Vector-borne protozoal infections of the CNS: cerebral malaria, sleeping sickness and Chagas disease

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Bullet points

- There is limited data on the accuracy of rapid diagnostic and polymerase chain reaction-based tests for *P. falciparum* malaria.
- PfEMP1, expressed in infected erythrocytes, underpins therapeutic and preventative advances in *P. falciparum* malaria.
- Chagas disease is a substantial risk factor for cardioembolic stroke, the prediction of which is facilitated by simple, point-of-care diagnostic tools.
- Tools for acute human African Trypanosomiasis and sleeping sickness diagnosis are evolving with the advent of polymerase chain reaction-based tests as well as field-adapted sleep studies.
- The recent introduction of oral therapy for HAT will improve care, quality of life, and contribute to eradication.
ABSTRACT

Purpose of the review: Malaria, Chagas Disease and Human African Trypanosomiasis are vector-borne protozoan illnesses, frequently associated with neurological manifestations. Intriguing but ignored, limited mainly to resource-limited, tropical settings, these disorders are now coming to light because of globalisation and improved diagnosis and treatment. Enhanced understanding of these illness has prompted this review. Recent findings: Methods of diagnosis currently transition from blood smear examinations to immunological assays and molecular methods. Tools to assess neurological involvement, such as magnetic resonance imaging, are now increasingly available in regions and countries with high infection loads. Sleep and other electrophysiological technologies (EEG, actigraphy) are also promising diagnostic tools but requiring field-validation. Access to treatments was formerly limited, even as limitations of agents used in the treatment are increasingly recognised. Newer agents are now being developed and trialled encouraged by improved understanding of the disorders' molecular underpinnings. Summary: Prompt diagnosis and treatment are crucial in ensuring cure from the infections. Attention should also be due to the development of globally applicable treatment guidelines, the burden of neurological sequelae and elimination of the zoonoses from currently endemic regions.

Key words: Plasmodium, Trypanosoma, Neurological manifestations, treatment
Introduction

Many disease-causing organisms are transmitted from animal to humans or from humans to other humans. The diseases are termed vector-borne diseases and the transmitting agents, vectors. The infecting organisms transmitted include viruses, bacteria, protozoans and helminths. Here, we review infections by protozoan pathogens. Each of these pathogens can infest a variety of organs, and the CNS is frequently involved. This review focusses on neurological manifestations of protozoan infections (Table 1).

Vector-borne protozoan diseases are prevalent in tropical and subtropical environments of many low- and middle-income countries. The 2019 Global Burden of Diseases (GBD) cycle estimated 231 million malaria infections and 643,000 (350,000 in children) deaths due to malaria worldwide. Over 90% of the cases and most deaths were from sub-Saharan Africa (Fig. 1). Human African Trypanosomiasis is prevalent in at least 37 African countries but mostly in the Democratic Republic of the Congo, Angola, Central African Republic, Chad, Congo, Gabon, Cameroon, Guinea, Malawi and South Sudan. The GBD estimated 2020 new cases in 2019. In comparison, it estimated 173,000 new cases and 9490 deaths due to Chagas disease. These cases were mostly from Brazil, Bolivia, Venezuela, Colombia and Paraguay. Two other vector-borne parasitic disorders, Leishmaniasis and Babesiosis may involve the human nervous system. Neurological manifestations are, however, unusual and we will not discuss them further.
In countries with a high prevalence of vector-borne diseases, the emphasis should be as much on prevention and eradication as on case detection and treatment. Prevention encompasses a variety of vector control measures depending on the transmission characteristics of the pathogens and vector properties but also mainly behavioural changes in human hosts. Apart from the resource-limited settings, vector-borne protozoan diseases are relevant on a global scale on account of frequent travel and immigration.

**Malaria**

Malaria is a human parasitic infestation by mainly two Plasmodium species, viz *P. vivax* and *P. falciparum*. From the clinical standpoint, malaria can be either uncomplicated or severe and complicated; the distinction between the two is based on organ involvement. Severe malaria is mostly caused by *P. falciparum* but rarely, also by *P. vivax* and co-infection is characterised by severe anaemia, impending or overt respiratory failure or coma. It occurs when the parasitised red blood cells are sequestered within the microcirculation, leading to release of pro-inflammatory cytokines, endothelial damage and microvascular occlusion. Cerebral malaria, the most severe form, typically presents with hyperpyrexia, coma, seizures and status epilepticus, mostly in children, < 5 years of age. It is fatal in 20% cases and when not, may lead to long-term severe neurological and cognitive sequelae.

Two pathophysiological derangements, each linked and complementary to the other form the basis of the manifestations of cerebral malaria: (i) erythrocyte sequestaration and (ii) inflammation. Sequestration is the cytoadherence of...
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*Plasmodium*-infected erythrocytes to the endothelium of the cerebral microvascular. The *Plasmodium* surface membrane protein, PfEMP1, expressed in infected erythrocytes is a key molecule in the process. Various endothelial receptors, including intracellular adhesion molecule-1, vascular cell adhesion molecule, and differentiation molecule-36) are also implicated. Recent experiments point to the endothelial glycocalyx pathogenic role, which in health forms of a protective cover over the endothelial cells. Hence, it prevents the binding of parasitised erythrocytes to the endothelium. Sequestration leads to vascular occlusion, venous hypertension, cerebral oedema and hypoxia. It also promotes leukocyte recruitment and activation of the vascular endothelium, leading to the release of inflammatory cytokines and chemokines. The latter incite local inflammation, breakdown of the blood-brain barrier and vascular leakage. Treatments targeting these processes offer potential avenues for lessening the pathophysiological derangements in the brain in cerebral malaria.

The diagnosis of cerebral malaria can be challenging. Case definitions and criteria have been proposed and widely followed. An autopsy study from Malawi found that seven of 31 children with clinically-established cerebral malaria died from unrelated causes. The findings of malaria-specific retinopathy, which is highly sensitive and specific for complicated but not uncomplicated malaria, help bedside diagnosis. Findings include retinal whitening, retinal haemorrhages with central white spots. These, however, might be restricted to the periphery of the retina and require pupil dilatation and expertise for recognition. The pathophysiology of retinopathy is similar to that of cerebral malaria.
The laboratory diagnosis of cerebral malaria hinges on the parasite's demonstration in Giemsa-stained thin and thick smears of blood. The method is labour-intensive and requires trained technicians. In many parts of the world, the blood smear is replaced by rapid diagnostic tests by immunochromatography\textsuperscript{11,12}. The rapid tests are based on reactions to \textit{Plasmodium} antigens or enzymes, e.g., the histidine-rich protein-2 for \textit{P. falciparum} and lactic dehydrogenase for other species\textsuperscript{13}. Real-time polymerase chain reaction-based diagnosis is now increasingly available. It has high sensitivity and specificity in comparison to conventional microscopy for falciparum malaria and somewhat improved diagnostic accuracy in \textit{P. vivax} infections, mixed infections and in case of low levels of parasitaemia\textsuperscript{14}.

Magnetic resonance imaging (MRI) characterises the cerebral derangements in malaria but is sparingly accessible for clinical use in most malaria-endemic regions. Very few reports of MRI findings in cerebral malaria are available\textsuperscript{15-17}. These have emphasised the presence of venous infarcts and cortical diffusion abnormalities in severe illness\textsuperscript{15}. Early in the illness, however, diffusion-weighted imaging with apparent diffusion coefficient and perfusion maps point to the development of oedema and venous congestion with patchy cytotoxic edema\textsuperscript{17}. Most of these changes reverse with treatment.

The World Health Organization (WHO) recently updated the guidelines for the diagnosis and management of malaria. The mainstay of treatment is artemisinin-based combination therapies. Cerebral malaria is best managed in intensive care, emphasising the appropriate treatment of coma, prompt treatment of status epilepticus and the prevention of neurological sequelae. The recent emergence of
artemisinin-resistance, particularly in Southeast Asia, is a challenge, and newer agents are desirable\textsuperscript{18,19}. A spiroindolone, KAE609, which inhibits \textit{Plasmodium} plasma membrane protein, PfATP4 was effective and safe in a Phase 2 trial in Thailand\textsuperscript{20}. It is now being trialled in a multi-country initiative. Seizures and status epilepticus are managed according to existing guidelines. There is, however, no role for prophylactic antiseizure medications\textsuperscript{21}. Likewise, the routine use of mannitol or dexamethasone provides no clinical benefit.

**Chagas disease**

The protozoan, \textit{Trypanosoma cruzi} is transmitted to humans through the bite of kissing bugs (\textit{Triatoma, Panstrongylus, Rhodnius}) and causes Chagas disease\textsuperscript{22}. Infection initially comprises an acute stage with local (at the site of insect bite) and systemic symptoms such a fever and malaise but is not uncommonly asymptomatic. This acute phase is followed mostly a dormant stage, eventually leading in many people to overt chronic disease. Major manifestations in the chronic stage are in the cardiac and digestive systems. Chronic heart failure, arrhythmias and cardiac conduction defects, and dysphagia symptoms resulting from oesophageal dilatation and constipation from colonic involvement are most common. Chagasic cardiomyopathy develops in nearly a third of those with the chronic stage of infection. Neurological involvement might occur in up to a tenth of people and is usually in the form of stroke or a mild sensory polyneuropathy.
The association between ischaemic stroke and Chagas disease is now well established\textsuperscript{23-25}. In endemic regions, Chagas disease is a significant risk factor for ischaemic stroke, and the underlying mechanism is mainly cardioembolic. For instance, in endemic parts of Colombia and the Brazilian state of Bahia, nearly a quarter of all ischaemic strokes attending hospitals may be attributable to Chagas disease\textsuperscript{26,27}. The strokes usually occur in the fifth or sixth decade, often in people without significant vascular risk factors and multiple previous cerebral infarctions\textsuperscript{25}. In the United States, Chagas disease is considered a risk factor in presumed cardioembolic stroke among travellers and migrants from endemic countries.

A risk prediction tool for the occurrence of stroke has been developed in Brazil but needs further validation\textsuperscript{28}. Four predictors in this score are age\textgreater{}48 years, ST-segment changes on the ECG, and evidence of systolic heart failure with a left ventricular apical aneurysm on the echocardiogram. Over a third of people with Chagasic strokes also have atrial fibrillation\textsuperscript{29}. As the development of Chagasic cardiomyopathy is the primary mechanism underlying stroke, identifying early or impending cardiac involvement by the use of biomarkers has been investigated. One such biomarker, plasma microRNA-208a is useful. Magnetic resonance imaging is a valuable tool to assess myocardial function and fibrosis in particular\textsuperscript{30}.

Atrophy of the brain may occur in chronic Chagas disease\textsuperscript{31}. Presumably, this is secondary to cardiovascular disease. The possibility of primary brain involvement by the parasite, however, cannot be excluded. Whether Chagas disease is an independent risk factor for vascular dementia has not been adequately assessed.
Ostensibly, it may because multiple and micro-embolisations are frequent in Chagasic individuals.

The mainstay of primary and secondary prevention of stroke associated with Chagas disease is anticoagulation. Conventional agents are widely used, and there is anecdotal evidence for the use of newer oral anticoagulants\textsuperscript{32,33}. However, no Class I or Class II studies exist to support the administration of the newer agents.

Benefits of administering antiparasitic agents are clear in people with acute and early stages of the disease with high cure rates. Treatment of pregnant women prevents congenital infection in offspring. The benefits of antiparasitic treatment in people with established cardiomyopathy are less certain. A meta-analysis found only marginal benefits of treating individuals with established cardiomyopathy\textsuperscript{34}. Two drugs are used for antiparasitic treatment: benznidazole and nifurtimox. Both have issues as they frequently lead to side-effects. These are mainly skin rashes and peripheral neuropathies in the case of benznidazole and gastrointestinal side-effects with nifurtimox. Benznidazole is preferred on account of lesser side-effects and ease of availability in Latin America. The recommended duration of treatment varies but should be at least 30-60 days. Curiously, the reactivation of Chaga’s disease after autologous bone marrow transplant has been reported\textsuperscript{35}.

Human African Trypanosomiasis
The Trypanosoma genus parasite causes Human African trypanosomiasis (HAT) or sleeping sickness. It is transmitted to humans through bites of the tsetse fly (Glossina genus), putting 70 million people in sub-Saharan Africa at risk of the infection\textsuperscript{36-38}. HAT is fatal if not treated and affects several thousand people every year\textsuperscript{1}, many of whom lack access to diagnostic and treatment facilities\textsuperscript{39-41}. Together with generally weak national control programmes, this situation constitutes a limiting factor in HAT elimination efforts\textsuperscript{41-43}.

HAT presents in two forms each caused by a specific species of the parasite. The first is \textit{T. brucei gambiense} which causes the more prevalent and chronic (lasting several months to years) West and Central African form, constitutes 95-97\% of all cases. The second, \textit{Trypanosoma brucei rhodesiense} which causes the acute (a few weeks) East African form constitutes 3-5\% of the cases\textsuperscript{44-46}. \textit{Trypanosoma brucei rhodesiense}, although globally less prevalent, still carries epidemiological importance. It is responsible for about two-thirds of HAT cases amongst returning tourists who have visited countries in East Africa\textsuperscript{47,48}. Each clinical form of HAT evolves in two main stages: the hemo-lymphatic, referred to as stage 1 and meningoencephalitis, generally known as stage 2 disease, corresponding to the condition phases resulting from the crossing of the blood-brain-barrier (BBB) by the parasites to invade brain parenchyma. Cardiac involvement in the form of pericarditis with or without myocarditis might also occur in \textit{T. brucei rhodesiense} infection and can be a severe condition\textsuperscript{49}. 
HAT’s pathophysiology has not been well understood until recently but seems dependant on activity on either side of the BBB. It has now been suggested that pericytes, embedded within the endothelial cell-derived basement membrane of the blood-brain barrier, play an essential role in controlling endothelial transcytosis and tight junction opening. Trypanosomes from the blood pass into the brain through the outer parenchymal basement membrane depending on immune response molecules induced by the infection. The exact molecular mechanisms of parasite neuroinvasion are still to be understood. Contrast-enhanced MRI studies in animal models, however, suggest a progressive deterioration of BBB function starting soon after infection, which is mitigated by curative treatment. Neuroinvasion appears to depend on the degree of neuroinflammation resulting from the delicate balance of pro-inflammatory and anti-inflammatory mediators. Still, in animal models of HAT, it has been shown that pro-inflammatory mediators such as tumour necrosis factor (TNF)-α, interferon (IFN)-γ, and CXCL10 play an essential role in parasite CNS invasion. Conversely, the administration of interleukin (IL)-10, an anti-inflammatory molecule, has been shown to reduce the CNS parasite load and lessen the severity of the neuroinflammatory response symptoms. The neuroinflammatory reaction characterising stage 2 disease occurs in the choroid plexus, the circumventricular organs and the parenchymal vasculature. It involves astrocyte activation, inflammatory cell infiltration (macrophages, T cells, B cells, plasma cells, Mott cells).

Clinically, stage 1 disease features are generally non-specific and include headache, intermittent fever, arthralgia, and lassitude rendering the distinction from malaria rather difficult as both can be co-morbid. Later, individuals may develop lymphadenopathy, hepatomegaly, splenomegaly, pericarditis, haemolytic anaemia,
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pruritus, eye involvement and endocrine disorders as the process of systemic inflammation progresses\textsuperscript{51}. The clinical presentation in infected persons from non-endemic regions is usually characterised by fever and gastrointestinal symptoms (diarrhoea and jaundice) without lymphadenopathy\textsuperscript{62}.

The clinical hallmark of stage 2 HAT is the appearance of characteristic sleep disturbances, usually associated with other neurological and psychiatric manifestations. The sleep disorder in HAT consists of daytime sleepiness and nocturnal insomnia - the characterisation of the diurnal hypersomnolence and nocturnal sleep fragmentation, which gives the disease its name, "sleeping sickness" is now well established. The transition from stage 1 to stage 2 disease is generally insidious. Early-stage 2 symptoms may include irritability, lethargy, psychiatric, and behavioural disturbances. Later, pyramidal and extrapyramidal syndromes, headaches, speech disorders, cerebellar dysfunction, myelopathy, peripheral nerve disease and eye involvement may be seen. Progression to impairment of consciousness, incontinence, seizures, and eventually death occurs in most untreated individuals\textsuperscript{44}.

Reports of asymptomatic carriers of HAT exist. Some evidence suggests that parasite evades the host immune system by switching its variable surface glycoprotein surface coat. It may further develop serum resistance-associated protein (SRA) and \textit{T. b. gambiense}-specific glycoprotein (TgsGP) production to fight host defence molecules\textsuperscript{45}. 

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The diagnosis of HAT is confirmed by the finding of the parasite on a thin or thick peripheral blood smear for *T. b. rhodesiense* disease (high blood parasite levels) and less so for *T. b. gambiense*, which only intermittently exhibits high parasite levels\(^\text{45}\).

The Card Agglutination Test for Trypanosomiasis (CATT) is generally used for disease screening, especially in endemic areas, but it has the drawback of false positives in regions of low endemicity. Polymerase Chain Reaction (PCR) techniques have shown some field challenges while the Rapid Diagnostic Tests (RDTs) under development may improve the serologic diagnosis. According to WHO criteria, the diagnosis of stage 2 disease involves finding the parasite in cerebrospinal fluid or more than 5 WBCs/ml or both. On a cautionary note, the first criterion is not sensitive, and the second is arbitrary \(^\text{42,46}\).

Whilst the search for more reliable biological markers is ongoing, novel diagnostic modalities have evolved and are more adapted to field use and are non-invasive. Polysomnography studies have led to the description of a characteristic sleep pattern in HAT associated with sleep onset eye movement periods (SOREMPs). Still, again, this technique is not practicable in field conditions\(^\text{42,46}\). Actigraphy has recently been suggested as a pragmatic non-invasive tool for diagnosis, staging, and disease monitoring, especially with the development of an actigraphy sleep score\(^\text{53,54}\). This technique still needs validation in more extensive studies.

The treatment of HAT is summarised in Table 2. Untreated, HAT is fatal. There is an excellent response to therapy during stage 1 disease. In stage 2 disease, Melarsoprol is used but poses serious toxicity challenges. Post-treatment follow-up is necessary for up to 24 months to rule out relapse. The WHO recently provided
interim guidelines based on evidence-based recommendations. A new oral drug, fexinidazole has advantages of out-patient administration and utility in both disease stages in HAT- *gambiense*. Clinical trials of Fexinidazole are planned for *rhodesiense* HAT; however, a small qualitative study of expectations concerning the new therapeutic approach suggests that serious consideration be given to counselling and monitoring efforts. New therapies such as acoziborole, obtained through non-profit Product Development Partnerships, have also shown promising clinical trial results in the cure of both stages of the disease in a single oral dose. Overall, this new approach could improve individual retention in care with better chances of disease eradication.

**Conclusion**

Recent developments in understanding cerebral malaria, Chagas disease and African human trypanosomiasis have opened new directions with prospects for better therapies and preventative approaches. The *Plasmodium* surface membrane protein, PfEMP1, expressed in infected erythrocytes is a good case in point. It is driving the development of newer treatments and vaccines currently being trialled or in transition. The emphasis of clinical research is on field testing of rapid diagnostic and polymerase chain reaction-based tests and the development of diagnostic, therapeutic protocols and risk prediction tools to enable early diagnosis, treatment and certain cure.
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LEGEND TO FIGURE

Fig. 1. Global distribution of age standardised disability-adjusted life years (DALYs)/100,000 population, both sexes combined due to malaria, Chagas disease and human African trypanosomiasis*.

Foot note: Data adapted from Ref. 1; For malaria: High level > 768.2 DALYs/100,000 population, Medium = 77.3 – 768.2 DALYs/100,000 population and Low < 77.3 DALYs/100,000 population; For Chagas disease: High level > 45.9 DALYs/100,000 population, Medium = 12.5 – 45.9 DALYs/100,000 population and Low < 12.5 DALYs/100,000 population; For human African trypanosomiasis: High level > 29.9 DALYs/100,000 population, Medium = 3.5 – 29.8 DALYs/100,000 population and Low < 3.5 DALYs/100,000 population.