

Opinion

Do Cryptic Reservoirs Threaten
Gambiense-Sleeping Sickness
Elimination?

Informal Expert Group on Gambiense HAT Reservoirs

Philippe Büscher,^{1,*} Jean-Mathieu Bart,^{2,3} Marleen Boelaert,⁴ Bruno Bucheton,² Giuliano Cecchi,⁵ Nakul Chitnis,^{6,7} David Courtin,⁸ Luisa M. Figueiredo,⁹ José-Ramon Franco,¹⁰ Pascal Grébaut,² Epcó Hasker,⁴ Hamidou Ilboudo,¹¹ Vincent Jamonneau,² Mathurin Koffi,¹² Veerle Lejon,² Annette MacLeod,¹³ Justin Masumu,¹⁴ Enock Matovu,¹⁵ Raffaele Mattioli,¹⁶ Harry Noyes,¹⁷ Albert Picado,¹⁸ Kat S. Rock,¹⁹ Brice Rotureau,²⁰ Gustave Simo,²¹ Sophie Thévenon,^{2,22} Sandra Trindade,⁹ Philippe Truc,² and Nick Van Reet¹

***Trypanosoma brucei gambiense* causes human African trypanosomiasis (HAT). Between 1990 and 2015, almost 440 000 cases were reported. Large-scale screening of populations at risk, drug donations, and efforts by national and international stakeholders have brought the epidemic under control with <2200 cases in 2016. The World Health Organization (WHO) has set the goals of *gambiense*-HAT elimination as a public health problem for 2020, and of interruption of transmission to humans for 2030. Latent human infections and possible animal reservoirs may challenge these goals. It remains largely unknown whether, and to what extent, they have an impact on *gambiense*-HAT transmission. We argue that a better understanding of the contribution of human and putative animal reservoirs to *gambiense*-HAT epidemiology is mandatory to inform elimination strategies.**

Can Cryptic Reservoirs in Humans and Animals Compromise the Sustainable Elimination of *gambiense*-HAT?

HAT is caused by two closely related parasites that are transmitted by tsetse flies. *Trypanosoma brucei gambiense* is responsible for the Western and Central African form of the disease and *Trypanosoma brucei rhodesiense* occurs in Eastern and Southern Africa – both forms of the disease are usually fatal if untreated [1]. Between 1990 and 2016, a total of 437 971 cases of *gambiense*-HAT were reported, with a peak of 37 385 cases in 1998ⁱ. Thanks to large-scale deployment of a serological screening test (**CATT/T. b. gambiense**) (see Glossary), drug donations, and intense efforts by national and international stakeholders, this epidemic has been brought under control, with fewer than 2200 cases reported in 2016. This represents a marked reduction in human suffering caused by the disease. Inspired by this progress, the WHO has set **elimination** of *gambiense*-HAT as a target for the near future: **elimination as a public health problem** by 2020 and the interruption of transmission to humans by 2030ⁱⁱ.

The rationale to shift from HAT control to elimination is based on several arguments, such as the epidemiological vulnerability of *gambiense*-HAT as a presumed **anthroponotic** infection,

Highlights

gambiense-HAT is targeted for elimination with zero transmission in humans.

Innovative tools may contribute to the achievement of elimination; these tools include rapid diagnostic tests, improved tsetse-control tools, and an oral drug to treat both stages of disease.

Research is revealing associations between infection outcome, including self-cure, and mutations within genes involved in immune responses.

Patient-derived *T. b. gambiense* strains can cycle in animals and tsetse flies without losing infectivity to humans. Molecular and serological techniques facilitate new studies on naturally infected animals as putative reservoir hosts.

Mathematical modelling supports the hypothesis that human or animal reservoirs drive transmission, and they, or the tsetse vectors, could be targeted to swiftly impact transmission. Ongoing modelling will assess possible recrudescence via reservoirs.



historic examples of elimination in several West African foci, the availability of new medicines and diagnostics, the political will of endemic countries, and the commitment of national control programs [2]. Furthermore, a drug donation agreement between pharmaceutical companies and WHO has made treatment freely available to endemic countries.

gambiense-HAT control classically relies on three pillars: vector control, case finding, and treatment. HAT is a vector-borne disease, and the reduction of human–fly contact below a critical threshold would lead to zero transmission. Although vector control is critical to achieve the elimination/eradication goals, in practice, it will be hard to sustain control of all tsetse fly populations in all endemic countries. Vector control being only part of the solution, *gambiense*-HAT control will continue to rely to a great extent on surveillance, diagnosis, and treatment, both for reducing transmission and for monitoring progress towards these goals.

The introduction of individual **rapid diagnostic tests** (RDTs) for *gambiense*-HAT may increase serological screening coverage as they can be performed in remote dispensaries devoid of technical facilities. Thus, they facilitate the integration of passive screening in the health system and play a role in a sustainable surveillance system. However, RDTs also have limitations – like CATT/*T. b. gambiense*, they only detect antibodies, and their **specificity** is not 100% [3]. As a consequence, given the adverse effects and logistic constraints of current treatment, individuals who test positive in an RDT or in CATT must undergo microscopic examination of blood or lymph node fluid to confirm the presence of the parasite, followed by a lumbar puncture for **stage determination**, as different drugs are required to treat early- and late-stage disease [2]. In recent years, the highly toxic melarsoprol regimen, used to treat late-stage disease, has been replaced by a safer, though still rather complex, treatment requiring parenteral administration and hospitalisation. An oral treatment might become available in late 2018, and a single-dose treatment is entering phase III clinical trialsⁱⁱ [4].

Whereas HAT elimination by 2020, as a public health problem, seems within reach, the sustained global elimination of HAT appears more challenging. Indeed, as long as the knowledge gaps surrounding the **reservoir** of *T. b. gambiense* in interepidemic periods are not filled, the concept of **eradication** of *gambiense*-HAT cannot be considered.

We present the current research evidence about potential human and animal *T. b. gambiense* reservoirs and discuss their importance in the light of the *gambiense*-HAT elimination goals.

Human Reservoir

Mathematical models show that the sustained transmission of HAT can be explained if a fraction of the HAT cases is systematically missed by the screening operations [5]. Unfortunately, this is the case in many settings as a number of *T. b. gambiense* infections remain undiagnosed for several reasons [6]. First, not all infected people are reached by screening activities. Second, current diagnostic techniques do not pick up all *T. b. gambiense* infections due to lack of sensitivity of serological screening tests, of molecular techniques, or of the parasitological confirmation tests [7]. These undiagnosed, yet infected, people will act as a human reservoir of the parasite and might sustain transmission, forming a **maintenance population** [8]. Still another potential category of human reservoir may consist of **latent infections**, also called ‘healthy carriers’, who do not always progress to clinical disease, though the relative contribution of these individuals to parasite transmission still needs to be documented (Box 1). These latently infected people may carry trypanosomes for years or even decades, as was first described half a century ago in West Africa, and later in patients refusing treatment in Côte d’Ivoire [9,10]. More recently, a HAT case with a latent infection of at least 29

¹Department of Biomedical Sciences, Institute of Tropical Medicine, Nationalestraat 155, 2000 Antwerp, Belgium

²INTERTRYP, IRD, CIRAD, Univ Montpellier, Montpellier, France

³Centro Nacional de Medicina Tropical, Instituto de Salud Carlos III, Calle Sinesio Delgado 4, 28029 Madrid, Spain

⁴Department of Public Health, Institute of Tropical Medicine, Nationalestraat 155, 2000 Antwerp, Belgium

⁵Sub-regional Office for Eastern Africa, Food and Agriculture Organization of the United Nations, CMC Road, Bole Sub City, Kebele 12/13, P O Box 5536, Addis Ababa, Ethiopia

⁶Department of Epidemiology and Public Health, Swiss Tropical and Public Health Institute, Socinstrasse 57, Postfach, 4002 Basel, Switzerland

⁷University of Basel, Switzerland

⁸Université Paris Descartes, Institut de Recherche pour le Développement, Unité MERIT, Mère et enfant face aux infections tropicales, 4 avenue de l’Observatoire, 75006 Paris, France

⁹Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Avenida Prof Egas Moniz, 1649-028 Lisboa, Portugal

¹⁰Control of Neglected Tropical Diseases, Innovative and Intensified Disease Management, World Health Organization, Via Appia 20, 1202 Geneva, Switzerland

¹¹Institut de Recherche sur les Bases Biologiques de la Lutte Intégrée, Centre International de Recherche-Développement sur l’Élevage en zone Subhumide, 01 BP 454 Bobo-Dioulasso 01, Burkina Faso

¹²Université Jean Lorougnon Guédé, BP 150 Daloa, Côte d’Ivoire

¹³Institute of Biodiversity, Animal Health and Comparative Medicine, University of Glasgow, Henry Wellcome Building, 464 Bearsden Road, Glasgow, UK

¹⁴Département de Parasitologie, Institut National de Recherche Biomédicale, Avenue de la Démocratie, BP 1197 Kinshasa 1, République Démocratique du Congo

¹⁵College of Veterinary Medicine, Animal Resources and Biosecurity, Makerere University, P O Box 7062 Kampala, Uganda

¹⁶Animal Production and Health Division, Food and Agriculture Organization of the United Nations, Viale delle Terme di Caracalla, 00153

years was documented [11]. Whether latently infected persons transmit the parasite sexually [12], and whether sexual and congenital transmission plays a significant role in the epidemiology of *gambiense*-HAT [13], remains hypothetical. In Guinea, asymptomatic or latent infections were found to have consistently high titres in CATT/*T. b. gambiense* and to be positive in the **immune trypanolysis** test, although no parasites could be detected in blood or lymph node fluid during a 2-year follow-up period [14]. This observation is in line with the fact that trypanosomes can survive in the extravascular spaces of diverse organs such as the heart, the central nervous system, and the skin [15–17]. Experimental infections in animals confirmed that parasites may be undetectable in the blood but hidden in different organs and tissues [18–21], including the skin, from where they can be ingested by tsetse flies [22,23]. It was only recently that researchers began to investigate the underlying host–parasite interaction mechanisms responsible for those latent infections. Microsatellite profiles and genomic sequencing of parasites from latent infections and from clinical HAT patients are indistinguishable, suggesting that the latent infection phenotype is determined primarily by the host rather than by the parasite [24]. Studies on host genetic polymorphism show that *tumor necrosis factor- α -308A*, *HLA-G UTR-2*, *APOL1 N264K*, and *APOL1 G2* are associated with increased risk of infection or with disease progression, while *IL10₋₅₉₂ A*, *IL6₄₃₃₉*, *APOL1 G1*, and other polymorphisms in *HPR* and *APOL1* are associated with decreased risk of infection or with latent infection [25–30]. Other studies have found associations between the innate and the adaptive immune response and infection outcome, for example, **self-cure** and high levels of interleukin-8 (IL-8); latent infection and high levels of IL-6 or specific interferon- γ -producing T cells; disease progression and high levels of IL-10, TNF- α , and sHLA-G [31–33]. In view of the global elimination of HAT, it is of the utmost importance to clarify the extent to which these human reservoirs contribute to the transmission of the parasite and hence to *gambiense*-HAT persistence and potential resurgence.

Animal Reservoir

Compared to latent infections in humans, our current knowledge of *T. b. gambiense* infections in animals is very limited and fragmented. The presence of *T. b. gambiense* in animals has been demonstrated in several studies (Figure 1) [34,35]. Several authors have suggested that animals can act as a reservoir for *gambiense*-HAT [36–45]. In *rhodesiense*-HAT, sustained parasite transmission cycles exist in both livestock and wildlife, from which the parasite can spill over to humans [46]. For *T. b. gambiense*, despite early data generated on its infectivity and transmissibility in animals, the epidemiological significance of any animal reservoir is not well understood and may depend on the specific ecosystem of the **HAT focus**. Even if the parasite can be transmitted to and from animals, factors such as the proportion of blood-feeding on that species by tsetse will determine the epidemiological significance of the species to act as a maintenance population or part of a **maintenance community**. *T. b. gambiense* can infect a variety of domestic animals and wildlife, as shown in Table 1. Following infection, most of these animals remain asymptomatic and generally show low to very low parasitaemia. For instance, in pigs infected with a *T. b. gambiense* strain isolated from a human patient, only **xenodiagnosis** and blood culture succeeded in revealing an infection but conventional microscopy failed to detect parasites [47–51]. Moreover, experimental studies have shown that human-derived *T. b. gambiense* strains that were cyclically transmitted by tsetse flies between animals for more than a year remained transmissible to humans [48].

Studying natural *T. b. gambiense* infections in animals is challenging. Major drawbacks are the usually low parasitaemia and the necessity to distinguish *T. b. gambiense* from other trypanosome species such as *T. brucei brucei*, *T. congolense*, *T. vivax*, *T. suis*, and *T. simiae*. In particular, *T. b. gambiense* is morphologically identical to the nonhuman infective *T. b. brucei*.

Rome, Italy

¹⁷Institute of Integrative Biology, University of Liverpool, Liverpool L69 7ZB, UK

¹⁸Foundation for Innovative New Diagnostics, 9 Chemin des Mines, 1202 Geneva, Switzerland

¹⁹Zeeman Institute for Systems Biology & Infectious Disease Research, University of Warwick, Gibbet Hill Road, Coventry CV4 7AL, UK

²⁰Trypanosome Transmission Group, Trypanosome Cell Biology Unit, INSERM U1201 and Department of Parasites and Insect Vectors, Institut Pasteur, 25, rue du Docteur Roux, 75015 Paris, France

²¹Department of Biochemistry, Faculty of Science, University of Dschang, P O Box 67 Dschang, Cameroon

²²CIRAD, INTERTRYP, Montpellier, France

*Correspondence: pbuscher@itg.be (P. Büscher).

Box 1. Diversity in Outcomes of Human *Trypanosoma brucei gambiense* Infections

There is growing evidence that infection with *T. b. gambiense* does not always follow the classical course of the disease, that is, a first haemolympathic stage followed by a second stage with central nervous system involvement progressing to death if left untreated (Figure 1). These symptomatic HAT patients are characterised by the detection of parasites in any body fluid (P+), detection of specific antibodies against *T. b. gambiense* Variable Antigen Type LiTat 1.3 or LiTat 1.5 in immune trypanolysis (TL+), and the presence of clinical symptoms. However, long-term follow-up studies in West Africa have shown that a number of infected individuals do not develop the disease and can be classified as having latent infections (i.e., they are healthy carriers) [9]. They remain asymptomatic without detectable parasites (P-) for several years, although they are consistently positive in the immune trypanolysis test (TL+). Moreover, some of them may become immune trypanolysis-negative (TL-) over time, suggesting that they self-cured and therefore cannot transmit the parasite any more.

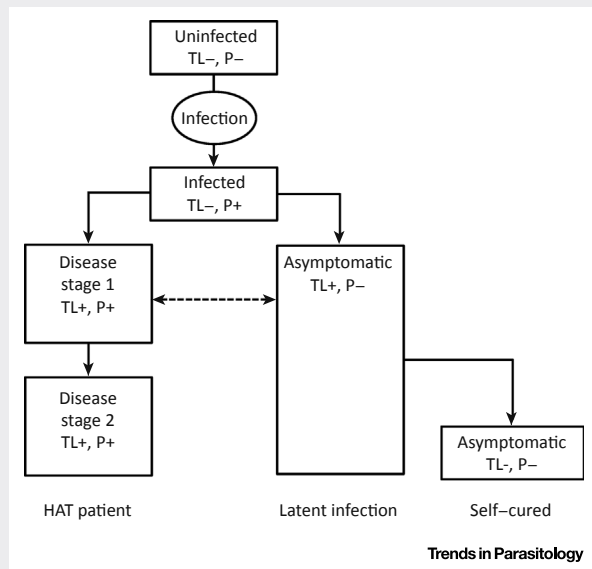


Figure 1. Outcomes of Human Infection with *Trypanosoma brucei gambiense*. get infected with *T. b. gambiense*. When naïve persons (uninfected), without specific antibodies (TL-) and without parasites (P-) become infected with *T. b. gambiense*, they undergo an early phase of the disease with detectable parasitaemia (P+) but without detectable specific antibodies. Thereafter, most of them develop the disease (HAT patient) and are characterised by specific antibodies (TL+) and detectable parasitaemia (P+). Some remain asymptomatic (latent infection) with detectable specific antibodies but without detectable parasites (TL+, P-). Evidence for self-cure comes from asymptomatic people who also eventually become negative for specific antibodies (TL-, P-).

Among the molecular tests, only those targeting the single-copy TgsGP gene are *gambiense*-specific, thus limiting their analytical sensitivity to >100 trypanosomes per ml of blood [52,53]. Biochemical assays, such as isoenzyme profiling, are only applicable on parasite strains that have been isolated and adapted to laboratory rodents or to *in vitro* cultures [54–56], and phenotypic assays such as the **blood incubation infectivity test** are only readily applicable on isolated strains and are not fully *gambiense*-specific [57]. Tests that detect antibodies against *gambiense*-specific antigens, such as the **variant surface glycoproteins** (VSGs) LiTat 1.3 and LiTat 1.5, may be more useful in revealing *T. b. gambiense* infections in animals. However, the immune trypanolysis test (TL), which is considered 100% specific in humans, still has to be validated in different species of animals. Ancillary information on the *T. b. gambiense* animal reservoir can be drawn from analysing *T. b. gambiense* infection in tsetse, in combination with its feeding behaviour, to assess the vectorial transmission of the parasite from the animal reservoir to humans [58]. In summary, there is a need to further improve our tools and increase our understanding regarding the importance of an animal reservoir in *gambiense*-HAT

Glossary

Anthropothonic disease: an infectious disease typically transmitted from human to human (including through an insect vector).

Blood incubation infectivity test: *T. b. gambiense* and *T. b. rhodesiense* have developed mechanisms to withstand lysis by normal human serum, in contrast to animal infective trypanosomes such as *T. b. brucei*, *T. congolense*, *T. vivax*. To confirm that an animal is infected with *T. b. gambiense* or *T. b. rhodesiense*, its blood, or trypanosomes isolated from that animal, are incubated with human blood or serum whereafter this mixture is injected into a susceptible animal. Only human serum-resistant trypanosomes will be able to initiate an infection in the susceptible animal.

CATT/*T. b. gambiense*: card agglutination test for trypanosomiasis is an agglutination test for the detection of *gambiense*-specific antibodies in blood. It was the first field-applicable serological test introduced in the 1980s for large-scale screening of populations at risk for *gambiense*-HAT.

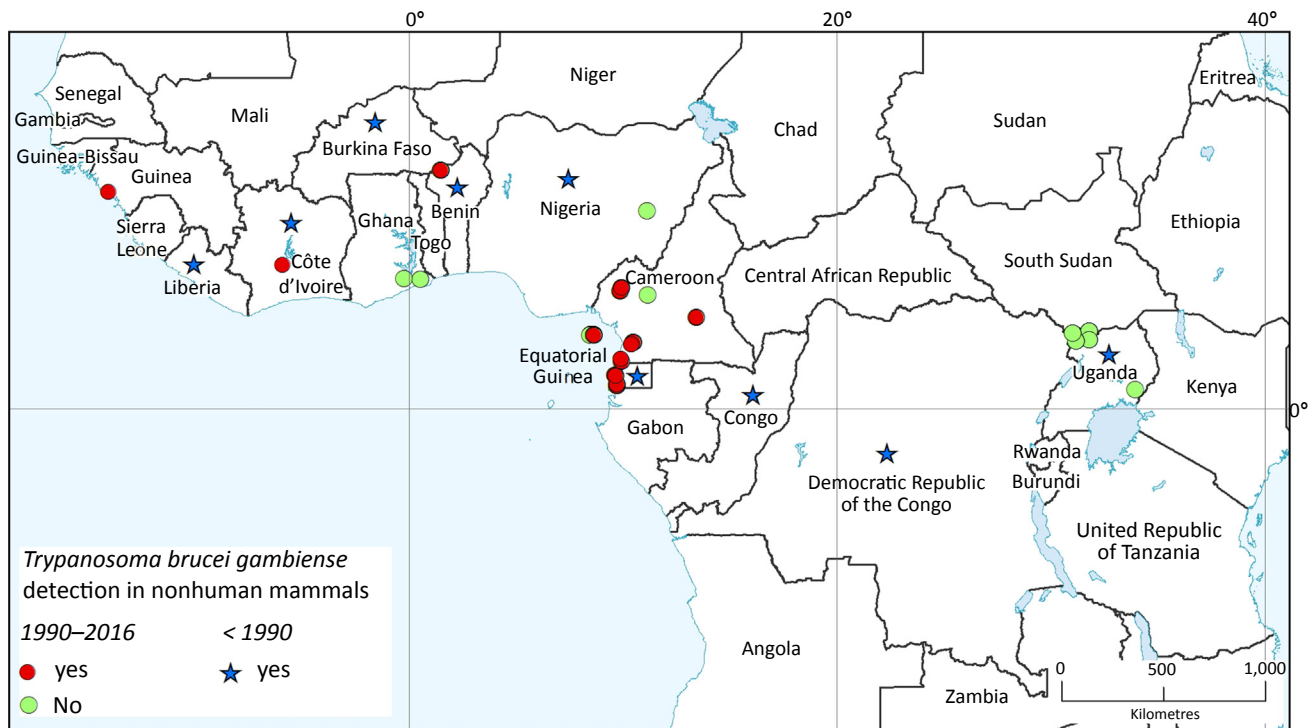
Deterministic mathematical model: deterministic models ignore the impact of random events, instead capturing average disease dynamics, so that multiple simulations with the same parameter values and initial conditions will lead to exactly the same outcome.

Elimination of *gambiense*-HAT: elimination is the reduction to zero of *gambiense*-HAT incidence in a defined area as a result of deliberate efforts; measures to prevent re-emergence are required.

Elimination of *gambiense*-HAT as a public health problem: 90% reduction in areas reporting more than 1 case in 10 000 compared to 2000–2004, and fewer than 2000 annually reported cases globally.

Eradication of *gambiense*-HAT: eradication is the permanent reduction to zero of the worldwide incidence of *gambiense*-HAT as a result of deliberate efforts; intervention measures are no longer needed.

HAT focus: a geographically defined zone where transmission of HAT occurs or has occurred, to which a



Trends in Parasitology

Figure 1. *Trypanosoma brucei gambiense* in Nonhuman Mammals. The map shows *gambiense*-human African trypanosomiasis in endemic countries and sites where *T. b. gambiense* infection in nonhuman mammals has been investigated with direct and indirect methods. Circles represent direct or indirect evidence of presence (red) and of absence (green) of *T. b. gambiense* in the period 1990–2016. For this period, data are mapped at the village/site level. (Blue) stars represent presence of detection in the years prior to 1990. For this period, data are mapped at the country level. All source references are provided in Tables S1 and S2 in the supplemental information online.

epidemiology. If further evidence indicates that an animal reservoir may threaten *gambiense*-HAT elimination, synergy with the control of animal African trypanosomiasis should be considered [59].

Filling the Knowledge Gaps

The presence of multiple reservoirs is a critical obstacle to the sustained elimination of any infectious agent [60]. For example, when the Guinea worm eradication programme was rolled out, the possibility of an animal reservoir was initially overlooked, but the recent finding of Guinea worm infections in dogs led to the hypothesis that dogs could have acted as a reservoir that caused the reappearance of human cases in Chad [61]. The existence of a human reservoir, in the form of post-kala-azar dermal leishmaniasis, and possibly also latent infections, is a challenge for the sustained elimination of visceral leishmaniasis (VL) from the Indian subcontinent [62].

The importance of investigating how HAT can re-emerge in so-called silent foci is clearly illustrated by the fact that a 9-year-old child was diagnosed with *gambiense*-HAT in Ghana in 2013, 10 years after the last detected case [63]. Also, the finding of a *gambiense*-specific PCR-positive squirrel in Equatorial Guinea on Luba island in 2014, where the last human HAT case was reported in 1995, is worrying [43]. Therefore, in the context of *gambiense*-HAT elimination, a key question is whether human and/or animal reservoirs are capable of maintaining

geographical name is given (locality, region, and river).

Immune trypanolysis: a highly accurate test for *gambiense*-specific antibodies, based on antibody-mediated complement lysis of trypanosomes exposing one single variant-specific antigen on their surface.

Latent infection: ongoing infection not progressing to clinical disease; it may remain undiagnosed.

Maintenance community: one or more populations which can transmit the pathogen and, together, can maintain the pathogen.

Maintenance population: individual populations which can transmit the pathogen and can also maintain the pathogen in the absence of other reservoir populations.

Rapid diagnostic test (RDT): serological antibody- or antigen-detection test, conditioned as individual test, compliant with the

Table 1. Animals Successfully Infected with *T. b. gambiense* Strains Isolated from Human Patients

Animal species	Origin of trypanosome strain ^a	Infectiveness to tsetse	Minimum observed duration of infection	Refs
Domestic animals				
Cat	Senegambia and Congo Free State	Not tested	12 days	[73]
Cattle	Nigeria	Yes	50 days	[66,74,]
Chicken	Unknown	Not tested	75 days	[75]
Dog	Senegambia and Congo Free State, Nigeria; Belgian Congo	Yes	109 days	[36,48,73]
Donkey	Senegambia	Not tested	14 days	[73]
Goat	Senegambia, Nigeria, Belgian Congo	Yes	13 months	[48,73,74]
Horse	Senegambia	Not tested	5 months	[73]
Pig	Côte d'Ivoire, Congo Belge, Nigeria	Yes	18 months	[47,51,76]
Sheep	Côte d'Ivoire	Not tested		[77]
Primates				
Agile mangabey (<i>Cercocebus galeritus agilis</i>)	Belgian Congo	Yes		[48]
Green monkey (<i>Cercopithecus callitrichus</i> , <i>C. aethiops tantalus</i>)	Congo Free State, Nigeria	Yes	3 months	[36,73]
Wolf's mona monkey (<i>Cercopithecus wolfi</i>)	Congo Belge	Yes	15 days	[47]
Patas monkey (<i>Erythrocebus patas patas</i>)	Nigeria	Yes	3 months	[36,78]
Rhesus macaque (<i>Macacus rhesus</i>)	Senegambia and Congo Free State	Not tested	1 month	[73]
Chimpanzee (<i>Pan satyrus</i> , <i>Pan troglodytes verus</i>)	Senegambia, Nigeria	Not tested	17 months	[73,78,79]
Dwarf galago (<i>Galagoides demidovii</i>)	République populaire du Congo	Not tested	28 days	[80]
Ungulates				
Bay duiker (<i>Cephalopus dorsalis</i>)	Belgian Congo	Yes	24 months	[48]
Waterbuck (<i>Kobus ellipsiprymnus</i>)	Uganda	Not tested		[50]
Reedbuck (<i>Redunca redunca</i>)	Uganda	Yes	15 months	[50]
Bushbuck (<i>Tragelaphus spekei</i>)	Uganda	Yes	22 months	[50]
Rodents				
Gambian pouched rat (<i>Cricetomys gambianus</i>)	République populaire du Congo	Yes	154 days	[37,80,81]
Thicket rat (<i>Thamnomys rutilans</i>), Jackson's praeomys (<i>Praomys jacksoni</i>), African marsh rat (<i>Dasymys incomtus</i>), Striped grass mouse (<i>Lemniscomus</i>)	République populaire du Congo	Not tested	131 days	[80]

ASSURED criteria (Affordable, Sensitive, Specific, User-friendly, Rapid and robust, Equipment-free and Deliverable to end-users); RDTs for *gambiense*-HAT detect antibodies against predominant *gambiense*-specific antigens.

Reservoir: a host where the pathogen can maintain itself and from where it can be transmitted to another host; a reservoir host is essential to sustain infection.

Self-cure: infection that is cleared by the host without treatment.

Specificity: the specificity of a diagnostic test is the probability that the test result is negative when the test person is not infected. It is usually expressed as a percentage and is calculated by dividing the number of test negatives by the number of true negatives x 100.

Stage determination: HAT develops from an early stage, with parasites in the peripheral tissues, towards a late stage, with parasite invasion into the central nervous system. Treatment is different for both stages, thus requiring stage determination before drug administration. Determination of the stage is achieved by examination of the cerebrospinal fluid for the presence of trypanosomes and the number of white blood cells.

Stochastic mathematical model: stochastic models include chance events so that two simulations with the same parameter values and initial conditions may lead to different outcomes. Chance events become more important at very low prevalences such as in pre-elimination or re-emergent settings.

Variation surface glycoprotein (VSG): in the vertebrate host, the cell surface of trypanosomes is covered with a layer of identical VSGs of one particular variant antigen type (VAT), that protects the trypanosomes against innate immune defence mechanisms of the host; VSGs are highly immunogenic, but periodic switches of the VAT of the VSG coat (antigenic variation) enable the trypanosome to escape the host humoral immune response; during the course of the infection, the host blood contains antibodies against a wide spectrum of different VATs.

Xenodiagnosis: diagnostic method based on detection of the parasite in

Table 1. (continued)

Animal species	Origin of trypanosome strain ^a	Infectiveness to tsetse	Minimum observed duration of infection	Refs
<i>striatus</i>), Rusty-nosed rat (<i>Cenomys hypoxanthus</i>), African brush-tailed porcupine (<i>Atherurus africanus</i>)				

^aFor reasons of traceability, we use the name of countries and the scientific name of animals as mentioned in the original publication: Senegambia = Senegal and The Gambia; Belgian Congo, Congo Free State and Congo Belge = Democratic Republic of the Congo; République populaire du Congo = Republic of the Congo.

susceptible vectors after they were fed on an individual suspected of being infected with the parasite; in HAT, the vectors used are teneral tsetse flies.

transmission and causing a resurgence of the disease in different geographical areas and epidemiological settings (see Outstanding Questions).

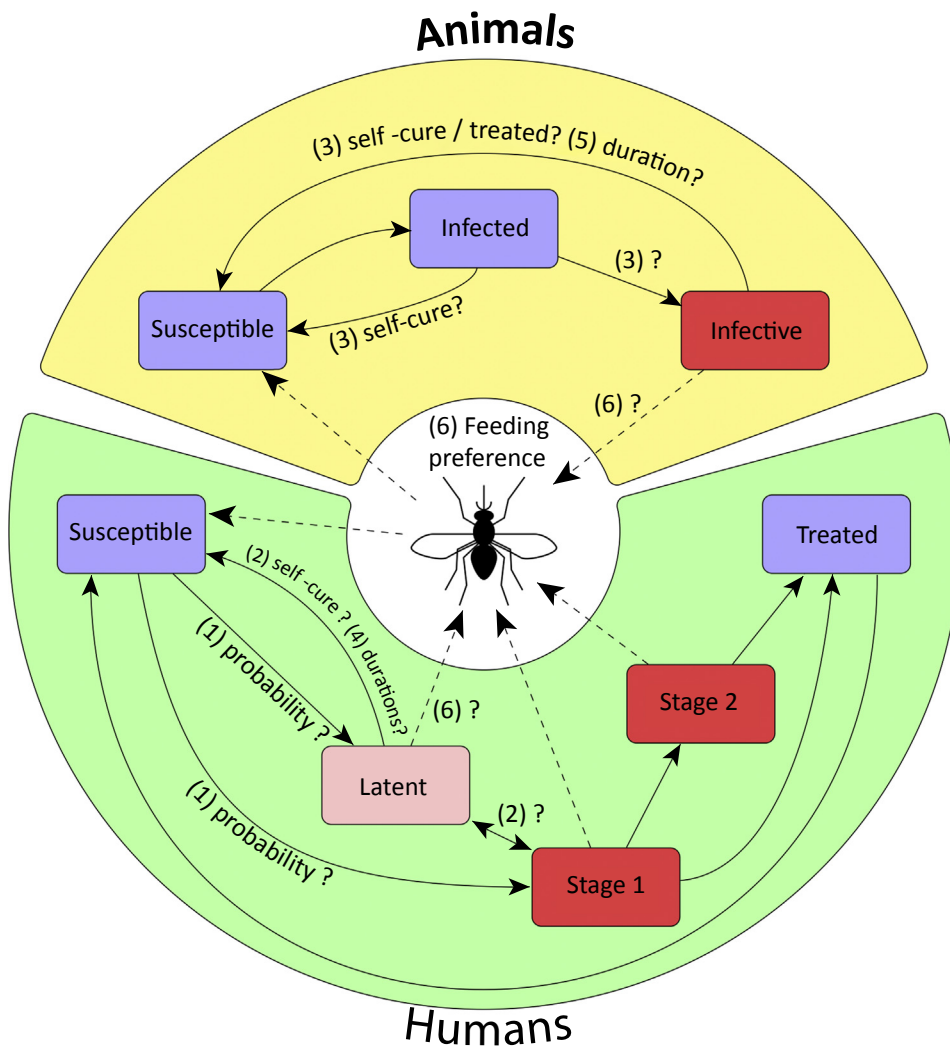
As with the mathematical modelling of other neglected tropical diseases [64], models on HAT epidemiology may help to improve our epidemiological knowledge and inform elimination strategies. Models can explore if, and how, animal and human reservoirs could sustain endemicity in HAT foci [65]. However, model predictions heavily depend on the availability of accurate information for their construction, parameterisation, and fitting. To date, a few models have attempted to infer the contribution of reservoirs in *gambiense*-HAT transmission maintenance by fitting to human epidemiological data. Funk *et al.* [66] suggested that animals were necessary for persistent transmission in Bipindi focus in Cameroon. Studies of existing *gambiense*-HAT models in a few foci (i.e., D. R. Congo, Guinea, and Chad) suggest that some type of additional infection reservoir is needed to match the observed dynamics of reported HAT cases [5,67,68]. This could arise from another human reservoir (including undiagnosed and latent infections), an animal reservoir, and/or heterogeneities in human risk exposure and surveillance coverage. A different modelling exercise considered the implications on transmission and control of whether animals function as reservoirs or as zoonophylaxis but did not address which was more likely [69].

Due to the current lack of knowledge surrounding latently infected people (including their frequency, disease progression, their relative infectivity to tsetse, and the duration of this infectious stage) modelling latent infections in humans is challenging, and these uncertainties will impact the models' predictions. In particular, latent infections have only been explicitly incorporated in one *gambiense*-HAT model, and the potential role of these individuals in maintaining transmission or hindering elimination has yet to be fully analysed [70]. Arguably, long-duration infections, which eventually progress to late-stage disease, are captured by the stage 1 exponential distributions used in many modelling frameworks, but modifications could better represent self-cure and nondetection of latent infections in active screening. Many recent modelling studies have concluded that existing vector-control methods have the ability to quickly reduce transmission to and from tsetse to all hosts, and may be critical for elimination in regions where reservoirs exist [67–72].

New data and investigations into latent human infections and animal infections will help shape the way in which future models are developed and parameterised by factoring in improved biological evidence. Some critical gaps in our knowledge, which influence modelling choices, are shown in Figure 2 (Key Figure). As well as refining formulation and parameterisation of the existing **deterministic** models, it is also clear that a new generation of models is needed.

Key Figure

Unknown Elements in Human African Trypanosomiasis Progression and Transmission



Trends in Parasitology

Figure 2. Solid lines represent progression between disease states, and dashed lines represent transmission of the parasites to and from the tsetse vector. Red boxes denote people or animals that may be infective to tsetse, with the darker shades denoting possible greater infectiveness. The figure highlights key unknown elements in disease progression and transmission including: (1) the probability of an infection leading to latent or stage 1 disease in humans – if, and how frequently; (2) self-cure of infected humans or (3) animals arises; (4) the duration of latent infection in humans, or (5) any infections in animals; and (6) the relative probability of transmission to tsetse from different types of infections (accounting for host feeding preferences).

Stochastic models are better suited to capture the chance events that determine the role of cryptic reservoirs and their implications for elimination. In conclusion, improved mathematical models on HAT epidemiology, combined with additional field and experimental data, are needed to help understand the respective roles of these reservoirs.

Concluding Remarks

We believe that attaining the elimination (zero transmission) target of *gambiense*-HAT by 2030 is feasible but, as observed for other neglected tropical diseases, latent infections – whether human or animal – may constitute cryptic parasite reservoirs and thus add another challenge to sustained elimination. To inform evidence-based elimination strategies, a better understanding of the contribution of these putative human and animal reservoirs on the epidemiology of *gambiense*-HAT is required, more in particular on (i) the frequency and duration of latent human infections and infections in animals, (ii) the infectiveness of latent human infections and animal reservoirs to tsetse flies, (iii) the ability of latent human infections or animal reservoirs to sustain transmission in interepidemic periods, and (iv) the possible existence of an animal transmission cycle in the absence of human transmission and its ability to seed a new transmission cycle in humans. To investigate these issues, we urgently need to improve our toolbox for the identification of latent and self-cured infections, including prognostic and diagnostic markers. Also, more accurate and preferably high-throughput tests to detect and monitor *T. b. gambiense* infections in animals should be developed, along with improved mathematical models for exploration of epidemiological hypotheses.

Acknowledgments

This work was supported by a grant from the Bill & Melinda Gates Foundation (OPP1150674). KSR gratefully acknowledges funding of the NTD Modelling Consortium by the Bill & Melinda Gates Foundation in partnership with the Task Force for Global Health under grant number OPP1053230. AML, BB, EM, GS, HI, MK, VJ, and VL are supported by TrypanoGen funded by the Wellcome Trust (grant number 099310/Z/12/Z). NC acknowledges funding from the Bill & Melinda Gates Foundation under grant OPP1156227. LMF is funded by Fundacao para a Ciencia e Tecnologia (IF/01050/2014). FAO contribution to this study was provided in the framework of the Programme against African Trypanosomiasis (PAAT), and supported by the Government of Italy (FAO Project 'Improving food security in sub-Saharan Africa by supporting the progressive reduction of tsetse-transmitted trypanosomiasis in the framework of the NEPAD', codes GTFS/RAF/474/ITA and GCP/RAF/502/ITA). The funders had no role in design, decision to publish, or preparation of the manuscript. The views, opinions, assumptions or any other information set out in this article are solely those of the authors.

Resources

- ⁱwww.who.int/gho/neglected_diseases/human_african_trypanosomiasis/en/
- ⁱⁱhttp://apps.who.int/iris/bitstream/10665/70809/1/WHO_HTM_NTD_2012.1_eng.pdf
- ⁱⁱⁱwww.dndi.org/diseases-projects/hat/portfolio/

Supplemental Information

Supplemental information associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.pt.2017.11.008>.

References

1. Büscher, P. *et al.* (2017) Human African trypanosomiasis. *Lancet* 390, 2397–2409
2. World Health Organization (2013) Control and surveillance of human African trypanosomiasis. *WHO Tech. Rep. Series* 984, 1–237
3. Jamonneau, V. *et al.* (2015) Accuracy of individual rapid tests for serodiagnosis of *gambiense* sleeping sickness in West Africa. *PLoS Negl. Trop. Dis.* 9, e0003480
4. Mesu, V.K.B.K. *et al.* (2017) Oral fexinidazole for late-stage African *Trypanosoma brucei gambiense* trypanosomiasis: a pivotal multicentre, randomised, non-inferiority trial. *Lancet* Published online November 4, 2017. [http://dx.doi.org/10.1016/S0140-6736\(17\)32758-7](http://dx.doi.org/10.1016/S0140-6736(17)32758-7)
5. Rock, K.S. *et al.* (2015) Quantitative evaluation of the strategy to eliminate human African trypanosomiasis in the Democratic Republic of Congo. *Parasit. Vectors* 8, 532
6. Robays, J. *et al.* (2004) The effectiveness of active population screening and treatment from sleeping sickness control in the Democratic republic of Congo. *Trop. Med. Int. Health* 9, 542–550

Outstanding Questions

How frequent are latent infections with *T. b. gambiense* in humans and in animals?

What is the duration of latent infection in humans and in animals?

How infective are latent human infections and animal reservoirs to tsetse flies?

Are latent human infections or animal reservoirs capable of sustaining transmission in interepidemic periods?

Is it possible to discriminate ongoing latent infection from self-cure in humans?

Do prognostic markers of latent infection outcome in humans exist?

What are the intrinsic and extrinsic factors that influence latent infection outcome in humans?

Can, and do, animal transmission cycles of *T. b. gambiense* exist in the absence of human transmission? If so, what is the likelihood that they could seed a new transmission cycle in humans?

7. Mumba Ngoyi, D. *et al.* (2014) Performance of parasitological and molecular techniques for the diagnosis and surveillance of *gambiense* sleeping sickness. *PLoS Negl. Trop. Dis.* 8, e2954
8. Viana, M. *et al.* (2014) Assembling evidence for identifying reservoirs of infection. *Trends Ecol. Evol.* 29, 270–279
9. Jamonneau, V. *et al.* (2012) Untreated infections by *Trypanosoma brucei gambiense* are not 100% fatal. *PLoS Negl. Trop. Dis.* 6, e1691
10. Fromentin, H. (1963) Nouvelles précision sur le *Trypanosome* sp souche FEO. *Ann. Soc. Belg. Méd. Trop.* 5, 797–800
11. Sudarshi, D. *et al.* (2014) 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.* 8, e3349
12. Rocha, G. *et al.* (2004) Possible cases of sexual and congenital transmission of sleeping sickness. *Lancet* 363, 247
13. Welburn, S.C. *et al.* (2016) Beyond tsetse – Implications for research and control of human African trypanosomiasis epidemics. *Trends Parasitol.* 32, 230–241
14. Ilboudo, H. *et al.* (2011) Diversity of response to *Trypanosoma brucei gambiense* infections in the Forécariah mangrove focus (Guinea): perspectives for a better control of sleeping sickness. *Microbes Infect.* 13, 943–952
15. Kristensson, K. and Bentivoglio, M. *et al.* (1999) Pathology of African trypanosomiasis. In *Progress in Human African Trypanosomiasis, Sleeping Sickness* (Dumas, M., ed.), pp. 157–181, Springer
16. Blum, J.A. *et al.* (2008) Cardiac involvement in African and American trypanosomiasis. *Lancet Infect. Dis.* 8, 631–641
17. McGovern, T.W. *et al.* (1995) Cutaneous manifestations of African trypanosomiasis. *Arch. Dermatol.* 131, 1178–1182
18. Trindade, S. *et al.* (2016) *Trypanosoma brucei* parasites occupy and functionally adapt to the adipose tissue in mice. *Cell Host Microbe* 19, 837–848
19. Giroud, C. *et al.* (2009) Murine models for *Trypanosoma brucei gambiense* disease progression—from silent to chronic infections and early brain tropism. *PLoS Negl. Trop. Dis.* 3, e509
20. Claes, F. *et al.* (2009) Bioluminescent imaging of *Trypanosoma brucei* shows preferential testis dissemination which may hamper drug efficacy in sleeping sickness patients. *PLoS Negl. Trop. Dis.* 3, e486
21. Biteau, N. *et al.* (2016) *Trypanosoma brucei gambiense* infections in mice lead to tropism to the reproductive organs, and horizontal and vertical transmission. *PLoS Negl. Trop. Dis.* 10, e0004350
22. Capewell, P. *et al.* (2016) The skin is a significant but overlooked anatomical reservoir for vector-borne African trypanosomes. *eLIFE* 10, 17716
23. Caljon, G. *et al.* (2016) The dermis as a delivery site of *Trypanosoma brucei* for tsetse flies. *PLoS Pathog.* 12, e1005744
24. Kabore, J. *et al.* (2011) Population genetic structure of Guinea *Trypanosoma brucei gambiense* isolates according to host factors. *Infect. Genet. Evol.* 11, 1129–1135
25. Courtin, D. *et al.* (2007) Association between human African trypanosomiasis and the IL6 gene in a Congolese population. *Infect. Genet. Evol.* 7, 60–68
26. Courtin, D. *et al.* (2006) Interest of tumor necrosis factor- α -308 G/A and interleukin-10 -592C/A polymorphisms in human African trypanosomiasis. *Infect. Genet. Evol.* 6, 123–129
27. Cuypers, B. *et al.* (2016) Apolipoprotein L1 variant associated with increased susceptibility to trypanosome infection. *mBio* 7, e02198-15
28. Courtin, D. *et al.* (2013) HLA-G 3' UTR-2 haplotype is associated with human African trypanosomiasis susceptibility. *Infect. Genet. Evol.* 17, 1–7
29. Hardwick, R.J. *et al.* (2014) Haptoglobin (HP) and haptoglobin-related protein (HPR) copy number variation, natural selection, and trypanosomiasis. *Hum. Genet.* 133, 69–83
30. Cooper, A. *et al.* (2017) APOL1 renal risk variants have contrasting resistance and susceptibility associations with African trypanosomiasis. *eLIFE* 6, e25461
31. Ilboudo, H. *et al.* (2016) Trypanosome-induced Interferon-gamma production in whole blood stimulation assays is associated with latent *Trypanosoma brucei gambiense* infections. *Microbes Infect.* 18, 436–440
32. Ilboudo, H. *et al.* (2014) Unravelling human trypanotolerance: IL8 is associated with infection control whereas IL10 and TNF α are associated with subsequent disease development. *PLoS Pathog.* 10, e1004469
33. Gineau, L. *et al.* (2016) Human Leukocyte Antigen-G: a promising prognostic marker of disease progression to improve the control of human African trypanosomiasis. *Clin. Infect. Dis.* 63, 1189–1197
34. Cecchi, G. *et al.* (2014) Assembling a geospatial database of tsetse-transmitted animal trypanosomosis for Africa. *Parasit. Vectors* 7, 39
35. Cecchi, G. *et al.* (2015) Developing a continental atlas of the distribution and trypanosomal infection of tsetse flies (*Glossina* species). *Parasit. Vectors* 8, 284
36. Yesufu, H.M. (1971) Experimental transmission of *Trypanosoma gambiense* in domestic animals. *Ann. Trop. Med. Parasitol.* 65, 341–347
37. Molyneux, D.H. (1973) Animal reservoirs and Gambian trypanosomiasis. *Ann. Soc. Belg. Méd. Trop.* 53, 605–618
38. Kageruka, P. (1989) Réservoir animal de *Trypanosoma (Trypanozoon) brucei gambiense* en Afrique Centrale. *Ann. Soc. Belg. Méd. Trop.* 69, 155–163
39. Van Hoof, L. *et al.* (1937) Sur le rôle du porc comme réservoir de *Trypanosoma gambiense*. *C. R. Soc. Biol.* 126, 72–75
40. Greggio, J. (1917) A propos de la trypanose des porcs dans la vallée de l'Inkisi. *Bull. Agr. Congo Belge* 1–2, 148–160
41. Jamonneau, V. *et al.* (2004) Mixed infections of trypanosomes in tsetse and pigs and their epidemiological significance in a sleeping sickness focus of Côte d'Ivoire. *Parasitology* 129, 693–702
42. Njiokou, F. *et al.* (2006) Wild fauna as a probable animal reservoir for *Trypanosoma brucei gambiense* in Cameroon. *Infect. Genet. Evol.* 6, 147–153
43. Cordon-Obras, C. *et al.* (2015) Molecular evidence of a *Trypanosoma brucei gambiense* sylvatic cycle in the human African trypanosomiasis foci of Equatorial Guinea. *Front. Microbiol.* 6, 765
44. Mehlitz, D. (1977) The behaviour in the blood incubation infectivity test of four *Trypanozoon* strains isolated from pigs in Liberia. *Trans. R. Soc. Trop. Med. Hyg.* 71, 86
45. N'Djetchi, M.K. *et al.* (2017) The study of trypanosome species circulating in domestic animals in two human African trypanosomiasis foci in Côte d'Ivoire identifies pigs and cattle as potential reservoirs of *Trypanosoma brucei gambiense*. *PLoS Negl. Trop. Dis.* 11, e0005993
46. Simarro, P.P. *et al.* (2010) The atlas of human African trypanosomiasis: a contribution to global mapping of neglected tropical diseases. *Int. J. Health Geogr.* 9, 57
47. Van Hoof, L. *et al.* (1940) Recherche sur le comportement de *Trypanosoma gambiense* chez le porc. *Ann. Soc. Belg. Méd. Trop.* 20, 203–228
48. Van Hoof, L.M.J.J. (1947) Observations on trypanosomiasis in the Belgian Congo. *Trans. R. Soc. Trop. Med. Hyg.* 40, 728–761
49. Corson, J.F. (1938) A third note on a strain of *Trypanosoma gambiense* transmitted by *Glossina morsitans*. *Ann. Trop. Med. Parasitol.* 32, 245–248
50. Duke, N.L. (1913) Antelope and their relation to trypanosomiasis. *Proc. R. Soc. Lond. B* 85, 156–169
51. Wombou Toukam, C.M. *et al.* (2011) Experimental evaluation of xenodiagnosis to detect trypanosomes at low parasitaemia levels in infected hosts. *Parasite* 18, 295–302

52. Radwanska, M. *et al.* (2002) Novel primer sequences for a polymerase chain reaction-based detection of *Trypanosoma brucei gambiense*. *Am. J. Trop. Med. Hyg.* 67, 289–295
53. Gibson, W. *et al.* (2010) Conserved sequence of the TgsGP gene in Group 1 *Trypanosoma brucei gambiense*. *Infect. Genet. Evol.* 10, 453–458
54. Gibson, W. *et al.* (1978) The identification of *Trypanosoma brucei gambiense* in Liberian pigs and dogs by isoenzymes and by resistance to human plasma. *Tropenmed. Parasitol.* 29, 335–345
55. Truc, P. *et al.* (1991) Multilocus isozyme identification of *Trypanosoma brucei* stocks isolated in Central Africa: evidence for an animal reservoir of sleeping sickness in Congo. *Acta Trop.* 49, 127–135
56. Zillmann, U. *et al.* (1984) Identity of *Trypanozoon* stocks isolated from man and a domestic dog in Liberia. *Tropenmed. Parasitol.* 35, 105–108
57. Rickman, L.R. (1976) Observations on the subspecific identification of *Trypanosoma (Trypanozoon) brucei* isolates from non-human hosts by the blood incubation infectivity test (BIIT). *Trans. R. Soc. Trop. Med. Hyg.* 70, 262–264
58. Simo, G. *et al.* (2008) Tsetse fly host preference from sleeping sickness foci in Cameroon: epidemiological implications. *Infect. Genet. Evol.* 8, 34–39
59. Diall, O. *et al.* (2017) Developing a progressive control pathway for African animal trypanosomosis. *Trends Parasitol.* 33, 499–509
60. Centers for Disease Control and Prevention (1993) *Recommendations of the International Task Force for Disease Eradication*. (42, RR-16 edn), 1–39
61. Callaway, E. (2016) Dogs thwart effort to eradicate Guinea worm. *Nature* 529, 10–11
62. Singh, O.P. *et al.* (2016) Elimination of visceral leishmaniasis on the Indian subcontinent. *Lancet Infect. Dis.* 16, e304–e309
63. Franco, J.R. *et al.* (2017) Monitoring the elimination of human African trypanosomiasis: update to 2014. *PLoS Negl. Trop. Dis.* 11, e0005585
64. Hollingsworth, T.D. *et al.* (2015) Quantitative analyses and modelling to support achievement of the 2020 goals for nine neglected tropical diseases. *Parasit. Vectors* 8, 630
65. Rock, K.S. *et al.* (2015) Mathematical models of human African trypanosomiasis epidemiology. *Adv. Parasitol.* 87, 53–133
66. Joshua, R.A. *et al.* (1983) Isolation of human serum resistant *Trypanozoon* from cattle in Nigeria. *Tropenmed. Parasitol.* 34, 201–202
67. Pandey, A. *et al.* (2015) Evaluating long-term effectiveness of sleeping sickness control measures in Guinea. *Parasit. Vectors* 8, 550
68. Mahamat, M.H. *et al.* (2017) Adding tsetse control to medical activities contributes to decreasing transmission of sleeping sickness in the Mandoul focus (Chad). *PLoS Negl. Trop. Dis.* 11, e0005792
69. Stone, C.M. and Chitnis, N. (2015) Implications of heterogeneous biting exposure and animal hosts on *Trypanosomiasis brucei gambiense* transmission and control. *PLoS Comput. Biol.* 11, e1004514
70. Rock, K.S. *et al.* (2017) Data-driven models to predict the elimination of sleeping sickness in former Equateur province of DRC. *Epidemics* 18, 101–112
71. Sutherland, C.S. *et al.* (2017) Seeing beyond 2020: an economic evaluation of contemporary and emerging strategies for elimination of *Trypanosoma brucei gambiense*. *Lancet Glob. Health* 5, e69–e79
72. Rock, K.S. *et al.* (2017) Predicting the impact of intervention strategies for sleeping sickness in two high-endemicity health zones of the Democratic Republic of Congo. *PLoS Negl. Trop. Dis.* 11, e0005162
73. Thomas, H.W. and Linton, S.F. (1904) A comparison of the animal reactions of the trypanosomes of Uganda and Congo Free State sleeping sickness with those of *Trypanosoma gambiense* (Dutton). *Lancet* 1337–1340
74. Moloo, S.K. *et al.* (1986) Cyclical development of *Trypanosoma brucei gambiense* from cattle and goats in Glossina. *Acta Trop.* 43, 407–408
75. Mesnil, F. and Blanchard, M. (1912) Infections des poules dues aux *Trypanosoma gambiense* et *Tryp rhodesiense*. *C. R. Soc. Biol.* 72, 938–940
76. Watson, H.J.C. (1962). The domestic pig as a reservoir of *T. gambiense* In 9th Meeting of the International Scientific Council for Trypanosomiasis Research and Control, Conakry, Guinea (Commission de Coopération Technique en Afrique au Sud du Sahara, ed), p. 327
77. Bouteille, B. *et al.* (1999) Experimental models for new chemotherapeutic approaches to human African trypanosomiasis. In *Progress in Human African Trypanosomiasis, Sleeping Sickness* (Dumas, M., ed.), pp. 289–300, Springer
78. Godfrey, D.G. and Killick-Kendrick, R. (1967) Cyclically transmitted infections of *Trypanosoma brucei*, *T. rhodesiense* and *T. gambiense* in chimpanzees. *Trans. R. Soc. Trop. Med. Hyg.* 61, 781–791
79. Lucasse, C. (1964) Fluorescent antibody test as applied to cerebrospinal fluid in human sleeping sickness. *Bull. Soc. Pathol. Exot. Fil.* 57, 283–292
80. Frézil, J.-L. (1979) Etude du comportement de *Trypanosoma brucei gambiense* sur petits rongeurs et lémurins du Congo. *Cah. ORSTOM Sér. Entomol. Méd. Parasitol.* 12, 119–126
81. Larivière, M. (1957) Réceptivité de *Cricetomys gambianus* au *Trypanosoma gambiense*. *C. R. Soc. Biol.* 151, 1349–1351