

Title: REVERSE-TRANSCRIPTASE PCR DETECTION OF *LEPTOSPIRA* IN RIO DE JANEIRO: POOR CORRELATION WITH SINGLE-SPECIMEN MICROSCOPIC AGGLUTINATION TESTING

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Abstract

Leptospirosis is a potentially fatal zoonotic disease caused by bacteria of the genus *Leptospira*. In the Americas, Brazil reports the majority of cases, though the incidence remains underestimated due to limitations in available diagnostics. The reference standard for the diagnosis of leptospirosis remains microscopic agglutination testing (MAT) on acute and convalescent serum. MAT is performed at the Centro de Referência Nacional para Leptospirose, Rio de Janeiro. However, paired specimens are rarely sent for testing, and positive MAT results from a single specimen (titer $\geq 1:800$) are often used to provide a presumptive diagnosis. The purpose of this study was to test serum samples from patients in Rio de Janeiro with a real-time reverse-transcriptase PCR (rRT-PCR) targeting the *Leptospira* 16S *rrs* gene and compare these results to detection with MAT. Improved analytical sensitivity of *Leptospira* detection was shown using rRT-PCR compared to optimized real-time PCRs with the same primers and probe. We then tested up to 55 archived samples per month from 2008, for a total of 478 samples. Thirty-five (7.3%) samples tested positive by rRT-PCR with no clear seasonality in the percent of cases detected. Clinician-reported day of disease information was available for 282 samples (18 positives). *Leptospira* was detected in samples collected as late as day 30, and cycle thresholds did not vary based on the day of disease of sample collection. The percentage of positive samples did not differ when samples were categorized as acute [≤ 7 days; 8/127 (6.3%)]; late acute [8 to ≤ 14 days; 6/69 (8.7%)]; or convalescent [>14 days; 4/86 (4.7%)]. Thirty-three (6.9%) samples tested positive by MAT using a regional panel of 19 *Leptospira* strains. Only three samples tested positive by both rRT-PCR and MAT, demonstrating poor agreement between these techniques. This finding was consistent regardless of how samples were categorized by day of disease at collection (acute, late acute, convalescent; ≤ 30 days and > 30 days). Based on these data, the use of single-specimen MAT, even with a conservative threshold, cannot be recommended for the diagnosis of leptospirosis.

Key-words: Leptospirosis, diagnosis, real-time reverse-transcriptase PCR

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