Exploring Genetic Architecture of Tick Resistance in South African Nguni Cattle

By

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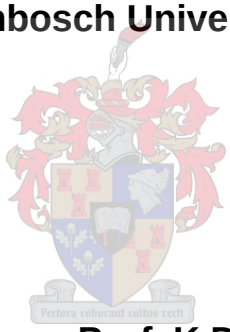
Dissertation presented in fulfilment of the requirements for the degree Doctor of Philosophy at the Department of Animal Sciences, Stellenbosch University



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December 2015

Declaration

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Summary

The broad objective of this study was to identify single nucleotide polymorphisms (SNP) markers associated with tick resistance in South African Nguni cattle and it was addressed by three specific objectives. The first objective was to assess tick load and prevalence in Nguni cattle in different agro-climatic regions of South Africa using tick count data collected monthly from 586 Nguni cattle reared under natural grazing conditions, over two years. Tick counts were assessed under natural challenge at ARC Roodeplaat and Loskop farms (warm climate), and Mukhuthali Nguni Community and University of Fort Hare farms (cool climate). The second objective was to estimate genetic parameters for tick counts in Nguni cattle. The third objective was to identify SNPs associated with tick resistance in Nguni cattle. Counts for each tick species were conducted on each animal in the herd once a month on different body locations, including the head, ears, neck, back, legs, belly, perineum and tail. Distribution of counts was determined using the PROC FREQ (SAS, 2002 - 2010). The tick counts were then analysed with the PROC GLM procedure using the two fixed effect models. Genetic parameters for log-transformed counts were estimated from univariate animal and sire models and bivariate sire models using the ASREML program. Animals were genotyped using Illumina BovineSNP50K assay. After Quality Control (call rate >90%, minor allele frequency > 0.02), 40 436 SNPs were retained for analysis. Association analysis for tick resistance was carried out using two approaches: genome-wide association (GWA) analysis using the GenABEL package and a Regional Heritability Mapping (RHM) analysis. Six tick species were identified: *Amblyomma hebraeum* (42%), *Rhipicephalus evertsi evertsi* (22%), *Rhipicephalus (Boophilus) spp*. (16%), *Rhipicephalus appendiculatus* (11%), *Hyalomma marginatum* (5%) and *Rhipicephalus simus* (4%). Tick infestation was significantly affected by location, season, year, month of counting and age of the animal. Loskop farm, as the warmest location, had the highest tick counts and also showed the largest variation in tick loads. Higher tick counts were also observed in the hot-dry (September to November) and hot-wet (December to February) seasons compared to the other seasons. *Amblyomma hebraeum* was the dominant tick species across all four locations. Heritability estimates for tick count varied according to season and trait (body part or tick species) and ranged from 0.01±0.01 to 0.26±0.01. Genetic correlations ranged from -0.79±0.33 to 1.00±0.00 among counts for different body parts and 0.00±0.00 to 0.99±0.00 among tick species. Phenotypic correlations ranged from 0.06±0.01 to 0.72±0.01 among body parts and 0.01±0.02 to 0.44±0.01 for tick species. Whole body count was highly correlated to the perineum and the belly. These two traits appear to be the most suitable surrogates for whole body count. Several genomic regions of interest were identified for different traits by both the GWA and RHM approaches. Three genome-wide significant regions on chromosomes 7, 10 and 19 were identified for total tick count on the head, total *A. hebraeum*

ticks and for total number of *A. hebraeum* in the perineum region. Suggestive significant regions were identified on chromosomes 1, 3, 6, 7, 8, 10, 11, 12, 14, 15, 17, 19 and 26 for several of the tick traits analysed. The GWA approach identified more genomic regions than did the RHM approach. These findings provide information that would be useful in developing strategies for genetic improvement of tick resistance through selection. The chromosome regions identified as harbouring quantitative trait loci (QTL) underlying variation in tick burden form the basis for further analyses to identify specific candidate genes related to cattle tick resistance and provide the potential for marker-assisted selection in Nguni.

Keywords: Nguni cattle, tick species, tick counts, genetic parameters, SNPs, and GWAS

Opsomming

Die doel van hierdie studie was om enkel nukleotied polimorfismes (ENPs) merkers te identifiseer wat verwant is aan bosluisweerstand in Suid-Afrikaanse Nguni beeste; dit is aangespreek deur drie doelwitte. Die eerste doelwit was om bosluislading en -voorkoms van bosluise in Nguni beeste in verskillende landbou-klimaatstreke van Suid-Afrika te bepaal deur die gebruik van bosluistelling data wat maandeliks van 586 Nguni beeste, grootgemaak op natuurlike weiding, oor 'n tydperk van twee jaar versamel was. Die tweede doelwit van die studie, was om die genetiese parameters te bepaal vir die bosluistellings in die Nguni beesras. Om hierdie doelwit aan te spreek, is vier verskillende datastelle onderskei in die bosluistelling data wat oor die twee jaar periode versamel was. Genetiese parameters is derhalwe beraam vir die telling van bosluise om sodoende die beste seisoen te identifiseer vir die insameling van bosluistelling data om ten einde strategieë te ontwikkel vir die genetiese seleksie vir vehoogde weerstand teen bosluise. Die derde doelwit was om ENP streke te identifiseer wat verband hou met bosluisweerstand in Nguni beeste. Verskillende bosluisspesies was getel op elke dier in die kudde een keer per maand op verskillende plekke op die liggaam, insluitend die kop, ore, nek, rug, bene, maag, perineum en stert. Bosluistelling data is ontleed met behulp van die SAS program om bosluislading variasie te bepaal. Genetiese parameter skattings vir log getransformeerde bosluistellings data was bereken vanaf twee-veranderlike vaar modelle en een-veranderlike dier- en vaar modelle met behulp van die ASREML program. Om ‘n genomiese wye assosiasie studie (GWAS) uit te voer, is DNS geïsoleer en genotipering gedoen met behulp van die Illumina BovineSNP50K toets. Na kwaliteit kontrole (oproep frekwensie>90%, klein alleelfrekwensie>0.02) is 40.436 ENPs behou vir ontleding. Assosiasie analise vir bosluisweerstand is uitgevoer met behulp twee benaderings, d.i. 'n genoom-wye assosiasie (GWA) analise met behulp van die GenABEL pakket en 'n plaaslike oorerflikheid karterings (POK) analise. Ses bosluisspesies is geïdentifiseer, d.i. *Amblyomma hebraeum* (42%), *Rhipicephalus evertsi evertsi* (22%), *Rhipicephalus* (*Boophilus*) spp. (16%), *Rhipicephalus appendiculatus* (11%), *Hyalomma marginatum* (5%) en *Rhipicephalus simus* (4%). Bosluis besmetting was beduidend beïnvloed deur die plek, seisoen, jaar, maand tel en ouderdom van die dier. Loskop plaas het die warmste weer ervaar en het die hoogste bosluis tellings en ook die grootste variasie in bosluislading gehad. Hoër bosluistellings is ook waargeneem in die warm droë (September tot November) en warm nat (Desember-Februarie) seisoene in vergelyking met die ander seisoene. *Amblyomma hebraeum* is geïdentifiseer as die mees dominante bosluisspesies oor al vier lokaliteite. Die voorkeur aanhegtingsarea vir die bosluise was onder die stert, perineum en maag areas op die liggaam. Die oorerflikheid beraming vir bosluistelling, soos beïnvloed deur die

seisoen en eienskap (d.i. deel van die liggaam of bosluisspesies), het gewissel van 0.01±0.01 tot 0.26±0.01. Genetiese korrelasies het gewissel van -0.79±0.33 tot 1.04±0.01 vir bosluistellings op verskillende liggaamsdele en tussen 0.00±0.00 en 0.99±0.19 vir bosluisspesies. Fenotipiese korrelasies was laag tot matig en het gewissel van 0.06±0.01 tot 0.72±0.01 vir liggaamsdele en 0.01±0.02 to 0.44±0.01 vir bosluisspesies. Die datastel D wat September-Januarie bosluistellings bevat het die hoogste genetiese variasie aangedui. Heel liggaam bosluistellings was hoogs gekorreleerd met bosluistellings rondom die perineum en maag. Hierdie twee lokaliteite blyk die mees geskikte plaasvervanger vir die heel liggaam bosluistelling te wees. Verskeie genoom gebiede van belang is geïdentifiseer vir die verskillende eienskappe van beide die GWA en RHM benaderings. Drie genoom-wye beduidende streke (op chromosome 7, 10 en 19) is geïdentifiseer vir die totale bosluistelling op die kop, totale *A. hebraeum* bosluise en vir die totale aantal *A. hebraeum* in die perineum streek. Aanbevelende beduidende streke is geïdentifiseer op chromosome 1, 3, 6, 7, 8, 10, 11, 12, 14, 15, 17, 19 en 26 vir 'n paar van die bosluis eienskappe wat ontleed was. Die GWA benadering identifiseer meer genoom gebiede as die POK benadering. Hierdie bevindinge bied nuttige inligting vir die ontwikkeling van strategieë vir die genetiese verbetering van bosluisweerstand deur seleksie. Die chromosome streke hier geïdentifiseer is skuiling kwantitatiewe eienskap loki (KEL) vir die onderliggende variasie in bosluislading en vorm die basis vir verdere ontledings vir spesifieke kandidaat gene te identifiseer wat verband hou met die vee bosluisweerstand en bied die potensiaal vir merkerbemiddelde seleksie in Nguni.

Keywords: Nguni beeste, bosluisspesies, bosluistellings, genetiese parameters, ENPs en GWAS

Acknowledgements

This dissertation would not have been possible without the help of Almighty God who had brought together a group of people from different institutions to collaborate and make this project possible. My gratitude goes to my promoters Prof K. Dzama, Prof A. Maiwashe, Prof M.D. MacNiel and Dr Banga who encouraged me and provided guidance throughout the study.

I would also like to express my gratitude to:

- ARC-Animal Produciton Institute Animal Breeding and Genetics research team for their support and encouragement;
- University of Fort Hare and the Mukhuthali community farms for providing animals that were used in the study;
- Prof Heather Burrow from University of New England in Australia for reviewing some of the manuscripts.
- Borlaug Fellowship scholarship that enabled me to undergo a short training visit to the University of Missouri with Dr JF Taylor;
- Dr O Matika, Dr V Riggio, Prof Bishop (deceased) from the Roslin Institute for assistance with the experimental design and data analysis;
- Red Meat Research and Development Trust of South Africa and Technology Innovation Agency (TIA), NRF-DST and NRF-THRIP provided funding for research; NRF-KIC funding provided the travel grant;
- ARC Biotechnology Platform for assistance with performing the genotyping

Finaly, the unwavering support of the most wonderful woman, my mother (Nyawasedza Thivharumi Mapholi), my son (Wavhudi Tshipuliso), my brother (Lawrence Mapholi) and my entire family is grealty appreciated.

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Chapter 1
Introduction

1.1 General Introduction

Ticks and tick-borne diseases are a global problem and considered as one of the major challenges to livestock health and performance. Economic losses due to ticks and tick-borne diseases in livestock have long been recognized by farmers, government and researchers (Hayward, 1981; Seifert, 1984b; de Castro, 1997; D'haese *et al.,* 1999; Ocaido *et al.,* 2009; Regitino *et al.*, 2010; Porto-Neto *et al.,* 2011). Heavy infestations cause loss in production and transmission of important tick-borne diseases. Some of the ixodid ticks also produce toxins, which cause sweating sickness in calves (Telford & Goethert, 2004; Dannis and Piesman, 2005). The estimated cost of tick challenges to the livestock industry in South Africa is more than R500 million per year (Mapholi *et al.,* 2014).

Numerous tick control strategies have been employed in Southern Africa (Bigalke *et al.,* 1976; De Vos, 1979; Purnell & Schroder, 1984). The most common method used is the application of acaricides. However, development of resistance to chemicals by ticks hampers the effectiveness of this tick control strategy. In addition, the use of acaricides as tick control, increases costs to the livestock producers and also leaves chemical residues in meat and milk products and the environment (Machado *et al.,* 2010). Vaccines have also been used to control tick-borne disease agents because of cost considerations and slower development of resistance compared to acaricides (Willadsen, 1997). However, the major disadvantage of some of the vaccines in current use is that they do not offer protection against multiple tick species. Pasture rotation and burning have been used to effectively control tick populations on pasture (Spicket *et al.,* 1992), but tick eggs hatching may miss the effect of fire while hiding underneath the soil surface, suggesting that pasture burning alone may not be an effective tick control method (Young *et al.,* 1988).

Hayward (1981) advocated the use of naturally resistant cattle for breeding selection programmes as a tick control method. While natural resistance to ticks is influenced by environmental factors (Adams and Templeton, 1998), a significant part of the variation in resitance to ticks is under genetic control (Davis, 1993; Burrow, 2001; Budeli *et al.,* 2009). Heritability estimate for tick resistance varies from 0.05 to 0.42 (Utech *et al.,* 1978; Budeli *et al.,* 2009; Mapholi *et al.,* 2014; Porto-Neto *et al.,* 2014), indicating that genetic improvement for tick resistance through selection should be feasible.

Genetic markers such as microsatellites have been developed and used for many decades to identify complex economic important traits, including tick resistance. For example, quantitative trait loci (QTL) associated with tick resistance were identified in F_2 Brazilian population explaining 13.1 to 18.4% of the phenotypic variation (Gasparin *et al.,* 2007). Recently, single nucleotide

polymorphisms (SNP) opened opportunities for unravelling the underlying genetic basis for host resistance to ticks through GWAS analysis (Barendse, 2007; Turner *et al.,* 2010). Detection of genetic markers or causal mutations could allow application of marker-assisted selection (MAS) as a method to improve resistance to ticks in cattle.

Indigenous breeds such as Nguni cattle are well-adapted to South African tropical environment and show excellent immunity to tick-borne diseases. The Nguni breed is endemic to Southern Africa and is amongst the most predominant cattle breed in the hands of the communal and emerging farming sectors in South Africa. The Nguni's excellent resistance to ticks and good immunity to tick-borne diseases provides an opportunity to improve other breeds that are susceptible to ticks (Spicket *et al.,* 1989; Rechav *et al*., 1991; Ramsay, 2000; Scholtz, 2005). However, little effort and attention has been devoted to understand the underlying genetic basis of tolerance to ticks in Nguni cattle.

1.2 Problem statement

In Southern Africa, environmental adaptation and tick-borne diseases are increasingly becoming severe problems in livestock production. About one million cattle were estimated to have died due to East Coast fever in Southern Africa in 1989 (Mukhebi *et al*., 1992). To date, the estimated losses due to tick-borne diseases are about R500 million in South Africa. It is reported that about 80% of the world's cattle suffer to some extent from the deleterious effects caused by ticks (Scholtz, 2005). Although efforts to eradicate ticks and tick-borne diseases using chemical control strategies have been implemented in South Africa, no tangible benefits have been attained except that these chemicals are costly and cattle susceptibility to ticks remain unchanged. Traditional genetic and phenotypic evaluation of tick resistance in conventional breeding schemes is rather ineffective and time consuming. There is evidence (e.g. Scholtz, 1991; Corbet *et al.,* 2006; Marufu *et al*., 2010) that indigenous Nguni cattle are more tolerant to ticks than *Bos taurus* breeds and this can form the basis of a framework for marker-assisted selection to improve tick resistance in the Southern Africa beef industry.

1.3 Objectives

To search for Quantitative Trait Loci associated with tick resistance in Nguni cattle.

The specific objectives were to:

1. Assess tick loads and prevalence in Nguni cattle in different agro-climatic regions of South Africa;
2. Estimate genetic parameters for tick count in Nguni cattle; and
3. Identify quantitative traits loci for tick resistance in Nguni cattle using Bovine SNP50K assay.

1.4 Hypotheses

The hypotheses tested were that:

1. Tick load vary according to agro-climatic regions in South Africa
2. Genetic variation increases when tick infestation is higher
3. Significant linkage disequilibrium exists between genetic markers (SNPs) and genes coding for tick resistance in Nguni cattle

1.5 Layout of chapters

The dissertation consists of six chapters; a general introduction, literature review followed by three research chapters and a general discussion and conclusion. Each research chapter has its own abstract, introduction, materials and methods, results, discussion, conclusion and references.

Chapter 1: General Introduction

This chapter provides a general introduction, problem statement, objectives, and hypotheses and gives a description of the layout of the dissertation.

Chapter 2: Literature Review

The literature review chapter discusses factors that affect host resistance for ticks (HGRT), breeding selection, immunology, and genomic approaches and their application to improve HGRT in order to enhance livestock production.

Chapter 3: Prevalence of tick loads in South African Nguni cattle reared in four different climates.

This chapter compared tick loads and prevalence among Nguni cattle in different climatic conditions, by measuring the level of tick infestation, identification of tick species, and effect of seasonal variation in tick loads and observation of the favourable attachment sites of ticks in different body locations of the host. This chapter provides useful information for the development of appropriate control strategies for ticks and tick-borne diseases in these provinces of South Africa.

Chapter 4: Genetic parameter estimates among seasonal tick counts from different body locations and tick species in South African Nguni cattle.

This chapter focuses on developing proper protocol for tick count data collection. Estimates of heritability, genetic and phenotypic correlation for whole body tick count, different body locations and tick species based on four different data sets are presented. This chapter provides information that would be useful in developing strategies for genetic improvement of tick resistance through selection.

Chapter 5: Genome-wide association study of tick resistance in South African Nguni cattle.

This presents results of the association analysis to identify genomic regions associated with host resistance to ticks in South African Nguni cattle. Two different analysis approaches (1) genome-

wide association (GWA) analysis using the GenABEL package and (2) regional heritability mapping (RHM) analysis were used to analyse genomic data. Discovered regions harbouring QTL underlying variation in tick burden will form the basis for further analyses to identify specific candidate genes related to cattle tick resistance and provide the potential for marker assisted selection in Nguni cattle.

Chapter 6: General discussion, conclusions and recommendations

This chapter presents, in a coherent manner, a comprehensive discussion of the results obtained in the research chapters. The main conclusions emanating from research conducted in this study are presented. Recommendations for future research considerations and practical control of ticks are put forth.

1.6 Papers and conference proceedings

1.6.1 Peer reviewed paper was published in international journal

Mapholi, N.O., Marufu, M.C., Maiwashe, A., Banga, C.B., Muchenje, V., MacNeil, M.D., Chimonyo, M. & Dzama, K., 2014. Towards a genomics approach to tick (Acari: Ixodidae) control in cattle: A review. Ticks Tick-Borne Dis. 5, 475–483.

1.6.2 Submitted paper for peer reviewed publication in international journals

Mapholi, N.O., Maiwashe, A., Matika, O., Riggio, V., Bishop, S.C., MacNeil, M.D., Taylor, J. F. & Dzama, K. Genome-wide association study of tick resistance in South African Nguni cattle. Submitted July 2015 to Ticks and Tick-Borne Diseases.

Mapholi, N.O., Maiwashe, A., Banga, C.B., Marufu, M.C., Muchenje, V., MacNeil, M.D. & Dzama, K. Prevalence of tick loads in South African Nguni cattle reared in four different climates. Submitted July 2015 to Experimental and Applied Acarology.

Mapholi, N.O., Maiwashe, A., Banga, C., Matika, O., Riggio, V., MacNeil, M.D. & Dzama, K. Genetic parameter estimates for seasonal tick counts from different body parts and tick species in South African Nguni cattle. Submitted November 2015 to Animal: An International Journal of Animal Bioscience

1.6.3 Presentations at national and international scientific congress

Mapholi, N.O., Maiwashe, A., MacNeil, M. D., Riggio, V., Matika, O., Taylor, J. F. & Dzama, K., 2015. Genome analysis for tick resistance in South African Nguni cattle. For 48th SASAS “Zululand Heritage Congress” to be held on 21st to 23rd September, in Empangeni, Kwazulu Natal, South Africa (accepted for September congress as oral presentation).

Mapholi., N.O., Maiwashe, A., Matika, O., Riggio, V., Bishop, S.C., MacNeil, M.D. & Dzama, K., 2015, 'Exploring Genetic Architecture of Tick Resistance in South African Nguni Cattle'

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Mapholi, N.O., Maiwashe, A., MacNeil, M. D. & Dzama. K., 2014. Assessments of tick dominance in South African Nguni cattle. 12th Biennial Conference of the Society for Tropical Veterinary Medicine (STVM) and the VIII International Conference on Ticks and Tick-borne Pathogens (TTP-8), 24th to 29th August, Cape Town, South Africa.

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Chapter 2

Literature Review

(Published in Ticks Tick-Borne Diseases. 2014 (5), 475–483)

Abstract

Ticks and tick-borne disease (TBD) are major challenges to cattle production in the tropics and sub-tropics. Economic losses associated with ticks amount to millions of dollars annually. Although efforts to eradicate ticks and TBD using chemical control strategies have been implemented in many developing countries for decades, these acaricides are costly and cattle susceptibility to ticks remains unchanged. Traditional breeding methods, where the farmer selected animals using records to improve the host genetic resistance to ticks (HGRT), are less than fully effective and time consuming. To date, solutions to fight ticks and TBD are still unclear. Development of single nucleotide polymorphisms (SNP) technologies have created an opportunity to estimate breeding values of animals from DNA samples. The use of SNP technology for genomic selection allows information retrieval from the genotype even before the gene is expressed; thus potentially giving farmers the ability to make selection decisions on HGRT at an earlier age. This review discusses factors that affect HGRT, breeding selection, immunology and genomic approaches and their application to improve HGRT in order to enhance livestock production.

Key words: Bos taurus africanus, host genetic resistance to ticks, single nucleotide polymorphisms, tick control, genomic selection

2.1. Introduction

There is greater demand for animal proteins worldwide, especially with the increasing affluence in the emerging markets of Brazil, Russia, India, China and South Africa. This has led to increased consumption of beef and dairy products and necessitated expansion of global cattle production. Ticks are a major challenge to increased cattle production affecting an estimated 1.4 billion cattle worldwide (FAO, 2011). On a global basis, ticks are one of the most important vectors of disease pathogens in livestock and companion animals (Ghosh *et al*., 2006). Tick-borne diseases (TBD) result in huge economic losses in both dairy and beef production systems, especially in tropical and sub-tropical areas (Rajput *et al*., 2006). Direct effects of tick infestation in cattle include the sucking of blood which causes anaemia and damage to the skin or hide, with downstream effects resulting in reductions in fertility, body weight and milk production, and in toxicoses, paralysis and mortality (Turton, 2001; Jongejan & Uilenberg, 2004; Kaufman *et al*., 2006).

Conventional tick control is based on application of acaricides. The use of these acaricides has increased the incidence of acaricide-resistant ticks and exacerbated the occurrence of environmental and food contamination (Parizi *et al.*, 2009). Together, these problems generate a rising economic and social demand for alternative approaches to reduce tick infestation and thereby enhance the contribution of cattle to the world economy. Natural immunity, developed by cattle in environments where ticks are endemic, shows promise for genetic tick control strategies which reduces expenditure on acaricides and other chemical control methods (Frisch, 1999; FAO, 2004). Tick control strategies targeted at the host's immunity requires that immunity can be assessed using an appropriate and accurate method.

Mapping of the bovine genome has opened new avenues for determining the genetic basis for HGRT, using technologies such as single nucleotide polymorphisms (SNPs) and copy number variants, and thus enabling their control in cattle (Piper *et al.*, 2008). The current review discusses the distribution of common tick species, their economic impact on cattle production and their control. Host resistance to ticks, breed variability in susceptibility to tick infestation, classical breeding and selection approaches to reducing susceptibility or increasing tolerance to ticks, as well as genomic tools to improve resistance are also discussed.

2.2 Geographical distribution of common ixodid ticks in Africa

2.2.1 Tick species distribution in Africa

There are approximately 879 known tick species worldwide. These species are grouped into three families: Argasidae or soft bodied ticks (186 species), Ixodidae or hard bodied ticks (692 species) and Nuttalliellidae (1 species) (Navas *et al.*, 2009). The present review focuses on ixodid ticks, as they have devastating impacts on cattle. In Africa, there are over 650 tick species spread across seven genera. Of the seven genera of hard-bodied ticks that affect livestock in Africa, four are of economic importance in cattle. These are: *Rhipicephalus*, *Amblyomma*, *Hyalomma* and the subgenus *Rhipicephalus* (formerly *Boophilus*) (Sonenshine, 1991; Walker *et al.*, 2003; Jongejan & Uilenberg, 2004).

2.2.2 Factors affecting tick distribution

Broad-scale factors that limit the ranges of tick species have not been definitively established. Given that most tick distributions are not limited by those of their host species, it can be inferred that a primary factor preventing expansion of tick species ranges is a direct effect of climate (Cumming, 2002). Olwoch *et al.* (2009) suggested that if global warming leads to temperature increases the incidence of ticks will further increase in regions where ticks are endemic. This

could have serious implications for seasonal variation in tick infestation, TBD incidence, and TBD control strategies. In South Africa, for example, increased environmental temperature is thought to have caused displacement of the indigenous African species *R*. *decoloratus* by the Asiatic intruder *R*. *microplus* (Tonnesen *et al.*, 2004; Nyangiwe *et al.*, 2013). Except for extremely cold and dry areas, *R*. *microplus* has extended its range and is now present in all warm and humid areas of the country. The bont tick (*A. hebraeum*) was reported to occur only in the warm, moist coastal areas of South Africa (Coetzer *et al*., 1994). However, it has recently been reported that *A*. *hebraeum*'s distribution is expanding to the inland semiarid areas of South Africa (Nyangiwe *et al*., 2011). The expansion in the distribution of the bont tick in South Africa may be associated with more intense periods of drought especially in the inland highlands areas as hypothesised by Estrada-Peña *et al*. (2008) for the bont tick in Zimbabwe.

The distribution and abundance of ticks are also impacted by factors other than climate such as, presence of alternative hosts, natural resistance, acaricide use and grazing management (Cumming, 2002) in Table 2.1.

Table 2.1 Life cycle, distribution and pathogens transmitted by common tick species in Africa

Tick species	**Life cycle**	**Pathogens transmitted**
Amblyomma hebraeum	Three-host	*Ehrlichia ruminantium*
Rhipicephalus decoloratus	One-host	*Babesia bigemina* *Anaplasma marginale*
Rhipicephalus microplus	One-host	*Babesia bovis* *Babesia bigemina* *Anaplasma marginale*
Rhipicephalus appendiculatus	Three-host	*Theileria parva*
Rhipicephalus evertsi evertsi	Two- or three-host	*Anaplasma marginale*

In situations where the natural hosts of ticks are sparsely distributed, alternative hosts tend to gain importance in influencing tick distribution. For example, in Zimbabwe, the only factor favouring the survival of the tick *A. hebraeum* in the low veld habitats where cattle densities are

low is the presence of alternative wildlife hosts (Norval *et al*., 1994). Alternative hosts also act as vehicles for moving ticks between distinct habitats further increasing their distribution (Ruiz-Fons & Gilbert, 2010). Intensive acaricide treatments over long periods coupled with the absence of alternative hosts may also alter tick distributions (Norval *et al*., 1994). These factors need to be considered when mapping the distribution of ticks and in prediction of the future distribution of ticks. As ticks continue to expand their range into previously uninhabited areas, outbreaks of TBD are also likely to increase negatively impacting cattle production. Additional or updated tick surveys need to be conducted to cater for these tick distributional dynamics.

2.3 Economic impact of ticks and tick-borne diseases on cattle production

2.3.1 Estimation of the economic impact of ticks and tick-borne diseases on cattle production

Apart from causing diseases, ticks cause substantial losses in terms of reduced productivity and fertility and often death, and are economically the most important ecto-parasites of cattle (Rajput *et al*., 2006). The lack of accurate data on the epidemiology of ticks and TBD makes it difficult to determine their impact. Table 2.2 shows the estimated costs of ticks and TBD to cattle production in different countries. Although fairly crude estimates, these values may help to comprehend the importance of ticks and TBD of cattle. These estimates expose the need for more studies on the determination of the economic impact of ticks and TBD on the cattle industry, especially in developing countries.

Table 2.2 Estimated economic losses due to ticks and tick-borne diseases

Country	**Cost in US$**	**References**
Global	7 billion	McCosker (1979)
Global	13-18 billion	deCastro (1997)
Africa	160 million	Dold and Cocks (2001)
Ethopia	1.5 million	Newson (1991)
South Africa	92 million	Farmer's weekly (1998)
Brazil	2 billion	Grisi *et al.* (2002)
Australia	184 million	Playford *et al.* (2005)
India	498.7 million	Minjauw and McLeod (2003)

2.3.2 Losses due to tick infestations

The economic losses due to ticks can be expressed either in terms of body weight or milk production lost per engorged tick or in terms of average financial loss (production loss plus cost of control) per animal per year (Jonsson, 2006). Each engorging female *R*. *microplus* tick causes the loss of 8.9 ml of daily milk production and 1.0 g of body weight in high yielding Holstein-Friesian cows (Jonsson *et al*., 1998). The loss of 14 % production rate of the lactation would result in a significant reduction in income and would be particularly a serious loss for livestock dependent systems (McLeod & Kristjanson, 1999).

Jonsson *et al*. (2008) stated that the loss per tick was similar in the different breed groups, however the total loss was a function of the number of ticks. Hence the losses in indicine cattle tend to be lower than those in taurine cattle. Breed-specific losses appear to result from breed differences in susceptibility to infestation. In South Africa, Scholtz *et al.* (1991) indicated that every engorged female *R*. *decoloratus* tick causes a reduction of 8.9, 8.0 and 8.6 g in the weaning masses of calves from Hereford (*Bos taurus*), Bonsmara (*Bos taurus africanus* X *Bos taurus* crosses) and Nguni (*Bos taurus africanus*) cows, respectively. However, the observed minor effect of infestation on the productivity of the Nguni cows was small due to their natural resistance. Norval *et al*. (1988) estimated losses of up to 4 g of body weight per engorging adult *R*. *appendiculatus* tick in *Bos taurus* steers. Sanga (*Bos taurus africanus*) cattle suffer less severe losses.

The damage caused by tick bites also diminishes the value of skins and hides for the manufacture of leather. Tick bite marks are among the different factors causing the non-availability of good quality raw material for the leather industry, causing between 20 and 30% depreciation in normal value in the market (Biswas, 2003). Hide damage is a function of the number of ticks infesting cattle and is probably independent of breed (Jonsson, 2006). Ticks with a long hypostome may induce abscesses because of secondary bacterial infection which in turn attracts myiasis producing flies, further compounding the arthropod related problems for cattle producers (Ghosh *et al.,* 2006).

2.3.3 Economic impact of ticks and TBD treatments

A large component of the economic cost of ticks in cattle is the application of control measures to reduce infestations (De Castro, 1997; Porto-Neto *et al*., 2011). Conventional tick control is based on the application of acaricides. There are few global reports on the costs involved in tick control and TBD treatments. Expenditures for tick control were estimated at US$ 8.43, 13.62 and 21.09 per animal per year for plunge dipping, hand spraying and pour-on, respectively (D'haese

et al., 1999). The mean annual cost of ticks and TBD control per animal in pastoral and ranch herds was estimated to be US$4.54 (Ocaido *et al*., 2009). There is need for more studies on the losses associated with tick and TBD control to ensure the accurate estimation of the total cost of these parasites on cattle production. The major component of economic cost of TBD, which can constitute up to 88% of total costs, is on their control (Ocaido *et al*., 2009). The control of TBD can be a large and regular part of the variable cost of beef farming in most infested areas, with control measures mainly involving a combination of acaricide and grazing management, together with a slowly growing interest in immunisation (Minjauw & McLeod, 2003).

2.3.4 Losses due to tick-borne diseases

Besides the losses due to the direct effects of ticks and their control, significant losses also arise indirectly due to the important role of ticks in the transmission of TBD. The economic impact due to TBD can be substantial, especially in cases of sudden outbreaks in susceptible herds. Losses that can be directly attributed to TBD include mortalities, chronic morbidity, cost of veterinary diagnosis and treatment, cost of vaccines, and costs arising from restrictions on movement of cattle (Jonsson *et al.*, 2008). Tick-borne diseases can cause downgrading of live animals at sales, and of meat, offal and hides (Tisdell *et al.*, 1999). Introduction of more tick-resistant cattle substantially reduces the costs associated with ticks and TBD. This is due to lowered manifestation of TBD, because fewer ticks are likely to attach per day due to reduced numbers of ticks in the field and because a smaller proportion of ticks that do develop to feed on infected cattle will in turn be infected (due to lower parasitaemia) (Jonsson *et al.*, 2008). There is a need for the investigation of alterative control measures that are effective, safe, economically and environmental acceptable (Webb & David, 2002). One such control measure is development of cattle that are resistant to tick infestation and (or) TBD.

2.4 Tick control

In regions where ticks are endemic, control methods include treatment with acaricides, pasture rotation, environmental modification, and integrated biological and chemical control management programmes.

2.4.1 Chemical tick control

Globally, chemical control has been the main strategy to combat tick infestations. The practice of intensive tick control spread rapidly throughout Africa following the introduction of imported cattle breeds and, in most southern African countries, it was enforced through legislation. Use of acaricides in many developing countries have been more expensive (de Castro & Newson, 1993). In tropical and sub-tropical countries, the control of TBD in susceptible exotic cattle breeds still

depends on intensive tick control using acaricides. However, regular dipping to prevent tick infestation and TBD infections is a costly exercise for the farmer as it results in increased veterinary and labour costs, possible tick resistance to acaricides, unnecessary animal movement and handling (Jonsson, 2006).

In Africa, intensive dipping and spraying programmes using acaricides have been ineffective in eradicating ticks and TBD. This is due to poor management of acaricides and improper legislations. For example, in Zimbabwe as a result of the civil war between 1973 and 1978, dipping services were interrupted in the communal areas with disastrous consequences (Norval, 1979). About one million head of cattle died of TBD. These deaths were most likely caused by a lack of immunity, resulting from cattle previously being too effectively dipped, and the natural disease challenge and maintenance of enzootic stability being disrupted. Small scale farmers use complimentary treatments including hand picking, household disinfectants such as Jeyes fluid and used car oil to control ticks (Masika *et al.*, 1997; Moyo & Masika, 2009). Most of these practices are not desirable in terms of animal and human health. One of the biggest problems with acaricides use in tick control is the selection of chemical-resistant tick strains which evolve faster than the development of new chemicals for tick control (Regitano & Prayaga, 2010). Moreover, many cattle breeds still remain susceptible due to the lack of predictability of TBD endemicity and misinformation about natural exposure. In addition, the use of acaricides increases costs to the farmers and also leaves chemical residues in meat, milk, hides and the environment (Machado *et al*., 2010). There is a growing public demand for residue free animal products (Regitano *et al*., 2008) and an integrated approach to tick control utilising alternative tick control methods is thus needed to reduce overdependence on acaricides and address the issue of residues in meat.

2.4.2 Tick vaccines

Vaccination with tick antigens is a safe alternative to the use of acaricides to control ticks in cattle (Kimaro & Opdebeeck, 1994). Vaccines are potentially important in the control of the disease agents, mainly for not being chemical agents, for being cheaper and because their resistance development is slower than for acaricides (Willadsen, 1997). Commercial tick vaccines for cattle based on the *Boophilus microplus* Bm86 gut antigen have proven to be a feasible tick control method that offers a cost-effective, environmentally friendly alternative to the use of acaricides (de la Fuente *et al*., 2007). Tick vaccines reduce the number of engorging female ticks, their weight and reproductive capacity, meaning that the greatest vaccination effect is seen as a reduced larval infestation in a subsequent generation (Willadsen, 2006). The delay in the ‘knock down’ effect of tick vaccines is the principal reason why vaccine use is often coupled with limited

acaricide application for short term control of unacceptable tick burdens. The major disadvantage of some of the tick vaccines in current use is that they may not offer protection against multiple tick species. However, controlled immunization trials conducted by de Vos *et al*. (2001) indicated that the *R*. *microplus* BM86-containing vaccines protect against other tick species. Tick infestation is rarely a one-species issue, and therefore, tick vaccines should aim at a more global protection against the main species of economical and epidemiological interest.

2.4.3 Grazing management

Pasture rotation combined with acaricide application is one economical method for controlling ticks on beef cattle and it reduces tick densities on a large scale. Areas with good vegetation and high rainfall, however, produce more ticks, than those with poor vegetation and erratic rainfall. Pasture burning can also be used to effectively control ticks as it significantly reduces tick populations on pasture (Spicket *et al.*, 1992). Annual pasture burning reduces tick loads on cattle (Baars, 1999). In some instances however, tick eggs hatching can miss the effect of fire while hiding in the soil surface suggesting that pasture burning alone may not be an effective tick control method (Young *et al.*, 1988). Another alternative technique to reduce ticks and TBD is to eliminate wildlife host of particular species from the livestock environment. For example, the separation of buffalo from cattle in Kenya reduced tick burdens and incidences of TBD in cattle (Young *et al.*, 1988). The same principle was used for *Ixodes scapularis* and white-tailed deer (Stafford *et al*., 2003).

2.5 Mechanisms of resistance to ticks in cattle

2.5.1 Natural resistance

The use of naturally tick-resistant cattle biotypes may be incorporated in tick control schemes as a means of biological control of tick infestations (Tatchell, 1992). An understanding of the biological intricacies underlying vector-host-pathogen interactions is required to innovate sustainable tick management strategies that can ultimately mitigate the impact of animal and zoonotic tick-borne diseases (Brake & Perez de Leon, 2012).The study of the mechanisms of resistance to ticks among different breeds of cattle can contribute to the development of alternative control methods (Gasparin *et al*., 2007).

2.5.2 Morphological coat traits

Tick infestation is affected by several innate morphological coat traits, most of which have high heritability (Regitano & Prayaga, 2010). Exhibition of coat characteristics that are unfavourable for tick attachment is an important mechanism of resistance to tick infestation in cattle. Phenotypic coat characteristics such as hair length, coat thickness, coat smoothness and coat colour have

an influence on tick counts and are related to HGRT in cattle on rangelands (Martinez *et al*., 2006; Foster *et al*., 2008, Marufu *et al*., 2011; Ibelli *et al*., 2012). Cattle with short, smooth and light coloured coats tend to have lower tick counts compared to those with long, rough and dark coloured coats (Verissimo *et al.,* 2002; Gasparin *et al*., 2007). Short and smooth hairs make it difficult for ticks to attach and easier for animals to groom themselves while dark coloured hairs act as a camouflage thus protecting ticks against predators, such as birds (Martinez *et al*., 2006).

2.5.3 Cutaneous hypersensitivity responses and cellular immunity

Cutaneous hypersensitivity reactions to tick antigens also influence host resistance to ticks (Kemp *et al.*, 1986). The development of a strong cutaneous delayed-type hypersensitivity (DTH) reaction to ticks has been associated with increased HGRT in cattle (Bechara *et al*., 2000; Piper *et al*., 2010; Marufu *et al*., 2013). The lack of a DTH in susceptible animals has been attributed to tick saliva induced suppression of protective immune responses during infestation (Ferreira *et al*., 2003; Brossard & Wikel, 2004). Apart from humoral responses, several cell types are thought to influence acquired resistance to ticks in cattle (Gill, 1984; Latif *et al*., 1991). Basophils and mast cells appeared to be the major effectors of acquired resistance at tick feeding sites in cattle. A vigorous granulocyte response especially in the earlier stages of infestations has been reported to be characteristic of the immediate type hypersensitivity reaction responsible for tick rejection in tick susceptible taurine cattle (Latif *et al*., 1991). An abundance of mononuclear cells, basophils and eosinophils is characteristic of a delayed type hypersensitivity reaction at tick feeding sites on the skin of highly resistant hosts following repeated infestations (De Castro & Newson, 1993; Szabo & Bechara, 1999).

Mast cells, and the histamine they contain inside cytoplasmic granules, are of fundamental importance to the self-grooming mechanism, which is thought to be critical to resistance of cattle to ticks (Verissimo *et al*., 2008). Resistant bovines have greater capacity than susceptible hosts to retain eosinophils in the lesion of adult tick-infested skin (Carvalho *et al*., 2010). Eosinophils are thought to be involved in the translocation of mast cell histamine to the tick attachment site resulting in increased grooming and tick rejection in cattle (Francischetti *et al*., 2009). Constantinou *et al*. (2010) observed the presence of consistently higher numbers of T cells in the resistant *Bos indicus* cattle and suggested that these cells might have a role in resistance to infestation. It was also supported by reports of Piper *et al*. (2009) that tick-resistant *Bos indicus* cattle develop a T-cell-mediated response to infestation which is absent in the *Bos taurus* cattle. Comparison of immune responses of resistant and susceptible cattle is thus a good strategy in the identification of candidate genes associated with resistance (Bram, 1983; Drummond, 1983;

Peter *et al.*, 2005). Factors affecting host responses to ticks should always be borne in mind during such comparison studies.

2.6 Host resistance, breeding and selection to increase host resistance

2.6.1 Factors affecting host genetic resistance to ticks

Several factors such as morphological, physiological and behavioural traits influence the resistance of cattle to ticks. Host body size affects tick abundance, with larger bodied animals being more heavily infested with ticks than smaller bodied ones, due to a greater surface area for tick infestation in the former. Morphological coat traits have been discussed earlier (Marufu *et al*., 2011) and influence HGRT in cattle. Heifers and cows are thought to be more resistant to ticks than bulls, and this could be attributed to testosterone, which reduces both innate and acquired resistance to tick feeding (Hughes & Randolph, 2001). Pregnant cows are more susceptible to ticks than non-pregnant cows due to the immunosuppressive effects of gestational hormones in the former. Younger animals carry lighter burdens of ticks than older animals (Swai *et al*., 2005), due to continuous selective grooming behaviour in the younger animals (Fivaz & de Waal, 1993). Preferential grazing in areas with shorter grass and less bush is a form of tick avoidance behaviour which may help to increase resistance to ticks in cattle (Meltzer, 1996).

2.6.2 Breed variability for host resistance

Resistance to ticks is under genetic control. The genetic basis for variation in HGRT, within and between breeds, has been recognised for many years (Wilkinson, 1955; Francis, 1966). *Bos indicus* (Zebu) cattle such as the Brahman are regarded to be generally more resistant to ticks than *Bos taurus* (European) cattle breeds such as the Angus and Hereford (Utech *et al.,* 1978; Madalena *et al.,* 1990; Frisch & O'Neill, 1998; Wambura *et al.,* 1998; Bianchin *et al*., 2007; da Silva *et al.,* 2007). European breeds (*Bos taurus*) were observed to carry up to 2.5 times more ticks than *Bos indicus* cross cattle under natural infestation (Seifert, 1971). Indigenous African cattle breeds (*Bos taurus africanus*), such as the Afrikander and Nguni, have also been shown to be more resistant to ticks than imported and local crossbred cattle (Scholtz *et al.*, 1991; Fivaz *et al*., 1992). In The Gambia, the N'Dama (*Bos taurus africanus*) breed was shown to possess a higher degree of resistance than Gobras and N'Dama x Gobra crosses against adult *A. variegatum*, *H. truncatum* and *H. marginatum rufipes* ticks (Mattioli *et al.,* 1993; Mattioli *et al.,* 1995). Much progress has already been made in crossbreeding to improve the productivity of tick resistant cattle breeds in countries like Australia and Brazil (Utech & Wharton, 1982; Frisch *et al.,* 2000). Moreover, backcrossing has been effective in introgression of desirable resistance traits into susceptible breeds. The Senepol composite beef breed was developed by crossing Red Poll

and N'Dama and has shown increased HGRT when compared to other pure taurine breeds (O'Neill *et al*., 2010).

There is an increasing role for crossbred bulls in developing countries to combine the desirable attributes of various breeds while maintaining a high degree of HGRT and environmental adaptation. Several studies quantify the level of HGRT in different cattle breeds against specific tick species (Table 2.3). In beef cattle production, problems with low resistance in exotic breeds can be avoided by crossing them with high resistance indigenous breeds. This can be only considered if productivity traits increased at the same level as that achieved by crossing to indigenous cattle.

In dairy production systems, the situation is far more complex than in beef production. Milk yield of the tropical breeds is much lower compared to that of the temperate dairy breeds. In Brazil, Furlong *et al*. (1996) reported a 23% reduction in milk production when Gir (*Bos indicus*) bulls were crossed with Holstein (*Bos taurus*) dairy cows to increase HGRT. The F_1 progeny were moderately resistant to ticks and had reduced milk production (Furlong *et al.*, 1996). The major challenge in dairy systems is that semen is obtained from donor bulls in temperate countries. These bulls have not been subjected to either artificial or natural selection for resistance to tick infestation. This results in crossbred progeny with only moderate resistance to ticks. It may be concluded that there is likely to be a serious challenge in crossbreeding with Holstein to achieve resistance to ticks without sacrificing milk production.

Table 2.3 Cattle breeds and their level of resistance to specific tick species

Cattle breeds	**Level of resistance**	**Tick species**	**References**
Brahman compared to Hereford	High	*Rhipicephalus microplus*	Rechav (1987)
Nelore compared to Taurine breeds	High	*Rhipicephalus microplus*	Gomes *et al*. (1988)
Nguni compared to Hereford & Brahman	High	*Rhipicephalus decoloratus*	Rechav *et al.* (1991)
Mashona compared Nguni and Brahman	High	*Amblyomma variegatum* *Rhipicephalus decoloratus*	Norval *et al*. (1996)
Boran compared to Tulis	High	*Rhipicephalus microplus*	Frisch & O' Neill (1999)
Gobra Zebu compared to N' Dama	Low	Amblyomma veriesgatum Hyalomma	Mattioli & Dempfle (1995)
Jersey compared to tropical breeds	Low to Avarage	*Rhipicephalus* sp *Amblyomma* sp *Hyalomma* And other African multi-host ticks	Utech *et al*. (1978); Spckett & De Klerk (1989); Norval *et al*. (1996); Solomon & Kaaya (1996)

2.6.3 Heritability estimates for host resistance to ticks

Heritability estimates of host resistance are summarised in Table 2.4. The mean is 0.27. The host genetic resistance to ectoparasites is thus thought to be approximately as heritable as milk yield

or growth, and resistance to ticks may be increased to very high levels by selection. Low tick infestations in cattle, and use of tick scores instead of tick counts could result in the lowering of heritability estimates (Prayaga & Henshall, 2005; Prayaga *et al*., 2009). Genetic variation in host resistance between cattle increases as natural infestation increases under extensive conditions (Budeli *et al*., 2009). In Australia, the Illawarra Shorthorn (*B. taurus*) dairy breed was selected for HGRT (Utech *et al*., 1978). Further, Utech & Wharton (1982) indicated that there is potential to select for higher resistance within the breed through culling animals that are susceptible to ticks. Frisch *et al*. (2000) developed a Hereford × Shorthorn (*B. taurus*) line of cattle that had high resistance. However, it was suggested that the high level of host resistance in this line of cattle was due to a single major gene (Frisch, 1994), but this was later confirmed not to be the case (Henshall, 2004).

Table 2.4 Heritability estimates of tick resistance

Cattle breed	**Location**	**Challenge**	**Tick trait**	**Heritability**	**Reference**
Shorthorn	Australia	Natural/artificial	count	0.39	Wharton & Roulston (1970)
Nguni	South Africa	Natural	count	0.26	Schoeman (1989)
Caracu	Brazil	Natural	count	0.22	Fraga *et al.* (2003)
Hereford Shorthorn line	Australia	Natural	count	0.44	Henshall (2005)
Holstein x Gir	Brazil	Artificial	count	0.21	Peixoto *et al.* (2008)
Bonsmara	South Africa	Natural	count	0.17	Budeli *et al.* (2009)
Brahman	Australia	Natural	score	0.15	Prayaga *et al.* (2009)
Gir X Holstein (F^2)	Brazil	Artificial	count	0.21	Machado *et al*. (2010)
B. taurus breeds (Dairy herds)	Australia	Natural	count	0.37	Turner *et al*. (2010)

Even in the Nguni breed, which is generally considered resistant to tick infestation, there is significant variation in tick counts and repeatability across months. Despite the generally moderate degree of additive genetic variation observed within various breeds implying scope for selection and a limited number of examples of the efficacy of selection for resistance, inability to accurately measure the trait of interest has hindered application of genetic evaluation systems (Regitano & Prayaga, 2010). It should be noted that selection for resistance to ticks should not hamper improvement in other productive traits such as growth, meat quality, and milk yield. Several studies have reported low and non-significant genetic correlation between tick count and various productive, adaptive and pubertal traits, and confirm that selection for HGRT may not have any unfavourable correlated response on other economically important traits (Davis, 1993; Prayaga *et al*., 2009).

Resistance of Holstein cows has been estimated in mid-lactation using artificial infestation with tick larvae and selection for resistance was found to be effective without compromising milk production (Jonsson *et al*., 2000). Recent findings of Turner *et al*. (2010), using genome-wide association study with 10k SNP markers, also indicate that selection for resistance to ticks would

not affect milk production. However, the innate level of tick susceptibility in Holstein cattle might limit the initial response to selection as any favourable alleles are likely to be in low frequency. Thus, opportunities for selection may be insufficient as cattle with high resistance to ticks and high milk production would be quite rare (Tuner *et al*., 2010).

2.7 Molecular approaches to improve host resistance

Difficulty in identifying animals with high or low resistance to ticks is a major limitation to traditional selection based on phenotype. Thus, molecular genetic approaches are an alternative approach for making selection decisions.

2.7.1 Candidate genes

Many of economically important traits in livestock production are complex traits controlled by many genes. Resistance to infestation by ticks is one such trait. However, a few of these many genes may have substantial effects. The first significant associations between tick load and serum amylase phenotypes were identified in late 1960s on bovine chromosome 3 and it was confirmed that cattle with serum amylase C were less infested than other genotypes (Ashton *et al*., 1968). Later, acute-phase reactants components of innate immune responses were also evaluated and found to associate the differences between susceptible and resistant breeds of cattle Holstein (*B. taurus*) to Nelore (*B. indicus*) animals under natural infestation (Carvalho *et al*., 2008). The results from these studies suggested that the difference in serum concentration of some proteins (e.g. haptoglobulin and transferrin) and could be potentially used as biomarkers to monitor the level of tick infestation.

In the early 1980s, the bovine leucocyte antigens (BoLA) group were identified and found to be associated to tick load. The detection of BoLA markers were done in the micro-lymphocyto-toxicity tests using different composite breeds under natural and artificial tick infestations (Stear *et al*., 1984; 1989; 1990). Although these studies mapped of resistance alleles to the bovine major histocompatibility complex (MHC) locus, the results were not consistent between studies, because the same BoLA allele was not always associated with increased HGRT. Following the BoLA marker findings, DNA microsatellite markers were selected as Class II BoLA microsatellite polymorphisms and were found to be associated with susceptibility of three quarter *taurine* cattle to B. microplus (Acosta-Rodriguez *et al* ., 2005). In addition, an association was also found between BoLA marker- DRB allele 3.2, 18, 20 and 27 for lower tick number in a reference Holstein × Gir F2 population in Brazil (Martinez *et al*., 2006), also another significant association to tick were found in DRB1 and DBR3 (Untalan *et al*., 2007). These findings also confirmed the location of genetic variation affecting the host resistance to ticks on bovine chromosome 23 (BTA23).

The results from gene expression profiles obtained after the tick challenge on the resistant versus susceptible cattle give promise of an alternative method of identification of candidate genes. Wang *et al*. (2007), using cDNA microarrays, described 66 genes with differential expression in tick-challenge skin of resistant versus susceptible Adaptaur cattle. Among these genes, Type I, III, and V collagen genes show higher expression in resistant animals than in susceptible animals and Keratin genes were more suppressed after challenge in susceptible than in resistant animals. These results suggested that some of the genetic variation of HGRT can be explained by genes related to skin structure.

2.7.2 Quantitative trait loci (QTL)

Several studies have identified QTL associated with particular phenotypes, including immunity in livestock production. Identified QTL that affect economic important traits including tick resistant are available on QTL database (www.animalgenome.org). In Brazil, a *Bos taurus* x *Bos indicus* F_2 population was developed from 1999 to 2005 by Embrapa and 382 individuals were measured for tick load (scoring and counting). From 382 F_2 animals, microsatellites panels were used to scan all chromosomes for QTL for tick load, and positive associations were found on chromosome 2, 4, 5, 7, 10, 11,14, 18 and 23 (Gasparin *et al*., 2007; Regitano *et al*., 2008; Machado *et al*., 2010). All detected QTL were dependant on seasons in which the phenotype was measured. In total, all the above QTL mapped on the 382 F2 animals explained 13.1% of phenotypic variation in the rainy season and 18.4% in the dry season. The only QTL significant in both seasons was detected on BTA23 (Machado *et al*., 2010) in a same genomic region containing the BoLA genes which had been previously known to associated with tick burden. Machado *et al*. (2010) also fine mapped the QTL on BTA 10 and BTA 11 reducing the confidence interval associated with the QTL. However, the candidate gene associated with host susceptibility or host resistance due to the large size of the QTL region could not be clearly identified.

2.7.3 Genome-wide association studies (GWAS)

Development of genetic technologies, such as high density SNP panels, provides an opportunity to evaluate individual animals based on their genotype. Knowledge of the location of loci linked to genes causing a variation in traits of economic importance can be exploited to increase effectiveness of selection (Georges *et al*., 1995), given an appropriate reference population for training a prediction equation that quantifies the relationship of genotype and phenotype (Meuwissen *et al*., 2001). This genome scan approach has been used effectively for complex traits where several genes are likely to contribute to the variability (Schnabel *et al.,* 2005; Williams, 2005; Machado *et al*., 2010).

Recently, a GWAS was conducted which found scores of SNPs significantly associated with tick burden (Turner *et al.*, 2010; Port Neto *et al*., 2011). A QTL for tick burden was identified on BTA3 in the region of the BoLA marker from previous studies (Porto-Neto *et al.,* 2010, 2011). An additional 13 chromosomal locations were identified associated with tick load. Turner *et al*. (2010) using 10k SNP panel found a low correlation between the allele effects for milk composition and tick burden further suggesting that selection based on markers used to increase HGRT might not cause an undesirable response in milk traits. The majority of markers explained a small proportion (~1%) of the phenotypic variation. Porto-Neto *et al.* (2010; 2011) complemented the previous reports with additional markers located in the same genomic regions. The markers were analysed for associations with tick burden as single markers and as SNP haplotypes. However, both initial QTL on BTA3 and BTA10 were confirmed in dairy and Brahman cattle and the QTL intervals were reduced and the Integrin Itga11 candidate genes was also identified (Porto-Neto *et al*., 2010; 2011). The location of a QTL affecting tick burden on BTA10 were close to the ITGA11 gene position, when using 17 SNP panel from BTA10 in Brahman and taurine cattle. Barendse (2007) using SNP assay, reported that several genes influencing the immune system are linked to HGRT.

2.8. Conclusion

An understanding of the mechanisms of genetic resistance to ticks and tick-borne diseases (TBD) could improve breeding programmes to develop cattle that are more resistant and productive. Genetic variation in resistance of livestock should be quantified within and across breeds so that appropriate strategies are adopted in breeding programmes. For breeds with moderate to high resistance, selection based on an index that combines breeding values for resistance and production traits will achieve desirable results; however, in low resistance breeds, introgression of major genes would be the way to improve these breeds with reasonable time period. The development of genetic marker panel and high density SNP chips have provided an opportunity to evaluate individual animals based on their DNA genotype. The application of marker-assisted selection and genomic selection promises great benefits since conventional breeding for resistance to ticks and tick-borne diseases by analysing tick counts and scores (phenotypes for resistance) in a large number of animals is not practical in commercial breeding schemes. Genome-wide association studies are opening a way to identify SNPs of interest within the population. The host and tick genomics and their proteomics, such as gene expression profiles, are likely to facilitate studies addressing the sequencing, annotation and functional analysis of their entire genomes. This could provide valuable information for improving tick control.

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Chapter 3

Prevalence and tick loads in South African Nguni cattle reared in four different climates

Abstract

Ixodid ticks are among the most economically important ecto-parasites of livestock in tropical and sub-tropical countries. Although South African Nguni cattle are adapted to harsh environments, their resistance to ticks and tick-borne diseases in different climatic conditions is unknown. The objective of the current study was to compare tick load and prevalence among Nguni cattle under different climatic conditions. Tick counts were conducted once a month under natural challenge over two years on 586 Nguni cattle located at ARC Roodeplaat and Loskop farms (warmer climate), and Mukhuthali Nguni Community and University of Fort Hare farms (cooler climate). The following tick species (relative prevalence) were observed: *Amblyomma hebraeum* (42%), *Rhipicephalus evertsi evertsi* (22%), *Rhipicephalus* (*Boophilus*) *spp*. (16%), *Rhipicephalus appendiculatus* (11%), *Hyalomma marginatum* (5%) and *Rhipicephalus simus* (4%). Tick infestation was significantly affected by location, season, year, month of the tick counting and age of the animal. Loskop farm, which is in the warmest location, had the highest tick counts and also showed the largest variation in tick count. Higher tick counts were also observed in the hot dry (September to November) and hot wet (December to February) seasons compared to the other seasons. *Amblyomma hebraeum* was the dominant tick species across all four farms followed by *R. evertsi evertsi*. The most favoured tick attachment sites were the perianal region (under the tail head), perineum and belly body locations. These results provide useful information for the development of appropriate control strategies for ticks and tick-borne diseases in these provinces of South Africa. Further work is required to investigate the feasibility of of genetic improvement for tick resistance.

Key words: *Amblyomma hebraeum*, warmer climate, Nguni cattle, tick count.

3.1 Introduction

Global warming has led to a growing interest in farming with cattle that are adapted to challenging environments, especially in the tropical and sub-tropical regions of Africa (Scholtz *et al*., 2013). High incidence of diseases and parasites in these environments result in large economic losses (Seifert, 1984b; Naser, 1985; de Castro, 1997; Frisch & O'Neill, 1998; Jonsson, 2001). It is thus important that farmers use animals that are well-adapted to these environmental conditions in order to maximize production efficiency. Ticks are among the most economically important ecto-parasites and vectors of disease pathogens in livestock production (Roberts, 1968; Bonsma, 1981; Latif *et al.,* 1991; Scholtz *et al*., 1991; Budeli *et al*., 2007 Machado *et al*., 2010; Mapholi *et al*., 2014). In South Africa, about 10 ixodid ticks are considered to be of major economic importance in livestock production (Walker *et al*., 2003; Coetzee & Tustin, 2004; Spickett & Williams, 2011; Spickett, 2013). The most economically important tick genera affecting cattle production in South Africa are *Rhipicephalus* (includes the genus formerly known as *Boophilus*), *Amblyomma*, and *Hyalomma* (Marufu *et al*., 2010; Mapholi *et al*., 2013; Nyangiwe *et al*., 2013; Spickett, 2013). These tick genera have an impact on animal productivity directly through heavy infestation and indirectly through transmission of tick-borne diseases (Dold & Cocks, 2001; Ghosh *et al*., 2006). They transmit diseases and produce toxins, of which the most important diseases affecting cattle production in South Africa are heartwater, babesiosis (redwater), anaplasmosis (tick-borne gallsickness) and theileriosis (corridor disease). Apart from blood feeding, which causes wounds and infections, ticks affect production through decreases in weight gain and milk yield (Jongejan & Uilenberg, 2004; Kaufman *et al*., 2006; Turton, 2001; Frisch, 1999; Spickett, 2013). Bites from the long mouth part ticks leave scars which cause depreciation of quality and price of leather products. Control strategies such as chemical control, tick vaccination and grazing management to eradicate ticks have been used; however these strategies may not permanently control ticks.

It has been suggested that if global warming leads to temperature increases, the abundance of ticks will increase in some regions where ticks are endemic (Olwoch *et al.,* 2009). This could lead to serious implications for tick infestation, tick-borne disease incidence, and tick-borne disease control strategies in tropical environment. Agro-ecological conditions, seasonal variations and host species differences influence tick infestations (Randolph, 1998; Regitano & Prayaga, 2010; Kabir *et al*., 2011; Katiyatiya *et al*., 2014). Therefore, climatic factors such as temperature, rainfall and humidity influence tick loads, especially of those tick species that prefer warmer and humid conditions. The displacement of *R. decoloratus* by *R. microplus* has been associated with warm

and humid conditions of some parts of South Africa (Nyangiwe *et al.,* 2013) and *A. hebraeum* distribution is escalating to the inland semi-arid areas of South Africa and has also been associated with intense periods of drought, especially in the inland highlands areas (Nyangiwe *et al*., 2011). These findings have brought concerns in the livestock industry about finding appropriate control strategies for these rising tick infestations.

The genetic basis for variation in tick resistance within and between breeds exists and has been well-recognised (Wilkinson, 1962; Wharton *et al*., 1970; Utech & Wharton, 1982; de Castro, 1991; Burrow, 2001; Prayaga *et al*., 2009). There are a number of exotic cattle breeds in South Africa that are susceptible to ticks and tick-borne diseases (Scholtz *et al*., 1991). Most of these breeds have high production potential. However, their production is compromised by tick-borne diseases. Indigenous breeds and some of the locally developed breeds in South Africa are adapted to harsh tropical conditions. Nguni cattle are known for their adaptation to tropical and semi-arid regions of the Southern African region and are extensively used by commercial and emerging farmers (Scholtz *et al*., 1991; Muchenje *et al.,* 2008; Ndlovu *et al.,* 2008). Spickett *et al*. (1989) reported that Nguni cattle are more resistant to natural tick infestation than Bonsmara and Hereford cattle. Rechav and Kostrzewski (1991) reported that Nguni cattle were more resistant to infestation by *R. decoloratus* than five other breeds of cattle considered in their study. Marufu *et al.* (2011) also highlighted that Nguni cattle are more resistant to ticks than non-descript cattle under rangeland grazing.

Although Nguni cattle carry lower tick loads than crossbred and exotic cattle, little is known about the extent of variation in tick loads within this locally adapted breed. Furthermore, it is expected that climate change will induce differences in the prevalence of different tick species (Scholtz *et al*., 2013). It is, therefore, important that tick loads and prevalence of ticks should be assessed at animal and species level to gain a better understanding of the implications of climate change on livestock production. Knowledge of agro-climatic and animal factors influencing tick load and prevalence in Nguni cattle is also important. Thus, the objective of the current study was to assess tick loads and prevalence of ticks in Nguni cattle in the different agro-climatic regions of South Africa.

3.2 Materials and methods

3.2.1 Experimental cattle

Nguni cattle of both sexes were randomly selected from four different pedigree herds in different provinces of South Africa. Each selected herd was managed in their original farms. The age of cattle and their physiological status varied in each location.

3.2.2 Sampling areas

Tick count data were collected from 586 Nguni cattle over a 2-year period (May 2012 to April 2014) from four locations: the Agricultural Research Council (ARC) Loskop Research Farm located in the Limpopo Province of South Africa (n = 124); ARC-Roodeplaat Research Farm located in Gauteng Province (n = 143); Mukhuthali Nguni Community Farm located in the Kwa-Zulu Natal Province (n = 224); and the University of Fort Hare Farm in Alice located in the Eastern Cape Province (n = 95). These farms are located in different agro-climatic zones (Table 3.1) and the map of the four farms is presented in Figure 3.1. The cattle were exposed to natural tick infestation at all four farms. Counts and identification of tick species were conducted every month on all farms from May 2012 to April 2014 (May 2012 to April 2013 = Year 1 and May 2013 to April 2014 = Year 2). All cattle were spray dipped with a flumethrin pour-on formulation "Drastic Deadline®" immediately after tick count data collection each month.

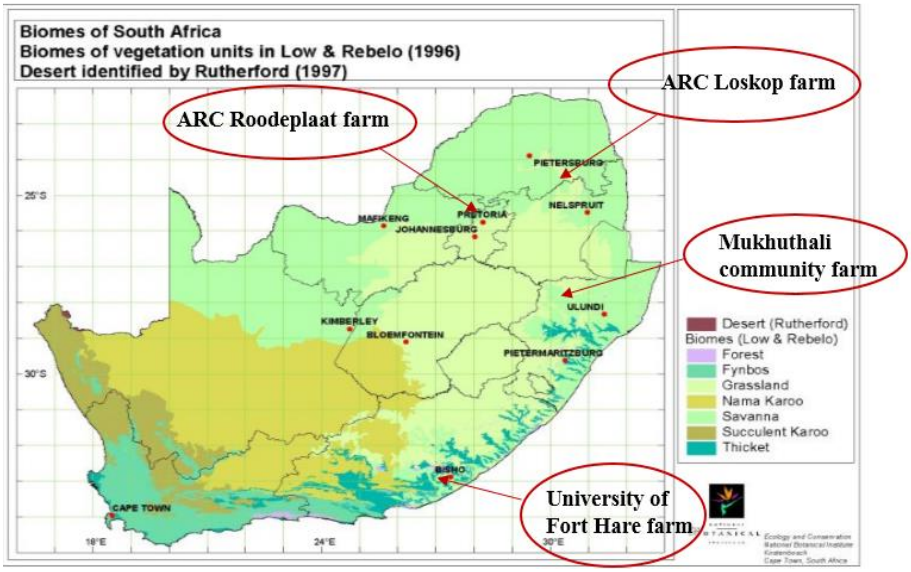

Figure 3.1 Location of the four experimental farms considered for tick counting

Table 3.1 Summary of the climatological and vegetation characteristics of the four farms used in the study

Farm (Province)	Minimum and Maximum Monthly rainfall (Annual Rainfall)	Average daily temperature range	Vegetation
Loskop (Limpopo)	0 mm in June and 92 mm in November (506 mm)	19 °C in June to 29 °C in January and annual average of 26 °C	Tropical forest, dense bush and shrubs to semi-desert areas (Acocks, 1975)
Roodeplaat (Gauteng)	0 mm in June and 110 mm in January (573 mm)	19 °C in June to 27 °C in December and annual average of 24 °C	Open savannah veld and bush and thornveld (Acocks, 1988)
Mukhuthali (KwaZulu-Natal)	3 mm in June and 122 mm in December (688 mm)	19 °C in June to 27 °C in January and annual average of 23 °C	Tall bush grass with mostly acacia trees (Mucina & Rutherford, 2006)
Fort Hare (Eastern Cape)	8 mm in July and 56 mm in March (480 mm)	20 °C in June to 26 °C in February and annual average of 24 °C	False thorn trees with some savannah vegetation type (Ngambu *et al.,* 2012)

3.2.3 Tick counting

Tick species were identified during counting at each location. The same group of trained technicians collected the data throughout the experiment. Two people counted one animal at each time, with each individual counting and identifying tick species on half of the body. Once per month, adult ticks were counted on each animal by counting and identifying tick species on different body parts, including the head (excluding the inside ears), ears (inside the ears), neck (including the gullet), back, legs, belly (including the udder or testicles), perineum and tail (including underneath the tail). Only adult ticks for each tick species were collected for the current study.

3.2.4 Statistical analysis

Data was analysed using SAS Version 9.3 (SAS, 2002-2010). Distribution of counts was determined using the PROC FREQ (SAS, 2002 - 2010). The tick counts were then analysed with the PROC GLM procedure using the following fixed effect models. Model (i) determined the fixed effects influencing tick counts in all the study areas. Model (ii) accounted for monthly and yearly variations in the tick counts:

$$Y_{ijklmn}=\mu+ L_i + N_j + (L*N)_{ij} + b(A_m) + e_{ijklmn} \quad \text{i}$$

$$Y_{ijklmn}=\mu+ L_i + M_j + R_k + S_l + (L*M)_{ij} + b(A_m) + e_{ijklmn} \quad \text{ii}$$

where: Y_{ijklmn} is the tick count;
μ is the overall mean;
L_i is the effect of the ith location (i=1, 2, 3, 4);
N_j is the effect of the jth season (i=1, 2, 3, 4);
M_j is the effect of the jth month (j=1, 2, 3 …12);
R_k is the effect of the kth year (k=1, 2, 3)
S_l is the effect of the lth sex (l=1, 2);
$(L*M)_{ij}$ is the interaction effect of the ith location and jth month;
b is the partial regression coefficient of age on tick count
A_m is the effect of the mth age of the animals;
e_{ijklmn} are the random residuals.

Pairwise comparisons of least square means were performed using the PDIFF option.

3.3 Results

The tick species observed were *Amblyomma hebraeum* (42%), *Rhipicephalus evertsi evertsi* (22%), *Rhipicephalus* (*Boophilus*) spp. (16%), *Rhipicephalus appendiculatus* (11%), *Hyalomma marginatum* (5%) *and Rhipicephalus simus* (4%). *R. simus* was the least frequently encountered species and was only found at the University of Fort Hare farm. Summary statistics of the tick count data for the four locations are given in Table 3.2. Loskop farm had the highest mean tick count of 30.69 with the standard deviation of 19.79 compared to other three farms. The maximum tick count per animal was also highest in Loskop. Highest tick load variation was observed in Roodeplaat farm followed by Loskop farm.

Table 3.2 Summary statistics of tick counts data for four locations

Location	Mean	SD	CV (%)	Min	Max
Loskop	30.69	19.79	64.52	0	198
Roodeplaat	25.97	18.66	71.85	0	118
Fort Hare	18.23	11.74	63.38	0	86
Mukhuthali	18.19	10.89	59.87	0	75

Abbreviation: SD= Standard deviation; CV= coefficient of variation; Min= minimum tick count; Max= maximum tick count. #the mean and standard deviation were calculated form back transformed tick count data

3.3.1 Influence of location and season on tick counts

Location had a significant effect on total tick count per animal. Figure 3.2 shows the least squares means for the different locations. The ARC Loskop Research Farm had the highest average tick count per animal, followed by Roodeplaat, Mukhuthali and Fort Hare.

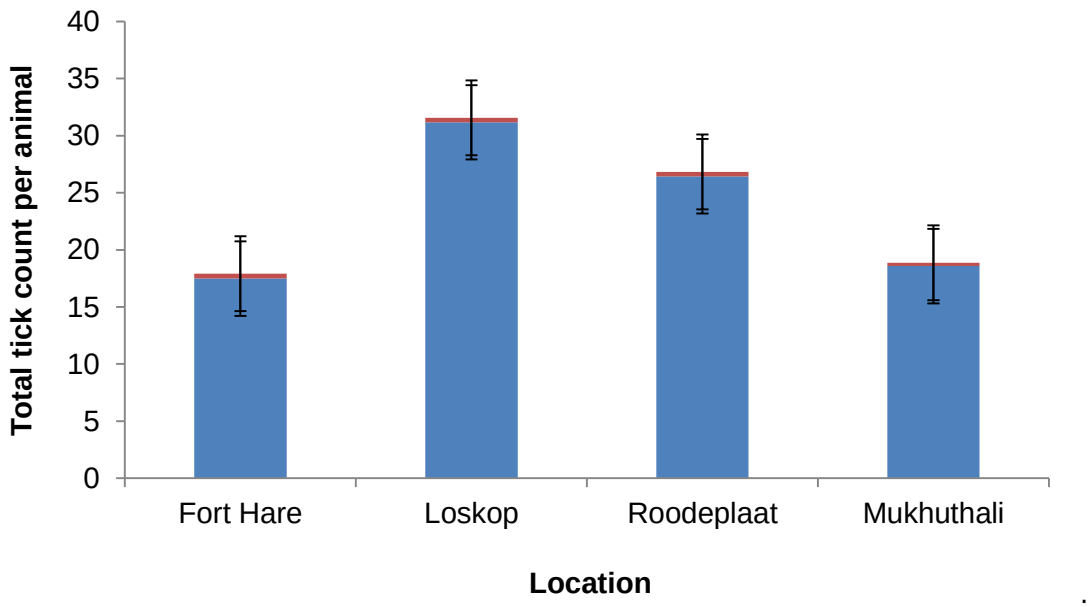

Figure 3.2 Least Square means for total tick count per animal for each location

Season had a significant effect on the prevalence of tick species (Figure 3.3). Most of the species had lowest count in the cool dry season, which gradually increased over the cool wet and hot wet season, and peaking in the hot dry season. *A. hebraeum* ticks were prevalent in all seasons.

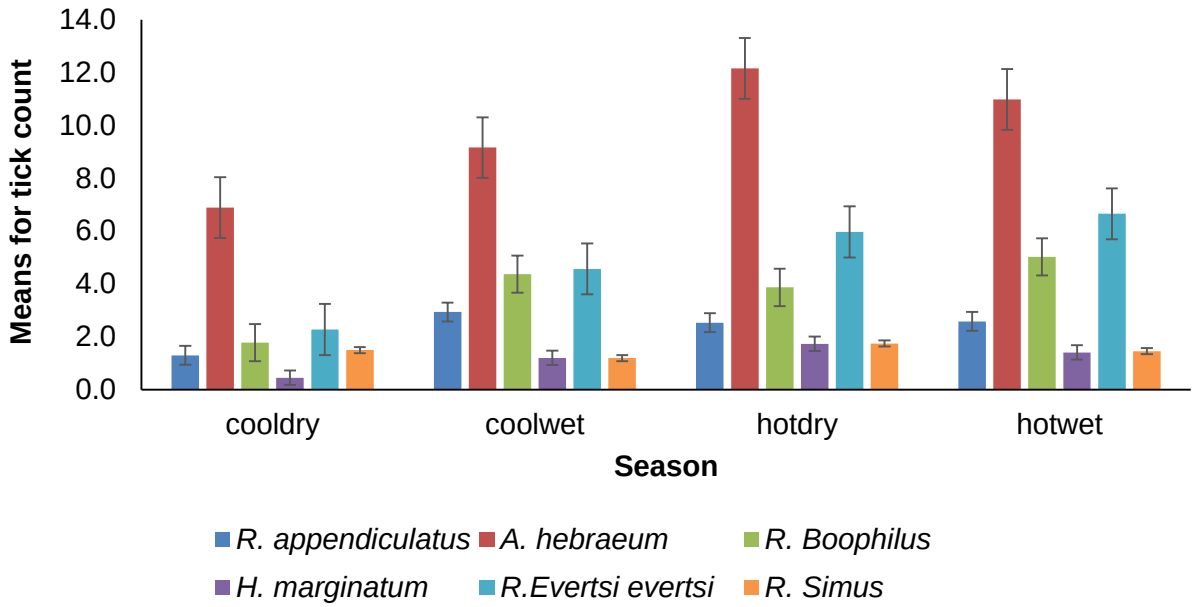

Figure 3.3 Seasonal variation in mean tick count of tick species over two years

Month and year had a significant effects on tick counts. May 2012 to April 2013 were reported as Year 1 and May 2013 to April 2014 as Year 2. Higher tick infestation was observed in November and lowest in June of Year 1 for all species. In Year 2 tick infestations was highest in December and lowest in June. *Amblyomma hebraeum* tick load was higher between October and January in both years while *R. evertsi evertsi* had higher tick load in December of both years. *R. Boophilus* ticks had low counts in Year 1, but much higher tick loads in Year 2. Tick count distributions for each tick species show different patterns in monthly tick count over the period of two years of tick count (Figure 3.4).

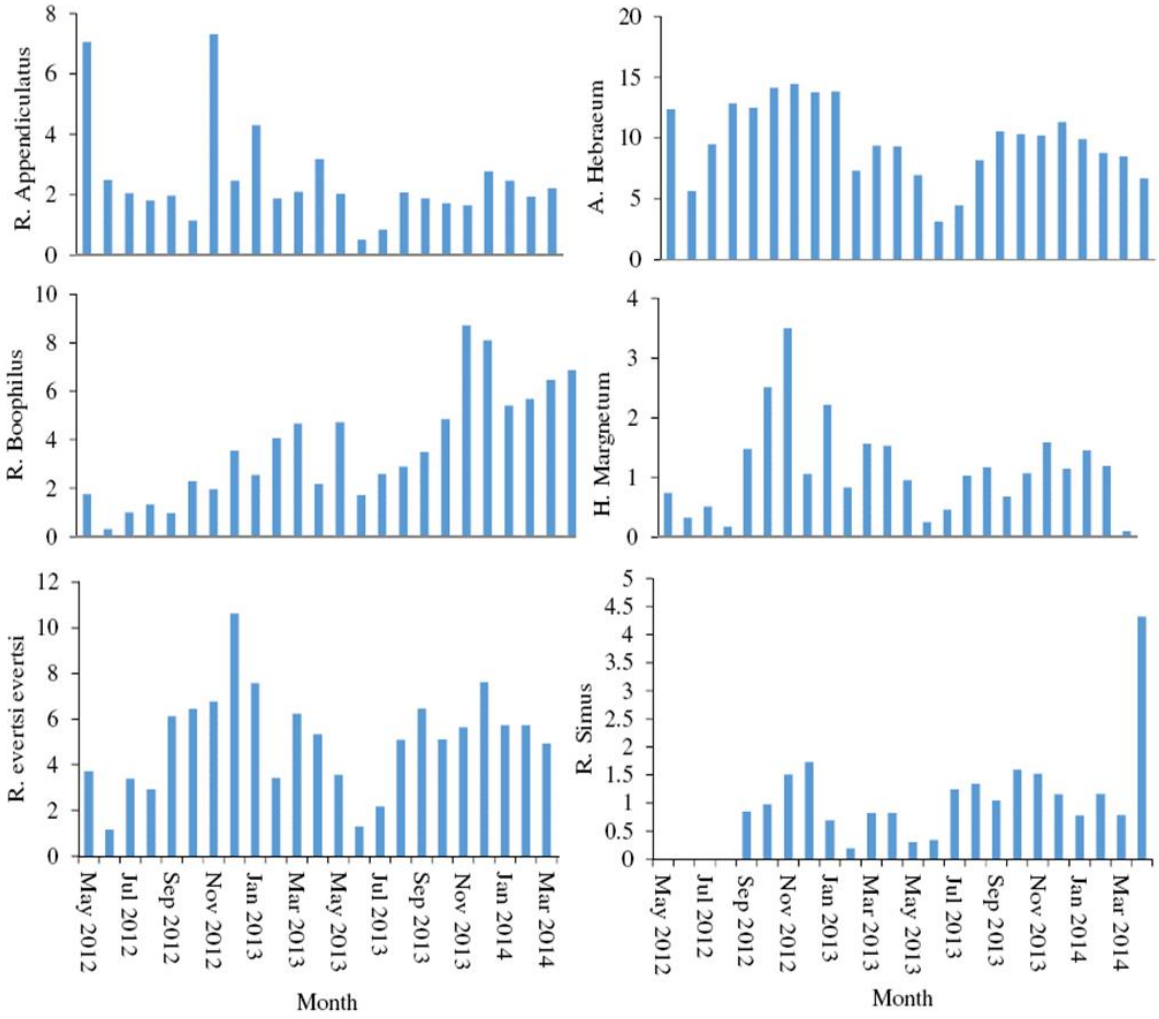

Figure 3.4 Monthly average tick counts per species over two years

Interaction between location and season was significant (P<0.001) for tick count (Figure 3.5). The magnitude and ranking of differences in tick count, among the different farms, varied from season to season. For example, Roodeplaat farm had the highest tick count in the cool wet season, whereas Loskop farm had the highest count in all other seasons.

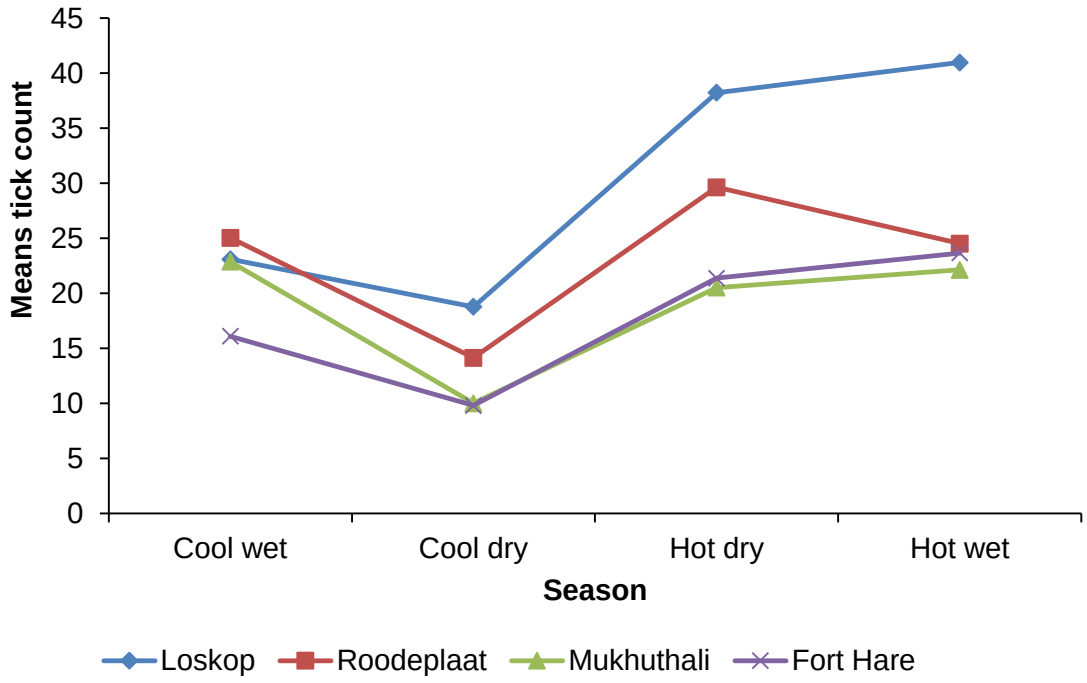

Figure 3.5 Interaction of location by season in all four farms

3.3.2 Species prevalence

A. hebraeum and *R. boophilus* were the most prevalent species at Loskop farm (Figure 3.6). The *A. hebraeum* tick burden at Loskop farm resulted in an outbreak of heartwater disease between November and December 2012. At Roodeplaat farm *A. hebraeum* and *R. evertsi evertsi* had significantly greater counts compared to the other four species. In Mukhuthali community farm, *A. hebraeum* had highest counts followed by *R. evertsi evertsi* and *R. appendiculatus*. At Fort Hare farm, *A. hebraeum* and *R. simus* had greater ($P<0.05$) counts compared to the other four species.

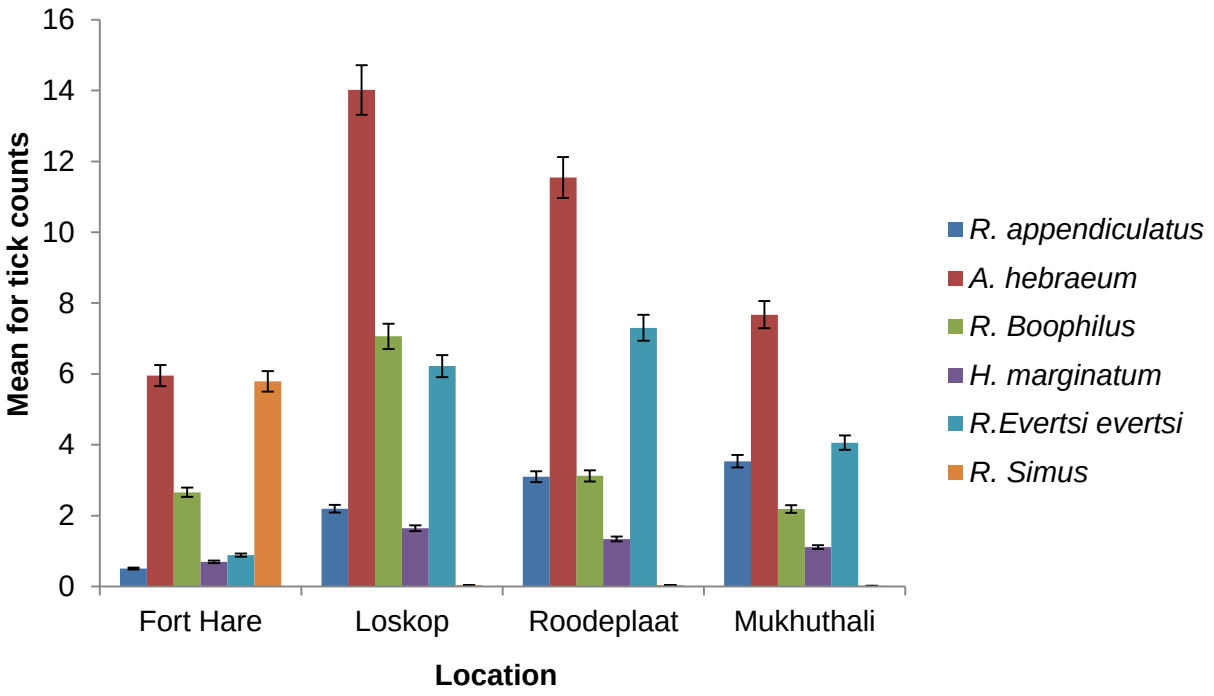

Figure 3.6 Tick loads for different species per location

3.3.3 Tick distribution on host body

The distribution of ticks on different body locations is shown in Figure 3.7. Under the tail, perineum and belly were the most preferred sites for attachment. The under tail region had the greatest infestation (36%) followed by the perineum (22%), belly (22%), and inside ear (11%). Other body locations accounted for less than 10% of the total infestation. *A. hebraeum* were located across the body, with the highest occurrence on the belly. Higher counts for *R. evertsi evertsi* were obtained under the tail, while *R. appendiculatus* were most prevalent inside the ears.

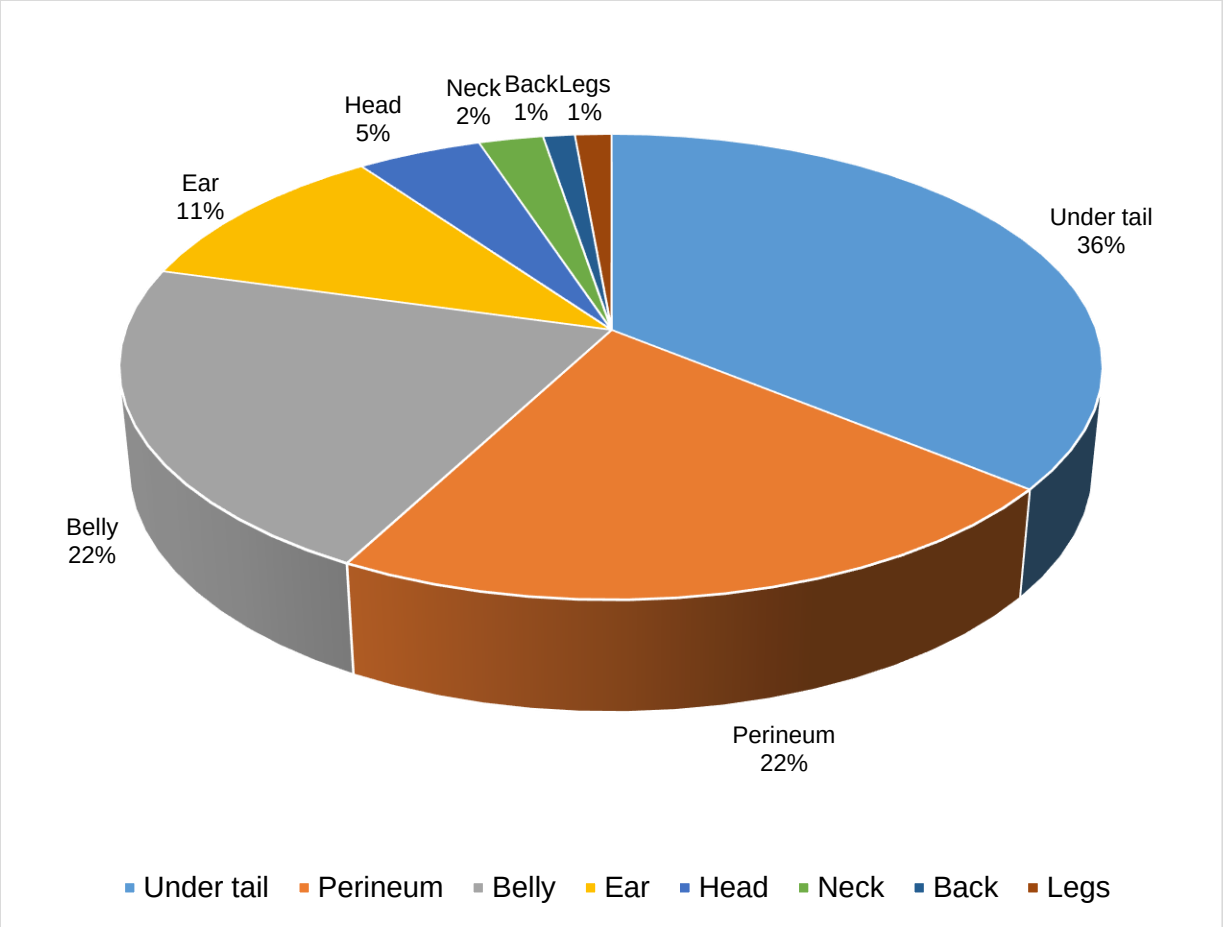

Figure 3.7 Percentage of total tick count per body location of the animal

The distribution of total tick per species onn different body locations is presented in Figure 3.8. Under the tail, *R. evertsi evertsi* (49%) was the most prevalent, followed by *A. hebraeum* (34%). On the belly, 69% were *A. hebraeum* ticks followed by 16% of *R. boophilus*. In the perineal region, 58% were *A. hebraeum* followed by 18% of *R. boophilus.* In the ear, 93% of ticks found were *R. appendiculatus*. On the head, 77% of the ticks found were *R. boophilus*. Thus, most tick species appear to have a preferred location for attachment to the host.

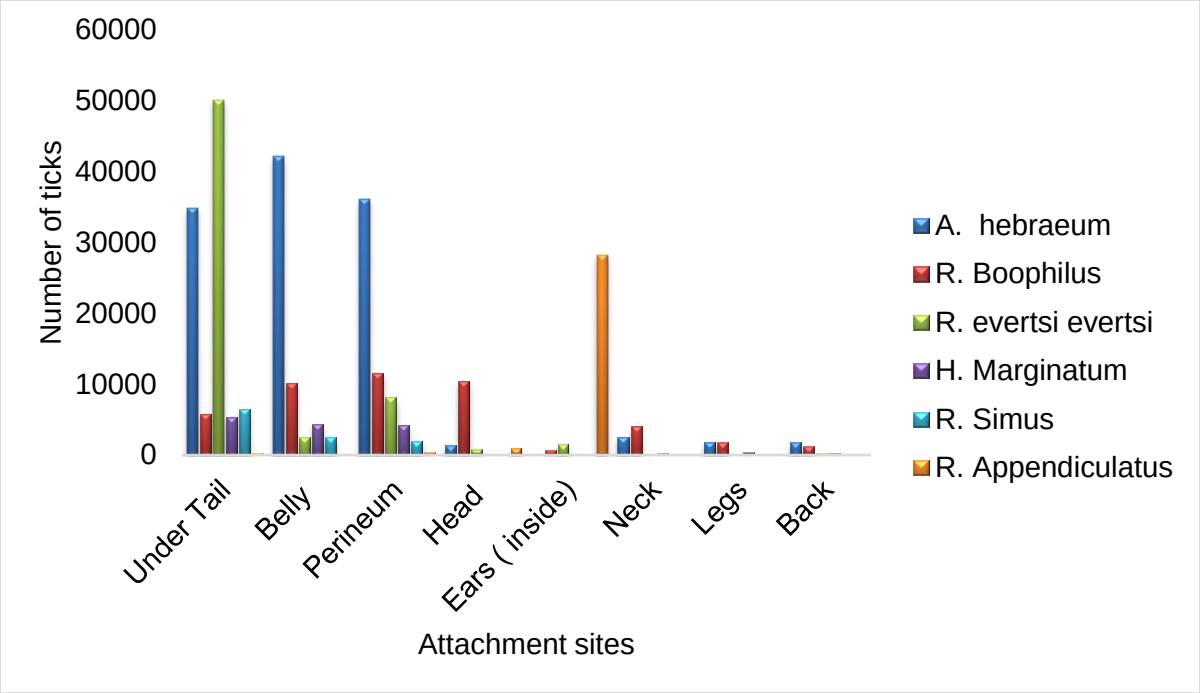

Figure 3.8 Distribution of total tick per species in different body locations

Tick distribution patterns on the different body locations in each month are presented in Figure 3.9. Higher tick loads on the belly were observed in November while lower tick loads were evident in June for both Years 1 and 2. The belly, perineum and tail had very similar distribution patterns for the observed years.

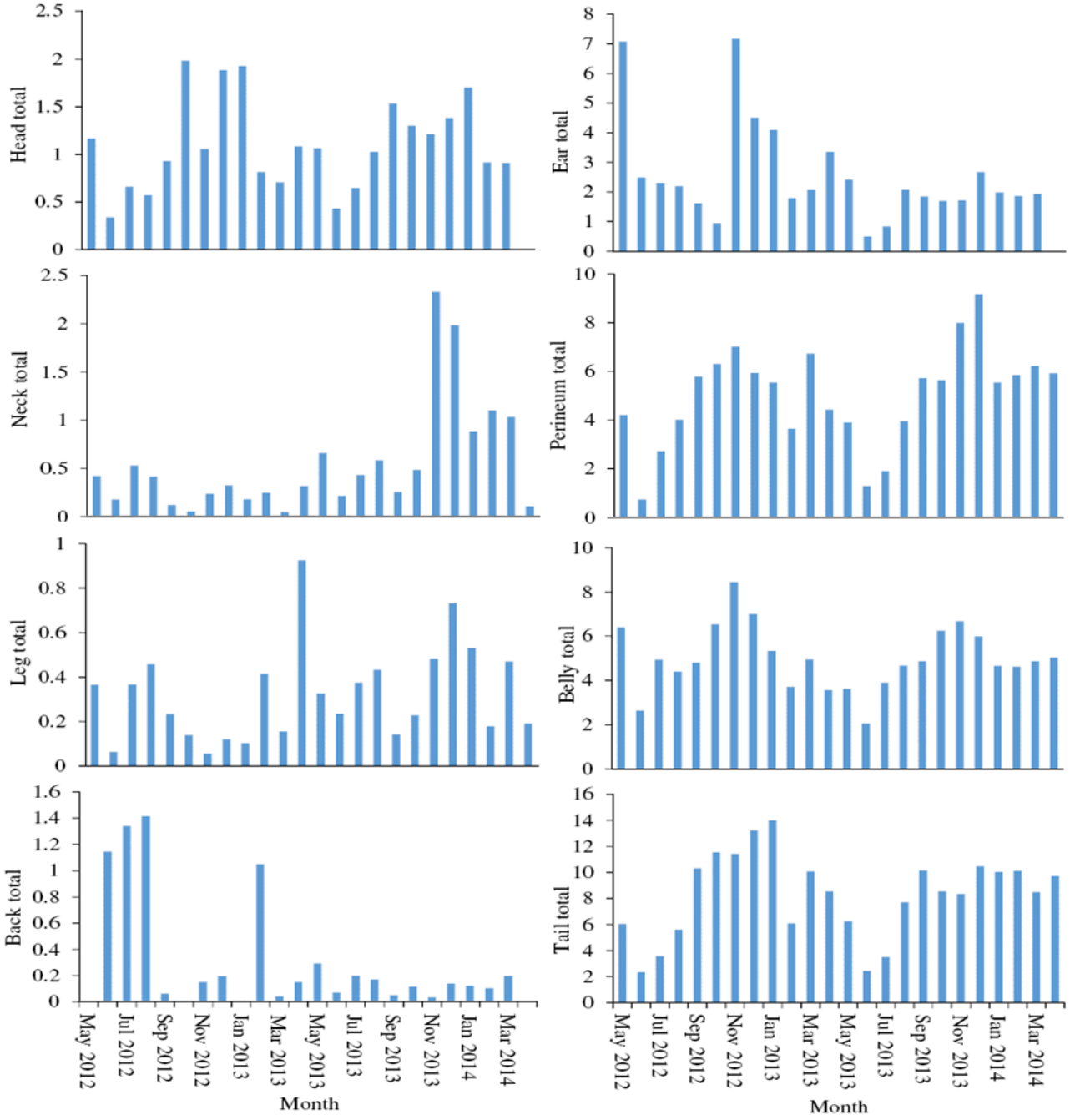

Figure 3.9 LS means for tick load distributions on body locations in monthly counts

3.4 Discussion

The distribution of tick species infesting cattle in the four locations considered in the current study shows large variation from one area to another. The species observed were *A. hebraeum, R. evertsi evertsi, R. boophilus spp., R. appendiculatus, H. marginatum* and *R. simus.* These were the same species identified by Marufu *et al*. (2011) and Katiyatiya *et al.* (2014), in the Eastern Cape Province of South Africa and by Assefa (2004), Tessema *et al*. (2010), Tiki & Addis. (2011) in Ethopia. *R. simus* was only observed at University Fort Hare farm in the Eastern Cape Province and its recent infestations in cattle were also reported by Nyangiwe *et al* (2011) and Katiyatiya *et al* (2014). Previously, this tick species was known to infest dogs, cats, cheetah, lions, sheep and goats, and mostly found in moist areas (Horak *et al.,* 2000; Walker *et al.,* 2000). Occurrence of *R. simus* at Fort Hare in the current study might be due to the fact that this farm is located in a moist region of the Eastern Cape and mixed grazing practices with small stock.

Amblyomma hebraeum was the most prevalent and widely distributed tick species. Similar observations were recorded in Limpopo Province of South Africa (Tonnesen *et al.,* 2004). This tick is usually found in warm and hot dry harsh areas and also occurs where cattle are dominant as the most important domestic host (Norval, 1994). The abundance of *A. hebraeum* was also observed in southern African buffalos (Anderson *et al.,* 2013). Heavy infestation of this species is known to cause losses in cattle production and damage to hides and skins, due to the long mouth part, which damages these commodities decreasing their value on the world market (Solomon & Kassa, 2001). Mapiye *et al*. (2007) reported that the value of Nguni hides drops due to tick bite damage and decreases the quality of skin hide products in South Africa. The bite wounds caused by this tick species become a favourable site for secondary bacterial infections such as *Dermatophilus congolensis*. *A. hebraeum* tick burden at Loskop farm resulted in an outbreak of heartwater between November and December 2012, during the course of the current study. Heartwater was only observed in the Angus x Nguni cross and resulted in 25% mortality of this crossbreed in Loskop farm. However, there was no incidence of heartwater mortality observed in Nguni cattle used in the current study. Mortality due to tick-borne diseases was observed over the years, and studies in host-resistance to tick infestations in South Africa cattle were presented (Bonsma, 1981).

Ripicephalus evertsi evertsi was the second most abundant tick species. The abundance of this tick species was previously recorded in the Eastern Cape province of South Africa (Marufu *et al.,* 2010; Nyangiwe & Horak, 2007; Nyangiwe *et al.,* 2013) and also in the various regions of Ethiopia

(Assefa, 2004; Tiki & Addis, 2011). *Ripicephalus evertsi evertsi* was also reported as the most prevalent species in the genus *Rhipicephalus* in Africa (Walker *et al*. 2000). This species is known to be found mostly in the savannah areas. Its other target hosts are zebra and small ruminants (Tessema *et al*., 2010) and it is found in all seasons of the year (Pegram *et al*., 1981). *Rhipicephalus evertsi evertsi* is a vector of *Anaplasma marginale* which causes *Anaplasmosis* and is known to cause considerable economic loss to both the dairy and beef industries worldwide.

Rhipicephalus (Boophilus) spp including *R. microplus* and *R. decoloratus* was the third most abundant tick species. *Rhipicephalus. microplus* was reported as the source of *R. decoloratus* displacement in the Eastern Cape province of South Africa by Nyangiwe *et al.* (2013). The distribution of *R. decoloratus* ticks was reported in South Africa (Scholtz *et al.,* 1991) and in other African countries (Gebre *et al*., 2001; Tessema *et al*., 2010) and *R. microplus* abundance was reported in Brazil (Regitino *et al*., 2008; Machodo *et al*., 2010) and Australia (Roberts, 1968a; Wharton *et al*., 1970; Wagland, 1975, Utech *et al*., 1978). Resistance of *R. decoloratus* to acaricide was observed by Baker (1982), Solomon (1983) and Walker (1991). This tick species transmits *Babesia bovis*, *B.bigemina* and *Anaplasma marginale* to cattle and heavy infestations can cause tick worry and anaemia (Mekonnen *et al*., 2001). The fourth most abundant tick species was *R. appendiculatus*, also known as the brown ear tick, was found to prefer the inside of the ear (93%) for attachment. Severe infestations of *R. appendiculatus* were observed to cause substantial damage to the ears of animals. *Hyalomma* and *R. simus* were the least prevalent species.

The differences in tick load among the four locations may be attributable to differences in latitude and hence average daily temperatures amongst the locations. Loskop and Roodeplaat farms are located more towards the northern part of South Africa, which is warmer, while Mukhutali community and Fort Hare farms are close to the cooler south coastal region. Higher temperatures at Loskop are the probable reason for the higher tick count compared to the other farms. Variation in vegetation may partly explain the differences in tick load among the four farms. In the current study the main biomes observed in all four farms were savannah and grassland, which are often associated with increased tick loads (Trollope *et al*., 2003). Scholtz *et al.* (1991) noted that Loskop research farm is a tick endemic area, because of its forest trees with tall grass and hot dry climatic conditions which attract ticks.

Higher tick loads were observed in the hot dry and hot wet seasons than in the cool wet and cool dry seasons. Similarly, Scholtz *et al.* (1991), Webb & David (2002), Wesonga *et al.* (2006) and Muchenje *et al*. (2008) reported a higher tick load in the hot wet season when comparing different breeds of cattle in South Africa. In Brazil, hot and rainy seasons were also associated with higher tick infestations (Regitano *et al*., 2008; Machado *et al*., 2010). This is probably due to hot and wet conditions being favourable to tick proliferation and survival. Tick proliferation is normally enhanced when there are high temperatures and humidity (Chilton *et al.,* 2000; Zeleke & Bekele, 2004). In addition, the magnitude and ranking of differences in tick count, among the different farms were not consistent across the seasons. The interaction probably arises due to the tick count at Loskop being similar to the other locations in the cool-wet season and being very much greater than all other locations during the hot wet season. The decrease, relative to the preceding hot dry season, in tick numbers at Roodeplaat observed during the hot wet season might have contributed to the significance of the interaction. This is evident from the constant to slightly increasing numbers of ticks at the other three locations.

The observed significant effect of age of animals on tick counts was in agreement with the report of Marufu *et al*. (2011) who also observed lower number of ticks on younger animals compared to older animals. The age effect is attributed to some form of innate protection that declines with age (Wickel & Bergman, 1997).

Ticks appear to have preferred attachment sites on the host body. More ticks were found under the tail, followed by the perineum and belly. *Amblyomma hebraeum* were located in most body locations, with the highest occurrence in the belly. Higher counts for *R. evertsi evertsi* were obtained under the tail, while *R. appendiculatus* were most prevalent inside the ears. The higher infestations under the tail could be due to the fact that ticks prefer warm, moist, hidden sites with a good vascular supply and thin skin and it is also thought to be an attractive effect of the anal odours to ticks (Marufu *et al.,* 2010; Muchenje *et al.,* 2008). The external genitals and inguinal/groin regions of the body are highly supplied with blood (Tessema *et al.,* 2010). Body parts with softer or thinner skin and short hair are preferred areas of attachment by ticks, as they allow easy penetration of mouth parts into the rich vascular areas for feeding (Muchenje *et al*., 2008; Sajid, 2007).

Environmental factors such as location, month, and season influence tick infestation. Tick prevalence was observed to be generally high and a major challenge in these four locations.

Several studies showed the same challenge in the Eastern Cape province of South Africa (Katiyatiya *et al.,* 2014; Nyangiwe *et al*., 2011; Marufu *et al*., 2011b; Muchenje *et al*., 2008) while Scholtz *et al*. (1991) raised the same concern in the Loskop area. The high tick loads in the hot dry and hot wet seasons result in the use of acaricides during these periods to prevent major cattle production losses. There is, however, a need to develop sustainable and more cost-effective strategies for tick control that can counter the effects of global warming or tick burden in livestock production. An integrated approach, incorporating genetic improvement through methods such as traditional or marker-assisted selection, is a plausible option. It is, therefore, necessary to determine the extent to which the large variation in tick count observed in the current study is under genetic control.

3.5 Conclusions

All four different experimental farms had the same tick species prevalence, excluding *R. simus.* Tick load in Nguni cattle varies according to these agro-ecological zones, with warmer locations tending to have higher tick loads and *A. hebraeum* being the most prevalent and widely distributed species. Year, season and month of counting and age of the animal also influenced tick loads. Tick count was significantly higher in the hot dry and hot wet compared to cool wet and cool dry seasons. There were within breed variations of tick counts and adaptation of animals to different agro-climatic conditions.

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Chapter 4

Genetic parameter estimates for seasonal tick counts from different body parts in South African Nguni cattle

Abstract

Resistance of cattle to ticks is important because it affects their productivity and welfare in tick-endemic regions of the world. Genetic selection offers an opportunity to improve such resistance. The objective of the study was to estimate genetic parameters for tick resistance in South African Nguni cattle, to assess the scope of improvement through selection. Tick count data from 586 Nguni cattle, from four herds located in different provinces of South Africa, were analysed to determine genetic variation of resistance to six different tick species under natural infestation. Data were divided into four data sets according to month of tick count. Genetic parameters for log-transformed counts were estimated from bivariate sire models using the ASREML program. All models included the fixed effects of location, month, sex, year, and age as well as location by month interaction effects. Heritability estimates for tick counts varied with season and trait (body part or tick species) and ranged from 0.01±0.01 to 0.26±0.01. Genetic correlations ranged from -0.79±0.33 to 1.00±0.00 among counts for different body parts and 0.00±0.00 to 0.99±0.00 among tick species. Phenotypic correlations were low to moderate ranging from 0.06±0.01 to 0.72±0.01 among body parts and 0.01±0.02 to 0.44±0.01 for tick species. Tick counts that were recorded from September to January showed the greatest genetic variation. The large genetic correlations between whole body count and most of the body location counts suggest that it may not be necessary to conduct whole body counts. Counts of ticks from the belly and perineum body location appear to be the most suitable surrogate traits for whole body count, due to the remarkably high genetic correlations. These findings provide useful information for developing strategies for genetic improvement of tick resistance through selection.

Keywords: tick count, data set, Nguni cattle, heritability estimates, genetic and phenotypic correlations

4.1 Introduction

Different tick species contribute to tick-borne diseases. Economic losses in livestock production due to ticks and tick-borne diseases have long been a major concern to livestock producers in tropical and sub-tropical regions (Seifert, 1984). A large portion of the cost associated with ticks

is in implementation of control measures, mainly chemical acaricides (Porto-Neto *et al* 2011), to reduce tick loads (de Castro, 1997). Increasing prices of acaricides and resistance of tick to these acaricides are increasing problems and real economic burdens to the livestock producers (Mukhebi *et al*., 1992; Rajput *et al*., 2006). Hence, there is a pressing need for alternative ways to reduce tick infestations in livestock. One possibility is the identification and use of cattle that are naturally resistant to ticks (Hayward, 1981). Resistance to tick infestation varies among individuals and breed of cattle (Utech & Wharton, 1982). However, imported breeds may need to adapt to additional environmental challenges, such as susceptibility to disease, during their lives (Prayaga *et al*., 2009).

Host resistance refers to an animal's ability to prevent maturation of large numbers of ticks and having immunity to tick-borne diseases (Roberts, 1968). Such resistance may be measured by counting or scoring the numbers of ticks on the animal following either artificial or natural infestation (Porto-Neto *et al*., 2011). Use of artificial infestation with known numbers of tick larvae, followed by counting of engorging adult females, has been suggested as the most appropriate method to measure tick resistance (Regitano *et al*., 2006). Host resistance to ticks is under genetic control (Hewetson, 1972) and genetic variation in tick resistance varies within and among breeds (Seifert, 1971; Utech *et al*., 1978; Mapholi *et al.,* 2013). Zebu cattle (*Bos indicus*) in Brazil and Australia have greater tick resistance when compared to European cattle (*Bos taurus*) (Utech & Wharton, 1982; Madalena *et al.,* 1990; Frisch & O'Neill, 1998; Mwangi *et al*., 1998; Wambura *et al*., 1998; da Silva *et al*., 2007). Indigenous breeds in South Africa are also more resistant to ticks than European cattle (Spicket *et al*., 1989; Scholtz *et al*., 1991; Latif, 2006).

Resistance of cattle to ticks is highly heritable and responsive to selection (Turner *et al.*, 2010; Burrow, 2001). Heritability estimates for resistance to ticks range from 0.05 to 0.42 (Warthon *et al.,* 1970; Burrow, 2001; Prayaga & Henshall, 2005; Peixoto *et al.,* 2008; Prayaga *et al*., 2009; Budeli *et al*., 2009; Porto-Neto *et al.,* 2014; Ayres *et al*., 2015). There could be a number of reasons for the wide variability in heritability estimates. Low heritability estimates obtained from some of these studies might have been due to different sampling methods or low natural tick infestation challenge in the field. Use of a scoring system for infestation rather than tick counts may also affect heritability estimate due to the subjectivity of this method and difficulty in consistent application across studies (Prayaga & Henshall, 2005; Prayaga *et al*., 2009). Environmental factors that affect the intensity of natural infestations, breed of cattle and immune status of the animal should be accounted for when estimating genetic parameters (Porto-Neto *et*

al., 2011). Season also plays an important role in the prevalence of ticks and could, therefore, influence heritability estimate (Wharton *et al*., 1970).

Reliable estimates of genetic parameters are a prerequisite for using selection to genetically improve any trait. Thus, the primary objective of the current study was to estimate genetic parameters for tick counts in South African Nguni cattle. A secondary objective was to determine the most appropriate season for counting ticks to facilitate selection for resistance to them.

4.2 Materials and methods

4.2.1 Data collection

Counts of adult ticks from six species, including *Amblyomma hebraeum, Rhipicephalus evertsi evertsi, Rhipicephalus (Boophilus) spp., Rhipicephalus appendiculatus* and *Hyalomma marginatum* were used. The data collection methods were described in section 3.3.1 to 3.3.3. A total of 63 traits, comprising a combination of tick species and animal body part, were defined; however only 11 of these were analysed for the study (Table 4.1).

Table 4.1 Definition and abbreviation of traits

Trait	**Definition**
ALLTBC	Whole body tick count
Headtot	Total tick count on the head
Bellytot	Total tick count on the belly
Perineum	Total tick count on the perineum
Tailtot	Total tick count on the tail
TBCAmbly	Whole body count of *A. hebraeum*
TBCReve	Whole body count of *R. evertsi evertsi*
TBCRapp	Whole body count of *R. appendiculatus*
TBCBoo	Whole body count of *R. boophilus*
TBCHylom	Whole body count of *H. marginatum*
TBCRsim	Whole body count of *R. simus*

4.2.2 Data sets

To assess genetic variation of tick infestations in different seasons, the data were divided into four data sets as shown in Table 4.2. This classification was based on the levels of tick infestation in different months as shown in Figure 4.1.

Table 4.2 Description of the tick count data sets

Data set	Tick count	Season
(a)	Full tick count data	All seasons
(b)	Full tick count data excluding June to August	Data excluding winter
(c)	Data with only September to January tick counts	Hot dry to hot wet
(d)	Data with only November to January tick counts	Hot wet

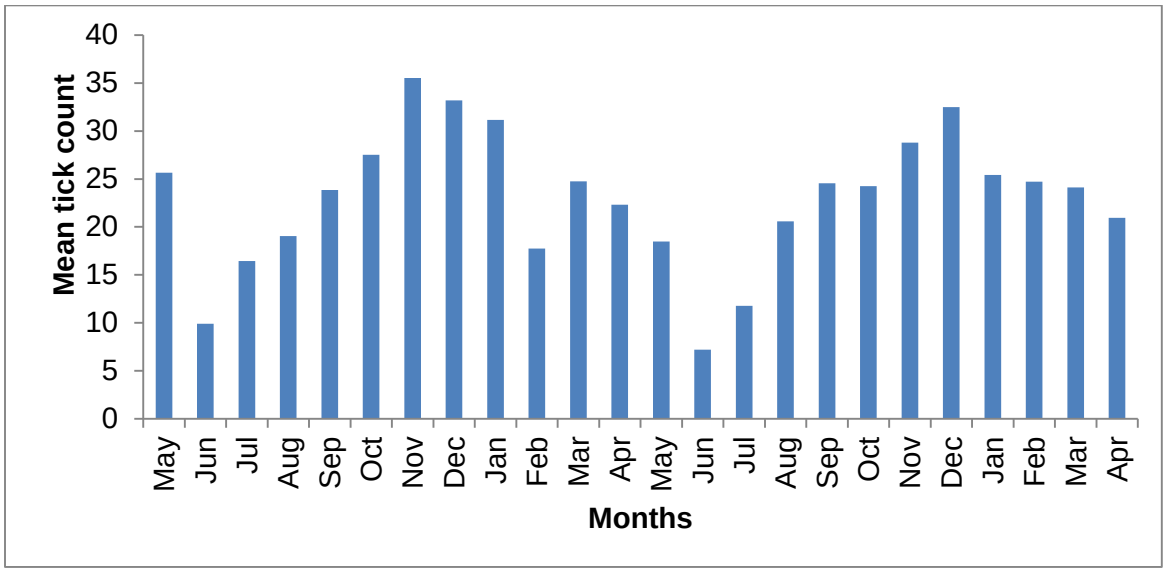


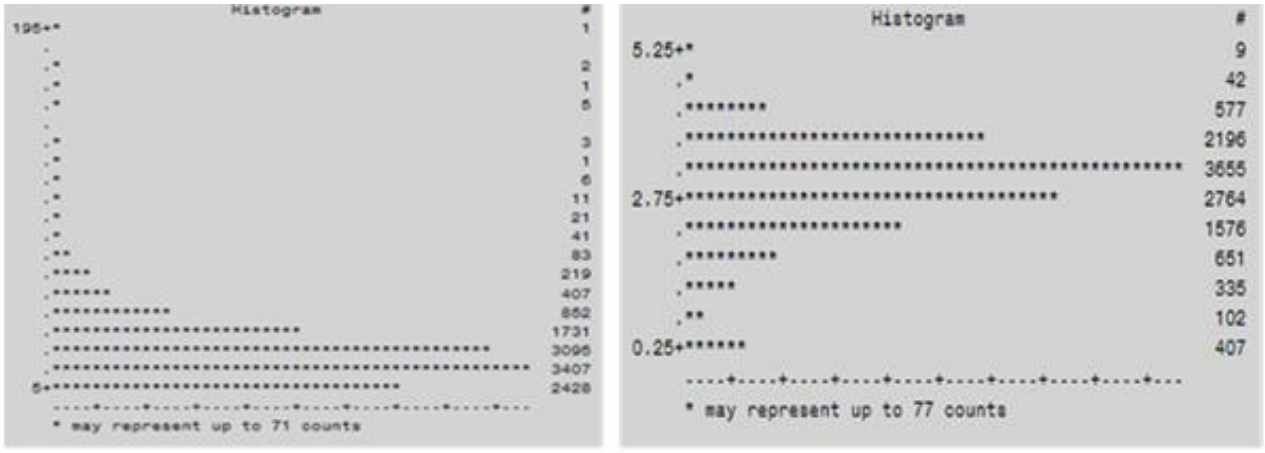
Figure 4.1 Distribution of whole body tick count (ALLTBC) over two years

4.2.3 Pedigree file

The pedigree file in this study comprised of 806 South African Nguni animals with three known generations.

4.2.4 Statistical analyses

Disccriptive statistics were obtained using SAS Version 9.3 (SAS, 2002-2010). Tick counts were transformed through base 10 logarithim to ensure normality. Examples of frequency distributions of the data before and after transformation are given in Figure 4.2.



Untransformed data Transformed (log10 (x + 1)) data

Figure 4.2 Histogram of untransformed and transformed (log_{10} (x + 1)) total body tick counts (ALLTBC)

Variance components and genetic parameters for log transformed tick counts were estimated by bivariate sire models using the ASREML software (Gilmour *et al.,* 2009). The sire model was preferred due to the fact that the pedigree data was incomplete but with enough sires. The general model equation used was as follows: **y** = **Xb** + **Zs** + **e**, where **y** is a vector of observations (log transformed tick count), **b** is a vector of fixed effects of location, sex, age of animal and first order interaction of location by month, **s** is a vector of random additive sire genetic effects, **e** is a vector of random residuals effects and **X** and **Z** are incidence matrices relating the fixed and random effects respectively to **y**.

4.3 Results

Descriptive statistics, for each data set, for actual and log transformed tick counts are presented in Table 4.3. The mean for whole body tick count ranged from 23.13 to 31.04. The highest mean count (31.04) was observed for Data set D (November to January), followed by data set C (September to January) with a mean count of 28.63. In addition, Data set D also showed highest tick count variation as indicated by the standard deviation, while the full data set (Data set A) had the lowest variation.

Table 4.3 Summary statistics for actual tick count and log transformed counts (in brackets)

Traits	No. of animals	Mean	SD	CV (%)	Min	Max
Data set (a)	586	23.13 (2.90)	16.46 (0.88)	71.17 (30.21)	0	198 (5.29)
Data set (b)	586	26.08 (3.05)	16.99 (0.83)	65.15 (27.15)	0	198 (5.29)
Data set (c)	586	28.63 (3.13)	18.59 (0.87)	64.94 (27.75)	0	198 (5.29)
Data set (d)	586	31.04 (3.19)	20.78 (0.91)	66.96 (28.52)	0	198 (5.29)

Abbreviation: SD = standard deviation; Min = minimum tick count; Max = maximum tick count.

4.3.1 Variance components estimates

Estimates of variance components and heritability for all four data sets are presented in Tables 4.4 and 4.5. Heritability estimates ranged from 0.01±0.00 for TBCRapp ticks using data set A to 0.26±0.01 for total tick count in the perineum for data set C. Bellytot and perineum generally had the highest heritability estimates, across data sets. Heritability estimates for tick species, TBCAmbly, were the highest across all data sets compared to other five tick species.

Table 4.4 Heritability estimates for tick count data set A and B

Full data (a)				
Trait	**σ^2_s**	**σ^2_e**	**σ^2_P ±se**	**h^2 ±se**
ALLTBC	0.07±0.02	0.55±0.01	0.57±0.01	0.12±0.03
Headtot	0.01±0.01	0.33±0.01	0.34±0.01	0.03±0.01
Bellytot	0.12±0.03	0.67±0.01	0.73±0.01	0.17±0.04
Perineum	0.07±0.02	0.67±0.01	0.69±0.01	0.11±0.03
Tailtot	0.04±0.02	0.68±0.01	0.69±0.01	0.06±0.02
TBCAmbly	0.08±0.02	0.63±0.01	0.65±0.01	0.12±0.03
TBCReve	0.02±0.01	0.65±0.01	0.68±0.01	0.03±0.01
TBCRapp	0.03±0.01	0.55±0.01	0.55±0.01	0.01±0.01
TBCBoo	0.04±0.01	0.63±0.01	0.64±0.01	0.07±0.02
TBCHylom	0.01±0.01	0.41±0.01	0.41±0.01	0.02±0.01
TBCRsim	0.08±0.01	0.08±0.01	0.10±0.01	0.02±0.01

(b) Full data set excluding June-August

ALLTBC	0.05±0.02	0.58±0.01	0.60±0.01	0.09±0.03
Headtot	0.01±0.01	0.37±0.01	0.37±0.01	0.03±0.02
Bellytot	0.13±0.04	0.72±0.01	0.75±0.01	0.17±0.05
Perineum	0.10±0.03	0.74±0.01	0.77±0.01	0.13±0.04
Tailtot	0.04±0.02	0.71±0.01	0.72±0.01	0.06±0.02
TBCAmbly	0.07±0.01	0.66±0.01	0.68±0.01	0.11±0.04
TBCReve	0.02±0.01	0.70±0.01	0.70±0.01	0.03±0.01
TBCRapp	0.01±0.01	0.59±0.01	0.59±0.01	0.02±0.01
TBCBoo	0.04±0.01	0.68±0.01	0.69±0.01	0.06±0.01
TBCHylom	0.01±0.01	0.47±0.01	0.48±0.01	0.03±0.02
TBCRsim	0.07±0.01	0.08±0.01	0.10±0.01	0.02±0.00

Abbreviations: σ^2_s = sire genetic variance; σ^2_e= residual variance; σ^2_P = phenotypic variance; h^2 = heritability; s.e. = standard error

There was an apparent increase in the heritability of tick count with increase in tick infestation levels, based on seasonal mean counts. The September to January data set (data set C), which had the highest tick infestations, generally had the highest heritability estimates for both body locations and tick species. Phenotypic variance varied among the traits and TBCRsim had the lowest variance for tick species.

Table 4.5 Heritability estimates for data sets C and D

Data set C				
Trait	**σ^2_s**	**σ^2_e**	**σ^2_P ±se**	**h^2 ±se**
ALLTBC	0.07±0.01	0.47±0.01	0.49±0.01	0.14±0.01
Headtot	0.02±0.01	0.41±0.01	0.41±0.01	0.05±0.03
Bellytot	0.12±0.04	0.68±0.01	0.71±0.02	0.18±0.04
Perineum	0.16±0.01	0.58±0.01	0.62±0.01	0.26±0.01
Tailtot	0.08±0.03	0.75±0.01	0.77±0.02	0.11±0.04
TBCAmbly	0.10±0.03	0.67±0.01	0.69±0.02	0.14±0.05
TBCReve	0.08±0.03	0.74±0.01	0.76±0.02	0.10±0.04
TBCRapp	0.01±0.01	0.60±0.01	0.60±0.01	0.04±0.01
TBCBoo	0.07±0.03	0.65±0.01	0.67±0.01	0.11±0.04
TBCHylom	0.02±0.01	0.50±0.01	0.51±0.01	0.04±0.01
TBCRsim	0.15±0.03	0.07±0.01	0.11±0.01	0.02±0.01

Data set D

ALLTBC	0.04±0.03	0.69±0.02	0.70±0.02	0.05±0.02
Headtot	0.02±0.02	0.45±0.01	0.45±0.01	0.05±0.01
Bellytot	0.10±0.04	0.70±0.02	0.73±0.02	0.13±0.06
Perineum	0.17±0.06	0.85±0.02	0.89±0.03	0.19±0.07
Tailtot	0.03±0.03	0.80±0.02	0.81±0.02	0.04±0.01
TBCAmbly	0.07±0.04	0.72±0.02	0.73±0.02	0.09±0.05
TBCReve	0.04±0.03	0.82±0.02	0.83±0.02	0.05±0.01
TBCRapp	0.01±0.01	0.69±0.02	0.69±0.02	0.03±0.01
TBCBoo	0.06±0.04	0.76±0.02	0.78±0.02	0.07±0.04
TBCHylom	0.04±0.02	0.53±0.01	0.54±0.01	0.03±0.01
TBCRsim	0.26±0.05	0.07±0.01	0.13±0.01	0.02±0.01

Abbreviations: σ^2_s = sire genetic variance; σ^2_e= residual variance; σ^2_P = phenotypic variance; h^2 = heritability; s.e. = standard error

4.3.2 Genetic and phenotypic correlations

Estimates of genetic and phenotypic correlations among traits are presented in Tables 4.6 and 4.7 for body location and tick species, respectively. Data set C generally had higher genetic and phenotypic correlations compared to the other data sets. Most of the genetic correlations were not estimable for data set D, due to the small number of records.

Whole body count (ALLTBC) had high genetic correlations (≥0.80) with all body location traits, except Headtot. All body location traits, with the exception of Headtot, were highly correlated with each other genetically. Bellytot and perineum had an exceptionally high (close to 1) genetic correlations with whole body count and between each other.

Phenotypic correlations were generally less than genetic correlations and varied widely, ranging from 0.06±0.01to 0.72±0.01 for body location traits. The trend was however similar, with ALLTBC being highly correlated phenotypically with all body location traits except Headtot. Estimates were also highest between Bellyot and Perineum and generally lowest for Headtot and the other body location traits.

Table 4.6 Genetic (above the diagonal) and phenotypic (below the diagonal) correlations among body location traits

	ALLTBC	Bellytot	Perineum	Tailtot	Headtot
Data set (a)					
ALLTBC		0.97±0.03	0.98±0.00	0.80+0.10	0.26±0.27
Bellytot	0.55±0.01		0.95±0.05	0.66±0.15	0.36±0.25
Perineum	0.59±0.01	0.28±0.01		0.74±0.14	0.11±0.30
Tailtot	0.68±0.01	0.17±0.01	0.29±0.01		0.17±0.33
Headtot	0.30±0.01	0.06±0.01	0.07±0.01	0.18±0.01	
Data set B					
ALLTBC		0.95±0.05	0.98±0.00	0.80±0.12	0.08±0.12
Bellytot	0.55±0.01		0.93±0.6	0.64±0.18	0.23±0.27
Perineum	0.63±0.01	0.30±0.01		0.80±0.13	0.10±0.29
Tailtot	0.72±0.01	0.22±0.01	0.32±0.01		0.08±0.34
Headtot	0.31±0.01	0.08±0.01	0.09±0.01	0.21±0.01	
Data set C					
ALLTBC		0.98±0.02	1.00±0.00	0.83±0.12	0.07±0.39
Bellytot	0.59±0.01		0.98±0.02	0.71±0.16	0.20±0.32
Perineum	0.61±0.01	0.33±0.01		0.86±0.11	0.05±0.32
Tailtot	0.74±0.01	0.29±0.01	0.36±0.01		-0.12±0.36
Headtot	0.32±0.01	0.09±0.01	0.10±0.01	0.23±0.01	
Data set D					
ALLTBC		-	-	0.92±0.08	-
Bellytot	0.59±0.01		-	-	-0.41±0.45
Perineum	0.66±0.01	0.33±0.02		-	-0.79±0.33
Tailtot	0.72±0.01	0.30±0.02	0.32±0.02		-
Headtot	0.32±0.02	0.11±0.02	0.08±0.02	0.22±0.02	

Note that (-) represent inestimable genetic correlations

4.3.3 Tick species correlations

Table 4.7 Genetic (above the diagonal) and phenotypic (below the diagonal) correlations among tick species traits for different data sets

	TBCAmbly	TBCReve	TBCBoo	TBCRapp	TBCHylom	TBCRsimus
Data set A						
TBCAmbly		0.80±0.15	0.50±0.18	-	0.63±0.23	0.26±0.17
TBCReve	0.30± 0.01		0.99±0.00	-	0.86±0.01	0.74±0.17
TBCBoo	0.17±0.01	0.17±0.01		-	0.97±0.02	0.74±0.12
TBCRapp	0.20±0.01	0.08±0.01	0.06±0.01		-	-
TBCHylom	0.15±0.01	0.15±0.01	0.16±0.01	0.12 ±0.08		0.52±0.27
TBCRsimus	0.04±0.01	0.07±0.01	0.05±0.01	0.01 ±0.01	0.01±0.01	
Data set B						
TBCAmbly		0.82±0.00	0.62±0.18	-	0.72±0.20	0.36±0.17
TBCReve	0.31±0.01		0.92±0.01	-	0.89±0.01	0.69±0.18
TBCBoo	0.21±0.01	0.19±0.01		-	0.95±0.0.01	0.68±0.15
TBCRapp	0.21±0.01	0.12±0.01	0.05±0.01		-	0.17±0.89
TBCHylom	0.18±0.01	0.14±0.01	0.15±0.01	0.13±0.01		0.57±0.25
TBCRsimus	0.07±0.01	0.09±0.01	0.05±0.01	0.02±0.01	0.02±0.01	
Data set C						
TBCAmbly		0.95±0.05	0.55±0.20	-	0.54±0.28	0.65±0.14
TBCReve	0.44±0.01		0.78±0.18	-	0.87±0.01	0.84±0.10
TBCBoo	0.26±0.01	0.25±0.01		-	0.86±0.01	0.71±0.13
TBCRapp	0.20±0.01	0.14±0.01	0.01±0.01		0.00±0.00	-
TBCHylom	0.19±0.01	0.15±0.01	0.20±0.01	0.15±0.01		0.40±0.30
TBCRsimus	0.07±0.02	0.10±0.02	0.08±0.02	0.02±0.01	0.03±0.02	
Data set D						
TBCAmbly		0.89±0.01	0.55±0.31	-	0.48±0.34	0.84±0.13
TBCReve	0.41±0.01		-	-	-	-
TBCBoo	0.27±0.02	0.27±0.02		-	0.00±0.00	0.89±0.11
TBCRapp	0.23±0.02	0.15±0.02	0.01±0.02		-	-
TBCHylom	0.18±02	0.14±0.02	0.21±0.02	0.18±0.01		0.60±0.24
TBCRsimus	0.13±0.03	0.16±0.03	0.15±0.03	0.05±0.02	0.06±0.03	

Note that (-) represent inestimable genetic correlations

Genetic correlations were generally high among most tick species, with the highest estimate (0.99) being obtained between *R. evertsi evertsi* and *R. boophilus* for data set A. Estimates were consistently high (≥0.80) between *A. hebraeum* and *R. evertsi evertsi,* and the highest (0.95) was obtained for data set C (September to December). Genetic correlations of *R. appendiculatus* to other tick species were inestimable in all four data sets, due to the small number of records of this species.

Phenotypic correlations among tick species were mostly lower than the corresponding genetic correlations; however they followed more or less the same trend.

4.4 Discussion

Recorded tick counts in the current study ranged from 0 to 198 ticks per animal indicating that tick infestations encountered by the animals were sufficient to allow genetic expression of each individual immunity or susceptibility. Mean tick counts were lower than that of 37.58 reported by Budeli *et al.* (2009) for in South African Bonsmara cattle. Corbet *et al.* (2006) also obtained a mean of 37 ticks in Bonsmara and Belmont Red cattle breeds in South Africa. These results are consistent with the fact that the Nguni is known to be more resistant to ticks than the Bonsmara and Hereford cattle (Scholtz *et al.,* 1991). Turner & Short (1972) compared tick infestation among different cattle breeds in Australia and observed higher mean tick counts from 20 to 30 per side for Afrikaner and Brahman cattle on natural infestation, while Shorthorn cattle carried between 75 and 100 ticks per side. On the other hand, Ayres *et al.* (2015) found a lower mean tick count of 11.6 in Nellore and Nellore x Herford crosses per side from natural tick infestation. These results suggest breed variation in resistance to ticks and also indicate high resistance for the Nguni and Nellore x Hereford crosses. This is to be expected, as Nguni and Nelore are indigenous tropical breeds and are therefore adapted to tick infestations.

Estimates of heritability were low to moderate and comparable to those from previous studies (Prayaga & Henshall, 2005; Peixoto *et al.,* 2008; Budeli *et al*., 2009; Prayaga *et al.,* 2009; Porto-Neto *et al.,* 2014; Cardoso *et al.,* 2015). They were, however, less than estimates in the range of 0.37 to 0.42 reported by other researchers (Wharton *et al*., 1970; Burrow, 2001; Turner *et al.,* 2010; Porto-Neto *et al.,* 2014). Disparity of estimates among studies may be partly attributed to the fact that tick counts were conducted in different seasons and tick count methods were not consistent. Higher levels of tick infestation, which normally occur in the hot seasons, appear to elicit more genetic variation in tick resistance. For example, Wharton *et al*. (1970) observed the

increase in heritability estimates for tick burden in summer and a low to zero estimates in winter season. Budeli *et al.* (2009) also reported moderate heritability estimates when the mean tick count was ≥25 and suggested that tick count data should be collected when the level of tick infestations is high. This is consistent with the relatively high estimates obtained for the hot and high infestation season (September to January) in the current study. Other studies in South Africa (Scholtz *et al*. 1991; Marufu *et al.* 2011) also reported higher infestations in the hot and dry seasons and recommended that genetic parameters for tick resistance should be estimated during this time of the year.

Seasonal changes might influence the sensitivity of ticks to some mechanisms of host resistance. Some researchers have emphasized that tick count data should be collected when animals have had sufficient exposure to ticks (higher tick infestation), in order to ensure that resistance has been acquired (Hewetson, 1968; Henshall, 2004; Latif, 2006). It therefore appears compelling to strategically collect tick count data for genetic evaluation in the hot season (September to January) in South Africa. Besides capitalising on the relatively high genetic variation in tick resistance realised during that time of the year, it also minimises the costs of data collection.

The high genetic correlations between whole body count and most of the body location counts suggest that it may not be necessary to conduct whole body counts. Bellytot and perineum appear to be the most suitable surrogate traits for whole body count, due to the remarkably high genetic correlations. The equally high correlation between Bellytot and perineum implies that either of these two traits can be used as reliable indicators of whole body tick counts. This might be due to the fact that both two traits have softer skin with short hair. Thus, tick count data can be collected more simply and cheaply by utilising either one of these body location traits. Moreover, perineum body location may be the most suitable indicator for tick count to avoid difficulty of tick count compare to the belly body location.The generally high genetic correlations among tick species, across the data sets, suggest that a uniform data collection approach can be used for all tick species.

The relatively high correlations observed for data set C reinforces the rationale for recommending collection of tick count data in the hot season.

4.5 Conclusion

There is sufficient genetic variation to warrant improvement in tick resistance through selection, thereby complementing other tick control methods. Such genetic variation appears to be expressed more during seasons with higher levels of tick infestation. It is, therefore, recommended that collection of tick count data for genetic selection be conducted during these seasons. Tick counts from either the perineum or belly may be used as reliable indicators of whole body count.

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Chapter 5

Genome-wide association study of tick resistance in South African Nguni cattle

Abstract

Ticks and tick-borne diseases are among the main causes of economic loss in the South African cattle industry through high morbidity and mortality in herds. Current control strategies depend on methods such as use of insecticides, vaccination against tick-borne diseases and grazing management. However, concerns for the presence of chemical residues in foods of animal origin may tarnish the perception of these practises from the perspectives of food safety and environmental health. The primary objective of this study was to identify SNP markers associated with host resistance to ticks in South African Nguni cattle. Tick count data were collected monthly from different body locations of 586 Nguni cattle reared in four herds under natural grazing conditions over a period of two years. Twelve traits, representing tick counts from the different animal body locations and the total number of identified tick species were analysed. Data were explored for normality and where appropriate, transformed using $\log_{10}(x + 1)$. DNA was extracted from hair and blood samples and genotyped using the Illumina BovineSNP50 assay. After quality control (call rate >90%, minor allele frequency > 0.02), 40 436 SNPs were retained for analysis. Genetic parameters were estimated and association analysis for tick resistance was carried out using two approaches: a genome-wide association (GWA) analysis using the GenABEL package and a regional heritability mapping (RHM) analysis. The Bonferroni genome-wide ($P<0.05$) corrected threshold was 1.24×10^{-6}, with 2.47×10^{-5} as the suggestive level (i.e., one false positive per genome scan) in the GWA analysis, whereas likelihood ratio test (LRT) thresholds for genome-wide and suggestive significance were 13.5 and 9.15 for the RHM analysis. Six ixodid tick species were identified, with *A. hebraeum* being the dominant species. Heritability estimates from both the animal and sire model ranged from 0.02±0.00 to 0.17±0.04 for the transformed tick count data. Several genomic regions of interest were identified for different traits by both the GWA and RHM approaches. Three genome-wide regions on chromosomes 7, 10 and 19 were identified for total tick count on the head, total *A. hebraeum* ticks and for total number of *A. hebraeum* in the perineum region. Suggestive regions were identified on chromosomes 1, 3, 6, 7, 8, 10, 11, 12, 14, 15, 17, 19 and 26 for several of the traits that were analysed. The GWA approach identified more genomic regions than the RHM approach. The chromosomal regions identified here as harbouring QTL underlying variation in tick burden form the basis for further analyses to identify

specific candidate genes related to cattle tick resistance and provide the potential for marker-assisted selection in Nguni cattle.

Key words: SNP markers, indigenous cattle, genomic analyses, tick count

5.1 Introduction

Ticks are important because they have adverse effects on livestock production. They parasitize a wide range of vertebrate hosts and transmit a wide variety of pathogenic agents; more so than any other group of arthropods (Tiki & Addis, 2011). Ticks transmit protozoan, bacterial, rickettsia and viral diseases and decrease livestock productivity (Dold & Cocks, 2001; Jongejan & Uilenberg, 2004; Kaufman *et al*., 2006; Anne & Conboy, 2006; Jonsson, 2006; Tadesse & Sultan, 2014). Control of ticks is largely based on the use of acaricides; however, this use increases the cost of production and also results in chemical residues in meat, milk, hides, and the environment (Machado *et al*., 2010; Regitano *et al*., 2010). These problems create rising economic and social demands for different approaches that alleviate the effects of ticks on cattle production and thereby enhance the cattle industries' contribution to the world economy (Porto-Neto *et al*., 2011; Mapholi *et al.,* 2014).

Cattle vary greatly in their tick loads, and much of this variation is known to be controlled by the genetic composition of the host. Studies have shown that some cattle breeds carry fewer ticks than do others under the same environmental conditions (Wharton *et al.*, 1970; Seifert, 1971; Utech & Wharton 1982; Latif *et al*., 1991; Burrow, 2001; Budeli *et al*., 2009; Marufu *et al*., 2011) and such variation in natural resistance is caused by the animals' ability to respond immunologically to tick infestation. Nguni cattle have been shown to have a higher potential to develop tick resistance than the Bonsmara and Hereford cattle in South African environment (Spickett *et al.,* 1989; Scholtz *et al*., 1991). Tick control by using selected tick resistant animals has been demonstrated in various cattle breeds (Riek, 1962; Wilkinson, 1962; Ayres *et al*., 2013; Rodriguez-Valle *et al*., 2013). Therefore, the use of naturally tick resistant cattle biotypes may be incorporated into tick control strategies as a means of biological control of tick infestations (Tatchell, 1992). Studying the mechanisms of resistance to ticks among different cattle breeds may therefore contribute to the development of alternative control methods (Gasparin *et al*., 2007).

Host resistance to ticks is known to be under genetic control. However, historically, the genetic control of tick resistance has been studied without identifying the genes or gene variants that underlie the observed phenotypic variation, but has focused on selection using estimated breeding values calculated from phenotypic and pedigree information (Goddard & Hayes, 2009). Interrelationships between genes influencing both tick resistance and animal productivity are not yet well understood. The first identified bio-marker associated with tick burden was serum amylase, which is encoded by a locus on chromosome (BTA) 3 (Ashton *et al*., 1968). This finding was followed by the detection of associations with the Bovine Lymphocyte Antigen (BoLA) markers using the microlymphocytotoxicity test on animals from different composite breeds exposed to natural and artificial tick infestations (Stear *et al*., 1984; 1989; 1990), however, these tests were found to produce inconsistent results in several studies, because the same BoLA allele was not consistently associated with increased tick resistance (Porto-Neto *et al*., 2011). Using the candidate gene approach, the BoLA microsatellite II polymorphisms were associated with tick load in cattle (Acosta-Rodriguez *et al*., 2005; Martinez *et al.*, 2006; Untalan *et al*., 2007).

Recent approaches combining molecular biology and quantitative genetics have enabled an improved understanding of genetic mechanisms underlying tick resistance. Linkage analysis has been used to identify several quantitative trait loci (QTL) associated with tick resistance on BTA 2, 10 and 23 during the dry season and BTA 5, 11, 23 and 27 during the wet season, in an F_2 Holstein × Gir population from Brazil, using 180 microsatellite markers (Gasparin *et al*., 2007; Regitano *et al*., 2008; Machado *et al*., 2010). However, these QTL explained only 13% and 18% of the phenotypic variance during the dry and wet seasons, respectively. The QTL identified on BTA 23 that influenced tick burden in both seasons was located in a genomic region containing the BoLA gene complex which had previously been associated with tick burden (Machado *et al*., 2010).

Recently, single nucleotide polymorphisms (SNPs) assayed using high-density SNP arrays have been used to identify genetic variants associated with complex traits. SNPs distributed throughout the genome can be used to detect and map the mutations underlying variation in target traits by a process called genome-wide association (GWA) analysis. This approach tests each marker independently for an association with the trait of interest while controlling for population stratification caused by pedigree or breed composition differences among animals. The expectation is that the variance explained by each marker is related to the size of the effect of the underlying causal polymorphism on the trait, the extent of the association between the marker

and the polymorphism, and the experimental error associated with trait measurement. This approach requires a large number of markers genotyped in a large sample of individuals to enable the analysis to possess a significant power to detect associations. Few GWA studies of tick resistance in cattle have been reported in the literature. In Australia, several QTLs associated with tick burden were thus identified in Brahman beef cattle and dairy cattle (Barendse, 2007; Porto-Neto *et al*., 2010; Turner *et al*., 2010). From these identified regions (BTA 1, 2, 3, 10, 11, 13, 14, 19 and 26), the greatest overlap among regions associated with tick resistance was found on BTA 2, 10, 13 and 19. The regions on BTA 3, 10 and 11 were further investigated by Porto-Neto *et al*. (2011) to identify candidate genes associated with tick burden. Recently, Sollero *et al*. (2014) identified SNPs associated with tick resistance on BTA 5, 11 and 15 in half-sib populations of Brazilian Hereford and Braford cattle using the Illumina BovineSNP50 (50K) SNP assay and a GWA analysis.

Another approach that can be used to identify genomic regions of interest using genomic data is regional genomic relationship mapping or regional heritability mapping (RHM) (Nagamine *et al*., 2012; Riggio *et al.,* 2013). RHM is a variance component based approach for mapping genomic regions influencing complex traits, which combines information across contiguous SNPs. This method has been advanced as being more powerful than single SNP analysis for capturing the underlying genetic effects influencing disease traits (Nagamine *et al*., 2012; Riggio *et al*., 2013). It provides heritability estimates that are attributable to small genomic regions and has the power to detect regions containing multiple alleles that may individually contribute to variance that are not detected by GWA studies.

There are currently no published studies that have identified SNP markers associated with tick resistance in African cattle. Due to the high levels of tick challenge in most African countries, there is a considerable need to explore these technologies, using breeds that are known to be tick resistant or tick tolerant. These approaches may provide tools that will allow selection towards host resistance. Therefore, the objective of this study was to identify SNP markers associated with tick resistance in Nguni cattle, genotyped with the 50K BovineSNP50 assay.

5.2 Materials and methods

5.2.1 Animals and tick count data collection

The tick count data was collected for adult ticks of six species including *Amblyomma hebraeum, Rhipicephalus evertsi evertsi, Rhipicephalus (Boophilus) spp., Rhipicephalus appendiculatus* and

Hyalomma marginatum were used and described in full in chapter 3.3.1 to 3.3.3 of chapter 3. A total of 63 tick-related traits were recorded, however only 12 traits are reported in this study (Table 5.1) based on the realised tick number distribution.

Table 5.1 Abbreviations and full identification for each of the analysed traits

Trait	**Trait full name**
ALLTBC	Whole body tick count
Headtot	Total tick count on the head
Bellytot	Total tick count on the belly
Perineum	Total tick count on the perineum
Tailtot	Total tick count on the tail
BAmbly	Total *A. hebraeum* ticks on the belly
PAmbly	Total *A. hebraeum* ticks on the perineum
TAmbly	Total *A. hebraeum* ticks on the tail
Pboo	Total *R. boophilus* ticks on the perineum
Treve	Total *R. evertsi evertsi* on the tail
TBCAmbly	Whole body tick count of *A. hebraeum* tick
TBCReve	Whole body tick count of *R*. *evertsi evertsi*

5.2.2 DNA sample collection

Blood and hair root samples were collected from all animals. Genomic DNA was isolated from both the blood and hair root samples by using proteinase-K digestion followed by phenol: chloroform: isoamyl alcohol extraction and ethanol precipitation (Sambrook *et al*. 1989). The isolated DNA was quantified by measuring the nucleic acid concentration using a Thermo Scientific NanoDrop 2000 spectrophotometer and the concentrations were verified using a Qubit® 2.0 Fluorometer. The extracted DNA samples were next normalised to 50 ng/µl. A minimum DNA concentration of 45 ng/µl was used with minimum optical density ratios of 2.0 (260/280) and 1.9 (260/230).

5.2.3 Genotyping

Of the 586 animals with tick count data only 500 (44 males and 456 females) were genotyped using the BovineSNP50 assay (Illumina Inc., San Diego, CA, USA) at the Agricultural Research Council Biotechnology Platform in South Africa (www.arc.agric.za). The bovine 50K SNP chips were scanned using an Illumina iScan and analysed using GenomeStudio software version

2011.1. Genotypes were inferred based on the forward (positive) strand output from GenomeStudio. The SNP genotype data were subjected to quality control (QC) measures to assure minor allele frequencies (MAF) >0.02 and SNP call rate >90%. Deviation from Hardy–Weinberg equilibrium was not considered in filtering the SNPs. Of the 54,606 SNPs on the bovine bead chip, 40,436 SNPs passed QC filtering. In addition, SNP markers on the X chromosome were included in the GenABEL analysis but were removed for the RHM analysis to avoid reducing the number of animals included in the analysis.

5.2.4 Statistical analysis

Descriptive statistics for tick count and the determination of fixed effects influencing tick conunt were analysed by the Statistical Analysis System (SAS, 2002-2010). Variance components and genetic parameters for tick count traits were estimated using ASREML software (Gilmour *et al.,* 2009). Animal and sire models were fitted with animal and sire considered random in the respective analyses. The fitted models accounted for the fixed effects of location, month, sex, year, and age as well as an interaction term for location by month.

Association analysis was conducted using two approaches: fitting single SNPs or SNP regions. The single SNP GWA analyses were conducted in the R environment using the GenABEL package (Aulchenko *et al.*, 2007). To overcome the limitations of fitting repeated records in GenABEL, animal (cow) average tick counts from the repeatability models obtained using ASReml with the previously described fixed effects were used as phenotypes. Association was tested using the mixed model function of mmscore (Chen & Abecasis, 2007) after correcting for relatedness. In this mixed model analysis, relationships among animals were accounted for by estimating the pair-wise identity by state genomic kinship matrix using autosomal SNPs. After Bonferroni correction, the genome-wide ($P<0.05$) and the suggestive (i.e., one false positive per genome scan) significance thresholds were $P<1.24 \times 10^{-6}$ and 2.47×10^{-5}, respectively. The p-values were corrected for the genomic inflation factor λ to account for population substructure.

The second approach used to analyse the genotype data was RHM, where the genome was divided into 804 windows, and each chromosome was divided into windows of a pre-defined number of SNPs and the variance attributable to each window was estimated. The window size was initially 100 contiguous adjacent SNPs, and the window was sequentially moved along the chromosome in steps of 50 SNPs. The genomic relationship matrix was estimated using all autosomal SNPs and the regional genomic additive effect was estimated using a regional

genomic relationship matrix constructed from consecutive adjacent SNPs from each region. Heritability in the RHM analysis was estimated using an animal model in ASREML. To test for regional differences in variances in RHM, a likelihood ratio test (LRT) was used to compare a model fitting variance in a specific window against the null hypothesis of no variance in that window. The test statistic was assumed to follow a mixture of $\frac{1}{2}\chi^2_{(1)}$ and $\frac{1}{2}\chi^2_{(0)}$ distributions (Self & Liang, 1987). After Bonferroni correction to account for multiple testing, the LRT thresholds for genome-wide and suggestive significance were 13.5 and 9.15, respectively.

5.3 Results

5.3.1 Descriptive statistics

The mean, standard deviation, minimum and maximum tick count of 12 analysed traits with multiple monthly counts over a period of two years are presented in Table 2.1. The monthly average of ALLTBC was 23, whereas the average TBCAmbly ticks were about 10 ticks per animal, higher than the other 10 traits. The largest count observed was 198. ALLTBC had greater variation compared to other traits.

Table 5.2 Summary statistics for tick counts traits analyzed

Traits	Mean	SD	Min	Max
Tick Traits based on location on the animal's body				
ALLTBC	23.13	16.46	0	198
Headtot	1.1	1.97	0	25
Bellytot	4.99	5.22	0	57
Perineum	5.04	5.6	0	71
Tailtot	8.33	7.23	0	60
Tick Traits based on tick species				
BAmbly	0.14	0.77	0	14
PAmbly	2.93	3.81	0	60
Tambly	2.83	3.62	0	30
Pboo	0.93	2.81	0	62
Treve	4.07	4.75	0	36
TBCReve	5.14	5.5	0	44
TBCAmbly	9.8	8.25	0	81

Abbreviation: SD= standard deviation; Min=minimum tick counts; Max= maximum tick counts; #the mean and standard deviation were calculated form back transformed tick count data.

5.3.2 Genetic parameters for tick count traits

The h^2 estimates for tick resistance traits obtained using animal and sire models applied to the transformed data are presented in Tables 5.3 and 5.4, respectively. The h^2 estimates from both models ranged from 0.02±0.00 to 0.17±0.04. Of the traits recorded the number of ticks on the belly (Bellytot) had the greatest heritability (0.17±0.04) based on the classical quantitative genetics models. The heritability estimates from the GWA analysis ranged from 0.00 to 0.17, while the regional heritability estimates h^2_w from the RHM analysis ranged from 0.05 to 0.12 using the transformed tick count data.

Table 5.3 ASREML estimates of heritability for the transformed tick count data using an animal model

Traits	σ^2_a	σ^2_e	σ^2_P ±se	h^2 ±se
Tick traits based on location on the animal's body				
ALLTBC	0.05±0.02	0.51±0.01	0.57±0.01	0.09±0.01
Headtot	0.01±0.01	0.32±0.01	0.34±0.00	0.04±0.01
Bellytot	0.09±0.01	0.64±0.01	0.72±0.01	0.12±0.01
Perineum	0.03±0.02	0.65±0.01	0.68±0.01	0.04±0.01
Tailtot	0.04±0.02	0.65±0.01	0.69±0.01	0.06±0.01
Tick traits based on tick species				
BeAmbly	0.08±0.02	0.56±0.01	0.64±0.01	0.12±0.01
PAmbly	0.02±0.01	0.59±0.01	0.61±0.01	0.04±0.02
Tambly	0.03±0.01	0.64±0.01	0.67±0.01	0.05±0.01
Pboo	0.01±0.01	0.32±0.01	0.32±0.01	0.02±0.00
Treve	0.03±0.01	0.61±0.01	0.65±0.01	0.05±0.01
TBCAmbly	0.05±0.02	0.59±0.01	0.65±0.01	0.08±0.01
TBCReve	0.03±0.01	0.65±0.01	0.68±0.01	0.05±0.01

Abbreviations: σ^2_a = Animal variance; σ^2_e= residual variance; σ^2_P = phenotypic variance; h^2 = heritability; s.e. = standard error;

Table 5.4 Heritability estimates for the transformed tick count data using a sire model

Traits	σ^2_s	σ^2_e	σ^2_P (s.e.)	h^2 (s.e.)
Tick traits based on location on the animal's body				
ALLTBC	0.02±0.01	0.55±0.01	0.57±0.01	0.12±0.03
Headtot	0.01±0.01	0.33±0.01	0.34±0.00	0.03±0.01
Bellytot	0.03±0.01	0.7±0.01	0.73±0.01	0.17±0.04
Perineum	0.02±0.01	0.67±0.01	0.68±0.01	0.10±0.03
Tailtot	0.01±0.01	0.68±0.01	0.69±0.01	0.06±0.02
Tick traits based on tick species				
BeAmbly	0.02±0.01	0.62±0.01	0.64±0.01	0.14±0.04
PAmbly	0.01±0.01	0.60±0.01	0.61±0.01	0.06±0.02
Tambly	0.01±0.01	0.67±0.01	0.67±0.01	0.02±0.01
Pboo	0.01±0.01	0.32±0.01	0.33±0.00	0.10±0.03
Treve	0.02±0.01	0.64±0.01	0.64±0.01	0.02±0.01
TBCAmbly	0.02±0.01	0.63±0.01	0.65±0.01	0.12±0.03
TBCReve	0.01±0.00	0.68±0.01	0.68±0.01	0.02±0.00

Abbreviations: σ^2_s = Sire variance; σ^2_e = residual variance; σ^2_P = phenotypic variance; h^2 = heritability; s.e. = standard error.

5.3.3 Genome-wide association analysis

5.3.3.1 Single SNP association results

The GWA analyses revealed two traits with SNPs that reached the genome-wide significance threshold, these were for head totals (Body location) and TBCAmbly (Tick species) (Table 5.5).

Table 5.5 Summary of tick resistance QTL segregating in Nguni cattle from genome-wide association analysis

Log_{10}(Trait+1)	**BTA**	**SNP name**	**Position (bp)**	**Pc1df**
Tick Traits based on location on the animal's body				
ALLTBC	10	*rs420979558*	28,070,182*	$5.12x10^{-6}$
	10	*rs43634842*	55,422,184**	$1.44x10^{-5}$
Headtot	7	*rs 29015334*	58,781,492	$1.05x10^{-6}$
	14	*rs 41665272*	25,912,898	$1.96x10^{-5}$
	18	*rs 110439061*	26,527,686	$4.53x10^{-5}$
	26	*rs42097850*	25,657,642	$5.85x10^{-5}$
	14	*rs41665024*	21,406,039	$6.13x10^{-5}$
Bellytot	14	*rs 41661020*	40,121,065	$2.13x10^{-5}$
	6	*rs110810914/ rs81167292*	87,281,196	$2.27x10^{-5}$
	1	*rs29015093*	96,382,717	$2.37x10^{-5}$
	11	*rs109474265*	6,243,988	$2.98x10^{-5}$
Perineum	10	*rs 420979558*	28,070,182*	$3.55x10^{-5}$
	10	*rs43634842*	55,422,184**	$3.85x10^{-5}$
Tailtot	10	*rs43634842*	55,422,184**	$5.81x10^{-5}$
	14	*rs109807031*	10,235,805	$6.03x10^{-5}$
	11	*rs109162468*	104,415,459	$6.94x10^{-5}$

Tick Traits based on tick species				
Pboo	26	*rs 42235074*	8,093,918	$4.66x10^{-6}$
	8	*rs42962717*	15,665,796	$7.83x10^{-6}$
	7	*rs109278195*	110,608,386	$2.60x10^{-5}$
	5	*rs 41576996*	19,931,600	$2.77x10^{-5}$
	11	*rs110540697*	92,692,190	$6.33x10^{-5}$
PAmbly	15	*rs 110611723*	23,738,373	$2.90x10^{-6}$
	19	*rs41901233*	16,400,722	$2.90x10^{-5}$
	17	*rs43092821*	43,990,974	$3.72x10^{-5}$
	17	*rs43092810*	44,000,618	$4.30x10^{-5}$
BeAmbly	1	*rs109236741*	146,632,014	$3.12x10^{-5}$
	11	*rs109474265*	6,243,988	$1.46x10^{-5}$
	12	*rs 110152589*	12,141,029	$2.31x10^{-5}$
Tambly	3	*rs110306387/ rs110306386*	108,483,066	$6.92x10^{-5}$
Treve	11	*rs109162468*	104,415,459	$2.89x10^{-5}$
	17	*rs 43499108*	7,118,768	$3.66x10^{-5}$
	14	*rs109807031*	10,235,805	$7.78x10^{-5}$
TBCReve	17	*rs 43499108*	7,118,768	$7.31x10^{-6}$
	17	*rs29011077*	7,165,500	$4.57x10^{-5}$
TBCAmbly	10	*rs420979558*	28,070,182*	$4.23x10^{-7}$
	10	*rs41660143*	31,692,125	$3.80x10^{-5}$
	10	*rs43634842*	55,422,184**	$6.28x10^{-5}$
	1	*rs1100893722*	74,643,836	$9.53x10^{-5}$

Abbreviations: BTA = *Bos taurus* chromosome; SNP= single nucleotide polymorphism, Pc1df = P-value corrected for the genomic inflation factor □. */** =SNPs that are similar but identified from different traits

These QTLs were located on BTA7 ($P = 1.05 \times 10^{-6}$) for Headtot and on BTA10 ($P = 4.23 \times 10^{-7}$) for TBCAmbly. The corresponding Manhattan plots and the QQ plots are presented in Figures 5.1 and 5.2, respectively.

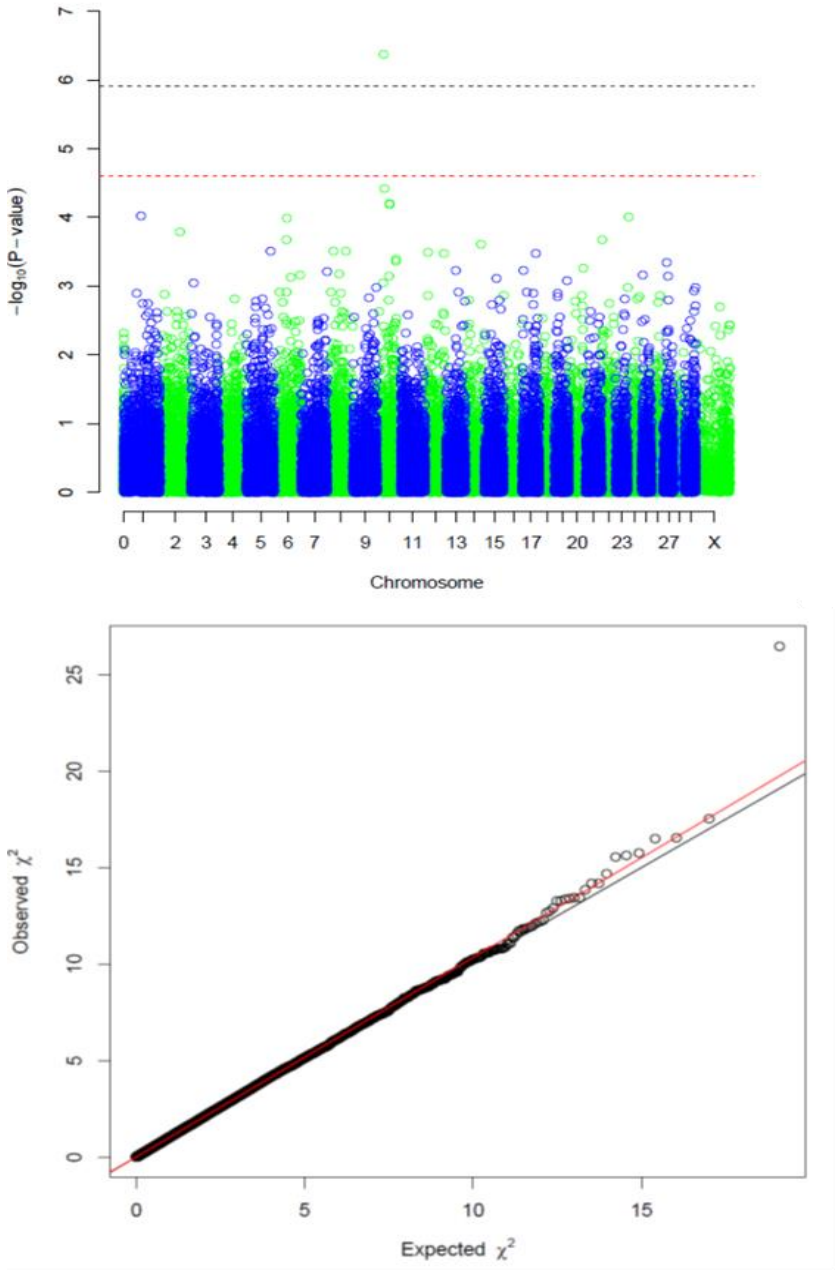

Figure 5.1 Manhattan plot displaying the GWA results (-$\log_{10}$(P) of the corresponding Pc1df, P-values corrected for the genomic inflation factor □) and Q–Q plot (below) of observed P-values against the expected P-values for $\log_{10}$(TBCAmbly + 1). Genome-wide P<0.05 (black dashed line) and suggestive (red dashed line) thresholds are shown.

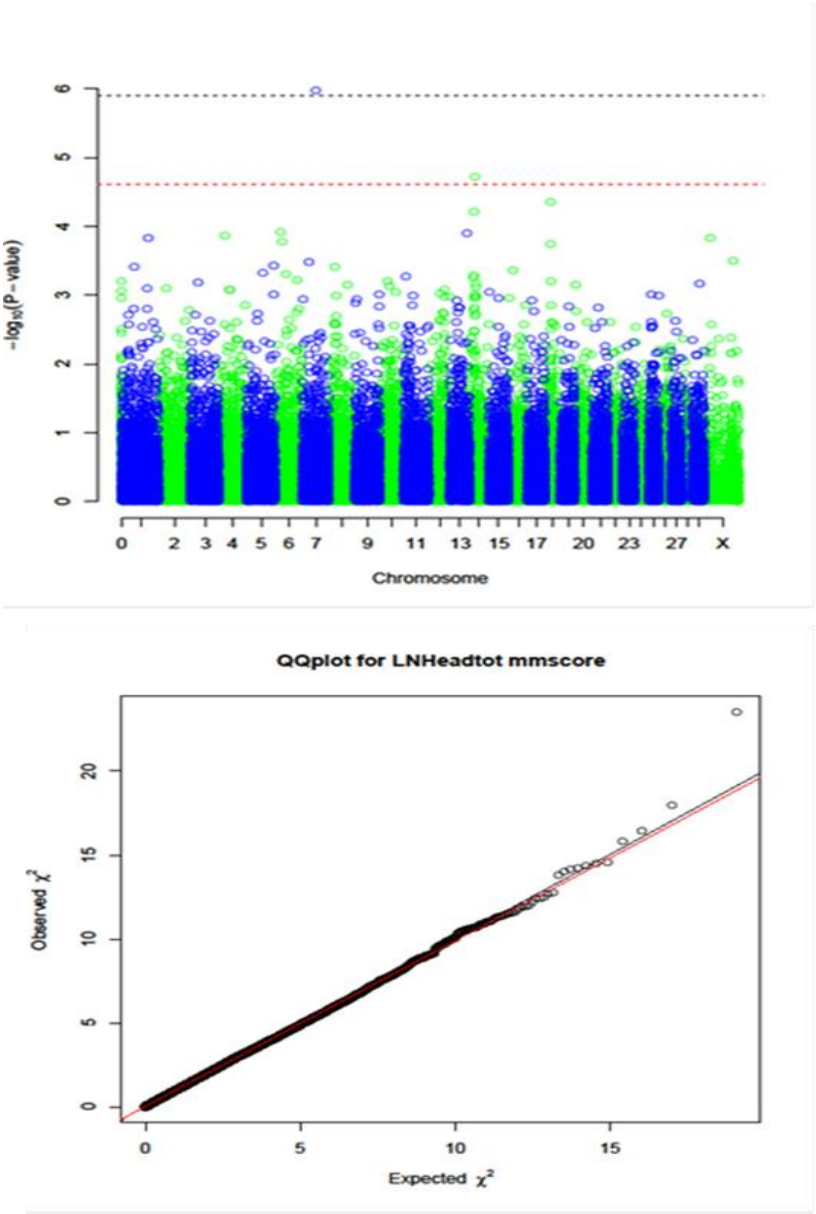

Figure 5.2 Manhattan plot displaying the GWA results ($-\log_{10}(P)$ of the corresponding Pc1df, P-values corrected for the genomic inflation factor □) and Q–Q plot (below) of observed P-values against the expected P-values for $\log_{10}$(Headtot + 1). Genome-wide P<0.05 (black dashed line) and suggestive (red dashed line) thresholds are shown.

However, we also found many SNPs throughout the genome that were associated with tick resistance at the suggestive level, which supports a polygenic pattern of inheritance for this trait. It is interesting to note that the QTL on BTA10 was also detected by other SNP reaching the suggestive threshold levels for body location traits such as ALLTBC, Perineum and Tailtot. These

included SNPs *rs42079558* and *rs43634842* associated with ALLTBC on BTA10 (P = 5.12 x 10^{-6}; Figure 5.3), which were also identified for TBCAmbly (*rs42079558* at the genome-wide significance threshold) and Perineum and Tailtot for total tick counts in the perineum and tail body region.

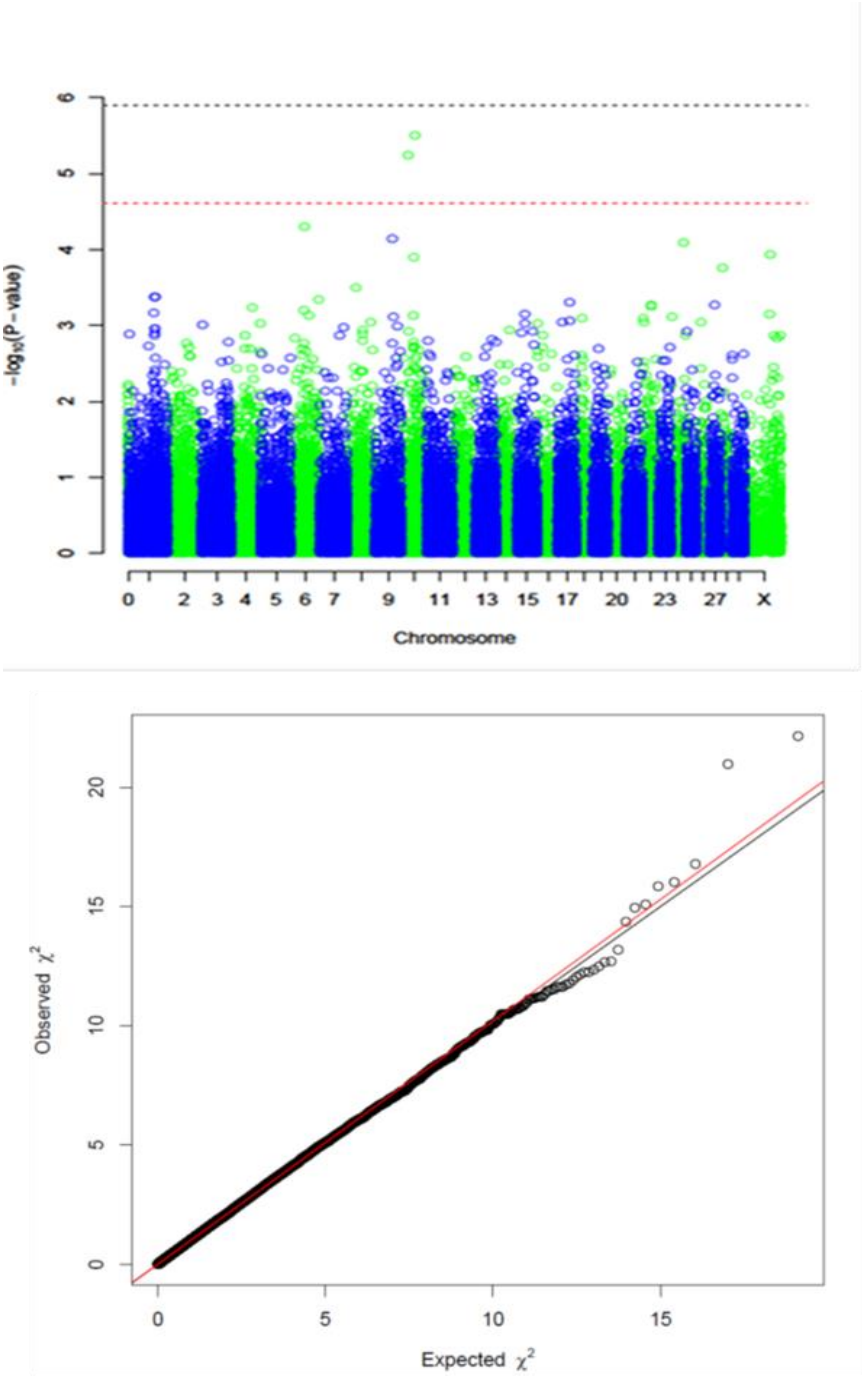

Figure 5.3 Manhattan plot displaying the GWA results (-$\log_{10}$(P) of the corresponding Pc1df, P-values corrected for the genomic inflation factor λ) and Q–Q plot (below) of observed P-values

against the expected P-values for $\log_{10}$(ALLTBC + 1). Genome-wide P<0.05 (black dashed line) and suggestive (red solid line) thresholds are also shown.

SNPs *rs42235074* identified on BTA26 (P = 4.66 x 10^{-6}) associated with *R. Boophilus* tick count in the Perineum, and the same chromosomal region was also identified as being associated with Headtot where there was a higher count of *R. Boophilus* ticks. The SNP *rs43499108* (P = 7.31 x 10^{-6}) on BTA17 associated with the number of *R. evertsi evertsi* ticks found for TBCReve was also identified as associated with Treve and PAmmbly traits, but at below the suggestive significance threshold. The region on BTA14 associated with tick resistance was also identified as being associated with the total numbers of ticks found in different body regions including the head (suggestive significance), belly and tail (at just below suggestive significance). Most of the associated regions found in the GWA analysis overlapped between traits.

5.3.3.2 Regional heritability mapping

The RHM analysis identified most of tick species traits as having one QTL significant at the genome-wide significance level and several regions at the suggestive significance level. Summary of RHM results showing the regions identified as being associated with tick resistance is presented in Table 5.6.

Table 5.6 Regional heritability mapping for tick count traits with windows significant at both genome wide level and the suggestive level

Log_{10}(Trait+1)	**BTA**	**window**	**SNP start-end** **Position start-end**	**LRT**	**h^2_w**
Tick Traits based on location on the animal's body					
Perineum	10	9	*rs110000217- rs110892901** 26,700,563 – 32,677,973	12.76	0.11
Tick Traits based on tick species					
PAmbly	19	18	*rs108957186- rs109163413* 53,499,697 – 57,714,094	14.44	0.09
	10	8	*rs110324502 - rs109116907* 20,899,486 – 29,375,348	9.74	0.12
	10	9	*rs110000217- rs110892901** 26,700,563 – 32,677,973	10.16	0.10
Tambly	3	35	*rs110576426- rs41255305* 105,994,898 – 111,430,815	10.95	0.07
	3	36	*rs109061240 - rs110592209* 108,427,681 – 114,191,841	11.24	0.08
Pboo	7	36	*rs41658051 - rs42309197* 110,161,093 – 112,628,884	9.34	0.05

Abbreviations: BTA = *Bos taurus* chromosome; LRT = Likelihood ratio test; h^2_w = Regional heritability. * = SNPs that are similar but identified from different traits

The region on BTA19 was significant (LRT = 14.44) at the genome-wide significance level ($P<0.05$) for the PAmbly trait. This region differed to that on BTA19 identified for PAmbly in the single SNP analysis. A QTL mapped by the RHM analysis at the suggestive significance level on BTA10 for PAmbly and Perineum appeared to be the same QTL as identified in the single SNP analysis at the suggestive level for the body location traits of ALLTBC and Perineum, and at the genome-wide significance level for the TBCAmbly tick species trait. The LRT profiles for the whole genome (a) and close up 100 SNP windows on BTA19 (b) are presented in Figures 5.4 and 5.5.

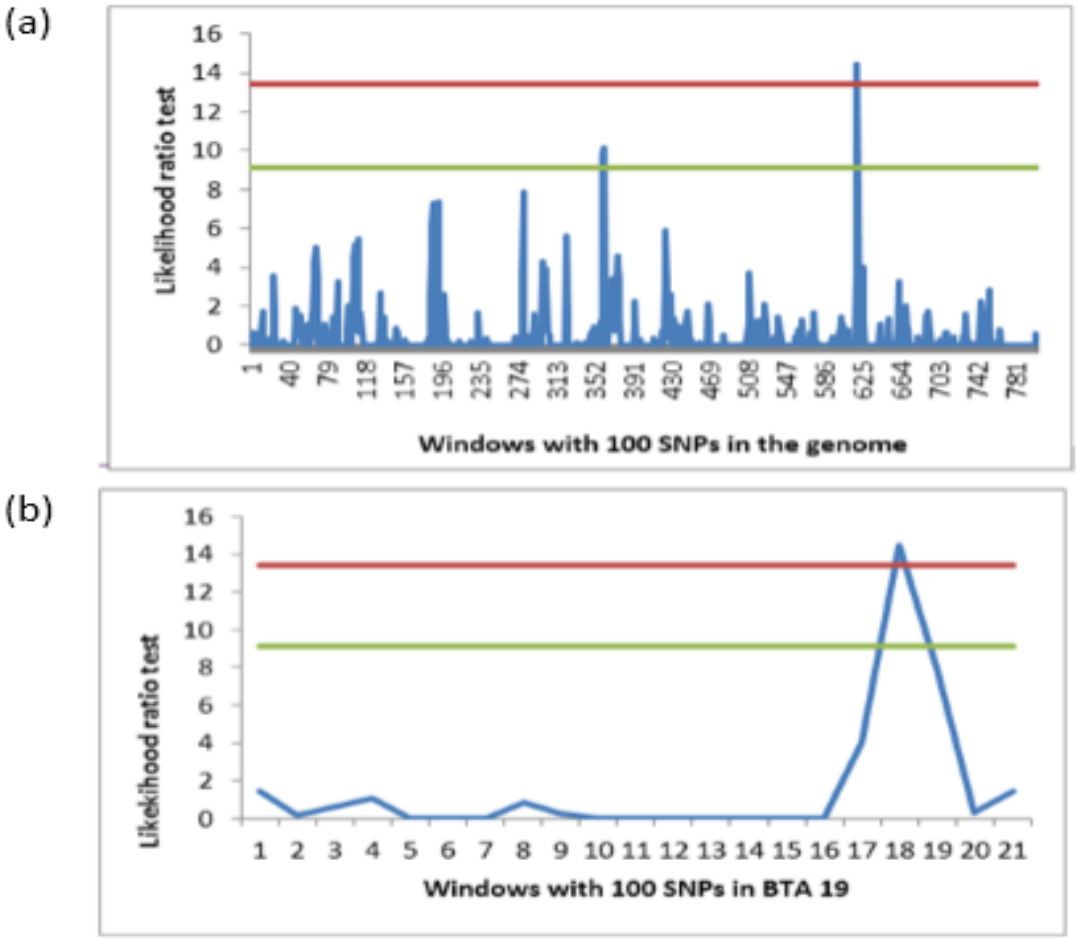

Figure 5.4 Plot of the LRT across the genome (a) and for BTA19 (b) for the RHM analysis of $\log_{10}$(PAmbly + 1). Genome-wide P<0.05 (red) and suggestive (green) thresholds are shown.

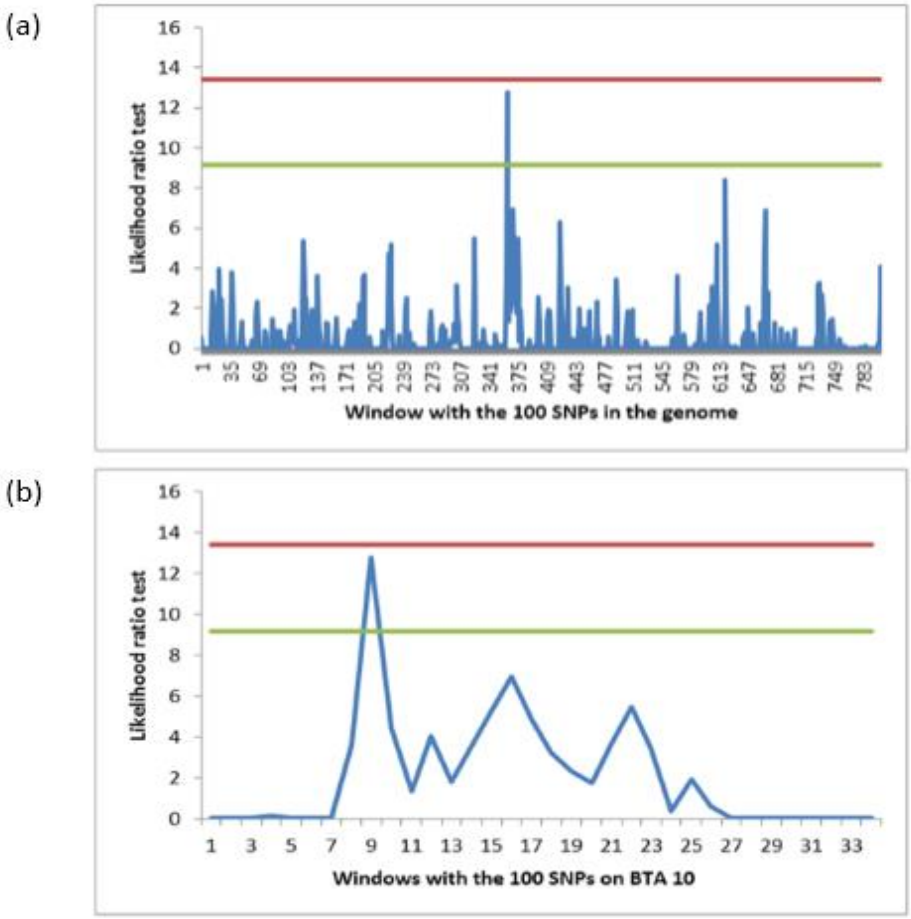

Figure 5.5 Plot of the LRT across the genome (a) and for BTA19 (b) for the RHM analysis of $\log_{10}$ (Perineum + 1). Genome-wide P<0.05 (red) and suggestive (green) thresholds are shown.

Other regions identified at the suggestive significance threshold by the RHM analysis included a region on BTA3 for TAmbly, which was the same region that was identified in the single SNP analysis for the same trait. The QTL on BTA7 for Pboo was also identified in the single SNP analysis. The region on BTA7 was associated with Headtot at the genome-wide significance threshold.

5.3.3.3 Genes associated to the identified GWAS regions.

The regions associated with tick resistance in this study were further investigated to locate candidate genes that were likely to underlie the association and these are reported in Table 5.7.

Table 5.7 Potential candidate genes located in the identified BTA regions associated with tick resistance

SNP	BTA	Position	Gene (gene symbol)
rs110576426	3	105,994,898	Potassium voltage-gated channel, KQT-like subfamily, member 4 (*KCNQ4*)
rs41255305	3	111,430,815	Small integral membrane protein 12 (*SMIM12*)
rs110592209	3	114,191,841	Transient receptor potential cation channel, subfamily M, member 8 (*TRPM8*)
rs110810914	6	87,281,196	Casein alpha-S2 (*CSN1S2*)
rs109278195	7	110,608,386	Fer (fps/fes related) tyrosine kinase (*FER*)
rs42962717	8	15,665,796	Leucine rich repeat and Ig domain containing 2 (*LINGO2*)
rs41658051	10	110,161,093	uncharacterized LOC101906833 (*LOC101906833*)
rs109162468	11	104,415,459	Calcium channel flower domain containing 1 (*CACFD1*)
rs109807031	14	10,235,805	Protein EFR3 homolog A (*LOC100336574*)
rs41665024	14	21,406,039	Uncharacterized *LOC101905779*
rs29011077	17	7,165,500	LPS-responsive vesicle trafficking, beach and anchor containing (*LRBA*)
rs43499108	17	7,118,768	LPS-responsive vesicle trafficking, beach and anchor containing (*LRBA*)
rs110439061	18	26,527,686	Solute carrier family 38, member 7 (*SLC38A7*)
rs108957186	19	53,499,697	Uncharacterized *LOC101907438*
rs109163413	19	57,714,094	G protein-coupled receptor 142 (*GPR142*)
rs42235074	26	8,093,918	Protein kinase, cGMP-dependent, type I (*PRKG1*)

Abbreviations: BTA = *Bos taurus* chromosome; SNP= single nucleotide polymorphism

5.4 Discussion

The highest average tick count observed for ALLTBC were lower to those reported by Schoeman (1989) in Nguni cattle. The heritability estimates generated in this study were low to moderate and comparable to the previous reports of 0.09 in Brahman cattle by Port Neto *et al.* (2014), 0.13 in composite breeds involving Indian, African and European cattle breeds by Prayaga & Henshall (2005), 0.15 in Brahman cattle by Prayaga *et al*. (2009), 0.17 in Bonsmara cattle by Budeli *et al.*

(2009), 0.19 in Braford and Hereford cattle by Cardoso *et al*. (2015) and 0.21 in a Hereford Shorthorn line by Peixoto *et al.* (2008). However, other investigators have found higher heritability estimates for tick resistance including 0.37 in *B. taurus* dairy breeds by Turner *et al.* (2010), 0.39 in Shorthorn reported by Wharton *et al*. (1970), 0.41 in a tropical composite breed by Port Neto *et al.* (2014) and 0.42 in a composite breed of tropical beef cattle by Burrow (2001). The lower heritability obtained here might have been caused by the small number of animals (n = 500) used in the current study or due to the reduced variation as a results of Nguni cattle being naturally resistant. Tick count varied considerably in this study between 0 and 198 ticks per animal across all measures.

We used both the GWA and RHM analysis approaches to allow for the possibility that the RHM analysis might be capable of identifying some genomic regions in which variation was due to several segregating alleles that may not have been detected by the GWA analyses (Riggio *et al.,* 2013). In both analytical approaches, the highest estimate of heritability was 0.17 for the Perineum trait based on the GWA analysis and 0.12 for the PAmbly trait using the RHM analysis.

Both analytical approaches identified genomic regions associated with tick resistance, with the GWA analysis identifying more regions than the RHM analysis. For the Perineum, ALLTBC, and Pboo traits, both the GWA and RHM analyses identified similar chromosomal regions associated with tick resistance. The GWA approach identified two SNPs at genome-wide significance, while the RHM approach identified one region associated with tick count at genome-wide significance. The RHM analysis appeared to improve the resolution for identifying regions explaining trait variation beyond that of the GWA approach. For example, a region identified on BTA19 for PAmbly was below the threshold for suggestive significance using the GWA analysis, but reached genome-wide significance in the RHM analysis at a different SNP from the same BTA. This suggests that the RHM analysis has increased power for identifying QTLs with more than two alleles and genomic regions that harbor multiple loci that contribute to variation in the trait of interest.

The QTL region on BTA10 that reached genome-wide significance level was for TBCAmbly, which was a total tick count for *A. hebraeum* ticks on the whole body. However, we found the same QTL for ALLTBC, Perineum and Tailtot which may be indicative that the body spread of this tick may not be uniform across the animal's body or that in mixed species infestation, the resistance mechanisms evoked by *A. hebraeum* ticks are the predominant ones. Previously reported tick

resistance QTL in this region of the genome, were identified in a Brazilian F_2 population using microsatellite markers (Regitano *et al.,* 2008; Machado *et al.,* 2010), and this QTL explains about 3.3% of the tick burden phenotypic variance during the dry season. Turner *et al.* (2010) also identified QTL associated with tick burden on BTA10 in Brahman cattle. This QTL was later confirmed by Port Neto *et al.* (2011) who reduced the size of the interval harbouring the QTL and identified a candidate gene using Australian dairy and Brahman cattle. However, the identified candidate gene did not contain any obvious mutations that might be expected to cause any differences in tick burden phenotype. The RHM study identified the same QTL for PAmbly which represent *A. hebraeum* ticks on the perineum with the QTL accounting for h^2 of 0.12.

The second region that reached the genome wide significance level was on BTA7 for headtot. Although a single SNP was also identified for Pboo (*R. boophilus* ticks on the perineum), it was situated 51.8 Mb upstream from the single simple analysis. However, the RHM identified a window covering the same region as identified in the single SNP analysis as the second region for Pboo and it accounted for 0.05 of h^2. This QTL was also mapped on a suggestive threshold ($P < 0.10$) chromosome-wide associated to tick burden on BTA 7 for the dry season in Brazilian F_2 experimental population (Gasparin *et al.,* 2007).

The third important result was the region segregating on BTA19 that was obtained from RHM. This region is among other genomic regions associated with tick resistance that were identified on BTA 1, 3, 11, 13, 14, 19 and 26 in Australian dairy and Brahman cattle using SNPs markers (Barendse, 2007; Turner *et al.,* 2010; Port Neto *et al*., 2011) and BTA 5, 7, 11, 14 and 15 in Brazilian cattle for dry and wet season tick burdens using microsatellite markers (Gasparin *et al.,* 2007; Regitano *et al.,* 2008; Machado *et al.,* 2010; Sollero *et al*., 2014). These findings indicate that there is independent support for the genomic regions associated with tick resistance in different breeds of cattle.

The two regions meeting genome-wide significance on chromosomes 10 and 19 in both the GWA and RHM analyses appeared to be associated with *A. hebraeum* ticks. However, this may have been influenced by the greater numbers of *A. hebraeum* ticks found on the animals in this study compared to other species. The region identified on BTA19 using RHM analysis harbours the G protein-coupled receptor 142 (*GPR142*) gene in which mutations have been described as causing skin defects in human and animals. The genome-wide significant regions identified on BTA7 for transformed Headtot and the suggestive regions on BTA 5, 8 and 26 for transformed Pboo appear

to be associated with *R. Boophilus* ticks and there were more *R. Boophilus* ticks found on the head than ticks of other species. The SNP *rs42097850* on BTA26 is located within the Protein kinase, cGMP-dependent, type I (*PRKG1*) gene. *PRKG1* is known to play a crucial role in the relaxation of vascular smooth muscle by lowering the intracellular levels of calcium.

The genomic region on BTA17 meeting suggestive significance appears to be associated with *R. evertsi evertsi* ticks and this region has not previously been identified as associated with tick resistance. This may be due to the fact that the *R. evertsi evertsi* tick is primarily distributed throughout African countries. Although this region was also identified for transformed PAmbly, its effect on this trait was very small based on the fact that it did not reach the suggestive significance threshold. The BTA17 region contains the LPS-responsive vesicle trafficking, beach and anchor containing (*LRBA*) gene. This gene is associated with protein kinase A and is assumed to be involved in leading intracellular vesicles to activated receptor complexes, which supports the secretion and/or membrane deposition of immune effector molecules. Defects in this gene are associated with common variable immunodeficiency (CVID) in human and mouse. Other genes such Small Integral Membrane Protein 12 (*SMIM12*) tagged by *rs41255305* on BTA3, Fer (fps/fes related) tyrosine kinase (*FER*) tagged by *rs109278195* on BTA7, Leucine rich repeat and Ig domain containing 2 (*LINGO2*) tagged by r*s42962717* on BTA8 were also found to be candidates for tick resistance QTLs in this study. However, it is unclear what roles any of these genes may have on conferring resistance or susceptibility to ticks.

An important finding of the current study is that we identified three regions achieving genome-wide significance threshold that were associated with tick resistance in Nguni cattle. In addition, there were many regions that achieved suggestive significance level. Since we are dealing with many tick species and body location, more studies will need to explore if resistance pathways are common across tick species or the apparent polygenic nature we observe in our results is indicative of independent pathways. The use of both GWAS and RHM analyses had the advantage of identifying regions with individual and potentially compound effects on the trait of interest. The findings of this study using the separate approaches to identify genomic regions of interest provide additional knowledge to evaluate the potential use of genomics to identify genes that control resistance to ticks in cattle. In future, this may provide new applications for cattle farmers to control ticks and tick-borne diseases in African cattle production systems.

5.5 Conclusion

The resistance of cattle to ticks is important because it is associated with their productivity and welfare in tick-endemic regions of the world. There is significant genetic variation among cattle for tick counts. In this study, the GWA analysis detected SNPs associated with variation in tick count, while the RHM analysis also identified genomic regions with large effects using contiguous SNPs to define tested regions. The chromosomal regions found to harbour loci associated with tick count in Nguni cattle were consistent with previously reported regions associated with tick resistance in other cattle breeds. Meta-analysis of published studies appears to be a useful next step to validate the identified chromosome regions associated with tick resistance before genomic information is used for genetic testing by cattle farmers.

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Chapter 6
General discussion, conclusions and recommendations

6.1 General discussion

Resistance of cattle to ticks is associated with their productivity and welfare in tick-endemic regions. Increases in the incidence of ticks and tick-borne diseases in cattle populations, and the extensive distribution of these diseases in the tropical and sub-tropical countries, including South Africa, is putting pressure on farmers and researchers to develop more effective and cost-eeffective alternative tick control methods. Genetic improvement of host resistance to ticks appears to be one of the most appealing options. An understanding of the genetic basis of resistance to ticks and tick-borne diseases in cattle could improve breeding programmes to develop animals that are more resistant and productive. It is important to quantify the genetic variation of host resistance to ticks within and between adapted or indigenous cattle breeds before they can be used in a selection programme to improve host resistance to ticks. Therefore, this the current study generate knowledge required to develop a sound breeding programme for tick control.

The first objective was to assess the variation in tick loads and prevalence of tick species in Nguni cattle using tick count data collected from four different climatic regions of South Africa. All four different experimental farms have the same tick species prevalence, excluding *R. simus,* which was only found at the University of Fort Hare farm. Tick loads in Nguni cattle differs in the agro-ecological zones considered in the current study, with warmer locations tending to have higher tick loads and *A. hebraeum* being the most prevalent and widely distributed tick species. Year, season and month of counting and age of the animal also influenced tick loads. Tick count was significantly higher in the hot-dry and hot-wet compared to cool-wet and cool-dry seasons. The most preferred tick attachment sites were under the tail, perineum and belly body locations. The tick species identified in this study will also assist in predicting tick-borne diseases expected in these regions.

The second objective was to estimate genetic parameters for tick count in Nguni cattle, based on data sets representing different seasons. Results indicate that there is sufficient genetic variation for tick resistance in Nguni cattle. Such genetic variation appears to be expressed more during seasons with higher levels of tick infestation. Data set D, containing September to January counts,

showed the highest genetic variation. Heritability estimates for tick count varied (0.01±0.01 to 0.26±0.01) according to season and trait. The observed high genetic correlations between whole body count and most of the body location counts suggests that it may not be necessary to conduct whole body counts. Bellytot and perineum appears to be the most suitable surrogate traits for whole body count, due to the remarkably high genetic correlations. These results provide a good basis for developing strategies for genetic improvement of tick resistance through selection.

The third objective was to perform a genome wide association study, based on tick count data collected over a two year period, together with genotypic data, in order to identify genomic regions associated with tick resistance in Nguni cattle. GWA and RHM analyses were applied. The GWA analysis detected single SNPs associated with tick count, while the RHM analysis identified genomic regions with large effects using contiguous SNPs to define tested regions. Genome-wide significant regions on chromosomes 7, 10 and 19 were identified for total tick count on the head, total *A. hebraeum* ticks and for total number of *A. hebraeum* in the perineum region. Suggestive significant regions were also identified on chromosomes 1, 3, 6, 7, 8, 10, 11, 12, 14, 15, 17, 19 and 26 for several of the traits that were analysed. The chromosomal regions identified in this study as harbouring QTL underlying variation in tick count form the basis for further analyses to identify specific candidate genes related to cattle tick resistance and also provide the potential for marker-assisted selection in Nguni cattle. The markers identified here can be validated and used to improve host resistance in beef cattle production systems.

There is scope for developing cattle that are naturally resistant to ticks, through well thought-out genetic improvement programmes in tropical and subtropical regions. Knowledge of the genetic basis for tick resistance in cattle is strongly warranted, given that 70% of global beef production and dairy production occurs in tropical and subtropical regions.

6.2 Practical considerations for tick control in the South African beef industry

In order to achieve tick control in a more sustainable, environmentally friendly and cost-effective manner in South Africa, an integrated approach combining the different methods available is recommended. South Africa is a tick endemic country, with ticks affecting livestock production and wildlife; however levels of tick infestation vary according agro-climatic zone.

Chemical methods of controlling ticks and tick-borne diseases impose a heavy financial burden on both commercial and emerging livestock producers. The current study explored alternative tick

control methods, which are more sustainable and environmentally friendly. The following are recommendations for incorporating genetic improvement in tick control programmes, to South African beef producers.

6.2.1 Protocol for tick load assessment

To develop proper protocol for tick assessment in tick endemic regions of South Africa, knowledge of geographical distribution and prevalence of ticks is needed to provide a good basis for the implementation of any tick control method.

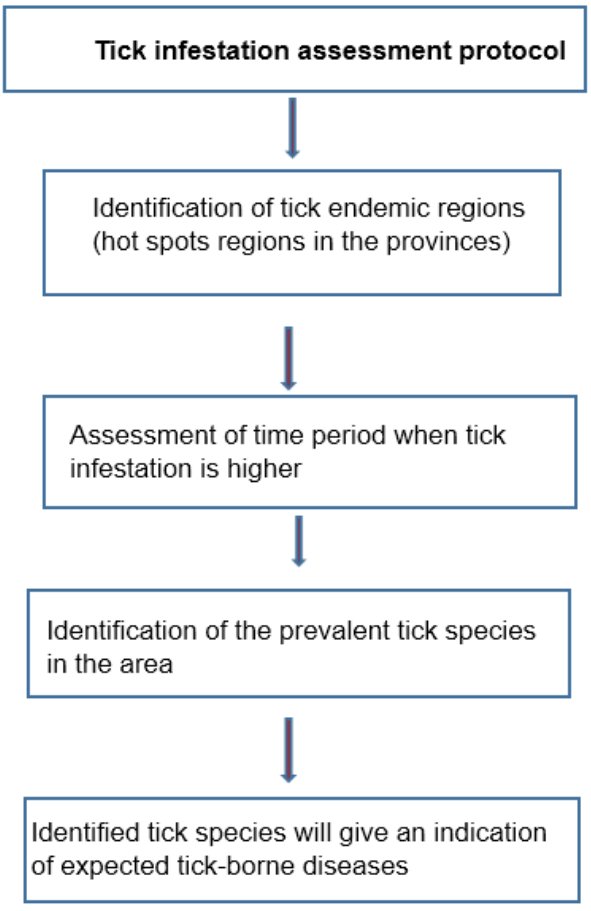

Figure 6.1 Steps for tick assessment in order to implement tick control methods

6.2.2 Selection of natural resistant animals as a method for tick control

In order to quantify variation in tick load, beef farmers need to determine the tick load on each breeding animal by means of a tick count, during spring and summer seasons (especially the September to January period) when the level of tick infestation is high. It is advised to count adult

ticks in the perineal area or the belly of the animal, every three weeks, during the above period. In order to measure resistance, it is important to enumerate the adult ticks because they have already fed on that specific host. The recorded tick count data can be used to estimate genetic variation for tick resistance within the cattle population. Such data can be used in breeding programmes or genomic analysis for tick resistance.

6.2.3 The application of genomics to improve tick resistance

Genetic markers associated with tick resistance have been identified in the current study. These markers will be validated across the cattle breeds in order to develop a bovine SNP panel for tick resistance. Breeding bulls and cows could therefore be screened with this panel to estimate the resistance status. Further investigation of major genes related to tick resistance from the identified marker regions should provide an opportunity to understand the genetic basis of resistance across the breeds. Cattle that have been tested and found to be resistant can then be used as breeding stock, through the inclusion of tick resistance in the breeding objective. Alternatively, resistant Nguni bulls can be used to improve breeds that are tick susceptible, in the South African beef industry, through marker assisted introgression.

The above practical recommendations can be used in any production system; however they need to be underpinned by a sound strategic plan for sustainable tick control. This is applicable to both commercial and emerging farmers, where good management practices are followed.

6.2.4 Future research topics

1) To validate identified regions between and within other cattle breeds in South Africa order to develop SNP panels for tick resistance and used in genetic evaluation
2) To further investigate major genes related to tick resistance from the identified marker regions
3) Application of transcriptomics to characterise the gene expression profiles which mediate beef cattle resistance to ticks will provide the opportunity to develop tick control measures for known tick species
4) Assessment of LD in Nguni cattle population for the characterisation of genetic architecture