Monitoring the natural and artificial infection of triatomines by quantitative real time PCR

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INTRODUCTION: Chagas disease constitutes the fourth most important tropical disease, supplanted only by malaria, tuberculosis, and schistosomiasis. Microscopical examination is a classic method to describe the natural infection of triatomines by *Trypanosoma cruzi*, but it presents some limitations, such as low sensitivity and reproducibility. **OBJECTIVE:** In this context, the application of a quantitative Real Time PCR (qPCR) assay can improve sensitivity, specificity and reproducibility, in order to investigate the development of *T. cruzi* in triatomine vectors. **METHODOLOGY:** In this work, we developed a qPCR TaqMan multiplex assay targeting the *T. cruzi* nuclear satellite DNA and the triatomines 12S rRNA gene, which is capable to detect and quantify absolute levels of *T. cruzi* in triatomine samples. Also, to quantify only viable parasites, a RT-qPCR was developed, targeting the *T. cruzi* GAPDH gene. **RESULTS:** Our multiplex qPCR reaction presented a linearity ranging from 10^5 to 0.5 parasite equivalents, for the *T. cruzi* target, and from 3 to 0.00015 triatomine equivalents, for the insect target. It was also possible to determine that qPCR Limit of Detection (LOD) was 0,41 parasite equivalents. To validate the assay, 13 samples from field triatomines, positive for *T. cruzi* infection, were evaluated by qPCR, with parasite loads from 10^2 to 10^{11} par. Eq./triatomine Eq. Furthermore, to follow *T. cruzi* (Dm28c) development in *Rhodnius prolixus* digestive tract, total DNA and RNA were extracted from insect samples under increasing periods after feeding with blood containing *T. cruzi*. After a peak at day 9, it was possible to observe a time-dependent decreasing on the viable parasite quantity during the time course of the experiment. **CONCLUSION:** The qPCR and RT-qPCR assays developed in this study are suitable to determine the infection rate of sylvatic triatomines and they can contribute to understand the vectorial competence of triatomines and the incidence of Chagas disease.

Key Words: Chagas Disease, *Rhodnius prolixus*, qPCR