THERYA NOTES 2024, Vol. 5 : 162-166 DOI: 10.12933/therya_notes-24-165 ISSN 2954-3614

Molecular detection of *Trypanosoma cruzi* in *Mus musculus* in a rural town in Mérida, Yucatán, México

Detección molecular de *Trypanosoma cruzi* en *Mus musculus* en una localidad rural de Mérida, Yucatán, México

Jesús Alonso Panti-May[1], Marco Torres-Castro[1], Javier Escobedo-Ortegón[1], Elsy B. Canché-Pool[1], and Hugo A. Ruiz-Piña[1]*

[1]Centro de Investigaciones Regionales "Dr. Hideyo Noguchi", Universidad Autónoma de Yucatán. Avenida Itzáes 490, C. P. 9700, Mérida. Yucatán, México. E-mail: alonso.panti@correo.uady.mx (JAP-M); antonio.torres@correo.uady.mx (MT-C); eortegon@correo.uady.mx (JE-O); elsy.canche@correo.uady.mx (EBC-P); rpina@correo.uady.mx (HAR-P).
*Corresponding author

American trypanosomiasis is a disease caused by the protozoan *Trypanosoma cruzi* and affects approximately 6 million people in the Americas. Commensal rodents are a food source for the vector and potential reservoirs of *T. cruzi*. The objective of this study was to estimate the prevalence of *T. cruzi* in commensal rodents that inhabit the rural town of Molas, Yucatán, México. Rodents were captured in households using Sherman traps. DNA extracted from heart samples from the captured rodents was used to detect *T. cruzi* using a PCR test. The prevalence of *T. cruzi* was compared by considering the sex and age of the rodents with the Chi-square test. A total of 114 *Mus musculus* mice were captured. The prevalence of *T. cruzi* was 21.1 % (n = 24) in the individuals examined. The comparison of the prevalence of *T. cruzi* between males and females and between adults and juveniles of *M. musculus* did not show statistically significant differences ($P > 0.05$). The prevalence of *T. cruzi* in *M. musculus* was high compared to other previous studies in México, and infection was observed regardless of rodent sex and age. These results show that *M. musculus* participates in the biological cycle of *T. cruzi* at Molas. Further studies are needed to understand the type of involvement (*e.g.*, reservoir) of these rodents in the transmission dynamics of this parasite.

Key words: American trypanosomiasis; commensal rodents; molecular biology; southeastern México; tropical.

La tripanosomiasis americana es una enfermedad causada por el protozoario *Trypanosoma cruzi* y afecta aproximadamente a 6 millones de personas en las Américas. Los roedores comensales son una fuente de alimento para el vector y potenciales reservorios de *T. cruzi*. El objetivo del presente estudio fue estimar la prevalencia de *T. cruzi* en roedores comensales de la localidad rural de Molas, Yucatán, México. Se capturaron roedores en viviendas usando trampas Sherman. El DNA extraído de muestras de corazón de los roedores capturados fue utilizado para la detección de *T. cruzi* por medio de la técnica de PCR. Se comparó la prevalencia de *T. cruzi* considerando el sexo y edad de los roedores con la prueba de Chi-cuadrada. Un total de 114 ratones *Mus musculus* fueron atrapados. La prevalencia de *T. cruzi* fue 21.1 % (n = 24) en los individuos examinados. La comparación de las prevalencias de *T. cruzi* entre machos y hembras y entre los adultos y juveniles de *M. musculus* no arrojó diferencias estadísticas significativas ($P > 0.05$). La prevalencia de *T. cruzi* en *M. musculus* fue alta, en comparación con otros estudios previos en México, y la infección se presentó independientemente del sexo y la edad de los roedores. Estos resultados demuestran que *M. musculus* participa en el ciclo biológico de *T. cruzi* en Molas. Es necesario realizar mayores estudios para entender el tipo de participación (*e.g.*, reservorio) de estos roedores en la dinámica de transmisión de este parásito.

Palabras clave: Biología molecular; roedores comensales; sureste de México; tripanosomiasis americana; tropical.

American trypanosomiasis, known as Chagas disease, is caused by the flagellated protozoan *Trypanosoma cruzi*. It is a life-threatening disease that has been neglected, which affects approximately 5,742,167 people and puts 70,199,360 people at risk of infection in Latin America (WHO 2015). *Trypanosoma cruzi* is transmitted through hematophagous hemipteran insects (vectors), such as *Rhodnius prolixus*, *Triatoma dimidiata*, and *Triatoma infestans*, accounting for 80 % of reported cases (OPS 2018). Vector transmission occurs when an infected vector feeds on a mammal and, after feeding, defecates on it, contaminating the feeding site or adjacent mucous membranes with parasites (WHO 2015). Other modes of transmission are transfusion of contaminated blood, congenital (vertical), transplantation of infected organs, and consumption of food contaminated with the parasite (Coura 2014).

Trypanosoma cruzi has been reported in more than 100 species of wild mammals, including marsupials, rodents, bats, armadillos, and primates (Jansen *et al.* 2017). In wild environments, rodents have been suggested to play a secondary role as reservoirs for this parasite due to the low infection frequencies reported (usually 0.4 %–5 %) compared to other mammals such as marsupials (11 %–90 %; Jansen *et al.* 2017). In anthropized environments, there are rodents known as commensals, such as the house mouse *Mus musculus* and the black rat *Rattus rattus*, which take advantage of food sources and shelter that favor large populations and infestations in households throughout the year (Battersby *et al.* 2008). This has suggested that commensal rodents may play an important role in domestic and peridomestic transmission of *T. cruzi* (Pinto *et al.*

2006; Ayaqui and Ruelas 2020). There are reports of *T. cruzi* infection in *M. musculus* and *R. rattus* in these environments in Ecuador and Chile, with contrasting results. Although *M. musculus* was the rodent most frequently captured (n = 79) in households in Guayas, Ecuador, all specimens tested negative, while in *R. rattus* (n = 61), the second most captured species, the infection rate was 11.5 %. In Coquimbo, Chile, *R. rattus* (n = 46) was captured more frequently than *M. musculus* (n = 5), but both species showed similar infection rates, 83.6 % and 83.3 %, respectively.

In the city of Mérida, Yucatán, México, and its rural surroundings, people positive for *T. cruzi*, have been reported, as well as vectors (Guzmán-Tapia *et al.* 2007; García-Montalvo 2011) and the rodents *M. musculus* and *R. rattus* (Panti-May *et al.* 2017) also positive for *T. cruzi*. Although experimental infections with this protozoan in *M. musculus* are abundant, few studies have reported the natural infection rate in this rodent compared to *R. rattus* (Jansen *et al.* 2017). Therefore, the present study aimed to determine the prevalence of *T. cruzi* infection in *M. musculus* living in a rural town in Yucatán, México.

As part of a multidisciplinary study on zoonotic pathogens (Pacheco-Castro *et al.* 2013), commensal rodents were sampled in the rural town of Molas (20° 48' 55.42" N, 89° 37' 48.40" W), *ca*. 8 km south of the city of Mérida, in the municipality of Mérida, Yucatán, México (Figure 1). During 2009–2010, 200 Sherman traps were placed monthly in 20 households for 3 consecutive nights. The bait used was a mixture of oat flakes and artificial vanilla essence. The captured rodents were euthanized with sodium pentobarbital administered intraperitoneally (Leary *et al.* 2020). Subsequently, a mid-thoracic incision was performed to remove the heart, which was preserved in 10 % buffered formalin for subsequent molecular testing and histopathology analyses. The species, sex, and age of each rodent specimen were recorded; age was determined according to Panti-May *et al.* (2012). All field and laboratory protocols were approved by the Bioethics Committee of the Campus of Biological and Agricultural Sciences, Autonomous University of Yucatán (protocol no. CB-CCBA-L-2009-001).

From each heart sample, a 0.5 g section was obtained and placed in 200 µL of phosphate buffer (PBS) and incubated at room temperature for 24 hr. Afterward, the PBS was discarded, and the DNA was purified with the InstaGene™ Matrix resin (Bio-Rad®, Hercules, California) following the manufacturer's protocol. For the detection of *T. cruzi*, primers 121 and 122 were used, which amplify a region of the kinetoplast DNA (kDNA) of *T. cruzi* of approximately 330 base pairs (Wincker *et al.* 1994). Each PCR reaction included 50-100 ng of DNA, 10 µL of GoTaq Green Master Mix (Promega, Madison, Wisconsin), 200 ng of each primer and nuclease-free water to a final volume of 20 µL.

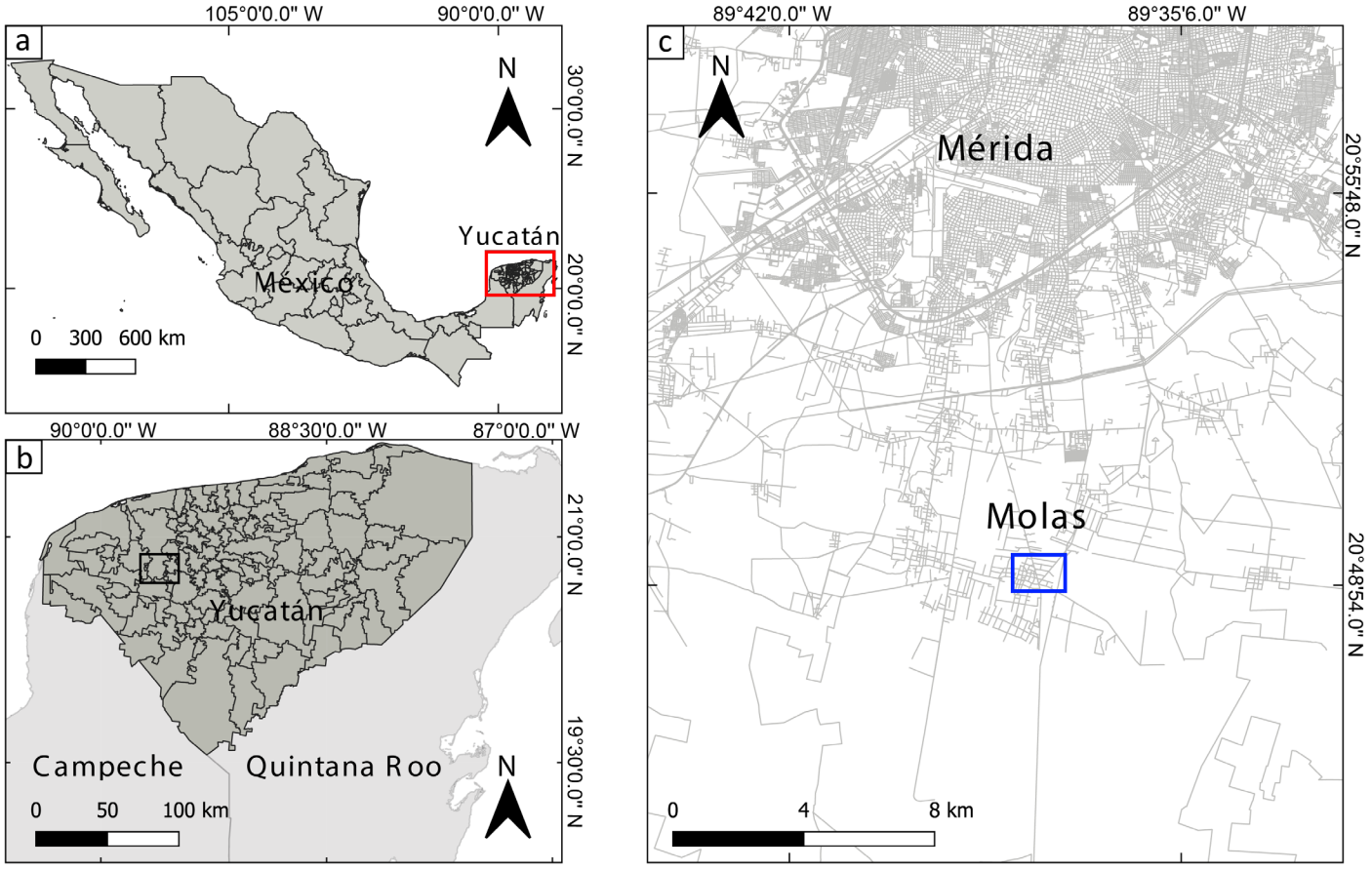

Figure 1. Geographic location of the town of Molas, Yucatán, México. a) Location of the state of Yucatán in México (red box); b) location of the city of Mérida (black box) in Yucatán; c) location of the town of Molas (blue box) in relation to the city of Mérida.

The amplification conditions were 5 min at 94 °C; 35 cycles of 94 °C for 60 seconds, 57 °C for 60 seconds, and 72 °C for 60 seconds; and a final extension at 72 °C for 10 minutes. Positive controls (*T. cruzi* DNA) and negative controls (molecular-grade water) were included in each reaction. Electrophoresis of PCR products was performed on 1.5 % agarose gels stained with ethidium bromide. The results were visualized and recorded on a photo-documenter (Bio-Rad®, Hercules, California).

The prevalence of *T. cruzi* infection and its 95 % confidence intervals (CI) were estimated with the Quantitative Parasitology 3.0 program (Rózsa *et al.* 2000). Likewise, the prevalence of infection was compared between host sexes and between age groups using the Chi-square test and the program mentioned above.

A total of 114 individuals of *M. musculus* were captured, including 47 females and 67 males; of these, 89 were adults and 25 were juveniles. Twenty-four samples of *M. musculus* (21.1 %, CI = 14.4 %–29.7 %) tested positive for *T. cruzi* (Figure 2). The comparison of the prevalence of *T. cruzi* between males (25.4 %, CI = 16.3 %–37.2 %) and females (14.9 %, CI = 7.1 %–28.5 %), was not significantly different ($\chi 2 = 1.8$, d.f. = 1, $P = 0.18$). In addition, no significant differences were found ($\chi 2 = 3.3$, d.f. = 1, $P = 0.07$) in the infection rate with *T. cruzi* between adults (27.7 %, CI = 16.7 %–34.8 %) and juveniles (8 %, CI = 1.5 %–25.6 %).

The presence of *T. cruzi* in *M. musculus* through molecular methods has been reported in some regions of the Americas. The prevalence of *T. cruzi* found in *M. musculus* in Molas (21.1 %) is higher than the 6.8 % reported in the United States of America (Herrera *et al.* 2015), but lower than 83.3 % in Chile (Yefi-Quinteros *et al.* 2018). In México, a 7.9 % prevalence of *T. cruzi* in this rodent was reported in the state of Morelos (Ramsey *et al.* 2012). Particularly in the city of Mérida, Panti-May *et al.* (2017) determined the infection of *M. musculus* with *T. cruzi* in an urban and a suburban area located *ca.* 11 km and 8 km north of Molas, respectively. In the urban area of Mérida, no specimens of *M. musculus* were positive for *T. cruzi*, while in the suburban area, the infection rate was 15.7 %. Another study conducted in Yucatán investigated *T. cruzi* infection in *M. musculus* and *R. rattus* living in a rural community in the municipality of Cenotillo; no specimens of *M. musculus* were positive (Hernández-Cortazar *et al.* 2018). This shows the wide variability of infection with *T. cruzi* in commensal rodents, even within the same region.

The present study recorded infection with *T. cruzi* in *M. musculus* regardless of rodent sex or age. This involves the exposure of this rodent to the parasite through several transmission routes, such as vectorial, oral, vertical, and sexual. Blood from *M. musculus* has been found in the gut of vectors such as *T. dimidiata* in Guatemala

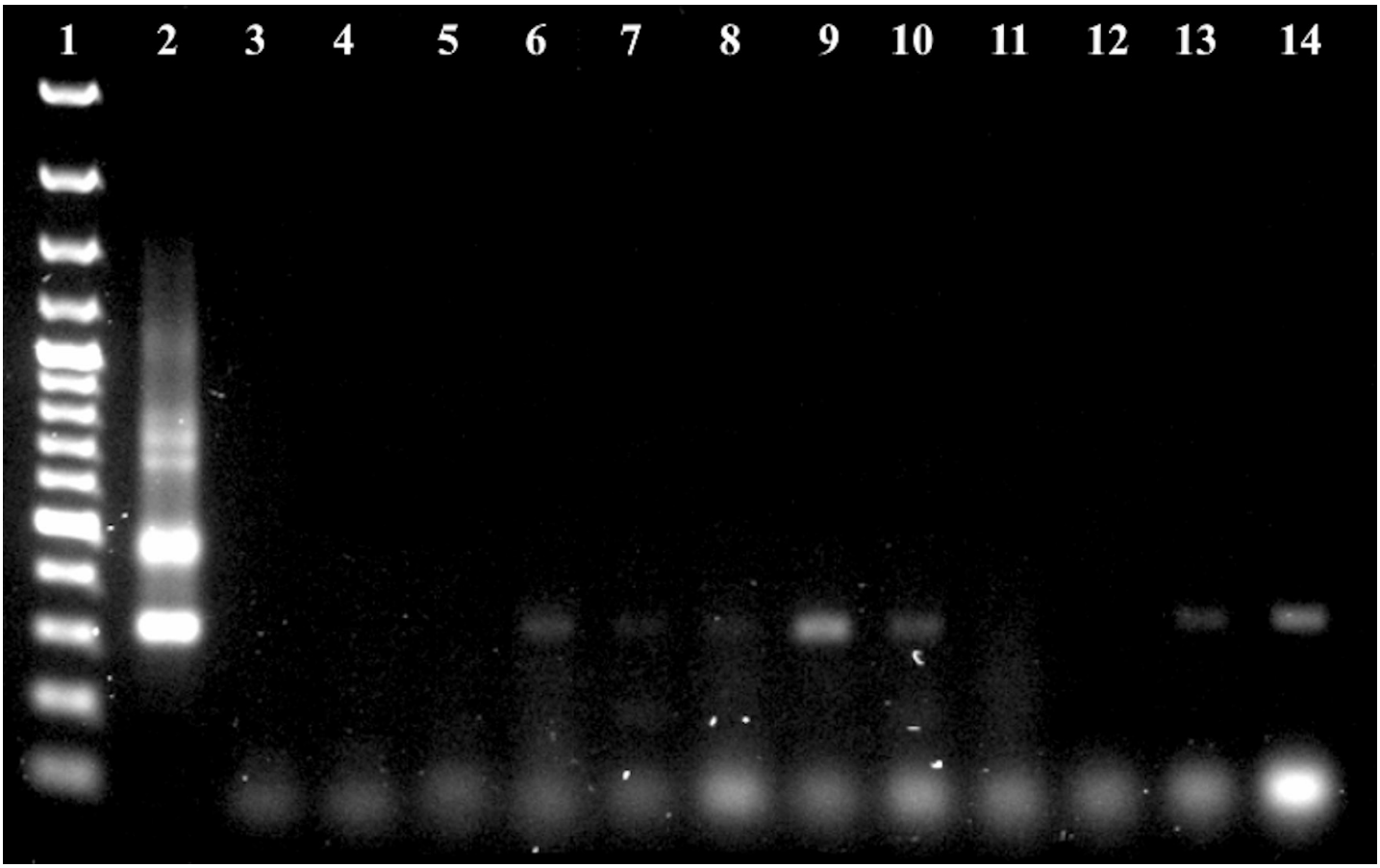

Figure 2. 1.5 % agarose gel showing the amplified 330 base pairs (bp) corresponding to the *Trypanosoma cruzi* kDNA in *Mus musculus* in Molas, Yucatán, México. Lane (L) 1, 100-bp molecular weight marker; L2, positive control; L3, negative control; L4, L5 and L12, negative samples; L6-L10, L13, L14, positive samples.

(Bustamante *et al.* 2014) and México (Torres-Montero *et al.* 2012) and *Panstrongylus geniculatus* in Venezuela (Segovia *et al.* 2023). The foregoing shows that *M. musculus* is a food source for vectors, which can promote vector-borne transmission. Experimental studies have shown that *M. musculus* can be infected through oral inoculum with blood and metacyclic trypomastigotes of *T. cruzi* (Dias *et al.* 2013), which may favor the maintenance of the parasite in rodents through the consumption of vectors that carry the metacyclic forms and contact with the blood of mice infected with trypomastigotes during fights. Vertical and sexual transmission routes have been experimentally demonstrated in *M. musculus*, but it has been concluded that they do not contribute significantly to the transmission and maintenance of the parasite (Rios *et al.* 2018; Faral-Tello *et al.* 2023).

This study used heart tissue to detect *T. cruzi* DNA in naturally infected mice. Infection with *T. cruzi* is characterized by an acute phase in which the parasite is found in the blood and various tissues, including the heart, and a chronic phase in which the parasite infects several tissues, such as the heart, skeletal muscle, kidney, liver, and spleen (Cencig *et al.* 2011; Caldas *et al.* 2012). Although *T. cruzi* can parasitize multiple tissues, the parasite load can vary in the different infection stages, depending on the parasite strain, the inoculum, and reinfections (Andrade *et al.* 1999; Cummings and Tarleton 2003; Cencig *et al.* 2011).

Commensal rodents have been identified as potential sentinels of vector transmission of *T. cruzi* because they are susceptible to infection, can be a source of food for vectors, are abundant, easy to capture, and live in proximity with the local inhabitants (Pinto *et al.* 2006; Bustamante *et al.* 2014; Panti-May *et al.* 2017; Ayaqui and Ruelas 2020). *Mus musculus* is the most frequently reported commensal rodent species in the city of Mérida (Panti-May *et al.* 2012, 2016). However, *R. rattus* may be more abundant in areas with tree vegetation due to its semi-arboreal behavior (Battersby *et al.* 2008). Therefore, *R. rattus* should be considered in studies addressing pathogens in commensal rodents. The results obtained in the present work highlight the need to study the participation of *M. musculus* in the biological cycle of *T. cruzi* in the city of Mérida and the rest of the Yucatán state.

Acknowledgements

The authors wish to thank W. I. Moguel-Chin for his assistance in the preparation of Figure 1. Thanks also to the Secretariat of Public Education for financing through the project "Estudio Multidisciplinario para la identificación de variables asociadas a la transmisión de enfermedades zoonóticas y ETVs en Yucatán" (Multidisciplinary study for the identification of variables associated with the transmission of zoonotic diseases and VBDs in Yucatán), project no. 103.5/09/1258. The comments of two anonymous reviewers improved the first version of this note. M. E. Sánchez-Salazar translated the manuscript into English.

Literature cited

Andrade, L. O., *et al.* 1999. Differential tissue distribution of diverse clones of *Trypanosoma cruzi* in infected mice. Molecular and Biochemical Parasitology 100:163-172.

Ayaqui, R., and N. Ruelas. 2020. Infección de *Rattus rattus* por *Trypanosoma cruzi* como indicador del control vectorial en Arequipa, Perú. Revista Veterinaria 32:73-78.

Battersby, S., R. B. Hirschhorn, and B. R. Amman. 2008. Commensal rodents. Pp. 387-419 *in* Public Health Significance of Urban Pests. World Health Organization. Copenhage, Swiss.

Bustamante, D. M., *et al.* 2014. Ecological, social and biological risk factors for continued *Trypanosoma cruzi* transmission by *Triatoma dimidiata* in Guatemala. PLoS One 9:e104599.

Caldas, S., *et al.* 2012. Real-time PCR strategy for parasite quantification in blood and tissue samples of experimental *Trypanosoma cruzi* infection. Acta Tropica 123:170-177.

Cencig, S., *et al.* 2011. Parasitic loads in tissues of mice infected with *Trypanosoma cruzi* and treated with AmBisome. PLoS Neglected Tropical Diseases 5:e1216.

Coura, J. R. 2014. The main sceneries of Chagas disease transmission. The vectors, blood and oral transmissions - A comprehensive review. Memórias do Instituto Oswaldo Cruz 110:277-282.

Cummings, K. L., and R. L. Tarleton. 2003. Rapid quantitation of *Trypanosoma cruzi* in host tissue by real-time PCR. Molecular and Biochemical Parasitology 129:53-59.

Dias, G. B. M., *et al.* 2013. Evolution of infection in mice inoculated by the oral route with different developmental forms of *Trypanosoma cruzi* I and II. Experimental Parasitology 135:511-517.

Faral-Tello, P., *et al.* 2023. *Trypanosoma cruzi* isolates naturally adapted to congenital transmission display a unique strategy of transplacental passage. Microbiology Spectrum 11:1-21.

García-Montalvo, B. 2011. *Trypanosoma cruzi* antibodies in blood donors in Yucatan state, Mexico. Revista Médica del Instituto Mexicano del Seguro Social 49:367-372.

Guzman-Tapia, Y., M. J. Ramírez-Sierra, and E. Dumonteil. 2007. Urban infestation by *Triatoma dimidiata* in the city of Mérida, Yucatán, México. Vector-Borne and Zoonotic Diseases 7:597-606.

Hernández-Cortazar, I., *et al.* 2018. Frequency of *Trypanosoma cruzi* infection in synanthropic and wild rodents captured in a rural community in southeast of Mexico. Veterinary Medicine International 2018:1-7.

Herrera, C. P., *et al.* 2015. Genotype diversity of *Trypanosoma cruzi* in small rodents and *Triatoma sanguisuga* from a rural area in New Orleans, Louisiana. Parasites and Vectors 8:123.

Jansen, A. M., S. C. C. Xavier, and A. L. R. Roque. 2017. Ecological aspects of *Trypanosoma cruzi*: wild hosts and reservoris. Pp. 243-264 *in* American Trypanosomiasis Chagas Disease. Elsevier. Amsterdam, Netherlands.

Leary, S. *et al.* 2020. AVMA Guidelines for the euthanasia of animals: 2020 edition. American Veterinary Medical Association. Schaumburg, U.S.A.

Organización Panamericana de la Salud (OPS). 2018. Guía para el diagnóstico y el tratamiento de la enfermedad de Chagas. Organización Panamericana de la Salud. Washington, U.S.A.

Pacheco-Castro, J., *et al.* 2013. Estudios multidisciplinarios de las enfermedades zoonóticas y ETVs en Yucatán. Universidad Autónoma de Yucatán. Mérida, México.

Panti-May, J. A., *et al*. 2012. Abundance and population parameters of commensal rodents present in rural households in Yucatan, Mexico. International Biodeterioration and Biodegradation 66:77-81.

Panti-May, J. A., *et al*. 2016. Population characteristics of human-commensal rodents present in households from Mérida, Yucatán, México. MANTER: Journal of Parasite Biodiversity 5:1-6.

Panti-May, J. A., *et al*. 2017. A survey of zoonotic pathogens carried by house mouse and black rat populations in Yucatan, Mexico. Epidemiology and Infection 145:2287-2295.

Pinto, C. M., *et al*. 2006. Infection by trypanosomes in marsupials and rodents associated with human dwellings in Ecuador. Journal of Parasitology 92:1251-1255.

Ramsey, J. M., *et al*. 2012. Ecological connectivity of *Trypanosoma cruzi* reservoirs and *Triatoma pallidipennis* hosts in an anthropogenic landscape with endemic Chagas disease. PLoS One 7:e46013.

Rios, A., *et al*. 2018. Can sexual transmission support the enzootic cycle of *Trypanosoma cruzi*? Memórias do Instituto Oswaldo Cruz 113:3-8.

Rózsa, L., J. Reiczigel, and G. Majoros. 2000. Quantifying parasites in samples of hosts. Journal of Parasitology 86:228-232.

Segovia, M. *et al*. 2023. Vector mapping and bloodmeal metabarcoding demonstrate risk of urban Chagas disease transmission in Caracas, Venezuela. PLoS Neglected Tropical Diseases 17:e0010613.

Torres-Montero, J., *et al*. 2012. House infestation dynamics and feeding sources of *Triatoma dimidiata* in Central Veracruz, Mexico. American Journal of Tropical Medicine and Hygiene 86:677-682.

Wincker, P., *et al*. 1994. Use of a simplified polymerase chain reaction procedure to detect *Trypanosoma cruzi* in blood samples from chronic chagasic patients in a rural endemic area. American Journal of Tropical Medicine and Hygiene 51:771-777.

World Health Organization (WHO). 2015. Chagas disease in Latin America: an epidemiological update based on 2010 estimates. Weekly Epidemiological Record 6:33-43.

Yefi-Quinteros, E., *et al*. 2018. *Trypanosoma cruzi* load in synanthropic rodents from rural areas in Chile. Parasites & Vectors 11:171.

Associated editor: Beatríz Bolívar Cimé.
Submitted: February 28, 2024; Reviewed: May 22, 2024.
Accepted: June 12, 2024; Published on line: June 19, 2024.