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IDENTIFICATION OF *Trypanosoma cruzi* BY DNA AMPLIFICATION USING 18S PRIMER IN POLYMERASE CHAIN REACTION (PCR)

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Abstract

American trypanosomiasis/Chagas disease is an infection caused by the protozoan *Trypanosoma cruzi*. Parasitological methods are effective for identifying the parasite; however, in the chronic phase, they show low sensitivity due to subpatent parasitemia in the patients' peripheral blood. To overcome this limitation, Polymerase Chain Reaction (PCR) has emerged as an effective alternative for improving parasite detection and diagnosis. This study aimed to identify *T. cruzi* from different DTUs through DNA amplification using newly designed PCR primers targeting the 18S gene. The primers were designed based on the DNA sequences of *T. cruzi* reference strains, clones, and isolates deposited in the NCBI GenBank. The primers (5' – CAAGCGGCTGGGTGGTTATT – 3' and 3' – CACGGATTTCCCACAAAGGC – 5') were designed using Primer-Blast and tested both *in silico* and *in vitro*, yielding a 104-bp fragment. The standardized PCR conditions were: one cycle of initial denaturation (94ºC/4'), 35 cycles of denaturation (94ºC/30"), annealing (60ºC/1'), and extension (72ºC/1'). Next, the analytical sensitivity was evaluated across different DTUs, DNA concentrations, and parasite loads in the blood. Additionally, the primers were tested on blood samples from different host species. The primers detected the parasite at concentrations ranging from 0.1 fg to 1000 fg in TcI (Col 1.7), 1 fg to 1000 fg in TcII (Y), and 10 fg to 1000 fg in TcIII (CBS56). In blood, the primers detected *T. cruzi* DNA at concentrations of 1000 p/mL in TcI and TcII, and from 10 p/mL to 1000 p/mL in TcIII. Thus, the primers demonstrated high sensitivity in detecting low concentrations of *T. cruzi* from DTUs I, II, and III. Furthermore, parasite DNA was identified in all culture samples of triatomine isolates. However, *T. cruzi* identification was less efficient in human blood samples and in samples from domesticated animals such as *Canis familiaris, Ovis aries,* and *Capra hircus*.

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