

## Background.

Quantitative polymerase chain reaction (qPCR), despite cost and logistical challenges, has the potential to provide accurate and timely diagnosis for leptospirosis at the point-of-care in endemic areas. We studied optimal sample types for qPCR, timing of sampling, and clinical manifestations in relation to quantitative leptospiremia.

## Methods.

A new qPCR assay using pathogenic *Leptospira*-specific 16S ribosomal RNA (rRNA) gene Taqman primers and an optimized temperature stepdown protocol was used to analyze patient blood samples. Serum was compared with whole blood as sample source. Quantitative leptospiremia was compared with clinical manifestations of leptospirosis and outcome.

## Results.

The diagnostic sensitivity of qPCR of whole blood and serum was 18.4% (95% confidence interval [CI]: 9.97%–31.4%) and 51.0% (95% CI: 37.5%–64.4%) respectively. The qPCR on suspected cases confirmed infection in 58 of 381 cases (15.2%). Of these, 6 cases confirmed by nested polymerase chain reaction (PCR) and sequencing were serologically negative using a standard but not regionally optimized microscopic agglutination test panel. The bacterial load in serum/blood ranged from  $10^2$  to  $10^6$  *Leptospira*/mL. Median leptospiral load for uncomplicated, renal failure, myocarditis, and multi-organ failure patients were 8616, 11 007, 36 100, and 15 882 *Leptospira*/mL respectively. The qPCR window of positivity ranged from day 2 to day 15; sensitivity of qPCR was not affected by the length of the interval between the onset of symptoms and sample collection (P 5 .328).

## Conclusions.

Quantitative PCR shows potential as a valid diagnostic test with a wider window of positivity than previously thought. Quantitative leptospiremia in serum/whole blood samples did not directly correlate with clinical manifestations of outcome in this patient population.