Chagas Disease in the United States: a public health approach

Caryn Bern[1], Louisa A. Messenger[2], Jeffrey D. Whitman[1], James H. Maguire[3],

[1]University of California San Francisco School of Medicine, San Francisco, CA; [2]London School of Hygiene and Tropical Medicine, London, UK; [3]Brigham and Women's Hospital, Harvard Medical School, Boston, MA.

Corresponding author: Caryn Bern, MD, MPH, Department of Epidemiology and Biostatistics, University of California San Francisco, 550 16th Street, San Francisco, CA 94158.
Caryn.Bern2@ucsf.edu

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SUMMARY

Trypanosoma cruzi is the etiological agent of Chagas disease, usually transmitted by triatomine vectors. An estimated 20-30% of infected individuals develop potentially lethal cardiac or gastrointestinal disease. Sylvatic transmission cycles exist in the southern United States, involving 11 triatomine vector species and infected mammals such as rodents, opossums and dogs. Nevertheless, imported chronic *T. cruzi* infections in migrants from Latin America vastly outnumber locally-acquired human cases. Benznidazole is now FDA-approved, and clinical and public health efforts are underway by researchers and health departments in a number of states. Making progress will require efforts to improve awareness among providers and patients, data on diagnostic test performance and expanded availability of confirmatory testing, and evidence-based strategies to improve access to appropriate management of Chagas disease in the United States.

INTRODUCTION

Trypanosoma cruzi is the causative agent of Chagas disease (1, 2). Infection is lifelong without treatment; thus, prevalence can be high despite low incidence. Current estimates of 6 million infections and 1.2 million cases of cardiomyopathy place Chagas disease first in disease burden among parasitic diseases in the Americas (3)(4). *Trypanosoma cruzi* is transmitted when infected vector feces enter the bite site or mucous membranes of a mammalian host. Transmission can also occur through blood component transfusion, organ transplantation, food or beverages contaminated by the vector or vector feces, and in utero from mother to fetus (5).

The classic setting for Chagas disease is rural Latin America, where adobe houses and the presence of domestic animals favor domestic and peri-domestic vector infestation (2). However, transmission in many rural areas has decreased due to vector control programs, and infected individuals have migrated to Latin American cities (6), the United States and Europe (7, 8). Unlike Europe, the United States has well-described enzootic *T. cruzi* transmission, involving 11 triatomine species and a range of mammalian hosts (9). Nevertheless, the vast majority of *T. cruzi*-infected individuals in the United States are Latin American immigrants infected in their countries of origin. We will review clinical, epidemiological and public health aspects of Chagas disease in the United States, with a focus on the most recent relevant publications.

BIOLOGY AND TRANSMISSION OF *TRYPANOSOMA CRUZI*

In 1909, Carlos Chagas, a young physician working in rural Brazil, demonstrated the etiological agent, its vector, several of its reservoir hosts and the salient manifestations of the disease that now bears his name, a feat unrivaled in medical history (10). He named the parasite in honor of his mentor, Oswaldo Cruz (11). Chagas proceeded to isolate *T. cruzi* from the blood

of a domestic cat, and finally from a symptomatic toddler. This “first patient” remained infected for life, but never developed chronic manifestations of Chagas disease, and died at 73 from unrelated causes (12). Finally, Chagas fulfilled Koch’s postulates by reproducing the infection experimentally in laboratory animals (11).

In the years since 1909, the life cycle has been more fully characterized. In order to successfully colonize the mammalian host and triatomine vector, *T. cruzi* assumes three distinct morphological forms at different developmental stages (Figure 1) (13). Amastigote and epimastigote forms replicate by binary fission in mammalian cells and the hindgut of the triatomine vector, respectively. Trypomastigote forms are non-replicative and are present at two distinct life cycle stages: (i) in the bloodstream of the mammalian host (bloodstream-form trypomastigotes) and (ii) in the rectum and feces of vectors (infective metacyclic trypomastigotes).

Infective metacyclic trypomastigotes are deposited on the skin of the mammalian host in fecal droplets extruded by a blood-feeding triatomine bug. Parasites enter through the bite site, skin abrasions or mucosa such as the conjunctiva. This mechanism, via the vector feces rather than mouthparts, is known as stercorarian transmission. Once internalized, motile trypomastigotes invade nucleated cells via both lysosome-dependent and independent mechanisms (reviewed by (14, 15)). The parasite is then taken up into a membrane-bound (parasitophorous) vacuole, which subsequently fuses with a lysosome; exposure to decreasing pH stimulates parasite differentiation to the intracellular amastigote form and its concomitant release into the cytosol over a period of 4-5 days. Here, amastigotes multiply asexually to form pseudocysts, which can arise in a variety of host tissues, but predominantly in cardiac, smooth and skeletal muscles and reticuloendothelial cells in the liver, spleen and lymphatic system.

Within pseudocysts, amastigotes differentiate into trypomastigotes that, upon cell lysis, can either infect adjacent tissues to initiate new replicative cycles, or disseminate throughout the bloodstream and lymph. Without antitrypanosomal treatment, infection persists for the duration of the mammalian host's life.

Triatomine bugs feeding on an infected host may ingest extracellular trypomastigotes, which pass to the midgut where transformation to an intermediate spheromastigote form occurs. Differentiation of spheromastigotes into epimastigotes occurs in response to decreasing environmental glucose levels as the blood meal is digested (13). Epimastigotes multiply by binary fission in the hindgut and migrate to the rectum where they attach hydrophobically to the waxy gut cuticle by their flagella and transform into infective metacyclic trypomastigotes, thus completing the life cycle.

Routes of transmission

Vector-borne transmission. Vector-borne transmission remains the predominant route of new human infections in endemic regions. Historically, vector-borne transmission has occurred in ecologically determined areas throughout continental Latin America, from Mexico to the northern 50-60% of the territories of Argentina and Chile (16). Infected vectors and reservoir animals are not infrequent in the southern half of the continental United States, but vector-borne transmission to humans is rarely detected (reviewed in multiple sections that follow).

Congenital transmission. Reported vertical transmission rates are variable, ranging from 0% in some studies to more than 15%; the pooled transmission risk in a recent meta-analysis was 4.7% (17). Factors associated with a higher risk include younger maternal age (reflecting more recent infection), maternal immunological responses, higher maternal parasitemia, twin births and HIV

co-infection (18-21). Infected infants are regularly detected in screening programs in Spain, and sporadically in other countries with Latin American immigrant populations (22-24).

Blood-borne transmission. In the early 1990s, *T. cruzi* infection was found in 1 to 60% of donated blood units in Latin American blood banks (25). Since then, blood donation screening has been established as a major component of Chagas disease control programs (26). With the addition of Mexico in 2012, screening of blood components for *T. cruzi* is now required in all endemic countries in Latin America, and reported donor prevalence has markedly decreased (26, 27).

Organ-derived transmission. Transplantation of an organ from a *T. cruzi*-infected donor can transmit *T. cruzi* to the recipient, but the risk varies by organ type. In cohorts of kidney recipients from infected donors in the U.S. and Argentina, transmission occurred in 13% and 19%, respectively (28, 29). The transmission rate among 10 U.S. liver transplant recipients was 20% (28). The risk from heart transplant in the same U.S. series was 75% (3 of 4); use of the heart from an infected donor is contraindicated (28, 30).

Oral transmission. Outbreaks of acute *T. cruzi* infection due to contaminated fruit or sugar cane juice have been reported in several countries of Latin America (31, 32). Most case clusters are small, affecting family groups in the Amazon and attributed to fruits such as açaí (33). The largest reported outbreak was associated with a 10% attack rate among students and staff at a school in Caracas; home-pressed guava juice was implicated (34).

EPIDEMIOLOGY AND ECOLOGY

Global burden of Chagas disease

The Chagas disease control initiatives instituted throughout Latin America since 1991 constitute a major public health success story. Thanks to vector control programs, blood bank screening, and in some countries, congenital Chagas disease screening programs, global estimates have decreased from 18 million in 1991 to less than 6 million infected individuals in 2010 (Table 1) (3, 35). Incidence estimates have fallen from 500,000 in 1991 to 30,000 new *T. cruzi* infections per year in 2010 (3). As vectorial transmission has come under increasing control, the proportion attributable to other routes has grown: currently, 22.5% of incident infections are estimated to occur through congenital transmission, and in some areas, oral transmission may be more frequent than the traditional vector-borne route (3, 31).

Triatomine vector biology

More than 130 triatomine species have been reported in the Western Hemisphere, many known to carry *T. cruzi* (16, 38). However, a few species are disproportionately responsible for *T. cruzi* transmission to humans, due to their propensity to colonize human houses and/or the peridomestic environment. These include *Triatoma infestans* and *Panstrongylus megistus* in the Southern Cone, *T. dimidiata* in southern Mexico and Central America, and *Rhodnius prolixus* in Central America and northern South America (16). These species have been the major targets of the regional control initiatives. Elimination of *R. prolixus* from Central America and the near elimination of domestic *T. infestans* from much of the Southern Cone are responsible for the steep decline in new infections and very low prevalence in children throughout most of the historic endemic zone (39, 40). However, in the Gran Chaco, an ecological zone that straddles southern Bolivia, northeastern Argentina and parts of Paraguay, the prevalence of house

infestation and transmission remain very high (41, 42).

The domestic environment is rich in blood meal sources, both human and animal. Crevices in adobe walls and dark spaces within animal corrals and poultry nests provide safe diurnal refuges for triatomines. *Rhodnius* species, which nest in palm crowns in the sylvatic environment, can infest thatch roofs. Triatomines of both sexes require at least one blood meal during each of the five nymphal stages, and females need a blood meal to lay eggs. Thus, both male and female nymphs and adults may carry *T. cruzi*, with infection rates increasing with age. Only adults have wings. Most domestic triatomine species feed nocturnally, and complete their blood meals without waking the host (38). The major Latin American vectors defecate during or immediately after taking a blood meal (43). Many sylvatic triatomine species colonize the nests of their blood meal sources, and are found in close association with specific rodent or marsupial species (16, 38). Sylvatic triatomine adults may be attracted by light to invade human dwellings, and lead to sporadic human infections (44, 45). Some triatomine species, such as *T. dimidiata*, can infest both domestic and sylvatic sites (46).

Triatomine distribution in the United States

Eleven triatomine species have been reported in the United States: *Triatoma gerstaeckeri, T. incrassata, T. indictiva, T. lecticularia, T. neotomae, T. protracta, T. recurva, T. rubida, T. rubrofasciata, T. sanguisuga,* and *Paratriatoma hirsuta* (Figure 2 and Table 2) (9, 38). Triatomines are present from coast to coast, across the southern two-thirds of the continental US (Figure 3). In field collections, vectors are often found in specific microenvironments (woodpiles, rock piles, rodent nests, livestock pens, dog kennels) (9). Natural *T. cruzi* infections have been documented in all species except the rarely collected *T. incrassata* and *P. hirsuta* (9, 47).

The two species with the widest geographic distribution in the United States are *T. sanguisuga* and *T. protracta*. The former has been reported from Texas to the Atlantic coast and as far north as Illinois, the latter from Texas to California (9, 48). A recent review by the Wheeling-Ohio County West Virginia Health Department turned up 10 specimens of *T. sanguisuga* archived since 1969, and adds this state (long assumed to have the vector) to the confirmed list (48). *T. sanguisuga* was also recently reported in Delaware (49). *T. protracta* has been extensively collected in association with its favored blood meal hosts, the woodrats (*Neotoma* spp); the prominent above-ground nests of these rodents makes sylvatic collection relatively straightforward for this species (9, 50). *T. protracta* includes three morphologically distinct subspecies in the United States, *T. protracta protracta* in California, Nevada, Utah, Arizona and New Mexico, *T. protracta woodi* in Texas and *T. protracta navajoensis* in the Four Corners area (51). Thousands of specimens of *T. sanguisuga* and *T. protracta* have been reported in literature dating back to the 1930s, and these species were found in or near the residences of humans with locally acquired *T. cruzi* infection in Tennessee, Louisiana, Mississippi (*T. sanguisuga*) and California (*T. protracta*) (9, 52-55). In field collections, both species frequently have *T. cruzi* infection, with rates generally in the 15-30% range (9, 50).

T. gerstaeckeri has a more limited range, encompassing south-central Texas and southeastern New Mexico, but is one of the most frequently collected species, perhaps in part because of its propensity to infest dog kennels and other peridomestic structures (56). *T. gerstaeckeri* constituted more than 70% of several thousand vectors submitted through a citizen science project based at Texas A&M University (57). Collections of *T. gerstaeckeri* show high rates of *T. cruzi* infection, often >60% (9, 57). Infected *T. gerstaeckeri* were collected in the house of a child with acute *T. cruzi* infection in south Texas in 2006 (58).

Texas and the southwestern states have the highest triatomine species diversity, with at least seven species in Texas and six in Arizona (9, 56) (Table 3). A spatial analysis of bugs submitted through the Texas citizen science initiative showed geographic overlap among species, but with *T. gerstaeckeri* predominantly in south-central Texas, *T. sanguisuga* in the eastern portion, *T. rubida* in west Texas and *T. indictiva* in a small area of central Texas (56). *T. gerstaeckeri* reports showed earlier seasonality than *T. sanguisuga*, possibly because of the earlier arrival of high temperatures in the southern part of the state. Like all passive surveillance, there may be reporting biases in these data. The authors observe that they received few submissions from west Texas (and perhaps for this reason, few *T. protracta*). They attribute this to lower human population density and/or less effective outreach (56), but lower rates of internet access in rural counties could also play a role.

The ranges of all United States species extend into Mexico with the exception of *T. rubrofasciata* (51, 59). *T. rubrofasciata* is associated with rats, and is thought to have been carried from North America globally on sailing ships in the 18th century (60). In the United States, this species has been reported in Jacksonville, Florida and Honolulu, Hawaii, consistent with its predominant distribution in ports.

Allergic reactions to triatomine antigens

T. gerstaeckeri, T. protracta, T. recurva, T. rubida and *T. sanguisuga* have been implicated in allergic reactions in the United States (61). Such reactions are due to vector salivary antigens, not the infection status of the vector. Most reactions consist of a pruritic welt where the bite occurred. Severe reactions may involve angioedema, urticaria, dyspnea, gastrointestinal symptoms and/or anaphylaxis (61). Severe reactions may necessitate treatment with epinephrine (62). Reports are most frequent in Arizona and California; the most commonly

identified species are *T. protracta* and *T. rubida,* and the most frequent scenario is house invasion by an adult triatomine (61). In a study in southern California, allergic reactions consistent with those provoked by triatomine exposure were reported by 13% of residents of desert areas with frequent triatomine sightings, compared to 4% of those living in suburban Los Angeles county (63).

Wild and domestic animal reservoirs

Wildlife reservoirs. *Trypanosoma cruzi* infection has been reported in more than 150 species of mammals from eight orders, and it is widely believed that all mammals are susceptible (64). Birds and cold-blooded vertebrates are refractory to infection (65). The epidemiological importance of particular species is highly variable, depending on local ecology and parasite transmission dynamics. Maintenance reservoirs have persistent infection, while amplifier reservoirs are those that display characteristics that favor transmission, such as high parasitemia levels (66). As with humans, most infected animals are chronically infected, and therefore detection may be reliant on a combination of examination of peripheral blood smears, culture isolation, serological testing and PCR; relative ease of trapping as well as variable performance of diagnostic assays contribute to bias in reported prevalence levels. Across the endemic range, *Dasypus novemcinctus* (nine-banded armadillo) and *Didelphis* species (opossums) are prominent sylvatic reservoirs and amplifiers of infection. *Trypanosoma cruzi* is able to infect almost all tissues in its mammalian hosts, including atypical sites, such as the cornea of *Thrichomys apereoides* (spiny rat) (67) and the anal scent glands of *Didelphis* species (68), enabling the latter to function as both host and vector. In addition to vector-borne transmission, many sylvatic mammals are prone to alternate transmission routes, including oral infection via ingestion of infected vectors, congenital infection and exposure to contaminated bodily secretions (69). These

biological features may predispose such hosts to infection with multiple strains, due to high transmission intensity and efficiency (70, 71).

In the US, *T. cruzi* infection has been demonstrated in more than 24 wildlife species, including raccoons, opossums, armadillos, foxes, mice, squirrels, coyotes, skunks and wood rats (9). Recent studies have expanded this list to include additional rodent (72, 73), bat (74) and deer species (75). Reported seroprevalence rates fluctuate quite widely within species, ranging in raccoons from 15 to 90% (72, 76, 77), skunks, 9 to 100% (72, 78), opossums, 8 to 33% (78, 79), and woodrats and other rodents, 20 to 76% (72, 80-82). The prevalence varies depending on ecology, local diversity and density of vector species, and in some cases between sexes, with female denning activities associated with increased triatomine contact (78, 83). High infection rates in some mammals, such as wood rats and raccoons, may result from frequent insectivory (84). Experimental infections studies and the high attack rates in human outbreaks of orally transmitted Chagas disease suggest that ingestion of infected vectors or vector fecal material is a very efficient transmission route (34, 84). In contrast, consumption of raw *T. cruzi*-infected meat did not result in experimental infection in one study (84).

Canine Chagas disease. Dogs are important in peridomestic cycles in Latin America, both as vector blood meal sources and *T. cruzi* infection reservoirs (85, 86). In the hyperendemic Chaco region of Argentina, dogs have been shown to be highly infective to vectors and are thought to be a key reservoir sustaining transmission to humans (87). In the United States, *T. cruzi*-infected dogs have been reported from Tennessee, South Carolina, Georgia, Virginia, Louisiana, California, Oklahoma and Texas (reviewed in (9)). Infected dogs may develop acute and chronic manifestations similar to those in humans, including acute myocarditis, arrhythmias, chronic dilated cardiomyopathy, congestive heart failure and sudden death (89). Several recent surveys

in Texas demonstrate widespread canine *T. cruzi* infection, especially in working dogs and those living in kennels (90-93). The prevalence in these surveys varied widely. Some studies demonstrated significant discordance between diagnostic tests, and a substantial number of dogs whose infection status was unresolved with the performed testing (92). The highest infection rate, 71% by serology, was reported in the investigation of a Texas kennel where several dogs had sudden death suspected to be due to acute Chagas disease (90). Triatomines collected in dog kennels and near houses in Texas show high prevalence of canine blood meals and *T. cruzi* infection (90, 94), suggesting that dogs may be an important peridomestic host.

Transmission potential in the United States

With the exception of the rarely collected species *T. neotomae, T. incrassata* and *P. hirsuta*, all US vector species have been reported to invade human dwellings (56, 62). A total of 2,883 specimens of 7 different vector species were submitted to the Texas citizen science project (56). Of these, 17% were collected inside human dwellings; the highest proportions were for *T. rubida* and *T. protracta*. Houses with refuges such as woodpiles, rock piles and brush, and those with structural gaps through which vectors can pass, are more vulnerable to vector invasion (52, 62, 95). Rarely, the presence of *T. protracta* or *T. recurva* nymphs has been reported inside houses, suggesting possible colonization (62). Window screens, air conditioning and caulking of gaps in house construction may be protective against such invasion (62).

Detection of human blood meals is frequent in tested triatomines, especially those collected in and around human dwellings, and in other spaces where humans congregate. In a recent study in Texas, human blood was detected in 59% (30/51) of *T. gerstaeckeri*; the second most frequent blood meal was canine (17/51, 33%), followed by more than a dozen other vertebrate hosts (96). In this study, collection sites were largely domestic or peridomestic, and

human blood meals were found in 77% (17/22) of *T. gerstaeckeri* collected inside homes; mixed blood meals were frequent. In another study from Texas, vectors were collected in dog kennels and woodrat nests, as well as domestic settings (94). In this study, dogs (10/33, 30%) were the predominant blood meal source for *T. gerstaeckeri,* followed by woodrats (*Neotoma micropus*) (7/33, 21%); human blood was identified in a single bug. All 40 *T. protracta* were collected in woodrat nests and had fed exclusively from *Neotoma micropus* (94). In a Louisiana study conducted near the house of the 2006 autochthonous human infection, 43 *T. sanguisuga* were collected; 53% had fed from American green tree frog, 49% from humans and 30% from raccoons; detection of blood from multiple host species was frequent (97). In the Arizona-Sonora Desert Zoo, human blood was detected in all 7 *T. rubida* tested; 5 of 7 had other blood meal sources detected, including pig, sheep or goat, dog, mouse, rat or woodrat (98). Human blood was also detected in 2 of 3 *T. recurva* collected elsewhere in southern Arizona (98).

The coincidence of human blood meals and *T. cruzi* infection in triatomines has been described as indicating the "potential for Chagas disease" in the United States (96-98). Clearly, transmission to humans occurs; more investigation is needed to quantify the risk since most infections likely go undetected. However, the small number of locally acquired *T. cruzi* infections detected in humans stands in contrast to the moderate to high *T. cruzi* prevalence rates in dogs, raccoons, opossums and woodrats. Compared to major South American vectors such as *R. prolixus* and *T. infestans*, North American vectors appear to have somewhat longer time intervals from blood meal to defecation, and may be less likely to defecate on the host (99-102). Vectors rarely colonize houses in the United States, and well-constructed houses with window screens provide effective barriers against domestic invasion. Perhaps most importantly, stercorarian transmission is inefficient; mathematical models based on data from the Gran

Chaco, where transmission to humans is the highest in the world, estimate that a single human *T. cruzi* infection requires on average 900 to 4,000 contacts with infected vectors (71).

MOLECULAR EPIDEMIOLOGY

***Trypanosoma cruzi* genotypes**. *Trypanosoma cruzi* is a highly genetically diverse parasite, estimated to have diverged from its most recent common ancestor 3-4 million years ago (103). Scientific consensus currently defines a minimum of six genetic lineages or discrete typing units (DTUs: TcI – TcVI) (104), plus a potential seventh, bat-associated genotype (TcBat), most closely related to TcI (105-108). Multiple molecular markers confirm a largely clonal population structure, which maintains the identity of major DTUs, interspersed with recombination events (109). TcI through TcIV form monophyletic clades, while TcV and TcVI resulted from recent hybridization of TcII and TcIII (103, 110). Genomic data support this evolutionary model. TcI to TcIV display substantial allelic homozygosity resulting from long-term, recurrent and dispersed gene conversion, whereas TcV and TcVI have natural heterozygosity and minimal distinction, with shared intact alleles from their parental DTUs (103, 110-114).

Each *T. cruzi* DTU is characterized by distinct but often overlapping transmission ecologies (115). TcI, TcII, TcV and TcVI are commonly associated with domestic cycles and are the genotypes found in most human infections. Investigators have long observed that gastrointestinal Chagas disease is more frequent in the Southern Cone than further north in Latin America, and hypothesized a connection to different circulating *T. cruzi* strains (116). However, there remains no clear, unequivocal evidence of influence of particular lineages on progression or clinical outcome of human Chagas disease (reviewed in (117)). Domestic TcI is distributed from the Amazon Basin northwards and is the principal DTU found in humans in Venezuela, Ecuador and

Colombia (118-120). TcI also circulates in arboreal ecotopes between *Didelphis* species and the triatomine tribe *Rhodniini* (121, 122), with secondary cycles among rodents and sylvatic *Triatoma* species in highland valleys in Bolivia, Peru and Chile (123-126). Sylvatic TcI populations are characterized by high levels of genetic diversity (127-133), while human infections are associated with divergent, more genetically homogenous strains (131, 134-136). By contrast, TcII, TcV and TcVI appear less variable overall (103) and are predominant in domestic cycles in the Southern Cone (115, 137, 138). However, recent whole genome sequencing of clinical TcII isolates has revealed more extensive intra-DTU diversity than previously reported (113). Sylvatic reservoirs of TCII, TcV and TcVI are less well delineated than for TcI, but TcII has been increasingly detected in Brazilian primates (133, 139, 140). In addition, TcV and TcVI have been demonstrated in domestic dogs from Argentina to as far north as Colombia (110, 141-144). TcIII is transmitted by *P. geniculatus* to *D. novemcinctus* and other burrowing mammals in terrestrial transmission cycles from the Amazon Basin to Argentina (145-147). The known host range of this DTU has expanded to include dogs, grisons and foxes in Brazil (148). TcIV, perhaps the most neglected DTU, circulates sympatrically with TcI in wild primates in the Amazon (149), and raccoons and dogs in the United States (150). TcIV can invade the domestic environment in Venezuela (116, 119) and has been isolated from oral outbreaks in the Brazilian Amazon (149, 151-154) and Colombia (155). Finally, TcBat has been isolated from Chiroptera species across Brazil (105), Panama (106), Colombia (108) and Ecuador (107), and is potentially infective to humans (156).

Trypanosoma cruzi molecular epidemiology in the United States

The majority of genotyping activities have concentrated on vectors and reservoir hosts. In an extensive analysis of U.S. vectors and mammalian hosts, all five autochthonous human cases were reported as TcI (online supplement in (122)). In a more recent series of presumed autochthonous chronic *T. cruzi* infections in Texas blood donors, authors were limited by genetic marker resolution and therefore unable to distinguish among TcII, TcV and TcVI (157).

To date, TcI and TcIV are the only DTUs detected among the six examined triatomine species, with no absolute associations between parasite genotype and vector (Table 4). Higher proportions of TcIV have usually been identified in *T. sanguisuga*, *T. indictiva* and *T. lenticularia*, compared to a predominance of TcI in *T. gerstaekeri*, *T. protracta* and *T. rubida* (80, 90, 92, 122, 158-160). However, except for studies of vectors collected by the Texas citizen science initiative (159, 160), samples sizes were far too small to make any meaningful extrapolations. The observation of potential TcII/V/VI autochthonous human infections in Texas is noteworthy but challenging to interpret without clear evidence of these genotypes circulating in local vector species (157). Similarly, a study of *T. protracta* collected in California encountered issues distinguishing among TcII/V/VI and was unable to establish the presence of infections with these lineages (161). Further investigations are warranted to confirm the presence of these DTUs in the United States, using a larger panel of more highly resolutive markers, in conjunction with phylogenetic analyses incorporating all representative *T. cruzi* DTUs; neither of these studies examined parasite sequence homology to TcI (157, 161).

Among reservoir hosts, TcI and TcIV are the principal DTUs identified in United States (Table 5). Similar to vector surveys, sample sizes are insufficient to reveal any strict correlations between host and parasite genotype; current data demonstrate both lineages circulating among

mammalian hosts in variable proportions. Finally, a few studies in Louisiana reported rodents harboring TcII, alongside other mixed TcI, TcIV and TcVI infections (80). Additional sampling efforts will be necessary to delineate the frequency and ecology of TcII/V/VI in the United States.

Issues underlying *T. cruzi* genotyping data collection

To accurately interpret *T. cruzi* genotypic data, biological and logistical limitations relating to both parasite infection dynamics and genotyping methodologies must be considered. *Trypanosoma cruzi* genotyping can be conducted on clinical samples (blood or tissue) or parasite axenic cultures, obtained through hemoculturing or xenodiagnosis. Due to low levels of peripheral parasitemia, especially in chronically infected patients, direct genotyping is insensitive, but may be improved if multiple specimens are tested (162). The principal limitation of parasite isolation is selection bias for specific clones, due to faster growth rates or culture conditions to begin with (163-165), and subsequently by loss of diversity from long-term maintenance in axenic culture or animals (166-170). Hemoculture recovery rates are usually less than 30% among chronic patients (171) and dependent upon parasite load and distribution in the initial inoculum. Xenodiagnosis can generally recover more parasite strains but biases may result from variable vector permissibility to specific strains (172-174). Furthermore, circulating clones isolated by hemoculture or xenodiagnosis may be different from those sequestered in tissues due to differential strain tropisms (175-177) and can vary even between sequential blood samples (178). Similar biases affect sylvatic *T. cruzi* sampling, with certain reservoir species more heavily represented in survey data due to their relative ease of capture and presence of detectable parasitemia.

The most commonly used genotyping techniques for clinical and field specimens involves analysis of size polymorphisms in multi-copy genetic markers, particularly the nuclear spliced-

leader intergenic region (SL-IR), 24α rRNA and 18S rDNA (179, 180) (Tables 4 and 5). The major confounder associated with the use of any multi-copy gene is the level of intra-clone copy number and undefined chromosomal orthology, which can prevent direct comparability between strains. The SL-IR is present in many hundreds of copies per parasite genome; the copies are not necessarily identical, and may instead comprise a predominant haplotype accompanied by a low abundance of minor paralogous sequence types more closely related in identity to other DTUs, likely resulting from their shared evolution (113, 181, 182). In this scenario, it is virtually impossible to distinguish between a monoclonal DTU infection containing multiple divergent SL-IR sequences and a polyclonal infection consisting of different major DTU parasites. This issue is minimized when using conventional PCR, as generally the most common gene sequence is amplified in a reaction. However, recently, a number of deep sequencing studies reported results based on sequencing millions of copies of the SL-IR locus that seem to indicate the occurrence of almost all DTUs in infected rodents and primates in the United States (183, 184). Parallel deep sequencing of appropriate biologically-cloned controls to exclude low abundance haplotypes, has thus far yielded equivocal evidence; further investigations are essential to define the applicability of this technique to characterize natural multiclonal infections (185, 186).

CLINICAL MANIFESTATIONS

Acute *T. cruzi* infection

The acute phase begins one to two weeks after vector-borne transmission and lasts approximately 8 weeks. Patients are most commonly asymptomatic or experience mild, non-specific symptoms such as fever. A *T. cruzi* abscess or chagoma may occur at the site of inoculation. Parasite entry via the conjunctiva may result in unilateral eyelid swelling (the

Romaña sign) (187). However, eyelid swelling can be caused by an allergic reaction to triatomine salivary or fecal antigens; confirmed diagnosis of *T. cruzi* infection is obligatory, even in the setting of vector exposure and an apparent Romaña sign. Severe acute Chagas disease, including myocarditis, pericardial effusion, and/or meningoencephalitis, is rare, but when it occurs, mortality risk is high (5, 188). In the absence of the Romaña sign or severe manifestations, individual infections are seldom diagnosed during the acute phase.

Orally-transmitted *T. cruzi* infection has been reported to cause more severe acute morbidity and higher mortality than vector-borne infection (190, 191). Micro-epidemics appear to be fairly frequent in the Amazon basin, due to sylvatic vectors contaminating produce such as *açaì* or sugarcane (31). In the Caracas outbreak, mentioned above, 103 people were infected, of whom 59% had ECG abnormalities, 20% were admitted to hospital and one person died from acute Chagas myocarditis (32, 34). Alterations in *T. cruzi* surface glycoproteins caused by exposure to gastric acid may increase parasite invasiveness, providing a possible explanation for the increased severity of orally acquired Chagas disease (192, 193).

Congenital Chagas disease is acute infection in the newborn. Most infected infants are asymptomatic or have mild findings, but a small percentage present with severe disease or die *in utero* (18, 194). Manifestations may include low birth weight, prematurity, low Apgar scores, hepatosplenomegaly, anemia and thrombocytopenia (18, 194). Severely affected neonates may have meningoencephalitis, gastrointestinal megasyndromes, myocarditis, pneumonitis and/or respiratory distress (18). Women who receive antitrypanosomal therapy prior to conception are significantly less likely to transmit *T. cruzi* to their infants (23, 195).

Chronic *T. cruzi* infection

One to two months after infection, parasitemia falls below levels detectable by microscopy, and the patient passes into the chronic phase of *T. cruzi* infection (2, 5). Chronic *T. cruzi* infection without signs or symptoms of Chagas disease is designated the indeterminate form (2, 5, 196). Over a period of years to decades, an estimated 20-30% of infected individuals develop cardiomyopathy (2, 5). A retrospective cohort analysis of Brazilian blood donors estimated progression to cardiomyopathy to occur at a rate of 1.85% per year (200). Chagas cardiomyopathy features chronic inflammation in all chambers and damage to the conduction system and cardiac muscle (199). The most frequent early signs are right bundle branch block or left anterior fascicular block, and segmental left ventricular wall motion abnormalities (188, 198, 199). Later manifestations appear decades after infection, and include ventricular arrhythmias, sinus node dysfunction and bradycardia, persistent or intermittent complete heart block, an apical aneurysm usually in the left ventricle, thromboembolic phenomena and progressive dilated cardiomyopathy. Patients may experience palpitations, syncope, systemic and pulmonary emboli, with high risk of sudden death or death from progressive heart failure. (188, 198, 199).

Gastrointestinal involvement is much less frequent than cardiomyopathy. Esophageal manifestations range from asymptomatic motility disorders through mild achalasia to megaesophagus (204). Patients may experience dysphagia, odynophagia, esophageal reflux, weight loss, aspiration and regurgitation. Patients with colonic involvement may have prolonged constipation, fecaloma, volvulus, bowel ischemia or megacolon. Symptomatic gastrointestinal disease, like symptomatic cardiac disease, usually appears several decades after infection.

Chagas disease in the immunocompromised host

Organ-derived infection. Acute *T. cruzi* infection in organ transplantation recipients may lead to a relatively severe clinical spectrum, with manifestations that include acute myocarditis and congestive heart failure (205). In recent years, as screening of donors has become more frequent, most donor-derived infections have been detected by PCR monitoring prior to symptom onset, allowing prompt antitrypanosomal treatment and favorable outcomes (28). Current recommendations suggest monitoring the recipient of an organ from an infected donor for at least 6 months, at which point the frequency can be decreased (Table 6) (30).

Reactivation in cardiac transplant recipients. Cardiac transplantation is an accepted treatment for end-stage Chagas cardiomyopathy (197, 206). In a large Brazilian cohort, survival of patients transplanted for Chagas cardiomyopathy was better than among those with idiopathic or ischemic cardiomyopathy and *T. cruzi* reactivation was a rare cause of death (207, 208). Data from a smaller cohort of patients transplanted for end-stage Chagas cardiomyopathy in the United States also demonstrated survival similar to that among patients transplanted for other etiologies (209). The most common manifestations of reactivation are fever and acute myocarditis in the transplanted heart. Patients may also develop inflammatory panniculitis and cutaneous nodules (205). Central nervous system (CNS) involvement occurs infrequently. All patients with dilated cardiomyopathy and a history of significant residence in continental Latin America should be screened (210). For those found to be infected, post-transplant monitoring should include histopathology of the explanted heart and subsequent endomyocardial biopsies, and serial peripheral blood monitoring by quantitative PCR (Table 6) (210).

Reactivation Chagas disease in HIV-co-infected patients. The most common clinical manifestation of *T. cruzi* reactivation in HIV-coinfected patients is meningoencephalitis with or

without a mass lesion (211). The case-fatality rate for CNS reactivation is very high. The presentation is often confused with CNS toxoplasmosis (212, 213); *T. cruzi* should be considered in the differential diagnosis of CNS mass lesions in HIV-infected patients (214, 215). Acute reactivated myocarditis is another frequent manifestation and may be obscured by pre-existing chronic cardiomyopathy (216). New arrhythmias or conduction system abnormalities, pericardial effusions or cardiac decompensation should prompt testing for reactivation.Subcutaneous nodules resembling erythema nodosum and parasitic invasion of the peritoneum, stomach or intestine can occur but are uncommon (217). Five cases of *T. cruzi* reactivation in HIV-infected Latin American immigrants have been reported in the United States since 1992; all presented as CNS syndromes and were treated initially as toxoplasmosis (212, 213, 218-220).

DIAGNOSTIC TECHNIQUES

The choice of modality to diagnose Chagas disease is determined by the clinical setting and suspected phase of infection. In general, techniques to detect the parasite are used in the acute phase and suspected reactivation, whereas IgG serology is the mainstay of diagnosis in the chronic phase (Table 6).

Microscopy. In acute, congenital or reactivated infection, trypomastigotes may be detectable by light microscopy in thick and thin smears from whole blood or buffy coat with routine staining (e.g. Giemsa) (221). When acute or reactivation meningoencephalitis is suspected, cerebrospinal fluid samples should be concentrated by thin-layer cell preparation technique, stained and examined by light microscopy. Microscopy is useful due to fast turnaround time, wide availability and high specificity, but its sensitivity is lower than that of molecular techniques (222, 223).

Molecular techniques. The highest sensitivity primer sequences originate from satellite or kinetoplast minicircle DNA (224-226). A recent publication from CDC outlines an algorithm that incorporates testing by multiple primer sets in a quantitative assay to optimize performance and reliability (225). Several recent initiatives have addressed standardization of extraction, and conventional and quantitative PCR for clinical use (224, 227). In acute or early congenital infection, PCR has substantially higher sensitivity than microscopy and is the diagnostic test of choice (194, 226). PCR results are variably positive in chronic *T. cruzi* infection, depending on specimen volume, primers, extraction methods and experience of the laboratory (227). Blood clot or buffy coat preparations may provide higher sensitivity than whole blood, but these preparations may not be widely available in routine clinical laboratories (225, 228). PCR has recently been utilized in several clinical trials as an early indicator of treatment failure; use in this setting requires rigorous standardization and criteria for patient inclusion (for example, positive results by PCR in at least one of 3 pretrial 10-cc specimens) (229, 230). In chronically infected patients at risk because of immunosuppression, a rise in parasite load by quantitative PCR in serial specimens is the earliest indicator of reactivation, enabling treatment before onset of symptoms (231, 232).

Diagnostic serology: Diagnosis in the chronic phase of Chagas disease relies on detection of host IgG against *T. cruzi* antigens (16). Currently, the main methods in use are ELISA, immunofluorescence assays (IFA), and immunochromatographic strip or cassette tests. Confirmed diagnosis requires positive results by at least two assays, preferably based on different antigens (for example, parasite lysate and recombinant antigens) (16). The sensitivity and specificity of the available assays are not sufficient for a single assay to be used alone for diagnosis, especially in a low prevalence setting where pretest probability is not high. The IgG trypomastigote excreted-secreted antigen immunoblot (TESA-blot) is used as a confirmatory test in blood banks and

clinical practice in Brazil (233, 234). Preparation of the test strips using antigen from trypomastigotes in cell culture requires more specialized infrastructure and may be prone to inter-batch and laboratory variability. The banding pattern may differ by *T. cruzi* DTU, suggesting different antigenic characteristics between strains (235). Conventional serology in cord and infant blood reflects transferred maternal IgG until around 9 months of age.

IgM-based assays have been evaluated for the diagnosis of acute *T. cruzi* infection, with a special focus on use for congenitally infected infants in settings where molecular assays are not available (233). IgM TESA-blot showed sensitivity of 58% compared to a consensus definition of infection, and 80% compared to PCR in congenitally infected infants in Bolivia; in these two analyses, the specificity was 98% and 94% respectively (194, 236). The specificity of IgM assays utilizing whole *T. cruzi* lysate was <30% in a similar population of congenitally infected infants (237). In the United States, PCR is the assay of choice for the diagnosis of acute and congenital *T. cruzi* infection (223).

Human cells, tissues and tissue-based products (HCT/P). Serological screening is recommended for donors of HCT/P with epidemiological risk factors, for example, those who were born or lived in endemic areas, or whose mothers were born in such areas. Two assays are approved by FDA for living and cadaveric donor screening, Ortho ELISA (Ortho-Clinical Diagnostics, Inc, Raritan, NJ) and Abbott PRISM (Abbott Diagnostics, Abbott Park, IL) (238). These tests are currently available only in blood donor testing laboratories. The Organ Procurement and Transplantation Network/United Network for Organ Sharing (OPTN/UNOS) Disease Transmission Advisory Committee also recommends the use of an FDA-cleared diagnostic ELISA to test living donors (239, 240). For living donors, a positive screening test should prompt referral for appropriate diagnostic testing and clinical evaluation (196).

Histopathology. *Trypanosoma cruzi* causes tissue damage through cellular lysis, inflammatory response, and fibrotic replacement (241). The spectrum of histopathology related to *T. cruzi* infection has been the subject of several recent reviews (242-246). The most important target organ is the heart, where chronic pathology includes multichamber damage, most prominent in the ventricles and often severe enough to form an apical aneurysm (242, 243). The spectrum of microscopic pathology includes myofiber degeneration, interstitial fibrosis, and patchy inflammation predominantly comprised of lymphocytes, macrophages, plasma cells, and eosinophils; neutrophils are not commonly observed. The patterns of inflammation and fibrosis can be focal or diffuse throughout the layers of the myocardium. Fibrotic plaques may be observed on the epicardium (242, 243). Intracellular amastigote pseudocysts are rarely observed in chronic pathology, especially with limited tissue sampling, but demonstrable parasite persistence appears to be associated with higher grade inflammation in the chronic phase (243, 247, 248). Histopathology can play an important diagnostic role in the setting of suspected reactivation. Careful examination of endomyocardial biopsies for nests of intracellular parasites can help distinguish rejection from *T. cruzi* reactivation in cardiac transplant recipients (210, 249). In immunosuppressed patients with suspected skin manifestations of *T. cruzi* reactivation, histopathology may reveal the parasite and confirm the diagnosis (245). The diagnosis of *T. cruzi* reactivation was made on brain biopsy in a patient with HIV and cerebral lesions of unknown etiology (212).

ETIOLOGICAL TREATMENT AND CLINICAL MANAGEMENT

Antitrypanosomal drugs. Benznidazole and nifurtimox are the only drugs with proven efficacy against Chagas disease (2). Benznidazole is considered the first line treatment, because of better tolerance and more comprehensive efficacy data (1, 250). Benznidazole is a prodrug, which requires

metabolism by parasite enzymes to become active; metabolites appear to act through multiple mechanisms to interrupt *T. cruzi* glutathione and trypanothione pathways (250). Dermatologic side effects are frequent, and consist of rashes and photosensitization (251, 252). Dermatitis occurs with significantly higher frequency in females than males (251). Most rashes are mild and can be managed with antihistamines or topical steroids without interrupting treatment (253). Treatment should be suspended immediately for severe or exfoliative dermatitis, or dermatitis associated with fever and lymphadenopathy. The peripheral neuropathy is dose-dependent, usually occurs late in the course of therapy and should prompt immediate treatment interruption; resolution may take months. Bone marrow suppression is rare, and requires immediate interruption of treatment. Clinical and laboratory monitoring for side effects should occur regularly throughout the course of treatment. Benznidazole tolerance is substantially better in children than adults, and in children younger than 7 years compared to older children (254). This better safety profile correlates directly with more rapid elimination of the drug in younger age groups (255).

Benznidazole was approved by the US Food and Drug Administration (FDA) in August 2017 (256), and became commercially available in the United States as of May 14, 2018. The drug is marketed in the United States by Exeltis, a US-based division of Insud Pharma (previously called Chemo Group) (257). The approval covers treatment of *T. cruzi* infection in children 2 to 12 years of age (257); usage for other age groups is off-label. Prescriptions require submission of a completed order form, available at http://www.benznidazoletablets.com/ or by contacting Foundation Care (Phone: 877-303-7181; Fax: 877-620-2849; Email: FastAccess@Exeltis.com). Urgent requests for benznidazole should be made by telephone.

Nifurtimox, a nitrofuran, impedes *T. cruzi* carbohydrate metabolism through the inhibition of pyruvic acid synthesis. The most common side effects are anorexia and weight loss, experienced by up to 70% of patients. Other frequent adverse effects include nausea, vomiting, irritability and insomnia (258, 259). . Rarely, patients develop peripheral neuropathy,

usually manifest as paresthesias. The peripheral neuropathy is dose-dependent, appears late in the course of therapy, and requires cessation of the drug. Nifurtimox is better tolerated by children than adults. Nifurtimox is not approved by FDA, but is provided by the CDC under investigational protocols (404-718-4745; email parasites@cdc.gov), CDC Drug Service (404-639-3670), and, for emergencies outside of business hours through the CDC Emergency Operations Center (770-488-7100).

Several new drug candidates (posaconazole and the ravuconazole prodrug E1224) have been tested in recent trials, but so far, none has shown acceptable efficacy (229, 230). All of the participants in these trials were from the Southern Cone. Although the posaconazole trial was carried out in Spain, 75 of the 78 subjects acquired their infections in Bolivia (230). Recent reviews have called for the inclusion of patients from diverse locations within Latin America, representing all of the major *T. cruzi* strains that infect humans (260). A novel aspect of these trials was the use of carefully standardized PCR assays to document treatment failure (261). Of those treated with posaconazole, 80 to 90% had detectable parasitemia by 12 months post-treatment, compared to benznidazole failure rates of 6% (per protocol) to 38% (intention to treat) (230). Similar results were demonstrated in a Bolivian trial of E1224, a related drug (229). These trials demonstrated that, with rigorous standardization, PCR may be useful as an early indicator of treatment failure, at least in populations of patients with infection acquired in the Southern Cone.

Acute and congenital *T. cruzi* infection. Acute infection has been an absolute indication for treatment since the drugs first became available in the 1970s (262). In acute and early congenital *T. cruzi* infection, antitrypanosomal therapy reduces the severity of symptoms, shortens the clinical course and decreases the duration of detectable parasitemia (262, 263). In severe acute

disease, treatment can be life-saving. Cure rates in acute and congenital infection are estimated at 80 to 99% (262-265).

Treatment of chronic *T. cruzi* infection. Evaluation of antitrypanosomal drug efficacy in chronic *T. cruzi* infection is challenging. PCR, while a potential indicator of treatment failure, is not a true test of cure, since many persons with chronic *T. cruzi* infection will have circulating parasite levels below the threshold of detection of the assay. Conventional IgG serology is considered the only sensitive indicator of infection, but requires years to decades to revert to negative after successful treatment (266). The longer the duration of infection the more durable the antibody response, with women treated after age 15 taking a median of 27 years to revert to negative serology (195). Age is often used as a proxy for infection duration, since in endemic communities most infections are acquired in childhood. Experimental lytic antibody assays convert to negative results more quickly than conventional serology, but still require years, even in children (267). In the 1990s, two placebo-controlled trials of benznidazole treatment in children with chronic *T. cruzi* infection showed approximately 60% cure rates based on lytic antibody assays 3-4 years after treatment (268, 269). These studies made early diagnosis and antitrypanosomal drug therapy the standard of care for children and prompted establishment of large-scale pediatric screening programs in high prevalence locations (16, 270).

Treatment of chronic infection in adults remains a topic of debate (271, 272). The fundamental question is whether antitrypanosomal therapy decreases the risk that an infected person will develop cardiac morbidity from *T. cruzi*. Observational data published in 2006 suggested that benznidazole treatment significantly decreased progression of Chagas cardiomyopathy in adults (273). Since progression only occurs in 20-30% of those with infection, and takes decades to become clinically evident, the ideal trial would require large

study populations followed for 20 years or more, a virtually impossible clinical trial design. The design of the BENEFIT trial (Benznidazole Evaluation for Interrupting Trypanosomiasis; ClinicalTrials.gov identifier, NCT00123916), a randomized, double-blinded, placebo controlled trial, was based on the observation that patients who already have cardiac morbidity are more likely to have further progression than those with normal cardiac status (274). Eligible patients were required to have cardiac findings consistent with established Chagas cardiomyopathy, and the primary outcome consisted of any of the following: death, resuscitated cardiac arrest, sustained ventricular tachycardia, insertion of a pacemaker or implantable cardioverter–defibrillator, cardiac transplantation, new heart failure, or other thromboembolic event. To the disappointment of many in the Chagas disease community, the trial showed no significant difference for the primary composite outcome, despite significantly higher conversion to negative PCR results in the treatment group compared to the placebo group (275).

The patient populations in the observational and trial populations differed substantially. The non-randomized study subjects had a mean age of 39 and two-thirds had normal cardiac function at baseline (273). In contrast, the BENEFIT trial population had a mean age of 55, all had cardiac damage based on electrocardiographic abnormalities and nearly half had decreased ejection fraction at baseline, indicating ventricular dysfunction (275). The question of whether treatment provides clinical benefit for those with no or very early cardiac signs therefore remains unanswered (276). The only clear take-away messages are that the younger the patient the higher the probability of benefit, and that active screening is essential to identify infected individuals before they become symptomatic. As in earlier publications (196), treatment recommendations remain stratified by age and clinical status, and require balancing risk of adverse effects with the probability and uncertainty of benefit (Table 7).

Management of the immunocompromised host. In organ transplant recipients with reactivation, a standard course of benznidazole or nifurtimox is effective in ameliorating clinical symptoms and shortening the duration of microscopically detectable parasitemia. Prior treatment or post-transplant prophylaxis has not been shown to decrease the risk of reactivation; post-transplant prophylaxis is not generally administered in heart transplant centers in Latin America (277). As no reliable test of cure exists, treated patients are considered to remain at risk for reactivation (232). Organ recipients at risk of reactivation should have regular monitoring of blood by quantitative PCR, with treatment based on demonstration of rising parasite load in blood (Table 6) (232, 278). *T. cruzi* reactivation should be included in the differential diagnosis of febrile episodes and apparent rejection crises, and endomyocardial biopsies should be examined for evidence of *T. cruzi* myocarditis in the transplanted heart. Reactivation in an HIV-coinfected patient is treated with standard courses of antitrypanosomal treatment and optimization of antiretroviral therapy (279). The utility of and optimal regimen for secondary prophylaxis are unknown.

HUMAN CHAGAS DISEASE IN THE UNITED STATES

Disease burden among Latin American immigrants

No population-representative data exist to make an unbiased estimate of *T. cruzi* infection prevalence in the United States. Several studies of *T. cruzi* seroprevalence in convenience samples of Latin American immigrants have been conducted. In Los Angeles, 59 (1.24%) of 4,755 Latin-American-born residents had confirmed *T. cruzi* infection (280). The prevalence was higher among participants older than 40 compared to those 18-40 (1.42% vs 0.95%), and higher among immigrants from El Salvador than those from Mexico (3.45% vs 0.79%) (280). A community health clinic-based program to screen Latin American immigrants in East Boston reported an overall prevalence of 0.87% (19/2183), with prevalence rising with age (0/101 [0%]

for age <20, 10/1562 [0.64%] for age 20-39, and 9/507 [1.78%] among those 40 years or older) (281).

Based on the reported number of immigrants from Chagas disease-endemic countries of Latin America and estimated national *T. cruzi* seroprevalence in their countries of origin, there were an estimated 240,000 to 350,000 infected persons in the United States in 2010; the upper end of the range includes an estimate for undocumented immigrants, whereas the lower does not (7). All estimates of Chagas disease burden in the United States have major uncertainties, and the method used for these estimates carries several potential biases. The demographics of Latin American immigrants in the United States may not reflect those of the general population in their countries of origin, and their significant exposure risk ended when they left their endemic home countries years earlier (282). Chagas disease prevalence is highly heterogeneous in endemic countries; depending on geographic sources of immigrants, the prevalence in immigrants could be either higher or lower than the national average. For example, in Spain, Bolivian immigrants appear to have a higher prevalence of Chagas disease than the estimated national prevalence, possibly because they are more likely to come from high prevalence departments such as Cochabamba and Santa Cruz than from low prevalence departments such as Oruro or Potosí (3, 8). Similar systematic information for Mexican and Central American immigrants in the United States is lacking, although data from Los Angeles support the notion that infection prevalence is higher among immigrants from some Mexican states than others (280). Finally, the composition of migrant populations entering the United States has changed in recent years, with a higher proportion of families and children from Central America, compared to earlier migrations in which adult men from Mexico predominated (282). National *T. cruzi* prevalence is higher in El Salvador, Guatemala and Honduras than in Mexico (3), but the younger age of migrants would

731 have the effect of decreasing the likely prevalence in migrants compared to earlier waves of
732 older migrants. The success of vector control programs has dramatically decreased the
733 prevalence of *T. cruzi* infection among children in Latin America over the past 30 years, and the
734 limited data from Boston suggest that pediatric Chagas disease is infrequent in Latin American
735 immigrants in the United States (281).

736 **Autochthonous vector-borne transmission to humans**

737 Available data indicate that autochthonous vector-borne *T. cruzi* transmission to humans
738 is rare in the United States (283, 284). A longitudinal study in repeat blood donors yielded a
739 point estimate of zero incidence, with an upper 95% confidence limit of 0.61 per million (284).
740 House colonization is rare, and vector-human contact occurs primarily in peridomestic areas,
741 when vectors invade houses, or when humans spend time in sylvatic sites with enzootic *T. cruzi*
742 transmission (9, 285).

743 Prior to initiation of blood bank screening in 2007, all reported vector-borne infections in
744 the United States were detected because of acute symptoms and/or the presence of a vector in the
745 vicinity of the case (Table 8). Four infections were reported in Texas, and one each in
746 California, Tennessee and Louisiana. Five of seven cases occurred in infants or small children,
747 and six were in the acute phase at the time of detection. In retrospect, one of the Texas
748 infections, reported to be in a 2- to 3-week old infant with no other details provided, may
749 actually have been congenital (286). In California, a contemporaneous survey demonstrated
750 positive complement fixation results in 6 (2.5%) of 241 residents of the community of the 1982
751 case tested compared to one (0.2%) of 637 persons surveyed in a major urban area (55).

752 Since 2007, blood bank screening has resulted in the publication of an additional 35
753 putative autochthonous *T. cruzi* infections (52, 287-292). All were in the chronic phase and

detected on serological screening by blood centers. States postulated to be sources of infection include Mississippi, Texas, Louisiana, Arizona and California, but with the exception of the CDC investigation (52), all were either individual case reports or focused on a single state, raising the likelihood of sampling bias. The CDC investigation estimated that locally acquired *T. cruzi* infection was likely to account for between 5.5% and 7.5% of confirmed positive blood donations (52). Unlike earlier case reports, all of these putative autochthonous infections were in adults in the chronic phase; thus, the location and timing of transmission events are unknown. Some reports speculate on potential sources of triatomine contact, including hiking, camping, hunting and peri-domestic woodpiles and brush (52, 289, 290, 292). In other cases, infected individuals had exposure in multiple states with known sylvatic cycles and no hypotheses could be formed as to which was the most likely site of acquisition (287, 289).

Blood donor screening and transfusion transmission

Prior to institution of blood donor screening, five transfusion-associated *T. cruzi* infections were documented in the United States, two in 1988, one in 1989, one in 1997 and one in 2002 (Table 9) (9, 293). Look-backs at the recipients of blood components from infected donors identified two additional transmission events in 2004 and 2006, from separate donations from the same donor (294). Most infected recipients had underlying malignancies and were immunosuppressed (9, 294). Donors from the Southern Cone were implicated in 5 of the 6 cases where the source was known. In two cases, the blood component was unknown; in all others, the implicated units were platelets. Based on tracing of 350 recipients of blood components from infected donors, the risk associated with a platelet unit was estimated to be 13.3% (95% confidence interval (CI) 5.6 - 25.7) compared to zero for packed RBCs (95% CI 0 - 0.15) and frozen plasma/cryoprecipitate (95% CI 0 - 3.7) (293). Several of the acutely infected recipients

had severe manifestations of Chagas disease, including acute myocarditis, acute atrioventricular block, severe congestive heart failure, pericarditis with *T. cruzi* in the pericardial fluid and possible meningoencephalitis.

Blood donation screening began in January 2007 using the Ortho ELISA, which had been licensed by FDA the previous month, with the radioimmune precipitation assay (RIPA) as the confirmatory test (295). The FDA licensed the Abbott PRISM chemiluminescent immunoassay (ChLIA) as a screening test in 2010, and the Abbott ESA as a supplemental test in 2011. Guidance from the FDA in 2010 recommended screening of all donations, regardless of previous screening results (296). Universal screening enabled an analysis of results from two or more serial specimens from 4.22 million repeat donors representing 6.06 million person-years of follow-up (284, 297). No incident *T. cruzi* infections were detected, corresponding to zero autochthonous transmission incidence, with an upper 95% confidence limit of 0.61 incident case per million person-years (284). In 2017, final FDA guidance endorsed a one-time screening approach, recommended against use of donor questions to assess risk based on low sensitivity, and required further testing of screen-positive donations with the Abbott ESA (298, 299). Screening was estimated to cover 75 to 90% of the U.S. blood supply as of 2008 (301).

Several ancillary studies were conducted during the early years of blood donor screening. In an analysis of approximately 14 million blood donations in 2008, the overall seroprevalence was 1/27,500, with the highest rates in Florida (1/3800), followed by California (1/8300) (301). Of 104 *T. cruzi*-infected donors with epidemiological data, 29 (28%) were born in Mexico, 27 (26%) in the United States, 17 (16%) in El Salvador and 11 (11%) in Bolivia; the remaining 20 donors were born in nine other countries of Central and South America. In a subsequent study of 22 million donations collected between 2007 and 2011, 717 donations were confirmed

seropositive by RIPA, corresponding to a seropositive rate of 1/31,000 (222). Among 263 donors who provided 30-cc blood specimens, 18 (6.8%) had positive results by hemoculture and 17 had parasite genotyping results. Only two (1.3%) of 157 donors from areas where TcI predominates (Mexico, Central America and northern South America) had positive hemocultures (both TcI). By contrast, *T. cruzi* grew in cultures from 13 (34.2%) of 38 donors from the Southern Cone (one TcII, ten TcV, one TcVI, one not typed). Three donors born in the United States had positive results by hemoculture, two TcV and one TcVI; no data were available to determine the likely source of their infections. Together with the predominance of Southern Cone donors implicated in recognized transfusion transmissions, these data support the hypothesis that TcII/V/VI infections result in higher parasite loads, and therefore higher blood-borne transmission risk, compared to TcI (222, 293).

As of August 1, 2019, a total of 2434 confirmed seropositive donors in 47 states have been detected in screening (302). Over the period 2007 to 2016, the mean prevalence in first-time donors was 64 per million donors overall and 3.64 per 10,000 donors in southern California, and showed a non-significant decreasing trend (284). The highest number of positive donations by calendar year was 420 in 2008; since 2014, yearly detections have ranged from 84 to 98 (302).

Organ donor-derived transmission and organ donor screening

A total of 14 investigations, involving organs from 14 *T. cruzi* infected donors transplanted to 32 recipients between 2001 and 2011, were reported in a recent review (28). Transmission occurred to 9 recipients of organs from six donors; no transmission occurred from the remaining 8 donors (Table 10) (28). Transmission risk differs by organ type: 3 (75%) of 4 heart, 2 (20%) of 10 liver and 2 (13%) of 15 kidney recipients became infected.

824 The earliest reported instances of transmission in 2001 and 2006 were not suspected until
825 at least one recipient presented with symptomatic acute Chagas disease (303, 304). More
826 recently, some organ procurement organizations have begun selective or universal screening of
827 donated organs (30, 305). Four subsequent published transmission events (in a liver recipient in
828 2006, two heart recipients in 2006 and 2010, and a bilateral lung recipient in 2011) were detected
829 through systematic laboratory monitoring. Three of these patients were treated and survived
830 their *T. cruzi* infection (28). The bilateral lung transplant recipient died two years post
831 transplantation from respiratory failure; his *T. cruzi* PCR was intermittently positive despite
832 prolonged benznidazole therapy, and Chagas disease was considered a possible contributing
833 factor in his death (28, 306).
834 In the US, screening of donors has been based largely on risk assessment; donors born in or
835 with significant periods of residence in Latin America, born of women from Latin America and/or
836 noted to have clinical findings such as cardiomegaly consistent with Chagas disease, should be
837 screened by IgG serology (30). Some organ procurement organizations contract with a blood bank
838 for donor testing, as the Abbott PRISM and Ortho ELISA have approval for use in specimens from
839 living and cadaveric organ donors (238).
840 The recipient of an infected organ donor should be monitored by microscopy and/or PCR
841 in serial specimens (28, 30). Seroconversion may be delayed or never occur in
842 immunocompromised individuals. Positive results by PCR occur days to weeks before parasites
843 are detectable by microscopy (225). The incubation period of transplant-transmitted *T. cruzi*
844 infection is typically 2-3 months, but detection may be delayed as long as 6 months (28). A
845 frequently recommended monitoring schedule consists of weekly specimens for two months,
846 every 2 weeks up to 4 months, then monthly afterwards (Table 6) (28, 30). In the absence of

847 other indications and assuming no evidence of infection has been detected, the monitoring
848 interval can be lengthened after six months post-transplantation.

849 **Chagas cardiomyopathy and heart transplantation for Chagas heart disease in the US**

850 Chagas heart disease has been recognized in U.S. health care facilities for nearly 30 years
851 (307). Ten years ago, we estimated that there were 30,000 to 45,000 patients with Chagas
852 cardiomyopathy in the United States (308). Recent studies have confirmed that Chagas disease is
853 frequent among Latin American-born patients with cardiac signs: 13% (5/39) of patients with left
854 ventricular ejection fraction (LVEF) <45% without evidence of ischemic heart disease in New
855 York City (309); 19% (25/135) of patients with LVEF <40% without evidence of ischemic heart
856 disease in Los Angeles (310); 5.3% (17/327) patients with any bundle branch block in Los Angeles
857 (311); 7.5% (6/80) patients with pacemaker implantation in Los Angeles (312). As in studies
858 from Latin America (313, 314), the most common conduction system abnormalities were right
859 bundle branch block, left anterior hemiblock and the combination of the two (311, 315), and
860 Chagas cardiomyopathy was associated with more rapid progression than other cardiac etiologies
861 to severe disease requiring transplantation or resulting in death (310, 315, 316). Data from 17
862 Texas blood donors suggest that locally-acquired Chagas disease can also result in
863 cardiomyopathy, but data are insufficient to assess the relative risk for autochthonous vs imported
864 infection (288).

865 In Brazil and Argentina, heart transplantation is an accepted modality to treat end-stage
866 Chagas cardiomyopathy, and survival for those transplanted for Chagas heart disease is the same or
867 better than that of recipients of cardiac transplants for other etiologies (207, 208, 317, 318). The
868 incidence of *T. cruzi* reactivation in Latin American heart transplant cohorts varies widely, from
869 20-90% (232). In the United States, data are published for 40 patients who underwent heart

transplantation for end-stage Chagas cardiomyopathy since 2006 (206, 232). In one review from a Los Angeles medical center, 31 of 405 patients who received heart transplants between 2006 and 2012 were born in Latin America; 20 of the 31 had serological testing for *T. cruzi* and 11 (2.7% of the total number of 405) had positive results (206). Only two of the *T. cruzi*-infected transplant recipients received their diagnosis prior to the transplant, both in their home countries. Two (18.2%) patients were diagnosed with *T. cruzi* reactivation when they experienced dysfunction of the transplanted heart; their infections had not previously been suspected, and one died of cardiogenic shock. One additional patient was asymptomatic but treated based on the finding of parasites in the explanted heart. One of the patients in the Los Angeles cohort is included in the CDC's comprehensive review of 31 patients that underwent heart transplantation for Chagas cardiomyopathy from 2012-2016; 19 (61%) had reactivation (232). In the CDC review, reactivation was defined by rising parasite load by quantitative PCR in peripheral blood, a finding that precedes both microscopically detectable parasitemia and development of symptoms (225). Only one instance of reactivation was symptomatic at the time of diagnosis, and all patients with reactivation were alive at the end of follow-up.

Congenital Chagas disease in the United States

Based on births to Latin American-born women in the United States and *T. cruzi* infection prevalence in their home countries, it was estimated that between 60 and 315 congenitally infected infants are born in the United States each year (308). Nevertheless, only two congenital infections have been reported, both in infants of Bolivian women (319, 320). The first infant was delivered by caesarian section at 29 weeks gestation because of fetal hydrops (a classic presentation of severe congenital Chagas disease) (18). The diagnosis was not suspected until the mother reported a prior diagnosis of Chagas disease (320). The second infant was also delivered by caesarian section at 30

weeks due to placental abruption, had a pericardial effusion, ascites and respiratory distress requiring intubation until the 10th day of life (319). The diagnosis was made on histopathological examination of the placenta. Both infants were successfully treated with antitrypanosomal therapy.

SPECIAL CONSIDERATIONS IN THE US

An adequate public health response to Chagas disease in the United States faces critical challenges, including limited patient and provider awareness of the disease; societal, economic and health system barriers to patient access; and sparse diagnostic options with an inadequate evidence base to assess performance.

Physician awareness. Surveys indicate that the majority of physicians practicing in the United States have limited knowledge of Chagas disease and seldom consider the diagnosis, even when caring for Latin American-born patients at high risk or with typical clinical syndromes (321, 322). In one survey, 23% of cardiologists, 47% of obstetrician/gynecologists and 25% of transplantation specialists reported that they had never heard of Chagas disease (321). In a survey of more than 400 obstetrician/gynecologists, 78% reported that they never considered the diagnosis of Chagas disease in their Latin American patients and fewer than 10% were cognizant of the risk of vertical transmission to the infant (322).

Patient awareness and access. Awareness and knowledge of Chagas disease are also limited among those at risk. Of Latin American immigrants interviewed in community outreach settings in southern California, 86% had never heard of Chagas disease, even though 62% reported having seen triatomines in their countries of origin (323). Patients with Chagas disease encounter multiple barriers to health care access in general, not just for Chagas disease: these patients disproportionately live below the poverty line, have less than a high school education, lack health

insurance, and have difficulty taking time off from work for medical appointments (324). Even legal immigrants have lower access than citizens to publically funded health insurance in most states; concerns about revealing immigration status may make patients reluctant to seek care (324).

Diagnostic issues. There are currently four commercial IgG serological tests cleared by FDA for diagnostic use in the United States, three ELISA kits (Hemagen [Hemagen Diagnostics, Waltham MA], Chagatest Recombinante 3.0 [Wiener Laboratories, Rosario, Argentina] and Ortho [Ortho-Clinical Diagnostics, Inc, Raritan, NJ]) and one point-of-care test (ChagasDetectPlus [InBios International, Seattle, WA]) (325). With the exception of the Ortho ELISA, which is also licensed for blood donor screening (222, 284, 326), there is a paucity of data on the performance of these assays in specimens from populations in the US, or in the likely predominant countries of origin of infected U.S. residents (Mexico, El Salvador, Guatemala, Honduras) (280, 301, 308). Discordant serology has been reported as a particular problem in Mexico (327). Some recombinant tests with excellent performance in the Southern Cone show discordance or low sensitivity when applied in some TcI-predominant areas (328, 329). In addition, no commercial laboratory in the United States currently offers more than one validated IgG serological assay. The diagnosis of chronic *T. cruzi* infection requires positive results by two distinct IgG assays and is therefore not possible with commercial testing alone (16). Currently, the only laboratory that conducts multiple IgG serological assays under CLIA is the CDC Division of Parasitic Diseases and Malaria (DPDM) laboratory.

FDA approval of benznidazole and resulting changes. Until May 2018, when benznidazole became commercially available, prescribing antitrypanosomal treatment necessitated a consultation with CDC epidemiologists (330). Confirmation of the diagnosis, recommendations for treatment, and advice on side effects monitoring and management were necessary components of these

consultations, and reporting of adverse events was mandatory under the investigational protocol. Ensuring appropriate diagnostic confirmation, judicious treatment decisions and adequate side effects monitoring going forward will require efforts to raise provider awareness and knowledge about Chagas disease and antitrypanosomal therapy.

From October 2011 to May 2018, CDC released benznidazole for 365 patients with confirmed *T. cruzi* infection (330). Four (1.1%) patients had acute phase infection, two organ-derived, one congenital and one presumed to be vector-borne. Treatment was administered for *T. cruzi* reactivation in 35 (9.6%) patients, comprising 29 organ transplant recipients, five HIV-co-infected patients and one on chemotherapy for malignancy. The vast majority of patients were adults, 236 (64.7%) aged 19-50 and 97 (26.6%) older than 50 years. Only 2 (0.5%) of 365 treated patients were aged 2-12 years, the age range for which FDA approved benznidazole.

Insud Pharma's approach to FDA approval and drug marketing represents a new paradigm in the United States, with patient access and affordability as central concerns (257). The FDA Priority Review Voucher (PRV) program was established in 2008 to provide an incentive for new drug development for neglected tropical diseases (NTD), but has had limited impact and unintended negative consequences (331). In one recent example, FDA approval of miltefosine, a drug used for leishmaniasis, was followed by an astronomical price increase, and the company ceased production after receiving and selling the PRV, leading to a global shortage (332). In contrast, the Insud Pharma / Exeltis Patient Assistance Program, funded in part by the PRV, ensures that the cost to the patient will not exceed $60 per course (257).

Public health surveillance and response. As of 2017, Chagas disease was a reportable condition in six states, Arizona, Arkansas, Louisiana, Mississippi, Tennessee and Texas (333); Utah added Chagas disease to its notifiable disease list recently. All of these states except

Arkansas and Utah have had published reports of locally-acquired *T. cruzi* infection (52-54, 287, 289, 290). Most of the states focus on autochthonous transmission and incident cases, and several have provisions for submission of triatomines for identification (333). In Texas, the Department of State Health Services (DSHS) provides extensive on-line guidance and data to health care providers and the public, including a summary of reported Chagas disease case data; of 124 cases reported from 2013-2017, all were chronic infections and 22 were judged to have resulted from local transmission (334). These figures likely overlap substantially with the published locally acquired infections detected in blood donors in recent years (289, 290). The Texas Chagas Taskforce, which includes the DSHS and several universities, addresses many public health aspects of Chagas disease, including provider and patient resources, and educational materials on local vectors (335).

PUBLIC HEALTH APPROACHES

Public health approaches to Chagas disease comprise primary prevention (prevention of transmission), secondary prevention (early treatment of infection to prevent sequelae) and tertiary prevention (medical and surgical management of morbidity to improve survival and quality of life) (Table 11) (318). In Latin America, primary prevention through vector control is responsible for the vast majority of the decrease in estimated annual incidence from 500,000 in 1991 to 30,000 today (35). Primary prevention through vector control is virtually impossible in the United States since the vectors are sylvatic; however, barriers to house invasion, elimination of microhabitats close to houses, and improved awareness among those living in areas of risk could help decrease the likelihood of autochthonous transmission. Blood bank screening has also been highly

985 successful in both Latin America and the United States. The last detected blood component-
986 derived infection in the United States occurred in 2006, before screening was instituted (284, 294).
987 In Latin America, secondary prevention efforts include congenital Chagas disease
988 screening programs, and mass testing and treatment of children in high prevalence zones (270,
989 336). Prenatal and congenital screening programs are attractive for several reasons. Cure rates are
990 >90% in infected infants and drug tolerance is excellent (264, 265); treatment of infected women,
991 once lactation ends, significantly decreases the risk of transmission in future pregnancies (195);
992 and detection of maternal infection provides a screening opportunity for her other children, who
993 are also at risk for *T. cruzi* infection (196). However, congenital screening programs are also
994 complicated. With current diagnostic modalities, effective congenital Chagas disease screening
995 requires a multistep algorithm consisting of prenatal serology in women and testing of infants of
996 seropositive mothers 2 or 3 times (parasitological or molecular testing in the first months of life,
997 and for those who test negative, serological testing at 9-12 months) (223). Even in Bolivia, where
998 infection prevalence in women is often 15% or higher and awareness is high, congenital Chagas
999 disease detection is challenging, because of low sensitivity of microscopy and >80% loss to
1000 follow-up for the 9-12 month visit (337).
1001 A pilot study in a hospital in Houston screened 4000 women, of whom 75% were born in
1002 Latin America (338). Ten (0.25%) women had confirmed *T. cruzi* infection; no infected infants
1003 were detected. A recent analysis concluded that in the United States, universal congenital Chagas
1004 disease screening and treatment would be cost-saving with congenital transmission rates $\geq$ 0.001%
1005 and maternal prevalence >0.06% (339). The results vary substantially depending on the cost and
1006 performance of the maternal screening test; thus, it will be essential to ascertain the currently
1007 unknown sensitivity of available serological assays in at-risk populations in the United States.

The effectiveness of secondary prevention strategies depends strongly on the effectiveness of antitrypanosomal treatment to prevent development and progression of cardiac disease, which remains a controversial topic without a clear answer (276). Current practice in Latin America prioritizes diagnosis and treatment of children 15 years old or younger, but the vast majority of infected individuals in the United States are adults. In the sparse available community screening data, the *T. cruzi* prevalence in Latin American adults 40 years or younger was 0.64 to 0.95% compared to 1.42 to 1.78% among those older 40 years (280, 281). In limited community screening to date, no infections have been detected among children (281).

Tertiary prevention has had a major impact on the survival and quality of life of persons living with *T. cruzi* infection in Latin America (340), and the experience of a dedicated center of excellence based in the cardiology service of a large Los Angeles hospital confirms this model in the United States (310, 311, 341-343). Expanding this effort will require outreach efforts to cardiologists and primary care physicians, and making accurate diagnostic testing more widely available.

CONCLUSIONS

Chagas disease causes disease of the heart and/or gastrointestinal tract in 20 to 30% of those infected by *T. cruzi*. The southern half of the United States contains enzootic cycles of *T. cruzi*, involving 4 major and 7 minor triatomine vector species. *T. cruzi* infection has been detected in multiple mammalian species, including raccoons, opossums, woodrats and dogs. Locally acquired Chagas disease has been increasingly recognized in the United States over the past 10 years, largely due to screening of blood donations and investigations of infected blood donors without exposure in Latin America. Nevertheless, imported chronic *T. cruzi* infections in

migrants from Latin America vastly outnumber autochthonous human cases, and locally acquired infection is rarely detected in the acute phase. Benznidazole is now FDA-approved, and clinical and public health efforts are underway by researchers and some state health departments to broaden access to diagnosis and treatment.

Making progress will require work on many fronts, including innovative ways to improve the knowledge base among providers, expand availability of high quality diagnostic and confirmatory testing, and pilot public health screening data to develop evidence-based targeting strategies. However, increased awareness of Chagas disease is crucial to all aspects of this effort. Providers with awareness of the disease can screen those at risk when they present for clinical care, with the highest priority for children and women of child-bearing age, since the benefit of antitrypanosomal therapy is clear for these groups. The appropriate index of suspicion saves lives when reactivation of chronic infection and donor-derived *T. cruzi* are recognized in a timely fashion. Diagnosis of *T. cruzi* infection and follow-up for onset or progression of cardiomyopathy or gastrointestinal disease can mitigate morbidity and improve survival and quality of life.

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FIGURE LEGENDS

Figure 1. *Trypanosoma cruzi* morphological forms: (A) The replicating epimastigote form in culture (Giemsa stain). (B) Trypomastigote in a peripheral blood smear from a patient with acute Chagas disease (Giemsa stain). (C) Nest of amastigotes within a cardiac myocyte in a patient with chronic Chagas disease (hematoxylin-eosin). Courtesy of the Division of Parasitic Diseases and Malaria, US Centers for Disease Control and Prevention.

Figure 2. Photographs of U.S. triatomine species of the genera *Triatoma* and *Paratriatoma*. Image size relative to the scale bar represents the average length of each species. Courtesy of E. Barrera Vargas (*Triatoma incrassata*), R. Hoey-Chamberlain and C. Weirauch (*T. recurva, Paratriatoma hirsuta*), G. Lawrence (DPDM/CDC) (*T. protracta protracta*); S. Kjos (all other species).

Figure 3. Range of the four most frequent triatomine species in the continental U.S. Based on references provided in Table 2.

Table 1. Countries endemic for Chagas disease, and estimates of national seroprevalence and number of infected inhabitants. Vector-borne *T. cruzi* transmission occurs, or occurred until recently, in parts of these countries.

		Estimated *T. cruzi* infection prevalence[1]	
Region	Country	%	N
North America	United States	NDA	240,000 to 350,000[2]
	Mexico	0.779%	876,458
Central America	Belize	0.330%	1,040
	Costa Rica	0.170%	7,667
	El Salvador	1.298%	90,222
	Guatemala	1.230%	166,667
	Honduras	0.918%	73,333
	Nicaragua	0.523%	29,300
	Panama	0.515%	18,337
South America	Argentina	3.641%	1,505,235
	Bolivia	6.104%	607,186
	Brazil	0.606%	1,156,821
	Chile	0.700%	119,660
	Colombia	0.956%	437,960
	Ecuador	1.380%	199,872
	French Guyana & Surinam	0.839%	12,600
	Paraguay	2.130%	184,669
	Peru	0.440%	127,282
	Uruguay	0.238%	7,852
	Venezuela	0.710%	193,339
Total		1.056%	5,742,167[3]

[1]Disease burden estimates are for the year 2010, based on references (3, 7). NDA = no data available.
[2]The figure for the United States reflects the estimated number of infected immigrants from endemic countries of Latin America. No estimate of locally-acquired infections is currently available.
[3]Excluding the United States.

2244 Table 2. Triatomine vectors in the United States[1]

Species	Frequency of collection	Range[2]	Ecological associations	*T. cruzi* prevalence
T. sanguisuga	Frequent	AL, AR, DE, FL, GA, IL, IN, KS, KY, LA, MD, MO, MS, NC, NJ, OH, OK, PA, SC, TN, TX, VA, WV	Highly diverse; woodrats, other rodents, armadillos, opossums, dogs, chickens, horses; frequent in peridomestic settings; invades houses	Moderate prevalence, very widespread
T. gerstaeckeri	Very frequent	Eastern NM, central TX	Sylvatic and peridomestic settings, dog kennels, rodent burrows; frequently invades houses	High prevalence, especially in dog kennel collections
T. protracta	Frequent	AZ, CA, CO, NM, NV, west TX, UT	Close association with woodrats (*Neotoma* spp); attracted by lights	Moderate prevalence, widespread
T. rubida	Frequent	AZ, southern CA, NM, southwest TX	Woodrat nests, disturbed environments in AZ and Mexico; reports of house colonization in Sonora, Mexico	Usually low, but focal collections with high prevalence
T. lecticularia	Infrequent	FL, GA, MO, NM, OK, SC, TN, TX, UT[3]	Houses, dog kennels, woodrat nests in TX; peridomestic settings	Can be high in collections from woodrat nests
T. indictiva	Infrequent	AZ, NM, TX	Found in woodrat nests and near lights	Moderate in sparse data
T. recurva	Infrequent	Southern half of AZ	Associated with rodents, especially rock squirrels	Low to moderate
T. neotomae	Rare	TX	Found in woodrat nests	Can be high in collections from woodrat nests
T. incrassata	Rare	Southern AZ	Unknown	No naturally infected specimen reported
P. hirsuta	Rare	CA, AZ, NV	Found in woodrat nests, near lights, and invading houses	No naturally infected specimen reported
T. rubrofasciata	Rare	Jacksonville FL, Honolulu HI	Roof rats (*Rattus rattus*); found in houses in FL and HI, chicken coops in HI	2 infected bugs in HI report

2245 [1]Based on our review of literature from 1939 to 2011 (9) plus new data in (48, 49, 344).
2246 [2]Frequency and range based on published reports; absence of reports from a given area often reflects lack of field research rather than true absence of vectors. Ranges of
2247 all species except *T. rubrofasciata* extend into Mexico.
2248 [3]Several other states are listed for *T. lenticularia* in (47) and reproduced by (285), but were not confirmed in our 2011 review (9). We follow the approach advocated by
2249 Ryckman (51) in which reports prior to Usinger 1944 are treated with caution in the absence of later verification. UT added based on the recent report in (344).

2250 Table 3. Triatomine vectors and *Trypanosoma cruzi*-infected mammals by state in published reports.

State	Vectors reported	*T. cruzi*-infected vectors	*T. cruzi*-infected wildlife	*T. cruzi*-infected dogs
AL	*T. sanguisuga*	Yes	Raccoon, opossum	
AR	*T. sanguisuga*			
AZ	*T. protracta, T. rubida, T. indictiva, T. recurva, T. incrassata, P. hirsuta*	Yes	Raccoon, ringtail, skunk, woodrats, other rodents	
CA	*T. protracta, T. rubida, P. hirsuta*	Yes	Skunk, woodrats, other rodents	Yes
CO	*T. protracta*			
DE	*T. sanguisuga*			
FL	*T. sanguisuga, T. lecticularia, T. rubrofasciata*	Yes	Raccoon, opossum, skunk, gray fox	
GA	*T. sanguisuga, T. lecticularia*	Yes	Raccoon, opossum, skunk, gray fox, bobcat, coyote, feral swine	Yes
HI	*T. rubrofasciata*	Yes		
IL	*T. sanguisuga*			
IN	*T. sanguisuga*	Yes		
KS	*T. sanguisuga*	Yes		
KY	*T. sanguisuga*		Raccoon, opossum	
LA	*T. sanguisuga*	Yes	Opossum, nine-banded armadillo	Yes
MD	*T. sanguisuga*		Raccoon, opossum	
MO	*T. sanguisuga, T. lecticularia*	Yes	Raccoon	
MS	*T. sanguisuga*	Yes		
NC	*T. sanguisuga*		Raccoon, opossum	
NJ	*T. sanguisuga*			
NM	*T. lecticularia, T. protracta, T. gerstaeckeri, T. rubida, T. indictiva*	Yes	Woodrats, other rodents	
NV	*T. protracta, P. hirsuta*			
OH	*T. sanguisuga*			
OK	*T. sanguisuga, T. lecticularia*	Yes	Raccoon, opossum	Yes
PA	*T. sanguisuga*			
SC	*T. sanguisuga, T. lecticularia*		Gray fox	Yes
TN	*T. sanguisuga, T. lecticularia*	Yes	Raccoon	Yes
TX	*T. sanguisuga, T. lecticularia, T. protracta, T. gerstaeckeri, T. rubida, T. indictiva, T. neotomae*	Yes	Raccoon, opossum, nine-banded armadillo, skunk, American badger, coyote, woodrats, other rodents, bat	Yes
UT	*T. protracta, T. lecticularia*			
VA	*T. sanguisuga*	Yes	Raccoon, opossum, coyote	Yes
WV	*T. sanguisuga*			

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2252 Table 4. *Trypanosoma cruzi* genotypes reported in triatomine vectors in the United States

Location	Vector species	Total examined	*T. cruzi* + N (%)	Typed N	TcI n (%)	TcIV n (%)	TcI/IV n (%)	Genotyping method	Reference/source
TX	*T. gerstaekeri*	16	16 (100)	16	10 (63)	4 (25)	2 (13)	SL-IR; TcSC5D and SNPs in subset	(90)
TX	*T. gerstaekeri*	897	574 (64)	548	294 (54)	189 (34)	65 (12)	TcSC5D; SL-IR on subset	(159)
S. TX	*T. gerstaekeri*	18	9 (50)	9	6 (67)	1 (11)	2 (22)	SL-IR	(92)
TX	*T. gerstaekeri*	11	1 (9)	NR	100%		NR	SL-IR	(160)
TX	*T. gerstaekeri*	NR	NR	3	2 (67)	0	1 (33)	SL-IR, 24S α rRNA, 18S rRNA	(122)
TX	*T. gerstaekeri*	19	13 (100)	13	13 (100)	0	0	18S rRNA sequencing	(73)
TX	*T. gerstaekeri*			1	1 (100)	0	0	SL-IR	(345)
TX	*T. indictiva*	67	32 (48)	28	9 (32)	17 (61)	2 (7)	TcSC5D; SL-IR on subset	(159)
TX	*T. lenticularia*	66	44 (67)	42	9 (21)	25 (60)	8 (19)	TcSC5D; SL-IR on subset	(159)
TX	*T. lenticularia*	2	2 (100)	2	2 (100)			18S rRNA sequencing	(73)
TX	*T. protracta*	19	3 (16)	2	2 (100)	0	0	TcSC5D; SL-IR on subset	(159)
Southwest[3]	*T. protracta*	14	1 (7)	1	1 (100)	0	0	TcSC5D; SL-IR on subset	(159)
N. CA	*T. protracta*	29	16 (55)	13	13 (100)	0	0	RFLP (HPS60, GPI), SL-IR, 24S α rRNA, sequencing of Rb19, TR and COII-ND1	(50)
S. CA	*T. protracta*	68	21 (31)	9	7 (78)	2 (22)	0	RFLP (HPS60, GPI), SL-IR, 24S α rRNA, sequencing of Rb19, TR and COII-ND1	(50)
S. CA	*T. protracta*	161	34 (21.1)	2[5]	0	0	0	24S α RNA	(161)
TX	*T. protracta*	9	4 (44)	NR	100%		NR	SL-IR	(160)
TX	*T. rubida*	64	11 (17)	7	6 (86)	1 (14)	0	TcSC5D; SL-IR on subset	(159)
Southwest[4]	*T. rubida*	40	7 (18)	5	5 (100)	0	0	TcSC5D; SL-IR on subset	(159)
S. TX	*T. rubida*	2	0	0	0	0	0	SL-IR	(92)
TX	*T. rubida*	299	69 (23)	NR	100%		NR	SL-IR	(160)
W. TX	*T. rubida*	39	24 (62)	24	24 (100)	0	0	TcSC5D	(158)
TX	*T. sanguisuga*	20	13 (65)	13	2 (15)	9 (69)	2 (15)	SL-IR; TcSC5D and SNPs in subset	(90)
TX	*T. sanguisuga*	315	158 (50)	135	21 (16)	107 (79)	7 (5)	TcSC5D; SL-IR on subset	(159)
Southeast[1]	*T. sanguisuga*	45	12 (27)	12	2 (17)	10 (83)	0	TcSC5D; SL-IR on subset	(159)
Midwest[2]	*T. sanguisuga*	7	4 (57)	3	0	3 (100)	0	TcSC5D; SL-IR on subset	(159)
FL, GA	*T. sanguisuga*			4	4 (100)	0	0	SL-IR, 24S α rRNA, 18S	(122)

LA	*T. sanguisuga*	12	8 (67)	6	6 (100)	0	0	rRNA SL-IR, 24S α rRNA, 18S rRNA	(80)

[1]AL, FL, GA, KY, LA, NC, TN, VA. [2]IN, KS, MO, OH, OK. [3]AZ, CA, NM. [4]AZ, NM. [5]Typed as II/VI

2254 Table 5. *Trypanosoma cruzi* genotypes reported in mammalian hosts in the United States

Locations	Host species	Total Genotyped	TcI (%)	TcIV (%)	TcI/IV (%)	Other reported	Genotyping method	Reference/source
CA (2), LA (1), TX (2)	Humans	5	5 (100)	0	0		SL-IR, 24S a rRNA, 18S rRNA	(122)
TX	Humans	6	0	0	0	TcII-V-VI (4), TcI/TcII-V-VI (2)*	PCR-RFLP (SL-IR, 24S a rRNA, 18S rRNA), sequencing	(157)
CA (1), OK (1), SC (2), TN (1), Unknown (2)	*Canis lupus familiaris* (domestic dog)	7	0	6 (86)	1 (14)		SL-IR, 24S a rRNA, 18S rRNA	(122)
TX	*Canis lupus familiaris* (domestic dog)	2	1 (50)	0	1 (50)		SL-IR	(92)
TX	*Canis lupus familiaris* (domestic dog)	15	9 (60)	5 (33)	1 (7)		SL-IR, sequencing of TcSC5D	(90)
TX	*Canis lupus familiaris* (domestic dog)	4	4 (100)	0	0		SL-IR	(345)
TX	*Canis lupus familiaris* (domestic dog)	6	5 (83)	1 (17)	0		SL-IR, 24S a rRNA, 18S rRNA, COII	(91)
FL (16), GA (45), MD (1), TN (1), SC (1)	*Procyon lotor* (raccoon)	64	2 (3)	61 (95)	1 (2)		SL-IR, 24S a rRNA, 18S rRNA	(122)
GA	*Procyon lotor* (raccoon)	5	0	5 (100)	0		SL-IR, confirmatory sequencing	(72)
TX	*Procyon lotor* (raccoon)	11	10 (91)	0	1 (9)		TcSC5D	(77)
TX	*Procyon lotor* (raccoon)	2	0	2 (100)	0		SL-IR, 24S a rRNA, 18S rRNA, COII	(346)
IL	*Procyon lotor* (raccoon)	5	0	5 (100)	0		SL-IR, 24S a rRNA, confirmatory sequencing	(347)
KY	*Procyon lotor* (raccoon)	2	0	2 (100)	0		SL-IR, 24S a rRNA, confirmatory sequencing	(347)
MO	*Procyon lotor* (raccoon)	1	0	1 (100)	0		SL-IR, 24S a rRNA, confirmatory sequencing	(347)

GA	*Lemur catta* (ring-tailed lemur)	3	0	3 (100)	0	SL-IR, 24S a rRNA, 18S rRNA	(122)
GA (1), Unknown (1)	*Macaca mulatta* (rhesus macaque)	2	1 (50)	0	1 (50)	SL-IR, 24S a rRNA, 18S rRNA	(122)
Tx	*Macaca mulatta* (rhesus macaque)	33	18 (55)	13 (39)	2 (6)	SL-IR, 24S a rRNA, 18S rRNA, COII	(346)
AL (1), FL (6), GA (6), LA (2)	*Didelphis virginiana* (opossum)	15	15 (100)	0	0	SL-IR, 24S a rRNA, 18S rRNA	(122)
TX	*Didelphis virginiana* (opossum)	4	4	0	0	SL-IR, 24S a rRNA, 18S rRNA, COII	(346)
GA (1), LA (2)	*Dasypus novemcinctus* (nine-banded armadillo)	3	2 (67)	1 (33)	0	SL-IR, 24S a rRNA, 18S rRNA	(122)
GA	*Mephitis mephitis* (striped skunk)	1	0	1 (100)	0	SL-IR, 24S a rRNA, 18S rRNA	(122)
GA	*Mephitis mephitis* (striped skunk)	4	1 (25)	3 (75)	0	SL-IR, confirmatory sequencing	(72)
TX	*Mephitis mephitis* (striped skunk)	2	1 (50)	1 (50)	0	SL-IR, 24S a rRNA, 18S rRNA, COII	(346)
TX	*Neotoma micropus* (Southern plains woodrat)	23	10 (43)	13 (57)	0	SL-IR, confirmatory sequencing	(72)
TX	*Neotoma micropus* (Southern plains woodrat)	1	1 (100)	0	0	18S rRNA sequencing	Aleman
GA	*Sigmodon hispidus* (hispid cotton rat)	2	0	2 (100)	0	SL-IR, confirmatory sequencing	(72)
GA	*Otospermophilus variegatus* (rock squirrel)	1	0	1 (100)	0	SL-IR, confirmatory sequencing	(72)
TX	*Peromyscus leucopus* (white-footed mouse)	3	3 (100)	0	0	18S rRNA sequencing	(73)
TX	*Chaetodipus hispidus* (hispid pocked mouse)	1	1 (100)	0	0	18S rRNA sequencing	(73)
TX	*Sigmodon hispidus* (hispid cotton rat)	1	1 (!00)	0	0	18S rRNA sequencing	(73)

TX	*Baiomys taylori* (northern pygmy mouse)	1	1 (100)	0	0		18S rRNA sequencing	(73)
TX	*Liomys irroratus* (Mexican spiny pocket mouse)	1	1 (100)	0	0		18S rRNA sequencing	(73)
LA	*Peromyscus gossypinus* and *Mus musculus* (mouse spp)	20	16 (80)	0	0	TcII (2), TcI/TcII (1), TcII/TcIV (1)	SL-IR, 24S a rRNA, 18S rRNA	(80)
LA	*Neotoma floridana*	3	2 (67)	0	0	TcII/TcIV (1)	SL-IR, 24S a rRNA, 18S rRNA	(80)
TX	*Nycticeius humeralis* (evening bat)	1	1 (100)	0	0		SL-IR, 24S a rRNA, 18S rRNA, COII	(74)

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Table 6. Chagas disease diagnostic testing by clinical context

Clinical Scenario	Testing modalities, specimen and schedule
Suspected chronic *T. cruzi* infection	
All persons (symptomatic or symptomatic) with epidemiological risk factors[1]; high priority to screen children and women of child-bearing age especially if pregnant or planning pregnancy	IgG serology[2] by two distinct assays, preferably based on different antigens (16)
Persons at risk for acute *T. cruzi* infection	
Suspected contact with infected vector	PCR (microscopy)[3] in blood between 2 and 8 weeks post exposure, IgG serology at 6 to 8 weeks
Infant of *T. cruzi*-infected mother	PCR (microscopy)[3] in blood at birth and 1-3 months; IgG serology at 9-12 months (223)
Recipient of blood components, organ or tissue from infected donor	Serial PCR in blood: Months 1-2: weekly, months 3-4: every 2 weeks, months 5-6: monthly, then based on clinical scenario (30)
Laboratory accident	Serial PCR (microscopy)[3] in blood weekly for 6-8 weeks, IgG serology at 6 to 8 weeks (348)
Persons at risk for *T. cruzi* reactivation	
Prospective organ or tissue recipient with risk factors	IgG serology by two distinct assays (349, 350)
Transplant recipient with chronic *T. cruzi* infection	Serial quantitative PCR in blood[4]: Months 1-2: weekly, months 3-4: every 2 weeks, months 5-6: monthly, then based on clinical scenario (210) For heart transplant patients, histology in endomyocardial biopsy, especially in setting of suspected rejection
HIV-*T. cruzi* co-infected patient with signs of reactivation	PCR[4], microscopy in tissue, blood, CSF as clinically indicated
T. cruzi infected patient with iatrogenic immunosuppression (chemotherapy, corticosteroids) and signs of reactivation	PCR[4], microscopy in tissue, blood, CSF as clinically indicated

[1]Epidemiological risk factors for *T. cruzi* infection include birth or residence, or maternal birth or residence, in a country with endemic vectorial transmission (see Table 1); residence in areas of the US with high rates of vector-human contact, especially if the patient reports triatomine bites and/or house invasion.
[2]Plasma is not an approved biospecimen for some FDA-cleared tests; serum is acceptable for all FDA-cleared tests.
[3]PCR is substantially more sensitive than microscopy in peripheral blood.
[4]Positive PCR in blood does not diagnose reactivation; rising parasite load in blood is generally the first indication. Positive PCR in CSF indicates reactivation.

Table 7. Recommendations for antitrypanosomal drug treatment according to Chagas disease phase and form, patient age, and clinical status.

Antitrypanosomal drug treatment by Chagas disease phase, form and demographic group	Strength of recommendation; quality of evidence[1]
Should always be offered	
Acute *T. cruzi* infection (including congenital infection in first months of life)	Strong; moderate
Children ≤ 12 years old with chronic *T. cruzi* infection	Strong; high
Children 13 - 18 years old with chronic *T. cruzi* infection	Strong; low
Reactivated *T. cruzi* infection in immunosuppressed patient	Strong; moderate
Reproductive-age women planning future pregnancies	Strong; moderate
May be offered with consideration of potential risks and benefits, uncertainties and patient preferences	
Adults with normal ECG and cardiac function	Discretionary; weak
Adults with early signs of cardiomyopathy	Discretionary; weak
Recommendation against treatment	
During pregnancy	Strong; weak
During lactation	Weak; weak
Patients with advanced cardiomyopathy	Strong; moderate
Patients with gastrointestinal Chagas disease that impairs absorption	Weak; weak

[1]Based on the Grading of Recommendations Assessment, Development and Evaluation (GRADE) system (351). The GRADE system offers only two grades of recommendations: "strong" and "weak" or "discretionary". Strong recommendations are provided when the balance of desirable vs undesirable effects is clear. Weak or discretionary recommendations require an assessment of the evidence, and decision-making based on a consideration of potential risks and benefits, uncertainties and patient preferences (352).

2270 Table 8. Reported autochthonous vector-borne *Trypanosoma cruzi* infection in the United States

Residence State	Age	Sex	Diagnosis	Phase	Detection year	Infection year	Evidence of autochthonous vector-borne origin; putative state of acquisition	Reference/source
TX	10 months	F	microscopy of peripheral blood	acute	1955	1955	Peridomestic infestation; TX	(353)
TX	2-3 weeks	M	not reported	acute	1955	1955	No details provided - perhaps congenital, given reported age; TX	(286)
CA	56 years	F	microscopy of peripheral blood	acute	1982	1982	Adult uninfected *T. protracta* in house; CA	(55, 354)
TX	7 months	M	histology of cardiac tissue	acute	1983	1983	No vectors found, but search made in winter; house in poor condition; TX	(355)
TN	18 months	M	T. cruzi PCR in peripheral blood	acute	1998	1998	*T. cruzi*-infected *T sanguisuga* found in child's crib; TN	(54)
TX	12 months	M	microscopy of pericardial fluid	acute	2006	2006	Mother uninfected; *T. cruzi*-infected *T. gerstaeckeri* near house; TX	(58)
LA	74 years	F	Serology and hemoculture	chronic	2006	unknown	*T. sanguisuga* infestation; 10/18 positive by *T. cruzi* PCR; LA	(53)
MS	44 years	M	Blood donor screening	chronic	2007	unknown	*T sanguisuga* found on property; also extensive hunting of known Tc reservoir species; MS	(52)
NR[1]	NR[1]	NR[1]	14 cases detected on blood donor screening	chronic	2006-2010	unknown	Blood donors not from Latin America and not primarily Spanish speaking	(52)
TX	59 years	M	Blood donor screening	chronic	2007	unknown	rural TX including deer hunting in a place with infected *T. gerstaekeri*	(289)
TX	69 years	M	Blood donor screening	chronic	2007	unknown	rural TX, some travel to Mexico	(289)
TX	47 years	F	Blood donor screening	chronic	2007	unknown	residence in rural TX and LA	(289)
TX	72 years	M	Blood donor screening	chronic	2010	unknown	residence in rural TX	(289)
TX	21 years	M	Blood donor screening	chronic	2011	unknown	extensive camping in TX and MO	(289)

TX	83 years	M	Blood donor screening	chronic	NR[1]	unknown	former/current residence considered high risk; TX	(290)
TX	61 years	F	Blood donor screening	chronic	NR[1]	unknown	former/current residence considered high risk; TX	(290)
TX	71 years	M	Blood donor screening	chronic	NR[1]	unknown	occupation considered high risk; TX	(290)
TX	NR[1]	NR[1]	Blood donor screening	chronic	NR[1]	unknown	camping considered likely risk; TX	(290)
TX	19 years	M	Blood donor screening	chronic	NR[1]	unknown	former/current residence considered high risk; TX	(290)
TX	60 years	M	Blood donor screening	chronic	NR[1]	unknown	former/current residence considered high risk; TX	(290)
TX	56 years	F	Blood donor screening	chronic	NR[1]	unknown	former/current residence considered high risk; TX	(290)
TX	52 years	M	Blood donor screening	chronic	NR[1]	unknown	current residence, occupation, hunting considered moderate risk; TX	(290)
TX	25 years	F	Blood donor screening	chronic	NR[1]	unknown	former/current residence considered high risk; TX	(290)
TX	51 years	F	Blood donor screening	chronic	NR[1]	unknown	former/current residence considered high risk; TX	(290)
TX	52 years	F	Blood donor screening	chronic	NR[1]	unknown	former/current residence considered high risk; TX	(290)
CA	19 years	M	Blood donor screening	chronic	2009	unknown	lack of international travel, extensive camping history; TX	(292)
TX	28 years	M	Blood donor screening	chronic	2014	unknown	Reported vectors near childhood home in AZ; TX resident at time of detection	(291)
AZ	16 years	F	Blood donor screening	chronic	NR[1]	unknown	T. cruzi infected *T. rubida* found near home; AZ	(287)

[1]NR, not reported

2273 Table 9. Published reports of transfusion-related *Trypanosoma cruzi* transmission in the United States.

Year of transmission	State	Recipient characteristics	Implicated blood component, donor origin	Reference/source
1988	NY	11-year-old girl with Hodgkin lymphoma, developed fever and pericarditis, trypomastigotes seen on blood smear; treated with nifurtimox and recovered	Platelets, Bolivia	(357)
1988	CA	17-year-old male post bone marrow transplant with fulminant acute Chagas disease	Not specified, Mexico	(358)
1989	TX	59-year-old female with metastatic colon cancer on chemotherapy, granulocytopenic, disseminated intravascular coagulation; developed fever, pulmonary infiltrates, bradycardia and AV block; parasites seen on bone marrow aspirate; died within 36 hours of diagnosis	Unknown; had received >500 units including RBC, platelets	(359)
1997	FL	60-year-old female with multiple myeloma; *T. cruzi*-infected donor unit detected during research study; recipient asymptomatic, treated with nifurtimox; died of underlying disease several years later.	Platelets, Chile	(360)
2002	RI	3-year-old female with Stage 4 neuroblastoma on chemotherapy, neutropenic, fever, trypomastigotes seen on blood smear; treated with nifurtimox but died of her underlying disease	Platelets, Bolivia	(361)
2004	NY	64-year-old male with non-Hodgkins lymphoma and chemotherapy induced thrombocytopenia; found on serological testing during look-back study	Platelets, Argentina	(294)
2006	NY	62-year-old male found on serological testing during look-back study	Platelets, Argentina	(294)

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2277 Table 10. Published reports of organ transplant-derived cases of Chagas disease in the United States.
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Year	State of organ harvest	Donor origin	Implicated organ	Recipient characteristics and outcome	Reference/source
2001	GA	El Salvador	Kidney-pancreas	37-year-old female with fever 6 weeks post transplant and *T. cruzi* on blood smear, died of Chagas myocarditis 7 months post transplant despite prolonged course of nifurtimox	(303)
2001	GA	El Salvador	Kidney	69-year-old female, asymptomatic, *T. cruzi* hemoculture positive; diagnosis sought because of recipient 1 above; treated with nifurtimox, survived.	(303)
2001	GA	El Salvador	Liver	32-year-old female, asymptomatic, *T. cruzi* hemoculture positive; diagnosis sought because of recipient 1 above; treated with nifurtimox but died of unrelated causes.	(303)
2005	CA	US-born (mother from Mexico)	Heart	64-year-old male with anorexia, fever, diarrhea diagnosed with organ rejection treated with steroids; 8 weeks post-transplant *T. cruzi* found on blood smear. PCRs became negative on nifurtimox. Died of rejection 20 weeks post-transplant.	(304)
2006	CA	El Salvador	Heart	73-year-old male with fever, fatigue, rash, *T. cruzi* on blood smear 7 weeks post-transplant; parasitemia cleared with nifurtimox; switched to benznidazole because of tremors. Died of heart failure 25 weeks post-transplant.	(304)
2006	PA	Bolivia	Liver	56-year-old male detected on PCR monitoring; died from GI bleed 244 weeks post-transplant.	(28)
2006	PA	Bolivia	Bilateral kidney	73-year-old female detected on PCR monitoring; died from kidney failure 15 weeks post-transplant.	(28)
2010	NY	Mexico	Heart	20-year-old female detected on PCR monitoring and successfully treated with benznidazole; survived at least to 24 months post-transplant.	(28)
2011	TN	El Salvador	Bilateral lung	36-year-old male with cystic fibrosis; detected on PCR monitoring. Completed course of benznidazole but intermittent post-treatment positive PCR. Chagas disease possible contributing factor to death 2 years post-transplant.	(306)

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2281 Table 11. Public health screening options for Chagas disease in the United States

Target population	Screening Methods	Primary goal	Secondary goal	Intervention details and effectiveness	Published estimates of screening yield
Blood donors	Serologic screening	Prevent transmission	Refer infected persons for management	Discard screen-positive donations; highly effective	~1/15,000 first-time donors, up to 1/2700 in high risk area (284)
Organ donors	Screening, serologic or risk based	Prevent transmission		Heart from infected donor not used; use of other organs with appropriate monitoring; highly effective	0.9% in combined risk-based and serologic donor screening (305)
Pregnant women from Latin America; infants of infected women	Maternal serology, serial testing of infants; serology in siblings	Detect infected infants early in life	Refer infected women and their other children for treatment	Early treatment of infants; treatment of women after lactation ends; treat infected siblings; highly effective in infants and children, moderate in young women	~10 mothers, <1 infected child per 4000 high risk women (majority born in Latin America) (338)
Latin American immigrants	Serological screening and confirmatory testing	Detect asymptomatic infected individuals		Treatment of infected individuals; effectiveness high in children, uncertain in adults	0.5 to 1% in high risk populations; many of those detected were >50 in whom treatment not generally recommended (280, 281)
Patients from Latin America with non-ischemic cardiac syndromes	Serological screening and confirmatory testing	Detect symptomatic infected individuals		Standard cardiac management; if transplant recipient, prospective monitoring for reactivation; effective in improving survival and quality of life	Latin American-born patients with bundle branch blocks, 5%; pacemakers, 7.5%; depressed LV ejection fraction, 13-19% (309-312)

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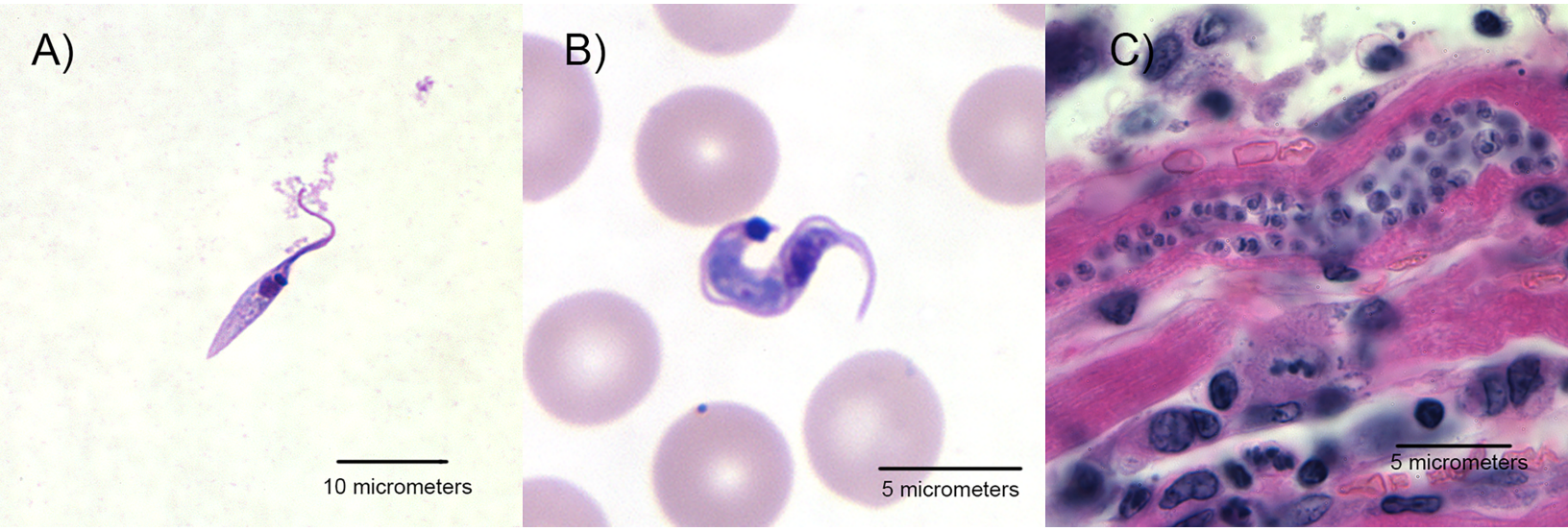
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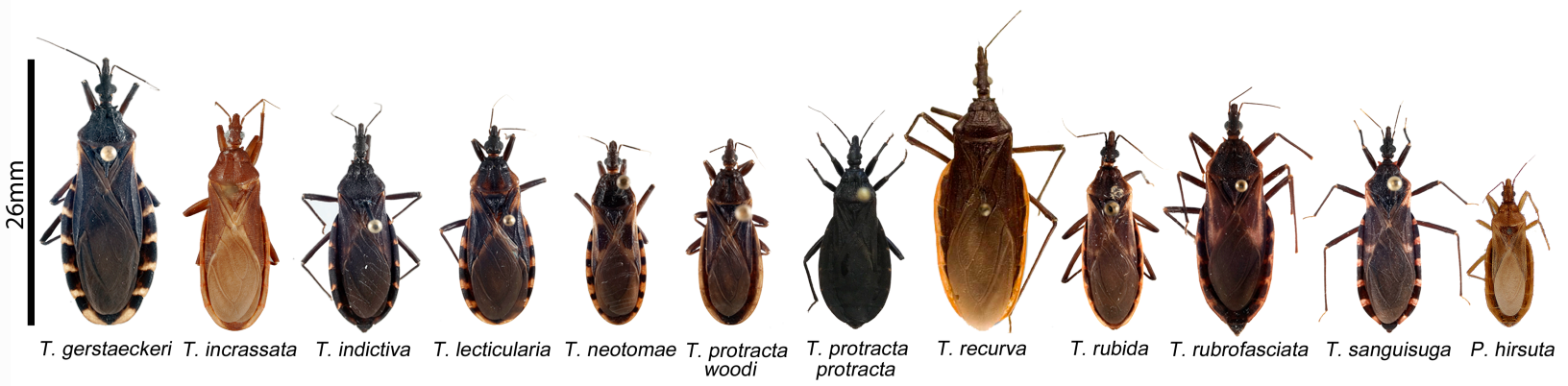
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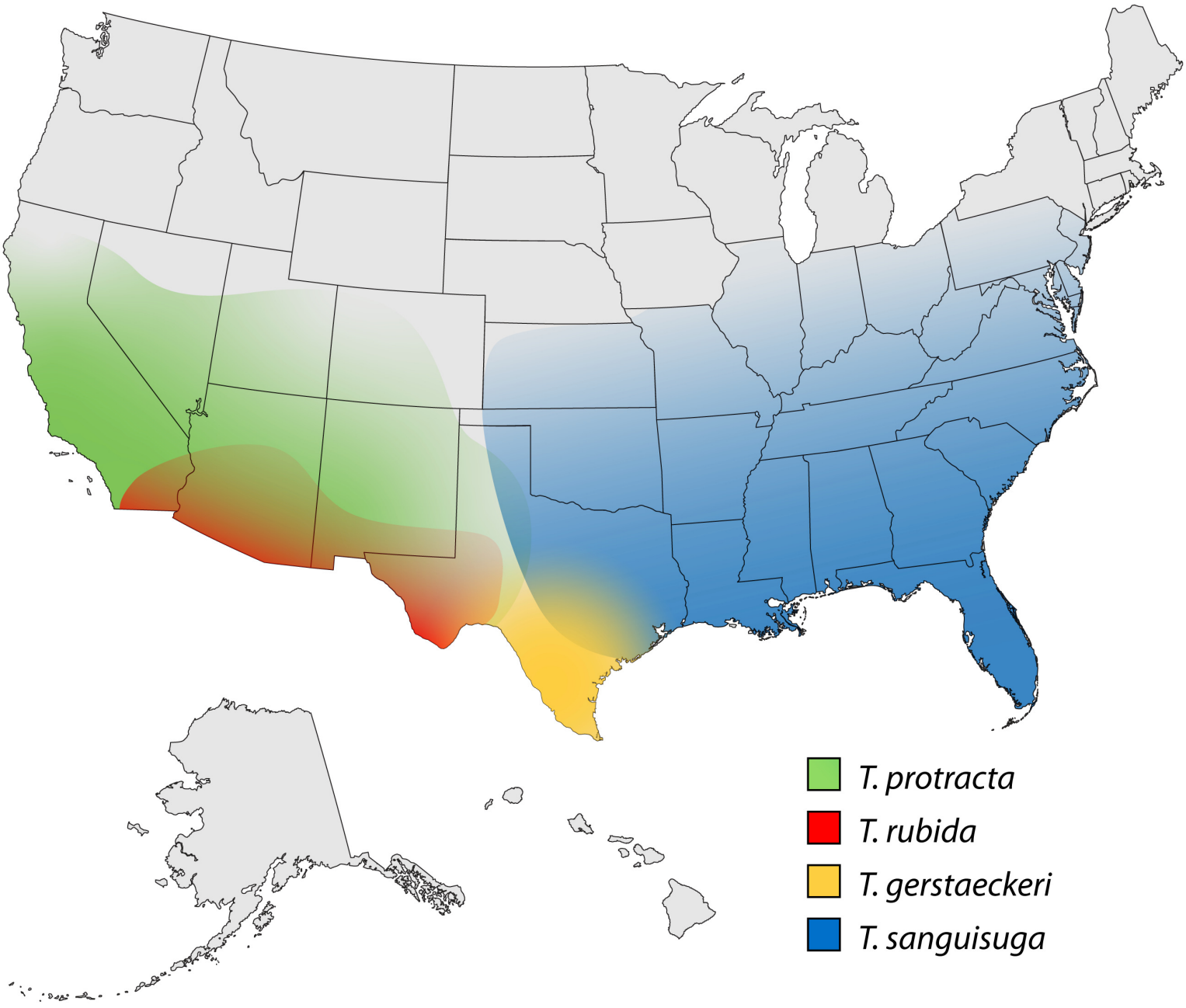
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T. rubida
T. gerstaeckeri
T. sanguisuga

AUTHOR BIOGRAPHIES

Caryn Bern is Professor of Epidemiology and Biostatistics in the School of Medicine, University of California, San Francisco. She holds an MD from Stanford University School of Medicine and an MPH from the Johns Hopkins University School of Public Health, and is board-certified in internal medicine. She was a medical epidemiologist at the Centers for Disease Control and Prevention from 1990 to 2011 and worked for 15 years in the CDC Division of Parasitic Diseases and Malaria. Dr. Bern has worked on clinical and epidemiological aspects of Chagas disease since 2003, with projects in Peru, Bolivia and the United States. Dr. Bern's current research focuses on diagnosis, treatment, epidemiology and control of Chagas disease and visceral leishmaniasis.

Louisa A. Messenger is an Assistant Professor in the Department of Disease Control of the London School of Hygiene and Tropical Medicine. She holds a BA from the University of Cambridge and an MSc in Medical Parasitology and PhD in Molecular Biology from the London School of Hygiene and Tropical Medicine. She has been a recipient of the L'Oréal-UNESCO For Women in Science UK and Ireland Fellowship and an American Society for Microbiology/Centers for Disease Control and Prevention Fellowship. Dr. Messenger has lived and worked extensively in South America and Africa to improve methods of Chagas disease and malaria control, respectively.

Jeffrey Whitman is currently a postdoctoral research fellow in the Department of Laboratory Medicine at the University of California, San Francisco School of Medicine. He has an MS in biochemistry and molecular biology from the University of California, Riverside and MD

from the David Geffen School of Medicine at UCLA. His research interests include improving diagnostic disparities in global health settings with a methodological focus on mass spectrometry-based metabolomics and proteomics. He is currently pursuing research related to improving Chagas disease diagnostics.

James H. Maguire is Professor of Medicine, Harvard Medical School, and Senior Physician, Brigham and Women's Hospital, Boston, MA. He completed his MD from Harvard Medical School and MPH from Harvard School of Public Health, and is board-certified in internal medicine and infectious diseases. He was the chief of CDC Parasitic Diseases Branch from 2001 to 2005. He was Professor and Head, Division of International Health, Department of Epidemiology and Preventive Medicine, University of Maryland School of Medicine from 2005 to 2008. Dr. Maguire performed seminal research in the epidemiology and cardiac manifestations of Chagas disease in rural Brazil in the 1980s, and collaborated on studies of Chagas disease in Peru and Bolivia from 2003 to the present.