, to eliminate gambiense HAT by reducing the incidence of infection to zero in a defined geographical area.

The previous report of the WHO Expert Committee on this disease followed a meeting in 1995. Intensive, coordinated efforts against HAT during the intervening 18 years have resulted in a decrease in incidence to a point at which elimination is considered feasible. This report provides information about new diagnostic approaches, new therapeutic regimens and better understanding of the distribution of the disease with high-quality mapping. The roles of human and animal reservoirs and the tsetse fly vectors that transmit the parasites are emphasized. The new information has formed the basis for an integrated strategy with which it is hoped that elimination of HAT will be achieved. The report also contains recommendations on the approaches that will lead to elimination of the disease.

2. Epidemiology of human African trypanosomiasis

Human African trypanosomiasis is transmitted only in sub-Saharan Africa, where there are suitable habitats for the tsetse fly vector. The 31 species and subspecies of *Glossina* have a wide range of environmental requirements, resulting in discontinuous distribution of the vector across the African tsetse belt. The interrelationships among the host, vector and parasite are complex, and the geographical distribution of HAT is highly focal.

2.1 Two diseases, two parasites, two epidemiological patterns

Two subspecies of *Trypanosoma brucei* are pathogenic for humans: *T. b. gambiense* and *T. b. rhodesiense*. Early work on sleeping sickness at the turn of the past century led to identification of the causative agent. The cause of "Trypanosoma fever" in The Gambia was reported in 1902 by Forde (*1*) and described by Dutton (*2*) as *T. gambiense*. In 1903, both Castellani (*3*) and Bruce and Nabarro (*4*) found an association between the occurrence of trypanosomes and sleeping sickness in Uganda. *T. rhodesiense* was first described as a separate parasite in present-day Zambia in 1910 (*5*). Infection with either *T. b. rhodesiense* or *T. b. gambiense* is termed "HAT" or "sleeping sickness"; however, biologically, clinically, therapeutically, geographically and, most importantly, epidemiologically, these parasites present as distinct entities and cause, in effect, separate diseases (*6*). Appreciation of the differences is essential to understanding the options for HAT control.

T. b. rhodesiense causes an acute, rapidly progressive infection in eastern and southern Africa, whereas *T. b. gambiense* infection is found in West and Central Africa and progresses at a more indolent pace than that of *T. b. rhodesiense*. The geographical separation of these two forms of infection may, however, change in the future, because *T. b. rhodesiense* has been spreading towards north-west Uganda, where the two forms could overlap (*7*). Throughout the past century, the vast majority of HAT cases were due to *T. b. gambiense*, and this trend continues, *T. b. gambiense* infection accounting for 97% of reported cases in the decade 2000–2009 (*8*). Nevertheless, *T. b. rhodesiense* has always had epidemic potential; the largest recorded epidemic killed more than 250 000 people over 15 years between 1900 and 1915 (*9*). There has been confusion about the identity of the causative organism in the early epidemics, however, because *T. b. rhodesiense* had not yet been formally described when they occurred (*10*, *11*).

2.2 Transmission cycle

2.2.1 Gambiense human African trypanosomiasis

While the life-cycle of the parasite is important in understanding its biology (see section 3 for more details), the transmission cycle is important in understanding its epidemiology. *T. b. gambiense* is transmitted to humans through the bite of an infected tsetse of the genus *Glossina*. There are 31 species and subspecies of tsetse, which are classified into three groups mainly according to habitat: fusca group (forest), morsitans group (savannah) and palpalis group (riverine and forest) (*12*) (see section 4 for more details). Under laboratory conditions, many *Glossina* species transmit *T. b. gambiense*, but, in nature, the infection is carried almost exclusively by flies of the palpalis group, especially *G. fuscipes* and *G. palpalis*. Both male and female flies feed on blood and are capable of transmitting infection. After the fly has fed on an infected host, the parasite undergoes several differentiation steps in the fly midgut, and, after 18–35 days, infective forms appear in the salivary glands. The interval required for maturation depends on several factors and is particularly sensitive to temperature (*13*). The risk for becoming infected is highest in newly hatched tsetse flies that are taking their first blood-meal—so-called teneral flies (*14*). Once infected, a fly remains so for life. Because mainly young flies are susceptible to infection and because the majority of ingested trypanosomes fail to develop, the prevalence of naturally acquired mature *T. b. gambiense* in tsetse is low, and less than about 0.1% of flies carry a transmissible infection (*15*, *16*). The infectious dose of *T. b. gambiense* has not been determined but is thought to be small. *T. b. rhodesiense* infection was transmitted to all vervet monkeys exposed to a single bite from a single infected fly (*17*).

Although the predominant mode of transmission is by vectors, other routes of transmission exist. Since 1933, 13 reports have been published of cases of congenital *T. b. gambiense* transmission diagnosed within 5 days of birth, and three additional cases were identified in children born to infected mothers who had left the endemic area before delivery (*18*). Congenital infection was thought to be uncommon, because of the small number of documented cases and the fact that *T. b. gambiense* infection can cause infertility. This mode of transmission, although not a major route of acquiring infection, is, however, undoubtedly more common than is currently recognized. A low index of suspicion, lack of routine pregnancy testing of HAT patients and the substantial number of deliveries that occur outside health centres are likely to contribute to under ascertainment. Furthermore, the clinical manifestations in neonates may not be apparent within the 5-day post-birth interval that is used to define congenital infection. Additional cases of HAT diagnosed in neonates older than 5 days and in very

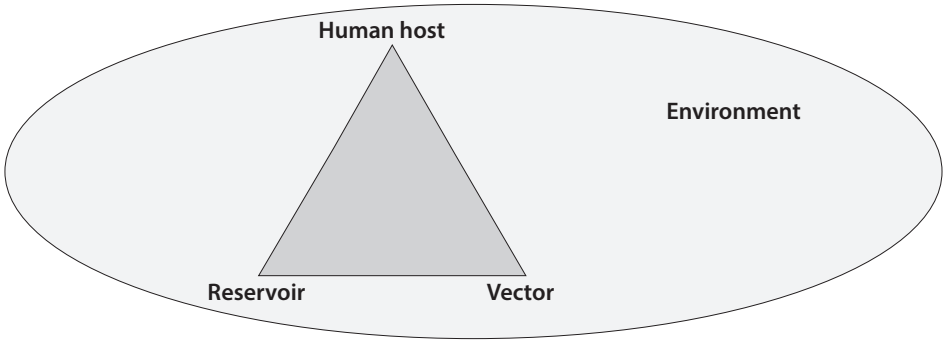
young infants have been reported, and congenital transmission was assumed (*18*). In a programme in which 200–400 new HAT patients were treated annually, one or two congenital infections were diagnosed (*19*).

Other modes of transmission are possible. Theoretically, the infection can be acquired through blood transfusion or organ transplantation, although such cases have not been reported. Transmission of the parasite through close personal or sexual contact was documented in a woman who had never visited an endemic country and who had no risk factors for infection other than exposure to her partner, a man with confirmed gambiense HAT (*20*). Accidental transmission in laboratories has also occurred occasionally (*21*).

2.2.2 Rhodesiense human African trypanosomiasis

Infection with *T. b. rhodesiense* occurs through the bite of an infected tsetse, as described for *T. b. gambiense*, above. The transmission cycle involves obligate biological development in the fly, although there is a small risk for mechanical transmission by a tsetse if parasitaemia is high enough (*22*). Congenital infection cannot be excluded in infants (*23*), although the severity of rhodesiense HAT disease, which is different from that induced by *T. b. gambiense*, makes this unlikely. A single bite by an infected tsetse fly is sufficient to cause infection in the mammalian host (*17*); equally, low parasitaemia resulting in a tsetse taking up only one parasite in a feed is sufficient to result in infection of the fly (*24*). In both forms of HAT, infection requires association of the three elements of the "epidemiological triangle": human host, reservoir and tsetse fly in an appropriate environment. This is illustrated in *Figure 2.1*. For gambiense HAT, the main reservoir is other human beings. Rhodesiense HAT, however, is a zoonosis, a "disease or infection naturally transmitted between vertebrate animals and humans" (*25*). Further, the rapid onset of severe symptoms in infected humans is

Figure 2.1
Epidemiological triangle for the transmission cycle of human African trypanosomiasis



likely to affect the epidemiological dynamics, as patients are less likely to be in the tsetse fly-infested environment that serves as a reservoir of infection of the vector. The transmission cycle of *T. b. rhodesiense* thus involves mainly transmission between non-human reservoirs by tsetse flies, with occasional animal–tsetse–human transfer.

2.3 Reservoirs of infection

2.3.1 Gambiense human African trypanosomiasis

It is generally accepted that humans constitute the epidemiologically important reservoir of *T. b. gambiense*. Although the vector competence of tsetse flies is relatively low, the long duration of human infection, about 3 years (*26*), is more than sufficient to maintain a human–fly–human transmission cycle. Furthermore, gambiense HAT control measures aimed solely at the human reservoir have been highly successful in reducing HAT transmission. A single round of population screening and treatment of cases typically reduces the prevalence of infection by several fold, and a very low prevalence can be reached after several annual rounds of population screening, without the use of vector control. Elimination of gambiense HAT was achieved in the Luba focus in Equatorial Guinea through active and passive case detection, treatment of confirmed cases and limited treatment of seropositive individuals (*27*); vector control was not used.

In other foci, however, active case detection conducted over many years has not led to elimination, and transmission continues (*28*) or has been reactivated (*29*, *30*). The persistence of transmission may be due in part to the fact that the effectiveness of active case detection is limited by incomplete attendance at population screening and by diagnostic inaccuracy. Model-based analysis of mass screening data has shown that the rate of under detection of cases may be up to 50% in most scenarios (*31*), and, even in well-resourced programmes, 20–50% of prevalent infections are not identified during active case detection (*32*).

An increasing body of data suggests the existence of an additional, unrecognized reservoir of infection. A genetic study of the parasite population conducted in Côte d'Ivoire and Guinea showed that the size of the parasite clonal population is larger than the prevalence observed by active case finding (*33*). One hypothesis to account for the additional parasite population is the existence of chronic, asymptomatic carriers, i.e. individuals who are seropositive but in whom parasites are not detectable by the most sensitive field methods. Self-cure and asymptomatic carriage have been reported in the past (reviewed in *34*), but interpretation of these observations was difficult because the diagnostic methods used at the time lacked both sensitivity and specificity. More recent data support the existence of "trypanotolerance" in humans (*35*). Many different infection outcomes were observed during long-term follow-up (5–15 years) of patients

parasitologically confirmed in first-stage HAT who refused treatment (*36*). While in this study the infection progressed to second-stage in most patients, as expected, a subset remained asymptomatic, became aparasitaemic by microscopy and polymerase chain reaction (PCR) and had a decreasing serological response, consistent with resolution of infection. A second subset of patients, although asymptomatic and aparasitaemic, showed continued seroreactivity and might be chronic carriers of infection. Additional data to support a chronic carrier state are the observations that aparasitaemic seropositive individuals are reactive in the highly specific immune trypanolysis test (*37*), and, although parasites cannot be found by microscopy, PCR gives intermittent positive results (*38*). Better understanding is needed of the immune response in these individuals to determine whether they play a role in maintaining transmission, so that appropriate strategies for sustainable control and elimination are used.

T. b. gambiense has been found in a variety of domestic and wild animals (*39–44*), and animals can be infected experimentally (*45, 46*). The exact role of these animal hosts as reservoirs in the epidemiology of human infection, however, remains uncertain. In some foci, mixed infections with *T. congolense*, *T. vivax* and *T. brucei* sensu lato (s. l.), including *T. b. gambiense* group 1, are found in livestock, most often in pigs, although this is not observed consistently. Other foci in which human infection is present show no evidence of *T. b. gambiense* group 1 in domestic animals (*41*, *47*). In a focus in Côte d'Ivoire, the genotypes of *T. brucei* s. l. in pigs differed from those of *T. b. gambiense* group 1 circulating in humans, suggesting that the pig may not be a reservoir for human infection in this focus (*15*). It has been noted that the prevalence of *T. b. gambiense* infection in domestic animals declines after human case detection activities (*42*), and the parasite is not found in wild animals at sites where there are no human cases (*43*). Using field data from Uganda, Davis, Aksoy and Galvani devised mechanistic models for the basic reproduction number R_0, with sensitivity analyses to examine the biological parameters important for determining R_0. They found that the most important factor was the proportion of blood-meals taken from humans by *G. f. fuscipes*, suggesting that exposure of humans to tsetse flies is fundamental to the distribution of *T. b. gambiense* (*48*). A modelling study with animal and human data from a Cameroon focus showed, however, that transmission of *T. b. gambiense* could not be maintained ($R_0 < 1$) at the known human prevalence, and the contribution of an animal reservoir was postulated (*49*). Additional data are needed to clarify whether an animal reservoir is involved in maintaining transmission at low human infection prevalence.

2.3.2 Rhodesiense human African trypanosomiasis

Rhodesiense HAT is a zoonotic disease. By definition, a zoonotic infection requires a non-human reservoir for maintaining its population. A reservoir for a zoonosis is often defined as an animal that harbours a human disease, where

the host is the reservoir; however, this simplistic definition does not account for important biological aspects. For rhodesiense HAT and other zoonoses, a reservoir has an epidemiological role in transmission of the agent to humans, which is different from a host species in which the parasite might be found but plays no epidemiological role in propagating the infectious agent. A reservoir is, therefore, a species or group of species that can permanently maintain the pathogen and from which the pathogen is transmitted to the target population (*50–52*). Understanding the reservoir role of a host is an essential element in the design of intelligent, cost-effective control.

The requirement for an epidemiologically relevant reservoir, the presence of a vector and the occurrence of humans, all in one environment in which transmission can occur, effectively make rhodesiense HAT a focalized problem: flies of the genus *Glossina* may be present across the tropical belt of Africa (see section 4 for more details), but infected reservoirs are not, so that transmission cannot always occur. Additionally, within a potentially suitable environment, micro-scale landscape elements play a role in delimiting areas of potential occurrence and spread; these are usually areas of greater suitability for the presence of tsetse (*53*) and may also be sites of intense contact with domestic animal (*54, 55*) or wild animal (*56–58*) reservoirs. These so-called "landscape determinants of risk" can be modelled and mapped spatially, providing information on current or future risk.

An important finding with these methods is that, despite intensive control to prevent large-scale transmission (i.e. epidemics), rhodesiense HAT foci tend to be stable over time, and their total elimination is unlikely without drastic environmental changes (*59, 60*). The focal nature of the occurrence of rhodesiense HAT means that a distinction must be made between the spread of the disease and the expansion of existing foci, during outbreaks or nascent epidemics. Within a focus, some factor (e.g. environmental or human) may change the intensity of transmission (*61, 62*), such that the incidence rises in that focus. Spread, however, is represented by translocation of infection to a previously unaffected location and the establishment of a new focus. Recently, with the increasing importance of livestock as reservoirs for *T. b. rhodesiense* in some foci and with the movement of livestock for social and economic reasons in modern Africa, new rhodesiense HAT foci have been found (*63, 64*) and, importantly, have been the source of further spread (*65*). In such situations, rhodesiense HAT control requires a "One Health" approach, in which human and animal health activities are integrated, involving institutions with remits for animal and for human health (*66*).

Uganda is the only country in sub-Saharan Africa with a recognized, extensive problem of both forms of HAT. Continued movement of the domestic animal reservoir and the establishment of new rhodesiense HAT foci are likely to lead, in the absence of continued control, to geographical overlap of the

two parasites in that country (*7*), which may have significant therapeutic and diagnostic implications. Rhodesiense HAT now occurs in the same areas from which small outbreaks of gambiense HAT were once eliminated, linked to the migration of populations (*67*). Human migrants from the Democratic Republic of the Congo (where *T. b. gambiense* is endemic) entering western United Republic of Tanzania (where there are active foci of *T. b. rhodesiense*) may create opportunities for geographical overlap of the two forms of HAT.

Several animal species have been identified as reservoirs or hosts of trypanosomes infective to humans. While much "natural history" research has been carried out in this area and trypanosome carriage has been diagnosed in a range of species, an important distinction must be drawn, as noted above, between *infection or carriage* and an *epidemiological role in transmission*, particularly with respect to transmission to humans. The latter is extremely difficult to ascertain in the absence of large-scale studies, few of which have been carried out in wildlife. Thus, the hosts of trypanosomes are discussed below, but no statement is made about the role or otherwise of these species as reservoirs.

The bushbuck (*Tragelaphus scriptus*) has been considered the archetypal animal host for *T. b. rhodesiense*, largely because a human infective trypanosome was first isolated from this species by Heisch and others (*68*). Other wild animals, especially bovids, may also be hosts (*69–71*); even lions (*Panthera leo*) have been found to be infected (*72*), although the infections were probably caught during feeding on infected animals, leading to mechanical transmission (*73–75*). Robson et al. (*76*) assessed the possible host status of wild carnivore and bovine and porcine species in the Lambwe Valley, Kenya, using the blood incubation infectivity test, and Geigy et al. (*77*) conducted similar studies in the Serengeti region of the United Republic of Tanzania. Because of a combination of difficulty in capturing and sampling wild animals and difficulty in sub-specific identification of trypanosomes, reports of the confirmed carriage of *T. b. rhodesiense* by wild animals are rarer than might be expected. Various studies have confirmed carriage of *T. b. rhodesiense* by the following species: bushbuck (*Tragelaphus scriptus*) (*68, 78*), duiker (*Cephalophus* spp., *Philantomba* spp. and *Sylvicapra* spp.) (*78*), giraffe (*Giraffa camelopardalis*) (*78*), hartebeest (*Alcelaphus buselaphus*) (*79, 80*), hyena (family Hyaenidae) (*70, 81, 82*), impala (*Aepyceros melampus*) (*78*), lechwe (*Kobus leche*), lion (*Panthera leo*) (*73, 75, 78*), oribi (*Ourebia ourebi*) (*81*), reedbuck (*Redunca* spp.) (*76, 82, 83*), warthog (*Phacochoerus africanus*) (*84, 85*), waterbuck (*Kobus ellipsiprymnus*) (*78, 85*) and zebra (*Equus* spp.) (*86*) (see *Annex 1*).

While in early studies (e.g. between 1950 and 1980) few tools were available to distinguish *T. b. rhodesiense* from *T. b. brucei*, more modern tools were used in later retrospective studies (*82*). In the 1940s, it was suggested that the presence of *T. b. rhodesiense* in humans during epidemics of the disease in the United Republic of Tanzania probably represented spill-over infections from

wild animal reservoirs (*87*), and, in areas with abundant wildlife, this is probably still true. There have been several reports of *T. b. rhodesiense* in tourists on safari holidays (*88–92*), most recently in Kenya's Masai Mara (*57, 58*). Blood-meal analysis of tsetse flies in the Serengeti National Park in the United Republic of Tanzania (*93*) showed that warthogs (*Phacochoerus africanus*), for example, are a preferred source of food for the flies; like pigs (*94*), they are good maintenance hosts of *T. brucei* s. l. Warthogs have also been found to be infected in other ecosystems, such as in the Luangwa Valley, Zambia (*78, 95*). Land-use pressure is resulting in an increasing overlap of grazing areas of wildlife and domestic animals (*96*), and transmission of human infective trypanosomes from wildlife to humans or to their livestock thus continues to be a public health issue. Essentially, within the tsetse belt of eastern and southern Africa, areas of abundant wildlife should be considered at risk for *T. b. rhodesiense* transmission.

Although the existence of a reptilian host for the maintenance of certain tsetse vector populations is certain, a reptilian contribution to the maintenance of trypanosomes is unlikely. Under experimental conditions, *Varanus niloticus* maintained a patent infection of *T. brucei* for 2 weeks (*97*), but in the wild these species are unable to maintain a constant body temperature, and it is highly unlikely that the parasites would survive.

In many African countries, there has been a drastic change in land use with pressure to grow food. MacKichan (*98*) observed that wild game animals were plentiful in the mid-1940s in Uganda, while presently most of the country supports very little wild game and correspondingly few wild species of bovids (*99*). Work in south-west Ethiopia also suggests that anthropogenic changes have affected the incidence of HAT, and game animals are being hunted out to make room for domestic stock (*100*). In areas where wild animals are no longer abundant, domestic hosts have become the principal reservoirs (*101*). In one area of Uganda during an outbreak of HAT in humans, 18% of the cattle population was found to be infected with *T. b. rhodesiense* (*102*); in western Kenya, although there had been no reports of human cases for several years, 1% of livestock tested had *T. b. rhodesiense* infection, pigs being at significantly greater risk (*103*). Domestic pigs also play a reservoir role in other locations (*94*).

2.4 Risk factors for infection

2.4.1 Gambiense human African trypanosomiasis

Transmission of gambiense HAT depends on the site, intensity and frequency of contact between tsetse flies and humans. The transmission rate is influenced by many factors. For *Glossina*, some of these factors are vector competence, proportion of blood-meals taken from humans, longevity and dispersal. For humans, they include place of residence, environmental characteristics and

agricultural and other occupational behaviour. Within the tsetse belt, the fly distribution, behaviour and potential for repeated contact with humans are not uniform, and the specific risk factors for infection depend on the ecological setting. The highest risk for transmission occurs when tsetse habitats are restricted, for example, by seasonal drought or by destruction of habitats by farming or logging, especially if there are few alternative hosts.

In humid forest habitats, *Glossina* spp. are widely distributed, with a considerable choice of hosts. In this setting, human–fly contact is casual, and infection is associated with activities such as hunting (*104*); however, forest clearings used for agriculture or other activities may be high-risk transmission sites. For example, the HAT epidemics in Côte d'Ivoire followed the pioneer front, as the forest was progressively replaced by cocoa and coffee plantations.

In the drier woodland savannah and along forest galleries, the fly is found close to rivers and streams. Risk for transmission has been associated with fetching water, domestic activities such as washing clothing or food (*105*) and fishing (*106*). In the transitional vegetation zones between forest and woodland savannah, which are often used for farming, the islands of vegetation provide tsetse habitats from which hosts are sighted, and farming has been implicated as a risk factor (*29*).

Transmission associated with mangrove swamps is less well understood than that in other settings but is now being characterized. Mangroves are apparently a favourable habitat for tsetse flies, and large populations have been found in genetic studies (*107*). Elevated risks for transmission are associated with encampments, cleared areas at the interface of the mangrove used for rice cultivation and use of pirogue jetties (*108*). Infection in this setting is also associated with walking long distances daily (*108*).

Although gambiense HAT is regarded as a rural disease, transmission has also been observed in urban settings. The transmission sites appear to be the suburban outskirts of cities, where there is a suitable tsetse habitat (*109*), but can also occur within a city when certain types of relic vegetation provide habitats for tsetse (*110*). In Kinshasa, infection was associated with travel to rural areas for agricultural work and for cultivating fields at the periphery of the city (*105*). The small number of alternative hosts in this setting may be a factor in the elevated risk. In entomological studies, a high degree of human–fly contact was found, with 67% of blood-meals from humans (*111*).

Gambiense HAT is predominately a disease of adults. Age-specific prevalence rates peaked in the young-to-mid-adult range in most case finding surveys *(29, 104, 112*). Rates in children are usually less than half those in adults, reflecting less exposure to flies during daily activities (*29, 112, 113*). The sex distribution depends on behaviour and activities in specific epidemiological settings. In places where at-risk activities include mining, hunting and fishing,

the prevalence of infection in males is greater than that in females; however, in transitional vegetation zones between forest and woodland, where infection is associated with cultivation and activities at water points, a similar prevalence of infection was found in males and females in case detection surveys (*29, 105, 112*).

The risk of short-term travellers from nonendemic areas for acquiring HAT is low and is much lower for *T. b. gambiense* than for *T. b. rhodesiense* infection, because tourists seldom visit the rural areas in which gambiense HAT is transmitted. Nevertheless, *T. b. gambiense* infections have occasionally been imported to Europe and North America by immigrants and expatriate residents who have lived in areas at risk (*114*). Between 2000 and 2010, 26 cases of gambiense HAT were reported to WHO from non-endemic countries (*115*). Diagnostic delay was common among these patients, because of the nonspecific clinical signs and symptoms, the low index of suspicion by treating physicians and the limited availability of serological and molecular diagnostic tests.

2.4.2 Rhodesiense human African trypanosomiasis

The age groups at highest risk for rhodesiense HAT are active, working-age groups who are most likely to venture to tsetse habitats, for example to collect firewood or honey, to herd cattle, to farm in peripheral areas and to fish and hunt, and rangers in natural protected areas (*116, 117*). The burden of infection on society as a whole is therefore high, affecting the income-generating capacity of families, apart from the effect at individual level. There is seasonal variation in transmission intensity, linked to the ecology of *Glossina*, with a peak in population densities after the rainy season and a lag time of 1–3 months after the rainy season before the appearance of human cases. There is some evidence of peridomestic behaviour among some tsetse species, e.g. *Glossina f. fuscipes* in certain regions (*118*) and in the savannah elsewhere (*119*), but peri-domestic transmission is likely to be secondary to transmission in habitats more suitable for tsetse flies. Transmission intensity is therefore linked to proximity to preferred tsetse habitats (see section 4 for more details). Some studies have demonstrated familial aggregation of infection with *T. b. rhodesiense* (*120*) and with *T. b. gambiense*, probably because of shared behavioural and spatial risk factors.

The fact that *T. b. rhodesiense* has a wildlife reservoir means that proximity to habitats shared by wildlife and tsetse flies is a risk factor for transmission. This is also the case for livestock kept in proximity to wildlife habitats (*83, 121*). Livestock may act as liaison hosts in such settings and also directly transmit the infection to people, including tourists visiting national parks. Several cases from eastern and southern African countries linked to exposure in wildlife reserves have been reported during the past few decades (*122–124*). In foci with a principally wildlife reservoir, interaction of indigenous populations with tsetse-

infested habitats in which wildlife are also abundant is key in transmission. Such exposure tends to be occupational, involving hunting, honey and wood collection and other activities involving the exploitation of natural resources.

In foci with a principally livestock reservoir, the tripartite interaction of humans, their animals and tsetse-infested habitat defines the risk profile. During endemic periods, HAT is a disease of the least franchised members of the population, and those living on the periphery of peripheral villages are at greatest risk (*53, 120*). These are also the members of the population with the poorest access to the health care system, compounding the burden of the disease.

2.5 Trends in numbers of cases reported

2.5.1 Gambiense human African trypanosomiasis

Like rhodesiense HAT, a salient characteristic of gambiense HAT is its ability to break out in epidemic form. Major epidemics have devastated large tracts of West and Central Africa in the past (*125*). At the turn of the twentieth century (1896–1906), gambiense HAT is estimated to have killed 800 000 people in equatorial Africa (*126*). The disease resurged in the 1920s, threatening both West and Central Africa; the outbreak lasted for a decade in some localized areas and for much longer in larger countries. This great epidemic occurred during a period of economic and social development, accompanied by increased population mobility, which facilitated the spread of infection. The colonial governments responded to the crisis by implementing vector control and dispatching mobile teams to conduct systematic population screening and treatment of cases, once effective chemotherapy became available. This was a successful strategy, and, although it required a sustained effort over two decades, the approach reduced the annual number of cases reported to near elimination, with around 4000 cases in Africa by 1960 (*127*).

A variety of explanations have been put forward to explain the origin of gambiense HAT outbreaks and their tendency to recur (*128*). Many factors are probably involved; they include sudden modifications to the environment (e.g. deforestation and the introduction of intensive agriculture for cash crops), mobility and displacement of populations and degradation of the economic environment that results in human behaviour change. Conflict and sociopolitical instability are associated with the persistence and resurgence of infection (*62*). There is some evidence that people develop immunity to *T. b. gambiense* (*129*), and it has been suggested that the inter-epidemic intervals observed in the twentieth century occurred while the population of susceptible individuals was increasing to a size that could support another epidemic.

In recent decades, trends in prevalence have been associated largely with the presence or absence of effective control programmes. The nadir of

HAT prevalence occurred just as disease-endemic countries were gaining independence. Surveillance for gambiense HAT decreased and was curtailed in many areas as public health authorities in the newly independent countries turned their attention and resources to other priorities. In addition, conflicts and insecurity constrained disease control interventions (*130, 131*). An analysis of the period 1974–2004 showed that cases of HAT occurred significantly more often in settings with political turmoil and internationalized civil war, with a 10-year interval between onset of conflict and peak prevalence (*132*). By the 1990s, epidemic disease had returned to central Africa (*113, 130, 133, 134*). By 1995, the reported annual number of cases of gambiense HAT had reached levels not seen for half a century, which was particularly alarming because only a small fraction of the areas at risk were under surveillance, and the true magnitude of the epidemic was unknown. The WHO Expert Committee on HAT control and surveillance in 1995 estimated that the true prevalence was 300 000 cases (*135*).

Continuing bilateral and multilateral assistance to countries endemic for the disease supported control during this period and probably prevented an even worse resurgence. WHO responded to the new epidemic with a campaign to coordinate the international partners working to improve control, garner political will and raise new resources from public and private sectors. This resulted in more technical and logistical support for country programmes, better drug availability and access to treatment, better monitoring of control activities and increased support for the development of new drugs, diagnostics and epidemiological tools. The result has been a substantial reduction in the annual number of cases reported. Between 2000 and 2009, the annual case detection rate decreased by 63% at the same time as geographical and population coverage by active screening increased (*8*). This promising trend continued in 2010 and 2011. In 2011, the number of new cases reported to WHO was 6631 (*136*). The Democratic Republic of the Congo currently bears most of the disease burden (*Table 2.1*).

The current trend in the reported number of cases of gambiense HAT is promising, but it should be regarded with some caution. The infection affects mainly remote rural communities, where the health infrastructure is basic, and cases may be unrecognized or unreported. Some of the known active foci are difficult to access because they are insecure or the topography is challenging. When active case detection is conducted in previously inaccessible or neglected foci, gambiense HAT can be found, often at a substantial prevalence (*137*). Additionally, assessment of foci in which no cases have been reported for years or decades is incomplete. Despite these gaps, knowledge of the current epidemiological situation has improved considerably in the past decade. In 1995, the gap between the numbers of reported and actual cases was estimated to be a

Table 2.1

Total number of new cases of gambiense human African trypanosomiasis reported to WHO by disease endemic countries, 1995–2012

Country	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012
Angola	2 441	6 726	8275	6 610	5 351	4 546	4 577	3 621	3 115	2 280	1 727	1 105	648	517	247	211	154	70
Benin	0	0	0	0	20	0	0	0	0	0	0	0	0	0	0	0	0	0
Burkina Faso	13	12	1	15	15	0	0	0	0	0	0	0	0	0	0	0	0	0
Cameroon	21	17	10	54	32	27	14	32	33	17	3	15	7	13	24	16	15	7
Chad	315	178	122	134	187	153	138	715	222	483	190	276	97	196	510	232	276	197
Central African Republic	676	492	730	1 068	869	988	718	572	539	738	666	460	654	1 194	1 054	395	132	381
Congo	475	474	142	201	91	111	894	1005	717	873	398	300	189	182	87	87	61	39
Côte d'Ivoire	326	240	185	121	104	188	92	97	68	74	42	29	13	14	8	8	10	9
Democratic Republic of the Congo	18 182	19 342	25 094	26 318	18 684	16 975	17 322	13 853	11 481	10 369	10 269	8 023	8 162	7 326	7 183	5 629	5 595	5 983
Equatorial Guinea	37	46	67	62	28	16	17	32	23	22	17	13	15	11	7	8	1	2
Gabon	20	32	11	6	38	45	30	26	26	49	53	31	30	24	14	22	17	9
Gambia	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Ghana	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Guinea	33	38	88	99	68	52	72	132	130	95	94	48	69	90	79	68	57	70

Table 2.1 (continued)

Total number of new cases of gambiense human African trypanosomiasis reported to WHO by disease endemic countries, 1995–2012

Country	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012
Guinea Bissau	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Liberia	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Mali	11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Niger	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Nigeria	0	0	0	0	27	14	14	26	31	10	21	3	0	0	0	2	3	2
Senegal	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Sierra Leone	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0	–	–
South Sudan[a]	56	157	737	1 726	1 312	1 801	1 919	3 121	3 061	1 742	1 853	789	469	623	373	199	272	317
Togo	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Uganda	1 062	981	1 123	971	1 036	948	310	604	517	378	311	290	120	198	99	101	44	20
Total reported	**23 671**	**28 736**	**36 585**	**37 385**	**27 862**	**25 865**	**26 117**	**23 836**	**19 963**	**17 130**	**15 644**	**11 382**	**10 473**	**10 388**	**9 685**	**6 978**	**6 637**	**7 106**

[a] South Sudan became an independant State on 9 July 2009. All the cases reported up to that date in former Sudan correspond to the current South Sudan.

Data for 2010, 2011 and 2012 include revisions of previously published figures (*136,140*)

factor of 12 (*135*); by 2006, it had decreased to a factor of three (*138*). Thus, the true annual incidence of gambiense HAT is estimated to be fewer than 20 000 cases. In view of the recent dramatic success in control of the disease, gambiense HAT was included in WHO's "roadmap on neglected tropical diseases". A 2020 target date was set for elimination of the infection as a public health problem (*139*), with complete interruption of transmission in Africa targeted for 2030 (*140*).

2.5.2 Rhodesiense human African trypanosomiasis

Rhodesiense HAT can occur in both endemic and epidemic forms. The endemic state is the norm, with stable, low incidence. This is the case in areas with both wildlife and livestock reservoirs. In areas with wildlife, cases may be reported only every several years; for example, extensive active surveys in the Serengeti area of the United Republic of Tanzania revealed no cases of infection (*84*), but several human cases (*56, 92, 123*) were reported in tourists during a similar period. Essentially, in such environments, intimate contact with transmission settings will result in a small number of cases.

In areas with livestock reservoirs, cases may occur more regularly (*53, 141*), as there are more opportunities for interaction with reservoirs and transmission environments. A changing physical environment may, however, affect the risk for transmission: for example, although western Kenya was once an active focus, few cases have been reported over the past decade (*117*), even though the parasite is still detectable in domestic reservoirs (*103*). Environmental changes, particularly increases in population density and expansion of farmed areas, have been largely responsible for the decrease in incidence. Elsewhere, including neighbouring southern Uganda, the environment has been modified to a lesser extent; therefore, habitats for transmission are still more extensive.

In both wildlife and livestock reservoir zones, civil instability and other major social disruption may result in an increase in the number of cases (*62*). Such situations often lead to more frequent exploitation of marginal lands, exposing people to tsetse flies. In Uganda, there is good evidence that the return of stability after a period of civil conflict led to large-scale movements of livestock as part of economic regeneration (*6*) and subsequent importation of rhodesiense HAT to new areas, where it then became established during a now decade-long epidemic. Livestock movements and restocking in the early 1900s may also have been partly responsible for the large Busoga epidemic in Uganda (*11*). It is possible that extensive contemporary livestock movements, resulting from price differentials across the region affected by *T. b. rhodesiense* (and between the gambiense HAT and rhodesiense HAT regions), will result in extensive mixing of animals from different sources and potential long-range spread of the parasite in coming years.

As for gambiense HAT, current trends in the number of cases reported by countries endemic for rhodesiense HAT are encouraging (*Table 2.2*), although it

Table 2.2
Total number of new cases of rhodesiense human African trypanosomiasis reported to WHO by disease endemic countries, 1995–2012

Country	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012
Botswana	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Burundi	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Ethiopia	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Kenya	0	2	5	14	22	15	10	11	0	0	0	1	0	0	1	0	0	2
Malawi	15	8	7	10	11	35	38	43	70	48	41	58	50	49	39	29	23	18
Mozambique	–	–	–	–	–	–	–	1	–	1	–	–	–	–	–	–	–	–
Namibia	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Rwanda	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Swaziland	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
United Republic of Tanzania	422	400	354	299	288	350	277	228	113	159	186	127	126	59	14	5	1	4
Uganda	497	178	217	283	283	300	426	329	338	335	473	261	119	138	129	112	82	71
Zambia	1	3	–	–	15	9	4	5	15	9	7	6	10	13	4	8	3	6
Zimbabwe	–	–	9	–	–	–	–	–	–	–	3	–	–	0	3	2	4	9
Total reported	**935**	**591**	**592**	**606**	**619**	**709**	**755**	**617**	**536**	**552**	**710**	**453**	**305**	**259**	**190**	**156**	**113**	**110**

Data for 2010, 2011 and 2012 include revisions of previously published figures (*136*)

is difficult to make firm predictions about changes in the incidence of rhodesiense HAT. As long as reservoirs and environments for transmission exist, there will be a risk, and unpredictable ecological or social changes may result in extensive transmission.

2.6 Geographical distribution and population at risk

Establishing an evidence base for making appropriate decisions is crucial if policy, planning and financial resources are to be distributed appropriately (*142*, *143*). In order to understand the magnitude of the problem presented by a particular disease, the population at risk for that disease must be known. For example, the incidence of pandemic influenza A might be calculated as a function of the total global population, while the population at risk for lung cancer might be those exposed occupationally to asbestos (*144*). Correct identification of the population at risk is essential, as disease incidence, which depends on this population, is important in establishing the need for interventions. Establishing a population at risk can, however, be problematic. With respect to HIV/AIDS, for example, while the majority of the population might be at low risk, certain behaviour can result in high risk for certain subsectors (*145*). In the case of malaria in Africa, the population at risk is essentially the total population of countries with malaria transmission (*146*, *147*), because the risk is general in endemic areas, although this is not the case for forecasting specific malaria epidemic situations (*148*). For schistosomiasis, the population at risk is estimated as the total population living in areas where "transmission is known to occur", and estimates of the population at risk for filariasis have been based on the population living in areas where the climate is suitable for transmission (*149*).

When the risk for disease transmission depends on biogeographical parameters, such as climate or altitude, there may be no clear boundary between people who are exposed and those who are not. The population at risk for onchocerciasis, for example, is determined, mainly for the purposes of interventions, by rapid field assessments of prevalence (*150*, *151*). The population potentially affected by focal diseases must be assessed on a finer scale because the disease is limited to areas in which the environment is conducive to transmission, with the reservoir hosts, vectors and susceptible population found together. Detailed parameters (e.g. detailed incidence figures) for every focus of transmission are usually lacking on a continental scale. Rather, appropriate generalizations must be made that are both reasonable and transparent. HAT is a focal disease. The previous estimate that 60 million people in the tropical belt of Africa are at risk for HAT is a "best guess" based on estimates of the total population of the tsetse-infested areas of the continent (*135*, *152*).

A major recent advance in the spatial analysis of HAT was the preparation of the *Atlas of human African trypanosomiasis* (*153–155*). This required extensive

work with WHO Member States to obtain all potential data and to locate cases geographically, which was an essential initial step (*156*), and to present them spatially. The *Atlas* was initiated in 2007 as a collaborative project between WHO and the Food and Agriculture Organization of the United Nations (FAO) in the framework of the programme against African trypanosomosis. The *Atlas* is an excellent basis for monitoring changes in distribution and epidemiology and for using spatial analytical methods to estimate the size and location of populations at risk.

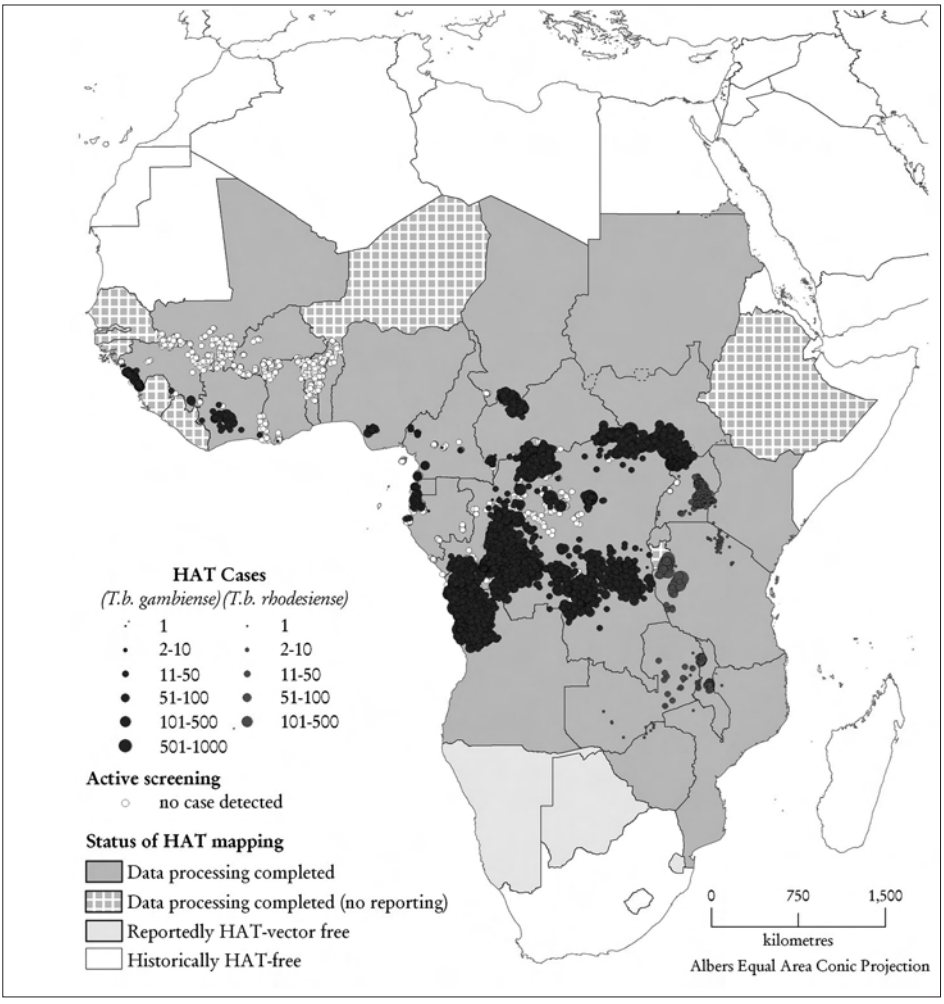
The distribution of disease can be presented on various scales, from local to continental. Examples of the distribution of gambiense HAT in Central and West Africa are given in *Annexes 2* and *3*, and the distribution of rhodesiense HAT in East and south-eastern Africa is given in *Annex 4*. The *Atlas* initially covered the period 2000–2009, with 86.2% of reported cases and 79.3% of reported locations presently mapped (*Figure 2.2*). However, the *Atlas* is a dynamic tool with continuous improvement of mapping accuracy and completeness. Regular updating is also planned, and epidemiological data for 2010–2012 are being processed.

The geographical distribution of both gambiense and rhodesiense HAT is uneven. During a meeting in December 2012 on HAT elimination (*140*), the list of foci was updated. In the absence of environmental change, these foci of endemicity tend to remain spatially stable over time, the transmission intensity and geographical coverage waxing and waning. Environmental modifications and human or livestock population movements can, however, result in shifts in geographical location and extent. Thus, disease mapping has always been a key element of HAT control (*157, 158*).

The *Atlas* promises to be extraordinarily useful for HAT control and research. The maps and background database can be used by programmes to plan and monitor control activities and to identify epidemiological trends. Combining HAT maps with other geospatial data sets may provide new insights into the relations between HAT distribution and livestock population, vegetation and tsetse distribution.

In the first application of this type, data from the *Atlas* were combined with spatially explicit population layers to generate current, evidence-based estimates of populations at risk (*155*). The risk was characterized into low, moderate and high, and the population in each category was quantified. A total of 57 million people distributed over 1.38 million km^2 are estimated to be at risk for gambiense HAT (*Table 2.3*). Areas of high and very high risk are defined as those with an annual incidence of at least 1 new case per 1000 people; the population of these areas is 5 million. Areas of moderate risk have an incidence of 1 case per 1000–10 000 people; these areas cover 14.5 million people. In low and very low risk

Figure 2.2
Distribution of human African trypanosomiasis (HAT), 2000–2009



Adapted from reference *154*. HAT database version: 25/06/2012

areas, the incidence is 1 new case per 10 000–1 000 000 people, and these areas have 37.5 million inhabitants. The Democratic Republic of the Congo has the most people at risk (36 million) and the largest area at risk (790 000 km^2) (*Table 2.4*), but South Sudan and Angola also have sizeable populations at risk. In West Africa, the areas of greatest endemicity are classified as at moderate risk and are located in central Côte d'Ivoire and coastal Guinea (*Figure 2.3*).

Table 2.3
Population at risk for *T. b. gambiense* infection in West and Central Africa

Country	Total country population (no. people × 10^3)[b]	Population at risk (no. people × 10^3)[b]				
		Very high and high	Moderate	Low and very low	Total	% of total country population
Angola	12 799	740	749	3 278	4 767	37.2
Cameroon	18 879	–	28	603	631	3.3
Central African Republic	4 511	69	130	237	435	9.6
Chad	10 329	109	114	243	465	4.5
Congo	4 013	113	451	2 002	2 566	63.9
Côte d'Ivoire	20 617	–	230	2 442	2 672	13.0
Democratic Republic of the Congo	68 693	3 569	10 767	21 911	36 247	52.8
Equatorial Guinea	633	2	27	14	43	6.8
Gabon	1 515	2	21	780	803	53.0
Guinea	10 058	–	187	2 420	2 606	25.9
Nigeria	149 229	–	–	2 182	2 183	1.5
Sierra Leone	5 132	–	1	170	170	3.3
South Sudan	6 996	416	453	401	1 270	18.2
Uganda	32 370	142	1 275	707	2 124	6.6
Other endemic countries[a]	103 673	–	–	–	–	–
Total	**449 447**	**5162**	**14 431**	**37 390**	**56 983**	**12.7**

[a] Countries at marginal risk: Benin, Burkina Faso, Gambia, Ghana, Guinea-Bissau, Liberia, Mali, Niger, Senegal and Togo

[b] Source for calculation of country population and population at risk: Landscan 2009. Population Dataset created by University of Tennessee and Battelle Memorial Institute, under limited liability partnership, the management and operating contractor of the Oak Ridge National Laboratory acting on behalf of the United States Department of Energy, an agency of the United States Government.

Table 2.4
Areas at risk for *T.b. gambiense* infection in West and Central Africa

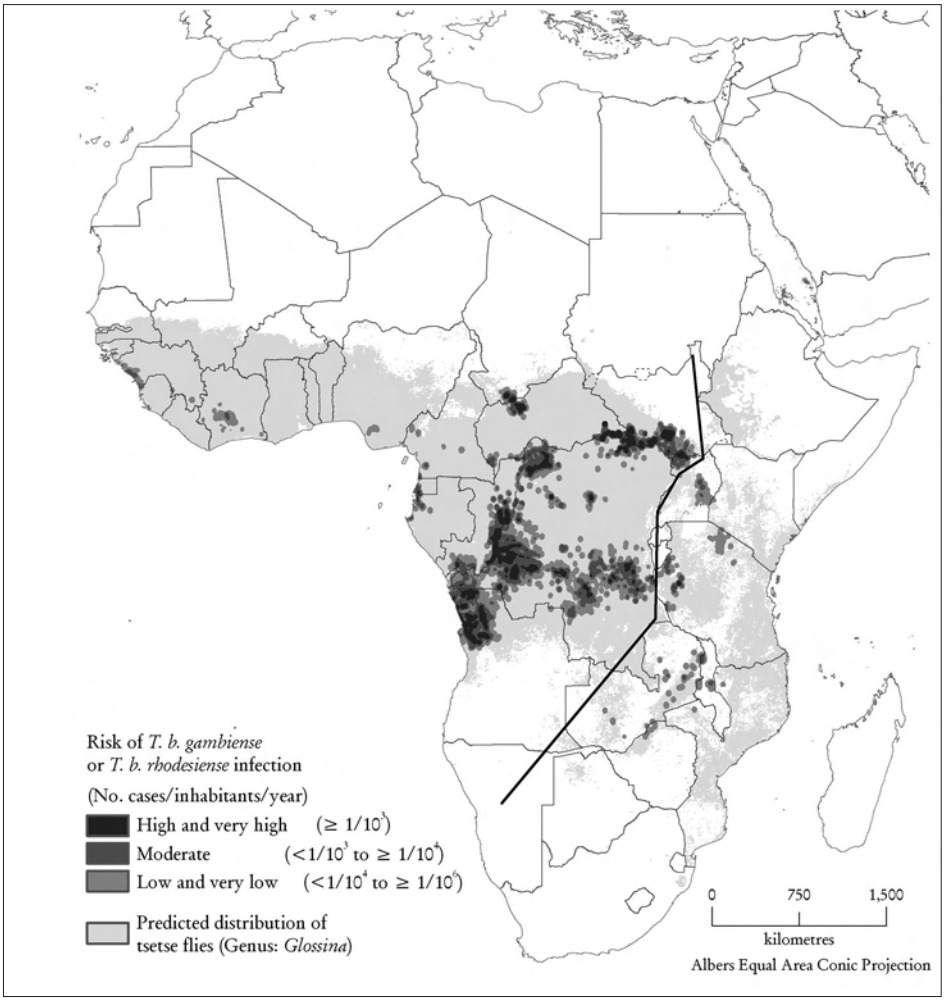
Country	Total country area ($km^2 \times 10^2$) [b]	Area at risk ($km^2 \times 10^2$)[b]				
		Very high and high	Moderate	Low and very low	Total	% of total country area
Angola	12 538	568	597	638	1,803	14.4
Cameroon	4 664	–	22	150	173	3.7
Central African Republic	6 244	196	204	258	659	10.6
Chad	12 725	33	34	75	142	1.1
Congo	3 385	220	388	554	1 162	34.3
Côte d'Ivoire	3 214	–	23	264	286	8.9
Democratic Republic of the Congo	23 041	1 023	2 717	4 162	7 902	34.3
Equatorial Guinea	270	4	37	24	65	24.1
Gabon	2 660	6	57	104	167	6.3
Guinea	2 461	1	42	141	184	7.5
Nigeria	9 089	–	–	71	70	0.8
Sierra Leone	728	–	–	18	18	2.5
South Sudan	6 334	281	379	341	1 001	15.8
Uganda	2 055	13	91	70	175	8.5
Other endemic countries[a]	60 316	–	–	–	–	–
Total	**149 722**	**2 346**	**4 591**	**6 871**	**13 808**	**9.2**

[a] Countries at marginal risk: Benin, Burkina Faso, Gambia, Ghana, Guinea-Bissau, Liberia, Mali, Niger, Senegal and Togo

[b] Area of surface water bodies depicted in the Shuttle Radar Topography Mission–River-Surface Water Bodies dataset (*159*) is not included.

The estimates are somewhat more precise for *T. b. gambiense* than for *T. b. rhodesiense*, since the presence of other infected humans, rather than animal reservoirs, is a key determinant in the former; nonetheless, some sensible extrapolations can be made, leading to an estimate of 12.3 million people at risk for contracting *T. b. rhodesiense* over an area of 0.171 million km^2 (*Tables 2.5* and *2.6*), with 88% at low to very low risk and 12% (around 1.5 million people) at moderate to high risk (*Figure 2.3*) (*155*).

Figure 2.3
Distribution of risk levels for human African trypanosomiasis



Adapted from reference *155*

Table 2.5
Population at risk for *T. b. rhodesiense* infection in East and south-eastern Africa

Country	Total country population (no. people × 10^3)[b]	Risk (no. people × 10^3)[b]				
		Very high and high	Moderate	Low and very low	Total	% of total country population [b]
Burundi	9 511	–	–	38	38	0.4
Kenya	39 003	–	–	1 124	1 124	2.9
Malawi	15 029	–	194	716	910	6.1
Mozambique	21 669	–	–	58	58	0.3
United Republic of Tanzania	41 049	22	373	1 429	1 824	4.4
Uganda	32 370	–	847	7 029	7 877	24.3
Zambia	11 863	–	14	401	416	3.5
Zimbabwe	11 393	–	–	93	94	0.8
Other endemic countries[a]	101 420	–	–	–	–	–
Total	**283 306**	**22**	**1 429**	**10 891**	**12 341**	**4.4**

[a] Countries at marginal risk: Botswana, Ethiopia, Namibia, Rwanda and Swaziland

[b] Source for calculation of country population and population at risk: : Landscan 2009 Population Dataset created by University of Tennessee and Battelle Memorial Institute, under limited liability partnership, the management and operating contractor of the Oak Ridge National Laboratory acting on behalf of the United States Department of Energy, an agency of the United States Government.

Table 2.6
Areas at risk for *T.b. rhodesiense* infection in East and south-eastern Africa

Country	Total country area ($km^2 \times 10^2$)[b]	Area at risk ($km^2 \times 10^2$)[b]				
		Very high and high	Moderate	Low and very low	Total	% of total country area
Burundi	251	–	–	2	2	0.8
Kenya	5 749	–	–	31	31	0.5
Malawi	948	–	33	105	138	14.6
Mozambique	7 791	–	–	39	39	0.5
United Republic of Tanzania	8 863	16	125	515	657	7.4
Uganda	2 055	–	45	243	288	14.0
Zambia	7 425	–	33	445	478	6.4
Zimbabwe	3 884	–	–	78	78	2.0
Other endemic countries[a]	25 685	–	–	–	–	–
Total	**62 650**	**16**	**236**	**1 459**	**1 711**	**2.7**

[a] Countries at marginal risk: Botswana, Ethiopia, Namibia, Rwanda and Swaziland

[b] Area of surface water bodies as depicted in the Shuttle Radar Topography Mission—River-Surface Water Bodies dataset (*159*) is not included.

2.7 Global environmental change

The *Atlas* is potentially useful for designing models to predict the future spread and risk of HAT, particularly if combined with geospatial datasets that include environmental data. The geographical distribution of HAT is expected to be sensitive to population growth and climate change, which alter the relationships between humans, tsetse flies and the environment. It is predicted that major environmental changes will occur in parts of Africa in the coming decades.

The population of Africa increased nearly fivefold during the past 60 years (*160*). Continued increases in human density often degrade vegetation. Although riverine tsetse can co-exist with a relatively high human density, drought may exacerbate landscape degradation and affect the fly distribution. This phenomenon has already been documented in West Africa, where the northernmost limit of fly distribution moved 200 km southwards during the past century in response to population growth and drought (*161*). In Burkina Faso, 70 000 km^2 of tsetse fly habitat have been lost since 1949, with the elimination of past foci of HAT (*162*). Although tsetse habitats have been lost and HAT has disappeared in some foci, global change has also resulted in new sites of transmission. Increasing urbanization in Africa has led to new peri-urban epidemiological settings for gambiense HAT (*105, 109, 111, 163*), and increasing numbers of visitors to wildlife zones have resulted in more cases of rhodesiense HAT among tourists.

Attempts to predict the future distribution of HAT and tsetse in the face of climate change (*164*) were limited by lack of a robust database for current infection and fly distributions, and it is clear that the impact of population growth and climate change will differ by region. Future modelling will benefit from the HAT *Atlas*, although better estimates of population growth and improved climate simulations will also be needed.

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3. The parasite

The biology of trypanosomes is interesting for many reasons. It is intrinsically interesting to understand the biological specificities that distinguish these organisms from others in the panoply of nature, and numerous phenomena first described in trypanosomes have pointed to important aspects of biology in general. More importantly, those features that distinguish trypanosomes from their mammalian hosts and offer targets for drugs must be understood in order to ensure a sustainable selection of drugs to meet the goal of elimination of HAT.

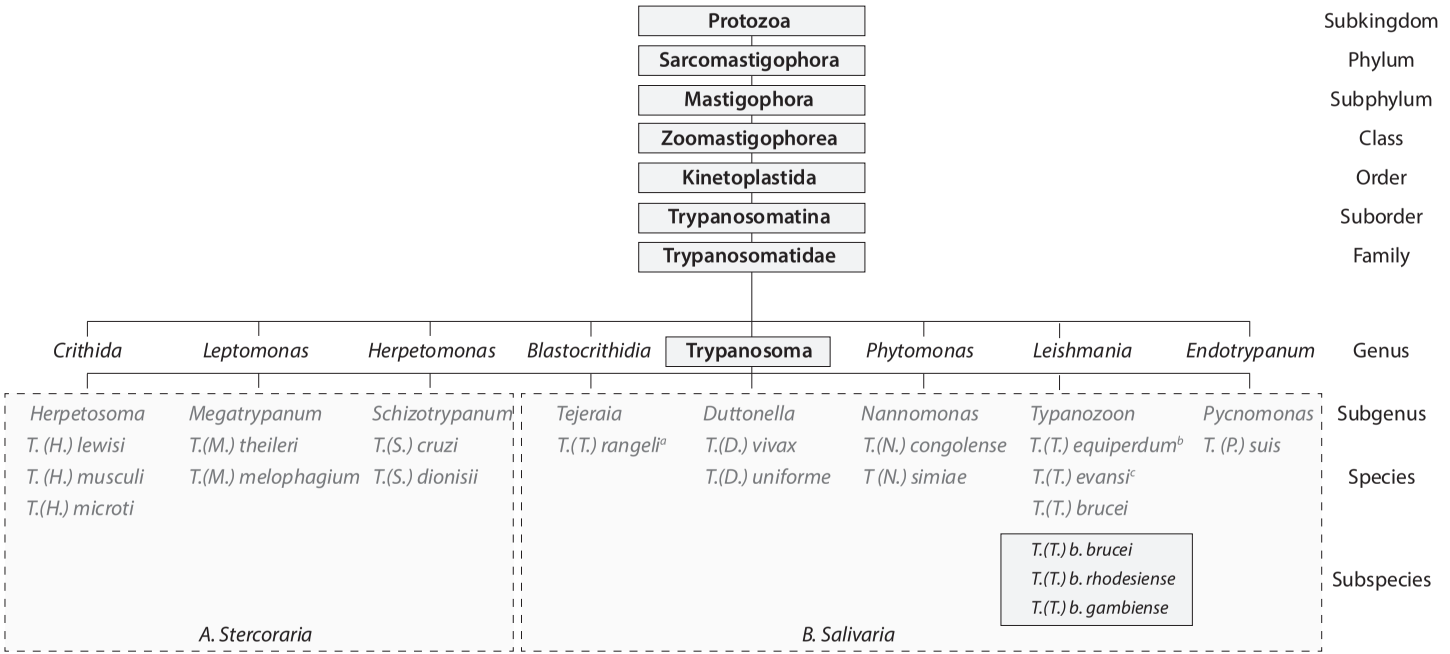
3.1 Taxonomy of human infectious African trypanosomes

The African trypanosomes are classified as part of the early branching phylogenetic order Kinetoplastida in the Protozoan subkingdom (*1*). They belong to the salivarian section of the genus *Trypanosoma* and are injected into mammalian blood via the bite of a tsetse fly (or other biting flies for some non-human infective trypanosome species). This distinguishes them from the stercorarian section, which includes the American trypanosome, *T. cruzi*, which is transmitted in the faeces of its reduviid bug vector (*Figure 3.1*).

There are many species of African trypanosome; however, only those of the *T. brucei* group are known to infect humans. In West and Central Africa, *T. b. gambiense* causes a chronic form of sleeping sickness, while *T. b. rhodesiense* causes an acute form in East and southern Africa (*4*). These subspecies cannot be differentiated by microscopy, as they are morphologically identical, and other methods are needed, as the diseases they cause are treated with different drugs and regimens and the diagnostic algorithms differ to increase the precision with which the parasites are detected in host tissues. *T. b. rhodesiense* has been differentiated from related parasites by a number of methods. Firstly, geographically, *T. b. rhodesiense* is present in eastern Africa to the east of the Rift Valley, while *T. b. gambiense* is found to the west of the Rift Valley (although this distribution may change as the parasites encroach on each other's classically defined zones).

Within its range, *T. b. rhodesiense* was, until the late 1960s, distinguished solely by its ability to infect humans; isolation of the parasite from non-human hosts was confirmed by experimental infection of human volunteers (*5*). Later, the blood incubation infectivity test, in which trypanosomes are exposed to human blood in vitro and then inoculated into mice to determine whether they establish infection, was used (*6, 7*). This was superseded by isoenzyme electrophoresis methods (*8, 9*). Later, a solely in vitro method for assessing human infectivity was introduced (*10*). More recently, a specific molecular marker, the serum resistance-associated (*SRA*) gene, was identified (*11, 12*), and its presence has been confirmed in all *T. b. rhodesiense* isolates (*13, 14*), proving its value as a diagnostic marker. *T. b. gambiense* was also characterized primarily by its geographical location. The identification of a specific molecular marker in

Figure 3.1
Classification of mammalian trypanosomes



Source: reference *2*

[a] *T. rangeli* has been removed from the subgenus *Herpetosoma* and placed in the subgenus *Tejeraia* in the salivarian section. See reference *3*.

[b] *T.(T.) equiperdum* is a sexually transmitted parasite of horses.

[c] *T.(T.) evansi* is mechanically transmitted by biting flies (tabanids).

type 1 *T. b. gambiense* (*TGSGP*) (*15*) has provided a means for identification and will be increasingly useful in ascertaining the full host range of *T. b. gambiense*. A second *T. b. gambiense* lineage defined as human infectious trypanosomes found in West and Central Africa that does not contain the *TGSGP* gene was classified as type 2 *T. b. gambiense*. This type may not, however, contribute significantly to the burden of disease: type 2 *T. b. gambiense* was last isolated in 1992 (*16*).

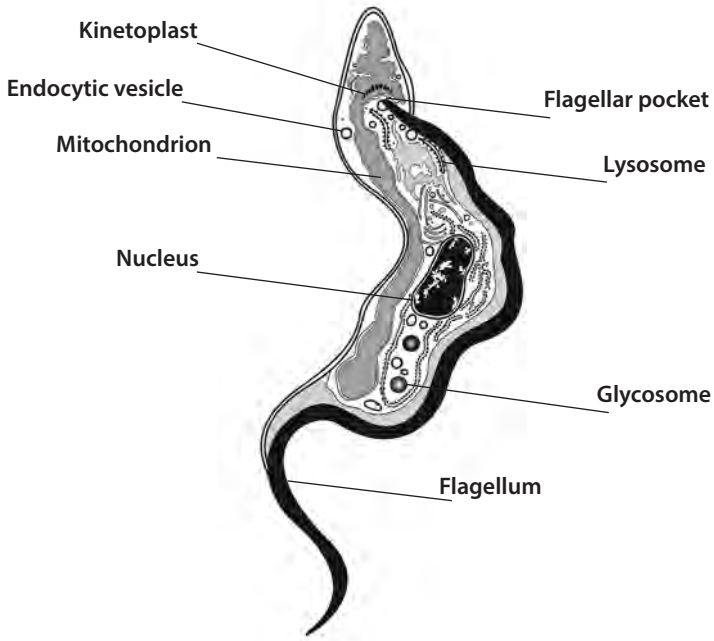
Many mechanisms have evolved in different host systems to combat microbial infections. Humans appear to have evolved mechanisms to destroy infectious trypanosomes by lysis with plasma-borne factors. Human infectious trypanosome subspecies can, however, avoid this non-immune lytic process, which kills other trypanosome species. The killing is mediated by several trypanolytic factors, including apolipoprotein L1, which is part of the high-density lipoprotein fraction of plasma. The particles also contain a haptoglobin-related protein that is recognized as a ligand by a specific receptor (hpr), which pulls the complex into trypanosomes (*17*). A second trypanolytic factor is less well understood but also critical in non-immune trypanolysis (*18*). *T. b. rhodesiense* can avoid trypanolysis by virtue of the so-called serum resistance-associated (*SRA*) protein, which binds to and neutralizes apolipoprotein L1 (*15*). *SRA* is a truncated version of a mutant variant surface glycoprotein (*VSG*), which is the protein that usually covers the parasite's surface, protecting it against complement-mediated lysis (see below). *T. b. gambiense* can also resist lysis, possibly by reduced uptake of apolipoprotein L1 (*19*), while *TGSGP* itself has also recently been shown to mediate human serum resistance (E. Pays, personal communication). The key role of apolipoprotein L1 in trypanolysis is supported by the finding that people deficient in this protein can also be infected by other trypanosome species, e.g. *T. evansi*, which is not usually associated with human infections (*20*).

3.2 Morphology and cell structure

African trypanosomes are single-celled extracellular parasites that live free in the bloodstream or other body fluids, including lymph and cerebrospinal fluid (CSF). They can be identified microscopically, particularly when stained with Giemsa, which clearly labels the two principal nucleic acid-containing organelles, the nucleus and the kinetoplast (*Figure 3.2*). Other staining techniques, e.g. with acridine orange for visualization by fluorescence microscopy, have also proven useful in demonstrating the parasites in clinical isolates. The parasites can be cryopreserved for long-term maintenance of viable cells, either in blood of patients, inoculated rodents or in laboratory cultures (*Annex 5*).

African trypanosomes are about 20 μm long and 5 μm wide. A flagellum is characteristically present. This is an organelle that is motile and can assist mobility if parasites are removed from the vasculature and also apparently helps to maintain a flow of fluid over the parasite surface. It exits from the posterior part of the slender bloodstream form trypomastigote (*Figure 3.2*) at an opening,

Figure 3.2
Structure of trypanomosomes



Drawing: Caroline Morris

termed the flagellar pocket, that is directly associated with the kinetoplast. The flagellum remains attached to the cell body before protruding from the front end of the cell. The kinetoplast is the parasite's mitochondrial genome and the eponymous structure after which the Kinetoplastida order is named. This unusual structure is significantly larger than the mitochondrial genomes in other eukaryotic cells, hence its prominent staining with DNA-specific dyes. It comprises a series of intercatenated circular DNA molecules, including maxi-circles (about 20 kb in size) that encode the mitochondrial protein coding genes and mini-circles (1–2 kb in size) that encode guide RNAs. The latter are small RNAs used in the remarkable process of RNA editing (*21*), in which the basic genetic code present within maxi-circle genes is altered by the addition (or, more rarely, removal) of U-residues to the encoded RNA. This process of RNA editing, in which the genetic code is modified before being translated into proteins, is quite extraordinary; although some other cells (e.g. plants) have various types of RNA editing, this extensive processing is unique to Kinetoplastid organisms.

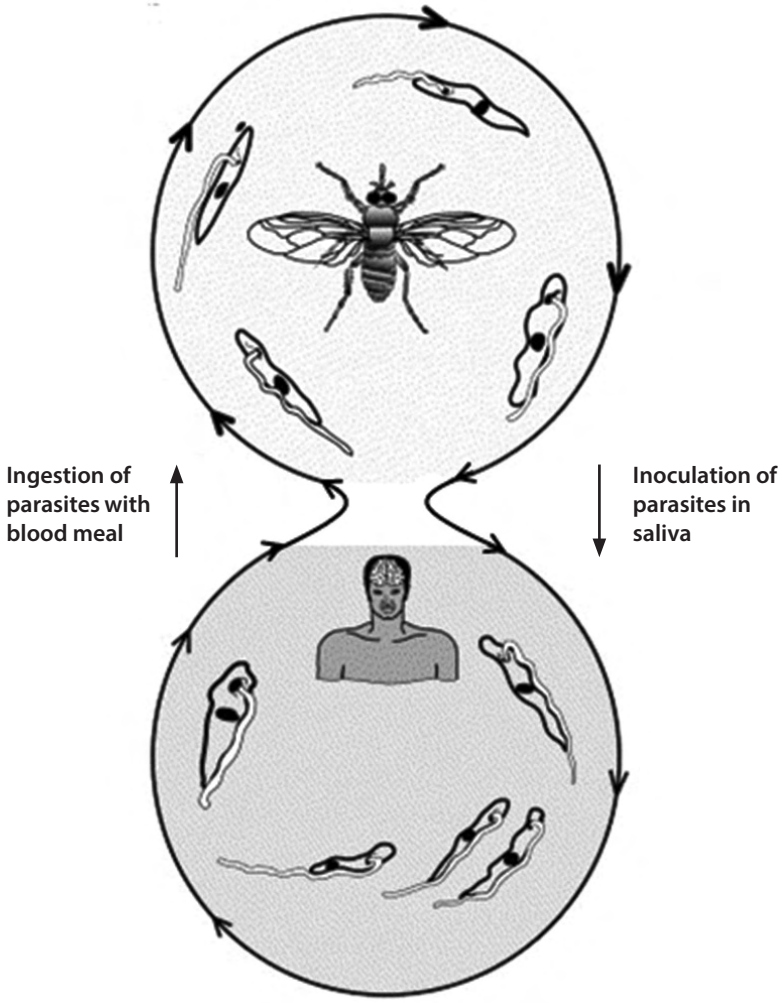
The shape of the trypanosome is described as corkscrew-like (the word "trypanosome" itself is derived from the Greek word *trupanon*, a screw-borer type device). It is maintained by an array of subpellicular microtubules, which are built from proteins into a cage-like structure. The cell biology of trypanosomes is unique in many other ways when compared with other eukaryotic cells, such as typical mammalian cells (*22*), which has inspired cell biologists to consider that these structures could be exploited in the development of drugs to treat the disease. Pragmatically, however, use of such information to develop new drugs has not reached fruition.

In addition to the kinetoplast, which resides within its single mitochondrion, the parasite has a wealth of organelles not usually associated with "model" eukaryotes such as yeast and mammalian cells. These include glycosomes, which are related to peroxisomes but contain the first seven enzymes of the principal energy-generating glycolytic pathway in addition to a variety of other biochemical pathways. The segregation of these pathways from other parts of the metabolic network confers an advantage, allowing these parasites to use glucose as their sole energy source at relatively high rates. Acidocalcisomes have a role in other important cellular functions, such as pH and cation balance, and many proteins inside the cell are susceptible to fluctuations in these parameters (*23*). The flagellum itself, in addition to a possible role in the mobility of the parasites (when they are in sites beyond the vasculature where the forces of blood flow vastly exceed those that the flagellum can create), plays a role in microfluidic dynamic sweeping of surface material (*24*). This is proposed to be particularly important when antibodies have bound to the surface of the parasite; surface flow generated by the flagellum leads to endocytosis of these antibodies, followed by their destruction within the phagolysosomal system, effectively cleansing the parasite's surface (*24*). The predominant feature of the surface is the *VSG* coat, which protects the parasites from complement-mediated lysis and can be changed periodically, so that antibodies raised against one variant coat will not destroy the next. The rate of endocytosis in bloodstream-form trypanosomes, the process whereby material that binds specifically to receptors on the cell surface is carried into the cell, exceeds that in most other cell types. This is reflected in a rich intracellular vacuolar system that interacts with the cell surface at the flagellar pocket, the only part of the cell not covered by the subpellicular microtubule array and thus a region where endocytic vesicles can form and pinch off from the surface and enter the cell.

3.3 Life-cycle

T. brucei group trypanosomes are transmitted by tsetse flies of the genus *Glossina*. The life-cycle is relatively complex, with various different stages and distinctive morphology and biochemical physiology in mammalian hosts and insect vectors (*Figure 3.3*).

Figure 3.3
Life-cycle of trypanosomes



The life-cycle starts with the injection of metacyclic forms, adapted for life in the bloodstream through a tsetse fly bite. Metacyclic forms differentiate into proliferating, long, slender bloodstream forms, which proliferate in the bloodstream and other body fluids. These forms can invade the central nervous system, leading to second-stage disease. Slender forms also differentiate into a non-replicative stumpy form, which is pre-adapted to the environment offered by the tsetse fly after uptake during a blood-meal. In the tsetse fly midgut, stumpy forms differentiate to replicative procyclic trypomastigotes, which can migrate to various anatomical sites in the tsetse fly, differentiating to other forms and eventually re-establishing the metacyclic forms in the salivary gland.

The procyclic form, which replicates in the insect's midgut, migrates to the salivary gland, where it transforms into the infectious metacyclic form, characterized by acquisition of the protective surface glycoprotein coat that will enable it to survive after transmission to the human host. Other forms exist in the tsetse fly, adapted to the various environments there, but they are difficult to cultivate in the laboratory and little is known about them. Metacyclic forms are injected during a tsetse's blood-meal, and the trypanosomes, injected subdermally, initially proliferate at the site of injection, often forming the characteristic trypanosomal chancre. They differentiate into long, slender bloodstream forms carried by the draining lymph nodes to the bloodstream, where they replicate. In time, the parasites can also leave the vasculature to invade other organs, most notably the brain and central nervous system (CNS). *T. b. rhodesiense* establishes itself within the CSF within a few weeks, while *T. b. gambiense* takes an average of 300–500 days to manifest in the CNS (*25, 26*). Other organs are invaded also, and organ failure (e.g. heart) associated with parasite invasion is occasionally the cause of death in rhodesiense HAT.

Parasitaemia is generally very low in *T. b. gambiense* infections (often < 100 parasites per ml of blood), complicating identification by microscopy, while in *T. b. rhodesiense* infections parasitaemia mounts more rapidly. The parasitaemia is also notable for its fluctuations, which are due partly to antigenic variation and host immune responses, as an antibody response is raised to the prevailing antigen type and these parasites are wiped out; however, an induced differentiation pathway is also active in the parasites. A quorum sensing process has been proposed, whereby parasites release a "stumpy induction factor", which triggers the production of cell cycle-arrested (non-replicating) stumpy forms. These are biochemically pre-adapted to differentiate further in the tsetse if taken up in a blood-meal. They re-establish the life-cycle when these forms further differentiate into the replicative procyclic form (*27*). Stumpy forms that are not taken into tsetse flies cannot replicate, so that parasitaemia falls before a new replicative variant emerges, contributing to the undulating parasitaemia that characterizes the disease. Multiple issues, including host immunity and parasite-related factors, combine to regulate parasitaemia (*28*).

Sexual reproduction is not obligatory in trypanosomes. It can, however, occur in tsetse flies, and it was shown recently that this probably takes place between the midgut and the salivary gland and by production of classical haploid gametes (*29*). Genetic exchange is important, as it allows rapid evolution and transmission of important traits such as drug resistance and human pathogenesis. For example, as genes for resistance to one drug are selected, the opportunity arises to transfer those genes to other genetic backgrounds. This ability to exchange genetic information can affect the population dynamics of the parasites with regard to various key traits. Several studies have indicated

that *T. b. rhodesiense* demonstrates relatively frequent genetic exchange, with sporadic clonal expansion of particular genotypes during epidemics. To date, the population structure of *T. b. gambiense* appears to be relatively stable, indicating that genetic exchange is less frequent (*30*).

3.4 The *Trypanosoma brucei* genome

The biological blueprint of an organism is encoded in its genome. Within the past decade, the sequencing of entire genomes has become relatively simple, and unravelling the genome sequence of *T. brucei* has provided great insight into how these organisms function. Furthermore, by coupling genomic information with phenotypic traits (such as virulence, immune evasion, drug sensitivity and resistance), it has become possible to identify the molecular processes that underpin the ability of these organisms to survive within humans and to cause disease. The *SRA* and *TGSGP* genes mentioned above are examples of subspecies-specific genes that can be used to distinguish *T. b. rhodesiense* and *T. b. gambiense*, respectively, and also provide insight into how these parasites avoid lysis by the trypanosome lytic factors of human serum.

T. brucei possesses a nuclear genome and a mitochondrial genome (or kinetoplast, discussed above). The principal nuclear chromosomes, of which there are 11, are diploid; however, the genome also contains numerous intermediate-sized chromosomes (300–900 kb) and many mini-chromosomes (50–100 kb), which appear to serve as repositories of *VSG* genes. The intermediate and mini-chromosomes do not follow the standard diploid condition. The total genome size is estimated to be 35 Mb per haploid genome; the full genome, the sequence of which was published in 2005 (*31*), is available for analysis on the GeneDB (http://www.genedb.org/Homepage/Tbruceibrucei927) and TritrypDB (http://tritrypdb.org/tritrypdb/) websites. Genome sequences for many strains of *T. b. brucei* and representative strains of *T. b. gambiense* and *T. b. rhodesiense* are now available. The advent of next-generation sequencing permits rapid, efficient sequencing of whole genomes for different strains, which can be rapidly assembled for comparison with reference strains. These genomic approaches will be increasingly valuable in, for example, distinguishing differences associated with drug resistance or other traits, such as virulence.

The genes of the trypanosomatid organisms follow a similar pattern, which is distinct from that in other eukaryotes (*32*). The genes are generally not interrupted by introns, and all genes appear to be transcribed constitutively. Large polycistronic transcripts (comprising multiple genes) are processed by addition of a polyA tail at the 3' end and a spliced leader at the 5' end; RNA stability depends on signals in the 3' untranslated region. Hence, genes, the products of which are differently expressed at different life-cycle stages, tend to be regulated

by their ability to translate messages, which is in turn related to RNA stability and processing (*33*). In addition to the intrinsic interest of the trypanosome molecular basis as a variation on the theme of gene expression in eukaryotic cells, unusual aspects can offer targets suitable for new drugs. The trypanocidal compound sinefungin, for example, prevents spliced leader RNA molecules (unique to these cells) from being methylated, a process required to stabilize RNA molecules and which leads to death of the parasites.

The genome of African trypanosomes is also relatively plastic, in that the gene copy number can expand and contract at relatively high frequency. This may be due to the evolution of the process of antigenic variation. In this case, the parasites sequentially express one of the thousands of *VSG* genes they have, usually by duplicating a basic copy of the gene and translocating the duplicate to an expression site, where it is expressed with the *VSG* genes. This is necessary to ensure the presence of a single *VSG* at the surface of the parasite (*34*). The process of gene conversion appears also to lead to rapid production of multiple chimaeric genes, in which part of one *VSG* gene is inserted into another, resulting in rapid evolution of the *VSG* family. Genome plasticity might also result in rapid evolution of beneficial traits, such as the emergence in *T. b rhodesiense* of the *SRA* gene, which confers resistance to apolipoprotein L1 (discussed above) and appears to be the product of duplication of a truncated *VSG* gene.

An emerging field is the study of parasite-derived factors that effect virulence (*35*). Classically, gambiense and rhodesiense disease are classified as slowly developing and rapidly developing, respectively. It is becoming clear, however, that there is substantial heterogeneity in the infectivity traits of subspecies; for example, rhodesiense infections often progress relatively slowly over a long time in Malawi but progress rapidly in Uganda (*36*). In studies in rodents, virulence manifested as splenomegaly, anaemia and other inflammatory symptoms differs according to the infecting strain (*35*). In gambiense infections in rodents, at least three grades of infection were identified (*37*). The levels of expression of the secreted protease, brucipain, appear to affect the ability of trypanosomes to traverse the blood–brain barrier (*38*). Classical genetic crosses of different trypanosome strains, followed by mapping of the genetic loci that are inherited with virulence phenotypes, led to the identification of regions associated with virulence, and other markers can be expected to emerge soon (*35*). Other parasite-specific factors at the cell surface (or secreted) that appear to regulate immunity have been described (*39*), and the complex interplay between host and parasite at the molecular level is beginning to be revealed.

The kinetoplast genome is even more extraordinary than the nuclear genome, in that many of the protein coding genes are in fact present in an incomplete format (*40*). These pre-genes are transcribed into RNA messages,

which are then processed by editing of the gene to yield RNA that can be translated into a protein. This RNA editing involves a complex structure, the editosome, many components of which are unique to the trypanosomatids (*41*). Some plants have various types of RNA editing, but the extensive editing process found in trypanosomes and related Kinetoplastid protozoa appears to be unique to these parasites. To date, no satisfactory explanation has been offered for why these organisms undergo what appears to be such an elaborate process to express information in their genes.

3.5 Immune evasion

In order for a microbial pathogen to survive in its host, it must avoid those processes that have evolved within the host explicitly to destroy microbes. Human infectious trypanosomes can avoid the immune-independent activities of trypanosome lytic factors in blood, as described above. They are, however, highly immunogenic and stimulate immunological responses in the form of antibodies, of the immunoglobulin (Ig)M and IgG classes in particular. It has been known for many years that the surface of the trypanosome is covered in a dense glycoprotein coat (about 15 nm thick), where dimerized glycoproteins (*VSG*s) provide protection. The *VSG* coat keeps antibodies and the complement system away from the parasite plasma membrane and the key nutrient acquisition and communication molecules within it. As it is the coat protein that is displayed at the parasite surface, most antibodies are directed to these highly immunogenic antigens. The coat protein is turned over rapidly in a manner that ensures that antibodies or other host material bound to the surface is regularly recycled. Eventually, an overwhelming antibody response to a particular surface coat will disrupt its homogeneous protective sheath effect, and the parasites will die; however, with up to 2000 individual *VSG* genes, including pseudogenes, as well as a propensity to evolve new variants by producing chimaeric versions by shuffling small regions of individual genes, the parasites can ward off immunological assault more or less ad infinitum. The genetic processes that allow antigenic variation have been studied in great detail and are one of the most interesting examples of monoallelic gene expression in eukaryotes (*32, 42*), as described in the previous section.

In addition to antigenic variation, trypanosomiasis is associated with general induction of immunosuppression, albeit by mechanisms that are not well understood, which is presumed to enhance the parasite's ability to survive in their mammalian host (*28, 43*). A recently described protein, the trypanosome-suppressive immunomodulatory factor (*39*), has been postulated to play a role in immunosuppression, although more work is required to understand how trypanosomes regulate host immunity.

3.6 Biochemistry and modes of drug action

The biochemistry of trypanosomes has also been the subject of much analysis, motivated mainly by the notion that differences in biochemical physiology between host and parasite offer an opportunity to develop selective chemical inhibitors of parasite metabolism, which could then be used to develop useful drugs. For example, the polyamine biosynthetic pathway is inhibited by the drug eflornithine (difluoromethylornithine, marketed as Ornidyl®) (*44*) (see section 7 for more details). The drug is a suicide, irreversible inhibitor of the enzyme ornithine decarboxylase. Loss of the enzyme leads to loss of synthesis of its product, putrescine, which in turn causes a reduction in the biosynthesis of spermidine. Spermidine has multiple roles in trypanosomes, as in other cell types. It is a highly abundant polycation and binds to many cellular anions (e.g. DNA, RNA, phospholipids), where it has a stabilizing role. In trypanosomes, spermidine has an additional, novel role: it conjugates with two molecules of cellular thiol glutathione to create N^1N^8-bisglutathionyl spermidine, commonly referred to as trypanothione (*45*). Trypanothione performs many of the roles in redox homeostasis in trypanosomes that glutathione plays in mammalian cells; hence, loss of its production, through reduced spermidine synthesis, affects the cell in this way. Other aspects of the polyamine pathway, such as the methionine cycle and production of *S*-adenosylmethionine (a widely used cellular methyl donor) and its decarboxylated derivative that provides the aminopropyl groups in polyamine synthesis, have also been studied. The emergence of metabolomic platforms for analysing metabolic perturbation induced by drugs has been used to confirm the modes of action eflornithine, and active metabolites of nifurtimox were identified by this route (*46*). The metabolomics platform thus promises to be useful in identifying the modes of action of drugs (*47*).

Other biochemical pathways in trypanosomes that are of particular interest are the glycolytic pathway, which operates at high flux rates in bloodstream forms of *T. brucei*, glucose being the only substrate capable of providing a supply of energy (mitochondrial metabolism, the tricarboxylic acid cycle and the respiratory chain being repressed in these forms) (*47*). The first seven steps of the glycolytic pathway occur in a membrane-bound organelle, the glycosome, which allows the enzymes to act without the allosteric regulation common in other cell types, as phosphate and redox balance are maintained within the organelle (*48*). Many attempts have therefore been made to develop drugs that inhibit glycolytic pathway enzymes, although another tier of complexity is added by the possibility that the enzymes might form a tight complex in situ and the fact that drugs must cross the glycosomal membrane as well as the plasma membrane and possibly the intestinal and blood–brain barrier (*49*). The pentose phosphate pathway, a second pathway of glucose metabolism, is also operative in bloodstream-form trypanosomes, and several enzymes in this pathway have been proposed as useful

drug targets. Glucose is also an important precursor of many of the carbohydrate moieties that enter cellular glycoconjugates, including the *VSG* that coats the cell.

Like all parasites, trypanosomes cannot produce the purine ring de novo and rely on a series of high-affinity transporters to acquire purines from their host and then use a range of purine salvage enzymes to acquire the full complement of purines required for growth (*50*). The purine uptake system has been used successfully in drug development (*51*). The P2 aminopurine transporter (encoded by the *TbAT1* gene) is a portal for the uptake of both the melaminophenylarsenical (e.g. melarsoprol) and the diamidine (e.g. pentamidine) classes of drug, and its loss correlates to the emergence of drug resistance (*52*). A second plasma membrane permease, aquaglyceroporin 2, was identified in a screen for genes loss of the function of which underlies resistance (*53*) and appears to be associated with the high-affinity pentamidine transporter, which is known to be a secondary transporter of both pentamidine and melaminophenylarsenicals, the loss of which is required for high levels of resistance to these drugs (*54*). As pyrimidines are synthesized by trypanosomes, the pyrimidine biosynthetic machinery might also be targeted by drugs (*55*).

Lipid metabolism in trypanosomes has also been studied (*56*). Inositol incorporated into the glycosylphophatidyl inositol anchor of the *VSG* and bulk inositol used in phosphatidyl inositol biosynthesis are derived from separate sources. The myristate groups that are added to *VSG*s as part of the anchoring process and to other membrane proteins are exchanged via *N*-myristoyl transferase, and this enzyme is a potential drug target (*57*). Highly potent inhibitors have been shown to be potently trypanocidal, and the fatty acid elongases, which are responsible for most fatty acid elongation, are further drug targets, as are the choline and ethanolamine pathways (*58*).

Cofactors such as biopterin and folate appear to be critical to trypanosomes that are vulnerable to inhibition of their synthesis. As these enzyme systems have been useful drug targets in other organisms, they continue to be targeted in trypanosomes (*59*). Better appreciation of the biochemistry of these organisms will enhance our ability to understand how drugs work against these parasites and further the development of new drugs.

3.7 Drug resistance

Resistance to antimicrobial drugs is a cause of concern in all areas in which there is infectious disease. As only a handful of drugs are available to treat the various types of HAT and the different stages of the disease, losing any of the current drugs to resistance would be a serious impediment to efforts to eliminate the disease. Significant progress has been made in understanding how resistance to drugs used in trypanosomiasis chemotherapy comes about (*53*).

The melaminophenylarsenical drug melarsoprol (and its active metabolite melarsen oxide) is known to enter trypanosomes mainly via the P2 aminopurine transporter (*60, 61*). A secondary route is proposed to be the high-affinity pentamidine transporter, which was initially characterized as a transporter for the diamidine drug pentamidine but was subsequently reported to mediate arsenical uptake as well. Loss of the P2 transporter was shown to be associated with resistance to melarsen oxide, and a number of studies have corroborated the central role of this carrier in resistance (*62*). Gene knockout experiments showed, however, that loss of the P2 transporter alone (encoded by the *TbAT1* gene) was insufficient for high-level resistance (*63*), while loss of high-affinity pentamidine transporter plus P2 resulted in high-level resistance to both pentamidine and melarsen oxide. It was subsequently shown with an RNA interference approach, in which libraries of gene fragments the expression of which as double-stranded RNA can lead to down-regulated expression of cognate genes, that loss of aquaglyceroporin-2 can also cause cross-resistance to melarsoprol and pentamidine (*53, 54*). Aquaglyceroporin thus appears to be related to high-affinity pentamidine transporter and drug resistance; in fact, the two entities are probably the same molecule. Overexpression of a multidrug resistance-type protein TbMRPA could also stimulate resistance to melarsoprol in vitro (*64*), and the loss-of-function RNA interference screen also revealed a number of candidates (*53*).

Eflornithine resistance was selected in the laboratory, and a metabolomics approach showed that metabolism in resistant cells was not changed, although the amount of eflornithine entering the cells was diminished (*65*). Subsequently, one of the parasite's 40 or more amino acid transporters, TbAAT6, was shown to be lost in two independently selected lines (*65*). Moreover, RNA interference experiments showed that down-regulation of TbAAT6 caused resistance, while its expression in the resistant line that had deleted the gene caused a reversion to eflornithine sensitivity. The RNA interference loss-of-function approach confirmed that loss of TbAAT6 was responsible for resistance to eflornithine, and no other mechanism of derived resistance has been shown (*66, 67*). As *T. b. rhodesiense* appears to be naturally refractory to eflornithine, alternative mechanisms have been proposed for this lack of sensitivity (*68*), including differences in the half-life of the ornithine decarboxylase enzyme (*67*), differences in uptake of drug and enhanced uptake of putrescine in refractory lines, allowing by-pass of the inhibition (*69*). Comparison of the genomes of *T. b. rhodesiense* and *T. b. gambiense* reference lines, however, has not yet given an obvious explanation for why the parasites have different sensitivities to the drug. This raises the question of the suitability of each parasite strain for use in drug screening. Thus, compounds should be tested against a panel of strains both in vitro and in vivo in order to select the compounds most likely to be active against human infectious parasites in the field.

Resistance to suramin has not been reported in human cases in the field, although the drug is also used in veterinary trypanosomosis. *T. evansi*-resistant clones were easy to derive, as were trypanosome lines in the laboratory. The uptake of suramin into trypanosomes was long thought to occur via receptor-mediated endocytosis (*70*). This was corroborated in the RNA interference resistance screen, which showed that loss of many parts of the cellular endocytic system could confer resistance to suramin (*53*), which appears to enter via endocytosis with the invariant surface glycoprotein ISG75 as a ligand for surface-binding. Other changes to biochemical pathways, including the polyamine pathway and *N*-acetyl glucosamine metabolism, could also affect the endocytic pathway.

Nifurtimox is the nitroheterocycle now used in combination with eflornithine. The drug is active after reduction by an unusual nitroreductase, followed by further metabolism to an active trinitrile derivative (*71*). Down-regulation of the nitroreductase can induce resistance, as can loss of enzymes involved in biosynthesis of the flavin adenine dinucleotide cofactor that is necessary for nitroreductase activity. Alarmingly, cross-resistance between nifurtimox and another nitroheterocycle, fexinidazole, occurs, indicating that down-regulation of the nitroreductase (either by mutation or by deprivation of the necessary cofactor) can also cause resistance to that drug (*72*). The ease with which resistance to all currently used trypanocidal drugs can be selected emphasizes the importance of sustaining a robust pipeline of drugs that work through novel modes of action.

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4. The vector

Tsetse flies (Diptera: Glossinidae) have a series of biological and demographic characteristics that make them unique among medically important vectors. Their life-cycle is particularly unusual, as they do not lay eggs; instead, a single larva develops within the female uterus. The larva feeds from the uterine glands of the mother ("adenotrophic viviparity") and is larviposited as a mature larva (third instar) in humid soil. The larva then quickly burrows into the soil surface, where it pupates, and the adult emerges 20–80 days later, depending on temperature and humidity (pupal development is not completed below 16 °C and above 36 °C). Thus, each female produces only one offspring at a time and may produce no more than three to five offspring during its total life in nature, which lasts about 3 months for females and 2 months for males as a result of various natural mortality factors, such as predation and starvation. As a result, the intrinsic rate of tsetse population growth tends to be low, the maximum rate of increase being no more than 10–15 times per year. Thus, even a small increase in the average daily mortality rate can cause a population to decline. Newly emerged flies have few resources and tend to be less discriminatory about the host for their first blood-meal than for subsequent feeds. The female is mated 1–3 days immediately after emergence; the only exception is *G. pallidipes*, which mates at 7–10 days of age. Recent observations confirm that, in contrast to long-standing assumptions, tsetse flies can be infected at any time of their life with trypanosomes, including *T. brucei*, if they have been starved. Tsetse flies are the only cyclical vectors of human and animal trypanosomes, which are responsible for HAT and *nagana* (African animal trypanosomosis), and thus provide an important link between the parasite and the mammalian hosts. Unlike mosquitoes, both female and male flies feed on vertebrate blood and are thus both vectors. Interestingly, so far, no resistance to insecticides has been reported in tsetse flies.

Hence elimination of the vector provides an important strategy for reducing disease levels in humans or animals. For effective sustainable vector control, detailed knowledge about the biology and ecology of the flies is essential. In addition, knowledge of the current vector control methods is needed. This section provides an overview of these. The days when tsetse control specialists advocated only one or another method are gone, and the use of several methods in an integrated disease and vector management strategy is recommended. An integrated pest management approach is preferable because it exploits all the weaknesses in fly behaviour and enables synergies of the methods that increase the intensity of the control effort.

4.1 Classification

Tsetse flies are Brachycera Cyclorrhapha Diptera, Schizophora and Calyptratae, close to Muscidae. The Glossinidae family was created for these insects, comprising a single genus, *Glossina*.

Both sexes of this genus are haematophagous. They are close to Stomoxyinae, and they differ from other Muscoidea by the adaptation of their mouthparts to blood-sucking. They have a characteristic mode of reproduction, which brings them closer to pupiparous haematophagous Diptera (*Hippobosca*, *Melophagus*). Thirty-one species and subspecies have been described. Their distribution, which extends over approximately 10 million km², is restricted to sub-Saharan Africa, north of the Namibian and Kalahari deserts. They are not found on islands east of the African continent, except for the island of Unguja in Zanzibar, where *G. austeni* was present but has been eradicated (*1*). Some individuals of *G. morsitans* and *G. fuscipes*, were also recorded in south-western Saudi Arabia *(2)*.

Tsetse flies are long, robust, brown–black to brown but never metallic-coloured. Their length, without the proboscis, is between 6 and 16 mm. Males are generally smaller than females. The wings, which are hyaline or slightly smoked in colour, are, at rest, crossed above the abdomen (like scissors), and their posterior end exceeds its extremity. The mouth parts are long and sharp; located at the base of the head, they are directed forwards at rest and are protected by maxilla palpi of the same length.

The first species (*G. longipalpis* and *G. palpalis*) were formally described in 1830, but their role in transmission of African trypanosomiasis was identified only in 1895. The trypanosomes they transmit belong to the section of Salivaria, with an anterograde cycle.

Classification of the genus *Glossina* is based on external morphological characteristics (e.g. colour, shape of the antennae, presence of bristles on thoracic pleura) but mainly on the shape of the male and female genitalia, geographical distribution and some bio-ecological features (*3*). The genus was divided into three groups of species, which are now considered subgenera (see *Table 4.1*):

- subgenus *Nemorhina* (Robineau-Desvoidy, 1830) (palpalis group)
  - standard species: *G.* (*N.*) *palpalis* (Robineau-Desvoidy, 1830)

- subgenus *Glossina* s. str. (sensu stricto) (Zumpt, 1935) (morsitans group)
  - standard species: *G.* (*G.*) *longipalpis* (Wiedemann, 1830)

- subgenus *Austenina* (Townsend, 1921) (fusca group)
  - standard species: *G.* (*A.*) *brevipalpis* (Newstead, 1910).

Table 4.1
Species and subspecies of *Glossina*

Subgenus	Species	Subspecies
***Nemorhina* (palpalis group)**		
	G. palpalis	*G. p. gambiensis**
		*G. p. palpalis**
	G. fuscipes	*G. f. fuscipes**
		*G. f. quanzensis**
		G. f. martinii
	G. pallicera	*G. p. pallicera*
		G. p. newsteadi
	G. caliginea	
	G. tachinoides	
***Glossina* s. str. (morsitans group)**		
	G. morsitans	*G. m. submorsitans*
		*G. m. morsitans**
		*G. m. centralis**
	*G. pallidipes**	
	*G. swynnertoni**	
	G. longipalpis	
	G. austeni	
***Austenina* (fusca group)**		
	G. fusca	*G. f. fusca*
		G. f. congolensis
	G. nigrofusca	*G. n. nigrofusca*
		G. n. hopkinsi
	G. medicorum	
	G. brevipalpis	
	G. hanningtoni	
	G. fuscipleuris	
	G. longipennis	
	G. schwetzi	
	G. tabaniformis	
	G. nashi	
	G. vanhoofi	
	G. frezili	
	G. severini	

[a]Modified from reference *4*

* Major vectors of sleeping sickness

The grouping of species of the *Glossina* genus into three subgenera is justified by common characteristics and morphology due to bio-ecological and even genetic similarities. The common characteristics are summarized below.

4.1.1 Subgenus *Nemorhina*

In species of medium (8–10 mm) or small (6–8 mm) size, the abdomen is black–brown or has dark spots on a clear greyish layer. All the segments of the tarsus of the posterior legs have dark brown or black bristles.

Almost all the species of this subgenus are found in West and Central Africa, from Lake Victoria and the Tanganyika basin to the Atlantic coast, in vegetation close to water (forests, small island forests, gallery forests, "sacred" woods, river thickets, banks of lakes, mangroves, *niayes*), and these species of the subgenus are also called "riverine" tsetse. Some species colonize coffee, cocoa, mango and banana plantations. In addition, *G. palpalis* appears to colonize wet savannahs invaded by *Chromolaena odorata* (Laos grass) in Côte d'Ivoire. *G. palpalis* and *G. fuscipes*, the main vectors of sleeping sickness, are increasingly described in medium to large towns, such as Abidjan, Conakry, Kinshasa, Libreville and Brazzaville (*5*), where peri-urban transmission of sleeping sickness has been demonstrated (*6*).

The species of this subgenus are the main vectors of HAT in West and Central Africa. They are also becoming increasingly important for the transmission of African animal trypanosomosis as a consequence of demographic growth, as they have been shown to be able to adapt to important human density, in contrast to tsetse flies of the morsitans group (*7–9*).

The possibility of the existence of cryptic species has been raised, and it has been suggested that subspecies be elevated to the rank of species, especially within *G. palpalis* s. l. (*sensu lato*) and *G. fuscipes* s. l., on the basis of morphological, genetic and geographical differences, differences in vectorial capacity and some hybrid sterility between subspecies (*10, 11*). The advantages and drawbacks of changing the current classification are not, however, clear. As few research teams are working on the topic, no major change is likely in the coming years, unless new tools and insights into the relations among tsetse species emerge. The same applies for morsitans group species (see below).

4.1.2 Subgenus *Glossina* s. str.

The species is of medium size (8–11 mm), and the abdomen is generally coloured, with dark spots on a clear yellowish layer. Only the last two segments of the tarsus of the posterior legs are covered with black bristles (except *G. austeni*).

These species are less restricted to watercourses than the species of the *Nemorhina* subgenus, although they can be abundant in these areas. They occur mainly in savannah woodland, dense thickets or clear forests and are often called

"savannah tsetse". Their distribution is linked, in particular, to the presence of wild fauna and cattle. Species of this subgenus are very efficient vectors of African animal trypanosomosis, and some are involved in the transmission of *T. b. rhodesiense*, such as *G. swynnertoni*, *G. pallidipes* and *G. morsitans* spp.

In the same way as for species of the palpalis group, questions have arisen about the status of subspecies, especially within *G. morsitans* s.l. and *G. swynnertoni* (reviewed in *12*), but the classification has so far remained unchanged.

4.1.3 Subgenus *Austenina*

This large species (11–16 mm) has an abdomen of a more or less uniform brown. The tarsus of the posterior legs is brown–black, sometimes only the last two segments being black.

The species of this subgenus live in forest belts (rainforest, evergreen rainforest, forest–savannah mosaics, coastal mosaics, relic forest remainders, broad and dense gallery forests), except for *G. longipennis* (arid savannahs of Kenya and Somalia) and *G. brevipalpis* (thickets in East and southern Africa). Increasing human activity in forests is tending to make them disappear. Some are excellent vectors of animal trypanosomosis, but there are few cattle in the areas in which they occur. They have not been reported to be vectors of HAT.

4.2 Reproductive system

The formation and development of the reproductive system and spermatogenesis occur during the pupal period. The adult male hatches with its stock of spermatozoids, which are not renewed during imaginal life. This biological characteristic forms the basis of the genetic control campaigns involving release of irradiated sterile males (*13*).

Female tsetse flies are larviparous, and their reproductive system is adapted to this mode of reproduction (adenotrophic viviparity). It is composed of a right and a left ovary, each with an internal and an external ovariole, and two short oviducts meeting in a common oviduct, which leads to the antero-superior part of the uterus. Two spermathecae (or seminal receptacles), globulous, brown and each with a canal, emerge behind the common oviduct, at the top of the uterine papilla (*14*).

The ovaries are much larger than those of other insects and are dissymmetrical and polytrophic, each egg having its own feeder cells. Each ovariole is composed of a germarium (germinal epithelium), which forms a follicle. After synchronous divisions, the follicle periodically includes an ovocyte and its feeder cells surrounded by a follicular epithelium. In a female that has just hatched, the four ovarioles are at different stages of development and will reach maturity in the same order: the right internal, then the left internal, the right external and finally the left external ovariole. There is never more than one follicle

per ovariole at the same time. Ovulation results in the appearance of a follicular relic, corresponding to the remaining degenerated feeder cells and the torn and retracted follicular tube. The first ovulation takes place on about the 8th or 10th day of imaginal life. Subsequent ovulations take place every 9 to 10 days, in the first hours that follow the laying of the preceding larva. This regular ovulation, in a predetermined sequence, makes it possible to determine the physiological age of a female, by dissecting its genital organ, examining the order of each ovariole in decreasing size and grouping these numbers following the spatial position of the four ovarioles. The number obtained added to the number of follicular relics is the age of a female up to the seventh ovulation, i.e. approximately 80 days.

The mature follicle is fertilized by spermatozoids from spermathecae at the moment it penetrates the uterus, and embryonic development, which lasts approximately 3 days, leads to the formation of a first-stage larva. From this stage, which lasts 26–36 h, the uterine gland is active and the larva is fed by its secretions. The second-stage larva is released after a moult. The latter, which has partially developed respiratory lobes, will grow considerably and distend the abdomen of the mother. Two to two and a half days after the first moult, a new moult leads to the third larval stage, which is larger and harbours entirely developed polypneustic lobes, becoming black at the end of the intra-uterine lifetime. The mature larva is laid 3 days after the second larval moult. The next ovulation takes place 30–90 min later, so that the uterus remains empty for a very short time; in a population, most females are thus always carrying a larva.

4.3 Reproduction

The principal characteristic of tsetse flies is their mode of reproduction, which is encountered in only a small number of Diptera, grouped in the biological group of pupiparous insects (Hippoboscidae, Streblidae, Nycteribiidae). In this group, females have a pocket analogous to the uterus of the mammal, in which it preserves its larva until maturity. During its intra-uterine life, the larva is fed by secretions of lactiferous glands annexed to the uterus. Hence, the absence of eggs, a free larval stage in nature and the fact that pupal development occurs in the soil make the adult fly the only phase that is directly accessible for control purposes. The only difference between tsetse flies and true pupiparous insects is the mobility of the tsetse larva, which can move actively after its expulsion from the uterus, whereas the larva of pupiparous immobilizes itself immediately for pupation.

When the tsetse imago hatches, its genital organs are already formed, and it can mate with subsequent fertilization in the hours that follow. In natural conditions, almost all females are fertilized as soon as they leave the puparium. Males can mate approximately 10 times if mating is adequately spaced. Mating is long (0.5–3 h), because during this period the male forms a spermatophore

(globulous and gelatinous mass of approximately 500 µm which contains sperm), which it deposits at the bottom of the uterus. Migration of sperm towards the spermathecae starts at once and continues well after the male has left the female, which will reject the empty envelope of the spermatophore a few hours later. One insemination is generally sufficient to enable the female to produce larvae for several months; spermatozoids can survive for nearly 200 days in the spermathecae. Some young females can accept several sexual unions to fill their spermathecae, but, under experimental conditions, only 0.7% of females accept more than one sexual union beyond the tenth day.

The free larva appears as a whitish maggot 5–8 mm long and is mobile, apodal, with a ringed body divided into 13 segments, the last of which carries two chitinized, black, bulky respiratory lobes (or polypneustic lobes), characteristic of the genus.

In riverine species, larviposition sites vary by season: they are generally located near water in the dry season and more distant from water in the rainy season. They follow or precede the rising and falling water levels of rivers. Savannah species are more diffuse and more difficult to detect and are all the more dissimulated when the temperature is high. During the dry season, larviposition sites are restricted to shady places back from rivers, tree boles and warthog burrows. After a free life of a few minutes to 2 h on the ground, the larva buries itself a few centimetres deep, generally in clay–sandy ground, always in a shady place. The burying depth depends on the structure, compaction, temperature and moisture of the ground. Generally, pupae are located at 2–8 cm of depth, but in the wet season some are found on the surface, under dead leaves. In forest areas, pupae can be found at the base of feather-grass or palm trees or in tree trunks containing humus. In savannah areas, they can be found under large roots, in holes, burrows, under fallen trunks, etc.

The entire process of metamorphosis to a mature adult insect occurs within the puparium. The duration depends on the species, sex and climatic conditions, in particular minimal and maximum ground temperatures. Pupae require sufficient ground moisture (over 60%), but flooding is fatal; deforestation, bush fires and compaction of the ground are also disadvantages. Predation can also occur. Within the critical temperature range for survival, the duration of pupation increases as temperature decreases (between 20 days in the hot season and 80 days in the cold season). At 25 °C, it ranges from 25 to 30 days, females having shorter pupations than males by 2–3 days. During this period, the pupa lives only on the food stocks that the larva constituted during its intrauterine life. This parameter is crucial for vector control with insecticide spraying, such as aerial spraying, as successive spraying operations must be done according to the timing of adult emergence (*15*). When metamorphosis is completed, the young fly leaves its puparium by breaking a circular slit in the anterior end. This detaches,

like a cap, under the combined action of the expansions and contractions of the ptilinum and movements of the head and thorax. The ptilinum retracts back into the head at the end of emergence. The wings are then spread, the abdomen inflates, and the proboscis straightens to a horizontal position. Chitin hardens, and the imago can fly within just a few hours of emergence. Its body is still tender (thus the term "teneral" fly, from the Latin *tener*: tender), and its vitality depends on its fat reserves. The imago takes its first blood-meal a few hours after emergence to develop its musculature for 7 days in the immature phase (males) and 10 days (females).

4.4 Lifespan and population dynamics

Tsetse flies are typical "k" strategists, with traits associated with living at densities close to carrying capacity, and are strong competitors in crowded niches that invest in few offspring, each of which has a relatively high probability of surviving to adulthood. Most other insects produce large numbers of eggs, have a high growth rate and exploit less-crowded ecological niches and are classified as "r" strategists (*16*). The maternal care given by the female tsetse fly to each larva results in a high rate of survival.

The lifespan of tsetse flies has been studied by capture–mark–recapture methods, determination of the physiological age of females by dissection of the ovaries, observation of abrasion of the posterior edge of the wing (wing fray method) and measurement of pteridines in the eyes (*17*), as this fluorescent material accumulates in the head of flies according to age. Age composition must be known before vector control operations and then followed up in order to assess the impact of operations.

Generally, females live longer than males. Records of 12-month survival were obtained in the laboratory, but in natural conditions the maximum seldom exceeds 5–7 months. The lifespan varies according to season: it is optimal in the rainy season (4–5 months), decreases in the cold (3–4 months) and is especially short in heat (1–2 months). Young individuals are more sensitive to the harmful effects of severe climatic conditions than adults, and, despite a sex ratio close to 1 at birth, females are generally more numerous in populations because of their longer lifespan. The apparent density of tsetse populations depends on biotic and abiotic environmental factors, varies widely from one place to another and is related mainly to the hatching rates of pupae and the lifespan of adults, which themselves are governed by climatic factors and host availability.

In areas with marked seasons, in West and Central Africa, the apparent density of riverine species increases relatively rapidly at the beginning of the rainy season and generally falls significantly in the middle of the rainy season due to the destructive effect of floods on the resting places of pupae and probably

the presence of more predators and parasites, the importance of the latter largely depending on density (*18*). A second peak occurs towards the end of the rainy season, followed by a decrease with the onset of the dry season, due to ageing of adults and dispersion of hosts. The decrease continues during the dry and cold seasons (due to lengthening of the pupation duration, increased pupal mortality and hatching of young imagos deprived of fat stocks). The hot season leads to a significant decrease in tsetse density, due to high temperatures and reduced humidity and vegetal cover, causing high mortality among adults and pupae. The population starts to grow again with the arrival of the first rains, moderate temperatures allowing better survival. In forest and pre-forest areas, where the dry season is short and not too pronounced, there is less variation. The density of the savannah species is strongly increased by the first rains and is at a maximum during and especially at the end of the rainy season; the density decreases significantly in the dry and hot seasons. The density curves vary with the duration of the dry season.

4.5 Diet

On average, males take one blood-meal every 3 days, at variable intervals. Females generally take three significant blood-meals during gestation: one immediately before the intrauterine moult between the second and third larval stages, the second at a variable time during gestation and the third immediately after laying the larva. Whatever the sex, a fly that has just hatched takes its first meal 12–24 h later. This first meal is always smaller than those that follow. Feeding is one of the most dangerous moments for a tsetse fly, as it can be killed by defensive movements of the host. Furthermore, because of their low reproductive rate, any additional mortality among adults represents a threat to the population. It is therefore not surprising that they tend to bite hosts on parts where they are less likely to be reached by defensive movements, e.g. on the lower parts of the anterior leg or on the belly of cattle. This behaviour has led to a proposal that the application of insecticides be restricted to this area of the body of cattle, for greater cost–effectiveness (*19, 20*).

After the tsetse fly lands on its host (human or animal), it lowers its proboscis and inserts it into cutaneous tissue, the maxillary palpi remaining horizontal. The skin is pierced by fast alternate movements of the labella, which causes intra-tissue trauma, leading to the formation of micro-haematomas into which the saliva is injected from the end of the hypopharynx. The saliva prevents blood coagulation, has a vasodilatory effect and contains infective trypanosomes if the fly has a mature trypanosome infection; when a fly has a mature *T. brucei* infection, the trypanosomes are located in its salivary glands. In this case, an inoculation chancre can appear at the place of puncture. The duration of the

blood-meal is variable but generally lasts 20–30 s. The amount of blood absorbed depends on the species, sex and nutritional stage of the fly (in particular the fat reserve stocks). The hungrier and more dehydrated the fly, the more blood it takes. Just after the meal, the fly eliminates its excess water via the anus in the form of a large clear drop, which can contain trypanosomes if the fly is infected. The abdomen of the unfed tsetse fly is flattened, and the crop contains only one small air bubble. During the blood-meal, the abdomen inflates when the jabot fills and becomes sharp red by transparency. The jabot starts emptying 5–10 min after the end of the meal. Vigorous pulsations push blood towards the proventriculus, which directs it towards the gut, where digestion takes place. Blood makes its way through the endoperitrophic space and becomes black under the effect of proteolytic enzymes secreted by the intestinal epithelium. Water is absorbed by intestinal cells (in < 3 h), and the residual blood-meal progresses through the middle and posterior segments of the midgut, where it becomes viscous and then semi-solid. Digestion is thus achieved after 24–72 h. The digestive waste is evacuated through the anus in the form of a dark-brown semi-fluid paste. The duration of the hunger cycle (interval between two consecutive meals) depends on local climatic conditions, the availability of hosts, physiological stage (especially in females) and fly activity. The survival rates are minimal in the hot season (low fat reserves, unfavourable climatic conditions) and maximal in the rainy season (high fat reserves, good climatic conditions). Depending on the species and sex, the hunger cycle is 2–4 days but can increase to 8–10 days under favourable environmental conditions. Adult female flies that were nutritionally stressed by feeding only once a week produced pupae with a significantly lower weight and offspring with a significantly lower fat content and lower baseline immune peptide gene expression. Moreover, in experimental infection, emerging teneral flies were significantly more susceptible to a *T. congolense* or *T. b. brucei* infection than those emerging from non-starved adult females (*21*).

4.6 Geographical distribution of the main vector species

All species of tsetse fly are potentially cyclical vectors of trypanosomes; however, only a few species are important vectors of human and animal trypanosomes, because of their specific distribution area and their behaviour. The transmission of trypanosomes infective to humans is due principally to close contact. In West and Central Africa, the riverine species are thus the more important vectors of human trypanosomes. *T. b. gambiense* is transmitted by species of the *Nemorhina* subgenus: *G. fuscipes fuscipes, G. f. quanzensis, G. palpalis gambiensis, G. p. palpalis* mainly. In East Africa, *T. b. rhodesiense* is mainly transmitted by savannah species of the subgenus *Glossina* s. str., and also by *G. fuscipes* (of the *Nemorhina* subgenus) in Kenya and Uganda.

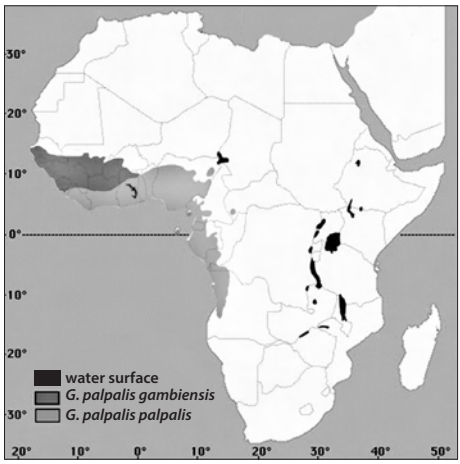
Conversely, virtually all tsetse species are infected by animal trypanosomes, but savannah species (subgenus *Glossina* s. str.) are the main vectors of cattle trypanosomosis. Thus, although all species can be infected by trypanosomes, only some will be vectors of the disease. In addition, the increasing fragmentation of habitat due to human development is reducing the role of tsetse flies of the *Glossina* subgenus (*8, 22*), and they are being replaced by riverine species in West Africa. For instance, *G. morsitans submorsitans* is being replaced, as a vector of *nagana* by *G. p. gambiensis* and *G. tachinoides*. Species of the fusca group are also disappearing with forest degradation due to human pressure, for instance in Côte d'Ivoire, and are being replaced by the very dangerous *G. palpalis* vectors.

4.6.1 Vector species of the *Nemorhina* subgenus

(a) Glossina palpalis

The distribution area of the two subspecies *G. p. gambiensis* and *G. p. palpalis* follows the Atlantic coast from Senegal to Angola (*Figure 4.1*). *G. p. gambiensis* is found in riverine vegetation (forest galleries of hydrographic networks of dry and wet savannahs, Guinean and Sudano-Guinean ecotypes) from Senegal to Benin, whereas *G. p. palpalis* predominates in degraded forests in the area from the coast to the humid forest area and to the savannah–forest transition area (*23*), including peridomestic habitats from the south of Côte d'Ivoire to Angola. Both subspecies are found in mangroves along the Atlantic coast.

Figure 4.1
Geographical distribution of *Glossina palpalis* subspecies



Modified from reference *24*

The main habitats of *Glossina palpalis* spp. are:

- dense vegetation of natural gallery forests with clean river beds in savannahs;
- cocoa and coffee plantations in forested areas;
- *niayes*, wet depressions with dense vegetation associated with oil palm trees in north-west Senegal;
- mango tree orchards and quickset Euphorbiaceous hedges (*Euphorbia balsamifera*);

– areas around human settlements in the forest belt, high densities being associated with pig rearing;

– medium and large towns in which there are suitable vegetation and hosts (zoological parks and forest relics in the main African towns, such as Abidjan, Conakry and Kinshasa); and

– mangroves along the Atlantic coast.

The two subspecies are believed to be the vectors of the parasites that cause 100% of cases of *T. b. gambiense* in western Africa, and they are also among the most important vectors of animal trypanosomosis.

(b) Glossina fuscipes

The three subspecies, *G. f. fuscipes, G. f. martini* and *G. f. quanzensis*, are present in the Congo and neighbouring basins (between the 8–9th northern and the 10–12th southern parallels), from Cameroon and Chad to Lakes Victoria and Tanganyika (*Figure 4.2*). In this area, they live in riverine and lacustrine habitats in forest–savannah mosaics. They are major vectors of HAT in Central Africa and in some countries of East Africa (Uganda, Kenya and South Sudan). Thus, *G. fuscipes* s. l. is the vector of at least 80% of all currently reported cases of HAT. They can also act as animal trypanosomosis vectors.

4.6.2 Vector species of the *Glossina* s. str. subgenus

(a) Glossina morsitans

Two subspecies, *G. morsitans morsitans* and *G. m. centralis*, occur in woodland savannahs of East and Central Africa, particularly in the *miombos* (in which *Julbernardia* and *Brachystegia* are the predominant vegetal species) and the mopane (open woodland with *Colophospermum mopane*), where they can be vectors of *T. b. rhodesiense* (*Figure 4.3*). The other subspecies, *G. m. submorsitans*, lives in various woodland savannah ecotypes as well as in clear forests of *Isoberlinia doka* in West and Central Africa. This species is highly sensitive to human growth and disappears when the human population increases. In Burkina Faso for instance, *G. m. submorsitans* has disappeared from most of the country and remains only in protected areas where there is still wildlife, such as national parks.

(b) Glossina pallidipes

The distribution of *G. pallidipes* is patchy, from Ethiopia to Mozambique (*Figure 4.4*). It is commonly sympatric with *G. m. morsitans*, for instance in the *mopane* woodlands of Zambia and Zimbabwe (*25*). *G. pallidipes* can transmit *T. b. rhodesiense*.

(c) Glossina swynnertoni

This species lives in open savannahs. Its geographical distribution is restricted to southern Kenya and northern United Republic of Tanzania (*Figure 4.5*), where it is suspected to be the major vector of *T. b. rhodesiense*, especially in national game parks. These species are also excellent vectors of animal trypanosomes

4.6.3 Vector species of the *Austenina* subgenus

Most of these species are found in dense forests, mainly in West and Central Africa; however, *G. medicorum* lives in gallery forests of woodland savannahs in West Africa, *G. brevipalpis* in the dense rainfed thickets of East Africa and *G. longipennis* in very arid areas (thorn-bush) and sometimes in dense thickets bordering water, from southern Ethiopia and Somalia to Mozambique and the northern part of South Africa.

Tsetse flies of this subgenus are not vectors of HAT. They are very efficient vectors of animal trypanosomes, but their importance is moderated by their location, which is often far from grazing areas.

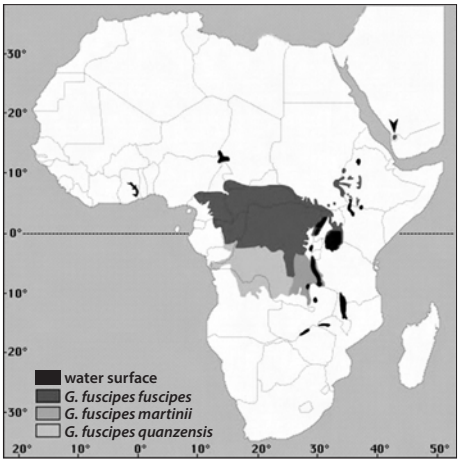
4.7 Tsetse flies in their natural environment

4.7.1 Main habitats

To live, tsetse flies need the particular temperatures and humidity that are encountered only in tropical areas. This ecoclimate corresponds to that of wooded areas receiving > 600 mm of annual rainfall. The mean average hygrometrical optima vary between 50% and 60% relative humidity for savannah species and 65–85% for riverine and forest species. The thermal optimum is about 25 °C for all species. In general, pupae and adults suffer as soon as the temperature exceeds 36 °C and die at temperatures that exceed 38–40 °C. Adults are paralysed by the cold, and pupae cannot develop normally in temperatures lower than 16 °C. Hot and dry climates (Sahelian or sub-Saharan) and cold climates in which the annual average temperature is below 20 °C (highlands and southern Africa) are incompatible with imaginal needs and prevent pre-imaginal stages from reaching the adult stage. It has been suggested that climate change, in particular temperature increase, in these regions of highlands and southern Africa could lead to invasion of new habitats by tsetse flies in the future (*26*). Conversely, in Burkina Faso, tsetse flies have lost 70 000 km^2 from their northern limit since 1949, due to a combination of human growth and droughts (*27*).

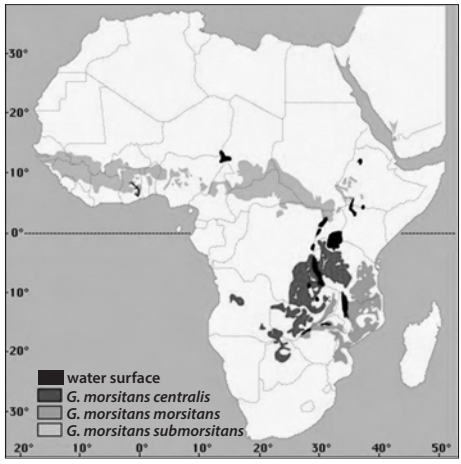
Tsetse flies are thus closely related to vegetation, which constitutes a protective screen from solar radiation and the desiccating effects of wind, and which itself depends on the presence of surface or underground water, which increases the local humidity of both atmosphere and soils. The type and density

Figure 4.2
Geographical distribution of *Glossina fuscipes* subspecies



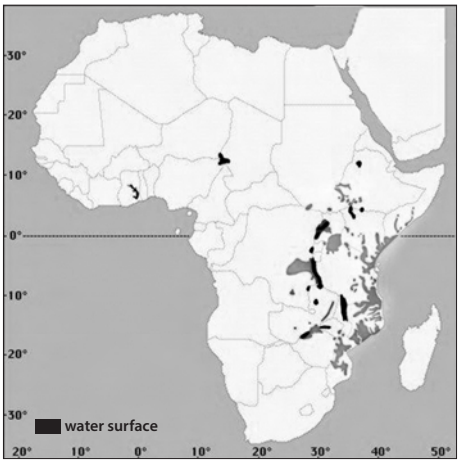
Modified from reference *24*

Figure 4.3
Geographical distribution of *Glossina morsitans* subspecies



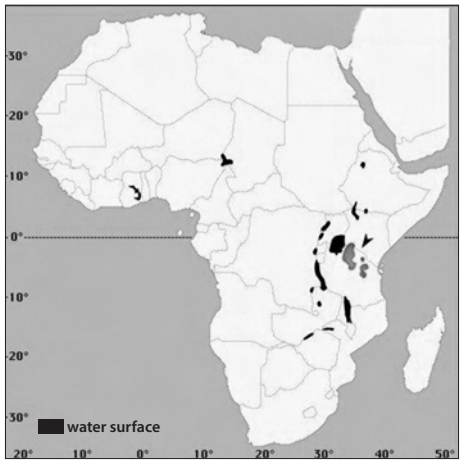
Modified from reference *24*

Figure 4.4
Geographical distribution of *Glossina pallidipes*



Modified from reference *24*

Figure 4.5
Geographical distribution of *Glossina swynnertoni*



Modified from reference *24*

of canopy and underwood influence temperature, humidity, luminosity, ease of flight and the presence of animals that constitute hosts on which tsetse flies feed. Host abundance, availability and attractiveness determine the maintenance and density of tsetse fly populations. Thus, particular vegetation types form habitats that tsetse flies colonize during an entire year or for a single season only. The flies' requirement for these particular conditions (vegetation-determined humidity and host availability) guided the earliest vector control operations, which were based on game elimination and habitat destruction (*28*). These methods are no longer used because of their damaging effects on the broader ecosystem.

The species of the subgenera *Nemorhina* and *Austenina*, which in general require higher relative humidity, depend on the ligneous vegetation of gallery forests or large forests. Those of the subgenus *Glossina* s. str., which are more xerophilous, disperse widely in savannah woodlands during the rainy season but come closer to vegetation near water in the dry season. Within these habitats, flies choose places where the microclimate is the most favourable. In these vegetal associations, generally located at the border between two vegetation types of which at least one is woody, they find resting and reproduction places and hunting areas.

4.7.2 Movements

The daily life of tsetse flies is divided into two unequal periods: one of rest and one of flying activity. The latter is always short and is devoted to searching for food, favourable resting places, larviposition sites for females and young females for males. The level of flight is low (0.5 m approximately) and the speed high (up to 25 km/h). Displacements last a few consecutive seconds or minutes, in small successive jumps. This is certainly due to the metabolism of the flies, which relies on the amino acid proline. In one day, males fly for approximately 30–50 min, whereas females move for only a few minutes, which explains the noticeable differences between sexes of the abrasion of the posterior edge of the wings, a method used to evaluate the average age of a population.

4.7.3 Looking for hosts

The displacement of a tsetse fly to find a host is the result of complex mechanisms with various factors, some specific to the insect (species, sex, age, pregnancy and nutritional status), the others specific to the host. The visual and olfactory organs of the fly help it to find a suitable host, and visual and/or olfactory attractive systems have been used extensively to trap tsetse flies (*29, 30*). Chemosensitive bristles on the antennae are stimulated by puffs of odour emitted by the host; the fly follows the fluctuating (direction and speed of the wind) trail of odours against the direction of the wind by detecting odours produced by cutaneous secretions, faeces, urine (various phenols), carbon dioxide and odours found in

the host's breath (acetone, octenol). Over short distances (10–15 m), visual factors become predominant: form, size, movement, contrast, especially the colour and reflectivity in the ultraviolet range. Important differences have been described between tsetse flies of the *Nemorhina* and *Glossina* subgenuses with regard to olfaction and vision: the species of the *Glossina* subgenus appear to respond much more to olfactory stimuli than those of the *Nemorhina* subgenus, the latter relying more on vision. Whereas it was long believed that tsetse flies are visually attracted to large, horizontal, oblong structures, it has now been shown that *G. p. palpalis* is more attracted to vertical shapes and that several species of *Nemorhina* are highly responsive to small targets. This opens new prospects for more cost-effective tools based on insecticide-impregnated traps or targets (*31–33*).

4.7.4 Feeding preferences

The feeding preferences of tsetse flies appear to rely not only on the nutritional value of the blood of their hosts, as some savannah species (*G. m. morsitans, G. m. centralis, G. m. submorsitans*) have been reported to feed preferentially on suidae (warthog and bush pig), whereas others (*G. pallidipes, G. longipalpis*) sometimes feed exclusively on bovidae (buffalo, bushbuck and other antelope) or hippopotamuses in some areas. When warthogs and antelopes disappear from an area that has no cattle, savannah tsetse flies become scarce. Species of the *Nemorhina* subgenus are believed to be more opportunistic and can adapt to local host availability, including humans, which has various epidemiological consequences, in particular for HAT. For instance, in Côte d'Ivoire, *G. palpalis* feeds mainly on humans in settlements in the absence of pigs, but takes 75% of its blood-meals on pigs when they are abundant. In the hot season, when humans, small antelopes and *G. palpalis* approach water, the fly takes 40% of its blood-meals on bushbuck and 35–55% on humans. In the cold season, it feeds mainly on reptiles. In the Central African Republic, up to 83% of the blood-meals of *G. f. fuscipes* are taken on reptiles during the dry season (monitor lizards, crocodiles). Riverine tsetse fly populations (*G. palpalis, G. fuscipes, G. tachinoides*) that inhabit habitats around villages in wet areas can feed exclusively on pigs bred by villagers. In many situations in West Africa, riverine species such as *G. palpalis gambiensis* and *G. tachinoides* appear to feed on reptiles such as monitor lizards, crocodiles and snakes.

Some wild animals (kob, zebra, wildebeest, oryx) that are common in tsetse habitats are rarely bitten by tsetse flies, possibly because their colour is less attractive (e.g. zebra) or because their skin contains repellent substances, as shown recently for waterbuck (*34*).

Humans are bitten by tsetse flies, as they contract sleeping sickness; however, it has long been known that human odour repels morsitans group tsetse flies (*35*). The reasons why humans are bitten, in spite of these repellents,

are beginning to be investigated in areas of rhodesiense HAT (*25*). In West and Central Africa, where *T. b. gambiense* is transmitted by *G. fuscipes* and *G. palpalis*, these vectors are attracted to human odour (*36*). This has led to the design of attractive devices that are more vertical (*33*), simulating humans (biconical or Vavoua trap), as opposed to those developed for morsitans flies, which are typically horizontal and thus simulate ungulates (see section 4.11 et seq.).

4.7.5 Activity cycles

Tsetse flies are active for a few minutes per day only when the climatic conditions are favourable. Most species are active only during the day, although some species in the *Austenina* subgenus are active at nightfall and sometimes even during the night. In savannah areas and during the hot season, tsetse flies are especially active in the morning and again at the end of the afternoon (*37, 38*). They do not leave their resting places during the hottest parts of the day and show positive phototactism at < 32 °C, which becomes negative beyond this temperature. At 35 °C, they are at rest. They are less active in the rain or in windy conditions. In the cold season, they become active only later in the morning and during the warmer hours of the afternoon. Activity is more uniformly distributed throughout the day in rainy seasons. Species of gallery forests generally have a similar activity cycle: bimodal in the hot season, very sharply unimodal in the cold season and uniform in the rainy season. In forest areas, where climatic variations are more uniform, activity is more homogeneous. Hunger stimulates activity: the response threshold to visual or olfactory stimuli increases with the starving level. Females show peak activity on day 1 of the gestation cycle just after larviposition and on days 6–7 during passage from the second to the third larval stage.

4.7.6 Dispersal

Flight movement generally involves short distances. Riverine species disperse mainly linearly, following a river by backward and forward motions, upstream or downstream, over short distances (200–300 m). In the dry season, displacements are longitudinal inside the galleries (*39*). In the rainy season, tsetse flies can fly from riverine vegetation to neighbouring savannah and may be able to cross from one hydrographic basin to the next. In the hot season, some individuals can fly long distances (17 km in 3 days for *G. tachinoides*; 22 km in 5 days for *G. p. gambiensis*) along rivers. The savannah species move more randomly, generally over short distances (between 175 m per day for *G. pallidipes* in Kenya to < 1 km for *G. m. submorsitans*) (*40*). At the beginning of the rainy season, however, successive waves of dispersal can occur, involving significant numbers of individuals. For example, cleaned areas are recolonized at a rate of 5–15 km per annum by *G. m. submorsitans* and of 3–10 km per annum by *G. palpalis* (*41*).

Tsetse flies can follow cattle herds or humans or be transported passively over long distances on vehicles, boats or trains, which can constitute sources of reinvasion and dispersal of trypanosomes.

4.7.7 Resting places

Knowledge of the diurnal resting places of tsetse flies allows efficient control by selective ground-spraying of insecticide (*39, 42*). These places are characteristically where temperature, humidity and light are within the most favourable limits, generally at levels that differ greatly (microclimate) from ambient conditions (macroclimate). The flies rest for the greater part of the day, generally close to the ground, on the living woody parts of vegetation. Early in the morning, they choose the sunny sides of plants, but, as soon as the temperature rises, guided by negative phototactism, they take refuge in the shady parts of their living area such as the lower faces of branches or brushwood, holes in tree trunks, shaded ground relief and under large roots. In these places, the temperature is often 8–10 °C lower than the ambient temperature. Their preference tends towards trunks or branches of diameters greater than 20–30 cm (*G. tachinoides, G. morsitans*) or below 10 cm (*G. p. gambiensis*). The height of the resting places is closely related to temperature: as soon as the ambient temperature rises, tsetse flies approach the ground. The resting places are closer to the ground during the day than at night and in dry and hot seasons than in rainy seasons. The resting height is below 3 m in cooler temperatures and below 50 cm in the hot season: for example, 90% of *G. tachinoides* are < 20 cm from the ground in this season (*42*). In the same way, the transverse distribution of resting places in a gallery depends on temperature. Flies regroup close to rivers during the hot season: about 60% of *G. p. gambiensis* concentrate close to rivers during the day, in a band of vegetation < 1 m wide. In Sudano-Guinean areas, 90% of flies are located in a narrow part of the gallery representing one sixteenth of its width. At nightfall, tsetse flies leave their diurnal resting places and appear to rise in the vegetation, landing on the tops of green sheets at heights of 2.5–6 m but sometimes up to 10 m. These night resting places are the main targets of sequential insecticide aerial treatment, which is usually applied at night.

4.8 Population genetics and geometric morphometrics

One objective of analysing the genetics of tsetse flies for vector control is to determine gene flow between populations in order to assess population isolation, which has direct consequences on the design and implementation of tsetse control measures (see reference *43* for a review).

In recent studies with remote sensing techniques, in areas subject to human encroachment in East and West Africa, tsetse fly populations become fragmented and, in some cases, isolated (*44, 45*). Finding such "biological

islands" by assessing their genetic isolation will undoubtedly help to target these populations for sustainable vector control and possibly even eradication. Such populations must, however, first be identified and characterized. Molecular and modern morphometric techniques (*46*) appear to have the potential for rapid identification of the levels of population sub-structuring in tsetse vectors. In studies with microsatellite DNA markers and geometric morphometrics of the wings, a population of *G. palpalis gambiensis* on the Loos Islands near Conakry (Guinea) was shown to be isolated from two others in the nearby mangrove habitat (*47*), and, in Senegal, *G. p. gambiensis* in the Niayes region were shown to be genetically isolated from those in the nearest area with another tsetse belt, 70 km to the south (*48, 49*). In these two cases, eradication is the objective of the vector control operations that are under way. Conversely the *G. p. palpalis* populations of Abidjan are not isolated from each other and should be considered a single, panmictic unit (*50*). The importance of conducting genetic studies before control operations is now widely accepted, especially (but not only) when tsetse eradication is the objective, with area-wide integrated pest management (*51*). This is the case in areas targeted by projects of the Pan African Tsetse and Trypanosomiasis Eradication Campaign.

4.9 Genomics of tsetse flies

An international consortium, the International *Glossina* Genomics Initiative, which brings together scientists in sequencing laboratories and research laboratories of the north and the south, was established by the UNDP/World Bank /WHO Special Programme for Research and Training in Tropical Diseases (TDR) to undertake genome sequencing of tsetse flies, as it was recognized that a fully sequenced genome would make a significant contribution to current and future vector control and to regeneration of a supporting scientific base. It is anticipated that the annotated full genome sequence will be available by the end of 2013.

This project has already made it possible to sequence and analyse > 80 000 expressed sequence tags from various tissues (midgut, salivary glands, body fat) of *G. morsitans* and *G. palpalis.* Comparison of these sequences with those of other organisms resulted in the identification of many new tsetse genes, most of which match *Drosophila* genes. It is anticipated that growing knowledge of such genes and their products, especially the putative immunity genes and those involved in physiological pathways that might be targeted for control, will help to understand better tsetse–trypanosome interactions and to find new control methods involving genetic manipulation of the tsetse fly or its symbionts.

4.10 Tsetse flies as cyclical vectors

As tsetse flies are the only cyclical vectors of African trypanosomes, their involvement in the epidemiology of human trypanosomiasis in Africa is obvious. Vertical transmission of *T. b. gambiense* has also been reported since 1933 (reviewed in reference *52*).

Cyclical transmission of trypanosomes by tsetse flies is a highly complex phenomenon, in particular for *T. b. gambiense* and *T. b. rhodesiense*, with a developmental cycle in the tsetse of about 25 days. Many factors influence the susceptibility of the flies to trypanosome infection, including long co-evolution between the tsetse, the trypanosomes and the symbionts found in tsetse. Several symbionts occur in every tsetse species, which may provide opportunities to reduce the vector competence of tsetse (see reference *53* for a review). Other bacteria have been identified in tsetse midgut that could play an additional role in tsetse–trypanosome interactions (*54*). Understanding of these factors and their mechanisms is still rudimentary and will not be discussed here; recent reviews on the subject have been published elsewhere (see reference *55*). Although several models have been designed to elucidate *T. brucei* transmission in the field (e.g. *56, 57*), two aspects of the cyclical transmission of sleeping sickness remain poorly understood:

– The rate of infection of mature tsetse in the field (i.e. flies that harbour trypanosomes in their salivary glands and can thus transmit them) with *T. b. gambiense* and *T. b. rhodesiense* is generally between 1/100 and 1/1000, even with use of the most sensitive tools for detecting trypanosomes (see reference *58* for *T. b. gambiense* and *59* for *T. b. rhodesiense*) and even in the most active foci of sleeping sickness. This suggests that these trypanosomes (at least *T. b. gambiense*) would rarely be transmitted to a human host if additional evolutive mechanisms, such as vector manipulation (e.g. *60*), did not exist. This certainly explains why none of the models of *T. b. gambiense* transmission, some of which are biologically robust (*56, 57*), has provided a satisfactory explanation of transmission.

– The focal nature of gambiense HAT has also never been clarified and thus has not been taken into account in any of the mathematical models. Thus, every place in which the disease occurs has quite clear spatial limits, beyond which the disease does not occur. This limited space is called a "focus"; however, why such foci exist has not been explained and has been poorly investigated.

4.11 Vector control strategies

Transmission of trypanosomiasis requires three interacting organisms: the mammalian host, human or animal, who are the victims of the disease but can also act as reservoirs of parasites; the insect vector and its symbionts, which transmits the parasites and also acts as a reservoir, as it remains infected throughout life; and the pathogenic parasite, the trypanosome, which is the etiological agent of trypanosomiasis. *Glossina* are responsible for linking the parasite and the mammalian host, and any reduction in their number should significantly reduce transmission and hence contribute to both the implementation and the sustainability of control. Vector control strategies are thus an excellent adjunct to case detection and treatment for both rhodesiense and gambiense HAT, because (i) reducing vector density can rapidly reduce transmission of trypanosomes and (ii) techniques for vector control exist. Vector control remains the only available strategy capable of protecting humans from acquiring infection and is an important complement to case detection and chemotherapy, which are the main strategies for HAT control.

Several strategies and methods are availabe to control or eradicate tsetse flies, although a distinction must be made between the two objectives, and proper baseline data must be acquired and preliminary surveys undertaken.

4.11.1 Control

The aim of control operations is to lower the density of tsetse flies in order to lower the risk for transmission to an acceptable level. Interventions have been used to control both African animal trypanosomosis and some foci of HAT.

4.11.2 Eradication

The aim of eradication is to remove tsetse flies totally and definitively from a given area, whatever its geographical scale. To be successful, eradication must follow the principles of area-wide integrated pest management, which involves integration of several tactics to control an entire pest population in a delimited geographical area. The theoretical basis of this strategy, as defined by Knipling (*61*), is: "the uniform suppressive pressure applied against the total population of the pest for a period of generations will achieve greater suppression than a higher level of suppression on most, but not all, of the population each generation." Thus, to avoid reinvasion, the targeted population must be isolated, or isolable, by use of natural or artificial barriers (the latter including, for instance, impregnated traps and targets). Theoretically, eradication should save more money than control operations because it is undertaken only once (if successful), whereas control operations must be repeated. The question of whether tsetse flies should be controlled or eradicated and, if so, how, has been debated for nearly a century and is beyond the scope of this section.

The literature is full of eradication efforts that failed because of inadequate methods or unsustainability due to reinvasion. Furthermore, proper cost–benefit analyses must be undertaken, and due consideration must be given to the inevitable consequences for land use in considering fly eradication and control.

4.11.3 Preliminary surveys

Preliminary surveys are obligatory in any control operation, irrespective of its size and the procedures adopted. In general, surveys are conducted in the dry season the year before the control phase. Their role is to determine the current spatial distribution of tsetse, identify the tsetse species and their densities and locate more intense tsetse–human contact points. Mapping the distribution of tsetse also makes it possible to plan the exact position of trap barriers and indicates potential natural barriers that could isolate certain populations. The accuracy of the preliminary survey is of paramount importance, because any mistakes could reduce the impact of the intervention. In eradication programmes conducted according to the principles of area-wide integrated pest management, a population genetic study to measure gene flow (or to demonstrate its absence) must be undertaken to ensure that the target population is isolated or can be isolated. Eradication is usually achieved in a phased approach, in which target populations are initially reduced to a minimal level (suppression), followed by interventions such as the sterile insect technique used in Zanzibar (*1*) and the sequential aerosol technique used in the Okavango delta of Botswana (*15, 62*) to eliminate the residual populations.

4.12 Tsetse fly control methods

The days when tsetse control specialists advocated a single method are gone, and use of several methods in an integrated disease and vector management strategy is recommended. An integrated approach is preferable because it exploits all the weaknesses in fly behaviour and enables synergy of the methods that increase the intensity of control. The main issues in determining the choice of a control method are technical suitability, cost and environmental impact.

4.12.1 Bush clearing

Clearing vegetation, which tsetse use for resting and breeding, is the oldest way of reducing their populations quickly (*63*). After bush clearing, the hosts of the flies also move, thus contributing to the reduction in tsetse populations. When the vegetation reappears, however, the flies reinvade quickly. Discriminative clearing was used in Nigeria and Ghana to eradicate tsetse flies from rivers and streams. Large-scale bush clearing is not practised now because of environmental concerns, although it is commonly practised by local farmers.

4.12.2 Elimination of wild animal hosts

As both sexes of tsetse feed exclusively on vertebrate blood and as there is no diapause in fly life cycles, elimination of the wild animals on which flies feed can reduce or eradicate fly populations rapidly. A good example of game destruction was the Shinyanga experiment in the United Republic of Tanzania, in which large numbers of wild animals were shot over a 1000 km^2 region, resulting in a simultaneous decline in catches of tsetse (*64*). The method was later refined to eliminate only favoured host species known to form a significant proportion of the flies' diet. For example, it was shown that in many areas of Zimbabwe *G. m. morsitans* and *G. pallidipes* took over 75% of their blood-meals from just four mammals—warthog, bushpig, bushbuck and kudu—and elimination of these animals could significantly reduce fly catches, as was undertaken in Nagupande in Zimbabwe between 1960 and 1963 (*65*). Indiscriminate killing of animals was also undertaken in western Kenya and south-east Uganda until almost no animals were left. The days when game animals were slaughtered and huge areas of woodland destroyed are long gone because of environmental concerns. Moreover, as game recovers, so do fly populations.

4.12.3 Biological control of tsetse flies

Biological control, in which one organism is used to kill another, is not practised to reduce tsetse populations, as no specific predators or parasites for the adults or pupae are known.

4.12.4 Autonomous control of tsetse flies

Tsetse populations are subject to continuous demographic pressure and to an ever-widening human impact on the environment, which reduce the number of flies. Examples of such pressures include increasing human populations and economic growth, expansion of agriculture, expansion of roads and trade networks, deforestation, hunting and fragmentation of tsetse habitats (*8, 27*).

4.12.5 Indigenous tsetse fly control practices

Farmers and pastoralists have long used methods like fire, smoke and avoidance of grazing to limit contact between tsetse and cattle. Fulani in West Africa and the Masai in Kenya avoid tsetse-infested areas for grazing and graze their animals only during low tsetse activity. Livestock are, however, exposed to tsetse bites when they are taken to infested areas during the dry season in search of pasture and water.

4.12.6 Ground and aerial insecticide spraying

Ground spraying is spraying of tsetse resting and breeding sites by teams on the ground, while aerial spraying is done by fixed-wing aircraft or helicopters.

During the 1950s to early 1970s, ground insecticide spraying was the method of choice for controlling tsetse and was used extensively to reduce fly populations in many countries, including Botswana, Kenya, Nigeria, Somalia, Zambia and Zimbabwe. In Nigeria between 1955 and 1978, approximately 200 000 km^2 of land was cleared of tsetse by ground spraying with 570 tonnes of DDT, 176 tonnes of dieldrin and 77 tonnes of endosulfan (*66*). The area was subsequentially kept tsetse-free by high human population density and settlement in riverine areas. In Zimbabwe, ground spraying was used to clear flies over 40 000 km^2 of the northern fly belts. In both cases, however, the flies reinvaded the cleared areas.

In aerial spraying, the insecticide is applied as an ultra-low-volume spray (aerosol) in a series of five or six cycles at approximately 15-day intervals, known as the "sequential aerosol technique". Treatments are timed to coincide with the emergence of young tsetse, before they give birth to their first larvae, and are repeated until no more flies emerge from the pupae underground. The sequential aerosol technique was used in Botswana between 2001 and 2002 with the objective of eradicating *G. m. centralis* from the Okavango delta (16 000 km^2) by spraying deltamethrin at 0.2–0.3 g/ha at a cost of US$ 270 per km^2 (*15, 62*). In this operation, four crop-spraying aircraft modified with lights for night-flying and with rotary atomizers and a Satloc® guidance system were used in the spraying cycles. The operations thus benefited from a global positioning system (GPS), with computers to guide the aircraft to within a few meters of their target area (*62*). The sequential aerosol technique was used with a similar objective in Angola, Namibia and Zambia and also in national projects of the Pan African Tsetse and Trypanosomiasis Eradication Campaign in Burkina Faso and Ghana (*67*).

Insecticide spraying has many side-effects on non-target organisms, such as reptiles, small mammals, fish, birds and other insects. The sequential aerosol operations being undertaken in southern Africa have, however, been approved by environmental monitoring organizations, as most of the affected organisms recovered rapidly (*68*). So far, all such operations have been undertaken against savannah tsetse, except for the most recent one in Burkina Faso and Ghana, which targeted *G. p. gambiensis* and *G. tachinoides*. The sequential aerosol technique is not, however, a panacaea, as its use depends on the terrain, it being difficult for aircraft to operate in hilly areas. The danger of resistance to the insecticides, although not yet reported for tsetse, also exists.

4.12.7 Protecting zero grazing units by insecticide-impregnated netting

In "zero grazing", families contain livestock in an enclosed, shaded area and carry fodder and water to them instead of letting them wander in the open. Black mosquito netting impregnated with synthetic pyrethroids (a "livestock protective net fence") can be placed at a maximum height of 1.0 m around zero grazing

Table 4.2
Trap types used to capture various species of tsetse fly

Trap type	morsitans				palpalis				fusca	
	G. morsitans	*G. pallidipes*	*G. austeni*	*G. swynnertoni*	*G. p. palpalis*	*G. p. gambiensis*	*G. tachinoides*	*G. fuscipes*	*G. longipennis*	*G. brevipalpis*
Biconical		x			x	x	x	x	x	
Pyramidal					x	x	x	x		
Vavoua					x	x	x	x		
Lancien					x			x		
Epsilon	x	x							x	
F3		x								
H-trap			x							x
Ngu		x							x	
Sticky screen			x							x

units to protect dairy cows from tsetse bites (*69*). The cows attract flies, which are intercepted by the netting, from where they pick up a lethal dose of insecticide. In foci of sleeping sickness, this method can be used around pig-pens, as tsetse are generally strongly attracted to pigs (*70*).

4.12.8 Bait methods

Environmental concerns about spraying large areas with chlorinated hydrocarbons led to a search for simpler, cheaper, less damaging techniques for tsetse control. In particular, there was interest in developing devices that could kill or sterilize tsetse in the field and methods that could be used by local communities. Interest in bait techniques was also raised by the observation that only a few traps per square kilometer could drastically reduce tsetse populations, owing to their low reproductive rate (*71*).

Scientists have been designing traps for catching tsetse for over a century. In the early twentieth century, flies were caught with hand nets or sticky panels fixed to the backs of workers. In the past three decades, however, a variety of visual baits have been developed, based on the finding that host odours can enhance trap catches. Studies of the visual acuity of tsetse contributed to development of traps or simplified "targets" or "screens", in which the right fabric emits the wavelengths to which tsetse are most attracted (*72*). Traps can be used to catch live tsetse, which is useful for sampling flies and also for control. Traps and targets may also be impregnated with insecticides (in general pyrethroids) to increase their efficiency.

The first modern trap was the biconical trap, developed by Challier and Laveissiere in the early 1970s (*73, 74*), and most of the traps being used for tsetse control are based on it. *Annex 6* shows the traps used most commonly to catch tsetse. Basically, for riverine tsetse (palpalis group), the biconical (*73, 74*), pyramidal (*75*), Vavoua (*76*) and Lancien traps (*77*) are preferred. For savannah (morsitans) species, which readily enter traps, the epsilon (*78*), F3 (*78*), H-trap (*79*) and Ngu traps (*80, 81*) are preferred. For the fusca group, the traps used for the morsitans group are commonly used (*Table 4.2*).

The monoconical trap (also referred to as the Lancien trap) was a derivative of the biconical trap, in which the lower cone was replaced by blue streamers hanging vertically from the cone rim. The pyramidal trap was similarly derived from the biconical trap but is much simpler and cheaper to make. Instead of a lower cone or streamers, it has two diagonal blue and black screens. The Vavoua trap was designed for the control of riverine tsetse and can therefore easily be suspended in riparian vegetation.

For savannah tsetse, the F3 trap was developed in Zimbabwe for sampling *G. pallidipes* and *G. morsitans*, while the Ngu trap series was developed specifically for trapping *G. pallidipes*; Ngu traps are also efficient for catching

G. longipennis. The epsilon trap is an alternative to the F3 trap and easier to set up. The Nzi trap (*82*) is a variant of the Ngu trap and is efficient for sampling biting flies, although its efficiency for trapping *G. pallidipes* is no better than that of Ngu traps. The H-trap was developed to catch *G. austeni* and *G. brevipalpis*. Sticky screens (plastic with sticky adhesive) were designed to trap *G. austeni*, as this species is reluctant to enter traps.

The efficiency of traps differs by agro-ecological zone. Furthermore, the optimal attractivity and efficiency of a trap depend on the precise choice of site. Very young flies and females in the late stage of pregnancy are often not found in trap samples, perhaps because newly emerged flies are not as active as older ones and females in the later stages of pregnancy are more sensitive to cues that will lead them to larviposition sites. It is also rare to find fully fed flies in trap samples, as they are less active immediately after a feed.

For morsitans group species, "the biggest is the best", i.e. tsetse captures increase proportionally to the size of the attractive device, whereas tsetse of the palpalis group have different types of response to size. This observation led to the development of smaller targets and screens for *G. fuscipes* and *G. palpalis*, which offer a better cost–effectiveness ratio (*32*). These blue-and-black cloth screens (often known as "targets") impregnated with a biodegradable pyrethroid insecticide such as deltamethrin are simpler and cheaper for tsetse control and are easily transported. Their disadvantage is that the farmer cannot see the result, as flies pick up the insecticide and die elsewhere. The screens are also vulnerable to theft. Electric nets can be placed in front of traps and screens for sampling or catching tsetse that are reluctant to enter. Electric screens are also useful in evaluating traps, odour attractants and tsetse behaviour.

Tsetse traps are made of blue and black cloth because experiments on the responses of tsetse to different colours indicated that royal phthalogen blue was the most attractive (*83, 84*). Black was just as attractive and elicits a better landing response. Hence, the blue is used to attract flies to a trap, and the black encourages them to land on it. As the intention is to lead the flies into the trap and subsequently catch them, the black part of the trap is generally internal to some extent, so that flies landing inside the trap will fly up towards the light, leading them into a holding device or cage (escape response), where they die from heat stress. Most tsetse traps have a netting cone or inverted funnel above the body of the trap, designed to allow light to pass and elicit the escape response. The upper cones are therefore made of white mosquito netting. It is recommended that the blue fabric consist of a polyester–cotton mix that is resistant to general degradation on exposure to the sun and rain, does not fade too quickly and is dyed with the right pthalogen blue dye for minimum ultraviolet reflectance for attracting tsetse. The fabric should also be suitable for impregnation with insecticide in screens or targets.

4.12.9 Olfactory baits (attractants) for tsetse flies

The efficiency of traps and targets or screens can be increased by severalfold if chemicals that attract tsetse to their hosts can be placed in appropriately designed dispensers next to them. Most such compounds (kairomones) have been identified in breath (*85–90*), urine (91–93) and skin secretions of cattle (*94*) and are most effective for savannah tsetse. There is no universal odour-baited trap for all tsetse fly species.

In the breath of cattle, carbon dioxide, acetone, 1-octen-3-ol, 4-methylphenol, 3-*n*-propylphenol and 2-butanone (methyl-ethyl ketone) have been identified as potent attractants for the morsitans group of flies (but are not very effective for *G. m. submorsitans*). For *G. pallidipes*, a combination of the two phenols with acetone is very attractive, while for *G. m. morsitans* a combination of octenol and acetone is effective. Use of aged bovid urine instead of the phenols is preferred, as urine is widely available and free. Carbon dioxide is a good attractant for several tsetse species but is expensive and difficult to dispense under field conditions. Various combinations of the attractants for savannah tsetse have also been tested for the palpalis group; the blend consisting of 4-methylphenol, 1-octen-3-ol, para-cresol and acetone has consistently been the best, increasing captures of *G. palpalis* and *G. tachinoides* by two- to sixfold (*95*). Research is under way to identify attractants from cold-blooded monitor lizards and from domestic pigs, which are the preferred hosts for the riverine group. As the fusca group of flies play a minor role as vectors, little work has been done, other than on *G. brevipalpis*, which are attracted by acetone alone, and *G. longipennis*, which are attracted, by a combination of acetone and bovid urine.

The advantages and disadvantages of odour-baited traps and targets are listed in *Table 4.3*.

Odour-baited targets or traps have been used in many countries and can suppress the tsetse fly population by 99%. While the simplicity of the traps or targets lends them for use by local communities, they are sometimes used on such a small scale that control efforts are bound to be frustrated by reinvasion.

4.12.10 Live baits

Cattle sprayed with insecticide can serve as mobile baits from which flies can pick up the insecticide (*96*). The insecticide may be applied onto the animal as a spray, in dip tanks or as pour-ons (applied in a thin strip along the back or sides of the animals). Pour-ons are effective, however, only if sufficient numbers of animals are treated in relation to the desired level of control; otherwise, the kill rate may be too low. Density, placement and movement of cattle also affect the efficacy of pour-ons (*97, 98*). An advantage of pour-ons is that they can kill ticks. They are, however, expensive, beyond the budget of most poor farmers; furthermore, their widespread use might promote the development of resistance

Table 4.3
Advantages and disadvantages of odour-baited traps and targets

Advantages	Disadvantages
Relatively cheap	Theft, damage by wind, wild animals and fire
Can be used for both monitoring and control	Not sensitive enough to detect very low populations of some tsetse species
Few traps required per km^2 to suppress tsetse populations by > 90%	Efficiency depends on siting
Suitable for community control	May be biased towards certain sections of tsetse population
Provides standardized system of sampling	No universal trap is available; hence, traps and odour baits must be developed for each species.
Can operate over full activity period of the fly	Tracks and spaces must be cleared for their installation and maintenance, resulting in potential soil erosion and access for illegal poachers and hunters.
Can be used for species to which odour of humans is repellent, e.g. *G. pallidipes* and *G. morsitans*	Avoidance behaviour of elephants, antelopes and suids reported in some places
Insecticide is impregnated directly onto cloth and not the habitat.	Some tourists object to their presence in parks.
Can be used to develop effective barrier systems	Must be well constructed and maintained at regular intervals and can thus be labour-intensive in large operations

to insecticides. Dermal absorption of the insecticide after licking by treated animals and effects on other soil arthropods are major problems. It has been shown, however, that application of insecticides to the belly and legs of cattle at 2-week intervals can kill tsetse just as effectively as spraying the whole animal every month (*19*). Less insecticide is used, and the cost of control is considerably reduced. As this technique involves livestock keepers to a far greater extent than other tsetse control techniques used by farmers, such as traps and targets, they regard it as a "private" benefit for their own cattle rather than a general "public" benefit in the tsetse-infested area.

A good example of the use of live baits is the project to "stamp out sleeping sickness" in south-east Uganda with an integrated vector and disease control strategy. About 90% of the cattle population, which are asymptomatic carriers of human infective *T. b. rhodesiense*, are being treated with trypanocidal drugs (such as diminazene aceturate) to remove the parasite from the animal reservoir, while tsetse populations are being significantly reduced by restricted application of insecticides on tsetse feeding sites on cattle (*99*). It is hoped that this integrated approach will minimize sleeping sickness caused by *T. b. rhodesiense* in this part of Uganda. Moreover, restricting the application of insecticide will reduce its environmental impact and is less likely to interfere with the development of natural immunity to tick-borne diseases.

4.12.11 Sterile insect technique

The sterile insect technique may be the method that works best when the density of tsetse is low, whereas most of the methods described above are more efficient when the density is high and are less efficient when the density decreases (*100*). The sterile insect technique is a highly specific method of control in which male tsetse sterilized with gamma radiation are released into a wild tsetse population. Females inseminated by sterile males produce no viable offspring, resulting in a decline in the wild population. This technique relies on the fact that only the first mating is the fecund one. In order for the technique to succeed, wild populations are usually swamped with a large number of sterile males (approximately 10 sterile males to 1 female). In addition, tsetse populations are initially reduced by > 95% by other methods, such as traps, targets, live bait or insecticide spraying, before sterilized males are released. The sterile insect technique can be used in eradication programmes only if the targeted area is isolated; otherwise, reinvasion will occur rapidly. The technique was used successfully to eradicate *G. austeni* on Unguja Island in Zanzibar (*1, 100*) after initial suppression of the fly population with white sticky targets and insecticide-treated cattle (*13, 101*).

The sterile insect technique is thus environmentally safe, as it does not affect non-target species. The production and the logistics of release of sterile insects are, however, complex, and the technique is expensive and complicated to use (*102*). Its feasibility in areas where multiple tsetse species are present is also doubtful.

4.13 New developments and outlook

While much work has been done to develop attractive baits, work to identify potent repellents for tsetse was initiated only recently. Repellents from synthetic sources (*34*) and from un-preferred hosts like waterbuck, which are present in tsetse habitats but rarely fed on, have been identified. In field trials with these repellents, the disease incidence in cattle was reduced by nearly 90%. Repellent compounds are being optimized to significantly reduce biting rates of flies not only on cattle but also on humans. Repellent techniques can be integrated with other control techniques for improved integrated control strategies with less reliance on drugs. Repellents could also be used in developing effective barriers.

Recently, new, smaller targets for *G. fuscipes* and *G. palpalis* have been developed, which offer a better cost–effectiveness ratio (*31, 83*).

The genome resources now available for tsetse flies, their symbionts and the trypanosomes they transmit have dramatically increased the number of lines of scientific enquiry (*43*). Currently, the GeneDB and Vector base repositories contain comprehensive, annotated *Glossina* expressed sequence tag libraries, and annotation of the full tsetse genome is projected to be completed by 2013. This will allow the development of control strategies that directly target the fly or its

ability to transmit parasites. Identification of the genes related to host–parasite interactions is vital for genetically engineering flies that are unable to transmit the trypanosome. The genomics of tsetse fly symbiotic bacteria are also of interest, as the longevity and reproduction of tsetse are severely impaired in the absence of their gut flora (*55, 103, 104*). Two bacteria have been found to modify the vector competence of their host (*Sodalis glossinidius* and *Wigglesworthia glossinidia*), and a third symbiont, *Wolbachia*, can confer mating sterility. Paratransgenic refractory flies like these could be released into natural populations to replace their susceptible counterparts and hence reduce disease transmission. They could also be used immediately in sterile insect release programmes, thus reducing the cost of the projects and increasing the efficacy of application in areas endemic for HAT. Olfactory genes might also be exploited in the development of more potent attractants and repellents.

The Pan African Tsetse and Trypanosomiasis Eradication Campaign, which was established by Decision AHG/156 (XXXVI) of the African Heads of State and Government during the 36th Ordinary Summit of the Organisation of African Unity in Lome, Togo, in July 2000 to eradicate tsetse flies from Africa, has given great impetus to vector control activities and to effective control, elimination or eradication of the threat of tsetse.

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5. The disease

The clinical presentation of HAT depends on the parasite species, the stage of the disease and the host. The signs and symptoms that characterize sleeping sickness are generally the same for both forms of the disease but differ in their frequency, severity and kinetics of appearance. Rhodesiense HAT is usually an acute disease, which progresses to second-stage within a few weeks and to death within 6 months. Gambiense HAT is characterized by a chronic progressive course that is usually fatal if untreated (*1*).

The disease occurs in two stages, the first or haemo-lymphatic stage and the second or meningo-encephalitic stage with invasion of the CNS by the trypanosomes. Neurological signs and symptoms, including sleep disturbances, are characteristic of the second-stage; however, most of the symptoms of the two stages overlap, making distinction between the stages on the basis of clinical features unclear. The distinction therefore relies on analysis of CSF (see section 6 for more details). The clinical signs and symptoms are unspecific, and their frequency varies between individuals and between disease foci; they can thus provide only a hint for diagnosis.

5.1 Gambiense human African trypanosomiasis: clinical signs and symptoms

Gambiense HAT is characterized by a chronic progressive course, usually leading to death if untreated. According to models based on survival analysis, the estimated average duration of this form of HAT is almost 3 years, evenly split between the first and the second-stage (*2*). A trypanosome chancre at the site of the insect bite is observed only exceptionally. Chronic and intermittent fever, headache, pruritus, lymphadenopathy, weakness, asthenia, anaemia and, to a lesser extent, hepato-splenomegaly are the leading signs and symptoms of the first-stage. In this stage, the symptoms may not be severe, and patients often do not seek medical care. Therefore, information and active suspicion of the disease are important; without active case screening, the diagnosis may be delayed. In the second-stage, sleep disturbances and neuro-psychiatric disorders dominate the clinical presentation.

5.1.1 Lymphadenopathy

In gambiense HAT, enlargement of the posterior cervical lymph nodes (Winterbottom sign) is typical. The lymph nodes are firm, mobile, non-suppurate and painless. The incidence of lymphadenopathy is 56–95% (*3*–*6*), depending on the stage of disease (*Table 5.1*). Adenopathy has also been described in the axillar, inguinal and epitrochlear regions.

5.1.2 Fever

Fever is intermittent, with attacks lasting from 1 day to 1 week, separated by intervals of a few days to a month or longer. It appears in synchrony with waves of parasitaemia. Febrile episodes are more pronounced and frequent in the first than in the second-stage (*4*).

5.1.3 Headache

Headache is the most frequent complaint and becomes increasingly severe and persistent as the disease evolves (4).

5.1.4 Pruritus

Pruritus is a subjective complaint, and its frequency increases with the duration and severity of the disease (*4*). In many endemic areas, gambiense HAT and filariasis (*Loa loa, Onchocerca volvulus, Mansonella perstans*) overlap; as coinfections are frequent, they could be responsible for pruritus. Severe, long-lasting pruritus, often with scratch marks and occasionally nodular lesions on the skin, was found in about one third of first-stage patients and in one half of second-stage patients (*3, 5, 7*). Sensory disorders like hyperaesthesia were observed mainly in the second-stage. A distinction between subjective perceptions of hyperaesthesia or paraesthesia and pruritus could not be made in most studies because these medical terms could not be translated into local languages.

5.1.5 Musculoskeletal pain, hepatomegaly and splenomegaly

Musculoskeletal pain and enlargement of the liver and spleen are often observed, although these are nonspecific symptoms and signs of any infection.

5.1.6 Cardiac involvement

Cardiac involvement documented by electrocardiographic alterations is observed in over 50% of patients in first-stage gambiense HAT and increases to 70% in the second-stage (*8*); however, it rarely leads to clinically relevant heart failure (*9, 10*). The most frequent electrocardiographic changes are Q–Tc prolongation, repolarization changes and low voltage. Q–Tc prolongation comprises a risk for fatal arrhythmia, but relevant arrhythmia is documented only rarely in endemic countries because of a lack of technical capacity (*9*). Unpublished observations indicate that a minority of patients die suddenly of unexplained causes; in these cases, arrhythmia might be responsible (*11*). Treatment with corticosteroids has been recommended to suppress inflammatory responses in the conducting system (*11–14*), but this has not been evaluated in a controlled trial.

5.1.7 Gastrointestinal symptoms

Gastroenterological symptoms such as nausea, vomiting, abdominal pain and diarrhoea have not been reported in most studies. They were, however, reported to be significantly more frequent in gambiense HAT patients (12/60) than in healthy controls (3/60) ($p = 0.013$) in a prospective study (*15*). Gastroenterological problems have been described fequently during treatment with melarsoprol, eflornithine and nifurtimox (*16, 17*) and in travellers (*18*). It is possible that gastrointestinal symptoms were not included in most clinical studies and therefore overlooked. Their inclusion in questionnaires for future clinical studies would provide additional information.

5.1.8 Oedema

Facial oedema was observed in 13% of patients in one study (*7*) but has not been studied systematically. Oedema of the lower extremities is much rarer (3–5%) (*7, 9*). The causes of oedema are not clear; they may include malnutrition, disturbed appetite, chronic diarrhoea, heart failure (*9*) or—most likely—a combination of these factors. Ascites was occasionally described.

5.1.9 Sleep disorder

Sleep disorder is the leading symptom in second-stage disease, hence, the name "sleeping sickness". As early as the nineteenth century, it was noted that patients had frequent sleep episodes of short duration, during both the day and the night, although the total length of sleep remained equal to that of healthy people. Lhermitte described the sleeping episodes as follows: "Sleep overcomes the patient in a rapid and brutal way: the patient sleeps during a conversation without finishing the sentence or during a meal with a full mouth, the head sinks to the breast and the sleep is complete. During the first crisis it is possible to awake the patient, but in repeated crisis attempts to awake the patient are fruitless" (*19*). Somnographic studies have demonstrated that the disease causes dysregulation of the circadian rhythm of the sleep–wake cycle and fragmentation of the sleeping pattern rather than the frequently reported "inversion of sleep" (*20*). The structure of sleep, and especially the sequence of rapid-eye-movement sleep and non-rapid-eye-movement sleep, is altered and is characterized by episodes of sleep-onset rapid eye movement (*21, 22*).

5.1.10 Neuropsychiatric symptoms and signs

HAT causes meningoencephalitis involving various parts of the brain. Nonspecific neurological or psychiatric symptoms such as headaches and mood or behavioural changes are commonly found in both the first and the second-stage, but their intensity and persistence increase as the illness evolves (*4*). Once parasites

cross the blood–brain barrier and invade the CNS, the clinical manifestations are partly explained by the predominant location of the brain lesions, e.g. sleep disturbances due to involvement of supraoptic nuclei, extrapyramidal signs due to involvement of the striatum and deep sensory disturbances and hyperpathia due to involvement of the thalamus and related structures. Deep sensory disturbances are one of the characteristics of the disease. Even a small shock provokes intense pain. This feature has been termed Kerandel's sign. Sensory involvement is often described as hyperaesthesia, paraesthesia, anaesthesia or pruritus (*23*).

Disorders of tone and mobility and abnormal movements are common features in advanced disease, reflecting the location of lesions in the diencephalon and superior mesencephalon. Motor weakness, tremor, bradykinesia, slurred speech and walking difficulties are common symptoms and signs. Signs of extrapyramidal disorders sometimes predominate, with Parkinson-like rigidity and paratonia. Abnormal movements can be athetoic or choreic, predominantly involving the distal portions of the upper extremities. Cerebellar involvement can be suspected in patients with ataxia and abnormal gait. Hemiplegia is rare and is usually associated with very advanced stage. Primitive reflexes such as pout and palmo-mental reflexes may be present (*4, 23–25*).

Mental disorders can start early in the course of the illness (first-stage) and may lead to a wrong diagnosis of primary psychiatric illness (*18*). Common presentations are mood disorders with irritability or indifference, aggressive or antisocial behaviour, hyperactive or apathic attitudes, depression and delirium with hallucinations.

In the terminal stage, severe disturbances of consciousness, dementia and epilepsy are present, leading to incontinence, coma, malnutrition, cachexia, bedsores, superimposed bacterial infections (e.g. aspiration pneumonia) and death.

Most of the neuropsychiatric disturbances, including sleep alterations, can be reversed by anti-trypanosomal treatment (*20*). Histopathology also shows a predominance of potentially reversible inflammatory lesions. The improvement observed during hospitalization continues after discharge, although irreversible sequelae of various degrees may occur, especially if the disease was at an advanced stage when diagnosed and treated. The sequelae may be subtle, such as delayed sexual maturity or decreased academic performance, as observed in children with second-stage disease (*26*).

5.1.11 Endocrine disorders

Disorders of the thyroid and adrenocortex comprise hypo- and hyperfunction but rarely require specific treatment (*15*). Decreases in the pituitary gonadotropins (follicle-stimulating hormone and luteinizing hormone) have been reported, as have a decrease of oestradiol in 50% of women and of testosterone in 50% of men (*27*). Amenorrhoea, loss of libido and sexual impotence have also been observed (*27, 28*). The circadian rhythm of secretion of hormones, including prolactin, renin, growth hormone and cortisol, disappears in severe cases (*20*).

Table 5.1
Signs and symptoms of human African trypanosomiasis (HAT) according to stage, disease form and affected population

Sign or symptom		Gambiense HAT		Rhodesiense HAT	
		First-stage	Second-stage	First-stage	Second-stage
Incubation period	Natives	18 months (*2*)	18 months (*2*)	1–3 weeks	A few weeks
	Travellers	75% < 1 month	No data	< 3 weeks	> 4 weeks
Chancre	Natives	< 5% (*3, 5, 6*)	0% (*4, 29*)	5–26% (*30–34*)	0 (*30, 35*)
	Travellers	55.6% (*18*)	33% (*18*)	87.9% (*18*)	75% (*18*)
Trypanosomal rash	Natives	0 (*3, 5, 6*)	0 (*4, 29*)	0 (*31, 32, 34*)	0 (*30, 33*)
	Travellers	22.2% (*18*)	50% (*18*)	24.4% (*18*)	41.7% (*18*)
Fever (≥ 37.5 °C)	Natives	10–20% (*3, 5, 6*)	10–40% (*3–6, 29, 36–38*)	28–90% (*30, 34*)	18–37% (*30, 32, 33, 35*); 72% (*31*)
	Travellers	88.9%; 55.6% > 38.5 °C (*18*)	100%; 50% > 38.5 °C (*18*)	100%; 72.7% > 38.5 °C (*18*)	91.7%; 50% > 38.5 °C (*18*)
Lymphadenopathy	Natives	79–95% (*3, 5, 6*)	56–85% (*3–6, 29*)	21% (*30*)	51–80% (*30–33, 35*)
	Travellers	Generalized 33.3%(*18*) Satellite (to chancre) 22% (*18*)	Generalized 50% (*18*) Satellite (to chancre) 50% (*18*)	Generalized 6.1% (*18*) Satellite (to chancre) 30.3% (*18*)	Generalized 33.3% (*18*) Satellite (to chancre) 16.7% (*18*)
Sleeping disorder	Natives	Somnolence 18% Insomnia 73% (*5*)	Somnolence 29–41% (*4, 29*) Insomnia 25–57% (*4, 5, 67*)	Somnolence 25–33% (*30, 34*)	Somnolence 54–66% Insomnia 28–64% (*30–33, 35*)
	Travellers	Somnolence 0% (*18*) Insomnia 28.6% (*18*)	Somnolence 0% (*18*) Insomnia 16.7% (*18*)	Somnolence 0% (*18*) Insomnia 6.7% (*18*)	Somnolence 16.7% (*18*) Insomnia 8.3% (*18*)
Pruritus	Natives	29–33% (*3, 5, 7*)	17–57% (*3–5, 7, 29*)	0% (*30*)	6–53% (*31, 33, 35*)
	Travellers	22.2% (*18*)	16.7% (*18*)	3% (*18*)	8.3% (*18*)
Headache	Natives	51–80% (*3, 7*)	38–79% (*3–5, 7, 24, 29*)	96% (*34*)	51–80% (*30, 35*)
	Travellers	55.5% (*18*)	50% (*18*)	42.4% (*18*)	66.7% (*18*)
Hepatomegaly	Natives	1–20% (*3, 5, 7*)	7–17% (*5, 29*)	0–40% (*30*)	6–30% (*30, 35*)
	Travellers	22.2% (*18*)	50% (*18*)	15.6% (*18*)	25% (*18*)
Splenomegaly	Natives	9–27% (*3, 5, 7*)	5–19% (*3, 5, 29*)	0–36% (*30*)	16–58% (*30, 35*)
	Travellers	55.6% (*18*)	66.7% (*18*)	30.3% (*18*)	8.3% (*18*)
Tremor	Natives	5% (*6*)	19–21% (*29, 39*)	17–61% (*30*)	16–67% (*30, 35*)
	Travellers	14.3% (*18*)	0 (*18*)	0 (*18*)	16.7% (*18*)
Neurological disorder	Natives	< 20% (*3, 5*)	20–40% (*4, 5*)	< 20% (*30*)	50–58% (*30, 35*)
	Travellers	25% (*18*)	33.3% (*18*)	0% (*18*)	8.3% (*18*)
Psychiatric disorder	Natives	< 10%	25% (*4*)	17% (*32*)	15–22% (*30, 35*)
	Travellers	0% (*18*)	0% (*18*)	3.3% (*18*)	8.3% (*18*)
Kidney impairment	Natives	Rare (*40*)	Rare (*15, 40*)	Unknown (*30*)	Unknown
	Travellers	0 (*18*)	0 (*18*)	85% (*18*)	77.7% (*18*)

5.2 Rhodesiense human African trypanosomiasis: clinical signs and symptoms

Rhodesiense HAT is classically described as an acute disease progressing to a second-stage within a few weeks and death within 6 months (*41*). The clinical presentation is similar to that of gambiense HAT, but trypanosomal chancres are frequently seen as a primary lesion at the site of an infective bite (5–26%) (*30–34*). They appear a few days after the bite of an infected tsetse as erythematous, tender swellings, which later become indurated and may eventually ulcerate; they are often accompanied by satellite lymphadenopathy. Subsequent peripheral desquamation and hyperpigmentation usually occur, and the chancre subsides within 2–3 weeks.

The localization of enlarged lymph nodes tends to be submandibular, axillary and inguinal rather than posterior cervical, and oedema is observed more frequently than in the gambiense form (*31, 42*). Recent descriptions of the clinical presentation, however, show wide variation among foci, for as yet unknown reasons. Host genetics, previous infections with apathogenic trypanosome species (*43, 44*), coinfections or differences in the virulence of parasite strains have all been proposed (*45*).

Whereas fever and headache are the main symptoms in the first-stage in some foci (96%) (*34*), tremor (61%) and somnolence (58%) dominate in others (*30*). Fever is reported less frequently in the second-stage in most but not all studies (18–37%) (*30, 32, 33, 35*). Pruritus, sleeping disorders, reduced consciousness or neurological signs and symptoms such as tremor, abnormal movements or walking disability predominate in some foci. Thyroid dysfunction, adrenal insufficiency and hypogonadism are found more frequently than in gambiense HAT, and myocarditis is more severe and even fatal (*46–48*). Liver involvement with hepatomegaly is usually moderate, but jaundice, hyperbilirubinaemia and ascites have been observed (*49*).

5.3 Specific groups

5.3.1 Children

As vertical transmission of trypanosomes can occur, the neonates of HAT-infected mothers should be screened for HAT (*50*). Most symptoms and signs are seen at similar frequencies in children with first- and second-stage disease, including sleep disturbances. The presence of trypanosomes in cervical lymph nodes is less frequent in preschool children than in older children and adults (*51*). More infants are seen at the second-stage, most likely due to delayed diagnosis and the immaturity of the blood–brain barrier (*52*). In some studies, fever, hepatomegaly, splenomegaly and facial oedema were observed more frequently in children than in adults (*4, 7*).

5.3.2 HIV-coinfected patients

The prevalence of HIV was not higher in HAT patients than in healthy controls in several West and Central African countries, suggesting that HIV infection does not predispose people to an increased risk for HAT (*53–55*). Coinfection with HIV did not influence the clinical presentation of rhodesiense HAT (*35*). One study indicates the possibility that HIV influences treatment outcome in second-stage gambiense HAT: whereas 14 HIV-negative patients treated with melarsoprol left hospital in good health, four HIV-coinfected patients had an unfavourable outcome (*39*); however, these data were collected retrospectively and were not confirmed.

5.3.3 Human African trypanosomiasis in nonendemic countries

The symptomatology of patients from nonendemic countries is markedly different from the usual descriptions of African HAT patients. The onset of the disease is almost invariably acute and of the febrile type, regardless of the species involved (*18, 56*).

Rhodesiense HAT has a short incubation period of less than 3 weeks in travellers. It is an acute, life-threatening disease, with the cardinal symptoms of high fever, headache and a trypanosomal chancre (*57–65*). The incubation period for gambiense HAT in travellers is often < 1 month, but can be as long as 7 years in immigrants (*66*).

Fever is nearly always present in infections by both species and exceeds 38.5 °C in > 50% of cases (*18*). If the pyrexial episodes are left untreated, they become irregular (*56*). A trypanosomal chancre is seen in about 84% of patients with rhodesiense HAT and in 47% of those with gambiense HAT. A trypanosomal rash may appear in 25–35% of cases at any time after the first febrile episode, consisting of non-itching, blotchy, irregular erythematous macules with a diameter of up to 10 cm. A large proportion of the macules develop a central area of normal coloured skin, giving the rash a circinate or serpiginous outline. The rash is evanescent, fading in one place and reappearing in another over a period of several weeks (*56, 67*).

As most travellers in whom the disease has been diagnosed were in the first-stage and had a short duration of disease, sleep disorders and neuropsychiatric findings were not recorded, as they may not have developed at the time of the first clinical assessment. Therefore, the classical sleep disorders and neurological findings of HAT are not a hallmark in travellers, irrespective of the species with which they were infected. Sleep disorders were present in only a minority of cases of rhodesiense HAT and night-time insomnia in 21% of gambiense HAT patients. Apart from tremor and motor deficits, observed in 15% of *T. b. gambiense*-infected travellers, neurological and psychiatric findings were absent.

Headache, lymphadenopathy, hepatomegaly and splenomegaly are seen in about a quarter to half of patients infected with either species. Nonspecific gastrointestinal symptoms, such as nausea, vomiting and diarrhoea, are more prevalent in rhodesiense HAT patients. Interestingly, jaundice has been reported in 28% of *T. b. rhodesiense* infections. Electrocardiographic alterations due to myopericarditis (*68*) and conduction abnormalities such as transient second- and third-degree atrioventricular block (*69*), supraventricular tachycardia and ventricular premature capture (*70*) have been reported. In a few travellers, rhodesiense HAT was complicated by renal failure requiring haemodialysis (*64*), multi-organ failure (*60, 63*), disseminated intravascular coagulopathy (*63*) and coma, even with a fatal outcome (*61, 71*).

Gambiense HAT is the predominant form observed in immigrants (*66*). The clinical presentation is dominated by low-grade fever and neuropsychiatric disorders. As psychiatric symptoms predominate, some HAT patients have even been admitted to psychiatric clinics (*18*). Because of its long incubation period, HAT should be considered even if the patient left an endemic country many years previously.

5.4 Sequelae

The signs and symptoms may require several weeks to months to heal; however, there are few data on long-term sequelae, they are difficult to distinguish from the sequelae of adverse reactions to drugs, and no data are available for the new drug regimens.

In several geographical areas, patients treated for late-stage disease are still advised to observe an up to six-month rest period after treatment that excludes, for example, working in the fields and sexual intercourse. The origin of this unsubstantiated practise is unclear and there is no evidence that it benefits the patient. In addition, keeping it could prevent affected people from seeking diagnosis and treatment (*72*).

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6. Diagnosis

Clinical signs and symptoms, such as prolonged fever, swollen lymph nodes or neurological signs, can raise suspicion of HAT but are not sufficient to start treatment (see section 5 for more details). The classical diagnosis of HAT is therefore laboratory-based and consists of multiple steps (*1*). For the diagnosis of *T. b. gambiense* infection, relatively simple, reliable antibody tests can be used for screening. Identification of suspected *T. b. rhodesiense* infection relies mainly on the presence of suggestive signs or symptoms, as no reliable serological tests are currently available for this form of the disease. As clinical signs and symptoms or serological tests are not 100% specific, parasitological confirmation by demonstration of trypanosomes in body fluids is generally required. Once infection has been confirmed parasitologically, assessment of neurological involvement to determine disease stage by examination of CSF is compulsory for choosing the treatment and to diagnose relapse after treatment.

On the basis of the levels of suspicion described above, cases have been categorized according to the exact status of the individual:

Case definitions for surveillance and reporting of human African trypanosomiasis

Confirmed case: disease in an individual with an epidemiological risk for HAT and in whom trypanosomes have been observed microscopically in one or more body fluids;

Case suspected by serological detection: disease in an individual with an epidemiological risk for HAT in whom anti-trypanosomal antibodies have been detected with a validated serological test but in whom trypanosomes are not observed microscopically in body fluids;

Case suspected by molecular detection: disease in an individual with an epidemiological risk for HAT in whom trypanosome DNA or RNA has been detected in body fluids but in whom trypanosomes are not observed microscopically in body fluids.

As HAT occurs in rural environments in sub-Saharan Africa, only those diagnostic techniques that combine cost-effectiveness, rapidity, simplicity and diagnostic performance should be used in control activities (*2*). Research on new diagnostics is supported by the WHO HAT specimen bank, which provides clinical reference material to research institutions for the development and evaluation of new tests for the diagnosis of HAT (*3*).

6.1 Diagnosis of gambiense human African trypanosomiasis

6.1.1 Antibody detection

Trypanosomes elicit production of high concentrations of specific IgG and IgM antibodies, which can be detected in tests. The sensitivity and specificity of antibody detection tests is determined by the antigen or antigens used. Most tests for detecting antibodies of *T. b. gambiense* contain selected *VSG*s of variable antigen types LiTat 1.3 and 1.5. Despite the abundance of *VSG* genes (see section 3), most patients with *T. b. gambiense* infection appear to carry antibodies in their blood that react with at least one of the two *VSG*s.

For the diagnosis of *T. b. gambiense* infection, a rapid agglutination test is available that can be used in mass screening. Individual rapid diagnostic tests have also been developed, which are more appropriate for passive screening and are under evaluation. Tests based on immunofluorescence and enzyme-linked immunosorbent assays (ELISAs) are more appropriate for laboratory testing. The immune trypanolysis test is considered to be for use in reference laboratories.

Serological tests detect antibodies only 3–4 weeks after infection, which may be one of the reasons for false-negative reactions. Cross-reactivity with other parasitoses may occur, particularly in whole blood or at low serum or plasma dilutions. In some rapid diagnostic tests for HIV and malaria, cross-reactions with HAT blood specimens may occur, decreasing the specificity of those tests (*4, 5*).

(a) Card agglutination test for trypanosomiasis

The card agglutination test for trypanosomiasis (CATT) is a rapid, simple assay for the detection of specific antibodies in patients with gambiense HAT (*6*). Because of its simplicity, reliability and low cost, it is used in all control programmes for serological screening of populations at risk for *T. b. gambiense* infection. The introduction of the CATT for mass population screening has been a major breakthrough, limiting time-consuming parasitological examinations to cases with a positive result in a CATT.

The CATT antigen consists of complete bloodstream forms of *T. b. gambiense* variable antigen type LiTat 1.3. To prepare the antigen, trypanosomes are purified from infected rat blood, fixed, stained with Coomassie blue and freeze-dried. Kits contain the reagent, positive and negative control sera and materials for performing the test on whole blood (capillary tubes, test cards, stirring rods, suction bulbs, syringe and droppers).

For screening, the test is performed on undiluted whole blood (CATT-WB). The test cannot be used with CSF. A heparinized capillary tube is filled with finger-prick blood. For the agglutination reaction, one drop of CATT antigen is

put on a test zone of the card, and one drop of blood is added. The reaction mixture is spread over the test zone, and then the test card is rocked at 60 rpm. The results are read after 5 min. The presence of weak-to-very strong agglutination, visible by the naked eye, is considered positive; the absence of visible agglutination is considered negative.

The CATT test performed on plasma (or serum) dilutions (CATT-P) is more specific than the CATT-WB and is therefore used to reduce the number of false-positive reactions in the CATT-WB, often before parasitological examinations. Twofold dilutions of 1/2, 1/4, 1/8, 1/16 and 1/32 are prepared in CATT buffer in 96-well microplates. The CATT-P test is performed in the same way as the CATT-WB but with 25 µl of plasma dilution. People with a CATT-P positive reaction at a dilution of 1/4 or 1/8 or higher, depending on the diagnostic algorithm used by the national control programme, undergo parasitological examination. This reduces the number of parasitological examinations required and results in a significant gain in time and reduction in costs. The risk remains, however, that HAT in patients with low CATT-P titres will remain undiagnosed. The "cut-off" used should therefore be adapted to the epidemiological situation, and people suspected clinically to have HAT but with a negative CATT or enlarged glands should not be excluded from parasitological examination.

The CATT can be performed on blood-impregnated filter paper if testing must be delayed (*7, 8*). The drawback is reduced sensitivity.

The sensitivity of CATT-WB is about 91%, with a range of 78–99.8%, and negative predictive values as high as 99–100% have been reported in mass population screening (*9–18*). False-negative CATT results may be obtained for patients infected with strains of trypanosomes that do not express the LiTat 1.3 gene, resulting in lower sensitivity of CATT in some endemic areas (*19–21*). False-negative CATT results in the presence of high antibody concentrations may also be found because of prozone (disequilibrium between the relative amounts of antibody and antigen) when undiluted blood, plasma or serum dilutions below 1/4 are tested.

Despite a specificity of about 97%, the positive predictive value of the CATT (*Table 6.1*) remains limited when the test is used for mass screening in populations in which the overall prevalence of gambiense HAT is low (*16, 18, 22, 23*). False-positive results are found for patients with other parasitic diseases, such as malaria and filariasis, or a transient infection with *T. b. brucei*.

The CATT has some practical inconveniences. The reagents must be kept at 4 °C for long-term storage, requiring a cold chain. Vials contain 50 test doses, but, once opened and reconstituted, the reagents can be used only during 1 week when stored between 2 °C and 8 °C or up to 8 h at 37 °C. These inconveniences limit the use of the CATT for passive screening in health centres without a cold chain or attended by only a few suspected clinical cases.

Table 6.1
Positive predictive value (PPV) of the card agglutination test for trypanosomiasis (CATT) on various plasma or serum dilutions according to the prevalence of gambiense human African trypanosomiasis

CATT	Prevalence (%)	PPV (%)	Reference
Dilution ≥ 1:16	1.9	52	*23*
Whole blood	2.5	43	*12*
	10	65	
Dilution ≥ 1:16	1	22	*22*
Whole blood	0.07	4	*16*
Dilution ≥ 1:8	0.07	13	
Dilution ≥ 1:16	0.07	20	
Whole blood	9	61	
Dilution ≥ 1:8	9	67	
Dilution ≥ 1:16	9	75	
Dilution ≥ 1:8	0.1	9.3	*18*
Dilution ≥ 1:8	1	51	
Dilution ≥ 1:8	10	92	

(b) Individual rapid diagnostic tests

Rapid individual lateral flow immunochromatographic tests for serological screening of gambiense HAT have been developed and are being evaluated (*24–26*). Lateral flow tests are available in two formats. In the first, a "naked" test strip is dipped into a tube containing a mixture of blood, serum or plasma and a buffer. The kit contains 20 strips. This test remains to be evaluated further. The second consists of a plastic cassette containing a test strip, with a well to dispense a drop of blood, serum or plasma followed by drops of buffer. After 15 min of incubation, the presence of antibodies is revealed as one or two coloured test lines. The functionality of the test is demonstrated by the appearance of a control line; if the control line is not present, the test should be considered invalid, and the sample should be retested. As the cassette tests are packed individually and can be stored at ambient temperature, they are suitable for passive case detection.

If the CATT cannot be performed for active screening, it can be replaced by these individual rapid diagnostic tests. The kits usually contain all of the material needed to perform the test. Cassette tests can be designed for reading results with portable equipment and wireless data transfer. Rapid diagnostic tests have been evaluated in phase-II trials and show a sensitivity of 89–99% and a specificity of 95–99% (*25, 26*).

(c) Indirect immunofluorescence assays

Immunofluorescence assays have been used successfully in the control of gambiense HAT in Equatorial Guinea (*27*). Serum, plasma, filter paper eluates and CSF can be used (*28–30*).

In the test, slides are coated with whole trypanosomes, a diluted test sample (dilution 1:100–1:200) is allowed to react for 30 min, and remnants are washed off with phosphate-buffered saline. The slide is incubated for 30 min with fluorescently labelled anti-human IgG. After washing, the slides are mounted with glycerol-phosphate-buffered saline and a cover slip and read under a fluorescence microscope (40 x 10).

The advent of standardized, stabilized antigens (*T. b. gambiense* variable antigen type LiTat 1.3 or 1.5) has improved the reliability of the test (*17*). Depending on which antigen is used, the sensitivity of the test with serum is reported to be 75–95% (31–33), and the specificity is > 90% (*33*).

Immunofluorescence reagents are relatively stable at 4 °C, but a fluorescence microscope, large quantities of pure water and electricity are required. Immunofluorescence is therefore suitable mainly for surveillance and laboratory diagnosis. With the introduction of fluorescence microscopes and portable light-emitting diodes (LEDs) (*34–37*), immunofluorescence might become applicable in more remote settings.

(d) Enzyme-linked immunosorbent assays

Numerous indirect ELISAs for sleeping sickness have been described (*38–43*). In these tests, the trypanosome antigen is applied to a microplate, which is blocked with a protein solution (mainly bovine serum albumin or milk powder) before incubation with a diluted sample. The plate is washed, and an anti-human IgG enzyme conjugate is added. After a second wash, the substrate and chromogen solution are applied. In the presence of trypanosome-specific antibodies, a colour reaction occurs.

Serum, plasma, filter paper eluates, saliva and CSF can be tested. Usually, purified antigens, *T. b. gambiense* variable antigen type LiTat 1.3 or 1.5 *VSG* is used instead of crude trypanosome lysates. Use of recombinant or synthetic peptides is being investigated (*44*). Incorporation of an antigen-negative control well for each sample is important, as trypanosomiasis sera tend to stick to the plates.

The various test protocols of ELISAs have a sensitivity of 95–100% and a specificity of 97–100% (*38, 39, 41, 43, 45*).

As for immunofluorescence, the requirements for sophisticated equipment and large volumes of pure water remain drawbacks for field application of the test. Because of their high sensitivity and specificity, ELISAs, like indirect immunofluorescence, can be used for surveillance and laboratory diagnosis or for surveys to estimate the prevalence of HAT in certain regions (*46*).

(e) Immune trypanolysis tests

The immune trypanolysis test for antibody detection involves use of live bloodstream trypanosomes (*T. b. gambiense* variable antigen types LiTat 1.3 and 1.5). Its use is restricted to laboratories with the necessary facilities (liquid nitrogen and laboratory animals) to maintain cloned trypanosome populations.

The test is based on recognition of the *VSG* epitopes on the surface of live trypanosomes by the corresponding antibodies in the sample, resulting in antibody-mediated complement lysis. Currently, the test is performed with plasma or serum, but a protocol for use with whole blood on filter paper exists (*47*).

Immune trypanolysis is a highly specific reaction, and the tests can therefore be used to determine whether any contact with *T. b. gambiense* has taken place (*48*), e.g. for management of suspected cases by serological detection. The test is being evaluated for surveillance of HAT foci, mainly in West Africa, and is used as a reference test for the presence of *T. b. gambiense*-specific antibodies in quality control of serological testing in the field (*49*).

6.1.2 Parasite detection

Demonstration of the presence of parasites provides direct evidence of trypanosome infection and thus a definitive diagnosis of HAT. Most techniques, except for thick blood films, are based on visualization of trypanosomes by their motility. A parasitological diagnosis is made by microscopic examination of lymph node aspirate or blood. CSF is usually examined for disease staging but can be used to diagnose HAT in highly suspected cases when other parasitological examinations are negative.

Parasite detection can be labour-intensive. In practice, therefore, parasitological examination is limited to cases suspected clinically or serologically. Parasite loads are generally low in gambiense HAT infection and may be below the detection limit of the most sensitive parasitological methods. Failure to demonstrate parasites does not therefore exclude infection. Management of cases suspected serologically but which cannot be confirmed parasitologically remains a matter of concern and is discussed in section 8. For the diagnosis of gambiense HAT, it is recommended that concentration techniques that allow examination of larger sample volumes be used. Meta-analyses of studies of the diagnostic sensitivity of some parasite detection techniques have been reported (*18, 50*).

For successful parasitological examination, the time between sampling and examination must be minimal (< 1 h) to avoid immobilization and lysis of trypanosomes. If, for any reason, the examination must be delayed, trypanosomes survive longer when the sample is kept cool (4–8 °C). Special attention should be paid to proper maintenance of the equipment, in particular the microscope. For fresh preparations e.g. the lymph node aspirate, the micro-haematocrit

centrifugation technique (mHCT) or the mini-anion exchange centrifugation technique (mAECT) (see sections a, d and f, below), the microscope must be adjusted for maximum contrast by lowering the condenser and dimming the light.

The most sensitive techniques should be used for parasite detection. For gambiense HAT, these are direct examination of a lymph node aspirate, examination of blood by concentration techniques (preferably the mAECT on buffy coat) and examination of CSF by modified single centrifugation.

(a) Lymph node aspirates

When enlarged cervical lymph nodes are present, they are punctured, and fresh aspirate is expelled onto a microscope slide. A cover slip is used to spread the sample, and the fresh preparation is examined microscopically at a magnification of 40 x 10 for the presence of motile trypanosomes. Because of its simplicity and low cost, this technique is widely used. Its sensitivity is about 59% (43–77%) but depends on the proportion of HAT cases with enlarged cervical nodes, which can vary by focus (*51*).

(b) Thick blood films

When a centrifuge is not available, the thick blood film is the technique of choice for examining blood. It is simple to perform and cheap. Not only trypanosomes but also other parasites, such as microfilaria and *Plasmodium*, can be detected.

For preparation of a thick blood film, a small drop (about 20 µl) of finger-prick blood is spread over an area of 1 cm^2 on a microscope slide and defibrinated. The slide is dried in a horizontal position, protected from direct sunlight, and is stained with Giemsa before microscopic examination at 40 x 10 or 100 x 10 magnification. For a sample to be considered positive, at least four of five morphological characteristics of trypanosomes should be visible: a stained nucleus, stained kinetoplast, a stained flagellum, a cell body or the correct size. The detection limit of thick blood films is, in practice, only 5000–10 000 trypanosomes/ml. As a result, the diagnostic sensitivity is rather low (26–35%) (*52, 53*).

Thick blood film examinations are relatively time-consuming (reading time, 10–15 min per slide). Samples must be prepared properly to avoid artefacts: correct thickness and defibrination, pure, good-quality staining reagents and clean slides. Deformation of trypanosomes is often seen.

(c) Lysis of red blood cells

Lysis of red blood cells with ammonium chloride or a commercial lysis buffer facilitates discrimination of trypanosomes. Preliminary experiments show good detection of trypanosomes in blood with red blood cell lysis followed

by centrifugation and examination by microscopy of a thin or thick smear of the sediment, which is stained with either Giemsa or acridine orange (*37*). The technique should be further evaluated before use in control programmes.

(d) Micro-haematocrit centrifugation technique

The mHCT is also called the capillary tube centrifugation technique or Woo test (*54–56*). Capillary tubes containing anticoagulant are filled to three quarters (about 50 µl) with finger-prick blood. The dry end is sealed with plasticine or by flame, avoiding heating of the blood and killing the trypanosomes. Trypanosomes are concentrated in the same layer as the white blood cells (WBCs), between the plasma and the erythrocytes, by high-speed centrifugation (12 000 *g* for 5 min) in a haematocrit centrifuge. The capillary tubes are mounted in a special holder or between a microscope slide and a coverslip, and the empty space between the glass surfaces is filled with water to reduce diffraction. The capillary tubes are examined at low magnification (10 x 10) for mobile parasites at the junction of the WBC layer and the plasma layer. If available, use of 16x ocular lenses facilitates recognition of trypanosomes.

The detection limit of the mHCT is estimated to be about 500 trypanosomes/ml. To increase its sensitivity, examination of at least four capillary tubes per person is recommended. The mHCT is cheap, moderately time-consuming and has a discreet sensitivity of about 56% (39–80%). All the materials necessary to perform the mHCT are widely available.

The disadvantages of the mHCT include the requirement for a micro-haematocrit centrifuge. Furthermore, some experience in reading the results may be required, as trypanosomes appear very small at this magnification and are found within a rich milieu of WBCs. Moving microfilariae, when present, render observation of trypanosomes particularly difficult.

(e) Quantitative buffy coat test

The quantitative buffy coat test, initially developed for rapid assessment of differential cell counts, has been extended to use in the diagnosis of haemoparasites, including *Plasmodium* and *Trypanosoma* (*57, 58*) and has been used successfully to diagnose sleeping sickness (*58*). It combines the concentration of parasites by centrifugation with fluorescent staining of DNA in the nucleus and the kinetoplast of living trypanosomes by acridine orange.

After high-speed centrifugation of about 70 µl of blood in special capillary tubes coated with ethylenediaminetetraacetic acid (EDTA) and acridine orange and containing a small floating cylinder, mobile trypanosomes are identified by their fluorescent kinetoplast and nucleus between WBCs in the expanded buffy coat. Ultraviolet light is generated by a LED module (*59*) or a "cold light source" connected by a glass fibre to an objective containing the appropriate filter, which

can be mounted on most microscopes.

With a detection limit of < 500 trypanosomes/ml, the quantitative buffy coat test allows detection of HAT in more patients with low parasitaemia than the mHCT. It is reported to be as sensitive (77%, 69–92%) as older models of the mAECT (see below) (*60, 61*). As with the mHCT, detection of trypanosomes in the quantitative buffy coat test becomes difficult when microfilariae are present. Capillary tubes are relatively expensive, and their availability depends on commercial production. An initial investment must be made in a special centrifuge and light source. The relative sophistication and fragility of the material obviates daily transport during active screening sessions. A dark room and some experience are needed to read the results.

(f) Mini-anion-exchange centrifugation technique

At pH 8, blood cells are negatively charged, while trypanosomes remain neutral, so that they can be separated by anion-exchange chromatography (*62, 63*). For mAECT, 350–500 µl of blood are applied onto a column containing diethylaminoethyl cellulose. The blood cells stay on the gel, and the eluant containing the trypanosomes is collected in a tube. Trypanosomes are concentrated at the bottom of the tube by low-speed centrifugation (1000 g for 15 min), and the tip of the tube is examined in a special holder under a microscope (10 x 10 or ideally 10 x 16 magnification) for the presence of trypanosomes (*64*). The mAECT on buffy coat (mAECT-BC) is an adaptation in which 5 ml of blood are centrifuged and 500 µl of the obtained buffy coat are applied to the column (*65*).

The large blood volume used in the mAECT allows detection of fewer than 30 trypanosomes/ml, and fewer than 10 trypanosomes/ml in mAECT-BC, resulting in a high diagnostic sensitivity of 77% (68.8–92.1)% for mAECT and up to 96% for mAECT-BC (*18, 65*). If microfilariae are present, some might migrate through the column, but they disturb the reading less than in the mHCT or the quantitative buffy coat test.

The manipulations required to perform the mAECT are relatively tedious and time-consuming, and a table centrifuge is indispensable. The price of the columns may be prohibitive for large-scale application. Production of mAECTs is laborious and requires continuous quality control. As the market is limited exclusively to diagnosis of HAT, there is no commercial interest, and mAECT production is therefore vulnerable to discontinuation.

6.1.3 Molecular detection

Molecular tests to detect the DNA or RNA of trypanosomes are an interesting surrogate for parasite detection (*66, 67*), especially as they can be performed on stored samples (frozen blood is preferred over blood on filter paper

or in storage buffer). Most such tests are based on enzymatic amplification of a trypanosome-specific DNA or RNA sequence, with visualization of the amplified product. While the *Trypanozoon* group (*T. brucei, T. evansi* and *T. equiperdum*) can be detected with multi-copy DNA targets such as satellite DNA (*68*) and ribosomal RNA genes (*69*), *T. b. gambiense* identification is based on detection of the single-copy *TGSGP* gene (*70*). Because of the lower sensitivity of this test, *Trypanozoon*-specific tests are usually the first choice, and subspecies are identified when appropriate.

For most molecular tests, DNA or RNA from the clinical specimen must be purified to allow the detection of a single trypanosome in 1 ml of blood; however, these tests are too complex to be performed in the field and are restricted to reference centres and research laboratories, where they are often used to confirm an infection and to identify the *Trypanosoma* (sub)species. It is not recommended, however, that therapeutic decisions be solely based on the results of molecular tests, as they are indirect and can only identify suspected cases. Although the analytical specificity of molecular tests is generally high (*66, 71*), false-positive results can occur if contaminating nucleic acids are amplified. To minimize this risk, pre- and post-amplification manipulation should be done in separate laboratory spaces, and negative controls should be used in each test run to identify any contamination. As nucleic acids can be very stable, detection does not guarantee the presence of live trypanosomes, which might obviate accurate staging and assessment of treatment outcome (*69*). Furthermore, most of the current molecular tests are *trypanozoon*-specific and thus cannot exclude a transient infection with non-pathogenic trypanosomes such as *T. b. brucei* (*72*).

(a) Polymerase chain reaction

The PCR produces billions of copies of short target DNA sequence in < 2 h by an enzymatic reaction and thermal cycling. Multiple PCR assays have been developed for detection of the *trypanozoon* subgenus, and a *T. b. gambiense*-specific PCR targets the *TGSGP* gene (*66*). The average diagnostic sensitivity and specificity are 98.7% (95% confidence interval, 94.9–99.7%) and 97.4% (95% confidence interval, 91.7%–99.2%), respectively (*73*), but most PCR assays have been evaluated only in phase-I (development) trials. Generally, amplified DNA is visualized by electrophoresis in agarose gels followed by ethidium bromide staining. A real-time PCR assay has been developed that allows high-throughput *trypanozoon* DNA detection (*74*). Attempts have been made to simplify the detection of amplified DNA with use of a lateral flow test for standardized detection of PCR-amplified *trypanozoon* DNA (*75*).

(b) Loop-mediated isothermal amplification

The loop-mediated isothermal amplification (LAMP) assay allows isothermal

amplification of the target DNA sequence. LAMP tests have been developed for the amplification of *trypanozoon*-specific DNA sequences (*76, 77*) and the *T. b. gambiense*-specific *TGSGP* gene (*78*). A lateral flow dipstick has been developed for simple LAMP read-out (*79*), but the most convenient visualisation of the LAMP product is by colour change with hydroxynaphthol blue (*80*). The *trypanozoon*-specific LAMP has been transformed into a kit containing all the components necessary to perform the assay, which must be further evaluated before it becomes commercially available.

(c) Nucleic acid sequence-based amplification

The *trypanozoon*-specific nucleic acid sequence-based amplification assay detects the 18S ribosomal RNA of the parasite. Both real-time (*81*) and lateral flow (*82*) formats have been developed. The diagnostic sensitivity ranges from 90% to 97% and the specificity from 59% to 99% (*73*). No commercial kit is available.

6.2 Diagnosis of rhodesiense human African trypanosomiasis

6.2.1 Antibody detection

Diagnostic *VSG* genes have not yet been identified for rhodesiense HAT because of the high antigenic variation of this subspecies. No equivalent of the CATT is available for screening for rhodesiense HAT. Immunofluorescence is the most frequently used antibody detection test for serodiagnosis of *T. b. rhodesiense* infection. As for *T. b. gambiense*, indirect immunofluorescence with whole trypanosomes can be applied to serum, filter paper eluates or CSF. The sensitivity with serum for the diagnosis of rhodesiense HAT has been reported to be 71–92% (*30, 83, 84*). ELISAs for detecting antibodies to *T. b. rhodesiense* in patients are usually based on crude trypanosome extracts as antigen, with a risk for nonspecific reactions (*85, 86*). Use of invariant surface glycoproteins, which can be expressed as recombinant proteins, should be further explored (*24*).

6.2.2 Parasite detection

Parasitological diagnosis of *T. b. rhodesiense* infection is done mainly by microscopic examination of chancre aspirate or of blood. Parasite loads vary in this infection but are considered to be much higher than in gambiense HAT, reaching values up to 10 000 trypanosomes/ml. Diagnosis is usually performed with simple techniques for trypanosome detection, mainly thick blood films, although all the techniques described for the parasitological diagnosis of gambiense HAT are applicable and are more sensitive than the techniques described below, which are valid mainly for diagnosing *T. b. rhodesiense* infection and are not recommended for detecting *T. b. gambiense* because of the rare presence of chancre in this form of the disease and their low sensitivity with whole blood.

(a) Chancre aspirates

Microscopic examination of the chancre is the earliest way to diagnose trypanosome infection (*87*), as trypanosomes can be detected in a chancre a few days before they appear in blood. A chancre exudate is examined as a fresh or fixed and Giemsa stained preparation at a magnification of 40 x 10.

This technique is simple and cheap and gives a rapid result; however, it is seldom used, as a chancre sometimes never appears and has often already disappeared by the time of examination.

(b) Wet blood films

In wet blood films, about 5–10 µl of finger-prick blood are applied to a microscope slide and examined at 10 x 40 magnification under a cover slip. Any trypanosomes present are revealed by their movement among red blood cells: the agitation of surrounding erythrocytes often attracts attention.

The detection limit in wet blood films is > 10 000 trypanosomes/ml; thus, the sensitivity is low, but it might be sufficient to detect *T. b. rhodesiense* infections with high parasitaemia. The technique is still used because of its low cost, simplicity and immediate result.

(c) Stained thin blood films

For preparation of a thin blood film, about 5 µl of finger-prick blood are deposited on a slide and spread evenly. The slide is dried and fixed for 2–3 min with methanol, after which it is stained with Giemsa or Field stain. Slides are examined at 40 x 10 or 100 x 10 magnification.

The sensitivity of stained thin blood films is low, similar to that of wet blood films, but they conserve the morphology of trypanosomes better than thick blood films.

6.2.3 Molecular detection

The *trypanozoon*-specific PCR, LAMP and nucleic acid sequence-based amplification tests described for *T. b. gambiense* can be used for the detection of *T. b. rhodesiense* in clinical specimens. PCR (*88, 89*) and LAMP (*90*) assays to identify *T. b. rhodesiense* subspecies have been developed, which are based on the *SRA* gene.

Few data are available on the diagnostic accuracy of *T. b. rhodesiense* molecular tests. For *SRA*-PCR, 96–100% sensitivity and 100% specificity (95% confidence interval, 89–100%) have been reported (*88, 91*). The sensitivity of LAMP performed with blood on FTA® filter paper was reported to be 95% (*92*). PCR oligochromatography has a sensitivity of 81% and a specificity 92%, while those of nucleic acid sequence-based amplification oligochromatography are 84% and 99%, respectively (*93*).

6.3 Disease stage determination

Diagnosis of the stage of disease is a necessary step to complete a diagnosis of HAT and is vital for appropriate treatment. HAT progresses in two stages. Initially, trypanosomes disseminate and proliferate in lymph, blood and other tissues. This haemo-lymphatic period, which is the first or early stage, evolves into a second or meningo-encephalitic stage, in which trypanosomes invade the CNS. Progression into the second-stage occurs after a mean of 300–500 days in gambiense HAT (*94*), while in rhodesiense HAT brain invasion is estimated to take place after 3 weeks to 2 months of infection (*95*). For both forms of the disease, stage is determined by examination of the CSF (*96*). Lumbar puncture for staging is usually performed immediately after parasitological diagnosis of trypanosome infection or when indications of infection are present that justify this relatively invasive intervention (e.g. indicative clinical signs or strong serological suspicion). In the case of *T. b. rhodesiense* infection, staging is, in practice, often performed only after a dose of suramin has been administered, as it is considered that blood parasitaemia should be cleared before a lumbar puncture in order to avoid the risk for introducing the parasite into CSF in cases of traumatic lumbar puncture.

The disease stage is defined from the number of WBCs in the CSF and the presence of trypanosomes (*Table 6.2*). Although determination of the total protein concentration was recommended for staging in the past (*97*), it is now determined only rarely for staging HAT and has little impact on the staging decision. Furthermore, as the total CSF protein concentration is influenced by the high Ig levels in blood, it is already moderately increased in the first disease stage. Only in the case of dysfunction of the blood–CSF barrier, which is relatively rare in HAT, do protein levels become markedly abnormal (*98*).

Table 6.2
Criteria for staging human African trypanosomiasis from cerebrospinal fluid

	White blood cell count	
	0–5 /µl	**≥ 6 /µl**
Trypanosome negative	Haemo-lymphatic First-stage	Meningo-encephalitic Second-stage
Trypanosome positive	Meningo-encephalitic Second-stage	Meningo-encephalitic Second-stage

6.3.1 White blood cell count

Patients with ≤ 5 WBC/µl and no trypanosomes in the CSF are considered to be in the first-stage of the disease; those with > 5 WBC/µl or trypanosomes in the CSF are defined as in the second-stage. Gambiense HAT patients with 6–20 WBC/µl and no trypanosomes in their CSF are probably a mixture of patients with and without CNS involvement (*98*). They have been considered to be in the "early second stage" or "intermediate stage" and treated with pentamidine in order to avoid the toxicity of melarsoprol. Little, contradictory information is available about the effectiveness of increasing the threshold for treatment (*99–101*). Since introduction of nifurtimox–eflornithine combination therapy (NECT) or eflornithine alone as first-line treatment for second-stage gambiense HAT, however, use of pentamidine for patients with 6–20 WBC/µl in their CSF is no longer recommended, and the concept of an intermediate stage has lost its therapeutic significance. There is nonetheless general agreement that patients with > 20 WBC/µl should be included with those in the second-stage only for clinical trials of new drugs (*102*).

The increased WBC count in CSF in the second-stage of HAT consists mainly of B cells (*103*). The total WBC count usually remains < 1000/µl. Sometimes large plasma cells, morular or Mott cells, are observed in the CSF, which are filled with IgM-containing vacuoles. The presence of these cells is considered indicative of HAT (*104, 105*).

A correct CSF WBC count is necesary for patient management, and the cells should therefore be counted with maximal care. Because of the low number of WBC present in normal CSF, CSF should not be diluted even if it contains red blood cells—a haemorrhagic CSF sample. CSF cell counting chambers should contain a volume of at least 0.9 µl. In practice, Fuchs-Rosenthal (3.2 µl) and Neubauer (0.9 µl) counting chambers are most often used. Incorrect mounting of the cover slip or replacement of the original cover slip by an ordinary microscope cover slip are errors that are often made with reusable counting chambers; these lead to an incorrect volume of CSF and thus inaccurate cell counts. Such manipulation errors can be avoided by using disposable plastic counting chambers, which retain a fixed volume of CSF (*106*). The filled counting chamber is placed on the microscope stage, and the counting grid is brought into focus at low magnification (objective 10x, oculars 10x or 15x). Before counting is begun, the cells are allowed to settle for 5 min, during which time trypanosomes can be identified.

For better differentiation between WBC and red blood cells during cell counting, a 40x objective may be used. When the CSF WBC count is < 20/µl, it is recommended that another chamber be filled for a second counting. The accuracy of the procedure is increased by taking the average of the two counts.

6.3.2 Parasite detection in cerebrospinal fluid

The finding of trypanosomes in CSF allows immediate classification of a patient into the second-stage (*Table 6.2*). Direct detection of trypanosomes in a counting chamber is a simple, cheap technique but has low sensitivity. The sensitivity is increased by centrifugation. As modified single centrifugation is more sensitive and easier to perform (*106, 107*), it should be preferred over single and double (*108*) centrifugation of CSF. In modified single centrifugation, about 3.5 ml of CSF are centrifuged immediately (10 min, 1000 g) in a flame-sealed Pasteur pipette or in an mAECT collector tube (*64, 109*). After centrifugation, trypanosomes are trapped in the tip of the tube, where they can be detected directly in the reading chamber of the mAECT.

6.3.3 Other staging markers

A number of alternative markers for second-stage disease have been proposed but require further evaluation. As a consequence of strong intrathecal IgM synthesis, high IgM concentrations are present in the CSF of patients with second-stage disease (*98*), which can be quantified in an agglutination test (*110*). High neopterin concentrations appear to be equally powerful for detecting second-stage HAT (*111*), and a rapid diagnostic test for detecting neopterin is being developed. Increased B-cell numbers in the CSF of patients with second-stage disease have been demonstrated by the binding of their CD-19 marker to anti-CD-19-coated micro-particles (*112*).

6.3.4 Molecular tests

PCR and nucleic acid sequence-based amplification tests have been used with CSF for HAT staging, with a reported sensitivity of 89–100% and a specificity of 14–75% (*69, 82, 113–115*). Molecular tests were not more accurate for diagnosis than CSF centrifugation techniques. Given the low specificity of molecular tests for disease staging, the presence of parasite DNA or RNA in otherwise normal CSF should be interpreted with care.

6.4 Treatment outcome assessment

Initially, follow-up examinations after treatment, including CSF examination, were foreseen every 6 months for up to 24 months after treatment (*97*). The relapse rate for first-stage gambiense HAT observed after pentamidine treatment is, however, < 5% (*101, 116*). However, for second-stage gambiense HAT, since NECT is used as the first-line treatment in about 98% of patients, the relapse rate is < 2% (*117, 118*). In practice, compliance with follow-up is low, and patients seldom return spontaneously after their first follow-up visit, especially if they remain asymptomatic (*119*). Systematic follow-up therefore remains rare in

routine practice. Among patients treated for rhodesiense HAT, compliance with follow-up is at least as low as that for gambiense HAT (*120*); because of the acute character of the disease, symptoms may reappear soon in relapsing patients. In view of these limitations and the fact that symptomatic patients do present themselves for follow-up examinations, systematic follow-up after treatment for HAT can no longer be recommended. At the end of treatment, patients should therefore be encouraged to present themselves when clinical symptoms of HAT do appear. Follow-up, including CSF examination, should focus on symptomatic patients. These recommendations apply only for routine treatment outcome assessment and not for clinical trials of new drugs or new treatment regimens.

Although reoccurrence of symptoms may be indicative, detection of trypanosomes remains the absolute proof of relapse. As relapses occur mainly after second-stage disease, use of modified single centrifugation of CSF (see above) for follow-up is recommended, although parasitological examination of the blood should not be neglected. In the absence of trypanosomes, the evolution of the WBC count in CSF can be used as an indicator of treatment outcome.

For patients treated for second-stage HAT who present for follow-up, treatment outcome is assessed on the basis of a two-step algorithm proposed for gambiense HAT (*121, 122*). Six months after treatment, patients with a CSF WBC count ≤ 5/μl in the absence of trypanosomes are considered to be cured, while those with cell counts ≥ 50/μl or the presence of trypanosomes at 6 months are classified as in relapse. Patients with cell counts of 6–49/μl at 6 months should be followed up at 12 months or treated at the discretion of the clinician, taking into account clinical presentation and differential diagnoses. From 1 year after treatment, a WBC count of 20/μl can be used to decide whether a patient is cured or requires retreatment.

There is little evidence for use of WBC counts in the absence of trypanosomes in blood or CSF in assessing treatment outcome for follow-up of patients treated for first-stage HAT. These patients usually relapse with increased WBC counts in their CSF, rarely with detectable trypanosomes (*123, 124*). As a CSF WBC count > 20/μl is indicative of neurological involvement (*98*), patients treated for first-stage HAT with > 20 CSF WBC/μl during follow-up should be considered as in relapse and be treated. Those with 6–20 WBC/μl should be considered to be in uncertain evolution and should be followed up at 12 months or be treated, at the discretion of the clinician, on the basis of clinical presentation and differential diagnoses. Patients treated for first-stage gambiense HAT may relapse late: in a trial of a pentamidine–suramin combination, the interval between initial treatment and diagnosis of relapse was 5–52 months, with a mean of 16.2 months (*124*). This finding is in line with those of other studies, in which the median time to relapse among patients treated for first-stage gambiense HAT was 12–17.5 months (*102*).

The WBC count 3 months after treatment (0–4 months) does not provide reliable information on treatment outcome and should not be interpreted. Diagnosis of relapse at that time should be based on parasite detection only.

As there are no data on rhodesiense HAT, the criteria for assessing treatment outcome are based on those for gambiense HAT.

Table 6.3 shows the criteria for assessing treatment outcome on the basis of biological findings. In addition, the clinician must evaluate the clinical condition of patients. If there is marked deterioration in the clinical condition that is unlikely to be due to a disease other than HAT, the clinician might suggest rescue treatment, regardless of favourable biological findings. The use of alternative markers, such as CSF neopterin (*125*), might also facilitate follow-up but should be investigated further. Although the CSF IgM concentration is

Table 6.3

Criteria for assessing the outcome of treatment for human African trypanosomiasis in patients treated with drugs of known efficacy who present for follow-up

	Stage before initial treatment	
Time of follow-up[a]	**First-stage (adapted from reference *102*)**	**Second-stage (references *121* and *122*)**
3 months (0–4)	• T+: Relapse. Treat.	• T+: Relapse. Treat.
6 months (5–9)	• 0–5 CSF–WBC/µl, T–: Cure • 6–20 CSF–WBC/µl, T–: Uncertain evolution Follow–up at 12 months or treatment at the discretion of the clinician, taking into account clinical presentation • > 20 CSF–WBC/µl, T–: Relapse. Treat • T+: Relapse. Treat.	• 0–5 CSF–WBC/µl, T–: Cure • 6–49 CSF–WBC/µl, T–: Uncertain evolution Follow-up at 12 months or treatment at the discretion of the clinician, taking into account clinical presentation • ≥ 50 CSF–WBC/µl, T–: Relapse. Treat. • T+: Relapse. Treat.
≥ 12 months (10– …)	• 0–5 CSF–WBC/µl, T–: Cure • 6–20 CSF–WBC/µl, T–: Uncertain evolution Further follow-up or treatment at the discretion of the clinician, taking into account clinical presentation • > 20 CSF–WBC/µl, T–: Relapse. Treat. • T+: Relapse. Treat.	• 0–20 CSF–WBC/µl, T–: Cure • > 20 CSF–WBC/µl, T–: Relapse. Treat. • T+: Relapse. Treat.

[a](0–4), (5–9) and (10–…) represent time windows for assigning patient follow-up data to the times of 3, 6 and ≥ 12 months, respectively, after treatment (*102*).

T + or T- , presence or absence of trypanosomes in blood, lymph or cerebrospinal fluid; CSF–WBC, white blood cell count in cerebrospinal fluid.

valuable for staging, it returns to normal only slowly during follow-up and is therefore poorly suited for assessing treatment outcome (*121*). The detection of antibodies in blood should not be used for follow-up, as they can persist for up to 5 years after cure (*28, 126*). In addition, it has been shown that a CATT-negative result does not exclude treatment failure (*127*). The PCR test is not recommended for post-treatment follow-up because of its low sensitivity and specificity (*69*). LAMP and nucleic acid sequence-based amplification remain to be evaluated for use as tests of cure.

6.5 Quality control of diagnostic testing

Digital recording allows quality control of test results that cannot be kept for delayed reading. For example, the result of a serological test like the CATT or a rapid diagnostic test can easily be digitalized with a mobile phone camera or with portable readers designed to capture images. With a mobile phone or a digital camera, it is also possible to capture still images and movie clips of trypanosomes detected under the microscope in a non-fixed preparation, e.g. mHCT or mAECT, or in stained preparations.

The storage of specimens allows repeated testing or testing with more sophisticated tests in a reference laboratory. For serological tests, plasma or serum that is frozen as soon as possible after preparation and that remains frozen until it is re-tested is excellent. Less optimal but still useful are dried blood spots on filter paper, preferably plain cellulose filters. The impregnated filters must be allowed to dry as much as possible and packed in sealed plastic bags with a desiccant like silica gel. Under these conditions, the filters can be shipped and stored for months at ambient temperature (*Annex 7*). Filters with dried blood spots are also convenient for molecular testing by PCR, although loss of sensitivity has been observed. It is better to freeze blood samples and keep them frozen at least at –20 °C until extraction of nucleic acid (RNA or DNA), or to mix one volume of blood (e.g. 500 µl) with the same volume of a commercially available stabilizing buffer so that it can be stored for months at ambient temperature before DNA extraction (*Annex 8*). Alternatively, a home-made guanidine hydrochloride–EDTA buffer can be used to stabilize blood for long-term storage at 4–8 °C (*Annexes 9 and 10*).

6.6 New developments and outlook

Important progress in the field of HAT diagnosis, mainly for the gambiense form, has been made in the past years. The availability of individual rapid diagnostic tests has been a major step forwards in answering the needs of increased integration of HAT control into the health system. Additional studies on the performance, cost-effectiveness and ease of implementation of these rapid diagnostic tests are being conducted (*26, 128*) to better understand their optimal implementation in different settings. In order to further avoid nonspecific reactions and to

standardize and facilitate the test production process, the possibility of replacing the native proteins used as antigens in these tests by recombinant proteins or synthetic peptides is being investigated (*24, 129*). The development of the LAMP assay represents important progress and, if the format is simplified further, could bring molecular diagnostics closer to the district or reference hospital level (*13, 130*). Studies are ongoing to determine the diagnostic accuracy of LAMP in clinical samples, and its impact and cost-efficacy relative to the standard parasitological tests in order to support its implementation (*26, 67*). For second-stage gambiense HAT, the newly discovered CSF marker neopterin was shown to be highly accurate for disease staging and treatment outcome assessment (*111, 125*), but its role in patient management remains to be established. Development of a rapid diagnostic test for the detection of neopterin in CSF for staging of gambiense HAT is ongoing (*125*) and will facilitate its application in a clinical setting.

Discovery research is being undertaken to tackle the diagnostic challenges that have remained. So far, relatively little progress has been made in the screening of rhodesiense HAT. However, suitable antigens for antibody detection and plasma biomarkers for *T. b. rhodesiense* infection are being searched for (*24, 131, 132*). If successful, these may allow development of rapid diagnostic tests for rhodesiense HAT as well. For both disease forms, stage determination and treatment outcome assessment still require CSF examination. Similar to biomarker research in CSF (*133*), discovery research on blood or urine biomarkers for staging and treatment outcome assessment has been initiated. If sufficiently accurate, such newly discovered non-invasive biomarkers might eliminate the need to perform a lumbar puncture. A new generation of molecular diagnostics is being developed based on the detection of specific RNA sequences in the trypanosome. RNA is considered to be a better marker for live trypanosomes than DNA. Detection of the trypanosome's RNA may thus provide greater potential as a test of cure (*67*). With decreasing HAT prevalence, the relative number of suspected cases by serological detection will increase and will constitute an increasing diagnostic problem in the field. Transient infection with non-pathogenic trypanosomes might be a possible cause of positivity in antibody and DNA detection tests. RNA detection might therefore also offer a solution for this problem, as well as new biomarkers specifically for *T. b. gambiense* contact.

Finally, the ability to translate successful discovery research into a sensitive, specific, simple, rapid, robust, affordable and field-adapted diagnostic test will be of crucial importance for its implementation.

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7. Treatment

Because of the historically strategic importance of sleeping sickness, research on the cause and treatment of the disease was encouraged and sponsored around the beginning of the twentieth century. At that time, drug research was highly innovative and was undertaken by prominent researchers such as Paul Ehrlich (*1*) and Louise Pearce (*2*). After the independence of African countries, interest in the disease dwindled, and economic constraints forced the new authorities to focus on other priorities. Consequently, HAT became one of the most neglected diseases while its prevalence resurged. Trypanosomes served as a highly interesting model organism for basic molecular research, but the drug pipeline remained empty (*3*). The substances developed in the first half of the past century must be considered major developments in the treatment of a formerly 100% fatal disease, but all have major disadvantages: they are either highly toxic (melarsoprol) or are reasonably tolerable but do not sufficiently pass the blood–brain barrier (suramin, pentamidine), and their use is restricted to treatment of first-stage disease. The last shortcoming creates the unique situation in which different drugs must be used for treating first- and second-stage disease and making lumbar puncture necessary in the diagnostic algorithm. First-stage sleeping sickness is still treated with pentamidine (gambiense HAT), developed in the early 1940s, and suramin (rhodesiense HAT), developed in the 1920s. Until recently, the first-line treatment for second-stage disease was melarsoprol, which was first used in 1948.

Treatment is still suboptimal, but substantial achievements have been made during the past decade, and the treatment recommendations require urgent revision.

In the 1980s, an antineoplastic drug, eflornithine, received attention for its antitrypanosomal activity (*4*), and the compound was eventually registered by the United States Food and Drug Administration for this indication in 1990 (*5*). Because its administration is highly complex, however, requiring sophisticated logistics and nursing care, and because of its high price, its use was limited for a long time to emergency interventions by a few nongovernmental organizations. In addition, eflornithine is not active against *T. b. rhodesiense* (*6*), which restricts the treatment options for the second-stage of this form of HAT to melarsoprol. At the same time as eflornithine was introduced, nifurtimox, which had been developed against Chagas disease, was used experimentally mainly to treat cases refractory to melarsoprol. The compound had only limited activity when used alone and it caused significant adverse reactions (*4*).

In the mid-1990s the pharmacokinetics of melarsoprol was finally elucidated (*7*), which allowed the proposal of an abridged regimen for treatment of second-stage sleeping sickness with melarsoprol instead of the variety of

empirically derived, complex, lengthy schedules. The abridged schedule was recommended by the 27th International Scientific Council for Trypanosomiasis Research and Control in 2003 (*8*) as standard treatment for second-stage gambiense HAT. Treatment could be shortened from 25–36 to 10 days, which was a major socioeconomic advantage because of shorter hospitalizations and drug savings. A programme for improved application of melarsoprol (IMPAMEL) included the first large-scale clinical trial on treatment of HAT conducted according to good clinical practice. This trial demonstrated the feasibility of conducting modern clinical trials in resource-limited conditions in sub-Saharan Africa and paved the way for future clinical research in such settings. Nevertheless, the abridged melarsoprol regimen could not be regarded as a breakthrough, as the frequency of the major adverse drug reaction remained stable: encephalopathic syndromes continued to occur in 5–10% of patients treated with melarsoprol and resulted in the death of 10–50% of those in whom encephalopathy developed.

The first exploratory comparison of different treatments, including a drug combination, was carried out in 1998. The standard national schedule for melarsoprol (three series of doses of 3.6 mg/kg for 3 days with 7-day breaks), an adapted form of the abridged 10-day schedule (incremental doses of 0.6 and 1.2 mg/kg followed by 8 x 1.8 mg/kg); nifurtimox alone for 14 days (5 mg/kg orally three times a day); and consecutive 10-day melarsoprol–nifurtimox combination therapy (0.6 mg/kg melarsoprol on day 1, 1.2 mg/kg on day 2 and 1.2 mg/kg/day intravenously, combined with oral 7.5 mg/kg nifurtimox twice a day on days 3–10) were compared for safety and efficacy. The frequency of adverse drug reactions was similar in the four arms; however, the drug combination appeared to be considerably more effective: no relapses were reported, and more patients were considered to be cured (randomized patients minus deaths, relapses and unknown outcome) after 24 months of follow-up (*9*).

Hence, by the end of the past century, moderate progress had been made, but the treatment of sleeping sickness was in peril. The availability of the drugs was threatened by increasing price (pentamidine), halted production (eflornithine) or planned cessation of production (nifurtimox, suramin and melarsoprol) (*10*); in addition, an increasing number of cases in certain foci were refractory to melarsoprol (*11,12*).

In 1999, WHO created the "HAT treatment monitoring and drug resistance network" to ensure the availability and affordability of anti-trypanosomal drugs. The exercise resulted, in 2001, in a contract with two manufacturers of these drugs, which will be continued until at least 2017. In the agreement, the companies have committed themselves to ensure continued manufacture and donation of the essential drugs to treat HAT through WHO and funds to support control of the disease (*13*). The contract boosted the activities launched in the mid-1990s to control the disease by WHO and several nongovernmental organizations, and it allowed gradual replacement of melarsoprol with eflornithine. The use of

eflornithine remained below 20% until 2006, however, when a standardized kit was developed by WHO, simplifying the logistics and leading to a usage rate of 64% in 2009 (*14*).

The agreement between industry and WHO not only significantly improved the access of patients to treatment but also triggered a new era of research. In the 1990s, the arrival of newer compounds against HAT raised the interest of scientists in the possibilities of combination therapy. Large series of combinations of registered and experimental compounds were tested in animals (*15*), and several trials of combinations of eflornithine, melarsoprol and nifurtimox were conducted. In all the trials, the combinations were more effective than any of the drugs alone; however, all the combinations containing melarsoprol caused high frequencies of severe adverse reactions (*9, 16*) and were rapidly abandoned. Eventually, in a multi-centre trial, NECT was compared with standard eflornithine therapy in the Republic of the Congo and the Democratic Republic of the Congo (*17*). NECT reduces the required number of eflornithine infusions from 56 to 14, shortens hospitalization by one third and reduces the total amount of eflornithine required by half (*18*). In view of its favourable results, NECT was included for treatment of second-stage gambiense HAT into WHO's Essential Medicines List in May 2009 (*19*). One year later, the rate of use of NECT was about 88% and that of melarsoprol only 12% for administration in exceptional situations, e.g. areas in which training had not yet been conducted (*14, 20*). Today, there is no place for melarsoprol in the treatment of gambiense HAT, apart from the treatment of relapses.

NECT is a landmark improvement, but the complexity of its application still restricts its use to facilities with specially trained personnel and to second-stage disease. Lumbar puncture for diagnostic staging and long hospitalization are still required, and the targeted integration of HAT treatment into public health structures is still limited.

The situation of treatment of rhodesiense HAT remains far more constrained. The only substantial progress made in the past 60 years was recommendation of the abridged treatment regimen with melarsoprol by the International Scientific Council for Trypanosomiasis Research and Control in 2009 on the basis of the last trial of the IMPAMEL programme (*21, 22*). The number of compounds under research and development is limited, although there are more promising substances in the pipeline than ever before.

A summary of all clinical research on treatment for second-stage HAT therapy is available in a review by the Cochrane Collaboration, "Chemotherapy for second-stage human African trypanosomiasis" (*23*).

7.1 Pharmacology of drugs for treatment of first-stage human African trypanosomiasis

The treatment decision process for HAT has a unique component, in that lumbar puncture must be performed to determine whether the parasites have reached the CNS, established by the presence of trypanosomes or surrogate counting of WBCs. Trypanocidal drugs that do not cross the blood–brain barrier sufficiently to reach adequate levels in the CNS to eliminate trypanosomes in this compartment are limited to use against the first-stage of the disease. The drugs that can cure second-stage disease were for a long time extremely toxic and thus not used against the first-stage owing to the availability of alternatives. Administration of the new first-line treatment for gambiense HAT, NECT, is still complex; hence, treatment of first-stage disease remains restricted to pentamidine and suramin.

7.1.1 Pentamidine

Pentamidine, introduced in 1940, is a synthetic aromatic diamidine with a relative molecular mass of 340 g/mol (base), 533 g/mol (methanesulfonate) or 593 g/mol (isethionate) (*24*). It is chemically related to the antidiabetic drug phenformin. The basis for the recommended dose of pentamidine changed over time from the base to the salt moiety. Thus, there is a significant reduction in the amount of active molecule injected if the isethionate (Pentacarinat®) is given rather than the discontinued methanesulfonate (Lomidine®) (*25*). As the clinical efficacy of pentamidine continues to be excellent, this section covers current practice, rather than recommending formal adaptation.

The pKa of 11.4 indicates that the large majority of the drug is positively charged at physiological pH. Pentamidine is readily soluble in water: 1 g of the isethionate dissolves in 10 ml of water. It is not absorbed orally and is therefore administered either by slow intravenous infusion (*24*) or deep intramuscular injection in the treatment of HAT or under conditions with technical constrainst (*24*).

Several high-performance liquid chromatography (HPLC) methods have been developed for the determination of pentamidine (*26–33*), and a method with micellar electrokinetic chromatography has been described (*34*). Most investigations of the pharmacokinetics of the drug were performed with the dimesylate salt; however, no difference is expected between the salts, and results are considered valid for both forms (*35*).

The maximum plasma levels in patients with gambiense HAT treated with 10 intramuscular injections of pentamidine methanesulfonate were generally reached within 1 h and varied widely (214–6858 ng/ml). The median plasma concentration after the last dose was about five times higher than that after the

first. The median half-lives associated with the first, second and third phases of elimination were 4 min, 6.5 h and 512 h, respectively (*35*).

In a detailed pharmacokinetics study of a single dose, 2-h intravenous infusions of 3.0–4.8 mg/kg of pentamidine isethionate were given to patients with *T. b. gambiense* infection. A rapid distribution phase of 10 min was followed by a slower distribution phase and an elimination phase over weeks to months. The average terminal elimination half-life was 265 h. There was a threefold variation in the clearance and volume of distribution, which may reflect individual differences in metabolism. About 5% of an administered dose of pentamidine was found unchanged in urine (*36*).

Additional information on the pharmacokinetics of pentamidine was obtained by studying patients treated for *Pneumocystis carinii (jirovecii)* pneumonia. In AIDS patients with this disease, the elimination half-life after a first dose of 4 mg/kg was 9.4 h after intramuscular administration and 6.4 h after intravenous administration, the latter route yielding peak plasma concentrations three times higher than intramuscular injection (*37*). In patients who received multiple doses of 3 mg/kg of pentamidine, the drug accumulated, and trough concentrations increased progressively without achieving steady state throughout treatment. The elimination after the first dose followed a three-compartment model, and the terminal half-life was estimated to be 29 h. After the last of an average of 13 daily injections, the mean elimination half-life was prolonged to about 12 days, and pentamidine could still be found 6 weeks after the last application (*38*). The dose dependence of the half-life was corroborated in other work on AIDS patients and after bone-marrow transplantation (*39*).

The multiple-dose pharmacokinetics of pentamidine shows that three injections may be as effective as 7–10 injections (*35, 40*). TDR has initiated several clinical trials to compare the effectiveness and toxicity of a 3-day with a 7-day regimen (4 mg isethionate/kg per day intramuscularly) (register numbers: ISRCTN55042030 and ISRCTN 35617647).

Extensive binding of pentamidine to tissues has been suggested, and the extremely large apparent volumes of distribution of 11 850 l after a single dose (*36*) and 35 000 l after multiple doses (*38*) support this interpretation. Pentamidine is strongly and extensively bound to lysosomes (*41*) and is thought to be deposited in tissues, mostly the kidneys and the liver (*42*). Those observations are consistent with the reported large volume of distribution. The highest drug concentrations were found in the kidneys, followed by the liver and spleen. This finding is of interest as nephrotoxicity is the commonest adverse reaction of the drug (*40*); however, renal clearance accounts for about 2% (*38*) to 12% (*36, 40*) of the plasma clearance, and dose adjustment is not recommended for patients with renal impairment (*37*).

Pentamidine is converted to at least seven primary metabolites by the cytochrome P450-dependent oxygenases in rat liver homogenates and rat liver microsomes (*43*). The two main metabolites, the 2- and 3-pentanol analogues of pentamidine, were found to be conjugated with sulfate or glucuronic acid (*44, 45*). In later work, four additional metabolites were detected in Fischer rat liver microsomes (*32*).

The mode of action of pentamidine is unknown. Mechanisms that may play a role were reviewed by Wang (*46)*. In summary, binding to nucleic acids, disruption of kinetoplast DNA, inhibition of RNA-editing in trypanosomes and inhibition of mRNA trans-splicing may be involved. The drug has also been shown to inhibit trypanosomal S-adenosyl-L-methionine decarboxylase, thus interfering with polyamine biosynthesis (*47*), but other findings suggest that inhibition of this enzyme is not the main mode of action (*48*). Additionally, pentamidine interacts with nucleic acids, thus affecting DNA biosynthesis (*49*). It has also been shown that the drug inhibits the plasma-membrane Ca^{2+}-ATPase of the parasites (*50*). The drug is actively transported into bloodstream forms of *T. b. brucei*, leading to accumulation within trypanosomes (*51*). Resistance can be induced in the laboratory (*48, 52*) and is due primarily to loss of capacity to import pentamidine.

The cure rate is 93–98% and has not decreased for decades (*53*). The stable, very low relapse rate, despite extensive former use of pentamidine in prophylaxis programmes (*54*), may be due to the uptake of the molecule by three different transporters (*55*). A number of treatment failures in rhodesiense sleeping sickness were reported some decades ago (*56, 57*), which led to revocation of the recommendation of pentamidine for this form of the disease. New evidence from recent case series suggests, however, that pentamidine is effective in first-stage disease due to *T. b. rhodesiense* (*58, 59*), and this finding should be followed up.

7.1.2 Suramin

Suramin is a polysulfonated naphthyl urea that is strongly negatively charged at physiological pH. Its relative molecular mass is 1297 g/mol (acid) and 1429 g/mol (sodium salt). The compound is freely soluble in water. It deteriorates in air and must be injected immediately after preparation (*60*). Suramin is poorly absorbed from the intestine and causes intense local irritation when given intramuscularly. It is therefore given by slow intravenous injection in the treatment of trypanosomiasis (*60*) and by continuous infusion in experimental cancer treatment (*61*). Suramin was introduced in 1920 in Germany for the treatment of trypanosomiasis.

Several HPLC (*62–67*) and micellar electrokinetic chromatography methods (*68*) have been developed for determination of suramin in serum. Colorimetric quantification of the drug demonstrated high inter-patient variation

in pharmacokinetics very early (*69*). After a single 1-g dose, suramin was detected in plasma for 5–8 days, and, after a course of five to six doses, it could be detected for 150–250 days.

Suramin has one of the longest half-lives of all drugs used in humans. After a test dose of 200 mg, 1 g of suramin was given intravenously once a week for 5 weeks to patients with HIV/AIDS. The drug accumulated during administration and the concentration then diminished, with a half-life of 44–54 days. The total plasma level remained at > 100 μg/ml for several weeks. The volume of distribution was 38–46 l, and the total clearance was < 0.5 ml/min (*70, 71*). Renal clearance made an essential contribution to removal of the drug from the body. In patients enrolled in a trial for treatment of onchocerciasis, elimination was slow, with a median half-life of 91.8 days and a plasma clearance of 0.1 ml/min (*72*). No recent data are available on its use for trypanosomiasis patients.

A correlation was found between creatinine clearance and suramin renal clearance in one trial, but there was no correlation between creatinine clearance and total suramin clearance. Therefore, modification of the dose for renal or hepatic dysfunction is not recommended at this time (*73*). Drug metabolites have not been identified. About 99.7% of the drug is bound to plasma proteins (e.g. albumin, globulins, fibrinogen), making suramin one of the most extensively bound drugs.

Suramin inhibits numerous enzymes, including L-α-glycerophosphate oxidase (*74, 75*), glycerol-3-phosphate dehydrogenase (*76*), RNA polymerase and kinases (*77*), thymidine kinase (*78*), dihydrofolate reductase (*79*), hyaluronidase, urease, hexokinase, fumarase, trypsin (*80*) and reverse transcriptase (*81*), and the receptor-mediated uptake of low-density lipoprotein by trypanosomes (*82*).

Suramin slowly eliminates parasites from blood and lymph nodes 12–36 h after injection (*77*). As the highly charged molecule does not enter glycosomes, it was speculated that glycolytic enzymes might be inhibited in the cytoplasm, where they are synthesized. These enzymes have an average half-life of 48 h, and interference with their import into glycosomes may gradually decrease the enzyme concentration and result in loss of metabolic capacity (*46*). Suramin is taken up into trypanosomes by receptor-mediated endocytosis as a plasma protein-bound complex binding to the invariant surface glycoprotein ISG75 at the parasite surface (*83*). The accumulation of suramin in trypanosomes was hypothesized to be one of the reasons for the differential toxicity in the host and the parasite. Another factor might be the unusually high isoelectric point values for most of the glycolytic enzymes of *T. b. brucei*. It has not, however, been possible yet to assign a definitive mode of action to the drug.

7.2 Pharmacology of drugs for treatment of second-stage human African trypanosomiasis

7.2.1 Melarsoprol

Until the first half of the twentieth century, second-stage rhodesiense HAT was untreatable, and the introduction of melarsoprol in 1949 was a landmark in patient management. Melarsoprol has saved many lives, but the unacceptably high rate of severe adverse reactions limits its application to second-stage disease. The ambiguous success of the drug has led to descriptions ranging from "the most deadly poison ever used in Africa" to "by far the most active and effective drug providing maximal changes of definitive cure" (*84*). The availability of an alternative, NECT, makes melarsoprol obsolete for first-line treatment of gambiense HAT.

Melarsoprol (Mel B®, Arsobal®) was developed by adding the heavy metal chelator dimercaptopropanol (British anti-Lewisite) to the trivalent arsenic of melarsen oxide. The adduct was reported to be about 100 times less toxic than melarsen oxide but 2.5 times less trypanocidal (*85, 86*). Melarsoprol has a relative molecular mass of 398 g/mol. The pKa is 9.2, and the coefficient of partition between buffer and n-octanol at pH 7.4 is approximately 160 (*87*). The compound is insoluble in all common solvents and is marketed as a 3.6% solution in propylene glycol in 5-ml glass ampoules.

Various methods have been proposed for determining concentrations of melarsoprol. A bioassay (*88, 89*) yields total activity, including that of all active metabolites, whereas HPLC methods are specific for melarsoprol (*90, 91*). Determination of the arsenic moiety by atomic absorption spectroscopy has been described (*7, 87*).

Until the beginning of the 1990s, virtually nothing was known about the pharmacokinetics of melarsoprol. A variety of treatment schedules were used during the first 50 years after the drug's development, in all of which the drug was given in repeated series of three to four injections spaced by 7–10 days. They are empirical adaptations of those originally proposed by Friedheim (*86*), Neujean (*92*) and Dutertre and Labusquière (*93*).

The drug-free intervals between doses were unique in the treatment of infectious diseases. Pepin and Milord (*80*) asked why this should be better than giving lower doses of melarsoprol every day for 2–3 weeks with the same cumulative dosage. The origin of repetitive serial applications is in the treatment schedules of older arsenical drugs, which required administration over a long period and repetition of treatment series because of insufficient penetration of the CSF and drug resistance. Early investigations of achieved drug levels supported the serial use: < 20% of injected arsenic was found in the urine, and none was

detectable after 3 days. It was concluded that melarsoprol might be retained in the body by a saturable process of renal filtration, and it was suggested that no more than three injections be given in one series to prevent accumulation of the drug (*94, 95*). More than 20 years later, it was reported that melarsoprol is eliminated mainly in the faeces of rats and humans, and only marginal amounts are found in the urine. Six days after the last injection of treatment, 85% of administered melarsoprol had been eliminated (*96–98*). These challenging findings, however, did not receive sufficient attention to trigger investigations on adaptation of the treatment schedule.

The maximum serum concentrations of melarsoprol after the fourth injection of a therapy course, determined by biological assay and atomic absorption spectrometry, were 2.5–6 µg/ml, with considerable inter-patient variation. The mean concentration was 220 ng/ml (± 80 ng/ml) 120 h after the last injection. Elimination was biphasic, with a pronounced β phase. The mean terminal elimination half-life of melarsoprol determined by bioassay was in the range of 35 h, the volume of distribution was about 2 l/kg, and total clearance was 1 ml/min per kg (*7*). The concentrations of melarsoprol determined by HPLC (*99*) were, however, much lower than those determined by bioassay, except in samples collected immediately after injection. The average maximal concentration of melarsoprol after 15 min was 0.7 µg/ml when determined by HPLC and 4.9 µg/ml when determined by bioassay. With HPLC, the half-life of melarsoprol was calculated to be < 1 h, and, in samples taken 3 h after administration, no melarsoprol was found. Less than 1% of the drug was found in urine, and none could be detected in plasma or CSF 24 h after the fourth injection. The discrepancy between the results obtained by HPLC and by bioassay led to the suggestion that the drug is transformed into active metabolites (*100*).

CSF levels of the drug are generally very low, in the range of 2% of those in serum. CSF samples are obtained by lumbar puncture, and little information about the compartment is available for humans. The mean CSF levels in 19 patients 24 h after a last drug administration varied from below the limit of determination (9 ng/ml) to 100 ng/ml (*7*). In a study conducted with six infected vervet monkeys, the maximum serum level 15 min after administration of the last dose of melarsoprol was about 3 µg/ml, the calculated mean residence time in serum was 18 h, the volume of distribution was 3.6 l/kg, and the clearance was 3.5 ml/min per kg. In the CSF, the drug levels were generally very low, not exceeding 55 ng/ml, and the half-life of melarsoprol in CSF was about 120 h. Similar estimates were made in earlier investigations: 0.5–5% of simultaneous plasma levels in a bioassay (*101*) and 3.5% in an experimental ELISA (*102, 103*).

The results of those pharmacokinetics investigations were used to simulate possible alternative therapy schemes, leading to the proposal of an abridged, uninterrupted treatment schedule and respective clinical investigations (IMPAMEL) (*104*).

Observation of a substantial difference in the half-life depending on the method (i.e. 35 h by biological assay and 1 h by HPLC) triggered an investigation of melarsoprol metabolites (*100*). Melarsen oxide was the only product detected by HPLC analysis after microsomal incubation. Its short half-life of 3.9 h is due not only to rapid elimination but also to irreversible binding to plasma proteins > 20 kDa. The proteins commonly responsible for drug binding, albumin (66 kDa) and α-acidic glycoprotein (44 kDa), fall into this category. The drug may also bind to antibodies, tissue proteins or red blood cells. Because of the irreversible nature of the binding, the protein–drug product cannot be determined by HPLC, although its activity can be detected by bioassay. The pharmacokinetics calculated after determination of total activity by bioassay, confirmed by atomic absorption spectroscopy, indicated that all the active metabolites contain arsenic. No further metabolites were found by HPLC, but no definitive conclusion could be made about whether melarsen oxide alone or also melarsoprol and/or other, so far unidentified metabolites are bound to proteins (*87*).

Total serum protein binding of 79%, (albumin binding, 79%; α-acidic glycoprotein, 70%) was measured by ultrafiltration (*87*). The situation might be more complicated in vivo, because patients with sleeping sickness often have elevated IgG and IgM levels (*105*), which may result in an increased total protein binding. Additionally, concomitant diseases like malaria, hepatitis or malnutrition (*106*) may modify the plasma concentrations of albumin and α-acidic glycoprotein. Up to 10-fold differences in melarsoprol concentrations were determined by a biological assay at the same times in serum and in CSF (*7*).

The mechanisms of action of melarsoprol are not completely understood. Trypanothione (N^1,N^8-bis(glutathionyl) spermidine), which plays a major role in dithioldisulfide redox balance in trypanosomes, was reported to form an adduct with melarsen oxide. Therefore, this compound has been proposed as the primary target of melarsoprol (*107*). The idea that trypanothione is the sole drug target was, however, contested by Wang (*46*) on the basis of the high K_i value required for this target and a modest decrease in intracellular reduced trypanothione and glutathione. Additionally, melarsen oxide was found inside treated trypanosomes, where it inhibited pyruvate kinase (K_i > 100 μmol/l) (*108*), phosphofructokinase (K_i < 1 μmol/l) and fructose-2,6-biphosphatase (K_i = 2 μmol/l) (*109*). The drug inhibits mammalian glutathione reductase and *T. brucei* trypanothione reductase, with estimated K_i values of 9.6 and 17.2 μmol/l, respectively (*110*). In summary, melarsoprol appears to be a highly indiscriminate inhibitor of a large number of mammalian and trypanosome enzymes that contain dithiols. Wang postulated that the unusual P2 nucleoside transporter unique to trypanosomes (*111, 112*), which was found to be responsible for the uptake of melarsoprol, is the key to the selective toxicity of melarsprol for trypanosomes.

Melarsoprol clears trypanosomes from blood and lymph within 24 h in most patients (*113*). In several foci in Angola, the Democratic Republic of the Congo, South Sudan and Uganda, the rate of treatment failure, however, is up to 30% of those treated. The mechanisms of resistance have been characterized and are described in section 3.

7.2.2 Eflornithine

Eflornithine (α-difluomethlyornithie, difluoromethylornithine) is a white to off-white, odourless, crystalline powder. The formula weight of the base is 182 g/mol, and that of the hydrochloride is 237 g/mol. The pK is unknown (*114*). The compound is freely soluble in water and sparingly soluble in ethanol (*115*). Because of its physico-chemical properties, the drug may be given orally or intravenously. Eflornithine does not bind significantly to plasma proteins (*115*).

Specific HPLC methods have been described for the determination of eflornithine (*116–121*) and specifically for its enantionmers (*122*).

The pharmacokinetics of eflornithine was first studied in a trial in volunteers given intravenous doses of 5–10mg/kg and oral doses of 10–20mg/kg. After oral administration, peak plasma concentrations were reached within 6 h. Elimination followed first-order kinetics, and the mean half-life of all doses was 199 ± 6 min. Renal clearance accounted for 83% of drug elimination. The bioavailability of the 10 mg/kg dose was estimated to be 58% from urinary recovery and 54% from the area under the plasma concentration curve (*123*).

In another study, cancer patients were treated with either pulse injections (duration, 1 h) every 6 h over 96 h, continuous infusion over 96 h or oral administration every 6 h over 96 h (5–10 mg/kg). Plasma levels did not increase further after administration of more that 3.75 g/m^2 (corresponding to 90–120 mg/kg) in dose escalation studies (*124*), indicating non-dose linear kinetics at high doses. The maximally tolerated dose was 3.75 g/m^2 over 4 days.

The CSF:plasma ratio of eflornithine is 0.13–0.51 (*115*). One of the main determinants of successful treatment appears to be the CSF drug level reached during treatment; levels > 50 μmol/l must be reached to achieve consistent clearance of parasites. It was suspected that this concentration is not consistently attained in patients treated at 100 mg/kg per 6 h orally (*125*). Other authors found higher ratios at the end of a 14-day intravenous regimen: 0.91 in adults and 0.58 in children < 12 years of age (*125*). The mean steady-state serum concentration in children was only half that of adults, while their mean CSF concentration was only one third. The differences were hypothesized to be due to greater renal drug clearance in children. In contrast, neither the efficacy nor the safety of the drug was significantly different in a non-randomized comparison of 96 children treated with 150 mg/kg every 6 h (600 mg/kg per day) with 130 children who were treated with the standard dose (*126*).

A major drawback of intravenous eflornithine is that it is inconvenient to administer, especially in understaffed rural hospitals in disease-endemic countries. Therefore, oral administration of the drug was explored, although the major concern was that the levels attained in the CNS might be insufficient (i.e. < 50 µmol/l). A study was carried out on the pharmacokinetics and the relation between clinical response and the plasma pharmacokinetics and concentration in CSF of eflornithine at a dose equivalent to the recommended intravenous dose (100 mg/kg every 6 h for 14 days) in comparison with the oral dose equivalent to the maximal tolerated oral dose of 3.75 g/m^2 (500 mg/kg every 6 h for 14 days). Relapse occurred in 12 of 25 (48%) patients during the 12-month observation period (six in each group). In agreement with earlier publications (*124*), the plasma difluoromethylornithine concentrations did not increase proportionally to the dose when the dose increased from 100 mg/kg to 125 mg/kg every 6 h (60–70% of the expected increase), and the CSF concentrations at steady state varied from 22.3 µmol/l to 64.7 µmol/l (*127*). The authors concluded that oral eflornithine at the dose tested would not provide adequate therapeutic levels in plasma and CSF for patients with second-stage gambiense HAT. The participants in both groups had the known adverse reactions to eflornithine, but all of them also had diarrhoea (unpublished results). Later research revealed another drawback of oral eflornithine. In rat liver, L-eflornithine (L-difluoromethylornithine) was more effective in irreversibly inhibiting ornithine decarboxylase than D-eflornithine (D-difluoromethylornithine) (*128*). The mean bioavailability of L- and D-eflornithine in rats was 41% and 62%, respectively, indicating that eflornithine exhibits enantioselective absorption, the more potent L-isomer being less favoured (*129*).

Ornithine decarboxylase catalyses the conversion of ornithine to putrescine, the first and rate-limiting step in the synthesis of putrescine and of the polyamines spermidine and spermine (*130*). Polyamines are involved in nucleic acid synthesis and contribute to the regulation of protein synthesis. They are essential for the growth and multiplication of all eukaryotic cells (*131*). Eflornithine acts by inhibiting trypanosomal ornithine decarboxylase (*130*), changing it to a dormant state that is vulnerable to the host's immune attack. Trypanosomes are more susceptible to the drug than human cells, possibly because of the slow turnover of this enzyme in *T. b. gambiense* (*130*). The rapid turnover of ornithine decarboxylase is responsible for the innate resistance of *T. b. rhodesiense* to eflornithine (*132*). For more information, see section 3.

7.2.3 Nifurtimox

Nifurtimox is a 5-nitrofuran derivative with a relative molecular mass of 287 g/mol. Its pK is unknown. The substance is slightly soluble in water (*133*). The drug can be administered by the oral route.

Nifurtimox (Lampit®) was introduced in the late 1960s for treatment of Chagas disease (*134*). The drug is not registered for use in sleeping sickness, and its use was restricted to compassionate treatment in combination with other trypanocidal drugs of patients who did not respond to melarsoprol. It is now a component of NECT. Nifurtimox was tested empirically in HAT case series during the 1970s and 1980s, with conflicting results (*16*); the studies differed with regard to treatment regimen and evaluation criteria, making them difficult to compare.

A specific HPLC method has been described for the determination of nifurtimox (*135*). Formerly, thin-layer chromatography was used (*136*).

In healthy human volunteers given a single oral dose of 15mg/kg nifurtimox, average peak plasma levels of 751 ng/ml (range, 356–1093 ng/ml) were reached within 2–3 h. The drug has an apparent volume of distribution of about 755 l (approximately 15 l/kg) and a high apparent clearance of 193 l/h (about 64 ml/min per kg). Nifurtimox is quickly eliminated, with an average plasma elimination half-life of 3 h (range, 2–6 h) (*137*). The bioavailability in humans is unknown, but the drug is almost completely absorbed in experimental animals (*136*).

Nifurtimox has been reported to be extensively metabolized in animals and humans (*135, 136*). It has been suggested that it is biotransformed partially by cytochrome P450 but mostly by nicotinamide adenine dinucleotide phosphate (NADPH) P450 reductase. The formation of reactive metabolites near DNA and nuclear proteins was suggested to be responsible for the long-term adverse reactions caused by the drug (*138*). Experiments with radiolabelled drug in rats showed relatively high concentrations throughout the animals, including brain and spinal cord (*139*). The mechanism of action of nifurtimox is described in section 3.

7.3 First-line treatment

7.3.1 First-stage gambiense human African trypanosomiasis: pentamidine

Today, the schedule used in most national sleeping sickness programmes is daily injections of 4mg/kg of pentamidine isethionate for 7 days. The drug is usually given as a deep intramuscular injection because of the frequent occurrence of hypotension after intravenous administration. If it is given intravenously, bolus injection must be avoided, and infusions should be spread over 60–120 min. Pharmacological evidence and reports of cases in travellers (*58, 59*) indicate that pentamidine is also effective against first-stage rhodesiense HAT.

Pentamidine is generally well tolerated, although minor adverse reactions are common (*49*). The immediate adverse reactions include hypotension in

about 10% of patients, with dizziness and sometimes collapse and shock; after intravenous injection, the frequency of a hypotensive reaction can be as high as 75%. Other adverse reactions occasionally observed are nausea or vomiting and pain at the injection site. Sterile abscesses or necroses may occur at the site of intramuscular injection. The systemic reactions reported include azotaemia due to a nephrotoxic effect, leukopenia, raised liver function enzymes, hypoglycaemia and hyperglycaemia. Persistent manifestation of diabetes is a rare but feared event (*140*). Severe adverse events like anaphylaxis and acute pancreatitis occur extremely rarely. In practice, patients should be given a source of sugar (e.g. a sweet drink or food) before injection to prevent hypoglycaemia and should lie down for at least 1 h after injection to prevent symptomatic hypotension.

7.3.2 First-stage rhodesiense human African trypanosomiasis: suramin

Suramin is effective against the first stage of both forms of the disease. Today, its use is generally restricted to treatment of rhodesiense HAT, because it is active against onchocerciasis (*77*) which is more prevalent in gambiense HAT endemic areas and it can cause severe immunological reactions in co-infected patients. In addition, pentamidine is far more practical to administer.

The most commonly used dosage consists of a test dose of suramin at 4–5mg/kg on day 1, followed by five injections of 20 mg/kg every 7 days (*53*). The maximum dose per injection is 1g. Suramin is injected intravenously after dilution in distilled water. Intramuscular injection is highly irritating.

The adverse reactions of suramin depend on the nutritional status, concomitant illnesses (especially onchocerciasis (*77*)) and the general clinical condition of the patient. Life-threatening events and lethal outcomes have been described, but the drug is still one of the safest in the treatment of HAT. Adverse drug reactions are rare, with the exception of pyrexia and nephrotoxicity, as the drug reaches higher concentrations in the kidneys than in other organs. Nephrotoxicity is usually mild (*141*) and reversible. The first symptoms of renal impairment are albuminuria; cylindruria and haematuria may occur later. Regular urine checks during the course of treatment are therefore strongly advised.

Other adverse effects of suramin occur at frequencies < 5%, although there might be considerable underreporting in Africa (*141, 142*). The effects observed are early hypersensitivity reactions like urticaria and circulatory collapse (justifying the current practice of administering a test dose), late hypersensitivity reactions like exfoliative dermatitis and haemolytic anaemia, peripheral neuropathy and bone-marrow toxicity with agranulocytosis, thrombocytopenia and reactive encephalopathy (*143*).

7.3.3 Second-stage gambiense human African trypanosomiasis: nifurtimox–eflornithine combination therapy

A preliminary trial conducted in Omugo, Uganda, during 2001–2004 was designed to compare three combination treatment regimens: melarsoprol–nifurtimox, melarsoprol–eflornithine and NECT. Equal dosages of the drugs were given in each arm: melarsoprol at 1.8 mg/kg per day intravenously once a day for 10 days; eflornithine at 400 mg/kg per day as a slow intravenous infusion every 6 h for 7 days; and nifurtimox at 15 mg/kg per day for adults or 20 mg/kg per day for children < 15 years orally every 8 h for 10 days. The trial was suspended after enrolment of only 54 patients, for ethical reasons, because of the high fatality rate observed in the melarsoprol–nifurtimox arm and strong contrasts in overall toxicity between arms. An intention-to-treat analysis was performed, which yielded cure rates of 44.4% with melarsoprol–nifurtimox, 78.9% with melarsoprol–eflornithine and 94.1% with NECT. Large differences between the regimens were also observed in the number of fatalities (four with melarsoprol–nifurtimox, one with melarsoprol–eflornithine and none with NECT) and in the number of major adverse events (grades 3 and 4) (18 with melarsoprol–nifurtimox, nine with melarsoprol–eflornithine and five with NECT). The small number of observations obviated a demonstration of significance (*16*).

This trial was followed by a study of a case series of 31 patients treated with NECT at the same regimen in Uganda. All patients were discharged alive, and no relapse was seen during follow-up (*144*).

A multi-country trial of NECT was then carried out to compare standard eflornithine therapy with an abridged regimen of nifurtimox and eflornithine, in order to simplify treatment. Eflornithine has a short half-life, and its pharmacokinetics indicates that four daily doses are required. It was argued, however, that the short half-life of eflornithine might be balanced by the long-lasting pharmacodynamic effect on trypanosomes, due to the long time (18–19 h) required by *T. b. gambiense* to replenish ornithine decarboxylase after inhibition by eflornithine; therefore, two daily doses might be sufficient (*145*). In an exploratory trial, a short, 7-day course of eflornithine alone showed significantly lower efficacy (*146*); however, the trial with standard doses of eflornithine and nifurtimox had resulted in a very low relapse rate (*16*). Therefore, the regimen used in the multi-country NECT trial consisted of 200 mg/kg of eflornithine as an intravenous infusion over 1–2 h every 12 h for 7 days combined with nifurtimox at 5 mg/kg orally every 8 h for 10 days. This regimen reduces the number of infusions from 56 to 14 and the treatment duration from 14 to 10 days (*17, 18, 145*). Among the 286 patients enrolled, the relapse rate after 18 months of follow-up was 5.7% with eflornithine and 1.4% with NECT. Adverse events were frequent in both groups but were mainly mild to moderate; 41 (28.7%) patients in the eflornithine group and 20 (14%) in the NECT group had major (grade 3 or 4)

reactions, which was a significant difference ($p = 0.002$). Neurological events were the main major reactions, with nine cases of seizures in both treatment groups and one case of coma with NECT and three with eflornithine. As expected, gastrointestinal disorders were frequent (106 and 78 cases, respectively), but only two patients in each group had major events. Haematological reactions were significantly less frequent with NECT (21.0%) than with eflornithine (40.6%) ($p = 0.0003$). In particular, neutropenia was less frequent under NECT than under eflornithine (1.4% vs 7%) (*18*). The reduced bone-marrow toxicity, reflected by the lower frequency of neutropenia and anaemia in the NECT group than in the eflornithine group, is consistent with the halved exposure to eflornithine (control, 14 days; NECT, 7 days). The reduced exposure might also explain the lower overall frequency of infections, an effect reinforced by a reduction in the number of catheter-related infections expected with the substantial reduction in the number of infusions. The overall mortality of patients treated with NECT was low (1/143, 0.7%).

NECT has many advantages over eflornithine alone, as it is easier to administer, reduces the amount of drug, staff and logistic resources, requires a significantly shorter hospital stay and may provide some protection against the development of drug resistance. In vew of these advantages and the favourable results of the trials conducted, NECT was included for treatment of second-stage gambiense HAT on the WHO Essential Medicines List in May 2009 (*19*).

In order to obtain additional data on safety and efficacy, a field study and a pharmacovigilance programme were conducted. The field study was designed to assess the effectiveness of NECT under real conditions in a wide range of patients (including pregnant women and children) and to identify the limitations of its use in rural settings. The study involved analysis of 629 patients, including 100 children ≤ 12 years, 33 lactating women and 14 pregnant women. A total of 39 serious adverse events were reported in 32 patients (5.1%), including 10 deaths, resulting in a case fatality rate in hospital of 1.6% (*147*). Gastrointestinal adverse events (e.g. nausea and vomiting) were the most frequent (61%), followed by neurological (all, 34%; convulsions, 9%) and psychiatric (all, 16%; agitation, 6%) adverse events. A pharmacovigilance programme set up by WHO in 2010 showed an overall case fatality rate of 0.5% among 1735 patients treated with NECT in 22 centres in nine endemic countries (*148*). Data from both the field study and the pharmacovigilance programme showed that NECT was better tolerated by children than by adults, with notably fewer neuropsychiatric adverse events.

7.3.4 Second-stage rhodesiense human African trypanosomiasis: melarsoprol

Melarsoprol was used for over 50 years in a wide range of treatment regimens, with administration in repeated series of three to four injections spaced by 7–10 days. None of the schedules proved to have a significant advantage, none was based on

pharmacological evidence, and most were not validated in prospective clinical trials. In 2004, an abridged schedule for treatment of second-stage gambiense HAT with melarsoprol (2.2 mg/kg per day for 10 days) was recommended as the standard schedule (*8, 104, 149*). Owing to the availability of alternatives and the high frequency of adverse drug reactions, use of melarsoprol is restricted to treatment of second-stage rhodesiense HAT and to special situations.

The same abridged schedule was evaluated and recommended for use against *T. b. rhodesiense* infection by the 30th meeting of the International Scientific Council for Trypanosomiasis Research and Control in 2009 (*21, 22*). Among 107 patients treated with the abridged schedule in Uganda and the United Republic of Tanzania, the case fatality rate during treatment was 8.4%, while that among 300 controls who had previously received the standard schedule was 9.4%; 1% of the 98 survivors relapsed during follow-up.

The adverse reactions to melarsoprol may be severe and life-threatening. The most important effect is an encephalopathic syndrome, which occurs in 5–18% of all treated cases and which is fatal in 10–70% of these patients (*53, 80*). Clinically, the symptoms have been defined as either convulsions, rapid deterioration of neurological symptoms and progressive coma or psychotic reactions and abnormal behaviour (*80*). The syndrome has been given various names, indicating that the exact cause is not known. An immune reaction is generally thought to underlie the syndrome (*53, 80, 150*). Concomitant administration of prednisolone had a protective effect against encephalopathy in patients with gambiense HAT in one large-scale study (*151*), but its protective effect in rhodesiense HAT is unknown. Use of pretreatment with suramin is questioned, as it may be associated with a poorer outcome (*22*). Data from the IMPAMEL III trials, however, suggest that concomitant administration of prednisolone (10 mg/kg) reduces the frequency of encephalopathic syndrome (*22*).

Apart from reactive encephalopathy, which is the most feared adverse reaction, pyrexia, headache and general malaise occur frequently during treatment with melarsoprol. Gastrointestinal (nausea, vomiting, diarrhoea) and skin reactions (pruritus) are common; severe complications like exfoliative dermatitis occur in < 1% of cases (*53*). Cardiac failure is common during treatment and can be a frequent cause of death (*152*), but it is still unclear whether this is due to an adverse drug reaction or the well-known cardiac involvement of HAT itself (*153–155*). Other adverse reactions that have been reported occasionally are peripheral motor (palsy) or sensorial (paresthesia) neuropathy, renal dysfunction (proteinuria and hypertension) and hepatotoxicity (elevated liver enzymes, bilirubinaemia) (*142*).

Melarsoprol is soluble only in propylene glycol, which is highly irritating. Therefore, the drug must be given intravenously as a slow bolus injection, with

use of high-quality injection needles and minimal injury of the inner vein wall. Paravasal deposition of the drug must be strictly avoided, as it leads to severe local inflammation and skin necrosis.

7.4 Alternative treatments for second-stage gambiense human African trypanosomiasis

7.4.1 Eflornithine monotherapy

Eflornithine monotherapy at a daily dosage of 400 mg/kg divided into four intravenous infusions for 14 days is an alternative to NECT when nifurtimox is not available or is contraindicated, such as in patients with epilepsy or psychosis. When eflornithine was used alone in new cases, treatment for 14 days was more efficient than treatment for 7 days, with a probability of cure of 97% and 87%, respectively, in a multi-centre study (*146*). The rate of relapse within 24 months of treatment is 5–10%; it was 7.6% in the largest published cohort of 1055 patients treated in South Sudan. Use of a higher dose (600 mg/kg per day) in children < 12 years did not decrease the risk for relapse (*126*). The mode of action requires a sufficiently active immune system to achieve cure (*47, 156*). The case fatality rate during eflornithine treatment is consistently < 2% and is lower than that with melarsoprol (*157, 158*). Administration of four daily infusions for 14 days necessitates good nursing care, including strict aseptic precautions during catheter insertion and fixation, to prevent local (phlebitis) and disseminated (abscess, sepsis) infections. Frequent (> 10%) reported adverse events during treatment are fever, pruritus, hypertension, cough, anorexia, nausea or vomiting, diarrhoea, abdominal pain and headaches, but some of these symptoms may also be caused by HAT or concomitant infections (e.g. malaria). Seizures occur in 4–10% of cases but are generally isolated or respond to treatment; a clinical picture consistent with the encephalopathic syndrome, including prolonged generalized and treatment-resistant epilepsy or coma, occurs in < 1% of patients (*126*). Haematological reactions were more frequent with eflornithine (40.6%) than with NECT (21.0%). In particular, neutropenia was much less frequent under NECT than under eflornithine (1.4% vs 7%) (*18*).

7.4.2 Melarsoprol

The only remaining indication for melarsoprol (2.2 mg/kg per day in slow intravenous injections for 10 days) in the treatment of gambiense HAT is in the treatment of relapse after NECT. The toxicity profile of melarsoprol is described in section 7.3.4.

Table 7.1 summarizes anti-trypanosomal treatment for first- and second-stage HAT.

7.5 Treatment in pregnancy

Few published data are available on the safety of anti-trypanosomal treatment during pregnancy and lactation (*147, 159*). The following recommendations are based on clinical practice rather than on solid evidence. For first-stage gambiense HAT, pentamidine can be given after the first trimester; if the patient has not passed the first trimester, pentamidine should be withheld until the second trimester. For second-stage gambiense HAT, melarsoprol, eflornithine and nifurtimox are all theoretically contraindicated, and the timing of treatment depends on the general condition of the mother. If her general condition allows for watchful waiting, regular (e.g. monthly) clinical assessment is advised. Pentamidine at 4 mg/kg intramuscularly for 7 days should be administered, with the main objective of reducing the risk for vertical transmission. NECT should be administered after delivery. If the general condition of the mother is moderately or severely altered, treatment with eflornithine alone or NECT must be administered with the main objective of saving the mother's life. The benefits and risks must be clearly explained to the patient and her relatives. In view of the acute presentation and rapid clinical evolution of rhodesiense HAT, treatment with suramin (also theoretically contraindicated) for first-stage or melarsoprol for second-stage disease usually cannot be delayed until after delivery. After delivery, the newborn should be examined clinically and checked for the presence of circulating trypanosomes in the blood. Breastfeeding should continue during HAT treatment.

7.6 New developments and outlook

Huge progress was made during the past decade in the methodology of drug target research and whole-cell and high-throughput screening. Target validation and lead selection remain difficult in HAT, but several new targets were identified, and a number of compounds with promising activity were found in primary screening. Currently, only one molecule for the treatment of HAT is in preclinical evaluation, and two are in clinical development. Despite the advancements in HAT treatment in the past decade, all currently available options are suboptimal, and the development of new, safe compounds that are effective against both disease stages and are easy to use is a high priority.

7.6.1 Preclinical and clinical developments

The most advanced of the new drug candidate is fexinidazole, a 2-substituted 5-nitroimidazole that was originally synthesized in the 1970s as part of an

Table 7.1
Treatment for first- and second-stage human African trypanosomiasis (HAT)

Disease and stage	First-line treatment	Dosage	Alternative treatment(s)
Gambiense HAT			
First-stage	Pentamidine isethionate	4 mg/kg per day i.m. or i.v. (diluted in normal saline and given in 2-h infusions) x 7 days	
Second-stage	Nifurtimox + eflornithine combination therapy[a]	Eflornithine: 400 mg/kg per day i.v. in two 2-h infusions (each dose diluted in 250 ml water for injection)[b] x 7 days Nifurtimox: 15 mg/kg per day orally in three doses x 10 days	Eflornithine: 400 mg/kg per day i.v. in four 2-h infusions (each dose diluted in 100 ml water for injection)[b] x 14 days Second-line (e.g. treatment for relapse): Melarsoprol: 2.2 mg/kg per day i.v. x 10 days[c]
Rhodesiense HAT			
First-stage	Suramin	Test dose of 4–5 mg/kg i.v. (day 1), then 20 mg/kg i.v. weekly x 5 weeks (maximal dose/injection: 1 g) (e.g. days 3, 10, 17, 24, 31)	Pentamidine isethionate[e] 4 mg/kg per day i.m. or i.v. (diluted in normal saline and given in 2-h infusions) x 7 days
Second-stage	Melarsoprol	2.2 mg/kg per day i.v. x 10 days[d]	

i.m., intramuscularly; i.v., intravenously

[a] Based on inclusion in the WHO List of essential medicines in 2009 (*19*)

[b] Children weighing < 10 kg: dilute in 50 ml of water for injection. Children weighing 10 to 25 kg: dilute in 100 ml of water for injection. In the absence of water for injection, eflornithine can be diluted in 5% dextrose or normal saline.

[c] Based on evaluation and recommendation by the 27th meeting of the International Scientific Council for Trypanosomiasis Research and Control, 2003 (*8*)

[d] Based on evaluation and recommendation by the 30th meeting of the International Scientific Council for Trypanosomiasis Research and Control, 2009 (*21, 22*)

[e] Based on limited information from a few case series (*58, 59*)

anti-infective drug discovery programme and was shown to have trypanocidal activity (*160*). In 2006, it was "rediscovered" by the Drugs for Neglected Diseases initiative during an extensive data-mining project involving review of > 700 new and existing nitroheterocyclic compounds as candidates for treatment of second-stage HAT. The compound proved to be active against *T. b. gambiense* and *T. b. rhodesiense* when given orally to experimental animals and had an excellent safety profile. As it penetrates the blood–brain barrier, it could be effective in both stages of sleeping sickness. In September 2009, the first human phase-I study was initiated (*161*). Some authors have expressed concern about potential cross-resistance between nifurtimox and fexinidazole. Trypanosome strains selected for resistance to fexinidazole were 10-fold more resistant to nifurtimox than their parents (*162*). The implications of this finding will have to be explored further during development of the drug. Fexinidazole entered phase-II/III clinical trials in the fourth quarter of 2012 at a dose of 1800 mg once a day for 4 days, followed by 1200 mg once a day for 6 days (register number: NCT01685827).

A novel class of boron-containing molecules, benzoxaboroles, has been extensively examined for clinical activity against sleeping sickness. About 50 compounds were originally screened, yielding three distinct classes of molecules attractive for lead optimization. SCYX-7158 was identified as the lead candidate for development to an orally active drug for treatment of second-stage HAT (*163*). After successful conclusion of the preclinical programme (*163–165*), SCYX-7158 entered phase-I clinical evaluation in February 2012 (register number: NCT01533961).

In 2008, a new oral drug against first-stage HAT, DB289 or pafuramidine maleate, failed in late development. After successful, extensive phase-I–III clinical trials, unexpected liver and nephrotoxicity were encountered during an additional phase-I trial designed to provide additional safety data for registration of pafuramidine, and the clinical development programme for the drug was discontinued (*166, 167*). A number of other diamidine compounds, however, showed good activity in mouse models (*168*). Aza analogues of pafuramidine achieved cure in a monkey model mimicking first- (oral administration) and second-stage disease (intramuscular administration). Currently, CPD-0802 (DB-829) and its methoxy prodrug DB-868 are under preclinical investigation (*160*). Care will be taken to determine the risk for renal toxicity with the new cationic diamidines in view of the unexpected, delayed-onset renal insufficiency observed with pafuramidine. The novel aza analogue CPD-0802 accumulates in rat kidney to around one tenth of the concentration of furamidine (the active metabolite of pafuramidine) 48 h after a single intravenous dose of 10 µmol/kg (*169*). The lower exposure of the kidney to CPD-0802 may provide the necessary safety margin and favourable risk–benefit profile for developing these CNS-active diamidines to treat second-stage HAT.

7.6.2 Research on other compounds

The safety and efficacy of two melarsoprol cyclodextrin inclusion complexes was investigated in vitro and in a mouse model in the search for a new treatment of rhodesiense HAT. CNS-stage murine infections were cured when the compounds were administered orally once a day for 7 days, with no overt signs of toxicity (*170*).

A series of nine mono-, di- and tri-substituted derivatives of eflornithine were synthesized to determine their partition coefficients and to assess whether they deliver the parent drug to the plasma. After oral administration to rats, none of the derivatives delivered eflornithine to the plasma, indicating that they were either not absorbed from the gastrointestinal tract or not metabolized to the parent drug. Two of the mono-substituted derivatives were toxic for *T. brucei* bloodstream forms (*171*).

Screening of the "library of pharmacologically active compounds" (LOPAC 1280) for candidates against bloodstream forms of *T. b. brucei* with a resazurin-based cell viability assay identified 33 compounds with half maximal effective concentrations < 1 μmol. The compounds included μ- and k-opioid antagonists. Unfortunately, they did not cure infection in the mouse model, but the divergence of antinociceptive and antitrypanosomal activity may represent a promising start for further exploratory chemistry (*172*).

Various prodrugs of pentamidine were synthesized and examined in vitro and in vivo to increase the permeability of the blood–brain barrier to pentamidine. The prodrug principles used included conversion of amidine functions into amidoximes and *O*-alkylation of amidoximes with a carboxymethyl residue to increase the affinity of the prodrug for the transporters and to mediate active uptake via carrier systems by conjugation of amidoximes with compounds that improve the solubility of the prodrug. Eight prodrugs were evaluated systematically. The *N,N'*-bis(succinyloxy)pentamidine derivative was identified as the most promising prodrug in terms of solubility, activation, permeability and, consequently, oral bioavailability (*173*).

The doublet cordycepin–deoxycoformycin was assessed for its potential in the treatment of second-stage *T. brucei* ssp. infections. The compounds were selected by direct parasite viability screening of a library of 2200 nucleoside analogues. The minimal number of doses and the concentrations of the drugs for effective treatment of *T. brucei* ssp. infections in mice were determined. Oral, intraperitoneal or subcutaneous administration of the compounds was successful. The doublet was effective for treatment of second-stage experimental infections with human pathogenic *T. b. rhodesiense* and *T. b. gambiense* isolates, at a dose of deoxycoformycin similar to or lower than those used in leukaemia patients. The drug combination has been tested in clinical trials for other indications (*174*).

The compounds *N,N*-dimethyl-2-(phenanthro[3,4-*d*][1,3]dioxol-5-yl) ethanamine (1895) and 2-(piperidin-1-ylmethyl)indene[1,2,3-*de*]phthalazin-3-(2*H*)one (0020) were recently identified while screening a large chemical library as poisons of bacterial IA topoisomerase. *T. b. brucei* has an unusual IA topoisomerase that is dedicated to kinetoplast DNA metabolism. This enzyme has no orthologue in humans, and RNA interference studies have shown that it is essential for parasite survival, making it an ideal drug target. These compounds were found to be trypanocidal in the low micromolar range (*175*).

T. brucei N-myristoyltransferase was recently identified and validated as a new drug target, and associated compounds were identified that rapidly kill *T. b. brucei* and *T. b. rhodesiense* both in vitro and in vivo and cure trypanosomiasis in mice. These high-affinity inhibitors bind to the peptide substrate to identify small molecules that prematurely induce bloodstream-form trypanosomes to differentiate into procyclic (insect)-form parasites. The cell surface of the bloodstream form has a dense coat of *VSG*s, which is replaced by an equally dense coat of procyclins pocket of the enzyme. The compounds identified are reported to have promising pharmaceutical properties and could be developed as oral drugs (*176*).

Procyclic forms of trypanosomes (life stage form in tsetse flies) do not survive in the human host. A whole-cell reporter gene assay to identify small molecules that induce down-regulation of *VSG* synthesis of bloodstream-form trypanosomes has been developed. Currently, this assay is being used in high-throughput screening of 300 000 diverse small molecules to find hits that act by this new mechanism (R. Brun and I. Roditi, unpublished data; *177*).

7.7 References

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8. Control and elimination

There is no vaccine against trypanosome infection, and chemoprophylaxis is not used because of the toxicity of the available drugs and the low incidence of infection. Control thus relies on human treatment, vector control or control of infection in the animal reservoir. The optimal strategy depends on the subspecies of trypanosome and its biology. The control of trypanosomiasis involves reduction of parasite transmission from the reservoir to the vector and on to further reservoirs or hosts. The underlying concept of R_0, the basic reproduction number (*1*), is the number of new infections that occur as a result of one infected case in a susceptible population. If R_0 is < 1, each infected individual gives rise to an average of less than one new infection, and, in a given population over a certain period, an outbreak would not sustain itself. If R_0 is > 1, the opposite is true, as each infected individual gives rise to more than one new infection. Higher R_0 values make control more difficult (*2*). Strategies for control can target the vector, the animal reservoir or simply the human reservoir host. No single approach to control can be used for a disease like HAT, in which each focus may have quite different transmission characteristics. Control of both forms of HAT in a location requires understanding of:

- the environment for transmission by tsetse flies,
- the main reservoir species in the area and
- the human interactions with the environment, tsetse habitats and reservoir species, if appropriate

8.1 Detection of cases of gambiense human African trypanosomiasis

The control of gambiense HAT is based on two strategies. As humans are the epidemiologically significant reservoir hosts, detection and treatment of infected humans is the main method of control. This reduces the size of the infection reservoir and also reduces morbidity and mortality. It is based on methods devised and used extensively in Cameroon (*3, 4*) and later in other parts of West Africa (*5, 6*). The second strategy is vector control to reduce the tsetse fly population, which is usually used as a supplement to case detection rather than as an alternative approach.

Clinical diagnosis of gambiense HAT is difficult, because the signs and symptoms are relatively nonspecific. Cases are detected by laboratory methods, usually an initial antibody screening test, followed by confirmation by demonstrating trypanosomes in body fluids (see section 6 for details) (*7*). The case detection approach is most effective when cases are identified early in

infection. People with gambiense HAT may be asymptomatic or have only mild symptoms for many months before they seek care, and, once care is sought, the low index of suspicion by health care workers and difficulties in observing the parasite frequently result in diagnostic delay (*8*). For these reasons, active case detection by mobile teams is the mainstay of control.

8.1.1 Active case detection

In active case detection for gambiense HAT, specialized mobile teams conduct large-scale screening of populations at risk. The targeted communities usually consist of entire villages or urban neighbourhoods, in which the population is screened for suspected cases with the CATT (*9*), a simple, cheap agglutination test for specific antibodies. The test can be done with either whole blood or, if better specificity is required, dilutions of plasma or serum. Cases suspected serologically with a CATT are then confirmed by direct microscopy to detect parasites in lymph node fluid or blood. Disease staging is conducted either by mobile teams or on referral of people who test positive to an appropriate health centre. As microscopy is labour-intensive, programmes often confine the search for parasites to people with titres in the CATT at or above a dilution of 1:4. Several blood concentration methods (*10–12*) can improve the sensitivity of trypanosome detection, and molecular techniques can be used (*7*); these are, however, too sophisticated to be useful for mobile teams or at basic health centres (*13*). They are described in section 6, with additional information about serological testing.

Various diagnostic algorithms are used for finding cases. The choice of a field algorithm depends on the laboratory resources (e.g. the availability of blood concentration methods) and on the disease prevalence. A sample algorithm is shown in *Annex 11*. Screening with CATT is more cost-efficient than other approaches (*14*), and its performance is good, with a sensitivity of 87–98%, depending on the geographical area (*7*). Its specificity is about 95%, but, because the prevalence of HAT is usually low (less than a few percent in endemic foci) (*15–17*), the positive predictive value may be < 50%, even if plasma or serum dilutions are used (*18*). In areas with moderate or high prevalence, a CATT dilution threshold of > 1:4 is usually used; in areas with a low prevalence, a higher threshold (e.g. ≥ 1:8) is used to select the subset of seropositive patients who will undergo parasitological diagnosis. This will minimize the time required for microscopy for people who are likely to give false-positive results.

Active case detection can result in a substantial reduction in the prevalence of infection, and successive rounds have even achieved elimination (*19*). The effectiveness of this method, i.e. the fraction of cases detected during mass screening, has been studied with model-based approaches (*20, 21*). In a single round of screening, 50–80% of prevalent infections were estimated to have been detected (*21*), although the percentage of infections detected and treated

was < 50% in some localities (*20*). Under-detection was due in part to diagnostic inaccuracy; however, a significant contributing factor was incomplete attendance at screening sessions. Several barriers to screening have been identified in focus groups; they include giving higher priority to occupational activities, fear of lumbar puncture, suspicion that HIV tests will be performed and fear of lack of confidentiality during screening (*22*). Social mobilization and good communication between the mobile team and the community are important, therefore, to reduce transmission. Community education about sleeping sickness and a screening schedule that is adapted to the occupational activities of the population could improve attendance and would be important when successive rounds of screening are conducted. Participation in screening tends to decrease after previous rounds have lowered disease prevalence (*23*).

No optimal interval between rounds of active case detection has been recommended. Transmission is incompletely understood, and the requirement of a community for control depends on the prevalence of infection and the intensity of transmission. At typical endemic prevalence, screening is conducted annually (*24, 25*), although 6-month intervals have been used in some programmes (*26, 27*). The goal with respect to gambiense HAT is now shifting from control to elimination. In areas with transmission, the new elimination strategy calls for annual village screening until no new cases have been detected for 3 years. At that time, the screening interval will be increased to 3 years. If no cases are detected for 5 years, mobile teams will not operate, and only passive case detection will continue.

8.1.2 Passive case detection

Cases of gambiense HAT are detected at fixed health facilities when patients present with signs or symptoms suggestive of the infection. Only a few facilities are equipped with the CATT tests used by mobile teams, because of the requirement for a cold chain and the fact that the reagent is supplied in vials of 50 tests, which cannot be stored for > 1 week at 4–8 °C once reconstituted. The CATT can be performed on blood-impregnated filter paper (*28, 29*), which can be sent for testing at an appropriately equipped facility. In practice, follow-up of patients whose specimen has been sent to a laboratory is often problematic, and this approach is seldom used. Thus, serological screening is underused at peripheral health facilities, and cases are often diagnosed by direct microscopy or after referral of a patient to a centre equipped to diagnose and treat the infection. Several new tools may expand screening possibilities in peripheral health centres. Rapid diagnostic tests for individual use have been developed, which are based on lateral flow, have good performance characteristics, are stable at ambient temperature and are being evaluated (*30*). Additional information is given in section 6.

A key challenge in passive detection of infections is the nonspecific nature of the clinical signs and symptoms of gambiense HAT. Health care workers must be able to recognize the infection in order to select patients appropriately for further laboratory investigation; however, diagnostic delay is common. In a study of passively detected patients in endemic areas of the Democratic Republic of the Congo, where half of all cases are detected at health facilities, the mean diagnostic delay after seeking care was 3 months (range, 0.5–11 months), and patients often visited several facilities (*8*). It is likely, therefore, that a substantial number of cases are missed, as for rhodesiense HAT (*31*). Passive screening should be reinforced, particularly in areas of low or decreasing prevalence where the infection is rare, the index of suspicion is low and health care workers have limited experience with the infection. The predictive value of simple clinical algorithms in a symptomatic population has been evaluated (*32*): algorithms of three core symptoms—sleep problems, neurological problems and weight loss—with or without additional signs or symptoms such as oedema or cervical adenopathy led to identification of about 90% of cases, with a positive predictive value of about 8%. If clinical algorithms are validated, and perhaps tailored to local clinical presentations of HAT, their use by health care workers might improve passive detection of cases, with regular supervision and a strong referral system.

One option for managing patients suspected serologically of having HAT is through a referral system, in which specimens are sent to a district or reference laboratory for confirmation, as is done in some research projects. In addition to the highly sensitive, specific immune trypanolysis test (*33, 34*), molecular diagnostics are being developed (*35*) for assessing serologically suspected cases. Although field-testing is needed, LAMP assays for use in low-resource settings might become available (*35*).

Passive case detection has several limitations as a control strategy. The rates of attendance at health centres may be low; for example, annual attendance in the Democratic Republic of the Congo is < 0.15 visits per person (*36*). Furthermore, gambiense HAT is usually diagnosed in the second-stage of the disease, and patients do not seek care during the initial months of infection, when they continue to be a potential reservoir of infection.

8.1.3 Management of parasitologically unconfirmed seropositive cases

A growing body of evidence suggests that there are asymptomatic carriers of infection, whose parasitaemia remains low for a long time (37). The role these carriers may play in transmission has not been defined, but they will not be detected passively.

The level of parasitaemia in gambiense HAT can be at or below the detection limit of the methods used by case-finding teams; therefore, failure of parasitological confirmation of seropositive cases does not rule out infection.

The finding of cases of seropositivity in antibody detection tests that cannot be confirmed parasitologically remains a matter of concern and is a key point in long-term gambiense HAT elimination. The optimal strategy for managing serologically suspected cases has not been defined. Programmes are therefore faced with the choice of withholding therapy until the infection is confirmed or administering therapy to serologically suspected cases, with the risk of unnecessary treatment with toxic drugs. One option is to ask these people to return for serial examinations at regular intervals (*15, 16, 38*). In practice, however, this has not been an efficient approach, as compliance with follow-up is low (*15*), so that some infected individuals are not treated; they will remain potential reservoirs and their disease will progress.

A second approach is to treat a subset of seropositive individuals without confirming the diagnosis. In this strategy, CATT end-titres are used to identify patients at highest risk for infection (*15, 16*). This approach was successfully tested in Angola, Equatorial Guinea and Sudan (*15, 16, 19*). An end-titre of ≥ 1:16 has been proposed as the threshold for treatment in areas of high prevalence of gambiense HAT (> 1% among tested individuals), when access to care is poor or when the availability of the most sensitive parasitological diagnostic methods (e.g. mAECT) is limited (*15, 18*). In a study of the accuracy of various algorithms, however, the authors concluded that the lower specificity of treatment in the absence of a parasitological diagnosis would result in some degree of overtreatment (*18*). Further work is required to better define the role played by seropositive people in parasite transmission and to identify the best therapeutic approach. The immune trypanolysis test has been proposed for confirming the presence of *T.b.gambiense*-especific antibodies in non-parasitologically confirmed, CATT-positive individuals for appropriate management (*34*).

8.2 Detection of cases of rhodesiense human African trypanosomiais

As with gambiense HAT, the clinical diagnosis of rhodesiense HAT is difficult, because the signs and symptoms are relatively nonspecific. Inexperienced clinicians and diagnosticians and those working in areas where HAT is rarely diagnosed may overlook the disease during differential diagnosis. Unlike for gambiense HAT, there are no serological tests for easy identification. Parasitological diagnosis of clinically suspected cases is the main method used. In contrast to people infected with *T. b. gambiense*, those with *T. b. rhodesiense* infection have higher levels of parasitaemia, which facilitates parasitological diagnosis (see section 6 for more details). An essential point to consider in control is the nature of the transmission cycle of *T. b. rhodesiense*, which consists predominantly of transmission from an animal reservoir and rarely includes human–fly–human transmission, such

that treating humans will not control transmission. Treating infected people is a medical imperative, but it will not affect the R_0, the basic reproduction number.

8.2.1 Active case detection

During endemic periods, rhodesiense HAT is not only rare but its acute nature makes regular (usually annual) active detection of infected individuals extremely inefficient and cost-ineffective. Active detection can be justified during outbreaks, usually as a response to an increase in the number of passively reported cases from a particular locality. Such events might lead to targeted active detection to ensure maximum case-finding.

8.2.2 Passive case detection

Most human infections with *T. b. rhodesiense* are detected passively, among people attending fixed health care units. Prompt detection in health care settings is not always straightforward, however. The general nature of first-stage symptoms, lack of recognition of the disease by communities and social stigmatization (especially for second-stage infection) may delay treatment-seeking. Technological advances, such as rapid serological tests and improved parasitological diagnostic capacity, and trained personnel will improve the situation in endemic areas.

As few people are infected with *T. b. rhodesiense*, even clinicians and diagnosticians may not include HAT in their differential diagnosis. Further, clinical suspicion must be supported by parasitological diagnosis and staging of the infection in order to determine the appropriate treatment. In some settings, expertise is not available to collect lumbar puncture samples required for staging; in others, stains, a microscope and (most importantly) a trained operator are not available. In many instances, diagnosis of rhodesiense HAT is reliable in only a few referral centres.

Studies in several countries endemic for rhodesiense HAT (*31, 39–41*) showed that patients make up to seven visits to different health care providers before their disease is properly diagnosed. Such delays increase the chance of second-stage presentation, which is a public health issue, as the fatality rate among patients with second-stage rhodesiense HAT is 2.5 times higher than that of patients with first-stage disease (*42*). The efficiency of detection can be assessed crudely from the ratio of first- to second-stage cases detected, especially in passive reporting, as the disease progresses to the second-stage between 3 weeks and 2 months after initial infection (*42*). Delays in treatment-seeking and in diagnosis together result in under-detection of rhodesiense HAT. Up to 40% of cases may be undetected and therefore untreated (*39, 41, 43*). As untreated cases are invariably fatal (a hidden burden of disease in communities), under-detection should be addressed as a priority in health care systems in disease endemic countries.

8.3 Control of animal reservoirs

Although *T. b. gambiense* has been found in a variety of domestic and wild animals (*44–49*), and animals can be infected experimentally (*49, 50*), the exact role of animal hosts as reservoirs in the transmission of human infection remains uncertain. Therefore, it is generally accepted that humans constitute the epidemiologically important reservoir of *T. b. gambiense*, and control of animal reservoirs is not considered at present as an important issue for controlling gambiense HAT. Rhodesiense HAT, however, is a zoonosis, with both domestic and wild animals as the main reservoirs of the parasite. Therefore, control of transmission from the animal reservoir is required to control rhodesiense HAT.

8.3.1 Control in the domestic livestock reservoir

Human treatment of infection with *T. b. rhodesiense* is a 'fire-fighting' exercise, whereas targeting control at the reservoir will have a longer impact on transmission. In some areas, a very high prevalence of *T. b. rhodesiense* (e.g. 5–20%) can be found in cattle or pigs (*51–53*), and preventing transmission from these reservoirs to humans is an absolute priority in rhodesiense HAT control. In this context, the control of rhodesiense HAT requires the same "One Health" approach required for other zoonotic diseases (*54, 55*). Treating domestic animals has the added effect of improving animal health by affecting other, non-human-infective trypanosomes of livestock. This increases the chances that such methods will be adopted by livestock-keeping communities.

Chemotherapy for livestock is cheap and relatively easy to administer. It can include the use of curative (e.g. diminazine aceturate) or prophylactic (e.g. isometamidium chloride) trypanocides. While resistance to these drugs has been reported in animal trypanosomes (*56–58*), there is no evidence of resistance among *T. b. rhodesiense* isolates, except for some evidence of reduced sensitivity (*59*). The common mechanisms of uptake of veterinary and medical drugs indicate, however, that a degree of caution should be exercised or at least that potential resistance should be monitored (*60*). Livestock-targeted control can be done on farms; focusing control in livestock markets might also be a cost-effective approach if there is a risk that animal trade will spread the infection (*43*). For delivery of control, medical and veterinary authorities should coordinate their actions and develop a control policy in affected areas. Integration of animal and human control has been shown to be highly cost-effective for both rhodesiense HAT and other zoonoses (*61*).

Control in livestock might also involve livestock-targeted vector control, discussed below.

8.3.2 Control in the wildlife reservoir

Direct control of trypanosomiasis in wildlife is not a viable option. In most areas, animals are protected in their natural habitats, and large-scale interventions among wild species would be both unacceptable and extremely costly. Rather, appropriate avoidance of wildlife areas by humans and their livestock or use of other measures to reduce transmission, such as vector control, are better indicated. Personal protective measures might be appropriate for people venturing into tsetse fly-infested wildlife habitats.

8.4 Vector control

The objective of vector control is to diminish the tsetse fly population to a level at which transmission of infection is substantially reduced (see also section 4). Although vector control is the primary method for controlling animal trypanosomosis, when it is used to control *T. b. gambiense* infections in humans, it is usually combined with case detection and treatment.

8.4.1 Methods

The choice of method for vector control depends on the local environment and epidemiology, the human and financial resources available and the potential ecological impact. Clearing of vegetation and ground-spraying of insecticide at tsetse breeding sites are sometimes used (*62*), although persistent insecticides are no longer used because of environmental concerns.

Modern approaches to tsetse control usually involve use of live animal or artificial baits to attract and kill the flies. This strategy has the advantage that it can be done by the local population. Use of insecticide-treated cattle as bait has been effective in controlling rhodesiense HAT (*63*), although use of pour-on insecticides is more expensive than restricted application. As there are few cattle in many foci of gambiense HAT in Central and West Africa, tsetse control is based mainly on traps or insecticide-impregnated screens.

8.4.2 Traps and screens

Riverine tsetse flies are visually attracted to blue cloth and particularly to royal phthalogen blue. The cloth used in traps and screens should be chosen not only for its colour but also for the spectrum of reflectance (*64*). Blue cloth with low reflectance in the ultraviolet and green bands attracts significantly more flies than cloth that reflects ultraviolet radiation strongly (65). It is recommended that the cloth used for traps or targets be a cotton–polyester blend that can survive environmental conditions without fading rapidly.

Visual attractants are the baits mainly available for *T. b. gambiense* vectors. Morsitans group flies are responsive to chemicals in cattle odours (*66*), but these

compounds are less effective in attracting palpalis group flies (*67*). Compounds that occur in lizard odour are effective attractants for *G. f. fuscipes* and compounds in pig odour for *G. f. quanzensis* (*68*), but the specific chemicals have not been identified, and no olfactory bait is available for control of gambiense HAT. Various phenols are routinely used to attract *T. b. rhodesiense* vectors, and cow urine and its constituents are particularly attractive to certain species of tsetse fly (*69, 70*).

Traps are constructed from blue cloth to attract the flies and black cloth to induce them to land. Netting covers the top of the trap. The cloth panels lure the flies into the trap, where they fly upwards towards the light and are caught in a holding cage, where they die of heat stress and desiccation. Olfactory bait can be used in traps where appropriate. Insecticide, usually a pyrethroid, can be added to increase the efficiency of traps. Several varieties of trap are available, including pyramidal (*71*), biconical (*72*), Lancien (*73*) and Vavoua (*74*), which are described in detail in section 4.12.8 and shown in *Annex 6*.

Traps cost US$ 5–15 each (*74–76*). For the control of tsetse flies, enough traps must be posed per unit area to reduce the density of the population significantly. For example, Lancien (*77*) used 10 unbaited traps per km^2 in Uganda for *G. f. fuscipes* control, and Hargrove (*78*) estimated that four baited targets per km^2 would be necessary for the control of *G. pallidipes* in a closed area. Centralized organization is likely to be needed for deployment on this scale; e.g. a 10-km^2 village would need 100 traps, which would cost up to US$ 1500, which may not be a realistic sum for community control in low-income settings. Nevertheless, communities can take charge of trapping under the right circumstances (*79, 80*). Dransfield and Brightwell (*81*) found that such programmes must have more than simply nominal participation of communities in order to be successful.

Screens, also known as "targets", are flat panels made of blue and black cloth impregnated with insecticide. The standard screen is a 1 m x 1 m cloth with a black–blue–black vertical design. Observations on the effect of target size show, however, that small screens (25 cm x 25 cm) with flanking nets are more effective in killing flies than the standard screen (*82–84*). Investigations of screen shape preferences showed that square screens should be used for most *T. b. gambiense* vectors, although *G. p. palpalis* showed a preference for vertical oblongs (*84*). The new small square designs will be easier to deploy and will significantly reduce the cost of vector control. For *G. p. gambiensis* and *G. tachinoides*, smaller screens with netting were estimated to improve cost-effectiveness by sixfold (*83*).

8.4.3 Insecticide-treated cattle for control of rhodesiense human African trypanosomiasis

In areas where trypanosomes are co-endemic with other parasitic diseases, integrated control of tick-borne pathogens and tsetse flies has been used (*85*). The individual animals are protected from new infections if the vectors are killed

before they feed, and the animals become living, impregnated tsetse targets. This approach is certainly economically sensible, especially where rhodesiense HAT is transmitted in the same areas as livestock trypanosomes that cause *nagana* (*86*). As pour-ons and sprays of pyrethroid insecticides can be used directly by farmers, they are especially suitable for this approach. Extensive work has even been carried out on restricted application of insecticides to the parts of animals that are preferred by tsetse for feeding (*87*). Use of this type of control, which implies continuous commitment by farmers, is cost-effective (*88*) and is especially suitable in areas where veterinary services have been decentralized, as is the case in much of sub-Saharan Africa.

8.4.4 Implementation of vector control

For optimal selection of sites, the local epidemiology should be known, so that transmission sites can be targeted. Surveys to determine the tsetse species present and their spatial distribution should be conducted before control is begun. Sites that are suitable for traps and screens have some visibility in most directions; the sites that are selected might have to be improved and maintained by cutting vegetation. A study of the effect of vegetation re-growth on the performance of screens for catching *G. f. fuscipes* suggested, however, that regular maintenance may not be required, as catches decrease significantly only after the screen is obscured by 80% (*89*).

If control is conducted with traps, the design should be appropriate for the local tsetse species. Construction of traps by local workers helps to control costs. The choice of traps or screens depends on the human and other resources available, the extent of community involvement and the environmental setting. Traps are effective, reducing the tsetse population by about 90% over time (*83*), but they are more expensive and difficult to construct and use, and they are more fragile than screens and require regular maintenance. Nonetheless, use of trained village volunteers in deployment, maintenance and monitoring of traps increases awareness of the disease in the community and can increase attendance during case-finding surveys (*90*). Trap catches can be monitored to evaluate programmes and can be used for sampling. Screens are less costly, more durable and more easily transported and deployed, but they must be re-impregnated with insecticide after about 8 months of use, and they might have to be replaced if the cloth colour has faded significantly. A comparison of small screens with biconical traps for palpalis group flies showed that they were 8.6–37.5 times more effective in killing flies (*82, 91*).

Ideally, vector control should be maintained as long as new cases of infection are detected. In practice, programmes often find it difficult to sustain activities, because community enthusiasm tends to wane after the density of tsetse is reduced to a low level. Programmes may be more sustainable when low-cost, low-maintenance, small screens are used.

8.5 Elimination of gambiense human African trypanosomiasis

The past decade has seen momentum build for the elimination of sleeping sickness caused by *T. b. gambiense*. In 2001, during a resurgence of the infection, WHO and partners established a public–private partnership to bring the disease under control, which was effective within a short time. In 2004, therefore, the World Health Assembly passed a resolution (WHA57.2) to enhance control in order to move towards elimination. In 2011, in view of the 70% decrease in the number of cases reported to WHO in the previous decade, the elimination goal was judged to be feasible by the WHO Strategic and Technical Advisory Group for Neglected Tropical Diseases. The WHO "roadmap" on neglected tropical diseases (*92*) set 2020 as the target date for elimination of HAT as a public health problem.

In January 2012, a gathering of partners from the public and private sectors launched an unprecedented effort against neglected tropical diseases by signing the London Declaration (*93*). This marked a coordinated effort to control or eliminate 10 infections, of which HAT was targeted for elimination. A meeting was convened by WHO in late 2012 to prepare the strategy, indicators and benchmarks for elimination of gambiense HAT (*94*). The results of the meeting are outlined below. The initial strategy will incorporate other approaches and modifications as elimination progresses.

8.5.1 Rationale

The transition of the goal of programmes from control to elimination appears to be feasible on the basis of multiple considerations.

- *T. b. gambiense* is epidemiologically vulnerable. Humans are the significant reservoir, and the control strategies in use are effective. In an adequately resourced programme, a single round of active case detection and treatment through mass screening will result in identification of 50–80% of prevalent infections (*21*). Annual screening with or without supplementary vector control can reduce the prevalence to very low levels within a few years.
- Proof of principle exists for gambiense HAT. In the 1960s, near-elimination was achieved with tools inferior to those currently in use, when the annual incidence in Africa dropped below 5000 (*95*). Evidence that elimination is possible in some settings emerged more recently from Equatorial Guinea (*19*).
- Details of the geographical distribution of infection are available. Foci of gambiense HAT tend to be geographically stable in the absence of major environmental change, although the boundaries and intensity of transmission change with conditions and the extent of control.

Current and past data on known foci were collected and analysed to prepare the HAT *Atlas* and database (*96*), which incorporates data on nearly 90% of reported cases in 2000–2009 and makes available geo-referenced epidemiological data at village level and up-to-date maps.

- The scope and geographical distribution of infection are limited. Less than 10% of the land area of endemic countries is estimated to be at risk (*97*), and 97% of reported cases are in only five countries: Angola, the Central African Republic, Chad, the Democratic Republic of the Congo and South Sudan (*94*), with a substantial majority in the Democratic Republic of the Congo.
- New tools for case detection will extend the possibilities for diagnosis and surveillance in the health system. Previously, screening for gambiense HAT in peripheral health centres was limited, because the CATT tests used for screening require a cold chain. Two rapid tests (*30*) have now been developed and are being evaluated in the field.
- The availability of drugs for treatment has increased considerably due to generous donations by and support from pharmaceutical manufacturers.
- There is commitment and political will for the elimination of HAT.

8.5.2 Concept of elimination, indicators and benchmarks

The definition of elimination of gambiense HAT is based on ideas of the International Task Force for Disease Eradication, the Dahlem Workshop on the Eradication of Infectious Diseases (*98*) and the WHO Strategic and Technical Advisory Group for Neglected Tropical Diseases. The definition of elimination is thus "reduction to zero of the incidence of infection caused by a specific pathogen in a defined geographical area, as a result of deliberate efforts; continued actions to prevent re-establishment may be required." Thus, the objective of elimination is interruption of the transmission of *T. b. gambiense*.

The natural ecological and epidemiological unit for interventions is the focus. The concept of a focus was defined by the WHO Expert Committee in 1986 as "a zone of transmission to which a geographical name is given (locality, region, or river)" (*99*). The list of foci of gambiense HAT was recently updated (*94*). A focus can be "open" or "closed," depending on whether migration of humans or flies occurs to and from neighbouring foci. Closure is rare, and post-intervention surveillance will be required while there is still a risk for both, resurgence and/or reintroduction of infection from outside the focus.

Progress toward elimination will be measured by two quantitative indicators, which will be updated annually: the number of cases reported and the number of foci validated as eliminated.

The case definitions described in section 6 can be used in programmes for reporting the results of surveillance and monitoring. Although treatment decisions may be made on the basis of other criteria, programmes will be strongly encouraged to use these case definitions for reporting.

Secondary indicators, which will be updated every 2 years, will be used to assess the quality and extent of the activities of an elimination programme. These include the proportion of the population at risk that is covered by control and surveillance activities, the geographical extent of the infection and the populations at different levels of risk.

The proposed target date for elimination is 2030, when it is hoped that interruption of transmission and validation of elimination will have been achieved in all foci (*94*). The goal of the WHO roadmap is to eliminate gambiense HAT as a public health problem by 2020. This is an intermediate step, defined as less than one new case per 10 000 inhabitants in 90% of foci (*Table 8.1*).

Table 8.1
Quantitative indicators for gambiense human African trypanosomiasis elimination, 2012–2020[a]

Year	2012	2013	2014	2015	2016	2017	2018	2019	2020
Cases annually reported	6000	5500	5000	4500	4000	3500	3000	2500	<2000
Number of foci reporting less than 1 case per 10,000 inhabitants				10%	30%	40%	60%	80%	90%

[a] source: adapted from reference (*94*)

8.5.3 Strategies for elimination

Elimination campaigns involve use of the classical control methods for gambiense HAT, i.e. reducing the reservoir by active and passive case detection and reducing the tsetse population by vector control. The plan for elimination will include the activities listed below.

- Active case detection will continue to be conducted by mobile teams.
- Passive case detection in the health system will be expanded to include sentinel sites selected on the basis of population coverage and capacity. Training will be provided to staff at the sentinel sites,

and they will be equipped with individual rapid diagnostic tests for screening. Health care workers at peripheral health centres in the foci will be trained to recognize signs and symptoms suggestive of gambiense HAT so that suspected cases are appropriately referred to the sentinel site. A WHO collaborating centre will provide immune trypanolysis testing for individuals who are seropositive but have negative parasitological results, in order to confirm the presence of *T. b. gambiense*-specific antibodies and to determine whether repeated parasitological examinations are indicated.

- Vector control should be implemented in selected foci as an adjunct to case detection, depending on the epidemiological setting and knowledge about sites at which it should be used.

The intensity of disease transmission will determine how these interventions are combined and their detailed implementation. For the purposes of elimination, foci of gambiense HAT have been classified into three categories of transmission intensity.

- **High-intensity transmission** foci are those with an annual average of at least one new case per 1000 people during the previous 5 years. The threshold of reported incidence corresponds to that for high and very high risk for HAT, as defined by Simarro (*97*). Data for 2000–2009 indicate that foci with high-intensity transmission are found in parts of Angola, Chad, the Central African Republic, Congo, the Democratic Republic of the Congo, Equatorial Guinea, Gabon, Guinea, South Sudan and Uganda.

- **Moderate-intensity transmission** foci are those with an annual average of at least one new case per 10 000 people during the previous 5 years but fewer than 1 per 1000. Such foci are found bordering high-intensity transmission areas and in Cameroon, Côte d'Ivoire and other parts of Guinea (*97*).

- **Low-intensity transmission** foci are those with a 5-year annual average of one new case per 1 000 000 people but fewer than 1 per 10 000. The threshold of reported incidence corresponds to that for low and very low HAT risk, as defined by Simarro (*97*). Such foci are currently found bordering moderate-intensity foci and in Cameroon, Côte d'Ivoire, parts of Guinea and Nigeria (*97*).

Although the epidemiology of HAT has become better understood in the past decade, remaining foci require assessment. Some are known to have transmission but the incidence of infection is unknown because of security

problems or because the terrain makes access difficult. Other areas have reported no cases, but their transmission status is uncertain because there has been no surveillance.

A strategy for elimination has been proposed for each transmission setting.

Foci of high or moderate transmission (*Annex 12*)

- Active case detection is conducted annually in the area around villages in which there has been at least one case in the past 3 years and every three years in villages having reported at least one case during the past 5 years but no cases during the past three years.
- Sentinel surveillance is in place for all villages.
- Vector control is implemented at selected sites.

Foci of low transmission (*Annex 13*)

- Active case detection is conducted annually in the area around villages in which there has been at least one case in the past 3 years.
- Sentinel surveillance is in place for all villages.
- Vector control is implemented at selected sites.

In foci with no cases reported for ≥ 5 years (*Annex 14*), sentinel surveillance will be conducted. If one or more confirmed cases are diagnosed at the sentinel site after active case detection has stopped, sentinel surveillance will continue, and, in addition, screening by mobile teams will be reactivated. This will be done annually in the area in which cases were identified until no cases are detected for 3 years. External support will be given to sites in which mobile team expertise has waned.

HAT will be considered to be eliminated in foci in which adequate surveillance has been in place for 5 years and in which no cases are detected in any village during the 5-year period. The country can then submit a technical report to WHO and request external verification of elimination. Such verification will be carried out by an international validation group in order to declare that the focus of gambiense HAT has been eliminated. The procedure for validation has not yet been determined.

Monitoring and evaluation of progress towards elimination will be based on screening by mobile teams and on surveillance data from the sentinel sites. Although the prevalence of HAT may be > 5% in epidemic conditions, the endemic prevalence is usually no more than a few percent. Therefore, a survey and sampling approach, of the type used in lymphatic filariasis elimination programmes (*100*),

would not be feasible for gambiense HAT. At such low prevalence, no sampling method will have sufficient statistical power for decision-making. Because of the central role played by the health system in surveillance, training and capacity-building are key elements in monitoring and evaluation. In a substantial number of foci, both sentinel surveillance and population screening by mobile teams will be in place. Incidence data collected by mobile teams will be used to assess the ability of sentinel sites to detect new cases of infection.

Xenomonitoring might be useful in monitoring and evaluation, but the low prevalence of infection in tsetse flies and the limited sensitivity of molecular detection of *T. b. gambiense* have been barriers to the development of this method. A pool screen strategy (*101, 102*) might overcome these problems, but only if an adequate number of tsetse can be collected to provide sufficient statistical power and if appropriate tools to identify *T. b. gambiense* in the vector are available.

8.5.4 Challenges

The challenges to elimination of gambiense HAT are similar to those in programmes to eliminate other neglected tropical diseases. Sustained political support and a national sense of ownership are required if elimination of gambiense HAT is to compete successfully with other health priorities. Adequate funding must be available, not only to implement programme activities but also to support operational research and tool development that could increase the efficiency and cost-effectiveness of the programme. A critical challenge for gambiense HAT elimination will be moving from a largely vertical programme to one that is integrated into the health system and strengthens it. Additional challenges are insecurity and instability in areas endemic for gambiense HAT, which can impede control activities.

Although the epidemiology of gambiense HAT is generally well understood, some scientific questions remain unanswered. Gaps in understanding the reservoir(s) of infection must be addressed as a priority as programmes move from control to elimination. It is important to determine whether asymptomatic, seropositive human carriers with undetectable parasitaemia play a role in maintaining transmission. Studies are needed to clarify whether animals can serve as reservoirs for human infection. The strategies used to achieve elimination will depend on what is learnt.

8.6 Elimination of rhodesiense human African trypanosomiasis

Rhodesiense HAT is a zoonosis, in which both domestic and wild animals serve as reservoir hosts. Therefore, total interruption of transmission is not feasible at this time, and elimination is not a goal.

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9. Recommendations

Epidemiology

- Effective surveillance and data collection should be strengthened.
- Surveillance and control of rhodesiense HAT should be coordinated with veterinary services in an "One Health" approach, particularly in areas of potential overlap with gambiense HAT.
- The epidemiological roles of people with parasitologically unconfirmed, serologically suspected HAT and of animals as reservoirs of *T. b. gambiense* should be explored.

Parasites

- Human infectious trypanosome species should be used more routinely in research.
- The full range of modern tools for biological analysis should be exploited to gain more insight into parasite biology and interactions between parasite and host.

Vectors

- The ecology of tsetse flies in various settings should be further investigated in order to optimize vector control.
- The feasibility of monitoring *T. b. gambiense* infections in vectors should be explored for use as a tool in elimination programmes.

Disease

- Simple clinical algorithms should be developed for detecting clinically suspected cases of HAT and other diseases that are part of the differential diagnosis.

Diagnostics

- The CATT remains the recommended test for use in active screening for *T. b. gambiense* infection. For passive screening of *T. b. gambiense*

infection, individual rapid diagnostic tests are recommended.

- Systematic follow-up after treatment is no longer recommended. Follow-up, including examination of CSF, should be confined to patients with clinical features suggestive of relapse.
- In the special case of clinical trials, post-treatment follow-up with regular control visits remains mandatory. The recommendations for clinical trials on HAT should be revised.
- At present, molecular methods should not be used for making therapeutic decisions.
- The development of rapid diagnostic tests for *T. b. rhodesiense* and blood or urine tests for stage determination in both forms of HAT is encouraged.

Treatment

- Safe, if possible oral, drugs that are active against both disease forms and are easy to use are required. Research towards these is strongly encouraged.
- The efficacy of NECT and the drugs under development against *T. b. gambiense* should be further investigated in appropriate animal models for *T. b. rhodesiense*.
- The efficacy of pentamidine against *T. b. rhodesiense* should be assessed in studies in humans.
- The safety and effectiveness of first-line treatments against *T. b. gambiense* should be studied in special populations, such as pregnant women and HIV-infected patients.

Control and elimination

- Operational research is required on integrating HAT into existing health systems and on optimizing passive case detection, surveillance and management of HAT in these systems.

- Vector control should be used as part of an integrated strategy with case detection and therapy for the elimination of gambiense and the control of rhodesiense HAT.
- Human resource capacity should be enhanced in all areas of HAT control, including for case detection, patient care, vector control, programme management and operational research.
- Coordination should be strengthened among people involved in control and in research to facilitate the development and validation of new control tools.
- Access to diagnostic facilities in HAT-affected areas should be improved, and systems for referral to facilities where HAT can be treated should be strengthened.
- Quality control of diagnostic proficiency is recommended.
- Additional indicators and modelling tools should be developed for estimating the proportion of undetected cases of both forms of HAT.

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The Expert Committee recognizes that the current situation, in which elimination of HAT is now feasible, was brought about by a concerted international effort involving many dedicated people and institutions. These include the national control programmes of endemic countries, WHO Member States, international organizations, nongovernmental organizations, the private sector, cooperation programmes, funding agencies across the globe and dedicated research workers in academic and private sector institutes. The skilled, dedicated staff in countries endemic for HAT deserve particular acknowledgement.

Annexes

Annex 1. Wild animal species documented as serving as hosts for *Trypanosoma brucei rhodesiense*

Species	Location	Technique (reference)
Bushbuck	Nyanza Province, Kenya	Human volunteer (*1*)
Bushbuck	Luangwa Valley, Zambia	Blood incubation infectivity test (*2*)
Duiker	Luangwa Valley, Zambia	Blood incubation infectivity test (*2*)
Giraffe	Luangwa Valley, Zambia	Blood incubation infectivity test (*2*)
Hartebeest	Serengeti, United Republic of Tanzania	Human volunteer (*3*)
Hyaena	Nyanza Province, Kenya	Isoenzymes (*4*)
Hyaena	Serengeti, United Republic of Tanzania	Blood incubation infectivity test and human volunteer (*3*)
Hyaena	Kenya (?)	(*5*)
Hyaena	Serengeti, United Republic of Tanzania	Blood incubation infectivity test and human volunteer (*3*)
Impala	Luangwa Valley, Zambia	Blood incubation infectivity test (*6*)
Impala	Luangwa Valley, Zambia	Blood incubation infectivity test (*2*)
Lechwe	Northern Botswana	Blood incubation infectivity test (*7*)
Lion	Luangwa Valley, Zambia	Blood incubation infectivity test (*2*)
Oribi	Nyanza Province, Kenya	Isoenzymes (*4*)
Reedbuck	Nyanza Province, Kenya	Blood incubation infectivity test (*8*)
Reedbuck	Nyanza Province, Kenya	Blood incubation infectivity test (*9*)
Reedbuck	Nyanza Province, Kenya	*SRA* polymerase chain reaction (*5*)
Warthog	Luangwa Valley, Zambia	Blood incubation infectivity test (*10*)
Warthog	Luangwa Valley, Zambia	Blood incubation infectivity test (*11*)
Warthog	Serengeti, United Republic of Tanzania	*SRA* polymerase chain reaction (*12*)
Warthog	Luangwa Valley, Zambia	Blood incubation infectivity test (*2*)
Waterbuck	Luangwa Valley, Zambia	Blood incubation infectivity test (*11*)
Waterbuck	Luangwa Valley, Zambia	Blood incubation infectivity test (*2*)
Zebra	Luangwa Valley, Zambia	Blood incubation infectivity test (*6*)

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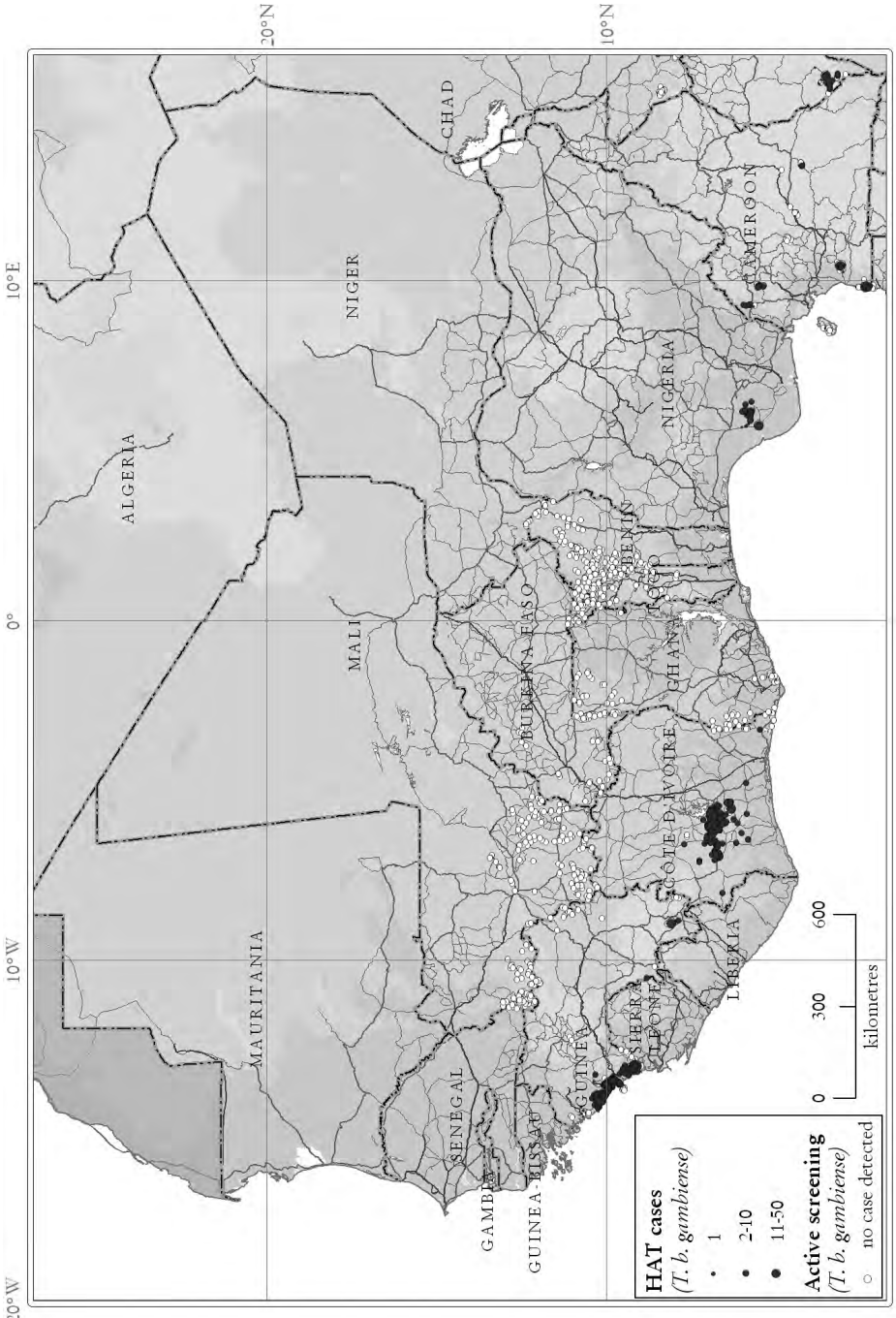
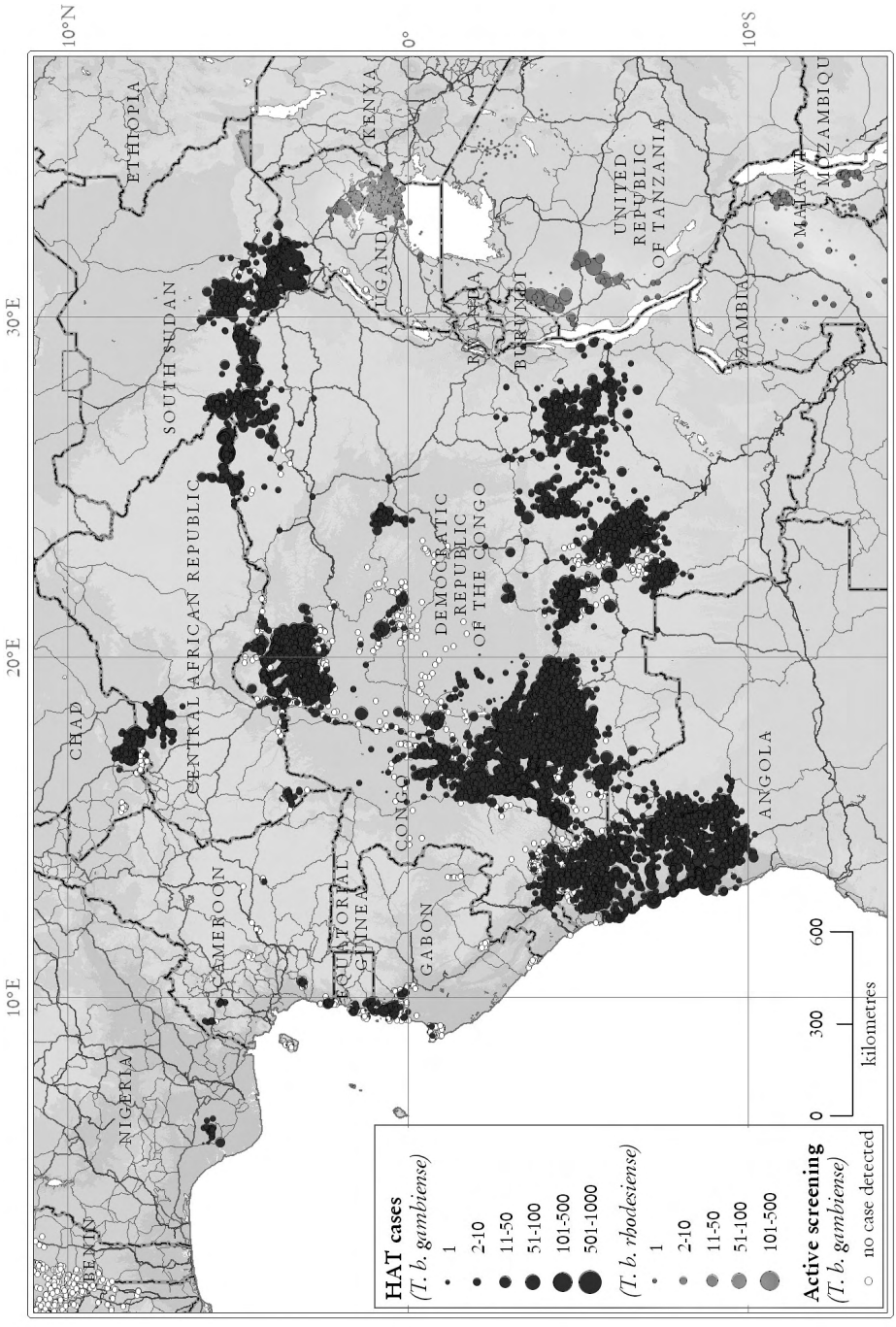
Annex 2. Distribution of human African trypanosomiasis in West Africa



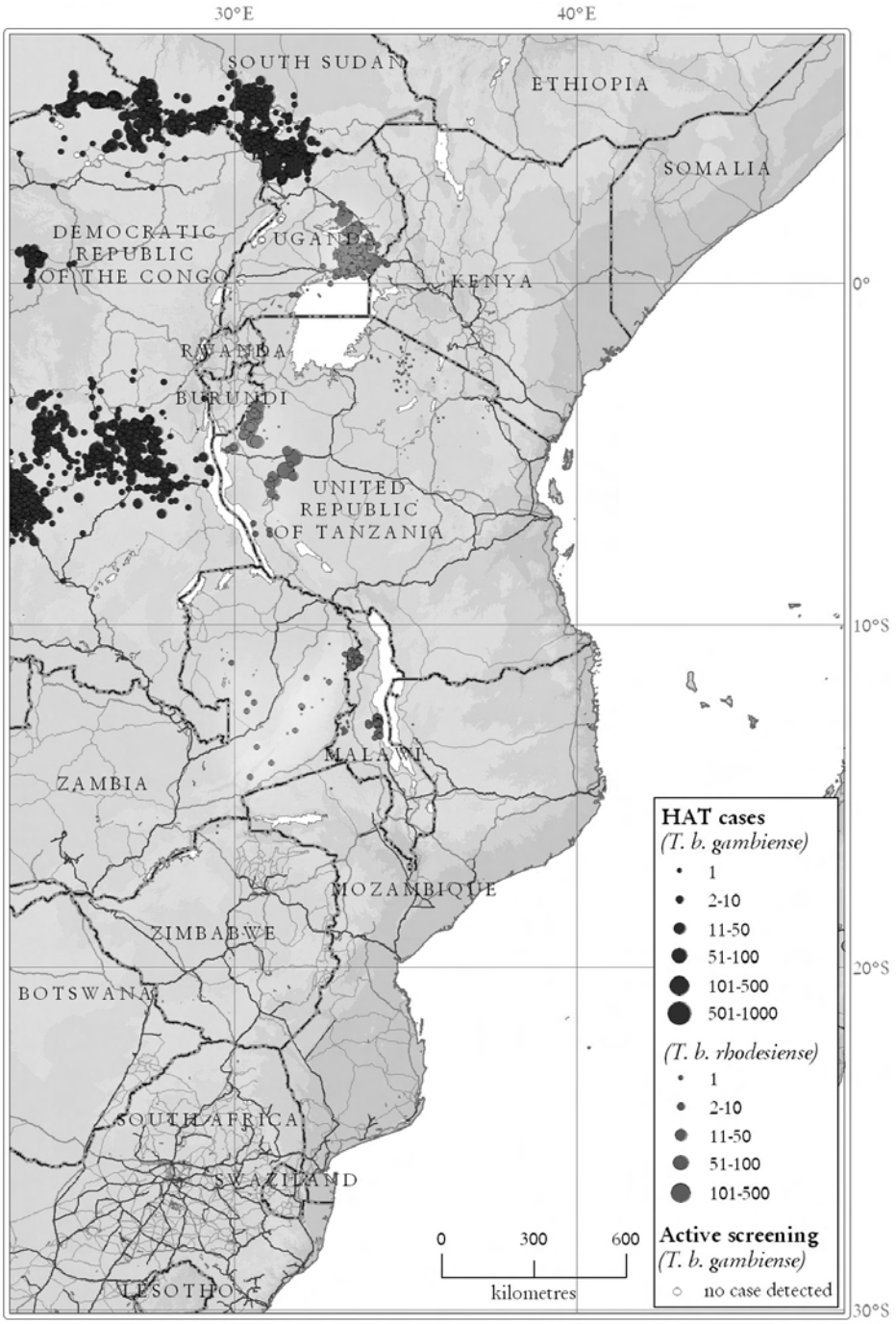
Adapted from: Simarro PP et al. The Atlas of human African trypanosomiasis; a contribution to global mapping of neglected tropical diseases. *International Journal of Health Geographics*, 2010, 9:57 (HAT database version: 25/06/2012).

Annex 3. Distribution of human African trypanosomiasis in Central Africa



Adapted from: Simarro PP et al. The Atlas of human African trypanosomiasis; a contribution to global mapping of neglected tropical diseases. *International Journal of Health Geographics*, 2010, 9:57 (HAT database version: 25/06/2012).

Annex 4. Distribution of human African trypanosomiasis in East and south-eastern Africa



Adapted from: Simarro PP et al. The Atlas of human African trypanosomiasis; a contribution to global mapping of neglected tropical diseases. *International Journal of Health Geographics*, 2010, 9:57 (HAT database version: 25/06/2012).

Annex 5. Cryostabilization

Cryostabilization of biological specimens for parasite isolation

To isolate trypanosomes from patients, the buffy coat prepared from blood or the sediment of centrifuged cerebrospinal fluid (CSF) can be cryopreserved in liquid nitrogen for subsequent inoculation into susceptible rodents. A protocol for isolating *Trypanosoma brucei gambiense* in *Grammomys surdaster* and immunodeficient mice was described by Pyana et al. (*1*). The basis of the cryomedium is a commercially available medium for cryopreserving bull sperm. To prepare the cryomedium, one volume of aseptically collected egg yolk is mixed with three volumes of bull sperm medium and three volumes of phosphate-buffered saline–glucose (7.5 g/l $Na_2HPO_4.2H_2O$, 0.34 g/l $NaH_2PO_4.H_2O$, 2.12 g/l NaCl, 10 g/l D-glucose, pH 8). This bull sperm medium–egg yolk–phosphate buffer mixture can be divided into 2-ml aliquots and kept frozen at –20 °C until use. To cryopreserve patient specimens, 1.5 ml of blood in heparin or of CSF are dispensed into microcentrifugation tubes and centrifuged for 5 min at 5200–7244 g. The plasma is removed from blood, and 250 µl of the buffy coat are transferred into 2-ml cryotubes filled with 250 µl of cryomedium. For CSF, the supernatant is removed, leaving about 300 µl of sediment in the tube. The sediment is mixed with 300 µl of cryomedium, and 500 µl are transferred into 2-ml cryotubes. Samples mixed with cryomedium are frozen in the vapour phase of liquid nitrogen for 1 h, whereafter they are dipped into the liquid phase of the gas until further use.

Cryostabilization of blood for long-term preservation of parasite strains

Parasite strains can by cryopreserved in liquid nitrogen after addition of glycerol or dimethylsulfoxide to blood or culture medium containing the trypanosomes. One volume of blood collected on heparin or ethylene diamine tetraacetic acid (EDTA) or one volume of culture medium is thoroughly mixed with three volumes of a 1:1 mixture of analytical grade glycerol (about 87%) and phosphate-buffered saline–glucose (7.5 g/l $Na_2HPO_4.2H_2O$, 0.34 g/l $NaH_2PO_4.H_2O$, 2.12 g/l NaCl, 10 g/l D-glucose, pH 8) to a final glycerol concentration of approximately 10%. The mixture of blood or culture medium with cryomedium is divided in 0.5–1-ml volumes in labelled cryotubes. If no glycerol is available, one volume of blood or culture medium can be mixed with three volumes of 20% dimethylsulfoxide (analytical grade) and divided into labelled cryotubes. After careful closure of the cryotubes to avoid entry of liquid nitrogen, the cryotubes are cooled at a rate of 1 °C per min to –40 °C, followed by a rate of 5 °C per min until the temperature reaches –100 °C, whereafter the tubes are transferred to liquid nitrogen. If no controlled freezing apparatus is available, these cooling rates can be approximated by keeping the tubes for 1 h in the vapour phase of the liquid nitrogen container, whereafter they are transferred into the liquid phase.

Reference

1. Pyana PP, et al. Isolation of *Trypanosoma brucei gambiense* from cured and relapsed sleeping sickness patients and adaptation to laboratory mice. *PLoS Neglected Tropical Diseases*, 2011, 5(4):e1025.

Annex 6. Traps or screens used for various species of tsetse flies

Biconical trap

Pyramidal trap

Vavoua trap

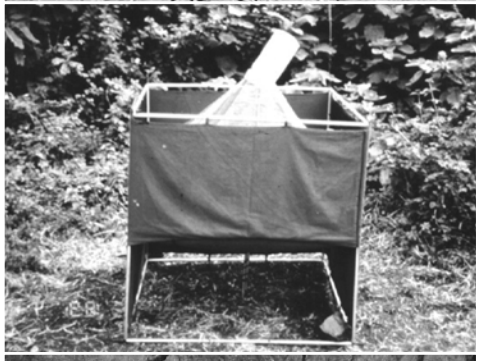
Lancien trap

Screen

Small screen

Epsilon trap

F3 trap



H trap

NGU trap

Annex 7. Protocol for storing blood samples on filter paper discs

Purpose

Storage of blood samples on filter paper discs for testing in molecular or serological diagnostics

Precautions

- Safety: All blood specimens should be treated as potential sources of hepatitis or HIV infection. Universal precautions should be followed.
- Avoid contamination. Always use disposable materials, and change protecting gloves often.

Background

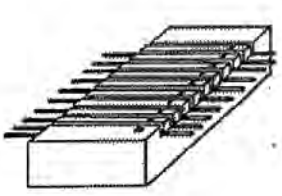
By drying blood on a filter paper, the quality of the DNA and antibodies can be maintained for a long time, as long as the paper remains completely dry.

Materials

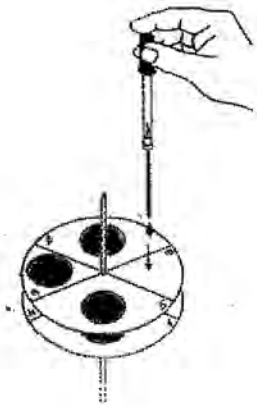
- filter paper discs, Whatman® No. 4, diameter 9 cm
- Silicagel
- material for blood collection: heparinized capillary tubes and blood lancets for finger-pick (as for the card agglutination test for trypanosomiasis, CATT)
- disinfecting solution (75% alcohol or similar)
- cotton
- latex gloves
- waste container
- envelopes for packing filters one by one
- marker
- plastic bags

Protocol

1. Collect blood from the finger and fill two heparinized capillary tubes per person.

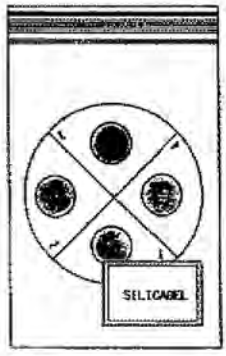


2. Write the code of the person on a filter paper disc and mount it on a lumbar puncture needle or similar on a standard. Without touching the filter paper disc, let fall four drops of blood on the filter paper. Place one blood specimen per section on the filter paper.



3. Let the filters dry in the shade, protected from flies and dust, for at least 1 h. **Do not expose to direct sunlight.**

4. Insert each filter in an envelope, and seal the envelope. Pack a maximum of 10 envelopes into a plastic bag, and add silicagel crystals. Store preferably in a freezer or a refrigerator.



Notes

- It is very important that the blood on the filter papers be allowed to dry completely and that it stays dry. Therefore, add enough silicagel crystals to each plastic bag, and make sure that the plastic bags are airtight.
- The filter papers containing blood should be separated by blank filters to avoid contamination of the specimen.

Annex 8. Storage of blood samples in AS1 buffer

Purpose

Storage of blood samples in AS1 buffer for DNA extraction

Precautions

- **Safety**: All blood specimens should be treated as if they were potential sources of hepatitis or HIV infection. Universal precautions should be followed.
- **Avoid contamination**. Molecular diagnostics are highly prone to contamination. Always use disposable materials, and change protecting gloves often.

Background

For molecular diagnostics such as polymerase chain reaction (PCR) or similar, the specimen should be collected in such a way as to avoids degradation of the target DNA before extraction from the specimen. Mixing an equal volume of blood with an equal volume of AS1 buffer will preserve good DNA quality for 3 months at ambient temperature.

Materials

- AS1 buffer (Qiagen), toxic material
- material for venous blood collection
- syringe
- tourniquet
- disinfecting solution (75% alcohol or similar)
- cotton
- latex gloves
- waste container
- box for 9 x 9 tubes of 2 ml
- microtubes of 2 ml with screwcap, filled with 0.5 ml AS1 buffer
- marker

Protocol

Collect venous blood and dispense it into the tube containing AS1 buffer up to one graduation line (0.5 ml). **Do not dispense more than 0.5 ml of blood**.

Close the tube tightly with the screw cap, and mix the blood with the AS1 buffer thoroughly.

Label the microtube with the person's code and the collection date.

Change latex gloves when contaminated with blood.

The tubes may be stored for a maximum of 3 months in a dark, dry place at ambient temperature. **Do not store in a refrigerator or freezer**.

Annex 9. Preparation of guanidine hydrochloride–ethylene diamine tetraacetic acid (EDTA) buffer

Purpose

Preparation of 500 ml of 6mol/l guanidine-HCl, 0.2mol/l EDTA, pH 8.0 buffer (GE buffer) for DNA extraction

Precautions

- Safety: Guanidine-HCl and NaOH are toxic. Protect yourself from contact with or inhalation of the reagents.
- Avoid contamination. Molecular diagnostics are highly prone to contamination. Always use disposable materials and molecular biology grade reagents and water. Use protective gloves.

Background

For molecular diagnostics like polymerase chain reaction (PCR) or similar the specimen should be collected in such a way as to avoid degradation of the target DNA before extraction from the specimen. Mixing an equal volume of blood with an equal volume of GE buffer will preserve good DNA quality for several months in a refrigerator (4–8 °C).

Reagents

- guanidine-HCl (Sigma G3272; molecular biology grade), toxic
- EDTA.H_2O (Sigma E5134, molecular biology grade)
- NaOH 10 mol/l solution, toxic
- water (molecular biology grade)

Protocol

In a 500-ml flask, dissolve 285 g of guanidine-HCl in 250 ml of water with agitation (guanidine-HCl takes 1–1.5 h to dissolve).

Prepare 0.5 mol/l EDTA pH 8 solution: dissolve 186 g of EDTA.H_2O in 800 ml of water; add NaOH 10 mol/l solution drop-wise to obtain pH 8 (10–15 ml required; the mixture should become clear at pH 5–6); adjust to 1000 ml with water.

Prepare 6 mol/l guanidine-HCl, 0.2M EDTA, pH 8,0 buffer (GE buffer): add 200 ml of EDTA 0.5 mol/l pH 8 solution to 250 ml of the previously prepared guanidine-HCl solution in the 500-ml flask, and adjust to 500 ml by adding water.

Store GE buffer for a maximum of 4 months at ambient temperature. Longer storage will cause irreversible precipitation of guanidine salts.

Annex 10. Protocol for storage of blood samples in guanidine hydrochloride-ethylene diamine tetraacetic acid (EDTA) buffer

Purpose

Storage of blood samples in guanidine-HCl-EDTA buffer (GE buffer) for DNA extraction

Precautions

- **Safety**: All blood specimens should be treated as if they were a potential source of hepatitis or HIV infection. Universal precautions should be followed.
- **Avoid contamination**. Molecular diagnostics are highly prone to contamination. Always use disposable materials, and change protecting gloves often.

Background

For molecular diagnostics like polymerase chain reaction (PCR) or similar, the specimen should be collected in such a way as to avoids degradation of the target DNA before extraction from the specimen.

Mixing an equal volume of blood with an equal volume of GE buffer will preserve good DNA quality for several months at 4–8 °C.

Reagents

- GE buffer prepared as described in *Annex 10*, necessary toxic materials
- material for venous blood collection
- syringe
- tourniquet
- disinfecting solution (75% alcohol or similar)
- cotton
- latex gloves
- waste container
- box for 9 x 9 tubes of 2 ml
- microtubes of 2 ml with screwcap, filled with 0.5 ml GE buffer
- marker

Protocol

Collect venous blood, and dispense into the tube with GE buffer up to one graduation line (0.5 ml). **Do not dispense more than 0.5 ml of blood**.

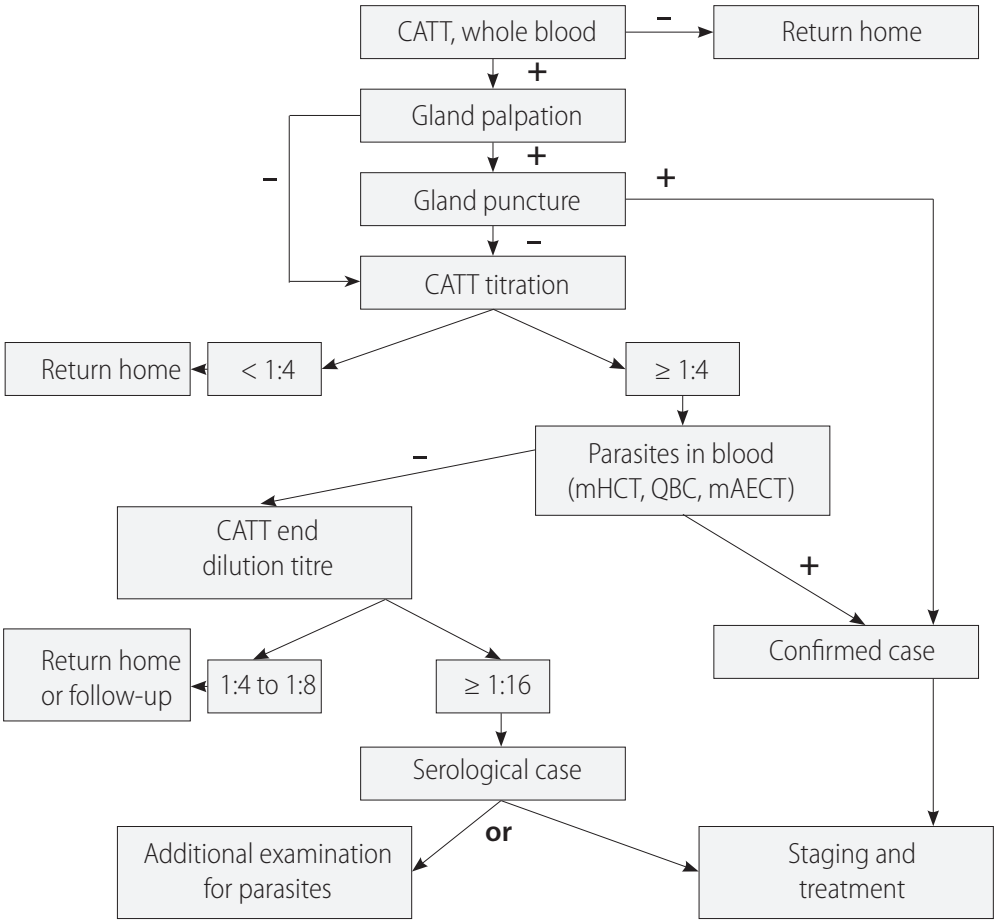
Close the tube tightly with the screw cap, and mix the blood with the GE buffer thoroughly.

Label the microtube with the person's code and the collection date.

Change latex gloves when contaminated with blood.

The tubes may be stored for several months in a refrigerator before DNA extraction.

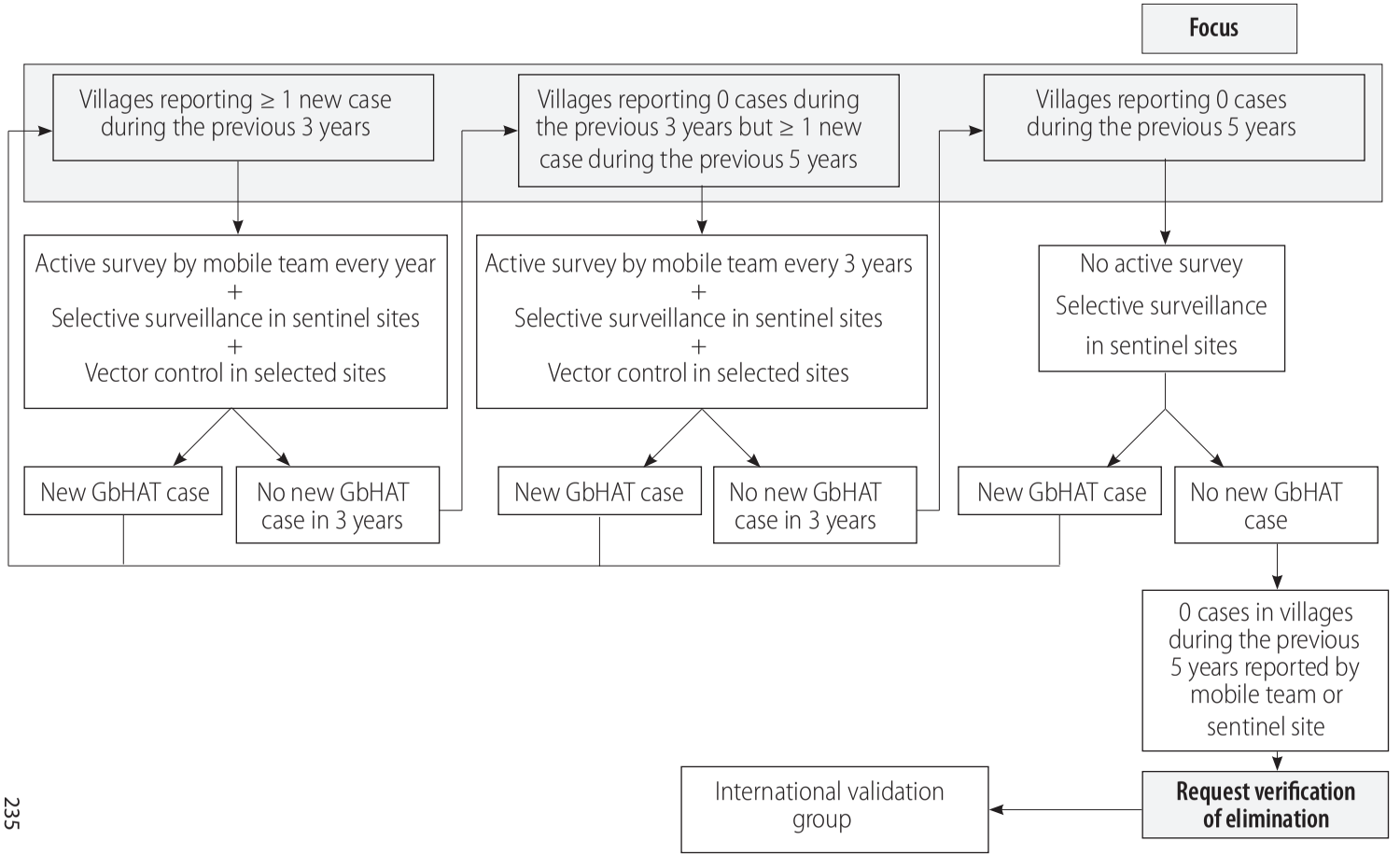
Annex 11. Example of a field algorithm for diagnosis of *Trypanosoma brucei gambiense* infection



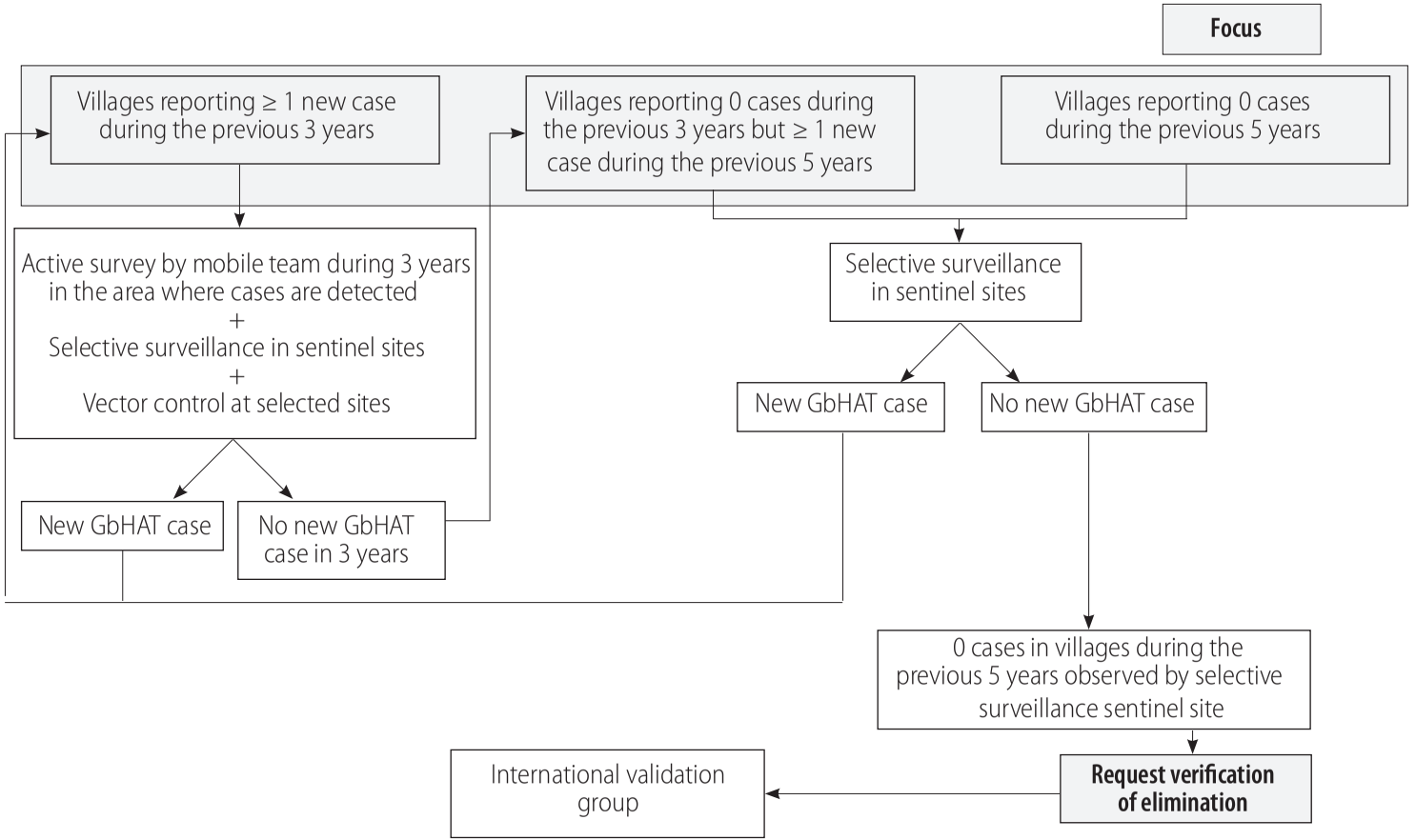
Source: Simarro P, Louis FJ, Jannin J. Sleeping sickness, forgotten illness: what are the consequences in the field? *Médécine Tropicale*, 2003, 63:231–235.

CATT, card agglutination test for trypanosomiasis; mAECT, mini-anion exchange centrifugation technique; mHCT, micro-haematocrit centrifugation technique; QBC, quantitative buffy coat

Annex 12. Strategy for the elimination of gambiense human African trypanosomiasis (GbHAT) in foci with high or moderate transmission (annual incidence > 1 case per 10 000 inhabitants in previous 5 years)



Annex 13. Strategy for the elimination of gambiense human African trypanosomiasis (GbHAT) in foci with low transmission (annual incidence > 1 case per 1 000 000 and < 1 case per 10 000 inhabitants in previous 5 years)



Annex 14. Strategy for the elimination of gambiense human African trypanosomiasis (GbHAT) in foci with no cases reported in previous 5 years