

Implementation of protocols for detection of *Trypanosoma vivax* in bovine animals by molecular diagnosis

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Trypanosomiasis disease, caused by *T. vivax*, is considered an endemic illness in the Pantanal region, but sporadic outbreaks have been reported in cattle herds in some Brazilian States, such as Paraíba and Pernambuco. The aim of this work was to elect an efficient method of DNA extraction considering two protocols proposed in the literature and then use the PCR technique to identify, from a molecular point of view, the presence of this hemoparasite in samples of bovine blood collected in different farms in the southern region of the State of Bahia in relation to which there was clinical suspicion of the disease. At first, we used 10 random samples for total DNA extraction considering the two proposed methods. These random samples were divided into two groups of five each. Protocol 1 was used in one of the groups (Lahiri and Nurnberg, 1991, adapted) and Protocol 2 (Madruga *et al*, 2006) in the other. After that, all DNA samples extracted (520) were quantified in spectrophotometer and subject to electrophoresis in 1.0% agarose gel stained with ethidium bromide for visualization in UV. The amplification reactions were performed in the samples for which the method applied presented a better standard of bands, namely, Protocol 2, using the primers described in literature, 18STnF2 (5' CAACGATGACACCCATGAATTGGGGA 3') and 18STnR3 (5' TGC GCGACCAATAATTGCAATAC 3'), whose amplification positive product features 710pb. Among the 520 samples analyzed from four farms in the region, 22,69% presented amplicon with molecular weight compatible with *T. vivax*. These results enabled us to elect a practical DNA extraction method for bovine blood and to perform the high sensitivity molecular diagnosis for detection of the above mentioned hemoparasite in different concentrations of this circulating agent in the blood, what could provide the health authorities of the state of Bahia with a parameter for implementation of prophylaxis measures and control of the disease in this region.

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