TITLE: APPLICATION OF THE ZINC SULPHATE FLOTATION METHOD TO IMPROVE THE SENSITIVITY OF PCR DETECTION OF TRYPANOSOMA CRUZI IN AÇAÍ (EUTERPE OLERACEA) PULP

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ABSTRACT

Chagas disease (CD) is a deadly, neglected tropical disease, for which the World Health Organization estimates roughly 6 million infected people worldwide. With the control of its vectorial transmission in Brazil, alternative forms of transmission have gained more epidemiological importance. In recent years, the main form of infection in humans has become the oral transmission. This form occurs through the ingestion of food contaminated with kissing bug wastes infected with the parasite *Trypanosoma cruzi*. According to the Brazilian Consensus on Chagas disease, the confirmation of oral transmission is done by excluding other forms of transmission and with epidemiological evidence of a contaminated food source as the origin of transmission. The artisanal açaí pulp is appointed as the main cause of CD outbreaks in northern Brazil, mainly in the states of Amapá, Amazonas and Pará. Even though the oral transmission of CD is the most prevalent transmission form, there are no laboratorial procedures for the detection of contaminated food sources. Thus, the objetive of this study was to develop a method of concentrating and detecting *T.cruzi* in one of the most common contaminated food sources, açaí pulp. We developed a methodology to detect *T. cruzi* contamination in açaí pulp using zinc sulfate to concentrate the parasites, followed by DNA extraction and PCR with TCZ and rDNA 28Sα genes as targets. This new procedure allowed detecting açaí pulp contaminations of the order of 1000 cells / mL using primers for the 28Sα rDNA gene and 0.1 cells / mL with primers for the TCZ gene.. In the PCR

assays, though TCZ presented a high sensibility, its specificity was inferior to 28Sα rDNA gene. Lastly, it was possible to amplify DNA either from parasites isolated from açaí or from their DNA dispersed in açaí pulp. The zinc sulfate flotation technique associated with PCR proved to be a simple and sensitive procedure to aid in the testing of suspected samples of açaí associated with CD outbreaks.

Keywords: Chagas disease, oral transmission, açaí, *Trypanosoma cruzi*, zinc sulfate

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