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COMMENTARY

# The Unfortunate Abundance of *Trypanosoma cruzi* in Naturally Infected Dogs and Monkeys Provides Unique Opportunities to Advance Solutions for Chagas Disease

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## Abstract

*Trypanosoma cruzi*, the protozoan parasite and cause of Chagas disease, is widely distributed in many vertebrate and triatomine species throughout North, Central, and South America. Variations in housing quality largely determines human infection risk in the Americas. However, the southern U.S. contains widespread, infected triatomine vectors and captive species and domesticated animals with active *T. cruzi* infection or at high risk of becoming infected and developing Chagas disease. There is a critical need for better detection and intervention strategies, principally focused on human infection throughout the Americas, but mainly in the U.S., for high-value dogs employed in government and other work. In addition to this economic impact, the concentration of largely unavoidable *T. cruzi* infections in U.S. dogs provides an incomparable opportunity to answer questions related to *T. cruzi* infection and Chagas disease that are impossible or unethical to address in humans. As the course of *T. cruzi* infection and Chagas disease, the immune response to infection, and the response to therapeutics are highly similar across the range of mammalian host species, information obtained from studies in other species can directly inform researchers on how to best detect, manage, and treat *T. cruzi* infection and Chagas disease in humans.

**Key words:** Trypanosoma, Pathogen, Dog, Host, Triatome, Vector

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*Trypanosoma cruzi* is a unicellular eukaryote that infects a wide range of vertebrates, including humans, and is transmitted by multiple species of triatomine insects. In vertebrates (primarily mammals), *T. cruzi* replicates as amastigote forms in the cytoplasm of a variety of host cell types, emerging as trypomastigotes following 3–6 days of replication and differentiation, and killing the host cell in the process. Trypomastigotes can invade new host cells and repeat the intracellular replication cycle or be acquired in a bloodmeal taken by triatomines. Therein, trypomastigotes convert to epimastigotes, which undergo a replication and differentiation phase entirely in the insect gut. Unlike most other arthropod-borne pathogens *T. cruzi* is not transmitted into hosts as part of the blood-feeding process,

but rather is released in the insect feces, frequently during or following a bloodmeal.

## INFECTION DYNAMICS

These life cycle properties have significant and often unappreciated implications for transmission dynamics and infection risks. The risk of *T. cruzi* infection is driven by the frequency and intensity of insect-to-vertebrate interactions. As nocturnal blood feeders, triatomines are attracted to sites where hosts are accessible and plentiful. In the natural environments, these include nests, burrows, and other animal resting sites. In human-modified landscapes, animals in dense collections in outdoor settings (kenneled dogs and zoo animals are two good examples) or indoors in housing that provides ready access (permanently open windows or doors) or nesting habitats (porous building materials) for insects are at higher risk. The extremely rare reports of insect-vectored human infections in the southern U.S., despite a locally high prevalence of infection (>50% in some cases) in both animals and triatomines, provides evidence that insect-vectored transmission to humans can be prevented nearly entirely by housing that largely averts triatomine invasion.

The risk of infection also correlates with animal behaviors. Animal grooming habits, including licking fur or insect bite sites, or directly ingesting bugs provide an increased opportunity for infection. In the case of ingesting infected bugs, these infections can originate with high infection doses and a greater risk of symptomatic acute infections. For humans, infection via insects is inadvertent. Specifically, insect-deposited feces may be rubbed into a mucosal site or break in the skin or otherwise unintentionally ingested in contaminated foods or drinks and are therefore much less common and frequently acutely asymptomatic. Infections can also result from maternal-to-fetal parasite transfer, although at a low frequency, or as for other blood-borne pathogens, via blood transfusion or transplantation.

*T. cruzi* is most often a life-long infection in vertebrates. However, the number of blood-circulating parasites fluctuates depending on the phase of the infection (acute, post-acute, or chronic) and is also highly variable between hosts [1]. Indeed, the often very low parasite levels achieved and maintained in immune competent hosts pose the major obstacle to dependable diagnosis of the infection, while also reducing the potential for transmission [2]. Thus, infected hosts are not all equally infectious for triatomines [3].

Although zoonotic, *T. cruzi* infection is an indirect zoonosis and the threat that infected non-human species pose for humans depends on the living conditions. In areas of Latin America where the prevalence of human *T. cruzi* infection is highest and housing quality is low, other animals, including dogs or guinea pigs, are a critical factor in the incidence of human infections. Dogs (frequently infected as a result of bug encounters outside the house) provide an infection source for bugs residing within the house, which may then be transmitted to humans. Accordingly, removing dogs from this setting greatly reduces human infections, even if insects are present [4,5]. In theory, infected animals (wild or domestic) are a direct infection risk to humans, but such transmission requires blood-to-blood contact and has not been reported. Indeed, surveys of hunters with potential exposure to infected animals revealed no evidence of such transmission [6]. Thus, the cohabitation of humans, triatomines, and animals with behaviors that facilitate high infection exposure, such as via ingestion of bugs, puts humans at high infection risk. The simple presence of infected dogs in or around a home from which triatomines are excluded, such as most structures in the U.S., does not pose a significant human infection risk.

A final set of points with respect to transmission is the broad overlap among parasite lineages that circulate in triatomines, humans, and other animals. While there may be parasite lineages that are only infrequently found in humans [7], this is more likely the result of a lack of opportunity (e.g., humans that have not encroached on an infection cycle) rather than species-restricted parasite lineages. Thus, there are no dog- or human-specific isolates that are behaviorally or genetically adapted to specific vertebrate species and unable to infect others. Finally, the breadth of vertebrate and triatomine species capable of harboring *T. cruzi* infection make it abundantly clear that this is a parasite that will not be eradicated. The impact of *T. cruzi* on humans and non-human animals can be drastically minimized.

## INFECTION CONTROL AND DISEASE DEVELOPMENT

Although differing in transmission dynamics, *T. cruzi* infection and the host response to *T. cruzi* infection are highly similar in humans and other mammals. In all species there is a broad range of infection and disease levels, as is the expected case for nearly all infectious agents. However, infections by *T. cruzi* are controlled but not fully eliminated in most cases, and the immune mechanisms involved in that control are not only similar in type but are also broadly overlapping in terms of the antigen specificity across species [see [8] and [9] for B and T cell responses, respectively]. The response to and effectiveness of anti-*T. cruzi* drugs in all animal species is nearly identical [8,10]. The tissues and host cell types that *T. cruzi* infects, then ultimately persist within (predominantly muscle) are the same and the site, impact, and clinical outcomes of infection (again predominantly muscle), are strongly comparable across species.

It has been argued that the term, Chagas disease, should refer only to the pathology observed in humans [11]. But considering the clear similarities in nearly all aspects of this infection and disease in these species, this restriction seems unnecessary and perhaps even misleading.

As in all species, the acute phase of *T. cruzi* infection in humans can present with a relatively high parasitemia, which in most cases is immunologically controlled within 2-3 months post-infection with an accompanying greatly reduced parasite load. Initially, the persistent chronic infection is clinically silent based on standard health screening methods. However, the long-term perseverance of *T. cruzi* (primarily in muscle tissues and despite a sustained immune assault) ultimately results in tissue damage due to direct parasitization and the inflammatory response to that infection. The continuing cell death and fibrosis over time drives a process of tissue remodeling, including hypertrophy, arrhythmias, and other dysfunctions, and in some cases, organ failure [12]. In chronic disease, electrocardiographic alterations develop, such as bradyarrhythmias (i.e., sinus node disease, atrioventricular rhythm disturbances, right bundle branch block, and left anterior hemiblock) or tachyarrhythmias with ventricular tachycardia and sudden death the most severe outcomes. Structurally, myocardial changes include dilatation of cardiac chambers, impaired systolic function, and segmental alterations of motility, such as hypokinesia, akinesia, and aneurysms [13]. It is generally agreed that up to 30% of chronically infected subjects develop readily detectable clinical symptoms, with cardiomyopathy being the most common. Gastrointestinal disorders may also develop, and vary clinically from motility disorders to megaviscera with megacolon and megaesophagus [14].

*T. cruzi* infection has been reported in many species of wild and captive non-human primates (NHPs). *T. cruzi* manifests similarly in NHPs as in humans, with acute and chronic stages of infection. Likewise, only a proportion of infected NHPs go on to develop disease, which is characterized most commonly by heart failure with conduction abnormalities and/or myocardial damage and cardiomyopathy, and less commonly by gastrointestinal disorders [15-18]. Much of what is known about the clinical disease in NHPs is the result of experimental infections [19], but there are reports of fatal heart disease resulting from natural infection as well [20-22]. Even in those NHPs without clinical signs, microscopic examination of the hearts of infected NHPs consistently reveals areas of lymphoplasmacytic inflammation within the myocardium. While NHPs housed with outdoor exposure in endemic areas are subject to infection pressure from local triatomine vectors that also interact with wildlife reservoirs, the low and inconsistent parasitemia identified among naturally-infected NHPs suggests the importance as a source of infection to triatomines is very low [23,24].

Likewise, dogs infected with *T. cruzi* exhibit acute and chronic stages of infection and cardiac symptoms that are comparable to those in infected humans. Acute infection in dogs are associated with a mild non-specific illness characterized by lymphadenopathy, splenomegaly, and fever. Some dogs develop cardiac abnormalities that can range from mild and transient-to-severe and life-threatening, especially in young dogs [25-27]. This tendency may be related to the higher initial parasite load related to ingestion of infected bugs or bug feces. Chronically infected dogs are often apparently healthy without clinical signs. However, in-depth diagnostic testing frequently reveals pathologic changes, the majority of which are complex combinations of conduction abnormalities and arrhythmias detected on electrocardiography. Ambulatory electrocardiographic recordings (Holter monitoring) increase the likelihood of identifying arrhythmias [26]. Myocardial damage, characterized by heart enlargement and systolic dysfunction, and detected on echocardiography, is observed in both acute infections or end-stage chronic infections. As a biomarker for myocardial damage, cardiac troponin I concentrations are more likely to be markedly elevated in severe acute myocarditis but are low-to-mildly elevated in chronically infected dogs. The prognosis varies greatly with many dogs that remain subclinical for life. The presence of ventricular arrhythmias and right heart enlargement are associated with shorter survival, and ventricular arrhythmias increase the risk of sudden death [26].

## *T. CRUZI* INFECTION IN NON-HUMANS IS A CRUCIAL PROBLEM AND AN INCREDIBLE OPPORTUNITY

Dogs in diverse ecologic settings in the southern U.S. across the geographic range of triatomine vectors are infected with *T. cruzi*. Any dog with vector exposure is at risk, with a review of recent medical records showing no strong breed or sex predispositions among infected dogs [28]. Congregate animal settings, such as group kennels and shelters, may be particularly attractive to triatomines. For example, previously uninfected hunting dogs that were group-housed in kennels of central and south Texas had a 30.7% risk of acquiring *T. cruzi* infection within 1 year [29]. Additionally, the high-value U.S. government working dogs, which are often kept in outdoor kennel environments, have had continuous diagnoses of infection, including both military working dogs [30] and the border patrol and port of entry dogs, leading to fatal Chagas myocarditis [31]. Dogs at animal shelters across variable ecoregions in Texas have an overall *T. cruzi* infection prevalence of ~18%, which is statistically indistinguishable from the heartworm prevalence in the same dogs [32]. A similar level of infection was reported among shelter dogs in Louisiana [33]. Much like the situation with human Chagas disease, there is increasing recognition of the translocation of infected dogs, often from the southern to northern U.S., where the veterinary community may have minimal awareness of Chagas disease [34].

Naturally occurring *T. cruzi* infections in captive NHPs are a major problem for biomedical research [35]. While animals occasionally die due to clinical progression of the disease, the primary issue is that significant inflammatory and immunologic alterations associated with *T. cruzi* infections confound data interpretation if infected animals are unknowingly included in research studies. In an

effort to eliminate this issue, there have been improved screening assays developed over the last decade to identify animals infected with *T. cruzi*. These animals are often removed from the general population, which contributes to the ongoing shortage of NHPs available for biomedical research. However, considering the lack of evidence for horizontal or vertical transmission occurring in NHP breeding colonies [36], some facilities have used this opportunity to develop well-characterized groups of naturally and chronically *T. cruzi* infected animals for use in treatment and other studies [8,10].

While the primary focus for interventions to prevent Chagas disease should be firmly on human infection and disease, in particular early detection and prompt and effective treatment, the study of Chagas disease in non-human species merits much more support and would potentially pay huge benefits for both animal and human health. Unfortunately, research support and commercial development have largely failed to deliver for both humans and non-humans.

NHPs in research facilities and working dogs on Texas ranches, in both cases infected as a result of natural environmental exposure and despite vector intervention efforts, are incredibly valuable substitutes for humans for gaining a better understanding of *T. cruzi* infection and Chagas disease. The fact that NHPs in facilities in the southern U.S. infected as a result of environmental exposure have similar ranges of age, gender, genetic background, length of infection, and diversity of parasite lineages as human populations, makes NHPs an exceptional, indeed it can be argued, obligatory step in drug testing prior to human clinical trials. Such trials can be conducted ethically and at reasonable cost and would likely avoid the adverse events and death that accompanied the most recent human clinical trial involving Chagas disease [37]. Indeed, in the one case of drug testing in *T. cruzi*-infected NHPs, parasite-free animals with much reduced likelihood of developing clinical signs were returned to the breeding colonies following successful treatment with a new clinical candidate [10].

Similarly, environmentally infected dogs in the U.S. present an incomparable opportunity to address questions that are ethically or pragmatically impossible to address in humans, but which warrant attention. The presence of large groups of dogs under very high infection pressure was an opportunity to test prophylactic and new treatment protocols, and have provided additional evidence for spontaneous cure early in *T. cruzi* infection and testing of novel parasite detection methods [8,38]. Additional studies could shed light on the link between parasite load and disease development and the level of resistance to re-infection provided by a prior drug-cured infection. Such studies have been argued to be uninformative because they are not being conducted in humans. However, this argument ignores the similarities of *T. cruzi* infection in these different species, as well as the fact that such studies will hopefully never be conducted in humans.

*T. cruzi*-infected canines are not just great study targets but legitimate populations needing infection detection and treatment. Commercial and government working dogs are expensive to breed and train and have incredibly high value [>$60,000/dog] [39]. The loss of companion dogs due to undiagnosed and untreated *T. cruzi* infection is no less impactful. The U.S. government and veterinary diagnostic and pharmaceutical industries have a timely opportunity to invest in research and assist in the development of tools to prevent these losses. These interventions in canines need to be improved and employed, not because they will necessarily save human lives, but because canine lives deserve saving.

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**CONFLICTS OF INTEREST**

The authors declare no conflicts of interest.

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