Comparison of *Trypanosoma cruzi* detection by PCR in blood and dejections of *Triatoma infestans* fed on patients with chronic Chagas disease

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**Abstract**

In this study, we compare the sensitivity of detecting *Trypanosoma cruzi* in dejections of *Triatoma infestans* nymphs that had fed on the blood of chronic chagasic patients, with detection of *T. cruzi* in peripheral blood, using a polymerase chain reaction assay (PCR-D and PCR-B, respectively). Fifty-seven chronic patients were evaluated who were positive (group I) or negative (group II) by xenodiagnosis (XD). Patients showed 84.8 and 75% positive PCR results in both kinds of samples in groups I and II, respectively. Six cases (10.5%) showed positive PCR-D and negative PCR-B, five of them belonged to group I. In contrast, five cases of group II showed negative PCR-D and positive PCR-B. Overall, the PCR-D assay gave positive results in 52 out of 57 samples (91.2%), while 51 out of 57 (89.5%) were positive by PCR-B. In comparison, only 57.9% were positive by XD (*p*= 0.0001). In conclusion, PCR performed in dejection or blood was more sensitive for the parasite detection than xenodiagnosis. All patients (100%) were detected positive when both, PCR-D and PCR-B, were applied.

**Keywords:** Chronic Chagas disease; Polymerase chain reaction; Xenodiagnosis

Chagas disease, caused by the protozoan parasite *Trypanosoma cruzi* (*T. cruzi*), is a major public health problem in Latin America. *T. cruzi* detection during the chronic phase is difficult due to the small number of circulating trypomastigote forms, which are recovered by xenodiagnosis (XD) only from a limited number of patients (Castro et al., 2002). The polymerase chain reaction (PCR) in human blood or serum has been considered an alternative technique for parasitological diagnosis (Britto et al., 2001). *T. cruzi* kinetoplast DNA detection by PCR has been applied in dejections of experimentally and naturally infected triatomines (Chiurillo et al., 2003), and in dejections of insect vectors fed with blood from infected patients (Silber et al., 1997). Kinetoplast DNA of *T. cruzi* is abundant and can be easily extracted (Junqueira et al., 1996). The use of the vector fecal drop as template for PCR assays was reported, although, others suggest that fresh tissue obtained by dissection of the bug is preferable (D’Alessandro-Racigalupo and Saravia,
PCR using these two types of samples for the detection of *T. cruzi* are equivalent. Also the detection capacity of *T. cruzi* by microscopy and PCR has been evaluated. PCR was significantly more sensitive than microscopy for the detection of *T. cruzi* (Dorn et al., 2001; Botto-Mahan et al., 2005). Other authors applied improved DNA extractions and PCR strategies for screening and identification of *T. cruzi* lineages directly from faeces of triatomines. They found that in the faecal sample was detected a mixed of two clones while in the culture sample only showed one of them. This fact demonstrates the importance of lineage identification of *T. cruzi* in field-collected triatomine (Marcet et al., 2006). The aim of the present work was to compare PCR sensitivity for detection of *T. cruzi* in dejections of *Triatoma infestans* (T. infestans) nymphs that had fed on the blood of chronic chagasic patients, with PCR performed in peripheral blood of the same patients. Oligonucleotides 121 (5′-AAATAATGTACGGG (T/G) GAGATGCATGA3′) and 122 (5′-GGTTGCGATTGGGTGGTGTAATATA3′), that amplify a 330 bp *T. cruzi* DNA fragment from minicircles were used (Wincker et al., 1994).

Fifty-seven chronic chagasic individuals, 25 females and 32 males (mean 22 years old) from endemic regions of Chile (IV and V regions) (Apt et al., 2003) were selected on the basis of positive serologic results (IFI and ELISA), and grouped as XD positive (group I, n = 33) or XD negative (group II, n = 24). The XD test was carried out using two wooden boxes with seven *T. infestans* nymphs stage III each (free of infection). Microscopic examination of insect dejection was performed at 30, 60 and 90 days after feeding, and a pool of all the insects dejection was collected to be used in the PCR assay, no matter whether the XD was positive or negative at these time (Schenone, 1999). As negative control, non-infected triatomines dejections were used for PCR. Two millilitres of whole blood was mixed with a similar volume of the buffer Guanidine HCl 6 M and EDTA 0.2 M, and DNA phenol extracted. PCR were made in triplicate in blood or dejection and performed as described (Solari et al., 2001). A positivity sample was considered when the three assays were positive. However, in case of discordance four other assays were performed. Positive samples were considered when at least five out of seven assays were positive. Results were analyzed using the χ² goodness of fit statistical test, and p-values ≤0.05 were considered to be significant.

Conventional parasitological methods rarely reach 100% sensitivity in the chronic phase of the Chagas disease using serology as gold standard, since, this parameter depends on the number of parasites in peripheral blood (Castro et al., 2002). Recently, molecular assays as PCR, which amplify certain repetitive sequences of trypanosome kDNA, have been proposed as a good alternative tool for detection of *T. cruzi* in human blood (Britto et al., 1995; Chiari, 1999; Zulantay et al., 2004). The PCR assays have shown a variable degree of efficiency, thus, a negative result does not necessarily mean that the chagasic patient is free of infection (Chiari, 1999).

In the present study, *T. cruzi* kDNA detection was considered positive when a band of 330 bp was detected. Fifty-seven chronic patients were evaluated who were positive (group I) or negative (group II) by xenodiagnosis. Patients showed 84.8 and 75% positive PCR results in both kinds of samples in groups I and II, respectively. Six cases showed positive PCR-D and negative PCR-B, five of these belonged to group I. In contrast, five cases of group II showed negative PCR-D and positive PCR-B (Table 1). Therefore, 10.5% of the cases were only detected by PCR-D and 8.8% only by PCR-B. The PCR-D assay gave positive results in 52 out of 57 (91.2%), while in 51 out of 57 (89.5%) were positive by PCR-B. In contrast, only 57.9% were positive by XD (p=0.0001).

Other authors reported 59.4% sensitivity with PCR in blood samples of serologically positive chronic chagasic individuals compared with 35.6% obtained by XD (Junqueira et al., 1996). However, PCR has been reported to provide 90% of *T. cruzi* detection efficiency with seropositive results for Chagas disease in other endemic regions (Britto et al., 1995). A study that detected *T. cruzi* DNA by PCR in dejection of insect vectors fed

### Table 1

**PCR-B and PCR-D in chronic chagasic patients with positive or negative xenodiagnosis**

<table>
<thead>
<tr>
<th>Group</th>
<th>Cases</th>
<th>XD</th>
<th>[PCR-B (+)</th>
<th>PCR-D (+)]</th>
<th>[PCR-B (+)</th>
<th>PCR-D (+)]</th>
<th>[PCR-B (+)</th>
<th>PCR-D (+)]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>I</td>
<td>33</td>
<td>+</td>
<td>28</td>
<td>84.8</td>
<td>5</td>
<td>15.2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>II</td>
<td>24</td>
<td>−</td>
<td>18</td>
<td>75</td>
<td>1</td>
<td>4.2</td>
<td>5</td>
<td>20.8</td>
</tr>
<tr>
<td>Total</td>
<td>57</td>
<td></td>
<td>46</td>
<td>80.7</td>
<td>6</td>
<td>10.5</td>
<td>5</td>
<td>8.8</td>
</tr>
</tbody>
</table>
with blood from infected patients using nuclear oligonucleotides BPI/BP2, found that this technique was only 10% more sensitive than XD (Silber et al., 1997).

Our results using 121 and 122 primers in these type of biological samples, showed a better overall sensitivity than XD. Our results demonstrate that PCR applied in both types of samples, peripheral blood or in dejection of triatomines, is significantly more sensitive for detection of T. cruzi than microscopically examination used in XD and confirm the importance of applying PCR in these biological samples. We have demonstrated that PCR applied in faecal samples is more precocious and sensitive than xenodiagnosis (manuscript in preparation).

The presence of T. cruzi in 100% of patients was only detected by PCR when using both samples. In 15.2% (XD+) and 4.2% (XD−) of the cases were PCR-B (+) and PCR-B (−), possibly for the presence of T. cruzi clones better adapted in triatomines than in human blood. Also, this result is likely due to the parasitemias being below the detection limit of the assay. We discarded the possibility of PCR inhibition in the DNA lysates obtained from these blood samples because a protocol was used as PCR internal control for amplification of the human β-globin gene sequences, as described (Saiki et al., 1985). On the other hand, 8.8% of the cases were PCR-B (+) and PCR-D (−) suggesting very low parasitemia, therefore, diminishing the T. cruzi detection. According to our results, we estimated that the detection limit was one parasite in 2 ml of blood samples, the volume of T. infestans feeding was 0.1 ml/triatomine (two boxes with seven nymphs each = 1.4 ml of blood) and the volume of blood used for PCR was 2 ml, therefore, the volume of samples were in the same order of magnitude.

T. cruzi populations appear to have a multiclonal structure (Barnabé et al., 2005), therefore, it is important to analyze parasites amplified in the XD test (Apt et al., 2003) because a probably selection of some T. cruzi genotypes and the amplification in T. infestans of minor parasite clones (Breniere et al., 1998). Recent studies of our group support this idea of a preferential selection of T. cruzi genotypes in vertebrate and invertebrate hosts (Coronado et al., 2006). In previous studies, PCR was used to analyze T. cruzi directly in biological samples: feces of infected Triatomine bugs, blood samples of experimentally infected mice and artificially infected human blood samples. The diagnosis of T. cruzi infection and simultaneous direct identification of the different natural clones, circulating in vectors and mammalian blood without isolation of the stocks was performed (Breniere et al., 1992). In other studies, PCR was employed to detect T. cruzi DNA in both insects fed on experimentally infected monkeys and insects. They found that monkeys with parasitemia levels below the limit of detection show delayed results than monkeys with higher parasitemia. These results suggest that PCR can be used to speed the xenodiagnosis results with great sensitivity (Russonando et al., 1996). Our results demonstrated that T. cruzi was detected in all patients only when both, PCR-D and PCR-B were applied. This fact show the importance of using XD dejection samples for PCR, specially in patients with negative PCR-B in order to evaluate chemotherapeutical efficacy and/or to improve diagnosis.

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References


