http://revistas.unam.mx/index.php/biocyt
BIOCYT Biología, Ciencia y Tecnología, 14: 1009-1020, 2021.
DOI: http://dx.doi.org/10.22201/fesi.20072082e.2021.14.77383
ISSN: 2007-2082
Publicada en la Facultad de Estudios Superiores Iztacala, Universidad Nacional Autónoma de México

FIRST REPORT OF AN INFECTED TRIATOMINE BUG IN AN URBAN AREA OF TUXTLA GUTIERREZ, CHIAPAS, MEXICO

PRIMER REPORTE DE UN TRIATOMINO INFECTADO EN UN ÁREA URBANA DE TUXTLA GUTIÉRREZ, CHIAPAS, MÉXICO

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ABSTRACT

Analyze the first report of an infected triatomine in an urban house in Tuxtla Gutiérrez, Chiapas; identifying the vector specie's, the virulence and the genetic group of *Trypanosoma cruzi* and a possible infection in the house inhabitants. The specimen was transferred to the laboratory where a stool sample was obtained by abdominal pressure. The virulence of *T. cruzi* was measured in mice and amplification of the mini exon gene was done to determine the lineage. In the inhabitants the ELISA test was performed to rule out Chagas disease. The specimen was identified as *Triatoma dimidiata* and it was infected with parasites belonging to the TcI lineage, which reached parasitaemia of $5.6X10^6$ parasites/ml of blood. The ELISA test were negative. *T. dimidiata* is the predominant species in the southern of the country and its finding in an urban area highlights the importance of having entomological surveillance programs. TcI lineage has a greater distribution in Mexico and no infection was found in the inhabitants.

Key words: triatomine; *Triatoma dimidiata;* Tuxtla Gutiérrez; *Trypanosoma cruzi*.

Manuscrito recibido el 11 de agosto de 2020, aceptado el 17 de septiembre de 2020.

RESUMEN

Analizar el primer reporte de un triatomino infectado dentro de una vivienda urbana en Tuxtla Gutiérrez, Chiapas; identificar la especie del vector, la virulencia y el grupo genético de *Trypanosoma cruzi* y la posible infección en los habitantes. El ejemplar se trasladó al laboratorio donde por presión abdominal se obtuvo una muestra de heces. La virulencia de *T. cruzi* fue evaluada en ratones y se realizó amplificación del gen mini exón para determinar el linaje. En los habitantes se realizó la prueba de ELISA para determinar anticuerpos contra *T. cruzi*. El ejemplar fue identificado como *Triatoma dimidiata*, positivo para *T. cruzi,* perteneciente al linaje TcI, con una parasitemia máxima de $5.6X10^6$ parásitos/ml de sangre. Las pruebas de ELISA resultaron negativas. *T. dimidiata* es la especie predominante en el sur del país y su hallazgo en una zona urbana pone de manifiesto la importancia de contar con programas de vigilancia entomológica. El linaje TcI tiene mayor distribución en México y no se encontró infección en los habitantes.
Palabras clave: triatominos; *Triatoma dimidiata;* Tuxtla Gutiérrez; *Trypanosoma cruzi*.

INTRODUCTION

The triatomines (Hemiptera, Reduviidae; Triatominae) are blood-sucking insects and epidemiologically important vectors of the Chagas disease parasite, *Trypanosoma cruzi* Chagas, 1909 (Jurberg and Galvao, 2006). This disease is endemic in Latin America and considered as one of the most important parasitic infections because it results in significant disability, just behind acute respiratory and intestinal infections, as well as AIDS (Guadalupe-Pérez et al., 2011). According to recent estimates, there are approximately eight million people worldwide with this disease and 25 million more at risk of contracting it (De Fuentes-Vicente et al., 2018).

The dissemination of Chagas disease is strongly linked to the distribution of hemiptera bug vectors from the subfamily Triatominae and it mainly affects rural areas with high poverty rates and social marginalization (Lee et al., 2013). The cracks of precarious houses made of adobe, palm, wood, or sheet metal, are ideal sites for the refuge of bug vectors, which feed on blood preferably at night (Gutiérrez-Cabrera et al., 2018). Some of the 140 species of bugs described so far have adapted to the inside or peripheral of dwellings, which increase the risk of human transmission (Ramirez-Sierra et al., 2010; Dumonteil et al., 2013). Among the most important vector species are *Rhodnius prolixus* Stål, 1859, *Triatoma infestans* Klug in Meyen, 1834, *Triatoma barberi* Usinger, 1939, *Triatoma dimidiata* Latreille, 1811 and *Meccus pallidipennis* Stål, 1872 (De Fuentes-Vicente et al., 2018). The last three are the most significant in the epidemiology of Chagas disease in Mexico (Salazar-Schettino et al., 2005).

The occurrence of these insects is mainly in rural areas near wild ecotopes. Nevertheless, urban areas with high population movements between both regions are also affected. Thus, travel equipment or transported products (e.g., wood, plants, and food bags) can be transport and infestation carriers of these bugs (Cécere et al., 1996). Although they hardly achieve their reproduction cycle in the virgin zone, they can represent a latent risk of infection for a certain time.

During 2017, Chiapas ranked second in acute Chagas disease, with an incidence rate of 0.58 per 100 000 population, only behind state of Guerrero with 0.97 per 100 000. This tendency continues, given that this Southeastern Mexican state occupied the second site until week 14 of 2019 (DGE, 2019). Health systems in state of Chiapas recognizes this disease as one of the major problems of the population. Within its state program to reduce and strengthen its surveillance, it establishes a specific plan for its prevention and control (PES, 2018). Despite these purposes and actions, Chagas disease as a neglected disease still prevails among the population of Chiapas. Therefore, it is necessary to review and improve the control actions.

This work reports the first occurrence of an infected triatomine inside a dwelling in an urban area of Tuxtla Gutierrez, Chiapas, Mexico. Thus, it is also described the species found, its parasitological and genetic characteristics, as well as the possible infection in the inhabitants of the dwelling. Finally, hypotheses of the specimen's origin are discussed.

MATERIALS AND METHODS

Collection and identification of the triatominae bug

Our working group was alerted to the presence of a possible triatomine bug by the inhabitants of a northwestern house of the city (16°46'30.1"N 93°07'44.4"W; 570) (Fig. 1). The collection site was georeferenced with the program ArcGis 10.7. The specimen was captured in August 2019 at noon by the head of the family and placed in a plastic bottle. The specimen was found in a room with a concrete wall and floor, with no characteristics of having been fed recently. Our work group searched for other specimens in the house, but no more were found. Subsequently, it was transferred to the Multidisciplinary Experimental Laboratory and Bioterium of the Universidad de Ciencias y Artes de Chiapas, where it was identified according to the dichotomous keys of Lent and Wygodzinsky (1979).

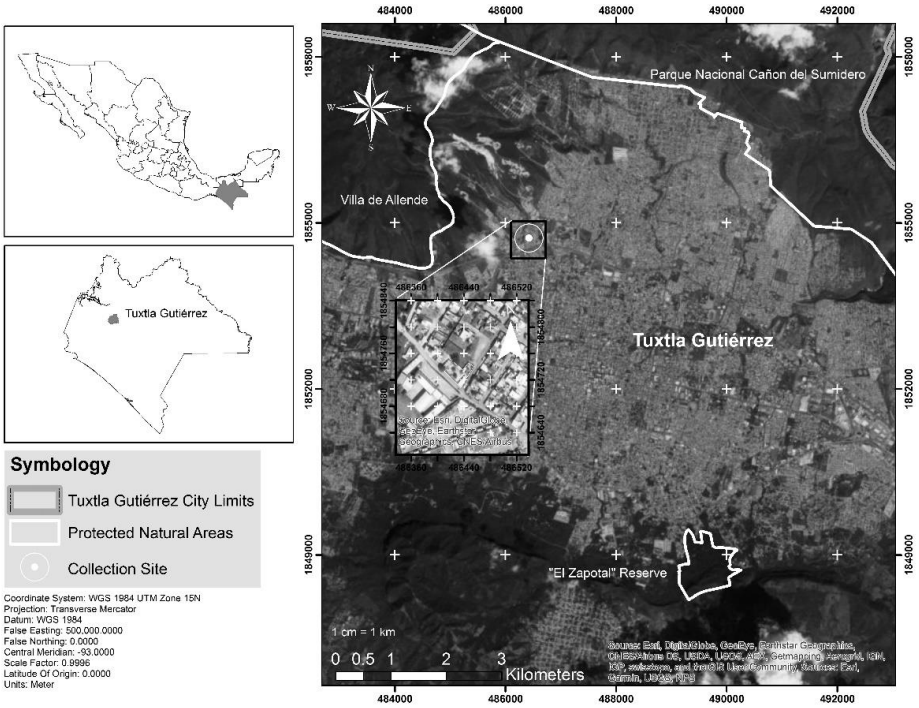

Fig. 1. Specimen collection site in an urban house of Tuxtla Gutiérrez, Chiapas (16°46'30.1"N 93°07'44.4"W; 570 m.a.s.l).

Rectal content examination and *T. cruzi* virulence

Through abdominal pressure of the insect, a rectal content sample was obtained and diluted to 1:1 with 0.9% physiological saline solution. The mixture was homogenized with a Vortex mixer (Thomas Scientific 945700, New Jersey, USA) for 1 min. 10 µl were then taken for presence of metacyclic trypomastigotes under optical microscopy at 40x magnification. We carried out the infection on CD-1 female mice for replication and isolation. Then, to evaluate its virulence and parasitemia, five female CD-1 mice with a weight of 18 g were inoculated with $5x10^5$ blood parasites via intraperitoneal puncture. The parasite quantification in blood was carried out every third day along 37 days in a Neubauer chamber using an optical microscopy with the 40x magnification. Mortality was recorded daily (Barretto, 1964; WHO, 1986). The mice were maintained at 26 ± 2° C temperature and 70% humidity. Handling of animals was according to the Official Mexican Standard NOM-062-ZOO-1999 (NOM, 1999).

Genetic characterization of *T. cruzi*

The insect was fed *ad libitum* with mouse blood (free of infection by *T. cruzi*) to collect feces and perform genetic tests. Thus, parasite DNA was isolated from the feces using the ZR Fecal DNA MiniPrep kit (Biasys) and following the manufacturer's specifications. The isolated lineage was determined with a pool of three primers for the amplification of the mini exon gene (Souto et al., 1996): [5-3´ GTGTCCGCCACCTCCTTCGGGCC (TCI, group 1-specific), 5′-CCTGCAGGCACACGTGTGTGTG (TCII, group 2-specific), and 5′-CCCCCCTCCCAGGCCACACTG (TC, common to groups 1 and 2). Following the reactions and amplifications conditions described by De Fuentes-Vicente et al., (2017). The products of the polymerase chain reaction (PCR) were analyzed by electrophoresis on a 2% agarose gel. As a negative control we used nuclease-free water, TcI control was Queretaro (Qro) strain, TcII control was Y strain (De Fuentes-Vicente et al., 2017).

Sample collection and determination of human IgG titer

Through an indirect ELISA test, the presence of anti-*T. cruzi* IgG antibodies was assessed in the dwelling inhabitants, composed of four adults and two minors under 16 years old. Prior informed consent, an interview was conducted to every inhabitant to determine their knowledge of the bug and identification of risk factors. Thereafter, 3 to 5 finger-prick blood spot samples were collected in filter paper. A crude extract of epimastigote antigens of *T. cruzi* from Chiapas, Mexico (De Fuentes-Vicente et al., 2017), was used as antigen. It was adjusted to 10 µL/mL in a solution of carbonate-bicarbonate buffer (pH 9.6). The microplate wells were coated with 100 µL of antigenic solution and incubated for 24 h at 4°C. To block any unfilled sites, it was added 200 µL of phosphate buffer saline (PBS), pH 7.2, containing 0.05% Tween 20 and 1% skim milk for 30 minutes. It was then added 100 µL of the dilution of blood eluates to 1:100 with PBS-1% milk and incubated at 37°C for 30 minutes. As a developing solution, 100 µL of o-phenylenediamine was used in citrate buffer, pH 5, and 10 µl of H_2O_2 at 30%. The optical densities were measured at 490 nm in a microplate reader (BioRad model 550). Readings above 0.180 units were considered as positive samples. As a negative control we used inactivated non-reactive human serum, and positive control was serum with high reactivity with respect to the cohort titer.

RESULTS

Triatominae bug identification

The collected specimen was an adult male insect identified as *Triatoma dimidiata* Latreille, 1811 (Fig. 2) according to the Keys of Lent and Wygodzinsky (1979). Its dimensions were 2.4 cm long and 0.9 cm wide (determined in the abdomen). The connexivum present a characteristic pale yellow color and with a dark central spot very extensive, forming an almost complete transversal band across hemelytron, Pronotum uniformly black, hemelytra surpassing apex of abdomen, legs uniformly dark. Abdomen color black, convex below, striate transversally. The abdomen of a triatomine cadaver was also found. However, its identification was not achieved due to its deterioration.

Fig. 2. *Triatoma dimidiata* adult male specimen captured in urban area of Tuxtla Gutiérrez, Chiapas. The connexivum present a characteristic pale yellow color with a dark central spot very extensive.

Trypanosoma cruzi virulence

The rectal content examination (feces) from Triatomine bug was positive for *T. cruzi*, we identified metacyclic trypomastigotes and transitional forms like "spheromastigotes" under optical microscopy at 40x magnification. Evaluation of virulence on mice, recorded that the prepatent period was five days and reached a maximum peak of $5.6x10^6$ per/ml at 27 days post-infection, after this time the number of parasites decreased (Fig. 3). No mice died during the experiment, which lasted 37 days.

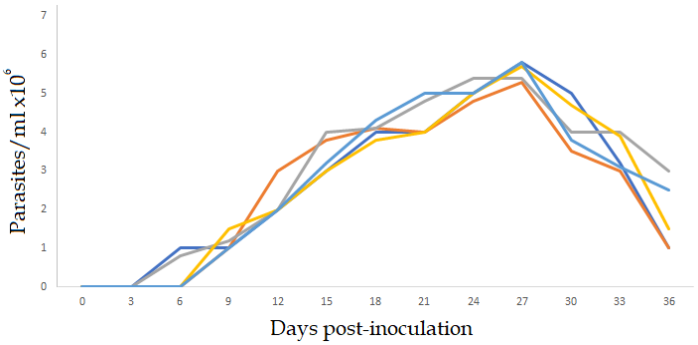

Fig. 3. Parasitaemia of the *T. cruzi* isolate. Mice (n=5) were inoculated with $5x10^5$ parasites per/ml. Parasite number in blood was determined by counting in a Neubauer chamber using an optical microscopy with the 40x objective.

Genetic characterization of *T. cruzi* isolate

With the amplification of the mini-exon gene, a product of 350 base pairs (bp) was obtained, which indicated that it belongs to the TcI group, same as TcI control Queretaro (Qro) strain (Fig. 4).

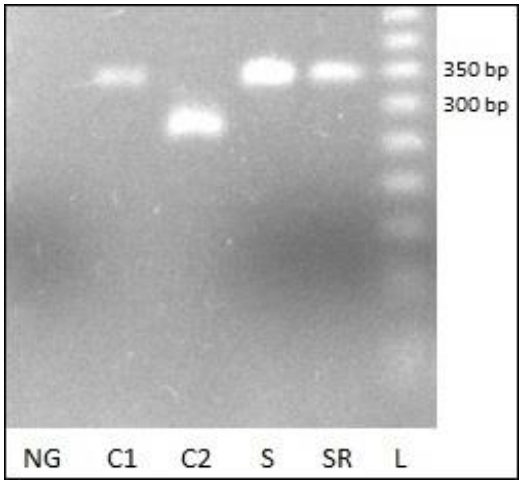

Fig. 4. Amplification of polymerase chain reaction products from the analysis of mini-exon genes. NG: negative control-nuclease-free water, C1: control 1-TCI control (Qro. strain); C2: control 2-TCII control (Y strain); S: sample-*T. cruzi* isolate on Tuxtla Gutiérrez, Chiapas); SR: sample repetition; L: 50-bp ladder.

Serological test and risk factors identification

Determination of human IgG in habitans was under 0.180 units, therefore they were considered as negative samples (Fig. 5). All inhabitants affirmed not to have been stung by the triatomine bug or to have presented skin reddening without apparent reason. They also mentioned not having seen the insect before or knowing about Chagas disease. The building materials of the house is completely concrete without cracks and all the windows had mosquito nets. They do not have domestic animals or free-range animals and there was no confinement of any material on the periphery of the house that could harbor triatomine insects.

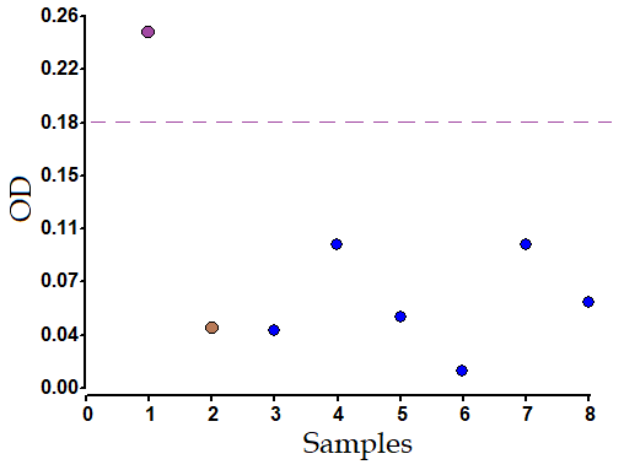

Fig. 5. Optical densities (OD) of the ELISA assay. Samples 1 and 2 correspond to positive and negative controls, respectively. From 3 to 8 corresponds to the inhabitants of the house. The test was done in duplicate. Values above 0.180 were considered seroreactive.

DISCUSSION

Chagas disease is one of the neglected diseases of Chiapas, which continues to register the highest rates of acute infection in the country. Previous studies have reported the sylvatic cycle of this disease in the ecological reserve El Zapotal (see Fig. 1), within the limits of Tuxtla Gutierrez, Chiapas. On this site, marsupials and other rodents have tested positive for *T. cruzi* through different techniques (Domínguez-Vázquez et al., 1990; Solís-Franco et al., 1997; Camacho, 2016). Despite multiple reports of triatomine bug sightings by neighboring villagers, they have not been yet proven (Solís-Franco et al., 1997). Thus, the presence of this triatomine in Tuxtla Gutierrez is unprecedented, given the urban character of the city.

The species found (*T. dimidiata*) is one of the most important in the epidemiology of Chagas disease in the Southeast of the country and Central America, its distribution reaches the north of Peru (Salazar-Schettino et al., 2005). This insect is responsible for most cases of transmission of *T. cruzi* in Chiapas and its presence occurs in localities with precarious housing conditions (Salazar-Schettino et al., 2005; Dumonteil et al., 2013). Although its distribution in the State is wide, *T. dimidiata* prefers altitudes between 600 and 1200 masl. The specimen was found in a room with a concrete wall and floor, with no characteristics of having been fed recently. In the absence of eggs, exuvias and previous sighting of these insects by the inhabitants, we discarded the triatomine infestation in the house.

The presence of these insects in urban areas is not surprising, since it has also been reported in other urban regions of Mexico and other countries (Barreto, 1976; Rodríguez-Bataz et al., 2011; Santana et al., 2011). In addition, it has also been suggested that this phenomenon is increasing (Dias et al., 2016). These events indicate that the Chagas disease has ceased to be a problem exclusive to public health in rural areas and has made its way to big cities, where their presence is explained by the parasite transmission through blood transfusion (Montgomery et al., 2014). The dissemination of this disease into urban areas has been demonstrated for *Meccus* complex at the level of insect population genetic structure, it was found two foci of triatomine wild populations in the edge of Guadalajara City through human migration (Breniére et al., 2012). In the state of

Morelos, Mexico, the urbanization of vector *Triatoma* (*Meccus*) *pallidipennis* is a fact, three-quarters of the state population reside in urban areas and >77% of them are infested (Ramsey et al., 2005).

It is challenging to determine the origin of the specimen reported here. In the interviews, it was registered that one inhabitant makes frequent trips to Olinala, state of Guerrero, whose records reveal that Chagas disease affects this population since almost half a century (Tay et al., 1980). Also, this state had the first place in acute American trypanosomiasis incidence (0.97 per 100 000 population) in 2017.

State of Guerrero has also documented the presence of *T. dimidiata* (Salazar-Schettino et al., 2005; Rodríguez-Bataz et al., 2011). Therefore, a hypothesis indicates that the chagasic bug could be passively transported by the inhabitant into the dwelling. A second hypothesis suggests that the insect arrive from a Protected Natural Area (PNA) known as Villa Allende, which borders the area where the specimen was found (Fig.1). This PNA borders the municipality of San Fernando, where the presence of *T. dimidiata* and positive cases of Chagas disease has been documented (Vidal-López et al., 2015). However, this is discouraged by the fact that triatomines have a short-range displacement by flight (Badel-Mogollón et al., 2017). In addition, according to predictions of niche expansion of infected insects, they are expected to have smaller ecological space than non-infected ones (Villalobos et al., 2019).

A useful tool to determine the specimen's origin is the genetic composition of triatomine bugs and *T. cruzi* parasite of different regions. Phylogenetic relationships and populations can be determined by using phylogenetic trees. The isolated parasite from the bug reported belongs to the TcI group, as the majority of the strains in our country (Bosseno et al., 2002), being *T. dimidiata* the most prevailing infection (Dorn et al., 2017). Furthermore, it belongs to the biodemo III, due to its slow growth and mortality in mice, characteristic of the strains of *T. cruzi (*Andrade and Magalhães, 1997). From an epidemiological point of view, these type of strains may be the least virulent and cause minor o no symptoms in humans, becoming an unnoticed disease.

The behavior of strains is influenced by the vector in an endemic area; in this case, *T. dimidiata* is the major vector in state of Chiapas, serial passages on mice allow the parasite to recover a virulence phenotype. *T. dimidiata* reduces the virulence of *T. cruzi* strains (Guzman-Marin et al., 2012), the parasitemia for "V" strain, isolated from *T. dimidata* showed a prepatent period of 12 days post-infection, the peak on day 28 with 190 x 10^5 parasites/ml, with 50% mortality at 45 days, different from values previously showed, this variation may be explained by endemism of vector, environmental factors, immunity and pathogenicity, it could influence the number of clinical reports in a region (Guzman-Marin et al., 2012).

Although a *T. cruzi* infection was not documented in the inhabitants, this finding highlights the importance of maintaining entomological surveillance systems active in both rural and urban areas. This approach will interrupt vector transmission of the parasite and prevent it from expanding. It is necessary to implement education and awareness campaigns among the population, allowing the involvement of society in the fight and prevention of Chagas disease in Mexico and Latin America, as seen in this report. The presence of *T. dimidiata* in an urban area highlights the epidemiological importance of this species, since it is an efficient vector of *T. cruzi* to human population, due to its antropophilia. A future study in Tuxtla Gutiérrez could explain if the vector has been translated accidentally or if a possible colonization from rural areas is achieved.

ACKNOWLEDGEMENTS

To Programa para el Desarrollo Profesional Docente (PRODEP-SEP) for the financing granted.

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