

Molecular Epidemiological Investigation of Theileriosis in Dairy Cattle in Kurunegala District, Sri Lanka

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Bovine theileriosis has a significant economic implication for dairy production globally. Investigating the prevalence of bovine theileriosis, the species and genotypes involved in dairy cattle, is crucial, particularly in the Kurunegala District of the Intermediate Zone, which houses the third largest cattle population in Sri Lanka. Sixty blood samples and questionnaire data were collected from three dairy farms in Galpokuna (n=20), Koulwewa (n=20), and Andigama (n=20) areas. Packed Cell Volume (PCV) analysis identified anaemic conditions, while Giemsa-stained blood smears facilitated microscopic analysis. A Fisher's Exact Test ($p < 0.05$) compared the prevalence of infection among age groups (adults and calves) and farms. For PCR, microscopically positive (n=9) and negative (n=9) samples were selected representing each farm. PCRs were conducted for *Theileria orientalis* and *Theileria annulata* using species-specific primers which are designed to amplify regions of *MPSP* and *18s rRNA* genes were conducted followed by sequencing, which allowed phylogeny of the species detected. Three farms practised semi-intensive farming with animals in a 'closed' setup, limiting movement. The overall theileriosis prevalence in dairy cattle was 55% (Koulwewa: 65%, Galpokuna: 50%, Andigama: 50%) based on microscopy in the Kurunegala District, with 33% (20/60) of cattle being anaemic based on haematocrit results. Anaemia was significantly higher in *Theileria*-tested-positive calves (45%) than in adults (25%; $p = 0.030$). PCR analysis revealed the presence of both *T. orientalis* (55%; 10/18) and *T. annulata* (22%; 4/18). The sequencing and phylogenetic data revealed that the *T. orientalis* genotype present in the Kurunegala District was *type 7*. This is the first report on *T. orientalis* and *T. annulata* in dairy cattle in the Intermediate zone of Sri Lanka. Among them, *T. orientalis* was more common compared to *T. annulata*. Comprehensive studies on *Theileria* pathogenicity in cattle are mandatory for a deeper understanding of potential health risks to livestock production.

Keywords: *Theileria orientalis*, *Theileria annulata*, Genotype 7, Prevalence, Anaemia
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