Human African trypanosomiasis

Philippe Büscher, Giuliano Cecchi, Vincent Jamonneau, Gerardo Priotto

Human African trypanosomiasis (sleeping sickness) is a parasitic infection that almost invariably progresses to death unless treated. Human African trypanosomiasis caused devastating epidemics during the 20th century. Thanks to sustained and coordinated efforts over the past 15 years, the number of reported cases has fallen to an historically low level. Fewer than 3000 cases were reported in 2015, and the disease is targeted for elimination by WHO. Despite these recent successes, the disease is still endemic in parts of sub-Saharan Africa, where it is a considerable burden on rural communities, most notably in central Africa. Since patients are also reported from non-endemic countries, human African trypanosomiasis should be considered in differential diagnosis for travellers, tourists, migrants, and expatriates who have visited or lived in endemic areas. In the absence of a vaccine, disease control relies on case detection and treatment, and vector control. Available drugs are suboptimal, but ongoing clinical trials provide hope for safer and simpler treatments.

Lancet 2017; 390: 2397–409

http://dx.doi.org/10.1016/S0140-6736(17)31510-6

Department of Biomedical Sciences, Institute of Tropical Medicine, Antwerp, Belgium (Prof P Büscher PhD); **Food and Agriculture Organization of the United Nations, Sub-regional Office for Eastern Africa, Addis Ababa, Ethiopia** (G Cecchi PhD); **UMR INTERTRYP, Institut de Recherche pour le Développement, Montpellier, France** (V Jamonneau PhD); **and World Health Organization, Control of Neglected Tropical Diseases, Innovative and Intensified Disease Management, Geneva, Switzerland** (G Priotto MD).

Correspondence to:
Prof Philippe Büscher, Institute of Tropical Medicine, Nationalestraat 155, 2000 Antwerp, Belgium
pbuscher@itg.be

Introduction

Human African trypanosomiasis is a neglected tropical disease that occurs in sub-Saharan Africa, within the distributional limits of the vector, the tsetse fly. Two forms of the disease exist: the slow-progressing form, caused by *Trypanosoma brucei gambiense*, which is endemic in western and central Africa; and, the faster-progressing form, caused by *Trypanosoma brucei rhodesiense*, found in eastern and southern Africa.[1]

Since the beginning of the 20th century, human African trypanosomiasis has killed millions of people. Today, the disease is rare, thanks to large-scale and efficient deployment of an—albeit incomplete—arsenal of control tools. Yet, cases are reported from more than 20 countries in Africa, where the disease causes substantial morbidity among the affected rural populations, and continues to pose the threat of severe epidemics.[2] In a globalised world, cases are also diagnosed outside endemic African countries among travellers, tourists, expatriates, and migrants.[3] In this Seminar, we discuss the epidemiology, cause, clinical features, diagnosis, and treatment of human African trypanosomiasis, and touch on epidemiological surveillance and methods of control and elimination.

Epidemiology

The trypanosomes that cause human African trypanosomiasis are classically transmitted by the bite of blood-sucking tsetse flies (Diptera, genus *Glossina*). *T brucei gambiense* can also be transmitted congenitally.[4-6] Other routes of transmission are possible but poorly documented, and are thought to be very rare (eg, sexual, laboratory accidents, blood transfusion, and organ transplantation).[6-9]

In the early 20th century, devastating epidemics occurred in, among other places, Uganda, Congo Free State (now emocratic Republic of the Congo), Cameroon, and other western African countries, which were probably triggered by ecological disruptions and forced population movements brought about by colonialism.[10] Since then, the intensity of control efforts and extent of *T brucei* transmission have been closely linked. In some endemic areas, changes in land use and climate dramatically reduced tsetse populations and interrupted *T brucei* transmission.[11] Neglecting human African trypanosomiasis, either because of social or political instability or the tyranny of success, will inevitably lead to resurgence. The last alarming peak in transmission occurred in the late 1990s, and robust and coordinated efforts were required to bring about disease control.

In 2015, 2804 cases of human African trypanosomiasis were reported to WHO, of which 2733 were caused by *T brucei gambiense* (90% reduction since 1999) and 71 were caused by *T brucei rhodesiense* (89% reduction since 1999); this number includes cases diagnosed in both endemic and non-endemic countries. The bulk of the case load of *T brucei gambiense* disease continues to be in the Democratic Republic of the Congo (86% of cases), followed by the Central African Republic and Chad (5% and 2%, respectively). As of 2015, these countries were the only ones to report more than 50 cases per year. However, the probable under-detection of cases of human African trypanosomiasis should be taken into account when reported incidence is assessed. For example, in South Sudan, an area of civil unrest, and Guinea, an area of Ebola virus disease outbreak, the

Search strategy and selection criteria

We searched PubMed on Dec 1, 2016, for publications written in English or French, using the keywords: "sleeping sickness", "*Trypanosoma brucei gambiense*", "*Trypanosoma brucei rhodesiense*", "CATT", "suramin", "pentamidine", "melarsoprol", "eflornithine", "tsetse", "*Glossina*", or "human African trypanosomiasis". Results were limited to those published between 2010 and 2016. Among the almost 3000 references, we selected those we judged relevant, prioritising those reporting applied research. An additional source was the Programme Against African Trypanosomosis Tsetse and Trypanosomosis Information Bulletin (2010–2015), edited by the Food and Agriculture Organization of the United Nations. Additional references were retrieved from the personal databases of all coauthors.

reported and actual incidence of disease might differ considerably. The case load of *T brucei rhodesiense* disease is concentrated in Malawi and Uganda, which account for 82% of cases.[12]

Although animal African trypanosomiasis, or *nagana*, is widespread in all tsetse-infested areas, human African trypanosomiasis is characterised by a markedly focal distribution (figure 1). This patchy distribution is the result of complex interactions between parasite, vector, host, and the environment, which are not yet fully understood. The disease is usually found in rural areas with suitable habitats for the tsetse fly vector and frequent human–tsetse contact. Periurban areas can also be affected, especially where riverine tsetse species have adapted to anthropic environments.[17–19] People can be infected while farming, fishing, hunting, collecting water or wood, or engaging in any other activity that exposes them to the bite of an infective tsetse fly. All age groups and both sexes are at risk, although prevalence is higher in adults, and sex distribution varies in relation to gender-specific at-risk activities (eg, the predominantly male activities of hunting and fishing, or the predominantly female activities of water fetching and small-crop growing).

Human beings are thought to be the main reservoir of *T brucei gambiense*, and domestic and wild animals the main reservoirs of *T brucei rhodesiense*. Although domestic and wild animals can also host *T brucei gambiense*, their epidemiological role remains unclear.[20] For people, *T brucei rhodesiense* infection leads to death within 6 months.

Exported cases of human African trypanosomiasis are reported from all continents,[3] with most cases being *T brucei rhodesiense* disease in tourists who have visited national parks and game reserves in Tanzania, but also in Kenya, Malawi, Uganda, Zambia, and Zimbabwe. Exported cases of *T brucei gambiense* disease are rarer, and include migrants, refugees, and long-term expatriates. Exceptionally long periods (up to 30 years, and possibly more) can separate infection and diagnosis;[21,22] thus, *T brucei gambiense* disease should be considered in differential diagnosis in all people who have ever lived in endemic countries.

Parasite and vector

T brucei belongs to the Trypanosomatidae family of exclusively parasitic organisms found in vertebrates and

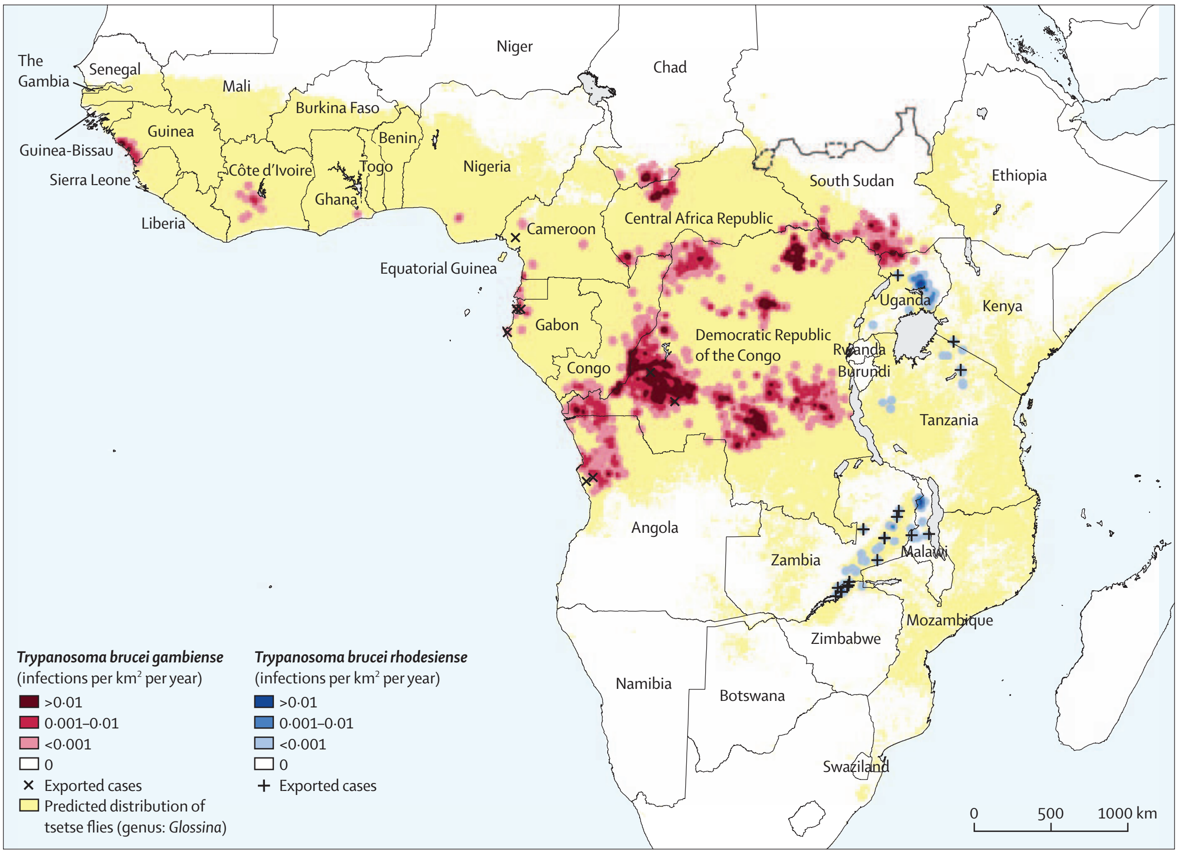

***Figure 1:* Geographical distribution of reported infections of human African trypanosomiasis (reporting period 2010–14)**
Trypanosoma brucei gambiense disease is found in western and central Africa, whereas *Trypanosoma brucei rhodesiense* disease is found in eastern and southern Africa. The source of reported infections is the WHO Atlas of human African trypanosomiasis.[1,13] The density of reported infections (ie, the number of reported infections per km² per year) was obtained from village-level data by kernel smoothing,[14] with a search radius of 30 km.[15] Exported cases (ie, those diagnosed in non-endemic countries) are mapped in the probable place of infection.[3] The predicted distribution of tsetse flies was provided by the Programme against African Trypanosomosis.[16]

insects worldwide.[23] These unicellular parasites have co-evolved with their hosts to such an extent that most are commensal rather than pathogenic.[24] *T brucei* includes three morphologically indistinguishable subspecies (figure 2): *T brucei brucei*, which causes animal African trypanosomiasis, and is not infective to human beings, whereas *T brucei rhodesiense* and *T brucei gambiense* can infect people because they are resistant to apolipoprotein A1 (a serum protein that triggers death in other trypanosomes).[26,27] All *T brucei* cells contain a nucleus, a mitochondrion that contains the kinetoplast, and a flagellum attached to the cell by an undulating membrane. During its lifecycle (figure 3), which alternates between a mammalian and an insect (tsetse fly) host, *T brucei* remains extracellular and undergoes important metabolic adaptations that are reflected by morphological changes.

In the blood and tissues of mammals, trypanosomes are most often observed as spindle-shaped cells that are 20–30 μm long (about three times the diameter of a human erythrocyte) and 2–5 μm wide, and are characterised by their wriggling movement. Sometimes, shorter forms are observed: these are metabolically preadapted to survive in the tsetse intestines (figure 4). In the mammalian host, the trypanosome cell membrane is covered by a dense coat of identical glycoprotein dimers that shields the underlying membrane against innate immunological attacks, such as by complement. These highly immunogenic glycoproteins induce a specific antibody response that triggers the destruction of all antibody-opsonised trypanosomes. To survive this antibody-mediated immune response, trypanosomes developed antigenic variation, whereby the glycoprotein coat is replaced by an antigenically different coat. Between 1×10^{-3} and 1×10^{-6} coat switches are estimated to occur per population doubling within the mammalian host.[28,29] The interplay between the immune response of the host and antigenic variation of the parasite results in irregular fluctuations in parasitaemia, reflected by intermittent fevers accompanying destruction of trypanosomes. *T brucei* infection usually induces polyclonal B-cell activation, resulting in extremely high IgM concentrations (up to 14 times normal values) and various non-trypanosome-specific antibodies, including autoantibodies. These antibodies, both specific and non-specific, take part in the pathogenesis of the infection and cause non-specific reactions in antibody detection tests for other infections.[30–32] Infection of mammalian hosts starts with the injection of metacyclic trypanosomes, together with tsetse saliva, into the skin (figure 2). After several days of local multiplication, the trypanosomes spread via the lymph and blood to various peripheral organs and tissues. The parasites then invade the brain parenchyma, where they trigger local inflammation and neurological damage.[33] The parasite's journey through the mammalian host is both accompanied and regulated by complex and diverse immunological reactions, some of which are pathogenic and induced by components of the parasite and tsetse fly saliva.[34]

Figure 2: **False-coloured scanning electronic microscopy image, 14 μm × 14 μm, showing trypanosomes (purple) and an adipocyte (green) in the ear dermis of a *Trypanosoma brucei*-infected mouse**
Adapted with permission from David Peréz-Morga and Marjorie Vermeersch (Université Libre de Bruxelles, Gosselies, Belgium), Guy Caljon (Antwerp University, Antwerp, Belgium), and Jan Van den Abbeele (Institute of Tropical Medicine, Antwerp, Belgium).[25]

T brucei depends on tsetse flies for its cyclical transmission (video 1). Both sexes are haematophagous (blood-feeding) and can transmit trypanosomes. Tsetse flies are viviparous, and the female deposits a fully developed larva that burrows into the soil, pupates, and emerges as an adult fly a month later. The 31 tsetse species and subspecies are classified as forest, riverine, or savannah, according to morphological differences and habitat preference.[35] *Glossina fuscipes* and *Glossina palpalis* (from the palpalis riverine group) are the main vectors of *T brucei gambiense*.[36,37] For *T brucei rhodesiense*, the main vectors are *G f fuscipes* (in Uganda) and the savannah-group species, which include *Glossina morsitans* and *Glossina pallidipes*.[38,39] Tsetse flies are infected with *T brucei* when they ingest trypanosomes in the blood or, as shown in experimental infections, in the skin of mammals.[25,40] Once ingested, the short stumpy trypanosomes undertake a complex journey through the fly tissues, until they reach the salivary glands and develop into the human-infective metacyclic forms, which are free-swimming and resemble the short stumpy form in shape.[41] In a natural population of tsetse flies, only a small proportion (about 0·01%) carry a mature infection of *T brucei* (ie, with metacyclic trypanosomes in the salivary glands[42,43]), but a tsetse fly, feeding every 3 days, can infect several people during its 2–3-month lifespan. Eliminating the tsetse or reducing contact between tsetse and human beings is one way to reduce or interrupt transmission of *T brucei*.

See **Online** for video 1

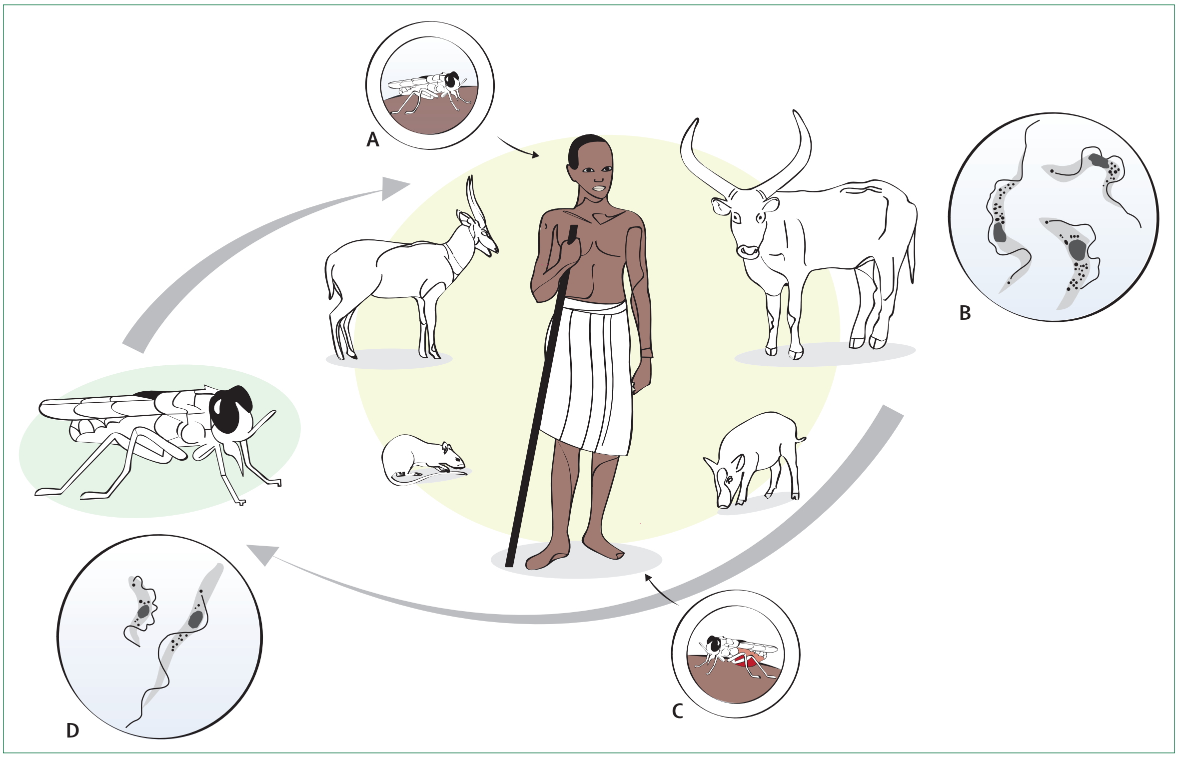

Figure 3: Lifecycle of *Trypanosoma brucei*
(A) Metacyclic trypanosomes are injected into the skin of a mammalian host, together with saliva containing anticoagulant factors. (B) Once in the mammalian host, trypanosomes transform into dividing long slender forms that, via lymph and blood, can infiltrate tissues and organs, including the brain parenchyma. Some transform into a non-dividing short stumpy form. (C) A tsetse fly is infected by taking blood from a human being or other mammal that contains stumpy trypanosomes. (D) After about 2 weeks, trypanosomes might have colonised the salivary glands, producing free-swimming metacyclic trypanosomes that can then be transmitted to the next mammalian host. Source: © Food and Agriculture Organization of the United Nations. Reproduced with permission.

Clinical features

The clinical presentation of human African trypanosomiasis depends on the *T brucei* subspecies, host response, and disease stage. Variations of virulence and pathogenicity are attributed to different strains of parasite.[44,45] Generally, both forms of the disease lead to death if untreated; although, for *T brucei gambiense* disease, healthy carriers and self-cure have been described.[46] *T brucei rhodesiense* disease is typically acute, progressing to second-stage disease within a few weeks, and death within 6 months.[47,48] *T brucei gambiense* disease follows a chronic progressive course, with a mean duration estimated at 3 years, albeit with high interpersonal variability.[49]

The disease goes through two stages: a haemolymphatic stage followed by a meningoencephalitic stage in which trypanosomes cross the blood–brain barrier and invade the CNS. Neurological disturbances, including sleep disorder, are typical of second-stage disease; however, most signs and symptoms are common to both stages.

A 3–4-cm dermal reaction at the site of the tsetse bite (inoculation chancre) appears within 2–3 days in 5–26% of Bantu people who contract *T brucei rhodesiense;* in people from other regions, this reaction occurs more frequently. This reaction is rarely seen in those with *T brucei gambiense* disease.[44,50]

First-stage *T brucei gambiense* disease presents predominantly with intermittent fever lasting 1 day to 1 week, separated by intervals of days or months, as well as headache, pruritus, and lymphadenopathy (mainly posterior cervical but also possible in the axillar, inguinal, and epitrochlear regions). Hepatosplenomegaly, oedema, and endocrine dysfunction (amenorrhoea, infertility, and miscarriage in women; reduced libido and impotence in men) present less frequently.

In second-stage disease, neuropsychiatric disorders accompany first-stage features, and fever becomes less frequent. The characteristic sleep disorder, from which the name sleeping sickness is derived, consists of daytime somnolence and sudden overwhelming sleep urges, and nocturnal insomnia. Polysomnographic records show a disruption of the sleep–wake cycle with frequent, short, sleep-onset rapid eye movement episodes that are equally likely to occur during day and night.[51–53]

Other neurological signs include: hypertonicity or hypotonicity; tremor of the hands and fingers; choreiform, athetoid, or oscillatory movements of limbs or trunk; fasciculation; motor weakness; ataxia; akinesia; and, speech disorders. Perioral and cheiro-oral reflexes are frequently seen. Mental changes are common and include emotional lability, attention deficit, indifference, apathy, aggressive behaviour, stereotypic behaviour, dissociative fugue, manic episodes, melancholia, confusion, and dementia. Neuropsychiatric symptoms increase in frequency and severity with disease progression.[54] Trypanosome infiltration of endocrine organs (mainly thyroid and adrenals) and the hypothalamic–hypophysial axis lead to disruption of circadian rhythms of hormonal secretion, including prolactin, renin, growth hormone, and cortisol[51] but generally do not require specific treatment. Cardiac alterations are common but do not have the same clinical relevance as in Chagas disease (American trypanosomiasis). Cardiac alterations develop early, with electrocardiogram abnormalities consistent with perimyocarditis (QT-interval prolongation, repolarisation changes, and low voltage) being most common.[55] In *T brucei gambiense* disease, these alterations are generally mild; in *T brucei rhodesiense* disease, earlier and more severe perimyocarditis and congestive cardiac failure are reported.[56]

The clinical features of *T brucei rhodesiense* disease are similar to those of *T brucei gambiense* disease, but trypanosomal chancre occurs more frequently, often with satellite lymphadenopathy. Fever presents in both disease stages, and more frequently in children than adults.[57] Enlarged lymph nodes tend to be submandibular, axillary, and inguinal in *T brucei rhodesiense* disease, rather than posterior cervical as in *T brucei gambiense* disease; additionally, oedema is reported more frequently in the former than the latter. Thyroid dysfunction, adrenal insufficiency, and hypogonadism are more common than in *T brucei gambiense* human African trypanosomiasis, and myocarditis is more severe and can be fatal. Liver involvement with hepatomegaly and jaundice are frequent but usually moderate, sometimes with ascitis, and generally occur less frequently in *T brucei gambiense* disease.[58] In southeastern African countries, in particular Malawi, a more chronic form has been reported: it has a longer first stage, with fewer neurological disorders and an absence of chancre.[44] Compared with locals, in travellers from non-endemic countries the incubation period is shorter (<3 weeks for *T brucei rhodesiense* human African trypanosomiasis and <1 month for *T brucei gambiense* human African trypanosomiasis) and the clinical picture is acute and febrile from the onset, regardless of the subspecies. A trypanosomal chancre is reported more frequently (88% in *T brucei rhodesiense* disease and 56% in *T brucei gambiense* disease) and a rash, consisting of non-itching, irregular erythematous macules of up to 10 cm in diameter often with a central area of normally coloured skin, develops in a third of cases.[59] The rash might last several weeks, vanishing and reappearing in different areas.[60] Headache, lymphadenopathy, hepatomegaly, and splenomegaly occur in a quarter to half of patients. In travellers with *T brucei rhodesiense* disease, gastrointestinal symptoms are more frequent, with jaundice reported in 28% of cases. Less frequent but severe complications include renal failure requiring haemodialysis, multi-organ failure, disseminated intravascular coagulopathy, and coma.[3,61]

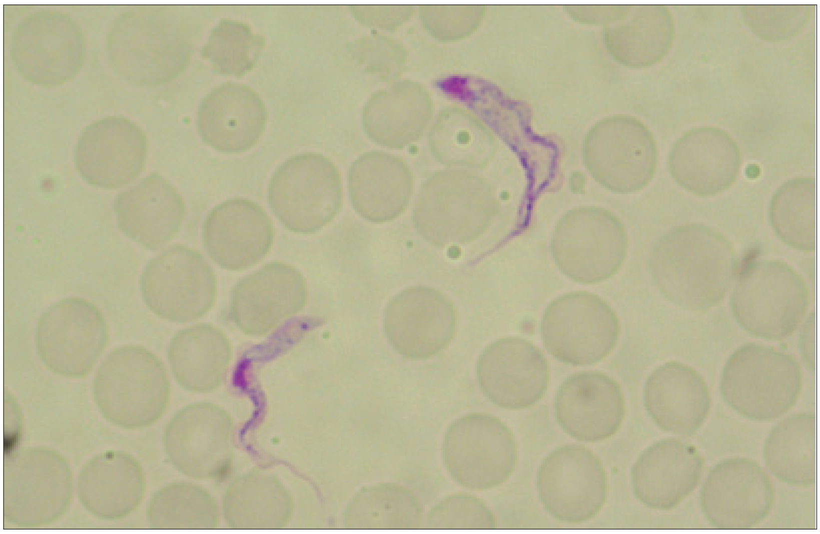

Figure 4: **Giemsa-stained thin blood film with one long slender (left side) and one short stumpy trypanosome (right side)**

Diagnosis

Clinical signs and symptoms of human African trypanosomiasis are unspecific and can easily be mistaken for those of other diseases; thus, they are often insufficient for diagnosis.

Reliable serodiagnostic tests exist only for *T brucei gambiense* infection, and are based on the detection of specific antibodies. The card agglutination test for trypanosomiasis (CATT),[62] developed almost 40 years ago, has been pivotal in the control of *T brucei gambiense* disease. CATT can be done with blood collected from a finger prick, plasma, or serum, and the agglutination reaction is scored visually after 5 min. It is particularly suited for screening of at-risk populations by mobile teams.[63]

Recently, rapid diagnostic tests for *T brucei gambiense* infection were developed and introduced: the HAT Sero-*K*-SeT (Coris BioConcept, Gembloux, Belgium) and the SD Bioline HAT 1.0 (Standard Diagnostics, Yongin, South Korea).[64–66] The major advantage, over CATT, is that these tests fully comply with the ASSURED (Affordable, Sensitive, Specific, User-friendly, Rapid and robust, Equipment-free and Deliverable to end-users) criteria;[67] therefore, they are more suitable for passive screening and surveillance in fixed health centres in endemic countries that often lack electricity and laboratory infrastructure.[68] Second-generation cassette and strip-format rapid diagnostic tests, including recombinant antigens, are under development.[69–71]

Although useful for screening of at-risk populations and identification of individuals as probably infected with *T brucei gambiense*, CATT and rapid diagnostic tests are not 100% specific.[72] Particularly when disease prevalence is low, their positive predictive value becomes critically low[73]—eg, with a specificity of 98% and a prevalence of 0·1%, the positive predictive value is 4·5%. Currently, in most human African trypanosomiasis foci, prevalence is far below 0·1% and serological screening tests yield about 99 false-positive results for every true positive.

Immune trypanolysis and ELISAs are applicable in laboratory conditions on serum, plasma, and dried blood spots.[31,72,74,75] Their high specificity and sensitivity, their applicability to dried blood spots, and adaptability to animal specimens make them excellent tools for large-scale surveys, post-elimination monitoring, and animal reservoir studies.[76,77]

No field-applicable serodiagnostic test exists for *T brucei rhodesiense* infection. Efforts to develop second-generation rapid diagnostic tests able to detect both *T brucei gambiense* and *T brucei rhodesiense* infection are ongoing, but the risk of cross-reaction with antibodies against non-human infective trypanosomes is ever-present.[68,69] Because cases of *T brucei rhodesiense* disease usually present with high levels of parasitaemia, antibody detection is less relevant.[36] Parasitological confirmation of *T brucei gambiense* infection is achieved by microscopic examination of a lymph node aspirate (video 2) or by concentration techniques applied on blood (mini-anion exchange centrifugation technique or microhaematocrit centrifugation technique) (video 3) or on cerebrospinal fluid (modified single centrifugation technique).[77–82] Importantly, to detect the colourless, motile trypanosome at low magnification (10×10, 16×10, or 10×40), the microscope should be adjusted for maximum light diffraction. The diagnostic sensitivity of these techniques is suboptimal (maximum 90%) although the analytical sensitivity of, for example, the mini-anion exchange centrifugation technique is less than 50 trypanosomes per mL of blood.[83] For *T brucei rhodesiense* infection, which usually presents with higher parasitaemia, stained blood thin film or thick drop, or chancre aspirate can be considered if the more sensitive concentration techniques are not available.

See **Online** for video 2

See **Online** for video 3

See **Online** for appendix

Stage determination (ie, assessment of neurological involvement) relies on the examination of cerebrospinal fluid collected by lumbar puncture.[84] Patients with five or fewer white blood cells per μL and no trypanosomes in the cerebrospinal fluid are classified as first stage, and those with more than five white blood cells per μL or trypanosomes in the cerebrospinal fluid as second stage.[36] Other markers for neuroinflammation (eg, intrathecal IgM and neopterin) have been proposed for improved stage determination; however, the added value of IgM is minimal and the quantification of neopterin is not currently possible under field conditions.[85,86]

Molecular diagnosis of human African trypanosomiasis, as a surrogate for microscopic parasite detection, has been the subject of numerous investigations but should be interpreted with caution in clinical practice, even for exported cases.[87] All formats have poor diagnostic accuracy (even for stage determination and post-treatment follow-up), poor reproducibility, and incompatibility with diagnostic facilities in endemic countries.[88–90] In some instances, most notably in the context of disease elimination, identification of the subspecies of *T brucei* (eg, in tsetse, animals, and human beings) might be useful since atypical infections with animal trypanosomes are possible.[37,91–97] *T brucei gambiense*-specific and *T brucei rhodesiense*-specific PCR assays exist, but they target single-copy genes; hence, their analytical sensitivity is poor.[98,99]

Because diagnosis of human African trypanosomiasis is a specialty and techniques are not common, technical assistance and reference testing can be sought from the two WHO Collaborating Centres for human African trypanosomiasis (ie, the Institute of Tropical Medicine in Antwerp, Belgium, and the Institut de Recherche pour le Développement, based at the Centre International de Recherche et Développement sur l'Élevage en zones Subhumides, in Bobo Dioulasso, Burkina Faso).[100]

Treatment

Five drugs are routinely used in the treatment of human African trypanosomiasis: pentamidine and suramin to treat first-stage disease, and melarsoprol, eflornithine, and nifurtimox for second-stage disease. All are donated by the manufacturers, and WHO ensures their worldwide distribution free of charge. The drugs can be obtained directly from WHO in Geneva or from the few institutes that stock them (appendix).[3] This accessibility means treatment of human African trypanosomiasis is unaffected by counterfeit and substandard drugs.

The earlier human African trypanosomiasis is treated, the better the prospects of treatment tolerability and cure. The choice of treatment depends on the causative agent and disease stage (table). Drugs for the treatment of first-stage disease will generally not cure second-stage disease. Similarly, the use of second-stage drugs is not justified in the treatment of first-stage disease because the second stage requires drugs that cross the blood–brain barrier, and such drugs tend to be more toxic and complex to administer than first-stage drugs.

First-stage treatment

Pentamidine isethionate is the first-line treatment for first-stage *T brucei gambiense* disease, and is an alternative treatment for *T brucei rhodesiense* disease; however, data on its efficacy in the latter role are limited.[59,101] The efficacy of pentamidine against *T brucei gambiense* disease (95–98%) has been stable for decades: it is given intramuscularly once daily for 7 days but can also be given as an intravenous infusion in saline over 2 h. In endemic countries, pentamidine is more commonly delivered via intramuscular injection. Administration should be preceded by the ingestion of sugar (10–20 g) to prevent hypoglycaemia, and followed by rest in the supine position for 1–2 h to prevent the effects of hypotension. Pentamidine is generally well tolerated. The intramuscular injection causes pain and transient swelling. Other adverse events include hypoglycaemia (5–40%), hypotension, abdominal pain, and gastrointestinal problems.[102]

Another treatment, suramin, is effective in the first stage of *T brucei gambiense* disease and *T brucei rhodesiense* disease, but is used only in the treatment of *T brucei rhodesiense* disease because of the risk of onchocerciasis co-infection in *T brucei gambiense*-endemic areas (ie, risk of allergic reactions arising from rapid killing of microfilaria), and because pentamidine administration is simpler. Suramin is administered as a slow intravenous infusion. It deteriorates rapidly in air and should be injected immediately after dilution. Recommended treatment schedules are complex and last up to 1 month. A test dose is administered before treatment because of the risk of acute hypersensitivity reactions. Adverse effects are frequent but mostly mild and reversible, and include pyrexia, nephrotoxicity, peripheral neuropathy, agranulocytosis, and thrombocytopenia.

	First-line treatment	Dosage	Alternative treatment and dosage
Trypanosoma brucei gambiense			
First stage	Pentamidine	4 mg/kg per day intramuscularly or intravenously (diluted in saline, in 2-h infusions) × 7 days	..
Second stage	Nifurtimox–eflornithine combination therapy	Nifurtimox 15 mg/kg per day orally in three doses × 10 days; eflornithine 400 mg/kg per day intravenously in two 2-h infusions (each dose diluted in 250 mL of water for injection)* × 7 days	Eflornithine 400 mg/kg per day intravenously in four 2-h infusions (each dose diluted in 100 mL of water for injection)* × 14 days; third-line (eg, treatment for relapse) is melarsoprol 2·2 mg/kg per day intravenously × 10 days
Trypanosoma brucei rhodesiense			
First stage	Suramin	Test dose of 4–5 mg/kg intravenously (day 1), then 20 mg/kg intravenously once per week × 5 weeks (maximum 1 g/injection—eg, days 3, 10, 17, 24, and 31)	Pentamidine 4 mg/kg per day intramuscular or intravenously (diluted in normal saline, in 2-h infusions) × 7 days
Second stage	Melarsoprol	2·2 mg/kg per day intravenously × 10 days	..

*Children weighing <10 kg: dilute in 50 mL of water for injection. Children weighing 10–25 kg: dilute in 100 mL of water for injection. If water for injection is unavailable, eflornithine can be diluted in 5% dextrose or saline.

***Table*: Standard treatment for human African trypanosomiasis by form and stage**

Second-stage treatment

The first-line treatment for second-stage *T brucei gambiense* disease is nifurtimox–eflornithine combination therapy (NECT). In 2009, NECT was included in the WHO Essential Medicines List. Compared with melarsoprol or eflornithine monotherapy, NECT has higher cure rates (95–98%), lower fatality rates (<1%), less severe adverse events, simpler administration, and is believed to avoid causing drug resistance of the parasite.[103–106] Nifurtimox is not licensed for African trypanosomiasis (only for American trypanosomiasis); thus, it can only be used to treat patients with African trypanosomiasis off label, subject to express authorisation and acceptance of responsibility by national authorities. WHO supplies endemic countries, free of charge, with a full NECT kit containing all drugs and materials needed for administration. NECT consists of nifurtimox delivered orally and eflornithine delivered intravenously. A dose of nifurtimox should be readministered if vomiting occurs within 30 min. With 14 infusions, instead of the 56 used in eflornithine monotherapy, NECT is easier to administer, demanding fewer hospital resources and reducing costs. Although the short half-life of eflornithine theoretically requires four daily infusions for a constant trypanostatic effect, infusions every 12 h are highly effective when combined with oral nifurtimox. The most common treatment-emergent adverse events are abdominal pain, vomiting, and headache.[103,104,106–109] The toxicity profile replicates that of nifurtimox and eflornithine monotherapies, but with lower frequency and severity, most likely because of the shorter drug exposure. NECT is better tolerated in children than in adults, and is generally better tolerated than eflornithine and melarsoprol monotherapies.

Eflornithine (α-difluoromethylornithine or DFMO) is given as a monotherapy for *T brucei gambiense* disease when the companion drug nifurtimox is unavailable or contraindicated. It is a cytostatic (ie, affects the host's cells) and trypanostatic (ie, affects trypanosome metabolism) drug. An active immune system is required to achieve cure: in patients who are immunocompromised, complete parasite elimination might not be achieved with eflornithine alone.[110] Eflornithine as monotherapy is given as a slow intravenous infusion for 14 days (56 infusions in total). In resource-poor settings this burdensome schedule is challenging and imposes specific care to prevent catheter-related infections. A 7-day regimen showed insufficient efficacy in children and adults,[111] and a higher dose (600 mg/kg per day *vs* 400 mg/kg per day) in children younger than 12 years did not improve effectiveness.[112] Higher doses were not tested in adults. Eflornithine monotherapy has proved effective against *T brucei gambiense* disease (90–95% cure rate) but is not recommended for *T brucei rhodesiense* disease.[103,113,114] Adverse events are frequent and similar to those of other cytostatics (including diarrhoea and neutropenia) but eflornithine is, on the whole, safer than melarsoprol, with fatality rates below 2%.[113] The main adverse events are fever, pruritus, hypertension, nausea, vomiting, diarrhoea, abdominal pain, headaches, myelosuppression (anaemia, leucopenia, thrombocytopenia), and, more rarely, seizures that are generally isolated and respond to treatment.

Another treatment option is melarsoprol, but owing to the high frequency of severe and life-threatening adverse drug reactions, and the availability of better alternatives, melarsoprol is restricted to the treatment of second-stage *T brucei rhodesiense* disease. In *T brucei gambiense* disease, the only remaining indication is the treatment of relapse after NECT or eflornithine monotherapy. The most important serious reaction is an encephalopathic syndrome that occurs in 5–18% of all treated cases and is fatal in 10–70% of affected patients.[115] Both the incidence and fatality rates of melarsoprol are higher in people with *T brucei rhodesiense* disease than those with *T brucei gambiense* disease,[116] with fatality rates of approximately 9%[117] and 6%,[118] respectively. Coadministration of prednisolone might have a protective effect against the immune reaction thought to be a component of the encephalopathic syndrome. The encephalopathic syndrome usually occurs between 7 and 14 days after the first injection and is characterised by fever and convulsions, rapid onset of neurological disorders, progressive coma, or abnormal behaviour.[119] Close monitoring of patients might allow detection of early signs, such as fever or headache, or both, leading to the cessation of melarsoprol and management with dexamethasone and diazepam.[120] Other frequent adverse reactions include pyrexia, headache, general malaise, gastrointestinal (nausea, vomiting, and diarrhoea), and skin reactions (pruritus); severe complications, such as exfoliative dermatitis, occur in less than 1% of cases.[119] Cardiac failure is common and can be fatal, but might be attributable to human African trypanosomiasis itself.[121]

Drug resistance

Mutations in the genome of *T brucei gambiense* that confer resistance to melarsoprol and pentamidine have been documented. In particular, melarsoprol resistance generated much concern at the turn of the century, when the failure rates rose in several human African trypanosomiasis foci.[122,123] The concern was alleviated with the introduction of NECT, which combines two drugs of different pharmacodynamics and modes of action, strongly decreasing the probability of resistance emergence.

Treatment in pregnancy

Although poorly studied, field experience has accumulated on the management of patients who are pregnant or lactating.[36] Pentamidine can be given after the first trimester of pregnancy. Nifurtimox, eflornithine, and melarsoprol, all of which are theoretically contraindicated, in practice are given when the mother is in advanced second-stage disease and her condition does not permit waiting. If postponement of treatment until childbirth is judged possible, a full course of pentamidine should be administered, principally to prevent vertical transmission. The benefits and risks should be clearly explained to the patient and her relatives. In *T brucei rhodesiense* disease, the acute clinical evolution usually precludes waiting until delivery, and suramin (also theoretically contraindicated) or melarsoprol are given. Newborn babies should be examined clinically, and their blood checked for trypanosomes. Breastfeeding should continue during treatment.

Post-therapeutic follow-up

The assessment of treatment outcome requires following up the patient for up to 24 months with laboratory examinations of body fluids, including cerebrospinal fluid, because parasites can remain viable for long periods and cause relapses. In rural Africa, such a follow-up plan is challenging and cannot be performed systematically; instead, patients are advised to consult their doctor if symptoms reappear.

New drugs in the pipeline

Two new molecules in clinical development could revolutionise human African trypanosomiasis treatment. These molecules are administered orally and are intended for treatment of both disease stages; thus, the need for stage determination is eliminated. Fexinidazole, a nitroimidazole taken orally once a day for 10 days, is in phase 2/3 clinical trials near conclusion,[124] and a benzoxaborole, called SCYX-7158, taken in a single oral dose has entered phase 2/3 clinical trials.[125]

Epidemiological surveillance

Surveillance is crucial for control of human African trypanosomiasis because of the disease's focal distribution, occurrence in remote rural areas, and capacity to re-emerge when control activities are relaxed. Control operations are resource-intensive and therefore require careful targeting. Surveillance is carried out by national control programmes, with support from WHO and other partners. Field data are collected through both active and passive case detection, and are assembled, harmonised, and geo-referenced at the village level in the Atlas of human African trypanosomiasis.[2,126,127] The atlas provides maps of disease occurrence, risk levels, control activities, exported cases, and health facilities with capacity for diagnosis and treatment.[1,3,5,128] Such maps provide crucial evidence to support the planning of control activities at national and subnational levels, and to monitor progress towards disease elimination.[129]

Importantly, human African trypanosomiasis is often under-diagnosed because of limited accuracy of diagnostic methods, insufficient staff capacities, incomplete community participation, and limited access to remote or insecure areas.

Control and elimination

In the absence of a vaccine or chemoprophylaxis, human African trypanosomiasis is controlled through case detection and treatment, and, to a lesser extent, vector control.

For *T brucei gambiense* human African trypanosomiasis, the most effective control strategy is case finding and treatment, which reduces the human reservoir and thus decreases *T brucei* transmission. Cases of *T brucei gambiense* disease are detected through active screening campaigns by mobile teams, consisting of up to eight people travelling in four-wheel drive vehicles or boats, and through passive screening in fixed health structures.[128] Diagnosis and treatment are resource-intensive activities and require specific training, which is difficult to ensure in all countries and all endemic areas. Although active mass screening has saved thousands of lives and led to a sweeping reduction in risk of human African trypanosomiasis, this labour-intensive strategy is no longer cost-effective in the numerous low-prevalence settings. Moreover, where the disease is no longer perceived as a threat, populations are reluctant to participate in repeated, time-consuming screening activities.[77,130,131] In low-prevalence settings, targeted door-to-door surveys focused on the immediate vicinities of former patients with human African trypanosomiasis may provide an alternative to mass screening, and complement passive case detection.[132] Active screening can also be performed by so-called light mobile teams, consisting of one or two people travelling on motorbikes, who can reach villages or camps that are inaccessible to four-wheel drive vehicles.[133]

In the current elimination context, it is also crucial to reinforce passive surveillance, integrating it into the general health-care system and focusing on self-presenting patients.[134,135] Because passive surveillance relies on clinical suspicion followed by serological tests, it mostly detects patients with second-stage disease, who are likely to have fed the transmission cycle for years before detection.[136] It is therefore necessary to carry out reactive screening campaigns in the probable areas of infection of these patients.

Although vector control in *T brucei gambiense* disease settings has been limited by the availability of better options, improved tools and strategies, such as low-cost small insecticide-treated screens (so-called tiny targets), have enhanced traditional disease control in some epidemiological settings.[137] Other tsetse control tools, such as insecticide-treated cattle, also exist and can be cost effective in the appropriate settings and in a One Health framework.[138,139] To date, insecticide resistance has not been reported in tsetse. For *T brucei rhodesiense* disease, control of the domestic animal reservoir is key. Blanket treatment of cattle, the reservoir, and amplifier closest to human beings, and insecticide application on these animals, have been used to contain epidemics.[140,141] Other methods include targeted bush clearing, aerial or ground spraying of insecticide, insecticide-impregnated nets and screens, fly traps, and release of sterile male tsetse. Integration of several methods in a combined approach is recommended.[142] By contrast, controlling the wild animal reservoir is far more challenging.

Travellers to endemic areas can take measures to prevent tsetse bites—eg, avoiding specific places known as tsetse habitats, travelling in vehicles with screens or closed windows, wearing clothes with long sleeves, and not wearing dark colours (especially blue and black). Insect repellents provide little protection.

In the context of steady progress against human African trypanosomiasis (85% reduction in cases reported in the past 16 years), WHO targeted the elimination of the disease as a public health problem by 2020. Beyond that, vulnerabilities in the transmission cycle and the focal distribution of *T brucei gambiense* disease make the interruption of transmission possible (WHO target for 2030). By contrast, the interruption of *T brucei rhodesiense* transmission does not seem attainable with the available tools.

Despite recent advances, the elimination process faces many challenges: sustaining the commitment of national authorities, partners and donors; overcoming the limitations of the current diagnostic and treatment tools; integrating disease control in peripheral health facilities; reaching populations living in or fleeing from areas of civil unrest; clarifying the role of and, if necessary, addressing the asymptomatic human carriers and the possible animal reservoir of *T brucei gambiense*; and, further developing tools and criteria to monitor, verify, and validate disease elimination at different geographical scales.

Panel: Research priorities

Treatment

Although there is hope that two safe drugs for the treatment of human African trypanosomiasis caused by *Trypanosoma brucei gambiense* will be available in the near future, the top research priority is improving therapies for disease caused by *Trypanosoma brucei rhodesiense*. Drug developers are confronted with such low numbers of cases that conducting clinical trials with sufficient statistical power is almost impossible.

Diagnosis

Improving the specificity of rapid diagnostic tests would transform the current complex diagnostic algorithm into a simple procedure, applicable at peripheral health facilities.

Asymptomatic carriers of *T brucei gambiense*

A fraction of people who are positive for *T brucei gambiense* following card agglutination test or rapid diagnostic tests cannot have the diagnosis confirmed with parasitological techniques. Some are false positives but others are not, and the latter can act as a human reservoir if left untreated. Nowadays, only trypanolysis can confirm the presence of *T brucei gambiense*-specific antibodies as a surrogate for contact with the parasite.[143–145] To eliminate *T brucei gambiense* disease, a high throughput alternative with the same specificity as trypanolysis would greatly facilitate the identification of human trypanosome carriers.

Animal reservoir of *T brucei gambiense*

Domestic and wild animals can be hosts of *T brucei gambiense*, and this reservoir might be the cause of human African trypanosomiasis re-emergence in eliminated foci.[146,147] Testing of animals, including tsetse, could become part of the toolbox for post-elimination monitoring to ensure sustained zero-transmission in controlled foci. It is therefore crucial to develop sensitive and *T brucei gambiense*-specific tools for such purpose.

Conclusions

Human African trypanosomiasis has long been a typical neglected tropical disease, characterised by suboptimal control tools and inadequate funding. Over the past 15 years, thanks to the efforts of a broad range of stakeholders, the situation has changed. Today, human African trypanosomiasis is a rare disease that is targeted for elimination. Drugs are available for free thanks to donations of the manufacturers, low-cost rapid diagnostic tests and vector control tools are on the market; safe oral drugs are expected to become available soon; and, the integration of human African trypanosomiasis diagnosis into peripheral health centres has begun. Yet, disease control might become the victim of its own success. History teaches us that falling case numbers can result in a decline in donor and control agency interest, opening the door to swift and severe recrudescence. Also, the progressive dismantling of highly specialised mobile teams entails the loss of expertise in diagnosis of human African trypanosomiasis, with grave consequences at the individual and community levels.

Despite the challenges, if current commitments and coordinated efforts can be sustained, human African trypanosomiasis could well become a disease of the past (panel). This effort will require the continuous provision of drugs, support by financial partners, adequate prioritisation and ownership of disease elimination at the national level, and coordination of the numerous actors involved in this laudable endeavour.

Contributors

PB coordinated the drafting of the manuscript. All authors contributed equally to the writing of the manuscript.

Declaration of interests

We declare no competing interests.

Acknowledgments

The Food and Agriculture Organization of the United Nations' contribution to this study was provided in the framework of the Programme against African Trypanosomosis, and supported by the Government of Italy (FAO Project Improving food security in sub-Saharan Africa by supporting the progressive reduction of tsetse-transmitted trypanosomosis in the framework of the NEPAD, codes GTFS/RAF/474/ITA and GCP/RAF/502/ITA). From the Institute of Tropical Medicine, Antwerp, Belgium, we thank Jan Van Den Abbeele and Luc Verhelst for video 1, and Epco Hasker for video 2. The boundaries and names shown and the designations used on the maps presented in this paper do not imply the expression of any opinion on the part of FAO or WHO concerning the legal status of any country, territory, city, or area, of its authorities, or concerning the delimitation of its frontiers or boundaries. The views expressed in this paper are those of the authors and do not necessarily reflect the views of FAO or WHO.

References

1 Simarro PP, Cecchi G, Paone M, et al. The atlas of human African trypanosomiasis: a contribution to global mapping of neglected tropical diseases. *Int J Health Geogr* 2010; **9:** 57.

2 Franco JR, Simarro PP, Diarra A, Jannin JG. Epidemiology of human African trypanosomiasis. *Clin Epidemiol* 2014; **6:** 257–75.

3 Simarro PP, Franco JR, Cecchi G, et al. Human African trypanosomiasis in non-endemic countries (2000-2010). *J Travel Med* 2012; **19:** 44–53.

4 Lindner AK, Priotto G. The unknown risk of vertical transmission in sleeping sickness–a literature review. *PLoS Negl Trop Dis* 2010; **4:** e783.

5 Lestrade-Carluer De Kyvon MA, Maakaroun-Vermesse Z, Lanotte P, et al. Congenital trypanosomiasis in child born in France to African mother. *Em Inf Dis* 2016; **22:** 935–7.

6 Rocha G, Martins A, Gama G, Brandão F, Atouguia J. Possible cases of sexual and congenital transmission of sleeping sickness. *Lancet* 2004; **363:** 247.

7 Mulumba MA, Kibonge MC, Mulumba MP, Musongela JP, Büscher P. Plaidoyer pour une nouvelle stratégie transfusionelle en zone endémique de la trypanosomiase humaine africaine. *Congo Médical* 2005; **4:** 99–106.

8 Herwaldt BL. Laboratory-acquired parasitic infections from accidental exposures. *Clin Microbiol Rev* 2001; **14:** 659–88.

9 Hira PR, Husein SF. Some transfusion-induced parasitic infections in Zambia. *J Hyg Epidemiol Microbiol Immunol* 1979; **23:** 436–44.

10 Lyons M. The colonial disease: a social history of sleeping sickness in northern Zaire, 1900–1940. Cambridge: Cambridge University Press; 1992.

11 Courtin F, Jamonneau V, Duvallet G, et al. Sleeping sickness in west Africa (1906–2006): changes in spatial repartition and lessons from the past. *Trop Med* 2008; **13:** 334–44.

12 WHO. Human African trypanosomiasis: epidemiological situation. 2017. http://www.who.int/trypanosomiasis_african/country/en/ (accessed June 14, 2017).

13 Franco JR, Cecchi G, Priotto G, et al. Monitoring the elimination of human African trypanosomiasis: update to 2014. *Plos Negl Trop Dis* 2017; **11:** e0005585.

14 Diggle PJ. Statistical analysis of spacial point patterns. London: Academic Press, 1983.

15 Simarro PP, Cecchi G, Franco JR, et al. Estimating and mapping the population at risk of sleeping sickness. *PLoS Negl Trop Dis* 2012; **6:** e1859.

16 Food and Agriculture Organization of the United Nations. Programme Against African Trypanosomosis (PAAT). 2014. http://www.fao.org/ag/againfo/programmes/en/paat/home.html (accessed June 14, 2017)

17 Courtin F, Dupont S, Zeze DG, et al. Human African trypanosomiasis: urban transmission in the focus of Bonon (Cote d'Ivoire). *Trop Med Int Health* 2005; **10:** 340–66.

18 Robays J, Ebeja KA, Lutumba P, et al. Human African trypanosomiasis amongst urban residents in Kinshasa: a case-control study. *Trop Med Int Health* 2004; **9:** 869–75.

19 Bilonda Mpiana A, Kabengele Mpinga E, Bukasa Tshilondan JC, et al. Risk factors of human African typanosomiasis in Mbuji Mayi, Eastern Kasai Province, Democratic Republic of the Congo. *Int J Trop Dis Health* 2015; **5:** 190–208.

20 Njiokou F, Nimpaye H, Simo G, et al. Domestic animals as potential reservoir hosts of *Trypanosoma brucei gambiense* in sleeping sickness foci in Cameroon. *Parasite* 2010; **17:** 61–66.

21 Sudarshi D, Lawrence S, Pickrell WO, et al. Human African trypanosomiasis presenting at least 29 years after infection—what can this teach us about the pathogenesis and control of this neglected tropical disease? *PLoS Negl Trop Dis* 2014; **8:** e3349.

22 Fromentin H. Nouvelles précision sur le trypanosome sp souche FEO. *Ann Soc Belg Méd Trop* 1963; **5:** 797–800.

23 Hoare CA. The trypanosomes of mammals. Oxford: Blackwell Scientific Publications, 1972.

24 Steverding D. The history of African trypanosomiasis. *Parasit Vectors* 2008; **1:** 3.

25 Caljon G, Van Reet N, De Trez C, Vermeersch M, Pérez-Morga D, Van Den Abbeele J. The dermis as a delivery site of *Trypanosoma brucei* for tsetse flies. *PLoS Pathog* 2016; **12:** e1005744.

26 Pays E, Vanhollebeke B, Uzureau P, Lecordier L, Perez-Morga D. The molecular arms race between African trypanosomes and humans. *Nat Rev Microbiol* 2014; **12:** 575–84.

27 Vanhollebeke B, Pays E. The trypanolytic factor of human serum: many ways to enter the parasite, a single way to kill. *Mol Microbiol* 2010; **76:** 806–14.

28 Horn D. Antigenic variation in African trypanosomes. *Mol Biochem Parasitol* 2014; **195:** 123–29.

29 Mugnier MR, Stebbins CE, Papavasilou FN. Masters of disguise: antigenic variation and the VSG coat in *Trypanosoma brucei*. *PLoS Pathol* 2016; **12:** e1005784.

30 Bisser S, Ayed Z, Bouteille B, et al. Central nervous system involvement in African trypanosomiasis: presence of anti-galactocerebroside antibodies in patients' cerebrospinal fluid. *Trans R Soc Trop Med Hyg* 2000; **94:** 225–26.

31 Lejon V, Büscher P, Magnus E, Moons A, Wouters I, Van Meirvenne N. A semi-quantitative ELISA for detection of *Trypanosoma brucei gambiense* specific antibodies in serum and cerebrospinal fluid of sleeping sickness patients. *Acta Trop* 1998; **69:** 151–64.

32 Lejon V, Mumba Ngoyi D, Ilunga M, et al. Low specificities of HIV diagnostic tests caused by *Trypanosoma brucei gambiense* sleeping sickness. *J Clin Microbiol* 2010; **48:** 2836–39.

33 Kristensson K, Masocha W, Bentivoglio M. Mechanisms of CNS invasion and damage by parasites. *Handb Clin Neurol* 2013; **114:** 11–22.

34 Stijlemans B, Caljon G, Van Den Abbeele J, Van Ginderachter JA, Magez S, De Trez C. Immune evasion strategies of *Trypanosoma brucei* within the mammalian host: progression to pathogenicity. *Front Immunol* 2016; **7:** 233.

35 Cecchi G, Mattioli RC, Slingenbergh J, de La Rocque S. Land cover and tsetse fly distributions in sub-Saharan Africa. *Med Vet Entomol* 2008; **22:** 364–73.

36 WHO. Control and surveillance of human African trypanosomiasis. *World Health Organ Tech Rep Ser* 2013; 1–237.

37 Grébaut P, Melachio T, Nyangmang S, et al. Xenomonitoring of sleeping sickness transmission in Campo (Cameroon). *Parasit Vectors* 2016; **9:** 201.

38 Solano P, Torr SJ, Lehane MJ. Is vector control needed to eliminate *gambiense* human African trypanosomiasis? *Front Cell Infect Microbiol* 2013; **3:** 33.

39 Shereni W, Anderson NE, Nyakupinda L, Cecchi G. Spatial distribution and trypanosomal infection of tsetse flies in the sleeping sickness focus of Zimbabwe (Zambezi escarpment, Hurungwe District). *Parasit Vectors* 2016; **9:** 605.

40 Capewell P, Cren-Travaillé C, Marchesi F, et al. The skin is a significant but overlooked anatomical reservoir for vector-borne African trypanosomes. *eLIFE* 2016; **10:** 17716.

41 Ooi CP, Bastin P. More than meets the eye: understanding *Trypanosoma brucei* morphology in the tsetse. *Front Cell Infect Microbiol* 2013; **3:** 71.

42 Auty H, Anderson NE, Picozzi K, et al. Trypanosome diversity in wildlife species from the Serengeti and Luangwa Valley ecosystems. *PLoS Negl Trop Dis* 2012; **6:** e1828.

43 Jamonneau V, Ravel S, Koffi M, et al. Mixed infections of trypanosomes in tsetse and pigs and their epidemiological significance in a sleeping sickness focus of Côte d'Ivoire. *Parasitology* 2004; **129:** 693–702.

44 MacLean LM, Odiit M, Chisi JE, Kennedy PG, Sternberg JM. Focus-specific clinical profiles in human African trypanosomiasis caused by *Trypanosoma brucei rhodesiense*. *PLoS Negl Trop Dis* 2010; **4:** e906.

45 Van Marck EA, Gigase PL, Beckers A, Wéry M. Experimental infections of laboratory rodents with recently isolated stocks of *Trypanosoma brucei gambiense*. 2. Histopathological investigations. *Z Parasitenkd* 1981; **64:** 187–93.

46 Jamonneau V, Ilboudo H, Kaboré J, et al. Untreated infections by *Trypanosoma brucei gambiense* are not 100% fatal. *PLoS Negl Trop Dis* 2012; **6:** e1691.

47 Odiit M, Kanshme F, Enyaru JCK. Duration of symptoms and case fatality of sleeping sickness caused by *Trypansoma brucei rhodesiense* in Tororo, Uganda. *East Afr Med J* 1997; **74:** 792–95.

48 Checchi F, Filipe JAN, Barrett MP, Chandramohan D. The natural progression of *gambiense* sleeping sickness: what is the evidence? *PLoS Negl Trop Dis* 2008; **2:** e303.

49 Checchi F, Filipe JAN, Haydon DT, Chandramohan D, Chappuis F. Estimates of the duration of the early and late stage of *gambiense* sleeping sickness. *BMC Infect Dis* 2008; **8:** 16.

50 Küpfer I, Hhary EP, Allan M, Edielu A, Burri C, Blum JA. Clinical presentation of *T.b. rhodesiense* sleeping sickness in second stage patients from Tanzania and Uganda. *PLoS Negl Trop Dis* 2011; **5:** e968.

51 Buguet A, Bourdon L, Bouteille B, et al. The duality of sleeping sickness: focusing on sleep. *Sleep Med Rev* 2001; **5:** 139–53.

52 Mpandzou G, Cespuglio R, Ngampo S, et al. Polysomnography as a diagnosis and post-treatment follow-up tool in human African trypanosomiasis: a case study in an infant. *J Neurol Sci* 2011; **305:** 112–15.

53 Njamnshi AK, Seke Etet PF, Perrig S, et al. Actigraphy in human African trypanosomiasis as a tool for objective clinical evaluation and monitoring: a pilot study. *PLoS Negl Trop Dis* 2012; **6:** e1525.

54 Blum J, Schmid C, Burri C. Clinical aspects of 2541 patients with second stage human African trypanosomiasis. *Acta Trop* 2006; **97:** 55–64.

55 Blum JA, Schmid C, Burri C, et al. Cardiac alterations in human African trypanosomiasis (*T. b. gambiense*) with respect to the disease stage and antiparasitic treatment. *PLoS Negl Trop Dis* 2009; **3:** e383.

56 Blum JA, Zellweger MJ, Burri C, Hatz C. Cardiac involvement in African and American trypanosomiasis. *Lancet Infect Dis* 2008; **8:** 631–41.

57 Kato CD, Nanteza A, Mugasa C, Edyelu A, Matovu E, Alibu VP. Clinical profiles, disease outcome and co-morbidities among *T. b. rhodesiense* sleeping sickness patients in Uganda. *PLoS One* 2015; **10:** e0118370.

58 Kouchner G, Bouree P, Lowenthal M. Hepatic involvement in *Trypanosoma rhodesiense* trypanosomiasis. *Bull Soc Pathol Exot Filiales* 1979; **72:** 131–35 (in French).

59 Urech K, Neumayr A, Blum J. Sleeping sickness in travelers–do they really sleep? *PLoS Negl Trop Dis* 2011; **5:** e1358.

60 Duggan AJ, Hutchinson MP. Sleeping sickness in Europeans: a review of 109 cases. *J Trop Med Hyg* 1966; **69:** 124–31.

61 Oscherwitz SL. East African trypanosomiasis. *J Travel Med* 2003; **10:** 141–43.

62 Magnus E, Van Meirvenne N, Vervoort T, Le Ray D, Wéry M. Use of freeze-dried trypanosomes in the indirect fluorescent antibody test for the serodiagnosis of sleeping sickness. *Ann Soc Belg Méd Trop* 1978; **58:** 103–09.

63 Lejon V, Büscher P. Serological diagnosis. In: Cattand P, Louis FJ, Simarro PP, eds. Sleeping sickness lectures. Gémenos: Association contre la Trypanosomiase en Afrique, 2013: 199–214.

64 Büscher P, Mertens P, Leclipteux T, et al. Sensitivity and specificity of HAT Sero-K-SeT, a rapid diagnostic test for serodiagnosis of sleeping sickness caused by *Trypanosoma brucei gambiense*: a case-control study. *Lancet Glob Health* 2014; **2:** e359–63.

65 Büscher P, Gilleman Q, Lejon V. Novel rapid diagnostic test for sleeping sickness. *N Engl J Med* 2013; **368:** 1069–70.

66 Bisser S, Lumbala C, Nguertoum E, et al. Sensitivity and specificity of a prototype rapid diagnostic test for the detection of *Trypanosoma brucei gambiense* infection: a multi-centric prospective study. *PLoS Negl Trop Dis* 2016; **10:** e0004608.

67 Peeling RW, Holmes KK, Mabey D, Rondon A. Rapid tests for sexually transmitted infections (STIs): the way forward. *Sex Transm Dis* 2006; **82** (suppl V)**:** v1–6.

68 Jamonneau V, Bucheton B. The challenge of serodiagnosis of sleeping sickness in the context of elimination. *Lancet Glob Health* 2014; **2:** e306–07.

69 Sullivan L, Fleming J, Sastry L, Mehlert A, Wall SJ, Ferguson MA. Identification of sVSG117 as an immunodiagnostic antigen and evaluation of a dual-antigen lateral flow test for the diagnosis of human African trypanosomiasis. *PLoS Negl Trop Dis* 2014; **8:** e2976.

70 Sternberg JM, Gierlinski M, Biéler S, Ferguson MA, Ndung'u JM. Evaluation of the diagnostic accuracy of prototype rapid tests for human African trypanosomiasis. *PLoS Negl Trop Dis* 2014; **8:** e3373.

71 Rooney B, Piening T, Büscher P, Rogé S, Smales M. Expression of *Trypanosoma brucei gambiense* antigens in *Leishmania tarentolae*. Potential for rapid serodiagnostic tests (RDTs). *PLoS Negl Trop Dis* 2015; **9:** e0004271.

72 Jamonneau V, Camara O, Ilboudo H, et al. Accuracy of individual rapid tests for serodiagnosis of *gambiense* sleeping sickness in west Africa. *PLoS Negl Trop Dis* 2015; **9:** e0003480.

73 Zhou X-H, Obuchowski NA, McClish DK. Statistical methods in diagnostic medicine. New York: John Wiley and Sons, 2002.

74 Camara O, Camara M, Lejon V, et al. Immune trypanolysis test with blood spotted on filter paper for epidemiological surveillance of sleeping sickness. *Trop Med Int Health* 2014; **19:** 828–31.

75 Van Meirvenne N, Magnus E, Büscher P. Evaluation of variant specific trypanolysis tests for serodiagnosis of human infections with *Trypanosoma brucei gambiense*. *Acta Trop* 1995; **60:** 189–99.

76 Guedegbe B, Verhulst A, Van Meirvenne N, Pandey VS, Doko A. Indications sérologiques de l'existence d'un réservoir sauvage du *Trypanosoma brucei gambiense* dans la réserve de la biosphère de la Pendjari en République du Bénin. *Ann Soc Belg Méd Trop* 1992; **72:** 113–20.

77 Kagbadouno MS, Camara M, Rouamba J, et al. Epidemiology of sleeping sickness in Boffa (Guinea): where are the trypanosomes? *PLoS Negl Trop Dis* 2012; **6:** e1949.

78 Büscher P, Mumba Ngoyi D, Kaboré J, et al. Improved models of mini anion exchange centrifugation technique (mAECT) and modified single centrifugation (MSC) for sleeping sickness diagnosis and staging. *PLoS Negl Trop Dis* 2009; **3:** e471.

79 Camara M, Camara O, Ilboudo H, et al. Sleeping sickness diagnosis: use of buffy coats improves the sensitivity of the mini anion exchange centrifugation test. *Trop Med Int Health* 2010; **15:** 796–99.

80 Miézan TW, Meda AH, Doua F, Djé NN, Lejon V, Büscher P. Single centrifugation of cerebrospinal fluid in a sealed pasteur pipette for simple, rapid and sensitive detection of trypanosomes. *Trans R Soc Trop Med Hyg* 2000; **94:** 293.

81 Woo PTK. The haematocrit centrifuge technique for the diagnosis of African trypanosomiasis. *Acta Trop* 1970; **27:** 384–86.

82 WHO. Trypanosomiasis control manual. Geneva: African Medical and Research Foundation Nairobi, Kenya, 1983.

83 Mumba Ngoyi D, Ali Ekangu R, Mumvemba Kodi MF, et al. Performance of parasitological and molecular techniques for the diagnosis and surveillance of *gambiense* sleeping sickness. *PLoS Negl Trop Dis* 2014; **8:** e2954.

84 Mumba Ngoyi D, Menten J, Pyana PP, Büscher P, Lejon V. Stage determination in sleeping sickness: comparison of two cell counting and two parasite detection techniques. *Trop Med Int Health* 2013; **18:** 778–82.

85 Lejon V, Legros D, Richer M, et al. IgM quantification in the cerebrospinal fluid of sleeping sickness patients by a latex card agglutination test. *Trop Med Int Health* 2002; **7:** 685–92.

86 Tiberti N, Hainard A, Lejon V, et al. Cerebrospinal fluid neopterin as a marker of the meningo-encephalitic stage of *Trypanosoma brucei gambiense* sleeping sickness. *PLoS One* 2012; **7:** e40909.

87 Migchelsen SJ, Büscher P, Hoepelman AI, Schallig HD, Adams ER. Human African trypanosomiasis: a review of non-endemic cases in the past 20 years. *Int J Infect Dis* 2011; **15:** e517–24.

88 Büscher P, Deborggraeve S. How can molecular diagnostics contribute to the elimination of human African trypanosomiasis? *Expert Rev Mol Diagn* 2015; **15:** 607–15.

89 Deborggraeve S, Büscher P. Recent progress in molecular diagnosis of sleeping sickness. *Expert Rev Mol Diagn* 2012; **12:** 719–30.

90 Deborggraeve S, Lejon V, Ali Ekangu R, et al. Diagnostic accuracy of PCR in *gambiense* sleeping sickness diagnosis, staging and post-treatment follow-up: a 2-year longitudinal study. *PLoS Negl Trop Dis* 2011; **5:** e972.

91 Cecchi G, Paone M, Feldmann U, Vreysen MJ, Diall O, Mattioli RC. Assembling a geospatial database of tsetse-transmitted animal trypanosomosis for Africa. *Parasit Vectors* 2014; **7:** 39.

92 Cecchi G, Paone M, Argiles HR, Vreysen MJ, Mattioli RC. Developing a continental atlas of the distribution and trypanosomal infection of tsetse flies (*Glossina* species). *Parasit Vectors* 2015; **8:** 284.

93 Deborggraeve S, Koffi M, Jamonneau V, et al. Molecular analysis of archived blood slides reveals an atypical human *Trypanosoma* infection. *Diagn Microbiol Infect Dis* 2008; **61:** 428–33.

94 Anderson NE, Mubanga J, Fèvre EM, et al. Characterisation of the wildlife reservoir community for human and animal trypanosomiasis in the Luangwa Valley, Zambia. *PLoS Negl Trop Dis* 2011; **5:** e1211.

95 Cordon-Obras C, Rodriguez YF, Fernandez-Martinez A, et al. Molecular evidence of a *Trypanosoma brucei gambiense* sylvatic cycle in the human african trypanosomiasis foci of Equatorial Guinea. *Front Microbiol* 2015; **6:** 765.

96 Truc P, Büscher P, Cuny G, et al. Atypical human infections by animal trypanosomes. *PLoS Negl Trop Dis* 2013; **7:** e2256.

97 Cordon-Obras C, Garcia-Estebanez C, Ndong-Mabale N, et al. Screening of *Trypanosoma brucei gambiense* in domestic livestock and tsetse flies from an insular endemic focus (Luba, Equatorial Guinea). *PLoS Negl Trop Dis* 2010; **4:** e704.

98 Radwanska M, Claes F, Magez S, et al. Novel primer sequences for a polymerase chain reaction-based detection of *Trypanosoma brucei gambiense*. *Am J Trop Med Hyg* 2002; **67:** 289–95.

99 Radwanska M, Chamekh M, Vanhamme L, et al. The serum resistance-associated gene as a diagnostic tool for the detection of *Trypanosoma brucei rhodesiense*. *Am J Trop Med Hyg* 2002; **67:** 684–90.

100 WHO. Human African trypanosomiasis: WHO's collaborating network. 2017. http://www.who.int/trypanosomiasis_african/surveillance/collaborating_network/en/ (accessed June 14, 2017).

101 Simarro PP, Franco J, Diarra A, Postigo JA, Jannin J. Update on field use of the available drugs for the chemotherapy of human African trypanosomiasis. *Parasitology* 2012; **139:** 842–46.

102 Pohlig G, Bernhard SC, Blum J, et al. Efficacy and safety of pafuramidine versus pentamidine maleate for treatment of first stage sleeping sickness in a randomized, comparator-controlled, international phase 3 clinical trial. *PLoS Negl Trop Dis* 2016; **10:** e0004363.

103 Priotto G, Kasparian S, Mutombo W, et al. Nifurtimox-eflornithine combination therapy for second-stage African *Trypanosoma brucei gambiense* trypanosomiasis: a multicentre, randomised, phase III, non-inferiority trial. *Lancet* 2009; **374:** 56–64.

104 Franco JR, Simarro PP, Dia A, Ruiz-Postigo JA, Samo M, Jannin JG. Monitoring the use of nifurtimox-eflornithine combination therapy (NECT) in the treatment of second stage *gambiense* human African trypanosomiasis. *Res Rep Trop Med* 2012; **3:** 93–101.

105 Lutje V, Seixas J, Kennedy A. Chemotherapy for second-stage human African trypanosomiasis. *Cochrane Database Syst Rev* 2013; CD006201.

106 Alirol E, Schrumpf D, Amici HJ, et al. Nifurtimox-eflornithine combination therapy for second-stage gambiense human African trypanosomiasis: Medecins Sans Frontieres experience in the Democratic Republic of the Congo. *Clin Infect Dis* 2013; **56:** 195–203.

107 Checchi F, Piola P, Ayikoru H, Thomas F, Legros D, Priotto G. Nifurtimox plus eflornithine for late-stage sleeping sickness in Uganda: a case series. *PLoS Negl Trop Dis* 2007; **1:** e64.

108 Priotto G, Fogg C, Balasegaram M, et al. Three drug combinations for late-stage *Trypanosoma brucei gambiense* sleeping sickness: a randomized clinical trial in Uganda. *PLoS Clin Trials* 2006; **1:** 1–8.

109 Schmid C, Kuemmerle A, Blum J, et al. In-hospital safety in field conditions of nifurtimox eflornithine combination therapy (NECT) for *T. b. gambiense* sleeping sickness. *PLoS Negl Trop Dis* 2012; **6:** e1920.

110 Bitonti AJ, Bacchi CJ, McCann PP, Sjoerdsma A. Uptake of alpha-difluoromethylornithine by *Trypanosoma brucei brucei*. *Biochem Pharmacol* 1986; **35:** 351–4.

111 Pépin J, Khonde N, Maiso F, et al. Short-course eflornithine in Gambian trypanosomiasis: a multicentre randomized controlled trial. *Bull World Health Org* 2000; **78:** 1284–95.

112 Priotto G, Pinoges L, Fursa IB, et al. Safety and effectiveness of first line eflornithine for *Trypanosoma brucei gambiense* sleeping sickness in Sudan: cohort study. *BMJ* 2008; **336:** 705–08.

113 Balasegaram M, Harris S, Checchi F, Hamel C, Karunakara U. Treatment outcomes and risk factors for relapse in patients with early-stage human African trypanosomiasis (HAT) in the Republic of the Congo. *Bull World Health Organ* 2006; **84:** 777–82.

114 Priotto G, Pinoges L, Badi Fursa I, et al. Safety and effectiveness of first line eflornithine for *Trypanosoma brucei gambiense* sleeping sickness in Sudan: cohort study. *BMJ* 2008; **336:** 705–08.

115 Chappuis F, Udayraj N, Stietenroth K, Meussen A, Bovier PA. Eflornithine is safer than melarsoprol for the treatment of second-stage *Trypanosoma brucei gambiense* human African trypanosomiasis. *Clin Infect Dis* 2005; **41:** 748–51.

116 Seixas JBA. Investigation on the encephalopathic syndrome during melarsoprol treatment of human African trypanosomiasis. PhD thesis, Universidade Nova de Lisboa, 2004.

117 Kuepfer I, Schmid C, Allan M, et al. Safety and efficacy of the 10-day melarsoprol schedule for the treatment of second stage *Rhodesiense* sleeping sickness. *PLoS Negl Trop Dis* 2012; **6:** e1695.

118 Schmid C, Richer M, Bilenge CM, et al. Effectiveness of a 10-day melarsoprol schedule for the treatment of late-stage human African trypanosomiasis: confirmation from a multinational study (IMPAMEL II). *J Infect Dis* 2005; **191:** 1922–31.

119 Pépin J, Milord F, Khonde A, Niyonsenga T, Loko L, Mpia B. Gambiense trypanosomiasis: frequency of, and risk factors for, failure of melarsoprol therapy. *Trans R Soc Trop Med Hyg* 1994; **88:** 447–52.

120 Brun R, Blum J, Chappuis F, Burri C. Human African trypanosomiasis. *Lancet* 2010; **375:** 148–59.

121 Adams JH, Haller L, Boa YF, Doua F, Dago A, Konian K. Human African trypanosomiasis (*T.b. gambiense*): a study of 16 fatal cases of sleeping sickness with some observation on acute reactive arsenical encephalopathy. *Neuropathol Appl Neurobiol* 1986; **12:** 81–94.

122 Graf FE, Ludin P, Wenzler T, et al. Aquaporin 2 mutations in *Trypanosoma brucei gambiense* field isolates concur with decreased susceptibility to pentamidine and melarsoprol. *PLoS Negl Trop Dis* 2013; **7:** e2475.

123 Munday JC, Eze AA, Baker N, et al. *Trypanosoma brucei* aquaglyceroporin 2 is a high-affinity transporter for pentamidine and melaminophenyl arsenic drugs and the main genetic determinant of resistance to these drugs. *J Antimicrob Chemother* 2014; **69:** 651–63.

124 Tarral A, Blesson S, Mordt OV, et al. Determination of an optimal dosing regimen for fexinidazole, a novel oral drug for the treatment of human African trypanosomiasis: first-in-human studies. *Clin Pharmacokinet* 2014; **53:** 565–80.

125 Jacobs RT, Nare B, Wring SA, et al. SCYX-7158, an orally-active benzoxaborole for the treatment of stage 2 human African trypanosomiasis. *PLoS Negl Trop Dis* 2011; **5:** e1151.

126 Cecchi G, Paone M, Franco JR, et al. Towards the Atlas of human African trypanosomiasis. *Int J Health Geogr* 2009; **8:** 15.

127 Lumbala C, Simarro PP, Cecchi G, et al. Human African trypanosomiasis in the Democratic Republic of the Congo: disease distribution and risk. *Int J Health Geogr* 2015; **14:** 20.

128 Simarro PP, Cecchi G, Franco JR, et al. Mapping the capacities of fixed health facilities to cover people at risk of *gambiense* human African trypanosomiasis. *Int J Health Geogr* 2014; **13:** 4.

129 Simarro PP, Cecchi G, Franco JR, et al. Monitoring the progress towards the elimination of *gambiense* human African trypanosomiasis. *PLoS Negl Trop Dis* 2015; **9:** e0003785.

130 Mpanya A, Hendrickx D, Vuna M, et al. Should I get screened for sleeping sickness? A qualitative study in Kasai province, Democratic Republic of Congo. *PLoS Negl Trop Dis* 2012; **6:** e1467.

131 Hasker E, Lutumba P, Chappuis F, et al. Human African trypanosomiasis in the democratic republic of the congo: a looming emergency? *PLoS Negl Trop Dis* 2012; **6:** e1950.

132 Koffi M, N'Djetchi M, Ilboudo H, et al. A targeted door-to-door strategy for sleeping sickness detection in low-prevalence settings in Cote d'Ivoire. *Parasite* 2016; **23:** 51.

133 Hasker E, Lumbala C, Mpanya A, et al. Alternative strategies for case finding in human African trypanosomiasis in the Democratic Republic of Congo. European Congress on Tropical Medicine and International Health; Basel, Switzerland; 6–10/09/2015. *Trop Med Int Health* 2015; **20:** 339.

134 Mitashi PM. Novel diagnostic tests for human African trypanosomiasis: what is their role in primary health care services? PhD thesis, University of Antwerp, 2014.

135 Franco JR, Simarro PP, Diarra A, Ruiz-Postigo JA, Jannin JG. The journey towards elimination of *gambiense* human African trypanosomiasis: not far, nor easy. *Parasitology* 2014; **141:** 748–60.

136 Kambire R, Lingue K, Courtin F, et al. La Trypanosomose Humaine Africaine dans l'espace ivoiro-burkinabé: optimisation des stratégies de surveillance épidémiologique. *Parasite* 2012; **19:** 389–96.

137 Courtin F, Camara M, Rayaisse JB, et al. Reducing human-tsetse contact significantly enhances the efficacy of sleeping sickness active screening campaigns: a promising result in the context of elimination. *PLoS Negl Trop Dis* 2015; **9:** e0003727.

138 Shaw AP, Wint GR, Cecchi G, Torr SJ, Mattioli RC, Robinson TP. Mapping the benefit-cost ratios of interventions against bovine trypanosomosis in Eastern Africa. *Prev Vet Med* 2015; **122:** 406–16.

139 Shaw AP, Torr SJ, Waiswa C, et al. Estimating the costs of tsetse control options: an example for Uganda. *Prev Vet Med* 2013; **110:** 290–303.

140 Wendo C. Uganda revises cattle treatment to protect humans from sleeping sickness. *Lancet* 2002; **359:** 239.

141 Magona JW, Walubengo J. Mass-treatment and insecticide-spraying of animal reservoirs for emergency control of Rhodesiense sleeping sickness in Uganda. *J Vector Borne Dis* 2011; **48:** 105–8.

142 Vreysen MJ, Seck MT, Sall B, Bouyer J. Tsetse flies: their biology and control using area-wide integrated pest management approaches. *J Invertebr Pathol* 2013; **112** (suppl 1)**:** S15–25.

143 Jamonneau V, Bucheton B, Kaboré J, et al. Revisiting the immune trypanolysis test to optimise epidemiological surveillance and control of sleeping sickness in west Africa. *PLoS Negl Trop Dis* 2010; **4:** e917.

144 Bucheton B, MacLeod A, Jamonneau V. Human host determinants influencing the outcome of *Trypanosoma brucei gambiense* infections. *Parasite Immunol* 2011; **33:** 438–47.

145 Ilboudo H, Jamonneau V, Camara M, et al. Diversity of response to *Trypanosoma brucei gambiense* infections in the Forecariah mangrove focus (Guinea): perspectives for a better control of sleeping sickness. *Microbes Infect* 2011; **13:** 943–52.

146 Molyneux DH. Animal reservoirs and Gambian trypanosomiasis. *Ann Soc Belg Med Trop* 1973; **53:** 605–18.

147 Simo G, Rayaisse JB. Challenges facing the elimination of sleeping sickness in west and central Africa: sustainable control of animal trypanosomiasis as an indispensable approach to achieve the goal. *Parasit Vectors* 2015; **8:** 640.