Susceptibility of Mepraia spinolai and Triatoma infestans to different Trypanosoma cruzi strains from naturally infected rodent hosts

Ricardo Campos a, Mariana Acuña-Retamar b, Carezza Botto-Mahan c, Sylvia Ortiz a, Pedro E. Cattan b, Aldo Solari a,∗

a Program of Cellular and Molecular Biology, Institute of Biomedical Sciences, Faculty of Medicine, University of Chile, Santiago, Chile
b Department of Biological Sciences, Faculty of Veterinary Sciences, University of Chile, Santiago, Chile
c Department of Ecological Sciences, Faculty of Sciences, University of Chile, Santiago, Chile

Received 23 March 2007; received in revised form 25 June 2007; accepted 10 July 2007
Available online 25 July 2007

Abstract

Trypanosoma cruzi is the causative agent of Chagas disease, a zoonosis involving domestic and sylvatic mammalian reservoirs. Since scarce information has been published about the susceptibility of T. cruzi lineages to other triatomine species besides Triatoma infestans, we evaluate the susceptibility of T. infestans and Mepraia spinolai to different T. cruzi lineages, originated from naturally infected Octodon degus rodents as mammal host. Xenodiagnosis-PCR methods to detect T. cruzi positive rodents and genotyping to differentiate T. cruzi lineages (TcI, TcIIb, TcIId and TcIIe) identified singly and mixed T. cruzi infections. More infections and nearly all mixed infections were identified using the wild vector M. spinolai than T. infestans.

© 2007 Published by Elsevier B.V.

Keywords: Triatome; Reduviidae; Chagas disease; Insect vector; Chile

1. Introduction

Chagas disease is a zoonosis involving a large number of domestic and sylvatic mammalian reservoirs. In Chile, two insect vectors propagate the protozoan parasite Trypanosoma cruzi, the causative agent of the disease. Triatoma infestans (Reduviidae: Triatominae) is the domestic vector, so far controlled (Lorca et al., 2001). Meantime, Mepraia spinolai is a peridomestic and sylvatic vector, found in corrals of domestic animal, stony hills and rock crevices, often inhabited by the sylvatic rodent reservoir Octodon degus (Botto-Mahan et al., 2005a). This wild vector is an endemic species, inhabiting ecologically isolated areas by the presence of the Andes Mountains and the Atacama Desert.

The taxon T. cruzi is divided into two groups, Tcl and TclI. T. cruzi I corresponds to zymodeme Z1, and T. cruzi II has been proposed to be subdivided into five sublineages, one corresponding to Z2 (TclIb), another to Z3 (TclIa), and the hybrids TclIc, TclId and TclHe (Brisse et al., 2001). According to direct parasite characterization methods, these lineages can be found singly or mixed as described for Bolivia and Chile (Bosseno et al., 1996; Breniere et al., 1998; Solari et al., 2001; Coronado et al., 2006). The relationship between the T. cruzi diversity and its vectorial transmissibility with
different insect vectors is not well known. *T. cruzi* populations differ in their ability to multiply and differentiate inside the insect vector (Schaub et al., 1989; Mello et al., 1996). However, the susceptibility of different triatomine species to *T. cruzi* infection has been studied by means of xenodiagnosis, that is, the use of uninfected bugs to detect *T. cruzi* flagellates on reservoirs (Schenone, 1999). Several reports have described methods to improve diagnosis using different triatomine species or modified xenodiagnosis protocols (de Carvalho Moreira and Perlowagora-Szumlewicz, 1997; Campos et al., 2007). It has been suggested that indigenous vectors from the same geographic area present a greater susceptibility (Dias, 1940), emphasizing the superiority of sylvatic vectors over domestic ones to detect *T. cruzi* (Perlowagora-Szumlewicz et al., 1988, 1990; Campos et al., 2007). However, scarce information has been published about the susceptibility of *T. cruzi* lineages to other triatomine species besides *T. infestans* (de Lana et al., 1998). In this study, we evaluate the susceptibility of *T. infestans* and *M. spinolai* to different *T. cruzi* lineages, originated from naturally infected *O. degus* rodents as mammal host. In order to maximize *T. cruzi* detection, we use a recently described method by Campos et al. (2007), which combines xenodiagnosis and PCR assays.

2. Materials and methods

2.1. Trapping site

The collecting site for *O. degus* specimens is located at Las Chinchillas National Reserve (IV Region; 31° 30’ 03” S; 71° 06’ 20” W), located about 300 km north from Santiago, Chile, and 60 km east from the Pacific coast. The reserve is in a mountainous terrain of 4229 ha with semi-arid climate and sporadic precipitation concentrated during austral winter months (di Castri and Hajek, 1976). Several reports indicate the presence of *M. spinolai* populations in this area (Botto-Mahan et al., 2002, 2005a), with up to 46.2% of *T. cruzi* infection (Botto-Mahan et al., 2005b). In this study, we captured 35 *O. degus* (Rodentia: Octodontidae) using Sherman traps in diurnal and nocturnal periods. Captured rodents were carried out to the laboratory and kept in a biotherium at 25 °C, 60% relative humidity with 12:12 h light:dark photoperiod, and individually housed in plastic cages (31.0 cm × 37.0 cm) with water and alfalfa pellet *ad libitum*. After experiments all *O. degus* specimens were returned to the collecting site. Experiments were conducted with permission of the Ethical Committee of the Faculty of Medicine, University of Chile.

2.2. Xenodiagnosis-PCR analysis

For xenodiagnosis-PCR purposes, *O. degus* specimens were intramuscularly anesthetized with 50 mg/kg of ketamine and placed in a tray with 10–20 uninfected first and second instar nymphs of each vector species. *T. infestans* and *M. spinolai* nymphs were obtained from established insect colonies at the Faculty of Medicine and Faculty of Sciences (University of Chile), respectively. Nymphs from both vector species were reared under optimal growing conditions inside a climatic chamber at 26°C, 65–70% relative humidity, and 14:10 h light:dark photoperiod. Parental insects of the *T. infestans* colony were captured in the decade of 1950 from different localities in the IV region (Chile). Approximately, the nymphs used in this study correspond to the 50th generation in captivity. The *M. spinolai* colony was established in 2005 from parental insects captured at Las Chinchillas National Reserve (see location above), and the nymphs used in this study correspond to the 2nd generation in captivity. Sixty days post feeding, a pool of feces from all the bugs of each vector species fed with each *O. degus* individual was obtained through abdominal extrusion and examined under a light microscope (Schenone, 1999). Each pool of total intestinal contents was mixed with 200 μl of PBS buffer, centrifuged at 10,000 × g, and frozen at −20°C for PCR assay. A sample of 1–5 μl of this crude 10 min-preboiled extract was used as DNA template. Intestinal contents were free of fresh blood, therefore, no DNA extraction was required. PCR was performed as previously reported, using primers 121 and 122 directed to amplify the variable region of minicircle kinetoplast DNA (Wincker et al., 1994; Campos et al., 2007). Each run included positive and negative controls. Samples were tested in triplicate, and an animal was considered positive when at least two out of the three assays turned out positives. PCR products were analyzed by electrophoresis on 2% agarose gels and visualized by ethidium bromide staining. A 330 base pairs (bp) product represents a positive assay. For confirmation of positive PCR, Southern analyses were performed using 10 μl of each PCR assay. PCR products were electrophoresed, transferred onto Hybond N+ nylon membranes (Amersham), and cross-linked by ultraviolet light for DNA fixation. After transferring PCR products, membranes were pre-hybridized for at least 2 h at 55°C, and finally hybridized with total kinetoplast DNA labeled with P32 (1 × 106 cpm/membrane) as probe. After hybridization, membranes were washed three times for 30 min each with 2× SSC, 0.1% SDS at 55°C, and later exposed in the Molecular Imager FX.
No inhibitors were detected in negative PCR samples.

2.3. Trypanosoma cruzi genotyping

For T. cruzi genotyping, four different T. cruzi clones (sp 104c11, CBBcl3, NRcl3 and v195c11) corresponding to TcI, TcIIb, TcIId and TcIIe, respectively, were used to generate specific probes. Construction of minicircles probes was performed as previously described (Veas et al., 1991), and radiolabeled as described for total kinetoplast DNA. Southern analyses were performed with each genotype specific probe.

3. Results

Light microscope examination detected two T. cruzi positive rodents (nos. 5 and 18) with both vectors. Results from xenodiagnosis-PCR analyses using M. spinolai and T. infestans showed that eight and two O. degus were T. cruzi positives, respectively (chi-square = 4.2, d.f. = 1, \( P = 0.04 \)). The amount of blood extracted by each pool of bugs from T. cruzi positive O. degus did not differ between the two vector species with an average amount of 128.8 and 142.2 mg in M. spinolai and T. infestans, respectively (t-test; \( F_{1,14} = 0.155, P = 0.699 \); Table 1). T. cruzi genotyping was performed on fecal samples from insects fed with O. degus that resulted positives (Fig. 1A–E).

Results from genotyping shown in Fig. 1B–E indicate that four O. degus were singly infected with TcI, two were infected with two strains (TcI and TcIId), and two other rodents were infected with three T. cruzi strains (TcI, TcIIb/TcIId, and TcIIe). These results indicate the presence of mixed infection in four rodents, three of them only detected with the wild vector M. spinolai.

![Fig. 1](image)

<table>
<thead>
<tr>
<th>Octodon degus specimens</th>
<th>Ingested blood in mg (number of nymphs fed)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Triatoma infestans</td>
</tr>
<tr>
<td>5</td>
<td>120(10)</td>
</tr>
<tr>
<td>9</td>
<td>80(6)</td>
</tr>
<tr>
<td>10</td>
<td>90(6)</td>
</tr>
<tr>
<td>12</td>
<td>105(5)</td>
</tr>
<tr>
<td>13</td>
<td>115(5)</td>
</tr>
<tr>
<td>15</td>
<td>100(6)</td>
</tr>
<tr>
<td>18</td>
<td>250(6)</td>
</tr>
<tr>
<td>19</td>
<td>280(6)</td>
</tr>
</tbody>
</table>

Numbers in parentheses indicate the total number of nymphs fed on O. degus.

Even though, our results for xenodiagnosis-PCR suggest a higher susceptibility of M. spinolai to different T. cruzi genotypes compared to T. infestans, not statistical differences were detected (chi-square = 4.2, d.f. = 2, \( P = 0.12 \)). Finally, O. degus with high parasitemia (i.e., microscope positives) were only infected with TcI as detected with both vector species.

4. Discussion

Experimental studies in chronic Chagas disease using genetically defined T. cruzi strains demonstrated that transmission efficiency could be associated with
the triatomine species used (Perlowagora-Szumlewicz et al., 1988, 1990). More recent studies on T. infestans susceptibility to T. cruzi naturally infected patient from Minas Gerais, Brazil, concluded that this species has low transmissibility compared to other triatomines (Nirschl et al., 1994; de Carvalho Moreira and Perlowagora-Szumlewicz, 1997). Results from our study follow the same tendency, that is, triatomine susceptibility to local T. cruzi populations circulating in the wild rodent O. degus varies between vector species. The native triatomine species M. spinolai presents a higher susceptibility compared to the domestic species T. infestans.

It can be hypothesized that T. infestans is less adapted to transmit local T. cruzi lineages than M. spinolai, especially if we consider the recent colonization of T. infestans in Chile after its migration from the origin in Cochabamba, Bolivia (Schrofield, 2000). More recently, it has been determined by experimental infections with well-defined T. cruzi lineages, that T. infestans has the best transmissibility for the TcI lineage, a minimal for TcIIb, and an intermediate for TcIIId (de Lana et al., 1998). Our results using naturally infected O. degus suggest that even though T. infestans was able to amplify the TcI lineage, only it was able to detect it from two heavily infected O. degus, as determined by classical xenodiagnosis. The rest of TcI-infected rodents, besides rodents 5 and 18, were only detected by M. spinolai. Three other rodents infected with mixed lineages (TcI, TcIIb/TcIIId and TcIIe) were exclusively detected by M. spinolai. These results show the higher capacity of M. spinolai to host and reproduce different T. cruzi lineages compared to T. infestans. This observation is interesting considering the low probability of T. cruzi transmission by M. spinolai due to defecation delay when compared to T. infestans, implying a lower transmission efficiency (Canals et al., 1999). However, this aspect is attenuated since T. cruzi infection reduces defecation time in infected insects (Botto-Mahan et al., 2006).

In this study, we detect lower infection rates in O. degus compared with previous reports of infection levels for the same geographic area (Botto-Mahan et al., 2005b). This fact may be explained by natural oscillations in parasite–host systems. Probably, the lack of statistical differences between the ability of both vectors to host and reproduce one or more T. cruzi strains is the result of this low infection levels in O. degus populations, which translates into a small sample size. However, our results show a tendency pointing that mixed infections could be better detected by M. spinolai than T. infestans, an aspect relevant when improving xenodiagnosis sensitivity. It is worth to point out that the direct characterization method used here, by PCR and Southern analysis, allows detection and genotyping without parasite isolation and cultivation, procedures that could select some T. cruzi clones from a mixture.

Acknowledgments

This study was supported by FONDECYT grant 1040762 to A. Solari. Additional support was obtained from FONDECYT grants 1040711 and 3050033 to P.E. Cattan and C. Botto-Mahan, respectively.

References


