

## DETECTION OF BOVINE THEILERIOSIS IN JAFFNA, SRI LANKA, USING POOLED MOLECULAR ANALYSIS

**M.M.M.M.S. Kumari<sup>1</sup>, T.H.P.S. Amarasingha<sup>1</sup>, P. Gajavathany<sup>2</sup>, M. Rajeshwaran<sup>3</sup>, R.S. Rajakaruna<sup>1</sup> and P.K. Perera<sup>1\*</sup>**

<sup>1</sup>Department of Zoology, University of Peradeniya, Peradeniya, Sri Lanka.

<sup>2</sup>Department of Zoology, University of Jaffna, Jaffna, Sri Lanka.

<sup>3</sup>Veterinary Surgeon Office, Tellipalai, Jaffna, Sri Lanka.

\*piyumali.perera@sci.pdn.ac.lk

Bovine theileriosis is a vector-borne parasitic disease that causes significant financial burden due to its detrimental effects on cattle health, particularly in Jaffna, Sri Lanka, where livestock remains an essential source of food and income. DNA pooling has not been utilised as a diagnostic strategy for *Theileria* detection in Sri Lanka with limited research available. This study aimed to utilise DNA pooling as a rapid, cost-effective technique to assess *Theileria* infection statuses of cattle herds in selected Jaffna farms. Blood samples ( $n = 60$ ) and tick samples ( $n = 27$ ) were collected from three cattle herds in Jaffna. Giemsa-stained blood smears were prepared from all samples for morphological analysis. Blood samples were pooled into three groups based on farm location, and molecular analysis targeting the MPSP gene was conducted on pooled and individual samples. A Chi square test was used to determine associations between prevalence and age, gender and farm locations. Microscopic analysis revealed an overall prevalence of 50.0% (30/60) for *Theileria* spp. whereas molecular analysis revealed a statistically higher prevalence (81.7%) (49/60,  $\chi^2 = 13.374$ ,  $p = 0.00025$ ). The presence of *T. orientalis* (genotype 5, 7) and *T. annulata* were confirmed by bands at 776 bp and 785 bp, respectively. No significant association was found between prevalence and age ( $\chi^2 = 2.456$ ;  $p = 0.117$ ), gender ( $\chi^2 = 0.0223$ ;  $p = 0.881$ ) and farm locations ( $\chi^2 = 1.684$ ;  $p = 0.431$ ). Two tick species were identified: *Haemaphysalis bispinosa* (24/27: 88.9%) and *Rhipicephalus linnei* (3/27: 11.1%). Pooled molecular analysis detected *Theileria* spp. in all three farms with a 12-fold cost reduction compared to individual PCR, with no false positives in negative pools. These findings suggest that molecular analysis of pooled blood samples be used to cost-effectively detect *Theileria* infections in cattle herds, enabling timely treatment and improved disease control.

Financial assistance from University of Peradeniya (Grant No. URG/2022/64/S) and Postgraduate Institute of Science (Grant No. PGIS/2022/01) are acknowledged.

**Keywords:** Cattle, Jaffna, *Theileria*, Tick-borne diseases