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Trypanosoma cruzi I–III in southern Brazil causing individual and mixed infections in humans, sylvatic reservoirs and triatomines

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ABSTRACT

The aim of this study was to characterise Discrete Typing Units (DTUs) of 28 isolates of *Trypanosoma cruzi* from humans (15), triatomines (9), and opossums (4) in the state of Paraná, southern Brazil. For this purpose, we analysed the size polymorphism at the 3′ end of the 24Sα ribosomal RNA gene (rRNA) and the restriction fragment length polymorphism (RFLP) of the partial 5′ sequence of the mitochondrial Cytochrome Oxidase subunit II gene (COII). Band patterns of the isolates were compared with reference samples of *T. cruzi* I (Silvio X10 and Col 17G2), *T. cruzi* II (Esmeraldo and JG), *T. cruzi* III (222 and 231), *T. cruzi* IV (CAN III), *T. cruzi* V (SO3 cl5), and *T. cruzi* VI (CL Brener). Our results confirmed that rRNA analysis is of limited use for assessing *T. cruzi* DTUs. COII RFLP analysis was suitable for screening, but for one isolate it was necessary to determine the COII partial sequence to identify the DTU. Only one of the isolates from humans belonged to *T. cruzi* I; 13 isolates belonged to *T. cruzi* II and one to *T. cruzi* III. The four isolates from opossums and five isolates from triatomines were identified as *T. cruzi* I. Four isolates from triatomines showed patterns of both *T. cruzi* I and II, indicating mixed infections. This study contributes to the characterisation of the dynamics of *T. cruzi* populations in southern Brazil.

1. Introduction

Trypanosoma cruzi, the etiological agent of Chagas' disease, circulates in nature among humans, vectors, domestic, and sylvatic reservoirs. At present, eight to nine million people are infected by this parasite in Latin America, and 25–90 million are at risk of infection (WHO, 2007; Hotez et al., 2008; Rassi et al., 2010). Classically, the interaction of this parasite with sylvatic triatomines and mammalian reservoirs is known as the sylvatic transmission cycle, and its circulation between humans and domestic animals as the domestic transmission cycle (WHO, 2002; Macedo et al., 2004).

Recently, based on different molecular markers, the Second Satellite Meeting (Zingales et al., 2009) recommended that *T. cruzi* should be classified into six Discrete Typing Units (DTUs – *T. cruzi* I–VI). *T. cruzi* I and *T. cruzi* II are frequently associated with different hosts and transmission cycles, but they have also been reported from the same host, indicating a mixed infection (Bosseno et al., 1996; Spitzner et al., 2007; Steindel et al., 2008; Ramírez et al., 2010). *T. cruzi* III is associated with terrestrial transmission cycles, armadillo reservoir hosts, and human infections (Freitas et al., 2006; Llewellyn et al., 2009). *T. cruzi* IV includes the strains belonging to the previously described zymodeme 3 (Miles et al., 1981) and has been found in sylvatic primates, *Rhodnius* spp., and humans with Chagas' disease associated with oral transmission (Marcili et al., 2009). *T. cruzi* V and *T. cruzi* VI are prevalent among isolates obtained from humans, and have wide geographical distributions (Barnabé et al., 2000; Burgos et al., 2010).

Nowadays it is well known that *T. cruzi* I shows high genetic diversity (Ia-Ie), through sequence analyses of the intergenic region of the mini-exon gene. These different genotypes have wide distributions in the Americas, and have been found in domestic, peridomestic, and sylvatic transmission cycles (Cura et al., 2010; Herrera et al., 2009; Ramírez et al., 2011; Guhl and Ramírez, 2011). TcI has consistently been isolated from marsupials of the genus *Didelphis*, which lives in both arboreal and terrestrial sylvatic and peridomestic ecotopes in the Paraguayan Chaco region (Yeo et al., 2005) and the Amazon Basin (Marcili et al., 2009). Gaunt and Miles (2000) suggested that *T. cruzi* I has an evolutionary history associated with *Didelphis*, and possibly with triatomines of the tribe Rhodniini and with palm trees. Nevertheless, the finding that the same DTUs of *T. cruzi* infect mammals of different orders in

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sylvatic transmission cycles confirms that the association of DTU with mammals is far from absolute (Yeo et al., 2005; Marcili et al., 2009). In contrast, *T. cruzi* II predominates in the domestic transmission cycle in southern South America (Argentina, Brazil, Bolivia, Chile, Paraguay, and Uruguay) (Chapman et al., 1984), but the natural hosts for this DTU are still unclear and controversial. Lisboa et al. (2004) associated this DTU with primates.

The relationship of *T. cruzi* DTUs I and II to the pathology of Chagas' disease remains unclear. At least in Brazil, *T. cruzi* II strains are primarily responsible for the tissue lesions that are seen in the chronic phase of the infection (Freitas et al., 2005); whereas in regions where *T. cruzi* I predominates, these lesions are less frequent (Miles et al., 1981). In Colombia, *T. cruzi* I is more prevalent, but *T. cruzi* II has also been reported. Patients infected with *T. cruzi* I demonstrated a higher prevalence of cardiac alterations than those infected with *T. cruzi* II (Ramírez et al., 2010). These two DTUs behave differently with respect to their virulence, capacity for cell invasion, infectiveness (reviewed by Mortara et al., 2005; Andrade et al., 2010), capacity for vector transmission (Lana et al., 1998), and resistance to chemotherapeutic agents (Toledo et al., 2003).

In previous publications from our laboratory, these two DTUs (*T. cruzi* I and *T. cruzi* II) were found together in the triatomines *Triatoma sordida* and *Panstrongylus megistus* from northwestern Paraná state in southern Brazil (Spitzner et al., 2007). This finding led us to investigate whether these mixed infections occur only in triatomines, or whether they can also be found in sylvatic reservoirs or in humans from the same region. The aim of this study was to identify the DTUs and the occurrence of mixed infections in isolates of *T. cruzi* obtained from sylvatic reservoirs, triatomines, and humans from northwestern Paraná, using two molecular markers recommended for studies of *T. cruzi* populations (Anonymous, 1999; Zingales et al., 2009). We analysed nuclear DNA through the polymorphism in the 3′ end of the 24Sα rRNA gene (Souto et al., 1996, reviewed by Macedo et al., 2004) and restriction fragment length polymorphism (RFLP) of the 5′ region of the mitochondrial Cytochrome Oxidase subunit 2, COII gene (Freitas et al., 2006). In the case of one isolate, it was necessary to sequence the partial COII gene. The isolates were compared with *T. cruzi* reference samples for the different DTUs, and the results were analysed in an epidemiological context.

2. Materials and methods

2.1. Isolates of T. cruzi

We analysed 28 isolates from different hosts. Fifteen isolates were obtained from humans infected for more than 10 years, i.e., in the chronic phase of Chagas' disease, who reside in northern and northeastern Paraná and who came from endemic areas (eight from Minas Gerais, five from Paraná, two from São Paulo). Four isolates were obtained from sylvatic reservoirs (*Didelphis albiventris*), and nine from triatomines (*T. sordida* and *P. megistus*) in northeastern Paraná. The *T. cruzi* isolates, hosts, and their geographical origins are shown in Table 1. The band patterns of the 28 *T. cruzi* isolates obtained by rRNA and COII/RFLP analyses were compared with the bands of nine reference samples of the six DTUs (*T. cruzi* I–VI; TcI–TcVI), according to the Second Satellite Meeting (Zingales et al., 2009), Silvio X10 and CO1 17G2 (TcI), Esmeraldo and JG (TcII), 222 and 231 (TcIII), CAN III (TcIV), SO3 cl5 (TcV), and CL Brener (TcVI).

2.2. Growth of parasite

T. cruzi isolates and reference samples were maintained by serial passage every 2 or 3 days in LIT medium at 28 °C. To obtain the parasite mass, LIT medium was added cumulatively to the cultures until they reached 10^9 cells. The cells were washed by

Table 1
Hosts, geographical origins, and Discrete Typing Units (DTUs) of *Trypanosoma cruzi* isolated in northeastern Paraná, based on the polymorphism of the 3′ extremity of the 24Sα rDNA gene and restriction fragment length polymorphism (RFLP) of the 5′ region of the mitochondrial Cytochrome Oxidase subunit 2 and the partial sequencing of the COII gene.

International code	Abbreviated code	Hosts	Origins	DTUs
MHOM/BR/94/PR379	PR-379	*Homo sapiens*	Londrina/PR	*T. cruzi* II
MHOM/BR/94/PR209	PR-209	*Homo sapiens*	Três Corações/MG	*T. cruzi* II
MHOM/BR/94/PR328	PR-328	*Homo sapiens*	Congonhinhas/PR	*T. cruzi* II
MHOM/BR/94/PR1256	PR-1256	*Homo sapiens*	União dos Palmares/PR	*T. cruzi* II
MHOM/BR/94/PR184	PR-184	*Homo sapiens*	Montes Claros/MG	*T. cruzi* II
MHOM/BR/94/PR2052	PR-2052	*Homo sapiens*	Mirasselva/PR	*T. cruzi* III
MHOM/BR/94/PR150	PR-150	*Homo sapiens*	Januária/MG	*T. cruzi* I
MHOM/BR/94/PR1921	PR-1921	*Homo sapiens*	Paraguaçu/SP	*T. cruzi* II
MHOM/BR/94/PR458	PR-458	*Homo sapiens*	Florinea/SP	*T. cruzi* II
MHOM/BR/94/PR149	PR-149	*Homo sapiens*	Montes Claros/MG	*T. cruzi* II
MHOM/BR/94/PR076	PR-076	*Homo sapiens*	Coração de Jesus/MG	*T. cruzi* II
MHOM/BR/94/PR2259	PR-2259	*Homo sapiens*	Virgem da Lapa/MG	*T. cruzi* II
MHOM/BR/94/PR399	PR-399	*Homo sapiens*	Primeiro de Maio/PR	*T. cruzi* II
MHOM/BR/94/PR427	PR-427	*Homo sapiens*	Terra Branca/MG	*T. cruzi* II
MHOM/BR/94/PR402	PR-402	*Homo sapiens*	Poté/MG	*T. cruzi* II
MDID/BR/95/G1	G1	*Didelphis albiventris*	Sarandi/PR	*T. cruzi* I
MDID/BR/95/G2	G2	*Didelphis albiventris*	Doutor Camargo/PR	*T. cruzi* I
MDID/BR/95/G3	G3	*Didelphis albiventris*	Maringá/PR	*T. cruzi* I
MDID/BR/97/G249	G249	*Didelphis albiventris*	Floresta/PR	*T. cruzi* I
ITRI/BR/95/A316A	A316A	*Triatoma sordida*	Sarandi/PR	*T. cruzi* I + *T. cruzi* II
IPAN/BR/95/N120B	N120B	*Panstrongylus megistus*	Doutor Camargo/PR	*T. cruzi* I
ITRI/BR/95/A21A	A21A	*Triatoma sordida*	Paiçandu/PR	*T. cruzi* I + *T. cruzi* II
IPAN/BR/95/PMARA31	PMARA31	*Panstrongylus megistus*	Arapongas/PR	*T. cruzi* I + *T. cruzi* II
IPAN/BR/95/PMARA40	PMARA40	*Panstrongylus megistus*	Arapongas/PR	*T. cruzi* I + *T. cruzi* II
ITRI/BR/95/F3	F3	*Triatoma sordida*	Paiçandu/PR	*T. cruzi* I
ITRI/BR/95/N914A	N914A	*Triatoma sordida*	Floresta/PR	*T. cruzi* I
IPAN/BR/95/PMARA38	PMARA38	*Panstrongylus megistus*	Arapongas/PR	*T. cruzi* I
IPAN/BR/95/PMARA67	PMARA67	*Panstrongylus megistus*	Arapongas/PR	*T. cruzi* I

T. cruzi I, II and III refers to the nomenclature established by the Second Satellite meeting described by Zingales *et al.* (2009). Abbreviations of Brazilian states: MG, Minas Gerais; PR, Paraná; SP, São Paulo.