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Review

Reactivation of Chagas Disease: Implications for Global Health

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Reactivation of Chagas Disease (CD) is a global public health issue. Reactivation of disease can affect the management of CD and its clinical outcome, adding pressure to global health systems because it exacerbates symptoms, leading to misdiagnosis and delays in the administration of correct treatments. Concurrent infections complicate the issue of reactivation, because there are various parasites and disease treatment regimens that are able to influence or suppress the immune system of the host, reactivating disease within infected individuals. The effect of delayed symptoms of chronic CD and the potential for disease reactivation are of great importance to nonendemic regions of the world, where knowledge about CD is lacking and the potential for vectorial transmission is not known.

The Global Significance of Chagas Disease

CD is a complex parasitic disease that is caused by infection with the protozoan *Trypanosoma cruzi*. The main identifiable symptoms of chronic disease progression are observed in the heart and organs of the gastrointestinal tract; however, the parasite has the potential to infect and cause damage within any host tissue [1]. CD is endemic to Latin America, but is increasingly found in other parts of the world, including countries previously considered free of disease, such as Japan and Australia (Figure 1). In Latin America and the Caribbean region, the burden of CD, as measured by disability-affected life years, is five times greater than that of malaria [2]. The natural disease progression for CD involves an acute phase that is followed by an asymptomatic indeterminate phase, where infection is present but chronic symptoms have not yet developed, and, in one-third of cases, a final chronic phase [3]. The acute phase of disease can result in death from virulent strains, although other factors, such as inoculum size and host genotype, age, and immune state, may also have a role [4,5]. Comparatively, however, the burden of disease caused by the chronic phase of infection is more detrimental to the public health systems of countries both where CD is endemic and those where it is nonendemic [6].

In countries considered nonendemic for CD, there is a general lack of familiarity with tropical parasitic infections, in particular the identification of their symptoms and disease progression, which is further complicated by the long indeterminate phase of infection, in which symptoms are not present. While numerous serological tests are often utilized to confirm diagnosis, the efficiency of such tests is largely dependent on the quality of the antigen used in the assay [5,7]. This often results in a poor rate of diagnosis and complications from further disease progression, because medical practitioners simply may not suspect a *T. cruzi* infection. In addition, a lack of preventative measures may have important biosecurity implications in nonendemic areas, particularly those areas that contain native vectors capable of transmitting

Trends

Reactivation of CD is a global health issue, and is of particular importance in areas nonendemic for CD, where health systems are unprepared for cases of reactivation due to the lack of experience of local clinicians and the lack of drugs.

With global migration already changing the epidemiology of CD, the identification of native vectors within areas nonendemic for CD that may support vectorial transmission is of particular concern.

Animal models have improved methods used for drug development and the identification of drug targets for efficacy studies. In a similar way, experimental models studying reactivation of disease may allow us to expand on our current knowledge of alterations to both disease progression and the clinical management of disease.

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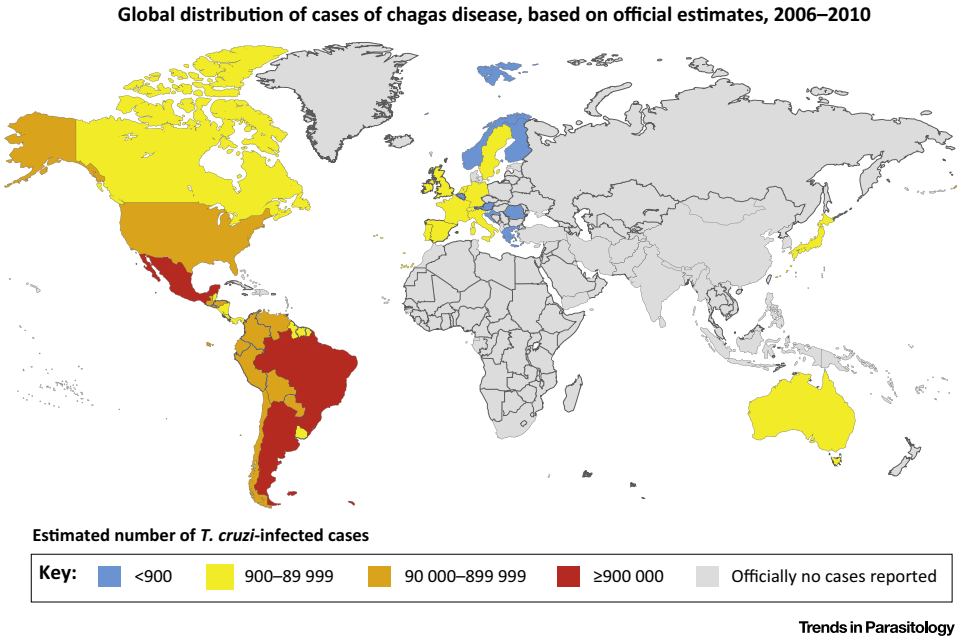

Figure 1. The Global Distribution of Cases of Chagas Disease (CD). Global migration has led to an increasing incidence of CD across the world within regions previously thought to be nonendemic for infection. The spread of CD throughout these areas may be problematic due to the presence of native vectors that may support transmission of infection. Data from [65].

infection [8]. Additionally, there is the potential for congenital transmission to occur within nonendemic areas and, currently, only Spain, Italy, and Switzerland carry out screening for congenital transmission of *T. cruzi* where at-risk populations reside [8].

The delayed onset of symptoms in chronically infected hosts within nonendemic areas for CD is concerning because symptoms can escalate rapidly and patient prognosis can decline [9]. The impact of chronic disease also extends to an increasing pressure on public health systems to cope with chronically ill patients and an increase in organ transplant requirements [10]. Also of growing concern are the potential implications that reactivation of disease may have in areas without preventative measures, such as blood screening and monitoring of infected patients.

Reactivation of CD

Reactivation of disease is the recurrence of acute symptoms that occurs when the immune system of a chronically infected host is interrupted or suppressed, resulting in the reduced ability of the host to control the *T. cruzi* infection [11,12]. Reactivation of disease is commonly linked to immunosuppressive treatments that are administered in the lead up to organ transplants [13], which are often required as a result of CD. In addition, corticosteroid therapies that are administered for immunological conditions, such as the autoimmune disease lupus [14], and chemotherapeutic treatments also have the potential to reactivate CD [15]. Historically, several cases have been reported during treatment of patients with cancer, where reactivation of CD has occurred resulting in fatality [15,16]. This has led to the recommendation that patients with positive *T. cruzi* serology be closely monitored during chemotherapy.

Polyparasitism (i.e., concurrent infections with HIV or other species or strains of parasites) [17] has also been associated with reactivation [14]. Polyparasitism is ubiquitous [18,19] and has the

potential to complicate the natural disease progression of CD due to parasite–parasite interactions [18]. *Trypanosoma cruzi* isolates differ in terms of their virulence, infectivity, tissue tropism, progression of disease, and drug susceptibility [20]. When compared with single *T. cruzi* infections, mixed *T. cruzi* infections are known to result in significant differences in disease progression, organ tropism, and drug susceptibility, which can all affect the clinical management of disease [20]. In areas known to be endemic for CD, various other opportunistic parasites are known to coexist [2,21–24], highlighting the importance for understanding interactions between parasite species within the host. In studies using *T. cruzi* and the mouse hepatitis virus type 3 (MHV-3) as concurrent infections, severe immunosuppression was observed compared with single infections with either parasite [25]. Various case studies involving reactivation of CD have been reported [13,26–28]; however, the effect of concurrent infections on reactivation of CD remains a relatively under-researched topic.

Concurrent infections may increase the risk of reactivation of CD because of an immunosuppressive effect. During the acute phase of a *T. cruzi* infection, *in vitro* observations of lower interleukin-2 production and *in vivo* observations of a lower humoral response against specific and nonspecific parasite antigens are characteristic of an immunosuppressive response [29]. If, for example, an infected host experiences a secondary exposure to *T. cruzi*, once disease has already had the chance to develop, there is the potential for the immunosuppressive response that occurs following *T. cruzi* infection to take place and, thus, in this case, the potential for reactivation of disease. Immunosuppression may also occur through concurrent infections with *T. cruzi* and other parasites. The main immunosuppressive infection associated with CD is HIV, a disease with global significance. Indeed, there are cases where reactivation of CD has led to diagnosis of HIV/AIDS and reactivation has even been suggested as a potential diagnostic marker for AIDS [30]. In addition to a link between CD reactivation and HIV infection, several case studies of CD reactivation have shown the presence of other parasites, such as cytomegalovirus, hepatitis, *Cryptococcus*, and *Mycobacterium*, within the host [31].

Timeline of Reactivation

Following the acute phase, if death has not occurred, there is the potential for reactivation within the various phases of disease that may follow (Figure 2, Key Figure). The indeterminate phase is perhaps the least-understood stage of disease progression, with the factors determining whether a host proceeds onto the digestive or cardiac chronic forms of CD, or maintains a permanent indeterminate form, largely unknown. Of all the stages of disease, infected individuals within the indeterminate phase of infection are at the greatest risk from reactivation, because, as a result of their lack of symptoms, there may be no preventative measures in place to monitor disease. Whether the disease progresses into one, or a combination of the two forms of chronic disease (cardiac and digestive) appears to be influenced by genetic factors of both host and parasite [32]. Individuals with the cardiac form of chronic CD are at risk of reactivation from immunosuppression due to treatment regimens or infections, with case studies predominantly linking reactivation to organ transplants [28]. While there is no evidence to suggest it cannot occur, we were not able to find any case studies linking the digestive form of CD to reactivation. However, we know that significant genetic diversity occurs across and within each of the discrete typing units into which *T. cruzi* is classified, and that this genetic diversity has been correlated to parasite pathogenicity as well as resistance or varying levels of susceptibility to drug treatments [33]. As such, more research into isolate diversity is required to determine the effect of this factor on the potential for disease reactivation to occur and assess the propensity of isolates that cause the digestive form of disease to reactivate.

Diagnosis of Reactivation

Reactivation of CD is most commonly diagnosed by the recrudescence of parasitemia; however, in some cases, parasitemia is only detectable months after other symptoms, such as rapid

Key Figure

A Timeline of Reactivation

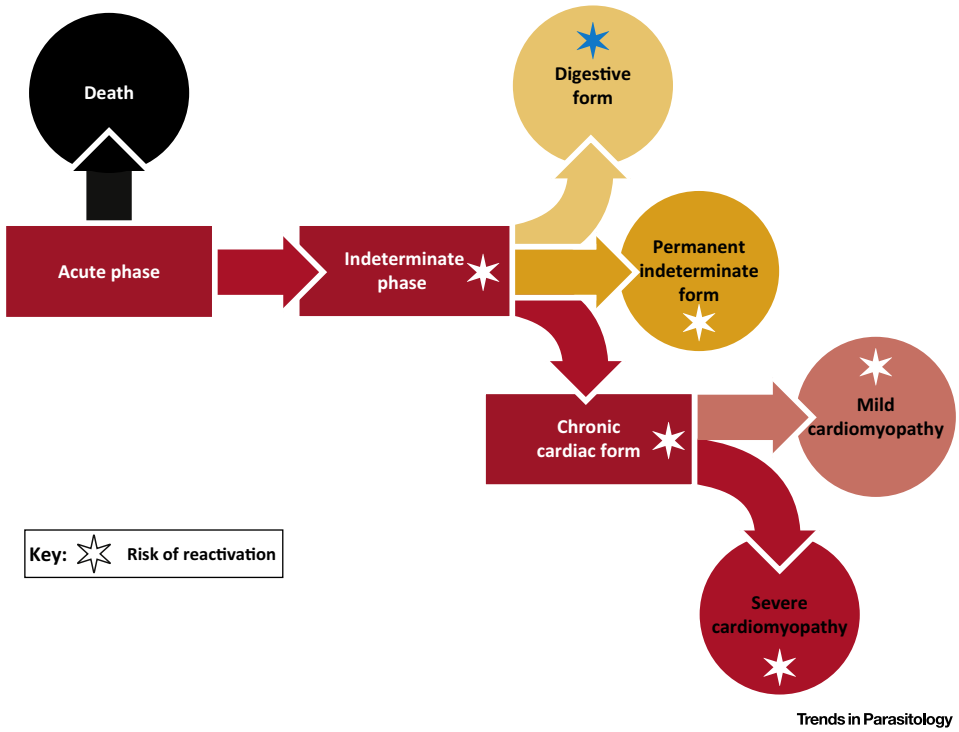

Figure 2. Upon infection with *Trypanosoma cruzi*, an acute phase develops and presents in one of three forms: as an asymptomatic infection; as a vague infection with seemingly harmless symptoms that go undetected by the host before subsiding; or as a severe acute infection that may result in death. In the event of a nonfatal acute infection, symptoms subside and the host enters into an asymptomatic indeterminate phase in which the parasite remains present but dormant within host organs. Disease can remain permanently within this indeterminate phase, with individuals remaining asymptomatic and living for years without suspecting infection [66]. Alternatively, disease can progress into chronic Chagas disease (CD) and take on either the cardiac or digestive form, or, in some instances, a combination of both [66]. Although the direct cause is not well understood, cardiac CD can range from mild to severe cardiomyopathy [67] and the digestive form of disease may take the form of dilated digestive organs, causing digestive issues [68]. Reactivation has been observed in both the indeterminate and chronic cardiac forms of disease, although not, as far as we are aware, in the digestive form (as indicated by the blue star).

weight loss, malaise, myocarditis, and neurological manifestations, have become apparent [30]. Due to the exacerbation of symptoms, a correct and prompt diagnosis is essential to survival, because patient health can deteriorate rapidly and death can arise from incorrect treatments or lack of action. Symptoms of myocarditis and neurological manifestations can take the form of slowed pulse, cardiac rhythm disturbances [28], confusion, nausea, seizures, stupor, and, in extreme cases, coma and death [14]. Myocarditis can be observed upon endomyocardial biopsy and neurological manifestations can be observed using magnetic resonance imaging, as well as by microscopically examining patient cerebrospinal fluid (CSF) [3,34]. Unfortunately, both the examination of CSF and myocardial biopsy are invasive and not available in all cases. Additionally, both clinically and radiographically, neurological manifestations of disease reactivation within HIV-infected individuals can present as toxoplasmosis in the form of tumor-like cerebral lesions [30], leading to misdiagnosis and delays in drug administration [35]. In cases where organ transplant has occurred, symptoms of reactivation must be differentiated from

transplant rejection to avoid misdiagnosis and mistreatment [36]. Painful skin plaques that are rarely seen in acute infections have also been observed following reactivation of disease [3]. In these situations, diagnosis can be aided by microscopy, identifying trypomastigotes in biopsies of the affected lesions [13]. By exacerbating natural disease progression, reactivation of disease further complicates the clinical management of CD.

Treatment of Reactivation

Drug efficacy studies are complicated by the toxicity of both benznidazole and nifurtimox, which leads to severe adverse effects and poor patient compliance [37]. Additionally, both drugs are recognized for their curative effects during acute infection, although their efficacy against chronic infections is still being examined [38]. Drug efficacy with a mono *T. cruzi* infection varies on a case-by-case basis and is thought to be dependent on the genetic diversity of the parasite and the host [39,40]. In the case of disease reactivation, drug efficacy is difficult to determine due to delays in the promptness of treatment and the inconsistent state of the health of the host [13,14]. Cases involving reactivation of disease following heart transplantation showed that allopurinol alone and in combination with conventional treatment for heart failure, was able to reduce symptoms associated with reactivation [41]. The fact that heart failure treatments were successfully used in conjunction with allopurinol is encouraging, due to the known cardiac issues associated with CD [42,43].

While there are reports of drug efficacy in reactivation scenarios involving organ transplantation, reactivation cases involving concurrent infections are not well documented. Patients co-infected with HIV/AIDS often show increased parasitemia during reactivation and, thus, while there is no officially recommended method of treatment for cases of reactivation, drug administration is often advised in patients who show clinical symptoms and no contraindications [44]. Even with the presence of contraindications, such as pregnancy, there have been case studies where vertical transmission of *T. cruzi* infection has been avoided and this is thought to be due to the administration of drugs, on a compassionate basis, after reactivation of disease [45]. However, due to the exacerbation of disease symptoms, there is not always a favorable response to drug treatment, and concurrent infections or other medical conditions may further complicate treatment regimens [46].

Implications for Nonendemic Regions

Diagnosis and Treatment

In nonendemic areas, public health systems appear unprepared for the possibility of reactivation of CD and the presentation of a delayed onset of symptoms as a result of chronic CD. Screening assays lack the specificity to guarantee clinical diagnosis and general practitioners within nonendemic regions typically lack the knowledge, experience, and ability to identify CD, let alone the exacerbated symptoms characteristic of chronic or reactivated CD, where a timely diagnosis is critical. Clinicians in endemic areas can also be mistaken; therefore, patient history is of utmost importance to identify previous travel or habitation within endemic areas. In 2010, a Brazilian man who had lived in Japan for 7 years died suddenly and unexpectedly from what was determined upon autopsy to be chronic CD [9]. More recently, in 2013, a woman was found to have complications from chronic CD 16 years after migrating to Japan [43]. Case studies such as these reiterate the importance of pathologists understanding the potential involvement of chronic CD in sudden and unexpected deaths, in addition to the importance of monitoring patients from areas known to be endemic for CD.

In cases where chronic disease is diagnosed or where reactivation occurs, nifurtimox and benznidazole, the drugs used for treatment of CD, are not registered in nonendemic countries. For example, in some countries, such as Italy, there is no official source for the drugs, while in

others, such as Australia, New Zealand, Spain, Portugal, and France, drugs are only available through governmental sources or special access schemes [8,47,48]. With patient prognosis relying so heavily on prompt drug intervention, direct access is more preferable.

Biosecurity

In regions close to centers of endemism, such as North America, CD has frequently been reported. Historically, however, the absence of an insect vector that was able to complete the life cycle, and living conditions that do not support close interactions between humans and vectors [49], meant that, while transmission was possible via other modes, it was improbable via the natural life cycle of the parasite. In recent years, there has been an increasing number of reports of CD in regions far removed from Latin America, and with the presence of vectors potentially suitable for *T. cruzi* transmission, reactivation of CD poses a risk in these areas.

Ninety eight cases of CD were reported in New Zealand in 2006 [8], 1500 cases in Japan in 2008 [50], 5000 cases in Canada in 2010 [50], and, more recently, Australia reported 1928 cases in 2011 [8,50]. There have been 138 triatomine species described throughout the world, with approximately half of these shown to be vectors for *T. cruzi*. Known vectors for the transmission of *T. cruzi* have been found in increasing numbers in North America [51]. Additionally, triatomine species have been identified in Africa (*Triatoma rubrofasciata*) [52], South-East Asia (seven species of *Triatoma*) [52], India (six species of *Linshcosteus*) [53], Vietnam (two species of *Triatoma*) [54,55], and within northern parts of Australia (*Triatoma leopolodi*) [17]. There is also the potential for the presence of additional vectors within areas nonendemic for CD that have not yet been discovered. All of these countries fall outside areas previously known to be endemic for CD and the reactivation of disease in the presence of a potential vector for transmission is a public health concern. Given that most clinicians in nonendemic regions are unfamiliar with symptoms of CD due to its association with developing countries, delays in treatment might enhance the risk of transmission to a suitable vector. Alarmingly, recent studies have shown that the common bed bug (*Cimex lectularius*), which is a global health problem, has the ability to act as a competent vector for the transmission of *T. cruzi* infections to naïve murine hosts [56]. Thus, the potential for other nontriatomid vectors in nonendemic regions needs to be considered.

Some, but not all, nonendemic countries have policies in place that help to identify migrants infected with *T. cruzi*. Spain has the highest number of cases of CD outside of North and South America [57] and, as such, antibody testing of all migrants is carried out [43]. In the UK, France, Spain, and Switzerland, blood donors at risk of being infected are tested before donations [8]. In Australia and New Zealand, donors are screened using a questionnaire before each donation and, while there are limitations on blood-donor eligibility based on the donor's country of origin, at-risk individuals are not tested before donating [8]. In Australia, migrants from endemic areas of the world are not tested for CD; neither is there any ongoing monitoring of the health of migrants from Latin America or children born of a mother migrating from Latin America [8]. Therefore, the exact impact of CD in countries such as Australia remains largely unknown [8]. Although those travelling to areas of the world where CD is endemic may be aware of preventative measures to protect against vector-borne disease transmission, upon returning home they may be complacent when suffering the vague symptoms associated with CD. Additionally, once they return home, few countries have follow-up monitoring programs for travellers who have entered regions where CD is endemic, unless they seek out medical advice.

Models of Study

In mixed or concurrent infections where an increased burden on the host exists [58], the effect of reactivation is not well known and, thus, requires further research. Given the difficulties associated with the confirmation of mixed infections, case studies of reactivation in this situation are

rare. Laboratory models may provide a way forward. Experimental CD models are primarily murine and used for studies on drug development, immune regulation and pathogenesis. They act as a crucial intermediate step between identifying *in vitro* drugs with efficacious pharmacological profiles and clinical trials. Murine models also enable us to predict what will happen in humans [59–63] and, thus, may help us to better understand the impact of reactivation and concurrent infections.

One of the larger issues with reactivation is that the severity of symptoms and the effect on the host can be unpredictable. *Trypanosoma cruzi* infections in general have a high degree of variability in terms of their overall effect on the host, and this is thought to be due to both host and parasite factors [62]. Under the same experimental conditions and using the same inoculation route, different isolates often display different biological behavior [20]. The diversity observed across different isolates may be of benefit to studying the different aspects of disease progression within a host. Isolates that previously have not attracted much attention due to their lack of observable parasitemia *in vivo* may be used to study disease reactivation with the introduction of immunosuppressive drugs, such as cyclophosphamide, or even immunosuppressive pathogens,such as HMV-3, cytomegalovirus, and HIV. In this way, the development of animal models able to assess reactivation of disease may elucidate key information in terms of the infection dynamics within a host under immunosuppressive pressures, thus building on our current understanding of this phenomenon. From these models, we may be able to better predict the outcome of reactivation of CD in humans, assisting with the development of specific treatment guidelines.

There is the possibility that the propensity for disease reactivation may differ among isolates, although this has not been well studied. In one study of disease reactivation within patients who were HIV positive, no correlation was found between reactivation and any particular *T. cruzi* genotype [64]. However, given the effect that genetic diversity has on the severity of disease progression and drug efficacy [1,62], we believe that the genetic diversity of *T. cruzi* has the potential to affect reactivation of disease by altering the likelihood of reactivation occurring and the severity of disease reactivation observed.

Outstanding Questions

Is drug efficacy affected by reactivation of disease and the exacerbation of symptoms?

Can polyparasitism alter disease progression, drug efficacy, and survival rates in cases of disease reactivation?

Does parasite diversity affect the way in which disease reactivation occurs?

Are there effective laboratory models for studying the reactivation of disease?

Can strains with lower virulence that have previously been difficult to study, be useful for studying disease reactivation?

Concluding Remarks

The lack of awareness to recognize and the lack of knowledge to suspect a diagnosis of CD are issues of great importance to global health, due to the potential implications of reactivation and the indeterminate phase of disease. The increasing movement of infected individuals into regions previously thought to be nonendemic brings these issues to light, and is further complicated by the presence of potential vectors in these regions. More research into the implications of reactivation is needed (Outstanding Questions Box) to enable us to build on our understanding of disease progression and how it can be altered by immunosuppression and concurrent infections.

Acknowledgments

We would like to thank Mark Preston for the production of the images used within this publication.

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