CHAPTER 1

General Introduction

1.1 Background

Corridor disease, caused by *Theileria parva*, is a controlled disease in South Africa and strict measures are applied to control outbreaks and prevent the spread of the parasite. It is the form of theileriosis that persists in South Africa after the eradication of East Coast fever (ECF) and it poses a threat to the cattle farming industry in this country (Stoltsz, 1989). *Theileria parva* is transmitted transstadially by the three-host ticks, *Rhipicephalus appendiculatus, Rhipicephalus. zambeziensis* and *Rhipicephalus duttoni* (Lawrence et al., 1983; Norval et al., 1992). The main vector, *R. appendiculatus*, is wide spread in South Africa (Horak et al., 1995; 2007; 2009). The African buffalo (syn. Cape buffalo) (*Syncerus caffer*) is the natural reservoir host of *T. parva* and infections in buffalo are usually asymptomatic but are acute, and usually fatal in cattle (Lawrence et al., 1994). Cattle get infected by sharing grazing land with infected buffalo in the presence of the tick vectors (Uilenberg, 1999).

Infection by *T. parva* limits the movement of cattle between countries and can result in production losses and high mortality in infected animals (Allsopp et al., 1993). After foot-and-mouth disease, Corridor disease is the most important disease transmitted from the African buffalo to cattle and is therefore a major constraint to the introduction of buffalo in cattle-rearing areas (Lawrence et al., 1994). It is also a constraint on the importation of new cattle breeds and improved stock as it is most severe in recently introduced, naïve animals (CFSPH, 2009). In South Africa, infected populations of buffalo exist in the Kruger National Park and in the KwaZulu-Natal parks (Collins et al., 2002; Mashishi, 2002). These have been declared as Corridor disease endemic areas and sporadic outbreaks occur in these areas when susceptible cattle share grazing land with infected buffalo (Stoltsz, 1989). Movement of buffalo outside of these areas is strictly controlled by the veterinary authorities in South Africa.

1.1.1 The importance of proper diagnosis and characterization of *Theileria* infections of buffalo in South Africa

African buffalo also play an important role in the epidemiology of several other livestock diseases in South Africa, including foot-and-mouth disease, bovine brucellosis and bovine tuberculosis (Collins et al., 2002; Mashishi, 2002). In order to limit the spread of infection and protect susceptible cattle from infection, infected buffalo are isolated by use of approved fences in national and provincial game parks as well as in a limited number of buffalo breeding projects. Farming of buffalo and cattle on the same farm is not allowed. The major reason for imposing these restrictions on the movement of buffalo is the threat of "transformation" of buffalo-associated to cattle-associated theileriosis, resulting in the re-emergenge of ECF (Stoltsz, 1989) which is a readily transmitted and more virulent form of theileriosis.

In South Africa, buffalo are important for eco-tourism and this has led to an increasing demand for buffalo (Collins et al., 2002). There are several breeding projects in South Africa, regulated by the veterinary authorities, that breed "disease-free" buffalo and it is compulsory to test all buffalo for *T. parva* and other infectious diseases prior to translocation in order to protect susceptible cattle and buffalo from infection (Collins et al., 2002).

Control measures rely on the ability to detect *T. parva* in infected cattle and buffalo. These animals undergo a series of parasitological, serological and molecular diagnostic tests before they are certified "disease-free" and fit for translocation (Collins et al., 2002). The tests have to be sensitive and specific for accurate detection of the parasite. As *T. parva* usually co-occurs with non-pathogenic and mildly pathogenic *Theileria* species in infected animals (Stoltsz, 1989), it is important to characterize and differentiate between the different species and variants of a species so that it is possible to develop highly specific and sensitive diagnostic tests for the diagnosis of *T. parva*.

Earlier diagnosis of *T. parva* involved microscopic and serological methods. The former is based on morphological differences between the different stages of the parasite, and the latter detects serum antibodies to parasite schizont or sporozoite antigens (Katende et al., 1998; Ogden et al., 2003; Oura et al., 2004; Billiouw et al., 2005). However, these tests lack specificity and sensitivity as *T. parva* cannot be easily distinguished from co-infecting *Theileria* spp. Furthermore, the morphology of the parasites can vary during the course of infection and this renders morphological methods unreliable (Norval et al., 1992). Other disadvantages of the two methods include the inability to

detect carrier animals, cross reactivity and lack of standardization of the assays (Allsopp et al., 1993; Katende et al., 1998; Billiouw et al., 2005).

Advances in molecular biology have resulted in the development of more sensitive and specific diagnostic tests based on the detection of minute amounts of the parasite DNA. This has decreased the subjectivity that usually occurs in the interpretation of diagnostic results, and allows for inter- and intra-specific detection and characterization of the different species (Zarlenga and Higgins, 2001; Monis et al., 2005). The development of a highly sensitive and specific assay for the detection of *T. parva* requires prior identification and characterization of known and novel *Theileria* species in buffalo (Zweygarth et al., 2009).

1.2 Problem Statement

The identification of species-specific regions in the parasite 18S rRNA gene has improved the specificity of diagnostic tests and has allowed for discrimination between *Theileria* species. A sensitive, *T. parva*-specific quantitative real-time PCR (qPCR) assay based on this gene (Sibeko et al., 2008) has superseded the assay of Allsopp et al. (1993) in the diagnosis of *T. parva* infections in cattle and buffalo in South Africa. The South African buffalo is known to harbour different *Theileria* spp. (Stoltz, 1989; Young et al., 1978), but no comprehensive molecular characterization studies of these species had been done when this study was initiated, although Mans et al. (2011) recently reported on sequence variation in the hypervariable (V4) region of the 18S rRNA gene. Preliminary sequencing studies of the full-length 18S rRNA gene in our laboratory have revealed sequence differences within the 18S rRNA gene among *Theileria* sp. (buffalo) isolates. However, the extent of this variation is unknown and if strains exist with sequences even more similar to *T. parva* than those currently known, this may compromise the specificity of the assay. In addition, hitherto uncharacterized *Theileria* species could exist which could compromise the specificity of the currently used qPCR assay. This could lead to inaccurate diagnostic results.

1.3 Objectives of the study

In view of the above, the following were the main objectives of the study:

- Identification of pathogenic, mildly pathogenic and benign *Theileria* spp. of the African buffalo from different localities in South Africa.
- Characterization (by cloning and sequencing) of partial and full-length sequences of the 18S rRNA genes of *Theileria* spp. of the African buffalo.
- Evaluation of a recently developed quantitative real-time PCR assay based on the cytochrome c oxidase III (cox III) gene for the simultaneous identification and differentiation of *Theileria* spp. in buffalo.

1.4 Thesis overview

Chapter 1 - provides a general introduction and outlines the rationale and objectives of the study.

Chapter 2 - provides a detailed review of the literature on *T. parva* and other *Theileria* parasites of buffalo and cattle in South Africa.

Chapter 3 - The aim of this chapter was to characterise the 18S rRNA genes of *T. parva* and *Theileria* sp. (buffalo), and to determine whether all identified genotypes can be correctly detected by the qPCR assay. The reverse line blot (RLB) hybridization assay was used to screen buffalo blood samples collected from different geographical regions of South Africa, and in Mozambique. Based on the RLB results, the 18S rRNA genes of selected samples that were positive for *T. parva* and/or *Theileria* sp. (buffalo) were cloned and sequenced, and the resulting clones were analysed.

Chapter 4 - The mildly pathogenic *Theileria mutans* and non-pathogenic *Theileria velifera* often co-occur with *T. parva* in infected cattle and buffalo. In order to determine whether these species, or their variants can compromise the specificity of the 18S rRNA qPCR in the detection of *T. parva*, we cloned and sequenced their full-length 18S rRNA genes. New oligonucleotide probes were developed from the novel *T. mutans* genotypes identified for inclusion in the RLB hybridization assay. We also report on the presence of *Theileria* sp. (strain MSD) in the African buffalo. This species was first identified from a naturally infected bovine in Pretoria, South Africa (Chae et al., 1999) but no further attempt has been made to clarify its identity.

Chapter 5 - We characterized the full-length 18S rRNA gene and complete internal transcribed spacer (ITS1-5.8S-ITS2) region of *Theileria buffeli* of the South African buffalo in order to determine their genetic diversity and establish their phylogeny based on these sequences.

Chapter 6 - This chapter is a continuation of the studies on the characterization of the 18S rRNA gene of *Theileria* species of buffalo in South Africa in an attempt to identify novel genotypes that might interfere with the diagnosis of *T. parva* infections in cattle and buffalo in when using the 18S qPCR assay. In view of the genetic diversity that was observed in the previous chapters, we cloned and sequenced the V4 hypervariable region of the 18S rRNA gene from additional buffalo and cattle samples in order to determine the extent of sequence variation in this area of the gene. The results obtained were compared with those of a recent similar study by Mans et al. (2011).

Chapter 7 - The limitations of the 18S rRNA qPCR assay in the specific diagnosis of *T. parva* have been outlined in the previous chapters. In order to improve the diagnosis of *T. parva* in cattle and

buffalo in South Africa, alternative assays based on molecular markers that can effectively differentiate between *T. parva* and co-infecting species are needed. A qPCR assay based on the cytochrome oxidase III (cox III) gene was recently developed and evaluated for the simultaneous detection and differentiation of *Theileria* species of cattle (Janssens, 2009). We evaluated this assay for the simultaneous detection and differentiation of *Theileria* spp. of the African buffalo. The results obtained were compared with those of the RLB assay for simultaneous detection and differentiation of *Theileria* spp. in buffalo, and with those of the 18S rRNA qPCR assay for the specific detection of *T. parva*.

Chapter 8 - provides a general discussion, conclusions and recommendations emanating from this study.

1.5 References

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