Serogroup cross-reactivity in acute leptospirosis: Evidence from a serological study in Sri Lanka's North Central Province

Aswaddumage DD[1], Sewwandi AHM[1], Menike WGYDK[1], Premalal WHAT[1], Kappagoda CN[1], Agampodi TC[4], Senevirathna I[3], Warnasekara YPJN[1], Jayasundara JMDD[2], Agampodi SB[5]

Introduction and Objectives: Leptospirosis remains underdiagnosed in hyper-endemic Sri Lanka. The Microscopic Agglutination Test (MAT) is the recommended method for confirming infection and predicting the likely infecting serogroup. However, cross-reactivity between serogroups can complicate epidemiological interpretation. This study aimed to evaluate serogroup cross-reactivity in laboratory confirmed acute leptospirosis cases using a locally optimised MAT panel.

Methods: Serum samples submitted for diagnosis of leptospirosis from Teaching Hospital, Anuradhapura and Base Hospitals, Medirigiriya, Thambuththegama, and Kabithigollawa to the Public Health Research Laboratory between May 19th 2022, and July 1st 2025 were tested. An optimised MAT panel comprising 11 *Leptospira* serovars representing nine pathogenic serogroups and one saprophytic serogroup was used. A positive MAT was considered presumptive evidence when a single serum sample showed a titre ≥1:400 in a clinically suspected patient with exposure history. Acute leptospirosis was confirmed by paired samples showing a four-fold rise or seroconversion. Cross-reactivity was defined as titres ≥1:50 against two or more serovars within the MAT panel.

Results: Seventy-one samples showed titres ≥1:400, with 58 exhibiting presumptive positivity in acute-phase samples and 13 confirmed by paired sera. Serovar Icterohaemorrhagiae was the most frequently detected, observed in 54 patients (76.1%), while serovar Bratislava showed the highest titre (≥1:3200) in 18 patients (25.4%), making it the predominant serovar with the highest titres. Among the 71 samples, 18 (25.4%) reacted to a single serovar, whereas 53 (74.6%) exhibited cross-reactivity, with titres ranging from ≥1:50 to ≥1:3200. Cross-reactivity involved Icterohaemorrhagiae in 48 patients (67.6%), followed by Canicola (44, 62.0%), Patoc (40, 56.3%), Bataviae (39, 54.9%), Bratislava (36, 50.7%), Weerasinghe (34, 47.9%), Mankaraso and Georgia (26 each, 36.6%), Ceylonica (25, 35.2%), Pyrogenes (9, 12.7%), and Wolfii (2, 2.8%). Notably, one patient's serum demonstrated cross-reactivity with all reactive serovars.

Conclusions: Frequent cross-reactivity and possible paradoxical serological reactions during the acute phase limit serogroup-specific interpretation and complicate accurate identification of the infecting serovar using MAT alone. While updating serodiagnostic panels to better reflect local strain diversity may improve surveillance, titres remain influenced by host factors and inter-laboratory variability, and definitive identification of infecting serovars still requires isolation from culture.

***Keywords**: Cross-reactivity, MAT, Leptospira, Serogroups*

[1]*Department of Community Medicine, Faculty of Medicine and Allied Sciences, Rajarata University of Sri Lanka*
[2]*Department of Microbiology, Faculty of Medicine and Allied Sciences, Rajarata University of Sri Lanka*
[3]*Department of Biochemistry, Faculty of Medicine and Allied Sciences, Rajarata University of Sri Lanka*
[4]*Epidemiology, Public Health, Impact Unit, International Vaccine Institute, Seoul, 08826, Republic of Korea*
[5]*Innovation, Initiatives & Enterprise Development Department, International Vaccine Institute, Seoul, 08826, Republic of Korea*

Address for correspondence: Mr. Digantha Dinujaya Aswaddumage: Department of Community Medicine, Faculty of Medicine and Allied Sciences, Rajarata University of Sri Lanka; Telephone: +94711862267
Email: diganthadinujaya@gmail.com; *https://orcid.org/0009-0000-5792-6339*