Human African trypanosomiasis: What are the prospects for control?

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ABSTRACT: Human African trypanosomiasis (HAT) is in decline, thanks to sustained control efforts in recent decades. Yet, its complexity as a disease at the animal–human interface and its potential for resurgence represent a significant concern for the final elimination goal. Understanding the challenges underlying HAT control involves engaging deeply with the epidemiology, ecology, and evolution of trypanosomes and their hosts. Dissecting the importance of parasite-intrinsic biological factors, vector life-history contribution, and host immunological aspects, requires integrated efforts across disciplines. The prospects for control of HAT, reviewed here, comprise a spectrum of developments, from new tools for disease diagnosis and staging, tsetse control, and prevention of transmission, to more effective and non-toxic treatment options, including immune therapies. Although fundamentally pathogenic, trypanosomes can also be carried asymptomatically by their hosts. Trypanotolerance, recently recognized as an important factor in persistence of disease foci, especially for *Trypanosoma brucei gambiense*, remains an under-studied aspect of HAT epidemiology. With advancing technologies, a better cellular and molecular resolution of trypanosome infection processes is now possible. Integrating these data with quantitative models, and linking mechanistically different aspects of disease across biological scales, could bring key novel insights into HAT control strategies.

KEYWORDS: Human African trypanosomiasis, epidemiology, control, treatment, new frontiers, biological scales

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Introduction

Human African trypanosomiasis (HAT), also known as sleeping sickness, is a neglected disease affecting around 36 countries in sub-Saharan Africa. This disease is caused by protozoan parasites of the genus Trypanosoma and transmitted via the bite of the tsetse fly. Recent estimates suggest that there are 70 million people at risk of contracting this disease.[1] Over the last 15 years, sustained control efforts against HAT have reduced substantially the number of new cases,[2] tending towards meeting the World Health Organization (WHO) goal of HAT elimination.[3] In 2009 the number of cases reported fell below 10,000 for the first time in five decades, and in 2014 there were 3796 cases recorded.[4,5] In the last 10 years, over 70% of reported cases have occurred in the Democratic Republic of the Congo (DRC) alone, a country also affected by political unrest and instability.

There are many subspecies of trypanosoma parasites. However, the infection to humans can only be caused by two of them: *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense*.[6] The first form of HAT (gHAT) is more common in West and Central Africa[i], and is responsible for about 98% of cases, whereas the second form of HAT (rHAT) is less common, responsible for only about 2% of cases, and is found in East and Southern Africa.[5] African trypanosomes pose a severe problem not only due to the pathogenic effects of their infections in humans, but also because of the socio-economic losses resulting from the disease.[7] Moreover, disease can also affect livestock in rural areas,[8] typically when infected by other species such as *T. congolense, T. vivax, T. b. brucei, T. simiae, T.evansi*, and *T. equiperdum*. The subspecies *T. b. brucei* occurs throughout sub-Saharan Africa, but is not infective to humans because it is rapidly lysed in the human serum,[9] unlike *T. b. gambiense* or *T. b. rhodesiense*.

Despite the remarkable achievements in the control of HAT over the past decade,[2] leading to a decline in the number of cases, these reductions need to be taken with caution, as under-reporting and difficult logistics to access remote affected regions remain a significant concern.[10] Parasite transmission at the human–animal–tsetse interface in remote rural regions can lead to the emergence and re-emergence of the disease in the form of epidemics, whenever there are suitable habitats for its vector. Therapeutic options against HAT are scarce and with several limitations, including high reported rates of therapeutic failure.[11] Antigenic variation of the parasite within host also hampers prospects for vaccine development.[12,13]

However, in a promising outlook for the future, multi-sectorial partnerships are active with joint initiatives for HAT prevention, surveillance, and control. Moreover, significant technological and molecular advances in recent years are contributing to a deeper understanding of trypanosome biology,[14] which may outline new directions for diagnostic tools and drug development, both for gHAT and rHAT. Depending on how much of this progress will ultimately reach the field, improving the quality of local health systems and trypanosomiasis control, the current positive trends in the decline of the disease will continue and get stronger. Although the challenges of controlling *T. b. rhodesiense* infections are likely to persist, the ambitious WHO goal of stopping transmission of *T. b. gambiense* by 2030[2] seems within reach.

Epidemiology of HAT

The two forms of HAT show significant differences, and hence vary in their prospects for control.[6] *T. b. gambiense* infection causes a chronic, slowly progressing disease which can be asymptomatic for months or even years, and ultimately leads to death if untreated. This is known as *sleeping sickness*, occurring primarily in West and Central Africa,[6] and transmitted by tsetse flies of the genus Glossina, of the *Palpalis* group (riverine tsetse). Both male and female flies feed on blood, and all species are efficient vectors of pathogenic trypanosomes.[15] Humans constitute the principal reservoir of *T. b. gambiense*, and although it is thought that their predominant mode of transmission is via the vector, vertical, sexual, and congenital transmission have also been reported.[5,16]

T. b. rhodesiense is zoonotic, rHAT presenting thus a different challenge to gHAT, due to a large portion of the pathogen population's lifespan spent cycling between wild animals and vectors. Persistence of this pathogen subspecies is thus largely driven by the sylvatic reservoir,[17] including zebra, lions, hartebeests, bushbucks, gazelles, and hyenas, and very little by the human population. Human infection is typically incidental, where the parasite causes an acute form of the disease, fatal within weeks if untreated, although more recent estimates suggest that more than 80% of deaths occur within 6 months of illness.[18] *T. b. rhodesiense* occurs east of the Rift Valley[i], and its vectors are flies from the *Morsitans* group (savannah tsetse). The predominant reservoir of *T. b. rhodesiense* are wild animals and cattle, and depending on vector–host proximity and vector contact with these reservoirs, humans get exposed to the disease.[5]

While control measures typically aim at a reduction of disease incidence, prevalence, morbidity, and mortality to a locally acceptable level, elimination of a disease is a stronger goal, namely reduction to zero of the incidence of a specified disease in a given population in a given region as a result of deliberate efforts. Elimination of infection, on the other hand, aims at reduction to zero of the incidence of infection caused by a specific agent, which requires continued interventions and measures to prevent re-establishment of transmission and asymptomatic infection. When considering the trypanosoma transmission system, prospects for elimination are unrealistic for rHAT, due to its large sylvatic reservoir, whereby only reduction in disease cases and reduction of infection by *T. b. rhodesiense* may be attempted. For gHAT instead, prospects for elimination are more feasible, once transmission is reduced below critical levels between the epidemiological compartments.

Uganda is known as the only country where both forms of HAT, *T. b. gambiense* and *T. b. rhodesiense*, occur. Despite having separate foci, there has been concern that the two subspecies may overlap because of the transport of infected cattle to Western Uganda.[19] The possible overlap of the two variants in the same region compromises patient treatment, which is different for the two diseases, complicated by the difficulty of distinguishing between these two subspecies via microscopy alone. In response to the threat of the overlap of the two diseases, a public–private partnership, Stamp Out Sleeping Sickness (SOS), was formed in 2006, with the aim of undertaking mass trypanocidal treatment and insecticidal spraying of cattle in five districts in Northern Uganda.[20] Successful chemotherapeutic treatment of domestic livestock reduces the prevalence of the parasite in the cattle, which are the primary animal reservoir of trypanosoma in countries such as Uganda, and in other areas where the wildlife populations are decreasing.

Three major HAT epidemics have been documented in the past century: the first around 1896 and 1906, which comprised equatorial Africa and killed around 800,000 people, the second around 1920–1940, which led to increased control efforts by the colonial powers in terms of surveillance and vector control, and a third around the 1990s, following the independence in many endemic African countries, which was accompanied by turmoil and collapse of control activities against HAT.[3,21] This collapse led to an increase in the number of cases in the DRC, Angola, Southern Sudan, and Uganda. Following such resurgence, control efforts were intensified again, which enabled a drastic reduction in the incidence of HAT disease over the last decades, especially gHAT.[2] Sporadic cases of HAT have also been reported in USA and Europe,[22] typically in travelers who have recently returned from visits in African countries. Most HAT cases outside Africa have been caused by *T. b. rhodesiense*.

As they are tsetse-transmitted, trypanosomes undergo a critical part of their lifecycle in the vector.[21] The fly first feeds on an infected mammalian host, after which the parasites gain entry into the fly's digestive tract. During a period of 3–5 weeks in the fly, trypanosomes differentiate in steps, until they migrate to the salivary glands, where they develop into the infective parasite form, which will be passed on to the next host. Successful completion of the parasite maturation process in the fly is difficult, thus infection in flies is rare in the field, especially with *T. b. gambiense*, reaching only about 0.1%. Although sexual reproduction is not obligatory in trypanosomes, it can occur between parasites in tsetse salivary glands,[23] enabling genetic exchange and the rapid transmission of crucial life-history traits such as drug resistance and virulence. While genetic exchange is relatively rare in *T. b. gambiense*, it happens much more frequently in *T. b. rhodesiense*.[24] Laboratory studies have shown that *T. b. rhodesiense* is easily transmitted in the lab and salivary gland infections are readily found in samples collected from the field. In contrast, *T. b. gambiense* is very difficult to transmit in the lab, and fly infections are found rarely in the field. These differences challenge our understanding of how disease foci are maintained.[25]

Clinical manifestation of sleeping sickness

When a human becomes infected, the disease develops first from a hemolymphatic stage with mild symptoms, including headaches, fever, joint pains, and itching, into a second more severe stage, where the parasites cross the blood–brain barrier and establish a cerebral infection.[11] However, it is difficult to discern the actual duration of stage 1 and stage 2 infection,

particularly regarding the period in which a patient remains infectious.[26] Using survival analysis models, the estimated average duration of *T. b. gambiense* infection is around 3 years, where the first half corresponds to stage 1 disease and the second half to stage 2.[27] In the case of *T. b. rhodesiense* instead, disease is acute, and death occurs within weeks or months, although in some surveys from Malawi, also a more chronic form has been reported.[28] While a greater diversity of infection profiles and clinical manifestations are associated with *T. b. gambiense* infection, less diversity and more canonical severe infection profiles are seen with *T. b. rhodesiense*.[28]

These differences in infection stages and progression affect parasite opportunities for onward transmission and also the strategies for treatment of disease. The term 'sleeping sickness' by which HAT is commonly known, refers to the symptoms of the neurological phase of infection, which include reduced coordination, disruption of the sleep cycle, and fatigue punctuated with manic episodes leading to daytime torpor and nighttime insomnia. However, the symptoms of the disease start in a milder way, first with a chancre formed at the site of tsetse bite, between 5 and 15 days after the human–fly contact, and a trypanosomal rash, typically more severe in *T. b. rhodesiense* than *T. b. gambiense* infection.[29] These symptoms have also been observed to be stronger and more abnormal in travelers than in locals from endemic countries.[30]

Following infection, several hematological disorders and immunological inflammatory reactions occur due to parasite extracellular replication in the blood and lymphatic organs. These include lymphadenopathy, enlargement of the spleen, cardiac problems, endocrine disruption, alopecia, and hepatomegaly.[31] The second stage, also known as the central nervous system (CNS) stage, is characterized by meningoencephalitis and an exacerbated host immune response. In this phase, the cerebral white matter is invaded with lymphocytes, macrophages, and plasma cells, leading to neuropathogenesis.[31] All these biological processes in late stage disease are associated with psychiatric, motor, sensory, and sleep disorders, eventually progressing to seizures, somnolence, coma, and death of the host.

The neuropathogenesis of HAT disease when parasites cross the blood–brain barrier is complex, and the understanding of the processes involved has advanced substantially in recent years, both from human examinations and animal models.[32] Yet, at present, the mechanisms by which trypanosomes are able to enter and persist in the CNS remain elusive. Recent reports focusing on virulence profiles across human *T. b. rhodesiense* infections are elucidating host factors responsible for variation in infection progression, in particular the role of systemic cytokine responses such as interferon (IFN)-γ and tumor necrosis factor (TNF)-α, as well as their interactions with parasite genotype and geographic location.[28,33]

Diagnosis and its challenges

It is recommended that diagnosis of HAT disease be made as urgently as possible, to avoid progression of the infection to the neurological stage[ii]. Typically due to the long, relatively symptom-free first stage of *T. b. gambiense* infection, a comprehensive, active screening of the entire population at risk should be performed, in order to diagnose patients in early stage, which can last from a few months to a few years[26] and can even be asymptomatic.[34,35] In rHAT instead, progression to stage 2 disease is considerably faster, ranging between a few weeks to a few months post-infection.[28,33] Active case detection and treatment has also population benefits because it reduces onward transmission by removing infective individuals from the reservoir. However, exhaustive screening requires major human and financial resources, often lacking in endemic countries, particularly in remote rural areas affected by the disease. As a consequence, some infections may proceed to death before they can ever be diagnosed and treated.

In recent years, WHO recommendations for disease management[iii] outline three steps.

- **Screening for potential exposure.** This step involves using serological tests (currently limited to *T. b. gambiense*) and searching for clinical signs, especially swollen cervical lymph nodes in the patient. The card agglutination test for gHAT (CATT) has been developed in the late 1970s, and can be performed in a fast and practical manner on serum or capillary blood obtained from a finger prick. It has an 87–90% sensitivity and 93–95% specificity.[21] In contrast, there are no serological screening tests available for *T. b. rhodesiense*. Polymerase chain reaction (PCR) approaches have also been attempted to increase sensitivity and specificity of HAT diagnostic testing for early stage disease,[36] with promising results, but they remain too advanced for many existing facilities in field conditions.
- **Diagnosing parasite presence in body fluids.** The parasitological confirmation entails microscopic examination of lymph node and/or blood aspirates. The sensitivity of this test varies between 40% and 80% and is generally better suited to diagnose stage 1 infection.[37,38] Parasitological confirmation is easier for *T. b. rhodesiense* infection because the density of parasites circulating in blood is higher than for *T. b. gambiense* infection, which is characterized by cyclical waves of parasitemia and frequent low parasite numbers below 100 parasites per milliliter of blood.[5] After diagnosis, treatment is recommended depending on the results. In areas of high seroprevalence (>1%), if CATT titers are high after a test (>1:16), treatment for the patient is recommended even if parasitological examination is negative.[11] In areas of low endemic prevalence, this strategy is not recommended due to the potential adverse effects of treatment.
- **Disease staging to establish the state of disease progression.** Since treatment for CNS-stage disease can be very toxic and with severe side effects, diagnostic staging to correctly classify patients into early versus late stage is

crucial but remains problematic.[32] This staging entails examining the cerebrospinal fluid (CSF) of the patient, a sample of which can be obtained by lumbar puncture. According to the WHO,[39] second stage disease is defined by the presence in CSF of more than 5 white blood cells per microliter, trypanosomes, or elevated protein content (>370 mg/L), although total protein content indicators remain controversial. More recently, increased IgM concentration in CSF and neopterin have been proposed as an early and specific marker of CNS invasion.[40] Yet, there is still no clear consensus for staging criteria, and some clinicians adopt a higher threshold for white cell count, e.g. 20 cells per microliter,[41] especially for diagnosing and staging gHAT.

Further development of these diagnostic approaches is ongoing,[11,42] with the aim of creating better tools that can more accurately diagnose HAT disease and staging, on the basis of immunological biomarkers,[43,44] proteomics,[45] and non-invasive techniques such as those examining sleep structure[46] or MRI data from patients.[47] These tools must be made reliable, practical, economically feasible and easy to administer. A challenge remains to use current diagnostic information or augment it, in order to parametrize effective treatment models and biomarker-guided therapies, and to interpret more deeply clinical responses in patients.

Prospects for control

For a multi-host parasite with a complex lifecycle like the trypanosome, control is inevitably difficult.[48] Several aspects of the lifecycle can be targeted, from applying prevention and vector control, to drug treatment and vaccines. Ultimately, the optimal ways in which to devise HAT control policies, when considering the differing biology and epidemiology of the parasites, depend on the wider context of control, including governments, international organizations, and the responsibilities of individuals.[49]

Prevention and vector control

These are the oldest pillars in HAT control,[50] especially in gHAT, focused on reduction of the number of bites by tsetse flies. Even though the prevalence of infection in flies is typically below 0.1%,[21] the density of flies influences the incidence and spread of both Gambian and Rhodesian sleeping sickness, and also animal trypanosomiasis. Tsetse flies are attracted to dark colors, such as blue and black, and also to vehicles in motion. Vector control, via the use of fly traps or screens,[51] in combination with odors preferred by the flies, or insecticide spraying of tsetse habitats helps to reduce fly density. If the combined density of infected humans and flies falls below the critical limits, transmission cannot be sustained.[52] When epidemiological parameters are known and integrated in a transmission model, including transmission rates, human, fly, and animal reservoir densities, these critical thresholds can be determined analytically, in a fashion similar to other multi-host parasites.[48] However, this epidemiological goal seems more likely to be reached for a disease that has a small and defined reservoir such as *T. b. gambiense*, rather than for *T. b. rhodesiense* parasites, typically sustained by transmission among wild animals and tsetse vectors, in particular in East- and South-African settings where wildlife numbers are high and transmission networks more complex.

Sequential aerosol techniques, ground spraying and sterile insect techniques[53] are also available measures for prevention of infections to humans, by reducing the number of tsetse flies. Each of these measures comes with varying costs for its implementation, which have to be carefully balanced with local needs, planning, and infrastructure.[7] For example, sequential aerosol techniques (SAT) are estimated to cost between US$285.81 and 628.79 per km^2 of application. Sterile insect techniques as a method of vector control cost more, and are generally used in settings of low fly numbers, typically following SAT or other vector control strategies that first reduce vector density. They are based on mass release of sterile male tsetse flies, which then compete with non-sterile males to mate with females, resulting in adult female flies unable to produce offspring.

On the biological side, focusing on vector life-history traits, research is ongoing to uncover mechanisms and compounds that could alter vectorial capacity to acquire or transmit infection,[54] for example exploring natural endosymbionts of tsetse flies,[55] or accounting for the special role of parasite VSG coat in tsetse competence.[56] Vector–parasite interactions are critical in the transmission and persistence of HAT, and could be exploited in disease control strategies. Indeed, cost-effectiveness analysis for HAT control must rely on quantifying the relative role and importance of different epidemiological parameters on HAT incidence and prevalence.[52]

Treatment of domestic livestock

Insecticide treatment of cattle is another way of controlling HAT, whereby reducing tsetse bites in the animal reservoir close to the human population reduces the risk of animal–vector–human transmission. Until now this measure has been mainly applied to control animal trypanosomiasis (also known as *nagana*), but in East Africa, this form of treatment has also contributed to control HAT cases in humans, because cattle are an important reservoir for *T. b. rhodesiense* in areas where populations of wildlife are decreasing. Cost estimates for this type of treatment, when restricted only to the legs and bellies of cattle, where the tsetse are more likely to feed, range around US$14.55 km^{-2}.[7]

Since 2006, a successful public–private partnership, SOS[20] has been in place, applying in areas of Northern Uganda treatment of domestic livestock. This includes not only application of restricted insecticide to domestic cattle, but also deployment

of curative drugs that remove infection from the domestic reservoir, and prophylactic drugs, which protect the animal against trypanosome infections for up to 3 months. Upon the launch of this programme, approximately 250,000 cattle in five districts in Northern Uganda, were treated successfully, reducing the prevalence of the sleeping sickness parasite in the cattle by close to 70%[iv]. SOS is estimated to have saved up to 400 million in human health care costs, in particular related to rHAT. This intervention has also generated increased productivity of 25 per head of cattle per year in the rural communities involved, thus constituting for local farmers a great incentive for continued participation.

HAT chemotherapy

While treatment of cattle is relatively cheap and easy to apply, chemotherapy for infected human hosts remains extremely complex and expensive. Despite years of drug research, chemotherapeutic options for HAT are limited and with severe side effects. Specific drugs need to be applied for the different disease stages, as well as for the subspecies of parasite involved.[21] Very few drugs are currently available in the treatment of HAT,[57] the main impediment being the cost of developing drugs for diseases like HAT, afflicting primarily people from the poorest countries and remotest areas in the world. Even with the progress of recent years, there is much need for research into new anti-parasite compounds.[3]

Early stage rHAT is treated with intravenous suramin,[21] first used in the 1920s, which although usually effective, can result in potential complications for the patient, such as renal failure and bone marrow toxicity. Pentamidine is a diamidine drug available since the 1940s, administered via intramuscular injection, effective only against *T. b. gambiense*, also applied exclusively in the early stage of the disease. This drug accumulates inside the parasite by nucleoside transporters and then binds multiple intracellular targets, causing a variety of detrimental effects.[58] The loss of these transporters is critical in the emergence of pentamidine resistance in the parasite and in cross-resistance with other drugs.[59,60]

Melarsoprol until recently was the only licensed drug for effectively treating both subspecies of HAT in the advanced disease stage.[57] It is an arsenical drug, whose uptake by the trypanosome leads to rapid lysis of the parasite. Besides melarsoprol injections being extremely painful for the patient, the drug's usefulness is constrained due to a severe side effect: arsenical encephalopathy, a series of permanent brain injuries which develop in 10% of treated patients, leading to an overall mortality from treatment of around 5.9%.[61] In addition to these deleterious treatment effects with melarsoprol, drug-resistance and treatment failures have also been observed.[62,63] For treatment of late stage *T. b. rhodesiense* infection, a 10-day regimen with melarsoprol is recommended.

In 1990, eflornithine was first licensed for use against *T. b. gambiense*, and in 2009, NECT (nifurtimox and eflornithine combination therapy) also emerged as an effective drug.[64] NECT has become the standard first-line treatment for *T. b. gambiense* CNS-stage HAT,[11] and has been applied successfully, reducing treatment duration from 14 to 10 days, and the number of needed intravenous drug doses by 75%.

Elucidating the mode of action of different drugs, alone and in combination, remains critical for optimizing therapies and for preventing the emergence of resistance and potential treatment failures.[63] It is, however, important to realize that despite the central role of chemotherapy in alleviating the disease burden of HAT and its pathogenic effects, drug treatment alone will not be able to eliminate the disease and stop transmission, because of the role played by asymptomatic untreated carriers in *T. b. gambiense* persistence.[34,65] To stop transmission, these infected individuals harboring subclinical trypanosome infections need to be detected by active screening and treated in order to reduce their contribution to the transmission cycle of the pathogen.

New frontiers towards HAT elimination

Trypanotolerance, host genetics, and epigenetic effects

Recently it is starting to be recognized that host trypanotolerance may play a major role in HAT epidemics, especially in the persistence of gHAT. Trypanotolerance is the ability to control parasite density or to limit the pathological effects of infection, and is widely employed in the context of animal African trypanosomiasis.[66–68] In humans, trypanotolerance is reflected in the wide range of the course of infection with *T. b. gambiense*, which can reach up to 7 and 29 years, and in the description of infections that self-cure.[35,69,70]

Although tsetse flies are historically associated with HAT, for *T. b. gambiense*, field data do not seem to support this link.[71] gHAT shows foci of disease independent of tsetse distribution or infection prevalence rates in flies. To address this puzzling observation, in a recent review,[25] the origin of gHAT epidemics was examined, and a hypothesis associating human genetic factors for infection susceptibility and resistance with HAT epidemiology were presented. Welburn et al.[25] propose that gHAT epidemics occur when trypanotolerant silent carriers of disease are stressed, particularly by factors such as famine and malnutrition, releasing epigenetic phenomena and causing epidemic cycling across generations.

Up until now, human genetics has received little attention in understanding HAT epidemiology,[72,73] but it could be explored more in the future, especially given the recent discoveries on the genetic resistance to *T. b. rhodesiense*, due to mutations in the apolipoprotein gene (ApoL1).[74] This gene has been shown to be the trypanolitic factor in the human serum, responsible for protection against most trypanosome species, and may even be involved in anti-Leishmania protection.[75] It has been suggested that the distribution of ApoL1 gene mutations throughout sub-Saharan Africa may explain spatial patterns of co-occurrence and competitive exclusion of the two subspecies *T. b. rhodesiense* and *T. b. gambiense*.[74] Uncovering human genes

involved in resistance and tolerance to these parasites, and quantifying their distribution, will be crucial to inform epidemiological interventions in a heterogeneous human host population.

Chronic silent carriers, infected with *T. b. gambiense*, have been reported in several studies, likely harboring levels of parasitemia that are too low for detection by conventional techniques or PCR.[34,65] These individuals, despite being seropositive, are usually not treated in control programs, acting as asymptomatic carriers and contributing to transmission. Thus, it is suggested that silent carriers may be the norm, rather than the exception, for how *T. b. gambiense* is endemically transmitted, with congenital transmission from infected mothers to children,[16] also playing a significant role. These new studies raise important questions about the strategies of disease elimination, suggesting differential approaches depending on the mechanisms by which epidemics are generated.

For *T. b. gambiense*, epidemics are more likely tied to a dynamic of genetic tolerance to parasites and a population of human silent carriers. Such tolerance could be overturned by famine, exposure to stress and epigenetic modification.[76,77] This model implies that control of gHAT is best achieved through diagnosis and treatment of the human reservoir of disease,[5] with special attention on pregnant women and children, in order to stop maternal transmission.[25] For *T. b. rhodesiense* instead, epidemics are much more tightly linked to tsetse populations feeding on infected wildlife or livestock, thus control is achieved by lowering tsetse population densities, and by treatment of the animal reservoir.[78,79] These new perspectives need further evidence and validation, especially in what concerns diagnostic tools for asymptomatic pathogen carriers.[80,81] Similarly, how nutritional and other forms of stress shape HAT susceptibility calls for further attention. As host tolerance is found also in other trypanosomatid species,[82] parallels across systems could be studied transversally and exploited in the context of HAT elimination.

Mathematical models for multi-scale understanding

Trypanosome parasites evade host immune responses via antigenic variation.[12,13] The protein responsible is the variant surface glycoprotein (VSG), encoded by a family of around 2000 genes in the parasite genome. As one wave of parasites in the bloodstream rises and generates variant-specific host immunity, which then acts to clear it, another wave of parasites that have switched to expression of new VSGs begins to take over, requiring different variant-specific host response. This process, known as antigenic variation, occurs both in *T. b. rhodesiense*[83] and *T. b. gambiense*, but is more important in the latter due to the long chronic nature of the disease.

Such a large repertoire of VSG genes available for sequential expression (>20% of the coding genome), provide an enormous potential for parasite survival within host, and enable the establishment of chronic infection. Chronic parasite persistence depends on a trade-off between transmission and virulence, which needs to be resolved by the parasite upon each infection.[84,85] Higher parasite numbers within-host increase the chances of transmission to the tsetse fly on one hand, but also risk killing the host on the other, rapidly interrupting the chain of transmission. So the parasite must seek a fine-tuning of replication and virulence within host. To understand how exactly this balance within-host is reached or breached,[86] mathematical models can be useful. They can integrate under the same framework many infection processes: antigenic variation,[12] parasite differentiation from slender to stumpy morphological forms,[87] the host immune response,[88] and parasite dissemination among host body compartments. Such formulations can provide systematic tools by which to investigate different aspects of infection dynamics, alone and in combination, and quantify their onward consequences for parasite transmission, infection stages, and host health. In trypanosome research mathematical models have thus far been under-exploited.

Despite several theoretical studies of antigenic variation and within-host infection dynamics,[89–93] frameworks integrating models with empirical data from animal experiments,[94] or data from human patients[33] are missing, except for a few studies.[95] Thus, there is a need for data-driven mathematical models, to enhance our understanding of infection dynamics. These can be used to estimate biological parameters consistent with empirical data of ever-increasing resolution,[94,96] and to evaluate treatment efficacy and outcomes, considering alternative drugs and their pharmacological modes of action.

There are many questions for which models could be useful.

- *Can the mechanisms of asymptomatic carriage observed with T. b. gambiense be explained?*
- *Can the antigenic variation machinery of the parasite be broken down, to interrupt chronic infection?*
- *Can treatment regimes be optimized based on patient data, including VSG composition, parasite numbers, and host cytokine profiles upon diagnosis?*
- *Are there critical within-host parameter combinations to determine the transition from stage 1 to stage 2 disease?*
- *Is it possible to find algorithms that predict the ordered sequence of antigenic variants over infection, at least probabilistically?*

In addition to their immediate application in medical intervention, within-host dynamic models can also be nested into realistic eco-epidemiological frameworks to study the larger-scale effects on disease transmission and epidemics, and to compare alternative control measures at the population level.[97] As recently highlighted,[25] there are multiple routes of transmission of *T. b. gambiense*, including vertical, vector, and sexual transmission. Host heterogeneity also spans multiple directions, including the vector subspecies, human susceptibility and resistance profiles, and symptomatic versus asymptomatic

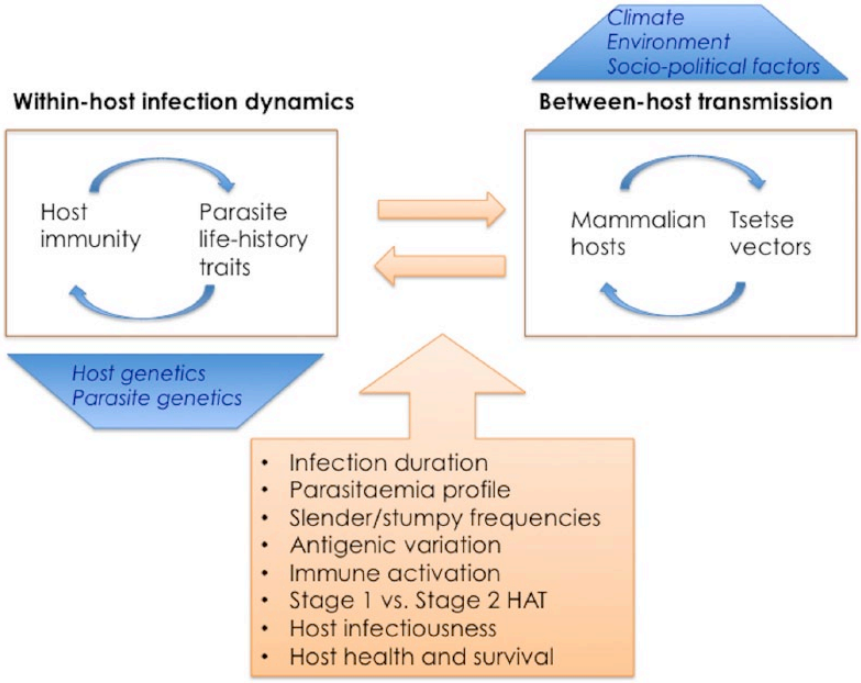

Figure 1. An integrated multi-scale approach to understanding and controlling African trypanosome dynamics and sleeping sickness. Mathematical models can be useful to quantify critical parameters and inform optimal interventions at the within- and between-host level. The arrows indicate intrinsic feedbacks arising in one biological scale and between biological scales. At the interface lie within-host infection features such as total parasitaemia infection duration, and host survival, which determine host and parasite fitness. Bottom-up processes such as host and parasite genetics ultimately interact with top-down epidemiological drivers, including environmental and socio-political conditions. Over time, selection pressures arising at the within-host and epidemiological level shape host and parasite life-history evolution.

carriers. Furthermore, when modeling rHAT, species barriers and reservoirs for *T. b. rhodesiense* must also be considered in transmission of this species, requiring multi-host epidemiological formulation.[48] When the disease approaches elimination, stochastic effects in epidemiological spatial patterns also need to be accounted for, and to be interpreted correctly, in order to quantify the real progress toward the HAT control goal.[2]

Integrating all of these processes requires flexible modeling frameworks, to capture the critical nonlinear feedbacks and the multiple host species, shaping trypanosome dynamics.[98] With mathematical models and multi-scale approaches (Figure 1), the key parameters, whether host-intrinsic, parasite-intrinsic, vector-intrinsic, or environmental, that drive pathogen persistence could be identified. As we gain a deeper understanding of the relative importance of each parameter, control strategies will be better informed.

Future research: host immunity and drug development

Trypanosome biology and epidemiology have become an exciting area of research, thanks to the latest developments in molecular technologies, genomic tools, and bioinformatic approaches.[14] From antigenic variation characterization, to the analysis of the trypanosome lifecycle and cell structure, to gene expression patterns, the parasite interaction with the host immune system, and to evolutionary dynamics, the last 25 years have seen an explosion of studies and impressive findings. Translating this body of knowledge into accurate diagnostics, better control measures for natural infections, and into drugs for HAT disease is the next step. High-throughput screening for possible drug compounds[99] is also providing additional power to explore more effective and cheaper therapeutics, and shed lights on new areas of parasite biology such as resistance to drugs.[100] Recently, several new drugs are being considered, with potential oral routes of administration,[101,102] and shorter treatment duration. However balancing toxicity and effectiveness remains a challenge, especially for treatment of *T. b. rhodesiense* late-stage disease.[32] In gHAT instead, new tools that can identify very low levels of parasite densities, and that can reveal the biological mechanisms underlying silent carriage, such as immunologically privileged within-host sites, will be crucial. Connecting the dots from parasite biology to host immunity will quantify how the asymptomatic infective state is reached, how asymptomatic individuals contribute to overall transmission of HAT, and most importantly, how this asymptomatic state loses its stability to cause disease.

African trypanosomes within host are constantly confronted with the host's immune defences ranging from innate parasite control mechanisms to adaptive and more specific responses. In addition to the prevalent view that anti-VSG antibodies dominate the immune response against

parasites in the bloodstream,[103] another more integrative view of host factors involved in resistance and pathology is recently emerging, where multiple host immunity mechanisms activated during trypanosome infection are receiving attention.[104] Research is unraveling to great resolution the innate and adaptive components of the host's immune system that are involved in direct resistance and killing of parasites, as well as in the net immunopathology or tolerance of an infection.[105] These include progression to and severity of anaemia, blood–brain invasion dynamics, and general inflammation, ultimately leading to pathogenicity and death if infection is left untreated. For example, activities of immune regulation mediated by innate cells, in particular macrophage-like M cells have been reported.[106] The role of interleukin (IL)-10 in reducing IFN-γ-mediated pathology in trypanosome infections has also been elucidated in *T. brucei*,[107] suggesting a major role of this anti-inflammatory cytokine in the transition from parasite density control to infection pathology control.

Although immune intervention therapies have not been considered until now, most current drugs focusing on parasite-intrinsic traits such as uptake of nutrients or specific organelles, in the near future, manipulation of host responses to control parasite growth and infection-induced damage could be another alternative.[104] This will require a more detailed quantitative understanding of host-derived factors implicated in the persistence of inflammation, and careful extrapolation of findings from murine models to human models of trypanosomiasis.

Reaching the field

The continued control and eventual elimination of HAT requires the expansion and integration of multi-sectorial activities,[5] from scientific research in the lab to ultimate delivery of services in the field. A key organization coordinating HAT initiatives globally remains the WHO. Partners in the public sector include academic institutions and governments, and in the private sector industry and non-governmental organizations. They are already engaged in joint initiatives for the active epidemiological surveillance, mapping of risk,[71] analysis of HAT, chemotherapeutic delivery,[20] and for the production of current drugs. Although the disease is in decline,[2] the control efforts must not be abated, given its potential for resurgence following conflicts and political instability. A stable supply of funds must be maintained to support the ongoing functionality of treatment and rehabilitation centers in epidemic foci and in endemic areas. To ensure sustainable control, public health infrastructures in afflicted zones must be supported, equipped properly, staffed with trained personnel, and empowered with research facilities.

Given the complexity of HAT transmission at the animal–human–vector interface, an integrated approach, combining veterinary medicine services and wildlife management services is also needed, especially for rHAT.[5] As the disease becomes less prevalent, HAT surveillance could shift gradually to more passive systems, which are deemed more cost-effective. However a shortcoming of this approach is that HAT patients would be diagnosed in the second and more severe disease stage, probably years after infection, in the case of *T. b. gambiense*. On one hand, this strategy would certainly help alleviate the burden of disease. On the other hand, it could miss the identification of asymptomatic carrier individuals, with great significance for transmission in persistent endemic foci,[25] particularly so for gHAT, the dominant disease form. In such a context, adapting surveillance and control strategies to account for trypanotolerance remains a key aspect of HAT elimination.

Author Contributions

EG reviewed and approved the final manuscript.

RESOURCES

i. http://www.who.int/trypanosomiasis–african/country/en/
ii. http://www.who.int/trypanosomiasis–african/diagnosis/en
iii. http://www.who.int/mediacentre/factsheets/fs259/en
iv. http://www.stampoutsleepingsickness.com

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