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ORIGINAL ARTICLE

***Trypanosoma cruzi*-infected *Triatoma gerstaeckeri* (Hemiptera: Reduviidae) from Nuevo León, México, and pathogenicity of the regional strain**

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Introduction: Four species of triatomines have been reported in Nuevo León, northeast (NE) México, but *Triatoma gerstaeckeri* has only been recorded from a peridomestic dwelling.

Objectives: To assess the natural infection index (NII) of *Trypanosoma cruzi* in triatomines and the infestation index (II) of *T. gerstaeckeri* collected in a suburban locality, and to collect histopathological data to understand tissue tropism of the regional *T. cruzi* strain (strain NE) obtained from the vectors collected after an experimental inoculation in *Mus musculus*.

Materials and methods: Triatomines were collected from 85 houses and peridomiciles in Allende, Nuevo León. Stool samples were obtained to determine the *T. cruzi* NII and were used in an experimental mice infection.

Results: A total of 118 *T. gerstaeckeri* were captured, and 46 (adults and nymphs) were collected inside the same house (II=1.17%). Thirty-seven reduvids were infected with *T. cruzi* (NII=31.3%). Tissue tropism of the *T. cruzi* NE strain was progressive in skeletal muscle, myocardial, and adipose tissues and was characterized by the presence of intracellular amastigotes and destruction of cardiac myocells.

Conclusions: The presence of naturally infected domiciliary vectors is an important risk factor for public health in the region considering that these vectors are the principal transmission mechanism of the parasite. The *T. cruzi* NE strain has similar virulence to that of other Mexican and Texan strains and caused chagasic infections in 11 of 12 mice.

Key words: *Trypanosoma cruzi*, *Triatoma*, pathology, virulence, Chagas disease.

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***Triatoma gerstaeckeri* (Hemiptera: Reduviidae) infectada con *Trypanosoma cruzi* en Nuevo León, México, y capacidad patógena de la cepa regional**

Introducción. En Nuevo León, localizado en el noreste de México, existen cuatro especies de triatomíneos, de las cuales *Triatoma gerstaeckeri* ha sido la única reportada en peridomicilios.

Objetivos. Evaluar el índice de infección natural de *Trypanosoma cruzi* en los triatomíneos y el índice de infestación de *T. gerstaeckeri* en una localidad suburbana, y obtener datos histopatológicos para comprender el tropismo tisular de la cepa regional (cepa NE) de *T. cruzi* obtenida de los vectores recolectados después de la infección experimental en *Mus musculus*.

Materiales y métodos. La recolección de triatomíneos se llevó a cabo en 85 casas y peridomicilios de Allende, Nuevo León, México. Se obtuvieron muestras de las deyecciones para conocer el índice de infección natural por *T. cruzi* y, con estas, se hicieron inoculaciones experimentales en ratones.

Resultados. Se capturaron 118 especímenes de *T. gerstaeckeri*, 46 (adultos y ninfas) en el mismo domicilio (índice de infestación=1,17 %). Treinta y siete reduvidos estaban infectados con *T. cruzi* (índice de infección natural, 31,3). El tropismo tisular de la cepa NE de *T. cruzi* fue progresivo en músculo esquelético, miocardio y tejido adiposo, y se caracterizó por la presencia de amastigotes intracelulares con destrucción de células cardíacas.

Conclusiones. La presencia de vectores domiciliarios naturalmente infectados con *T. cruzi*, es un factor de riesgo importante para la salud pública de la región, considerando que este es el principal mecanismo de la transmisión del parásito y que la cepa NE de *T. cruzi* tiene una virulencia similar a la de otras cepas mexicanas y texanas, y causó infección chagásica en 11 de los 12 ratones inoculados.

Palabras clave: *Trypanosoma cruzi*, *Triatoma*, patología, virulencia, enfermedad de Chagas.

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Author's contributions:

Zinnia J. Molina and Lucio Galaviz designed the study, prepared the samples, and conducted the epidemiological study.

Daniel P. Molina and Roberto Mercado entomologically classified the captured insects, performed the histopathology, analyzed and interpreted the data, and drafted and edited the manuscript.

American trypanosomiasis, or Chagas disease, is a major health problem in rural, urban, and suburban areas of the Americas (1). Few studies of Chagas disease have been conducted in north-eastern México, which was considered free of *Trypanosoma cruzi* infection until 2009, because no reports of human cases were recorded. We recently demonstrated a mean seropositivity of 2.8% (2), but we did not address whether transmission of *T. cruzi* could occur from intradomiciliary or peridomiciliary hematophagous vectors.

Thirty-four triatomine species have been reported in México (3); however, only four species exist in the NE region of the country (4), where *Triatoma gerstaeckeri* has been collected most frequently from sylvatic habitats in association with the woodrat, *Neotoma micropus* (5). Few reports have shown infestations in domiciliary or peridomestic dwellings. Furthermore, no reports have focused on the tissue tropism of *T. cruzi* strains from NE Mexico and their public health implications.

Thus, we assessed the infestation index (II) and natural infection index (NII) of *T. cruzi* in *T. gerstaeckeri* collected in a suburban locality and gathered histopathological data to understand the tissue tropism and clinical importance of this *T. cruzi* strain (hereafter the *T. cruzi* NE strain). We also used the vectors collected for an experimental inoculation of *Mus musculus*.

Materials and methods

Study area

Sampling was carried out in the municipality of Allende, Nuevo León State, México, which shares its northern border with Texas, USA, and is located at 25°13' N and 100°08' W (figure 1) at the foot of the Sierra Madre Oriental, with valleys and hills ranging from 300 m above sea level in the northeastern region and up to 1,640 m above sea level in the southeastern region of the municipality. The mean annual temperature is 21.2°C, with a maximum of 40.1°C and a minimum of -2.4°C. The

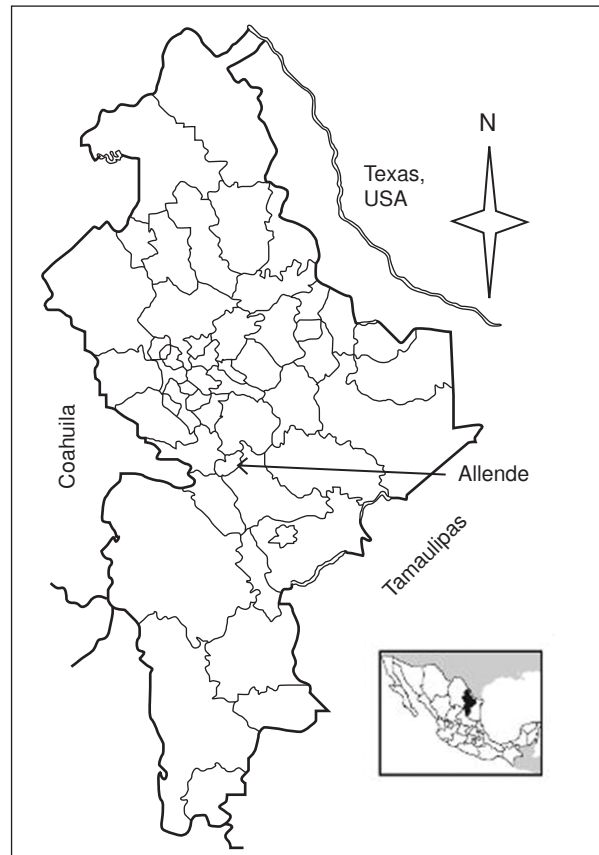


Figure 1. Map of the municipality of Allende, Nuevo León State, México

mean annual precipitation in the municipality is 1,055 mm (according to the Instituto Nacional de Estadística y Geografía) (6).

Specimen collection, infestation index, and natural infection index

This study was conducted from February to August, 2013. The sample size was determined using an estimated vector prevalence of 2.3% reported recently by our group (7). Eighty-five domiciles were visited of 7,500 houses in the municipality, with an absolute precision of 1.96, confidence level of 95%, and $E=1.96$, which exceeded the calculated sample size of 35 houses. Triatomines were collected in intradomiciles and peridomiciles according to a systematic random-stratified model. Inclusion criteria included a community socio-economic index, which was developed using household characteristics (wall, roof, and floor materials). The domiciles were assigned numerical values of 0, 1, or 2, representing three economic strata. Two to 10 housing blocks were selected for sampling randomly from maps (6), according to the

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density of each population. The selection procedure for the houses to be sampled involved enumerating each house in the block and selecting five houses on each side (6). Consent of the householder was the final inclusion criterion. If consent was refused, the house was replaced with a house nearby (8,9).

The timed manual collection consisted of searching for bugs inside dwellings and peridomestic habitats. The intradomiciliary searches focused on objects where triatomines could hide, and cracks and crevices in the walls, floors, and roofs, as well as areas around tables and chairs in the kitchen and bedrooms, were checked for 30 min by two searchers per house (10). Peridomestic annexes, including hen houses, corrals, storerooms, and wood-piles, were searched by two searchers for 30 min (10). Light traps were set up for collections in peridomestic and sylvatic areas using UV light-trap devices and plastic containers (model GT-200; Gardned Products, Horicon, WI, USA). The surveys were performed from 18:00 to 6:00 h (11,12).

All captured specimens were placed in glass containers covered with nylon mesh, and vertically folded cardboard was placed inside as a substrate. Each container was labeled according to the collection site and transported to the laboratory for identification (3,4). Stool samples were obtained by abdominal compression to determine the *T. cruzi* NII and to perform the experimental infection. A fresh stool aliquot was examined using an optical microscope (model DM 750; Leica, Ballerup, Denmark) to observe the trypomastigote forms *in vivo* (5).

Experimental infection

An experimental infection was performed using 5-week-old NHI male mice (*M. musculus*). Two groups (A and B) of six mice each were kept under standard conditions. Each mouse was injected intraperitoneally with 1×10^5 metacyclic trypomastigotes of the *T. cruzi*NE strain. Parasitemia

was evaluated every 3 days using a hemocytometer (Cat. 1490; Hausser Scientific Co., Horsham, PA, USA), and the values are expressed as parasites/ml (13). In addition, one group of 10 mice was monitored daily for survival over 100 days. Infected mice were euthanized by exsanguination under anesthesia on day 20-55 post-infection (dpi). Tissue samples were processed for histopathology and stained with hematoxylin and eosin.

The data of the arithmetic means for the pre-patent period, parasitemia values, dpi, tissue lesions, predominant tropism, and percent survival between the two groups were analyzed using Student's t-test and the variance to detect differences using SPSS ver. 17 software (SPSS, Inc., Chicago, IL, USA).

Ethics statement

This study protocol was in strict accordance with the *Universidad Autónoma de Nuevo León* ethics committee recommendations. All mice were maintained under pathogen-free conditions in the animal facility at the *Facultad de Ciencias Biológicas*. The animals had free access to food and water and were handled in compliance with European standards. The mice were euthanized in a CO₂ chamber, and all efforts were made to minimize suffering.

Results

Natural infection and infestation indices

A total of 118 *T. gerstaeckeri* reduvids were collected (19 stage III nymphs, 36 stage IV nymphs, and 63 adults). Seventy-two reduvids were collected in peridomiciles. Forty-six triatomines (adult and nymphs) were collected from inside the same house (table 1), with an II of 1.17%.

Thirty-seven (37/118, 31.3%) houses were infected with *T. cruzi*. The greatest II (40.2%) was in a peridomicile where 72 triatomines were collected.

Table 1. Examination of the *Triatoma gerstaeckeri* and *Trypanosoma cruzi* infection indices in intradomicile and peridomicile, Allende, N.L., México

Development status	Intradomicile*			Peridomicile**			Total		
	Exam	Pos	NII (%)	Exam	Pos	NII	Exam	Pos	NII
Adults	17	5	29.4	46	22	47.8	63	27	42.8
Nymphs (III)	--	--	---	19	1	5.2	19	1	5.26
Nymphs (IV)	29	3	10.3	7	6	85.7	36	9	25
Total	46	8	17.3	72	29	40.2	118	37	31.3

(*) Only one house was infected.

(**) Thirty-one triatomines were collected with light traps.

Exam: examined; Pos: positive; NII: natural infection index

Five adult triatomines (5/17, 29.4%) and three nymphs (3/29, 10.3%) were infected with *T. cruzi* (NI=17.3%) inside the same house. The other entomological indices could not be calculated because it was impossible to determine whether the light trap-captured triatomines were sylvatic or peridomiciliary.

***Trypanosoma cruzi* NE strain pathogenicity and tissue tropism**

The experimental infection revealed a mean pre-patent period of 7 dpi. The highest and earliest parasitemia peak in mice from groups A and B was observed between 16 and 25 dpi ($p \leq 0.08$) (figure 2). The mice showed hindquarter atrophy during the acute phase and paralysis on 28-29 dpi for the next 5 days, during which the parasitemia persisted in both groups. Peripheral-blood parasite counts peaked at $5-7 \times 10^5$ trypomastigotes per ml in both groups. No parasites were observed in mouse 5B. The differences in tissue lesions, predominant tropism, and the percent survival between the two experimental groups were not significant ($p \leq 0.669$), and the variances were similar.

The anatomopathological damage caused by *T. cruzi* did not differ between the groups. Histopathological analyses of mice infected with *T. cruzi* strain NE showed mononuclear infiltrated cells and progressive myositis in skeletal muscle, myocardial, and adipose tissues, which were observed in mice euthanized 20 dpi. Intracellular amastigote nests and destroyed myocells accompanied by perivascularitis and adipose tissue degeneration were observed, which became more intense 30 dpi. Additionally,

intense myocarditis was characterized by interstitial inflammation with predominant mononuclear infiltrates due to myocardial invasion.

The intramyocytic presence of amastigote nests, interstitial inflammation, disorganized muscle fibers (figure 3), and an inflammatory infiltrate of perivascular adipose tissue (figure 4) were evident in skeletal muscle 40-50 dpi. The smooth muscle layer in the digestive tract showed a few nests of amastigote forms with an intense inflammatory reaction. Amastigote nests were also observed in Kupffer cells. No mortality was observed during the survival experiment.

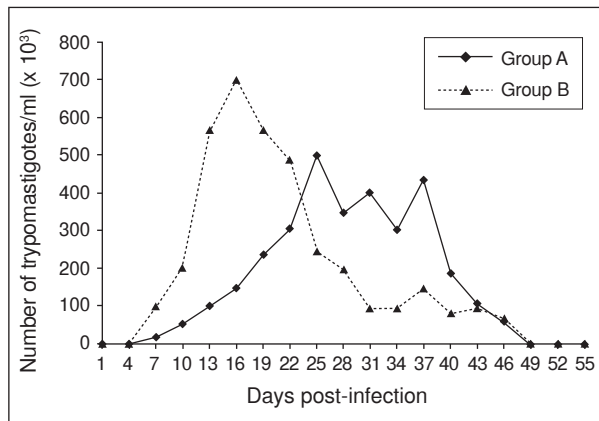


Figure 2. Parasitemic profile observed in the peripheral blood of experimental mice (NHI) groups A and B. Group B had the highest parasitemic peak of 699.9×10^3 trypomastigotes/ml, and Group A peaked at 500×10^3 trypomastigotes/ml.

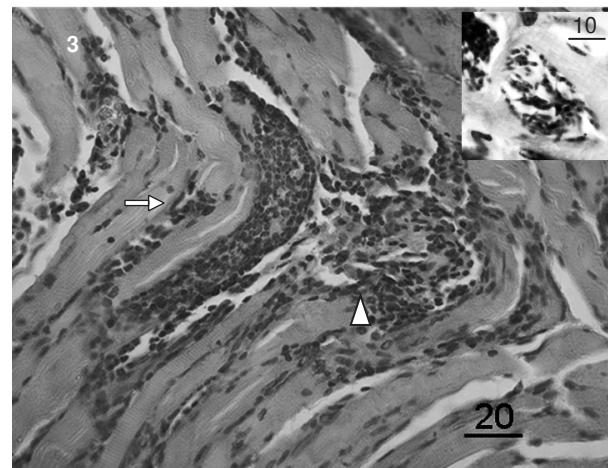


Figure 3. *Trypanosoma cruzi* myocardial histopathology. Intense myositis (arrow) and disruption of muscle fibers (triangle) are observed. A nest of amastigotes is present (square) (hematoxylin and eosin, 100X). Scale bar = 20 μ m

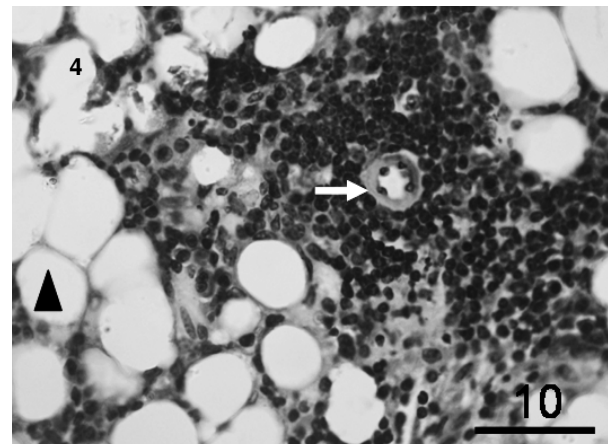


Figure 4. *Trypanosoma cruzi* myocardial histopathology. The myocardial tissue has regions of adipositis (triangle) and perivascularitis (arrow) with a dense inflammatory infiltrate (hematoxylin and eosin, 100X). Scale bar = 10 μ m

Discussion

Natural infection and infestation indices

The distribution of *T. gerstaeckeri* extends from central México through Texas and New Mexico, USA, (14) and is commonly associated with pack rat (*Neotoma* spp.) burrows as a sylvatic species (5,15). Some reports have described *T. gerstaeckeri* as a human and livestock pest species but the adults are frequent invaders of rural houses in southwestern USA, which agrees with our findings.

This is the first report of *T. gerstaeckeri* nymphs and adults inside a dwelling in Nuevo León, México, but reports of humans being bitten are common in southwestern USA (15). Another study reported possible colonization based on observations of a large number of immature stage bugs in a Cameron (Texas, USA) residence where 31 triatomines were collected after three pet dogs died from Chagas cardiomyopathy (24 contained *T. cruzi*) (15). These findings highlight the importance of epidemiological studies of *T. gerstaeckeri* in this geographical region.

Our results are considered an additional case, as we collected 46 triatomines inside a single home, and three employees of the field house were later confirmed to be anti-*T. cruzi* antibody seropositive. A 1992 survey of some villages in the municipality of General Terán, N.L., which is 90 km from our study site, revealed that 181 of 192 (94%) *T. gerstaeckeri* specimens were found in or around houses, with an I of 24% and an NII of 28% (16). Furthermore, this particular species was frequently associated with human dwellings in 2009, when 113 (63%) of 180 *T. gerstaeckeri* specimens collected were found in domestic settings.

Indeed, infected *T. gerstaeckeri* specimens were recovered recently from the southern Texas residence of a child with acute Chagas disease (17). Similarly, the distribution of *T. gerstaeckeri* was extended through the central region of México in 2008 to San Luis Potosí, Veracruz, Querétaro (18), and Hidalgo, as well as inside human dwellings (19). The presence of *T. gerstaeckeri* nymphs inside houses suggests that this triatomine is adapting to houses and that colonization has occurred, based on the presence of nymphs in Texas, USA (14,15,17) and Querétaro (18). Thus, vectorial transmission has increased, and thus the risk of Chagas disease.

Tissue tropism and pathogenicity

Similar low infection peaks ($\sim 1-1.5 \times 10^6$) have been observed in Central American (11,20) and other Mexican strains (21). We elucidated the myotropic

behavior of the *T. cruzi* NE strain by examining invasive capacity into the muscle and adipose tissue of the myocardium. The infective capacity of a Venezuelan isolate of *T. cruzi* was demonstrated in fatty connective tissue and skeletal and cardiac muscle (13); however, future studies are needed to determine the phylogenetic lineage of the *T. cruzi* NE strain (22).

The predominance of *T. cruzi* and its pathogenicity, histopathological analyses, and strain virulence have been described in eight of the 32 Mexican states, showing that *T. cruzi* exhibits considerable intraspecific diversity, as exemplified by differences in morphology of blood forms, virulence, and pathogenicity (23). Mexican strains that are preferentially cardiomyotropic have been reported in Jalisco, Guerrero, and Zacatecas; the Tetitlán strain infects nerves, stomach, brain, and striated muscle, limiting mice survival to 26-30 dpi, whereas the Tepechitlán strain (Zacatecas) causes death in 100% of mice 15-20 dpi (24). The Querétaro strain results in 100% mortality, whereas no mortality was observed in mice infected with the Ninoa strain (25). We report a similar lack of mortality in our survival experiment. Furthermore, both strains produce extended lymphocytic infiltrates in cardiac tissue (25).

The histotropic behavior and pathogenicity of the *T. cruzi* strains differ between areas in México (19), but the most frequently reported tissues infected with *T. cruzi* are the visceral organs, peritoneum, and central nervous system (24,26). The NE strain of *T. cruzi* established itself in the experimental host and caused the development of a chagasic infection in 11 of 12 experimental mice. The third experimental group of mice showed parasitemia peaks that reached 699.9×10^3 parasites/ml, which could be the lowest parasitemia level among the strains that have been analyzed in México (21,25). A *T. cruzi* strain established in southeastern USA was analyzed by Hall in 2010 (13), and their tropism and low virulence results agree with our observations from the third experimental group.

These results contrast with the tropism and infectivity of some populations of *T. cruzi* strains widely documented in Central and South America (27,28), which have higher public health significance because they can cause "silent" disease. Our findings suggest that the presence of a vector naturally infected with *T. cruzi* is an important public health risk factor for the region, considering that it is the principal transmission mechanism.

Therefore, programs should be developed to interrupt vectorial transmission. In addition, it is necessary to promote recognition and identification of the vector in the community because Chagas disease is currently viewed as an exotic illness by public health personnel (29).

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Conflicts of interest

None declared.

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