

30. Evaluation of two new targets for MLST in *Leishmania*

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Leishmaniasis is a protozoal disease caused by parasites of the genus *Leishmania*. The clinical manifestation goes from the less severe forms (cutaneous leishmaniasis) to the visceral form which is severe and can be deadly if remained without treatment. Several different species of *Leishmania* have been described throughout South America. In Argentina two species, *Leishmania (V.) braziliensis* and *L. (L.) infantum*, have recently emerged. Because of the high variability and diversity in this genus, it is necessary to have an accurate method for typing. Multilocus Enzyme Electrophoresis (MLEE) is the reference method suggested by the WHO for identification and typing of *Leishmania*. However, this technique is laborious and time-consuming, and also different allozyme may have coincidental mobility. DNA analysis methods, therefore, have been proposed without the disadvantages mentioned. These methods stand out Multilocus Sequence Typing (MLST), which originally was proposed for bacteria typing but recently have been used for *Leishmania* and other diploid organisms. The first MLST scheme proposed is difficult to accomplish, because it is necessary to develop a new strategy with new targets much shorter and capable to identify the different species of *Leishmania*. In this work we evaluated two new targets, Aspartate Aminotransferase (ASAT) and 6 Phosphogluconate Dehydrogenase (6PGD). Two primers were designed for each gene, which amplified a sequence of 877bp for ASAT and 700 bp for 6PGD. Both genes were tested over 16 samples (8 reference strains and 8 strains from Argentina).

Conclusion: Both genes PGD and ASAT showed considerable polymorphism and a high power of discrimination, which made them excellent candidates for *Leishmania* isolates typing.