Short Note [Nota corta]

PREVALENCE OF *Trypanosoma cruzi* IN BACKYARD MAMMALS FROM A RURAL COMMUNITY OF YUCATAN, MEXICO†

[PREVALENCIA DE *Trypanosoma cruzi* EN MAMÍFEROS DE TRASPATIO EN UNA LOCALIDAD RURAL DE YUCATÁN, MÉXICO]

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SUMMARY

Trypanosoma cruzi the causative agent of Chagas disease, can be found in more than 150 different species of domestic and wild mammals in the American continent. Domestic mammals like dogs, and commensal rodents, have been frequently implicated as hosts and/or reservoirs in the domestic and peridomestic cycle of transmission, however, little attention have been paid to backyard mammals as potential maintaining hosts of *T. cruzi* in the peridomicile. In the present work, we reported the first data of prevalence of infection with *T. cruzi* in the backyard mammals of households in a rural community of Yucatan, Mexico. A total of 84 animals, including swine, sheep, horses, cattle and rabbits, were sampled. Blood samples were collected and processed for DNA extraction of parasite and analyzed by PCR technique. From all animals tested, 75% of the pigs (21/28), 85.71% of the sheep (6/7) and 100% of the horses (8/8), were positive for *T. cruzi*. Cattle (28) and rabbits (13) were negative. The results obtained constitute an important preliminary evidence to implicate backyard mammals as potential maintaining hosts of *T. cruzi* in the peridomestic habitat of rural communities in Yucatan.
Key words: *Trypanosoma cruzi;* prevalence; backyard mammals; Yucatan.

RESUMEN

Trypanosoma cruzi, el agente causal de la enfermedad de Chagas, se puede encontrar en más de 150 especies diferentes de mamíferos domésticos y silvestres en el continente americano. Mamíferos domésticos como perros y roedores comensales, han sido frecuentemente implicados como hospederos o reservorios en el ciclo de transmisión doméstico y peridoméstico; sin embargo, se ha prestado poca atención a los mamíferos de traspatio como posibles hospederos de *T. cruzi* en el peridomicilio. En el presente trabajo, reportamos los primeros datos de la prevalencia de infección con *T. cruzi* en los mamíferos de traspatio de una comunidad rural de Yucatán, México. Se muestrearon un total de 84 animales, incluyendo cerdos, ovejas, caballos, vacas y conejos. Las muestras de sangre se recolectaron y procesaron para la extracción de ADN del parásito y se analizaron mediante una técnica de PCR. De todos los animales analizados, el 75% de los cerdos (21/28), el 85.71% de las ovejas (6/7) y el 100% de los caballos (8/8) fueron positivos para *T. cruzi*. El ganado (28) y los conejos (13) fueron negativos. Los resultados obtenidos constituyen una evidencia preliminar importante para implicar a los mamíferos de traspatio como posibles hospederos de *T. cruzi* en el hábitat peridomiciliar de las comunidades rurales de Yucatán.
Palabras clave: *Trypanosoma cruzi*; prevalencia; mamíferos de traspatio; Yucatán.

INTRODUCTION

American Trypanosomiasis, also known as Chagas disease, is a parasitic disease caused by *Trypanosoma cruzi* and is endemic to the American continent, from the south of the United States of America to Argentina and Chile. It is considered a serious health problem with an estimate of 15 million people infected (OMS, 2007). The Chagas disease is considered endemic in several regions of Mexico (Ramsey et al., 2003). The infection is maintained by more than 150 domestic and wild animals and transmitted from infected to

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susceptible animals through the bite of triatomine insects (Días, 2000). The backyard of the houses, especially in rural communities of Mexico, represents a very complex system where plants and animals are kept for consumption of families (Ruiz-Piña y Reyes-Novelo, 2012). Many different animal species can be found in the system but domestic fowl and pigs are the commonest, with cattle, sheep, horses and other minor species like rabbits are host as well (Acosta-Casanova, 2004). The role that backyard mammals of rural households could play in the maintaining of peridomestic transmission of *T. cruzi*, has not been investigated in Yucatan; the habitat conditions found in the rural backyard system clearly favor the presence of the triatomine vector, probably representing an important source of blood, and consequently a risk for people living in the same environment (Ruiz-Piña et al., 2013). Previous studies in the Yucatan peninsula have reported the circulation of *T. cruzi* in rural peridomicile, both in synanthropic mammals as opossums, and in livestock kept in backyard (Ruiz-Piña and Cruz-Reyes, 2002; Duarte-Ubaldo, 2005; Jiménez-Coello et al. 2012).

The objective of this study was to determine the prevalence of infection in mammals kept in the backyard system, in order to go further into the knowledge of dynamics of *T. cruzi* transmission in households of rural communities of Yucatan, Mexico.

MATERIALS AND METHODS

Area of study

The study was carried out in the community of Molas, Yucatan, Mexico, located 16 km South of Merida capital city of the state of Yucatan, at 20° 48' 58.54" LN and 89° 37' 45.43" LW.

Diagnostic tests were carried out in the "Laboratorio de Zoonosis y otras Enfermedades Transmitidas por Vector" of the Centro de Investigaciones Regionales (CIR) "Dr. Hideyo Noguchi", of Universidad Autonoma de Yucatan.

Study population

As part of a larger study on zoonosis diseases in a rural community of Yucatan, Mexico, 156 out of 300 houses in the community were visited, 33 of them had backyard animals but only in 16 of them it was possible to obtain blood samples from animals. In some cases, owners did not authorize to include their animals in the study.

A total of 84 backyard animals distributed in 16 houses were sampled. Five mililiters of blood were obtained from cattle (28), sheep (7), horses (8) and pigs (28) from the jugular vein, but in the case of rabbits (13) one mililiter was obtained from the auricular vessels. Blood was placed in tubes with EDTA and kept refrigerated (4 ºC) until the tests were performed. All procedures were followed accordingly with NOM-062-ZOO-1999 for animal care.

Diagnostic test

To detect the presence of *T. cruzi* in blood samples a polymerase chain reaction test (PCR) was carried using the methodology described by Monteón *et al*. (1994). This methodology uses primers KNS1 and KNS2 designed from kinetoplast sequence, this reaction has proved higher sensitivity than other methods, with 100% sensitivity and 86% specificity.

DNA was extracted from the samples using common method previously described. Briefly, 80 µl of sterile water (Baxter) were mixed with 20 µl of sample (dilution 1:5), then the mixture was heated at 95 °C for 10 minutes and then centrifuged to maximum (approximately 15000 x *g*) in an microfuge for 5 min; afterwards, 8 µl of supernatant were placed in a new PCR tube with 10 µl of the master mix (Gotaq Green Master Mix 2x, Promega, Madison, WI, USA)) and 1 µl (10 pmol) of each of the primers to give a final volume of 20 µl. After that, the amplification program, consisting of 35 cycles of 92 ºC for 1 minute, 56 ºC for 2 minutes and 72 ºC for 1 minute was run in a Bio-RadiCycler thermal cycler (Bio-Rad Laboratories, Hercules, CA, USA). This program also includes a denaturing step before de cycles at 95 °C for 3 min and an additional step after, at 72 °C for 10 min.

PCR products were electrophoresed in a molecular biology grade agarose gel (1.2 %) and stained with ethidium bromide (10 µg/ml). A DNA weight marker Sigma ΦX174 DNA/Hae III Marker (Sigma-Aldrich Mexico, DF, Mexico) was included on each electrophoresis run. Electric current was applied at 100 volts for 25 minutes and fragments visualized in a UV light transilluminator (UVP, Upland, CA, USA) at 330 nm and the image documented with EDAS 290 1D Gel documentation system v. 3.5 from Kodak (Scientific Imaging Systems, Rochester, NY, USA).

Data Analysis

The prevalence of infection for each animal species was obtained using formula described by Thrusfield (2007).

RESULTS AND DISCUSSION

Thirty five out of 84 samples were positive (42 %) for the amplification of PCR products corresponding to *T. cruzi* (Figure 1). Total results for *T. cruzi* infection prevalence in all mammal species analyzed are

presented in Table 1. Cattle and rabbit samples were negative in the PCR test for *T. cruzi*.

Novel and preliminary findings were obtained in the present study, especially regarding sheep and horses with the highest *T. cruzi* infection prevalence (85.7% and 100% respectively). According to Noireau (1999) these animal species generally present low indices of infection and do not play an important role as hosts; however, despite the small number of animals tested in this study, our results obligate us to continue the research in order to determine if that these species could play a part in the maintenance of *T. cruzi* in the backyard system. Pigs also shown a high prevalence of *T. cruzi* infection, the only previous report of pigs naturally infected in Mexico was published by Salazar-Schettino *et al.* (1997) and Jiménez-Coello et al. (2012), Nonetheless, the later study showed low seroprevalence in pig farms, making the finding of this study relevant, since backyard pigs apparently had higher prevalence, probably because of *T. cruzi* cycle stablished in the peridomicile, including other domesticated and synanthropic mammals and infected *T. dimidiata* vectors in the locality (Koyoc-Cardeña et al., 2015). The potential role of horses, pigs and sheep in the cycle of *T. cruzi* in the backyard system of rural communities in Mexico, needs further investigation.

In relation to the relative importance of larger mammals as hosts for *T. cruzi*, Noireau (1999) argued that there is a negative correlation between body size and infection index with *T. cruzi*, suggesting short infection rates and duration in large compared to small animals. Other researchers claim that cattle could be a host for the parasite (Fujita *et al.*, 1994). The different prevalence data found for different animal species could also have been influenced by the feeding preferences of the insect vector. Many animal species have been found to be a source of food for *Triatoma dimidiata*, the main vector for *T. cruzi* in Yucatan, Mexico (Reyes-Novelo *et al*., 2011). Chickens, turkeys, cattle, horses, pigs, dogs, rats, opossums and humans have been reported as source of food for the insect (González-Angulo and Ryckman, 1967; Christiansen *et al.*, 1988). Considering the generalist feeding behavior of *T. dimidiata* (Zeledón, 1981), backyard animals support peridomicile populations acting as feeding hosts, and at the same time, potential hosts for the maintenance of *T. cruzi* peridomicile infection (Reyes-Novelo et al., 2013; Koyoc-Cardeña et al., 2015).

Table 1. Prevalence of infection by *Trypanosoma cruzi* in backyard mammals of rural households from Molas, Yucatan, Mexico.

Backyard Mammal species	Tested animals (number)	Positive (number)	Prevalence
Pigs	28	21	75%
Sheep	7	6	85.71%
Horses	8	8	100%
Cattle	28	0	0%
Rabbits	13	0	0%
Total	84	35	42%

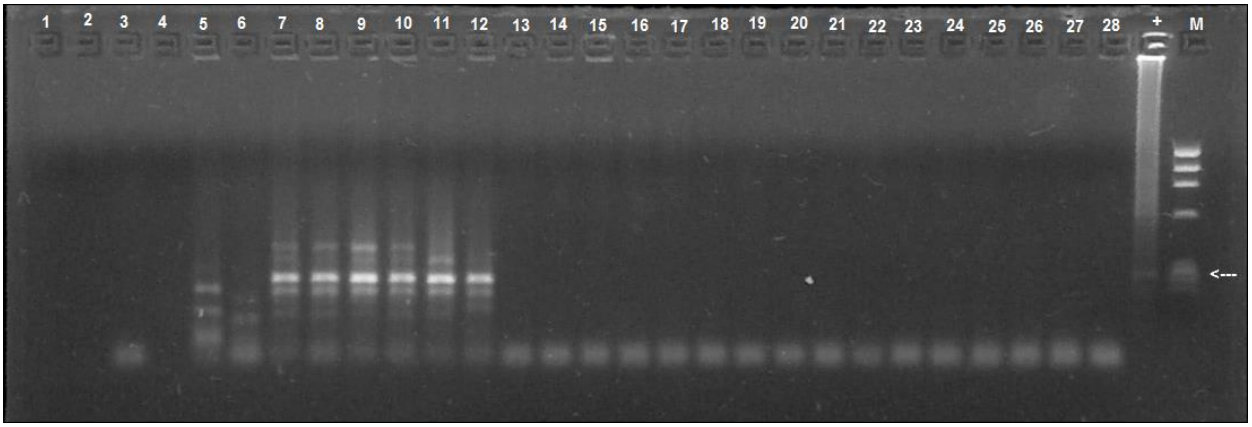

Figure 1. PCR results for *Trypanosoma cruzi* from backyard animal species of the community of Molas, Yucatan, Mexico. DNA bands of 188 bp were considered positive. Columns 1-4 are from negative rabbits, 5 is from a negative sheep, 6 from negative cattle, 7-12 from positive pigs and 18-28 from negative cattle, (+) positive control, (M) molecular weight marker (arrow).

All animal species analyzed and others like sheep, cats and rabbits are present in the backyard system of Molas, Yucatan, and are raised in close proximity to human beings. More studies on the specific role of

infected animal species regarding the cycle of *T. cruzi*, are necessary to understand the rural epidemiology of Chagas disease in the backyard system. Special attention must be paid for future research in the potential role of pigs and horses because their economic importance and abundance in the backyard of rural houses in Yucatan (Gutiérrez-Ruiz et al., 2013).

CONCLUSION

The results obtained constitute an important preliminary evidence to implicate backyard mammals as potential maintaining hosts of *T. cruzi* in the peridomestic habitat of rural communities in Yucatan.

REFERENCES

Acosta-Casanova, M.I. 2004. Caracterización del subsistema de ganadería de traspatio en 33 comunidades rurales del estado de Yucatán. Tesis de Licenciatura. Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma de Yucatán, Mérida, México.

Christensen, H.A., Sousa, O.E., Vázquez, A.M. 1988. Host feeding profiles of *Triatoma dimidiata* in peridomestic habitats of Western Panamá. American Journal of Tropical Medicine and Hygiene. 38(3): 477-479.

Días, J.C.P. 2000. Epidemiology. *Trypanosoma cruzi* and Chagas disease, 2ª ed. Rio de Janeiro. pp 55-58.

Duarte-Ubaldo, I.E. 2005. Aspectos zoosanitarios de la interacción entre fauna silvestre y animales domésticos en una comunidad maya de la región de Calakmul. Tesis de Maestría. El Colegio de la Frontera Sur. Campeche, México. pp 87-88.

Fujita, O., Sanabria, L., Inchaustti, A., De Arias, A.R., Tomizawa, Y., Oku, Y. 1994. Animal reservoirs for *Trypanosoma cruzi* infection in an endemic area in Paraguay. Journal of Veterinary Medical Science. 56(2): 305-308.

González-Angulo, W. Ryckman, R.E. 1967. Epizootiology of *Trypanosoma cruzi* in Southwestern North America. Part IX. An investigation to determine the incidence of *Trypanosoma cruzi* infections in triatomine and man on the Yucatán peninsula of Mexico. Journal of Medical Entomology. 4 (1): 44-47.

Gutiérrez-Ruiz, E., Aranda-Cirerol, F.J., Rodríguez-Vivas, R. I., Bolio-González, M., Cámara-Gamboa, E., Ramírez-González, S., Estrella-Tec, J., Acosta-Casanova, M. 2013. Características del subsistema de producción animal de traspatio en Yucatán, México. In: Pacheco Castro J, Lugo Pérez JA, Tzuc Canché L, Ruiz Piña H, editores. Estudios multidisciplinarios de las enfermedades zoonóticas y ETVs en Yucatán. Mérida: Universidad Autónoma de Yucatán. p. 184-94.

Jiménez-Coello, M., Acosta-Viana, K.Y., Guzmán-Marín, E., Ortega-Pacheco, A. 2012. American Trypanosomiasis infection in fattening pigs from the South-east of Mexico. Zoonoses and Public Health. 59(S2): 166-169.

Koyoc-Cardeña E, Medina-Barreiro A, Escobedo-Ortegón FJ, et al (2015) Chicken coops, Triatoma dimidiata infestation and its infection with Trypanosoma cruzi in a rural village of Yucatan, Mexico. Rev Inst Med Trop Sao Paulo 57:269–272 .

Monteón-Padilla, V.M., Reyes-López, P.A., Rosales-Encina, J.L. 1994. Detección de *Trypanosoma cruzi* en muestras experimentales por el método de reacción en cadena de la ADN polimerasa. Archivos del Instituto de Cardiología México. 64:135-143.

Noireau, F. 1999. La enfermedad de Chagas y sus particularidades epidemiológicas en Bolivia In: La enfermedad de Chagas en Bolivia-Conocimientos científicos al inicio del programa control 1998-2002, OMS/OPS, La Paz. pp 33,38 y 39. En: http:www.ops.org.bo/textocompleto/ncha29350.pdf.

NOM-062-ZOO-1999. México. Secretaria de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación. Especificaciones técnicas para el cuidado y uso de los animales de laboratorio. Ciudad de México. Diario Oficial de la Federación.

Organización Mundial de la Salud. 2007. Reporte sobre la enfermedad de Chagas. Grupo de trabajo científico, Buenos Aires, Argentina. pp 1-5.

Ramsey J M, Ordonez R, Tello L A, Pohls J L, Sánchez V, Peterson A T (2003) Actualidades sobre la epidemiología de la Enfermedad de Chagas en México; iniciativa para la vigilancia y el control de la Enfermedad de Chagas, en la República Mexicana. Instituto Nacional de Salud Pública de México, Salud Pública de México, 142p

Rey, J., Kobylinski, K., Rutledge Connelly R. 2006. La Tripanosomiasis Americana – Mal de Chagas, UF University of Florida. pp 1-4

Reyes-Novelo, E., Ruíz-Piña, H., Escobedo-Ortegón, J., Rodríguez-Vivas, R.I., Bolio-González, M., Polanco-Rodríguez, A., Manrique-Saide, P. 2011. Situación actual y perspectiva para el estudio de las enfermedades zoonóticas emergentes, reemergentes y olvidadas en la península de Yucatán, México. Tropical and Subtropical Agroecosystems. 14 (1): 35-54.

Ruiz-Piña, H.A., Cruz-Reyes, A. 2002. The opossum *Didelphis virginiana* as synanthropic reservoir of *Trypanosoma cruzi* in Dzidzilché, Yucatán, Mexico. Memorias del Instituto Oswaldo Cruz, 97(5): 613-620.

Ruiz-Piña H, Reyes-Novelo E. El huerto familiar yucateco y las zoonosis. In: Flores JS, editor. Huertos familiares de la Península de Yucatán. Mérida: Universidad Autónoma de Yucatán; 2012. p. 359-74.

Ruiz-Piña HA, Reyes-Novelo E, Escobedo-Ortegón F, Barrera-Perez M. Mamíferos sinantrópicos y la transmisión de enfermedades zoonóticas en el área rural de Yucatán. In: Pacheco Castro J, Lugo Pérez JA, Tzuc Canché L, Ruiz Piña H, editores. Estudios multidisciplinarios de las enfermedades zoonóticas y ETVs en Yucatán. Mérida: Universidad Autónoma de Yucatán. p. 184-94.

Sabino, E. C., Ribeiro, A. L., Lee, T. ., Oliveira, C. L., Carneiro-Proietti, A. B., Antunes, A. P., … Busch, M. P. (2015). Detection of T. cruzi DNA in Blood by PCR is associated with Chagas cardiomyopathy and disease severity. European Journal of Heart Failure, 17(4), 416–423. http://doi.org/10.1002/ejhf.220

Salazar-Schettino, P.M., Bucio, M.I., Cabrera, M., Bautista, J. 1997. First case of natural reservoirs in México. Memorias del Instituto Oswaldo Cruz. 92(4): 499-502.

Tay, J., Schenone, H., Sanchez, J.T., Robert, L. 1992. Estado actual de los conocimientos sobre la enfermedad de Chagas en la República Mexicana. Boletín Chileno de Parasitología. 47: 43-53.

Thrusfield, M. 2007. Veterinary Epidemiology, 3ª ed. Blackwell Science Ltd. Edinburgh. pp 53.

Zeledón, R. 1981. El *Triatoma dimidiata* (Latreille, 1811) y su relación con la Enfermedad de Chagas, Editorial Universidad Estatal a Distancia, San José, Costa Rica, 146 pp.