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Research paper

Chagas' disease in Aboriginal and Creole communities from the Gran Chaco Region of Argentina: Seroprevalence and molecular parasitological characterization

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ABSTRACT

Most indigenous ethnias from Northern Argentina live in rural areas of "the Gran Chaco" region, where *Trypanosoma cruzi* is endemic. Serological and parasitological features have been poorly characterized in Aboriginal populations and scarce information exist regarding relevant *T. cruzi* discrete typing units (DTU) and parasitic loads. This study was focused to characterize *T. cruzi* infection in Qom, Mocoit, Pit'laxá and Wichi ethnias (N = 604) and Creole communities (N = 257) inhabiting rural villages from two highly endemic provinces of the Argentinean Gran Chaco.

DNA extracted using Hexadecyltrimethyl Ammonium Bromide reagent from peripheral blood samples was used for conventional PCR targeted to parasite kinetoplastid DNA (kDNA) and identification of DTUs using nuclear genomic markers. In kDNA-PCR positive samples from three rural Aboriginal communities of "Monte Impenetrable Chaqueño", minicircle signatures were characterized by Low stringency single primer-PCR and parasitic loads calculated using Real-Time PCR.

Seroprevalence was higher in Aboriginal (47.98%) than in Creole (27.23%) rural communities (Chi square, $p = 4.e^{-8}$). A low seroprevalence (4.3%) was detected in a Qom settlement at the suburbs of Resistencia city (Fisher Exact test, $p = 2.e^{-21}$).The kDNA-PCR positivity was 42.15% in Aboriginal communities and 65.71% in Creole populations (Chi square, $p = 5.e^{-4}$). Among Aboriginal communities kDNA-PCR positivity was heterogeneous (Chi square, $p = 1.e^{-4}$). Highest kDNA-PCR positivity (79%) was detected in the Qom community of Colonia Aborigen and the lowest PCR positivity in two different surveys at the Wichi community of Misión Nueva Pompeya (33.3% in 2010 and 20.8% in 2014).

TcV (or TcII/V/VI) was predominant in both Aboriginal and Creole communities, in agreement with DTU distribution reported for the region. Besides, two subjects were infected with TcVI, one with TcI and four presented mixed infections of TcV plus TcII/VI. Most minicircle signatures clustered according to their original localities, but in a few cases, signatures from one locality clustered with signatures from other village, suggesting circulation of the same strains in the area. Parasitic loads ranged from undetectable to around 50 parasite equivalents/mL, showing higher values than those generally observed in chronic Chagas disease patients living in urban centers of Argentina. Our findings reveal the persistence of high levels of infection in these neglected populations.

1. Introduction

Chagas' disease represents a devastating health and social threat to around eight million infected people in 21 Latin American countries, and is emergent in non-endemic countries introduced by extensive global migrations and perpetuated by means of vertical transmission (Rodrigues Coura and Viñas, 2010). The infection mainly affects rural and neglected populations, such as Aboriginal groups, since they are highly exposed to the risk of vectorial transmission, as a consequence of poverty and lack of sanitized households. Many Creole populations resulting from miscegenation with European colonists and immigrants coexist within these native communities.

In Argentina, the Chagas' disease National Program has achieved important decreases in the rates of parasitic transmission by blood transfusion, vectorial pathway and prenatal care for congenital transmission, especially among non-indigenous communities. Deficient spraying and discontinuity of surveillance favors persistence of highly endemic

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areas (Zaidenberg et al., 2004). Aboriginal houses are much more likely to be infested with *Triatoma infestans* than Creoles' houses; indigenous people mostly tend to thatch the roofs and often sleep on the floor, providing the vector with a more primary habitat than just mud walls. Moreover, the lack of timely reporting of re-infestation opens the way for renewed transmission (Dell'Arciprete et al., 2014). Besides, among indigenous groups, culture and language differences make control efforts more difficult (Dell'Arciprete et al., 2014).

By year 2010, according to the Survey of Indigenous Population of the National Institute of Statistics and Census (Censo, 2010), there were 955,032 people in Argentina who self-recognized and/or identified themselves as belonging to an indigenous community minority. At the present time, ten well-defined indigenous ethnias are recognized in the country, 90% of them living within "the Gran Chaco Americano" region.

Three different indigenous ethnias inhabit the province of Chaco, namely Qom, spanish-called Toba, that includes 30,000 individuals living in urban and rural areas of central and southeastern plains of the province; Wichis, spanish-called Mataco, that comprises approximately 6500 individuals living in rural communities in the northwestern corner of the province called "Monte Impenetrable Chaqueño" and Moquoít, spanish-called Mocoví, that includes approximately 6500 individuals living in the southwestern area of the province. Moreover, in the province of Formosa, the Pilagá, originally named Pit'laxá, are nowadays situated in 19 settlements. Indigenous populations mostly live in rural locations with severe housing constrictions and with limited access to health care services. In many locations there are no medical facilities within or close to the settlements and there is also lack of medical staff trained to understand their cultural characteristics and languages (Petherick, 2010).

Currently, *Trypanosoma cruzi* is partitioned into six discrete typing units (DTUs) renamed by consensus as TcI–TcVI (Zingales et al., 2009, 2012). Epidemiologically relevant *T. cruzi* DTUs and genetic parasite diversity infecting native populations remain to be determined. Most DTU typing methods have used DNA from cultured parasites, which may underestimate the diversity of natural populations due to subpopulation selection during culturing procedures (Diosque et al., 2003; Macedo et al., 2004). Moreover, parasite cultures from blood samples of chronically infected cases have very low sensitivity. In the last years, sensitive PCR strategies have been developed for typing of *T. cruzi* in clinical samples (Burgos et al., 2007, 2010; Zingales et al., 2012; Monje-Rumi et al., 2015; Cura et al., 2015). This has opened the possibility to deepening our understanding of parasite genetic diversity in native communities.

In this context, this work aimed to characterize serological and parasitological features of affected Aboriginal and Creole communities residing in rural neglected areas of the Argentinean Gran Chaco.

2. Materials and methods

2.1. Subjects and samples

Between August 2010 and March 2014, *T. cruzi* infection was searched in a total of 861 individuals inhabiting the Provinces of Chaco and Formosa, in the Argentinean Gran Chaco Region. A total of 604 out of 861 individuals belonged to eight Aboriginal communities at a) Province of Chaco: seven rural villages of Colonia Aborigen, N = 35 and Las Hacheras, N = 60 (ethnia Qom); Miraflores, N = 46 (ethnias Wichi and Qom); Misión Nueva Pompeya survey 2010, N = 55 Misión Nueva Pompeya survey 2014, N = 110 (ethnia Wichi, different individuals studied in each survey) and Villa Berthet, N = 115 (ethnia Mocoit) and the urban Mapic settlement in the suburbs of Resistencia city, known as "Gran Resistencia", N = 108 (ethnia Qom) and b) Province of Formosa: rural communities of Estanislao del Campo, N = 53 (ethnia Pit'laxá) and El Potrillo, N = 22 (ethnia Qom) (Fig. 1 and Table 1).

Furthermore, 257 out of 861 individuals belonged to Creole rural populations of the Province of Chaco: Concepción del Bermejo (N = 49), Las Breñas (N = 67), Las Garcitas (N = 54) and Lapachito (N = 87) (Fig. 1 and Table 1).

The study was approved by the bioethical Committee of The Institute of Regional Medicine of the Northeastern National University (UNNE), Resistencia, Chaco and IDACH (Chaco Aboriginal Institute) upon written informed consents of adult individuals or parents/tutors in pediatric cases.

2.2. Serological examination

Conventional serological tests were assayed in the Immunological Department of the Institute of Regional Medicine or in the Parasitology School of the Faculty of Biochemistry of the UNNE. Indirect hemagglutination (Chagatest® Wiener Lab, Argentina; cut-off 1:32) and Ig G-ELISA test (*ELISA Chagatest®*, Wiener Lab, Argentina) were performed following the manufacturers´ instructions. In case of discordant findings, a third serological test was carried out using indirect immunofluorescence (Biocientífica, Argentina), following the manufacturers´ instructions. Subjects were considered infected if at least two tests were positive.

Seropositive individuals were notified to the Provincial Public health Chagas program, in coordination with the Health Care program of the UNNE (UNNE-SALUD), for clinical and cardiological examination and treatment with trypanomicidal drugs following the normative in Argentina. However, these data were not available for the present study. Digestive megasyndromes were not studied.

2.3. PCR detection of bloodstream T. cruzi DNA

PCR based detection of the 330-bp minicircle variable region of parasitic kinetoplastid DNA (kDNA-PCR) was carried out in blood samples of seropositive subjects using primers 121 and 122, as reported (Schijman et al., 2011). Seven hundred μL of peripheral blood were collected in EDTA tubes and stored at −20 °C until DNA extraction. The DNA was purified with CTAB (Hexadecyltrimethyl Ammonium Bromide) as previously reported (Escalante et al., 1997; Lucero et al., 2007).

2.4. Quantification of parasitic loads using TaqMan Real Time PCR (qPCR)

Parasitic loads were determined in a group of kDNA-PCR positive samples by means of TaqMan Real Time PCR (qPCR) targeted to a 166-bp segment from *T. cruzi* satellite DNA (SatDNA) using FastStart Universal Probe Master Mix (Roche Diagnostics GmbHCorp, Mannheim, Germany) in a final volume of 20 μL and the following reagents: 0.75 μM of primers Cruzi 1 (5′-ASTCGGCTGATCGTTTTCGA-3′) and Cruzi 2 (5'AATTCCTCCAAGCAGCGGATA-3′), and 0.05 μM of specific TaqMan probe Cruzi 3 (5′-Fam-CACACACTGGACACCAA-NFQ-MGB-3′). Thermocycling conditions were a first step of 10 min. at 95 °C followed by 40 cycles at 95 °C for 15 s. and 58 °C for 1 min (Ramírez, J.C. et al., 2015).

For quantification, a standard curve was prepared from DNA obtained using CTAB reagent from a seronegative blood sample spiked with 10^5 CL Brener parasites/mL. The DNA was mixed with DNA obtained by the same way from a seronegative blood sample in order to create a series of dilutions ranging from 0.1 to 10^4 parasite equivalents in 1 mL of blood (par.eq/mL).

2.5. Analytical validation of DNA extraction and PCR

DNA extraction with CTAB was validated for conventional PCR using external control panels provided by a referral Laboratory of Molecular Diagnosis at INGEBI-CONICET in the context of an International PCR harmonization study (see LbP/2 in Schijman et al., 2011).

To validate Sat DNA-qPCR serial dilutions of seronegative EDTA-blood spiked with 0.05 to 10^5 par.eq./mL of cultured CL Brener were