Molecular prevalence of equine piroplasms in horses in mainland Portugal: preliminary results

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Objectives: Equine piroplasmosis, caused by *Babesia caballi* and *Theileria equi*, is a tick-borne disease that threatens equine health, performance, and economic interests through increased mortality, movement restrictions, and trade limitations. Although both parasites may be present in endemic regions, *T. equi* tends to exhibit greater prevalence and pathogenicity. The aim of this study was to determine the molecular prevalence and geographic distribution of these pathogens in mainland Portugal.

Materials and Methods: A stratified sampling scheme, proportional to the registered equine populations across Portugal's NUTS II regions, was employed. Genomic DNA was extracted from whole blood samples, and the presence of *Babesia/Theileria* spp. was assessed by PCR targeting the *18S rRNA* gene. Twenty percent PCR positive samples were randomly selected, following the same stratified scheme, for sanger sequencing and phylogenetic analysis.

Results: A total of 270 horses were included in this study, of which 44.1% tested positive by PCR, with the highest prevalence being found in Setúbal Peninsula (51%), followed by Alentejo (50%) and Algarve (50%). Sanger sequencing of selected amplicons (n=24) revealed the exclusive presence of *T. equi,* with no evidence of *B. caballi*.

Conclusions: This study suggests that *T. equi* is the predominant piroplasm infecting horses in mainland Portugal, with higher prevalence of piroplasm infection in the southern regions. Given the impact of equine piroplasmosis on animal health and international trade, these findings highlight the need for continued surveillance and targeted control measures in Portugal.

Keywords: *Theileria equi*, *Babesia caballi*, horses, molecular epidemiology, mainland Portugal

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