

PP-06: Development and validation of an in-house ELISA to detect *Leishmania* parasites in dogs

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Introduction: Leishmaniasis, a vector-borne parasitic disease, caused by *Leishmania donovani* is endemic in Sri Lanka. Inconclusive evidence suggests the possibility of dogs being a reservoir for Leishmania. Identification of animal reservoirs is crucial to control the infection. The objective of this study was to develop and validate a low-cost ELISA method to screen for Leishmania in dogs.

Methods: An experimental study was conducted. Leishmania parasites were cultured, and crude protein lysate was extracted and quantified. The ELISA was optimised using checkerboard titration and was validated using a commercial kit. Accuracy, precision, linearity, specificity and sensitivity of the in-house ELISA were determined. The crosstab analysis was performed to determine the sensitivity and specificity of the in-house method. Blood samples were collected from 71 dogs: 60 from Kilinochchi and 11 from Mirigama. The collected serum samples were analysed using the optimised in-house ELISA.

Results: An antigen concentration of 5 µg/ml and 1/400 primary antibody dilution were chosen as the best combination for the ELISA. The cut-off absorbance value to detect positive cases was identified as 0.212. The sensitivity and specificity of the in-house method were 92.9% and 90.9%, respectively. The accuracy, intra-assay precision and inter-assay precision were 91.6%, 2.6%, and 3.5%, respectively. The dilutional linearity was up to 0.9904 of absorbance. Out of 71 dogs, 14 were positive for leishmaniasis according to the developed method and confirmed by the commercial kit.

Conclusion: The developed assay is a valid low-cost method to determine the presence of Leishmania in dogs. The non-specificity of the antigen used in crude lysate is a limitation. The results add evidence to suggest that dogs are potential reservoirs for Leishmania in Sri Lanka. However, it cannot be generalised due to the small sample size obtained from the limited number of hotspots. Further molecular and epidemiological studies are needed to confirm our findings.

Keywords: leishmaniasis, dogs, ELISA, development, validation