Short Report: Concurrent Detection of Trypanosoma cruzi Lineages I and II in Domestic Triatoma dimidiata from Guatemala

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Abstract. The agent of Chagas disease, Trypanosoma cruzi, is phylogenetically divided into two lineages, T. cruzi I (TCI) and II (TCII). TCI is found in sylvatic and domestic habitats across South America. Despite a high prevalence of TCI in domestic habitats in South America, it has been rarely found in domestic habitats in Central America and Mexico. This may be caused by limitations in detection tests previously used. A modified semi-nested polymerase chain reaction assay was developed to improve detection of TCI and TCII mini-exon genes. This method detected TCI and II concurrently in 84% of 44 cultured isolates from Triatoma dimidiata specimens collected inside homes across the disease-endemic area in Guatemala.

Trypanosoma cruzi, the agent of Chagas disease, is divided into two genetically and biologically distinct lineages, T. cruzi I (TCI) and II (TCII).1–6 TCII is subdivided into five discrete typing units, IIa–e.7,8 TCIIa and IIe were derived from an ancestral hybridization between TCI and TCIIb. TCIIId and TCIIe were derived from a hybridization between TCIIb and TCIIc.9 TCI is associated with sylvatic and domestic environments across South America, having a strong association with arboreal marsupial reservoirs.3–5,10–11 TCI is associated with domestic environments in South America, with sylvatic raccoons in North America and sylvatic armadillos in South America.3,12–19

Studies on T. cruzi diversity in Central America and Mexico suggest that TCI is the primary lineage circulating in domestic and sylvatic environments.15,20–26 In most of these studies, parasites were isolated from human blood or triatomine vectors through passage in mice and/or culture.20,22–25 Some studies in Mexico typed parasites directly in triatomine blood meals by polymerase chain reaction (PCR).21,26 Cultured isolates were typed by isoenzyme analysis and molecular markers such as the mini-exon gene.15,20,22–25 Among these studies, those that used the mini-exon marker to type TCI and TCII used a multiplex PCR procedure was adapted to amplify the TCI and TCII mini-exon genes.15,20–26 The modified PCR was tested on T. cruzi isolates cultured from the intestines of Triatoma dimidiata. Triatomines were collected in domestic environments across the disease-endemic area of Guatemala during a baseline entomologic survey (2000–2005). Houses were randomly selected (n = 7,271) in 572 randomly chosen villages across the departments of Zacapa, Jutiapa, Santa Rosa, and Chiquimula. The house sample size was estimated on the basis of the 1994 National Census and on parameters of an estimated 30% infestation level (5% precision, 95% confidence interval, 80% statistical power). Two entomology technicians searched each house for 15–30 minutes. Collected triatomines were microscopically screened for T. cruzi metacyclic infection and selected positive live specimens were aseptically dissected. The intestinal contents were homogenized in 1 mL of penicillin/streptomycin (3,000 U/mL), incubated overnight at 4°C, and cultured at 28°C in 3 mL of liver infusion tryptose liquid medium supplemented with 10% fetal bovine serum. DNA was extracted from positive cultures with the IsoQuick kit according to manufacturer’s instructions (Orca Research Inc., Bothwell, WA). A semi-nested PCR was designed as an adaptation of previously published primer sequences (Figure 1A). The external PCR was performed in a final volume of 20 µL with 1 µL of DNA, 0.2 µM of each primer KMe1F (5¢-TGTCGTGATCATAATGGGTA-3¢) and KMe1R (5¢-CAATAATAGTACAGAACTG-3¢), 0.15 mM of each dNTP, 1.5 mM MgCl2, 0.04 µL of Taq polymerase, 75 mM Tris-HCl, pH 8.5, 20 mM (NH4)2SO4, and 0.1% Tween 20 (CLP, San Diego, CA). An initial 5-minute denaturation at 94°C was followed by 30 cycles of denaturation at 94°C for 30 seconds, annealing at 48°C for 30 seconds, extension at 72°C for 30 seconds, and a final extension of 72°C for 10 minutes. For the second reaction, 1 µL of the first reaction was added to a master mixture containing the same reagent conditions as before, except for a different combination of primers, each at 0.15 µM.20 This multiplex detects TCI (200 basepairs bp), TCII (250 basepairs), Zymodem 3 (150 basepairs) and T. rangeli (100 basepairs) using the primers: TC1F (5¢-ACACCTTTTCTGGGCGTCTG-3¢), TC2F (5¢-TTGGCTCGACACATCGGTCTG-3¢), TC3F (5¢-CCGGC GAAACAACCCTAATAAAAATG-3¢), TRR (5¢-CCTATT GTGATCCCACTTCTGG-3¢), and MRR (5¢-TACCATAT AGTACAGAAACTG-3¢). The PCR conditions were the same as those for the external reaction except for an annealing at 55°C. The external and internal reactions were performed in different laminar flow hoods and mineral oil was used to prevent product aerosols. Negative reagent controls were included with all reactions. Products were separated by electrophoresis...
The presence of hybrid strains circulating in domestic environments. Finally, there may be a high prevalence of hybrid strains circulating in domestic environments.

Molecular evolution studies estimate that both lineages may have diverged 3–88 million years ago.4,31 One hypothesis proposes that an ancestral lineage was distributed in North and South America 84.4 million years ago, when both continents were still connected.7 After the Cenozoic Era, South America separated from North America and TCI developed in South America, associated with marsupials.2 TCII would have developed in North America in association with placentals, subsequently entering South America during the Oligocene Epoch (38 million years ago) with the island hoppers (primates and rodents) or during the Pliocene-Pleistocene Boundary (5 million years ago) through the great faunal exchange by the Panama isthmus.

Another hypothesis suggests that both lineages evolved in South America after the Cretacic Period and Cenozoic Era.7 In this case, TCI would have been associated with arboreal marsupials and TCII with cave-dwelling edentates such as armadillos.10,32 Cave-dwelling placentals that migrated to South America after the Oligocene Epoch would have become infected with TCII upon entry into these niches. We propose that, regardless of the origin of these lineages, the great faunal exchange would have enabled both lineages to enter Mesoamerica.

Thus, the high proportion of TCI/TCII strains could have important epidemiologic and evolutionary implications. Given that TCI and TCII show different susceptibilities to drugs, the resistance profiles of Central American strains should be evaluated and compared with those of South American strains.33 Isoenzyme analysis of Central American strains previously showed some evidence of genetic exchange, with lack of deviation from Hardy-Weinberg equilibrium at one of three loci.34 This finding may be explained by the presence of mixed infections. The characterization of cloned strains and the use of microsatellites are currently underway and should help elucidate whether these strains are mixed or hybrid strains. Future work will include subtyping of the TCII samples. On the basis of these results, we propose that the isthmus provided an environment that enabled interaction between hosts carrying both lineages during faunal exchanges, leading to mixed infections and/or hybrid formation in cave-dwelling triatomine species such as *T. dimidiata*.

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