Vol. 88 (1): 163-165, jan./mar. 1993
ISSN = 0074-0276

Research Note

COPY NUMBER DIFFERENCES IN THE 195 BP REPEATED SATELLITE DNA FROM *TRYPANOSOMA CRUZI* AND *TRYPANOSOMA RANGELI:* POTENTIAL USE FOR EPIDEMIOLOGIC SURVEYS

S. F. BRENIERE; M. F. BOSSENO; C. BARNABE; S. URDANETA-MORALES* & M. TIBAYRENC

UMR CNRS ORSTOM 9926 "Génétique Moléculaire des Parasites et des Vecteurs", ORSTOM, 911, Av. Agropolis B. P. 5045, 34032 Montpellier cédex 1, France *Universidad Central de Venezuela, Centro de Biologia Celular, Apdo. 47058, Caracas 1041A, Venezuela

Trypanosoma cruzi, the agent of Chagas' disease, and the apparently non-pathogenic species *Trypanosoma rangeli* can be both encountered in human and in the same mammiferous hosts. On fresh isolates, the discrimination between both species can be sometimes difficult. Hence the development of reliable molecular markers able to distinguish between the two species is sorely needed (F. Guhl et al., 1987, *Parasitol., 94:* 475-484). Toward reaching this goal, we report here further characterization of the 195 bp DNA repeat previously described in *T. cruzi* (P. Sloof et al., 1983, *J. Mol. Biol., 167:* 1-21). A closely related satellite DNA sequence was evidenced in *T. rangeli,* but apparently with a much lower number of repeats, hence providing a potential epidemiological tool for discrimination between the two taxa.

Fifteen different *T. cruzi* stocks as well 7 *T. rangeli* stocks were studied. These stocks represent for each taxon a high genetic variability, as verified by isoenzyme analysis at 11 polymorphic loci, according to the methods described by M. Tibayrenc & F. J. Ayala, (1988, *Evolution, 42:* 277-292). Oligonuclotide primers TCZ1 and TCZ2 previously described by D. R. Moser et al. (1989, *J. Clin. Microbiol., 27:* 1477-1482), were used for DNA amplification of the 195 bp DNA units from purified total DNA (F. Veas et al., 1991, *Cell. Mol. Biol., 37:* 73-84), according to the PCR amplification procedure described by S. F. Brenière et al. (1992, *Am. J. Trop. Med. Hyg., 46:* 335-341).

As expected, all *T. cruzi* stocks gave a major amplified band of 195 bp on ethidium bromide-stained 2% agarose gels. For the 7 *T. rangeli* stocks studied, PCR products exhibiting a comparable molecular weight were obtained (Fig. 1). These 195 bp length PCR fragments were not observed with other Kinetoplastida species used as control (*Leishmania, T. brucei*), a result already noted by D. R. Moser et al. (1989, *loc. cit.*). The minimal amount of template DNA required to obtain a positive PCR amplification was 10 fg for one *T. cruzi* stock and 10 pg for one *T. rangeli* stock.

The sequence homology of *T. cruzi* and *T. rangeli* was evaluated by cross-hybridization of *T. cruzi* and *T. rangeli* probes with sequential diluted PCR products, electrophoresed and transfered onto nylon membranes (S. F. Brenière et al., 1992, *loc. cit*). Both probes were prepared as follows: the PCR fragments of one *T. cruzi* stock and one *T. rangeli* stock were purified by electrophoresis on 0.8% preparative low melting ultra pure agarose gels (Bethesda Research Laboratories, Uxbridge UK). The fragments were eluted from the agarose using Glass beads (Gene Clean kit) according to instructions of the manufacturer (Bio 101, La Jolla, CA USA). Non radioactive labeling of purified products was performed by using ECL gene detection system based on enhanced chemiluminescence (Amersham, Buckinghamshire, UK). Briefly membranes were incubated at 42 °C in hybridization buffer (0.1 ml/cm^2) for 15 mn and during the same time, each purified probe was labeled for 10 mn at 37 °C. The hybridization was performed at 42 °C overnight in a rotative oven. A similar sensitivity was obtained with either homologous or heterologous hybridizations (Fig. 2a, b, c). This result favours the hypothesis of strong sequence homology between 195 bp PCR products from *T. cruzi* and *T. rangeli.*

This work was supported by a grant from TDR Chagas' disease n° 880190.

Received 13 August 1992.
Accepted 22 October 1992.

O.R.S.T.O.M. Fonds Documentaire
N° : 39.862 ex 1
Cote : B
05 SEP. 1994

PN 297

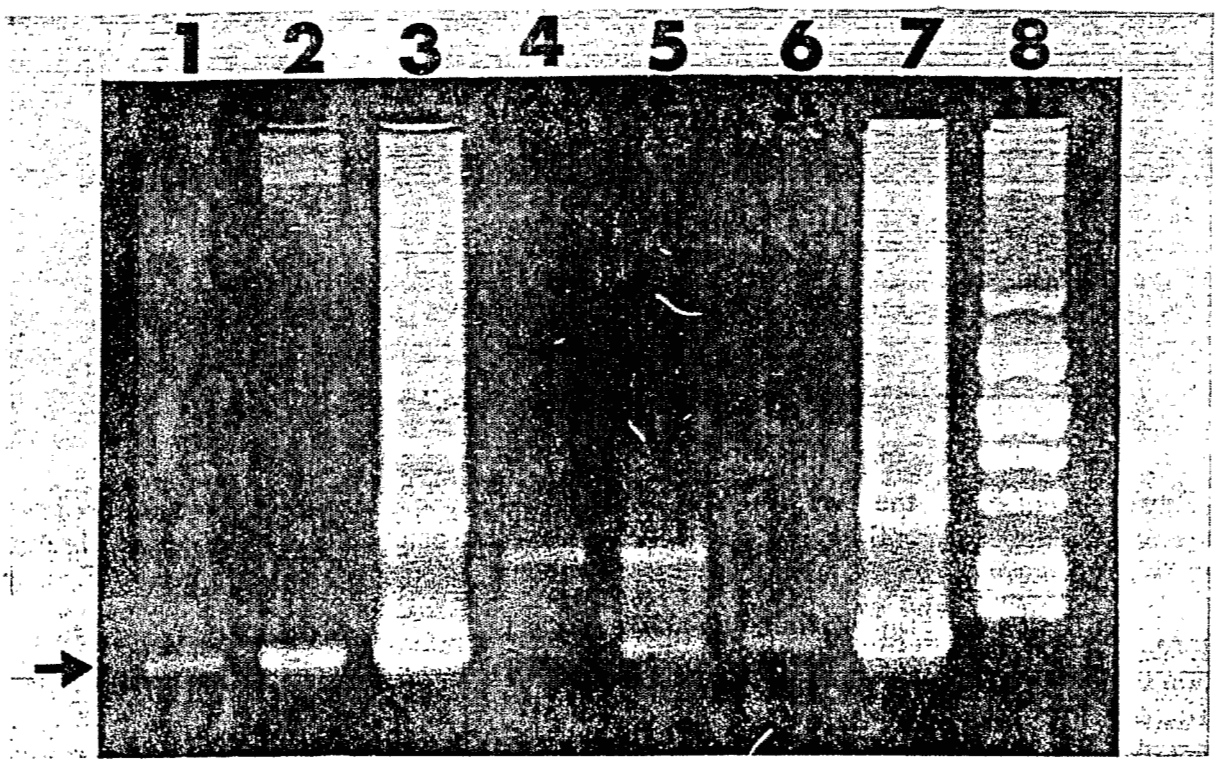

Fig. 1: electrophoresed and ethidium bromide stained 2% agarose gel exhibiting PCR products from: lanes 1, 2, 4 and 5, 1 µg of total DNA of reference *Trypanosoma rangeli* stocks (R2008, Basel, Riera and R2010 respectively); lanes 3 and 7, 1 µg of total DNA of *T. cruzi* stock SC43 cl 1; lane 6, 100 pg of total DNA of *T. cruzi* stock OPS21; lane 8, molecular weight marker PBR 322- *Alu* I. The arrow indicate the major amplified band of 195 bp.

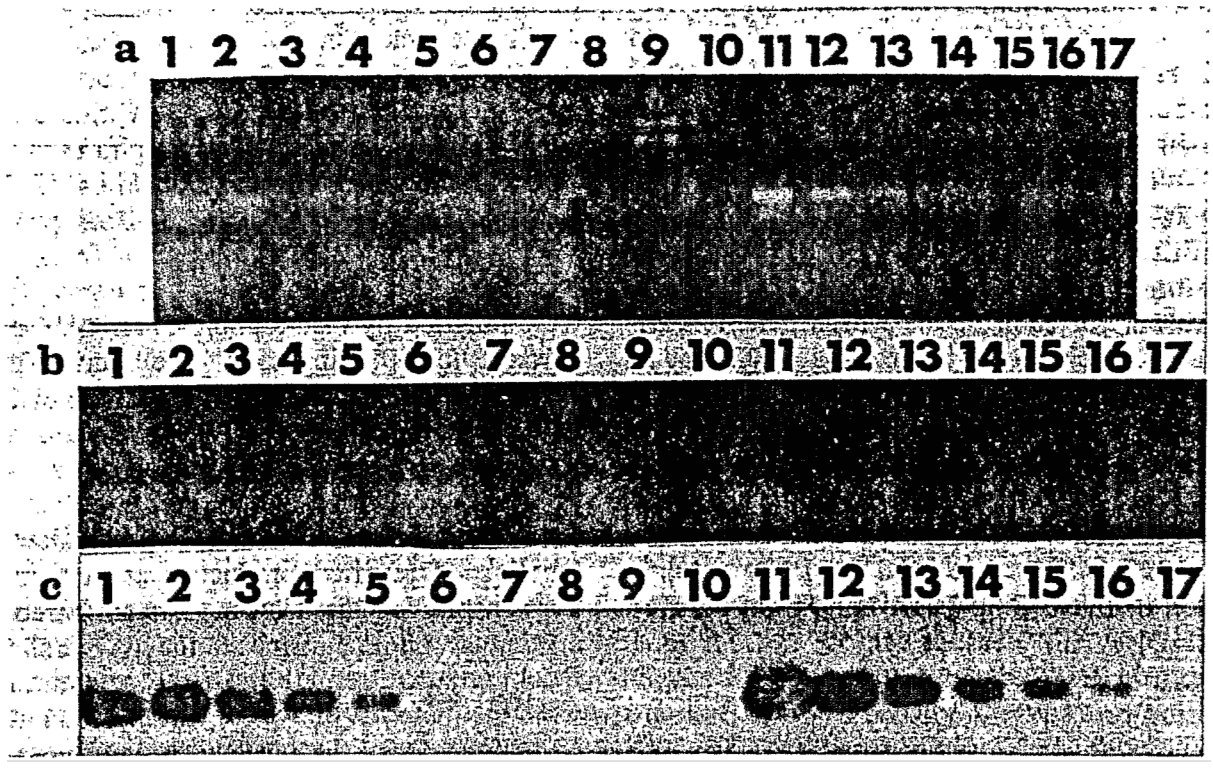

Fig. 2: a – Electrophoresed and ethidium bromide stained 0.8% agarose gel exhibiting – lane 1 to 8: serial dilutions (1/1, 1/2 to 1/128) of the PCR products from *Trypanosoma rangeli* Basel stock total DNA (1 mg); lanes 11 to 17 and 10: serial dilutions (1/1, 1/2 to 1/128) of the PCR products from *T. cruzi* NR stock total DNA (100 fg) respectively; lane 9: molecular weight marker PBR 322- *Alu* I. b and c – hybridizations of the blotted gel with NR *T. cruzi* and Basel *T. rangeli* non radioactive probes (5 ng/ml and 20 ng/ml respectively), high washing stringency was monitored twice in 6 M urea, 0.5 x SSC buffer for 20 mn at 42 °C and then twice in 2 x SSC buffer for 10 mn. The expositions were performed 10 mn on autoradiography films (Hyperfilm TM-MP Amersham, Buckinghamshire, UK).

We have dot-blotted serially-diluted total DNA extracts from the total range of 15 *T. cruzi* stocks and 7 *T. rangeli* stocks as well as one *T. cruzi marinkellei* stock, 2 *Leishmania* stocks and 3 *T. brucei* stocks, according to F. Veas et al. (1990, *loc. cit.*). All *T. cruzi* stocks gave a positive hybridization at a minimal concentration ranging from 0.01 to 10 ng, either with *T. cruzi* or *T. rangeli* probes (see above). Some *T. rangeli* stocks gave a positive hybridization but with a minimal concentration of 500 ng total DNA, while others remained negative, even with a concentration as high as 1 µg total DNA. With 500 ng total DNA, non hybridization was observed for the others species used as controls.

These results confirm the presence in *T. cruzi* of a satellite DNA composed of 195 bp repeat, and show that this DNA is constantly observed over a range of stocks of this parasite that are genetically highly diversified. Moreover, the presence of repeats that are either identical or similar was shown also on a set of *T. rangeli* stocks whose genetic heterogeneity is also very high. Nevertheless, the number os repeats appeared as far lower in the case of *T. rangeli* than for *T. cruzi;* we propose the hypothesis that the *T. rangeli* sequence is an ancestral one that has been duplicated and amplified in the species *T. cruzi.* Whatever be the explanation of it, this result (far higher number of repeats in *T. cruzi* than in *T. rangeli*) seems to be valid for any *T. cruzi* or *T. rangeli* stock, since it has been verified for two set of stocks belonging to the two taxa, that are genetically extremely diversified: this potentially provides a convenient tool to discriminate between the two species in the course of epidemiological surveys, by simple dot-blot hybridizations. Discrimination of both taxa by restriction endonuclease patterns of kDNA (A. C. C. Frasch, 1981, *Mol. Biochem. Parasitol., 4:* 163-170; A. M. Gonçalves et al., 1991, *Mem. Inst. Oswaldo Cruz, 86:* 477-478) and PCR amplification of mini-exon genes (V. K. Murthy, 1992, *Mol. Cell. Probes, 6:* 237-243) have been proposed. These authors do not contemplate the intraspecific genetic variability of both taxa which is particulary high (R. Kreutzer & O. E. Sousa, 1981, *Am. J. Trop. Med. Hyg., 30:* 308-317; M. Tibayrenc & F. J. Ayala, 1988, *loc. cit.*), nevertheless, the markers used in these experiments should be valid for discrimination of the taxa. Studies, in the light of population genetics, of the different proposed markers with extensive samples of stocks pertaining to both taxa, are highly recommended to investigate their possible correlation.

In our dot-blot experiments, the only *T. cruzi marinkellei* stock, which is considered a subspecies of *T. cruzi,* and hence, closely related to *T. cruzi sensu stricto* showed no hybridization signal. The close genetic relationship between *T. cruzi* and *T. cruzi mareikellei* is questioned by this unexpected result. This opens the more general and difficult problem of a reliable identification of the so-called *T. cruzi*-like organisms, whose epidemiological relevance and actual taxonomic relationship with *T. cruzi* remain unclear. Work is in hand to explore this problem by complete population genetics analysis.

Acknowledgement: to Prof. Dominique Le Ray (Tropical Medicine Institute in Antwerp, Belgium), who kindly provided three of the *T. rangeli stocks*).