Denipitiya, D.T.H. Chandrasekharan, N.V. Abeyewickreme, W. Hapugoda, M.D. PAPER

Detection of pathogenic Leptospira species in rat blood samples by molecular-based assays¹²

D.T.H. Denipitiya, Molecular Medicine Unit, University of Kelaniya

N.V. Chandrasekharan, Department of Chemistry, University of Colombo

W. Abeyewickreme, Department of Parasitology & Molecular Medicine Unit, University of Kelaniya

M.D.Hapugoda, Molecular Medicine Unit, University of Kelaniya

Background:

Leptospirosis is a worldwide zoonotic infection, caused by pathogenic species of the genus *Leptospira*. It was traditionally known as 'rat fever' in Sri Lanka, because rodents, especially rats, are considered to be the most important reservoirs or maintenance hosts of *Leptospira*. In 2012, the highest numbers of cases were reported in the District of Gampaha. The objective of this study is to detect pathogenic *Leptospira* species in rat blood samples by molecular based assays.

Method:

Rats (n=38) were trapped in a high risk area (Mirigama) in the District of Gampaha, from May 2012 to February 2013 by using live traps. Each rat was anesthetized by using diethyl ether and 2-3 ml sample of blood was collected from each rat. Blood samples collected from all rats were tested by molecular- based assays and a serological assay. Qualitative Polymerase Chain Reaction (PCR), real time PCR and Loop Mediated Isothermal Amplification (LAMP) were used as molecular-based assays which targetted conserved gene regions among pathogenic serovars of *Leptospira* species. Microscopic Agglutination Test (MAT), the Gold Standard assay for detection of anti *Leptospira* antibody was used as a serological assay.

Results and Discussion:

Of the 38 rat blood samples, molecular-based assays confirmed *Leptospira* infection in 5% (2/38), 16% (6/38) and 11% (4/38) by qualitative PCR, real time PCR and LAMP assay respectively. None of the samples was positive by MAT. After first infection, some *Leptospira* species live in the host animal as commensal bacteria. Therefore, host does not stimulate antibody production further and that may be below the detection level of the antibody by MAT.

Conclusions:

Results of molecular based assays showed that *Leptospira* are circulating among the rats tested in this study, although at the time of collection, their antibody levels were too low to detect by MAT, which had the lowest detection limit of 1:800.

¹² **Acknowledgements:** National Science Foundation, Sri Lanka (NSF/RG/2009/BT/01), International Centre for Genetic Engineering and Biotechnology (ICGEB CRP/ SRI 08-02) and International Atomic Energy Agency (IAEA SRI 5/042) are gratefully acknowledged.