# DIVERSITY OF *THEILERIA* PARASITES IN AFRICAN BUFFALO (*SYNCERUS CAFFER*) AND THE CHALLENGE OF DIFFERENTIAL DIAGNOSIS


**By**

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# DECLARATION

I declare that this thesis, which I hereby submit for the degree **Philosophiae Doctor** at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at any tertiary institution.

Mamohale M.E. Chaisi

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***For I can do everything through Christ, who gives me strength…….****Philippians 4:13*

# DEDICATION

**To my daughters Reitumetse and Refiloehape**

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| | |
|---|---|
| AEGP | Addo Elephant Game Park |
| BLAST | Basic local alignment search tool |
| Cox III | Cytochrome oxidase subunit III |
| DNA | Deoxyribonucleic acid |
| dNTP | Deoxynucleotide triphosphate |
| FRET | Fluorescence resonance energy transfer |
| GLTP | Greater Limpopo Transfronteir Park |
| HIP | Hluhluwe iMfolozi Game Park |
| ITS | Internal transcribed spacer |
| KNP | Kruger National Park |
| LAMP | Loop mediated isothermal amplification |
| $MgCl_2$ | Magnesium chloride |
| ml | Milliliter |
| µl | Microliter |
| OVI | Onderstepoort Veterinary Institute |
| PCR | Polymerase chain reaction |
| qPCR | Quantitative polymerase chain reaction |

| | |
|---|---|
| RFLP | Restriction fragment length polymorphism |
| RLB | Reverse line blot |
| rRNA | Ribosomal ribonucleic acid |
| $T_m$ | Melting temperature |

# THESIS SUMMARY

In South Africa, the diagnosis of *Theileria parva* in cattle and buffalo has been complicated by the presence of mildly pathogenic and non-pathogenic *Theileria* spp. This can lead to inaccurate diagnostic results and confuse the epidemiology of theileriosis. The aims of this study were to identify and characterize the 18S rRNA genes of novel *Theileria* spp. of the African buffalo, as well as to test new gene targets that will allow for the development of more accurate diagnostic tests for the identification of *T. parva* infections in cattle and buffalo.

Buffalo blood samples originating from different geographical regions in South Africa and from Mozambique were screened for the presence of *Theileria* spp. by the reverse line blot (RLB) hybridization assay. A total of six *Theileria* spp., namely *T. parva*, *Theileria* sp. (buffalo), *Theileria mutans*, *Theileria velifera* and *Theileria buffeli*, were identified from the buffalo samples. These occurred mainly as mixed infections. Some of the samples hybridized only with the *Theileria*/*Babesia* genus specific probe that is used in the RLB assay, and not with any of the species-specific probes used, suggesting the presence of novel genotypes or species.

The full-length 18S rRNA genes of parasites from selected samples were characterized by cloning and sequencing. In addition to the identification of 18S rRNA gene sequences that were similar to published *Theileria* spp. of cattle and buffalo, we identified *Theileria* sp. (bougasvlei), and novel 18S rRNA gene variants of *T. mutans*, *T. velifera*, *T. buffeli*. This variation explained why the RLB hybridization assay failed to detect these species in some of the analysed samples. As extensive variation was observed within the *T. mutans* group, specific RLB oligonucleotide probes were designed from the V4 hypervariable region of the *T. mutans*-like 1 and 2/3 18S rRNA gene sequences. Unfortunately these cross-hybridized with *T. mutans* target DNA and could not be used to screen buffalo samples to determine the occurrence of these genotypes in buffalo in South Africa. This problem could be solved by designing probes from a more variable area of the 18S rRNA gene of the *T. mutans* groups. Alternatively, a quantitative real-time PCR (qPCR) assay could be used for differentiation of these genotypes as it is more sensitive than the RLB assay.

Despite the variation observed in the full-length *T. parva* 18S rRNA gene sequences, the area in the V4 hypervariable region where the *T. parva* RLB and real-time PCR hybridization probes were developed was relatively conserved between sequences obtained in this study. The existing *T. parva*-specific qPCR assay was able to successfully detect all *T. parva* variants identified in this study and, although amplicons were obtained from *Theileria* sp. (buffalo) and *Theileria* sp. (bougasvlei) DNA, these species were not detected by the *T. parva*-specific hybridization probes. The sequences of the other *Theileria* spp. and the novel genotypes identified in this study under the probes were also different from that of *T. parva* and therefore these species do not compromise the specificity of the *T. parva* 18S qPCR assay.

In order to determine the sequence variation and phylogenetic positions of *T. buffeli* spp. of the African buffalo, we cloned and sequenced their 18S rRNA gene and complete internal transcribed spacer (ITS). We identified novel *T. buffeli*-like and *T. sinensis*-like 18S rRNA and ITS genotypes from buffalo originating from two different geographical regions in South Africa. There was extensive sequence variation between these novel South African genotypes and known *T. buffeli*-like and *T. sinensis*-like genotypes. The presence of organisms with *T. buffeli*-like and *T. sinensis*-like genotypes in the African buffalo is of significant importance, particularly to the cattle industry in South Africa as these animals might act as sources of infections to naïve cattle.

Recently, a qPCR assay based on the cox III gene was developed for the diagnosis of *Theileria* spp. in cattle. This test detects and differentiates six *Theileria* spp. in cattle. We evaluated the use of this assay for the detection of *Theileria* spp. in buffalo. The results of the cox III qPCR were compared to those of the RLB and 18S qPCR for the simultaneous detection and differentiation of *Theileria* spp. of the African buffalo, and for the specific detection of *T. parva*, respectively. The cox III genes from selected samples with non-specific melting peaks were characterized by cloning and sequencing. Extensive sequence variation in the cox III gene was observed between and within species. The *T. mutans* group was the most variable. The qPCR assay could be further improved by designing new primers and probes using all known cox III gene sequences of *Theileria* spp. of buffalo and cattle.

This study highlights the complexity of the diagnosis of *T. parva* in cattle and buffalo in South Africa. It provides invaluable information towards the development of an improved molecular diagnostic assay for *T. parva* and co-infecting species in cattle and buffalo in South Africa which will assist the veterinary regulatory authorities in the control of Corridor disease in South Africa.