Current Tropical Medicine Reports (2022) 9:140–149
https://doi.org/10.1007/s40475-022-00260-x

EMERGING VECTOR BORNE DISEASES IN THE U.S. (JK PETERSON, SECTION EDITOR)

Chagas Disease in the Southeastern USA

Samuel B. Jameson[1,2] · Erin Cloherty[3,4] · Berlin Londono-Renteria[1,2] · Dawn M. Wesson[1,2]

Accepted: 30 May 2022 / Published online: 12 September 2022

Abstract

Purpose of Review In this review, we provide an update on recent research surrounding Chagas disease in the southeastern USA. We identify current gaps in knowledge, suggest areas for future research, and provide short reports of previously unpublished work.

Recent Findings *Trypanosoma cruzi* has a greater genetic diversity in the region than previously thought, including genotypes previously thought to be confined to South America. *Paratriatoma lecticularia*, a regional triatomine bug vector of *T. cruzi*, has undergone a genus-level reclassification. Deep sequencing technologies in *T. cruzi* genetics and blood meal analyses have shown promise as a method for examining complex ecological cycles. Fatal Chagas disease in imported exotic animals in Mississippi was identified, and the lack of record keeping of human Chagas disease cases in the region has been investigated finding that 94.8% of confirmed human Chagas cases voluntarily reported from blood banks were not recorded by any state or federal public health agency in the region. Additionally, an ecological framework for the kissing bug *Triatoma sanguisuga* as a nidicolous insect is proposed.

Summary Recent investigations of *T. cruzi* and its insect vectors in the southeastern USA have furthered the understanding of this native vector-borne disease system in the region. New technologies and conceptual frameworks are generating new questions and routes of inquiry that may lead to a more comprehensive understanding of Chagas disease in the Southeast.

Keywords *Trypanosoma cruzi* · Southeastern USA · Chagas disease · Triatomine bugs · *Triatoma sanguisuga* · *Paratriatoma lecticularia*

Introduction

A great deal about Chagas disease in the southeastern USA remains uncovered. Gaps in regional Chagas disease knowledge extend across the entire disease system: from parasite to insect vector to animal and human disease. In the Southeast, there has been a steady, albeit small, stream of research in this topic for more than 100 years [1–6]. However, in the past decade the body of research focusing on all aspects of this system has accelerated. This review covers each aspect of the Chagas disease system and provides updates and previously unpublished reports in some key areas. For the purposes of this article, the southeastern USA is defined as the states of Arkansas, Louisiana, Mississippi, Tennessee, Kentucky, Alabama, Georgia, Florida, North Carolina, South Carolina, Virginia, and Maryland (Fig. 1). Data from outside of the region are included only when necessary, and the term "kissing bug" is used as a general term for any member of the sub-family Triatominae.

Trypanosoma cruzi in the Southeastern USA

Trypanosoma cruzi is native to regions spanning both American continents, including the southeastern USA. In this region, *T. cruzi* naturally infects the triatomine bug species *Triatoma sanguisuga* and *Paratriatoma lecticularia* as well as a wide

This article is part of the Topical Collection on *Emerging Vector Borne Diseases in the U.S.*

✉ Samuel B. Jameson
sbishop@tulane.edu

[1] Department of Tropical Medicine, Tulane University, New Orleans, LA, USA

[2] Vector-Borne Infectious Disease Research Center, Tulane University, 1430 Tulane Ave, New Orleans, LA 70112, USA

[3] Termite, and Rodent Control Board, City of New Orleans - New Orleans Mosquito, New Orleans, LA, USA

[4] Department of Entomology, Louisiana State University, Baton Rouge, LA, USA

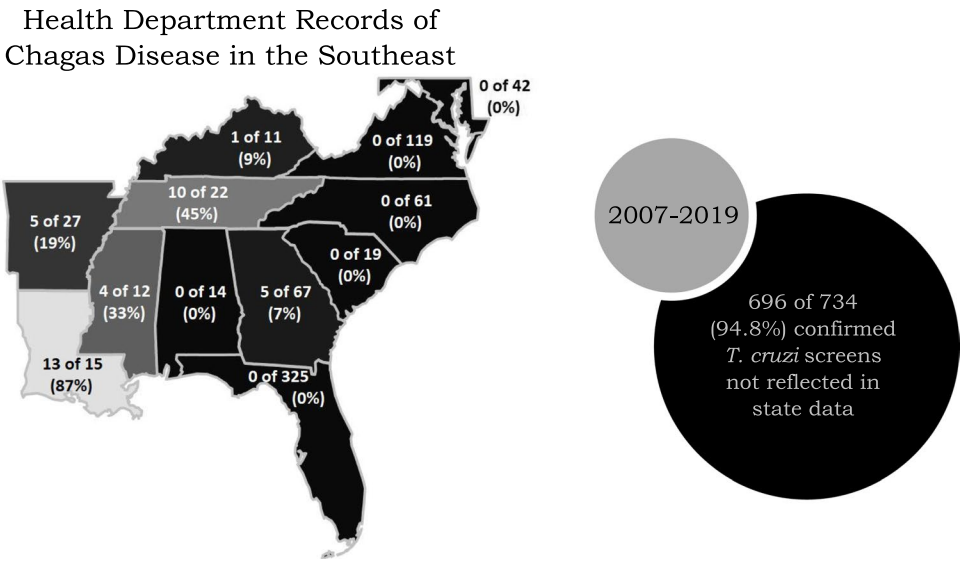

Fig. 1 A choropleth map of the southeastern USA demonstrating the number of human Chagas disease cases known to that state's health department, the number of confirmed human Chagas disease cases documented by the AABB Chagas Biovigilance Network, and the resulting percentage of health department identified cases. Florida and Virginia rank 2nd and 5th highest, respectively, in the nation-wide AABB confirmed Chagas case counts between 2007 and 2019. Neither state maintains public health records of any of these cases

range of mammals (covered in subsequent sections) [7, 8]. In recent years research into the genetics of *T. cruzi* in the USA has increased, particularly in the Southeast. The most significant recent development in this area is the identification of discrete typing units (DTUs) that were previously considered absent in the USA.

T. cruzi is currently classified into DTUs rather than clades [9]. Although the potential for genetic exchange exists and some degree of genetic recombination within DTUs has been observed, *T. cruzi* is considered to be a clonal organism given that its observed means of replication is through binary fission [10, 11, 12]. Additionally, *T. cruzi* exhibits a reticulate evolutionary pattern. This reticulate evolutionary pattern coupled with low levels of genetic exchange renders the classification of this parasite into clades impossible (9). However, there are identifiable, stable genetic groupings of *T. cruzi* that are deemed DTUs. Currently, *T. cruzi* is divided into DTUs classified as TcI through TcVI and a seventh group named TcBAT. It was previously believed that each DTU largely followed confined geographic distributions (9). However, this understanding is currently being reevaluated. The first identification of TcII in the USA was from a rodent collected near New Orleans, LA, in 2015 [7]. Increased diversity of circulating DTUs in the wider USA was the confirmed in locally acquired human Chagas cases in Texas in 2017 [13].

Research in *T. cruzi* DTUs took a significant step forward in 2020 with a next-generation deep sequencing approach. Deep sequencing is the process of sequencing the same region of a nucleic acid target hundreds or thousands of times. This technology increases the ability of researchers to identify infrequent sequence variations withing a sample. A deep sequencing workflow was used to identify circulating *T. cruzi* parasites in the Southeast from TcI, TcII, TcIV, TcV, and TcVI [14••, 15•, 16, 17, 18••, 19]. This DTU diversity is notable given that less than 10 years ago TcII, TcV, and TcVI were thought to be confined to the Southern Cone countries of South America [9]. Increased primary sample collections and sub-DTU genetic classification are likely to aid in identifying independent or overlapping cycles of *T. cruzi* transmission within a given sylvatic system. A better understanding of these sylvatic transmission cycles will clarify the risk to human and animal health posed by this parasite in the southeastern USA.

Insect Vectors of *T. cruzi* in the Southeast

Three competent vectors of *T. cruzi* are known to occur in the Southeast: *Paratriatoma lecticularia*, *Triatoma sanguisuga*, and *Triatoma rubrofasciata*. The latter species will not be covered here due to its limited distribution in southern Florida.

Paratriatoma Lecticularia

While this species has long been recognized as a triatomine bug species native to the USA, our understanding of its genetics, distribution, and animal associations have undergone a significant shift in recent years.

The most significant change occurred in 2021 with the formal assignation of *Triatoma lecticularia* to the genus *Paratriatoma* [20••]. This genus-level change is the result of years of consistent research on the genetics of North American Triatominae. Since Lent and Wygodzinsky's seminal "Revision of the Triatominae" published in 1979, *P. lecticularia* has been considered a member of the Lecticularia

species complex [21, 22]. In 2014, phylogenetic analysis based on six separate molecular sequences found that *P. lecticularia* grouped with the southwestern species *Paratriatoma hirsuta* rather than with the *Triatoma* spp. [23]. That same year, modern genetic and morphometric methods were used to suggest that the Lecticularia species complex be dissolved, and its members be included in more similar species complexes. Under this proposed system *P. lecticularia* was not able to be classified into a *Triatoma* species complex [24]. Additional evidence for the reclassification was found in the karyotypes of each species. *P. lecticularia* and *P. hirsuta* are the only kissing bug species in the USA that have an undivided X chromosome (20 autosomes (A) + XY). The *Triatoma* spp. found in the USA all have a divided X chromosome ($20A + X_1X_2Y$ or $22A + X_1X_2Y$) (25).

In addition to its taxonomic reclassification, the recognized distribution of *P. lecticularia* has been updated. In 2019, CDC distribution maps were published that removed eight of the sixteen states previously reported to contain this species [25]. The updated distribution illustrates the Southeast as a significant portion of the distribution of *P. lecticularia*. Systematic field collections targeting the species are needed in Arkansas, Louisiana, Mississippi, and Alabama to confirm its presence or absence. Currently, these states constitute a void in the middle of *P. lecticularia*'s documented range.

In addition, more data are needed on the basic ecology of the species. There are just a handful of papers regarding *P. lecticularia* ecology, many of which contain sparse data and potentially outdated information. For example, an important and frequently cited source from 1967 used to support *Neotoma* spp. (woodrats) as the primary hosts for *P. lecticularia* do not provide any data relating *P. lecticularia* to *Neotoma* spp. and mention the bug species only as being present in a hollow log [26]. More recent reports of *P. lecticularia* which found this species associated with *Neotoma* spp. and dogs in Texas [27, 28] might be more accurate sources of information on *P. lecticularia* ecology, but more studies on the species in other parts of the region are needed in order to fully characterize its epidemiologic importance.

Triatoma Sanguisuga

T. sanguisuga is the most widely distributed Chagas disease vector species in the USA, as well as the predominant species in the Southeast [29]. Given the wide geographic range of *T. sanguisuga*, this species has been studied for more than 100 years [1]. Recent advances have been made in two of areas of this kissing bug's biology that have long been poorly understood: [1] the ecological niche inhabited by *T. sanguisuga* in the Southeast and [2] the feeding habits of the species in the region.

Throughout the literature, reports of the natural history of *T. sanguisuga* are biased toward anecdotal evidence from experiences with adult specimens. The bias toward adult specimens misses much of the natural history of the species because the time between eclosion and the final molt into the adult stage can last up to 2 years [2, 30]. The length of time spent in immature stages is dependent on what time of year eclosion occurs and the proportion of the year that is warm enough to allow for feeding [2]. While no empirically determined feeding threshold is available in the literature, Grundemann found *T. sanguisuga* to be active and feeding from mid-March to mid-November in Manhattan, KS. Climate records indicate that the average daily temperature at the beginning and end of this active season is approximately 13 °C [31]. During this immature period, the wingless nymphal stages require access to vertebrate blood approximately every 30–45 days during the active season [2].

An unpublished observational report from Bayou Sauvage National Wildlife Refuge in Orleans Parish, LA, has been instrumental in shaping the authors' conceptions of *T. sanguisuga* in nature. Briefly, a long-abandoned honeybee hive near Bayou Sauvage was identified as containing more than 100 T. *sanguisuga* across every stage of development. The bugs were located in the base of the hive, in cracks in the wood, and in the bedding of what appeared to be a woodrat nest. The hive was removed from over the rodent bedding and roughly half of the observable *T. sanguisuga* were collected. Return trips were made weekly to the site with continued collections of bugs. Over four sequential weeks of collections, *T. sanguisuga* nymphs were located in the rodent bedding despite being left open to the elements and, presumably, without their rodent host. Weekly systematic searches up to 20 feet away from the hive site did not locate any kissing bugs dispersing.

The observation of *T. sanguisuga* nymphs remaining in the nest even when exposed to the elements and without a host taken together with the documented nutritional requirements of the species suggests that new insights might be gained by considering *T. sanguisuga* using a nidicolous ectoparasitic species framework with late nymphal and adult stages that may disperse to find a new nest to inhabit [2, 30]. By adopting an animal nest-based constraint for *T. sanguisuga*, it is possible to reevaluate and potentially improve ecological niche models for this species. An active area of research is improving upon the current published species distribution model for *T. sanguisuga* in the Southeast (Jameson 2017, Ibarra-Cerdeña, et al. 2009) as this model relies on published occurrence data from regions that are not representative of the full range of the species. Moreover, the model is a composite model of the former *lecticularia* complex (*P. lecticularia*, *T. indictiva*, *T. incrassata*, and *T. sanguisuga*) [22] resulting in an ecological niche model that does not include southern Louisiana despite hundreds

of primary collections of the species in the region [7, 14••, 32, 33, 34, 35]. The absence of *T. sanguisuga* from coastal areas, like southern Louisiana, in the published model is likely the result of few collection points from the southeastern USA coupled with the use of the Genetic Algorithm for Rule-set Prediction (GARP). The dataset used to generate the GARP model included published *T. sanguisuga* collections from 1955 to 2006 in the USA. There were few published occurrences of *T. sanguisuga* in southern Louisiana prior to the publication of an autochthonous case of human Chagas disease in New Orleans, LA, in 2007 [6]. Given the limited available dataset, the GARP algorithm was unlikely to produce accurate ecological niche models. While GARP is a powerful tool for generating presence-only distribution models, when it is applied to regions with little or no collection data the resulting model reflects the potential suitable habitat for the species in the new region based on the sampled region [36]. These factors resulted in a model that was as accurate as possible at the time of analysis and would benefit from the addition of recent collection data. Adopting a nidicolous ectoparasitic framework for this species may allow for improved species distribution modeling of *T. sanguisuga* by constraining these models to the geographic overlap of suitable habitat for *T. sanguisuga* and its associated nest-dwelling mammals.

T. sanguisuga: Using Blood Meal Analyses to Understand its Behavioral Ecology

Research suggests that *T. sanguisuga* is an opportunistic feeder with generalist tendencies. Blood meal analysis has demonstrated that *T. sanguisuga* feeds on amphibians (including bullfrogs and treefrogs), reptiles (including skinks, geckos, and anoles), birds (including chickens and kites), and mammals (including humans, squirrels, pigs, cows, cats, raccoons, opossums, mice, and rats; [2, 14••, 16, 35]. It is important to note that amphibians, reptiles, and birds are refractory to *T. cruzi* infection and do not contribute to sylvatic transmission cycles [14••].

In the past 5–10 years, interest has increased in using molecular analysis of kissing bug blood meals to infer triatomine bug behavior and expose potential *T. cruzi* transmission dynamics within an environment. The underlying idea of this approach is that by characterizing *T. cruzi* DTUs and blood meals from a large number of sylvatic triatomine bugs, a network model can be constructed that approximates animal-triatomine interactions and, consequently, the potential for passage and maintenance of *T. cruzi* in a sylvatic environment.

Multiple methods of varied resolution are used for identifying blood meal sources, and an increasingly nuanced picture of triatomine bug feeding tendencies has emerged. In 2013, Kjos et al. utilized vertebrate and mammalian cytochrome b primers to identify blood meals from *T. sanguisuga* in Texas using conventional PCR [37]. This study was followed in 2014 by Waleckx et al. who achieved increased sensitivity compared to Kjos et al. by amplifying a highly conserved and species-specific mitochondrial gene and sequencing the amplicons to identify the animal source of the genes [34]. In 2017, Jameson demonstrated the potential for high-throughput blood meal analysis from *T. sanguisuga* by amplifying a species-specific mammalian gene in a high-resolution melt analysis assay to identify clusters of identical amplicons. A single sample from each cluster was sequenced to identify the species of the blood meal for that cluster [35]. In 2020, Dumonteil et al. achieved vastly increased sensitivity compared to previous *T. sanguisuga* blood meal analysis methods by leveraging Illumina's MiSeq platform. Briefly, tagged sequences of the 12S rRNA gene were deep sequenced at a depth of 1000–100,000 reads per marker. These data were able to detect multiple blood meal sources and the relative abundance of each source from single bugs. This wealth of data was used to construct network models that demonstrated both feeding and *T. cruzi* transmission networks [18••]. In all cases, the results of the blood meal analyses were strongly biased by the environments from which the bugs were collected. For example, Jameson found cow blood meals in *T. sanguisuga* collected next to a cow pasture and raccoon and cat blood meals from *T. sanguisuga* collected near human houses [35]. Similarly, Dumonteil et al. found that dogs were the primary blood meal source from *T. sanguisuga* collected from an animal shelter and humans were the primary blood meal source from *T. sanguisuga* collected from human homes [14••]. These findings suggest that blood feeding habits of adult *T. sanguisuga* may be driven by opportunity rather than by specific host-seeking behaviors.

T. sanguisuga: Epidemiologic Importance

Despite early reports of "painful" and "dangerous" bites, the bite of *T. sanguisuga* is painless [1, 38], though repeated exposure to bites may increase immune sensitivity to the saliva and cause increasingly severe allergic and hypersensitivity reactions [38]. This species can harbor high rates of *T. cruzi* infection; multiple studies have confirmed that between 55.1 and 62.4% of *T. sanguisuga* in southeastern Louisiana harbor *T. cruzi* [32, 33, 34]. Taken together, there exists the potential for vector-borne transmission of *T. cruzi* from *T. sanguisuga* in the region. Delayed and hypersensitive reactions to bites may complicate epidemiologic investigations into potential exposures.

Despite a lack of records of *T. sanguisuga* colonizing human homes [39], there are reports within the past 5 years of *T. sanguisuga* feeding on humans in Mississippi, South Carolina, and Delaware [40, 41, 42]. In wooded suburban

and rural areas of the southeast, adult *T. sanguisuga* disperse on summer nights and are attracted to artificial lights [2]. The dispersing adults wander in search of a blood meal and, potentially, shelter. The bugs may feed on companion animals or livestock sleeping outdoors or, conversely, they may be predated on by companion animals or livestock. In either case, peridomestic animals can become infected with *T. cruzi* if they come into contact with the feces of a *T. sanguisuga* carrying the parasite. Dispersed *T. sanguisuga* may also enter homes and feed on sleeping humans. Although the use of air conditioning does not eliminate the risk of *T. sanguisuga* invading a given home, air conditioning has been found to be significantly negatively associated with the presence of this species in homes in southeastern Louisiana [33].

Regardless of the environment, *T. sanguisuga* has been found containing human DNA in multiple studies [14••, 34, 35, 37]. While this species undoubtedly opportunistically feeds on humans, blood meal analysis results demonstrating human DNA should be viewed with increased scrutiny given the ubiquity of ambient human DNA and the potential for contamination. Unfortunately, the potential for contamination is not always considered [34]. Reliance on strict protocol adherence to avoid contamination may not be sufficient, as erroneous human DNA can be amplified during arthropod blood meal analysis studies in controlled laboratory settings [43]. An excellent example of contextualizing suspicious human blood meal results from kissing bugs can be found in Kjos et al. [37]. If protocol controls and contextualization cannot provide sufficient clarity, a final option for detecting human DNA contamination is human DNA typing. Miniature short tandem repeats (mini-STR) from human positive meals have been found to be satisfactory for this purpose [44]. Mini-STR profiles for human blood meal samples can be generated using high-resolution melt analysis or capillary electrophoresis. By comparing the obtained profiles, contamination can be easily identified (e.g., the same profile appearing in geographically disparate sites). Additionally, control profiles can be easily generated for individuals involved in field collections and laboratory procedures as well as for residents of homes concerned about insect bites without the need for generating or storing sequence-level data.

While some newer molecular methods like high-resolution melt analysis and Illumina's MiSeq platform require significant resources and infrastructure, routine *T. cruzi* detection [45], DTU assignment [46], and blood meal amplification [47] can all be performed with traditional PCR reactions. Mini-STR profiles [44] may be generated using traditional PCR products separated by polyacrylamide gel electrophoresis. Though prices will change over time and by location, published estimates of the cost to use the different technologies are provided below to provide context for the breadth of cost differences for different methods (costs do not include the price of the instrument): using traditional *Taq*-based PCR as reference [48]: conserved target PCR followed by sequencing—approximately 4 × reference cost per sample (traditional PCR + 1 forward reaction and 1 reverse reaction needed for each sequence); high-resolution melt analysis—approximately 1.02 × reference cost per sample (difference in instrument cost with traditional PCR is substantial); Illumina MiSeq—approximately 475–630 × reference method per run. Tagging systems can be used to add multiple samples to a single run to reduce costs [49].

Trypanosoma cruzi as a Pathogen of Veterinary Importance

There are over 100 mammalian hosts capable of harboring *T. cruzi*, and the parasite has a long documented history of infecting both sylvatic and domestic animal species in

Table 1 Summary of *Trypanosoma cruzi* prevalence in wild and domestic animals in the Southeast

	Prevalence	References
Wild		
Raccoon (*Procyon lotor*)	12.1% (FL)–66.7% (TN)	(3, 72)
Opossum (*Didelphis virginiana*)	8.3% (NC)–51.9% (FL)	(73, 74)
Eastern woodrat (*Neotoma floridana*)	73.3% (LA)	(16)
Mouse (*Mus musculus*, *Peromyscus gossypinus*)	77.3% (LA)	(16)
Coyote (*Canis latrans*)	7% (GA)–9.5% (TN)	(58, 75)
White-tailed deer (*Odocoileus virginianus*)	0.3% (TX)	(76)
Nine-banded armadillo (*Dasypus novemcinctus*)	1.0 (LA)–37.5% (LA)	(4, 5)
Gray fox (*Urocyon cinereoargenteus*)	0% (GA)–18.2% (VA)	(74, 77)
Bobcat (*Lynx rufus*)	3.2% (GA)	(74)
Feral swine (*Sus scrofa*)	0% (GA)	(74)
Domestic		
Dog (*Canis familiaris*)	15.7% (LA)	(59)
Cat (*Felis catus*)	24.6% (LA)	(19)

the USA [50]. Bern et al. and Hodo and Hamer reviewed the published literature and summarized the prevalence of *T. cruzi* among mammals within many southeastern states [50, 51], so here we provide a few brief updates on *T. cruzi* prevalence in a few key animal species (Table 1), as well as two unpublished cases of *T. cruzi* infection in imported exotic animals in the Southeast.

Raccoons and opossums are often found to be infected with *T. cruzi* in the southeastern USA [14••, 52•, 53]. Given the widespread presence of the parasite in these animal species and their presence in blood meal analysis studies of *T. sanguisuga*, it is possible that these animals serve as sylvatic reservoirs for the parasite [14••, 52•]. Further studies examining the transmission dynamics of *T. cruzi* in sylvatic populations of raccoons and opossums will be needed to determine if these species act as maintenance reservoirs for *T. cruzi* in the Southeast.

White-tailed deer (*Odocoileus* virginianus) in Texas have been identified as carrying *T. cruzi*. Although the overall prevalence was very low (0.3%, $n=314$), the public health implications are significant in that there were 8.1 million white-tailed deer hunters in 2016 [54]. Transmission of *T. cruzi* could pose a risk to hunters during the field dressing process of an infected deer, as it has been found that hunters and trappers infrequently use gloves or other personal protective equipment during this process [55]. Studies of deer in southeastern states will be needed to determine the potential *T. cruzi* infection risk posed by deer to hunters in the region.

An important distinction that has been noted in the veterinary field, as in human medicine, is the apparent test discordance between diagnostic serologic tests and molecular methods like PCR and deep sequencing. *T. cruzi* infections in dogs are well-documented throughout the Southeast, with infections historically ranging from 1.0% in Virginia to 16% in Louisiana depending on the method of diagnosis [56, 57•]. In one study, a sample of 540 dogs from 20 shelters across southern Louisiana demonstrated 7% seropositivity for *T. cruzi* using commercial serology tests, whereas nearly 16% of the same samples tested positive by PCR [57•]. In addition, further analysis of these samples using deep sequencing found that many dogs with discordant serology and PCR results harbored infections with multiple DTUs or TcI parasites with different sequences, suggesting that parasite diversity or multiclonal infections might impact the effectiveness of these serologic tests [18••]. A similar negative effect of *T. cruzi* diversity on serological assays was previously described in human Chagas patients [58].

Cats have also been shown to harbor *T. cruzi* infections in southern Louisiana. In one study, nearly 25% of cats ($n=284$) evaluated by PCR tested positive for *T. cruzi* despite a seropositivity rate of 7.3% [19]. In both dogs and cats, deep sequencing and phylogenetic analyses revealed the presence of mixed and multiclonal infections with TcI, TcII, TcIV, TcV, and TcVI DTUs, further demonstrating the high degree of *T. cruzi* diversity that is present in the USA [18••, 19].

A unique case of Chagas disease was recently described in Louisiana, where *T. cruzi*-associated megaesophagus was identified in a domestic llama (*Lama glama*; 61). The llama was born and raised in a rural area of New Orleans, which is also where the first autochthonous human *T. cruzi* case in Louisiana was identified [6]. *T. cruzi* DNA was positively identified in several blood and tissue samples from the llama. These findings raise an important public health question about Chagas pathology in the southeastern USA, given that gastrointestinal mega syndromes are not typically observed in the region. Locally acquired Chagas disease in the USA has traditionally been associated with cardiac manifestations, while gastrointestinal megasyndromes are believed to be confined to the southern half of South America [50]. It is also possible that the unusual pathology of megaesophagus in this llama was related to the biology of the host animal. Passive surveillance of livestock in the area continues in an ongoing effort to identify more veterinary *T. cruzi* cases and gain more clarity on the topic.

A second recent unpublished event occurred in 2021 when a small private zoo located next to established woodlands in Mississippi reported the unexpected deaths of one 4-month-old sloth (*Choloepus didactylus*) and one 9-week-old Asian small-clawed otter (*Aonyx cinereus*). A medical record review found that the sloth had a history of cachexia and died due to *T. cruzi* myocarditis, and the otter died 4 weeks after arriving from a seller in Georgia after high levels of circulating parasitemia compromised multiple organs. Given the very young age of the animals at death and limited time both animals spent at the Mississippi facility, it is likely that both animals were infected with *T. cruzi* prior to arriving at the facility in MS, either from congenital transmission or infection shortly after birth. Health records for each animal's mother were not available for review. A survey of the site was performed by the authors at the invitation of and with the help of the Mississippi State Department of Health. No kissing bugs were found on the property. However, the zoo is in an area where *T. sanguisuga* would be expected. This case exemplifies a potential means of *T. cruzi* introduction from distant regions.

Human Chagas Disease in the Southeast

A major barrier to any intervention is the lack of broad epidemiological studies on the prevalence of Chagas disease in the USA [59, 60]. Current estimates of Chagas disease cases in the USA are based on focal samples. New population-based studies are needed to determine the true prevalence of this disease and to inform appropriate interventions where

possible. Below we describe what is known about autochthonous transmission in the southeastern USA, followed by Chagas disease contracted in other endemic countries prior to living in the USA.

Vector-borne transmission of Chagas disease in the USA is considered rare. The US Food and Drug Administration considers any disease affecting fewer than 200,000 individuals a rare disease [61]. There are approximately 76 case reports of presumed or suspected autochthonous human Chagas disease in the USA documented in the literature [62••, 63]. Adjusting this definition to the population of the Southeast, the region would need to exceed 54,400 cases of autochthonous Chagas disease to not be considered rare [64]. While there are no available data documenting the true prevalence of Chagas disease in the Southeast, Cantey et al. used blood donor screening data to calculate a preliminary estimate of the prevalence of autochthonous Chagas disease in the USA. The authors found that prevalence to be 1 in 354,000 blood donors. Prevalence estimates based on blood bank prevalence are obviously an imperfect indicator, as a sample of voluntary, healthy blood donors is not representative of the wider population. Additionally, the cases were identified relatively shortly after blood supply screening was implemented, so the estimate does not serve as an indicator of incidence, given the chronic nature of untreated Chagas disease [65].

Connecting an autochthonous vector-borne case of Chagas disease in the USA to any specific event or risk factor is complex and rarely possible because such events are subject to both recall bias and imprecise clinical diagnostics. A major shortcoming of current diagnostics is the inability to determine how long ago an individual was infected [66]. Better diagnostic tests are needed given that there is currently no "gold standard" test. The practical implication of this lack of a standard is that multiple tests, often with conflicting results, must be used and reconciled to reach a diagnosis [59].

In an attempt to provide a current picture of Chagas disease in the Southeast, state health departments in the region were contacted by the authors and asked to share reports of human Chagas disease cases. These data were compared to the AABB Chagas Biovigilance Network's data [67]. It was found that nearly 95% of AABB confirmed *T. cruzi* screens were not reflected in state health department data. This gap in data is summarized in Fig. 1.

While there have been tens of cases of autochthonous Chagas disease cases in the USA, current estimates suggest approximately 300,000 recent immigrants are living with Chagas disease [29]. Some researchers estimate this number to be as high as 1,000,000 [60]. It has also been estimated that up to 16% of the non-ischemic heart disease in at-risk US Latino populations is caused by *T. cruzi* [59]. Furthermore, congenital Chagas is believed to be the primary mode of *T. cruzi* infection in non-endemic areas like the Southeast [9]. Although data specifically for the Southeast do not exist, Buekens et al. estimated that in the USA in 2004 there were 3780 pregnant women with Chagas disease, resulting in 189 cases of congenital Chagas disease [68].

Conclusions

While rapid progress has been made in the understanding of Chagas disease in the southeastern USA in the recent years, there remain many unanswered questions. Future studies will be needed to investigate *T. cruzi* genetics and transmission dynamics and systematic sylvatic surveys for triatomine bugs and their associated animals. Improved human and veterinary Chagas diagnostics will be needed to inform public health guidance and health education campaigns to reduce the morbidity and mortality associated with *T. cruzi* in the southeastern USA and other endemic regions.

Declarations

Conflict of Interest The authors declare no competing interests.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

References

Papers of particular interest, published recently, have been highlighted as:
• Of importance
•• Of major importance

1. Kimball BS, editor *Conorhinus sanguisugus*: its habits and life history. Transactions of the Annual Meetings of the Kansas Academy of Science; 1893: JSTOR.
2. Grundemann AW. Studies on the biology of *Triatoma sanguisuga* (Leconte) in Kansas (Reduviidae, Hemiptera). J Kansas Entomol Soc. 1947;20(3):77–85.
3. Schaffer GD, Hanson WL, Davidson WR, Nettles VF. Hematotropic parasites of translocated raccoons in the southeast. J Am Vet Med Assoc. 1978;173(9):1148–51.
4. Yaeger RG. The prevalence of *Trypanosoma cruzi* infection in armadillos collected at a site near New Orleans, Louisiana. Am J Trop Med Hyg. 1988;38(2):323–6.
5. Barr SC, Brown CC, Dennis VA, Klei TR. The lesions and prevalence of *Trypanosoma cruzi* in opossums and armadillos from southern Louisiana. J Parasitol. 1991;77(4):624–7.
6. Dorn PL, Perniciaro L, Yabsley MJ, Roellig DM, Balsamo G, Diaz J, et al. Autochthonous transmission of *Trypanosoma cruzi*. Louisiana Emerg Infect Dis. 2007;13(4):605–7.
7. Herrera CP, Licon MH, Nation CS, Jameson SB, Wesson DM. Genotype diversity of *Trypanosoma cruzi* in small rodents and

Triatoma sanguisuga from a rural area in New Orleans. Louisiana Parasit Vectors. 2015;8:123.
8. Beard CB, Young DG, Butler JF, Evans DA. First isolation of Trypanosoma cruzi from a wild-caught Triatoma sanguisuga (LeConte) (Hemiptera Triatominae) in Florida, USA. J Parasitol. 1988;74(2):343–4.
9. Zingales B, Miles MA, Campbell DA, Tibayrenc M, Macedo AM, Teixeira MM, et al. The revised *Trypanosoma cruzi* subspecific nomenclature: rationale, epidemiological relevance and research applications. Infect Genet Evol. 2012;12(2):240–53.
10. Carranza JC, Valadares HM, D'Avila DA, Baptista RP, Moreno M, Galvao LM, et al. *Trypanosoma cruzi* maxicircle heterogeneity in Chagas disease patients from Brazil. Int J Parasitol. 2009;39(9):963–73.
11. Gaunt MW, Yeo M, Frame IA, Stothard JR, Carrasco HJ, Taylor MC, et al. Mechanism of genetic exchange in American trypanosomes. Nature. 2003;421(6926):936–9.
12. Ocana-Mayorga S, Llewellyn MS, Costales JA, Miles MA, Grijalva MJ. Sex, subdivision, and domestic dispersal of *Trypanosoma cruzi* lineage I in southern Ecuador. PLoS Negl Trop Dis. 2010;4(12):e915.
13. Garcia MN, Burroughs H, Gorchakov R, Gunter SM, Dumonteil E, Murray KO, et al. Molecular identification and genotyping of *Trypanosoma cruzi* DNA in autochthonous Chagas disease patients from Texas, USA. Infect Genet Evol. 2017;49:151–6.
14.• Dumonteil E, Pronovost H, Bierman EF, Sanford A, Majeau A, Moore R, et al. Interactions among Triatoma sanguisuga blood feeding sources gut microbiota and Trypanosoma cruzi diversity in southern Louisiana. Mol Ecol. 2020;29(19):3747–61. **Thiswork demonstratesthepowerofdeepsequencingtechnologiestoelucidatecomplexfeeding andecologicalnetworksofcollectedinsects.Themethodsusedtomultiplexthesequencingreads greatlyincreasethedatayieldandwill certainlyfindapplicationsinfuturestudies inthisand othervector-bornediseasesystems.**
15.• Majeau A, Herrera C, Dumonteil E. An improved approach to Trypanosoma cruzi molecular genotyping by next-generation sequencing of the mini-exon gene. Methods Mol Biol. 2019;1955:47–60. **This protocol paper is an important workflow that describes a next-generation sequencing approach for Trypanosoma cruzi that is likely to become a standard method as sequencing prices continue to decline.**
16. Pronovost H, Peterson AC, Chavez BG, Blum MJ, Dumonteil E, Herrera CP. Deep sequencing reveals multiclonality and new discrete typing units of *Trypanosoma cruzi* in rodents from the southern United States. J Microbiol Immunol Infect. 2020;53(4):622–33.
17. Herrera C, Majeau A, Didier P, Falkenstein KP, Dumonteil E. *Trypanosoma cruzi* diversity in naturally infected nonhuman primates in Louisiana assessed by deep sequencing of the mini-exon gene. Trans R Soc Trop Med Hyg. 2019;113(5):281–6.
18.•• Dumonteil E, Elmayan A, Majeau A, Tu W, Duhon B, Marx P, et al. Genetic diversity of Trypanosoma cruzi parasites infecting dogs in southern Louisiana sheds light on parasite transmission cycles and serological diagnostic performance. PLoS Negl Trop Dis. 2020;14(12):e0008932. **This work identifies potential pitfalls for diagnostic tests for canine Chagas disease. Poor assay performance in humans is well documented, and this paper demonstrates that the issue is likely fundamental to the parasite and its various lineages.**
19. Dumonteil E, Desale H, Tu W, Duhon B, Wolfson W, Balsamo G, et al. Shelter cats host infections with multiple *Trypanosoma cruzi* discrete typing units in southern Louisiana. Vet Res. 2021;52(1):53.
20.•• de Paiva VF, Oliveira J, Galvao C, Justi SA, Landa JMA, Rosa JAD. Formal assignation of the kissing Bug *Triatoma lecticularia* (Hemiptera: Reduviidae: Triatominae) to the genus Paratriatoma. Insects. 2021;12(6). **This is the formal notice of reclassification of Triatoma lecticularia to Paratriatoma lecticularia. The authors are authorities in this field and do a clear and convincing job at explaining the need for reclassification.**
21. Lent H, Wygodzinsky PW. Revision of the Triatominae (Hemiptera, Reduviidae), and their significance as vectors of Chagas' disease. Bull Am Mus Nat Hist. 1979;163(3):123–520.
22. Ibarra-Cerdena CN, Sanchez-Cordero V, Townsend Peterson A, Ramsey JM. Ecology of North American Triatominae. Acta Trop. 2009;110(2–3):178–86.
23. Justi SA, Russo CA, Mallet JR, Obara MT, Galvao C. Molecular phylogeny of Triatomini (Hemiptera: Reduviidae: Triatominae). Parasit Vectors. 2014;7:149.
24. de la Rua NM, Bustamante DM, Menes M, Stevens L, Monroy C, Kilpatrick CW, et al. Towards a phylogenetic approach to the composition of species complexes in the North and Central American *Triatoma*, vectors of Chagas disease. Infect Genet Evol. 2014;24:157–66.
25. Centers for Disease Control and Prevention. *Triatoma lecticularia* 2021 [Available from: https://www.cdc.gov/parasites/chagas/gen_info/vectors/t_lecticularia.html. Accessed 16 May 2022
26. Ryckman RE, Ryckman JV. Epizootiology of Trypanosoma cruzi in southwestern North America XII Does Gause's rule apply to the ectoparasitic Triatominae? (Hemiptera: Reduviidae) (Kinetoplastidae: Trypanosomidae) (Rodentia: Cricetidae). J Med Entomol. 1967;4(3):379–86.
27. Kjos SA, Snowden KF, Olson JK. Biogeography and *Trypanosoma cruzi* infection prevalence of Chagas disease vectors in Texas, USA. Vector Borne Zoonotic Dis. 2009;9(1):41–50.
28. Williams GD, Adams LG, Yaeger RG, McGrath RK, Read WK, Bilderback WR. Naturally occurring trypanosomiasis (Chagas' disease) in dogs. J Am Vet Med Assoc. 1977;171(2):171–7.
29. Klotz SA, Dorn PL, Mosbacher M, Schmidt JO. Kissing bugs in the United States: risk for vector-borne disease in humans. Environ Health Insights. 2014;8(Suppl 2):49–59.
30. Hays KL. Longevity, fecundity, and food intake of adult *Triatoma sanguisuga* (Leconte) (Hemiptera: Triatominae). J Med Entomol. 1965;2(2):200–2.
31. National Oceanic and Atmospheric Administration. Climate at a glance 2022 [Available from: https://www.ncdc.noaa.gov/cag/statewide/time-series. Accessed 16 May 2022
32. Cesa K, Caillouet KA, Dorn PL, Wesson DM. High *Trypanosoma cruzi* (Kinetoplastida: Trypanosomatidae) prevalence in *Triatoma sanguisuga* (Hemiptera: Redviidae) in southeastern Louisiana. J Med Entomol. 2011;48(5):1091–4.
33. Moudy RM, Michaels S, Jameson SB, Londono B, Lopez V, Caillouet KA, et al. Factors associated with peridomestic *Triatoma sanguisuga* (Hemiptera: Reduviidae) presence in southeastern Louisiana. J Med Entomol. 2014;51(5):1043–50.
34. Waleckx E, Suarez J, Richards B, Dorn PL. *Triatoma sanguisuga* blood meals and potential for Chagas disease, Louisiana, USA. Emerg Infect Dis. 2014;20(12):2141–3.
35. Jameson SB. Population genetics and natural history of *Triatoma sanguisuga* in southeastern Louisiana. [Doctoral dissertation, Tulane University]: ProQuest Dissertations Publishing; 2017.
36. Peterson AT, Papes M, Eaton M. Transferability and model evaluation in ecological niche modeling: a comparison of GARP and Maxent. Ecography. 2007;30(4):550–60.
37. Kjos SA, Marcet PL, Yabsley MJ, Kitron U, Snowden KF, Logan KS, et al. Identification of bloodmeal sources and *Trypanosoma cruzi* infection in triatomine bugs (Hemiptera: Reduviidae) from residential settings in Texas, the United States. J Med Entomol. 2013;50(5):1126–39.

38. Shields TL, Walsh EN. Kissing bug bite. AMA Arch Derm. 1956;74(1):14–21.
39. Curtis-Robles R, Hamer SA, Lane S, Levy MZ, Hamer GL. Bionomics and spatial distribution of triatomine vectors of *Trypanosoma cruzi* in Texas and other southern states, USA. Am J Trop Med Hyg. 2018;98(1):113–21.
40. Kuehn B. Delaware kissing bug. JAMA. 2019;321(22):2156.
41. Dye-Braumuller KC, Evans CL, Lynn MK, Forsyth CJ, Gomez C, Nolan MS. Domestic *Triatoma sanguisuga* human exposure in the South Carolina coastal region. Am J Trop Med Hyg. 2020;103(4):1487–9.
42. Mississippi State Department of Health. 2019.
43. Humair P-F, Douet V, Cadenas FM, Schouls LM, Pol IVD, Gern L. Molecular identification of bloodmeal source in *Ixodes ricinus* ticks using 12S rDNA as a genetic marker. J Med Entomol. 2007;44(5):869–80.
44. Lopes V, Andrade L, Carvalho M, Serra A, Balsa F, Bento A, et al. Mini-STRs: a powerful tool to identify genetic profiles in samples with small amounts of DNA. Forensic Sci Int: Genetics Supplement Series. 2009;2(1):121–2.
45. Russomando G, Figueredo A, Almiron M, Sakamoto M, Morita K. Polymerase chain reaction-based detection of *Trypanosoma cruzi* DNA in serum. J Clin Microbiol. 1992;30(11):2864–8.
46. Burgos JM, Diez M, Vigliano C, Bisio M, Risso M, Duffy T, et al. Molecular identification of *Trypanosoma cruzi* discrete typing units in end-stage chronic Chagas heart disease and reactivation after heart transplantation. Clin Infect Dis. 2010;51(5):485–95.
47. Mota J, Chacon JC, Gutiterrez-Cabrera AE, Sanchez-Cordero V, Wirtz RA, Ordoez R, et al. Identification of blood meal source and infection with *Trypanosoma cruzi* of Chagas disease vectors using a multiplex cytochrome b polymerase chain reaction assay. Vector-Borne and Zoonotic Diseases. 2007;7(4):617–27.
48. Schlatter RP, Matte U, Polanczyk CA, Koehler-Santos P, Ashton-Prolla P. Costs of genetic testing: supporting Brazilian public policies for the incorporating of molecular diagnostic technologies. Genet Mol Biol. 2015;38(3):332–7.
49. Quail MA, Smith M, Coupland P, Otto TD, Harris SR, Connor TR, et al. A tale of three next generation sequencing platforms: comparison of Ion Torrent, Pacific Biosciences and Illumina MiSeq sequencers. Bmc Genomics. 2012;13.
50. Bern C, Kjos S, Yabsley MJ, Montgomery SP. *Trypanosoma cruzi* and Chagas' disease in the United States. Clin Microbiol Rev. 2011;24(4):655–81.
51. Hodo CL, Hamer SA. Toward an ecological framework for assessing reservoirs of vector-borne pathogens: wildlife reservoirs of *Trypanosoma cruzi* across the southern United States. ILAR J. 2017;58(3):379–92.
52.• Majeau A, Pronovost H, Sanford A, Cloherty E, Anderson AN, Balsamo G, et al. Raccoons as an important reservoir for Trypanosoma cruzi: a prevalence study from two metropolitan areas in Louisiana. Vector-Borne and Zoonotic Diseases. 2020;20(7):535–40. **This work sheds light on the issue of urban raccoon populations infected with Trypanosoma cruzi. This is important from both a human-animal contact perspective and a disease transmission dynamics perspective.**
53. Yabsley MJ, Noblet GP. Seroprevalence of *Trypanosoma cruzi* in raccoons from South Carolina and Georgia. J Wildl Dis. 2002;38(1):75–83.
54. United States Census Bureau. Quick facts from the 2016 National Survey of Fishing, Hunting, and Wildlife-Associated Recreation 2016 [Available from: https://www.census.gov/library/visualizations/2016/demo/fhw-16-nat.html.
55. Garcia MN, Hotez PJ, Murray KO. Potential novel risk factors for autochthonous and sylvatic transmission of human Chagas disease in the United States. Parasit Vectors. 2014;7:311.
56. Rosypal AC, Smith T, Alexander A, Weaver M, Stewart R, Houston A, et al. Serologic survey of antibodies to *Trypanosoma cruzi* in coyotes and red foxes from Pennsylvania and Tennessee. J Zoo Wildl Med. 2014;45(4):991–3.
57.• Elmayan A, Tu W, Duhon B, Marx P, Wolfson W, Balsamo G, et al. High prevalence of Trypanosoma cruzi infection in shelter dogs from southern Louisiana, USA. Parasit Vectors. 2019;12(1):322. **Current identification of canine Chagas disease in shelter dogs in the Southeast. This expands the scope of the issue from shelter-based surveys conducted in more western states.**
58. Herrera C, Truyens C, Dumonteil E, Alger J, Sosa-Estani S, Cafferata ML, et al. Phylogenetic analysis of *Trypanosoma cruzi* from pregnant women and newborns from Argentina, Honduras, and Mexico suggests an association of parasite haplotypes with congenital transmission of the parasite. J Mol Diagn. 2019;21(6):1095–105.
59. Montgomery SP, Starr MC, Cantey PT, Edwards MS, Meymandi SK. Neglected parasitic infections in the United States: Chagas disease. Am J Trop Med Hyg. 2014;90(5):814–8.
60. Hotez PJ, Dumonteil E, Betancourt Cravioto M, Bottazzi ME, Tapia-Conyer R, Meymandi S, et al. An unfolding tragedy of Chagas disease in North America. PLoS Negl Trop Dis. 2013;7(10):e2300.
61. United States Food and Drug Administration. Rare diseases at FDA 2021 [Available from: https://www.fda.gov/patients/rare-diseases-fda.
62.•• Lynn MK, Bossak BH, Sandifer PA, Watson A, Nolan MS. Contemporary autochthonous human Chagas disease in the USA. Acta Trop. 2020;205:105361. **Important and timely review and update of the state of autochthonous human Chagas disease in the United States.**
63. Turabelidze G, Vasudevan A, Rojas-Moreno C, Montgomery SP, Baker M, Pratt D, et al. Autochthonous Chagas disease - Missouri, 2018. MMWR Morb Mortal Wkly Rep. 2020;69(7):193–5.
64. United States Census Bureau. 2019 [Available from: https://www.census.gov/quickfacts.
65. Cantey PT, Stramer SL, Townsend RL, Kamel H, Ofafa K, Todd CW, et al. The United States *Trypanosoma cruzi* Infection Study: evidence for vector-borne transmission of the parasite that causes Chagas disease among United States blood donors. Transfusion. 2012;52(9):1922–30.
66. Montgomery SP, Parise ME, Dotson EM, Bialek SR. What do we know about Chagas disease in the United States? Am J Trop Med Hyg. 2016;95(6):1225.
67. AABB. Chagas Biovigilance Network [Available from: https://www.aabb.org/news-resources/resources/hemovigilance/chagas-biovigilance-network.
68. Buekens P, Almendares O, Carlier Y, Dumonteil E, Eberhard M, Gamboa-Leon R, et al. Mother-to-child transmission of Chagas' disease in North America: why don't we do more? Matern Child Health J. 2008;12(3):283–6.
69. Panzera F, Perez R, Panzera Y, Ferrandis I, Ferreiro MJ, Calleros L. Cytogenetics and genome evolution in the subfamily Triatominae (Hemiptera, Reduviidae). Cytogenet Genome Res. 2010;128(1–3):77–87.
70. Jurberg J, Costa JM. Studies on the resistance to fasting and nutritional aspects of *Triatoma lecticularia* (Stal, 1859) (Hemiptera, Reduviidae, Triatominae). Mem I Oswaldo Cruz. 1989;84(3):393–9.
71. Thompson JM, Habrun CA, Scully CM, Sasaki E, Bauer RW, Jania R, et al. Locally transmitted *Trypanosoma cruzi* in a domestic llama (*Lama glama*) in a rural area of Greater

New Orleans, Louisiana, USA. Vector Borne Zoonotic Dis. 2021;21(10):762–8.
72. Herwaldt BL, Grijalva MJ, Newsome AL, McGhee CR, Powell MR, Nemec DG, et al. Use of polymerase chain reaction to diagnose the fifth reported US case of autochthonous transmission of *Trypanosoma cruzi*, in Tennessee, 1998. J Infect Dis. 2000;181(1):395–9.
73. Karsten V, Davis C, Kuhn R. *Trypanosoma cruzi* in wild raccoons and opossums in North Carolina. J Parasitol. 1992;78(3):547–9.
74. Brown EL, Roellig DM, Gompper ME, Monello RJ, Wenning KM, Gabriel MW, et al. Seroprevalence of *Trypanosoma cruzi* among eleven potential reservoir species from six states across the southern United States. Vector-Borne and Zoonotic Diseases. 2010;10(8):757–63.
75. Gates M, Gerhold RW, Wilkes RP, Gulsby WD, Maestas L, Rosypal A, et al. Parasitology, virology, and serology of free-ranging coyotes (*Canis latrans*) from central Georgia, USA. J Wildl Dis. 2014;50(4):896–901.
76. Gunter SM, Cordray C, Gorchakov R, Du I, Dittmar B, Brown EL, et al. Identification of white-tailed deer (*Odocoileus virginianus*) as a novel reservoir species for Trypanosoma cruzi in Texas, USA. J Wildlife Dis. 2018;54(4):814–8.
77. Rosypal AC, Tripp S, Lewis S, Francis J, Stoskopf MK, Larsen RS, et al. Survey of antibodies to Trypanosoma cruzi and Leishmania spp in gray and red fox populations from North Carolina and Virginia. Journal of Parasitology. 2010;96(6):1230–1.