Molecular characterization of *Babesia caballi* and *Theileria equi*, the aetiological agents of equine piroplasmosis, in South Africa

By

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Dedicated to the memory of my late father,

Vasantrai Bhoora

DECLARATION

I declare that the 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 this or any other tertiary institution.

Raksha Bhoora
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THESIS SUMMARY

In an attempt to develop quantitative real-time PCR (qPCR) assays for the detection of equine piroplasms, sequence heterogeneity in the V4 hypervariable region of the 18S ribosomal RNA (rRNA) gene sequences within both *Theileria equi* and *Babesia caballi* from South Africa was discovered. A molecular epidemiological survey of the protozoal parasites that cause equine piroplasmosis was therefore carried out using horse and zebra samples from different geographical locations around South Africa.

We evaluated the ability of a recently developed *T. equi*-specific qPCR assay in detecting all *T. equi* 18S rRNA variants identified in South Africa. We further present the first report on the development and application of a TaqMan minor groove binder (MGB™) qPCR assay, targeting the 18S rRNA gene, for the detection of *B. caballi* infections in equine blood samples.

Despite the ability of the 18S rRNA *T. equi*- and *B. caballi*-specific qPCR assays to detect all known 18S rRNA gene sequence variants thus far identified in South Africa, the existence of as yet undetected variants in the field cannot be overlooked. Other qPCR assays targeting alternative genes could be developed which, used in conjunction with the 18S rRNA qPCR assays, may provide better confirmation of test results. A *T. equi*-specific qPCR assay targeting the equi merozoite antigen gene (*ema-1*) was recently developed for the detection of *T. equi* parasites in the midgut of *Rhipicephalus (Boophilus) microplus* nymphs. This assay was not able to detect *T. equi* in all South African samples that were confirmed positive by other molecular and serological assays. Sequence characterization of the *ema-1* gene from South African isolates revealed the existence of variation in the regions where the qPCR primers and probes had been designed. Based on these observations, a conserved region of the *ema-1* gene was selected and targeted in the development of an *ema-1*-specific TaqMan MGB™ qPCR assay, which was shown to have a higher sensitivity than the previously reported *ema-1* qPCR assay.

The rhoptry-associated protein (*rap-1*) gene from South African *B. caballi* isolates was also characterized following the failure of a *B. caballi*-specific competitive-inhibition enzyme-linked immunosorbent assay (cELISA) to detect *B. caballi* antibody in the sera of infected horses from South Africa. The genome walking PCR technique was used to amplify the complete *rap-1* gene sequence from two South African *B. caballi* isolates. Significant heterogeneity in the *rap-1* gene sequences and in the predicted amino acid sequences was found. Marked amino acid sequence differences in the carboxy-terminal region, and therefore the probable absence of the monoclonal antibody binding site, explains the failure of the cELISA to detect antibody to *B. caballi* in sera of infected horses in South Africa.

This is the first comprehensive molecular study of the parasites that cause equine piroplasmosis in South Africa. Our results add further to the existing knowledge of piroplasmosis worldwide and will be invaluable in the development of further molecular or serological diagnostic assays.