Chagas Disease, Need and Progress Made in Implementing Donor Blood Screening in the United States.

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Background: Chagas disease is a parasitic infection caused by *Trypanosoma cruzi* affecting 11 million people worldwide. The condition can be acquired via exposure of a wound or mucosa to infected feces from a reduviid bug, blood transfusion or congenitally. It is estimated that there are between 50,000-100,000 infected individuals in the United States.

Methods: This is a narrative review of medical and veterinary literature published in peer reviewed journals in either English or Spanish language. The specific aims of this review are to evaluate the risks for acquiring Chagas disease, needs, options, and associated cost for the implementation of a blood donor screening program for *T. cruzi* in the United States.

Results: There have been fifteen documented cases of acute Chagas disease in the United States, ten of which were associated with blood product transfusions or solid organ transplants. The FDA approved a *T. cruzi* ELISA test for blood and solid organ donor screening in the US but has not approved any confirmatory test to this date.

Conclusions: The availability of a single screening test for *T. cruzi* allows blood banks to screen for *T. cruzi* without giving them the ability to re-introduce false-positive donors into the donor pool. Education of the general public as well as the medical community is needed as Chagas disease is rarely seen in the US.

TABLE OF CONTENTS

I

INTRODUCTION

Chagas disease or American trypanosomiasis is a parasitic infection caused by *Trypanosoma cruzi*, affecting millions of people in the Americas. The condition was first described in Brazil by Dr. Carlos Chagas in 1909. It is estimated that there are 11 million people infected with *T. cruzi* in Central and South America [1], where it is associated with poor living conditions in rural areas. *T. cruzi* is primarily transmitted to humans by infected Reduviidae family bugs (cone nosed or kissing bug; see Apendix-1). The parasite can also be acquired by consumption of infected food, blood transfusion, organ transplant, congenital transmission, or laboratory accidents. [2]

Individuals may acquire the infection during their childhood and not be aware they have chronic Chagas disease as the condition may remain asymptomatic for several decades after its acute phase. The complications associated with chronic Chagas disease mostly involve the cardiac and gastrointestinal systems, and can be lethal as it can induce sudden cardiac death in as many as 30% of individuals in whom the parasite has invaded the heart. [3] Chronic Chagas disease can reactivate and present with signs and symptoms similar to those seen during the acute phase if the infected individual becomes immunosuppressed as is the case for organ transplant recipients and AIDS patients. [4]

Large population movements from Central and South America are expanding the distribution of Chagas disease to the northern half of the United States (US), Canada and European countries. The vector-borne parasitic infection is gaining interest in the US as an increasing number of people from *T. cruzi* endemic areas immigrate into the country

every year. There are rising concerns regarding American trypanosomiasis being an emerging infectious disease in the US with potentially infected blood being introduced into our blood banks.

Specific Aims

1. Evaluate the risk and common modes of transmission of *T. cruzi* in the US. There is a need to evaluate the risk of getting Chagas disease since it is considered enzootic in several mammals in the southern US. [5-8] Although the socio-economic conditions and living standards in the US are most likely not conducive to domestic infestation with the vector for *T. cruzi*, there have been several cases of transfusion and transplant associated *T. cruzi* infections reported in the US. [9-14] No cases of congenital Chagas disease have been reported in the US, and its prevalence in highly endemic areas has been cited to be between 1-10%. [15, 16]

2. Evaluate the need for a *T. cruzi* blood donor screening program in the US. Many Latin American immigrants may be infected with *T. cruzi* and not be aware of it due to the infection taking place during their childhood or they only developed mild non-specific signs and symptoms. As these individuals integrate into the local communities they may donate blood and introduce the risk of transfusion-transmitted *T. cruzi* into the US. [14] These individuals can also become organ donors and infect the recipients of the harvested organs with *T. cruzi*. [9, 10]

The recent introduction of a screening test for *T. cruzi* in the US makes it possible to start screening blood donors for antibodies against *T. cruzi*. However, implementation of a

nationwide screening program requires careful planning and training of health care providers and the community at large. An important issue is the identification of the population at risk and accurate estimation of the prevalence, given the fragmented data. These estimates will facilitate the evaluation of the different alternatives for such a program and the calculation of the test predictive values.

3. Perform a cost benefit analysis of the current strategies for implementing a blood screening program for *T. cruzi* in the US. Until the recent introduction of a screening test for *T. cruzi* in the US, it was not possible to make a realistic cost analysis of a blood screening program in the US. The added cost to the current blood screening process should help define the most beneficial strategies for the development of a blood screening program for *T. cruzi* in the US.

II

BACKGROUND AND SIGNIFICANCE

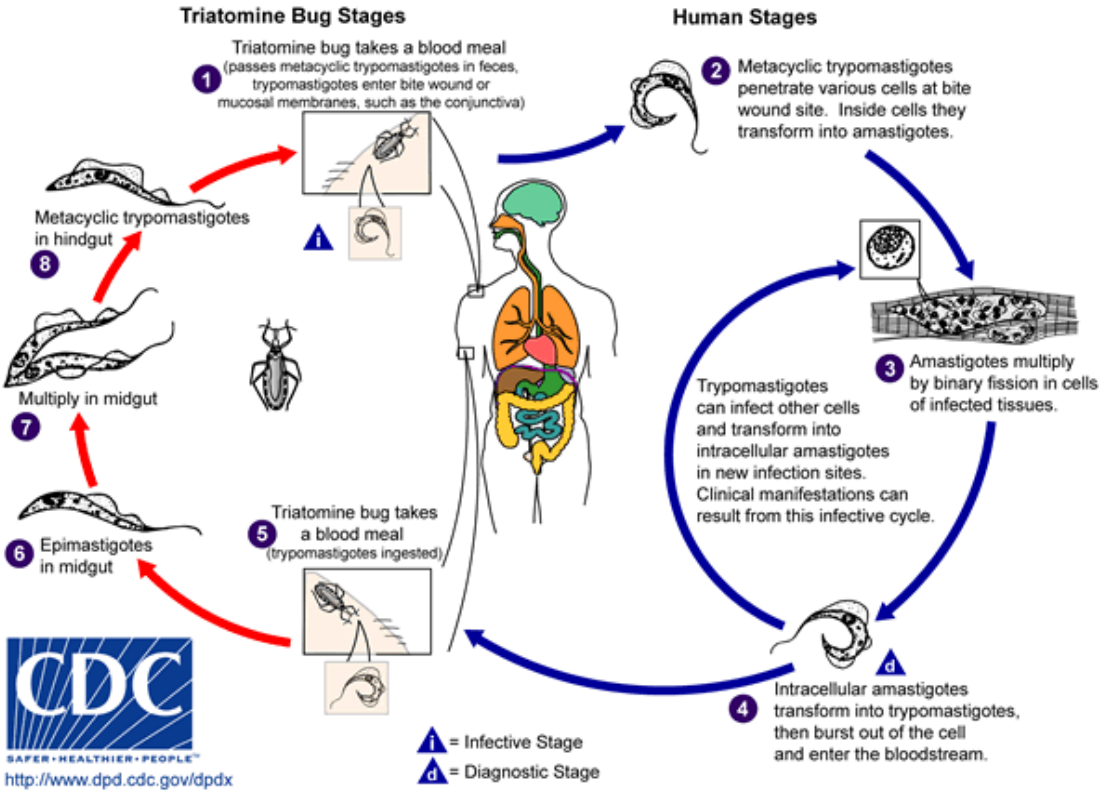
Chagas disease affects millions of individuals throughout Central and South America. The prevalence of this condition is estimated to be between 16–18 million with an incidence of 200,000 new cases per year. The population at risk of acquiring Chagas disease in Latin America is estimated to be 120 million. The primary form of infection in *T. cruzi* endemic areas is through exposure to its vector with blood transfusion associated infections as the second most important etiology. [17, 18] Large population movements into the US and Canada have introduced the risk for blood product transfusion-associated *T. cruzi* infection into these countries. [18]

Trypanosoma cruzi is a flagellated protozoan parasite of the order Kinetoplastida and is one of the two pathogenic *Trypanosoma* species affecting humans. The epimastigotes develop in the midgut of the reduviid bug and are transmitted through exposure to contaminated feces. There are primarily two different strains of *T. cruzi*: *T. cruzi* I is mostly found in the Amazon region and shows primarily a sylvatic life cycle while *T. cruzi* II is associated with the domestic infection cycle and is found throughout South and Central America. [18] In 1994, the World Health Organization (WHO) Special Programme for Research and Training in Tropical Diseases (TDR), the World Bank and the United Nations Development Program initiated a series of research initiatives known as the *T. cruzi* Genome Project. The goal of the project is to identify genetic characteristics that could be used to identify new pathways that could be targeted by new drugs, improved testing techniques and understanding of how the parasite interacts with the host. [18]

Humans are not the natural reservoir of *T. cruzi*. They are infected as they move into areas where the parasite is enzootic. They can come in contact with the reduviid as they work around the insect nest area or via domestic exposure as the vector moves into the houses as a result of the loss of its natural habitat. The reduviid bugs are nocturnal warm weather insects. Some species are capable of adapting to urban conditions and colonize domestic structures. Another important characteristic of this vector is its feeding habits. Reduviid bugs are obligatory blood feeders and are capable of fasting for up to 200 days between blood meals. Species of reduviid bugs are found between the parallels 45° S and 40° N and up to altitudes of 1500 meters above sea level. [18]

Domestic animals such as dogs and chickens as well as wild mammals can become infected with *T. cruzi* and become reservoirs.

Tripanosoma cruzi life cycle



An infected triatomine (reduviid) insect vector (or "kissing" bug) takes a blood meal and releases trypomastigotes in its feces near the site of the bite wound. Trypomastigotes enter the host through the wound or through intact mucosal membranes, such as the conjunctiva ***(1)***. Common triatomine vector species for trypanosomiasis belong to the genera *Triatoma*, *Rhodinius*, and *Panstrongylus*. Inside the host, the trypomastigotes invade cells, where they differentiate into intracellular amastigotes ***(2)***. The amastigotes multiply by binary fission ***(3)*** and differentiate into trypomastigotes, and then are released into the circulation as bloodstream trypomastigotes ***(4)***. Trypomastigotes infect cells from a variety of tissues and transform into intracellular amastigotes in new infection sites. Clinical manifestations can result from this infective cycle. The bloodstream trypomastigotes do not replicate (different from the African trypanosomes). Replication resumes only when the parasites enter another cell or are ingested by another vector. The "kissing" bug becomes infected by feeding on human or animal blood that contains circulating parasites ***(5)***. The ingested trypomastigotes transform into epimastigotes in the vector's midgut ***(6)***. The parasites multiply and differentiate in the midgut ***(7)*** and differentiate into infective metacyclic trypomastigotes in the hindgut ***(8)***. CDC/Alexander J. da Silva, PhD/Melanie Moser. Public domain. [19]

Reduviid bugs live in holes dug by small animals or in the cracks of substandard houses in rural areas. The risk factors for Chagas disease include sleeping in poorly constructed houses and living in rural areas within an endemic region. The term "Chagasic house" has been used to describe dwellings constructed from mud, adobe or thatch with roofs made of straw and with dirt floors.

Reduviid bugs can become infected through the ingestion of human or animal blood that contains circulating parasites. In the bug gut, parasites reproduce and differentiate, creating infective metacyclic trypomastigotes. During subsequent feeding on another vertebrae host, trypomastigotes are released in insect feces adjacent to the bite wound. Host infection occurs through non-intact skin or through conjunctiva or other mucous membranes. Once infection has occurred, trypomastigotes invade host cells and differentiate into intracellular amastigotes, which proliferate and differentiate into trypomastigotes. Trypomastigotes then enter the blood stream and remain dormant until the *T. cruzi* enter a new host or are consumed by another vector. [19] Once infected via contamination of a skin wound, mucosa, blood transfusion, organ transplant or congenitally there will be an incubation period of 5-14 days after which symptoms can develop.

The classic signs associated with Chagas disease are the Romaña sign, a swelling of the eyelids of the affected eye (parasite point of entry after rubbing eyes with contaminated hands) and the chagoma, an induration at the site of entry with associated erythema and local lymphadenopathy. The initial presentation can vary from asymptomatic to severe and lethal; the acute phase can last weeks to a couple of months.

[20-22] In most cases the symptoms are limited to fever or non-specific complaints while more severe ones may present with myocarditis or meningoencephalitis. When the condition remains untreated during the acute phase, 70–85 % of the cases will develop chronic Chagas disease of which 10-30% will develop symptomatic complications. [23] The death rate in severe acute cases of Chagas disease is estimated to be around 10%. [2] During the chronic phase some people may develop cardiomyopathy, megaesophagus or megacolon. Individuals with chronic Chagas disease become reservoirs for *T. cruzi* for decades before developing cardiac or gastrointestinal complications. Many of them will be unaware they are infected with *T.* cruz*i*, and once the complications develop Chagas disease may not be considered in the differential as it is not endemic to the US. The treatment for some of its cardiac and esophageal complications is similar to idiopathic or more common etiologies. [23]

Chagas disease can be diagnosed during its acute phase by microscopic examination of fresh anticoagulated blood (or buffy coat) for motile parasites or thin and thick blood smears stained with Giemsa. Blood cultures for *T. cruzi* take several weeks, making them impractical. Serology testing in the US is limited as there is only one commercially approved enzyme-linked immunosorbent assay (ELISA) in the US for the diagnosis of Chagas disease. [23] An indirect fluorescent antibody (IFA) test is available through the Centers for Disease Control (CDC). Patients with a past history of leishmaniasis, a protozoan parasite of the order Kinetoplastida, have high false positive rates due to serologic cross-reactivity. [23]

The treatment for Chagas disease is mostly limited to the acute phase of the disease; there are only two medications (Nifurtimox and Benznidazole) available for the treatment of acute cases of Chagas disease. The efficacy of these medications is 60%, and only children under the age of 18 can be treated once the patient has reached the chronic phase. [21] The use of several antifungal medications for the treatment of Chagas disease has been studied in the past. These medications showed minimal anti-trypanosomal activity, and their use for the treatment of Chagas disease is not warranted. [23]

WHO estimated the total deaths for Chagas disease to be approximately 13,000 deaths for the year 2002. [24] The World Bank estimates Chagas disease accounts for 649,000 disability-adjusted life years (DALYs) in the American continent. [25] Analysis of migration patterns from the 2000 US Census estimates that over a thousand people a day come into the US across the Mexico-US border, of whom 5-10 are expected to be infected with *T. cruzi*. According to Leiby et al (2002), national seroprevalence for *T. cruzi* antibodies among blood donors in the USA is estimated to be 1/25,000. [26]

III

METHODS

A narrative review of the medical and veterinarian literature was performed using the Medline search engine. The literature review included articles and case reports published in peer reviewed journals, and government or World Health Organization agencies reports, as well as recent infection/tropical disease conferences. Medical textbooks were utilized for the review of the classic clinical presentation signs and

symptoms, pathophysiology, diagnosis and treatment of Chagas disease. Searches were also accomplished in the CDC and World Health Organization Web sites for reports on Chagas disease. Keywords used for searching the literature included *Trypanosoma cruzi*, Chagas disease, American trypanosomiasis and United States. These terms were used to find articles pertinent to the incidence, prevalence, epidemiology, and management strategies of Chagas disease in the United States. The languages included in the literature review were English and Spanish. Most of the articles published in Spanish were also available in English and Portuguese versions through their publisher. Government reports from Latin American countries were the only literature published exclusively in Spanish, none of which were included in this paper.

Articles were collected until March 2007. All references regarding case reports and prevalence studies performed in the US were reviewed regardless of the date of publication. These references were needed to analyze the progression in the understanding of the prevalence of Chagas disease in the US. A total of 122 articles were selected after performing an abstract review of the literature identified through Medline searches, of which 46 articles were referenced in this paper. Illustrations of *T. cruzi* life cycle, vector and an example of a "Chagasic house" were obtained through the CDC web site. These illustrations are public domain, and their use does not represent a violation of copyright laws.

Chronic Chagas disease is a growing health problem in the US due to the risk of trypomastigotes transmission via infected blood and/or organs. However, information

regarding the nature and extent of the Chagas disease problem and national prevention efforts is limited and fragmented.

IV

FINDINGS

Risk and common modes of transmission for acquiring *T. cruzi* in the US

American trypanosomiasis is considered a zoonosis in the US and is enzootic in several mammals in the southern US. [5-8] Its etiologic agent, *T. cruzi*, was first reported in California in 1916 by C. Kofoid in reduviid bugs. [27] There are twelve species of reduviid bugs in the US, all of which are capable of transmitting the disease and are found in the southern portion of the country. Among the most important of these reduviid bugs are *Triatoma sanguisuga*, *Triatoma gerstaeckeri*, *Triatoma rubida* and *Triatoma protracta*. *Triatoma sanguisuga* is considered the most important and is primarily found in the eastern US. *Triatoma gerstaeckeri* primarily affects Texas and New Mexico while *Triatoma rubida* and *Triatoma protracta* tend to be found in the western regions of the country, mainly California and Arizona. [5] All of these triatoma species are sylvatic with *Triatoma gerstaeckeri* being the only one to also be peridomestic. Although the above species are considered to be of primary importance, additional *Triatoma sp.* are present and can be vectors of the disease. There is a species of Reduviid bugs, *Triatoma lecticularia*, present across the southern US which has been

found in domestic environments. [18] Although *Triatoma lecticularia* is capable of colonizing houses, it has not been described as a significant vector in the transmission of *T. cruzi* in urban areas in Central or South America. A significant finding is the absence of *T. dimidiata* in the US, an important species in the domestic cycle throughout Latin America, including Mexico. The ability of *Triatoma dimidiata* to colonize urban areas and transmit *T. cruzi* at low density levels makes it important epidemiologically since domestic exposure is the main factor in increasing the risk of *T. cruzi* transmission to humans as reported by WHO. [18] Another aspect in which the *Triatoma sp.* found in the US differs from those in Latin America is their defecation patterns after a blood meal. The vectors in the US exhibit delayed defecation habits, rather than defecating immediately after a meal as is observed in reduviid bugs found in *T. cruzi* endemic countries. [8] This behavior decreases the probability of contaminating the insect bite wound or mucosa when the individual scratches or rubs the skin after getting bitten by the vector.

The natural reservoirs of *T. cruzi* in the US are mainly wildlife animals, among them raccoons and opossums. The presence of *T. cruzi* among raccoons has been documented as far north as Maryland by Walton *et al.* as early as 1956. The prevalence of *T. cruzi* among these mammals in the US has been reported to be as low as 2% in northwestern Florida to as high as 62% in Oklahoma (1958). [8] In addition to high prevalence among wild animals, genetic studies of *T. cruzi* samples in the US showed divergence patterns suggesting the parasites are indigenous to the local fauna and not imported. [28] These studies indicate *T. cruzi* is well established in the southern US.

Review of the veterinary literature shows domestic animals are also affected by *T. cruzi* in this country. The first cases of American trypanosomiasis among domestic dogs in the US were diagnosed in 1972 and were published along with 8 other cases in 1977. Of these nine cases only one was associated with infected reduviid bugs colonizing the dog's kennel, while the others were believed to have acquired the condition by eating the bugs or the tissue of infected animals. [7] A study involving 300 domestic and stray dogs in Oklahoma found the prevalence of *T. cruzi* among canines to be 3.6%. The prevalence among a subgroup of the population, coonhounds, was found to be 15% and the source of infection was attributed to mucosal exposure from biting an infected animal. [29] The findings were more compatible with a sylvatic infectious cycle rather than a peridomestic/domestic cycle since no *T. cruzi*-infected reduviid bugs were found in most cases. Cases of domestic infection among dogs have been documented as recently as 2003. Beard et al reported a case of domestic infection in a dog involving *T. gerstaeckeri* in southern Texas. Infected vectors colonized the kennel and were also found under cement slabs and a detached garage but none in the family house. [5] These finding are compatible with the known ability of *T. gerstaeckeri* to infect peridomestic structures.

The first group of documented human cases of Chagas disease in the US consists of autochthonous infections with *T. cruzi*. There are five documented cases of autochthonous Chagas disease in the US since 1955. Four of these cases involved pediatric patients less than two years old, and a 56-year-old woman accounted for the only documented adult case. The distribution of these cases were limited to Texas (three infants), California (one adult), and Tennessee (one toddler). The fifth case took place in

a rural area of Tennessee in 1998 and involved an eighteen month-old-boy whose mother had found reduviid bugs in the toddler's crib. The parents had the insects analyzed due to concerns with their resemblance to insects previously seen in a television program regarding insects that prey on mammals. The insects were identified as *T. sanguisuga* infected with *T. cruzi* trypomastigotes and epimastigotes. At the time the insects were found in the boy's crib, he was described as healthy. Within two weeks of finding reduviid bugs in the home, the boy developed mild fever with non-specific physical findings. Polymerase chain reaction (PCR) studies performed at the CDC were positive for *T. cruz*i approximately one week after the child's fever onset. In this case the home was not colonized with *T. sanguisuga* but several raccoons and one of the family's dogs were found to be positive for *T. cruzi* antibodies. [21] This case represents a sylvatic transmission cycle within a domestic environment due to the temporary translocation of the vector into the child's room.

A study performed in the late 80s among cardiac surgery patients found a non-Hispanic patient who tested positive for *T. cruzi* antibodies in the US. The patient was a 55-year-old male from Corpus Christi, Texas who had no past history of risk factors for Chagas disease and for which reason the authors suspected it was a case of autochthonous infection; however, it could not be confirmed. [30]

The second group of documented acute Chagas disease in the US involves cases of individuals who acquired *T. cruzi* via transfusion of blood products. Transfusion-associated Chagas disease is the second most important form of transmission in Latin America. [31] There have been five documented cases of transfusion-associated acute

Chagas disease in the US and two in Canada. Four of the US cases received platelet transfusions from donors who were found to be *T. cruzi* antibodies positive; no specific blood component or donors were identified as the source of infection for the fifth case. In the cases where donors were identified, all were from countries where Chagas disease is endemic, had been living is the US for over a decade, and none were aware of ever having acute Chagas disease or of the chronic status of their illness. All of the seven cases of transfusion-associated acute Chagas disease in the US and Canada were immunocompromised patients. [14] Several of these patients had overwhelming acute Chagas disease as a result of their immunocompromised status. [12, 13] Their clinical presentation made it possible for their providers to identify *T. cruzi* in the patient's blood. In two of the US cases the patients presented no symptoms of acute Chagas disease and the diagnosis was incidental. This includes the most recent case concerning a 3 ½ year old patient with a history of neuroblastoma undergoing chemotherapy, status post laparotomy two weeks prior to presenting to a teaching hospital with a low grade fever (2006). In this case trypomastigotes of *T. cruzi* were identified in a blood smear performed on the patient due to the hospital policy to perform complete blood counts with manual differentials for all patients with a history of neutropenia. [14]

No cases of transfusion-associated acute Chagas disease have been reported in recipients of PRBC in North America. [12-14] Look back studies performed on recipients of blood products other than platelets from *T. cruzi*-positive blood donors (identified on seroprevalence studies or from clinical cases) have identified any new

cases of Chagas disease in the US. [32] No cases of blood products transfusion-associated chronic Chagas disease have been documented in the US.

There have been several cases of solid organ transplant-associated *T. cruzi* infections reported in the US. [9-11] In Latin America, the transmission of *T. cruzi* within solid organ recipients had only been observed among kidney transplant cases. Barcan *et al* reported in 2005 the number of transplant-associated primary acute Chagas disease in Latin America to be twelve. The Latin American cases differ from those in the US because the donors in Latin American countries have been screened for Chagas disease. Kidneys from 34 donors who were seropositive for *T. cruzi* antibodies were transplanted to *T. cruzi* naive individuals with a 35% transmission rate. [11] A study of seroprevalence of Chagas disease among deceased organ donors in southern California published in 2005 found a prevalence of 0.25% (n=404). Previous studies failed to identify any evidence of Chagas disease among deceased solid organ donors. [33] In 2001 the first case of acute Chagas disease was reported in a liver transplant patient in the US. The donor, an immigrant from Central America, transmitted *T. cruzi* to all of the recipients of his/her organs, who had positive blood cultures for the protozoan. A 37-year-old female received a kidney and pancreas from the infected individual, and eventually died from acute Chagasic myocarditis. The liver was transplanted into a 32-year-old female. The patient acquired acute Chagas disease and had recurrent acute Chagas disease after completing a four month course of Nifurtimox and eventually died from liver and kidney failure not related to Chagas disease or its treatment. The other kidney was transplanted into a 69-year-old female, who had completed one course of

therapy with Nifurtimox without further complications. [9] The fourth US case was a 64-year-old male who received a heart in December 2005 from a US-born donor who had a history of traveling to *T. cruzi* endemic areas of Mexico. The blood samples from the donor were found to be seropositive for *T. cruzi* antibodies. Treatment with Nifurtimox was started, but the patient died four months after his surgery from acute rejection of the transplanted organ. Three other recipients of the donor's liver and both kidneys were reported to be seronegative for *T. cruzi* antibodies by IFA and no evidence of parasitemia was found by PCR. [10] The fifth case of solid organ transplant-associated acute Chagas disease in the US involved a second case of heart transplant on a 73-year-old male in January 2006. A month later the patient was re-admitted to the hospital due to fever and a skin rash. *T. cruzi* trypomastigotes were observed on a blood smear done during his evaluation; blood cultures and PCR were also found to be positive for *T. cruzi* but were found to be seronegative for *T. cruzi* antibodies. The patient was treated with Nifurtimox and remained seronegative by IFA. He died five months after his surgery from cardiac failure. The donor was a US resident who was born in El Salvador and was found to be seropositive for *T. cruzi* antibodies by radioimmonoprecipitation assay (RIPA). The other solid organ recipients from the same donor showed no evidence of infection by IFA or PCR. [10]

No cases of congenital Chagas disease have been reported in the US, and its transmission in highly endemic areas has been cited to be between 1-10%. [15, 16] A seroprevalence study performed in Waco, Texas published in 1999 found two US-born individuals with no risk factors for Chagas disease to have antibodies for *T. cruzi* by

enzyme immunoassay (EIA) and RIPA. Both individuals had strong family histories of cardiac illness compatible with complications associated with chronic Chagas disease among the females in their maternal family trees where the grandmother or great-grandmother came from a *T. cruzi* endemic area. Attempts by the research team to test the family members to confirm a possible congenital transmission were unsuccessful. [26] Hence there have been no confirmed cases of congenital Chagas disease reported in the US to this date.

Another source of infection is accidental exposure in laboratory workers. A review of such exposures by Herwaldt published in 2001 revealed there have been eight cases of acute Chagas disease in the US as a result of laboratory accidents. [34] This kind of transmission represents an occupational hazard associated with biological research and is outside the scope of this paper.

Sylvatic and domestic autochthonous infectious cycles are possible and have been documented in the US, where both of these forms of infection have only resulted in a small number of documented cases during the 20th century. The emergence of blood product transfusion and solid organ transplant as the primarily route for acquisition of *T. cruzi* infection and a twofold increase in the number of documented cases within the past 20 years makes the latter the most important forms of infection in the US.

The need for a *T. cruzi* blood donor screening program in the US

Research reports have identified *Triatoma sanguisuga*, *Triatoma gerstaeckeri*, *Triatoma rubida* and *Triatoma protracta* as the most important species for insect

transmission to humans in the US. Although individual reduviid insects have been found occasionally in the southern states of the US, colonization of homes by local vectors has not been reported. However, the presence of zoonotic vectors in the southern US suggests the need for monitoring the risk for a possible domestic transmission cycle. In addition to cats, dogs, wild mammals and domestic rodents, Chagas disease reservoirs include humans. Strategies to control the vector include elimination of the vector and housing improvement. However, the vector species found in the US and the small number of reported cases of autochthonous Chagas disease show the risk for domestic transmission cycle in the US to be very low. Therefore the need for implementation of vector control programs such as the Southern Cone Initiative as seen in Latin America to decrease the risk of Chagas disease is not warranted. However, the increased number of cases of blood transfusion and organ transplant-associated acute Chagas disease within the US in the past two decades shows the prevention and control of this disease needs to focus on blood screening rather than vector control.

With an estimate of 12.4 million cases of Chagas disease in Latin America, it is the population of immigrants from *T. cruzi* endemic areas who represent the largest risk for transmission of this protozoan via blood and solid organs in the US. Transfusion-associated transmission of *T. cruzi* is the second most important form of infection in Latin America. It is estimated that 15% of all *T. cruzi* transmission in endemic areas is due to blood product transfusions. [17] Because of the insidious course in most cases of chronic Chagas disease, many Latin American immigrants may be infected with *T. cruzi* and not be aware of it. These individuals are reservoirs for the condition and are

introducing the risk of transfusion-transmitted *T. cruzi* into the US and other non-endemic countries. [14] They can also become organ donors and infect the recipients of the harvested organs with *T. cruzi* as discussed in the previous section.

The risk of infection associated with the transfusion of a contaminated 500 ml unit of whole blood has been estimated to be between 12 and 20% (but there have been reported values as low as 1.4 and as high as 48%). [18, 35] In the US researchers have taken three different approaches in their efforts to determine the risk for infection with *T. cruzi* via transfusion. The first has been the evaluation for the presence of risk factors for Chagas disease among volunteer blood donors. The second approach is the measurement of *T. cruzi* antibodies in blood donors. Some studies tested only those donors who had a history of risk factors for Chagas disease while other smaller-scale studies tested all of the samples regardless of their history. A third approach tested a large population of individuals that had received blood product transfusions by looking for evidence of transfusion-associated *T. cruzi* infection.

The first estimate of the number of cases of chronic Chagas disease in the US was performed in 1987 in a study involving 205 Salvadorian and Nicaraguan immigrants. The prevalence of chronic Chagas disease among the studied population was found to be 5% and based on this finding the author was able to estimate the number of chronic Chagas disease cases among the US immigrant population to be between 50,000 to 100,000 cases. [36] Since then several approaches have been used to study prevalence of chronic Chagas disease and which associated risk factors are present among the infected blood donors in the US.

One of the fulminant transfusion-associated acute Chagas disease cases in the US prompted the American Red Cross (ARC) to perform a series of studies to determine the seroprevalence of *T. cruzi* antibodies among volunteer blood donors. A study looking at the presence of antibodies for *T. cruzi* performed in Los Angeles involving 988 donors found a seroprevalence of 0.1%. [37] Another study with blood donors from the southeastern US (mostly rural areas of Florida, Georgia, Alabama, Mississippi, and Louisiana) did not find any confirmed cases of chronic Chagas disease among 6013 ARC Gulf Region blood donors. [38] However a study performed between 1994 and 1995 involving almost 300,000 donors from the Los Angeles and Miami ARC regions showed a seroprevalence rate of approximately 1/8800. [39] The authors continued collecting data from these two ARC regions from May 1994 until September 1998 and in 2001 published a second study in which 1.1 million Los Angeles and 181,000 Miami blood donors were screened for history of risk factors for Chagas disease. Those individuals found to have risk factors for Chagas disease were then tested for *T. cruzi* antibodies. The seroprevalence of antibodies for *T. cruzi* was found to be 0.19% in a study of 7500 participants living in Los Angeles and 0.08% in 9000 Miami residents.

A breakdown of the data per individual year showed a unique pattern in the seroprevalence of antibodies for *T. cruzi* in the Los Angeles population. The authors found the seroprevalence of *T. cruzi* antibodies was increasing steadily among the Los Angeles group. The percentages were 0.010 for 1996, 0.014 for 1997 and 0.018 for 1998, with seroprevalence rates of 1/9850, 1/7200 and 1/5400, respectively. The authors attributed the changes observed in the Los Angeles population not only to changes in the

demographics of the Hispanic population but to enhanced blood donor recruitment efforts among Hispanics in the Los Angeles region at the time their study was underway. [40]

Studies looking into the prevalence of risk factors for Chagas disease alone among blood donors have found it to be much higher than those seen with the aforementioned seroprevalence studies. Researchers in California found the prevalence among Los Angeles blood donors of having at least one risk factor for Chagas disease to be 1/340 (2.4.%). [41] Another study also performed in Los Angeles found 15.5% of their eligible blood donor population (n=3492) had at least one risk factor for Chagas disease of which only 4.4% were considered by history to be high risk for Chagas disease. [42]

A large prospective multi-center study involving cardiac surgery patients from hospitals in Baltimore and Houston looked for evidence of transfusion-associated infections among those subjects. The study involved 12219 patients between 1985 and 1991 of which 9811 received a total of 127,035 blood product transfusions. [30] This is currently the largest study in the US looking for evidence of asymptomatic transfusion-associated infection of *T. cruzi.* The authors reported six cases of Chagas disease, none of which were found to be transfusion-associated; however, cases of the disease in its chronic stage were detected with one possible case of autochthonous infection in a non-Hispanic white male. These findings correlate with the look back results in all examined studies evaluating the seroprevalence of Chagas disease among volunteer blood donors in the US. No cases of transfusion-associated transmission have been found among recipients of blood products, including platelets, from repeat donors who were found to

have Chagas disease. [37, 39-41] Although these finding can be interpreted as reassuring, the occurrence of transfusion-associated Chagas disease in the US can not be discarded as demonstrated by the case reports discussed in previous sections.

Blood screening efforts in the US

Various strategies for the screening of blood donors in the US have been proposed in the medical literature. The use of questionnaires to screen donors who could be considered high risk for Chagas disease has not been found to be an efficient screening method in several studies. [39-41, 43] In one of the studies a *T. cruzi*-infected donor answered no to having any risk factors for Chagas disease and was allowed to donate blood. He was later found to be infected after being enrolled and eventually tested as part of the control group. [39] This screening method can result in the deferral of a large number of healthy willing blood donors to self-report and recall bias [40, 44]. A second proposed screening method is testing only those individuals with positive risk factors for Chagas disease. There are advantages and disadvantages associated with this strategy as it would decrease costs as compared to a universal screening program, but would increase time and complexity of the pre-donation screening process. A variation of the selective screening is the use of a one-time screening of all blood donors for *T. cruzi* antibodies; those with negative results would not be required to be tested during subsequent donations. This strategy will not be seen as discriminatory compared to the screening of those with positive risk factors which largely target individuals born in endemic countries of Latin America. However, the logistical requirements to keep track of the donor

screening status can be complicated and fragmented between the blood bank groups managing the national blood supply. The use of universal screening of blood donors for *T. cruzi* antibodies is considered the simplest, most logistically feasible and easiest to standardize across blood banks of all proposed strategies; however, it carries the disadvantage of higher cost. [43, 44]

The 2002 WHO expert committee recommendations advise the use of two different serologic tests for the diagnosis of chronic Chagas disease. For the screening of blood-bank inventories WHO recommends the use of a single ELISA test. [18] With the introduction of the first FDA-approved blood and organ donor screening test for the detection of antibodies for *T. cruzi* in the US on December 2006, known as the ORTHO *T. cruzi* ELISA Test System, it is now possible to develop national policies and screening programs aimed at preventing transfusion and transplant associated Chagas disease in the US. This ELISA test was found to be 97.7% sensitive and 100% specific for *T. cruzi* in a US sample population (Ortho-Clinical Diagnostics list sensitivity of 100% and specificity of 99.997%). [35] A high specificity is essential for the screening of *T. cruzi* in the US due to the low prevalence of the condition in this country. While a single test would be adequate for the screening of blood from volunteer donors in accordance with the WHO recommendations, a confirmatory test is needed due to the known cross-reactivity of the *T. cruzi* ELISA test with *Leishmania* antibodies. At the present time, there is no other approved test for the detection of *T. cruzi* antibodies that could be used as a confirmatory test for cases with positive results with ELISA. The lack of an approved confirmatory

test also precludes the development of policies and guidelines to enable blood banks to reintegrate false positive cases into the donor pool.

Cost benefit analysis

The addition of a screening test for *T. cruzi* to the processing of blood and solid organs will represent an added cost for blood and solid organs banks across the US. It is the patients and blood banks that will ultimately absorb this extra cost. The ARC estimates the cost of testing a unit of blood to be between 5 and 10 dollars per unit depending on the volume of the blood center (e-mail communication with ARC officials). Recent published data on blood collection and transfusion in the US for 2001 from the 2001 National Blood Data Resource Center (NBDRC) survey shows the total number of units collected to be approximately 15.3 million units (including autologous donations). The percentage of units from first time donors in blood centers was reported to be 38%, which represents a significant increase from the 20% seen in previous surveys. The article fails to provide information on the demographics of the donor population beyond the percentage of units from new donors. [45] The data available through this survey showed that the cost associated with a universal *T. cruzi* screening strategy is estimated to fall between 76.5 and 153 million US dollars per year. The use of a screening strategy where donors need only to be tested once would decrease the cost of screening by its second year. Calculations using the 2001 percentage of new donors revealed that the added annual cost for the screening of blood would be between 29.1 and 58.1 million dollars. However, if a more traditional figure of 20% is used for the number of first time

donors, then the cost is expected to be between 15.3 and 30.6 million dollars per year. The lack of information regarding the percentage of blood donors with risk factors for Chagas disease precludes the calculation of an accurate estimate for a screening strategy limited to the testing of a high risk population. The use of the estimated prevalence for the general population nationwide would result in unrealistic numbers when compared to those found in cities such as Los Angeles and Miami. Using estimates for the general population also shows how implementing such a strategy could disproportionately increase the cost of processing blood units in cities with large percentages of Latin American immigrants. A more general approach would be to apply the rate of units of blood donated per 1000 available donors (85 units per 1000 age eligible US adults) to the estimated number of immigrants from *T. cruzi* endemic areas in the US (12.4 million). The annual cost of screening a targeted population could then be estimated to be between 5.3 and 10.5 million dollars.

The decrease in the incidence of Chagas disease through the interruption of the sylvatic and transfusion-associated transmission cycles in the countries comprising the Southern Cone Initiative in Latin America has resulted in savings of approximately US$17 for each dollar spent. [46] Similar analyses can not be performed for the US at this time since the prevalence of Chagas disease is currently unknown. Reported cases of blood transfusion and organ transplant associated acute Chagas disease are seen as sentinel cases and not representative of the true incidence among those receiving blood products. Many researchers believe there are unreported and therefore untreated cases of acute Chagas disease in the US that may have acquired the condition through the

transfusion of blood components. Some of these individuals will develop chronic Chagas disease which will have an economic impact on our society as a result of the disability-adjusted life years (DALY) associated with the condition. The loss of healthy living years will impact how productive these individuals can be and how much they can contribute to the community. The savings achieved in avoiding the cost of the treatment of complications associated with Chagas disease outweighs the monetary cost associated with the screening of a blood unit for *T. cruzi*.

V

DISCUSSION AND CONCLUSIONS

The presence of *T. cruzi* in the US has been well documented in the veterinary literature. The identification of *T. cruzi* infected vectors in several states during the mid 20th century confirms its presence in the US. This is not as a result of increased immigration from Latin American countries during the latter half of the century. Despite the presence of this protozoan and its vector in the southern half of the country, only a small number of cases have been documented in the US medical literature. Several factors are believed to contribute to this low incidence. The species of reduviid bugs found in the US colonize sylvatic or peridomestic structures. The absence of vectors with the ability to colonize domestic structures such as *T. dimidiata*, a vector of significant importance in the epidemiology of Chagas disease in Latin America, is among the most important factors for the low incidence of American trypanosomiasis in the US. The socio-economic conditions and living standards in the US also contribute to the low incidence of autochthonous Chagas disease as they are not conducive to domestic

infestation with reduviid bugs. Another important finding accounting for the low incidence of this condition in the US is the defecation habits after a blood meal of the local species of reduviid bugs. These vectors do not defecate immediately after a blood meal; their delayed defecation pattern decreases the risk of contaminating a wound or mucosa with the metacyclic trypomastigotes found in the feces of an infected reduviid bug.

Although low, the risk for the autochthonous transmission of *T. cruzi* is present in the US and the most recent autochthonous case of acute Chagas disease in the US illustrates several important aspects of the disease. First, even while *T. sanguisuga* is considered a sylvatic species it can temporarily invade well-built and maintained dwellings, or be introduced accidentally into a home. Second, the characteristically mild clinical presentation would not have raised the child's physician's suspicion at the time of initial presentation, and adequate therapy would most likely not been given if the provider did not know in advance of the infant exposure to *T. cruzi*. This demonstrates the importance of education to the public. Third, education of the general community is essential in the prevention of Chagas disease in areas were *T. cruzi* is known to be enzootic. Need of education can not be limited to the general public; health care workers and practitioners are in greater need because Chagas disease is almost unknown to many.

The highest incidence of acute Chagas disease in the US during the past 25 years has been among patients who received blood products or solid organ. There have been ten documented cases in the US during that period of time versus five cases of autochthonous transmission in the past 50 years. Blood product transfusions and solid

organ transplants are currently the most likely source of transmission for Chagas disease in the US. Transplants were considered a low threat as a mode of transmission in the past due to the low incidence of autochthonous vector transmission in the country. The first case of blood transfusion-associated acute Chagas disease in the mid 1980s prompted the medical community and US governmental agencies to re-evaluate the safety of the blood supply in the nation. The US cases of acute Chagas disease in patients who received solid organs from an infected donor showed different patterns from those previously observed in Latin America in that the transmission of *T. cruzi* had only taken place among kidney recipients. The need to screen blood and solid organ donors has been the subject of multiple studies taking place in the US during the past 25 years. Most of these studies have involved volunteer blood donors throughout different regions in the southern portion of the country. The seroprevalence of *T. cruzi* antibodies among blood donors in the US has been found to vary according to the percentage of the population with history of known risk factors for Chagas disease. Studies done in areas with low numbers of Latin American immigrants within the southern US did not show an increased prevalence as compared with Los Angeles and Miami. [32] What is not clear at the present time is the prevalence of Chagas disease nationwide; studies estimate the prevalence of Chagas disease among blood donors to be approximately 1/25000, while studies in Los Angeles and Miami have found the prevalence to be 1/8800 with the most recent data showing a prevalence of 1/5400 for Los Angeles in 1998. Although the FDA has not mandated the testing of blood in the US, one of the major stakeholders in the safety of the nation's blood supply, the American Association of Blood Banks (AABB), recommends the

testing of blood donors by its members. The AABB has developed the Chagas Biovigilance Network based on the program used for tracking new cases of West Nile virus to monitor any reported ELISA positive cases of Chagas disease among blood donors. ARC and United Blood Services, who combined handle approximately 65% of the nation's blood supply, are currently testing blood donors for Chagas disease and reporting any positive results to the AABB through the Chagas Biovigilance Network. The AABB has recognized the need for a confirmatory test and coordinated for all donors with positive ELISA results to be tested using a RIPA test for Chagas disease. The Chagas RIPA Test has been the standard confirmatory test for researchers in the US. The use of RIPA, which is a non-FDA approved confirmatory test, may be temporary as new screening and confirmatory tests are being evaluated by the FDA for their potential use in the US. The data collected through the AABB Chagas Biovigilance Network will provide the data necessary to determine the sensitivity, specificity and predictive values of the ORTHO *T. cruzi* ELISA Test System under use by blood banks nationwide. The data will provide a more accurate estimate of the prevalence of Chagas disease among blood donors and the risk for the transfusion-associated transmission of the condition in the US.

At the present time the most effective strategy is the implementation of a universal screening policy. Such an approach, in combination with the AABB Chagas Biovigilance Network, will provide government agencies with the data needed to determine the most efficacious screening policy that can protect the nation's blood supply without creating any unnecessary burden for blood banks. The use of universal screening

simplifies the screening process for blood bank personnel. In addition, it will avoid any misperception of targeting minority groups, as this could be the case if the selective screening approach utilizing risk factors was implemented. Such a program could have a negative impact among individuals born in Latin America or those that have lived in *T. cruzi* endemic areas, since they may not continue donating blood due to perceived discrimination. The loss of potential donors may be substantial in communities such as Miami and Los Angeles where many of the current studies took place. In the past, the use of a selective screening policy as a mode of prevention seemed to be an adequate strategy in the US. Chagas disease is a rare condition, and until the 1980s only a handful of cases had been reported, all of which were the result of autochthonous vector transmission. The recognition of the presence of transfusion-associated transmission of Chagas disease in the US requires that responsible governmental agencies such as the CDC, Federal Drug Administration (FDA), and the medical community develop better methods to protect patients receiving a blood transfusion or solid organ transplant. The shift from a high risk prevention strategy (use of questionnaires to screen out potential blood donors with risk factors for Chagas disease) to a population screening strategy (all blood donors are tested for serological evidence of *T. cruzi* infection) will provide a more effective method to protect individuals from acquiring Chagas disease. There are additional benefits to implementing this strategy; the use of universal screening will simplify blood bank processes needed to safeguard the donor's health information as required by Health Insurance Portability and Accountability Act (HIPAA). Although

this strategy is more costly for the end user, the cost is minimal and the benefits clearly outweigh the cost.

The diagnostic and treatment challenges of Chagas disease represent areas of much needed research. The high cost associated with the development of new drugs and the limited success seen with some of the antifungal medications for the treatment of Chagas disease limit the medical treatment to Nifurtimox and Benznidazole. Both of these medications require treatment for prolonged periods of time and are not frequently tolerated by the patients due to their side effects. There is a need for the development of newer drugs with higher cure rates, less side effects, and shorter treatment courses. Traditionally, the treatment of Chagas disease has been limited to its acute phase on young individuals. Newer recommendations allow for the treatment of adult patients with chronic Chagas disease as long as the transmission cycle has been interrupted as would be the case for individuals who have migrated to the US. Treatment is not recommended for chronic Chagas disease at risk for re-infection due to the high incidence and severity of side effects associated with these medications. However, the difficulties associated with the use of the current medications still remains, limited availability (through CDC), long treatment courses, and high incidence of side effects. Treating individuals found to have chronic Chagas disease would not clear them to become blood/organ donors as they would not be considered cured until they become seronegative. The cross reactivity of *T .cruzi* antibodies with *Leishmania* antibodies represents a problem with most screening and diagnostic testing used to diagnose Chagas disease. The *T. cruzi* Genome Project and the *Leihsmania* Genome project have provided

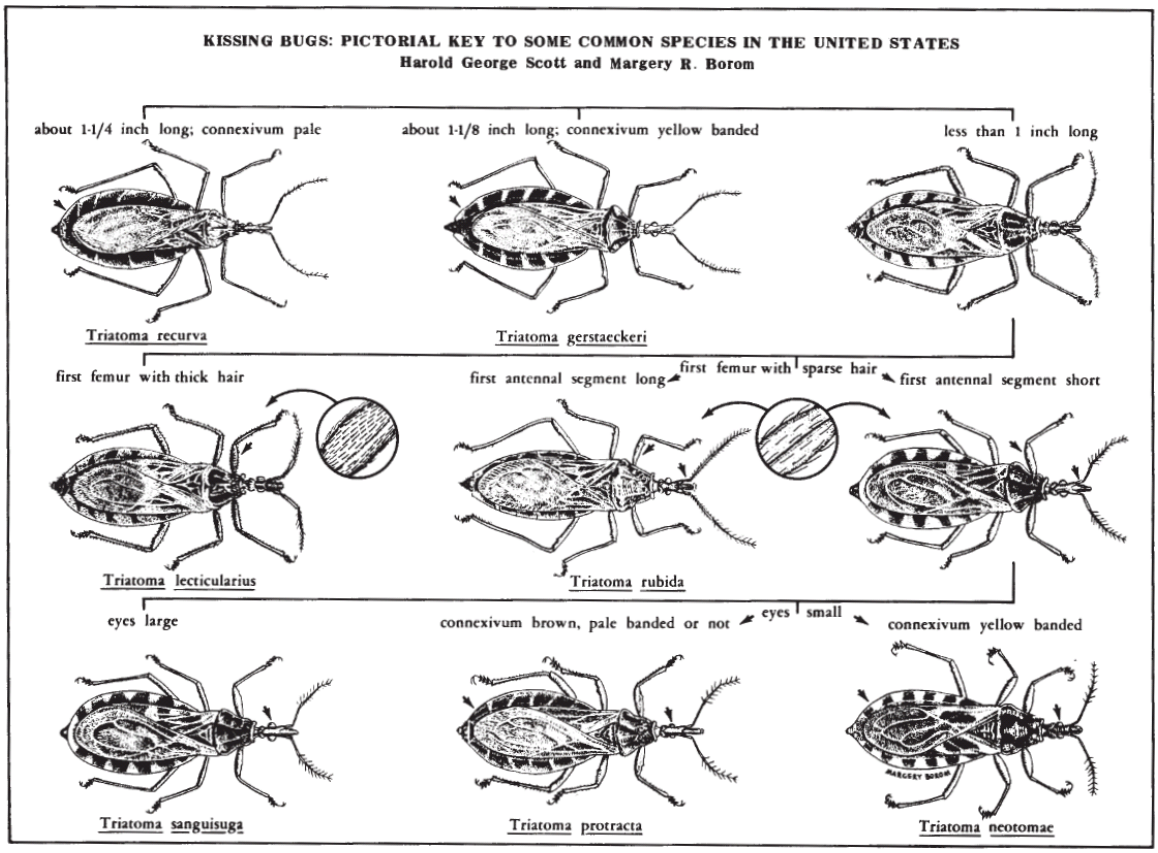
researchers new areas for study which could lead to the development of new tests and treatments for these conditions.

To this date no education campaign on Chagas disease has been developed. There is a need for educating the general public on the condition, its signs and symptoms, complications, modes of transmission and treatment options. The educational efforts also need to include primary care providers since they will be the ones answering the patient's concerns after the patient has been notified of having a positive test for *T. cruzi*. Education of health care providers may be conducted in collaboration with the American Medical Association, American College of Physicians, American Academy of Pediatrics and the American Academy of Family Practice, American Academy of Nurse Practitioners, American Nurse Association, and the CDC, as these organizations can reach large numbers of primary care providers in the US.

There are several limitations that can be identified with this review. The development of different techniques for detecting *T. cruzi* antibodies affects the ability to compare some of the studies reported. Although some of the studies involved large study populations over several years using the same testing technique, they are being compared with older studies where different testing methods were used, and/or the study populations were not representative of the general population. There are also new screening and confirmatory tests currently under evaluation by the FDA. These tests may provide the components currently missing for the development of an efficient blood and solid organ donor screening program in the US.

There have been substantial achievements in decreasing the incidence and prevalence of Chagas disease throughout Latin America thanks to the efforts of WHO and local government efforts in the past 20 years. DALYs have decreased from over 2 million to 649,000, and the vector transmission of *T. cruzi* has been reduced thanks to programs like the Southern Cone and Central American Initiatives. It may be possible that these efforts will help decrease the risk of blood product transfusion and solid organ transplant associated transmission in the US in years to come as the prevalence of Chagas disease among the Latin American immigrant decreases.

Appendix-1
Common *Triatoma sp.* Found in the United States



Source: CDC. Public domain

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