DOI: 10.22055/ivj.2025.551126.2854

Molecular Detection of Tick-Borne Hemoparasites (Theileria, Babesia and Anaplasma) in Stray Dogs using Nested PCR

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Received: 04.10.2025 Accepted: 07.12.2025

Abstract

Haemoparasitic infections are frequently observed in dogs from tropical regions, including Iraq. Numerous dogs become infected with several blood parasites, resulting in more serious diseases than a singular infection. This investigation was designed in Wasit Province, Iraq to conduct a comprehensive molecular detection and characterization of haemoparasites in Infested Dogs. This cross-sectional study was performed from the beginning of May 2024 to the end of December of 2024. Totally 280 stray dogs were examined in different areas in waist, Iraq. The blood sample were obtained from the jugular vein of infested dogs was used for both microscopic and molecular analysis. Thin blood smears were prepared, detected by giemsa staining and screened for piroplasms Babesia, Theileria and Anaplasma. Total DNA was extracted followed by nested PCR using primer targeting16S *rRNA* gene to detect for Anaplasma spp. and 18SrRNA for Babesia spp and Theilleria spp. PCR products were confirmed by agarose gel electrophoresis. Nucleotide sequencing verified the authenticity of the amplified genes, whose sequences were compared with reference sequences of the *16S rRNA* and *18S rRNA* genes, and the isolate sequences from this work were posted in GenBank. A microscopic analysis of thin Diff-quick-stained blood smears identified big intra-erythrocytic *Babesia sp., Theileria sp*., and *Anaplasma sp*. in thirty four dogs. The PCR investigation revealed *Anaplasma sp*. in 77 dogs (27.5%), *Babesia sp*. in 55 dogs (19.6%), and *Theileria sp*. in 63 dogs (22.5%). The identification and similarity scores between the isolates of this investigation and the reference strains were 100% identical. The findings of this study indicate that stray dogs are reservoirs of *Anaplasma spp*., *Babesia spp.*, and *Theileria spp.*, potentially playing a significant role in the epidemiology and dissemination of blood parasites, hence posing a substantial threat to the cattle industry.

Key words: Iraq, Blood-Borne Parasites, Stray dog, Nested PCR assay

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