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Improved molecular diagnostics and characterization of *Theileria parva* isolates from cattle and buffalo in South Africa

By

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A thesis submitted to the Faculty of Veterinary Science, University of Pretoria, South Africa, in fulfillment of the requirements for the degree

Philosophiae Doctor

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DEDICATION

I dedicate this thesis to the memory of my late grandmother,

Mrs Ethel Magaret Sibeko,

who has been a source of inspiration and a pillar of strength,

without whose unlimited support I would not have realized this dream;

I will be forever grateful for everything she has been to me.

DECLARATION

I hereby declare that this thesis is my own work. It is submitted in fulfillment of the degree, **Philosophiae Doctor**, in the University of Pretoria, South Africa. It has not been submitted before for any degree or examination in any other University, nor has it been prepared under the aegis or with the assistance of any other body or organization or person outside the University of Pretoria, South Africa, other than as indicated in the acknowledgements which follow.

Kgomotso P. Sibeko

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THESIS SUMMARY

The aim of this study was to improve the official diagnostic test package in South Africa for detection of *Theileria parva* infections in cattle and Cape buffalo (*Syncerus caffer*) and to investigate the presence of cattle-type *T. parva* parasites in buffalo and cattle in South Africa. To improve diagnosis of *T. parva* infections, a *T. parva*-specific real-time polymerase chain reaction (PCR) assay based on hybridization probe technology was developed. Oligonucleotide primers and hybridization probes used in the assay were designed based on the 18S ribosomal RNA (rRNA) gene. The primers amplify *T. parva* and *Theileria* sp. (buffalo) DNA but the hybridization probes specifically detect *T. parva* amplicons. Because of the high sequence similarity between the *T. parva* and *Theileria* sp. (buffalo) 18S rRNA genes, amplification of *Theileria* sp. (buffalo) DNA could not be avoided; no other bovine blood pathogens tested were amplified by these primers. The real-time PCR assay demonstrated superior sensitivity compared to other molecular tests used in detection of *T. parva* infections, reliably detecting the parasite in carrier animals with a piroplasm parasitaemia as low as $8.79x10^{-4}$% with minute template DNA input. The assay requires less time to perform with a low risk of contamination because of the closed-tube system that does not require handling of amplicons for post-PCR analysis.

The presence of cattle-type *T. parva* parasites in buffalo and cattle was investigated using restriction fragment length polymorphism (RFLP) profiles of PCR products and sequences of the parasite genes which code for the antigenic proteins p67, p104, and the polymorphic immunodominant molecule (PIM). Cattle-type p67, p104 and PIM alleles were identified from three *T. parva* samples obtained from cattle from a farm near Ladysmith in the KwaZulu-Natal Province. These cattle-type alleles were identical to those previously identified from a cattle-derived *T. parva* stock, *T. parva* Muguga, a parasite stock that causes East Coast fever (ECF) in Kenya; however, ECF was not diagnosed in animals in this farm. Cattle-type alleles identical to those previously reported were not identified from *T. parva* buffalo samples, but variants of p67 allele 1 as well as p104 allele 1, both previously obtained from *T. parva* Muguga, were identified. It is not known if parasites that possess these variants can cause disease, and the risk of their adapting to cattle as in the case of ECF and January disease needs to be evaluated. Furthermore, these findings suggest that cattle-like alleles may not be exclusively associated with cattle-derived *T. parva* parasites. Most of the p67, p104 and PIM gene sequences obtained in this study were not identical to known sequences; furthermore, novel alleles were identified, demonstrating extensive genetic diversity in the

South African *T. parva* parasite population in buffalo. The significance of the parasites that possess 'novel' alleles in the epidemiology of theileriosis in South Africa still needs to be determined. The identification of variants and novel alleles reveals that p67, p104 and PIM gene PCR-RFLP profiles are more complex than previously thought and the classification of buffalo- and cattle-derived *T. parva* parasites in South Africa based on p67, p104 and PIM gene profiles would not be possible. Identification of more reliable markers that can be directly associated with the theilerial disease syndromes remains a challenge.