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# Detection and quantification of *Trypanosoma vivax* by real-time PCR in experimentally infected cattle

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## Abstract

*Trypanosoma vivax* spreads in herds worldwide mainly by the introduction of animals in chronic phase of the infection, where parasitaemia is low and intermittent, which makes diagnosis challenging. Direct *T. vivax* visualization still the more widely used method for the disease diagnosis, but is inaccurate in low parasitaemia. The aim of the present study was compare Brener technique (BT) and real time PCR (qPCR) for *T. vivax* detection and parasitemia quantification during the course of the disease. Three Girolando cows were experimentally infected with 2.0 x $10^7$ trypomastigotes of *T. vivax* and whole blood in EDTA were collected of each cattle, on days -7, 0, 1 and then every seven days up to 119 days after infection (DAI). BT was performed using 5 µL of whole blood in a microscope slide under a 22 x 22 mm coverslip and, trypomastigotes were counted in 50 microscopic fields at 40X objective. qPCR was conducted using the TaqMan system. Reactions were performed using 5 µL of genomic DNA (except for negative control), 200 nM of each primer, 160 nM of *T. vivax* probe, 120 nM of TLR-8 probe, buffer 2X (6 nM MgCl2, 0.8 mM dNTPs, 1 U Taq DNA polymerase) in a final volume of 25 µL. Amplifications were conducted in duplicate in a CFX96 thermal cycler (initial denaturation at 95 °C/10 min, followed by 45 cycles of 95 °C/30 s, 61 °C/1 min, and ended with 72 °C/1 min). The analytical sensibility was tested with gBlock® Gene fragments targeting sequences for *T. vivax* ITS-1 region and serial dilutions were made to construct patterns with different concentrations of gBlock® targets (2.0 x $10^7$ to 2.0 x $10^0$ copies/µL). The copies number was determined according to the formula (Xg/µL DNA/ [gBlock® size (bp) x 660]) x 6.022 x $10^{23}$ x copies of gBlock®/µL). BT detected 31.48% (17/54) of known positive samples, of which 76.47% (13/17) were detected within 7 to 35 DAI for all three cows and the parasitaemia range was 8,100 up to 16,248,600 parasites/mL. All samples were positive for the endogenous gene (TLR-8), indicating that extractions were efficient, and giving reliability to the results obtained by qPCR, which detected 61.11% (33/54) of known positive samples and parasitaemia range was 19.2 up to

270,000,000 parasites/mL. Positive correlation between the two techniques was found ($P < 0.0001$ and Spearman $r = 0.8757$). This is the first time that qPCR was used for detection and quantification of American *T. vivax* isolates. These results suggest that qPCR can be used as an accurate tool for *T. vivax* detection and quantification, even between acute and chronic phases, where low and intermittent parasitemia lead to false negative results in direct parasitological tests.