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Prevalence and Epidemiological Insights of *Theileria orientalis* in Cattle from Karachchi and Kandawala Veterinary Range in Kilinochchi District in Sri Lanka: An Integrated Analysis using Microscopy and PCR

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Haemoparasitic infections represent major constraints to livestock productivity and welfare in tropical regions, causing anemia, immunosuppression, and reduced performance in affected animals. These vector-borne pathogens, primarily transmitted by ticks, include species of *Theileria*, *Babesia*, and *Anaplasma*, which collectively threaten bovine well-being and rural livelihoods. In Sri Lanka, such infections are widespread, particularly in the dry zones, where high tick abundance facilitates pathogen transmission. The Kilinochchi district in the northern province, characterized by its tropical dry climate and predominantly extensive and semi-intensive cattle management systems, provides a highly favorable environment for the spread of tick-borne haemoprotozoa such as *Theileria orientalis*. The present study, conducted from May to October 2025, aimed to determine the prevalence of *T. orientalis* in cattle from two veterinary ranges, Karachchi and Kandawalai, within the Kilinochchi district. We randomly collected 160 samples from 50 farms across both veterinary ranges. The farms were selected based on previous reports of infection and their management practices to ensure representative sampling. Of the 160 samples, indigenous cattle included 35 from Karachchi and 39 from Kandawala, while crossbred cattle included 45 from Karachchi and 41 from Kandawala. This distribution allows a valid comparison of prevalence between breeds and locations that were examined microscopically, with *Theileria* piroplasms observed in 49 samples, corresponding to an overall microscopy prevalence of 30.6%. These 49 smear-positive samples were subsequently subjected to species-specific PCR targeting the major piroplasm surface protein (MPSP) gene of *T. orientalis* using primers TO-MPSP-F (5'-CTTTGCCTAGGATACTTCT-3') and TO-MPSP-R (5'-ACGGCCAAGTGGTGAGAACT-3') resulting in the confirmation of 19 PCR-positive samples. PCR confirmed *T. orientalis* infection in 40% of samples from Karachchi and 37.5% from Kandawalai. The chi-square results show that there was no significant difference in infection rates between the two veterinary ranges or between the different breeds. The very low χ^2 value for ranges (0.06) means that infections were almost the same in both locations. For breeds, the χ^2 value (≈ 2.14) was also not high enough to show a real difference. This means that under the current farm conditions, neither the location nor the breed had a clear effect on the occurrence of infection. This study confirms the molecular presence of *T. orientalis* in Kilinochchi, underscoring its subclinical circulation and the need for integrated diagnostics, effective tick control, and improved herd management strategies.

Keywords: Blood smear; cattle; haemoparasite; PCR; *Theileria orientalis*