SHORT COMMUNICATION

A Simplified Method for Sample Collection and DNA Isolation for Polymerase Chain Reaction Detection of *Trypanosoma rangeli* and *Trypanosoma cruzi* in Triatomine Vectors

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Due to the overlapping distribution of Trypanosoma rangeli *and* T. cruzi *in Central and South America, sharing several reservoirs and triatomine vectors, we herein describe a simple method to collect triatomine feces and hemolymph in filter paper for further detection and specific characterization of these two trypanosomes. Experimentally infected triatomines feces and hemolymph were collected in filter paper and specific detection of* T. rangeli *or* T. cruzi *DNA by polymerase chain reaction was achieved. This simple DNA collection method allows sample collection in the field and further specific trypanosome detection and characterization in the laboratory.*

Key words: *Trypanosoma cruzi* - *Trypanosoma rangeli* - polymerase chain reaction - triatomines - *Dipetalogaster maximus*

Trypanosoma cruzi Chagas (1909), the etiological agent of Chagas disease, affects more than 18 million people in America. Among distinct ways, *T. cruzi* is mainly transmitted by triatomine bug feces containing metacyclic trypomastigote forms of the parasite (WHO 1991). *Trypanosoma rangeli* Tejera (1920) infects several mammalian species including man in Central and South America. *T. rangeli* presents an overlapping distribution with *T. cruzi*, sharing animal reservoirs and triatomine vectors. Differently from *T. cruzi*, *T. rangeli* is considered pathogenic only for the triatomine bugs, being harmless to the vertebrate hosts. However, *T. rangeli* induces a humoral immune response in humans that strongly cross-reacts with *T. cruzi* (Grisard et al. 1999b). The sympatric distribution, allowing the occurrence of single and/or mixed infections in both vertebrate and invertebrate hosts, allied to the cross-reactivity in serological assays are of great importance for Chagas disease diagnosis, specially in the indeterminate form of the chronic phase (D'Alessandro & Saravia 1992).

Detection of *T. rangeli* and *T. cruzi* infections are based on the same serological and parasitological assays such as indirect immunofluorescence, enzyme linked immunosorbend assay, hemoculture and xenodiagnosis. However, none of these methods can specifically detect these trypanosomes. Recently, some authors have used molecular assays in order to specifically detect *T. rangeli* and *T. cruzi* in vertebrate and invertebrate hosts using distinct DNA extraction methods (Moser et al. 1989, Brenière et al. 1995, Russomando et al. 1996, Shikanai-Yasuda et al. 1996, Souto et al. 1999, Vallejo et al. 1999).

We now report here a polymerase chain reaction (PCR) for specific detection of *T. rangeli* and *T. cruzi* DNA in feces and hemolymph of the experimentally infected triatomine (*Dipetalogaster maximus*), using a simple method for sample collection and DNA isolation.

The *T. cruzi* CL strain, isolated from *Triatoma infestans* in Brazil (Brener & Chiari 1963) and *T.*

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rangeli SC-58 strain isolated from the naturally infected rodent *Echimys dasythrix* in Southern Brazil (Steindel et al. 1991) were used. These strains were respectively maintained in liver infusion tryptose (LIT) and in NNN+LIT medium at 26°C by weekly passages.

Fourth stage nymphs of *D. maximus* were used in this study. They were obtained from the Laboratório de Triatomíneos e Epidemiologia da Doença de Chagas, Centro de Pesquisas René Rachou-Fiocruz. Three groups of 30 triatomines were independently fed with LIT medium and with exponential growth phase cultures of *T. rangeli* and *T. cruzi* in artificial feeders. After the infective meal, triatomines were kept at 27°C, 70% humidity and fed on mice each 15 days. Hemolymph was obtained from each triatomine by section of a single leg. Daily observations for the presence of flagellates in feces and hemolymph of all triatomines were performed after ten days post feeding under light microscopy by both fresh and Giemsa stained smears. Also, feces and hemolymph were collected every ten days after the infective meal in sterile filter papers, air dried and stored at -20°C until use. Feces and hemolymph of all non-infected triatomines fed on LIT medium were also collected as PCR control.

Prior PCR analysis, 50 µl FPLC pure water was added to round filter paper pieces of 6 mm in diameter containing feces and hemolymph and boiled in eppendorf microtubes for 10 min. After cooling at room temperature and a short spin at 14,000 x g, 2 µl of the supernatant was directly applied to the PCR reaction. PCR for specific *T. rangeli* detection was performed according to Grisard et al. (1999a) using primers TrINT-1/2 and TrINT-3/2 that are directed to the mini-exon gene. For specific *T. cruzi* detection, PCR was performed according to Diaz et al. (1992) using primers Diaz-7/ Diaz-8 directed to a repetitive nuclear DNA sequence. Amplification products were resolved in polyacrylamide gel electrophoresis and revealed after silver staining (Santos et al. 1993).

After 15 and 21 days of infection, microscopic observation of feces and hemolymph of *T. rangeli* infected triatomines revealed the presence of parasites, respectively. For *T. cruzi* infected triatomines, parasites were detected in feces by microscopy after 15 days of the infective meal. Our results showed a complete agreement between the microscopic observation of *T. cruzi* in feces and *T. rangeli* in feces and hemolymph and the PCR detection up to 60 days after infection (Fig. 1a).

PCR detection of *T. rangeli* DNA in hemolymph and feces of infected triatomines was possible with both primer pairs up to 110 days after infection. The presence of the amplification products obtained with primers TrINT-1/2 and TrINT-3/2 was observed even when no parasites were detected by microscopy. Since TrINT-1/2 and TrINT-3/2 primers revealed the same results, the amplification products obtained with primers TrINT-3/2 are shown in Fig. 1a. The origin of the fragment of approximately 194 bp is under study, but it is still unknown. It is a *T. rangeli* strain dependent phenomenon and appears to indicate that different strains may have distinct arrangement types of the mini-exon gene (Grisard, pers. commun.).

As observed for *T. rangeli*, PCR reaction revealed the 195 bp amplification product (Fig. 1b) in all *T. cruzi* samples from 15 up to 110 days after the infective meal. As already described, we observed no PCR cross-reaction when using the specific primers and DNA from *T. cruzi* and *T. rangeli* (Diaz et al. 1992, Grisard et al. 1999a). Also, no reaction was observed when using both specific primers and DNA from non-infected triatomine feces and hemolymph (Figs 1a, 1b). Positive feces of both *T. rangeli* and *T. cruzi*-infected triatomines were mixed and used as DNA source for PCR, revealing no cross-reaction (data not shown). Our results are in agreement with Diaz et al. (1992) and Grisard et al. (1999a) descriptions, where no cross-reaction between *T. cruzi* and *T. rangeli* DNA was observed.

Our results confirm the feasibility of the PCR technique to detect single or mixed infections by *T. rangeli* and *T. cruzi* in triatomine vectors using a simple technique to collect samples and extract DNA for PCR. Formerly, the triatomine feces were collected in filter paper for the precipitin assays in order to identify their blood source. Using the same approach, triatomine feces and hemolymph collected in filter paper allowed us to specifically detect DNA of the two trypanosomes species infecting humans in Central and South America. This method proved to be easy, fast, effective, sensitive, low cost and reproducible, being of great importance for field works with triatomines, specially in areas where *T. rangeli* and *T. cruzi* coexist. Moreover, this method allows further strain typing by different molecular methods such as ramdomly amplified polymorphic DNA (Steindel et al. 1994), LSSP-PCR (Pena et al. 1994) or PCR amplification of specific genes such as the mini-exon (Grisard et al. 1999a).

In natural conditions, *D. maximus* is not a *T. rangeli* vector. This triatomine species has the ability to abort hemolymph infections by killing the parasite. Thus, our positive PCR results at 110 days after the infective meal did not mean active infec-

tion, but the presence of the parasite DNA. Other studies will be carried out with natural *T. rangeli* vectors which can longer sustain the parasite in the hemolymph. Due to the *T. rangeli* importance in the epidemiology of Chagas disease, the methodology proposed here may be of the outmost importance to study the *T. rangeli* distribution, allowing a simple sample collection and DNA isolation that can be used to detect single or mixed natural triatomine infections.

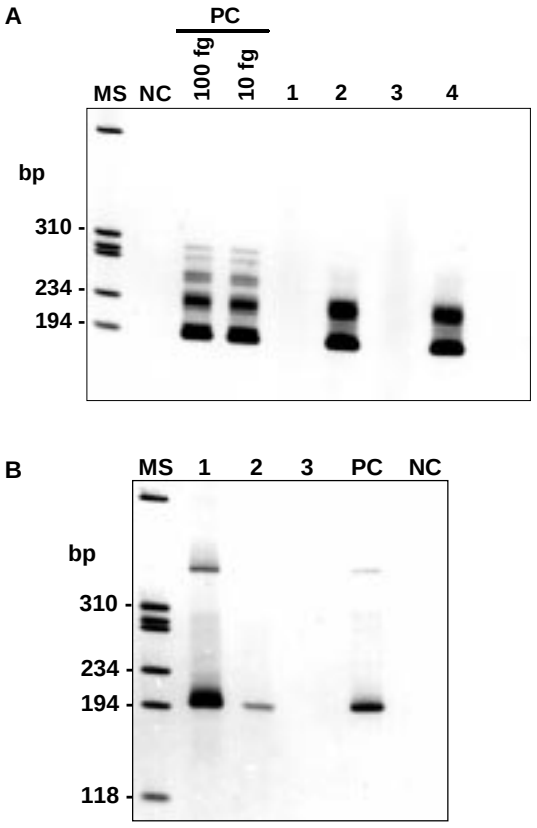

Fig. 1 - A: polymerase chain reaction (PCR) amplification of feces and hemolymph of *Dipetalogaster maximus*, experimentally infected with *Trypanosoma rangeli* SC-58 strain using primers TrINT-3/2. Samples were taken 60 days after the infective meal. PC: positive controls: 100 and 10 fg of total DNA from *T. rangeli* Choachi strain. Lanes 1 and 3 are feces and hemolymph of the non-infected triatomine group. Based on light microscopy, lanes 2 and 4 are positive feces and hemolymph, respectively; B: PCR amplification of feces and hemolymph of *D. maximus*, experimentally infected with *T. cruzi* CL strain using primers Diaz-7/8 at different days after the infective meal. 1: feces collected at 15 days; 2: at 110 days after infection; 3: feces from non-infected triatomines; PC: positive control: 100 fg of *T. cruzi* total DNA. MS: molecular size marker (ϕx 174 *Hae*III digested); NC: negative control (no DNA added); gels are 6% polyacrylamide gel, silver stained.

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