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Molecular characterisation of *Theileria* species in bovine theileriosis in Zimbabwe

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Several species of the tick-transmitted apicomplexans of the genus Theileria infect cattle and wildlife in eastern and southern Africa. Theileria (T.) parva, the most important economically, is the cause of a disease that is a heavy burden for livestock production in Zimbabwe. In recent years, the spatio-temporal patterns of theileriosis outbreaks have changed. Investigating the causative Theileria species and strains is key to understanding the infection process and adapting control measures. Samples were collected from suspected bovine theileriosis cases (n=158) in three provinces from November 2020 to April 2021. Frozen blood samples (n = 147) and organs (n = 22)were tested by a combination of pan-Theileria 18S rRNA PCR, specific T. parva PCR and Sanger sequencing to determine the *Theileria* species present. *Theileria* was detected in 83.3% of the sampled clinical cases. Theileria parva (63%), T. velifera (24%), T. taurotragi (2.3%), T. mutans (2.3%), and co-infections (3.1%) were detected in the positive samples. To characterise the T. parva strains, we sequenced the sporozoite surface antigen p67 and the CD8+ antigens Tp2 from 44 and 23 of the *T. parva* positive samples, respectively. Phylogenetic analysis showed that the *T.* parva present was cattle-derived, closely related to the Kenyan Muguga isolate which is part of the widely used *T. parva* vaccine cocktail. Dried blood spots (n=145) and faeces (n=87) were also screened by the 18S rRNA PCR to evaluate their suitability in Theileria diagnosis. Sensitivity was less in dried blood spots (59.3%) and faeces (12.7%) than frozen blood, whilst the specificity for blood spots and faeces was 64% and 85.7%, respectively. The use of non-invasive samples such as faeces can however be suitable to study the circulation of *Theileria* at population level, e.g. in a wild animal population. Overall, our data contribute to the knowledge of *Theileria* species circulating in Zimbabwe, informing control strategies and guiding future research.