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Natural infection with *Trypanosoma cruzi* in bats captured in Campeche and Yucatán, México

Infección natural con *Trypanosoma cruzi* en murciélagos capturados en Campeche y Yucatán, México

***Trypanosoma cruzi* in bats from Yucatán and Campeche.**

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Introduction: Bats have been reported as hosts of the *Trypanosoma cruzi* protozoan, etiologic agent of the American trypanosomiasis, an endemic zoonotic disease in México.

Objective: To describe the *T*. *cruzi* infection in bats from the states of Campeche and Yucatán, México.

Materials and methods: Captures were made from March to November 2017 at three sites in Yucatán and one in Campeche. Up to four mist nets for the capture on two consecutive nights were used. The bats' species were identified, and the euthanasia was performed to collect kidney and heart samples to be used in the total DNA extraction. The *T*. *cruzi* infection was detected by conventional PCR with the amplification of a fragment belonging to the *T*. *cruzi* DNA nuclear.

Results: 86 bats belonging to five families (Vespertilionidae, Noctilionidae, Mormoopidae, Phyllostomidae, and Molossidae) and 13 species (*Rhogeessa aeneus*, *Noctilio leporinus*, *Pteronotus davyi*, *P*. *parnellii*, *Artibeus jamaicensis*, *A*. *lituratus*, *A*. *phaeotis*, *Glossophaga soricina*, *Carollia sowelli*, *Chiroderma villosum*, *Uroderma bilobatum*, *Sturnira parvidens*, and *Molossus rufus*) were captured. The PCR showed an infection frequency of 30,2 % (26/86), detected only in the renal tissue. The infected species were *P*. *parnellii*, *G*. *soricina*, *A*. *lituratus*, *A*. *jamaicensis*, *S*. *parvidens*, *C*. *villosum*, and *R*. *aeneus*.

Conclusions: The results confirm the participation of several bats species as hosts in the *T*. *cruzi* transmission cycle in the region. Further studies are necessary to establish the importance of these animals in the zoonotic transmission of *T*. *cruzi.*

Key words: *Trypanosoma cruzi*; Chiroptera; infections; polymerase chain reaction; México.

Introducción. Los murciélagos han sido reportados como hospederos del protozoario *Trypanosoma cruzi*, agente etiológico de la tripanosomiasis americana, enfermedad zoonótica endémica en México.

Objetivo. Describir la infección con *T*. *cruzi* en murciélagos capturados en los estados de Campeche y Yucatán, México.

Materiales y métodos. Se realizaron capturas de marzo a noviembre de 2017 en tres sitios de Yucatán y uno de Campeche. Para la captura se emplearon hasta cuatro redes de niebla por dos noches consecutivas. Se identificó la especie de los murciélagos capturados y se les practicó la eutanasia para recolectar muestras de riñón y corazón, utilizadas en la extracción de ADN total. La infección con *T*. *cruzi* se detectó por la amplificación con PCR convencional de un fragmento perteneciente al ADN nuclear de *T*. *cruzi*.

Resultados. Se capturaron 86 murciélagos pertenecientes a cinco familias (Vespertilionidae, Noctilionidae, Mormoopidae, Phyllostomidae, Molossidae) y 13 especies (*Rhogeessa aeneus*, *Noctilio leporinus*, *Pteronotus davyi*, *P*. *parnellii*, *Artibeus jamaicensis*, *A*. *lituratus*, *A*. *phaeotis*, *Glossophaga soricina*, *Carollia sowelli*, *Chiroderma villosum*, *Uroderma bilobatum*, *Sturnira parvidens* y *Molossus rufus*). La PCR mostró una frecuencia de infección de 30,2 % (26/86), detectada únicamente en tejido renal. Las especies infectadas fueron *P*. *parnellii*, *G*. *soricina*, *A*. *lituratus*, *A*. *jamaicensis*, *S*. *parvidens*, *C*. *villosum* y *R*. *aeneus*.

Conclusiones. Los resultados confirman la participación de varias especies de murciélagos como hospederos en el ciclo de transmisión de *T*. *cruzi* en la región. Es necesario realizar más estudios para identificar la importancia de estos animales en la transmisión zoonótica de *T*. *cruzi*.

Palabras clave: *Trypanosoma cruzi*; quirópteros; infecciones; reacción en cadena de la polimerasa; México.

Trypanosoma cruzi (order Kinetoplastida) is a protozoan parasite recognized as the causative agent of Chagas disease or American trypanosomiasis, a zoonotic disease with relevance in areas with poverty conditions and social inequality, mainly in Central America countries (1).

In México, approximately 1,100,000 people may be infected with *T*. *cruzi* and 29,500,000 are at risk of infection (2). The Yucatán Peninsula (southeast of México), which includes the states of Campeche, Yucatán, and Quintana Roo, is an area with numerous cases, mostly due to the *Triatoma dimidiata* high abundance in the peridomestic environment and eventually inside houses (3). In relation to this, according to the *Dirección General de Epidemiología* of the *Secretaría de Salud* (SSA by its acronym in Spanish) of México, in 2017 seven cases of chronic American trypanosomiasis were registered in Campeche, while 86 cases of chronic American trypanosomiasis and one of the acute form were registered in Yucatán (4).

Trypanosoma cruzi infects more than 400 species of mammals (5). The most important natural reservoirs are armadillos, wild rodents, and opossums that help to support the wild transmission cycle (1,6). Other animals such as dogs (7), pigs, sheep, horses (8), and synanthropic rodents (9,10), have also been described as *T*. *cruzi* accidental hosts.

Bats are the second more diverse group (after rodents) of mammals worldwide, so they are distributed in all the México's natural areas (11). These animals have ecological and commercial relevance because they are consumers of insects known as pests, and they pollinate plants used in products for human consumption, respectively (12). However, bats are also recognized as natural

reservoirs of different viruses (13) and accidental hosts of bacteria (14) and parasites (15) with relevance in public and animal health.

Several studies have involved the bats in the *T*. *cruzi* transmission cycle, which has led to formulate the hypothesis that they are parasite ancestral hosts. Later, the parasite was genetically diversified and adapted to other vertebrate hosts (16).

In the American continent, investigations with bats from Ecuador (17), Colombia (18), Argentina (19), México (20), and the United States of América (USA) (21) have been conducted. In them are indicate the importance of these mammals in the transmission risk of *T*. *cruzi* to other animals and humans' populations (17-21).

The present study aims to describe the *T*. *cruzi* infection in bats from Campeche and Yucatán, México, and in this way collaborate with the understanding of the intervention of bats in the *T*. *cruzi* transmission cycle.

Materials and methods

Study sites

The captures were made in three sites of the Yucatán state and one of the Campeche state, México. The sites were selected due to their easy access, roads close to highways, and because they had the necessary infrastructure (electricity, clean running water, and ventilated rooms) to set up a field station.

Site I (Hobonil ranch) is in the Tzucacab municipality, Yucatán (20° 01' 00.9 " N and 89° 01' 11.8" W). It has a warm sub-humid climate with a summer rainfall with little thermal oscillation. The average annual temperature is 26,1° C and the average annual rainfall is 1,097 mm. Its average elevation is 40 m above mean sea level (MAMSL) (22). The ranch's vegetation is composed of medium sub-

deciduous forest areas with different use degrees and areas with introduced species for pasture and forage (23).

Site II (*Ich Ha Lol Xaan* Recreational and Ecotourism Center) is in the Hampolol town, Campeche (19° 56’ 16” N and 90° 22’ 21” W). It has a tropical climate with rains in summer with an average annual temperature of 26,6° C and an average annual rainfall of 1,088 mm. Its average elevation is 10 MAMSL. The vegetation is composed of several types of tropical forests (medium sub-deciduous, medium sub-evergreen, and low flood sub-evergreen), aquatic, and secondary vegetation (24).

Site III (San Francisco ranch) is in the Panabá municipality, Yucatán (21° 21' 48.2" N and 88° 19' 23.6" W). It has a warm sub-humid climate with rains in summer with an average annual temperature of 25,6º C and an average annual rainfall of 1,049 mm. Its average elevation is 17 MAMSL (25). The vegetation is predominantly deciduous rainforest; however, due to agricultural and livestock activities, it has been severely transformed (26).

Site IV (Campus of Biological and Agricultural Sciences [*CCBA* by its acronym in Spanish]) is in the *Cuxtal* Ecological Reserve, Mérida, Yucatán (20° 52′ 02″ N and 89° 37′ 29″ W). The climate is warm sub-humid with rains in summer with an average annual temperature of 26º C and an average annual rainfall of 984,4 mm. Its average elevation is 10 MAMSL (27). The surface is mainly covered with secondary vegetation in different regeneration stages (85 % of the total area); the rest (15 %) is land with agricultural (cornfields, grasslands, henequenals, or other crops) or livestock uses, family gardens, streets and houses (28).

Bats capture

The captures were carried out in March (site I), May (site II), August (site III), and November (site IV) of 2017. On each study site were install up to four mist nets (12 m wide x 2,5 m high) in two consecutive nights and located around water bodies, fruit trees, cave entrances, or abandoned buildings. The nets were open from 6:00 p.m. to 11:00 p.m. and checked every 20-30 min (depending on the bats' activity). All the captured bats were removed from the nets and were placed in a cloth bag. Subsequently, they were transported to the field station.

Obtaining individual variables and biological samples conservation

On the field station, euthanasia in all the studied bats, according to the guidelines described by the American Veterinary Medical Association was performed (29). After, the species of each captured individual as described by Medellín *et al*. (30) and Reid (31) was identified. Also, sex (male or female), reproductive status (mature or immature; in males, mature were individuals with descended testicles; in females, mature were pregnant or with alopecia areas around the nipples), and age (juvenile or adult, according to Torres-Castro *et al*. (15)) were registered. Heart (atria and ventricles) and kidney (cortex and pelvis) fragments from each bat were collected and deposited in a microcentrifuge vial (Eppendorf®; Germany) of 1,5 ml with 99 % ethanol. All the samples were stored at 4° C and were transferred to the Laboratory of Emerging and Re-emerging Diseases (*LEER* by its acronym in Spanish) of the Center for Regional Research "Dr. Hideyo Noguchi "(*CIR* by its acronym in Spanish) of the Autonomous University of Yucatán (*UADY* by its acronym in Spanish), where they were stored at -80° C until use in total genomic DNA extraction.

Total DNA extraction and quantification

Before the total DNA extraction, all tissues were washed to remove alcohol excess. Twenty-five mg of heart or kidney (cut into small fragments) were embedded in 25 µl of proteinase K (Omega Bio-tek Inc®, USA) and lysis buffer; the mixture was incubated at 56° C overnight. Next, a commercial kit (DNeasy Blood & Tissue Kit, QIAGEN®, Germany) was used following the commercial house's specifications. The DNA extraction was performed on 86 hearts and 70 kidneys (it was not possible to collect the kidney sample from the bats of the site I [Hobonil ranch]). The extracted DNA was evaluated on a spectrophotometer (NanoDrop2000TM, Thermo Scientific®, USA); after that was stored at -79° C.

Detection of Trypanosoma cruzi infection

The *T*. *cruzi* detection was performed in all collected tissues by conventional PCR using the oligonucleotides TCZ1 and TCZ2, which amplify a tandem repeated fragment of 188 base pairs (bp) belonging to a region of the nuclear DNA of *T*. *cruzi* (32).

The molecular reaction included the following reagents (final concentrations): 1X PCR buffer, 2,5 mM of $MgCl_2$, 0,2 µM of each oligonucleotide, 1 U Taq polymerase (Thermo Scientific®, USA), 0,2 mM of dNTP's, 3 µl of template DNA (heart or kidney), and molecular biology grade water sufficient for 25 µl (final volume). The conditions in the thermal cycler were: a five-minute stage at 94° C, followed by 35 cycles of ten seconds at 94° C, 30 seconds at 55° C, and 30 seconds at 72° C. The final extension was for five minutes at 72° C.

All reactions included positive (genomic DNA extracted from rodent organs experimentally infected with a *T*. *cruzi* lineage I) and negative controls (all reaction

reagents, but without template DNA). Electrophoresis was performed on 1 % agarose gels stained with ethidium bromide. After, the gels were visualized in a photo-documentation system (Bio-Rad®, USA) for recording results.

Statistical analysis

Descriptive statistic was used to determine the *T*. *cruzi* infection frequency and the frequency of each variable (age, sex, and reproductive condition) collected in the studied bats. Additionally, the association strength of each variable with the infection frequency with a chi-square test (□2) was explored. All data were analysed on the Epiinfo™ program (V 7.2.3.0) (CDC, USA). A $P < 0,05$ value was used for statistical significance.

Bioethical guidelines

The Ministry of Environment and Natural Resources of Mexico (*SEMARNAT* by its acronym in Spanish) approved the extraction of the captured animals (minutes: SGPA/DGVS/ 03705/17 and SGPA/DGVS/01186/17). The Bioethics Committee of the Faculty of Veterinary Medicine and Zootechnics (*FMVZ* by its acronym in Spanish) - *UADY* (minutes: CB-CCBA-I-2018-001) approved the capture, sacrifice, and biological sampling of the studied bats.

Results

Eighty-six bats of 13 distinct species belonging to five families were captured (table 1). In table 1 can be identified that the family with the greatest richness was Phyllostomidae with eight distinct species and that the species with the highest number of individuals captured was *A*. *jamaicensis*, which was presented at all study sites.

The PCR showed an infection rate overall of 30,2 % (26/86). All positive reactions corresponded to renal tissue extractions. The species with infected individuals were: *A*. *jamaicensis*, *G*. *soricina*, *C*. *villosum*, *P*. *parnellii*, *A*. *lituratus*, *R*. *aeneus*, and *S*. *parvidens*, each one with different infection frequencies concerning the number of captured individuals for each species (table 1). Of the infected bats, 16 were captured at site III (61,5 %) and 10 at site II (38,5 %).

Table 2 shows the values and frequencies of the individual variables collected in the bat studied population, as well as those from bats infected with *T*. *cruzi*.

The analysis with $\chi 2$ showed no significance for any of the evaluated cases ($P > 0,05$).

Discussion

Five families and 13 species of bats, which are 20,3 % of the total species found in the Yucatán Peninsula, were captured (33). The most abundant species (by capture) was *A*. *jamaicensis*, which has wide distribution in the Yucatán Peninsula because is tolerant to the ecosystem fragmentation and can colonize a wide variety of natural or artificial shelters (34).

In México, there are reports of the *T*. *cruzi* natural infection in several bat species (20,35,36). It is relevant to mention that in this work, five species in each state (Campeche and Yucatán) were found infected, being a total of seven distinct infected species in all the study sites (two states). This diversity of infected species captured at specific sites underlines the need to evaluate the *T*. *cruzi* distribution in the different epidemiological environments that could be influencing the transmission dynamics between bat populations, such as the abundance of other

vertebrate hosts and the circulating species of insect vectors, in addition to the bats' behavior and ecology (20,37,38).

In a first study conducted with bats from Molas, Yucatán, infection with *T*. *cruzi* was described in the *A*. *jamaicensis*, *A*. *lituratus*, and *S*. *parvidens* species (35). Taking this background as a reference, the records described here for the *C*. *villosum*, *G*. *soricina*, and *R*. *aeneus* species are the first in Yucatán state. Additionally, in other previous study performed with bats from Calakmul, Campeche, the *A*. *jamaicensis*, *A*. *lituratus*, *S*. *parvidens*, *S*. *ludovici*, *C*. *brevicauda*, and *Myotis keaysi* species were found infected (20); therefore, this work reports for the first time the *T*. *cruzi* infection in the *P*. *parnellii* and *G*. *soricina* species, captured in Campeche.

On the other hand, in the investigation carried out with bats from the state of Morelos (central México), the *T*. *cruzi* infection was described in the *A*. *jamaicensis*, *G*. *soricina*, *S*. *parvidens*, and *Choeronycteris Mexicana* species (36). According to these findings and the results presented in the studies with bats from Yucatán (35) and Campeche (20), this work presents the first evidence of the *T*. *cruzi* infection in the *C*. *villosum* species captured in Mexico. Previously, the *Pteronotus parnellii* species has been reported as parasitized by *T*. *cruzi* in Mexico (state of Morelos) (36).

In an international context, there are also reports of the *T*. *cruzi* infection in bats of different genera. For example, in Colombia, infected individuals of the *Carollia*, *Desmodus*, *Glossophaga*, *Noctilio*, *Peropteryx*, *Phyllostomus*, and *Artibeus* genera have been informed. In this same study, the *A*. *lituratus* and *C*. *villosum* species were suggested as a *T*. *cruzi* accidental hosts (18); a result that coincides with this research. Likewise, in Ecuador, infections in individuals of the *Artibeus* and *Myotis*

genera, and the *G*. *soricina* species have been reported (17); this last species was also detected as infected in this work. In Brazil, the *A*. *lituratus* and *G*. *soricina* species were registered as accidental hosts for *T*. *cruzi* (39). Also, in South America, specifically in Peru, infections in specimens of the *Phyllostomus*, *Diaemus*, *Trachops*, and *Desmodus* genera, were reported (37). However, none of the infected species previously described, coincide with the ones found in the bat population studied of Campeche and Yucatán; although, it is important to note that not all species circulate in both study regions (Peru and México).

There are several hypotheses about the *T*. *cruzi* transmission routes to bat populations. These mammals are usually established in caves, trees, or artificial constructions (buildings and houses), places where *T. dimidiata* and others triatomines (reported in Ecuador) such as *Cavernicola pilosa* (associated with *Myotis* sp.) and *Triatoma dispar* (associated with *Molossus molossus species*), have been detected (17); *C*. *pilosa* also has been found in roost sites of at least nine bat species within five families (40,41). These findings are relevant in human health, because, potentially, triatomines associated with bats species might opportunistically feed on and transmit trypanosomes to humans (17,38); also demonstrate that bats and triatomines share the same area and shelters, even when these last are considered to be restricted to sylvatic environments (17), therefore, exists a probability of *T*. *cruzi* vectorial transmission to bat populations (42,43). In this context, in a study in which massive sequencing (12S RNA gene) was used to analyse the abdominal contents of 14 triatomines (*T*. *dimidiata*) collected in Yucatán, at least 14 species of vertebrate animals as food sources for this insects were described, among them bats of the *Artibeus* genus; consequently,

the probability that this bat genus participates in the stability of the *T. cruzi* transmission cycle in the wild environments of the study region was established (44) and also contributes to the parasite enzootic expansion (5,16), since some *Artibeus* genus species (including *A*. *jamaicensis*) in the surroundings and inside rural houses from the Yucatán Peninsula have been captured (35).

The infections in bat species, belonging to the insectivorous trophic level, may have generated by the intake of triatomines with the parasite. Thomas *et al*. (45) confirmed this in individuals of the *Artibeus*, *Carollia*, *Glossophaga*, and *Molossus* genera, to which were offered triatomines experimentally infected with *T*. *cruzi* for consumption. Finally, the *T*. *cruz*i transmission during the gestation period (vertical or congenital) or the lactation stage has also been described in bats (46).

The presence of *T*. *cruzi* DNA was only detected in the renal tissue of the studied bats. Although unexpected because *T*. *cruzi* usually invades the cardiac cells (1), this could be due to many factors, among them the infectious strain virulence (19). In this context, it has been proved under experimental conditions that, depending on this characteristic, a limited distribution on the organism of the affected vertebrate hosts can be generated (19,47,48). Likewise, *T*. *cruzi* did not persist on the cardiac tissue of experimentally infected mice (49,50).

Further studies are necessary to determine the infective strain (s) in the bat populations in the region, as well as the distribution and the damage that the parasite could be caused in the different organs and tissues affected (16).

Finding infected bats with *T*. *cruzi* may have implications for the public health (5,16,21,37), considering that *T*. *dimidiata* lodges in cracks or dark spaces in rural homes to feed on its inhabitants (42,43). Regarding this, Ramírez *et al*. (38) have

shown (through cloning and blood culture) the infection with *T*. *cruzi* strains from bats in humans. Additionally, Villena *et al*. (37) suggest that bats with chronic infections in the salivary glands can contaminate with saliva (with viable parasites) fruits and vegetables, it allows through the intake of contaminated food the *T*. *Cruzi* transmission to human populations and other susceptible animals.

Conflicts of interest

The authors declare that no conflicts of interest exist.

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Table 1. Family, species, total number of captured (n) and infected bats by each study site in Yucatán and Campeche, Mexico.

n = number.

Family	Species	Site I	Site II	Site III	Sitio IV	n	n of infected and % of infected by species
Vespertilionidae	*Rhogeessa aeneus*	0	0	1	0	1	1 (100 %)
Noctilionidae	*Noctilio leporinus*	0	6	0	0	6	0 (0 %)
Mormoopidae	*Pteronotus davyi*	2	0	0	0	2	0 (0 %)
	Pteronotus parnellii	0	6	0	0	6	4 (66,7 %)
Phyllostomidae	*Artibeus jamaicensis*	6	7	12	20	45	10 (22,2 %)
	Artibeus lituratus	0	1	1	0	2	2 (100 %)
	Artibeus phaeotis	3	0	0	0	3	0 (0 %)
	Glossophaga soricina	0	1	3	1	5	4 (80 %)
	Carollia sowelli	0	2	0	0	2	0 (0 %)
	Chiroderma villosum	0	1	6	0	7	4 (57,1 %)
	Uroderma bilobatum	0	1	0	0	1	0 (0 %)
	Sturnira parvidens	0	1	0	0	1	1 (100 %)
Molossidae	*Molossus rufus*	5	0	0	0	5	0 (0 %)
Total		16	26	23	21	86	26

Table 2. Values and frequencies of the studied population of bats and the bats infected with *Trypanosoma cruzi*, in sites of Yucatán and Campeche, Mexico.

	Number of captured bats*	Number of bats infected with *Trypanosoma cruzi***
Study sites		
I	16 (18,6 %)	0 (0 %)
II	26 (30,2 %)	10 (38,5 %)
III	23 (26,8 %)	16 (61,5 %)
IV	21 (24,4 %)	0 (0 %)
Sex		
Male	48 (55,8 %)	10 (38,5 %)
Female	38 (44,2 %)	16 (61,5 %)
Age		
Juvenile	28 (32,6 %)	8 (30,8 %)
Adults	58 (67,4 %)	18 (69,2 %)
Reproductive condition		
Active	50	12 (46,2 %)
Inactive	36	14 (53,8 %)

*n = 86
**n = 26