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Vampire bats in Belize harbor multiple Trypanosoma cruzi genotypes: implications for parasite t domestic–human interface

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Abstract

Background Chagas disease, caused by *Trypanosoma cruzi*, is a neglected tropical disease with complex sylvatic and domestic vectors, mammalian hosts, and humans. The common vampire bat (*Desmodus rotundus*) is an obligate blood-feeding species tha and humans, yet their role in parasite maintenance in Central America remains poorly characterized.

Methodology/Principal Findings We analyzed 205 blood samples from vampire bats collected at two sites in northern Belize ov PCR screening revealed an overall *T. cruzi* prevalence of 41.5% and increasing infection risks over time. The amplicon-based nex parasite mini-exon locus identified 36 unique haplotypes belonging to five DTUs: TcI, TcIV, TcV, TcVI, and TcBat. TcBat was preser

TcBat and TcVI, were detected in Belize for the first time. Belizean TcBat haplotypes clustered closely with Colombian reference s Belize-specific clade, indicating previously unrecognized TcBat diversity in the region; in contrast, Brazilian TcBat sequences were

Conclusions/Significance Vampire bats in Belize harbor diverse *T. cruzi* genotypes, including DTUs with established roles in hur blood diet, frequent feeding on livestock, and occasional biting of humans, vampire bats may serve as bridge hosts linking sylvatic cycles. Together with a recent report of an acute human Chagas disease case in northern Belize, these results underscore the ne surveillance of bats, vectors, livestock, and humans to better evaluate and mitigate the risk of Chagas disease in Central America.

Author Summary Chagas disease, caused by the parasite *Trypanosoma cruzi*, is a major public health concern in the Americas regions, including Central America. The parasite is typically transmitted by kissing bugs, but wild mammals also serve as importan *rotundus*) are unique because they feed exclusively blood and frequently bite livestock, and occasionally humans, creating opport across different environments. In this study, we screened 205 vampire bats from northern Belize and found that over 40% were inf parasite DNA, we discovered a surprising diversity of DTUs, including TcI, TcIV, TcV, TcVI, and TcBat. The detection of TcVI is part DTU, associated with human infections in South America, had not been previously reported in Belize. TcBat haplotypes were high Our results show that vampire bats are important hosts of diverse *T. cruzi* genotypes in Belize and may act as bridge hosts betwe transmission cycles. Enhanced One Health surveillance across vectors, bats, domestic animals, and humans will be critical for un disease emergence in Central America.

Introduction

Chagas disease, caused by the protozoan parasite *Trypanosoma cruzi*, is a major neglected tropical disease in the Americas, affe people and placing nearly 70 million at risk of infection [1, 2]. Clinically, infection can lead to chronic cardiac, gastrointestinal, and together make Chagas disease a leading cause of cardiomyopathy in Latin America [3]. The parasite occurs from the southern U reflecting its remarkable ecological adaptability and capacity to persist across diverse host and vector species [1, 4]. Transmissio including vector-borne, oral, congenital, and transfusional pathways [4] that interconnect mammalian hosts and insect vectors, cre

epidemiological networks [5].

T. cruzi is genetically diverse, comprising seven discrete typing units (DTUs: TcI–TcVI and TcBat) that differ in their geographic dist clinical relevance [6, 7]. This remarkable heterogeneity is shaped by selective pressures from a wide range of mammalian hosts a biological and epidemiological differences among DTUs [8]. Bats are particularly important in this context: phylogenetic studies su whereby the common ancestor of *T. cruzi* emerged from a bat trypanosome, with TcI representing the first major lineage to expanc While studies in Central America have rarely documented *T. cruzi* infection in bats [11], evidence from Colombia and South Americ mammals in maintaining an extensive genetic diversity[12–14]. In Colombia, DTUs such as TcI and TcBat have been detected in f suggesting active transmission cycles involving diverse chiropteran hosts [12]. Similarly, research in Ecuador [13] and Brazil has s *Trypanosoma* species and contribute to the maintenance and dispersal of *T. cruzi* in fragmented landscapes [14]. The common va of particular concern because of its obligate hematophagy and frequent feeding on both livestock and humans [15, 16], positioning sylvatic and domestic cycles of parasite transmission.

Despite the ecological importance of bats, their role in *T. cruzi* transmission in Central America remains especially poorly understo sensu lato has historically been considered the primary vector of *T. cruzi*, with most populations found in sylvatic habitats and only reported in the Cayo and Toledo districts in the central and southern region of the country [17]. However, recent findings challenge transmission risk in the north of Belize. In 2020, the first confirmed autochthonous case of acute Chagas disease in Belize was dia the Corozal District. Molecular analysis revealed a multiclonal infection involving TcII, TcIV, and TcV DTUs, and triatomine vectors one of which tested positive for *T. cruzi* [18]. Subsequent entomological investigations identified a novel *Triatoma* species closely r the peridomestic environment of the same household. This vector also harbored *T. cruzi* TcIV, genetically matching the strain found transmission and the presence of previously unrecognized vector diversity [19].These findings underscore the need to reassess th diversity of *T. cruzi* in northern Belize.

Here, we used molecular diagnostic and amplicon-based next-generation sequencing (NGS) to investigate *T. cruzi* infection preva *rotundus* populations in the Orange Walk District of northern Belize. By characterizing parasite genotypes at high resolution, our g vampire bats in local transmission cycles and assess the implications for public health in this understudied region of Central Ameri

Methods

Study area and bat sampling

As part of a long-term study on vampire bat ecology and pathogen dynamics [20–23] , we analyzed blood samples from *Desmodu* field seasons: April 2019 (n = 136), November 2021 (n = 14), and April 2022 (n = 55). Sampling was conducted at two sites in the Belize, the Lamanai Archaeological Reserve (LAR) and Ka'Kabish (KK), which are separated by approximately 8 km and embedd agriculture, pasture, and forest fragments [24, 25]. The LAR represents a minimally disturbed 450-ha semi-deciduous tropical fore secondary growth forest (Figure 1).

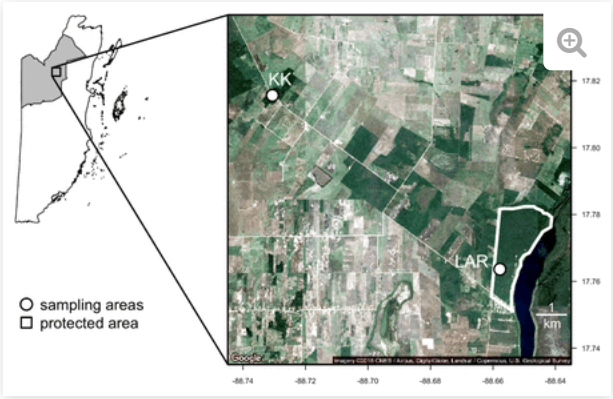

Figure 1.

Map of the two study sites in northern Belize where samples were obtained. Borders (in color) mark the boundaries of Ka'Kabish (KK) and the Lamanai Archaeological Reser

Bat captures were conducted during the dry seasons (2019, 2022) and the rainy season (2021). Roost abandonment prevented sa

bats using harp traps and mist nets positioned at roost exits and along flyways. Individuals were marked with 3.5 mm incoloy wing class, and reproductive status were recorded. Blood was obtained by lancing the propatagial vein with a 23-gauge needle, followe capillary tubes. Blood was stored on Whatman FTA cards with desiccant, stored at ambient temperature in the field, and later kept Oklahoma.

Bleeding was stopped using styptic gel, and bats were released at the capture site. All animal handling protocols followed guidelin Mammalogists and were approved by the AMNH (IACUC 20190129) and the University of Oklahoma (R21-021). Bat captures wer Department (FD/WL/1/19(09), FD/WL/1/21(12)) and the Belize Institute of Archaeology (IA/S/5/6/21(01)).

Trypanosoma cruzi *diagnostics*

We extracted genomic DNA from blood on FTA cards using QIAamp DNA Investigator Kits (Qiagen) as described previously, with manufacturer protocols [26, 27]. Two standard detection PCR assays using previously described primers, targeting a 330 bp minic (121/122) and a 188 bp fragment of a highly repetitive genomic satellite DNA present in *T. cruzi* (TCZ1/TCZ2), respectively [28, 29 samples. PCR products were then subjected to gel electrophoresis on an ethidium bromide–stained 2.0% agarose gel run for 50 r infection status. Samples were considered diagnostically positive for parasite presence if at least one of the two PCR reactions sh presence of a 188 bp or 330 bp band for the respective reactions. Extraction controls (blank punches from FTA cards) and negativ were included in all PCR reactions. In addition, positive control from culture, *T. cruzi* strain SC43, a well-characterized strain repre was included in diagnostic PCR reactions [30]. A small subset of 2019 *T. cruzi* positivity data (*n*=19) were published previously [31

Statistical analysis

We used the prevalence package in R to estimate global as well as site- and year-specific infection prevalence and 95% confiden risk factors for *T. cruzi* infection in vampire bats, we fit three generalized linear mixed models (GLMMs) using the *lme4* package [3 entire dataset (n = 205) and focused only on individual-level predictors, including bat sex, reproductive status, age, and all relevan vampire bats in KK were not sampled in 2021. To next test for site differences in *T. cruzi* infection without confounding site and sea only data from 2019 and 2022 (n = 191), including site, year, and their interaction as predictors alongside all individual-level main interaction terms from our first model. Lastly, to assess temporal variation in infection status, we fit a third GLMM to just data from year alongside the same individual-level predictors (and interactions) as the previous model. All GLMMs used binomial errors and as a random effect, owing to within- or between-year recaptures of 14 individual bats during the study period (representing 30 sam

Trypanosoma cruzi *genotyping*

We conducted genotyping on *T. cruzi*–positive samples with sufficient remaining DNA (n = 62) using PCR amplification of the mini- complementary PCR assays were used to maximize lineage assignment confidence: (i) the classical multiplex mini-exon assay of additional PCR amplifying a 450–500 bp mini-exon fragment (primers TrypME/TcCH) designed to provide greater phylogenetic res diversity by sequencing [34].

PCR reactions (25 μL) included template DNA, molecular-grade water as a negative control, and reference strains representing kn SC43 at 0.01 ng/μL). Amplicons were resolved with 2.0% agarose gels. We selected a subset of 10 high-quality samples for ampli sequencing (NGS) of the mini-exon locus. This subset provided sufficient read depth for robust haplotype recovery and phylogene sample, amplicons from the three PCR reactions targeting the mini-exon sequence were pooled and purified using the Invitrogen I Kit (Cat. No. K3100-02; Thermo Fisher Scientific Baltic UAB, Vilnius, Lithuania) according to the manufacturer's instructions. DNA pooled, purified product was measured with a NanoDrop™ 2000 spectrophotometer (Thermo Scientific™, Wilmington, DE, USA) p

Next-Generation sequencing and sequence analysis

Amplicon libraries of the mini-exon gene were prepared following end-repair and indexing, and sequenced on a MiSeq platform (Il Inc.) at the Tulane National Primate Research Center. Libraries were multiplexed, and sequencing produced between 134,000 and blood DNA sample after removal of low-quality and short fragments.

Raw Fastq sequences files were imported into Geneious 11 software for analysis, and quality filtering was applied prior to mapping set of *T. cruzi* mini-exon reference sequences representing each of the seven DTUs (TcI–TcVI and TcBat). Reference sequences u Tu18 (AY367125), TcIII: M5631 (AY367126), TcIV: 92122102r (AY367124), TcV: SC43 (AY367127), TcVI: CL (U57984) and TcBat: discard the presence of any other trypanosomatides in bats reads were also aligned against the mini-exon sequence of *Trypanosc* bat trypanosome within the *T. cruzi* clade. Assemblies to each reference DTU were then analyzed separately. Partial matches to a

analysis, and only assemblies covering the full-length of the expected mini-exon PCR products were considered to ensure specific trimmed of PCR primer sequences [35].Sequence polymorphisms and haplotypes were identified using the FreeBayes SNP/variar were retained, and variants accounting for <1% of total reads per sample were excluded to minimize background noise [36]. Mini-e have been deposited in GenBank (accessions PX660128-PX660163)

Phylogenetic analyses

Maximum likelihood phylogenies were built using PhyML as implemented in Geneious [37], and mini-exon sequences from referer were included additionally to sequences from triatomine *T. dimidiata* TcI (MW861785), TcIV (MW861768) [17], and human case fro (OM818340), TcV (OM818332–41) [18]. Resulting trees were then edited and visualized using FigTree (Interactive Tree of Life) [3

Results

Prevalence of* Trypanosoma cruzi *in vampire bats

Across 205 vampire bats sampled in our two sites and over three years, 85 bats (41.5%, 95% CI: 34.9–48.3%) tested positive for more positives (83/205, 40.5%) than the satellite DNA assay (52/205, 25.4%), with concordant amplification in 50 samples. Thirty- the kDNA assay, while only two were unique to the satellite DNA assay, consistent with the greater sensitivity of the kDNA target ir

Our first GLMM of individual-level risk factors did not identify effects of age, sex, reproductive status, or their interactions on the pr S1, $R^2 = 0.03$, $R^2 = 0.04$). When analyzing only data from 2019 and 2022 to assess spatial effects, we found significant main effec interaction (Table S2, $R^2 = 0.16$, $R^2 = 0.18$). After adjusting for individual-level covariates, vampire bats had higher odds of *T. cruzi* = 0.03) and in 2022 (OR = 3.75, $p < 0.01$). When assessing annual variation more explicitly in the LAR in our third GLMM (Table S that the odds of infection were greater in 2022 than in 2019 (OR = 3.91, $p < 0.01$) and in 2021 (OR = 6.78, $p = 0.01$), with no differ 0.58, $p = 0.41$), suggesting annual and seasonal variation in *T. cruzi* infection risks (Figure 2). More generally, these results suppo bats in northern Belize with *T. cruzi*.

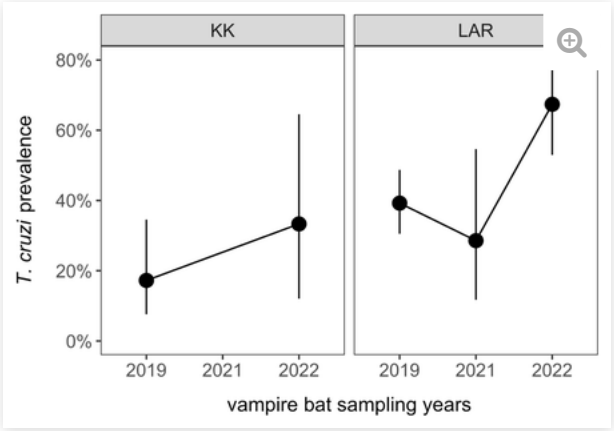

Figure 2.

Temporal and spatial variation in *Trypanosoma cruzi* infection prevalence among vampire bats in northern Belize.
Point estimates and 95% confidence intervals of *T. cruzi* infection prevalence are shown for (KK) and the LAR across three years (2019, 2021, and 2022).

Across all three GLMMs, individual bat identity had relatively weak contributions to variance explained (i.e., the difference betweer our small sample of 14 recaptured individuals, eight did not change infection status within or between capture years. Two bats exh short timescales (i.e., our two-week sampling periods), three bats gained infection between the wet and dry season (2021–2022), between 2019 and 2022 (Figure 3).

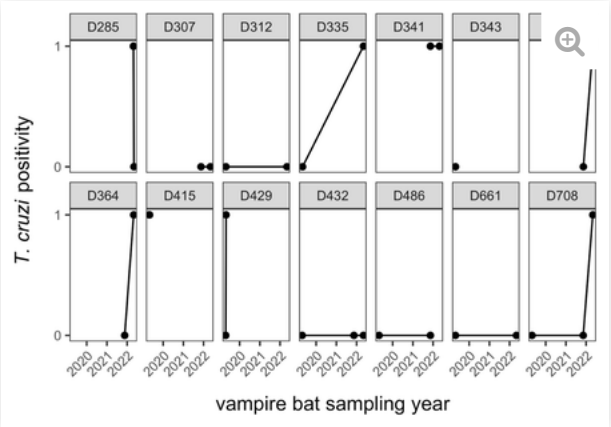

Figure 3.

Infection dynamics among resampled vampire bats in northern Belize.

T. cruzi *sequence diversity in vampire bats*

To refine DTU assignments and characterize intra-lineage diversity, we selected 10 representative *T. cruzi*–positive samples for an locus. Selection criteria included strong PCR amplification, high DNA quality, and representation across multiplex genotyping profil from three independent PCR reactions were pooled to maximize haplotype recovery.

NGS analysis showed 36 high-confidence haplotypes across the 10 samples, with bats carrying between 1 and 9 haplotypes per i given individual consistently belonged to the same DTU, indicating intra-DTU haplotypic diversity rather than mixed-DTU infections phylogeny (Figure 4), haplotypes belonged to five DTUs: TcI, TcIV, TcV, TcVI, and TcBat, with TcBat as the predominant lineage. A robustly within the TcIV clade. No haplotypes corresponding to TcII or TcIII were detected.

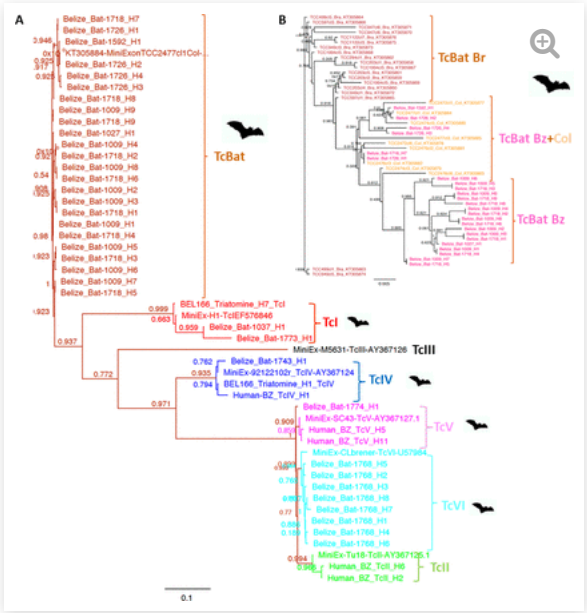

Figure 4.

Phylogenetic analysis of *Trypanosoma cruzi* haplotypes recovered from Belizean vampire bats.
(A) Maximum-likelihood phylogeny showing that Belizean haplotypes cluster within five DTUs (TcI, TcIV, TcV, TcVI, and TcBat). (B) TcBat haplotypes from Belize form two maj (Col) TcBat reference sequences, and another forming a distinct Belize-specific clade (Bz). The Brazilian (Br) TcBat lineage is more distantly related. DTUs are color-coded, and study.

Although individual bats frequently carried multiple haplotypes, all haplotypes within a given bat belonged to the same DTU, demo mixed-DTU) infections. TcBat included several Belize-specific haplotypes not closely matching available references, representing Central America. No sequences from other trypanosomatids were recovered.

Phylogenetic analysis of T. cruzi haplotypes

Maximum-likelihood phylogenetic analysis of the 36 haplotypes (Figure 4) confirmed their grouping within five DTUs: TcBat (68.6%
(2.9%), and a single TcV haplotype. All DTU assignments were supported by clustering with their respective reference sequences.

The TcBat haplotypes identified here formed a distinct, well-supported cluster that branched closest to TcI reference sequences, c
evolutionary origin of TcBat [6, 9, 39, 40]. Within this cluster, several Belizean haplotypes grouped tightly with Colombian TcBat se
TcBat lineage was more distantly related. A subset of Belizean haplotypes formed a separate, Belize-specific cluster (Figure 4B).

The single TcIV haplotype identified here clustered with 93% bootstrap support alongside TcIV sequences from *Triatoma dimidiata*
reported from an acute human infection in 2020, reinforcing the link between sylvatic bat cycles and potential human transmission

The eight TcVI haplotypes clustered within the TcVI clade and grouped with the CL Brener reference strain included in the analysis
dataset is limited, the Belizean TcVI haplotypes showed internal diversity within the clade, suggesting intra-DTU variation but not f
4A). This observation is similar to a recent report of TcVI in non-human primates [41, 42] and rodents in North America [43, 44],su
more broadly distributed in sylvatic reservoirs than previously appreciated.

Together, these results demonstrate that Belizean vampire bats harbor multiple *T. cruzi* DTUs with substantial haplotypic diversity,
epidemiological importance.

Discussion

Our findings provide new insights into the eco-epidemiology of *Trypanosoma cruzi* infections in *Desmodus rotundus* in Central Am
specifically. With an overall prevalence of 41.5%, common vampire bats are important sylvatic hosts within local *T. cruzi* transmiss
prevalence is consistent with, and in some cases exceeds, those reported in vampire bat populations from Peru, Brazil, and Mexic
estimated to range from 10–40% [13, 45–48]. These results support growing evidence that vampire bats play a significant role in r
wildlife communities and may contribute to sylvatic–domestic spillover dynamics, particularly in areas where human–wildlife interfa

In Central America, the contribution of bats to *T. cruzi* transmission has been underexplored, with most research focused on terres
armadillos, and rodents) and synanthropic vectors such as *Triatoma dimidiata* [51]. Our study fills a geographic and ecological gap
prevalence and substantial parasite diversity in *D. rotundus* from northern Belize, where Chagas disease remains endemic but un
Desmodus rotundus frequently feeds on domestic animals and occasionally humans [13, 15], its potential to serve as a host (and
mechanical bridge between sylvatic and domestic cycles warrants further attention [12, 50, 52–54].

A striking result of our study is the high diversity of *T. cruzi* DTUs detected in vampire bats. Haplotypes corresponding to TcI, TcIV,
using NGS of the mini-exon locus. This level of haplotypes diversity in a single host species, and within a relatively small geograph
complex transmission networks involving multiple parasite DTUs and vector species. TcI, the most widespread DTU in the America
sylvatic and domestic transmission cycles, including in human cases across Central America [6].

In addition to TcI, TcIV, TcVI, and TcBat, we also detected a TcV haplotype, a lineage typically associated with domestic transmissi
America [8]. The detection of TcV haplotypes in Belize aligns with recent evidence of a multiclonal human infection involving TcV [
are considered rare [8, 44], our findings suggest that vampire bats may contribute to the local maintenance of TcV. Identifying the
bats and other host taxa will be essential to assess its role in regional spillover or potential spillback from domestic or human trans
evidence of TcV involvement in an acute human case in northern Belize [18]. Resolving the frequency, host range, and directionali
for understanding its regional significance [17, 18].

TcBat was the most prevalent DTU in our sequenced samples and included several Belize-specific haplotypes, expanding its knov
sequences clustered closely with Colombian TcBat references, whereas the Brazilian TcBat lineage was more distantly related, co
variation. TcBat was long thought to occur predominantly in South American chiropterans [12, 54]. While TcBat has not yet been li
prevalence raises important questions about its evolutionary origin, host specificity, and ecological function [10, 13]. Likewise, the
TcVI, and TcBat across the study sites indicates that multiple DTUs circulate within the same bat populations. Although individual
haplotypes belonging to a single DTU rather than mixed-DTU infections, the co-circulation of these lineages at the population leve
opportunities for inter-lineage contact in vectors or other hosts. Despite detecting multiple DTUs across the two sampled vampire
evidence of mixed DTU infections in individual bats. This contrasts with reports of frequent coinfections in other mammalian hosts

Taken together, the NGS data indicate that infections in Belizean vampire bats consisted of multiple haplotypes per individual but c DTU, suggesting substantial intra-DTU variation rather than multi-DTU coinfections We also observed spatial and temporal variatic higher prevalence at the LAR compared to KK and higher prevalence in 2022 than in 2019 and 2021 (Figure 2). These differences livestock availability, and climate variability, which are known to influence vector populations, bat immunity, and host exposure in v 27, 56]. Such patterns may also reflect the impacts of increasing agricultural land conversion on host–host and host–vector contac seasonal effects on bat immunity [58, 59]. Although our study was not designed to robustly assess individual infection trajectories, infection gain and infection loss over relatively short periods; future longitudinal work will be essential to clarify infection persistenc *cruzi* in vampire bats (Figure 3).

Our study has some limitations. Sampling was restricted to two sites over three years, only partly assessing seasonal variation, wi midway through our sampling. Further, only a subset of PCR-positive samples was sequenced, limiting our ability to fully characte not measure vector abundance, blood meal sources, or host immune function, all of which would provide further insight into the ec transmission. Nevertheless, the high prevalence and DTU diversity detected in vampire bats emphasize the complexity of local *T.* hosts, and overlapping transmission pathways. Future studies integrating entomological surveys, blood meal analysis, and parasit mammals will be essential to construct a more holistic picture of transmission networks in Belize.

From a public health perspective, the detection of TcV and TcVI, DTUs strongly associated with human disease in South America, of TcI, highlights the potential for sylvatic spillovers into domestic environments. Belize remains one of the least studied countries i Chagas disease, yet recent events indicate that spillover is already occurring: in 2020, an acute case in a child in northern Belize v TcIV, TcV) and to infected triatomines within the household [18] Subsequent entomological work identified a novel *Triatoma* specie household [19]. Moreover, our previous work on *T. dimidiata* in southern Belize revealed clear evidence of local parasite differentia in vectors, together with frequent blood-feeding on humans and other mammals, highlighting a highly connected sylvatic–domestic overlap between DTUs detected in bats, vectors, and humans strongly suggests that vampire bats are part of broader regional tra infections detected in *T. dimidiata* in Mexico [55], the diversity found in Belizean vampire bats may represent an overlooked source strains.

The high prevalence of *T. cruzi in* Belizean vampire bats, together with the observation that individual bats carried multiple haploty repeated exposure to circulating parasite populations. Because *T. dimidiata* rarely feeds on vampire bats and primarily feeds on hu Belize, infection in *D. rotundus* may occur predominantly through feeding on infected prey rather than via vectorial transmission. C pathways in vampire versus non-vampire bats remain limited, underscoring the need for studies explicitly addressing transmission

Conclusions

Our study demonstrates that common vampire bats in northern Belize harbor a high prevalence of *T. cruzi* and an unexpected dive TcI, TcIV, TcV, TcVI, and TcBat. The detection of multiple DTUs in a single host species and geographic area highlights the comple region and underscores that Belize harbors a more dynamic *T. cruzi* landscape than previously recognized.

Similar findings of diverse DTUs in non-human primates, rodents, and other wildlife in North America further emphasize that paras broader than historically appreciated. These results reinforce the importance of sustained vigilance, since underestimating genetic spillover and transmission to humans.

Given that *Desmodus rotundus* feeds regularly on livestock and occasionally on humans, these bats represent potential bridge ho between sylvatic, domestic, and human cycles. From a One Health perspective, integrated surveillance that simultaneously monit and humans will be essential to anticipate and mitigate the public health consequences of Chagas disease in Central America. In typically linked to domestic transmission and human disease, raises the possibility of spillback from domestic or peridomestic sour further underscoring the need for coordinated cross-sector surveillance.

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Predictor	χ^2	df	p
Age	0.01	1	0.91
Sex	1.55	1	0.21
Reproductive status	0.62	1	0.43
Age * sex	1.89	1	0.17
Sex * reproductive status	0.41	1	0.52

Supplemental Table 1.

GLMM results for model 1 (individual-level predictors) of *T. cruzi* infection (n = 205).

Predictor	χ^2	df	p
Age	0.01	1	0.93
Sex	0.74	1	0.39
Reproductive status	0.28	1	0.60
Site	4.76	1	0.03
Year	10.08	1	0.002
Site * year	0.06	1	0.80

Supplemental Table 2.

GLMM results for model 2 (spatiotemporal predictors) of *T. cruzi* infection (n = 191).

Predictor	χ^2	df	p
Age	0.20	1	0.65
Sex	0.90	1	0.34
Reproductive status	1.45	1	0.23
Year	10.12	1	0.006

Supplemental Table 3.

GLMM results for model 3 (temporal predictors) of *T. cruzi* infection (n = 167).

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References

1.WHO. Weekly epidemiological record. *Chagas disease in Latin America: an epidemiological update based on 2010 estimates*. 2015. p. 33–44.

2.Bern C. Chagas' Disease. *N Engl J Med*. 2015;**373**(19):1882. doi:10.1056/NEJMc1510996. PubMed PMID: 26535522.

3.Rassi A, Jr., Rassi A. Predicting prognosis in patients with Chagas disease: why are the results of various studies so different? *Int J Cardiol*. 2010;**145**(1):64–5; author re 10.1016/j.ijcard.2009.04.034. PubMed PMID: 19428129.

4.Bern C, Messenger LA, Whitman JD, Maguire JH. Chagas Disease in the United States: a Public Health Approach. *Clin Microbiol Rev*. 2019;**33**(1). Epub 20191127. doi: 31776135; PubMed Central PMCID: PMCPMC6927308.

5.Dumonteil E, Pronovost H, Bierman EF, Sanford A, Majeau A, Moore R, et al. Interactions among Triatoma sanguisuga blood feeding sources, gut microbiota and Tryp *Mol Ecol*. 2020;**29**(19):3747–61. Epub 2020/08/05. doi: 10.1111/mec.15582. PubMed PMID: 32749727.

6.Zingales B, Miles MA, Campbell DA, Tibayrenc M, Macedo AM, Teixeira MM, et al. The revised Trypanosoma cruzi subspecific nomenclature: rationale, epidemiologic *Genet Evol*. 2012;**12**(2):240–53. Epub 20111227. doi: 10.1016/j.meegid.2011.12.009. PubMed PMID: 22226704.

7.Messenger LA, Miles MA, Bern C. Between a bug and a hard place: Trypanosoma cruzi genetic diversity and the clinical outcomes of Chagas disease. *Expert Rev Anti I* 10.1586/14787210.2015.1056158. PubMed PMID: 26162928; PubMed Central PMCID: PMCPMC4784490.

8.Zingales B, Bartholomeu DC. *Trypanosoma cruzi* genetic diversity: impact on transmission cycles and Chagas disease. *Mem Inst Oswaldo Cruz*. 2022;**117**:e210193. Epul 02760210193. PubMed PMID: 35544857.

9.Hamilton PB, Lewis MD, Cruickshank C, Gaunt MW, Yeo M, Llewellyn MS, et al. Identification and lineage genotyping of South American trypanosomes using fluores *Evol*. 2011;**11**(1):44–51. Epub 2010/10/30. doi: 10.1016/j.meegid.2010.10.012. PubMed PMID: 21029792.

10.Lima L, Espinosa-Alvarez O, Ortiz PA, Trejo-Varon JA, Carranza JC, Pinto CM, et al. Genetic diversity of Trypanosoma cruzi in bats, and multilocus phylogenetic and as an independent DTU (discrete typing unit). *Acta Trop*. 2015;**151**:166–77. Epub 2015/07/23. doi: 10.1016/j.actatropica.2015.07.015. PubMed PMID: 26200788.

11.Cottontail VM, Kalko EK, Cottontail I, Wellinghausen N, Tschapka M, Perkins SL, et al. High local diversity of Trypanosoma in a common bat species, and implicatio T. cruzi clade. *PLoS One*. 2014;**9**(9):e108603. Epub 20140930. doi: 10.1371/journal.pone.0108603. PubMed PMID: 25268381; PubMed Central PMCID: PMCPMC41824

12.Ramirez JD, Tapia-Calle G, Munoz-Cruz G, Poveda C, Rendon LM, Hincapie E, et al. Trypanosome species in neo-tropical bats: biological, evolutionary and epidemio 2014;**22**:250–6. Epub 20130702. doi: 10.1016/j.meegid.2013.06.022. PubMed PMID: 23831017; PubMed Central PMCID: PMCPMC7106241.

13.Pinto CM, Ocana-Mayorga S, Tapia EE, Lobos SE, Zurita AP, Aguirre-Villacis F, et al. Bats, Trypanosomes, and Triatomines in Ecuador: New Insights into the Diversit cruzi and Chagas Disease. *PLoS One*. 2015;**10**(10):e0139999. Epub 20151014. doi: 10.1371/journal.pone.0139999. PubMed PMID: 26465748; PubMed Central PMCID:

14.Jansen AM, Xavier S, Roque ALR. Trypanosoma cruzi transmission in the wild and its most important reservoir hosts in Brazil. *Parasit Vectors*. 2018;**11**(1):502. Epub PubMed PMID: 30189896; PubMed Central PMCID: PMCPMC6127949.

15.Streicker DG, Allgeier JE. Foraging choices of vampire bats in diverse landscapes: potential implications for land-use change and disease transmission. *J Appl Ecol*. 20 10.1111/1365-2664.12690. PubMed PMID: 27499553; PubMed Central PMCID: PMCPMC4950014.

16.Brown N, Escobar LE. A review of the diet of the common vampire bat (Desmodus rotundus) in the context of anthropogenic change. *Mamm Biol*. 2023:1–21. Epul 3. PubMed PMID: 37363038; PubMed Central PMCID: PMCPMC10258787.

17.Polonio R, Lopez-Dominguez J, Herrera C, Dumonteil E. Molecular ecology of Triatoma dimidiata in southern Belize reveals risk for human infection and the local *Int J Infect Dis*. 2021;**108**:320–9. Epub 20210618. doi: 10.1016/j.ijid.2021.05.083. PubMed PMID: 34098097.

18.Murray KO, Saldana MA, Gunter SM, Manzanero R, Zielinski-Gutierrez E, Herrera C, et al. Diagnosis of Acute Chagas Disease in a Belizean Child with Evidence of *J Trop Med Hyg*. 2022;**107**(5):992–5. Epub 20221114. doi: 10.4269/ajtmh.22-0338. PubMed PMID: 36395748; PubMed Central PMCID: PMCPMC9709023.

19.Gunter SM, Nelson A, Kneubehl AR, Justi SA, Manzanero R, Zielinski-Gutierrez E, et al. Novel species of Triatoma (Hemiptera: Reduviidae) identified in a case of ve northern Belize. *Sci Rep*. 2024;**14**(1):1412. Epub 2024/01/17. doi: 10.1038/s41598-023-50109-0. PubMed PMID: 38228608; PubMed Central PMCID: PMCPMC107921

20.Becker D, Lei, G. , Janech, M. , Bland, A. , Fenton, B. , Relich, R. and Neely, B. . Serum proteomics identifies immune pathways and candidate biomarkers of coronavir *Virology*. 2022. doi: 10.3389/fviro.2022.862961.

21.Becker DJ, Han BA, Kamath P. The macroecology and evolution of avian competence for Borrelia burgdorferi. *Global Ecology and Biogeography*. 2021;**30**(3):710–24.

22.Becker DJ BA, Bergner LM, Meza DK, Simmons NB, Fenton MB, Altizer S, Streicker DG. Temporal patterns of vampire bat rabies and host connectivity in Belize. *Tr* 10.1111/tbed.13754. . PubMed Central PMCID: PMCPMC8246562.

23.Becker DJ, Speer KA, Brown AM, Fenton MB, Washburne AD, Altizer S, et al. Ecological and evolutionary drivers of haemoplasma infection and bacterial genotype *Ecol*. 2020;**29**(8):1534–49. Epub 2020/04/04. doi: 10.1111/mec.15422. PubMed PMID: 32243630; PubMed Central PMCID: PMCPMC8299350.

24.Fenton MB, Bernard, E., Bouchard, S., Hollis, L., Johnston, D. S., Lausen, C. L., et al. . The bat fauna of Lamanai, Belize: roosts and trophic roles. *J Trop Ecol* 2001;**17**:51

25.Herrera JP, Duncan, N., Clare, E., Brock Fenton, M., and Simmons, N. Disassembly of Fragmented Bat Communities in Orange Walk District, Belize. *Acta Chiropterol* doi.org/10.3161/15081109ACC2018.20.1.011.

26.Volokhov DV, Becker DJ, Bergner LM, Camus MS, Orton RJ, Chizhikov VE, et al. Novel hemotropic mycoplasmas are widespread and genetically diverse in vampire Epub 2017/10/25. doi: 10.1017/S095026881700231X. PubMed PMID: 29061202; PubMed Central PMCID: PMCPMC6538534.

27.Becker DJ, Bergner LM, Bentz AB, Orton RJ, Altizer S, Streicker DG. Genetic diversity, infection prevalence, and possible transmission routes of Bartonella spp. in v 2018;**12**(9):e0006786. Epub 2018/09/28. doi: 10.1371/journal.pntd.0006786. PubMed PMID: 30260954; PubMed Central PMCID: PMCPMC6159870.

28.Moser MS, Fleischmann CJ, Kelly EA, Townsend RL, Stramer SL, Bern C, et al. Evaluation of InBios Chagas Detect Fast, a Novel Enzyme-Linked Immunosorbent Ass cruzi Antibodies. *J Clin Microbiol*. 2023;**61**(3):e0176222. Epub 20230228. doi: 10.1128/jcm.01762-22. PubMed PMID: 36853062; PubMed Central PMCID: PMCPMC100

29.Sturm NR, Degrave W, Morel C, Simpson L. Sensitive detection and schizodeme classification of Trypanosoma cruzi cells by amplification of kinetoplast minicircle disease. *Mol Biochem Parasitol*. 1989;**33**(3):205–14. doi: 10.1016/0166-6851(89)90082-0. PubMed PMID: 2565018.

30.DeCuir J, Tu W, Dumonteil E, Herrera C. Sequence of Trypanosoma cruzi reference strain SC43 nuclear genome and kinetoplast maxicircle confirms a strong gene discrete typing units. *Genome*. 2021;**64**(5):525–31. Epub 2020/10/22. doi: 10.1139/gen-2020-0092. PubMed PMID: 33086026; PubMed Central PMCID: PMCPMC80581

31.Vicente-Santos A, Lock LR, Allira M, Dyer KE, Dunsmore A, Tu W, et al. Serum proteomics reveals a tolerant immune phenotype across multiple pathogen taxa in 2023;**14**:1281732. Epub 2024/01/09. doi: 10.3389/fimmu.2023.1281732. PubMed PMID: 38193073; PubMed Central PMCID: PMCPMC10773587.

32.Bates D, Mächler, M., Bolker, B., and Walker, S. . Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software*. 2015;**67**(1):1–48. doi: 10.18637/jss.v06

33.Souto RP, Fernandes O, Macedo AM, Campbell DA, Zingales B. DNA markers define two major phylogenetic lineages of Trypanosoma cruzi. *Mol Biochem Parasitol*. 10.1016/s0166-6851(96)02755-7. PubMed PMID: 9027747.

34.Majeau A, Herrera C, Dumonteil E. An Improved Approach to Trypanosoma cruzi Molecular Genotyping by Next-Generation Sequencing of the Mini-exon Gene. 2019/03/15. doi: 10.1007/978-1-4939-9148-8_4. PubMed PMID: 30868518.

35.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 serological diagnostic performance. *PLoS Negl Trop Dis*. 2020;**14**(12):e0008932. Epub 20201217. doi: 10.1371/journal.pntd.0008932. PubMed PMID: 33332357; PubMed

36.Garrison E MG. Haplotype-based variant detection from short-read sequencing. . *Quantitative Biology*. 2012. doi:10.48550/arXiv.1207.3907.

37.Guindon S, Delsuc F, Dufayard JF, Gascuel O. Estimating maximum likelihood phylogenies with PhyML. *Methods Mol Biol*. 2009;**537**:113–37. Epub 2009/04/21. doi: 1 19378142.

38.Rambaut A. *FigTree v1.3.1*. In: Institute of Evolutionary Biology UoE, Edinburgh. , editor. 2010.

39.Lewis MD, Llewellyn MS, Yeo M, Acosta N, Gaunt MW, Miles MA. Recent, independent and anthropogenic origins of Trypanosoma cruzi hybrids. *PLoS Negl Trop Dis*. 10.1371/journal.pntd.0001363. PubMed PMID: 22022633; PubMed Central PMCID: PMCPMC3191134.

40.Llewellyn MS, Rivett-Carnac JB, Fitzpatrick S, Lewis MD, Yeo M, Gaunt MW, et al. Extraordinary Trypanosoma cruzi diversity within single mammalian reservoir hos selection. *Int J Parasitol*. 2011;**41**(6):609–14. Epub 2011/01/15. doi: 10.1016/j.ijpara.2010.12.004. PubMed PMID: 21232539; PubMed Central PMCID: PMCPMC3084450

41.Herrera C, Majeau A, Didier P, Falkenstein KP, Dumonteil E. Trypanosoma cruzi diversity in naturally infected nonhuman primates in Louisiana assessed by deep se *Trop Med Hyg*. 2019;**113**(5):281–6. Epub 2018/11/20. doi: 10.1093/trstmh/try119. PubMed PMID: 30452731; PubMed Central PMCID: PMCPMC6515898.

42.Dumonteil E, Herrera C, Marx PA. Safety and preservation of cardiac function following therapeutic vaccination against Trypanosoma cruzi in rhesus macaques. *J M* 20220929. doi: 10.1016/j.jmii.2022.09.003. PubMed PMID: 36210315; PubMed Central PMCID: PMCPMC10131272.

43.Pronovost H, Peterson AC, Chavez BG, Blum MJ, Dumonteil E, Herrera CP. Deep sequencing reveals multiclonality and new discrete typing units of Trypanosoma c States. *J Microbiol Immunol Infect*. 2020;**53**(4):622–33. Epub 2019/02/03. doi: 10.1016/j.jmii.2018.12.004. PubMed PMID: 30709717.

44.Gibson NL, Ghersi BM, Knudson B, Peterson AC, Riegel C, Tu W, et al. Potential vertical transmission of genetically diverse Trypanosoma cruzi in natural rodent p 2025;**19**(4):e0012930. Epub 2025/04/01. doi: 10.1371/journal.pntd.0012930. PubMed PMID: 40168428; PubMed Central PMCID: PMCPMC11978043.

45.Bergner LM, Becker DJ, Tello C, Carrera JE, Streicker DG. Detection of Trypanosoma cruzi in the saliva of diverse neotropical bats. *Zoonoses Public Health*. 2021;**68** 10.1111/zph.12808. PubMed PMID: 33484236; PubMed Central PMCID: PMCPMC8569697.

46.Franca DA, Langoni H. Prevalent zoonoses in Sao Paulo State, Brazil: the role of bats and molecular diagnosis. *Rev Inst Med Trop Sao Paulo*. 2025;**67**:e17. Epub 2025/ PubMed PMID: 40053003; PubMed Central PMCID: PMCPMC11883767.

47.Franca DA, Louro M, Zuquete S, Zanini DDS, Moraes GN, Rocha GDS, et al. Trypanosoma cruzi in Bats (Chiroptera; Mammalia) from the Brazilian Atlantic Forest, Epub 2024/05/25. doi: 10.3390/microorganisms12050945. PubMed PMID: 38792775; PubMed Central PMCID: PMCPMC11123848.

48.Lopez-Cancino SA, Tun-Ku E, De la Cruz-Felix HK, Ibarra-Cerdena CN, Izeta-Alberdi A, Pech-May A, et al. Landscape ecology of Trypanosoma cruzi in the souther

72. Epub 2015/07/30. doi: 10.1016/j.actatropica.2015.07.021. PubMed PMID: 26219998.

49.Coutinho CF, Souza-Santos R, Lima MM. Combining geospatial analysis and exploratory study of triatomine ecology to evaluate the risk of Chagas disease in a rura 20111012. doi: 10.1016/j.actatropica.2011.10.005. PubMed PMID: 22015423.

50.Jansen AM, Xavier SC, Roque AL. The multiple and complex and changeable scenarios of the Trypanosoma cruzi transmission cycle in the sylvatic environment. *Act* 10.1016/j.actatropica.2015.07.018. PubMed PMID: 26200785.

51.Dumonteil E, Gourbiere S. Predicting triatoma dimidiata abundance and infection rate: a risk map for natural transmission of chagas disease in the yucatan peninsul 2004;**70**(5):514–9. Epub 2004/05/25. PubMed PMID: 15155983.

52.Maeda FY, Alves RM, Cortez C, Lima FM, Yoshida N. Characterization of the infective properties of a new genetic group of Trypanosoma cruzi associated with bats 20110907. doi: 10.1016/j.actatropica.2011.09.001. PubMed PMID: 21925137.

53.Marcili A, Lima L, Cavazzana M, Junqueira AC, Veludo HH, Maia Da Silva F, et al. A new genotype of Trypanosoma cruzi associated with bats evidenced by phylogen and Histone H2B genes and genotyping based on ITS1 rDNA. *Parasitology*. 2009;**136**(6):641–55. Epub 2009/04/17. doi: 10.1017/S0031182009005861. PubMed PMID: 1

54.Marcili A, Lima L, Valente VC, Valente SA, Batista JS, Junqueira AC, et al. Comparative phylogeography of Trypanosoma cruzi TCIIc: new hosts, association with terr *Genet Evol*. 2009;**9**(6):1265–74. Epub 2009/07/28. doi: 10.1016/j.meegid.2009.07.003. PubMed PMID: 19632356.

55.Dumonteil E, Ramirez-Sierra MJ, Perez-Carrillo S, Teh-Poot C, Herrera C, Gourbiere S, et al. Detailed ecological associations of triatomines revealed by metabarco implications for triatomine behavior and Trypanosoma cruzi transmission cycles. *Sci Rep*. 2018;**8**(1):4140. Epub 20180307. doi: 10.1038/s41598-018-22455-x. PubMed PMCPMC5841364.

56.Chaves LF, Cohen JM, Pascual M, Wilson ML. Social exclusion modifies climate and deforestation impacts on a vector-borne disease. *PLoS Negl Trop Dis*. 2008;**2**(1):e 10.1371/journal.pntd.0000176. PubMed PMID: 18265876; PubMed Central PMCID: PMCPMC2238711.

57.Lock LR DK, Volokhov DV, Yang A, Fenton MB, Simmons NB, Becker DJ. Longitudinal impacts of habitat fragmentation on Bartonella and hemotropic Mycoplasma 2025. doi: 10.1101/2025.07.21.665613. PubMed Central PMCID: PMCPMC12330531.

58.DeAnglis IK, Andrews BR, Lock LR, Dyer KE, Yang A, Volokhov DV, et al. Bat cellular immunity varies by year and dietary habit amidst land conversion. *Conserv Phys* 10.1093/conphys/coad102. PubMed PMID: 38293641; PubMed Central PMCID: PMCPMC10823333.

59.Becker D DK, Lock L, Fenton B, Simmons N. . Habitat and seasonal drivers of leukocyte profiles within and across Neotropical bat species. . *Pre-Print bioRxiv*. 2025.