GENETIC DIVERSITY OF THE BOVINE LEUKOCYTE ANTIGEN (BoLA) AND ITS ASSOCIATION WITH RESISTANCE TO TICKS AND TICK-BORNE DISEASES IN SELECTED BEEF CATTLE BREEDS IN NAMIBIA

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LAVINIA HAIKUKUTU

201025051

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Main Supervisor: Dr. Theopoline Omagano Itenge

Co-Supervisor: Prof. Japhet Robert Lyaku

ABSTRACT

The bovine leukocyte antigen (BoLA) class II genes play a significant role in presenting processed peptides to CD4 $^{+}$ T lymphocytes. BoLA genes particularly class II are highly polymorphic, enhancing the number of peptides that an individual can recognise thereby triggering a cascade of immune responses. This study investigated the genetic diversity of the BoLA class II genes in 249 animals comprising of Nguni ecotypes, Bonsmara and Afrikaner cattle from Khomas, Omusati and Zambezi regions in Namibia. Molecular characterisation of the three cattle breeds was performed using four microsatellite markers (*DRB3, DRBP1, RM185, BM1815*) within the BoLA genes, or in close proximity, to assess genetic diversity and to determine the population structure. Ticks infesting the animals naturally were counted and identified. In addition, animals were screened for tick-borne infections (*Anaplasma/ Ehrlichia* and *Babesia/ Theileria*) using "catch-all" primers for *Anaplasma/ Ehrlichia* and *Babesia/ Theileria* cluster of species. A total of thirty seven alleles were identified across all breeds. Nguni cattle exhibited the highest level of genetic diversity (H_e = 0.728) and Bonsmara cattle had the lowest level of genetic diversity (H_e = 0.637). Extensive inbreeding (F_{IS} = 0.247) was observed in Afrikaner cattle while Bonsmara cattle showed evidence of outbreeding (-0.057). The Bayesian cluster at K = 3 revealed genetic admixture between breeds. The overall *Anaplasma/ Ehrlichia* and *Babesia/ Theileria* infection prevalence across all breeds was 85% and 53%, respectively. Ticks infesting the animals included species (relative prevalence) of *Hyalomma truncatum (35%)*, *Hyalomma turanicum* (2%), *Hyalomma rufipes* (29%), *Rhipicephalus evertsi evertsi (5%), Rhipicephalus evertsi mimeticus (9.5%), Rhipicephalus simus (16%), Rhipicephaluss appendiculatus (3%)* and *Amblyomma variegatum (0.5%).* Alleles associated with tick and tick-borne disease resistance were identified. Alleles DRB3-289, *DRB3*-290, *DRB3*-291, *DRB3*-292, *RM185*-93 and *BM1815*-145 were associated with tick and tick resistance were identified. Allele DRB3-290, DRBP1-120, DRBP1-122 and DRBP1-126 were associated with decreased incidence of *Anaplasma/Ehrlichia* infections in Namibian cattle breeds. Significant allelic association ($P < 0.15$) was found between three DRBP1 alleles (*DRPB1*-122, *DRBP1*-124*, DRBP1*-128), four alleles belonging to the RM185 locus (*RM185*-101, *RM185*-103, *RM185*-105, *RM185*-107) and resistance to *Babesia/Theileria* infections. These alleles can be used

as potential markers for the selection of cattle with tick and tick-borne disease resistance. The results of this study can aid in future marker-assisted selection (MAS) in breeding programs for animals with superior tick and tick-borne disease resistance.

Keywords: Beef cattle, BoLA, genetic diversity, tick resistance, tick-borne disease resistance

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ABBREVIATIONS

AF	allele frequency
AFR	Afrikaner
AMOVA	analysis of molecular variance
BGSAC	Bovine Genome Sequencing and Analysis Consortium
BON	Bonsmara
BONK	Bonsmara cattle sampled in Khomas region
BONO	Bonsmara cattle sampled in Omusati region
BP	Before present
BTA	Bovine chromosome
CI	Confidence interval
DART	Directorate of agricultural research and training
DNA	Deoxyribonucleic acid
EDTA	Ethylene diamine tetra acetic acid
EF	Etiological fraction
FAO	Food and Agriculture Organisation
FIS	Inbreeding coefficient of individuals within a subpopulation
FIT	Inbreeding coefficient of individuals within the total population
FST	The amount of genetic differentiation within the total population
GC	Guanine Cytosine
GDP	Gross Domestic Product
GWAS	Genome-wide association study
He	Expected heterozygosity
Ho	Observed heterozygosity

HWE Hardy-Weinberg equilibrium

ILRI International Livestock Research Institute

K Number of assumed populations

LD Linkage disequilibrium

LD-MAS Linkage disequilibrium marker assisted selection

LE-MAS Linkage equilibrium marker assisted selection

MAI Marker-assisted introgression

MAS Marker-assisted selection

MAWF Ministry of Agriculture, Water and Forestry

MET Ministry of Environment and Tourism

$MgCl_2$ Magnesium chloride

MIT Ministry of Industrialisation and Trade

N$ Namibian dollar

NCAs Northern Communal Areas

NCBAN Nguni Cattle Breeders Association of Namibia

NGU Nguni

NGU Nguni cattle sampled in Khomas region

NGUO Nguni cattle sampled in Omusati region

NGUZ Nguni cattle sampled in Zambezi region

OR Odds ratio

PCR Polymerase chain reaction

PIC Polymorphism information content

QTL Quantitative trait loci

RFLP	Restriction fragment length polymorphism
RST	Coefficient index under the step-wise mutation model
SNP	Single nucleotide polymorphism
SSA	Sub-Saharan Africa
TAE	Tris –acetate- EDTA
TBDs	Tick-borne diseases
UNAM	University of Namibia
UNDP	United Nations Development Programme
US$	United States Dollar
UV	Ultraviolet

PUBLICATIONS AND CONFERENCE PROCEEDINGS

JOURNAL PUBLICATIONS

1. L. Haikukutu, T. O. Itenge, L. Bosman, C. Visser & E. van Marle-Köster (2017). Genetic variability of the major histocompatibility complex (MHC) class II (DRB3) in South African and Namibian beef cattle breeds. *Advances in Animal Biosciences,* 8 *(1),* 19-21.

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3. *L. Haikukutu, T.O. Itenge & J.R Lyaku* (2016). Genetic variation of the BoLA-DRB3.2 region and its association with tick resistance in selected beef cattle breeds in Namibia. Poster presented at the 5th Regional Universities Forum for capacity building in agriculture (RUFORUM) biennial conference. University of Stellenbosch, South Africa.

4. *L. Haikukutu, T.O. Itenge, L. Bosman, C. Visser, J. Lyaku, F. Mausse and E. Van Marle-Koster* (2016). Genetic variability of the MHC class II (DRB.3) in South

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DEDICATION

This work is dedicated to Ottilie Joram. You have been a friend, a mother and indeed an invaluable source of optimism and support throughout the course of this study. I could not have done this without you.

Proverbs 3:6

Trust in the LORD with all thine heart; and lean not unto thine own understanding.

We must remember that intelligence is not enough. intelligence plus character – that is the goal of true education. - Martin Luther King, Jr.

DECLARATION

I, **Lavinia Haikukutu**, hereby declare that this study is my own work and is a true reflection of my research, and that this work, or any part thereof has not been submitted for a degree at any other institution.

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CHAPTER 1 : INTRODUCTION

1.1 Background

Agriculture is one of the dominant sectors in Namibia contributing to the county's economy. Agriculture accounts for about 3.8% of the country's gross domestic product (GDP) and employs 31% of the labour force (Namibia's Fifth National Development Plan [NDP5], 2017). Moreover, 70% of the Namibian population depend on agriculture as their main source of livelihood and food security (National Planning Commission [NPC], 2016). Lucrative export of beef to the European Union and other countries constitutes a significant portion of the agricultural contributions to the country's GDP. In 2010 alone, Namibia exported beef worth N$ 1.5 billion, mostly to South Africa and the European Union (Ministry of Trade and Industry & United Nations Development Programme [MTI & UNDP], 2011). However, the advent of drought coupled with the rapid expansion of other sectors have led to a steady decline in the economic contribution of (Ministry of Environment and Tourism & United Nation Development Programme [MET & UNDP], 2008) from 10% in 2004 (Els, 2004) to 5.1 % in 2012 (African Development Bank, 2014) and presently 3.8% in 2017 (Fifth National Development Plan [NDP5], 2017). Despite its marginal contribution to GDP, agriculture remains central to the livelihood of the majority of the Namibian population.

With a mean annual rainfall of approximately 270mm, Namibia is the most arid country in sub-Saharan Africa (SSA). Despite the aridity, Namibia has a rich diversity of livestock of about 2.9 million cattle, 2.0 million sheep and 1.9 million goats but the numbers fluctuates with the rainfall received (Ministry of agriculture, water and forestry [MAWF], 2015). The indigenous cattle breed of Namibia is known as Nguni, initially called Sanga (Nguni Cattle Breeders Association of Namibia

[NCBAN], 2011). Nguni cattle are widely distributed in the Northern and North-Eastern parts of the country and comprise half of the total cattle population in Namibia (Els, 2004). The Northern and North-Eastern parts of the country are mainly communal and more subsistence oriented. Based on the socio-economic criteria, farming systems in Namibia can be classified into commercial and communal (subsistence) farming. Although communal farming occupies only 41% of the land as compared to 44% of commercial farming land, 67% of the total cattle population is kept by communal farmers in Northern communal areas (NCAs) (Els, 2004).

The commercial farming sector employs more than 30,000 farmers and constitutes a major part of the contributions of agriculture to the country's GDP (Els, 2004; MET& UNDP, 2008). On the contrary, the communal farming sector is subsistence based and is vital for the livelihood of most rural households. There are at least 120,000 farmers involved in livestock farming in the communal sector (Els, 2004). A map of the distribution of farming systems in Namibia is displayed in Figure 1.1.

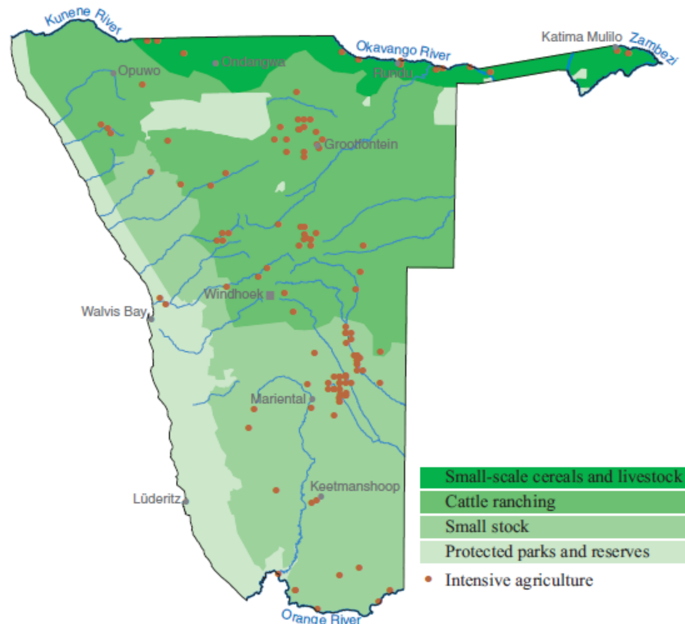


Figure 1.1. The distribution of farming systems in Namibia (Mendelson, 2006)

The demand for agricultural products in particular from livestock is continuously increasing with the growing human population (Food and Agriculture Organisation [FAO], 2011; Otten & Van den Weghe, 2011). In order to feed the growing population, local breeds are replaced with the high-yielding temperate breeds to increase production outputs. Unfortunately, replacing locally adapted breeds with exotic breeds threatens livestock diversity hence putting them at risk of extinction (FAO, 2015).

There is general concern that the genetic diversity within African indigenous cattle breeds is rapidly declining as a result of breed substitution and indiscriminate crossbreeding with exotic cattle such as Hereford, Santa Getrudis, Aberdeen Angus and Simmental (Scholtz *et al*., 2008; FAO, 2015; Mwai *et al*., 2015). For example, in South Africa, crossbred cattle constitutes 66% of the herds (Scholtz *et al*., 2008) and pure Nguni cattle numbers declined from 1.8 million in 1992 to 9,462 in 2003 (International Livestock Research Institute [ILRI], 2009). However, the 2014 census of the Nguni cattle released by the stud book annual logix beef report shows that the number of registered Nguni cattle was 75, 155 (54, 748 individual females and 20, 407 males). This figure excludes the Nguni cattle found in rural areas. The rapid decline in the number of indigenous cattle can result in extinction if no corrective action is taken to conserve indigenous breeds (FAO, 2011).

It is well known that indigenous cattle breeds possess valuable traits such as disease resistance, good walking ability, high fertility, and adaptability to limited water and poor quality feeds which are responsible for their adaptations to harsh and changing climatic conditions of Africa (Okomo-Adhiambo, 2002). Although Nguni cattle and their crosses make up 50% of the cattle population in Namibia, indiscriminate cross breeding with exotic breeds remains a challenge in the NCAs

where structured breeding programmes are not practised. In such areas, the genetic diversity of Nguni cattle is already being challenged by mortalities due to drought, wild fires and diseases, indiscriminate crossbreeding will cause an even further loss of genetic diversity hence reducing the valued adaptive traits of the Nguni if no steps are taken to conserve them (MET & UNDP, 2008).

Conservation of threatened indigenous cattle does not only maintain genetic diversity but also their contributions to household food security and ensures that their valuable traits remain available for future breeders (Nyamushamba, 2017). Furthermore, most indigenous breeds reflect cultural and historical identities of the communities that developed them (FAO, 2015). Thus, the need to conserve them and prevent the irreversible effects of genetic erosion. In efforts to conserve indigenous breeds worldwide, the FAO proposed molecular characterization of breeds in order to sustain and determine their genetic status (FAO, 2011). In Namibia, Nguni ecotypes have been characterized using microsatellite markers to determine their genetic status (Hanotte *et al*., 2000; Nortier *et al*., 2002). Microsatellite markers are powerful tools used to estimate genetic diversity between and within livestock breeds and allow identifications of genes involved in adaptive traits (Singh *et al*., 2014), such as tick and tick-bone disease (TBD) resistance (Acosta-Rodriguez *et al*., 2005; Untalan *et al*., 2007). These markers have been used in humans with a major success to identify a number of genes associated with diseases such as atopic asthma (Ober *et al*., 2000), tuberculosis (Greenwood *et al*., 2000), diabetes (Shao *et al*., 2003), Puumula hantavirus disease (Guivier *et al*., 2010) and Schizophrenia (Shibata *et al*., 2013).

1.2 Ticks: Biology and economic impact

Ticks are blood sucking ectoparasites belonging to the phylum Arthropoda. Ticks can be classified as either ixodid (hard ticks) or argasid (soft ticks). All ticks

have two body segments, the capitulum (mouth and feeding parts) and the idiosoma (body) comprising of most organs, anus and genital aperture (Walker *et al*., 2003). There are three active life stages of ticks: larva, nymph and adult (Jongejan & Uilenberg, 1994). The first life stage is called larva, which after hatching from the egg, seek for a host to feed by a behaviour called questing. Questing ticks swarm up grass blades and wait with their front legs outstretched (Figure 1.2). When a potential host brushes against the grass, they immediately grab onto it using their front legs and crawl over the skin to find a suitable place to attach and feed (Blagburn & Dryden, 2009). After feeding for about a week, larvae drop to the ground and moult to a nymph which after feeding for a few weeks also moult and turn into adults (Blagburn & Dryden, 2009) .

Figure 1.2. Questing tick larva on grass (Walker *et al*., 2003)

Some ticks feed on only one host throughout all three life stages and females drop off the host prior to laying eggs. These ticks are called one-host ticks (Coetzer & Justin, 2004). One-host life cycle occurs in all *Rhipicephalus* (*Boophilus)* species (Figure 1.3). This life cycle is usually rapid and takes about three weeks for feeding

and two months for egg-laying and larval development.

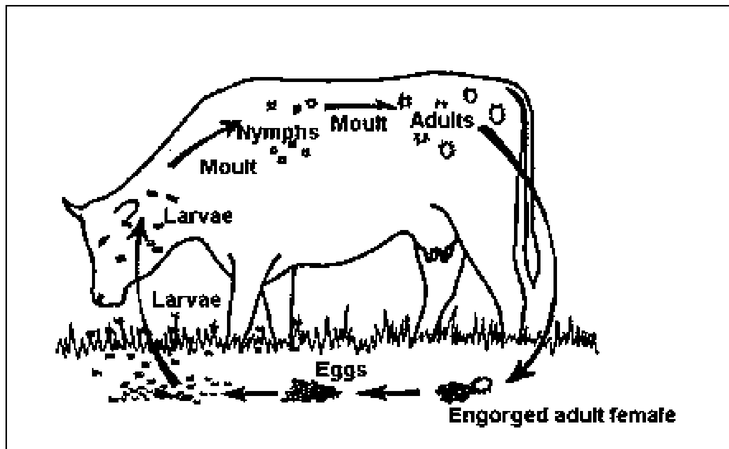


Figure 1.3. One-host tick life cycle (Coetzer & Justin, 2004)

Two-host ticks feed on the same host during the larval and nymphal stages and in the adult stage they feed on a different host (Figure1.4). *Rhipicephalus evertsi* and *Hyalomma detritum* are examples of two-host ticks. Others feed on a different host at each of the three active life stage and these are called three-host ticks (Figure 1.5). The three-host life cycle is the slowest and most common life cycle among hard ticks (Walker *et al.*, 2003).

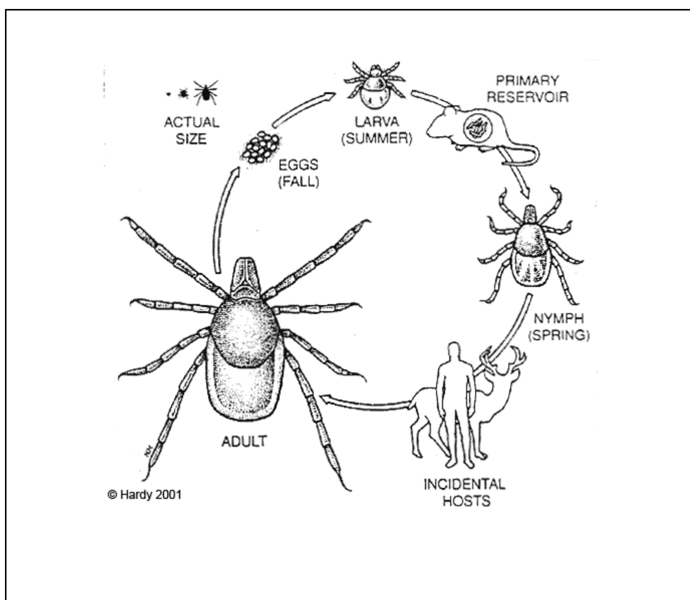


Figure 1.4. Two-host tick life cycle (Coetzer *et al.*, 2004)

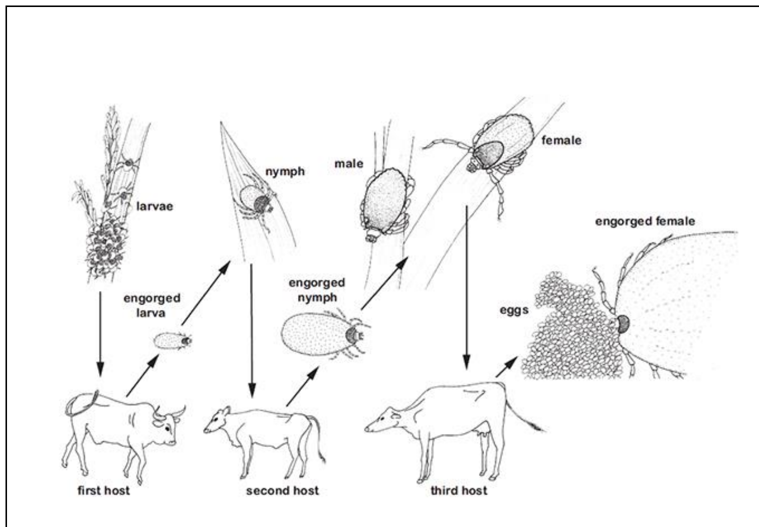


Figure 1.5. Three-host tick lifecycle (Walker *et al*., 2003)

Ticks, as gluttonous blood feeding parasites, can feed up to a hundred fold their unfed weight (Kaufman, 2007). As such, ticks cause blood loss, tick worry, damage to hides (Präffle *et al*., 2009) and transmit pathogens of viral, bacterial and protozoan diseases (Gubler, 2009). Thus, ticks are a major constraint to animal health and productivity in tropical and sub-tropical regions causing substantial losses in the livestock industry (Shyma *et al*., 2015). Consequently, a huge amount of money is spent on the management of ticks and tick-borne diseases. Two decades ago, the global economic losses caused by ticks and tick-borne diseases in the cattle industry was estimated to be in the range of US$ 13.9 billion and US$ 18.7 billion per year (de Castro, 1997). The overall losses caused by ticks were estimated to be over US$ 100 million dollars/year in Australia and one billion dollars/year in Central and South America (Sabatini *et al*., 2001). In Tanzania, total annual losses due to TBDs were estimated to be US$ 364 million in 2006 which included loss due to mortality of 1.3 million cattle (Kivaria, 2006). In Brazil, losses attributed to *Rhipicephalus microplus* in cattle were estimated to be US$ 3.2 billion in 2011 (Grisi *et al*., 2014. For these reasons, ticks have become a major focus in veterinary research.

1.3 Ticks of veterinary importance in sub-Saharan Africa (SSA)

Ticks are considered to be the most important ectoparasite of livestock in tropical and sub-tropical regions (Rajput *et al*., 2006). Approximately 900 species of ticks are recognized globally of which about 700 species are hard ticks and 200 species are soft ticks (Madder *et al*., 2013). The most notable ones belongs to the *Rhipicephalus*, *Hyalomma* and *Amblyomma* genera; these are responsible for causing significant economic losses in SSA (Walker *et al*., 2003).

Studies of ticks in Namibia date back to the 1900's with research in cattle (Biggs & Langenhoven, 1984), springboks, gemsbok, kudus (Horak *et al*., 1992) and elephant shrews (Fourie *et al*., 2005). In the early 1900, Howard (1908) identified five ixodid tick species in Namibia, amongst them *Rhipicephalus decoloratus*, which at the time he referred to as *Margaropus annulatus*. Later, Bedford (1932) identified three species of ticks, bringing the total number of ticks species identified by the 1930s to eight. By 1962, twenty eight tick species were identified. The significant contribution to tick identification was made by Theiler (1962). Walker *et al*. (2000) also contributed to the identification of tick species in Namibia. More recently, Nyangiwe *et al*. (2014) recorded the first observation of the pantropical blue tick *Rhipicephalus microplus* in Namibia. Ticks of veterinary importance in SSA are as shown in Table 1.1.

1.4 Ticks as vectors of pathogens that cause diseases in livestock

Ticks transmit numerous pathogens including viruses, bacteria and protozoa responsible for causing severe diseases which cause huge economic loses in the livestock industry (de la Fluente *et al*., 2008). Approximately 10% of the currently known 900 species of ticks are vectors of a range of pathogens (Jongejan & Uilenberg, 2004).

The most notable cattle TBDs in SSA include; Anaplasmosis, Babesiosis, Theileriosis and Heartwater (Madder *et al*., 2013).

Table 1.1. Ticks of veterinary importance in sub-Saharan Africa (SSA)

Tick species	Description	Pathogens transmitted	Life cycle
Rhipicephalus (*Boophilus*)	bluish tick short mouthparts faint/absent anal groove eyes small/absent	*Babesia bigemina*, *Babesia bovis*, *Anaplasma marginale*	One and two-host ticks
Rhipicephalus appendiculatus	Uniformly brown scutum short mouthparts reddish-brown legs	*Theileria parva* *Anaplasma bovis* *Theileria taurotragi*	three-host tick
Rhipicephalus evertsi evertsi	dark brown scutum shorth mouthparts medium sized beady-eyed ticks with reddish-orange legs	*Anaplasma marginale* *Borrelia theileri*	One-host tick
Hyalomma truncatum	dark-brown scutum long mouth parts banded legs eyes present	Toxins	Three-host tick
Amblyomma variegatum	brightly ornamented ticks beady eyes long mouthparts	*Ehrlichia ruminantium* *Ehrlichia bovis*	Three-host tick
Rhipicephalus decoloratus	Yellowish conscutum Short mouthparts Eyes present Pale yellow slender legs	*Babesia bigemina* *Anaplasma marginale* *Borrelia theileri*	One-host tick
Amblyomma hebraeum	brightly ornamented scutum flat eyes long mouthparts	*Ehrlichia ruminantum* *Theileria mutans* *Theileria velifera*	Three-host tick

Sources: Horak & Fourie (1991), Walker (1991) and Coetzer *et al.,* (1994).

Tick-borne pathogens are transmitted in various ways. Transovarial transmission involves the transmission of pathogens from the parent to the offspring via the ovaries (da Cruz *et al*., 2015). *Babesia bovis* and *Babesia bigemina* are transmitted in this manner by *Rhipicephalus (Boophilus)* species (Madder *et al*., 2013). Transstadial transmission occurs when parasites acquired by ticks at one life stage (nymph) are transmitted in the next life stage (adult). *Anaplasma* species are transmitted by *Dermacentor* ticks via trans-stadial means (Kocan *et al*., 2015). Other routes of transmission include co-feeding (uninfected ticks feeding with infected ones) and intra-stadial (within the same tick life stage, by males) transmission. Pathogens can also be transmitted when infected blood is transferred to susceptible animals via contaminated fomites or mouth parts of biting flies (Kocan *et al*., 2004). Table 1.2 shows the important economical TBDs in SSA, their vectors and causative agents.

Table 1.2. Vectors, causative agents and diseases of economic importance in sub - Saharan Africa

Vector	Disease	Causative agent	Method of transmission[1]
A. hebraeum	Heartwater	*Ehrlichia ruminantum*	TS, IS, TO
A. variegatum	Anaplasmosis	*Anaplasma bovis*	TS
H. dromedarii	Oriental theileriosis	*Theileria annulata*	TS
H. truncatum	Sweating sickness	*Toxin*	-
R. microplus	Babesiosis	*Babesia bigemina*	TO
R. appendiculutus	East coast fever	*Theileria parva*	TS
R. evertsi evertsi	Anaplasmosis	*Anaplasma marginale*	IS
R. zambensiesis	East coast fever	*Theileria parva*	TS

[1]TS-Transtadial transmission, TO-Transovarial transmission, IS-Intrastadial transmission

1.5 Tick and tick borne disease control methods

Control of ticks is essential in tick endemic areas to prevent production losses and the spread of TBDs. Farmers use different methods to control ticks including chemicals, tick vaccines, indigenous knowledge, biological methods and host resistance. Chemical control with acaricides plays a major role in the control of ticks worldwide. Acaricides were introduced in Africa in the 1890s and are still the most commonly practiced control methods used in tick eradication in most African countries despite their well-known drawbacks. One major drawback of acaricides is development of acquired, cross, or multiple resistance by ticks which rendering the drug less effective (Abbas *et al.,* 2014). *Rhipicephalus* (*Boophilus)* ticks are particularly known to be resistant to almost every available acaricide (Guerrero *et al*., 2012).

Besides being costly, acaricides can cause environmental contamination and may leave potentially harmful residues in meat and milk (George *et al*., 2004). Nevertheless, these chemicals have been effective in controlling tick infestations in the world and have improved the viability of cattle farming in tick-endemic areas (Brito *et al*., 2011). One should however be careful to avoid intensive acaricide use as it interferes with enzootic stability, rendering animals susceptible to diseases (Musisi & Lawrence, 1995).

Tick vaccines have proven effective in reducing tick infestations and the incidence of TBDs applications (Merino *et al*., 2013). Immunological control using anti-tick vaccines offers a cost-effective and environmentally sound tick control method (de la fluent *et al*., 2007) replacing and/or supplementing the use of chemical acaricides. In addition to being cheap, the development of tick resistance against vaccines is slower compared to acaricides (Mapholi *et al.,* 2014). The implementation

of anti-tick vaccines in integrated control programmes permits a reduction in the use of acaricides, which in turn decreases treatment costs. Major drawbacks of vaccines include, short shelf life, possible spread of silent pathogens such as bovine leukaemia virus, risk of reversion of virulence and vaccines may not offer protection against multiple tick species (Domingos *et al*., 2013).

Indigenous knowledge plays a major role in tick control. Several studies have documented ethnoveterinary use of plants by communal farmers to treat animal diseases in Namibia (Habeeb, 2010; Chinsembu *et al*., 2015). Herbs and indigenous medical practices were the disease control methods used long ago before pharmaceutical drugs were introduced to Africa and this knowledge has been passed on from generation to generation. In Namibia, the roots, leaves and bark extracts of *Terminalia sericia* (Muhonono in Lozi) are used by farmers to control ticks and TBDs (Mashebe *et al*, 2015). In their extensive review of plants used in South Africa for Ethnoveterinary medicinal purposes, McGaw & Ellof (2008) reported that the bulbs and roots of *Boophane disticha* are used to treat Redwater (Babesiosis) in cattle and *Mystroxylon aethiopian* is used to treat Heartwater. A survey done in the Buuri district in Kenya demonstrated the importance of *Cucumis aculeatus* fruits and roots of *Ehretia cymosa* in treating anaplasmosis (Gakuubi *et al*., 2012). A number of active compounds such as 1,8-cineole, camphor and borneol with 70-100% repellent efficacy have been isolated from *Lavendula augustifolia* (Mkolo & Magano, 2007). The major drawback of the medicinal plants as tick control methods is that they may be effective for some tick species and are also not be practical on large scale farming.

Biological control of ticks has also been reported to be effective. In several studies, entomo-pathogenic fungi have been shown to have the ability to kill ticks. Strains of *Beauveria bassiana* and/or *Metarhizium anisopliae* have been shown to be

pathogenic towards *B. microplus* (Frazzon *et al*., 2000), *R. appendiculatus* (Kaaya & Hassa, 2000; Nana *et al.,* 2012) and *Ixodes ricinus* (Wassermann *et al*., 2016). The efficacy of entomo-pathogenic fungi depends on tick species, tick stage and fungus strain. Some major drawback of biological control methods is that they may not be practical on large scale farming and are also susceptible to environmental conditions such as humidity, temperature and length of daylight.

It has been reported that indigenous cattle of Africa typically *Bos indicus* and N'dama cattle possess host resistance to ticks (Mattioli *et al*., 1995). Thus, minimal or no tick control is required when farming with these breeds. Given the many tick control methods, it is worth noting that there is no single ideal solution to control ticks but integrated control methods involving host resistance, vaccines, pasture management and minimal chemical application can be recommended.

1.6 The Major Histocompatibility Complex (MHC)

The Major Histocompatibility Complex (MHC) are special membrane proteins involved in antigen presentation of processed antigenic peptides to T cells (Janeway *et al*., 2001). MHC molecules are one of the main cell types involved in cell mediated immunity. The MHC was discovered in the 1940s by George Snell as the genetic locus whose products are responsible for rapid rejection of tissue grafts exchanged between inbred strains of mice (Abbas *et al*., 2007). The MHC consists of a set of closely linked genes clustered together within a single genomic locus hence the term 'complex' (Garrick & Ruvinsky, 2015). Many of the MHC genes encode molecules involved in antigen presentation. A major breakthrough in the understanding of the MHC was made by the Nobel Prize winners Zinkernagel and Doherty who showed that T lymphocytes (T cells) require antigens presented to cytotoxic T cells in the

context of self MHC antigens (Janeway *et al*., 2001). Antigens are degraded intracellularly and subsequently presented by MHC molecules in the form of short peptides to T cells for recognition. The bovine MHC is designated as BoLA (Bovine Leukocyte Antigen) whereas in sheep and goats it is referred to as *Ovar* (representing *Ovis aries*) and *Cahi* (representing *Capra hircus*), respectively (Ammils *et al*., 1998).

Historically, there are three classes of MHC molecules involved in antigen presentation; class I, class II and class III. These classes differ in function in that class I molecules present antigens derived from proteins synthesized in the cytosol to $CD8^+$ cells (cytotoxic T cells) and class II molecules bind and present antigenic peptides derived from intracellular vesicles to $CD4^+$ cells (T helper cells) eventually leading to elimination of infected cells. Class III molecules are involved in inflammatory responses and include members of the tumour necrosis family complement proteins and lympotoxin (Goldberg & Rizzo, 2015).

The most striking feature of the MHC is the high degree of polymorphism they exhibit in vertebrates which enables them to present a wide range of antigens to T-cells (Janeway *et al*., 2001). This polymorphism is thought to stem from evolution and not mutation (Ellis, 2004) and has therefore become a target of much research as it is useful in inferring evolutionary histories of many animal species and in studying variations in immune responsiveness of different animals to vaccinations and infections (Yasmeen *et al*., 2014). For these reasons, the MHC is considered a good candidate gene for genetic markers in parasite and disease resistance studies (Untalan *et al*., 2007).

1.7 Tick and tick-borne disease resistance in sub-Saharan African (SSA) indigenous cattle breeds and their crosses

The earliest cattle in Africa were *Bos taurus*. Today the straight-backed taurine cattle are predominantly found in the western and eastern part of the continent, while the *Bos indicus* (Zebu or humped cattle) that makes up the majority of cattle in Africa are found in the northern as well as eastern parts of Africa (Kim *et al*., 2017). The Sanga cattle (taurine-indicine hybrids) are predominantly found in the central and southern part of Africa. The majority of African indigenous cattle are still managed under traditional semi-extensive systems in communal areas and are subjected to strong environmental pressures and diverse disease challenges (Musisi & Lawrence, 1995). There is consistent agreement that *Bos indicus* cattle exhibit higher resistance to ticks than *Bos taurus* breeds (Piper *et al*., 2009; Constantinoiu *et al*., 2010). Utech *et al*. (1978) defined tick resistance as the ability of cattle to limit the number of ticks that survive to maturity. Hull (1912) suggested that cattle resistance to *Boophilus microplus* was hereditary. Subsequently, numerous studies have reported the heritability of tick resistance in different cattle breeds (Budeli *et al*., 2009; Porto Neto *et al*., 2011; Ayres *et al*., 2013).

The Nguni breed of South Africa is one of the most extensively researched Sanga cattle breeds. Numerous studies reported significantly high resistance of this breed to ticks compared to other South African cattle breeds (Spickett *et al*., 1989; Rechav & Kostrzewski, 1991; Marufu *et al*., 2011; Mapholi *et al*., 2016). Tick resistance in Afrikaner and Drakensberger breeds of Sanga cattle of South Africa has also been reported by Fourie *et al*. (2013) and attributed to coat score and hide thickness. Magona *et al*. (2011) demonstrated differences in tick resistance of Ugandan Nkedi Zebu cattle to *R. decoloratus*, *A. variagatum*, *R. appendiculatus*

suggesting that cattle can possess specie-specific resistance to ticks. A cohort study done in Tanzania assessed natural tick infestation on Zebu cattle in Tanzania based on geographical locations, animals were dipped once every two to three weeks to control the tick challenge. Results revealed that more animals ($P < 0.05$) were infested with ticks in Tarime district (96.1 %) than in Serengeti (61.7 %) suggesting that Serengeti Zebu herds exhibited high resistance to ticks than Tarime Zebu herds (Laisser *et al*., 2016).

Given *Bos taurus* breeds are more susceptible to ticks, genetic management for ticks in exotic breeds is mainly attained by crossbreeding *Bos indicus* with *Bos taurus* breeds thus introgressing tick resistance genes into improved breeds (Taberlet *et al*., 2008). The Bonsmara breed (Afrikaner-exotic crossbreed) has been reported to possess high levels of tick resistance in several studies (Spickett *et al*, 1989; Budeli *et al*., 2009; Nyangiwe *et al.,* 2011). The crosses between Horro (Zebu) and Jersey cattle in Ethiopia have also been reported to exhibit a high degree of tick resistance (Irvin *et al*., 1996).

In a recent publication, acquired immunity against *Theileiria parva* was demonstrated in two Tanzanian indigenous cattle breeds (Tarime and Sukuma cattle) in the Lake zone (Laisser *et al*., 2016). In Kenya, Zebu cattle from an East Coast Fever (ECF) endemic area were reported to have a better ability to control the course of the ECF disease and recover in a shorter period of time than Friesian (*Bos taurus*) cattle (Ndungu *et al*., 2005). In Zambia, over 200 000 cattle have been exposed to Babesiosis and Anaplasmosis (Makala *et al*., 2003). However, not all animals developed overt disease in particular the Sanga breeds due to enzootic stability suggesting high resistance of indigenous breeds to TBDs. This indicates that there are differences in susceptibility to ticks and TBDs between breeds. High natural

resistance implies that there may be a genetic basis to host resistance to ticks and TBDs which if characterised could be used in selecting for resistant breeds.

1.8 Statement of the problem

Namibia is richly endowed with beef cattle breeds including the Nguni and Afrikaner breeds of Sanga cattle and Bonsmara which have a major contribution to the country's economy. However, these breeds in particular the Nguni are threatened by indiscriminate crossbreeding with exotic breeds such as Hereford, Santa Getrudis, Aberdeen Angus and Simmental. This is of great concern in Africa as it causes genetic erosion which gradually leads to extinction of species. It is reported that approximately one to two breeds of domesticated animals are lost every week due to extinction thus decreasing the unique genetic resources they possess (Scherf, 2000). Twenty–two percent of the cattle breeds are believed to be extinct already in just the last century, while 32% is believed to be at risk of extinction as a result of loss of genetic diversity caused by reckless uncontrolled mating and other factors (Rege, 1999). Loss of genetic diversity in indigenous cattle reduces fitness components such as survival, reproductive output, growth rates and their ability to adapt to environments with harsh climatic conditions and to respond to ticks and disease challenges the animals are subjected to (Sommer, 2005). Immediate steps must therefore be taken to conserve indigenous cattle.

An important strategy in breed conservation and establishing breed risk status is genetic characterization using molecular markers (FAO, 2015). In Namibia, only a limited number of studies focused on the characterization of cattle breeds at a molecular level (Hanotte *et al.*, 2000; Nortier *et al.*, 2002) and no study has been carried out on the population structure and genetic diversity of the Namibian Bonsmara and Afrikaner cattle. Therefore, there is need to characterize and document

the genetic diversity of the cattle breeds in Namibia to aid in their conservation, management and understanding their evolutionary history. In addition, molecular characterization of variation with immune system genes can help elucidate the mechanisms of disease resistance observed in indigenous cattle breeds.

Ticks cause substantial losses in tropical cattle production in terms of diseases, reduced productivity, fertility and often death. It has been estimated that approximately 80% of the world's cattle population are affected by ticks and TBDs (Marcelino *et al*., 2012). The use of acaricides as the primary tick-control method continues to be limited by the emergence of acaricide-resistant strains, accessibility to resource-poor farmers, increasing costs, environmental and food-related impacts such as chemical residues in meat, milk and the environment and environmental pollution (Robbertse *et al*., 2016). Furthermore, a large component of the economic cost associated with ticks is attributed to acaricides (Kivaria *et al*., 2006). The short comings in the use of acaricides and vaccines indicate that attention should be directed to alternative methods. Several experimental studies have indicated that host genetic diversity influence resistance to ticks and TBDs (Porto-Neto *et al*., 2011). Genetic diversity of the BoLA has been associated with resistance and susceptibility to ticks and TBDs in cattle (Untalan *et al*., 2007; Duangjinda *et al*., 2013). Molecular markers measures genetic diversity and can be used to select cattle with resistance to ticks and TBDs in marker-assisted selection (MAS) breeding programs. Integrating current breeding programs with selection for resistance to ticks and TBDs in cattle seems to be the promising and effective method of controlling ticks. The information available indicates that no study have so far been carried out to identify BoLA genes associated with tick and TBD resistance in cattle breeds in Namibia hence the need for this study.

1.9 Aim and Objectives

The aim of this study was to identify the genetic diversity of three beef cattle breeds in Namibia namely, Nguni ecotypes, Afrikaner and Bonsmara cattle using four microsatellite markers (DRB3, DRBP1, RM185, and BM1815) associated with the BoLA genes locate on bovine autosome 23 (BTA 23). The level of genetic diversity was then used to test for association with tick and TBD resistance. This was achieved by setting the following objectives:

1. Identify the genetic diversity and population structure within and between the Nguni ecotypes, Bonsmara and Afrikaner cattle breeds in Namibia using BoLA microsatellite markers.
2. Compare seasonal variation of tick burden on the beef cattle (Nguni ecotypes, Bonsmara and Afrikaner) in Namibia.
3. Identify the tick species infesting the Nguni ecotypes, Bonsmara and Afrikaner cattle breeds in Namibia.
4. Screen for tick-borne pathogen infection in Nguni ecotypes, Bonsmara and Afrikaner cattle breeds in Namibia.
5. Identify the BoLA class II alleles associated with resistance to ticks and TBDs in beef cattle breeds in Namibia.

1.10 Hypotheses

H_{01}: There is no genetic diversity within the BoLA microsatellite loci in the Nguni ecotypes, Bonsmara and Afrikaner cattle breeds in Namibia

H_{02}: There is no significant association between the BoLA class II alleles and tick and TBD resistance is in beef cattle breeds in Namibia

H_{03}: There is no seasonal variation in tick burden infestation on the Nguni ecotypes, Bonsmara and Afrikaner cattle in Namibia

1.11 Significance of the study

This study will contribute information to establish the risk status of the cattle breeds in Namibia, such as inbreeding and extinction which is essential for *in situ* conservation of threatened breeds. Information on genetic diversity of the cattle breeds may serve as reference for genetic improvement, establishing conservation policies and in understanding the adaptation of these breeds to their local environments. The cattle's genetic diversity and its association with resistance to ticks and TBDs can be used in MAS programs to improve the herd's resistance to ticks and TBDs. At present, there is no available scientific information on cattle breeds in Namibia with regards to their resistance to ticks and TBDs and possible association with the BoLA genetic diversity.

This study has the potential to underpin future genetic research in cattle breeds in Namibia and ultimately aid future marker-assisted selective breeding programs for animals with superior disease resistance and productivity. The characterization of animal genetic resources is essential in assessing the value of African cattle breeds which can guide decision making in livestock development and breeding programmes. The results on tick species identification will be useful in understanding the distribution of tick species, the prevalence of *Anaplasma/Ehrlichia* and *Babesia/Theileria* parasites and the epidemiology of diseases caused by these pathogen in Namibia. It is envisaged that this research project will significantly add to the existing body of knowledge on the genetics of cattle breeds in Namibia.

CHAPTER 2 : LITERATURE REVIEW

2.1 Introduction

The origin of ancient and modern cattle in Africa is still a matter of debate among researchers. Mitochondrial and Y-chromosome DNA evidence suggests independent domestications of indicine or humped cattle in South Asia and of taurine or straight-backed cattle in southwest Asia (Loftus *et al*., 1994). It is hypothesized that taurine cattle were the first to arrive in Africa around 7000 BP from south west Asia (Brass, 2012) and spread to southern Africa around 2000 BP (Smith, 2000). Since their arrival in Africa, extensive crossbreeding occurred between taurine and indicine cattle resulting in Sanga cattle (Hanotte *et al*., 2002). Today, there are three groups of modern cattle in Africa; taurine, indicine (Zebu) and taurine-indicine (Sanga). Subsequent migration led to Sanga reaching the southern part of Africa as shown in the map in Figure 2.1. The approximate migration route and the origin of African domestic cattle is also shown in Figure 2.1.

Sub-Saharan Africa has a rich diversity of cattle with at least 212 breeds (FAO, 2015) of which 47 are considered to be at risk of extinction (FAO, 1999). These cattle breeds vary in their molecular or genetic diversity and advances in technology have enabled researchers to study these differences (FAO, 2015). Molecular characterization of breeds enables identification of breeds at risk of extinction and most importantly identification of breeds with favourable alleles for adaptation (Bishop & Morris, 2007). This chapter reviews the genetic diversity and tick resistance of *Bos taurus* and *Bos indicus* breeds, effects and control of ticks. A short review on the three cattle groups in Namibia is also included. Figure 2.1 shows the approximate migration route and the origin of African domestic cattle.

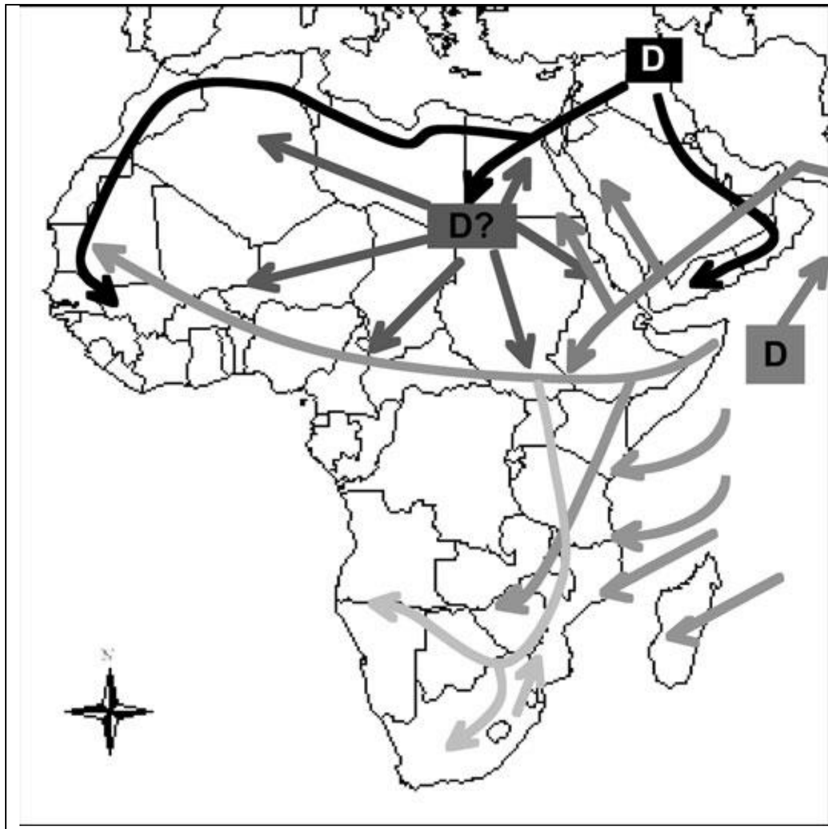


Figure 2.1. Migration route and two main centres of cattle domestication (indicated by a D) of African cattle. Migration of taurine cattle (black and/or darkest grey arrows), Zebu (lighter gray arrows) and Sanga cattle indicated by the lightest grey arrows (Gifford-Gonzalez & Hanotte, 2011).

2.2 Indigenous and locally adapted beef cattle breeds in Namibia

According to the 2014 livestock census there are 2.9 million cattle in Namibia; this indicates a 9% increase from 2013 (MAWF, 2015). Cattle farming is the most important agricultural economic activity in Namibia contributing largely to agricultural outputs (Els, 2004). Indigenous cattle meat is one of the top agricultural commodities produced in Namibia with an estimated value of production worth N$ 2.1 million (MAWF, 2012). Furthermore, indigenous Nguni ecotpyes of Sanga cattle reared on communal land comprise a large proportion of the cattle population in Namibia (Els, 2004). Popular cattle breeds reared in commercial farms include Afrikaner (Sanga), Brahman (Zebu), Bonsmara (Sanga-exotic) and Simmentaler

(taurine). Afrikaner and Bonsmara cattle are some of the few locally adapted breeds (MAWF, 2013). Figure 2.2 shows a map of cattle densities in Namibia.

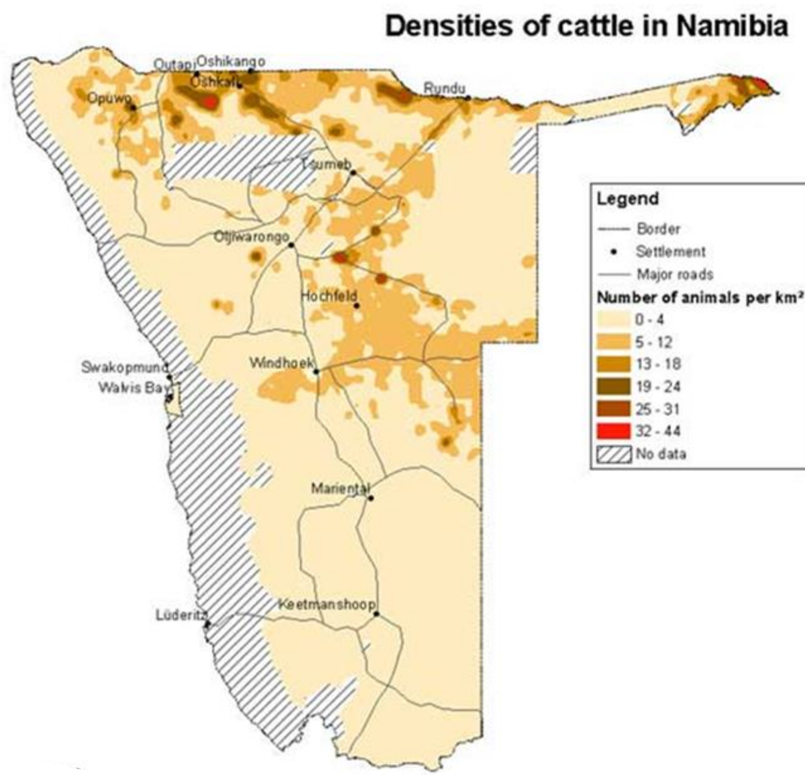


Figure 2.2. A map of cattle densities in Namibia (Ministry of environment and tourism, 2003).

2.2.1 Nguni breed

The Nguni breed is a subtype of Sanga and was introduced to southern Africa by nomadic people from north, central and west Africa (Bester *et al*., 2003). They are widely distributed in southern Africa including South Africa, Zambia, Mozambique, Malawi and Swaziland.Four Nguni ecotypes exist in Namibia, namely: Kunene also known as Kaoko, Ovambo, Kavango and Caprivi. The names relate to the tribes and geographical areas where they were initially distributed.

Nguni cattle have characteristic small to medium body frames, long-lyre shaped horns and black-tipped noses (Makina *et al*., 2014). Their soft, glossy and shiny coats take different colours and patterns which can either be whole black, red,

red roan, grey roan, red or black dappling on white in a matching fashion or on either side of the body (Porter, 1991). Nguni cattle have good depth, moderate width and characteristic long flexible tails that dislodge irritating insects. Also, Nguni cattle are well adapted to walking long distances between the water points and available grazing, often going two days without water (Bester *et al*., 2003).

Historically, the Ovambo and Kavango populations split first, and much later the Kavango and Kunene populations, which are on land the furthest apart (Els, 2002). Genetic analysis of the Namibian Nguni ecotypes suggest that the Caprivi ecotype split from the Kunene ecotype because of the small genetic distances (F_{ST} = 0.061) which also correlates with the gene flow (Nm = 11.578) between the populations (Nortier *et al*., 2002). The Owambo ecotype is the common ancestor and also the smallest of all the Nguni ecotypes in Namibia weighing about 160-225kg (Porter, 1991). Their small frame is attributed to the dry environments and low rainfall of Owamboland relative to Kunene, Kavango and Caprivi/Zambezi regions. The Kavango ecotype is the largest of the Namibian Nguni cattle types and possesses the heaviest bone structure (Els, 2002). The Kunene Nguni ecotype of Namibia is characterized by its long slender legs, large variation in horn shapes, and larger than normal hooves. Their coat colors are red, black, pied or line-backed (Figure 2.3) and have large horns approximately 100 cm (Porter, 1991). The Caprivi Nguni ecotype is characterized by its fine bone structure, even in mature animals. Previous research indicated that the Caprivi ecotype has high nitrogen retention in its body, which enables it to better digest poor quality grazing (Els, 2002). This probably contributed to their adaptation to the highly leached soils of the Caprivi. Phenotypic characteristics of the Nguni cattle in Namibia are shown in Figure 2.3.

Lepen (1996) demonstrated the excellent reproductive performance of the Namibian Nguni cattle i.e. high weaning weight, less prone to dystocia and high calving percentage compared to Afrikaner, Hereford, Simmentaler and Santa Getrudis cattle. Nguni cattle require low maintenance which make them an ideal breed to keep in low input communal areas (Mapiye *et al*., 2007). Furthermore, their good walking ability serves them well when walking long distances in search of water and grazing (Bester *et al*., 2003).

Nguni cattle have been found to have significantly low tick burdens of adult *H. rufipes, Rhipicephalus follis, R. appendiculatus, R. decoloratus, R. evertsi evertsi* and *R. microplus* ($P \leq 0.05$) compared to the Bonsmara cattle (Nyangiwe *et al*., 2011). These results concur with that of Rechav *et al*. (1991) and Marufu *et al*. (2011) confirming that Nguni cattle are more resistant to ticks than Bonsmara cattle. Scholtz *et al*. (1991) analysed the effect of tick infestation on the productivity of cows of three breeds (Nguni, Bonsmara and Hereford) and found that the productivity of Nguni cows, as measured by the weaning masses of their calves was also least affected compared to the Bonsmara and Hereford. Nguni cattle have been reported to be more resistant to ticks than Afrikaner cattle (Spickett *et al*., 1989). This implies that Nguni cattle are less susceptible to tick infestation than either the Bonsmara or Afrikaner cattle. Their ability to tolerate ticks and diseases has also been demonstrated by Marufu (2008) and Mapholi *et al*, (2016).

Temperamentally, Nguni are docile and possess good mothering abilities (Bester *et al*., 2003). Therefore their ability to grow and reproduce in harsh environments has increased their use as dam lines in crossbreeding systems for sustainable meat production (Scholtz & Theunissen, 2011). Nguni cattle produce the most kilograms of beef per hectare at the lowest cost (Nguni Cattle Breeders

Association [NCBA], 2008). Furthermore, the beef quality of Nguni finished on natural pasture is comparable to beef quality of European breeds (Muchenje *et al*., 2008). One of the breed standards of the NCBA aims is to improve the reproductive efficiency of Nguni cattle (NCBA, 2008). Nguni cows of Namibia were reported to have higher reproductive performance than Afrikaner and Hereford cows (Lepen, 1996). Their average age at first calving and calving interval is 34 months and 400 days respectively (Marciel *et al*., 2016). Research has revealed that Nguni cows are less prone to dystocia compared to Afrikaner and Hereford cattle (Lepen, 1996).

Nguni cattle are perceived as inferior due to their small frame sizes and as a result they are being replaced and crossbred with high yielding exotic breeds. In the process, their unique genetic attributes are lost especially those responsible for adaptations to the ever changing African environmental challenges (Mwai *et al*., 2015). Although the goal of crossbreeding is to increase production, uncontrolled crossbreeding can cause genetic erosion if no pure-bred population is maintained (Scholtz & Theunissen, 2010). Namibia has taken action to conserve the indigenous Nguni breed. For example, the Directorate of Agricultural Research and Training (DART) have herds of Nguni ecotypes for conservation at three research stations, namely; Sonop, Omatjenne and Sandveld located in Grootfontein, Otjiwarongo and Gobabis respectively. Furthermore, livestock development centres (Sachinga, Oshaambelo and Okapya) have been set up in the NCAs for *in situ* conservation and livestock improvement programmes (Els, 2004). The Nguni Cattle Breeders Association of Namibia (NCBAN) was established in 1994 to conserve and enhance the unique characteristics of the Nguni breed and to promote the production of the breed (NCBAN, 2011). So far the association has registered 40 breeders of which 30 are stud breeders. As the main indigenous cattle breed of Namibia, it is important to

conserve the Nguni to preserve their unique qualities such as disease resistance for optimal production under harsh environmental conditions of Namibia.

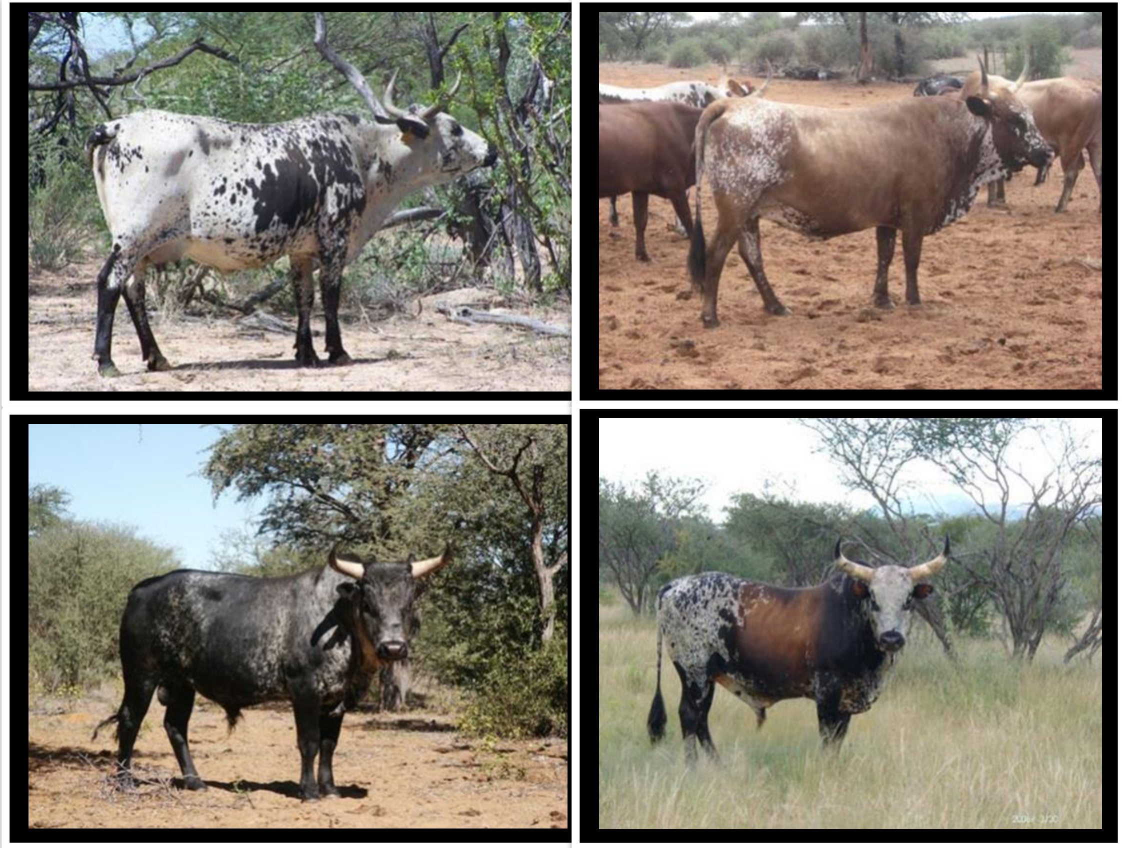


Figure 2.3. Different Phenotypic characteristics (coat colours) of the Nguni cattle in Namibia (Nguni Cattle Breeders Association of Namibia, 2011).

2.2.2 Afrikaner breed

Afrikaner is a locally adapted Sanga sub-type with a medium frame and characteristic yellow to red coloured smooth, glistening coats and a massively long coffin head with a broad brow and long oval horns spreading backwards, forwards and upwards (Porter, 1991). What was once known as the powerful, hardy, docile draught, rangy, long legged Hottentots cattle is now the modern Afrikaner (Figure 2.4). A mature female Afrikaner weights between 550kg and 730 kg. Afrikaner cattle have a first calving interval of 441 days and their age at first calving varies between 36 to 41 months (Collins-Lusweti, 2000). The breed was almost exterminated when

huge numbers died of rinderpest more than a hundred years ago during the South African war (Martin & Rafi, 2006). The Afrikaner was the first indigenous South African breed to form a breed society in 1912 (Makina *et al*., 2014). The breed has been bred according to the breeds standards and decades of selection has changed its shape, ranginess and colour which is now uniformly red (Porter, 1991).

The Afrikaner breed is widespread throughout southern Africa and is the main ranching breed in southern Africa. As an indigenous breed in southern Africa, Afrikaners are adapted to the harshest subtropical environments characterized by dry and low-nutrition grazing areas and limited water. Afrikaner cattle are reported to be more resistant to ticks than Bonsmara cattle (Spickett *et al*., 1989; Rechav *et al*., 1991).The mild temperament of this breed in addition to its grazing and browsing abilities makes it easy to handle and a good choice in utilising the rangeland available. Afrikaners are also acknowledged for their good quality meat being tender, tasty and succulent (Strydom *et al*., 2000). Other economically important adaptive traits of Afrikaners include and heat tolerance (Bonsma, 1949), draught power, disease resistance and good walking ability (Porter, 1991). These desirable traits have led to Afrikaner-exotic crossbreeds such as Bonsmara. In Namibia, Johan Holms crossed the Afrikaners with Brown Swiss to create the Holmonger breed (Porter, 1991). In addition, Namibian beef Nuras is a crossbreed of 25% Simmental and 25% Hereford and 50% Afrikaner. Figure 2.4 shows the phenotypic characteristics of Afrikaner cattle in Namibia.

Figure 2.4. Phenotypic characteristics of Afrikaner cattle in Namibia

2.2.3 Bonsmara breed

The most well-known Afrikaner-based breed is the Bonsmara. The uniformly red to light brown beef breed was created by Prof Jan Bonsma at Mara and Messina Research Station from 1937 to 1963 using 3/8 Milk Shorthorn or Hereford, and 5/8 Afrikaner cattle with the aim to produce a locally adapted beef breed (Bonsma, 1980). The name is of course derived from Professor Bonsma and the name of the farm where the breed was developed. Bonsmara cattle are medium framed and the adult males weight between 544kg and 950kg, while the female weight ranges from 300kg to700kg (Scholtz *et al*., 2010). Bonsmaras are bred and selected according to the Bonsmara cattle breeders society standards which promotes cow efficiency and and efficient production methods. This breed is widely acknowledged for the following traits; excellent meat quality (Muchenje *et al*., 2008), fertility (average of 414 days interval period and a calving interval of 401 days), milk production, growth and adaptability (Corbet *et al*., 2006; Scholtz *et al*., 2010). Tick resistance in Bonsmara cattle have been reported (Spickett *et al*., 1989; Rechav *et al*., 1991; Marufu *et al*., 2011; Nyangiwe *et al*., 2011). Genetic parameters of tick resistance in Bonsmara cattle were estimated and a heritability estimate of 0.17 was obtained

suggesting that genetic variation for tick count exists in the Bonsmara cattle (Budeli *et al*., 2009). This breed is adapted to warm bushveld and subtropical areas making it suitable for farming in harsh environmental conditions. Figure 2.5. shows the phenotypic characteristics of Bonsmara cattle in Namibia.

Figure 2.5. Phenotypic characteristics of Bonsmara cattle in Namibia

2.3 Molecular characterization of livestock genetic resources

Traditionally, breeds are characterized based on their phenotypic characteristics. Advances in technology have increased the capacity to characterize breeds using genetic information. In 1993, the FAO proposed a global programme for characterization of animal genetic resources including molecular genetic characterization (FAO, 2011). Molecular characterization is more informative than phenotypic characterization and allows inferences on the breeds risk status such as inbreeding, structure and provides some indication of the biological basis for the observed characteristics (FAO, 2015).

Molecular markers defined as "DNA locations presenting different detectable variants" (Toro *et al*., 2009) are the major tools used in molecular characterization as they reveal polymorphism at the DNA level (p.174). Also known as genetic makers,

these pieces of DNA can be part of the gene of interest or can be linked to it. Conversely, molecular markers should not be considered as normal genes as they usually do not have any biological effect (Ruane & Sonnino, 2007). Instead they should be considered as constant landmarks in the genome. Applications of molecular markers in genetic studies include; MAS, marker-assisted introgression (MAI), assessment of genetic variation and identification of disease carriers. Application of molecular markers in bovine genetics research includes markers in milk quality and production (Grisart *et al*., 2002; He *et al*., 2006), disease resistance (Coussens & Nobbis, 2002), thermo-tolerance in cattle (Hansen, 2004) and meat tenderness (Casas *et al*., 2006). They can also be used efficiently in breeding and management decisions in order to increase productivity. Many studies have focused on molecular characterization of European breeds leaving the molecular diversity of local indigenous breeds in Africa largely unknown (Engelsma *et al*., 2012; Miyasaka *et al*., 2012; Takeshima *et al*., 2015). Various molecular markers exist such as restriction fragment length polymorphisms (RFLPs), microsatellites and single nucleotide polymorphisms (SNPs) markers. These markers differ in many ways including costs, technical requirements, the amount of genetic variation detected and reproducibility.

2.3.1 Restriction fragment length polymorphism (RFLP)

First developed in 1980, RFLP uses restriction enzymes to cut sequences of DNA at specific sites, producing sets of fragments of different sizes (Uddin & Cheng, 2015). The traditional RFLP is based on a nucleic acid hybridization technique called Southern blotting. The procedures involve digestion of the DNA sample with restriction enzymes. The restriction enzymes cut the double-stranded DNA wherever an enzyme-specific sequence of bases occurs, thereby generating a mixture of discrete fragments which are separated on agarose gels by gel

electrophoresis. The separated DNA fragments are transferred as denatured (single-stranded) arrays to a filter membrane and are subsequently incubated with labelled probes (probe hybridization). The presence of restriction sites of individual samples may differ due to nucleotide sequence variation at the site or the distance between restriction sites may differ due to insertions or deletions of nucleotides allowing polymorphism to be detected among individuals (Edwards *et al*., 2004). Allelic variation for a gene or genotype at an RFLP locus can occur where the hybridizing targets are of unequal length, which can be recognized by a difference in the migration of the fragments through the gel. A simpler modern use of RFLPs approach is RFLP-PCR which involve digestion of PCR products by restriction enzymes hence generating DNA fragments of different sizes. The digested amplicons are generally resolved by electrophoresis.

Analysis of RFLP variation in genomes was a vital tool in human genome mapping and genetic disease analysis (Vignal *et al*., 2002). To determine the chromosomal location of a particular disease gene, the DNA samples are analyzed for RFLP alleles that show a similar pattern of inheritance as that of the disease. Once a disease gene was localized, RFLP analysis of other families would reveal who was at risk for the disease, or who was likely to be a carrier of the mutant genes (Marwal *et al*., 2014). This technique was the first molecular marker used to detect genetic variability in organisms. Moreover, it was used to perform the first large scale effort in mapping the human genome (Vignal *et al*., 2002). RFLP markers are well known for being stable and highly reproducible (Mburu & Hanotte, 2005). However, one major limitation of this technique is that in many cases, only two alleles at each locus are revealed and thus has low level of polymorphism (Ben-Ari & Lavi, 2012). In addition, RFLP markers are time-consuming and restriction enzymes tend to be

expensive. Simpler marker systems have subsequently been developed, many of which are based on satellite DNA sequences. Table 2.1 shows the recent reports on applications of RFLP-PCR (Restriction Fragment Length Polymorphism - Polymerase Chain Reaction) in cattle genetics research.

Table 2.1. Some recent reports on application of restriction fragment length polymorphism – polymerase chain reaction (RFLP-PCR) in cattle genetics research

Breed	**Application**	**Reference**
Sahiwal, Hariana	Milk production	Sharma *et al*., 2016
Holstein	Milk production	Velez *et al*., 2016
Chinese Holstein	Disease association	Liu *et al*., 2016
Holstein-Friesian	Milk production	Zaglool *et al*., 2016
Holstein	Disease association	Bagheri *et al*.,2016

2.3.2 Microsatellites markers

Restriction fragment length polymorphism markers lost their popularity to microsatellites which after 1990 transformed the science of molecular genetics (Ellegren, 1992). Microsatellites were the first markers to take full advantage of PCR technology (Litt & Luty, 1989). Microsatellites are short tandem repeats of about one to six nucleotides present throughout the genome (Toro *et al*., 2009). As such they are also known as simple sequence repeats (SSRs), short tandem repeats (STRs), simple sequence tandem repeats (SSTRs) and variable number tandem repeats (VNTR). The sequence CACACACACA and ATATATAT are examples of microsatellites. Microsatellites have high mutation rates which create variations or alleles that contribute to the adaptation potential of a population (King *et al*., 1997). Microsatellite loci are individually amplified by PCR using pairs of oligonucleotide primers specific to unique DNA sequences flanking the SSR sequence. The most common way to detect microsatellites is to design PCR primers that are unique to one locus in the genome and the base pair on either side of the repeated portion (Marwal

et al., 2014). The PCR-amplified products can be separated in high-resolution electrophoresis systems, for example, agarose electrophoresis (AGE) and polyacrylamide gel electrophoresis (PAGE), and the bands can be visually recorded by fluorescent labelling or silver staining. An automatic sequencing machine is used for allele size determination (Vignal *et al*., 2002). Microsatellite markers are one of the most popular markers in livestock molecular genetic characterization studies due to their many desirable attributes such as abundance of alleles per locus, co-dominance, high reproducibility, easy genome coverage, amenability to automation, high throughput coverage (Parida, *et al*., 2009). All these attributes enhance their information content over other types of markers (Singh *et al*., 2014; Vignal *et al*., 2002).

Despite their popularity, microsatellites have pitfalls some of which can be avoided by careful selection of loci. Mutation mechanism of microsatellite can be complex or unclear (Ellegren, 2004). Analyses such as genetic distances rely on mutational models, it is therefore important to know the exact mutational mechanism. Inconsistencies in allele size calling of microsatellites are a major challenge when comparing data from different laboratories (Vignal *et al*., 2002). Heterozygotes may be misclassified as homozygotes when mutation occurs in the primer region, leading to one allele being amplified (Mburu & Hanotte, 2005; Selkoe & Tooten, 2006). Some of the recent publications on application of microsatellites in cattle genetics research are shown in Table 2.2.

Table 2.2. Some recent reports on application of microsatellite markers in cattle genetics research

Breed	Application	Reference
Nguni	Molecular characterization of ecotypes	Sanarana *et al*., 2016
Malnad Gidda,	Molecular characterization	Ramesha *et al*., 2016
Yakutian, Busha	Molecular characterization	Iso-Touru *et al*., 2016
Zebu	TBDs association	Pothmann *et al*., 2016
Bachaur, Gangatiri	Molecular characterization	Sharma *et al*., 2016

2.3.3 Single nucleotide polymorphism (SNP)

Single nucleotide polymorphism (SNP) is a DNA sequence variation that results from a single base mutation which substitutes one nucleotide for another at a single location within the genome (Yang *et al*., 2013). More than half (2/3) of the SNPs involve a substitution of cytosine (C) with thymine (T). A variation can only be classified as a SNP if it occurs in at least 1% of the population. An example of an SNP is when the G in a gene containing ACA**G**A is substituted by an A to form ACA**A**A. If this variation occurs within a gene, the gene is described as having more than one allele. Because only about 3% to 5% of an organism's DNA codes for proteins, most SNPs are found outside the regions of genes of interest or in non-coding regions (Tak & Farnham, 2015). All the major SNP detection techniques depend on an initial PCR amplification of the target DNA segment (Jiang, 2017). The main categories of SNP genotyping techniques include direct hybridisation techniques from allele specific oligonucleotides to chips and techniques involving the generation and separation of an allele-specific product such as PCR – RFLP. Two commercial approaches, KASPar® and TaqMan® assays are used that allow affordable typing of a single to hundreds of SNP (FAO, 2011). The main advantage of SNPs is their high potential for an automated high throughput analysis (Chen & Sullivan, 2003).

Some SNPs are associated with disease resistance or susceptibility and these are of particular interest to researchers. SNPs are likely to substitute microsatellites as they continue gaining popularity. Amongst all markers, SNPs are the most abundant (Schork, 2000). Advantages that have contributed to the emergence of SNPs relative to microsatellites include stability, low mutation rates, very informative in the sense that they detect both neutral and functional genetic variation, re-combinational oddities i.e. they show patterns of linkage equilibrium which is essential in association studies (Toro *et al*., 2009; FAO, 2011; Kohn *et al*., 2006).

A number of caveats should however be noted when using SNPs. To derive a true DNA fingerprint, a large number of SNPs is required relative to microsatellites (about six SNPs are equivalent to one microsatellite) because microsatellite loci typically have many alleles, whereas SNP loci have two (Morin *et al*., 2004). In addition, previous knowledge of the sequence is required i.e. sequencing is necessary, specialized skills and computing infrastructures are required in high density SNP screens as it produce a large amount of data. Furthermore, SNPs are expensive to develop and can be time consuming. SNPs are usually biallelic, therefore the information content of a single SNP is limited. Some recent reports on their applications in animal genetics research are shown in Table 2.3.

Table 2.3. Recent reports on the application of single nucleotide polymorphism (SNPs) in cattle

Breed	Application	Reference
Brown Swiss	Genome Wide Study (GWS) of copy number variants	Prinsen *et al*., 2016
Sanga	Genetic characterization	Makina *et al*., 2016
Sarabi, Najdi	Genetic characterization	Karimi *et al*., 2016
Chillingham	Inbreeding	Williams *et al*., 2016
Canadian Angus	Feed efficiency trait	Li *et al*., 2016

2.4 Classical estimators of genetic diversity from molecular data

Quantification of genetic variation within and between breeds is very important in understanding the effects of mating and selection but most importantly in conservation of genetic resources (Oldenbroek, 2007). Given the progress and advances in computational population-genetic analysis, various strategies of analysing molecular diversity exist (FAO, 2011). The determination of genetic structure and analysis of genetic variation within and between breeds remain the gold standard of characterizing livestock genetic resources.

A common parameter in most molecular characterization of animal genetic resources studies is heterozygosity also known as gene diversity (Nei, 1973). Shete *et al*. (2000) defined heterozygosity as the probability that an individual chosen at random from the population is heterozygous at a locus. The FAO describes it as one of the basic analysis undertaken in nearly all studies of molecular diversity (FAO, 2011). Measures of heterozygosity vary between 0 and 1. While high values of heterozygosity suggest high genetic variation, low values indicate low genetic variation (Mpofu *et al*., 2006). The difference in observed and expected heterozygosity reportedly indicates non-random mating (Mburu & Hanotte, 2005) and selection of high heterozygosity (approaching 0.50) loci provides the greatest power for parentage analysis (Morin *et al*., 2004). Heterozygosity can be calculated using the following formula:

$$\boldsymbol{H = 1 - \sum_{i=1}^{k} Pi^2},$$

where Pi is the frequency of the ith of k allele (Shete *et al*., 2000).

The observed level of heterozygosity is usually compared to the expected level under Hardy-Weinberg equilibrium (HWE). The HWE law predicts that genetic variation (genotypic frequencies) will remain in equilibrium (remain the same) from

generation to generation in the absence of disturbing factors such as natural selection, non-random mating and division of population into subgroups (Hardy, 1908; Mayo, 2008). The polymorphism information content (PIC) is similar to the heterozygosity index but subtracts the probability of uninformative mating. PIC is a measure of how informative a marker is in genetic diversity analysis of breeds commonly used in linkage analysis (Botstein *et al*., 1980).

Allelic richness is defined as the mean number of alleles per locus. Allelic richness has been proven to be a more powerful tool than heterozygosity in measuring genetic diversity (Leberg, 2002). Additionally, it has been argued that allelic richness is more sensitive to bottlenecks and a better indicator of past demographic changes (Nei, 1975; Petit *et al*., 1998; Leberg *et al*., 2002). The problem of uneven sample sizes in allelic richness was statistically solved by Hurlbert (1971), who introduced the technique of rarefaction making allelic richness an even more useful tool. Petit *et al*. (1998) stressed that allelic richness should be prioritised as a measure of genetic diversity. In their study on the allelic richness of *Glacial refugia*, Widmer & Lexer (2001) demonstrated the importance of making use of both gene diversity (heterozygosity) and allelic richness in genetic diversity studies to avoid misleading results. Private alleles (unique alleles observed in only one population) can also be used to measure genetic distinctiveness (Kalinowski, 2004).

The Fixation index also known as F statistics introduced by Wright (1949) offers a convenient means of characterizing genetic differentiation between species and analysing structures of subdivided populations. The F parameters are correlation coefficients between uniting gametes. These parameters include F_{ST}, F_{IT} and F_{IS}. The parameter F_{IS} (where '$_{IS}$' refers to individuals within subdivision) is also known as the inbreeding coefficient and is a good indicator of inbreeding as it measures the

deficiency or excess of the average heterozygotes in a population (Weir & Cockerham, 1984). Fixation index ranges from 0 to 1 indicating no differentiation and maximum differentiation respectively (Ojango, 2011). F_{ST} (where '$_{ST}$' refers to subdivision within total) measures the degree of gene differentiation among populations in terms of allele frequencies, whereas F_{IT} (where '$_{IT}$' refers to individuals in total) measures the deficiency or excess of average heterozygotes in a group of populations. F_{ST} is always positive while F_{IS} and F_{IT} can be negative (Wright, 1949).

The fixation index formula introduced by Wright is as indicated below:

$$\mathbf{1-F_{IT} = (1-F_{IS})\ (1-F_{ST})}$$

Where,F is the correlation between alleles in uniting gametes.

F_{ST} is the correlation between random gametes, drawn from the same population relative to the total.

F_{IT} is the correlation between two uniting gametes to produce the individuals relative to the total population.

F_{IS} is the correlation between two uniting gametes to produce the individuals relative to the subpopulations.

Although Wright's F statistic is very useful in genetic diversity analysis, the formula has been made under the assumption of neutral genes and infinitely large subpopulations and may not apply in the presence of selection, migration and finite population. Several authors have since tried to reformulate the F-statistics, leading to F_{ST} analogues such as R_{ST} (Slatkin, 1995) and G_{ST} (Nei, 1987).

AMOVA (Analysis of Molecular Variance) computes one of the F_{ST} analogues called φ_{ST} as a ratio of variance components (Excoffier, 1992). This parameter quantifies partitioning of the total diversity in a within-breed and an among-breed component brought about by breed formation (FAO, 2011). One of the strong points

of AMOVA is that it uses different hierarchical levels of gene diversity such as geographical location of the breeds in its analysis (Meirmans, 2006). Typically, 50% to 90% of the total diversity corresponds to the within-breed component, depending upon the group of breeds sampled and the sources of variability considered (FAO, 2011). AMOVA differs from Analysis of Variance (ANOVA) in that it may contain different evolutionary assumptions without modifying the basic structure of the analysis and the driving hypothesis uses permutation methods that do not require the assumption of a normal distribution. Furthermore, the mean of squares in AMOVA are computed for grouping at all levels of hierarchy (Ojango, 2011).

Traditionally, individuals are grouped into populations based on physical characteristics, behavioural patterns and geographical locations. However, these groupings are not always consistent with the genetic information (Evanno *et al*., 2005). Advances in technology have made it possible to determine population structures from known and unknown populations based on their genotypes (Pritchard *et al*., 2000). Based on their genotypes, individuals can be clustered using either the distance-based method which calculates a pairwise distance matrix or model-based method which assumes that observations from each cluster are random (Pritchard *et al*., 2000).

The commonly used STRUCTURE program for analysing population structures utilises model-based clustering to analyse genetic structure of populations (Pritchard *et al*., 2000). This is achieved based on the pre-set number of clusters. The program may identify clusters of related breeds, clusters of individuals of the same breed or clusters that correspond to subpopulations within breeds. For each individual the proportion of the genome derived from the inferred clusters are calculated, which may reveal qualitatively introgression events. Furthermore, prior information on

ancestral populations can be introduced in the dataset and be used for supervised clustering.

2.5 Molecular characterization of sub-Saharan Africa indigenous cattle breeds using microsatellite markers

Microsatellites have been used with success in genetic characterization studies of indigenous breeds in SSA. Recently, twenty two microsatellites were used to characterize the Nguni ecotypes of South Africa, revealing a high genetic diversity of 199 alleles and expected heterozygosity values ranging from 69% to 72% (Sanarana *et al*., 2016). In Namibia, Nortier *et al*., (2002) characterized the local Nguni ecotypes using ten microsatellites. The author reported 30 alleles and an average heterozygosity ranging from 66.7% to 74.8%. The Afrikaner cattle have also been characterized using microsatellite markers and have been reported to possess moderate to high degree of genetic variation with an overall heterozygosity estimate of 56.8 % (Pienaar, *et al*., 2014).

The indigenous Mozambican cattle breeds, namely Angone, Landim and Bovino de Tete were characterized using 14 microsatellites by Bessa *et al*. (2009) exhibiting 66.0% to 69.0 % expected mean heterozygosity. Musimuko (2014) characterized the Zambian Angoni, Tonga and Barotse cattle using 32 microsatellites and found 274 alleles and expected heterozygosity values varying from 69.9% to 73.5%. Microsatellite markers have also been used to trace the origin of 69 SSA indigenous cattle breeds by analyzing the frequency of an indicine and a taurine *Y* specific allele. Results confirmed that indigenous breeds in SSA are indeed crosses of indicine and taurine cattle (Hanotte *et al*., 2000). The great genetic diversity exhibited by southern African cattle breeds is a valuable resource of genetic material because of their adaptations to harsh climatic environments.

2.6 The Bovine Leukocyte Antigen (BoLA) and its genetic variation

The MHC of cattle also known as the bovine leukocyte antigen (BoLA) is located on the short arm of bovine chromosome 23 (BTA23) hence distinguishing it from the human leukocyte antigen (HLA) located on chromosome 6 and that of buffalo on chromosome 2 (Fries *et al*., 1993). The MHC is one of the most important components of the immune system that have evolved to protect animals from invading pathogens and carcinomas. The bovine genome assembly has predicted 154 genes within the BoLA regions which include 60 genes within the class I region, 38 within the class IIa and class IIb regions, and 56 within the class III region (Bovine Genome Sequencing and Analysis Consortium, 2009).

The BoLA class II also known as the D region is divided into three sub-regions; DP, DQ, DR. Each of these sub-regions contains two functional genes, A and B encoding the α (DPA, DQA, DRA) and β (DPB, DQB, DRB) chains respectively. Interestingly, no functional DP gene has been identified (Garrick *et al*., 2014). For quite a long time, the DRA chain has been considered monomorphic because only one allele has been identified (Norimine & Brown, 2005), but recent evidence suggest that there is more than one allele at this locus (Zhou *et al*., 2007). Contrarily, the DRB chain in particular DRB3 (the only functional DR molecule) exhibit high levels of polymorphism and is indeed considered as the major source of diversity in DR molecules (Elsik, *et al*., 2009). Figure 2.6 shows the genetic linkage map of the MHC region in cattle.

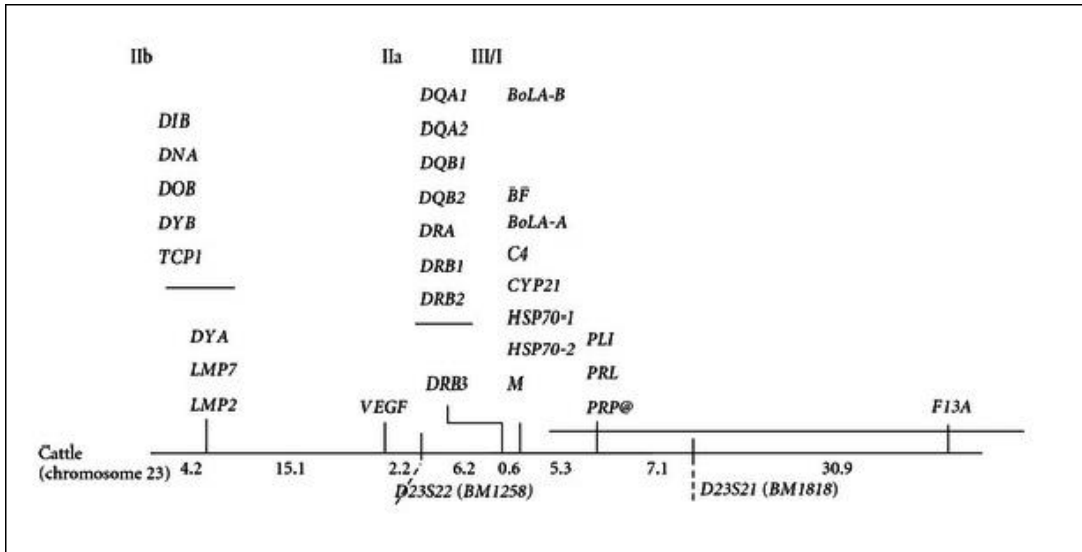


Figure 2.6. Genetic Linkage map of the BoLA region in cattle (Amills *et al*., 1998).

Polymorphism of BoLA genes is important given its function in the immune system and affects antigen recognition by T cells by influencing both peptide binding and the contacts between T cell receptor and MHC molecule (Behl *et al*., 2012). The product of each individual MHC allele can differ by up to 20 amino acids, resulting in distinct proteins (Janeway *et al*., 2001). MHC polymorphism appears to arise from evolutionary pressures and some genetic mechanisms such as point mutations and genetic recombination or gene conversion (Janeway *et al*., 2001). The DRB3.2 is the most researched BoLA gene and has been reported to be the most polymorphic locus (Behl *et al*., 2012). Microsatellite analysis of the BoLA-DRB3.2 region revealed an average of 10.13 alleles in the BoLA-DRB3 region (DRB3, DRBP1, BM1815, RM185) in portuguese cattle (Bastos-Silveria *et al*., 2008). Takeshima *et al*. (2002) reported a total of 21 DRB3 alleles in Japanese Shorthorn cattle, while 17 alleles were reported in Japanese Black (Miyasaka *et al*., 2011). A higher number of 28 DRB3 alleles was reported in Iranian Holstein cattle (Nassiry *et al*., 2005) compared to 19 alleles reported in Iranian Golpayegani cattle ((Mosafer & Nassiry, 2005). The

highly polymorphic nature of the MHC molecules is critical to its role in the immune response of cattle.

2.7 Effects of tick infestation on cattle

Ticks affect their host both directly and indirectly resulting in major losses in both beef and dairy production (Jonsson, 2006). Ticks cause physical damage with their sharp mouth parts and heavy infestations can result in poor meat and hide quality, retarded growth and mortality especially in calves. Most importantly, ticks affect their host indirectly as vectors of pathogens causing severe diseases which reduce production. There is substantial literature on the negative effects of ticks on productivity in beef (Frisch & O'Neill, 1998; Jonsson, 2006) and dairy production (Madalena *et al*., 1990; Jonsson *et al*., 1998; Perera *et al*., 2014).

2.7.1 Direct effects of tick infestation on cattle

Heavy infestation of ticks has major effects on animal health some of which are direct and others indirect. The direct effects of ticks on cattle caused through blood sucking include blood loss, tick-worry and damage to hides (Präffle *et al*., 2009). Heavy infestations often result in anaemia as ticks ingest large quantities of blood, amounting to several hundred times their unfed weight (Jongejan & Uilenberg, 1994). Experiments have shown that one female *B. microplus* tick can cause a daily loss of 1.37 g body weight in *Bos taurus* cattle (Jonsson, 2006). In another study, body weight loss due to infestations with *R. decoloratus* and *R. appendiculatus* were reported to be 1.5 g and 4.4 g per day respectively (Norval *et al.*, 1988).

Reductions in milk production due to ticks have also been reported. For example, in Holstein cattle, milk production was reduced by 8.9 ml per engorging *B. microplus* female (Jonsson, 1998). Rafique *et al.* (2015) reported a 0.5 L reduction in

milk production caused by *R. microplus* in Sahiwal, Red Sindhi and Dahni cows in Pakistan. In Brazil, losses caused by each female *R. microplus* were estimated to be 6.7 L (Rodrigues & Leite, 2013).

During feeding, ticks attach to their hosts' skin with needle like mouthparts called hypostome which cut and pierce the skin (Walker *et al*., 2003). This often results in a wound which later forms a scar after healing. Numerous tick bites result in a lot of scars so that when the skin is processed for leather blemishes appear hence deteriorating the leather quality (Gashaw & Mersha, 2013). Additionally, dermal necrosis which occurs at the site of tick bite coupled with innate inflammatory response can cause skin damage. In Ethiopia, losses due to damaged hides were estimated to be 1 million birr (Abunna *et al*., 2012). While feeding, *Demacentor andersoni* and *H. truncatum* inject toxins causing paralysis and sweating sickness respectively (Latif & Walker, 2016). Reck *et al*. (2014) demonstrated how *R.microplus* tick infestations increases the risk of myiasis in cattle to about four times more than those with a low parasite load. This happens when with cutaneous lesions formed by tick bites exudes tissue fluid and blood scent which attracts *Cochliomyia hominivorax* flies. Female flies lay their eggs in a mass at the edges of wounds and after they hatch, the larvae (maggots) also known as screwworms burrow head first into the living flesh, feed and grow hence enlarging the wound.

2.7.2 Tick-borne diseases of economic importance in sub-Saharan Africa

Tick-borne diseases (TBDs) are one of the most important causes of livestock losses in SSA. Approximately 70% of global beef cattle production and significant dairy production occurs in regions that have the highest prevalence of ticks (Porto-Neto *et al*., 2011). There is substantial literature on the negative effects of ticks on productivity in beef (Frisch & O'Neill, 1998; Jonsson, 2006) and dairy production

(Madalena *et al*., 1990; Jonsson *et al*., 1998; Perera *et al*., 2014). The most notable cattle TBDs in SSA include; Anaplasmosis, Babesiosis, Theileriosis and Heartwater (Madder *et al*., 2013).

2.7.2.1 Babesiosis (Redwater)

Bovine Babesiosis also known as Redwater is one of the most economically important TBDs that affect cattle worldwide but is more prevalent in tropical and subtropical regions of the world (Suarez *et al*., 2011). It is mainly caused by two obligate intra-erythrocytic protozoan parasites namely *Babesia bigemina* and *Babesia bovis* (Bock, 2004). The two species are prevalent in southern Africa and are transmitted by ticks of the genus *Rhipicephalus* which ingest the parasites during a blood meal from infected cattle (Marufu *et al*., 2010). *Bos taurus* breeds are particularly more susceptible to Babesiosis than *Bos indicus* breeds (Duangdjinda *et al*., 2013). The disease symptoms usually appear two to three weeks after tick infestation (Goès *et al*., 2007). Clinical signs of Babesiosis depend on the species and other host factors, however, major signs include anaemia, anorexia, high fever, red urine due to destruction of red blood cells, and in severe cases death (Mtshali & Mtshali, 2013).

Effects of Babesiosis on body weight have been demonstrated. For example in beef cattle, Babesiosis has been to reduce the live weight gain of cattle infected with *Babesia bovis* and *Babesia bigemina* by 45% (Solari *et al*., 1992). Diagnostic methods for *Babesia* parasites include serological diagnostic assays such as the enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR) which is the most highly specific and sensitive diagnostic method (Marufu *et al*., 2010). Figure 2.7 shows the blood stages during the development of *B. bovis* parasites in bovine erythrocyte. Control methods for Babesiosis include

immunisation, tick management, and anti-*Babesia* drugs (Bock *et al*., 2004). However, these control methods are limited by factors such as acaricide resistance

and the drawbacks of vaccines such as short shelf life and requiring a cold chain system of maintenance (Suarez & Noh, 2011).

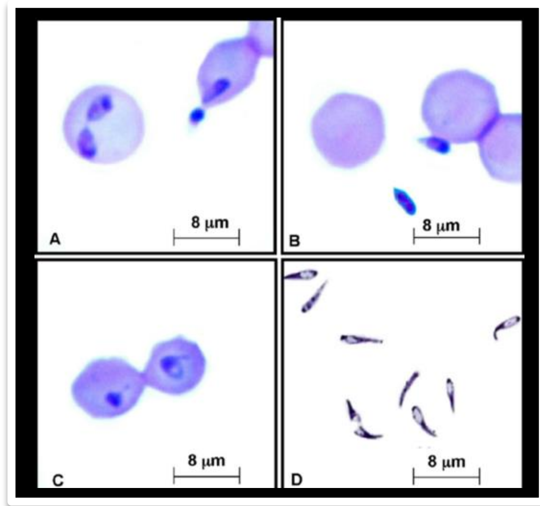


Figure 2.7. Stages of *Babesia bovis* parasites in bovine erythrocyte: (A) a mature intracellular *B. bovis* merozoite pair is shown on the left side of the Panel. (B) *B. bovis* free merozoite, and a free merozoite attaching to a bovine erythrocyte surface; (C) intracellular *B. bovis* trophozoites; (D) B. bovis kinetes isolated from the hemolymph of *Riphicephalus microplus* infected with *B. bovis* (Suarez *et al*., 2011).

2.7.2.2 Anaplasmosis

By definition bovine Anaplasmosis, also known as gall sickness, is an acute to sub-acute disease caused by *Anaplasma marginale* an obligate intracellular parasite that infects erythrocytes (Aktas & Ozubek, 2015). This disease is infectious but non-contagious and is mostly spread by *Rhipicephalus* (*Boophilus*) and *Hyalomma* tick species. *Anaplasma* can also be transmitted through the transfer of fresh blood from infected to susceptible cattle from biting flies or by blood contaminated fomites (Aubry & Geale, 2011). Like most TBDs, bovine Anaplasmosis occurs mostly in tropical and sub-tropical regions worldwide. The severity of this disease increases

with the age of the host; calves are therefore more immune to the disease than older cattle.

Clinically, Anaplasmosis is manifested in a number of forms, from subclinical to fatal, and major clinical signs may include fever, jaundice (icterus), weight loss, abortion, lethargy and often death in animals older than two years (Aktas *et al*., 2015). Traditionally, the acute phase of bovine Anaplasmosis is diagnosed by stained blood smears but this method has proven not to be reliable in detecting infection in carrier animals in which case PCR may be used (Aubry *et al*., 2011; Sharma, 2015). Figure 2.8 shows *Anaplasma* parasites infecting cells of a mouse and tick.

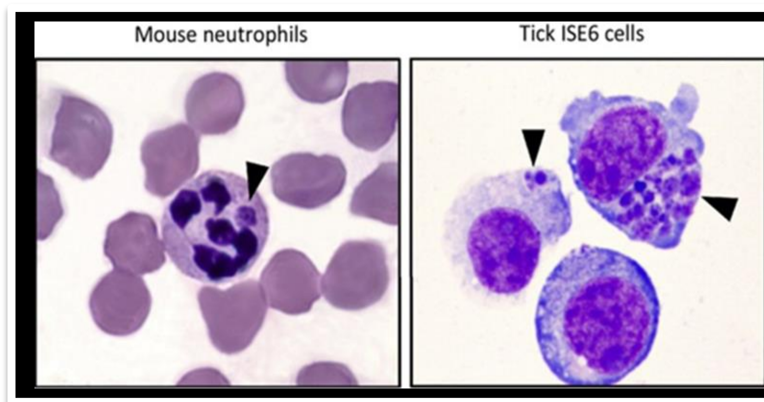


Figure 2.8. *Anaplasma phagocytophilum* infecting erythrocytes of a mouse and tick as indicated by the black arrows (de la Fluente *et al*., 2016).

2.7.2.3 Theileriosis

Theileriosis is a collective name given to infections caused by intracellular protozoan parasites of the genus *Theileria* that are transmitted by ixodid ticks (Perera *et al*., 2013). In 1989, losses due to Theleiriosis in SSA (Burundi, Zambia, Zimbabwe, Kenya, Malawi, Mozambique, Rwanda, Sudan, Tanzania, Uganda and Democratic Republic of Congo) were estimated to be U$ 168 million with 1.1 million cattle mortality (Mukhebi *et al*., 1992). Depending on the type of Theileriosis, a number of hard ticks belonging to the genera *Amblyomma*,

Haemaphysalis, *Hyalomma*, and *Rhipicephalus* can transmit *Theileria* (Jabbar *et al*., 2015). In Africa, *Theileria parva* causes a severe, potentially fatal disease in cattle called East Coast Fever, which is mainly transmitted by *Rhipicephalus appendiculatus* ticks (Olds *et al*., 2016). According to Bishop *et al*. (2009), clinical signs of Theileriosis include pyrexia, enlargement of lymph nodes, loss of weight, and condition, severe pulmonary distress due to oedema, and death in severe cases. The most sensitive method for diagnosing *Theileria* parasites is PCR. However, PCR assays to detect *Babesia* and *Theileria* species simultaneously have also been documented (Nijhof *et al*., 2003).

Effects of Theileriosis on milk production have been demonstrated. For example, Perera *et al*. (2014) assessed the effect of oriental Theileriosis on milk production in dairy cows and found that animals with oriental Theileriosis produced significantly less milk (288 L; $P < 0.001$), milk fat (16.8 kg; $P < 0.001$) and milk proteins (12.6 kg; $P < 0.001$) compared to healthy animals. Figure 2.9 shows schizonts and piroplasm of *Theileria* parasites. This proves that TBDs seriously impair health and productivity of cattle and is therefore important to research and develop effective control methods for ticks and TBDs.

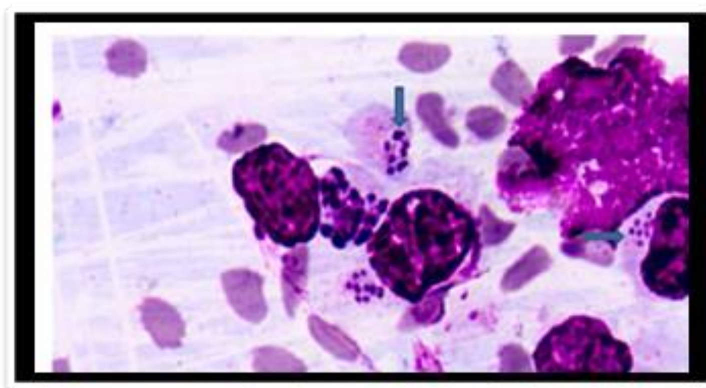


Figure 2.9. Schizonts and piroplasm of *Theileria* parasites indicated by blue arrows (Salih *et al*., 2015).

2.7.2.4. Cowdriosis (Heartwater)

Heartwater is a TBD caused by a rickettsial bacterium previously known as *Cowdria ruminantium* (Walker & Olwage, 1987), but recently reclassified as *Ehrlichia ruminantium* (Bekker *et al.,* 2001). The disease affects ruminants notably cattle, goats, sheep and some wild animals and is transmitted by ticks of the genus *Amblyomma* (Walker & Olwage, 1987). Lounsbury (1900) was the first to prove that this TBD was transmitted by *Amblyomma hebraeum* and *Amblyomma variegatum*. Heartwater is responsible for huge economic losses in SSA and has been suggested to be a potential emerging zoonosis (Sayler *et al*., 2015). While it can cause high mortality rates of up to 90 % in susceptible cattle (Dominique *et al*., 2012), the infection rates of ticks vary according to the season and locality. Heartwater is established in nearly all countries of SSA where its vectors are present. Clinically, acute forms are characterized elevated temperature, loss of appetite, heavy breathing, hanging head, stiff gait, depression, exaggerated blinking and chewing movements, anorexia, hyperaesthesia, lacrimation, convulsions, recumbency and death in severe cases (Allsopp, 2015).

The traditional method of diagnosing Heartwater is from a stained smear of brain tissue (Figure 2.10). Several serological tests have also been developed, these include the indirect immunofluorescences antibody test (IFAT) and the enzyme-linked immunosorbent assay (ELISA) of which is considered the serological “gold standard”. The ELISA has been reported to have low sensitivity resulting in false negatives and is thus recommended that the ELISA test should be repeated 1-2 weeks after the first antibody essay (Harrus *et al*., 2002). Many species-specific PCR assays for most species of tick-borne parasites including *Ehrlichia* have been developed. However, it is possible to simultaneously detect all protozoan and ehrlichial parasites

that could possibly be present in the blood of an infected host or vector using PCR. For example, PCR assays have been developed to detect *Anaplasma* and *Ehrlichia* species simultaneously by targeting the 16S ribosomal RNA gene (Bekker *et al*., 2002). The use of PCR and DNA sequencing is by far the most sensitive diagnostic method for detecting *Ehrlichia* (Biguezoton *et al*., 2016).

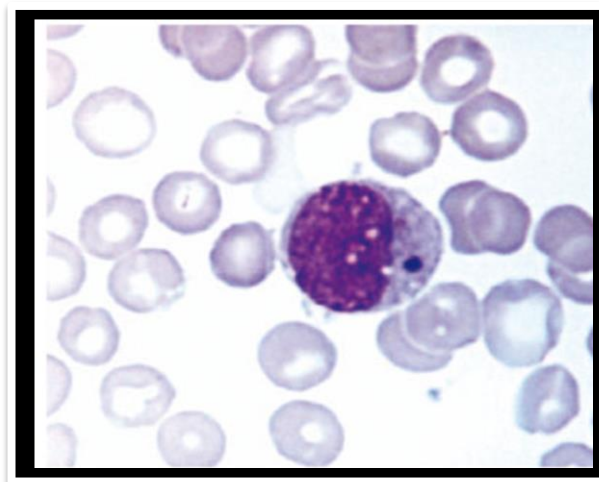


Figure 2.10. *Ehrlichia* parasite inside a macrophage of an infected animal (Vieira *et al*., 2011).

2.8 BoLA DRB3 and DQA genes as informative ticks and TBD resistance markers for assisted breeding selections

Marker-assisted selection is a process where selection of a trait of interest is based on a marker linked to the trait of interest. In other words, MAS is tool for genetic improvement of traits of interest (Yasmeen *et al*., 2014). High-density DNA marker maps provides the framework needed for applications of MAS. Genes affecting traits of interest can be detected by testing for statistical association between marker variants and traits of interest (Ruane & Sonnino, 2007). Some traits are controlled by single genes, while others are genetically complex controlled by multiple genes, in which case the latter is called quantitative trait loci (QTL), and environmental factors. The success of MAS is determined by the relationship between the marker the gene

of interest. The most favourable selection is when markers are located within the gene of interest termed gene-assisted selection. Although markers located in the gene of interest are the most favourable, they are also the most uncommon and difficult to find. Markers can also be in linkage disequilibrium in which case selection based on these markers is termed linkage disequilibrium marker-assisted selection (LD-MAS). These are commonly found in crossbreeds. Selection using markers not in linkage disequilibrium is called linkage equilibrium marker-assisted selection (LE-MAS). The use of molecular markers helps to address problems associated with traditional selection and thus help to select for genetically superior animals (Singh *et al*., 2014).

The first report on markers for tick resistance was by Francis & Ashton (1967) on blood protein polymorphism. Because of its role in the immune system, the BoLA region has been found to contain good candidate genes for ticks and TBD resistance (Taberlet *et al*., 2008). Since Francis & Ashton's pioneering work (Francis, 1967), significant association between markers, in particular those within the BoLA region, and host resistance to ticks has been demonstrated in numerous studies. For example, Untalan *et al*. (2007) identified significant associations between BoLA *DRB3*-174 and *DRB1*-118 alleles with resistance to the Lone Star tick (*Amblyomma americanum*). Acosta-Rodriguez identified four BoLA locus alleles (*DRB3*-184, *DRBP1*-128, BM1815-152 and *DRBP1*-130) associated with high tick burdens in Holstein- Zebu crosses (Acosta-Rodriguez *et al*., 2005). *DRB3* alleles have also been associated with TBDs (Anaplasmosis and Babesiosis) tolerance in dairy cattle in Thailand (Duangjinda *et al*., 2013).

BoLA-*DQ* molecules derived from inter-haplotype and intra-haplotype pairing of A and B chains have been demonstrated to be functional in presenting *Anaplasma marginale* peptides (Norimine *et al*., 2005) suggesting that the BoLA-*DQ* genes also

play important roles in TBD resistance. Recently, Mapholi, *et al*. (2016) performed a genome- wide study of tick resistance in South African Nguni cattle and obtained a low heritability estimate ranging from 0.02 ± 0.00 to 0.17 ± 0.04. These markers are important in selecting breeds that are resistant to ticks and TBDs and optimally adapted to harsh conditions in tick endemic areas of SSA (Medugorac *et al*., 2009). However, genetic components are not the sole factor that influence tick resistance, environmental factors such as rainfall and temperature and other phenotypic characteristics (coat colour, skin thickness) have also been reported to influence tick resistance (Greenfield *et al*., 2011).

CHAPTER 3 : MATERIALS AND METHODS

3.1 Introduction

Three cattle populations belonging to the indigenous Nguni, Afrikaner and Bonsmara breed from three different geographical areas were included in this study. Four microsatellite loci on bovine chromosome (BTA) 23, (*BM1815*, *DRBP1*, *DRB3* and *RM185*) were used to characterize the breeds at a molecular level. Ticks infesting the animals were counted and collected for identification. Molecular detection of tick-borne pathogen parasites (*Anaplasma*, *Babesia*, *Theileria* and *Ehrlichia*) was also carried out using "catch-all" primers. The alleles identified were analyzed for possible association with tick and TBD resistance.

3.2 Research design

The research design was a mixed model of both quantitative and qualitative methods. Qualitative data was obtained as infection status used to evaluate whether the animals were infected with *Anaplasma*, *Theileiria*, *Ehrlichia* or *Babesia* species as well as the tick species identified. DNA quality, genetic diversity (number of alleles and heterozygosity) and the number of ticks observed yielded quantitative data. The flow chart of the research design is shown in Figure 3.1.

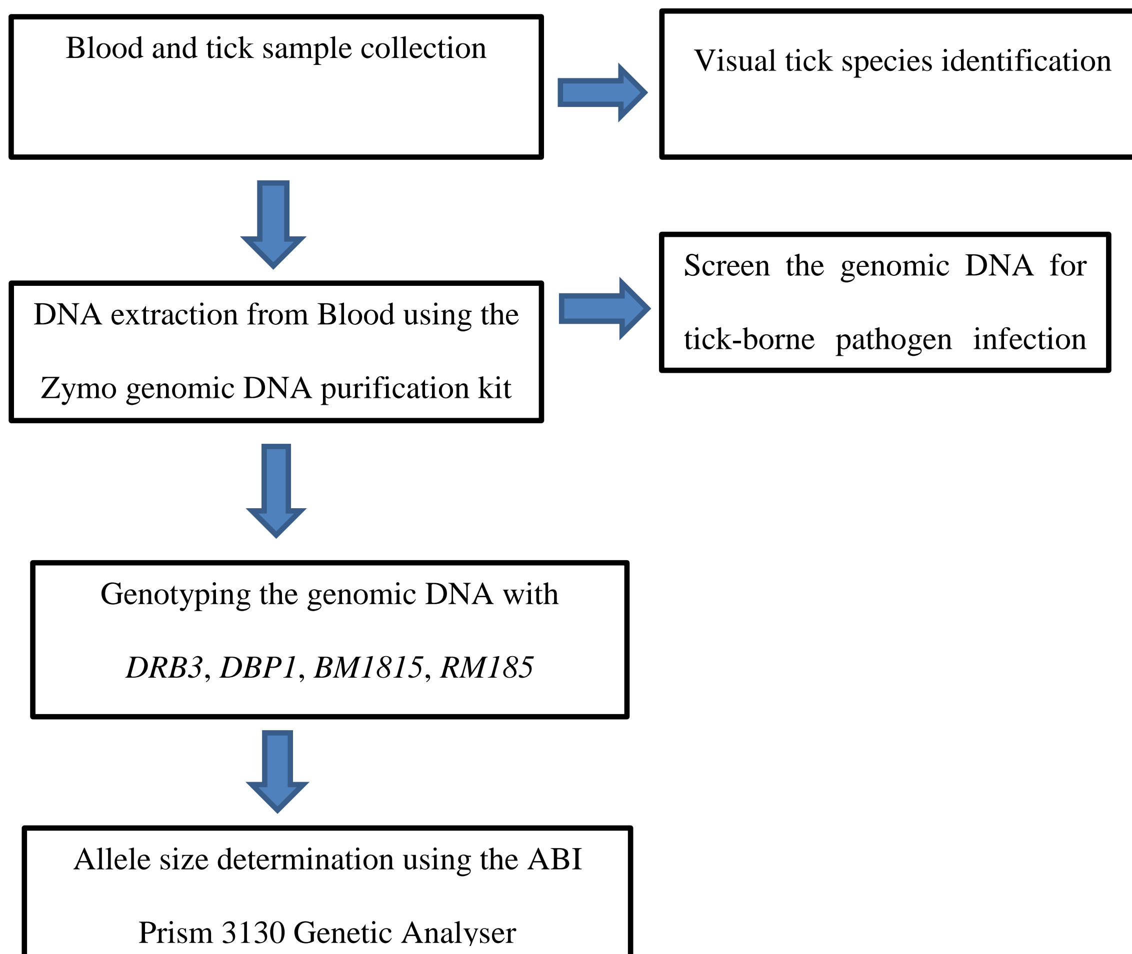


Figure 3.1. Schematic flow chart of the research design used during this study.

3.3. The study area and animals sampled

The ethical clearance (FANR/68/2015) was obtained from the University of Namibia prior to sample collection. Blood samples and ticks were collected from cattle between September 2015 and September 2016. A total of 4 sites in Khomas, Omusati and Zambezi (Caprivi) regions were sampled. The study areas are indicated in Figure 3.2 and Table 3.1. Figure 3.2 shows the map of Namibia indicating the location of farms where populations under study were sampled. A detailed description of the sampling locations is given in Table 3.1.

Figure 3.2. Sampling sites map for the Nguni (blue), Afrikaner (red) and Bonsmara (yellow) cattle in Namibia. Google earth (Version 7.1.5.1557)[software]. Mountain View, CA: Google Inc. (2017).

Table 3.1. Geographical descriptions of the sampling sites.

Region	Site	Coordinates	[1]n	Breed
Khomas	University of Namibia Neudamm experimental farm	22°30'14.8126'' S and 17°22'23.0441''E	42	Afrikaner
			54	Nguni
	Claratal stud farm	22°52'59.862''S and 16°53'47.5487'' E	38	Bonsmara
Omusati	University of Namibia Ogongo campus farm	17°40'40.3136''S and 15°17'36.4643''E	40	Nguni
			20	Bonsmara
Zambezi	Sachinga Livestock Development Centre	17°42' 33.8519'' S and 24°14'27.4052'' E	60	Nguni

[1]n-number of cattle sampled

A total of 249 animals were sampled for blood and ticks in accordance with the UNAM research ethics policy. A convenience sampling method was used. Four millilitres of blood was collected from the coccygeal (tail) vein into EDTA vacutainer tubes (Figure 3.3) and stored at 4°C until DNA extraction. Concurrently, ticks under the tail, were counted and removed from each individual animal using the hand-picking method. Ticks were removed as described by Needham (1985) by grasping as close to the skin as possible with protected fingers and pulling straight up with steady even pressure so as not to break the mouthparts. Animals from both sexes with different ages ranging from three to 10 years were sampled.

Figure 3.3. Blood sample collection from the coccygeal vein of a Nguni cow.

3.4. Tick collection and Identification

Data on tick counts was collected from the animals naturally exposed to ticks. Ticks scoring was done according to Herring (2014) as shown in Table 3.2. Actual numbers of ticks were also recorded.

Table 3.2. Tick scoring system

Scores	Tick resistance	Number of observable ticks under the tail
0	Clean	0
1	Very high	≤10
2	High	11-30
3	Average	31-80
4	Low	81-150
5	Very low	≥150

Female and male adult ixodid ticks were collected from under the tail of each animal twice in summer (September- April) and winter (May-August) respectively. Collected samples were placed in glass containers containing 70 % ethanol and labelled. The label indicated the name of the site, owner's name, animal identification code, date and month of collection. Ticks were identified based on morphological and structural differences of the adult ticks of each species. The grouping to their genus and species was made after their examination under a stereomicroscope (SMZ-171, Motic group, hong Kong, China) according to the methods developed by Walker *et al*. (2003).

Figure 3.4. Tick sample collection from under the tail of a Nguni

3.5. Genomic DNA extraction

Genomic DNA (gDNA) was extracted from fresh and frozen bovine blood using the ZR Zymo kit (Zymo Research, CA, USA) at the University of Namibia, Molecular Biology Laboratory according to the manufacturer's protocol. The DNA was immediately stored at -20 °C until PCR amplification was performed. The concentration of the DNA samples was determined by measuring the A260/A280 ratio with a NanoDrop spectrophotometer (ND-1000; NanoDrop Technologies, DE, USA).

3.6. Polymerase Chain Reaction (PCR)

Polymerase Chain Reaction was performed on all the samples in the Animal Breeding and Genetics Laboratory of the Department of Animal and Wildlife Sciences at the University of Pretoria. Three microsatellite markers mapped within the BoLA complex (*DRB3*, *DRBP1* and *BM1815*) and one other in proximity with the BoLA (*RM185*) were amplified. Microsatellites primer sequences are shown in Table 3.5. All forward primers were labelled with fluorescent dye at the 5' terminus, while all reverse primers were unlabelled. A 15 µL reaction volume was prepared with molecular water (nuclease-free water), ×10 buffer optimised with 50 mM $MgCl_2$ and 100 mM deoxynucleotides triphosphates, 5U Bioline MyTaq DNA polymerase® (Bioline USA, Inc.), 10 mol/µl primers (Applied Biosystems, Foster city, CA, USA) and 50–100 ng of gDNA. The volume of each component is shown in Table 3.3.

Amplification of DNA samples was performed using Perkin Elmer GeneAmpSystem® 9700 Thermal cycler (Applied Biosystems, Foster city, CA, USA) programmed to run as follows: 94 °C for 10 min, 33 cycles of denaturation at 94 °C for 45 sec, specific marker annealing temperature (*DRB3* 65°C, *DRBP1* 55°C, *RM185* 55°C and *BM1815* 55°C) for 80 sec and replication at 72 °C for 60 sec,

followed by a final extension at 72 ° C for minutes and held indefinitely at 4 °C. After amplification, the PCR products were visualized using 3 % agarose gel stained with ethidium bromide in Tris-acetate-EDTA (TAE) buffer (pH 8.3) and run at 220 V for 15 min and later visualised under Criterion stain free imager UV trans-illuminator (Bio-RAD laboratories, CA, USA). Only reproducible amplification pattern were considered. Amplification products were scored as the presence or absence of bands.

Table 3.3. Master Mix composition for one sample to amplify genomic DNA from bovine blood

PCR reagents (concentration)	Volume
Buffer: dNTPs (50mM $MgCl_2$ (100mM)	3µL
Nuclease-free water	6.1µL
Bioline Taq polymerase (5U/ µL)	0.3µL
Forward Primers (10pmol/µL)	0.3µL
Reverse Primers (10pmol/µL)	0.3µL
Genomic DNA (50-100ng)	5µL
Total	**15µL**

3.7. Polymerase Chain Reaction (PCR) amplification for pathogen infection screening

Pathogen infection screening was done using two sets of primers with matching melting temperatures and thus the same PCR thermos-cycling program was used for both reactions. PCR amplification of a variable region in the 16S ribosomal RNA gene (specific to *Ehrlichia* and *Anaplasma*) or 18S ribosomal RNA gene (specific to *Theileria* and *Babesia*) was performed. The primers were designed for specific amplification of the rRNA gene of the target pathogenic organisms and they are not complementary to the hosts or ticks rRNA genes resulting in a high specificity of the PCR reaction (Bekker *et al*., 2002; Nijhof *et al*., 2003). Catch-all primers were used

which allows for simultaneous detection of (*Ehrlichia* and *Anaplasma*) and (*Theileria* and *Babesia*) species.

The PCR amplification of related species of the *Ehrlichia*/*Anaplasma* cluster of species and the cluster of *Theileria*/*Babesia* species was performed on all of the bovine DNA samples collected in this study, making specific PCR reactions for each individual species obsolete. A 15 µL reaction volume consisted of 4.2 µL molecular grade water (nuclease-free water), 7.5 µL Lucigen econotaq (Lucigen Corporation, Middleton,USA) containing the Taq polymerase (5U/ µL), buffer optimised with dNTPs (2.5 mM each) and $MgCl_2$ (15 mM) , 0.15µL of 100 pmol/µl primers and 3 µL of 50-100 ng of genomic DNA (Table 3.4). The PCR program used was 5 min at 94°C initial denaturation and 33 cycles of 30 s at 94°C denaturation; 50 °C for 30 s annealing , 72 °C for 45 s extension and a post-run hold at 4 °C. PCR was performed with Perkin Elmer GeneAmp PCR System® 9700 Thermal cycler (Applied Biosystems, Foster city, CA, USA). PCR products were examined by 3% agarose gel electrophoresis at 220 V for 15 min. The amplified products were visualised and photographed with a gel documentation system (Image lab, BioRAD, USA). Table 3.6 shows the primer sequences used for pathogen infection screening.

Table 3.4. Master Mix composition for one sample for pathogen infection screening

PCR reagents (concentration)	**Volume**
Buffer: dNTPs	
Nuclease-free water	4.2µL
Lucigen econotaq: Taq (5 U/µL) Buffer, DNTPs , $Mgcl_2$ (2.5 Mm)	7.5µL
Forward Primers (10pmol/µL)	0.15µL
Reverse Primers (10pmol/µL)	0.15µL
DNA (50-100ng)	3µL
Total	**15µL**

3.8. Microsatellite typing

The PCR samples were diluted in a 1:10 ratio by combining 18 µL of molecular grade/nuclease free water with 2 µL of the PCR product. A master mixture containing 720 µL of Formamide and 10.1 µL Genescan Liz® size standard (Life technology and Applied Biosystems, Foster City, CA, USA) was prepared. Nine microliters (9µL) of Formamide-Liz mixture and 1 µL of the diluted PCR products were pipetted into each well of the 96 well plate. The amplified products were separated using the capillary electrophoresis ABI Prism 3130 Genetic Analyzer (Applied Biosystems, Foster city, CA, USA) at the Forestry and Agricultural Biotechnology Institute (FABI) department of the University of Pretoria. The output data from the ABI Prism 3130 Genetic Analyzer was analyzed using GeneMarker 1.95™ software (Applied Biosystems, CA, USA).

Table 3.5. Details of the microsatellite marker primers.

Micro-satellite	Forward primer(5'→ 3')	Reverse primer(5'→ 3')	Label dye	Annealing temperature (°C)	Reference
DRB3	ATC CTC TCT CTG CAG CAC ATT TCC T	CGC GCT CAC CTC GCC GCT G	6FAM (blue)	65	van Eijk *et al*., 1992
DRBP1	ATG GTG CAG CAG CAA GGT GAG CA	GGG ACT CAG TCT CTC TAT CTC TTT G	VIC (green)	55	Ihara *et al*., 2004
RM185	TGG CCT GTC TAT GCT TGC ATC	GAG TTT CCT TTG CAT GCC AGT C	NED (yellow)	55	Ihara *et al*., 2004
BM1815	AGA GGA TGA TGG CCT CCT G	CAA GGA GAC AAG TCA AGT TCC C	PED (red)	55	Ihara *et al*., 2004

Table 3.6. Details of the tick-borne pathogen infection primers.

Pathogen	Forward primer (5'→ 3')	Reverse primer (5'→ 3')	Annealing temperature (°C)	Reference
Babesia/Theileria	GAC ACA GGG AGG TAG TGA CAA G	CTA AGA ATT TCA CCT CTG ACA GT	50	Nijhof *et al*., 2003
Ehrlichia/ Anaplasma	GGA ATT CAG AGT TGG ATC MTG GYT CAG	CGG GAT CCC GAG TTT GCC GGG ACT TYT TCT	50	Bekker *et al*., 2002

3.9. Statistical analysis

Allele data was imported to the Microsatellite toolkit (Park, 2001) where descriptive statistics for each locus and population were calculated. Descriptive statistics included expected and observed heterozygosity values, PIC values, allele frequencies, total number of alleles, mean number of alleles and private alleles. Allele data was also imported to CONVERT software version 1.31 (Glaubitz, 2004) to reformat diploid genotypic data into compatible input files for POPGene (Alberta, Canada), Arlequin (Swiss Institute of Bioinformatics, Bern, Switzerland) and FSTAT (Lausanne, Switzerland). Genetic relationships between breeds were determined according to Nei's standards (Nei, 1987), using POPGene software version 3.2. The analysis of molecular variance (AMOVA) and test for deviation from HWE per locus was performed using Arlequin version 3.1(Excoffier *et al*., 2005).

A Bayesian clustering method was used to determine population structures in STRUCTURE programme version 2.3 (Pritchard *et al*., 2000). A burn-in period of 100,000 generations followed by Markov Chain Monte Carlo (MCMC) simulations of 500,000 iterations was used in all the runs. Five iterations for each K was performed; K was set from K= 2 to K= 10 assuming admixture.

Statistical difference in tick counts between summer and winter seasons were analyzed using a mixed-design analysis of variance (ANOVA) in SPSS (version 25) with season being a repeated measure, while breed and geographical location were the independent variables. This was followed by post hoc Tukey's Honest Significant Difference (HSD). The tick count data was transformed to $\log_{10}$ n + 1 so that the distribution could approximate normality before the mixed-design ANOVA was carried out. All analyses were tested at 5% level of significance. The infection

prevalence of *Babesia/Theileria* and *Anaplasma/ Ehrlichia* parasites was calculated as follows:

$$P = \frac{d}{n} \times 100$$

Where P represents the prevalence;

d represents the number of animals that tested positive for a particular TBD; and

n represents the total number of animals sampled.

Tick prevalence for each tick specie was calculated as described by Thrusfield, (1995).

Prevalence for each tick species was calculated as follows:

$$P = \frac{d}{n} \times 100$$

Where P represents the prevalence;

d represents the number of animals that tested positive for a particular tick species;

n represents the total number of animals sampled.

In order to test for the association between BoLA alleles and tick counts, animals were classified as susceptible or resistant depending on the number of adult ticks counted on the animal under the tail. Mean tick numbers for each breed for the summer season were used to classify the animals as susceptible or resistant to ticks. Animals were phenotyped as susceptible to ticks when having a number of ticks greater than or equal to the mean $\overline{(X)}$: Nguni ($\bar{X} \geq 7$), Bonsmara $\bar{X} \geq 26$) and Afrikaner cattle ($\bar{X} \geq 17$). Animals were classified as resistant to ticks when they yielded a number of ticks less than or equal to the mean of each breed. Allele frequencies were determined by Hi = $\sum ni/N$, where Hi is the frequency for allele i, ni is the number of allele i in a population and N is the total number of alleles in the

population. Alleles with a frequency less than 0.10 were discarded for allelic of association with ticks and TBDs.

A binary logistic regression was performed to test for allelic association with tick resistance and pathogen infections using SPSS (version 24). For tick resistance, tick resistance or tick susceptible was the response variable and allele, sex and age were used as predictors. An OR (odd ratio) was derived that takes these factors into account. The statistical model was as follow:

$$\ln\left(\frac{Pi}{1-Pi}\right) = \beta 0 + \sum \beta j G j + \sum \quad \beta m X m + \varepsilon ijkl$$

where Pi = the probability that cow i with genetics j (Nguni, Afrikaner, Bonsmara) is infected by a specific pathogen (0 = no infection or susceptible, 1 = infection or resistant); βo is the intercept;$\beta 1, \beta 2, \beta 3 \ldots \beta m$ are the regression coefficients for alleles 1, 2, 3, . . ., m; X_1, X_2. . ., X_m are the dummy variables for presentation of effects of alleles 1, 2, 3, . . ., m; and ε ij = random error term.

The probability of pathogen infections ($\hat{P}$) for each allele was estimated by

$$\widehat{Pi} = \frac{e^{\hat{\beta}0+\hat{\beta}m}}{1+e^{\hat{\beta}+\hat{\beta}m}}$$

where $\hat{\beta}$ is the intercept; $\hat{\beta}m$ is the regression coefficient for allele m estimated from (1); and *e* is the exponential constant.

CHAPTER 4 : RESULTS

4.1. Introduction

Microsatellite markers were used to characterize the Bonsmara, Nguni and Afrikaner cattle from different geographical locations in Namibia. The degree of polymorphism was measured by heterozygosity, PIC and allelic richness. All four microsatellite markers were found to be polymorphic in all the three cattle breeds analyzed. Considering all populations, the HWE test for deviation revealed that all microsatellite markers but one (DRB3) adhered to HWE. A total of 37 alleles were detected across all loci (Table 4.1) depicting significant amounts of genetic diversity exhibited by all cattle populations in this study.

Table 4.1. Observed alleles and Hardy Weinberg Equilibrium (HWE) test for deviation for four loci in three beef cattle breeds in Namibia

Locus	**Observed alleles**	**N[1]**	***P*-value HWE**
DRB3	289, 290, 291, 292	4	0.000
DRBP1	118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138	11	0.236
RM185	91, 93, 95, 97, 99, 101, 103, 105, 107	9	0.467
BM1815	145, 147, 149, 151, 153, 155, 157, 163, 165, 167, 169, 173, 175	13	0.351
Total		37	
Mean		9	

[1]N is the number of alleles

4.2. Microsatellite polymorphism and population differentiation

The level of heterozygosity and PIC values for each microsatellite marker are shown in Table 4.2. Analysis of results across all populations for all four loci indicated that all loci exhibited moderate to high levels of polymorphism except the DRB3 locus. Observed heterozygosity (Ho) ranged from 0.244 in DRB3 to 0.816 in RM185 with an overall mean of 0.616. Expected heterozygosity (He) values varied

between 0.428 in the DRB3 and 0.781 in the DRBP1 marker. With the exception of the RM185 marker, all markers had higher He than Ho. PIC values varied between 0.381(DRB3) and 0.743 (DRBP1) with an overall mean of 0.633.

Table 4.2. Heterozygosity levels and polymorphism information content (PIC) values of each of the four microsatellite markers

Locus	**Expected heterozygosity (He)**	**Observed Heterozygosity (Ho)**	**PIC**
DRB3	0.428	0.244	0.381
DRBP1	0.781	0.690	0.743
RM185	0.743	0.816	0.707
BM1815	0.747	0.713	0.700
Mean	0.675	0.616	0.633

The results of Wright's fixation indices (F_{IS}, F_{ST}, F_{IT}) for each loci across populations are presented in Table 4.3. The average deficit of heterozygote (F_{IS}) across all loci amounted to 11.6 %. Evidence of inbreeding was observed in three out of four markers namely; DRB3, DRBP1, BM1815. Limited levels of inbreeding were observed in the DRBP1 and BM1815 loci with F_{IS} values of 0.110 and 0.004 respectively, while the DRB3 marker indicated excessive inbreeding with an inbreeding coefficient of 0.425. An excess of heterozygotes was observed in the RM185 (F_{IS} = -0.106), showing evidence of outbreeding at this locus. The estimates of the degree of gene differentiation among populations (F_{ST}) varied from 0.021 (BM1815) to 0.084 (DRB3) with a mean of 0.058 indicating low genetic differentiation. The overall loci estimates of deficiency or excess of average heterozygotes in a group of populations (F_{IT}) ranged from 0.053 to 0.474 with a mean of 0.164. Estimates of gene flow varied from 2.714 (DRB3) to 11.863 (BM1815) with a mean of 5.614 indicating moderate gene flow.

Table 4.3. F statistics and estimates of gene flow for all loci across all population

Locus	F_{IS}	F_{IT}	F_{ST}	Nm[1]
DRB3	0.425	0.474	0.084	2.714
DRBP1	0.110	0.179	0.078	2.961
RM185	-0.106	-0.053	0.048	4.920
BM1815	0.037	0.057	0.021	11.86
Mean	0.116	0.164	0.058	5.614

[1]Nm-Gene flow estimated from $F_{ST} = 0.25(1-F_{ST})/F_{ST}$

4.3. Genetic diversity within and between three beef cattle breeds in Namibia

Within breed genetic diversity was quantified by measuring the expected and observed heterozygosity, average number of alleles per locus, PIC and richness of allelic variants. The F statistics were also calculated to characterize genetic differentiation between breeds. AMOVA values were calculated to quantify the partitioning of the total diversity in a within-breed and an among-breed component brought about by breed formation. Finally, the population structure of each breed was analysed assuming admixture.

4.3.1. Heterozygosity, PIC and allelic richness

Within breed genetic variation was observed, with expected heterozygosity ranging from 0.637 (Bonsmara) to 0.728 (Nguni) and a mean of 0.675 (Table 4.4). Observed heterozygosity was high in Nguni cattle (Ho = 0.676) and low in Afrikaner (Ho = 0.499) with a mean of 0.616. The average number of alleles per locus varied between 5.50 (Afrikaner) and 8.75 (Nguni) with a mean of 7.08. It should be noted that observed heterozygosity was lower than expected in the Nguni and Afrikaner breeds. PIC values varied slightly around a mean value of 0.633, from low values in Afrikaners of 0.598 to 0.698 in Nguni cattle, indicating the informativeness of these markers.

Table 4.4. Number of alleles, heterozygosity levels[1] and polymorphism information content (PIC)[2] for each of the three cattle breeds analysed at four loci.

Population	***N***[1]	[2]**He ± SD**	[2]**H_O ± SD**	[2]**PIC ± SD**	[2]**No. alleles ± SD**
Afrikaner	42	0.660±0.019	0.499±0.039	0.598±0.051	5.50±1.91
Bonsmara	58	0.637±0.131	0.673±0.032	0.602±0.252	7.00±2.16
Nguni	149	0.728±0.102	0.676±0.020	0.698±0.213	8.75±3.59
Mean		0.675±0.084	0.616±0.030	0.633±0.172	7.08±2.56

[1]*N* indicates the number of animals genotyped

[2]Expected heterozygosity (He) and observed heterozygosity (Ho) where SD is the standard deviation and No. is the number of alleles.

A total of six distinct private alleles were found, two in Afrikaner and four in Nguni cattle. No private alleles were found in the Bonsmara population. Three out of six private alleles belonged to the BM1815 marker. All private alleles were rare alleles with allele frequencies less than 3% (Table 4.5). The most frequent alleles (Appendix 1) at each locus were DRB3-292 with 86%, DRBP1-126 (49%), RM185-99 (52%) and BM1815-151 (39%).

Table 4.5. Private alleles and their frequencies

Breed	**Locus**	**Allele**	**Frequency**
Afrikaner	RM185	93	0.012
Afrikaner	BM1815	175	0.024
Nguni	DRBP1	132	0.026
Nguni	DRBP1	138	0.007
Nguni	BM1815	147	0.003
Nguni	BM1815	167	0.007

Allelic richness was also evaluated to measure the genetic diversity (Table 4.6). Allelic richness per locus calculated was observed to be highest in the Nguni breed with an average of 7.53 whereas the lowest number of alleles was observed in the Afrikaner breed with an average of 5.45.

Table 4.6. Allelic richness per breed and loci based on a sample size of 35 individuals per breed

Locus	Afrikaner	Bonsmara	Nguni
DRB3	3.00	3.74	3.87
DRBP1	5.00	8.02	10.3
RM185	6.83	6.95	7.96
BM1815	6.97	7.11	8.02
Mean	5.45	6.45	7.53

4.3.2. Nei's genetic distances among populations

The evolutionary relationship among cattle populations was determined from genetic distances according to Nei's standards (Nei 1987) using POPGene (version 1.32, Raymond & Rousset 1995). The pair-wise F_{ST} values of breeds ranged between 0.0071 and 0.322 thereby revealing close relationship between Bonsmara and Nguni cattle (F_{ST} = 0.071) and the highest divergence of 0.322 between the Bonsmara and Afrikaner cattle (Table 4.7).

Table 4.7. Pair-wise matrix of Nei's genetic distances for the three cattle breeds analysed

Population ID	Afrikaner	Bonsmara	Nguni
Afrikaner	****	-	-
Bonsmara	0.322	****	-
Nguni	0.181	0.071	****

4.3.3. Population differentiation

Wright's estimate of inbreeding (F_{IS}) indicated limited inbreeding (F_{IS} = 0.071) in the Nguni breed. A significant deficit of heterozygotes was observed in the Afrikaner breed as indicated by a significantly high F_{IS} value of 0.247 suggesting evidence of excessive inbreeding within this breed. An excess of heterozygotes (F_{IS} = -0.057) was observed in the Bonsmara cattle reflecting outbreeding in this breed. A

further breakdown of within-breed inbreeding estimate at each locus in the three cattle breeds under study is presented in Table 4.8.

Table 4.8. Inbreeding (F_{IS}) estimates per locus and breed

Locus	Afrikaner	Bonsmara	Nguni
DRB3	0.582	-0.036	0.489
DRBP1	0.313	-0.050	0.126
RM185	-0.056	-0.095	-0.137
BM1815	0.183	-0.033	-0.001
Mean	0.247	-0.057	0.071

The AMOVA test showed significant differentiation ($P < 0.05$) among populations, with 4.8% of the genetic variation due to differences between breeds while 95.2% was due to differences within breeds as presented in Table 4.9.

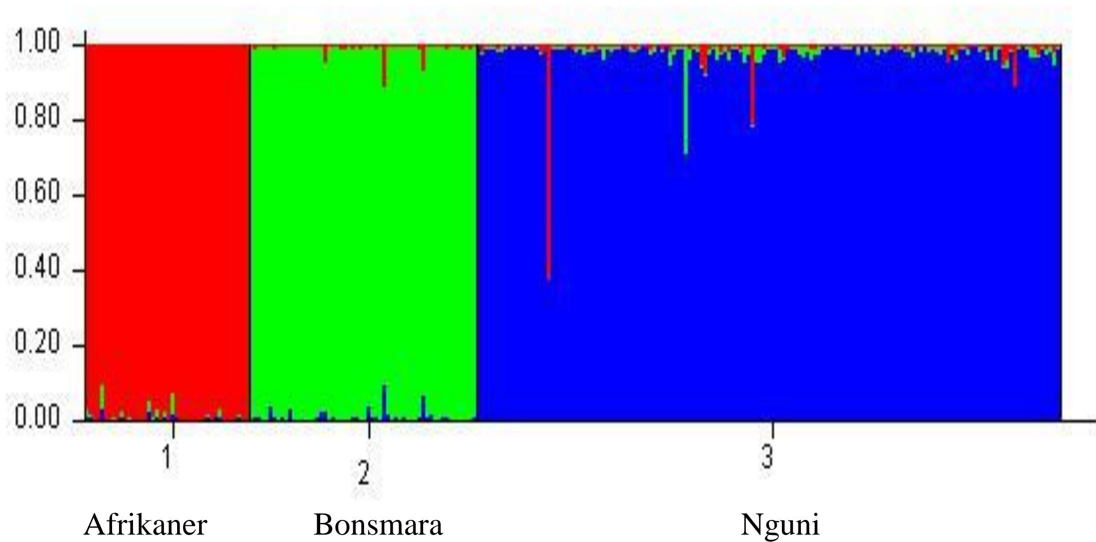
Table 4.9. Analyses of molecular variance (AMOVA) for the three cattle populations

Source of variation	Sum of squares	Variance components	Percentage variation	P- value
Among populations	19.5	0.064	4.75	0.001
Within populations	600.4	1.28	95.2	0.001
Total	619.9	1.34		

4.3.4. Population Structure analysis

Figure 4.1 shows the results of Bayesian cluster analysis (K = 3) which indicated three distinct clusters with a small degree of admixture between breeds. The size and colour correspond to the relative proportion of the animal genome corresponding to a particular cluster. The proportion of membership of each pre-defined population in each of the three clusters were as displayed in Table 4.10 with Afrikaner, Bonsmara and Nguni in clusters 1, 2 and 3, respectively**.** Table 4.10 shows that 99% of the Afrikaner breed was assigned to cluster one with 0.08% and 0.06% of their genome assigned to cluster two and three respectively. Ninety-eight percent of the Bonsmara genome was assigned to cluster two, whereas 97% of the Nguni genome was assigned

to cluster three. The results revealed that Afrikaner cattle were the least admixed.



Afrikaner Bonsmara Nguni

Figure 4.1: Bayesian clustering assignment of 249 animals representing three cattle populations at K = 3. Afrikaners (red), Bonsmara (green), Nguni (blue)

Table 4.10. Proportion of membership of each pre-defined population in each of the three clusters

Population	**Inferred clusters**		
	1	2	3
Afrikaner	**0.986**	0.008	0.006
Bonsmara	0.008	**0.980**	0.012
Nguni	0.014	0.013	**0.973**

4.4. Genetic diversity of the three cattle breeds based on their geographical location

The genetic diversity of the Nguni and Bonsmara cattle was analyzed based on their geographical location namely Omusati, Khomas and Zambezi regions. Afrikaner cattle was excluded from these analyses as they were only sampled from one location. The level of heterozygosity, number of alleles and PIC values for each herd were analysed as shown in Table 4.11.

Table 4.11. Heterozygosity levels[1], polymorphism information content (PIC) and number of alleles of the three beef cattle populations based on their geographical locations in Namibia

Population[2]	N^3	Loci typed	$^1H_e \pm SD$	$^1H_O \pm SD$	PIC	No. alleles ± SD
BONO	20	4	0.653±0.082	0.744±0.052	0.586±0.170	5.50±1.91
BONK	38	4	0.616±0.156	0.642±0.039	0.581±0.292	6.50±1.73
NGUK	49	4	0.743±0.079	0.648±0.035	0.695±0.185	8.25±3.10
NGUO	40	4	0.716±0.085	0.741±0.036	0.676±0.172	7.00±2.45
NGUZ	60	4	0.684±0.138	0.653±0.032	0.653±0.274	7.50±3.11
Mean			0.682±0.108	0.685±0.039	0.638±0.219	6.95±2.46

[1] He – Expected heterozygosity, Ho – observed heterozygosity, SD-Standard Deviation
[2]BONO - Omusati Bonsmara herd, BONK- Khomas Bonsmara herd, NGUK- Khomas Nguni herd, NGUO- Omusati Nguni herd, NGUZ- Zambezi Nguni herd
[3]N indicates the number of animals genotyped

4.4.1. Heterozygosity, PIC and allelic richness

All herds were found to be polymorphic, with the number of alleles ranging between 5.50 in Omusati Bonsmara herd (BONO) and 8.25 in Khomas Nguni herd (NGUK). While expected heterozygosity ranged from 0.616 in the Khomas Bonsmara (BONK) herd to 0.743 in Khomas Nguni herd with a mean of 0.682, observed heterozygosity varied around an overall mean of 0.685. The Zambezi Nguni herd (NGUZ) showed the lowest expected heterozygosity (He = 0.684) compared to all Nguni herds. Although the BONO herd exhibited the lowest number of alleles (5.50), it showed a higher expected heterozygosity (He = 0.653) than the BONK herd (He = 0.616). The Nguni and Bonsmara herds of Khomas region exhibited the highest genetic diversity compared to other regions. Observed heterozygosity was lower than expected heterozygosity in the Khomas and Zambezi Nguni herds. PIC values varied from 0.581 (BONK) to 0.695 (NGUK), with an overall mean of 0.638.

Allelic richness of the cattle breeds per geographical location was measured per locus based on a sample size of 17 individuals (Table 4.12). The number of alleles varied slightly among populations.It was observed that the Nguni herd of the Khomas region had the highest number of alleles of 6.67 in contrast to the lowest number of 5.43 observed in Omusati Bonsmara herd. The most polymorphic locus across all populations based on allelic richness was the DRBP1 with an overall mean of 9.09 and the lowest mean number of alleles was recorded in the DRB3 with 3.71 (Table 4.12).

Table 4.12. Allelic richness per locus and population[1]

Locus	**[1]BONO**	**[1]BONK**	**[1]NGUK**	**[1]NGUO**	**[1]NGUZ**	**Mean**
DRB3	3.00	2.98	3.38	3.91	2.68	3.71
DRBP1	6.94	6.93	9.18	8.50	9.47	9.09
RM185	4.94	6.51	7.36	6.64	7.05	7.22
BM1815	6.83	5.80	6.78	5.73	6.15	6.94
Mean	5.43	5.56	6.67	6.19	6.34	

[1]BONO - Omusati Bonsmara herd, BONK- Khomas Bonsmara herd, NGUK- Khomas Nguni herd, NGUO- Omusati Nguni herd, NGUZ- Zambezi Nguni herd

4.4.2. Population differentiation

Results of the inbreeding coefficients (F_{IS}) for each of the five populations across all loci are shown in Table 4.13. Three populations showed negative F_{IS}, ranging from -0.035 to -0.142 for the NGUO and BONO herd respectively suggesting excess of heterozygotes in the population. A clear deficit of heterozygotes was observed in the NGUZ and NGUK herd as evidenced by the positive F_{IS} values of 0.045 and 0.129 respectively suggesting limited levels of inbreeding. All Omusati herds (BONO and NGUO) showed evidence of outbreeding.

Table 4.13. Inbreeding estimates (F_{IS}) for the five cattle breeds based on their geographical location[1]

Locus	**[1]BONO**	**[1]BONK**	**[1]NGUK**	**[1]NGUO**	**[1]NGUZ**
DRB3	-0.286	0.131	0.742	-0.070	0.665
DRBP1	0.010	-0.095	0.048	0.084	0.172
RM185	-0.211	-0.072	-0.154	-0.096	-0.205
RM1815	-0.162	0.006	0.113	-0.083	-0.062
Mean	-0.142	-0.043	0.129	-0.035	0.045

[1]BONO- Omusati Bonsmara herd, BONK- Khomas Bonsmara herd, NGUK- Khomas Nguni herd, NGUO- Omusati Nguni herd, NGUZ- Zambezi Nguni herd

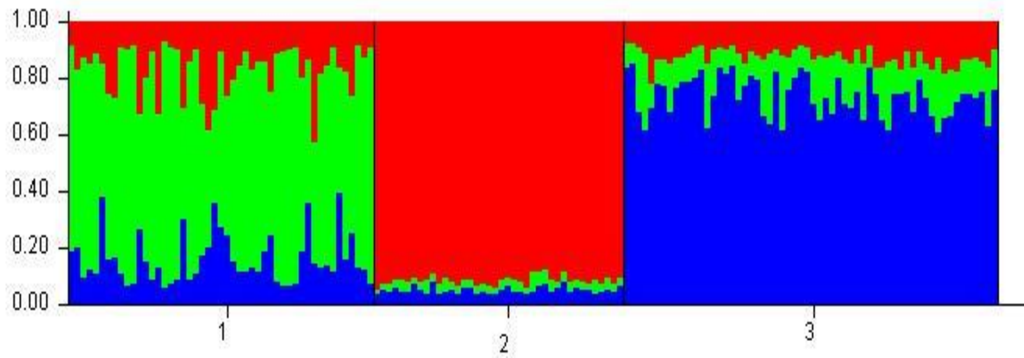
Calculation of AMOVA using the Arlequin programme indicated that 5.2 % of variation was caused by among population differences, with the remaining variation due to within population and individual variation (Table 4.14).

Table 4.14. AMOVA design and results across all five cattle populations

Source of variation	**Sum of squares**	**Variance components**	**Percentage variation**	**P-value**
Among populations	32.56	0.0685	5.16	0.001
Within populations	587.33	1.26	94.84	0.001
Total	619.89	1.33		

4.4.3. Population structure

The STRUCTURE program was used to determine the genetic structure of the Nguni ecotypes. Results revealed three distinct clusters (K=3). A clear admixture between ecotypes was observed as shown in Figure 4.2.



NGUO NGUK NGUZ

Figure 4.2. Cluster assignment of three Nguni ecotypes in Namibia using K=3
[1]NGUO- Omusati Nguni herd, [2]NGUK- Khomas Nguni herd. [3]NGUZ- Zambezi Nguni herd

Sixty seven percent (67%) of the unknown ecotype or NGUK herd was assigned to cluster two, the NGUO herd was assigned to cluster one and the NGUZ herd was assigned to cluster three (Table 4.15). The NGUK ecotype showed the highest level of admixture.

Table 4.15. Proportion of membership of the Nguni herds of Namibia

	Inferred clusters		
Population	**1**	**2**	**3**
[1]NGUK	0.169	**0.670**	0.161
[1]NGUO	**0.914**	0.033	0.053
[1]NGUZ	0.124	0.139	**0.738**

[1]BONO - Omusati Bonsmara herd, BONK- Khomas Bonsmara herd, NGUK- Khomas Nguni herd, NGUO- Omusati Nguni herd, NGUZ- Zambezi Nguni herd

4.5. Tick loads and species prevalence

According to the Herring (2014) tick scoring system ,Nguni cattle possessed very high resistance to ticks with an average of ≤ 10 ticks in summer season (Table 3.2, Figure 4.3). Of the Nguni cattle, 19 % were clean and had no ticks under the tail during summer indicating extremely high tick resistance in these individuals. Ten percent of the Afrikaner and none of Bonsmara cattle were clean during summer.

More than 50% of the Afrikaner and Bonsmara cattle had 11-30 observable ticks under the tail in summer, also suggesting high resistance to ticks based on Herring (2014) tick scoring system in Table 3.2.

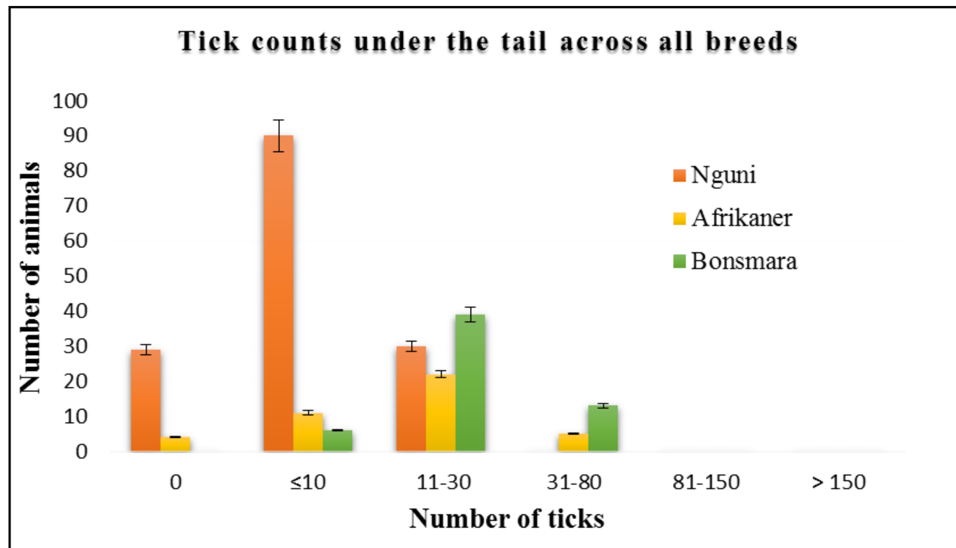


Figure 4.3. Tick counts under the tail ± Standard deviation (SD) for summer season per cattle breeds

Tick scores were also recorded during the winter season. All breeds had on average observable tick numbers ≤ 10 under the tail in winter (Figure 4.4). Twenty seven percent of the Nguni cattle had 0 ticks under the tail during the winter season. Actual tick numbers were also recorded and the mean number of ticks under the tail in summer ranged from 3 (Nguni) to 26 (Bonsmara) as shown in Table 4.16. Analysis done on actual tick counts showed significant difference across summer and winter season ($P < 0.05$) and significant difference between breeds ($P < 0.05$) in tick counts according to the mixed-design ANOVA results (Appendix 4). Mean tick counts differed significantly between breeds, with the Bonsmara cattle having a significantly high mean number of ticks than the Nguni and Afrikaner cattle.

Table 4.16. Mean number of ticks in summer and winter and minimum and maximum number of ticks counted in this study

Breed	Mean		Number of ticks	
	Summer	Winter	Minimum	Maximum
Afrikaner	17	6	0	45
Bonsmara	26	9	7	67
Nguni	3	3	0	15

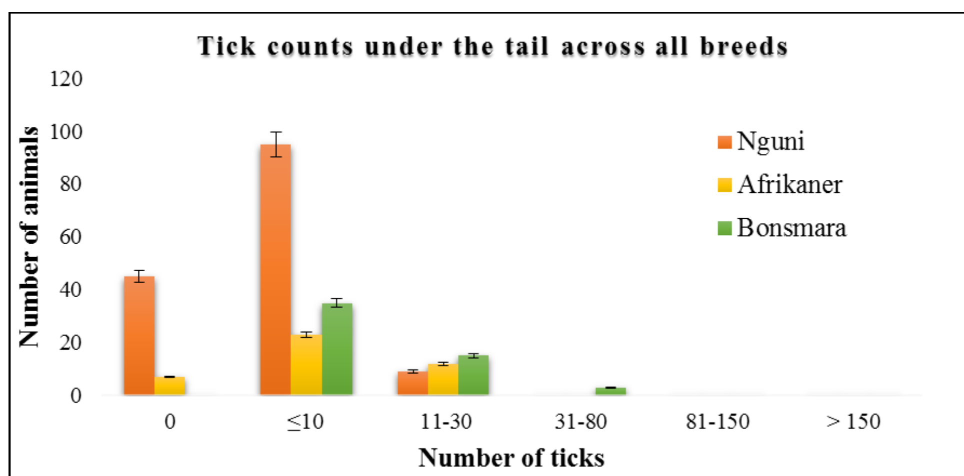


Figure 4.4. Tick counts under the tail ± standard deviation (SD) for winter season per catttle breeds

Tick scores recorded during the winter and summer season for Bonsmara and Nguni cattle were compared based on their geographical location. Bonsmara cattle were sampled from the Khomas and Omusati region. No interaction was found between the geographical locations of the animals and seasonal tick ($P > 0.05$). Seventy-nine percent and sixty-five percent of the BONK and BONO cattle had a tick score of 11-30 ticks, while ≤10 ticks were recorded for the majority of Nguni cattle from all Nguni herds during summer, however these differences were not significant. (Figure 4.6 and Figure 4.7). Tick loads on the NGUZ (last treated for ticks in February 2015 with Delete-all), NGUK (treated every year in March with Delete-all) and Omusati (treated yearly in June for ticks with Delete-all) were also compared. The NGUO herd had the lowest number of ticks, while the highest number was found

in the NGUK herd during both summer and winter (Table 4.17 and Table 4.18). All herds but one (BONO herd) had ≤10 ticks in winter suggesting low tick resistance of the BONO herd compared to other herds.

Table 4.17. Tick counts for Bonsmara and Nguni cattle based on geographical location recorded in summer

		Observable number of ticks per category (%)						
Location	**Breed**	**N**	**0**	**≤ 10**	**11-30**	**31-80**	**81-150**	**> 150**
Khomas	Nguni	49	14	57	29	0	0	0
	Bonsmara	38	0	13	79	9	0	0
Omusati	Nguni	40	30	63	8	0	0	0
	Bonsmara	20	0	0	65	35	0	0
Zambezi	Nguni	60	17	75	8	0	0	0

Table 4.18. Tick counts for Bonsmara and Nguni cattle based on geographical location recorded in winter

		Observable number of ticks per category (%)						
Location	**Breed**	**N**	**0**	**≤10**	**11-30**	**31-80**	**81-150**	**> 150**
Khomas	Nguni	49	24	67	8	0	0	0
	Bonsmara	38	0	63	37	0	0	0
Omusati	Nguni	40	45	50	5	0	0	0
	Bonsmara	20	0	25	75	0	0	0
Zambezi	Nguni	60	25	73	2	0	0	0

4.6. Tick-borne pathogen Infection status

The results of the number of individuals that showed positive on PCR for *Anaplasma/ Ehrlichia* and *Babesia/ Theileria* infection are displayed in Figure 4.5 and Table 4.19. The highest *Anaplasma/ Ehrlichia* infection prevalence was observed in Afrikaner cattle with 93%. Eighty-eight percent of the Nguni cattle were infected with *Anaplasma/Ehrlichia,* while the Bonsmara cattle had the lowest infection of relatively 71%. A significant difference in the *Anaplasma/ Ehrlichia* infection prevalence was found between the breeds ($P < 0.05$). The statistical analysis is shown in appendix 5.

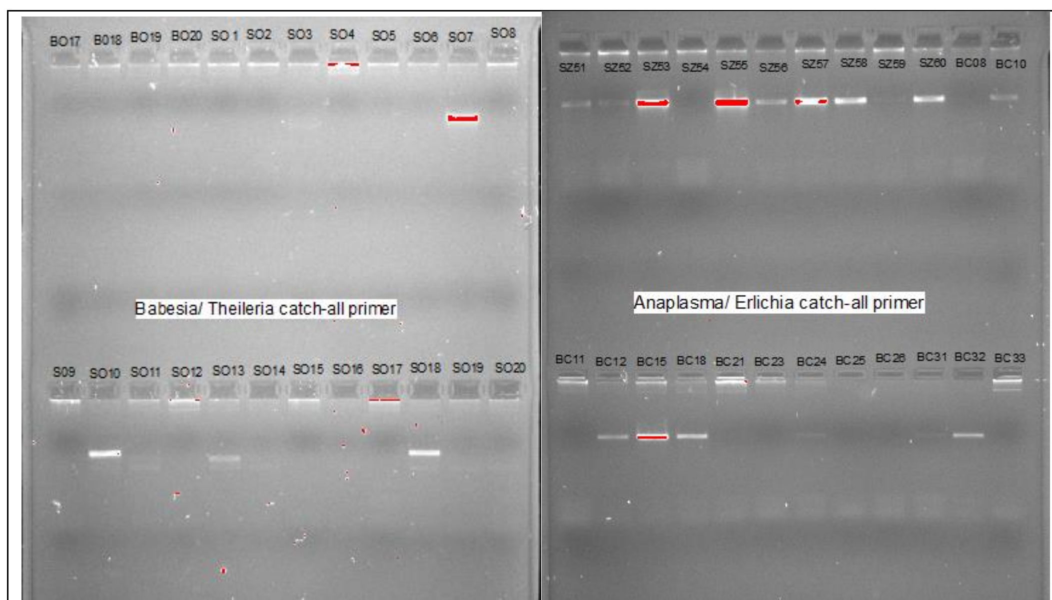


Figure 4.5. Agarose gel images showing bands of positive *Anaplasma/ Ehrlichia* and *Babesia/ Theileria* pathogen infection

Babesia/ Theileria infection were highest in Afrikaner cattle with 83%, followed by 68 % in Nguni and the lowest infection prevalence was observed in Bonsmara cattle with only 2% of the population infected. Notably, Afrikaner and Nguni cattle had high infection prevalence of both pathogens but low number of ticks compared to the Bonsmara cattle. A significant difference in the *Babesia/ Theileria* infection prevalence was found between the breeds ($P < 0.05$).

Table 4.19. Prevalence of *Anaplasma/Ehrlichia* and *Babesia/Theileria* infection per breed

Breed	***N***	***Anaplasma/Ehrlichia***	***Babesia/Theileria***	**Co-infection**
Afrikaner	42	93%	83%	76%
Bonsmara	58	71%	2%	3%
Nguni	149	88%	65%	57%

N- Total number of cattle per breed

Co-infection with *Babesia/Theileria* and *Anaplasma/Ehrlichia* within the cattle breeds was also examined (Table 4.19). The overall pathogen co-infection prevalence in all cattle breeds examined was 47% (118/249) comprising almost half the total

cattle population sampled in this study. The Afrikaner population exhibited the highest co-infection prevalence of 76%, while only 3% and 57% of the Bonsmara and Nguni cattle respectively were co-infected. Out of the 118 co-infected individuals, 85 belonged to the Nguni, 32 to the Afrikaner and 1 to the Bonsmara breed suggesting that the Nguni cattle contributed more to the overall co-infection between breeds.

The results of the infection prevalence per geographical location (region) across all breeds are displayed in Figure 4.6. The Omusati region had the highest prevalence values of *Anaplasma/Ehrlichia* infection. Almost all animals (58/60) sampled from this region tested positive for *Anaplasma/Ehrlichia*. High *Anaplasma/Ehrlichia* prevalence values were also recorded for Khomas and Zambezi region with 83% and 75%, respectively. However, the differences in prevalence of *Anaplasma/Ehrlichia* infections across all breeds were not significant between the three regions ($P > 0.05$).The highest prevalence of 78% for *Babesia/Theileria* was found in the Zambezi region. Only 11 samples out of 60 collected from Omusati region were positive for *Babesia/Theileria*, resulting in the notably lowest prevalence 18% recorded across all regions sampled. The prevalence of *Babesia/Theileria* infections across all breeds varied significantly between the three regions sampled ($P < 0.05$).

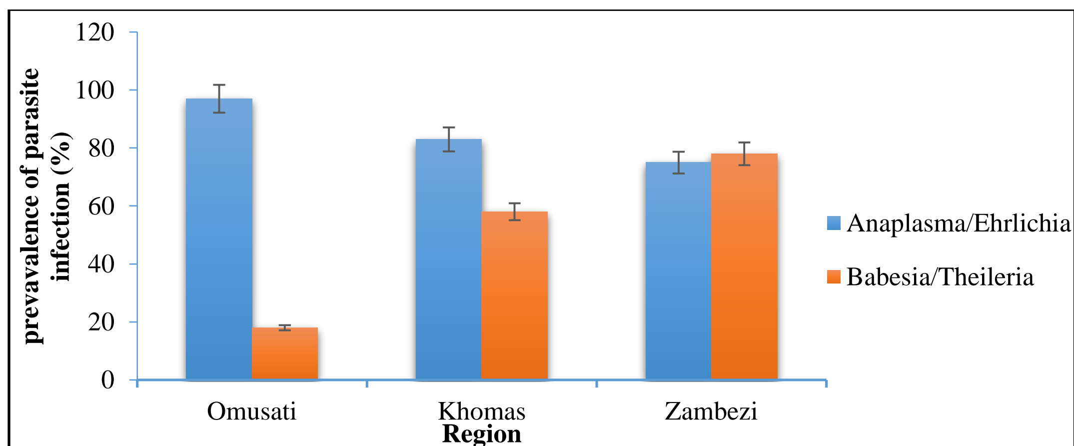


Figure 4.6. Prevalence of *Anaplasma/Ehrlichia* and *Babesia/Theileria* infections ± standard deviation (SD) across all breeds per geographical location

The prevalence of pathogen infection per cattle herd is shown in Table 4.20. While all BONO cattle (prevalence = 100%) were infected with *Anaplasma/Ehrlichia*, only 55% of the BONK were infected. *Anaplasma/Ehrlichia* infections were high in the NGUK herd with 96% and low prevalence of 75% was observed in the NGUZ herd. However, no significant difference in *Anaplasma/Ehrlichia* infection prevalence was found between the locations ($P > 0.05$).

The data demonstrated 28% of the NGUO herd harboured *Babesia/Theileria* parasites. Surprisingly, none of the cattle from the BONO herd were infected with *Babesia/Theileria*. However, 3% of the BONK herd tested positive for *Babesia/Theileria* infections. The highest *Babesia/Theileria* infections prevalence of 80% in NGUK cattle was observed and 78% was observed in the NGUZ herd. However, no significant difference in *Babesia/Theileria* infection prevalence was found between the locations ($P > 0.05$).

Table 4.20. Prevalence of *Anaplasma/Ehrlichia* and *Babesia/Theileria* infection per cattle herd

Pathogen Infection	Khomas Nguni herd	Khomas Bonsmara herd	Omusati Nguni herd	Omusati Bonsmara herd	Zambezi Nguni herd
Anaplasma/Ehrlichia	96%	55%	95%	100%	75%
Babesia/Theileria	80%	3%	28%	0%	78%

4.7. Tick identification

Ticks belonging to three genera i.e. *Hyalomma*, *Amblyomma*, *Rhipicephalus* were identified in Khomas, Omusati and Zambezi region (Table 4.20). Six tick species were found in Omusati region, five in the Zambezi region and four in the Khomas region.

The most abundant tick species observed across all regions were *H. truncatum* with a prevalence of 35% followed by *H. rufipes* with 29%, *R. simus* with 16% and *R. evertsi mimeticus* with 9.5%. The prevalence of *R. evertsi evertsi*, *R. appendiculutus* and *Hyalomma turanicum* were 5%, 3% and 2% respectively. The least frequently encountered tick with a prevalence of merely 0.5 % and only found in the Zambezi region was *A. variegatum*. Figure 4.8 and Figure 4.9 shows some of the *Hyalomma, Rhipicephalus* and *Amblyoma* tick species identified in this study.

Table 4.21. Tick species collected from Omusati, Khomas and Zambezi region

Tick specie	Location
Hyalomma truncatum	Khomas, Omusati, Zambezi
Rhipicephalus evertsi mimeticus	Omusati, Zambezi
Rhipicephalus evertsi evertsi	Omusati, Zambezi
Hyalomma rufipes	Zambezi
Amblyomma variegatum	Zambezi
Rhipicephalus simus	Khomas, Omusati
Rhipicephalus appendiculutus	Khomas, Omusati
Hyalomma turanicum	Khomas, Omusati

Hyalomma truncatum

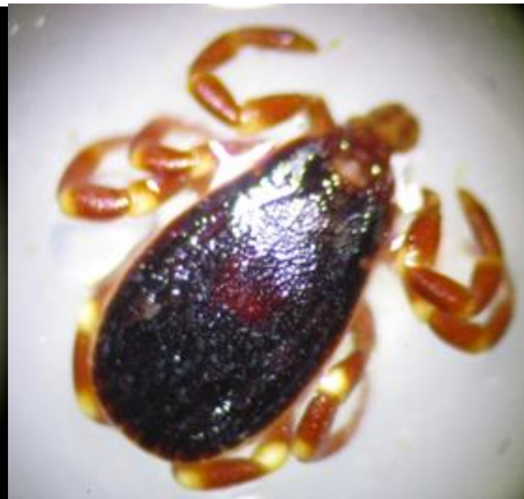
Hyalomma rufipes

Hyalomma turanicum

Amblyomma variegatum

Figure 4.7. *Hyalomma* and *Amblyomma* tick species found in Khomas, Zambezi and Omusati regions.

Rhipicephalus evertsi mimeticus *Rhipicephalus evertsi evertsi*



Rhipicephalus simus *Rhipicephalus appendiculutus*

Figure 4.8. *Rhipicephalus* male tick species found in Khomas, Omusati and Zambezi regions

4.8. Association of genetic variation with tick resistance

Four BoLA microsatellites (*DRB3*, *DRBP1*, *RM185*, *BM1815*) were analyzed for possible association with tick and TBD resistance. Analysis of BoLA class II alleles in Namibian beef cattle revealed that DRB3-292 (Allele frequency, AF = 0.66), *DRBP1*-126 (AF = 0.32), *RM185*-99 (AF = 0.31) and *BM1815*-155 (AF = 0.29) were the most frequent alleles (Table 4.22-Table 4.23) in tick resistant animals. Due to the polymorphic nature of BoLA class II microsatellite markers, only alleles with a frequency > 0.010 were considered for analysis of allelic association with tick resistance. Allele frequencies of the BoLA microsatellite loci in animals infected with *Anaplasma/Ehrlichia* and *Babesia/ Theileria* pathogens were also analyzed. The most frequent alleles in *Anaplasma/Ehrlichia* infected animals were *DRB3*-292 9AF = 0.67), *BM1815*-151 (AF = 0.33), *RM185*-99 (AF = 0.29) and *DRBP1*-126 (AF = 0.23), whereas allele *DRB3*-292 (AF =0.69), BM1815-151 (AF = 0.30), RM185-99 (AF = 0.26) and *DRBP1*-126 (0.18) were the most frequent alleles in animals infected with *Babesia/Theileria* parasites (Table 4.24-Table 4.26).

Table 4.22. Allele frequencies (AF) of *BoLA-DRB3* microsatellite loci amplified from Nguni, Afrikaner and Bonsmara cattle phenotyped as tick-resistant (R) and tick susceptible (S)

DRB3 locus				
Allele	**R**	**AF[a]**	**S**	**AF**
289	28	0.086	9	0.052
290	21	0.064	3	0.017
291	61	0.187	19	0.110
292	216	0.663	141	0.820

[a] AF-Allele frequency of tick-resistant (R) animals
[b] AF-Allele frequency of tick-susceptible (S) animals

Table 4.23. Allele frequencies (AF) of *BoLA-DRBP1*, *BM1815*, *RM185* microsatellite loci amplified from Nguni, Afrikaner and Bonsmara cattle phenotyped as tick-resistant (R) and tick susceptible (S)

Tick resistance														
***BM1815* locus**					***DRBP1* locus**					***RM185* locus**				
Allele	**R**	**AF[a]**	**S**	**AF[b]**	**Allele**	**R**	**AF[a]**	**S**	**AF[b]**	**Allele**	**R**	**AF[a]**	**S**	**AF[b]**
145	8	0.037	3	0.027	118	15	0.074	2	0.035	91	11	0.055	1	0.021
147	1	0.005	0	0.000	120	23	0.114	8	0.140	93	1	0.005	0	0.000
149	0	0.000	1	0.007	122	15	0.074	3	0.053	95	8	0.040	2	0.042
151	59	0.269	10	0.322	124	14	0.069	3	0.053	97	12	0.060	3	0.063
153	2	0.009	0	0.013	126	65	0.322	31	0.544	99	62	0.308	14	0.292
155	64	0.292	24	0.242	128	15	0.074	1	0.018	101	47	0.234	14	0.292
157	1	0.005	1	0.013	130	19	0.094	4	0.070	103	14	0.070	2	0.042
163	12	0.055	2	0.054	132	3	0.015	1	0.018	105	8	0.040	2	0.042
165	16	0.073	3	0.074	134	22	0.109	2	0.035	107	38	0.189	10	0.208
167	1	0.005	0	0.007	136	10	0.050	2	0.035					
169	53	0.242	9	0.228	138	1	0.005	0	0.000					
173	1	0.005	0	0.013										
175	1	0.005	0	0.000										

[a] AF-Allele frequency of tick-resistant (R) animals
[b] AF-Allele frequency of tick-susceptible (S) animals

Table 4.24. Allele frequencies (AF) of *BoLA-DRBP1*, *BM1815*, *RM185* microsatellite loci for *Anaplasma/Ehrlichia* pathogen infection status across all cattle populations

***Anaplasma/ Theileria* infections**														
BM1815 locus					**DRBP1 locus**					**RM185 locus**				
Allele	**positive**	**AF[a]**	**negative**	**AF[b]**	**Allele**	**positive**	**AF[a]**	**negative**	**AF[b]**	**Allele**	**positive**	**AF[a]**	**negative**	**AF[b]**
145	14	0.060	1	0.030	118	21	0.104	4	0.114	91	19	0.077	1	0.026
147	1	0.004	0	0.000	120	3	0.015	10	0.286	93	1	0.004	0	0.000
149	0	0.000	1	0.030	122	22	0.109	1	0.029	95	13	0.052	3	0.079
151	76	0.326	9	0.273	124	6	0.030	2	0.057	97	11	0.044	2	0.053
153	1	0.004	0	0.000	126	47	0.233	4	0.114	99	73	0.294	10	0.263
155	52	0.223	11	0.333	128	27	0.134	2	0.057	101	46	0.185	8	0.211
157	3	0.013	0	0.000	130	31	0.153	3	0.086	103	21	0.085	3	0.079
163	10	0.043	0	0.000	132	5	0.025	1	0.029	105	14	0.056	1	0.026
165	28	0.120	4	0.121	134	19	0.094	7	0.200	107	50	0.202	10	0.263
167	2	0.009	0	0.000	136	19	0.094	1	0.029					
169	45	0.193	7	0.212	138	2	0.010	0	0.000					
175	2	0.008	0	0.000										

[a] AF-Allele frequency of animals that tested positive for *Anaplasma/Ehrlichia* infections

[b] AF-Allele frequency of animals that tested negative for *Anaplasma/Ehrlichia* infections

Table 4.25. Allele frequencies (AF) of *BoLA-DRBP1*, *BM1815*, *RM185* microsatellite loci for *Babesia/Theileria* pathogen infection status across all cattle populations

***Babesia/ Theileria* infections**														
***BM1815* locus**					***DRBP1* locus**					***RM185* locus**				
Allele	**Positive**	**AF[a]**	**negative**	**AF[b]**	**Allele**	**positive**	**AF[a]**	**negative**	**AF[b]**	**Allele**	**positive**	**AF[a]**	**negative**	**AF[b]**
145	9	0.050	6	0.065	118	13	0.073	12	0.128	91	17	0.086	3	0.034
147	1	0.006	0	0.000	120	30	0.168	11	0.117	93	1	0.005	0	0.000
149	1	0.006	0	0.000	122	12	0.067	11	0.117	95	10	0.051	6	0.067
151	54	0.302	31	0.333	124	6	0.034	2	0.021	97	3	0.015	10	0.112
153	1	0.006	0	0.000	126	33	0.184	18	0.191	99	52	0.264	31	0.348
155	46	0.257	17	0.183	128	22	0.123	7	0.074	101	41	0.208	13	0.146
157	3	0.017	0	0.000	130	26	0.145	8	0.085	103	18	0.091	6	0.067
163	3	0.017	11	0.118	132	3	0.017	11	0.117	105	12	0.061	3	0.034
165	23	0.128	9	0.097	134	19	0.106	7	0.074	107	43	0.218	17	0.191
167	1	0.006	1	0.011	136	14	0.078	6	0.064					
169	36	0.201	16	0.172	138	1	0.006	1	0.011					
173	0	0.000	1	0.011										
175	1	0.006	1	0.011										

[a] AF-Allele frequency of animals that tested positive for *Babesia/Theileria* infections
[b] AF-Allele frequency of animals that tested negative for *Babesia/Theileria* infections

Table 4.26. Allele frequencies (AF) of *BoLA-DRB3* microsatellite loci for *Anaplasma/Ehrlichia* and *Babesia/Theileria* pathogen infection status across all cattle populations

Anaplasma/Ehrlichia					***Babesia/Theileria***				
Allele	**positive**	**AF[a]**	**negative**	**AF[b]**	**Allele**	**positive**	**AF[a]**	**negative**	**AF[b]**
289	35	0.086	2	0.023	289	13	0.047	24	0.108
290	22	0.054	2	0.023	290	14	0.051	10	0.045
291	79	0.194	1	0.011	291	58	0.210	22	0.099
292	272	0.667	83	0.943	292	191	0.692	166	0.748

[a] AF-Allele frequency of animals that tested positive for *Anaplasma/Ehrlichia* and *Babesia/Theileria* infections
[b] AF-Allele frequency of animals that tested negative for *Anaplasma/Ehrlichia* and *Babesia/Theileria* infections

The parameter estimates, standard error, *P* values and odds ratio of alleles associated with tick resistance are shown in Table 4.27. Breed had a significant effect on host resistance to ticks ($P < 0.05$), while age and gender had no significant effect ($P > 0.15$). The BoLA class II alleles influencing tick resistance in Namibian beef cattle breeds were determined. Effects of each BoLA allele identified in this study on tick resistance is shown in Appendix 6. The *DRB3*, *RM185* and *BM1815* were found to be potential markers for tick resistance ($P < 0.05$). Significant association ($P < 0.15$) was found between *DRB3* alleles (*DRB3*-289, *DRB3*-290, *DRB3*-291, *DRB3*-292) and resistance to ticks (Table 4.26). The *RM185*-93 allele showed a significant association ($P < 0.05$) to tick resistance with an OR of 4.315 (0.663 - 28.071). The *BM1815*-145 allele may also be involved in host resistance to ticks ($P < 0.15$). No allelic association was observed between tick-resistance and the *DRBP1* loci ($P > 0.15$).

Table 4.27. Parameter estimates, *P* values and odd ratio of the association of *DRB3, DRBP1, BM1815* and *RM185* alleles with tick-resistance

Allele	Parameter estimates	Standard error	X^2 statistic	df	*P-value*	Odds ratio	95% (Confidence Interval)
DRB3 locus							
289	-0.409	0.203	4.085	1	0.043	0.664	0.447 - 0.988
290	-0.546	0.251	4.722	1	0.030	0.580	0.354 - 0.948
291	-1.586	0.758	4.376	1	0.036	0.205	0.046 - 0.905
292	-0.713	0.417	2.924	1	0.087	0.490	0.216 - 1.110
RM185 locus							
93	1.462	0.955	2.341	1	0.126	4.315	0.663 - 28.071
BM1815 locus							
145	-1.302	0.738	3.111	1	0.078	0.272	0.064 - 1.156

X^2- Chi-square
df- degrees of freedom

The BoLA class II alleles associated with resistance to tick-borne pathogens (*Anaplasma/Ehrlichia*) were as shown in Table 4.28. Breed was found to have a significant association with resistance to *Anaplasma/Ehrlichia* pathogens ($P < 0.05$), while age and gender had showed no significant effect ($P > 0.05$) on resistance to *Anaplasma/Ehrlichia* pathogens (appendix 8).The *DRB3* and *DRBP1* loci were observed to be associated with lower tick burdens. Significant association ($P < 0.05$) was found between allele *DRB3*-290 and tick resistance. Three *DRBP1* alleles (*DRBP1*-120, *DRBP1*-122, *DRBP1*-126) showed association with resistance to *Anaplasma/Ehrlichia* infections. Allele *DRBP1*-120 showed a stronger association (OR = 2.710) compared to other alleles.

Table 4.28. Parameter estimates, standard error chi-square test statistics and odds ratios of the association of BoLA class II microsatellite alleles with *Anaplasma/Ehrlichia* infections across all cattle population

Allele	Parameter estimates	Standard error	X^2 statistic	df	*P-value*	Odds ratio	95% (Confidence Interval)
DRB3 locus							
290	-0.801	0.404	3.930	1	0.047	0.449	0.203 - 0.991
DRBP1 locus							
120	0.997	0.424	5.535	1	0.019	2.710	1.181 - 6.218
122	0.876	0.383	5.240	1	0.022	2.401	1.134 - 5.082
136	0.256	0.147	3.007	1	0.083	1.291	0.967 - 1.724

X^2- Chi-square
df- degrees of freedom

The *BoLA-DRB3, DRBP1, RM185* and *BM1815* alleles influencing host resistance to *Babesia/Theileria* infection in beef cattle breeds in Namibia were determined (Table 4.29). One DRB3 allele (*DRB3*-291) showed an association with *Babesia/Theileria* infections resistance ($P < 0.15$). Three *DRBP1* alleles (*DRPB1*-120, *DRBP1*-124, *DRBP1*-128) also showed a significant association with *Babesia/Theileria* infections resistance with *DRBP1*-120 showing the strongest association (OR = 1.785). Alleles belonging to the *RM185* locus (*RM185*-101, *RM185*- 103, *RM185*- 105, *RM185*- 107) may be involved in host resistance to *Babesia/Theileria* infections as shown by the significant association ($P < 0.15$) with OR values ranging from 1.244 (0.922 - 1.680) for *RM185*-105 to 1.643 (1.180 - 2.289) for *RM185*-103. The *BM1815* alleles (*BM1815*-145 and *BM1815*-163) also showed a significant association with resistance to *Babesia/Theileria* infections ($P < 0.15$). The breed, age and gender also influenced host resistance to *Babesia/Theileria* pathogens ($P < 0.05$) as shown in appendix 8.

Table 4.29. Parameter estimates, standard error chi-square test statistics and odds ratios of the association of BoLA class II microsatellite alleles with *Babesia/Theileria* infections across all cattle population.

Allele	Parameter estimates	Standard error	X^2 statistic	df	*P-value*	Odds ratio	95% (Confidence Interval)
DRB3 locus							
291	-1.189	0.738	2.599	1	0.107	0.304	0.072 - 1.293
DRBP1 locus							
120	0.579	0.403	2.069	1	0.144	1.785	0. 811 - 3.929
124	0.408	0.265	2.368	1	0.124	1.503	0. 894 - 2.527
128	0.247	0.162	2.316	1	0.128	1.280	0.931- 1.758
RM185 locus							
101	0.303	0.156	3.763	1	0.052	1.354	0.997 -1.840
103	0.497	0.169	8.620	1	0.003	1.643	1.180 - 2.289
105	0.219	0.153	2.037	1	0.154	1.244	0.922 - 1.680
107	0.250	0.108	5.317	1	0.021	1.284	1.038 - 1.588
BM1815 locus							
145	-1.620	0.988	2.686	1	0.101	0.198	0.029 - 1.373
163	-0.242	0.125	3.747	1	0.053	0.785	0.614 - 1.003

X^2- Chi-square
df- degrees of freedom

CHAPTER 5: DISCUSSION

Molecular characterization of livestock genetic diversity can facilitate breed improvement, and aid in the conservation of breeds. This characterization can assist in detecting inbreeding but most importantly it is the main tool for identifying genomic regions involved in disease resistance, adaptability and production traits (FAO, 2011). In the present study Nguni, Bonsmara and Afrikaner cattle from different locations of Namibia were characterized using four microsatellite markers. The polymorphism of these microsatellite markers which were all associated with the BoLA class II genes was further explored and associated with tick and TBD resistance in the three breeds. To date, only a limited number of studies on molecular characterization of the indigenous cattle breed (Nguni) in Namibia have been undertaken (Hanotte *et al*., 2000; Nortier *et al*., 2002) hence the importance of this study. Other studies (Stear *et al*., 1984; Acosta-Rodriguez *et al*., 2005) have reported association of the polymorphism of the BoLA genes with tick resistance; thus, it was the goal of this study to substantiate these claims in cattle breeds in Namibia.

5.1. Genetic diversity of BoLA class II genes and population structure of Nguni, Afrikaner and Bonsmara cattle in Namibia

The genetic diversity of the three cattle breeds was unravelled using four microsatellite markers; DRB3, DRBP1, BM1815, RM185. The DRB3 have been characterized in previous studies of the BoLA (Takeshima *et al*., 2003; Giovambattista *et al*., 2013; Takeshima *et al*., 2015). In this study, a total number of 37 alleles with a mean of 9.25 were detected across all four loci. A comparable mean number of alleles of 9.00 were reported by Sanarana *et al*. (2016) in Nguni ecotypes of South Africa. Lower mean numbers of alleles of 7.47 and 7.69 were reported in

Zambian indigenous breeds (Musimuko, 2014) and Mozambican indigenous cattle breeds (Bessa *et al*., 2009) respectively. Overall, the *DRBP1* locus was the most polymorphic (He = 0.781) and the most informative marker (PIC = 0.743) whereas the *DRB3* exhibited the lowest polymorphism (He = 0.428) and only 4 alleles could be amplified at this locus. The *DRB3* was however reported to be the most polymorphic locus with 12 alleles in European cattle (Acosta-Rodriquez *et al*., 2005) implying high genetic diversity at this locus as compared to Namibian cattle breeds.

Analysis of the genetic diversity of the cattle breeds revealed that Nguni cattle exhibited the highest mean number of alleles with 7.53 alleles and expected heterozygosity of 0.728 suggesting that this breed exhibit higher genetic diversity than the Afrikaner (He =0.660) and Bonsmara (He = 0.637) cattle. The genetic diversity of the Namibian Nguni herds (H_e = 0.728) was higher than the genetic diversity of South African Nguni ecotypes (He = 0.701) and Mozambican Angone (He =0.688) (Bessa *et al*., 2009; Sanarana *et al*., 2016). Four private alleles were found in Nguni cattle signifying the uniqueness of this breed amongst other breeds. Indigenous cattle are indeed reservoirs of genetic diversity and conservation of these breeds should be prioritised to maintain their genetic diversity (FAO, 2011). The high genetic diversity of the indigenous Nguni breed probably contributed to the adaptability of this breed to the country's harsh environmental conditions with some of this variation stemming from introduction of new alleles arising from new mutations or pre-existing segregating genetic variant (Maynard *et al*., 1974).

The lowest level of genetic diversity was found in Bonsmara cattle with He = 0.637. Makina *et al*. (2014) reported the lowest genetic diversity (He = 0.24) in South African Afrikaners relative to other cattle breeds which included Nguni and Bonsmara. No private alleles were found in the Bonsmara population, presumably

due to breed selection pressures. The Afrikaner breed exhibited the lowest number of alleles per locus of 5.50 while the highest number of 8.75 alleles was observed in Nguni cattle. Contrarily, Makina *et al*. (2014) reported the lowest number of alleles per locus in Nguni and the highest in Afrikaner cattle with 1.73 and 1.88, respectively. Although Afrikaner cattle exhibited high levels of gene diversity, they had a low number of alleles. A similar observation was reported in South African Afrikaners (Makina *et al*., 2014). It should be noted that the Afrikaner breed was wiped out by the outbreak of the Rinderpest pandemic more than a hundred years ago (Porter, 1991). It is probable that this event resulted in population bottlenecks contributing to the low number of alleles observed. Strong selection and small population size could have also attributed to the low number of alleles. New alleles should be introduced into the population by introducing new bulls from different gene pools in order to increase the genetic diversity of this breed.

High levels of inbreeding were detected in Afrikaners with an F_{IS} value of 0.247. The level of inbreeding recorded here is an important observation as it exceeds the acceptable value of 0.15 (Blackburn *et al*., 2011). This may be attributed to paucity of pure Afrikaner bulls, small population sizes and strong selection as this is the main breed used in crossbreeding in Southern Africa and have to conform to the Afrikaner cattle breeder's society standards. Afrikaners have also been reported to be at risk of inbreeding in South Africa (Makina *et al*., 2014) probably because of the selection pressure to breed pure Afrikaner animals for crossbreeding purposes. Inbreeding has negative effects which include reduced genetic diversity, milk production losses, reduced survival, decreased lactation length and decreased fertility as demonstrated in Holstein cattle (Thompson *et al*., 2000; McPaland *et al*., 2007). Inbreeding can also expose recessive deleterious alleles to natural selection through

increased homozygosity (Keller & Waller, 2002). These deleterious effects can be avoided by random mating and introducing sires from different gene pools.

An excess of heterozygotes was observed in Bonsmara cattle with an inbreeding coefficient of -0.057, this can be interpreted as possible signs of outbreeding. This suggests a positive effect of the managed breeding taking place for this breed. Makina *et al*. (2014) also reported an excess of heterozygotes (F_{IS} = -0.017) in South African Bonsmara. Low levels of inbreeding were observed in Nguni cattle with an inbreeding coefficient of 0.071. Inbreeding warrants close monitoring and should be assessed every five years to maintain acceptable levels (Makina *et al*., 2014).

Significant deviation ($P < 0.05$) from HWE assumptions was observed in the *DRB3* markers in all breeds. This is presumably a consequence of the presence of null alleles (caused by a mutation in one of the primer sites) or the presence of population sub-structure which leads to Wahlund's effect (Wittke-Thompson *et al*., 2005). This could also be due to typical, low levels of genotyping errors such as mis-scoring, replication slippage and allelic dropout as demonstrated by Phillip *et al*. (2009).

Nei's measures of genetic distances revealed shortest genetic distance between Bonsmara and Nguni with 0.0706 followed by Nguni and Afrikaner with 0.181. Interestingly, the greatest divergence of 0.322 was found between Bonsmara and Afrikaner cattle. A similar trend was observed by Makina *et al*. (2014). Considering that Bonsmara cattle are a cross breed of 5/8 Afrikaner and 3/8 Shorthorn, short genetic distance would be expected between Bonsmara and Afrikaner. This is likely to be due to the effects of genetic drift after admixture and strong selection of animals to conform to the standards and breeding objectives of the breed's society (Makina *et al*., 2014).

The results of Bayesian cluster analysis with STRUCTURE software revealed some admixture between breeds. Bonsmara cattle shared some genetic links with Nguni cattle, with about 1.2% of their genome derived from Nguni and 0.08% derived from Afrikaners. The genetic links between Afrikaner and Nguni was 1.4% which was the highest value of admixture between the three breeds. These results are in accordance with those obtained by Makina *et al*. (2014) in South African Afrikaners and Nguni. As suggested by Scholtz *et al*. (2011) this could be explained by the origin of the breeds and their introduction to Southern Africa. These results provide insight into the origin and evolution of cattle in Southern Africa.

All three cattle populations (Nguni, Afrikaner and Bonsmara) maintained a high level of genetic variation and a highly significant level of population differentiation of 95.2% attributed to within-populations differences as revealed by the analysis of molecular variance (AMOVA). The remainder (4.8%) was accounted for by differentiation among populations. It should be noted that the algorithms for calculating F_{ST} in ARLEQUIN can produce negative values for some loci when, as in the present study, sample sizes differ greatly among populations hence depressing the overall mean $F_{ST.}$ Sanarana *et al*. (2016) reported 4.8% population differentiation among South African Nguni ecotypes, 4.7 % was obtained among Mozambican indigenous breeds (Bessa *et al*., 2009) and 2.3% in Zambian indigenous breeds (Musimuko *et al*., 2014). In Indian cattle, 24% among breed variation was reported (Sharma *et al*., 2015) while only 1.3 % was reported in Ethiopian indigenous cattle populations (Dadi *et al*., 2008). In addition to their common historic origins, the high gene flow could theoretically be another possible explanation to the low population differentiation observed in the present study.

The genetic diversity of Bonsmara and Nguni cattle was analyzed based on their geographical locations. The highest expected heterozygosity of 0.743 was found in the NGUK herd, this value is slightly higher than those obtained in previous studies of Nguni ecotypes in South Africa (Sanarana *et al*., 2016) and Mozambique (Bessa *et al*., 2009). The NGUO herd had an expected heterozygosity of 0.716 which is comparable to the 0.717 of the Pedi Nguni ecotype of South Africa (Sanara *et al*., 2016). The NGUZ herd exhibited the lowest levels of expected heterozygosity in this study of 0.684 which is comparable to that of the Angone cattle breed (He = 0.688) of Mozambique (Bessa *et al*., 2009). Historically, Nguni cattle in Northern Namibia are divided into four ecotypes; Owambo, Kavango, Kunene and Caprivi. The difference observed in the genetic diversity of the Nguni herds could be attributed to the fact that these cattle belong to different ecotypes. The Omusati Nguni herd belong to the Owambo ecotype and the Zambezi Nguni to the Caprivi ecotype. The ecotype of the Nguni sampled at the Neudamm experimental farm in Khomas region could be either Owambo, Kunene, Kavango or Caprivi. The specific origin is not known. The high gene diversity of the NGUK herd could be attributed to the fact that the Neudamm experimental farm buys new bulls every six years, which could be the underlying reason for the high gene diversity observed. Contrary to the results herein, Nortier *et al*. (2002) reported high levels of gene diversity of 0.732 in the Caprivi ecotype compared to the 0.669 observed in this study. However a comparable number of alleles in the Caprivi ecotype was obtained in the two studies.

Population structure analysis revealed a signal of admixture and genetic relationships between the Nguni ecotypes. The unknown Nguni ecotype from Khomas region and the Caprivi ecotype showed the highest levels of admixture. Sixty seven percent (67%) of the unknown ecotypes genome was assigned to cluster two

while 16.9% and 16.1% of its genome was derived from Caprivi and Owambo ecotype respectively. The Caprivi ecotype also shared genetic links of about 12.4% with the Owambo ecotype. Almost fourteen percent of the Caprivi ecotype was derived from the unknown Nguni ecotype from Khomas region. It is probable that the unknown Nguni ecotype from the Khomas region belongs to the Kavango ecotype because of the comparable mean heterozygosity values and number of alleles reported by Nortier *et al*. (2002). The author reported 7.9 alleles and a mean heterozygosity of 0.710 while 8.2 alleles and a mean heterozygosity of 0.700 was observed in the current study. Also, the Kavango and Caprivi ecotype are closest on land which explains the high levels of admixture of 16.9% between the two ecotypes. Most importantly, the Kavango ecotype is large-framed (Els, 2002) compared to other ecotypes which could be one of the potential explanations as to why it has the highest proportion of admixture among all the ecotypes. Selection for its large frame must have led to its distribution in the central part of the country.

Differences in genetic diversity were observed in the two Bonsmara herds from Khomas and Omusati region. Expected heterozygosity was high in the Omusati herd (He = 0.653) than in the Khomas herd (He= 0.616). It should be noted that Omusati region is much drier with high temperatures, constantly affected by drought and floods, and is located in the NCAs which is considered disease-prone. Animals in this part (NCAs) of the country are constantly challenged with diseases, low quality feed and limited water. High genetic diversity is crucial in response to environmental and disease challenges and aids in the adaptation of livestock to their respective environments. This is probably one of the underlying reasons for the observed high heterozygosity in the Omusati region Bonsmara herd as compared to the Bonsmara herd in Khomas region. In addition, there were two alleles (*DRBP1*-136 and

BM1815-173) in the Omusati Bonsmara herd that were not found in the Khomas Bonsmara herd but were found in Nguni cattle. These could have been introduced in response to the harsh and dry conditions of Northern Namibia or in response to diseases. However, since Bonsmara cattle are crossbreed of Afrikaner and Shorthorn and/or Hereford the differences in genetic diversity could have also stemmed from the crossing. Considering differences in the geographical distributions of the cattle breeds, low levels of gene differentiation were observed (F_{ST} = 0.058). This suggest that the three breeds are not so different which is also correspond to the high gene flow value (Nm =5.614).

5.2. Tick loads and species prevalence

Tick counts significantly varied between breeds with the highest tick count recorded in Bonsmara cattle and the lowest in Nguni cattle ($P < 0.05$). Other authors (Spickett *et al*., 1989; Scholtz *et al*., 1991; Nyangiwe *et al*., 2011;) also reported low tick counts in Nguni cattle and higher tick counts in Bonsmara cattle. The differences in tick counts observed in the three breeds were most likely influenced by the genetics of the cattle (Acosta-Rodriguez *et al*., 2007), morphological traits such as coat colour and skin thickness (Marufu *et al*., 2011) along with environmental factors and vegetation composition (Schulz *et al*., 2014) which affect the exposure to ticks as well as their survival on the host. The Nguni breed which exhibited high genetic diversity (He = 0.728) had low tick burdens. On the other hand, the Bonsmara that exhibited the lowest genetic diversity (He = 0.637) in this study had high tick burdens. It is probable that there is a genetic component of variation in host resistance to ticks. Breeding for genetic resistance to ticks using the indigenous Nguni cattle is one of the promising ways to control ticks. Higher tick counts were recorded in the hot-wet summer (mean = 17 ± 7) than in the cool-dry winter (mean = 6 ± 4)

suggesting that warm moist climatic conditions in summer are more conducive for tick proliferation and survival given that there is abundance of grass for ticks to quest on and high humidity in summer than in winter. The significant seasonal difference in tick counts ($P < 0.05$), proves that environmental factors and vegetation cover influence the number of ticks infesting the animals. It should be noted that total tick populations (whole body tick counts) on each host was not enumerated in this study, only ticks under the tail area, ears and on the udder were counted.

H. truncatum was the most prevalent tick with 35% and was found in all sampling sites. This tick specie (*H. truncatum*) is prevalent in the central and northern Namibia and is present throughout South Africa, Zimbabwe and some parts of Botswana, Angola and most of Mozambique (Walker *et al*., 2003). *H. truncatum* was also found to be the most prevalent tick in Namibian black rhinoceroses (Horak, *et al*., 2017). Based on the survey by Nyangiwe *et al*. (2013), it could have been expected to find the recently introduced *R. microplus* in the Khomas region. We might have expected to find *R. decoloratus* as well as previously reported by Biggs & Langenhoven (1984), but these were not encountered in our tick sampling. This is possibly because the preferred attachment sites of these ticks which include the sides of the body, neck and dewlap were not the focus of this study. It is postulated that *R. microplus* was introduced in Namibia from South Africa through importation of cattle (Nyangiwe *et al*., 2013).

The geographical and cattle breed distribution of the eight tick species in this study varied, possibly due to different environmental conditions (temperature and rainfall), vegetation coverage, human activities, and photoperiods of the sampling sites as these have been previously reported to be some of the important factors influencing tick distribution (Dantas-Torres, 2015; Beckley *et al*., 2016).

5.3. Tick-borne pathogen infections in three beef cattle breeds in Namibia

Over 85% and 53% of the cattle in this study were exposed to *Anaplasma/Ehrlichia* and *Babesia/Theileria* infections. However, none of the animals showed clinical signs suggestive of Anaplasmosis, Ehrlichiosis, Babesiosis, Theileriosis, respectively, or any disease caused by these pathogens. This could be explained by enzootic stability which is an epidemiological state in which clinical disease is low despite high levels of infection in the population (Musisi & Lawrence, 1995; Makala *et al*., 2003). Jonsson *et al*. (2012) however strongly argued that enzootic stability may not be appropriate for babesiosis and is not very useful for planning disease control because it is rarely achieved cannot be effectively managed without impractical monitoring. Duangjinda *et al*. (2013) also reported higher proportions of *Anaplasma* infection (prevalence = 68%) compared to *Babesia* infections (prevalence = 48.3%) in cattle breeds in Thailand. There was a significant difference ($P < 0.05$) in proportion of infected animals between breeds for each pathogen which could have arisen from different factors such as the genetics of the cattle and the tick species infesting them.

Although Afrikaner cattle had the highest percentage (93%) of *Anaplasma/Ehrlichia* infections compared to the Bonsmara cattle (60%), higher tick numbers were recorded in Bonsmara (26 ± 10) compared to the Afrikaner cattle (17 ± 8). Thus it is surprising that Afrikaner cattle had low tick counts but high proportions of infection because indigenous breeds are known and have been proven to be resistant not only to ticks but also to TBDs (Nyamushamba *et al*., 2017). The contradicting results can be explained by two hypotheses. Since the tick counts did not correspond to the TBDs prevalence, the observed low tick numbers and high infection proportion may have arisen from enzootic stability. This means that these

cattle were infected as offsprings transplacentally as demonstrated by Grau *et al.* (2013). Also, Afrikaner cattle had high levels of inbreeding (F_{IS} = 0.247) amongst the three breeds hence rendering it susceptible to TBDs. While Bonsmara cattle had the highest tick counts compared to either Afrikaner or Nguni, only a 2% *Babesia/Theileria* infection rate was recorded. This may be attributed to the low prevalence of *H. rufipes* on Bonsmara cattle responsible for transmitting *Babesia* in cattle. Other tick species infesting Bonsmara cattle included *H. truncatum* and *R.simus* which do nottransmit neither *Babesia* nor *Theileria*.

The overall *Anaplasma/Ehrlichia* and *Babesia/Theileria* co-infection prevalence in all breeds was 47%. Differences in the prevalence of co-infection within breeds were observed with 76%, 3% and 57% pathogen co-infection prevalence in Afrikaner, Bonsmara and Nguni cattle, respectively. An Overall of 26 % co-infection of *Anaplasma, Theileria* and *Babesia* species has been reported in indigenous cattle breeds of Ghana (Beckley, 2013). The chi-square test revealed no significant associations ($P > 0.05$) between breed and co-infection prevalence hence ruling out genetic diversity as the underlying reason to the observed differences in co-infection prevalence between breeds. The prevalence of tick-borne pathogen co-infection is dependent on the geographical distribution of the vector.

5.4. Association of BoLA alleles with tick and tick-borne disease resistance

BoLA class II genes are of major interest in veterinary medicine and have been associated with resistance and susceptibility to ticks (Untalan *et al.*, 2007) and TBDs in cattle (Duangjinda *et al.*, 2013). Three BoLA microsatellite loci (*DRBP1, RM185, BM1815*) amplified from Bonsmara, Nguni and Afrikaner cattle in Namibia were evaluated for association with ticks and TBDs resistance. Even though the *DRB3*

locus deviated from HWE ($P < 0.05$) it was included in evaluation of allelic association with ticks or TBDs because the cause for deviation could not be established.

In this study, all four *DRB3* alleles (*DRB3*-289, *DRB3*-290, *DRB3*-291, *DRB3*-292). DRB3 alleles have been previously reported to be involved in tick resistance (Acosta-Rodriguez *et al*., 2005; Martinez *et al*., 2006; Untalan *et al*., 2007). One resistance allele namely *DRB3*-174 was reported in Simmental-Red poll crossbreeds (Untalan *et al*., (2007), while Acosta-Rodriguez *et al*. (2005) reported one *DRB3* susceptibility allele (*DRB3*-184) in Mexican Holstein-Zebu crossbreeds. Untalan *et al*. (2007) also reported allelic association between the *DRBP1* loci (*DRB1*-118 allele) and tick resistance. However, no association was found between the *DRBP1* alleles and tick resistance in this study. The *RM185*-93 allele showed a strong association with tick resistance (OR = 4.315) while the *BM1815*-145 allele showed a weak tick resistance association (OR =0.272).Selecting parents possessing allele *DRB3*-289, *DRB3*-290, *DRB3*-291, *DRB3*-292, *RM185*-93 and *BM1815*-145 for future generations should therefore increase the herd's resistance to tick infestation. It is important to emphasize that tick resistance is a polygenic trait controlled by many loci (Bishop & Woolliams, 2014) along with environmental and immunological factors (Budeli *et al*., 2012). Therefore good management practices such as pasture spelling, habitat modifications are advised even when farming with tick-resistant cattle breeds.

Previous studies that analyzed the same loci (Acosta-Rodriquez *et al*., 2005; Untalan *et al*., 2007), reported no association between tick resistance and the *RM185* locus. However, the *RM185* that lies outside of the BoLA complex showed the strongest association in Namibian cattle breeds. In contrast to the findings of Untalan

et al. (2007), the *BM1815* which lies between BoLA IIa and IIb clusters was associated with tick resistance. Other studies have found association between tick resistance and BoLA class I allele (Weigel & Freeman, 1990), and genes not part of the immune system, *ELTD1* gene (Porto-Neto *et al*., 2010). GWA studies of tick resistance have also been undertaken in South African Nguni (Mapholi *et al*., 2016), Gyr-Holstein crosses (Machado *et al*., 2010) and in Afrikaner, Nguni and Bonsmara cattle (Makina *et al*., 2015).

Alleles associated with resistance to *Anaplasma/Ehrlichia* infections in cattle were identified in this study on the *DRBP1* and *BM1815* locus. Allele *DRB3*-290, *DRBP1*-120, *DRBP1*-122 and *DRBP1*-126 were associated with decreased incidence of *Anaplasma/Ehrlichia* infections in Namibian cattle breeds. The *DRB3*-290 allele was found to be associated with both decreased incidence of *Anaplasma/Ehrlichia* and decreased tick counts. Overall *DRBP1* alleles, *DRBP1*-120 showed a stronger association (OR = 2.710) with resistance to *Anaplasma/Ehrlichia* infections.

One DRB3 allele (*DRB3*-291) showed an association with tick resistance ($P < 0.15$). BoLA-DRB3 alleles (allele *14 and *21) were reported to be significantly associated with *A. marginale*, a *B. bovis* (allele *14) and *B.bigemina* (allele *10 and *51) by (Duangjinda *et al*., 2013).The finding of Duangjinda *et al*. (2013) were consistent with the results of Martinez *et al*. (2006) in that allele*51 was reported to be associated with mastitis resistance in Holstein crossbreeds. Three *DRBP1* alleles (*DRPB1*-122, *DRBP1*-124, *DRBP1*-128) and four alleles belonging to the *RM185* locus (*RM185*-101, *RM185*-103, *RM185*-105, *RM185*-107) may be involved in host resistance to *Babesia/Theileria* infections. The breed, age and gender also was shown to also influence host resistance to *Babesia/Theileria* pathogens ($P < 0.05$). Resistant alleles for TBDs identified in this study could be used as potential genetic markers for

selecting TBD resistant animals. Allelic association with TBDs in cattle is not as well documented as is resistance to ticks themselves but one of the relevant results on the use of MAS for disease resistance was by Maillard *et al*., (2003). The author demonstrated how MAS can be used to reduce dermatophilosis prevalence.

CHAPTER 6: CONCLUSION AND RECOMMENDATIONS

This study presented a detailed analysis of the genetic diversity and differentiation of three beef cattle breeds from three different regions in Namibia. The four BoLA markers used in this study were effective in detecting genetic diversity and allelic association with ticks and TBD resistance in the three cattle breeds. The markers were also effective in detecting inbreeding in populations and in understanding the population structure of the three breeds. It was revealed that indigenous Nguni cattle of Namibia retain high levels of genetic diversity based on the results of the analysis of the four microsatellite markers. It will be worthwhile to assess the genetic diversity of other Nguni ecotypes (Kunene and Kavango) from different localities to get a good picture of the genetic diversity within the Nguni breed. The high genetic diversity of the Nguni suggests that this breed should be conserved as they form the backbone of relevant and sustainable cattle production in Namibia. In addition, the Nguni breed hold the potential for production in the ever increasing, harsh and fluctuating Namibian environments brought about by the notable climatic changes which is characterized by frequent droughts. Thus, this study may serve as an initial reference for establishing conservation policies.

In the present study, excessive inbreeding detected in the Afrikaners suggests the need for appropriate measures to be taken to avoid the negative effects of inbreeding. It may be more appropriate to sample more Afrikaner populations to establish their risk status and take necessary measures for their conservation such as introducing new bulls. Given its role in crossbreeding, it is imperative to conserve the Afrikaner breed to ensure utilization to its full potential in the era of climate change and disease epidemics.

The results obtained in this study confirm the potential of BoLA alleles as molecular markers for ticks and disease resistance in cattle. This is the first report on the association of BoLA alleles with tick and TBD resistance in cattle breeds in Namibia. Alleles identified in this study to be associated with ticks and TBDs can be used in breeding programs to select resistant animals. Integrating current breeding programmes with selection for tick and TBD resistance is the promising way to control ticks and increase productivity simultaneously. This not only could minimise the use of chemicals to control ticks which have deleterious effects on health and environment, but also reduces the susceptibility of a population in a given enzootic area.

Although the Nguni breed showed the highest resistance to ticks compared to Bonsmara and Afrikaner cattle, it is known to have low productivity due to its small frame. Since tick resistance is a heritable trait, Nguni can be used as dam lines in crossbreeding to improve productive beef breeds that are susceptible to ticks, thus introgressing desirable resistance attributes. The Australian Friesian Sahiwal was an excellent composite breed was created for high milk production and tick resistance using MAS.

In order for such endeavours to succeed, good management practises must be followed. In future, genomic scans for quantitative trait loci (QTL) controlling tick resistance using SNPs is recommended. Future studies can also focus on the linkage disequilibrium (LD) in Nguni cattle population for the characterization of genetic architecture.

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APPENDICES

Appendix 1: Allele frequency comparison over populations (within and between breeds)

DRB3	AFR	BON	NGU	**DRBP1**	AFR	BON	NGU	**RM185**	AFR	BON	NGU	**BM1815**	AFR	BON	NGU
289		10.00	6.57	118		9.62	9.63	91	3.57		6.60	145		5.36	5.59
290	17.14	1.82	2.55	120	4.76	34.62	15.93	93	1.19			147			0.35
291	30.00	1.82	17.52	122	3.57	9.62	7.78	95		3.57	5.56	149		0.89	0.35
292	52.86	86.36	73.36	124	30.95	0.96	3.33	97	7.14	8.04	5.21	151	39.29	25.89	32.52
				126	48.81	16.35	19.26	99	52.38	18.75	28.82	153	4.76		0.35
				128		0.96	11.11	101	16.67	43.75	19.79	155	38.10	33.93	25.87
				130		12.50	11.85	103	4.76	3.57	7.99	157	4.76		1.40
				132			2.59	105		3.57	5.56	163	4.76	8.04	3.50
				134	11.90	14.42	10.00	107	14.29	18.75	20.49	165		1.79	12.24
				136		0.96	7.78					167			0.70
				138			0.74					169	5.95	23.21	16.78
												173		0.89	0.35
												175	2.38		

AFR-Afrikaner

BON-Bonsmara

NGUN-Nguni

Appendix 2: Allele frequencies across all populations based on geographical locations

BONO - Omusati Bonsmara herd,
BONK- Khomas Bonsmara herd
NGUK- Khomas Nguni herd
NGUO- Omusati Nguni herd
NGUZ- Zambezi Nguni herd

Locus	Populations					
DRB3	**AFR**	**BONO**	**BONK**	**NGUK**	**NGUO**	**NGUZ**
289		23.5	3.9	3.3	15.8	2.8
290	17.1		2.6	2.2	6.6	
291	30.0	2.9	1.3	32.2	6.6	13.0
292	52.9	73.5	92.1	62.2	71.1	84.3
DRBP1	**AFR**	**BONO**	**BONK**	**NGUK**	**NGUO**	**NGUZ**
118		5.6	11.8	4.5	13.9	10.9
120	4.8	38.9	32.4	22.7	20.8	7.3
122	3.6	16.7	5.9	9.1	11.1	4.5
124	31.0		1.5	2.3	1.4	5.5
126	48.8	5.6	22.1	11.4	26.4	20.9
128			1.5	19.3	4.2	9.1
130		19.4	8.8	18.2	9.7	8.2
132				2.3		4.5
134	11.9	11.1	16.2	3.4	2.8	20.0
136		2.8		5.7	8.3	9.1
138				1.1	1.4	
RM185	**AFR**	**BONO**	**BONK**	**NGUK**	**NGUO**	**NGUZ**
91	3.6			16.7		2.6
93	1.2					
95			5.3	4.2	6.6	6.0
97	7.1	2.8	10.5	2.1	13.2	2.6
99	52.4	13.9	21.1	25.0	36.8	26.7
101	16.7	41.7	44.7	24.0	17.1	18.1
103	4.8	5.6	2.6	8.3	9.2	6.9
105			5.3	8.3	2.6	5.2
107	14.3	36.1	10.5	11.5	14.5	31.9

BM1815	AFR	BONO	BONK	NGUK	NGUO	NGUZ
145		5.6	5.3	2.1	3.9	9.5
147				1.1		
149			1.3			0.9
151	39.3	13.9	31.6	30.9	36.8	31.0
153	4.8			1.1		
155	38.1	44.4	28.9	33.0	25.0	20.7
157	4.8			3.2		0.9
163	4.8	2.8	10.5	1.1	10.5	0.9
165		2.8	1.3	14.9	1.3	17.2
167				1.1		0.9
169	6.0	27.8	21.1	11.7	21.1	18.1
173		2.8			1.3	
175	2.4					

Appendix 3: Hardy Weinberg Equilibrium

Key to Locus names

1- DRB3
2- DRBP1
3- RM185
4- BM1815

Hardy-Weinberg equilibrium: (Afrikaner)

Locus	Number of genotypes	Observed heterozygosity	Expected heterozygosity	*P*-value	Standard deviation	Steps done
1	35	0.171	0.288	0.038	0.000	1001000
2	42	0.452	0.656	0.027	0.000	1001000
3	42	0.714	0.677	0.487	0.000	1001000
4	42	0.571	0.698	0.155	0.000	1001000

Hardy-Weinberg equilibrium: (Bonsmara)

Locus	Number of genotypes	Observed heterozygosity	Expected heterozygosity	P-value	Standard deviation	Steps done
1	55	0.236	0.210	1.000	0.000	1001000
2	52	0.846	0.806	0.225	0.000	1001000
3	56	0.804	0.735	0.776	0.000	1001000
4	56	0.786	0.761	0.700	0.000	1001000

Hardy-Weinberg equilibrium: (Nguni)

Locus	Number of genotypes	Observed heterozygosity	Expected heterozygosity	P-value	Standard deviation	Steps done
1	137	0.153	0.166	0.300	0.000	1001000
2	135	0.770	0.881	0.004	0.000	1001000
3	144	0.931	0.819	0.007	0.000	1001000
4	143	0.783	0.782	0.005	0.000	1001000

Appendix 4: Tick count across breeds and geographical locations (mixed-design ANOVA)

Tests of Within-Subjects Contrasts						
Source	**Season**	**Type III Sum of Squares**	**Degrees of freedom**	**Mean Square**	**F**	**Sig.**
Season	Linear	1.866	1	1.866	18.233	0.000
season * breed	Linear	6.135	2	3.067	29.980	0.000
season * Location	Linear	0.200	2	0.100	0.977	0.378
Error(season)	Linear	24.863	243	0.102		

Tests of Between-Subjects Effects					
Source	**Type III Sum of Squares**	**Degrees of freedom**	**Mean Square**	**F**	**Sig.**
Intercept	113.441	1	113.441	1306.969	0.000
Breed	44.106	2	22.053	254.077	0.000
Location	0.413	2	0.206	2.377	0.095
Error	21.092	243	0.087		

Multiple Comparisons (Tukey HSD)

	Breed	Breed	Mean Difference	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	AFR	BON	-0.241*	0.0422	0.000	-0.341	-0.141
		NGU	0.534*	0.0364	0.000	0.448	0.620
	BON	AFR	0.241*	0.0422	0.000	0.141	0.341
		NGU	0.775*	0.0322	0.000	0.699	0.851
	NGU	AFR	-0.534*	0.0364	0.000	-0.620	-0.450
		BON	-0.775*	0.0322	0.000	-0.851	-0.699

Appendix 5: Binary logistic regression (TBD pathogen prevalence, breed & Location)

Variables in the Equation (*Anaplasma/ Ehrlichia*)

		B	S.E.	Wald	Degrees of freedom	Sig.	Exp(B)	95% C.I. for EXP(B)	
								Lower	Upper
Step 1[a]	Breed			20.375	2	0.000			
	Breed(1)	1.348	0.799	2.847	1	0.092	3.850	0.804	18.430
	Breed(2)	-1.355	0.384	12.445	1	0.000	0.258	0.122	0.548
	Location			1.212	2	0.545			
	Location(1)	-19.555	40193.741	0.000	1	1.000	0.000	0.000	.
	Location(2)	-19.133	40193.741	0.000	1	1.000	0.000	0.000	.

Variables in the Equation (*Babesia/theileria*)

		B	S.E.	Wald	df	Sig.	Exp(B)	95% C.I.for EXP(B)	
								Lower	Upper
Step 1[a]	Breed			29.341	2	0.000			
	Breed(1)	-0.580	0.627	0.854	1	0.355	0.560	0.164	1.915
	Breed(2)	-5.922	1.103	28.824	1	0.000	0.003	0.000	0.023
	Location			13.563	2	0.001			
	Location(1)	-19.014	40196.048	0.000	1	1.000	0.000	0.000	
	Location(2)	-20.900	40196.048	0.000	1	1.000	0.000	0.000	

Appendix 6: Allelic association with tick resistance (Binary logistic regression)

Variables in the Equation								
							95% C.I.for EXP(B)	
	B	S.E.	Wald	df	Sig.	Exp(B)	Lower	Upper
Breed			0.980	2	0.613			
Breed(1)	0.423	0.726	0.340	1	0.560	1.527	0.368	6.331
Breed(2)	0.533	0.565	0.891	1	0.345	1.704	0.563	5.153
Gender	0.012	0.495	0.001	1	0.980	1.012	0.384	2.668
DRB3 291	-1.586	0.758	4.376	1	0.036	0.205	0.046	0.905
DRB3 292	-0.713	0.417	2.924	1	0.087	0.490	0.216	1.110
DRB3 289	-0.409	0.203	4.085	1	0.043	0.664	0.447	0.988
DRB3 290	-0.546	0.251	4.722	1	0.030	0.580	0.354	0.948
DRBP118	0.121	0.559	0.047	1	0.828	1.129	0.377	3.380
DRBP120	0.084	0.262	0.104	1	0.747	1.088	0.651	1.818
DRBP122	0.073	0.207	0.125	1	0.724	1.076	0.717	1.614
DRBP124	0.092	0.171	0.291	1	0.590	1.097	0.784	1.534
DRBP126	-0.129	0.099	1.681	1	0.195	0.879	0.724	1.068
DRBP128	0.005	0.109	0.002	1	0.962	1.005	0.812	1.244
DRBP130	0.081	0.078	1.080	1	0.299	1.084	0.931	1.263
DRBP132	-0.151	0.158	0.917	1	0.338	0.860	0.631	1.172
DRBP134	-0.052	0.061	0.737	1	0.391	0.949	0.842	1.069
DRBP136	-0.058	0.074	0.618	1	0.432	0.943	0.816	1.091
RM91	0.730	0.740	0.972	1	0.324	2.074	0.486	8.845
RM95	-0.234	0.272	0.737	1	0.391	0.792	0.464	1.350
RM97	0.075	0.175	0.183	1	0.669	1.078	0.765	1.519
RM99	0.047	0.115	0.165	1	0.685	1.048	0.836	1.314
RM101	0.086	0.097	0.780	1	0.377	1.089	0.901	1.317
RM103	0.067	0.101	0.441	1	0.507	1.069	0.878	1.303
RM105	0.070	0.090	0.601	1	0.438	1.073	0.899	1.280
RM107	0.089	0.063	2.004	1	0.157	1.093	0.966	1.236
BM145	-1.302	0.738	3.111	1	0.078	0.272	0.064	1.156
BM151	-0.017	0.119	0.021	1	0.884	0.983	0.778	1.241
BM155	-.099	0.081	1.502	1	0.220	0.906	0.774	1.061
BM163	-0.041	0.079	0.271	1	0.603	0.960	0.822	1.120
BM165	0.020	0.068	0.085	1	0.771	1.020	0.892	1.167
BM169	0.008	0.041	0.043	1	0.836	1.008	0.931	1.092
AGE	-0.736	0.448	2.694	1	0.101	0.479	0.199	1.154

Appendix 7: Allelic association with *Anaplasma/ Ehrlichia*

Variables in the Equation								
	B	**S.E.**	**Wald**	**df**	**Sig.**	**Exp(B)**	**95% C.I.for EXP(B)**	
							Lower	**Upper**
Breed			8.244	2	0.016			
Breed(1)	2.113	1.275	2.747	1	0.097	8.274	0.680	100.699
Breed(2)	-1.712	0.878	3.803	1	0.051	0.181	0.032	1.009
Gender	0.355	0.786	0.204	1	0.652	1.426	0.306	6.648
DRB291	0.602	1.254	0.231	1	0.631	1.826	0.156	21.349
DRB289	0.462	0.346	1.777	1	0.183	1.587	0.805	3.129
DRB290	-0.801	0.404	3.930	1	0.047	0.449	0.203	0.991
DRBP118	-0.857	0.761	1.268	1	0.260	0.425	0.096	1.886
DRBP120	0.997	0.424	5.535	1	0.019	2.710	1.181	6.218
DRBP122	0.876	0.383	5.240	1	0.022	2.401	1.134	5.082
DRBP124	-0.221	0.260	0.723	1	0.395	0.802	0.482	1.334
DRBP126	-0.100	0.149	0.451	1	0.502	0.905	0.675	1.212
DRBP128	0.213	0.197	1.167	1	0.280	1.237	0.841	1.821
DRBP130	0.067	0.137	0.242	1	0.623	1.070	0.818	1.398
DRBP132	-0.083	0.157	0.282	1	0.596	0.920	0.677	1.251
DRBP134	-0.064	0.092	0.482	1	0.488	0.938	0.783	1.124
DRBP136	0.256	0.147	3.007	1	0.083	1.291	0.967	1.724
DRBP138	0.033	0.149	0.050	1	0.822	1.034	0.773	1.384
RM91	-0.460	1.471	0.098	1	0.754	0.631	0.035	11.273
RM95	-0.214	0.357	0.357	1	0.550	0.808	0.401	1.627
RM97	0.254	0.267	0.904	1	0.342	1.290	0.764	2.178
RM99	-0.023	0.181	0.017	1	0.897	0.977	0.685	1.393
RM101	-0.178	0.146	1.489	1	0.222	0.837	0.629	1.114
RM103	0.080	0.153	0.275	1	0.600	1.084	0.803	1.463
RM105	0.099	0.140	0.499	1	0.480	1.104	0.839	1.454
RM107	0.009	0.092	0.010	1	0.922	1.009	0.843	1.207
BM145	1.403	1.269	1.222	1	0.269	4.068	0.338	48.941
BM151	0.034	0.189	0.033	1	0.855	1.035	0.715	1.498
BM155	-0.133	0.127	1.102	1	0.294	0.876	0.683	1.122
BM163	0.051	0.119	0.182	1	0.670	1.052	0.833	1.328
BM165	0.082	0.112	0.538	1	0.463	1.085	0.872	1.350
BM169	-0.017	0.065	0.067	1	0.796	0.983	0.865	1.118
AGE(1)	1.000	0.624	2.570	1	0.109	2.719	0.800	9.235

Appendix 8: Allelic association with *Babesia/ Theileria*

Variables in the Equation *Babesia*

	B	S.E.	Wald	df	Sig.	Exp(B)	95% C.I.for EXP(B)	
							Lower	Upper
Breed			32.500	2	0.000			
Breed (1)	1.371	1.049	1.707	1	0.191	3.939	0.504	30.789
Breed (2)	-8.387	1.537	29.792	1	0.000	0.000	0.000	0.005
Gender	2.872	0.806	12.693	1	0.000	17.676	3.641	85.827
DRB291	-1.189	0.738	2.599	1	0.107	0.304	0.072	1.293
DRB292	0.224	0.469	0.227	1	0.633	1.251	0.499	3.139
DRB289	0.047	0.269	0.030	1	0.862	1.048	0.619	1.774
DRB290	-0.247	0.230	1.155	1	0.283	0.781	0.497	1.226
DRBP118	-0.685	0.789	0.753	1	0.386	0.504	0.107	2.368
DRBP120	0.579	0.403	2.069	1	0.150	1.785	0.811	3.929
DRBP122	0.354	0.303	1.363	1	0.243	1.425	0.786	2.583
DRBP124	0.408	0.265	2.368	1	0.124	1.144	0.894	2.527
DRBP126	0.076	0.154	0.242	1	0.623	1.078	0.798	1.457
DRBP128	0.247	0.162	2.316	1	0.128	1.280	0.931	1.758
DRBP130	0.075	0.122	0.373	1	0.541	1.078	0.848	1.370
DRBP132	-0.084	0.161	0.269	1	0.604	0.920	0.670	1.262
DRBP134	0.040	0.086	0.221	1	0.638	1.041	0.880	1.233
DRBP136	0.085	0.095	0.808	1	0.369	1.089	0.904	1.311
RM91	0.129	1.079	0.014	1	0.905	1.138	0.137	9.425
RM95	0.285	0.373	0.583	1	0.445	1.329	0.640	2.759
RM97	-0.123	0.251	0.242	1	0.623	0.884	0.541	1.445
RM99	0.210	0.185	1.286	1	0.257	1.234	0.858	1.775
RM101	0.303	0.156	3.763	1	0.052	1.354	0.997	1.840
RM103	0.497	0.169	8.620	1	0.003	1.643	1.180	2.289
RM105	0.219	0.153	2.037	1	0.154	1.244	0.922	1.680
RM107	0.250	0.108	5.317	1	0.021	1.284	1.038	1.588
BM145	-1.620	0.988	2.686	1	0.101	0.198	0.029	1.373
BM151	-0.205	0.185	1.221	1	0.269	0.815	0.566	1.172
BM155	-0.097	0.123	0.614	1	0.433	0.908	0.713	1.156
BM157	-0.256	0.246	1.079	1	0.299	0.774	0.478	1.255
BM163	-0.242	0.125	3.747	1	0.053	0.785	0.614	1.003
BM165	0.088	0.100	0.772	1	0.380	1.092	0.897	1.330
BM169	-0.005	0.063	0.005	1	0.942	0.995	0.880	1.126
AGE(1)	-1.968	0.961	4.195	1	0.041	0.140	0.021	0.919

ANNEXURE

Genomic DNA extraction

Genomic DNA (gDNA) from fresh and frozen blood was extracted and purified using the ZR Zymo kit (Zymo Research, CA, USA) at the University of Namibia, Molecular biology laboratory according to the following protocol; One hundred microliter of whole blood was pipetted into a microcentrifuge tube together with 5 µl of proteinase K and 95 µl of 2X digestion buffer. The mixture was immediately vortexed and incubated at 55 °C for 20 minutes. Genomic lysis buffer (700 µl) was thereafter added to the tube and the mixture was vortexed for thorough mixing. The mixture was then transferred into a Zymo spin column placed in a collection tube. This was followed by centrifugation at 10, 000 x g for one minute. DNA Pre-wash buffer (200 µl) was pipetted into the spin column in a new collection tube and centrifuged at 10,000 x g for one minute. Four hundred µL of g-DNA Wash buffer was subsequently added to the spin column and centrifuged at 10,000 x g for one minute. Finally, the spin column was transferred to a clean microcentrifuge tube and 100 µl of DNA elution buffer was added to the tube.

The tube was incubated 2-5 minutes at room temperature and later centrifuged at top speed for 30 seconds to elute DNA. The eluted DNA was immediately stored at -20 °C until PCR amplification was performed. The concentration of DNA samples were determined at the State Veterinary Biotechnology Laboratories in Windhoek, using a spectrophotometer (Thermo Fisher Scientific; Wilmington, DE) at 260 nm (260 A).The purity of extracted gDNA from the samples was determined by measuring A260/A280 and 260 A /230 A ratio with NanoDrop ND-1000.