

MED/OP/21

Diagnostic Implications of Sustained High MAT Titres for the Interpretations of Leptospirosis at Six Months Post-Infection.

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Background: Microscopic Agglutination Test (MAT) remains the key diagnostic tool during the immune phase of Leptospirosis. However, the long-term data on persistence antibody titres following acute leptospirosis is scarce, which may complicate the interpretation of MAT in endemic regions with previous exposure or past infection.

Objective: This study aimed to investigate the persistence of antibody titres over six months following acute leptospirosis and to assess their impact on the interpretation of MAT results for diagnosis.

Methods: In the Rajarata Leptospirosis Cohort, patients with acute-phase MAT titres $\geq 1:400$ (diagnostic cut-off for symptomatic cases) are followed longitudinally. Of the 46 patients who completed six months of follow-up, paired serum samples (acute and six-month) were successfully collected from 18 individuals. These were analyzed at the Public Health Research Laboratory, RUSL, using an optimized 11-serovar MAT panel under rigorous quality control.

Results: The maximum titres of the 18 acute samples were, 1:3200 (n=5), 1:1600 (n=4), 1:800 (n=8), and 1:400 (n=1). The most commonly reactive serovars in the acute samples were *Leptospira interrogans* serovar Bratislava (n=16), Icterohaemorrhagiae (n=16), Canicola (n=11), Bataviae (n=11) and *L. santarosai* serovar Georgia (n=11). Among these, serovar Bratislava was the predominantly reactive in 13 (72.2%) samples. At six-month follow-up, none had clinical features, but all 18 samples remained reactive with maximum titres of 1:3200 (n=1), 1:1600 (n=1), 1:800 (n=8), 1:400 (n=4) and 1:200 (n=4), with 10 (55.6%) had titres $\geq 1:800$ (cut-off without symptoms). Moreover, serovar Bratislava was the predominantly reactive serovar in 15 (83.3%) follow-up samples, while a change in the predominant serovar was observed in 2 (11.1%) samples.

Conclusion: Persistent high MAT titres at six months post-infection indicate a sustained immune response, potentially misclassification of past infections as acute leptospirosis. In endemic regions, MAT results should be interpreted with clinical suspicion to improve diagnostic accuracy, highlighting the need to redefine MAT cut-off titres for acute infection in high-endemic regions.

Keywords: Immune response, Leptospirosis, MAT