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Chagas disease: the challenge of polyparasitism?

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The debilitating zoonosis Chagas disease (CD) is caused by infection with the flagellate protozoan *Trypanosoma cruzi*. One century after its discovery, a curative agent remains elusive. Immune evasion by *T. cruzi* results in a poorly controlled infection in the host, which can end in either sudden death or a fatal chronic disease that often eventuates after years of an asymptomatic infection. Polyparasitism or mixed/concurrent infections occur more often than not and contribute to the high degree of variability observed across both disease progression and the success of therapeutic interventions. A thorough understanding of the effects of polyparasitism on CD is essential for improving the likelihood of containing, treating, and eventually eliminating CD.

Keywords: polyparasitism; Chagas disease; *Trypanosoma cruzi*; drug discovery
The impact of Chagas disease

Chagas disease (CD) in humans is caused by infection with the flagellate protozoan *Trypanosoma cruzi*. As a neglected disease, CD is associated with malnutrition, poverty, and inadequate sanitation 1 and 2. Poor living conditions in areas where CD is endemic allow the vector of *T. cruzi*, a triatomine insect, to infest housing, increasing transmission to both mammalian and non-mammalian hosts [3]. Education and vector control have helped contain the spread of infection; however, CD remains a debilitating zoonosis. It is the leading cause of death and morbidity in the Latin American and Caribbean regions1 and 4, with approximately 8 million people infected worldwide [5], more than 25 million people at risk of infection, and approximately 15 000 resultant deaths each year [6].

The acute phase may be completely asymptomatic or may result in a plethora of symptoms 7 and 8 that may lead to death over a variable time period 7 and 9. A consequence of increased immigration worldwide is that areas with a previously low prevalence of CD, such as the USA, Japan, and Australia, now report a growing number of cases 10 and 11. Additionally, the ramifications of delayed onset of symptoms in chronic infections could be that in coming years we can expect infected individuals to show symptoms, adding strain to global health systems. Thus, to ensure the containment and proper treatment of CD, it is of utmost importance that clinicians in countries of nonendemicity are able to recognise CD symptoms. An emerging issue is that of polyparasitism, where concurrent infections with different strains of *T. cruzi* and/or other parasites may be associated with variances in virulence, drug sensitivity, organ predilection, and clinical outcome [12]. As such, this will clearly have an impact on the clinical management of CD and, accordingly, a comprehensive
knowledge of the complexities arising from polyparasitism is crucial for effectively combatting CD.

**Diagnosis of *T. cruzi* infection**

The accurate diagnosis of *T. cruzi* infection is pivotal to the clinical management of CD. *T. cruzi* has a complex life cycle (Figure 1), and its ability to infect any nucleated cell complicates diagnosis [13]. The acute phase of infection is generally associated with parasites circulating throughout the bloodstream prior to migration into host organs. As such, infection is detectable via serology, haemoculture, PCR, or visualisation of the parasites in blood smears 5 and 14. Additionally, when symptomatic, the acute phase can manifest as signs specific to *T. cruzi* infection, such as unilateral periorbital swelling (known as Romana's sign), or ulcerative lesions at the site of inoculation (known as Chagoma). Alternatively and more commonly observed with the transmission of *T. cruzi* via modes other than through the insect vector, symptoms can be vague such as fatigue, fever, anorexia, diarrhoea, and vomiting [6].

The indeterminate phase of infection follows where parasites are not as readily seen circulating the bloodstream but rather sequester in host organs. In this phase, serology and/or histology are generally used for diagnosis because morphological changes of key organs are not yet detectable 6 and 15. Where CD is endemic, the choice among ELISA, indirect immunofluorescence (IIF), or indirect haemogluttination (IHA) for serological testing is based on availability 6, 15 and 16. Although PCR is highly sensitive, it is not recommended as a diagnostic tool for CD owing to the small sample sizes that are analysed. Parasite
detection from organ biopsies are more successful during chronic infection when parasites sequester within organs; however, this technique is often impractical because biopsies are not always feasible [16]. The chronic phase of infection is characterised by the enlargement of key organs (i.e., splenomegaly, hepatomegaly, and cardiomegaly), where detection of morphological changes, histology, and serology are used for diagnosis 6, 15 and 17. Additionally, there have been reported cases where parasite DNA is not detected in chronic infections, and much debate remains as to whether chronic damage associated with CD is the direct result of the parasitic infection or the subsequent effect of a host autoimmune response 6 and 18.

**Limitations of detection methods**

There are clear limitations with the specificity and sensitivity of current serological techniques used for diagnosing *T. cruzi* infection. Additionally, the absence of a ‘gold standard’ test that reliably and consistently detects the presence of a *T. cruzi* infection makes the evaluation of current methods difficult [19]. Crossreactivity between *T. cruzi* and *Leishmania* spp. is well documented and causes issues with detection 20 and 21. In Brazil, the sensitivity of ELISA kits was reported as 100%, but in Panama, assay sensitivity ranged between 75% and 100% [14]. In Brazil, these studies determined that with the inclusion of leishmaniasis cases, specificity ranged between 82.8% and 100%; however, when leishmaniasis cases were excluded, specificity ranged from 95.6% to 100%. Moreover, the false negatives and false positives associated with commercial kits used to confirm infection are a major concern to public health 6 and 16.
There is a definite need for more specific and sensitive techniques to diagnose CD, enabling the prompt treatment of those infected and the efficient evaluation of therapeutic intervention. However, organ tropism and the discovery that the most prevalent *T. cruzi* genotype present within the bloodstream can differ from the strain found sequestered within organs (Figure 2) further complicate diagnosis [22]. Additionally, not all *T. cruzi* strains are associated with an observable parasitaemia and the implications of undetected infections threaten the containment and clinical management of CD. Improvements of techniques used to diagnose any form of polyparasitism are required, with mixed infections likely to pose the biggest threat to public health and treatment success 23 and 24.

**T. cruzi strains and genetic diversity**

A panel of experts recently redefined *T. cruzi* into six discrete typing units (DTUs), TcI–TcVI, and a proposed seventh *T. cruzi* branch, referred to as Tcbat, based on genetic characteristics observed across strains [7]. Variability among strains and DTUs in terms of virulence, infectivity, tissue tropism (Box 1), progression of disease, and drug susceptibility, as well as particular geographical regions, virulence within different host species and transmission cycles within areas where CD is endemic has been well documented 7, 25 and 26. However, there is as yet no clear association between genetic variants of the parasite and these life history or epidemiological characteristics. Infectivity is highly variable, and in murine models infectivity of strains was found to be dependent on DTU and within DTUs, dependent on their area of isolation [27]. Preliminary studies found that in comparison to inoculation via intraperitoneal injection, infectivity of one representative of DTUs TcI and TcII via the oral route varied significantly with parasite genotype, number of parasites, inoculation volume, and developmental stages [28]; however,
with the highly heterogeneous nature of these genotypes, further study is required.

Additionally, there is evidence that particular strains that are poor at invading and infecting host cells display increased infectivity via the oral route [29], a route of infection that has become more significant in recent years [30]. Strains expressing high levels of gp90 isoforms, a metacyclic stage-specific molecule that negatively regulates the invasion process, are poor at invading and infecting host target cells. However, additional studies found that when gp90 isoforms that are susceptible to peptic digestion are ingested [31] and come into contact with gastric juices, these strains become highly invasive to host target cells and cause oral outbreaks, which are linked to more severe disease 21 and 32. More alarmingly, oral transmission is thought to be more efficient than the natural mode of transmission, and with strains that are linked to orally transmitted cases showing a high level heterogeneity, further study is required [33].

Box 1. The significance of tissue tropism/favourable niches

Trypanosomes display an apparent preference to infect and sequester within particular host organs. Whether this is true tissue tropism or whether it is simply the outcome of differential survival of the parasite in different tissues that it infects by chance is an open question. Although *Trypanosoma cruzi* has the capacity to infect any nucleated cell, particular cell types, including cardiac and skeletal muscle, enteric nerves, and adipocytes, are commonly associated with disease 26 and 59, and the question must be asked: what is it about these particular environments that encourage and support infection with particular strains of *T. cruzi*? Across *Leishmania* species, vast differences in the manifestation of disease are observed because of species-specific preferences for niches present within varying host organs [60]. In the case of Australian trypanosomes, two clades isolated from native wildlife displayed differential tissue distribution within infected hosts [61]. Additionally, two different genotypes within one of these clades showed varying abilities to infect rat skeletal muscle (L6), kidney epithelial cells (Vero), human ileocecal adenocarcinoma cells (HCT8), and a human monocytic cell line (THP1) that was differentiated into a macrophage-like cell (A. Botero et al., unpublished). Within these *in vitro* studies a clear preference for the infection of kidney epithelial cells was observed. The *in vivo* outcome of differing tissue tropism between strains of *T. cruzi* is the observation of variation in disease progression 60, 62 and 63. In a murine model using the Co11.7G2 clone and/or the JG strain of *T. cruzi*, organ tropism varied between single and mixed infections [63]; similarly, humans suffering from chronic infection had differential tropism [22]. Tissue tropism or favourable niches have the potential to have a significant effect on disease progression and the success of therapeutic interventions, yet its direct effects remain largely unknown.
Resistance is an issue with the current treatment options for CD, Nifurtimox and Benznidazole, and TcI strains are more resistant to antitrypanosomal agents in mice than TcII and TcV strains [34]. Interestingly, Benznidazole resistance has been observed among natural populations of *T. cruzi* in the Amazon without prior exposure to the drug6 and 35; however, further information on drug resistance and susceptibilities is required as there is no exact correlation across DTUs.

Within a number of geographical regions in South America, DTUs are linked to different transmission cycles, with TcI strains predominantly observed in sylvatic cycles and TcII strains prevailing in domestic cycles [36]. Genetic variability observed across DTUs and the ecosystem in which *T. cruzi* circulates within and outside the human host are key limitations to the treatment of CD and an impediment to drug discovery. Although the parasite genotype can affect drug susceptibilities and mixed infections can affect the success of drug interventions, many studies fail to consider the effect of parasite genotype in drug trials. Furthermore, the potential effect that host species has on the expression of parasite virulence and the outcome of infection in both laboratory induced and naturally occurring *T. cruzi* infection studies has been largely overlooked and requires further study.

**Limitations of drug treatments and clinical management of Chagas disease**

Neither Benznidazole nor Nifurtimox are effective at completely curing CD. Both compounds have shown promise by slowing disease progression and improving the health of infected individuals [37], with the efficacy of Benznidazole currently being reviewed in the Benznidazole Evaluation of Interrupting Trypanosomiasis (BENEFIT)[38]. However,
because they are expensive, have problems with resistance, require lengthy treatments, and are poorly tolerated by patients, both compounds fall short of the WHO recommendations for an ideal treatment [39]. Furthermore, drug treatments are still not always readily accessible, and the social stigma attached to the disease means that many governments struggle to acknowledge an incidence of CD, where compound toxicity and lengthy treatment regimens ensure that prophylactic treatments in those vulnerable to infection are unmanageable.

These issues have led to research focusing on drug development; yet, to date, a curative agent has not been generated, with few compounds progressing into clinical trials [40]. The question must be asked: is this due to the difficulties involved with determining drug efficacy, or is the complexity of the parasite–host relationship a major limiting factor in effective treatment?

*T. cruzi* has demonstrated an innate ability to adapt, evading the host immune system. Even when considering host and parasite genotype, there is no clear understanding of why infection with the same strain of *T. cruzi* can result in such variable disease progression and treatment success [26]. Although it is difficult to correlate chronic phase cure rates to individual parasite strains, failure of treatments during acute infection in a clinical study might have resulted from strain-dependent variation in drug sensitivity [34]. Thus, a better understanding of parasite genetic diversity and the effects of polyparasitism is crucial to drug discovery.
The complexity of polyparasitism

Polyparasitism has been widely reported among humans, vectors, and other parasite reservoirs 41 and 42. Areas where CD is endemic are associated with poverty and malnutrition and known to harbour multiple strains of *T. cruzi* along with other opportunistic parasites 2 and 43. Coinfections with different strains of *T. cruzi* are of concern because they can affect host susceptibility to acquiring CD, can cause variability in disease progression following infection, and can alter host responsiveness to treatments 44 and 45 (Figure 2). Although concurrent infections appear to be the norm rather than an anomaly, the precise interactions between parasites within a mixed infection, either synergistic or competitive, remain largely unknown 44, 46, 47 and 48. Compared with single strain *T. cruzi* infections, mixed infections in BALB/c mice were able to trigger a response that attenuates the damage caused by inflammation and disease severity [49].

To date, there have been various investigations into the incidence of natural mixed infections involving different parasite species within areas where CD is endemic. A study of intestinal protozoan and helminth parasites in Colombia, for example, found that 10.4% of children under 10 years of age were infected with two parasite species, whereas 52.1% were infected with three or more species [50]. In the case of mixed infections with *T. cruzi* and various helminths, reports of decreased helminth-associated death rates and a decreased ability for those infected to clear helminth infection suggest that mixed infections can be mutually beneficial to the parasites, allowing an increased risk of transmission [51]. For instance, intracellular protozoans such as *T. cruzi* initiate a T helper 1 (Th1)-like immune response that results in the production of interleukin-12 (IL-12), interferon (IFN), interferon-γ (IFN-γ), and tumour necrosis factor-α (TNF-α), whereas helminths initiate a Th2-like response resulting in
IL-4, IL-5, and IL-10 production [52]. The presence of a Th2 immune response in wild type mice contributes to the persistence of *T. cruzi* and exacerbates disease symptoms [53]. Additionally, induction of a Th1 response protected the susceptible BALB/c mouse strain against the challenge of infection from virulent parasite strains, whereas the induction of a Th2 immune response resulted in higher parasitaemia and mortality [54].

When polyparasitism is defined more broadly, it can include coinfection with viral pathogens, for example, with HIV. *T. cruzi*–HIV concurrent infections present a risk to public health as a recrudescence of parasitaemia is often observed due to the immunosuppressive nature of HIV [55]. Further understanding of *T. cruzi*–HIV infections will be beneficial, considering the increased morbidity and mortality, and longer periods of drug treatment compared with HIV infection alone [56].

In a mixed infection involving multiple strains of *T. cruzi*, treatment may do more harm than good, with the potential for a drug to successfully target one strain but in doing so alter the competitive suppression of virulence in a mixed strain infection, resulting in a host succumbing to the effects of the remaining strain [57]. In other scenarios, competition among strains for resources within a host is observed with a mixed infection[44]. This is thought to affect growth, reproduction, survival rate of the parasite, and the health of the host with multi-clonal population studies proving that natural selection favours the more virulent strain 57 and 58. The study of artificial mixed infections has shown that drug efficacy differs compared with single infections [45], but the direct effect of mixed infections on drug efficacy within a clinical setting has yet to be fully elucidated.
Although it is desirable to have one drug able to target every DTU, the more we learn about the complexity of the parasite, the more unrealistic this seems. In addition, the ideal drug would not only need to show efficacy in clearing parasites from the bloodstream and organs in individuals suffering from the early stages of organ infiltration but also in individuals suffering from years of chronic damage. With different strains sequestering within varying organs, the reality is that combination therapies utilising drugs that exert their antitrypanosomal effects via differing modes of actions may hold the answer.

**Concluding remarks: the future for drug discovery**

Unfortunately, the overall complexity of the relationship between *T. cruzi* and its human host is a limitation to drug discovery. There are understandable frustrations with the slow pace of advances with drug discovery and the complications associated with determining compound efficacy and cure in a clinical environment. Adding to these hindrances is the fact that with every gain in knowledge, another question surfaces (Box 2).

To date, parasite–host interactions have largely been explored in experimental mono-infected, murine models. Unfortunately, the correlation between drug efficacy in mice and humans is not strong, and thus there is a clear need for other *in vivo* models for determining drug efficacy. Additionally, teasing apart the complex interactions between multiple strains of *T. cruzi* within an individual human host is critical given that polyparasitism has the potential to significantly affect the direction of drug discovery efforts.
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Box 2.

Outstanding questions

- Are we able to develop a better understanding of the variability observed across multiple strains of *Trypanosoma cruzi*?

- To further elucidate host–parasite and parasite–parasite interactions, should studies focus on mixed infections?

- How can we address the apparent disconnect between drug efficacy observed in rodent models and the lack of success in human clinical trials?

- Are there alternative models of study that may allow us to develop a better understanding of alternative transmission routes?

- Are there superior *in vivo* models (i.e., other than rodent models) available for determining drug efficacy that could provide an improved correlation to the success of therapeutic interventions in humans?

- Do we fully understand how immunosuppressive diseases such as HIV and cytomegalovirus affect disease progression and reactivation?
References


Dias, G.B. et al. (2013) Evolution of infection in mice inoculated by the oral route with different developmental forms of Trypanosoma cruzi I and II. Exp. Parasitol. 135, 511–517


Hoft, D.F. et al. (2000) Involvement of CD4+ Th1 cells in systemic immunity protective against primary and secondary challenges with Trypanosoma cruzi. Infect. Immun. 68, 197–204


Botero, A. et al. (2013) Trypanosomes genetic diversity, polyparasitism and the population decline of the critically endangered Australian marsupial, the brush tailed bettong or woylie (Bettongia penicillata). Int. J. Parasitol. Parasit. Wildl. 2, 77–89


Figure 1. The life cycle of *Trypanosoma cruzi*. The life cycle for *T. cruzi* is complex and involves an insect vector and a mammalian or non-mammalian host. Firstly, the triatomine insect takes up a blood meal (A) containing blood form trypomastigotes from an infected host. The parasites differentiate into epimastigotes (B) and enter the midgut of the triatomine (C) where they replicate via binary fission. In the hindgut, the parasites differentiate into infective metacyclic trypomastigotes, which are deposited in the faeces of a triatomine at the bite site (D). The metacyclic trypomastigotes enter the bloodstream of a host through the insect bite site or by the host accidentally rubbing contaminated faeces into the eye or abraded skin, and infect host cells (E), and subsequently differentiate into amastigotes (F), replicate via binary fission (G), differentiates back into trypomastigotes (H), and burst out of cells into the bloodstream (I) to either infect additional host cells (J) or be taken up in a blood meal by the insect vector (A).
Figure 2. The possible effect of polyparasitism on the clinical outcome of Chagas disease. The clinical outcome of Chagas disease is dependent on the course of infection and both the availability and subsequent success of drug treatment. The course of infection may differ depending on variability in infectivity and pathogenicity observed across Trypanosoma cruzi strains, and although most of the damage from CD is observed in the heart, the bloodstream, and the intestine, the extent to which damage occurs is both host- and strain-dependent. Furthermore, the outcome of competitive interactions may be influenced by variable drug sensitivity between various strains of T. cruzi and/or immune effector mechanisms, which may be compromised by concurrent infections with gastrointestinal helminths or the immunosuppressive effect of cytomegalovirus or HIV infection. Variable sensitivity to different drugs A (Nifurtimox) and B (Benznidazole) may be observed depending on the strain of T. cruzi, tissue distribution, acute or chronic infection, and degree of inflammatory response.