Trypanosoma cruzi isolates from Mexican and Guatemalan acute and chronic chagasic cardiopathy patients belong to Trypanosoma cruzi I


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Trypanosoma cruzi is classified into two major groups named T. cruzi I and T. cruzi II. In the present work we analyzed 16 stocks isolated from human cases and four isolated from triatomines from diverse geographical origins (Mexico and Guatemala). From human cases four were acute cases, six indeterminates, and six from chronic chagasic cardiopathic patients with diagnosis of dilated cardiomyopathy established based on the left-ventricular end systolic dimension and cardiothoracic ratio on chest X-radiography and impaired contracting ventricle and different degree conduction/rhythm aberrations. DNA samples were analyzed based on mini-exon (ME) polymorphism, using a pool of three oligonucleotide for the amplification of specific intergenic region of T. cruzi ME gene.

All the Mexican and Guatemalan isolates regardless their host or vector origin generated a 350 bp amplification product. In conclusion T. cruzi I is dominant in Mexico and Guatemala even in acute and chronic chagasic cardiopathy patients. To our knowledge, this is the first study describing predominance of T. cruzi I in human infection for North and Central America.

Key words Trypanosoma cruzi - mini-exon - Mexico - lineage I - Guatemala

Trypanosoma cruzi is classified into T. cruzi I and T. cruzi II, this denomination aroused from a consensus reached by specialists based on different markers (Satellite Meeting 1999). T. cruzi I is mainly observed in wild mammals and more adapted to marsupials and sylvan triatomines, it is only occasionally isolated from humans, whereas T. cruzi II is apparently more associated with primates and it is usually found in human infections. Until now all parasites that have been isolated from seropositive individuals in Brazil belong to T. cruzi II (Fernandes et al. 1998, 1999, Zingales et al. 1998). Recently a published report paper show a predominance of lineage I in 56 Mexican T. cruzi stocks isolated from vectors, humans, and sylvatic triatomines using RAPDs, but the clinical status of human cases was not identified (Bosseno et al. 2002). In South-America also was found in 23 isolates from acute chagasic patients using ribosomal and mini-exon (ME) marker that 74% of them belonged to T. cruzi I (Anez et al 2004). It is known that the ME gene is presented in the nuclear genome of all Kinetoplastida in nearly 200 copies in tandemly-repeated sequences. This gene consist of three regions: exon, intron, and intergenic region. The exon is highly conserved, the intron is moderately conserved and the intergenic region or non-transcribed spacer is particular dissimilar. This feature has allowed the classification of T. cruzi in two main groups (Devera et al. 2003, Macedo et al 2004). In the present work we analyzed 16 stocks isolated from human cases and four isolated from triatomines all from with diverse geographic origins (Mexico and Guatemala). Seven came from Guatemala and 13 from Mexico.

Six were isolated from chronic chagasic cardiopathic (CCC) patients who were evaluated at Instituto Nacional Cardiología “I. Chávez” in Mexico. All of them have been diagnosed with dilated cardiomyopathy based on the left-ventricular end systolic dimension, cardiothoracic ratio on chest X-radiography and impaired contracting ventricle and different degree of conduction/rhythm aberrations and five were from indeterminate or blood bank donors, one of them showed an RBBB on his ECG record, four were symptom less subjects and one more from acute case. Two from triatomine vectors. All fourteen isolates came from Mexico.

Out of six Guatemalan isolates, three came from acute cases and one from asymptomatic subject and two from triatomine origin (Table). The CL-Brener strain was used as T. cruzi II control.

All parasites were culture in LIT 10% fetal calf serum enriched medium. The DNA extraction was performed with a mixture of phenol-chloroform-isoamyl alcohol, sodium acetate, and ethanol precipitation. Samples were analyzed based on ME polymorphism, using a pool of three oligonucleotide for the amplification of the intergenic region.

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of T. cruzi mini-exon gene: 5´GTGTCCGCCACC
TCCTTCCGGGC3´ (group 1-specific); 5´CCTGCAGGC
ACACGTGTGTG3´ (group 2-specific); and 5´CCCCCC
TCCCAGGCCACACTG 3´ (common to group 1 and 2) by
PCR as previously reported (Souto et al. 1996). In brief 10
ng of DNA were submitted to amplification in a 50 µl of
reaction mixture following this thermal profile: 94°C/1min;
27 cycles of 94°C/30 s, 55°C/30 s, 72°C/30 s; 72°C/10 min.
Amplification products were analyzed in 1.5% agarose
gels. T. cruzi I generates a 350 bp product whereas T. cruzi
II generates 300 bp product. All the Mexican and Guate-
malan isolates regardless their host or vector origin gen-
erated a 350 bp amplification product (Fig. 1), conse-
quently all of them belong to T. cruzi I in spite of their
broad geographic distribution, since stocks were isolated
from individuals living in Northwest of Mexico, Pacific
Coast, Central part of Mexico, Gulf of Mexico Coast, in-
cluding Guatemala. In previous paper it has been reported
that Mexican stocks from eight states out of 31 in Mexico
belonged to T. cruzi I (Bosseno et al. 2002). Now our data
confirm and extend previous findings in addition we dis-
close T. cruzi I may play a major role in human infection in
Mexico and Guatemala. Moreover they are involved in
CCC as well as acute cases (Table). These results con-
trast with the situation reported in Brazil, where parasites
belonging to T. cruzi II are preferentially associated with
human infection (Fernades et al. 1999) while T. cruzi I are
associated with the sylvatic cycle of the parasite. How-
ever, our data is in accordance to recently published pa-
sper where 74% of Venezuelan isolates from acute chag-
sisic patients were typed as T. cruzi I (Anez et al. 2004).

Although, the exact reason to explain these findings is
not completely understood, observational data sug-

gest that T. cruzi I predominates in human and sylvatic
cycle at least in Mexico and Guatemala. 

In order to confirm our results, DNA sequences of
PCR amplification products were confirmed by fluores-
cent DNA sequencing utilizing a Perkin-Elmer Genetic
Analyzer 310 DNA sequencer after the agarose DNA frag-
ments were cutt-off from the gel and purified utilizing
magnetic micro-beads (Dynal beads) following the
manufacture’s instructions (data not shown).

### TABLE

Main features of Mexican and Guatemalan Trypanosoma cruzi isolates

<table>
<thead>
<tr>
<th>Isolate name</th>
<th>Place and date</th>
<th>Human clinical findings</th>
<th>T. cruzi group mini-exon</th>
<th>Standardized nomenclature</th>
</tr>
</thead>
<tbody>
<tr>
<td>INC-1</td>
<td>Oaxaca, Mex 1994</td>
<td>AVB, LBBB, VE</td>
<td>I</td>
<td>MHOM/MX/1994/INC1 (T. cruzi I)</td>
</tr>
<tr>
<td>INC-5</td>
<td>Veracruz, Mex 1994</td>
<td>LBBB, VE, VT, CI and cardiomegaly</td>
<td>I</td>
<td>MHOM/MX/1994/INC5 (T. cruzi I)</td>
</tr>
<tr>
<td>INC-6</td>
<td>Oaxaca, Mex 2000</td>
<td>RBBB, CI cardiomegaly</td>
<td>I</td>
<td>MHOM/MX/2000/INC6 (T. cruzi I)</td>
</tr>
<tr>
<td>INC-7</td>
<td>Veracruz, Mex 2001</td>
<td>AVB, VT, VF cardiomegaly</td>
<td>I</td>
<td>MHOM/MX/2001/INC7 (T. cruzi I)</td>
</tr>
<tr>
<td>INC-8</td>
<td>Veracruz, Mex 2001</td>
<td>RBBB, CI and cardiomegaly</td>
<td>I</td>
<td>MHOM/MX/2001/INC8 (T. cruzi I)</td>
</tr>
<tr>
<td>INC-9</td>
<td>Guerrero, Mex 2001</td>
<td>RBBB, CI and cardiomegaly</td>
<td>I</td>
<td>MHOM/MX/2001/INC9 (T. cruzi I)</td>
</tr>
<tr>
<td>INC-10</td>
<td>Guanajuato, Mex 2002</td>
<td>Asymptomatic blood donor</td>
<td>I</td>
<td>MHOM/MX/2002/INC10 (T. cruzi I)</td>
</tr>
<tr>
<td>INC-12</td>
<td>Morelos, Mex 2003</td>
<td>RIBBB blood donor</td>
<td>I</td>
<td>MHOM/MX/2003/INC12 (T. cruzi I)</td>
</tr>
<tr>
<td>JJO</td>
<td>Jalisco, Mex</td>
<td>Asymptomatic</td>
<td>I</td>
<td>MHOM/MX/0000/JJO (T. cruzi I)</td>
</tr>
<tr>
<td>MOR5</td>
<td>Morelos, Mex</td>
<td>Asymptomatic</td>
<td>I</td>
<td>MHOM/MX/0000/MOR5 (T. cruzi I)</td>
</tr>
<tr>
<td>H1</td>
<td>Yucatan, Mex</td>
<td>Acute case</td>
<td>I</td>
<td>MHOM/MX/0000/H1 (T. cruzi I)</td>
</tr>
<tr>
<td>Nayarat</td>
<td>Nayarat, Mex</td>
<td>Triatome</td>
<td>I</td>
<td>PIR/MX/0000/Nayarat (T. cruzi I)</td>
</tr>
<tr>
<td>CIES</td>
<td>Chiapas, Mex</td>
<td>Triatome</td>
<td>I</td>
<td>PRX/MX/0000/CIES (T. cruzi I)</td>
</tr>
<tr>
<td>H38</td>
<td>Guatemala</td>
<td>Acute case</td>
<td>I</td>
<td>MHOM/GT/0000/H38 (T. cruzi I)</td>
</tr>
<tr>
<td>H31</td>
<td>Guatemala</td>
<td>asymptomatic</td>
<td>I</td>
<td>MHOM/GT/0000/H31 (T. cruzi I)</td>
</tr>
<tr>
<td>H7</td>
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<tr>
<td>H64</td>
<td>Guatemala</td>
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<td>I</td>
<td>MHOM/GT/0000/H64 (T. cruzi I)</td>
</tr>
<tr>
<td>T1131</td>
<td>Guatemala</td>
<td>Triatome</td>
<td>I</td>
<td>/GT/0000/T1131 (T. cruzi I)</td>
</tr>
<tr>
<td>T1147</td>
<td>Guatemala</td>
<td>Triatome</td>
<td>I</td>
<td>/GT/0000/T1147 (T. cruzi I)</td>
</tr>
</tbody>
</table>

DNA sequences from each *T. cruzi* isolated including reference CL-Brener strain confirmed that PCR products corresponded to mini-exon. Although small fragment was sequenced (88 to 96 bp), a BLAST analysis indicates identities between 93 to 97% in CL Brener respect to Tul 18, AFI, CL, SC43, MN, and IGRE strains. In the case of Mexican isolates identities were found in the following ranges 88 to 93% (data not shown).

In conclusion *T. cruzi* I is dominant in México and Guatemala even in human infections.

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