African Trypanosomiasis

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Learning Objectives

1. To understand the importance of innate immunity and factors associated with it in protection to infection.
2. To have an idea about the geographic distribution of different species and subspecies and their roles in disease causation.
3. To have a knowledge about the primary importance of serological tests in diagnosis.

Introduction

Trypanosomiasis is a disease that affects both humans and animals and has a detrimental impact on the socio-economy of numerous endemic countries. Trypanosomes are protozoan parasites mostly transmitted by blood-feeding vectors, which in many cases represent their primary obligate host. Two types of trypanosomes exist, stercorarian trypanosomes, released through insect faeces, and salivarian trypanosomes, transmitted through insect saliva. These two groups of trypanosomes are characterized by very distinct host-parasite interactions, and this chapter focuses on African trypanosomiasis only. From all the salivarian trypanosomes known to infect mammals, only three are to be considered zoonotic, all belonging to the subgenus *Trypanozoon*, and two of these might be even up for discussion when it comes to a zoonotic classification sensu stricto. The true zoonotic trypanosome is *Trypanosoma brucei rhodesiense*. This East-African trypanosome has an extended mammalian host reservoir that includes both game and domestic animals. The reservoir diversity is the main reason why eradication of HAT (human African trypanosomiasis) as such is considered unfeasible. *T. b. rhodesiense* causes an acute and most often deadly form of sleeping sickness. However, *T. b. rhodesiense* infections represent only a small fraction of all HAT cases reported, as

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Trypanosoma brucei gambiense parasite is responsible for an estimated 95–98% of all HAT cases. This infection is more chronic and has a much wider geographic distribution that covers West and Central Africa. *T. b. gambiense* infections are often considered to be anthroponotic. Indeed, it is now accepted that *T. b. gambiense* parasites represent a group of more diverse *Trypanozoon* organisms, of which the zoonotic infections might be much harder to control. Finally, *Trypanosoma evansi* is in general not considered to be a human parasite, despite the fact that several atypical human infections have been reported. These infections have only been reported outside Africa, but it is very well possible that due to a lack of surveillance, the number of aHT infections has been systematically under-reported.

History

T. evansi was the first salivarian pathogenic trypanosome to be discovered. The parasite was identified in the Indian subcontinent by Dr Griffith Evans in 1880, in horses and camels suffering from surra. David Bruce identified trypanosomes in the blood of infected cattle suffering from the African cattle wasting disease known as nagana between 1894 and 1910. While the name surra finds its origin in the Hindi word meaning 'rotten', nagana finds its origin in the Zulu language, meaning 'depressed' or 'low spirit', directly reflecting the clinical manifestation of this animal disease.

The first reports of HAT date back to the eighteenth century. However, it was only at the end of the nineteenth century, from 1896 until 1906, that the first properly recorded HAT epidemic occurred, coinciding with population displacements as a result of the colonial development of the Congo River basin. A second epidemic occurred during the 1920s. By the 1960s, transmission of HAT was nearly halted, helped by a combination of intense screening and treatment policies, as well as vector control. In the aftermath of decolonization, a loss of interest in the surveillance took place. In combination with the banishment of DDT insecticide, this led to a re-emergence of the disease in the 1970s. At the end of the twentieth century, the WHO estimated 300,000 people contracted the infection each year. After the renewed establishment of successful diagnosis and treatment programmes, this third epidemic now seems to be controlled. In 2019, less than 1000 *T. b. gambiense* infections were reported to the WHO, while the case report number for *T. b. rhodesiense* marginally surpassed 100.

Taxonomy

The taxonomical position of the genus *Trypanosoma* belongs to family Trypanosomatidae, order Trypanosomatida and class Kinetoplastida in the phylum Euglenozoa. *T. b. rhodesiense*, T. *b. gambiense* and *T. evansi* cause infections in humans.

Parasite Genomics and Proteomics

The genome of *T. brucei* has 11 megabase chromosomes (of 35 Mb total) as well as 5 intermediate (200–300 kb) and about 60–100 minichromosomes of sizes 30–150 kb. The genome contains 9068 predicted genes, including approximately 900 pseudogenes and approximately 1700 *T. brucei*-specific genes. Antigenic variation is one of the most interesting mechanisms that exhibited the trypanosomes to evade the host immune response. Upon injection by an infected tsetse fly, the metacyclic trypomastigotes reach the mammalian blood circulation, covered by unique surface glycoprotein called the metacyclic variant surface glycoprotein (mVSG). This protein acts as a defence layer against host antibodies and complement attacks and also helps in immune evasion. Large subtelomeric arrays contain 806 variant surface glycoprotein (VSG) genes. A single trypanosome has more than 1500 VSG genes, most of which are located in extensive silent arrays. Interestingly, most of these silent VSGs are pseudogenes, and ongoing studies are trying to understand how non-intact VSGs are recombined to produce genes encoding functional coats. Only 1 VSG is expressed at a

time from 1 of approximately 15 dedicated VSG expression site transcription units. Antigenic variation can be classified into two distinct types. VSG switching by recombination allows that VSGs are regularly being altered, while other genes associated with the expression site (ESAGs) remain unchanged. Alternatively, the active expression site is 'switched off', allowing mRNA elongation from a newly activated expression site. This changes the VSG, as well as the ESAGs. The latter is an advantage when the trypanosome needs to adapt to a new host, as will be outlined below in the case of adaptation to growth in human serum/blood.

Subspecies differentiation of *T. brucei* parasites is based on two specific 'resistance' genes that allow growth in human serum/blood. In *T. b. rhodesiense*, human serum resistance is linked to the presence of the SRA encoding gene (or serum resistance-associated gene). *T. b. gambiense* is mostly marked by the presence of the *TgsGP* gene. However, *T. b. gambiense* is not a homogeneous family of parasites and is currently being divided into two groups. The rather homogeneous Group 1 *T. b. gambiense* parasites show an invariable true NHS resistance phenotype, all having the *TgsGP* gene marker. Group 2 *T. b. gambiense* parasites are a much more heterogeneous, showing variable resistance, being much closer related to *T. b. rhodesiense* and *T. b. brucei* and representing the zoonotic side of gambiense HAT. There is no specific genetic marker for these parasites.

Although *T. evansi* was first discovered in India, it is generally accepted that the parasite is in fact a 'variant' of *T. brucei* that has lost the kinetoplast DNA (kDNA), which is essential for development in the gut of the tsetse fly. With respect to atypical HT, no genetic markers have been discovered so far that can explain how some *T. evansi* parasites have acquired a serum resistance mechanism. The notion that *T. evansi* mutations are far from understood has been made clear by the genetic analysis of a large group of *T. evansi* parasites found in a limited geographic location. Detailed microsatellite genotyping of parasites isolated in Kenya allowed grouping of *T. evansi* in at least four different clusters, with different evolutionary origins (Fig. 1). It is feasible that if similar studies would be done including *T. evansi* parasites of four different continents, an even more complex parasite ontology would be revealed.

Only a few studies have been done regarding the proteomic analysis of first- and second-stage HAT disease CSF protein profiles, showing that the number of differentially expressed proteins between the two stages is less than hundred. Two of these proteins, osteopontin and beta-2-microglobulin, were confirmed to be accurate markers of first and second stages of patients with sleeping sickness. The proteome of the insect stage and the human blood stage of the parasite have also been mapped. Comparing 4364 protein groups resulted in the identification of stage-specific proteins that can lead to a better

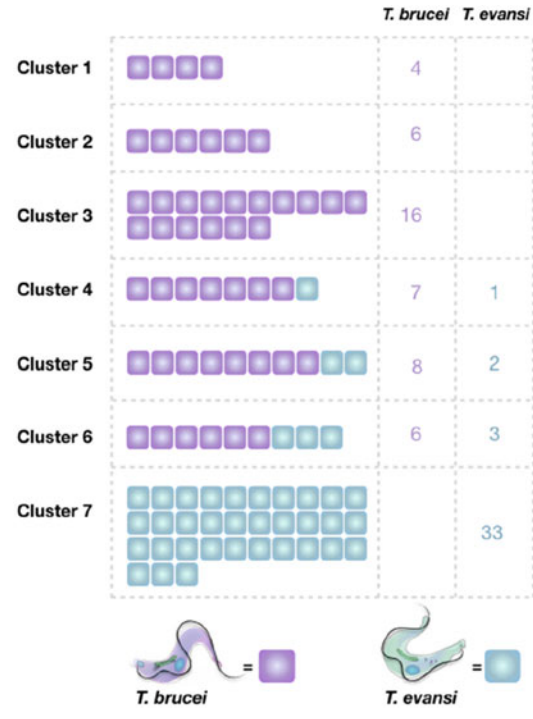

Fig. 1 Genetic clustering of a collection of Kenyan trypanosome field isolates. While some clusters contain distinct *Trypanosoma brucei* or *Trypamosoma evansi* parasites, other clusters contain a mix of the two with close genetic relation. Hence, different *T. evansi* parasites are considered to be derived from different *T. brucei* parasites

understanding of how parasites adapt to different hosts.

The Parasite Morphology

T. brucei is pleomorphic, with three main forms, all of which have a small kinetoplast and a conspicuous undulating membrane (Fig. 2).

Trypomastigotes or Long Slender Forms

These forms are 20–30 μm in length with a free flagellum, which may be up to one half of the length of the organism. The posterior end is pointed and the nucleus is central. The kinetoplast is situated in front of the posterior extremity. They are the proliferative stage of the parasite.

Metacyclic Trypomastigotes or Short Stumpy Forms

These forms may be 15–20 μm in length without a free flagellum. The kinetoplast is usually sub-terminal. In stained specimens, blue volutin granules are often present in the cytoplasm, often arranged in a line along the margin of the cell. They are the non-proliferative stage of the parasite.

Intermediate Forms

Intermediate forms of variable length, in-between two stages, are also found. In this form, a free flagellum is present. The nucleus is centrally placed. The posterior end is somewhat variable in shape, but is bluntly pointed. The kinetoplast is close to the posterior extremity. Volutin granules are occasionally present but neither as common nor as plentiful as in the short, stumpy forms.

The structural rigidity of the cell, in all these forms, is ensured by the microtubes that are aligned below the plasma membrane as longitudinal bundles. The only place where this structure is interrupted is at the level of the flagellar pocket, where a basal body anchors the single flagella and where all the endocytosis and exocytosis events take place. At the base of the flagellar pocket, the kinetoplast is located, which is made up of numerous circular DNA molecules. In the long slender form, the tip of the flagella is free and points towards the direction of motility of the trypanosome. The long slender form has a single simple mitochondrion extending anteriorly from the kinetoplast, and the cristae are short, few in number and tubular. The metacyclic stumpy form has larger mitochondrion extending anteriorly and posteriorly from the kinetoplast with numerous cristae and plate-like in appearance.

Cultivation of Parasites

In vitro cultivation methods are available for trypanosomes that allow a limited range of experiments to be executed, such as those that have been used in the context of the discovery of the quorum sensing regulation, i.e. parasite population density regulation. In short, most culture methods are based on the use of HMI-9 medium (Hirumi's modified Iscove's medium 9) that can be supplemented with 1.1% (w/v) methylcellulose, 15% (v/v) fatal calf serum and 5% (v/v) heat-inactivated human serum. Key to this medium choice is the presence of hypoxanthine (1 mM), bathocuproinedisulphonate (0.05 mM), β-mercaptoethanol (0.2 mM) and sodium pyruvate (1 mM). Procyclic cells can be grown in SDM-79 medium. In vitro, cell differentiation to the insect form (procyclic) parasites can be obtained by adding citrate and *cis*-aconitate (3 mM each) or 6 mM *cis*-aconitate and dropping culture temperature conditions from 37 °C to 27 °C. Cells need to be refreshed every 24–48 h.

Laboratory Animals

Most laboratory animal experiments have been performed in C57BL/6 mice (and many gene-deficient knockout variants) and BLAB/c mice. A smaller number of reports have documented results obtained in CBA/Ca mice, C3H/HeN

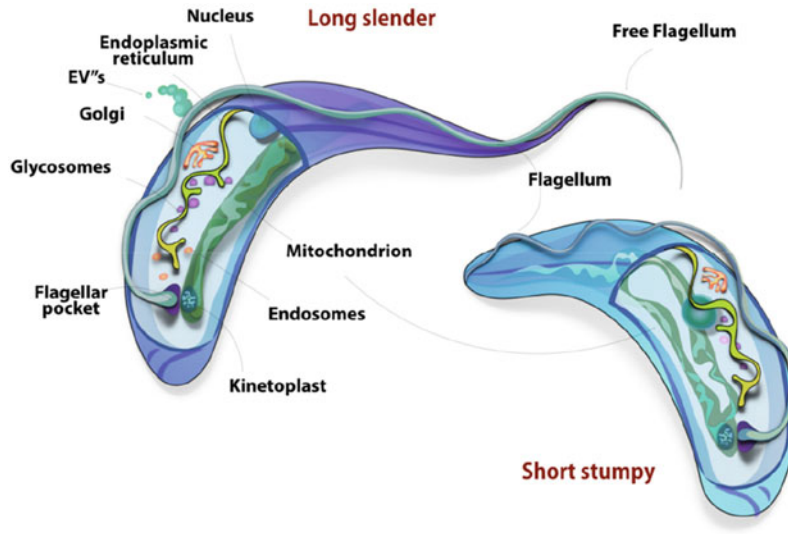

Fig. 2 *T. brucei rhodesiense* and *T. brucei gambiense* parasites are pleomorphic parasites, occurring in the blood of their mammalian host as both a long slender (LS) proliferating form and a short stumpy (SS) non-proliferating form. Both forms can be distinguished by their shape, the long free flagellum of the LS form and their motility, the latter being higher in the LS form. *Trypanosoma evansi* is only observed in the LS form in the blood of the mammalian host

(or J) mice or SWISS mice. F1 crosses have been mainly used in immunology hereditary determination experiments. Studies in AKR mice are of particular interest, as this stain has a natural C5 complement deficiency. The occurrence of 'regular' *T. brucei* parasitaemia control in this strain was the first indication that trypanosome growth in vivo can be regulated in large in a complement-independent manner. Experimental mouse infection is usually performed by intraperitoneal (IP) injection of around 5000 parasites (blood/PBS). This dose was determined based on the average trypanosome content of an infectious tsetse fly bite. If available, infections can be performed using infected tsetse flies, placed on the skin of mice in order to allow the flies to feed, resulting in natural disease transmission. Using intradermal needle injection can mimic some aspects of the natural bite transmission, but is only useful when the early onset of infection is being studied or when specific immunological skin features are being addressed. To study longer-term systemic host-parasite interaction events, the IP injection of parasites has been shown to deliver satisfactory results.

Fundamental understanding of trypanosomiasis-associated B cell destruction has been understood mainly from experimental murine infections with *T. b. brucei*.

Life Cycle of Trypanozoon Trypanosomes

Hosts

Primary Host

Tsetse fly (*Glossina* spp.)

Intermediate Host

Humans, domestic cattle and game animals like antelopes and wild buffaloes.

Infective Stage

Metacyclic form of the parasite.

Transmission of Infection

The life cycle of salivarian trypanosomes is completed in two hosts. Both *T. b. rhodesiense* and *T. b. gambiense* need a primary insect host of the genus *Glossina*, the tsetse fly, which is only present in Africa. Humans and some other animals get the infection from the insect vectors (Fig. 3).

Metacyclic stage of the parasite is introduced into the body of humans when the tsetse fly of the

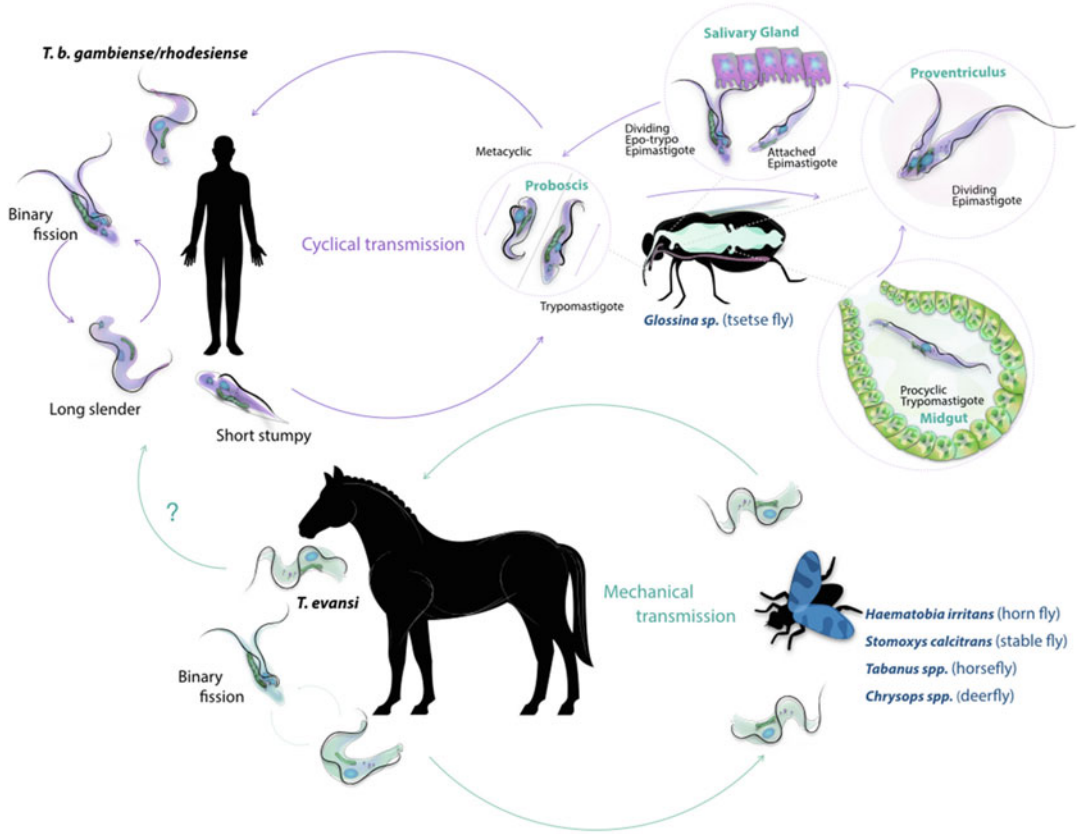

Fig. 3 The *Trypanozoon* life cycle. *Trypanosoma brucei rhodesiense* and *Trypanosoma brucei gambiense* both cause human African trypanosomiasis (HAT) and rely on tsetse fly-mediated cyclic transmission. The main host reservoir for *T. b. rhodesiense* consists of livestock and game animals. *T. b. gambiense* parasites are split into two groups. Group 1 is rather homogeneous and mainly anthroponotic. Group 2 parasites are much more heterogeneous and considered zoonotic. *Trypanosoma evansi* is an animal parasite that has lost the capacity to complete its life cycle in the tsetse fly. It can be passed between hosts by mechanical transmission. Several different biting fly species are responsible for disease transmission

genus *Glossina* takes a blood meal. The saliva of the fly contains this infective form coming from the salivary gland of the insect. The saliva also contains substance that inhibits blood clotting at the bite site. In the blood, the aflagellate metacyclic forms transform into flagellated trypomastigotes and start multiplying by longitudinal binary fission. The division starts at the kinetoplast followed by nuclear and cytoplasmic divisions. The long slender trypomastigotes are actively motile in the blood and lymph. In chronic infection, many invade the central nervous system where it continues to multiply. In the event of cessation of glycolysis and through quorum sensing, further division of trypomastigotes stops, and they transform into the short stumpy form after passing through a brief intermediate stage.

During the blood meal, the short stumpy forms enter the posterior section of the midgut of the fly where it multiplies into procyclic trypomastigote forms for about 10 days. Subsequently, they penetrate the peritrophic matrix that covers the gut epithelium and migrate to the ventriculus. The parasites can resist digestive enzymes and a strongly alkaline environment of the fly's gut. Then the slender forms migrate to the foregut, where they are found between the 12th and 20th days. They then move up to the oesophagus, pharynx and hypopharynx. Finally, they enter the salivary glands where they are transformed

into epimastigote form. After further asexual multiplications, they finally transform into metacyclic trypomastigotes. When feeding, a tsetse fly may inject up to several thousand parasites into the host. In the fly the whole cycle is completed in 15–35 days. During the saliva infection stage, the parasite downregulates the capacity of the fly to inject saliva containing anticoagulants and anti-platelet aggregation into the bite site. This results in a decreased feeding efficiency and in turn increases the likelihood of the tsetse feeding on multiple hosts, resulting in an increased chance of parasite transmission.

In comparison to *T. brucei*, *T. evansi* has a much simpler life cycle having lost the capacity to adapt to life within the tsetse fly vector. Hence, the long slender morphology is the only form seen in the bloodstream of the mammalian host. *T. evansi* efficiently relies on mechanical transmission. This non-tsetse fly-mediated transmission has allowed the parasite to move out of Africa and infect most parts of the rest of the world.

Pathogenesis and Pathology

In humans, trypanosomiasis is characterized by a first phase, the haemolymphatic stage, where the parasite invades the host's circulatory and lymphatic systems and causes immune-dysfunction. Initial infection is characterized by fever, weakness, enlarged lymph nodes and joint pains. If the parasite passes through the blood-brain barrier, the meningo-encephalitic stage begins, causing neuropsychiatric symptoms such as daytime sleepiness and nocturnal insomnia, due to the fragmentation of the circadian rhythm. Later symptoms lead to the death of the individual if left untreated. Those symptoms are also related to the popular name of human African trypanosomiasis, as sleeping sickness.

Symptoms of gambiense HAT are very similar to those for rhodesiense HAT, but the main difference is that it takes in general much longer for the disease to progress into the second stage. Both infections result in mild anaemia, but not to the extent that is being observed in animal trypanosomes causes by non-brucei trypanosomes.

Immunology

In contrast to many protozoan parasites that ensure optimal survival by hiding from the immune system inside host cells, salivarian trypanosomes remain extracellularly localized throughout the mammalian stage of their life cycle. Hence, these parasites are continuously exposed to attacks by the host innate immune system, as well as the adaptive immune system. To survive these attacks, salivarian trypanosomes have acquired multiple evolutionary strategies to evade and even destroy the host immune system. Evasion relies mainly on the system of antigenic variation exhibited by the parasites. Immune destruction involves the diversion and destruction of the host B cell response, resulting in an impairment of effective antibody production.

Immunity against animal trypanosomes in humans is provided by an innate system, the trypanosome lytic factors TLF1 and TLF2. Humans share this trypanolytic serum activity with gorillas and certain Old World monkeys. Human TLFs contain apolipoprotein A1, the primate-specific ion channel-forming protein apolipoprotein L-1 (APOL1) and the haemoglobin-binding protein haptoglobin-related protein (HPR). Uptake of TLF1 is mediated by the *T. brucei*-specific receptor TbHpHbR (haptoglobin-haemoglobin receptor). Interestingly, baboon APOL1 is much more potent than the human homologue. Hence, it confers resistant against all trypanosomes, even those causing HAT. Since *T. b. rhodesiense* is a human pathogen, it obviously acquired resistance against the human APOL1. This resistance is linked to the expression of a serum-resistant antigen (SRA), with SRA inhibiting the pore-forming capacity of APOL1 inside the acidic environment of the endocytic system of the trypanosome. In contrast, Group 1 *T. b. gambiense* parasites acquire their resistance by a more complex mechanism. This involves reduced uptake of TLF1 due to reduced expression of the HPHBR gene and a reduced ligand binding through mutations in the receptor protein sequence. Additional data has shown that in these parasites, the TgsGP molecule aids in APOL1 resistance by reducing trypanosomal

membrane fluidity. Finally, a third factor, not fully elucidated yet, relates to the action of a cysteine protease. For the heterogeneous Group 2 *T. b. gambiense* parasites, as well as for the human-infective *T. evansi* parasites, the APOL1 resistance mechanism remains to be elucidated.

Infection in Humans

The HAT is characterized by a first haemolymphatic stage phase, progressing towards a second meningo-encephalitic stage. If infections are left untreated, HAT most likely results in death.

First-stage HAT is not characterized by any specific symptoms, but may be accompanied by intermittent headache, fevers and joint pains. These symptoms may correspond with successive waves of parasitaemia and B cell and/or immune activation. Hepatomegaly, splenomegaly and lymphadenopathy are other manifestations. A range of other nonspecific symptoms that may be present include skin rash, weight loss and facial swelling. Neuroendocrine disturbances leading to amenorrhoea in women or impotence in men have been documented. The first stage of *T. b. gambiense* infections can last several years.

Second-stage HAT is characterized by CNS inflammation and an increase in cerebrospinal fluid IgM tires and white blood cell counts ($\geq$20 cells µl). This stage is characterized by the disturbance of the sleep cycle, resulting in night-time insomnia and daytime somnolence.

Infection in Animals

Domestic cattle as well as a wide range of wild animals including buffaloes and antelopes are the main reservoir hosts of *T. b. rhodesiense*. Cattle is a reservoir for the zoonotic transmission of Group 2 *T. b. gambiense*. *T. evansi*, because of the wide geographic spread of the parasite, as well as the increased virulence as compared to *T. brucei*, which is an important parasite of animals. In many host species such as horses, camel, cattle, dogs and even rats, *T. evansi* infections are characterized by anaemia, loss of appetite, weight loss, oedema, fever, salivations, lacrimation and abortion. Neuropathological features including paralysis of the hind limbs are observed with *T. evansi* infections particularly in horses.

Epidemiology and Public Health

In 2018, *T. b. rhodesiense* trypanosomiasis was reported in six sub-Saharan countries, including Kenya, Malawi, Uganda, Tanzania, Zambia and Zimbabwe (Fig. 4/Table 1). The same year, no cases were reported in Burundi, Ethiopia, Mozambique and Rwanda, all countries that in the past were considered endemic for the disease. Together, only 24 cases were reported to the WHO and its partners, representing only 2% of the overall HAT burden for that year. HAT cases were reported in South Africa, the Netherlands, China (each two cases) and France, Germany and India (each one case for the 2017–2018 period). In addition, rhodesiense HAT account for two-thirds of all tourist HAT cases.

T. b. gambiense HAT is still considered the major HAT infection problem, making up for 98% of all cases reported to the WHO and its partners. In total, 953 infections were reported in 2018, in 15 sub-Saharan countries (Fig. 4). Eight countries that are considered to be endemic for gambiense HAT did not report any cases in 2018, and two countries (Gambia and Liberia) did not report any surveillance activities. Cases of gambiense HAT have been dramatically reduced over the last 10 years, as back in 2009, there were still nearly 10,000 case reports. The important epidemiological features of salivarian trypanosomes are shown in Fig. 4.

As *T. evansi* is a mechanically transmitted animal parasite, it has moved out of Africa and can be found in South and Central America, various regions in Africa, the Middle East, China, the Indian subcontinent and the Southeast Asia. The main mammalian host in Africa and the Middle East is considered to be camels. In South America, the main host reservoir is found in horses and local animals such as capybaras. In Asia, *T. evansi* is mainly found in water buffaloes where it serves as a reservoir for parasite transmission to cattle, pigs and goats. More ‘exotic’

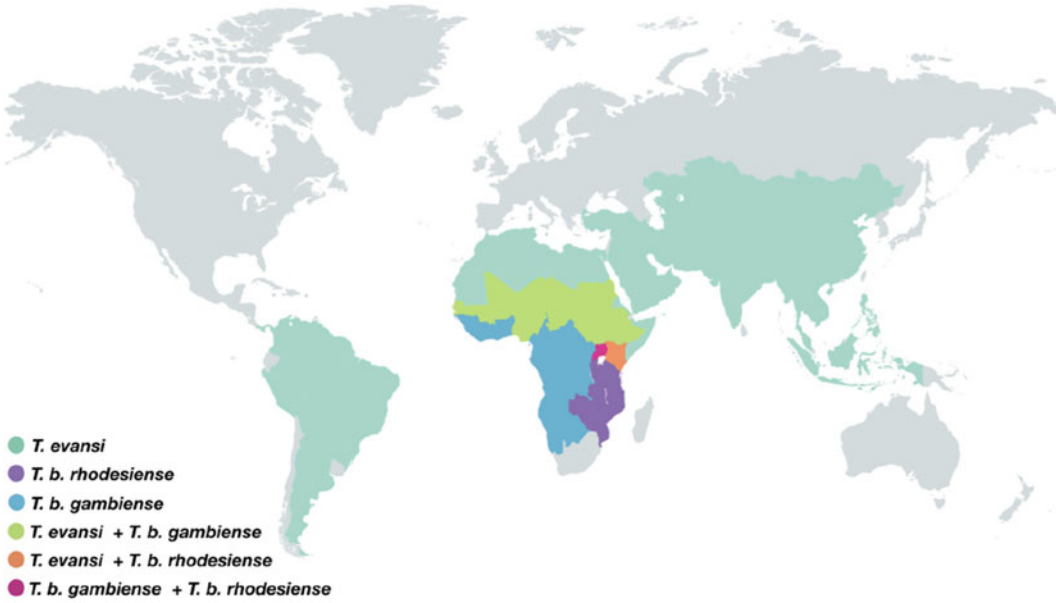

Fig. 4 Geographic distribution of *Trypanosoma brucei rhodesiense*, *Trypanosoma brucei gambiense* and *Trypanosoma evans*i. Due to the wide range of vectors involved in the spread of *T. evansi*, this parasite has moved out of Africa and is now present in large parts of the world. In contrast, the two human-infective *Trypanosoma brucei* subspecies are found only in the sub-Saharan African tsetse belt

animals such as elephants and deer are known to serve as a parasite reservoir in the wild. One of the problems with *T. evansi* is the fact that many infected animals hardly show any symptoms, resulting in the spread of infection through the transport of seemingly healthy animals. This has led to occasional outbreaks such as those reported in Spain and France, after the introduction of infected camels originating from the Canary Island.

Table 1 Epidemiological features of important salivarian trypanosomes

SN	Species	Host	Vector	Geographic distribution	Human infection
1.	*Trypanosoma brucei* subsp. *gambiense*	Humans, bovines	Tsetse fly (*Glossina* spp.)	West and Central Africa	Most common
2.	*Trypanosoma brucei* subsp. *rhodesiense*	Humans, bovines	Tsetse fly (*Glossina* spp.)	East and Southern Africa	2% of all HAT cases
3.	*Trypanosoma evansi*	Equines, bovines, camelids	Tsetse fly *Glossina* spp., stable fly (*Stomoxys* spp.), horse fly (*Tabanids* spp.), deer fly (*Chrysops* spp.)	Central and South America, North Africa, the Russian territories, the Indian subcontinent, China and Southeast Asia	Infrequent
4.	*Trypanosoma vivax*	Bovines, ovines, caprines, equines	Tsetse fly *Glossina* spp., stable fly *Stomoxys* spp., horse fly *Tabanids* spp.	Africa, South America	Extremely rare, lack of reliable reporting
5.	*Trypanosoma congolense*	Bovines	Tsetse fly *Glossina* spp.	Sub-Saharan Africa	Extremely rare, only reported as mixed infection with *Trypanosoma brucei gambiense*

Diagnosis

As the clinical signs of HAT in general are rather unspecific, the first-line diagnosis that relies on symptoms and epidemiological assessment is inefficient. Hence laboratory diagnosis (Table 2) is essential for the treatment of the condition.

Microscopy

Microscopic detection of the parasite is the definite technique for the diagnosis of HAT (Fig. 5). However, since the trypanosome concentration in blood is often below the detection limit of conventional microscopy, concentration techniques, such as buffy coat preparation, are necessary for high yield of parasites. Mini ion exchange chromatography (mAECT) is also used to evaluate parasites from blood samples, prior to microscopy. The use of fluorescent dyes that intercalates nucleic acids can result in high-sensitive detection of parasites by fluorescent microscopy. Microscopy analysis of aspirate fluid from swollen cervical lymph nodes is an alternative tool, used when parasites cannot be detected in the blood. Cerebrospinal fluid can be analysed to confirm the neurological second stage of the infection.

Serodiagnosis

As trypanosomes induce a strong humoral response in their mammalian host, antibody-based diagnostic tests are considered a primary screening tool. Their use has in large contributed to the recent successes in the control of *T. b. gambiense* infections.

The card agglutination test for trypanosomiasis (CATT) is used for the detection of *T. b. gambiense* and *T. evansi* infections (Fig. 5). No such test is available for rhodesiense HAT. HAT/CATT is based on the detection of antibodies that cross-react with particular VSG molecules of laboratory-cultured trypanosomes, i.e. the *T. b. gambiense* LiTat 1.3 and LiTat 1.5 clones. The test is characterized by high sensitivity and specificity, but a lower positive predictive value. This means that often a majority of CATT-positive individuals scores negative in a parasitological assay. However, the very high negative predictive value allows to exclude vast numbers of people from further microscopy screening, a technique that requires skilled analysts and is time-consuming. When found positive by both CATT and microscopy screening, patients have to undergo a 'staging' screening, meaning that the analysis of cerebrospinal fluid is needed to determine whether or not parasite has crossed the blood-brain barrier.

In recent years, several efforts have been undertaken to transform the principle of the CATT into more user-friendly lateral flow formats. These tests are currently still being improved and evaluated under various field conditions. In the end however, antibody-based test will always suffer the drawback of measuring exposure, rather than actual infection. Hence, there are several reasons why most antibody-based trypanosome tests will always have a low positive predictive value. First, there seem to be many individuals with cross-reacting anti-LiTat antibodies that have never suffered HAT, but might have other underlying conditions such as allergies that generate poly-reactive antibodies. Second, it is quite possible that individuals who live in *T. b. brucei* endemic areas are regularly exposed to bites by infected tsetse flies. There is no reason to assume that these infected bites cannot cause a cross-reactive host antibody response, upon efficient lysis of the *T. b. brucei* parasites by the human TLFs. Finally, anti-trypanosome treatment of infected individuals will result in the induction of anti-trypanosome antibodies that will remain in the circulation long after the parasite has been eliminated.

A diagnostic CATT test specifically targeting *T. evansi* has been available since the 1990s. It targets the RoTat1.2 VSG. Nevertheless, the test is unsuitable for *T. evansi* detection in regions where RoTat1.2-negative parasites occur, the so-called *T. evansi* Type B, or the RoTat1.2-negative *T. evansi* Type A, described in Kenya.

Molecular Diagnosis

With the prevalence of HAT decreasing due to the surveillance and treatment successes, there is a need for diagnostic tools that can directly detect the parasite or components released/secreted by

Table 2 Diagnostic methods for human African trypanosomiasis

Diagnostic approach	Samples/methods	Target	Remarks
Microscopy	Blood, bone marrow, CSF, lymph node aspirate. Concentration techniques in blood (mini ion exchange chromatography; micro-haematocrit concentration). Giemsa stain, fluorescent stain. Unstained preparation for motile forms	Trypomastigote form	Most definitive method Low detection limit
Immunological tests	Card agglutination test	Antibodies against variable antigen type LiTat 1.3/1.5	Useful for mass screening of whole blood for control/elimination for *Trypanosoma brucei gambiense* and *Trypanosoma evansi.* Not available for *Trypanosoma brucei rhodesiense*
Molecular diagnosis	PCR, LAMP	18S ribosomal RNA, RoTat1.2 VSG gene	No large-scale field application yet

the parasite. PCR is now increasingly evaluated and used for species-specific diagnosis of *Trypanosoma* species causing trypanosomiasis, both in humans and animals. While PCR is a sensitive technique for direct pathogen detection, this technique has significant limitations in point-of-care (POC) resource-poor field settings. As alternatives, isothermal PCR diagnostic solutions such as LAMP have been developed at experimental level but are still not being implemented on a large scale. Currently, POC tools with a very high positive predictive value are however becoming crucial when taking into account that in a zoonotic setting, asymptomatic animals can

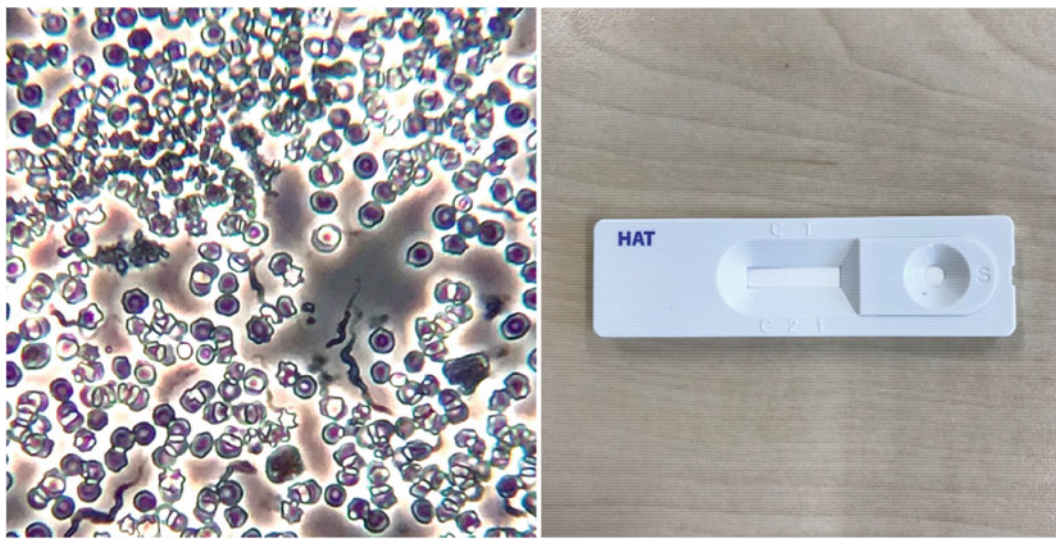

Fig. 5 Phase-contrast microscopy image of *Trypanosoma brucei rhodesiense*, parasite (unstained) as observed with a regular field microscope, using a 20× magnification and a cell phone adaptor for image capturing (left). Microscopy validation is still the only accepted method for true positive case determination. Rapid diagnostic tools, such as this SD Bioline HAT prototype, are rapidly replacing the CATT assay (right). These tests are based on anti-parasite antibody detection; hence they score trypanosome exposure rather than active infection

serve as an everlasting reservoir for human-infective parasites.

For *T. evansi* diagnosis, there is a drive to implement genetic testing that allows direct parasite detection. Here, PCR detection of the gene encoding the RoTat1.2 VSG allows for accurate detection of *T. evansi* in most geographic locations, as does LAMP. Recently, an alternative diagnostic test based on recombinase polymerase amplification (RPA) has been developed. It has been combined with lateral flow detection for easy and rapid result interpretation. The detection of *T. evansi* Type A is achieved through isothermal amplification at 39 °C of the *T. evansi* RoTat1.2 VSG gene. Results of the test are obtained within 20 min. For the detection of *T. evansi* Type B, which does not express RoTat1.2 VSG, loop-mediated isothermal amplification (LAMP) of DNA has been shown to be a more sensitive tool compared to classical PCR tests. The current RPA technology, however, has not been adapted yet for the detection of *T. evansi* Type B.

Treatment

Despite being used for nearly a century and despite inducing a string of severe negative side effects, suramin is still being applied as a major curative treatment for early stage *T. b. rhodesiense* HAT.

Melarsoprol is used as first-line curative treatment of *T. b. rhodesiense* HAT, irrespective of actual disease staging. Being an arsenical compound, the drug has high toxicity and severe side effects, inducing reactive encephalopathy resulting in fatal outcome in up to 10% of patients. Hence, in optimal circumstances, this drug needs to be restricted in its use for treatment of second-stage *T. b. rhodesiense* infection only.

Pentamidine has for long been the most used drug for the treatment of first-stage *T. b. gambiense* HAT. For the treatment of second-stage *T. b. gambiense* HAT, a combination of nifurtimox and eflornithine (NECT) has been introduced in 2009. This combination therapy reduces the complexity of the previously used eflornithine therapy. Both drugs are provided free by the WHO to endemic countries. A free-of-charge kit with all administration necessities is available.

Most recently, in 2018, fexinidazole has been made available as an oral therapy for *T. b. gambiense* HAT and has been incorporated in the WHO interim guidelines as one of the first-line treatments for HAT. The drug is also used to cure non-severe second-stage patients.

No standardized treatment strategy for the treatment of *T. evansi* HT is available so far. However, successful cure of *T. evansi* HT has been achieved by using the rhodesiense HAT treatment scheme using suramin.

Treatment of animal trypanosomiasis relies on the use of diminazene diaceturate. This compound is used effectively for the treatment of *T. evansi* infections in animals, but has not been registered for use in human due to severe side effects of the treatment in animal, including dogs. Diminazene does not cross the blood-brain barrier; therefore it is not effective in the case of CNS infections.

Prevention and Control

Currently, no universal methodology to control HAT is available. Nevertheless the 'National Sleeping Sickness Control Programmes' (NSSCPs) are supported by the WHO focusing on implementing control activities and capacity building through training. Control and surveillance rely on active and passive case finding, diagnosis, treatment, follow-up and control of the animal reservoir.

Controlling the spread of HAT also relies on vector control. Indeed, the control of the tsetse fly population by using nets and insecticidal spraying has helped reduce the number of HAT cases. However, control of the spread of *T. evansi* infections is much more difficult, due to the wide range of biting insects involved in parasite transmission. To avoid the spreading of surra, it has been suggested that equines should be bred several kilometres apart from cattle, which usually act as a reservoir. Monitoring of international

trading and quarantine measures are both essential to avoid introducing infected animals in non-infected countries. Failure to implement these rules can lead to unexpected disease outbreaks such as those that happened in Southern Europe in the recent past. Prevention of aHT by *T. evansi* is difficult. This is because these infections are rare; most are under-reported and occur in situations where humans live in close proximity to infected animals such as water buffaloes that can serve as asymptomatic trypano-tolerant reservoirs. In resource-poor areas, this is a risk factor that is very hard to control, in particular when multiple different insect vectors can be responsible for zoonotic transmission. Here, large-scale animal surveillance efforts and herd-treat livestock animals are crucial to limit the risk of disease transmission.

No vaccine is currently available for the prevention of either human or animal trypanosomiasis. One reason for this is the presence of the inexhaustible VSG gene repertoire encoding the major surface protein. However, in between the VSGs, there are a number of invariant surface glycoproteins present that have been the target of several alternative vaccination approaches. None of these, however, has resulted in any success.

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Case Study

As there are many typical case reports of both rhodesiense and gambiense HAT available, one report that stands out is the identification of an atypical *T. evansi* HT infection in 2015. This was the first HT infection diagnosed at both serological and molecular level in Southeast Asia. The report covers the case of a 38-year-old woman who presented to a healthcare facility in southern Vietnam. Her symptoms included non-species issues such fever, headache and joint pain. Interestingly, the report included the APOL1 measurement in the patient's blood, showing that there was no genetic deficiency that could easily explain the susceptibility to infection. This report followed a decade of APOL1 research, where a consensus grew that this molecule was indeed the most important factor in the trypanolytic activity of human serum. Hence, with full trypanolytic activity being present in this case, it remains to be discovered how some *T. evansi* trypanosomes survive in human blood, while being devoid of the known *T. b. rhodesiense* and *T. b. gambiense* resistance factors.

Research Question

1. Can anti-trypanosome immunity be induced by vaccination, and can vaccine-induced memory against any trypanosome target be recalled upon infection fast enough to stop the imitation of immune destruction by the parasite?
2. Does host pathology and inflammation contribute directly to the signals that drive quorum sensing during peak parasitaemia?
3. Which mechanisms allow *T. b. gambiense* Group 2 and *T. evansi* to avoid APOL1-mediated trypanolysis in aHT?
4. What is the mechanism of the uptake of the major trypanolytic factor, i.e. TLF2, and which resistance mechanism is operated by *T. b. gambiense* Group 1 parasites that allows survival in human serum?

Further Readings

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