New developments in human African trypanosomiasis
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\textbf{Purpose of review}
To review recent literature on human African trypanosomiasis, focussing on genome sequencing, diagnosis and drug discovery, and typing of trypanosomes.

\textbf{Recent findings}
The most important recent development has been the completion of the \textit{Trypanosoma brucei} genome which will greatly facilitate the discovery of new drug targets and genetic markers. Correct staging of the disease is of key importance for treatment. The analysis of sleep patterns is a promising new method to this end and has advanced enough to begin thorough clinical trials. In terms of novel drug candidates, dicationic molecules show the most promise with one oral diamidine in phase 3 clinical trials. New targets and classes of molecules which show in vitro trypanocidal activity are also described. Two new methods – MGE-PCR and microsatellites – allow analyses without parasite cultivation, eliminating a major impediment to efficient sampling for population studies. The finding that several wild animal species harbour \textit{T. b. gambiense}, and that parasite transmission is efficient even from very low parasitaemias, sheds a new light on the importance of animal reservoirs.

\textbf{Summary}
The use of \textit{T. brucei} as model system for molecular and cell biology is regularly producing new technologies exploitable for diagnosis and new drugs. Drug discovery and development experience a revival through new public-private partnerships and initiatives. The challenge remains to translate this progress into improvements for affected people in disease endemic areas.

\textbf{Keywords}
animal reservoir, chemotherapy, population genetics, sleeping sickness, strain distinction, \textit{Trypanosoma brucei}

\textbf{Introduction}
Human African trypanosomiasis (HAT) or sleeping sickness is caused by flagellated protozoan parasites (\textit{Trypanosoma brucei}) and transmitted by tsetse flies (\textit{Glossina} spp.). The disease is fatal if not treated and is among the most neglected diseases. It affects individuals in over 30 African countries, and resources are lacking to control the disease properly and to improve diagnosis and chemotherapy. Uganda is the only country harbouring both forms of HAT, caused by \textit{T. brucei gambiense} and \textit{T. brucei rhodesiense}, respectively. The two forms of disease do not overlap yet, but there are indications that this may change in the near future because both areas are expanding [1]. \textit{T. brucei rhodesiense} has recently moved from the south-eastern focus at Lake Victoria northward into Soroti and Kaberamaido [2]. As diagnosis is unreliable and active surveillance is lacking in many countries, it is likely that most cases go undetected. For example, Odiit \textit{et al.} [3*] calculated that approximately 90\% of HAT fatalities are not detected in epidemics in Uganda. Similar studies in \textit{T. brucei gambiense} areas should be encouraged. In the period under review, the first human case of a pathogenic \textit{Trypanosoma evansi} infection has also been reported from India [4*]. It is, however, not clear if this case involves a \textit{T. evansi} strain that has acquired human infectivity (the strain could not be isolated) or if the patient was lacking the trypanolytic factor in his blood plasma.

Diagnosis and chemotherapy are both problematical areas: diagnosis is either insensitive or laborious, whereas chemotherapy depends on drugs that are old, inefficient, toxic and expensive. The pharmaceutical industry is currently not pursuing research and development for new drugs for HAT. Fortunately, this gap can be filled partly by new initiatives such as the Tropical Disease Research/World Health Organization committee on Genomics and Discovery Research, or the Drugs for Neglected Diseases initiative. In addition, as a result of several special features of its biology, \textit{T. brucei} is an intensely studied model system for molecular, biochemical and cell biology. As a literature search reveals, only approximately a quarter of the published research articles on \textit{T. brucei} are medically relevant, whereas the majority is fundamentally molecular, biochemical, cell or microbiological research with no disease-oriented applied objectives (Fig. 1). This produces abundant information, however, to suggest new targets for diagnosis and treatment and a rich array of molecular tools that greatly facilitate applied research.
target proteins as serodiagnostic markers [16]. A new approach to HAT diagnosis is proteomic signature analysis [17,18*], which recognizes the typical fingerprint of HAT infections in the protein content of patient samples. The method is extremely sensitive and specific (100 and 98.6%), but requires expensive equipment and extensive expertise. It is therefore not directly applicable in the field, but may help identify specific proteins exploitable for serodiagnostics in the field.

There is an urgent need for better diagnostics for HAT stage determination. Buguet et al. [19**] demonstrated that entry into the second stage of the disease is associated with marked changes in the sleep pattern, observable with relatively simple devices. This is a very promising new avenue of research, especially as it appears that rats exhibit the same two-staged disease course [20*], so that an appropriate experimental system is at hand to develop this system further. Finally, a new dot-enzyme-linked immunosorbent assay for stage determination in the field has been described by Courtioux et al. [21*]. After validation it may replace or complement other available field assays [5*].

Chemotherapy

The search for new drugs is of paramount importance and can follow several strategies: the improved use of existing drugs, new drug combinations, therapeutic switching, and the search for new chemical entities that should be efficacious, safe and affordable for disease-endemic countries. A 10-day melarsoprol treatment schedule was validated in a multinational study in over 2000 patients [22**]. The cure rate was 94% 24 h after treatment and was still 86% after 2 years. The fatality rate was 5.9%, and 4% of treated patients died from an encephalitic syndrome. In a previous study [23], it was shown that the efficacy and safety of the 10-day treatment schedule are comparable to a standard 26-day treatment schedule in three courses. The new treatment schedule reduces treatment duration, the amount of drug and costs, and thus increases the capacity of hospitals for HAT treatments. On the basis of data from southern Sudan, Chappuis et al. [24] showed that eflornithine is a safe and effective drug for second-stage disease. Mortality during treatment and adverse effects were much less dramatic in patients treated with eflornithine than with melarsoprol. The authors recommend the use of this 14-day treatment schedule with four daily intravenous infusions not only in areas with high melarsoprol relapse rates but wherever possible. The feasibility of this eflornithine treatment schedule is, however, questionable in rural areas considering that more than 50 intravenous infusions are needed and the drug is enormously costly.

The most promising new compounds in the clinical or preclinical phase are the aromatic diamidines, which represent lead compounds against various protozoan
parasites and fungi [25**]. Several classes of dicationic molecules were recently synthesized and their in-vitro and in-vivo activity was demonstrated. Dicationic guanidine and reversed amidine derivatives were able to cure a T. brucei rhodesiense mouse model after multiple parenteral applications. Carbamate prodrugs could cure the mouse model even with an oral application [26**]. Biphenyl benzimidazole derivatives were synthesized and their DNA binding was measured [27]. All molecules showed strong DNA affinities and also a very high in-vitro activity against African trypanosomes and malaria parasites. Two compounds cured a T. brucei rhodesiense mouse model at 20 mg/kg with intraperitoneal application. Another interesting class of antitrypanosomal dications are the imidazopyridines [28]. Five parent compounds were able to cure the T. brucei rhodesiense mouse model at 20 mg/kg intraperitoneally. Prodrugs did not have the same efficacy, probably because of poor oral bioavailability. Athri et al. [29*] have generated three-dimensional quantitative structure–activity relationship maps based on a library of heterocyclic diamidines, and have built a model with additional descriptors for donor/acceptor and hydrophobic properties to help design new molecules with improved DNA binding characteristics and improved antiparasitic activity.

Progress has also been made on non-diamidine targets and lead compounds. Cordycepin (3'-deoxyadenosine) in combination with the adenosine deaminase inhibitor coformycin was found to cure a T. brucei brucei mouse model [30]. The authors noted that adenosine analogues are already on the market and that given the marker's nature, the results can not be amplified. This approach is that it requires only a single PCR, and is thus fast and relatively inexpensive. It apparently only amplifies Trypanozoon DNA and can thus be directly applied to field samples. The drawbacks of the method are that one does not know what exactly is amplified, and that given the marker's nature, the results can not be used for population genetic or phylogenetic inferences.

**Strain distinction and population genetics**

The ability to distinguish different genotypes (or strains) is key to understanding trypanosome population dynamics, parasitism, and host adaptation. This is important for understanding the role of parasites and viruses in their hosts, and for designing effective control strategies. The use of molecular markers for population genetic studies is crucial for understanding the dynamics of trypanosome populations in the wild.

All genotyping methods traditionally applied have major limitations, arguably the worst being that most require a large amount of parasite material and thus depend on parasite cultivation. Cultivation, however, is a complex and time-consuming process that has limited sample sizes to levels below those required for many population studies. Furthermore, because parasite genotypes vary widely in and how well they can be cultured, cultivation represents a selection process determining what genotypes are included in such studies [35], which introduces the possibility for a strong bias. Two new molecular markers promise to overcome these limitations and greatly improve our ability to study different T. brucei genotypes in the field. Simo et al. [36**] demonstrated the utility of mobile genetic element PCR (originally developed by Hide and Tilley [37]) to distinguish different strains of T. brucei across all three ‘subspecies’ and notably among T. brucei gambiense, which is at the same time the most medically relevant and the least genetically variable T. brucei subspecies. The beauty of this approach is that it requires only a single PCR, and is thus fast and relatively inexpensive. It apparently only amplifies Trypanozoon DNA and can thus be directly applied to field samples. The drawbacks of the method are that one does not know what exactly is amplified, and that given the marker’s nature, the results can not be used for population genetic or phylogenetic inferences.

To address this problem, Balmer et al. [38**] characterized a set of microsatellite markers. Microsatellites are extremely variable stretches of repeated DNA sequence that vary even among close relatives. Balmer et al. [38**] presented 14 microsatellite loci and demonstrated that they are highly variable and thus capable of distinguishing many different T. brucei genotypes. The great advantage microsatellites offer compared with all other available genotyping approaches is that they are selectively neutral and conform to a defined mutation model. They are thus ideal markers for population genetic analyses of historic and present population structure and the population dynamics of T. brucei. As the method is PCR based, it can be applied directly to field samples (host blood, tsetse flies) without relying on cultivation.
The main drawback of microsatellites is that their analysis is technically demanding and best performed with a DNA sequencer.

MacLeod et al. [39] have used microsatellites in another way to give a statistically robust demonstration that alleles segregate in a Mendelian fashion in T. brucei. Although their data, contrary to their explicit claim in the title, do not prove the involvement of meiosis, the results are very comforting to population geneticists because they confirm the validity of a key assumption for population genetic analyses.

The need to distinguish T. brucei genotypes further arises in laboratory studies investigating interactions between different genotypes and effects of multiple genotype infections. The most powerful way to do this is to use markers that make different genotypes phenotypically distinguishable. Balmer and Tostado [40] described genetic constructs that allow fluorescence expression in four different colours and notably in all parasite life stages. They demonstrate that these constructs are readily integrated into the genomes of various strains of T. brucei brucei and T. brucei rhodesiense (but not T. brucei gambiense, it seems) and that fluorescence expression is stable in all life stages. By transfecting two strains with two different colours they become distinguishable by eye. Relative population sizes can thus be followed under the fluorescence microscope or even more efficiently using a fluorescence-activated cell sorter, without the need to genotype or clone. The ability to use a fluorescence-activated cell sorter is a big step forward because it not only allows the very efficient daily tracking of large numbers of infections, but even allows the separation of strains again after experimental mixed infections.

Animal reservoir

The great significance of domestic and wild animal hosts for Rhodesian sleeping sickness is undisputed. As a result of the close proximity of livestock to humans and the relative ease with which they can be treated, Welburn et al. [41] propose that sleeping sickness would most effectively be controlled locally by fighting the parasite in livestock as part of farming practices. Ng’ayo et al. [42] show that domestic animals (here a sheep, a goat and a pig) harbour T. brucei rhodesiense even in areas with few recent cases of sleeping sickness. The role of animal reservoirs is much more controversial in Gambian sleeping sickness. An unbiased observer would be astonished how strongly workers in this field seem willing to dismiss the important role of animals in T. brucei gambiense epidemiology without much proper data from systematic surveys. Clearly, this mindset has not helped us gain a realistic view of sleeping sickness epidemiology in west Africa. Pigs have been shown repeatedly to harbour T. brucei gambiense [36]. Even though pigs can clear infections in less than 6 months, as Penchenier et al. [43] demonstrate, they are still capable of maintaining parasite populations outside the human cycle. Njiokou et al. [44] now show evidence that eight wild animal species (out of 36 species sampled) belonging to four orders (primates, artiodactyls, rodents, carnivores), host T. brucei gambiense group 1 parasites (Fig. 2). Even if these animal species play minor roles during epidemics, they probably hold the key to where the parasites are maintained between epidemics and where new epidemics start. Hopefully, the accumulated evidence on animals carrying T. brucei gambiense group 1 parasites will finally lead to the thorough rethinking of the role of animal hosts in Gambian sleeping sickness. Two next steps would be particularly desirable:

Figure 2 The greater white-nosed monkey, Cercopithecus nictitans, and its central and west African range

The recent discovery that this and several other wild animal species harbour Trypanosoma brucei gambiense group 1 parasites [44] strongly suggests that animal reservoirs must play a more important role in west African sleeping sickness than generally acknowledged. A better understanding of their role will help control efforts in the long run.

First, the identified *T. brucei gambiense* group 1 samples should be investigated with a wider array of genetic markers to eliminate any doubts about their identity, and to place them within the other isolates from the area to see if they are the same genotypes also found in humans. Second, a systematic geographical sampling independent of ‘foci’ would be desirable, also for *T. brucei rhodesiense* in east Africa, to assess how parasite presence and disease occurrence correlate.

Van den Bossche *et al.* [45**] further highlighted the potential impact of animal reservoirs by showing that the transmissibility of *T. brucei rhodesiense* to tsetse flies is independent of parasitemia and is efficient even at very low parasitemias. This means that each tsetse bite on an infected host has a similar probability of leading to a mature infection, and that even animals with very low parasite loads play an important epidemiological role, a result that is also of great importance for disease modelling.

**Conclusion**

Research in the field of HAT has progressed during the past 2 years in the areas of genomics, diagnosis and chemotherapy, but also in the development of new tools for the better characterization of trypanosomes. HAT largely benefits from basic research that uses *T. brucei* as a model organism to tackle genetic, molecular and biochemical questions. Research for diagnosis and drug discovery/development, abandoned by big pharmaceutical companies for the past 20 years, receives more attention through new public–private partnerships such as the Foundation for Innovative New Diagnostics, Geneva, the Tropical Disease Research/World Health Organization committee on Genomics and Discovery Research or the Drugs for Neglected Diseases initiative, Geneva. It can be expected that such non-profit organizations will deliver new tools for the diagnosis and treatment of HAT. For a long-term perspective it is also important that epidemiological work on the distribution of infected humans, vectors and animal reservoir hosts is intensified, especially in *T. brucei gambiense* areas.

**Acknowledgement**

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**References and recommended reading**

Papers of particular interest, published within the annual period of review, have been highlighted as: • of special interest **••** of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 495).


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4 A model-based study estimating that a large proportion of deaths go undetected in *T. brucei* rhodesiense epidemics in Uganda based on hospital records and early to late stage ratios.
6 This is the first report of a human infection of *T. evansi* causing disease. The significance of this is not clear, an epidemic in India is unlikely.
8 A good review of present field diagnosis methods for sleeping sickness.
10 An improved version of an earlier PCR marker to distinguish trypanosome species.
12 This study presents the *T. brucei* genome and compares metabolic pathways and some other cell biological aspects of *T. brucei* with those in *Leishmania major* [10**] and *T. cruzi* [11**].
14 A very interesting comparison of gene content and genome architecture of the three sequenced trypanosome genomes [8**,10**,11**].
16 A comparative view of gene expression in *T. brucei* [8**], *T. cruzi* [11**] and *L. major*.
18 This study discusses DNA replication, repair machinery and retrotransposons of *T. brucei* [8**], *T. cruzi* and *L. major* [10**].
23 This study presents a genetic map for the *T. brucei* genome strain, which will help identify genes coding for important traits.
27 A less technical introduction into the method presented in Ref. [17].
29 This work demonstrates that the analysis of sleep structure by portable devices may be a good diagnostic tool to define which disease stage a patient is in and to determine the success of treatment.
31 This study shows that *T. brucei*-infected rats exhibit two disease stages like humans, and thus represent a good model system for work on stage determination.
33 A new assay to detect *T. brucei* in cerebrospinal fluid. It needs more testing but could aid staging in combination with existing methods or replace them in certain areas.


25 Soeiro MN, De Souza EM, Stephens CE, Boykin DW. Aromatic diamidines as antiparasitic agents. Expert Opin Invest Drugs 2005; 14:957–972. This review covers aromatic diamidines as antiparasitic agents (trypanosomes, leishmanias, Plasmodium falciparum and Pneumocystis carinii) and summarises uptake, mode of action and antiparasitic activities.


28 Ismail MA, Brun R, Wenzler T, et al. Novel dicaticonic imidazo-[1,2-a] pyridines and 5,6,7,8-tetrahydro-imidazo-[1,2-a] pyridines as antiprotozoal agents. J Med Chem 2004; 47:3658–3664. This study presents an extended comparative molecular similarity indices analysis model to help design compounds with better antiprotozoal activity.

29 Athin P, Wenzler T, Ruiz P, et al. 3D QSAR on a library of heterocyclic diamidine derivatives with antiparasitic activity. Bioorg Med Chem 2006; 14; 3144–3152. This study presents an extended comparative molecular similarity indices analysis model to help design compounds with better antiprotozoal activity.


36 Simo G, Herder S, Njokou F, et al. Trypanosoma brucei s.l.: characterisation of stocks from central Africa by PCR analysis of mobile genetic elements. Exp Parasitol 2005; 110:369–362. This work is important because it demonstrates the utility of a simple molecular method to distinguish a number of T. brucei gambiense and many non-gambiense strains without a need to culture them.


38 Balmer O, Palma C, MacLeod A, Caccone A. Characterization of di-, tri- and tetranucleotide microsatellite markers with perfect repeats for Trypanosoma brucei and related species. Mol Ecol Notes 2006; 6:508–510. The microsatellites presented in this paper are ideal population genetic markers because they are extremely variable and thus distinguish many strains without the requirement for parasite cultivation.


40 Balmer O, Tostado C. New fluorescence markers to distinguish co-infecting Trypanosoma brucei strains in experimental multiple infections. Acta Tropica 2006; 97:94–101. This work demonstrates a very efficient method to distinguish and quantify different parasite strains visually in all life stages in co-infection experiments.


44 Njokou F, Lavessiere C, Simo G, et al. Wild fauna as a probable animal reservoir for Trypanosoma brucei gambiense in Cameroon. Infect, Genet Evol 2006; 6:147–153. This work reports that eight out of 38 wild animal species screened in Cameroon were infected with T. brucei gambiense group 1 parasites, suggesting that the animal reservoir may be much more important than assumed.

45 Van den Bossche P, Ky-Zerbo A, Brandt J, et al. Transmissibility of Trypanosoma brucei during its development in cattle. Trop Med Int Health 2005; 10:833–839. This study shows that infectivity to tsetse of T. brucei brucei-infected cows remains constant over time even as parasitemia decreases to very low levels. This implies that parasitemia is of limited importance for transmission.