Detection and quantification of *Trypanosoma cruzi* in dogs (*Canis lupus familiaris*) by Real-time PCR

Oliveira, G. A¹; Paiva Cavalcanti, M.²; Lorena, V. M. B.²; Brandão-Filho, S. P.²

¹Faculty of Veterinary Medicine, Cesmec University Centre, Maceió, Alagoas;
²Department of Immunology, Aggeu Magalhães Research Centre, Fiocruz, Recife, Pernambuco.

Considered the ideal experimental model for Chagas Disease (DC), dogs could play an important epidemiological role as reservoir. As the human host, the dogs have two clinical phases of the disease: acute and chronic. The acute phase is characterized by intense parasitaemia, where trypomastigotes are easily detected by parasitological methods. Already the chronic phase is characterized by scarce circulation parasites. The small amount of the parasite makes both diagnosis and prognosis of disease hard to achieve. Real-time PCR (qPCR) is such an essential tool for the advancement of researches having dogs as the experimental model. Due to the small number of researches concerning molecular biology and DC diagnosis, the present study aimed at developing a new system of primers for detecting and quantifying *Trypanosoma cruzi* in dogs to be used/applied in qPCR. With the aid of software Mega (version 6.0), a multiple alignment of the sequences of *T. cruzi*'s kDNA available in GenBank (http://www.ncbi.nlm.nih.gov) and in literature was carried out and target areas were identified. With Primer Express software (Applied Biosystems, version 2.0) primers for *T. cruzi* detection were outlined. Selected primers were then named MG F1 (5’ATTACGGGCTGTGGGTTATGG 3’) and MG 2R (5’ACAAACCTACATTATCACTACCC 3’) with GC percentage of 52.38 and 40, Tm 60.13 and 58.59, self complementarity 3.00 and 3.00 and self 3’ complementarity 0.00 and 0.00 respectively. The second pair of primers was named MPG 1F (5’GGGTTCGATTGGGGTTGGT 3’) and the MPG 2R (5’TCACTACCCATCTATAACATCA 3’) with GC percentage 57.89 and 57.19, Tm 59.92 and 57.19 self complementarity 4.00 and 3.00 and self 3’ complementarity 0.00 and 1.00 respectively; systems will be evaluated as to sensitivity, specificity and efficiency. After evaluation and validation, the diagnostic systems will be implemented in the routine of the Reference Service of Chagas Disease at Fiocruz-PE.

**Key Words**: Chagas disease; primers; kDNA.

**Acknowledgements**: Cesmec University Centre and CPqAM-FIOCRUZ