Research Article

New Scenarios of Chagas Disease Transmission in Northern Colombia

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Chagas disease (CD) is a systemic parasitic infection caused by the flagellated form of Trypanosoma cruzi. Córdoba department, located in the Colombian Caribbean Coast, was not considered as a region at risk of T. cruzi transmission; therefore CD is not included in the diagnosis of febrile diseases in hospitals and health centres. Additionally, due to the lack of knowledge about CD clinical symptoms, diagnosis in a setting where multiple infectious tropical diseases are present, such as tuberculosis, malaria, dengue, chikungunya, and Zika, is a challenge; therefore annual reports considered...
CD cases to be imported from other departments. In this article, we describe the first acute CD case in Salitral village in Sahagún, Córdoba, confirmed by microscopy and serological tests. Sampling area was located in Salitral village in the Sahagún municipality of Córdoba department, located in the northwest part of Colombia (8° 49' 47.9" N, 75° 31' 31.5" W, and 75 m a.s.l.) (Figure 1). This area has a tropical climate with an annual mean temperature between 27°C and 30°C and a relative humidity of 84%. Main economic activities are related to mixed extensive crop-livestock systems, usually linked to the proliferation of wild small mammals (rodents and marsupials) described as *T. cruzi* reservoirs.

The 16-year-old young male patient born and resident in Salitral village was referred to the emergency service of San Juan de Sahagún Hospital (ESE HSJS) with eight days of headache and high fever (38°C) associated with chills, generalized myalgia, asthenia, adynamia, choluria, severe epigastralgia without epistaxis, and gingivorrhagia. On his epidemiological history, the patient denied knowing triatomine bugs, having received any blood transfusion or organ transplant, or traveling outside Córdoba prior to the beginning of the symptoms. No inoculation point either in skin or periocular region suggesting vectorial transmission could be detected. The patient was alert, without respiratory distress or cardiovascular involvement (electrocardiogram). Abdominal palpation and ultrasound examination confirmed symptoms of a moderate spleen enlargement (splenomegaly). Thick blood smear examination was negative for *Plasmodium* spp. but positive for *T. cruzi* trypomastigotes (Figure 2).

Serological analysis by enzyme linked immunosorbent assay (ELISA) for CD was negative at the seventh day of hospitalization.

All experimental and sampling protocols were approved by the Ethics Committee of Universidad del Sinú according to national normativity for human populations studies and the NIH Guide for the Care and Use of Animals [6].

In order to determine the presence of triatomine bugs, active manual search was carried out by the professional staff and community members in walls, cracks in the walls and ceiling, mattresses, and floor of the patient’s house and other 24 houses in the neighborhood area according to OMS recommended methodology [7]. Additionally, live-baited traps [8] and Gómez-Nuñez boxes [9] were also placed in the intra and peri domicile of each selected household. Taxonomic identification of captured specimens was performed based on external morphology, according to Lent and Wygodzinsky [10]. Detection of *T. cruzi* infection in captured triatomines was confirmed by direct and molecular techniques examining intestinal contents and rectal ampulla. Small- and mid-sized mammals were also captured using 5 mist nets for bats and 20 Tomahawks and 40 Sherman traps. Captured mammals were taxonomically identified according to Emmons and Feer [11], Linares [12], Tirira [13], Gardner [14], and Patton et al. [15] and whole blood samples were taken for molecular identification of *T. cruzi*. Sampling was performed on 80 volunteers selected from the entire population. All participants filled out a clinical-epidemiological survey including identification variables and
Figure 2: *T. cruzi* trypomastigotes detected in thick blood smears of the infected patient (1000x).

Figure 3: Patient’s house infrastructure and peridomicile.

evidence of signs or symptoms according to case definition. All family members and other individuals related to the acute case patient were also analyzed. All samples were collected after obtaining the corresponding informed consent. The serological analysis included detection of IgG antibodies by ELISA and indirect immunofluorescence (IFI). For *T. cruzi* detection, human blood samples and triatomine bugs rectal ampulla were collected in a volume solution containing EDTA and guanidine 6M and stored at room temperature. A spin column-based nucleic acid purification kit was used to perform DNA extraction (High Pure PCR Template Preparation, de Roche®). Molecular detection was carried out through amplification of the variable region of kinetoplast DNA (kDNA) according to the methodology previously described by [16, 17] and tandem repeat satellite region from *T. cruzi* using the cruzi1 and cruzi2 primers described by [18]. Amplification cycles for kDNA were performed using a two-step procedure using an initial denaturation step at 94°C for 3 min; 5 cycles of denaturation at 94°C for 1 min, annealing at 68°C for 1 min, and extension at 72°C for 1 min, followed by 35 cycles at 94°C for 45 sec; annealing at 64°C for 45 sec, extension at 72°C for 45 sec, and final extension at 72°C for 10 min. Cycling conditions for cruzi1 and cruzi2 were initial denaturation at 94°C for 5 min and 40 cycles of denaturation at 94°C for 1 min, annealing at 64°C for 30 sec, extension at 72°C for 1 min, and final extension at 72°C for 10 min. Patient’s house was built with wood walls, palm roofs, and dirt floors surrounded by dense vegetation consisting of trees and palms (Figure 3). Among the 24 houses included in the study 32% were constructed with wooden walls, 40% with dirt floors, and 56% with thatch palm roof and 32% had unplastered walls. Conventional parasitological methods, serological screening, and molecular testing for detection of *T. cruzi* infection performed on blood samples of 80 voluntary patients showed negative results. In this particular community, serological test showed positive results only for the acute case described in this work. During the entomological sampling, seven individuals identified as *Rhodnius pallescens* and two classified as *Panstrongylus geniculatus* were captured. Most captured insects were collected by members of the community. Analysis of intestinal content and rectal ampulla confirmed the presence of *T. cruzi* in one specimen of each species. Analysis of blood samples of 29 specimens of small- and mid-sized mammals using molecular methods confirmed the presence of *T. cruzi* DNA in two specimens of *Didelphis marsupialis* and two specimens of *Heteromys anomalus*. The transmission scenario of CD in Córdoba still remains a challenge and must be addressed through clinical and ecoepidemiological studies since, as our results showed, a sylvatic cycle exists and accidental human cases might be occurring. In this particular case, signs and symptoms presented by the patient including prolonged febrile illness, epigastralgia, and absence of lesions in either the skin or the periocular region indicating the insect bite, together with patient statement of never being bitten by triatomines and never leaving Córdoba department, would suggest oral transmission as the most likely pathway of infection [19, 20].

Considering the *T. cruzi* detection in specimens of the triatomine bugs *Panstrongylus geniculatus* and *Rhodnius pallescens* and the mammals *Didelphis marsupialis* and *Heteromys anomalus*, there is an evident risk of infection to humans either. In Córdoba department previous studies reported the presence of several triatomine species [21]. In line with our findings, *E. cuspidatus, P. geniculatus*, and *R.
The authors declare that there are no conflicts of interest regarding the publication of this article.

Conflicts of Interest

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References

[1] J. R. Coura and P. A. Vías, “Chagas disease: a new worldwide challenge,” Nature, vol. 465, no. 7301, pp. 56–57, 2010.
[2] K. Rueda, J. E. Trujillo, J. C. Carranza, and G. A. Vallejo, “Transmisión oral de Trypanosoma cruzi: un nuevo escenario epidemiológico de la enfermedad de Chagas en Colombia y otros países suramericanos,” Biomédica, vol. 34, no. 4, 2014.
[3] J. F. Ríos, M. Arboleda, A. N. Montoya, E. P. Alarcón, and G. J. Parra-Henao, “Probable brote de transmisión oral de enfermedad de Chagas en Turbo, Antioquia,” Biomédica, vol. 31, no. 2, p. 185, 2011.
[4] W.H.O., Chagas disease (American trypanosomiasis): Fact sheet N°340. 2014.
[5] Ministerio de la Protección Social, I.N.d.S., Organización Panamericana de la Salud OPS/OMS, Guía para la Atención Clínica Integral del paciente con enfermedad de Chagas. 2010: p. 1–81.
[6] Care, I.o.L.A.R.C.o., U.o.L. Animals, and N.I.o.H.D.o.R. Resources, Guide for the care and use of laboratory animals. 1985: National Academies.
[7] M. D. Feliciangeli, M. Hernández, B. Suarez et al., “Comparación de métodos de captura intradoméstica de triatominos vectores de la enfermedad de Chagas en Venezuela,” Boletín de Malaria y Salud Ambiental, vol. 47, pp. 103–117, 2007.
[8] V. M. Angulo and L. Esteban, “Nueva trampa para la captura de triatominos en hábitats silvestres y peridomésticos,” Biomédica, vol. 31, no. 2, p. 264, 2011.
[9] A. Longa and J. V. Scorza, “Acrocomia aculeata (Palmaceae), hábitat silvestre de Rhodnius robustus en el Estado Trujillo, Venezuela,” Boletín de Malaria y Salud Ambiental, vol. 47, no. 1-2, pp. 213–220, 2007.
[10] H. Lent and P. Wygodzinsky, “Revision of the Triatominae (Hemiptera, Reduviidae), and their significance as vectors of Chagas disease,” Bulletin of the American Museum of Natural History, vol. 163, pp. 123–520, 1979.
[11] L. Emmons and F. Feer, Neotropical Rainforest Mammals: A Field Guide, University of Chicago Press, 1997.
[12] O. J. Linares, Mamíferos de Venezuela, Sociedad Conservacionista Audubon de Venezuela, 1998.
[13] D. Tirira, “Guía de campo de los Mamíferos del Ecuador. Ediciones ed. 2007, Quito, Ecuador: Publicación especial sobre los mamíferos del Ecuador 6. 576-576”.
[14] A. L. Gardner, Mammals of South America, Volume 1: Marsupials, Xenarthrans, Shrews, and Bats, University of Chicago Press, 2008.
[15] J. L. Patton, U. F. Pardiñas, and G. D’Elia, Mammals of South America, Volume 2: Rodents, University of Chicago Press, 2015.
[16] J. M. Burgos, J. Altcheh, M. Bisio et al., “Direct molecular profiling of minicircle signatures and lineages of Trypanosoma cruzi
bloodstream populations causing congenital Chagas disease," International Journal for Parasitology, vol. 37, no. 12, pp. 1319–1327, 2007.

[17] A. G. Schijman, M. Bisio, L. Orellana et al., "International study to evaluate PCR methods for detection of Trypanosoma cruzi DNA in blood samples from Chagas disease patients," PLoS neglected tropical diseases, vol. 5, no. 1, e931 pages, 2011.

[18] M. Bisio, C. Cura, T. Duffy et al., "Trypanosoma cruzi discrete typing units in Chagas disease patients with HIV co-infection," Revista Biomedica, vol. 20, pp. 166–178, 2009.

[19] B. Alarcón de Noya, J. Veas, R. Ruiz-Guevara et al., "Evaluación clínica y de laboratorio de pacientes hospitalizados durante el primer brote urbano de enfermedad de chagas de transmisión oral en Venezuela," Revista de Patologia Tropical, vol. 42, no. 2, 2013.

[20] L. Zuleta, “Enfermedad de Chagas: posible transmisión oral en trabajadores del sector hidrocarburos, Casanare, Colombia,” Biomédica Revista del Instituto Nacional de Salud, vol. 37, no. 2, pp. 2–12, 2014.

[21] F. Guhl, G. Aguilerl, N. Pinto, and D. Vergara, "Updated geographical distribution and ecoepidemiology of the triatomine fauna (Reduviidae: Triatominae) in Colombia," Biomedica, vol. 27, 2007.

[22] F. Guhl, G. Aguilerl, N. Pinto, and D. Vergara, "Actualización de la distribución geográfica y ecoepidemiología de la fauna de triatomínos (Reduviidae: Triatominae) en Colombia," Biomedica, vol. 27, no. 1, pp. 143–162, 2007.

[23] M. Reyes-Lugo and A. Rodriguez-Acosta, “Domiciliation of the sylvatic Chagas disease vector Panstrongylus geniculatus Latreille, 1811 (Triatominae: Reduviidae) in Venezuela,” Transactions of the Royal Society of Tropical Medicine and Hygiene, vol. 94, no. 5, p. 508, 2000.

[24] E. Dumonteil, M. J. Ramirez-Sierra, J. Ferral, M. Euan-Garcia, and L. Chavez-Nuñez, "Usefulness of community participation for the fine temporal monitoring of house infestation by non-domiciliated triatomines," Journal of Parasitology, vol. 95, no. 2, pp. 469–471, 2009.

[25] O. Cantillo-Barraza, E. Garcés, A. Gómez-Palacio et al., “Eco-epidemiological study of an endemic Chagas disease region in northern Colombia reveals the importance of Triatoma maculata (Hemiptera: Reduviidae), dogs and Didelphis marsupialis in Trypanosoma cruzi maintenance," Parasites and Vectors, vol. 8, no. 1, article no. 482, 2015.

[26] A. M. Mejía-Jaramillo, L. A. Agudelo-Uribe, J. C. Dib, S. Ortiz, A. Solari, and O. Triana-Chávez, "Genotyping of Trypanosoma cruzi in a hyper-endemic area of Colombia reveals an overlap among domestic and sylvatic cycles of Chagas disease," Parasites and Vectors, vol. 7, no. 1, article no. 108, 2014.

[27] F. Guhl, M. Restrepo, V. M. Angulo, C. M. F. Antunes, D. Campbell-Lendrum, and C. R. Davies, "Lessons from a national survey of Chagas disease transmission risk in Colombia," Trends in Parasitology, vol. 21, no. 6, pp. 259–262, 2005.

[28] J. Buendía, Guía de atención de la enfermedad de Chagas. Guías promoción la salud y prevención enfermedades en la salud pública, 2005: p. 1–48.