A Comparative Study in Ectoparasite Tolerance Between Purebred Brahman (*Bos indicus* Linnaeus), Sussex (*Bos taurus* Linnaeus) and Brahman x Sussex Crossbred Cattle in the Free State, South Africa

by

Marilie Esterhuyze

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Department of Zoology and Entomology
Faculty of Natural and Agricultural Sciences
University of the Free State
Bloemfontein
South Africa

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Supervisors: Ms. EMS van Dalen
Co-supervisor: Dr. S Brink

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Declaration

I declare that the thesis submitted by me, Marilie Esterhuyze, in fulfilment of the requirements for the degree Magister Scientiae in Entomology at the University of the Free State, is my own independent work accept for specific vegetation data received from Mr. Bertiaan Luyt. This thesis has not previously been submitted by me or anyone else at another university or faculty. I furthermore concede copyright of this thesis in favour of the University of the Free State.

Marilie Esterhuyze

“Ask, and it shall be given to you; seek, and ye shall find; knock, and it shall be opened unto you.”-Matthew 7:7, King James Bible.

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BRAHMAN
THANK YOU
BOERDERY
Omring deur God se goedheid.

TABLE OF CONTENTS

CHAPTER 1: INTRODUCTION

CHAPTER 2: MATERIALS AND METHODS

CHAPTER 3: ACARI INFESTATIONS ON DIFFERENT CATTLE BREEDS

CHAPTER 4: DIPTERA FAMILY ABUNDNANCE AND DIVERSITY

CHAPTER 5: CHARACTERISTICS AND EFFECT ON ECTOPARASITE INFESTATION

CHAPTER 6: CONCLUSION

ANNEXURES:

LIST OF TABLES:

LIST OF FIGURES:

LIST OF ABBREVIATIONS AND COMMONLY USED WORDS:

ANOVA	-	Analysis of variance
cm	-	centimetre
g	-	gram
HSD	-	Honest significant difference
ID	-	identification number
kg	-	kilogram
kraal	-	cattle yard
m	-	meter
mm	-	millimetre
nm	-	nanometer
ºC	-	Degree Celsius
SEM	-	Scanning electron microscope
µm	-	micrometer
UV	-	Ultra violet
veld	-	natural pasture where animals graze
W	-	Watts

SUMMARY

Crossbreeding *Bos indicus* with *Bos taurus* cattle was explored as a measure to manage ectoparasite infestation specifically comparing tick, mite, fly and lice resistance between Brahman, Sussex and Brahman x Sussex crossbreds. The study area was located in the central Free State on different farms, not more than 15km apart, where cattle breeds were followed and ectoparasites collected on a monthly basis from March 2014 to March 2015. The aim of the study was to determine if Brahman cattle have a natural ectoparasite resistance and if this resistance can be linked to certain breed characteristics when compared to other cattle breeds such as Sussex. A second aim was to establish if the resistance qualities identified are preserved in the cross bred generations. Ectoparasites were collected from both the on-host environment by inspecting 20 cattle from each breed every month as well as the off-host environment through tick drags, fluorescent light traps and sticky fly traps. Ectoparasite abundance regarding both the on-host and off-host environment were also compared to mean monthly temperature and rainfall numbers

A total of five Ixodidae species were collected from the animals over the study period including *Hyalomma rufipes* (3797), *R. evertsi evertsi* (596), *H. truncatum* (393), *R. decoloratus* (29) and *R. appendiculatus* (30). All of the tick species except for *H. truncatum* showed a higher affinity for the Sussex breed. The Sussex cattle groups also had the most tick infested individuals over the entire test period. Attachment areas for ticks showed Sussex cattle to have nine areas of tick attachment with Red Brahman two, Grey Brahman three and the crossbred cattle with four attachment areas, corresponding more to the Brahman breeds than the Sussex breed.

A greater overview was gained of Diptera diversity and abundance as well as the presence of veterinary important Diptera ectoparasites in the Kroonstad region. Rainfall however, seemed to be a factor influencing host preference, for during December 2015, with higher rainfall numbers and a significant higher Diptera species presence, no significant differences of Diptera abundance between any of the breeds were observed. If this is compared to December 2014, when a dry spell occurred, unfavourable conditions caused the presence of lower numbers of Diptera species, and a preference for the Sussex breed was observed.

No association was found between on-host and off-host ectoparasite abundance for all ectoparasite species collected, but this needs to be investigated more intensively in future. Seasonal factors like rainfall and temperature had an influence on ectoparasite abundance in the on-host and off-host environment with higher numbers found during the warmer months with higher rainfall.

From the results gained it is evident that certain breed characteristics can have an influence on ectoparasite load. Comparison of phenotipic characteristics showed Sussex cattle to have higher ectoparasite loads which correlated to longer, denser, coarser, and darker coats and higher body temperatures. The Grey- and Red Brahman groups had the lowest parasite loads accompanied by shorter and smoother coats and lower body temperatures. Tail length did not play a role in regulating ectoparasite loads.

Although Sussex cattle were statistically significantly heavier than the Brahman breeds, they still had the most ticks per kg body weight compared to the Brahman and cross breeds. However, the Brahman x Sussex crossbreed, had a mean weight only 22.9kg lower than those of the Sussex group and 115kg higher than the Brahman breeds with a significantly lower ectoparasite load. This indicated that crossbreeding could be integrated into herd management plans as an effective measure in controlling ectoparasite loads on cattle in both intensive and extensive production systems.

Key words: *Bos indicus*, *Bos taurus*, crossbred, Brahman, Sussex, ticks, mites, lice, flies.

CHAPTER 1

GENERAL INTRODUCTION AND LITERATURE REVIEW

The South African agricultural system is pressured not only to produce food for our local market but also for a growing continental population. The growing world population and in particular the growing African population, is expected to increase from an estimated 1 billion to 2 billion in 2050 (United Nations, Department of Economic and Social Affairs, Population Division, 2015). Recent studies indicated that the increase of middleclass consumers, are predicted to contribute to an increased demand for animal protein (Bureau for Food and Agricultural Policy; United Nations, Department of Economic and Social Affairs, Population Division, 2015). Furthermore, The Bureau for Food and Agricultural Policy estimates that the current average of 700 000 to 900 000 ton beef consumption in South Africa as estimated in 2010 to 2013, can increase with 20 to 25% in 2020 (Philips 2013; Stiftung 2014). However, due to the shortage of available productive land, land redistribution and restrictions to water accessible for agriculture in South Africa there are concerns of increasing the national herd without causing environmental damage, and thus the focus of improvement should be on existing productivities (Meissner *et al.* 2013). The main objectives for producing beef for exportation as well as local consumption are based on the principle of consumers' satisfaction while the industry stays competitive concerning price and lucrativeness in production (Strydom 2008). Thus, to be able to follow these principles, producers need to be able to reduce inset cost effectively as well as price for the consumers while producing the most tender, nutritious, tasteful beef as possible in the ever-demanding economy of our country.

The conversion of forage into food for humans by grazing ruminants is an economically important component of food production (Kashino *et al*. 2005). One of the factors that determines the efficiency at which animals convert forage into protein is health, and in turn health is influenced by the incidence of ectoparasites on livestock and the diseases they transmit. Losses in livestock production due to ectoparasite infestations are a reality commercial and communal producer's need to address daily. Various parasites can afflict cattle herds simultaneously, thereby increasing maintenance costs and reducing the overall productivity of the herd (Oliveira *et al*. 2013). Reduced performance and production result from blood loss, toxicosis, and arthropod borne diseases (Oliveira *et al*. 2013).

The feeding ectoparasites cause damage to the hide and subcutaneous tissue which can transmit a wide variety of pathogenic conditions. Feeding activity of the parasites is associated with pruritus, erythema, papules, excoriation, lichenification, crusting and scaling, generally causing trauma to the animal (Blagburn *et al.* 2009; Elshahawy *et al*. 2016). Wounds caused by ectoparasites can be subjected to secondary infestations and infections (Oberem *et al.* 2009). Salivary and fecal antigens produced by parasites during feeding can stimulate radical immune responses leading to hypersensitivity in vulnerable individuals (Oberem *et al.* 2009). According to Wall (2007) and Marufu *et al.* (2011), all of the described conditions lead to production loss in one form or another (contributing to weight loss, milk yield reduction and even abortion). Even more importantly, some ectoparasites also play a major role as vectors of bacteria, viruses, protozoans, cestodes and nematodes (Oberem *et al.* 2009). Diptera ectoparasites can transmit the following illnesses: bluetongue, pinkeye, epizootic bovine abortion, anaplasmosis, rift valley fever and hoof and mouth disease (Jonsson 2006). Ticks can transmit various diseases such as heartwater, redwater, African tick fever and Congo fever. Animals infested with lice are restless, they often scratch and bite at infected areas. Behavioural disturbances can also result from ectoparasite infestation. It has previously been recorded that animals increase behaviour such as rubbing due to ectoparasite infestation, that reduces the time spent grazing or ruminating and can even lead to self-wounding (Wall 2007; Oberem *et al.* 2009).

In 2000 annual worldwide losses associated with tick borne diseases alone were estimated to be about 18 billion US dollars (Mattioli *et al*. 2000). One engorged female tick can be responsible for as much as 0.25g live bodyweight loss in *Bos taurus* cattle, and to a lesser extent in *B. indicus* cattle (Jonsson 2006). According to Jongejan *et al.* (1994), when Ixodid ticks feed, 60% to 70% of surplus water in the blood meal is re-injected into the host animal through salivation. This means the actual consumption of blood can be two to three times the body weight of the engorged female tick.

Aside from ticks, Diptera (flies, midges and mosquitoes) has also been identified as some of the most economically important ectoparasites by producers and researchers alike (Banerjee *et al.* 2015). Irritation caused by these insects (larvae and adult stages) lead to gadding responses in livestock, resulting in possible injury and less time spent grazing (Oberem *et al.* 2009). Damage caused by the insects themselves, as well as the injury by the cattle due to self-harm behaviour in the attempt to rid themselves of the flies, results in a decrease in overall production potential (Marufu *et al*. 2011).

Acaricides are still being used as main defence against ticks in Africa, where in contrast Australia has already successfully utilised host resistance as an alternative (Ali *et al.* 1993). Chemical control measures for ectoparasites on livestock currently used include; plunge-dip, spraying, pour-on and belly bathing (Oberem *et al.* 2009). Easy application and all-round season suitability of pour-on acaricides makes this the most popular method used in extensive farming systems. The continued use of chemical agents leads to the development of resistance in parasites, as well as the presence of dangerous deposits in agro-ecosystems (Rajput *et al.* 2006). Environmental awareness and safety legislation concerning the use of "chemical agents" in agro-ecosystems are increasing globally (Shehani *et al*. 2014).

In order to avoid these negative outcomes, chemical control measures should be supplemented with other strategies, such as genetic resistance of host animals to parasites. Genetic tolerance against ectoparasites is considered to be a long-term resolution and possibly superior to chemical defence provided by pesticides. From the aforementioned, it is clear why a study describing ectoparasite tolerance by selected Zebu-and European (*B. indicus* and *B. taurus*) cattle as well as their crosses (*B. indicus* x *B. taurus*) is important for southern Africa.

Breed performance can be characterised by comparing different breeds under the same environmental conditions, or through crossbreeding parameters under different environmental conditions (Schoeman 1996). This is generally practiced in South Africa with consideration that breed performance is also subject to differences in management and environment (Schoeman 1996). As a main objective of this study crossbreeding effectiveness in ectoparasite resistance, between Brahman (*B. taurus*), Sussex (*B. indicus*) cattle breeds and their crosses, will be investigated.

1.1 FARMING PRACTICES

Ectoparasites pose a threat to cattle herds in both commercial and communal farming systems in the Free State. Most of the cattle sold by commercial or communal producers are bought by the feedlot industry. South African beef production is different to that of other countries in that more than 75% (1.35 million carcasses annually) of beef is produced through feedlots (Strydom 2008). In comparison, Australia produces 35% in feedlots and this percentage is even lower in Argentina, Brazil and New Zealand where beef is mainly produced from grazing on natural pastures, which South African producers do not have access to all year round (Strydom 2008).

The local feedlot industry has been placed under more pressure recently, due to higher grain prices as a result of the drought of 2014-2015, making it essential to be very strict concerning cattle selection for feedlot purposes (Strydom 2008). Only the cattle with the fastest growing rate and lowest maintenance cost should be selected, highlighting the reasoning behind breeding cattle with natural ectoparasite resistance.

Industrial crossbreeding allows for genotypic optimisation that can meet market demand and improve meat quality while being more tolerant to environmental factors. According to a well-known South African production animal scientist, animals better suited to a particular environment, have better reproduction and production achievements, opposed to animals experiencing environmental stress (Bonsma 1980).

1.2 VECTORS/ECTOPARASITES

Ectoparasites examined during this study belong to the orders; Acari, Diptera, and Phthiraptera. Each specific ectoparasite order's influence on the cattle breeds will be discussed. However, if a certain order was absent in the on-host or off-host environments it was not included further on in the study.

1.2.1 Acari (Ticks and mites)

Ticks are obligatory blood feeding parasites that have a long-standing history with humans, their domesticated animals and wild animals (Brites-Neto *et al.* 2015). They can cause severe blood loss as well as transmit various diseases to cattle and other livestock (Oberem *et al.* 2009). Ticks and mites belong to the Phylum Arthropoda. They are part of the Class Arachnida and can further be divided into two family groups; hard body ticks (Ixodidae) and soft body ticks (Argasidae) (Walker *et al.* 2003). The total dorsal surface of a male Ixodid tick is covered by a sclerotized dorsal shield known as the conscutum. Female Ixodid ticks have a smaller sclerotized shield referred to as the scutum. In Argasid ticks the dorsal shield is absent, and the outer surface of the body is leathery (Walker *et al.* 2003). In this study, the focus will be on Ixodidae ticks found on cattle in the Free State, as Argasidae ticks are more commonly associated with nest dwelling animals.

Species sampled in the Free State may be one-host, two-host or three-host ticks that feed on cattle and other wild animals. Some of the most important endemic tick species harboured by cattle and wildlife in the Free State are *Rhipicephalus appendiculatus*, *Rhipicephalus decoloratus* and *Rhipicephalus microplus* (Horak *et al.* 2015). *Rhipicephalus microplus* is a non-endemic species that was introduced from southern Asia to South Africa more than 100 years ago (Horak *et al.* 2015). Other tick species commonly associated with cattle are *Rhipicephalus evertsi evertsi*, *Hyalomma rufipes* and *Hyalomma truncatum* (Horak *et al.* 2015).

Rhipicephalus appendiculatus Neumann, 1901, is known as the brown ear tick because they are commonly found on the ears of bovids, wild ungulates, sheep and even dogs. This is a three-host tick species that is capable of completing their entire lifecycle on cattle as host species if abundantly available. The adult ticks are hunters, moving across the ground vegetation towards the suitable host species in the vicinity. After feeding, the engorged females drop into the vegetation and oviposit in a sheltered microhabitat. Larvae, hatching from the eggs will usually attach to the feet, legs, and muzzle of their hosts from where they will seek for a suitable feeding place, feed and drop into the vegetation where they hatch into nymphs. Nymphs will then seek for a suitable host where they can attach themselves to the feet, legs, sternum, groin and neck area of their host animals. Immature stages usually infest smaller host animals, feeding on small antelopes, hares and guinea fowl (Walker *et al*. 2003), but can also infest the same host animals as the adult ticks (Walker *et al*. 2003). In South Africa they are present in foci areas of the Free State, and more abundantly in North-West, Gauteng, Mapumalanga, KwaZulu-Natal and the Eastern Cape provinces (Horak *et al*. 2015). *Rhipicephalus appendiculatus* ticks transmit the veterinary important pathogen *Theileria parva*, causing east Coast fever in bovids as well as various strains of *T. parva* that causes corridor or buffalo disease. This tick species can also serve as vectors for strains of *Theilerai taurotragi* causing bovine theileriosis, *Anaplasma bovis*, causing bovine ehrlichiosis, and *Rickettsia conorii* bacteria causing typhus in humans (Walker *et al*. 2003).

Rhipicephalus decoloratus Koch, 1844, is generally referred to as the blue tick. This is a one-host tick species originally classified as a member of the full *Boophilus* genus (Walker *et al.* 2003). As a one host tick species, it only need to seek and infest a single host in the larval stage, completing its entire lifecycle divided into three stages; larval, nymphal and adult on this host (Walker *et al.* 2003) with only the fed female dropping into the vegetation for oviposition. Blue ticks are very abundant in most parts of southern Africa with a wide range of host species (Equidae, Ovidae & Bovidae). It serves as a vector for the protozoan *Babesia bigemina*, causing babesiosis (redwater) in cattle, *Anaplasma marginale*, causing gall sickness and *Borrelia theileri*, the cause of spirochaetosis in cattle, sheep, goats and horses (Walker *et al*. 2003).

Rhipicephalus evertsi evertsi Neuman, 1897, commonly known as the red-legged tick is widely distributed on livestock throughout South Africa. They have very distinct red-orange legs, beady eyes and heavily punctate scutum. This two-host tick species is commonly recorded on livestock including members of the families Equidae, Bovidae and Ovidae (Walker *et al*. 2003). *Rhipicephalus evertsi evertsi* transmits the piroplasmosis causative protozoans *Babesia caballi* and *Theileria equi* in horses. The bacterium *Anaplasma marginale* can be transmitted to cattle through this vector species, causing gall sickness. Feeding females of this species can produce a salivary toxin causing paralysis in calves and sheep known as spring lamb paralysis (Goppalraj *et al*. 2014).

Hyalomma rufipes Koch, 1844, generally known as the coarse legged tick is commonly found attached to cattle and wild ungulates (Hornok *et al.* 2012). It is the most wide spread *Hyalomma* species on the African continent. It is economically important as a vector of

Anaplasma marginale transmitted to cattle, causing losses in livestock through gall sickness. It can also transmit Crimean-Congo haemorrhagic fever virus in humans (Walker *et al*. 2003).

Hyalomma truncatum Koch, 1844, males have a visually smoother scutum surface when compared to *H. rufipes*. This two host tick species is widely distributed throughout Africa south of the Sahara (Walker *et al*. 2003). The long mouthparts of the ticks and group clustering on the animals' bodies, cause severe tissue damage that results in secondary infections or the formation of abscesses. They can cause lameness when attached to hooves/feet of smaller livestock such as sheep. *Babesia caballi*, the cause of equine piroplasmosis can be transmitted by this tick species (De Waal *et al.* 1990) as well as Crimean-Congo haemorrhagic fever in humans. Lastly specific strains of female ticks from this species can also release a toxin that causes sweating sickness in cattle calves (Walker *et al*. 2003).

Rhipicephalus microplus Canestrini, 1888, a non-endemic tick species in South Africa is commonly referred to as the cattle tick (Walker *et al.* 2003). This tick species, originally from Madagascar, has established itself in southern Africa, after being introduced by cattle imported from south east Asia. This species is a one host tick and can be confused with *R. decoloratus* in appearance, but can be distinguished by 4+4 columns of teeth arrangement on the hypostome compared to *R. decoloratus* that only has 3+3 teeth columns arrangement (Walker *et al.* 2003). These ticks attach to the underline, dewlap, flank and shoulder areas of cattle and wildlife hosts. They can reproduce in larger volumes and faster than *R. decoloratus*, making them successfully competing with *R. decolatus* ticks. This species commonly occurs in savanna climatic conditions with wooded grasslands. They are established in dispersed areas along the eastern and southern coastlines of the Eastern and Western Cape, KwaZulu-Natal, Mpumalanga, Northern provinces and the Free State (Walker *et al.* 2003). *Rhipicephalus microplus* can transmit the protozoans *Babesia bovis* and *B. bigemina* (causing redwater), the bacteria *A. marginale* (causing gall sickness/ anaplasmosis) and *Borrelia theileri* (causing spirocheatosis) (Walker *et al.* 2003). Large infestations will lead to production losses such as decreased weight gain, milk yield and hide damage (Oberem *et al.* 2009)

Additional to ticks, mange mites usually feed on exuding lymph from the bite wounds and dead cells or other debris leading to 'barn itch' with hair loss and skin crusts (Oberem *et al.* 2009). Parasitic mites feed by using their mouthparts and front appendages to dig into the outer epidermal layer of the host's skin, causing damage to the hide of the animal (Fentahun *et al*. 2012). Feeding activity, host immune system response to mite secretions and faecal matter, cause irritation that leads to extensive scratching, scabbing and secondary infection (Oberem *et al.* 2009). Veterinary important mite species in South Africa belongs to the Astigmata and Prostigmata orders. Furthermore, the mite species form part of the two veterinary important families Sarcoptidae and Psoroptidae. Mites from the Sarcoptidae family are obligate parasites that burrow into the skin of mammals, generally affecting the thinly haired parts of the animals' body (Williams 2010). In the family Psoroptidae (non-burrowing mites) there are three genera of veterinary importance, *Psoroptes*, *Chorioptes* and *Otodectes* (Williams 2010).

One of the most common species infecting wild and domesticated animals is *Sarcoptes scabiei* Linneaus, 1758. *Sarcoptes scabiei* var. *bovis* affect cattle worldwide (Williams 2010). *Sarcoptes Scabiei* mites mate on the dermis of a host (neck, tail head and back area) where males keep seeking unfertilized females (Kraalbøl *et al*. 2015). Female mites lay two to four eggs daily during their four to six week lifetime. Eggs are laid inside the epidermal layer of host animals with only about 10% resulting in mature mites. The larvae emerge two to four days after egg deposition, leaving the borrows to feed, molt and then matures in 14 to 17 days (Kraalbøl *et al*. 2015). Transmission between hosts occur with contact between host animals during the free-living nymph and adult stages (Oberem *et al*. 2009; Kraalbøl *et al*. 2015). Mite infestations can lead to alopecia and hyperkeratosis of the skin. *Sarcoptes* species infestations can also be associated with high morbidity and mortality of both wild and domestic host animals (Munang'andu *et al*. 2010).

1.2.2 Diptera (Flies, midges and mosquitoes)

As part of the Diptera order, many veterinary important species are responsible for losses in animal production due to the transmission of pathogens, irritation and painful bites (Oberem *et al.* 2009). The life cycle of these species often relies on the abundance of animal dung present in the environment, playing a key role in nutrient cycling in ecosystems (Aziz *et al.* 2016). House flies, gnats and stable flies are commonly found in a livestock farming environment (Oberem *et al.* 2009). Their populations in the Free State can reach large numbers in the summer months when the temperature and rainfall is at a peak, being associated with outbreaks of diseases such as Rift Valley Fever that lead to economic losses (Aziz *et al.* 2016). Species belonging to the following families are of importance in South Africa: Muscidae, Fannidae, Calliphoridae, Ceratopogonidae, Culicidae, Hippoboscidae, Tabanidae and Simuliidae (Oberem *et al.* 2009).

1.2.2.1 Muscidae

Musca domestica Linnaeus,1758, is generally referred to as the common house fly. Adult flies are 5-8mm long, bearing four longitudinal black stripes on the thorax with soft proboscis (Picker *et al.* 2004; Holm 2008). When conditions are optimum during warmer summer months these flies can complete their lifecycle in seven to ten days with distinct egg, maggot, pupal and adult stages (Sarvar 2016). They have a widespread distribution throughout South Africa leading to both irritation of production animals on farms and spreading of diseases. This fly spreads diseases via mechanical transmission such as brucellosis, eye infections and dysentery (Oberem *et al.* 2009).

Stomoxys calcitrans Linnaeus, 1758, are more or less the same size as the common house fly, but is easily distinguished by a piercing proboscis used to consume blood through piercing the skin of hosts (Picker *et al.* 2004; Holm 2008). The thorax has a grey colour with four longitudinal dark stripes, with a checkerboard pattern on the second and third segments of the abdomen (Picker *et al*. 2004). Both male and female flies are bloodsucking pests of livestock and wildlife most widely distributed in the western hemisphere, including South Africa (Showler *et al.* 2015). Eggs are usually deposited in decaying vegetation that can include manure. High loads of these flies on farms can lead to significant reductions in weight gain and milk production (Oberem *et al.* 2009). Aside from these negative effects, *S. calcitrans* has been identified as a vector of *Trypanosoma brucei* and *Trypanosoma vivax* causing trypanosomiasis or nagana, however, the distribution of these *Trypanosoma* parasites does not match the near cosmopolitan distribution of these flies (Masmeatatship *et al.* 2006). The involvement of *Stomoxys* in the transmission of *Trypanosoma* may be questionable. They can act as intermediate hosts for nematode species such as *Habronema* species commonly infecting horses (Showler *et al.* 2015).

1.2.2.2 Fannidae

Fannia canicularis Linnaeus, 1761, is a small uniform black fly (4-6mm) strongly resembling *M. domestica*. Three dark longitudinal stripes can be observed on the brown/grey thorax (Achiano *et al.* 2005). It is a veterinary important invasive pest species in poultry houses all over South Africa, originating from the northern hemisphere (Picker *et al.* 2011). The presence of these flies can be very irritating to both animals and humans (Achiano *et al.* 2005).

1.2.2.3 Calliphoridae

Chrysomya bezziana Villeneuve, 1914, is a metallic green to blue blow fly, with yellow eyes (Picker *et al.* 2004). The larvae are obligate parasites classified as Old-World screwworms and can accordingly be found throughout sub-Saharan Africa (Oberem *et al*. 2009). They can cause traumatic wound myiasis in both wildlife and livestock host animals in a three-week lifecycle (Obanda *et al.* 2013). Their eggs are laid in a superficial wound or mucous membrane on the animal. Larvae then hatch and borrow into the flesh of the host, where they feed on the living tissues of the animals causing wounds to become infected (Picker *et al.* 2004; Oberem *et al*. 2009).

Chrysomya albiceps Wiedemann, 1819, is a medium sized blow fly species identified by its uniformly metallic green colour, white face patterns and black abdominal bands (Picker *et al*. 2004). They are commonly distributed throughout southern Africa, with first instar larvae feeding mainly on decaying organic matter, in which they are laid as eggs (Verves 2004). Second and third instar larvae are predatory on other dipteran larvae. The larval instars can, however, cause wound myiasis leading to economic losses in livestock (Oberem *et al*. 2009).

Lucilia sericata (Meigen), 1826, known as the common greenbottle blowfly can be identified by the metallic green to blue body colouration and bronze colouration on thorax (Picker *et al*. 2004). Males from this species have three setae behind the eyes from which they can easily be distinguished from other botfly species (Picker *et al*. 2011). They are commonly distributed in temperate and tropical regions of the planet, including South Africa where they are an invasive species introduced from the Holartic region (Picker *et al.* 2011). This species occurs in the central and western parts of South Africa, with a low likelihood of occurring in the northern parts of the country (Williams *et al*. 2014a). These flies are primarily responsible for myiasis in sheep referred to as "sheep strike". They are also opportunistic wound parasites of cattle, sheep, horses and humans (Oberem *et al*. 2009; Choe *et al*. 2016).

Lucilia cuprina (Wiedemann), 1830, or the Australian sheep blow-fly appears to be very similar to *L. sericata*, but can be distinguished from each other by characteristics such as the number of paravertical setulae and the distance between the outer and inner vertical setae of females (Williams *et al.* 2014b). They mainly have a central and eastern distribution in South Africa, but can also occur in the northern parts of the country (Williams *et al.* 2014a). The females lay their eggs on wounds, where the larvae feed on the flesh of the host animal causing myiasis (Oberem *et al*. 2009).

1.2.2.4 Ceratopogonidae

One of the other most prominent veterinary Diptera families is known as Ceratopoginidae. *Culicoides* species biting midges that form part of the Ceratopogonidae family are small blood feeding insects that can impact both humans and animals negatively. There are about 120 *Culicoides* species identified in southern Africa, of which some has been investigated as possible vectors of disease (Meiswinkel *et al.* 2004; Labuschagne *et al*. 2007).

Culicoides bolitinos Meiswinkel,1989, and *Culicoides imicola* Kieffer,1913, are closely associated with livestock and game (Meiswinkel *et al.* 2004). They transmit several different viruses, protozoa and filarial nematodes while feeding on a wide variety of hosts (Oberem *et al*. 2009). *Culicoides* species midges play a role as disease vectors for bluetongue, African horse sickness, bovine ephemeral disease, epizootic haemorrhagic disease, orpouche virus, and Rift Valley Fever (Oberem *et al*. 2009; Lehmann *et al*. 2012). Other less veterinary important *Culicoides* species found in South Africa include *Culicoides magnus*, *Culicoides zuluensis*, *Culicoides gulbenkiani*, *Culicoides pycnostictus*, *Culicoides simillis*, *Culicoides macintoshi* and *Culicoides schultzei* (Meiswinkel 1996).

1.2.2.5 Culicidae

Diptera that belong to the Culicidae family (Anophelinae, Culicinae and Toxorhynchitinae) is commonly referred to as mosquitoes. The subfamily Culicinae includes all the veterinary important species in South Africa. Adult females are specialised for feeding on blood whereas the males feed on plant nectar (Triplehorn *et al.* 2005). Larval stages are restricted to aquatic environments such as stagnant waterbodies (Oberem *et al*. 2009). Culicinae need a water source to be able to deposit their eggs, and as medium for their larvae to mature; such habitats can be found in drinking troths or other water sources in pastures where livestock graze (Triplehorn *et al.* 2005).

The most influential species belonging to the genera *Aedes*, *Culex* and *Ochlerotatus* will show a high preference for livestock during years with heavy summer rainfall, leading to economic losses in the agricultural industry (Oberem *et al*. 2009; Tchouassi *et al*. 2016). These parasitic nematocerans can transmit devastating illnesses in livestock including the arboviral disease Rift Valley Fever, equine encephalitis, and west Nile virus (Jupp *et al.* 1990; Oberem *et al*. 2009; Tchouassi *et al*. 2016).

1.2.2.6 Hippoboscidae

The louse fly family includes both winged and wingless forms. These parasites are however rarely encountered on livestock (mainly on sheep) and is thus of a minimal veterinary importance (Triplehorn *et al.* 2005)

1.2.2.7 Tabanidae

Horse flies are usually uniformly brown/black/yellow in colour with big eyes. Females from this family has large mouthparts adapted to drink blood from a host animal. Males from this family feed on nectar and pollen. Bites from these flies are painful and can result in self harming behaviour of animals. Mechanical transmission of vectors such as bacteria is possible during feeding on a host animal (Triplehorn *et al.* 2005)

1.2.2.8 Simuliidae

The last veterinary important species, black flies, belong to the family Simuliidae. They are considered as pests along the Orange-, Vaal-, Great Fish-, Sundays- and Gamtoos rivers that have structures such as streams, dams with weirs and barrages, irrigation schemes and hydro-electrical plants (Myburg *et al*. 2003; Oberem *et al*. 2009). About 39 blackfly species are known to occur in South Africa. Five members belong to the genus *Paracnephia* and 34 to the *Similium* genus (Palmer *et al.* 1998; Yousseft *et al.* 2008), that contains the following economically important species: *Simulium damnosum sensu lato*, *Simulium chutteri*, *Simulium adersi* and *Simulium nigritarse sensu lato*. Species belonging to the Paracnephia genus are endemic to the southwestern Cape and of a minor veterinary importance in the Free State. These insects can among other pathogens, transmit the filarial nematode *Onchocera volvulus* (causing river blindness) to humans but is restricted to West Africa, simuliotoxicosis (blackfly fever), leucocytozoonosis, bovine onchocercosis and mechanical transmission of Rift Valley Fever (Myburg *et al.* 2003; Picker *et al.* 2004). Other than pathogen transmission and extreme irritation to animals, black fly bites casue painful open wounds on the animals (ears, eyes and udders), that can lead to secondary infection and death (Myburg *et al*. 2003).

Simulium chutteri Lewis, 1965, is considered to be the most significant species of blackfly in South Africa. They can be identified by their distinct humped thorax, specific wing venation, prominent eyes, segmented antennae and biting mouthparts (Yousseft *et al.* 2008). Females require an obligatory blood meal to deposit eggs. This species prefers to deposit their eggs in aquatic environments with fast flowing water such as dam weirs or streams. They generally occur along the Orange-, Vaal- and Great Fish Rivers. They are most abundant during spring and autumn months suggesting that they thrive in moderate weather conditions (Myburg *et al.* 2003).

1.2.3 Phthiraptera (Louse)

Louse belong to the order Phthiraptera, and is further divided into the suborders; Amblycera (biting), Ischnocera (biting), Rhynchophthirina (partly biting), and Anoplura (sucking). The various species classified as biting lice, feed on skin debris that leads to severe skin irritation (Oberem *et al*. 2009). These lice can generally be found on the neck, shoulders, back and rump of the host animals (Howell *et al.* 1978). In contrast, sucking lice pierce the skin and suck out the host's blood, causing anemia when present in large numbers (Oberem *et al*. 2009). The life cycles of these lice are similar: eggs are deposited by the female onto the hair shafts, the nymphs then undergo three molts on the host animal until they are adults. The life cycle generally takes two to three weeks to complete and is dependent on environmental conditions and food availability (Oberem *et al*. 2009). Lice are spread from one animal to another via direct contact (Oberem *et al*. 2009).

Linognathus vituli Linnaeus, 1758, referred to as the long-nosed louse, has piercing mouthparts ideal for sucking blood from host animals. The first lesions usually appear on the shoulders, neck and chest of cattle (Oberem *et al*. 2009).

Damalinia bovis Linnaeus, 1758, known as the red louse is an emerging economically important cattle parasite with biting mouthparts (Oberem *et al*. 2009). These are large enough to be seen with the naked eye on the animals hide. Lesions appear on the shoulders, backline, and tail head of host animals, but can spread to other areas of the body. These lice cause severe irritation, hair loss and weight loss in livestock (Oberem *et al*. 2009).

1.3 HOST ANIMALS

In the study area, mainly three cattle breeds are used for production purposes.

1.3.1 Purebred Brahman cattle

The Brahman breed's meat production ability, has contributed greatly to its success in South Africa. During personal communication sessions, various beef cattle producers have indicated that they believe the Brahman breed has been contributing to the sustainable increase in meat production in South Africa ever since they have been imported ([1]Mr. Sietze Smith, [2]Mr. Pieter Esterhuyze, Mr. [3]Willem Verhoef). These beliefs have contributed that this breed is now regarded in the national herd as the king of crossbreeding (Coetzer *et al*. 2007).

One of the most outstanding qualities of the Brahman is its meat production ability and high ectoparasite resistance, and has contributed greatly to its success in South Africa. An example of their ectoparasite resistance characteristics is their skin secretion (sebum), reported to have ectoparasite repellent qualities (Turner 1980).

Brahman cattle were selected for this study due to this popular belief that they have a certain level of ectoparasite resistance (Turner 1980). As reinforcement for the selection of this breed, it is commonly known that *Bos indicus* cattle and their crosses carry lower

[1] Mr. Sytze Smith, Breed director at Brahman Breeders Association of South Africa, April 2014.
[2] Mr. Pieter Esterhuyze, Producer from EFT Boerdery, March 2014.
[3] Mr. Willem Verhoef, member of Brahman Breeders Association of South Africa, January 2015.

tick loads than pure bred *Bos taurus* cattle (Jonsson 2006). Brahman cattle generally carry one tenth of the number of ticks found on European breeds such as the Hereford in the same environmental circumstances and infestation possibilities (Jonsson 2006). Tick resistance on cattle has also been proven to be a heritable trait, thus tick resistance qualities can be carried over to a following generation within each breed (Morris 2007).

In theory crossbreeding Brahman cattle with other less ectoparasite resistant cattle breeds should be an effective way of increasing tick resistance in the crossbred generations. In previous studies, both pure bred Brahmans and Brahman x Hereford were found to be more resistant to ticks when compared to pure bred Hereford cattle (Turner 1980). It was also found that the production losses due to *R. microplus* engorgement were 0.6g of live weight per engorged female tick in Brahman cattle and up to 1.5g by Brahman x European crossed cattle in Australia (Mattioli *et al.* 2000). Furthermore, the production weight loss of indigenous zebu type cattle associated with one engorged *A. variegatum* female ranged from 46g to 63g of live weight which is considerably more than that lost in Brahman cattle under similar environmental conditions (Mattioli *et al.* 2000). Infestations by male as well as immature stages, although to a lesser extent contributes to a reduced meat and milk production (Mattioli *et al.* 2000).

The modern type Brahman was bred in the Gulf area of the southern United States between 1854 and 1926. Brahman cattle was imported to Africa from America in 1945 (Coetzer *et al*. 2007). This was done with the goal of implementing breeding schemes by introducing cattle into South Africa that were both parasite and heat tolerant, utilises low feed intake, has high fertility ratings and at the same time produces good quality meat. It is possible that the indigenous cattle breeds in South Africa did not produce the same quality and meat yield at the time. The South African Brahman Breeders Association was founded in 1957 and accreditation from SA Studbook was received in March 1958 (Coetzer *et al.* 2007).

The Brahman breed quickly caught the attention of other South African cattle owners that wanted to improve beef production in harsh environmental conditions. Today, both grey and red colour variations of the Brahman breed is well established in South Africa and they are in demand for crossbreeding purposes (Coetzer *et al*. 2007).

1.3.2 Purebred Sussex cattle

The Sussex breed is one of the oldest cattle breeds in South Africa. The breed was developed in England, were they were used as draught animals. Only animals with suitable strength and temperament were selected to draw wagons. These animals had to survive harsh European winter conditions, with scarce fodder. British producers bred these cattle to dominate the export market with good quality meat, as this breed offers fine grained beef with an even fat covering and internal marbling (http://sussex.co.za/sussex-breed/history-of-the-sussex-breed/).

Another valuable breed trait is high fertility. Sussex bulls are known to have a good libido rate, hardiness and higher than average post weaning growth. Cows consistently achieve high conceiving rates, with an average of 90% calving rate (http://sussex.co.za/sussex-breed/history-of-the-sussex-breed/). Sussex cows are good mothers, with high butterfat content in the milk that leads to higher weaning weights and relative early maturity. The early selection for temperament makes them easy to handle, and are sought after by feedlots ([4]Mr. Pieter Esterhuyze).

The breed was imported into South Africa in 1903-1909 to produce more "stocky" built cattle when compared to South African indigenous breeds. They were distributed through the country in both hot and cold areas, where they were used for crossbreeding purposes to improve the national herd concerning both meat quantity and quality (http://sussex.co.za/sussex-breed/history-of-the-sussex-breed/). An example where *Bos indicus* cattle are effectively crossed with this *Bos taurus* breed in South Africa is between Sussex and Afrikaner cattle, where the Afrikaner's breed characteristics generally provide more heat and insect tolerance to the crossbred generation than the Sussex cattle breed characteristics (http://sussex.co.za/sussex-breed/history-of-the-sussex-breed/).

1.3.3 Brahman x Sussex cattle

The crossbred cattle used in this study are the result of Brahman (*B. indicus*) cattle cross bred with Sussex cattle (*B. taurus*). The crosses are usually 50% Brahman and 50% Sussex. *Bos indicus* x *Bos taurus* crosses are generally favoured by feedlots for their growth abilities as well as their low inset cost due to heat and parasite resistance (Bonsma

[4] Mr. Pieter Esterhuyze, Producer from EFT Boerdery, June 2014.

1980). They perform well as grass fed animals, with satisfactory production in terms of milk yield, fertility, as well as meat quality and quantity. These crossbred cattle ensure the profitability of a successful commercial beef enterprise in South Africa that depends on both productivity (growth) and a maternal component (reproduction and milk production) (Schoeman 1996).

Mr. P. C. Esterhuyze started crossbreeding Brahman bulls with Sussex heifers in northern Natal in 1988. The main reason for this particular crossbreeding combination is that the F1 crossbred generation performs very well as slaughter animals in South African feedlots and are commonly bred as a *B. indicus* and *B. taurus* type crossbreed by many beef producers throughout the country (https://www.farmersweekly.co.za/farm-basics/how-to-livestock/choosing-a-breed/). They have a docile temperament like the Sussex breed, fast growth factor, good quality meat, high fertility rates and resistance qualities to heat and parasites like the Brahman breed (http://sussex.co.za/sussex-breed/cross-breeding-kruisteteling-met-sussex-bul/jersey-sussex-cross-breeding/). These cattle bred by Mr. Esterhuyze performed very well under stressful environmental circumstances such as snow during winter, very warm summers and very high parasite loads in Natal.

During 2004 the Esterhuyze family moved to the Kroonstad area in the Free State, choosing to continue to farm with Sussex and Brahman stud herds and crossbreeding them with the goal of producing high quality commercial slaughter animals. This combination proved to be successful in the Free State environment ([5]Mr. Pieter Esterhuyze).

1.4 AIM OF STUDY

The focus of sustainable profitability on any cattle farm is on monetary gain but also on production of a quality product while being environmentally conscious. For this reason, clearly defined crossbreeding structures need to be adopted on both commercial and subsistence cattle farming systems aimed at exploring the best qualities of different cattle breeds as a measure towards ectoparasite control.

[5] Mr. Pieter Esterhuyze, Producer from EFT Boerdery, June 2014

In this study, the aim was to determine if Brahman cattle have natural ectoparasite resistance due to certain breed characteristics when compared to other cattle breeds such as Sussex. This was done by comparing phenotypical traits such as skin characteristics, hair characteristics, tail length and body temperatures with ectoparasite loads. A further aim was to establish if the resistance qualities identified are preserved in the crossbred generations.

1.5 OBJECTIVES OF STUDY

In order to achieve the aim of the study the following objectives were set:

- To compare abundance of ectoparasites on the three selected cattle breeds. **Null hypotheses:** All the selected cattle breeds have the same abundance of ectoparasites over the entire sampling period.

- To investigate if there is a correlation between on-host and off-host ectoparasite diversity and abundance. **Null hypotheses:** There is no positive correlation between on-host and off-host ectoparasite diversity and abundance.

- To determine if there is a correlation between weather conditions recorded during the test period and (on-host and off-host) ectoparasite abundance. **Null hypotheses:** There is no correlation between ectoparasite abundance sampled (on-host and off-host) and weather conditions.

- To establish if there is a correlation between the body scores recorded for the animals and the abundance of ectoparasites sampled in the on-host environment. **Null hypotheses:** There is no correlation between body scores recorded for the animals and abundance of ectoparasites sampled in the on-host environment.

- To determine if one or more of the selected breeds are prone to have more tick attachment sites than the other cattle breeds in this study. **Null hypothesis:** There is no difference between the number of tick attachment sites for the selected breed in this study.

- To investigate if there is a difference in skin and hide characteristics recorded between the different cattle breeds. **Null hypothesis:** There is no difference in skin and hide characteristics between the different cattle breeds.

- To determine if there is a positive correlation between average breed weight and ectoparasite load. **Null hypothesis:** There is no correlation between average breed weight and ectoparasite load.

- To determine if there is a positive correlation between average body temperatures recorded for the different breeds and ectoparasite load. **Null hypothesis:** There is no positive correlation between average breed body temperature and ectoparasite load.

- To determine if there is a difference in average tail length recorded between the breeds that might aid in fly repelling. **Null hypothesis:** There is no difference in the average tail length recorded between the different breeds that might aid in fly repelling.

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CHAPTER 2

MATERIALS AND METHODS

INTRODUCTION

The study was performed in an extensive production system where environmental variables such as parasites, disease, climatic conditions and nutrition cannot be closely controlled. This chapter describes the materials and methods used in this study to determine, on-host and off-host ectoparasite infestation of Acari, Diptera and Phthiraptera on the cattle from the host breeds, Brahman, Brahman x Sussex and Sussex as well as in the grazing. The cattle herds were followed and inspected on a monthly basis over 13 months as they were moved between different camps on different farms in the Kroonstad region. Methods to determine host characteristics that could influence host resistance, are also described as well as the means in which climatological information and field evaluations were obtained.

Farms and camps where animals grazed were not pre-determined by the study protocol but by the producer according to his farming practises, which were again determined mainly by food availability in the different camps during different seasons throughout the year.

2.1 STUDY AREA

2.1.1 Farms

The study sites were located in the central Free State, about 30km west from the town Kroonstad. The cattle were kept on and moved between the seven different farms indicated in Figure 2.1:

1. Blanquilla (S27° 39.458’, E027° 04.967’) -785ha.
2. Border (S27° 32.134’, E026° 53.393’) -312ha.
3. Hamiltonsrus (S27° 41.399’, E027° 03.319’) -583ha.
4. Theron A. (S27° 38.399’, E027° 05.261’) -517ha, Theron B-517ha.
5. Toggekry (S27° 40.799’, E026° 53.530’) -193ha.
6. Tweeloop (S27° 39.089’, E026° 57.500’) -268ha.
7. Welgegund (S27° 40.799’, E026° 53.530’) -249ha.

These farms were all located within 15 km of each other and are therefore all exposed to similar habitat types as well as rainfall and temperature conditions. The central Free state is an important beef cattle production area because it is also a very significant maize production region in South Africa. Therefore, high quality winter feed is available in the form of maize residue on various farms in the region. Various game farms are also located in the Kroonstad district. From a total of 13.4 million cattle in South Africa, 2271 is found in the Free State region as estimated in 2017. (http://www.daff.gov.za/Daffweb3/Portals/0/Statistics%20and%20Economic%20Analysis/Statistical%20Information/Abstract%202017.pdf).

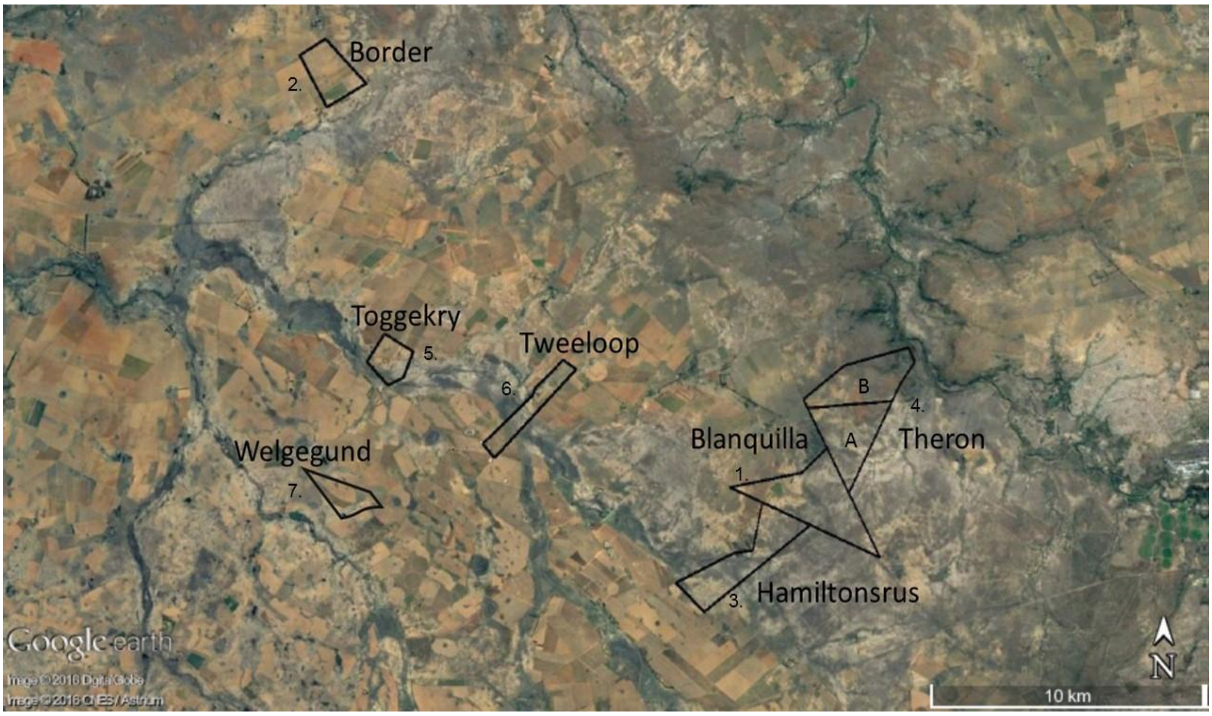


Figure 2.1: Google earth image of the seven farms where animals grazed from March 2014 to March 2015 in the Kroonstad district (Courtesy of Mr. Piet Human, local land surveyor).

2.1.2 Camps

An Etrex, Legend Cx, Garmin GPS device was used to record the coordinates for the camps where the different cattle groups were moved to. Coordinates were recorded as decimal degrees and decimal minutes to be compatible with google maps formatting. The coordinates obtained by the GPS device were searched in google and marked on the map. The camps of the different farms on which the animals grazed during the observation period are indicated in Figure 2.2.

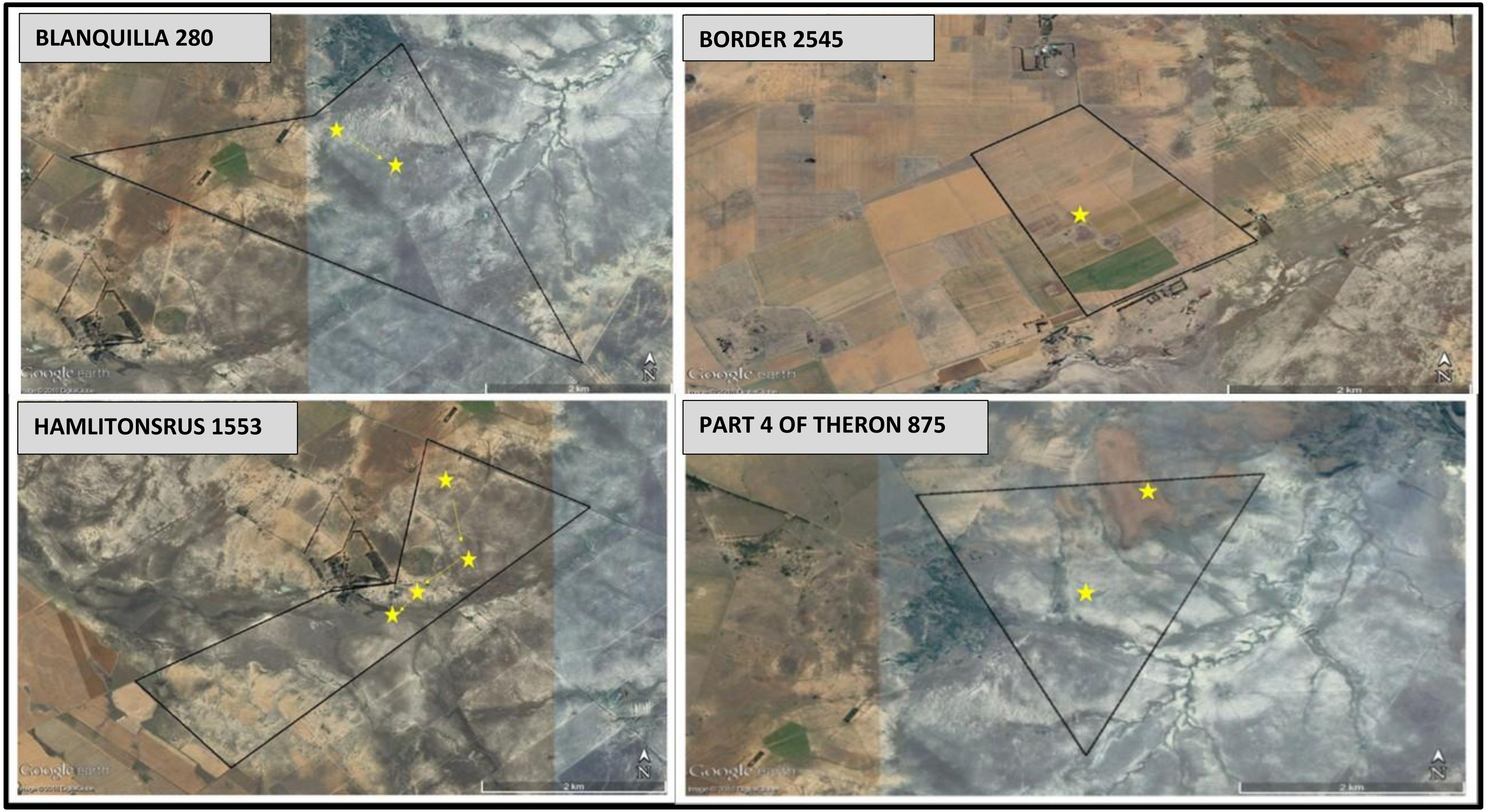


Figure 2.2: Farm Blanquilla, Border, Hamiltonsrus and Theron A in the Kroonstad district, with the stars indicating the camps on the farm that was used during the study period from March 2014 to March 2015.

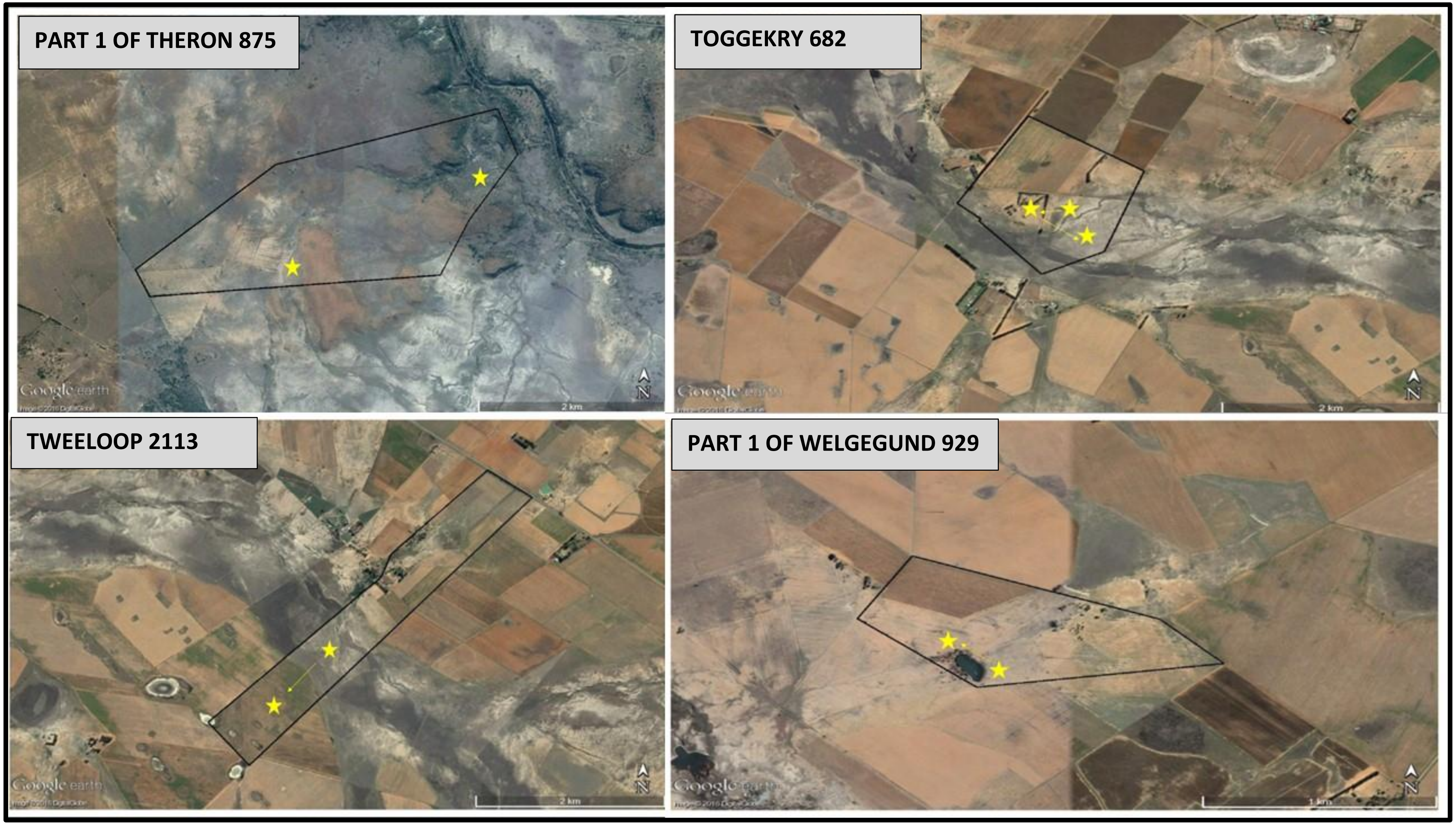


Figure 2.2 continued: Farm Theron B, Toggekry, Tweeloop and Welgegund, in the Kroonstad district, with the stars indicating the camps on the farm that was used during the study period from March 2014 to March 2015.

2.1.3 Vegetation type

The influence of vegetation cover and quality on both the cattle and ectoparasite population dynamics is important as spatial and temporal environmental conditions do have an effect on parasite survival and infestation on livestock.

The latest description of the Free State grassland biome done by Van Oudtshoorn in 2015 is shown in Figure 2.3. This report indicated four different types of biomes in the Free State including; grassland (72% of the province), Nama Karoo (22% of the province), Savanna (5.95% of the province) and forest (0.05% of the province). The study areas were located in the grassland biome (Van Oudtshoorn 2015).

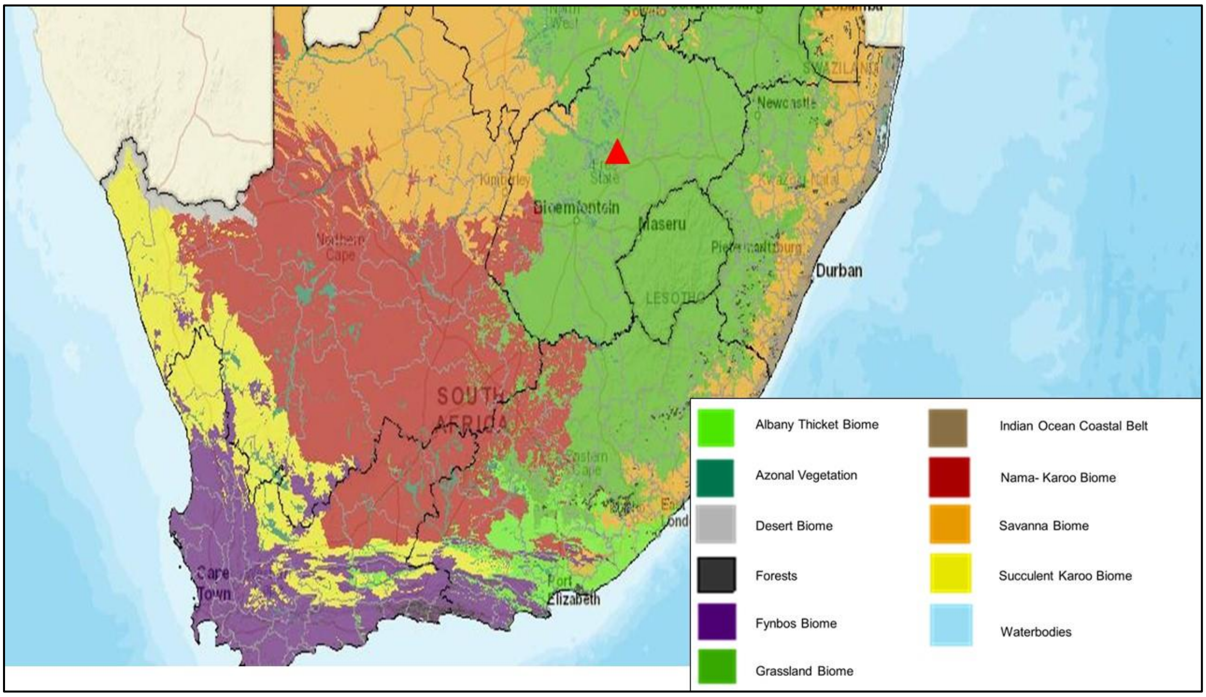


Figure 2.3: The Kroonstad area where the study was done is indicated by the red triangle, in the grassland biome of South Africa (https://www.sanbi.org/biodiversity-science/foundations-biodiversity/national-vegetation-map).

The grazing quality of the grass species on the farms Blanquilla and Yvonne as determined with the wheelpoint method (Roux 1963) feeding value, palatability, growing vigour, and digestibility are indicated in Table 2.1 (Mr. B. Luyt unpublished data). Grass species were identified as part of four ecological status groups; decliners, increaser I, increaser II and increaser III as described by Van Oudtshoorn (2002).

Although the primary focus of his study was on these two farms, the vegetation type on all the farms included in the current study, is regarded as being visually homogeneous to the investigated farms and therefore regarded as being representative of the study area observed in the host parasite interactions (Figure 2.4). The only exception is the farm Border where cattle grazed during the winter months on residue maize. Border is primarily used for maize production, therefore the amount of natural vegetation growth is limited.

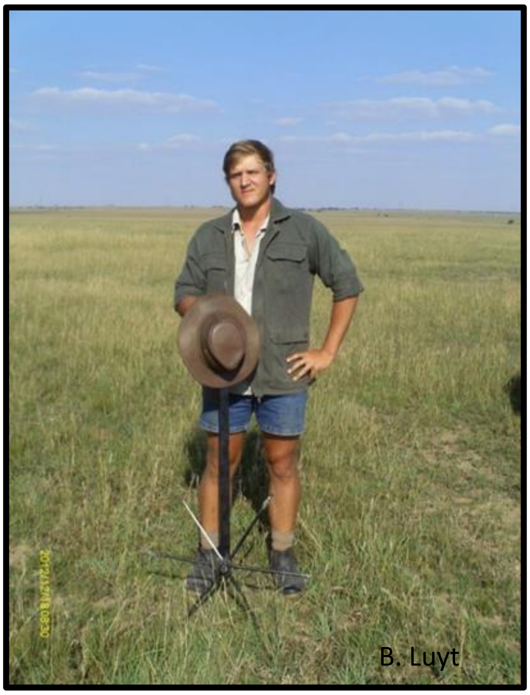


Figure 2.4: [1] Mr. Bertiaan Luyt collecting vegetation samples on Blanquilla representing the majority of vegetation type on the various farms in this study.

[1] Photo taken by Mr. B. Luyt, Kroonstad, March 2015.

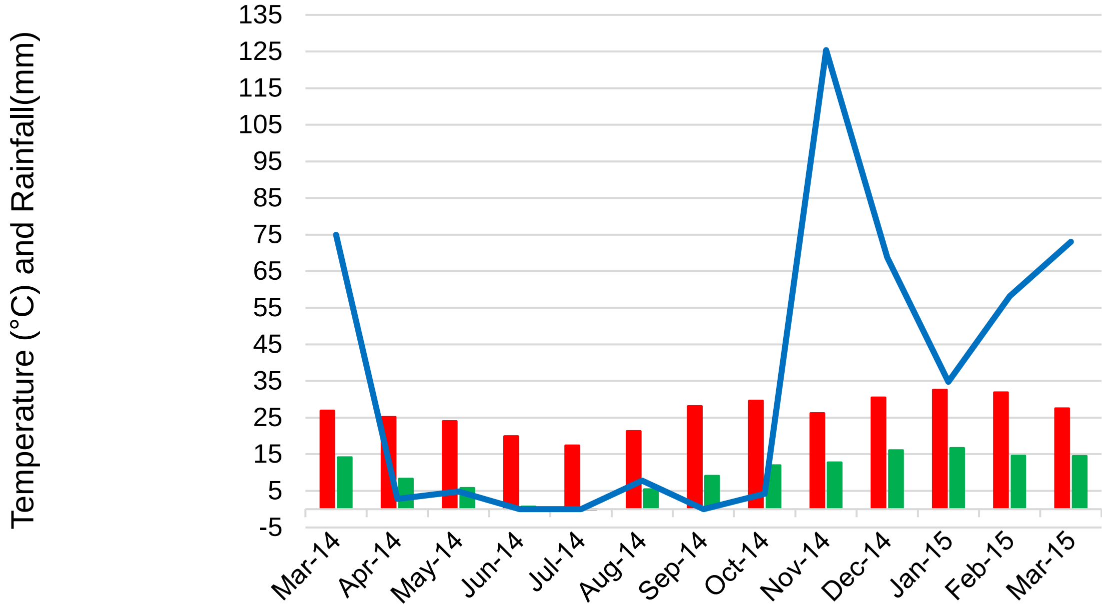
Table 2.1: Grass species identified on the farms Blanquilla and Yvonne in the study area ([2]B. Luyt. unpublished data).

Species	Grazing value	Succession	Ecological status
Aristida canescens	Low	Sub climax grass	Increaser II
Aristida congesta subsp. congesta	Low	Pioneer grass	Increaser II
Aristida diffusa	Low	Pioneer grass	Increaser II
Brachiaria euruciformis	Moderate	Pioneer grass	Increaser II
Chloris virgata	Moderate	Pioneer grass	Increaser II
Cymbopogon excavatus	Low	Climax grass	Increaser I
Cymbopogon plurinodis	Low	Climax grass	Increaser I & III
Cynodon dactylon	High	Pioneer grass	Increaser II
Digitaria argyrograpta	High	Climax grass	Decreaser
Digitaria eriantha	High	Climax grass	Decreaser
Echinochloa holubii	Moderate	Sub climax grass	Increaser II
Elionurus muticus	Low	Climax grass	Increaser III
Eragrostis bicolor	Moderate	Sub climax grass	Increaser II
Eragrostis chloromelas	Moderate	Climax grass	Increaser II
Eragrostis lehmaniana	Moderate	Climax grass	Increaser II
Eragrostis obtusa	Moderate	Sub climax grass	Increaser II
Eragrostis obtusa	Moderate	Sub climax grass	Increaser II
Eragrostis plana	Low	Sub climax grass	Increaser II
Eragrostis superba	Moderate	Sub climax grass	Increaser II
Eragrostis trichophora	Moderate	Sub climax grass	Increaser II
Heteropogon contortus	Moderate	Sub climax grass	Increaser II
Microchloa caffra	Low	Pioneer grass	Increaser II
Panicum schinzii	High	Pioneer grass	Increaser II
Setaria incrassata	High	Climax grass	Decreaser
Setaria sphacelata var. torta	Moderate	Sub climax grass	Decreaser
Themeda triandra	High	Climax grass	Decreaser
Tragus berteronianus	Low	Pioneer grass	Increaser II
Urochloa panicoides	Low	Pioneer grass	Increaser II

[2]LUYT, P. 2016. Weidingsleer. Unpublished data submitted as partial completion of requirements to obtain Diploma in mixed Agriculture, Agriculture College, Potchefstroom.

2.1.4 Temperature and rainfall

The study area was situated in a summer rainfall region that can be prone to drought. Temperature and rainfall data were obtained from the SA Weather Services via the Agricultural Department of the University of the Free State. Information was provided by the nearest weather station, (Skoonspruit weather station; -27.628; 26.594 >1285m above sea level). Data for the test period, March 2014 to March 2015, is shown in Figure 2.5.



	Mar-14	Apr-14	May-14	Jun-14	Jul-14	Aug-14	Sep-14	Oct-14	Nov-14	Dec-14	Jan-15	Feb-15	Mar-15
Mean maximum temp (°C)	26.9	25.2	24	19.9	17.4	21.3	28.1	29.6	26.2	30.5	32.6	31.9	27.5
Mean minimum temp (°C)	14.2	8.3	5.8	0.7	-0.1	5.4	9.1	12	12.8	16.1	16.7	14.6	14.5
Total rainfall(mm)	75	2.8	4.8	0	0	7.8	0	4.2	125.4	68.8	34.8	58.2	73

Figure 2.5: Mean maximum and minimum temperature (°C) per month and total rainfall (mm) recorded for the test area from March 2014 to March 2015 (courtesy of Skoonspruit weather station).

2.2 HOST ANIMALS

2.2.1 Breeds

Host animals observed consisted of Grey- and Red purebred Brahman cattle, Brahman x Sussex crossbred cattle and Sussex purebred cattle. All animals used in the study were reared in the study area to minimise the effect of adaptation of the host animals to the environment. Twenty animals from each breed was inspected each month (Grey Brahman, Red Brahman, Brahman x Sussex, Sussex). Purebred cattle herds included in this study were confirmed to be registered stud animals or part of a stud animal group to ensure trueness to each represented breed selected. The Sussex cattle were bought from Sussex stud breeders between 1990 and 2000, but the group was not registered as a different stud due to logistical reasons. However, all the Brahman cattle are currently registered at Studbook and the Brahman Breeders Association as part of the Top Gene Brahman herd managed by Esterhuyze Family Trust (Bpk). Ethical approval for the study was granted according to the animal protocol for Zoology and Entomology projects at the University of the Free State (Confirmation code for approval: UFS-AED2015/0003). The genus and species names of hosts used in the study, are those described by Heindleder *et al.* (2008).

2.2.1.1 Grey- and Red purebred Brahman cattle

Brahman cattle is light grey to dark red or spotted coated humped cattle (zebu type), with dark pigmented skin, loose skin around the brisket area and a recognisable sebum secretion produced from their skin glands (Figure 2.6).

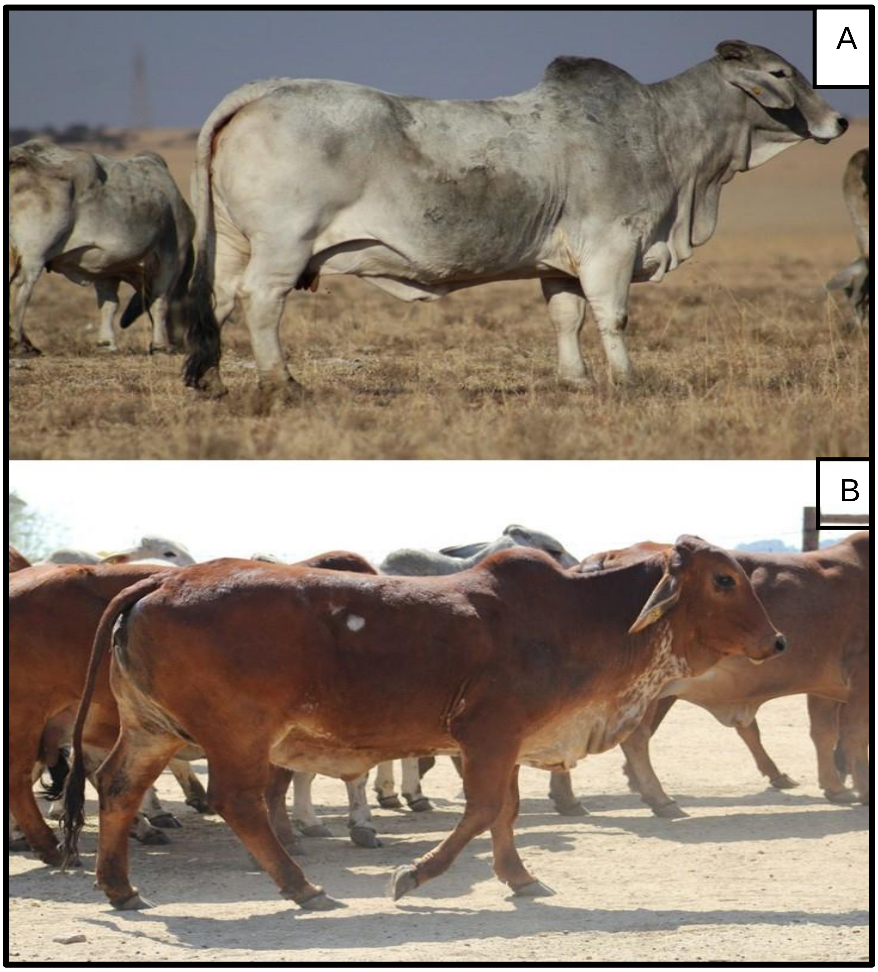


Figure 2.6: A: Grey Brahman cow, B: Red Brahman cow.

2.2.1.2 Brahman x Sussex crossbred cattle

These cattle strongly resemble the Brahman body type, but carry more meat. Crossbred cattle are generally brindle or tiger striped cattle representing brown or fawn colouring with lighter or darker streaks throughout (Figure 2.7).

Figure 2.7: Brahman x Sussex crossbred cow.

2.2.1.3 Sussex purebred cattle

This breed is medium frame, dark-red coated cattle with a white tail switch. These animals have a typical taurine body shape with a thick coat and a docile temperament (Figure 2.8).

Figure 2.8: Sussex cow.

2.2.2 Criteria for inclusion and sample size

Age dependent selection criteria was used to choose animals within each cattle breed (Brahman, Brahman x Sussex, Sussex). Only adult females over the age of 36 months were included in the study. At this age, the female animals are all physiologically developed and can be mated with a bull to produce offspring. They were all subjected to the same breeding program synchronising the majority of their hormonal cycles to the breeding program.

Observations of each breed group were done during the last week of each month. If the weather conditions did not allow for inspections to take place, it was moved forward with a week. This happened only once during the study at the end of June 2014 and the observations were moved to the beginning of July 2014. On each inspection day, all female animals from a specific production group that fit the criteria were selected into the kraal and from these animals, the first ten to enter the crunch were chosen as the group representatives for sampling on that specific day. This caused animals of each breed in a production group to not necessarily be the same for each inspection cycle. The cattle breeds in a kraal might also be mixed due to mixed production groups, for example crossbred animals and red Brahmans where in the same production group (group 4) causing them to be handled in the same kraal during inspection. When each individual was securely restrained in the crush and neck clamp, all of the body regions indicated in Figure 2.9 was inspected for ectoparasite infestation and body scoring.

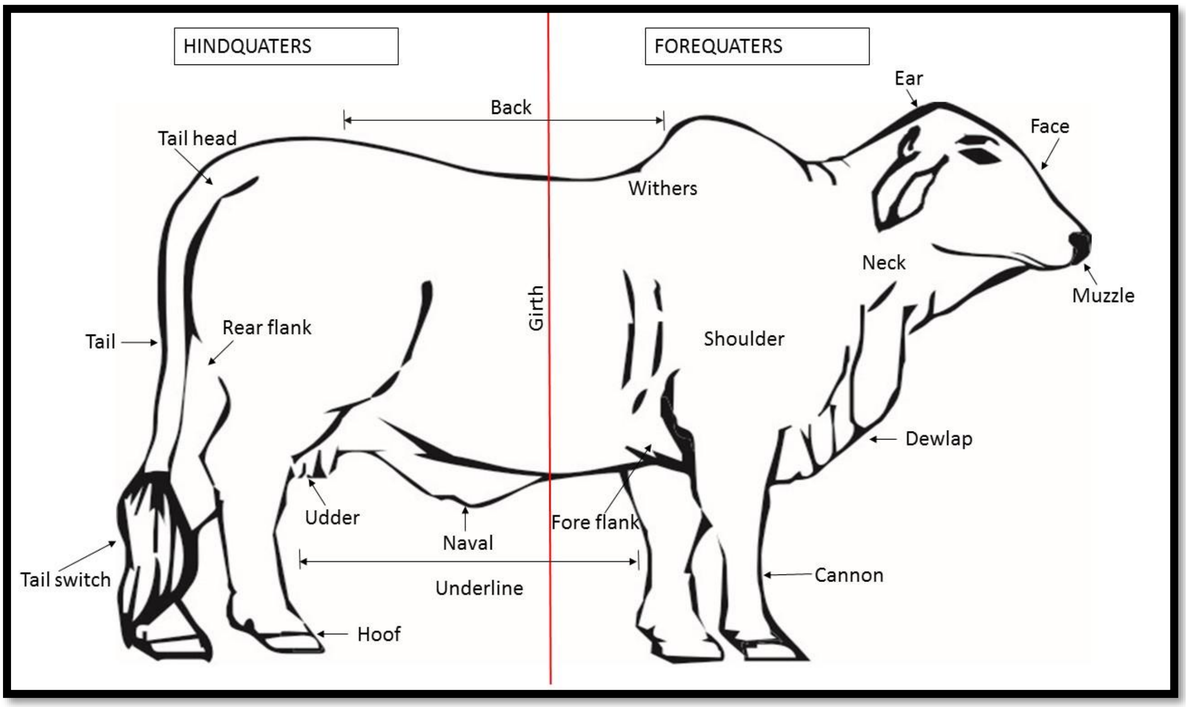


Figure 2.9: Anatomy of differential body regions of a cow (Sketch drawn by Marilie Esterhuyze).

2.2.3 Body condition scoring of cattle

A body condition score was given to each individual animal during every inspection to determine the well-being of the animal based on the amount of body fat that the animal carried. A quick and effective method to determine body condition was adapted from the American body condition scoring system (Table 2.2). The scores were determined by feeling for fat cover as well as visual confirmation. This was done on the following areas of the animals' body; back bone (spine and topline), short ribs, hip bones (hooks and pins) and tail head.

Table 2.2: Adapted version of the American body condition scoring system for cattle (http://www1.agric.gov.ab.ca/$department/deptdocs.nsf/all/beef8822).

Score	General description
Thin	
1	The vertebrae along the top line are prominent. No abundant muscle tissue. Individual vertebrae can be felt, as well as individual ribs.
Optimum	
2	Increased fat cover over ribs. Muscle tissue maximum or nearing maximum. Obvious fat deposits behind the front shoulder. Tail head is slightly rounded and feel spongy when palpated.
Fat	
3	Very fleshy squared appearance, due to excess fat over back, tail head and hindquarters. Individual short ribs cannot be felt even with firm pressure. Mobility of animal may be impaired

2.2.4 Body weight

Average breed weight was determined to be able to correlate ectoparasite load with body size. Ten animals from each breed were weighed on one occasion at the end of the study period, to make an estimation of mean weight for each breed. This mean weight was required to even out the difference in body weight when comparisons were made in total ectoparasite abundance on the breeds. Each animal was weighed in kilogram using a Micro T7E scale by encouraging her to move forward in the crush until climbing on the scale. The animal was then left to stand still on the metal plate while the weight, displayed on a digital screen, was recorded (Figure 2.10) and documented on a weight data document sheet (Annexure 2).

Figure 2.10: Weighing animals using a digital scale.

2.2.5 Selected ectoparasite resistance characteristics in cattle: Sample collection

Certain breed characteristics such as hair length and density, skin thickness, tail length and rectal temperature were previously described as characteristics used for ectoparasite resistance for specific breeds (Jonsson *et al.* 2014; Mubi *et al.* 2014). To be able to compare the effect of these characteristics on ectoparasite loads, the following breed characteristics were assessed during this study: hair characteristics, skin characteristics, tail length, and body region of ectoparasite collection as well as rectal temperatures.

2.2.5.1 Hair characteristics

2.2.5.1.1 Hair structure

Hair from ten animals of each cattle breed was collected on two occasions, once in winter (July 2014) and once in summer (January 2015). Hair was sampled between the 13th and 14th rib nearest to the spinal column of the animal. The hair was pulled out of the animals' body by grasping it with the blunt side of a scalpel and quickly pulling it directly away from skin in one smooth action to ensure the roots were still intact. The hair samples were placed into individual paper envelopes and labelled with Animal ID, date and location.

A simple hair structure grading test as described by Bonsma (1980), was done with all hair samples on site (Figure 2.11). Each sample was placed in the palm of the left hand and moistened slightly with water. The hair was then rubbed between both hands; if it felted into a tight mass, the animal was classified as having a wooly coat, and will never become sleek coated (Bonsma 1980). The hair samples of sleek coated animals do not felt when moistened and rubbed between the palms.

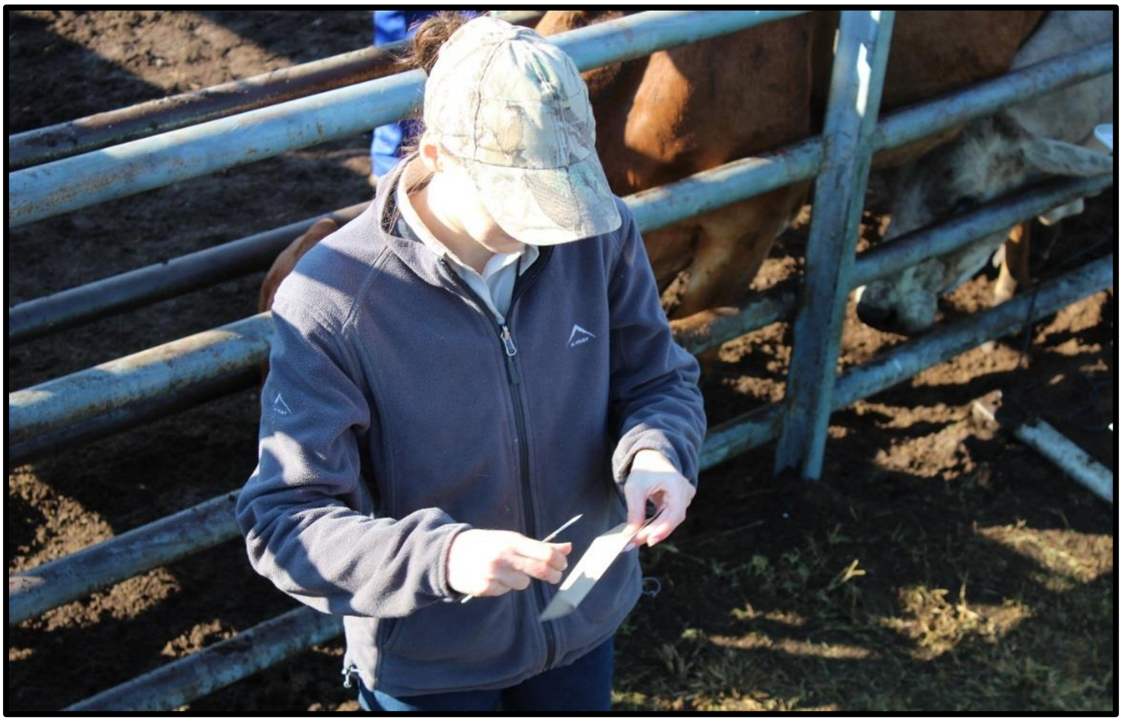


Figure 2.11: Rubbing hair samples between fingers to determine coat type.

Hair samples were also prepared for inspection under a SHIMADZU SSX-550 Superscan, scanning electron microscope to determine if there is significant difference in hair structure concerning hair thickness and scale pattern. Four hairs from 5 animals of each group were inspected. Measurement of hair width was made under 1000 x magnification utilizing 3 different locations on each hair to determine a mean diameter. Between scale measurements was made by measuring from the end of one scale margin to the beginning of another for a total of 8 scales on one hair to determine mean scale width at 3000 x magnification. These measurements were made with the SHIMADZU SSX computer program after inspection through the SEM, in μm and processed in SPSS to identify any significant differences between the groups.

2.2.5.1.2 Hair colour

Coat colours of each breed were classified as described by Foster *et al.* (2009) where coat colours were scored according to absorption rates to be: white, grey, yellow-fawn, light-red, red, dark-red, brown, dark-brown and black. The coat colours of the study breeds fell into the following groups as indicated by Figure 2.12 A: Grey Brahman (grey-white), B: Red Brahman (red), C: Brahman x Sussex (brindle-fawn), Sussex (dark-brown).

Figure 2.12: Hair colour chart adapted from Foster *et al*. 2009. A: Grey Brahman (grey-white), B: Red Brahman (red), C: Brahman x Sussex (brindle-fawn), Sussex (dark-brown).

2.2.5.2 Skin characteristics

2.2.5.2.1 Skin thickness and structure

Once-off single skin biopsies of 1cm x 1cm were taken from ten animals per breed (Grey Brahman, Red Brahman, Brahman x Sussex, Sussex) by a qualified veterinarian, [3]Dr. Danie van Zyl (Figure 2.13) during the January 2015 inspection cycle. The location where the samples were taken (between 13th and 14th rib) was numbed prior to the biopsy, where after a stitch was inserted to close the incision made followed by necessary wound treatment. The skin specimens were preserved in 4% buffered formalin inside plastic

[3] Dr. D. Van Zyl, Kroonstad Animal Hospital, January 2015.

screw cap containers labelled with the Animal ID, date and location. The containers were transported to the Veterinary Entomology laboratory, where they were stored until being delivered to Idex Laboratories in Pretoria, South Africa for further preparation.

Figure 2.13: Skin samples of a grey Brahman cow being taken by Dr. Danie van Zyl, Kroonstad Animal Hospital (A to D) during January 2015.

The specimens were prepared for histological analysis by Idex Laboratories and stained with hematoxylin and eosin. Slides were then inspected in the microscopy unit of the University of the Free Sate with a Nikon Eclipse TE2000-E microscope under 10 x magnification and 40 x magnification to determine if any morphological differences between the breeds are apparent. A Zeiss 475022 stereo microscope was used to count the number of hairs on a 0.5cm x 0.5cm skin sample and to measure the length (in mm) to determine average hair density and hair length of the cattle groups.

Skin thickness of all the breeds were determined on two occasions during inspections to evaluate breed related differences (June 2014 and January 2015). The measurements (cm) were taken by the same person each time, using a metal skin caliper placed between the 13th and 14th rib area. The skin was pinched and the fold placed between the calipers “arms”. The skin-fold was then gently squeezed between these “arms” until an accurate measurement could be taken (Gregory *et al*. 1998). Results were recorded on a data document sheet and later condensed in excel to obtain the average skin thickness for each breed.

2.2.5.2.2 Skin colour

Skin colour was described by means of pigment presence within epidermis cells and the colour was classified as light or dark from biopsy samples.

2.2.5.3 Tail length

Tail length (cm) was measured on one occasion (March 2014) from the base of the tail head to the tip of the tail switch to determine if there is a difference in tail length between the breeds that could serve as an advantage in fly repelling behaviour. This was done during the monthly inspection when animals were secured in the crush.

2.2.5.4 Body regions identified for ectoparasite inspection

The bodies of the animals were divided in to five specific regions for ectoparasite inspection as indicated by Figure 2.14. During each observation month, each of these regions were thoroughly checked for the specific ectoparasites.

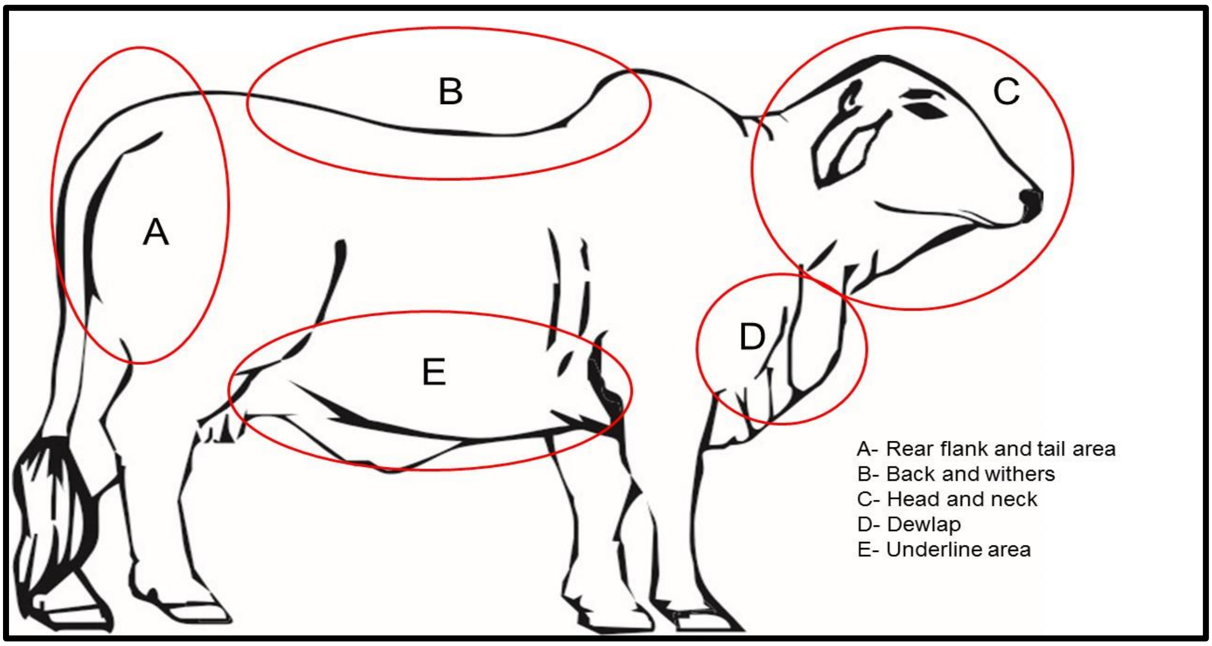


Figure 2.14: Body regions inspected for ectoparasites during inspection (Sketch drawn by Marilie Esterhuyze).

2.2.5.5 Rectal temperature

Average rectal temperatures of each breed were determined by recording the temperatures of 10 animals of each breed on a once-off basis. Each animals tail was lifted up and held to the side while secured in the crush. A digital thermometer was gently placed into the rectum of the animal and left there until the device indicated an accurate temperature was taken by emitting a beeping sound (Figure 2.15).

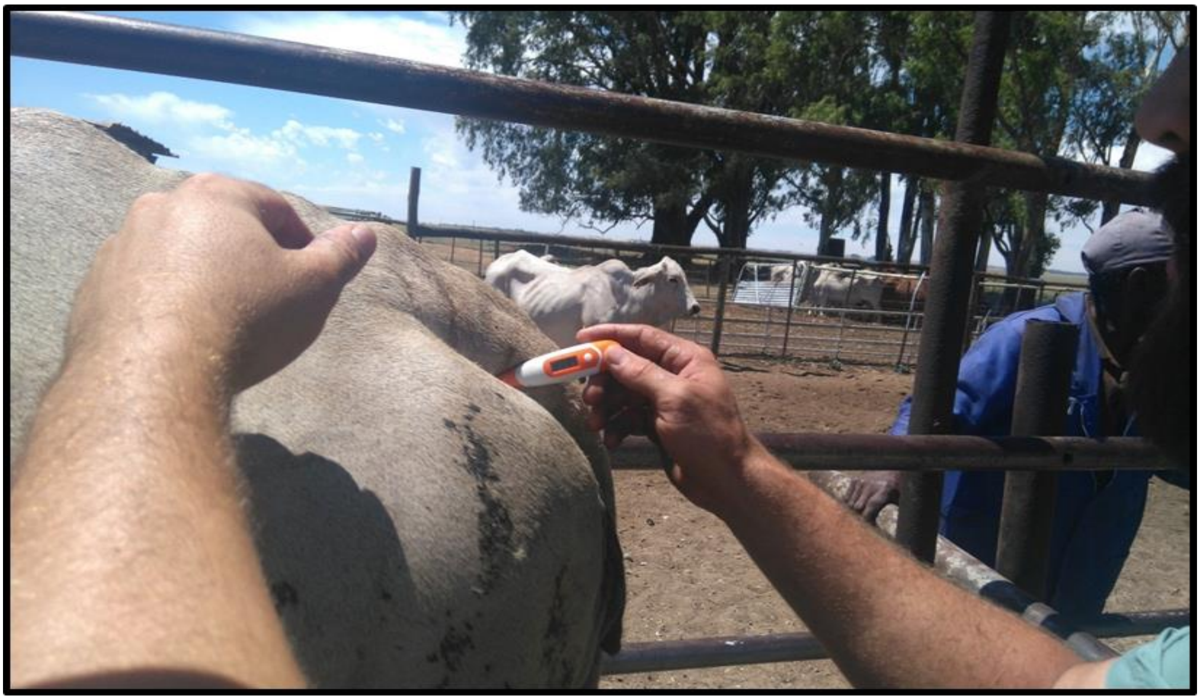


Figure 2.15: Digital thermometer inserted by cattle handles recording a cow's rectal temperature.

2.2.6 Treatment regime

The control program for parasite and disease treatment on the cattle was followed as instructed by the producer, Mr. P. C. Esterhuyze of Esterhuyze Family Trust Boerdery (Bpk). In the event of clinical cases of tick-borne and other diseases being reported it was recorded, as well as the treatment regime followed for such infected animals. Table 2.3 summarises the ectoparasite treatments throughout the study period.

2.2.6.1 Ectoparasite treatments

The following products were used by the producer as part of the routine dipping schedule to control ectoparasites for each production group during the study period.

- Ectoshield (Cipla-Agrimed)

Actives: Cypermethrin 1,5% m/v, Amitraz 1,75% m/v.

Description: Ectoparasiticide used as a pour-on dip. Two actives can be identified as Cypermethrin and Amitraz, for the effective control of flies, ticks and lice. Withdrawing period for milk is not necessary.
Dosage: 10mℓ Ectoshield per 100kg live mass.

- Coopers Tick Grease (Afrivet)

Actives: Delthamethrin 0.1%m/m, Piperunyl butoxide 0.5% m/m.
Description: Commonly used as patch/localized area treatment for the control of ticks at infestation site. No meat or milk withdrawal period required.
Dosage: Usually brushed in generous amounts under tail and genital area with paint brush.

- Doraject + AD_3E (Cipla-Agrimed)

Actives: Doramectin 1% m/v, Vitamin A 3.3 % m/v, Vitamin D_3 0.015% m/v, Vitamin E 5.0% m/v.
Description: Injectable anti-parasitic medication for cattle and sheep for the treatment of gastro-intestinal roundworms, parafilaria, screwworms and single host ticks with vitamins to prevent and treat vitamin deficiencies.
Dosage: 1 mℓ per 50 kg body mass.

- Decatix 3 (Afrivet)

Actives: Deltamethrin 2,5 % m/v
Description: External spray dip intended for the control of ectoparasites on cattle, sheep, goats and ostrich.
Dosage: Use 1.5 ℓ Decatix 3 to 1 000 ℓ water (average dip tank of 15 000 ℓ requires 22,5 ℓ concentrate to fill).

- LUMPYVAX™ (MSD Animal Health)

Actives: Each 1ml (1 dose) of vaccine contains 10^{4} $^{TCID}{}_{50}$ of freeze-dried, live, attenuated virus (SIS type).
Description: For the prophylactic immunisation of cattle against Lumpy Skin Disease.
Dosage: Approximately 5 ml of sterile diluent to the bottle containing the freeze-dried vaccine. Mix until all the powder is dissolved and then transfer this suspension back

to the remaining sterile diluent and again mix well using the sterile syringe. Shake the bottle before filling the syringe. Inject 1ml per animal subcutaneously.

Table 2.3: Summary of ectoparasite treatment of groups from March 2014 to March 2015.

	Grey Brahman A	Grey Brahman B	Red Brahman A	Red Brahman B	Brahman x Sussex A	Brahman x Sussex B	Sussex A	Sussex B
Mar-14	×							
Apr-14	Ectoshield and Coopers tick grease							
May-14	×							
Jun-14	×							
Jul-14	×							
Aug-14	Ectoshield and Coopers tick grease							
Sep-14	Ectoshield and Coopers tick grease							
Oct-14	Doraject + AD3E and Coopers tick grease							
Nov-14	Decatix 3 and Coopers tick grease							
Dec-14	×							
Jan-14	Decatix 3 and Coopers tick grease							
Feb-14	Ectoshield and Coopers tick grease							
Mar-14	Ectoshield and Coopers tick grease							

2.2.6.2 Other treatments

Animals with any other ailments were treated accordingly by the producer. Treatment of animals were only necessary for a limited number of individuals from the various production groups. During September 2014 Sussex breed group B was treated for skin mites with Ectoshield and Coopers tick grease. During the following month, October 2014, all of the production groups received Doraject intestinal deworming with AD3E as a vitamin supplement. During January 2015 the Grey Brahman group and the Red Brahman group was treated with Lumpyvax, Duplocillin and Finadyne for lumpy skin disease. One individual from the Sussex group was treated for an eye infection with Doxymycin eye powder during January 2015. Lastly two individuals from the Sussex group was treated for eye infections with the same medication during February 2015.

The following products were used by the producer as treatment for various ailments.

- Duplocillin (MSD Animal Health)

Actives: 150.000 IU Prociane penicillin and 150.000 IU benzathine penicillin per ml.
Description: Aqueous injectable suspension for the treatment and prevention of infections caused by bacteria sensitive to penicillin.
Dosage: 1ml per 25kg body mass, with a dosage interval of 72 hours.

- Finadyne (MSD Animal Health)

Actives: Flunixin 5.0 % w/v.
Description: Injectable solution for cattle, horses, and pigs, 50mg/ml used for the control of acute inflammation and fever.
Dosage: Injection of 2ml per 45kg bodyweight once daily for up to 5 days according to clinical response

- Doxymycin eye powder (Bayer)

Actives: 1% m/m Doxycycline, 5% m/m sulpha- cetamide sodium and 1% m/m cetrimide.
Description: Powdered medicine for ophthalmia in stock animals. Alleviates ophthalmia caused by bacteria, fungi, and viruses.
Dosage: Puff a little powder directly into eye twice daily until acute inflammation symptoms diminish.

2.3 CATTLE MOVEMENT

2.3.1 Production groups

The producer organised his herds into production groups consisting of sexually mature purebred Sussex, purebred Grey Brahman, purebred Red Brahman and crossbred cows. Brahman bulls were paired-off with the Sussex cow groups during mating periods whereas the Grey Brahman group B and Red Brahman group A and B were mated with Sussex bulls. The Grey Brahman group A is part of the stud breeding program whereas the Grey Brahman group B, and Red Brahman group A and B are kept for commercial breeding purposes. When the subsequent crossbred offspring heifers are weaned they are either sold or placed into the crossbred group where they will start to reproduce their own calves.

During the study period, the producer organised his herds into the following production groups:

1. Red Brahman group A, Crossbred group A and B and Sussex group A.

2. Sussex group B.
3. Grey Brahman group A.
4. Grey Brahman group B and Red Brahman group B.

These groups consisted of the same herds throughout the study period and each group moved from one camp to the following camp or farm as a unit.

2.3.2 Criteria for cattle movement

Farms and camps where animals grazed were not pre-determined by the study protocol but by the producer according to his grazing and breeding system, which were again determined mainly by food availability in the different camps at different seasons throughout the year. Nutritional requirements were also not prescribed by the study protocol, and supplementary feed was given to the animals on the discretion of the producer.

The producer constantly assessed the utilisation of natural grazing in the different camps, carrying the production groups. The decision to move each group to the following camp or farm was also made by the producer according to his aquired experience, when food availability and grazing capacity of a camp became insufficient.

2.3.3 Movement plan

The movement plan of all the cattle groups to the various camps and farms that was followed during the study period from March 2014 to March 2015 is shown in Figure 2.16. No other stock animals grazed in the camps throughout the test period.

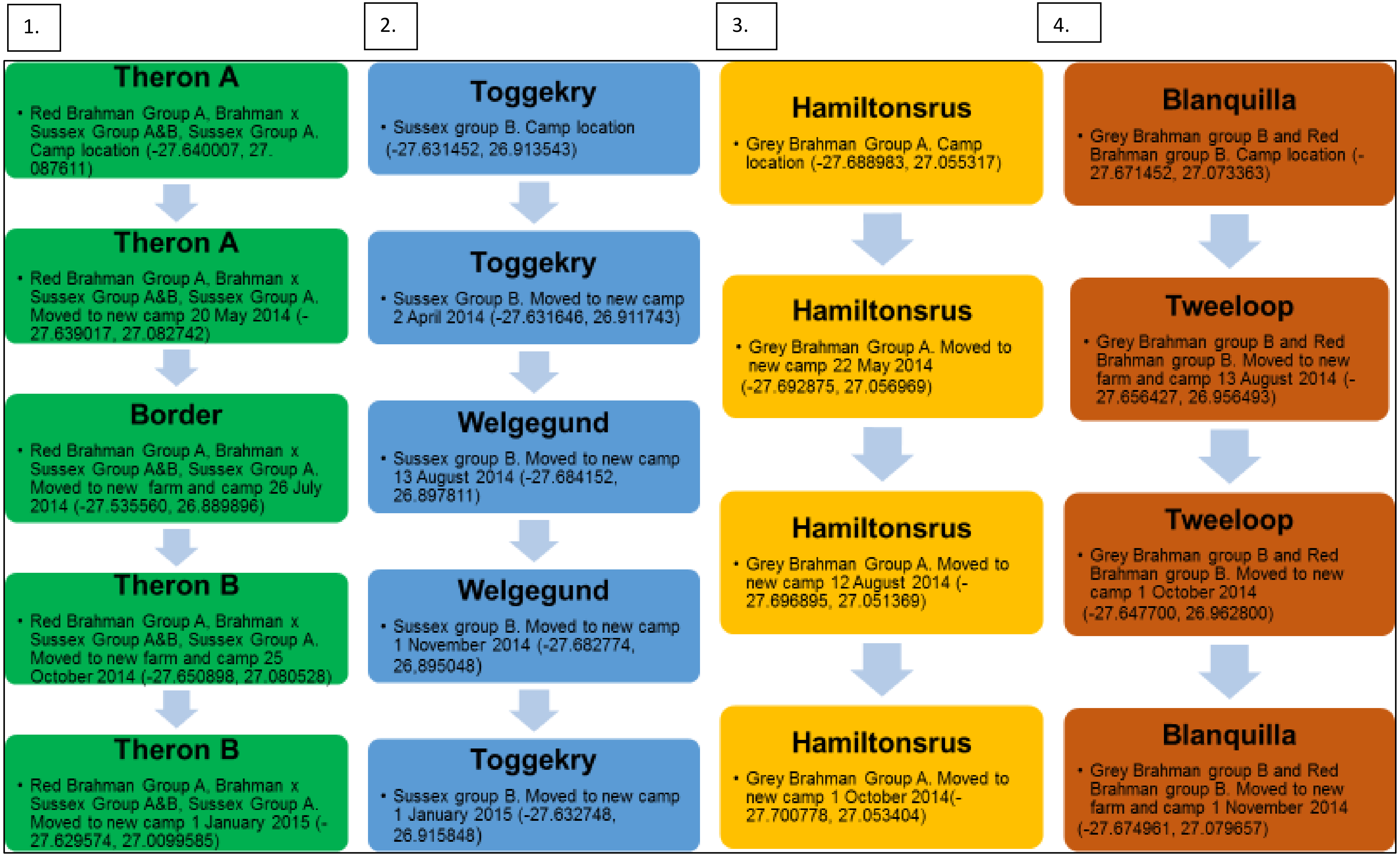


Figure 2.16: Movement plan of production and breed groups between the different camps and farms from March 2014 to March 2015

2.4 ECTOPARASITE COLLECTION

Ectoparasites can inhabit two different microhabitats namely on the cattle during feeding, representing a protected on-host habitat, or in the surrounding environment which is a more exposed off-host habitat. Both on-host and off-host collections were done to obtain an indication of possible influential factors on ectoparasite abundance or absence.

2.4.1 Acari collection (Ticks and Mites)

2.4.1.1 Tick collection

2.4.1.1.1 On-host collection

Ticks were collected on a monthly basis from March 2014 to March 2015 during the last week of each month from twenty cows of each breed (Grey Brahman, Red Brahman, Brahman x Sussex, Sussex). Specimens were obtained by restraining the animals in a crush and neck clamp mechanism to ensure requisite sampling and observations. Due to the visually low tick burden on the cattle, all ticks on an individual animal were removed with blunt forceps (Figure 2.17A and B). Tick attachment sites were also recorded according to body region of removal (Figure 2.14) to determine differences in attachment sites between the different breeds. Ticks sampled from individual animals were placed into a plastic screw top container in 70% ethanol and uniquely labeled with the animal ID, date of collection and location of collection.

After collection, all samples were transported to and stored in the Veterinary Entomology Laboratory, at the University of the Free State, Bloemfontein. Collected tick specimens were identified to genus and species level with a Zeiss 475022 stereo microscope at a 1.6 x magnification using keys compiled by Horak *et al*. (2008) (unpublished) and Walker *et al*. (2003).

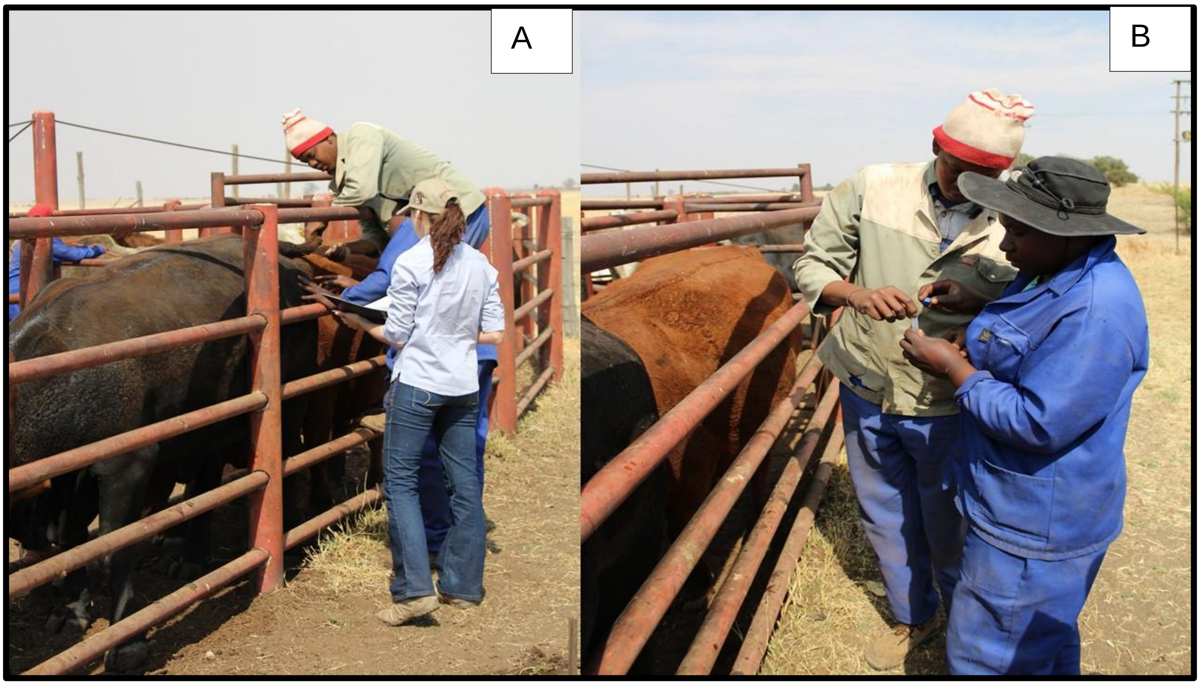


Figure 2.17: A: Inspecting cattle for ticks. B: Removing ticks and placing them in screw cap containers.

2.4.1.1.2 Off-host collection

Tick sampling in the environment was done by drag sampling (Spickett *et al.* 1991) in each of the camps where the different production groups (group one to four) grazed each month for 13 months (Figure 2.16). This entails a drag stick consisting of ten 100cm x 10cm flannel cloth pieces attached to a 120cm rod and an extension string that was dragged along the vegetation in the field. The cloth pieces were allowed to make full contact with the surface of the vegetation. As the vegetation in the camps had a homogeneous appearance, a total of six 100 meter drags were performed per camp at random locations throughout the entire camp to obtain a general surveillance of active questing ticks.

Drags of the vegetation were done once either in the morning before 11:00 or during the late afternoon after 15:00 to avoid sampling during the hottest time of the day when according to Spickett *et al*. (1991) less ticks exhibited questing behaviour. Wet vegetation with morning dew or after rain were avoided, as the water could influence the efficacy of the flannel strips.

After each 100 meter drag, the cloth pieces were inspected for any tick larvae or nymphs that could have attached themselves to the material. Any immature ticks attached to the cloth pieces, were removed with fine-point forceps and placed into appropriately labeled (Date, farm and camp location) screw cap plastic containers in 70% ethanol. These tick specimens were also counted and documented.

2.4.1.2 Mite collection

Mange lesions are described by Williams (2010) to first appear around the tail, anus, thighs, udder, legs and feet. These sites were therefore checked first when a mite infestation was suspected or noticed on any of the 20 cows from each breed inspected every month (Figure 2.14). However, the rest of the body was also checked for other areas of possible mite infestation. Five skin scrapings of each infected area were made using the method described by Yasine *et al*. (2015), by holding a scalpel blade at a 45° angle to the skin and scratching the surface of the skin until a very small amount of blood is visible. This is done to ensure that skin scrapings were made deep enough to sample mite species that burrowed into skin. This scraping motion was done at the edge of sites where mite infestations were located and formed wound areas. The scrapings were placed in screw cap containers in 70% ethanol for preservation and labeled appropriately (Animal ID, date and location).

Examination under a Zeiss 475022 stereo microscope at a 1.6 x magnification of skin scrapings was done to confirm mite presence and identify the mites up to genus and species level based on the morphological characteristics described by Horak *et al*. (2008).

2.4.2 Diptera collection (Flies, midges and mosquitoes)

2.4.2.1 Diptera sampled during the day

Commercial fly baited traps (3 litre REDTOP Flycatchers bought from Hinterland Kroonstad) were used to collect flies surrounding animals during the day. Four traps were initially baited with the bait sold with the traps. No breakdown of ingredients was given on the packaging accept that a non-toxic protein based dry powder was in the bait bag and should be mixed with water to make it especially attractive to female flies. These traps were hung around the kraal about 0.5 meters from the cattle in the kraal during the monthly inspection of the breed groups over the period of 13 months (Figure 2.18). However, this was found to be insufficient to sample all different visible flies around the cattle and the sampling method was adapted to attract more flies during daytime. The four modified traps replaced the original traps and were individually baited with cattle urine obtained from the cattle herds, chicken liver and dung obtained from the cattle herds. Water was used as a control during inspection each month. The urine and dung were used to simulate natural attraction products from cattle, ensuring a clear indication as to which fly species are attracted to body fluids of cattle. The liver was chosen as another attractant to simulate bloodshed or a wound on an animal. These traps were also placed within 0.5 meters from the animals in the kraal during the monthly inspections.

This decision was only made several months into the sampling period and therefore only three month's data was collected by means of the improved method. These proposed traps would need to be tested in future studies to prove their validity, thus these results were not included in this study. Flies sampled with commercial sticky fly traps (3 litre REDTOP Flycatchers) used during the controlled Diptera count experiment (heading 2.4.2.3) was however included in this study. Collection traps used to sample Diptera active at night was found to be effective, thus no improvements were made.

Fly species described by Picker *et al*. (2004) and Meiswinkel (1996), was used to identify all fly species sampled. Meiswinkel (1996) was mainly used to distinguish between blackflies (Simuliidae) and midges (Ceratopogonidae) as they can sometimes be confused with one another. Collected Diptera specimens were identified in the laboratory to family level under a Zeiss 475022 stereo microscope at a 2 to 3 x magnification.

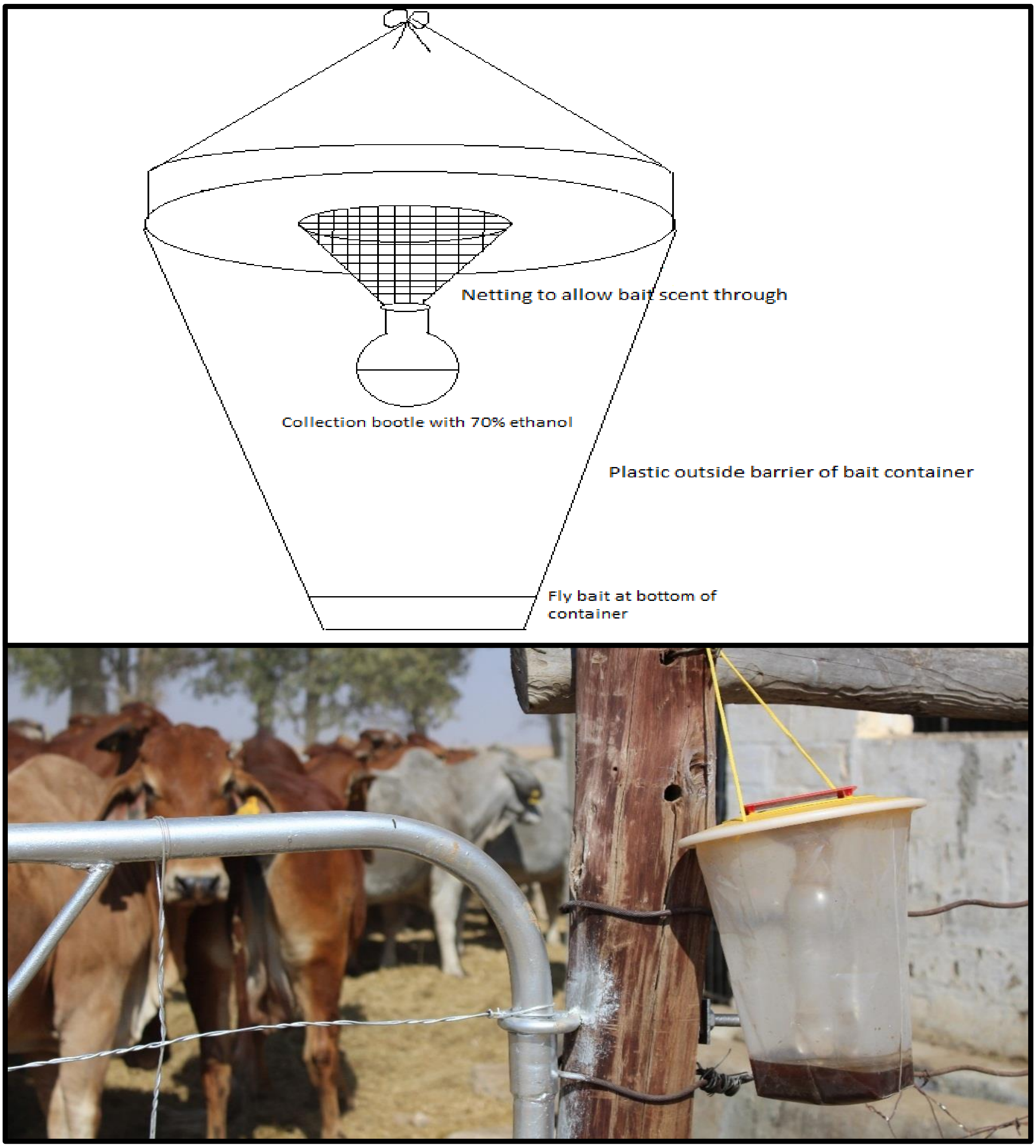


Figure 2.18: One of four (REDTOP Flycatchers) traps used to sample flies during the day, placed 0.5 meters from cows in the kraal during inspections each month.

2.4.2.2 Diptera sampled during the night

Two suction light traps of 220 Volt (Figure 2.19) were used each month for every production group making up a total of eight light traps in total per month for 13 months, as a source to collect night flying insects, particularly biting midges and mosquitoes. The fan captured the insects by sucking them into a collection bottle filled with 70% alcohol underneath the black light and fan. Netting with 3mm x 3mm holes was placed strategically to enclose the fan to prevent bigger insects such as Coleoptera from being caught. The lights used were 8W fluorescent tubes, emitting UV light in the 300 to 400nm light range. The traps were placed in the grazing camps near the cattle were the watering and feeding stations were located. The individual traps were activated for one night at each herd location, and samples were collected the following morning. In the event of rain, the trap was placed in the same location on the following night, or as soon as more favourable weather conditions have set in.

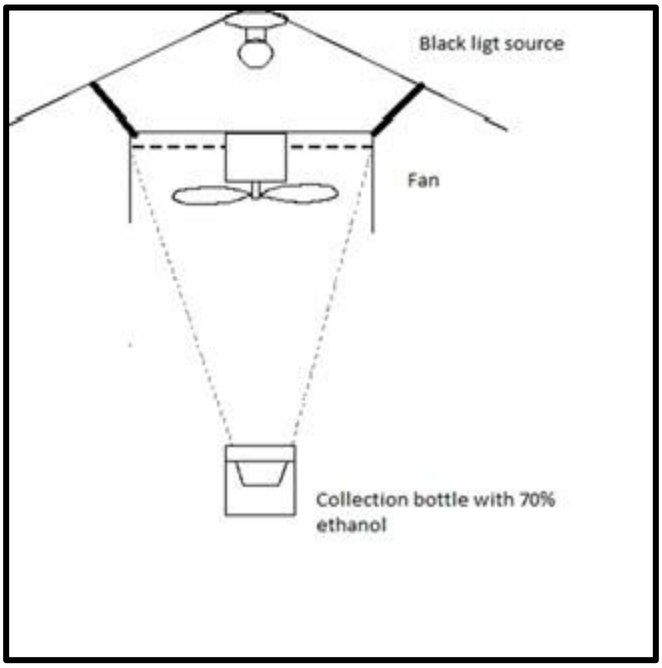


Figure 2.19: One of two fluorescent light traps used to sample Diptera at night from each production group for 13 months.

2.4.2.3 Controlled Diptera count experiment

The heritable trait in fly attractiveness resulting in total fly load, appears to be an innate resistance quality expressed by an individual that can possibly be linked to certain breeds (Pruett *et al*. 2003). This was tested by placing one individual from each selected breed into a holding pen for observation for a total of two uninterrupted days once in December 2014 and again in December 2015. These pens were directly next to each other with no more than two meters' open space between any two animals at any given time (Figure 2. 20). One side of the animal was observed as described by Castro *et al*. (2008), by scrutinising it from head to tail head including flank, foreleg and hind leg areas, for two intervals of 15 minutes at a time, twice a day. A total observation time of 30 minutes in the morning and 30 minutes in the afternoon for every selected individual representing the four breeds were thus obtained. During these observation periods, fly counts were recorded for each individual animal consecutively. A fly was counted the moment it landed on any part of the animal during a five-minute interval scan sample technique. Thus, each cow was observed for 6 scans during the 30-minute observation period. More replications of this experiment should be repeated in the future to ensure statistical accuracy.

Figure 2. 20: One individual from each selected breed in holding pens during controlled fly count experiment during December 2014 and December 2015.

During the recording time, four commercial sticky-tape fly traps (Victory's FLY catcher) (Figure 2. 21) were also placed, one near each cow, to get an indication of what fly species were more abundant in that particular environment at that time. This was done for two consecutive observation days in December 2014 and again in December 2015. This trap does not have any active ingredients, bait, poison or dangerous vapors. The flies collected with the sticky-tape traps were compared with the species and number of flies counted on the animals.

Fly repelling behaviour from each individual was recorded to determine the severity of irritation the animals experienced. Strong tail flicks to either side, head shakes, skin rippling and ear flicks were counted, whereas weak tail movements were not counted. Fly repellent behavior was divided into categories (ear flap, light tail switch, forceful tale switch, head shake and skin ripple) based on a study done by Eicher *et al*. (2001). The experiment was adapted to only house the animals for two days at a time to best suit the time constraints and cost related to feeding the animals while in the pens. This test was done on two separate occasions during summer (December 2014 and December 2015) when flies are the most active and proven to be a nuisance.

Figure 2. 21: Diptera collected using commercial (Victory's FLY catcher) sticky-tape fly traps placed in each group representatives pen.

The stalls were cleaned before the experiment began, and again every morning before counting started. This experiment should be repeated in future studies to explore various influencing factors such as the distance the cows were from each other for breed specific preference ques, the effect of sunlight and shade on the presence of flies on the animals and the presence of the observer near the cattle.

2.4.3 Phthiraptera collection (Louse)

Lice were collected with blunt steel forceps from each animal. Collected specimens were placed into plastic screw cap containers in 70% ethanol and labeled appropriately (Animal Id, date, location). The entire body of each cow was inspected. However, specific sites on the animal was focused on if lice infestation was suspected. These sites include the face, dewlap, back and tail head (Figure 2.14). The number of adult lice found per square cm at each site was recorded. Mean number per square cm was categorised as less than 5: very slight, 5 to 10: slight; 10 to 20: moderate; 20 to 50: severe; more than 50: very severe infestation. This can be used as a reference in future studies.

2.5 STATISTICAL ANALYSIS

Abundance and presence of the ectoparasites collected (both ticks and fly species) on the cattle and in the grazing pastures for each selected breed were analyzed in accordance with temporal and spatial environmental conditions. Descriptive statistics were used to define if purebred Brahmans and their F1 crosses with purebred Sussex individuals have a higher ectoparasite resistance than purebred Sussex cattle based on fly- and tick sampling from both live animals and the camps they grazed in from March 2014 to March 2015. Significant differences between on-host ectoparasite abundance was determined by using a confidence interval of 95% utilising the IBM SPSS, Version 22, computer program. Comparisons between the cattle groups ectoparasite resistance qualities and ectoparasite load was also made by utilising SPSS and Excel with a confidence level of 95%.

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CHAPTER 3

ACARI INFESTATIONS ON DIFFERENT CATTLE BREEDS

INTRODUCTION

Exploring the best ectoparasite resistance and tolerance qualities of different cattle breeds as a measure towards ectoparasite control, needs to be the focus of both commercial and subsistence cattle farming systems. For this reason, clearly defined crossbreeding structures must be adopted with not only an aim on monetary gain but also to produce a quality product while also being environmentally conscious. These crossbreeding structures also needs to aim for ectoparasite resistance to lower costs of chemical ectoparasite control and therefore compensate for possible loss in production mass.

As illustrated in Figure 3.1 tick infestation has an influence on the commercial- or subsistence cattle herds' productivity in the form of primary and secondary effects (Sutherst *et al.* 1987). As an example of this type of this knock-on effect (Figure 3.1), the calves born from cows with moderate to severe tick infestations will not achieve sufficient weight gain per month and will not be profitable (Sutherst *et al.* 1987).

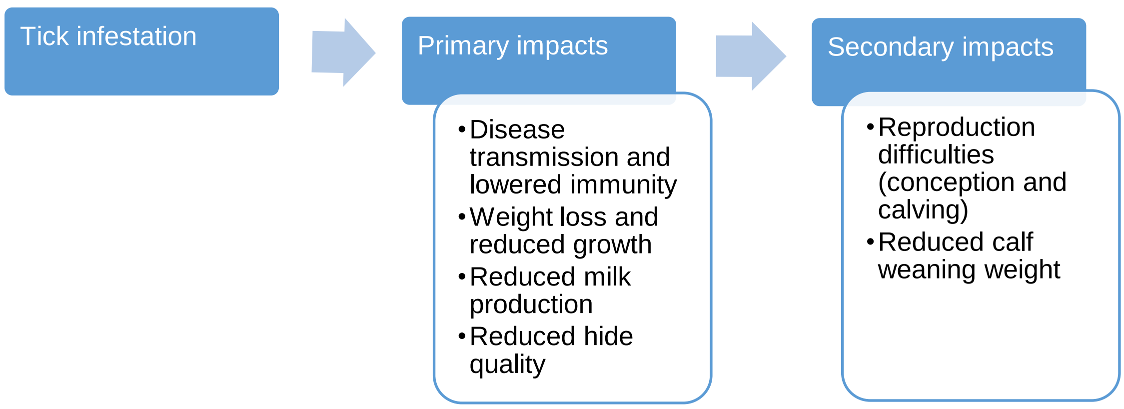


Figure 3.1: Primary and secondary effects on cattle production due to tick infestation (Adapted from Sutherst *et al*. 1987).

With regard to the influence of Acari on herd productivity the terms resistance and tolerance first need to be defined as a form of natural immunology for the purpose of this study. Resistance is defined as the measure of the host animals' ability to reduce parasite establishment whereas tolerance is described as the measure of the hosts ability to deal with a given parasite load (Baucom *et al.* 2011). For the purpose of this chapter investigated differential Acari resistance of the three different cattle breeds were investigated by analysing tick and mite presence and abundance on the animals.

Ticks utilise two different habitats, an on-host and off-host habitat with different microclimatic environments to complete their lifecycles (Greenfield 2011). The on-host environment can be influenced by coat characteristics such as hair length and hair density and this habitat is used by the ticks to feed and mate before dropping off into the more exposed off-host microhabitat to moult and survive (Estrada-Peña *et al.* 2014). In this more hostile environment, engorged female ticks deposit their eggs. Larvae hatch from the eggs and actively seek for suitable hosts. Vegetation type and quality plays a vital role in retaining optimum air humidity necessary for tick survival during off-host-periods (Schulz *et al*. 2014).

Mites in contrast with ticks, only utilise the on-host environment. They are passively transmitted from one host animal to the next through physical contact (Oberem *et al.* 2009). Mites expected to be sampled from the cattle, belongs to the order Acarina, suborder Astigmata. Mites of medical and veterinary importance in this suborder are finely sclerotid arthropods including the families; Sarcoptidae, Psoroptidae, Cheletoidea, Demodicidae, Cheyletiellidae and Tromiculidae (Yasine *et al.* 2015). The Demodicidae and Cheyletiellidae families only consist of parasites of companion animals with no significant contribution to livestock production.

As a main objective in this chapter, crossbreeding was explored as a measure to manage Acari infestation specifically comparing tick and mite resistance between Brahman, Sussex and Brahman x Sussex F1 crossbreds by determining Acari abundance and species composition for each breed.

Specific objectives investigated in this chapter therefore include:

- The comparison of ectoparasites (Acari) abundance on the three selected cattle breeds to determine if crossbreeding can be a means of controlling ticks and mites through breed resistance.

- Investigation of the association between on-host and off-host ectoparasite diversity and abundance.
- Determination of the influence of weather conditions on ectoparasite abundance.
- To determine if there is a relationship between weather conditions recorded during the test period and (on-host and off-host) ectoparasite abundance.
- To establish if there is a relationship between the body scores recorded for the animals and the abundance of ectoparasites sampled in the on-host environment.
- The determination of a relationship between the health issues recorded for the animals and the abundance of ectoparasites (Acari) sampled in the on-host environment.
- Comparing attachment sites between the different breeds.

3.1 MATERIALS AND METHODS

Selected cattle breeds were followed from camp to camp according to the farmer's movement program, determined by the availability of grazing material as described in detail in Chapter 2. The normal situation in an extensive farming system was therefore investigated and movement of cattle to suit research objectives could not be prescribed.

The study area, cattle breeds followed, ectoparasite presence and abundance for each area, as well as criteria for inclusion of animals and characteristics investigated to evaluate ectoparasite infestation, are extensively described in Chapter 2.

Collection of ticks and mites from 20 cows in each breed were done on a monthly basis from March 2014 to March 2015 as described in Chapter 2.

3.2 RESULTS

3.2.1 Tick attachment sites

The tick attachment sites on the different breeds are illustrated in Figure 3.2. Table 3.1 provides details on the tick attachment sites recorded for 10 individuals in each breed group namely Grey Brahman group A and B, Red Brahman group A and B, Brahman x Sussex group A and B, and Sussex group A and B for the entire test period as well as the tick species and numbers found during the study period.

No ticks were found on the forequarter section of the Grey and Red Brahman groups. Red Brahman cattle only exhibited ticks attached to two body locations, under the tail and the rear flank area, with grey Brahman showing an additional attachment site on the udder. With regards to Sussex cattle, ticks attached to most of the preferred attachment sites for each tick species and were collected from all attachment sites for 13 months. The Brahman x Sussex cattle showed less attachment sites compared to the Sussex groups, however they also had more than the Brahman groups with ticks also being found on the forequarters and flank of the animals (Figure 3.2).

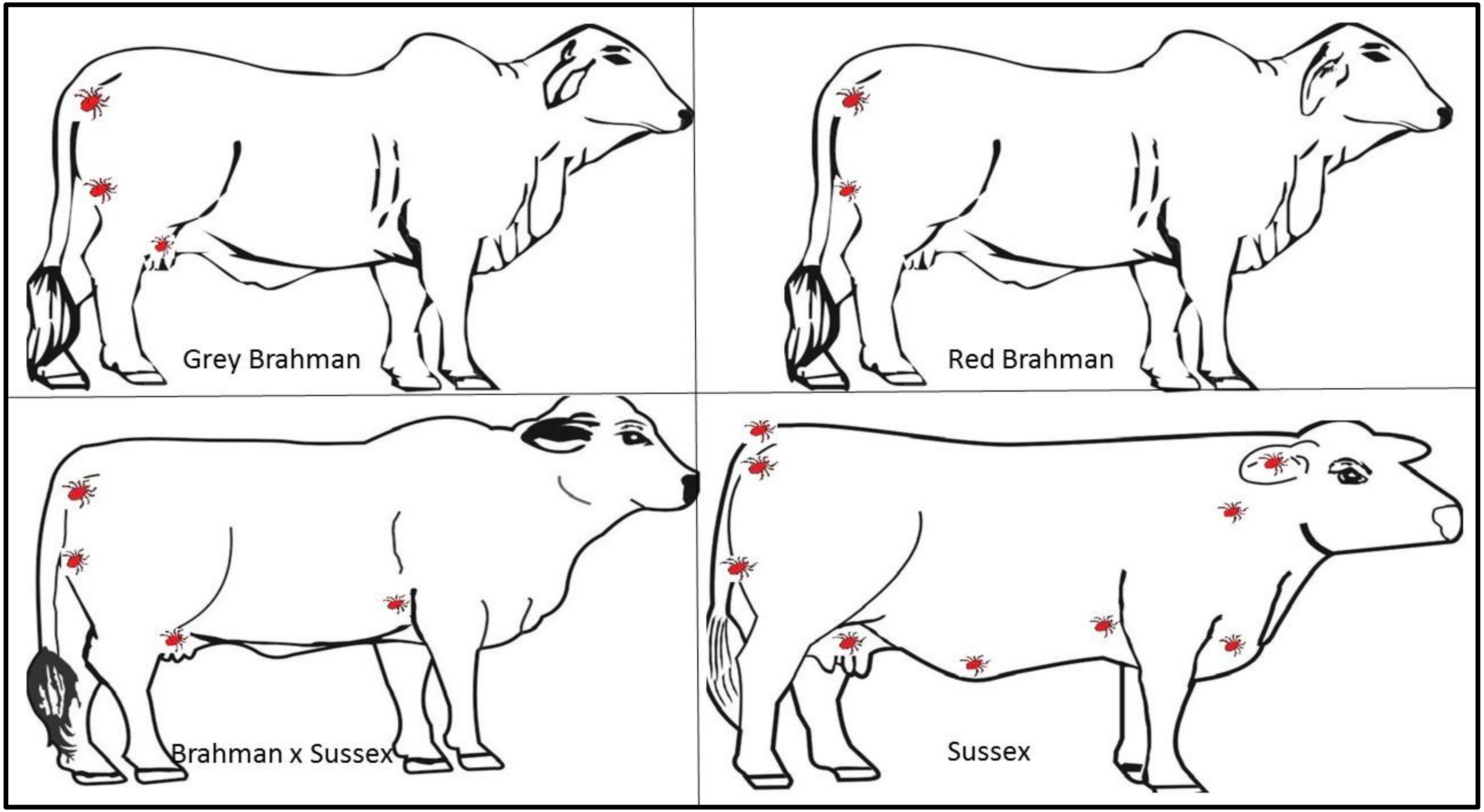


Figure 3.2: Body mapping of tick attachment sites recorded for each breed over 13 months from March 2014 to March 2015(Sketch drawn by Marilie Esterhuyze).

Table 3.1: Summary of results of monthly collections of tick species, total number of each species found on each breed and attachment sites recorded for each breed over the entire test period (March 2014 to March 2015).

	Under tail	Tail head	Rear flank	Udder	Naval	Fore flank	Dewlap	Shoulder	Neck	Ears	Tick species found	N	Total ticks
											Hyalomma rufipes	675	
											Rhipicephalus evertsi evertsi	102	
Grey Brahman	x		x	x							*Rhipicephalus appendiculatus*	1	919
											Rhipicephalus decoloratus	0	
											Hyalomma truncatum	141	
											Hyalomma rufipes	384	
											Rhipicephalus evertsi evertsi	96	
Red Brahman	x		x								*Rhipicephalus appendiculatus*	0	550
											Rhipicephalus decoloratus	1	
											Hyalomma truncatum	69	
											Hyalomma rufipes	702	
											Rhipicephalus evertsi evertsi	94	
Brahman x Sussex	x		x	x		x					*Rhipicephalus appendiculatus*	3	846
											Rhipicephalus decoloratus	3	
											Hyalomma truncatum	44	
											Hyalomma rufipes	2036	
											Rhipicephalus evertsi evertsi	304	
Sussex	x	x	x	x	x	x	x	x	x	x	*Rhipicephalus appendiculatus*	26	2534
											Rhipicephalus decoloratus	29	
											Hyalomma truncatum	139	
Total ticks												4849	

N-number of individuals.

3.2.2 Tick presence and abundance

3.2.2.1 Species abundance

Table 3.1 is also a representation of all the tick species sampled during the study period from all the selected cattle breeds. The most abundant species sampled was *H*. *rufipes* with 3797 individuals representing 79% of total Ixodidae sampled over the study period followed by *R. evertsi evertsi* with 12.3%, *H. truncatum* with 8.1%, *R. decoloratus* with 0.7% and *R. appendiculatus* was the least abundant species with a total of 30 individuals at 0.6%.

3.2.2.2 Seasonal occurrence

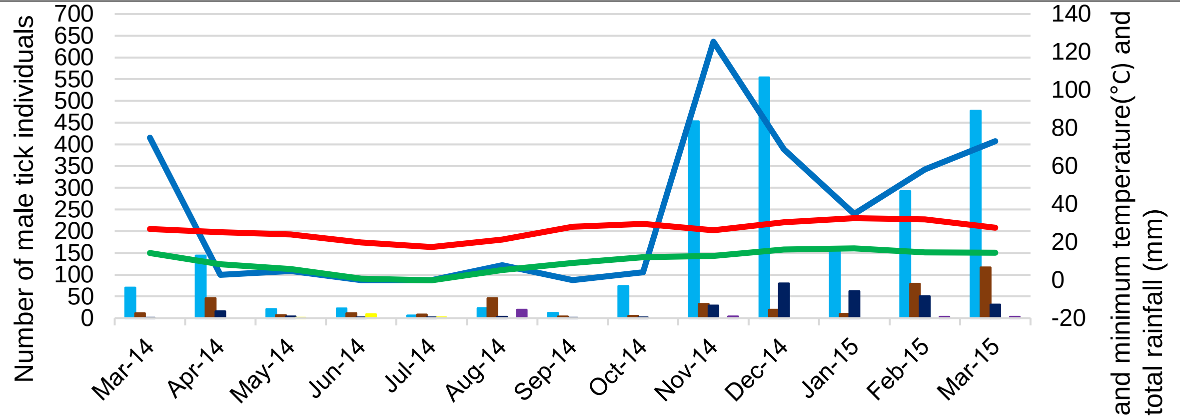
Both male and female Ixodidae abundance were influenced dramatically by rainfall and temperature fluctuations (Figure 3.3). Males and females in general were more restricted to warmer months as male abundance showed higher peaks from October 2014 to March 2014 and high numbers of females were found from October 2014 to January 2015 (Figure 3.3).

Hyalomma rufipes male and female individuals were not only the most abundant throughout the test period but the highest numbers were observed from March 2014 to June 2014 and again October 2014 to March 2015 for males and March 2014 to April 2014 and September 2014 to March 2015 for the females. These high numbers coincided with peak rainfall and temperatures measured during these months (Figure 3.3). Lower rainfall and temperatures during winter and autumn also coincided with lower abundance of male ticks, accept for *R. appendiculatus* and *R. decoloratus* which were only found during the colder months. *Rhipicephalus evertsi evertsi* males and females occurred throughout the year, not being limited by seasonal changes, although their numbers increased during warmer more humid months (Figure 3.3).

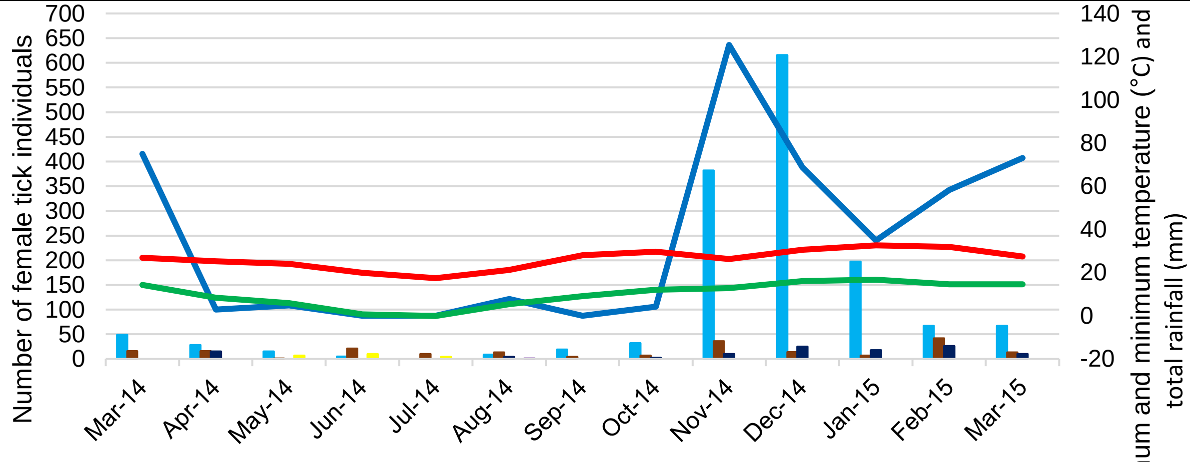
Only a small percentage of ticks collected during the study was identified as *H*. *truncatum*. Summer burdens of *H*. *rufipes* and *H*. *truncatum* on the animals were higher than compared to winter.

During winter months (May to July 2014), the total number of all tick species found on all groups of animals observed, was less than 100 individuals per sex. All tick species accept *R. decoloratus* showed a decreased in abundance during these months.

Rhipicephalus decoloratus was documented for the first time at this specific locality in the Free State and only from May to July 2014. They mostly occurred on the Sussex breed animals (29 of 33) and during a period of low rainfall and temperature conditions not usually described as ideal for these species. Dreyer *et al*. (1998) described *R. decoloratus* to be more abundant from February to June in their study, demonstrating that the peaks in adult infestation indicate the completion of a generation. Three peaks were recorded by them throughout the 13 month sampling period, during November/December, March/April and June respectively, indicating that *R. decoloratus* potentially produced three generations. In contrast to their findings only one peak of adult abundance of this species was recorded during the current study, limiting them to one completed generation in a 13 month period.



	Mar-14	Apr-14	May-14	Jun-14	Jul-14	Aug-14	Sep-14	Oct-14	Nov-14	Dec-14	Jan-15	Feb-15	Mar-15
H. marginatum rufipes	70	144	21	22	6	23	12	74	453	554	163	292	477
R. evertsi evertsi	11	46	7	11	8	46	4	5	33	19	10	79	117
H. truncatum	1	16	4	2	2	3	1	2	29	80	62	50	31
R. decoloratus	0	0	1	9	2	0	0	0	0	0	0	0	0
R. appendiculatus	0	0	0	0	0	19	0	0	4	0	0	3	3
Total rainfall(mm)	75	2.8	4.8	0	0	7.8	0	4.2	125.4	68.8	34.8	58.2	73
Mean max temperature(°C)	26.9	25.2	24	19.9	17.4	21.3	28.1	29.6	26.2	30.5	32.6	31.9	27.5
Mean min temperature(°C)	14.2	8.3	5.8	0.7	-0.1	5.4	9.1	12	12.8	16.1	16.7	14.6	14.5



	Mar-14	Apr-14	May-14	Jun-14	Jul-14	Aug-14	Sep-14	Oct-14	Nov-14	Dec-14	Jan-15	Feb-15	Mar-15
H. marginatum rufipes	49	28	15	5	0	9	19	32	382	616	197	67	67
R. evertsi evertsi	16	16	1	21	10	13	4	7	36	14	7	42	13
H. truncatum	0	15	0	0	0	4	0	2	10	25	18	26	10
R. decoloratus	0	0	7	10	4	0	0	0	0	0	0	0	0
R. appendiculatus	0	0	0	0	0	1	0	0	0	0	0	0	0
Totsl rainfall(mm)	75	2.8	4.8	0	0	7.8	0	4.2	125.4	68.8	34.8	58.2	73
Mean max temperature(°C)	26.9	25.2	24	19.9	17.4	21.3	28.1	29.6	26.2	30.5	32.6	31.9	27.5
Mean min temperature(°C)	14.2	8.3	5.8	0.7	-0.1	5.4	9.1	12	12.8	16.1	16.7	14.6	14.5

Figure 3.3: Total monthly numbers of male (A) and female (B) Ixodidae species collected to indicate abundance compared to mean maximum and minimum temperatures (°C) and total rainfall (mm) sampled from March 2014 to March 2015.

3.2.3 On-host and off-host tick abundance

When considering on-host tick abundance (Table 3.2), a clear preference for Sussex cattle was found. Sussex cattle had a total on-host tick abundance of 2534 over the entire test period, compared to Grey Brahman with 919, Red Brahman with 550 and Brahman x Sussex with 846. The on-host tick abundance for the Sussex group differed significantly from the Grey Brahmans ($p=0.028$), the Red Brahmans ($p=0.007$), and the Brahman x Sussex cattle ($p=0.022$), (Annexure 3: Table 3.1)

Further statistical analysis, testing for deviations from normality, found a normal distribution with group variance less than 3. A two-way ANOVA test done with total tick count as dependant variable and month of the year and breed as independent variables indicated significant differences in on-host tick abundance between various breeds during certain months with $F\ (36,988) = 14.675$, $p<0.05$, partial $\eta^2=0.348$ (Annexure 3: Table 3.2). The partial eta-square value (η^2) was used as an estimate of effect influence on tick abundance. As the partial eta-square value is not close to 1.00 it is a clear indication that although the p-value shows a significant difference between tick abundance, month and breed, independent variable influence on the outcome is moderate as the sample size was large. This indicates that the effect that "breed" had on total on-host tick abundance depended on which month they were sampled. In order to determine when and between what cattle groups significant differences were evident, simple main effects were analysed by running pairwise univariate test between tick abundance recorded for each group. Significant differences in terms of on-host tick abundance was mainly evident between Sussex cattle compared to the other groups (Grey Brahman, Red Brahman and Brahman x Sussex) with $p<0.05$ at a 95% confidence interval (Annexure 3: Table 3.2)

Figure 3.4 indicates the presence or absence of ticks on the different cattle breeds during the monthly observations and collections from each breed for the period from March 2014 to March 2015. It also indicates the number of ticks found on the different breeds.

These results will be discussed on a month to month basis to indicate the number of animals from each breed hosting ticks, tick burden and significance, immatures found on vegetation, movement of groups, health issues and dipping occurrence for each month.

Of the total number of *H. rufipes collected,* 53.6% were collected from the Sussex breed group, 18% from each of the Grey Brahman and Brahman x Sussex group and 10% from the Red Brahman group. The Sussex group also hosted the highest number of *R. evertsi evertsi* individuals with 51% followed by the Grey Brahman group with 17%, Red Brahman hosting 16% and the Brahman x Sussex 15% of this species. *Rhipicephalus. decoloratus* (33) and *R. appendiculatus* (30) individuals were also more prevalent on the Sussex breed group and less than three individuals in total were found on each of the other groups for the entire study period. The only species that showed a similar preference for the Sussex as well as the Grey Brahman group were *H. truncatum* (35% on each of these two groups) followed by the Red Brahman group (20%) and the Brahman x Sussex group (11%). The number of *H. truncatum* ticks collected were however only 8.10% of the total number of ticks collected during the entire study period.

The Sussex cattle groups also had the most tick infested individuals over the entire test period with 223 animals infested out of a total of 240 individuals checked for ticks. Although the Sussex group did have considerably less infested individuals (similar to the other breeds) during the colder months of May to Augustus 2014, Sussex cattle always had an infestation percentage above 45%. The Brahman x Sussex group were impacted to a lesser extent (188 of 240) followed by the Grey Brahman group (169 of 240) and Red Brahman group (151 of 240) over the observation period. During the hottest and most humid months, February and March 2015, all the cattle groups had more than 85% individuals ranging from 17 to 20 of each group infested with ticks.

The total number of ticks sampled in the off-host habitat by drag sampling was much less than that sampled from the cattle, with 12 individuals found on 4 July 2014 in the camp on the farm Toggekry where the Sussex group B grazed.

<u>March 2014</u>

The initial sampling made during March 2014 showed all the selected herds to have tick infestations (Figure 3.4A). The Sussex cattle groups were the most affected with all animals in the group hosting ticks, whereas the Grey Brahman herd was the least affected with 11 infested animals (13.8%). The Sussex herd carried a higher tick burden, 67 ticks, in comparison to the other herds; Grey Brahman 20 ticks, Red Brahman 24 ticks and Brahman x Sussex 33 ticks in total (Figure 3.4B). The tick burden between the breeds was not found to be significantly different (p=1.00) at a 95% confidence interval. No ticks were effectively collected from the drag sampling performed in the camps where all the groups grazed. No groups were moved to new camps, no general health issues were reported and no animals were treated for any ailments. Body condition scoring indicated all animals to be ideal with a score of two.

<u>April 2014</u>

During April 2014, the number of individual animals with tick infestations increased from the previous month within each selected breed. More than 80% of cattle in each group were affected by tick infestations (Figure 3.4C and D). Both the Sussex- and the Brahman x Sussex groups had a tick presence on all animals inspected in the groups (100%). The Sussex groups carried the heaviest tick burden of all, 88, however it was not significantly different from tick loads recorded for the other herds; Grey Brahman; 63 ticks, Red Brahman; 63 ticks and Brahman x Sussex; 51 ticks. Grey- and Red Brahman groups had more or less the same number of animals with a similar tick load with no significant intra group differences recorded (p=1.00). No ticks were successfully sampled through drag sampling in the camps where the cattle herds grazed. Sussex group B was moved to a new camp on the farm Toggekry on the 2nd of April. No animals were treated for any other ailments and body condition scoring was again found to be ideal. All of the cattle were dipped with Ectoshield and treated with Coopers tick grease from 21 to 24 April 2014 for ectoparasite control during the inspection as part of the routine dipping schedule.

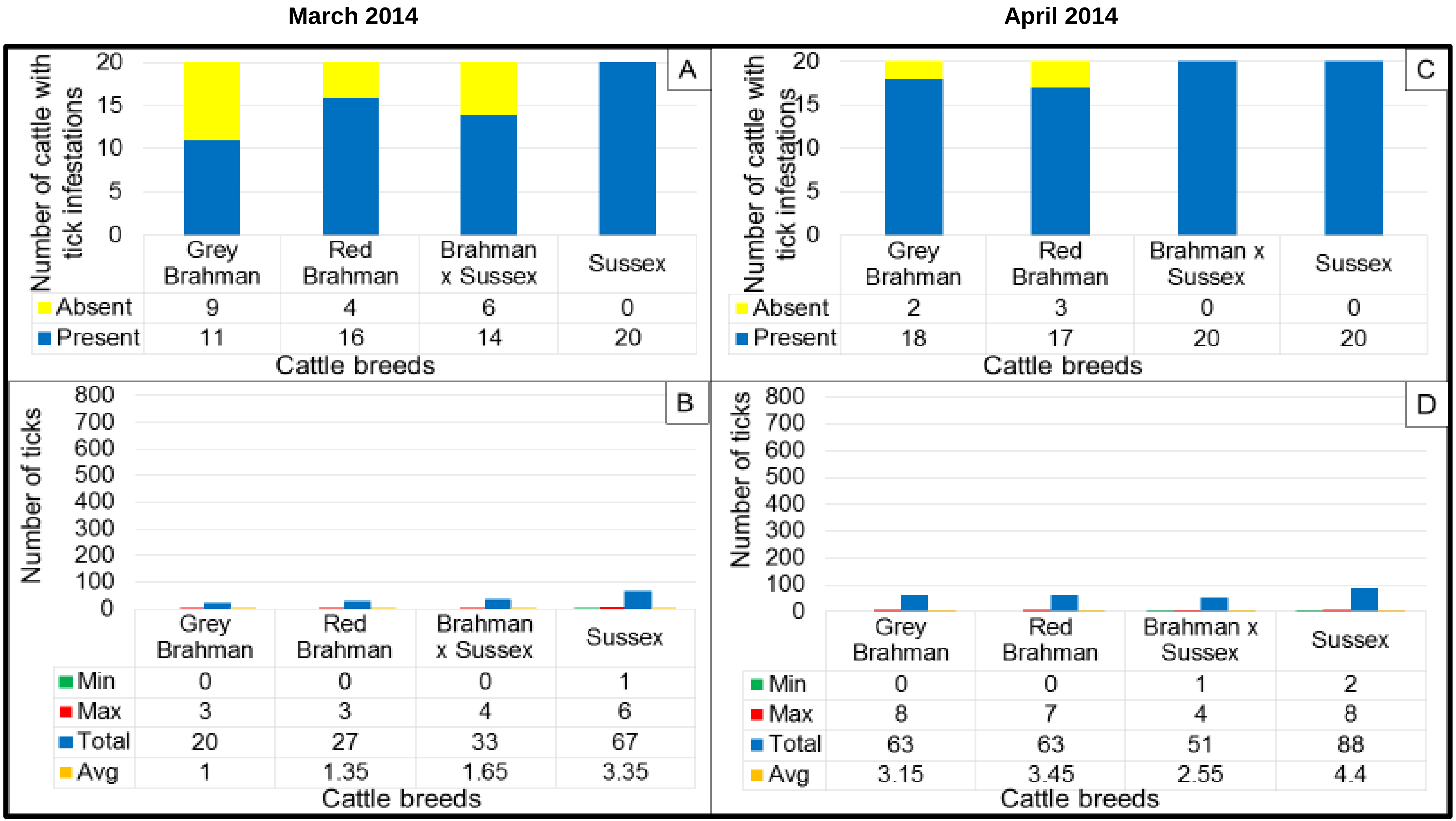


Figure 3.4: Presence and abundance of ticks sampled from cattle breed groups from March 2014 to March 2015.

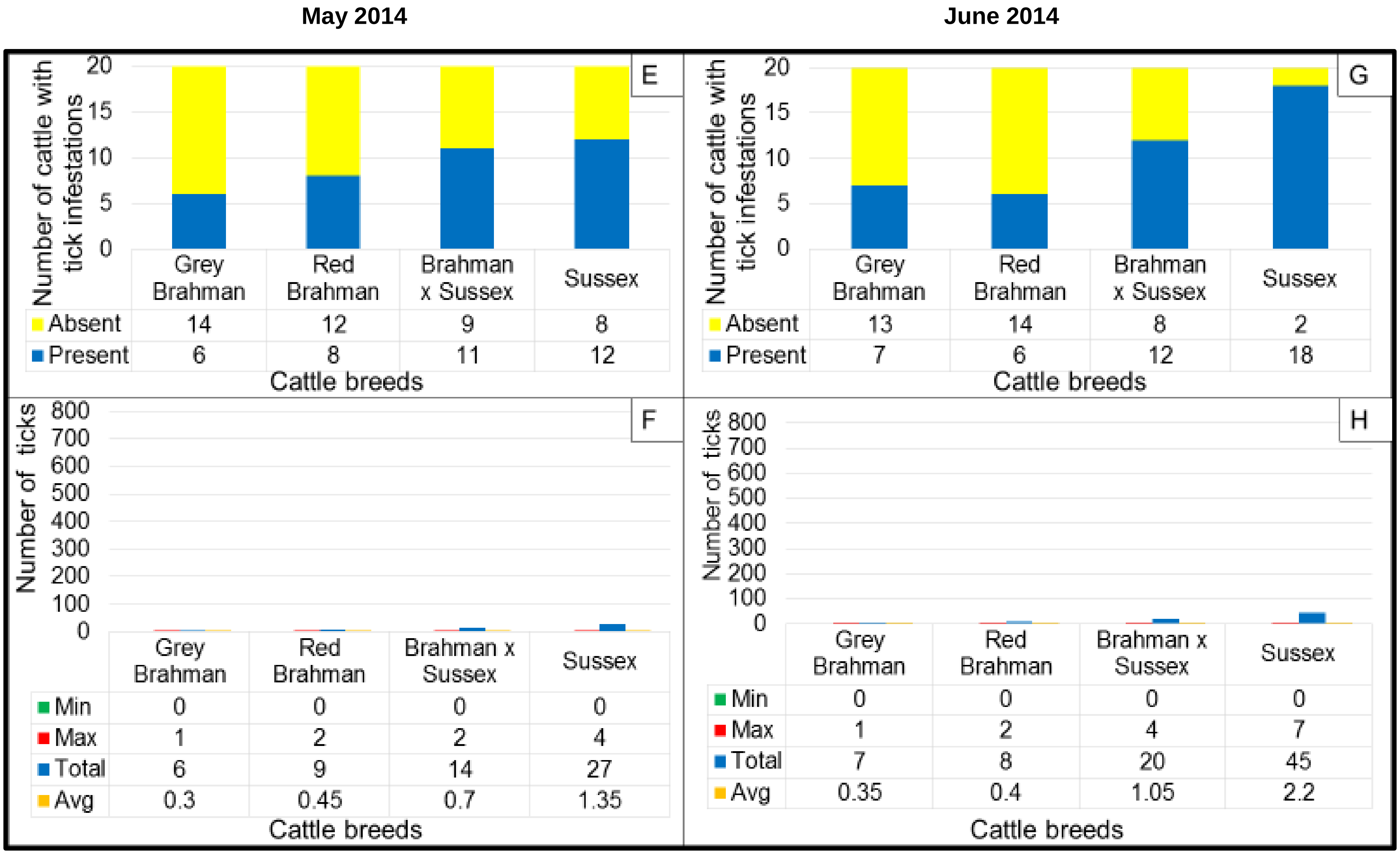
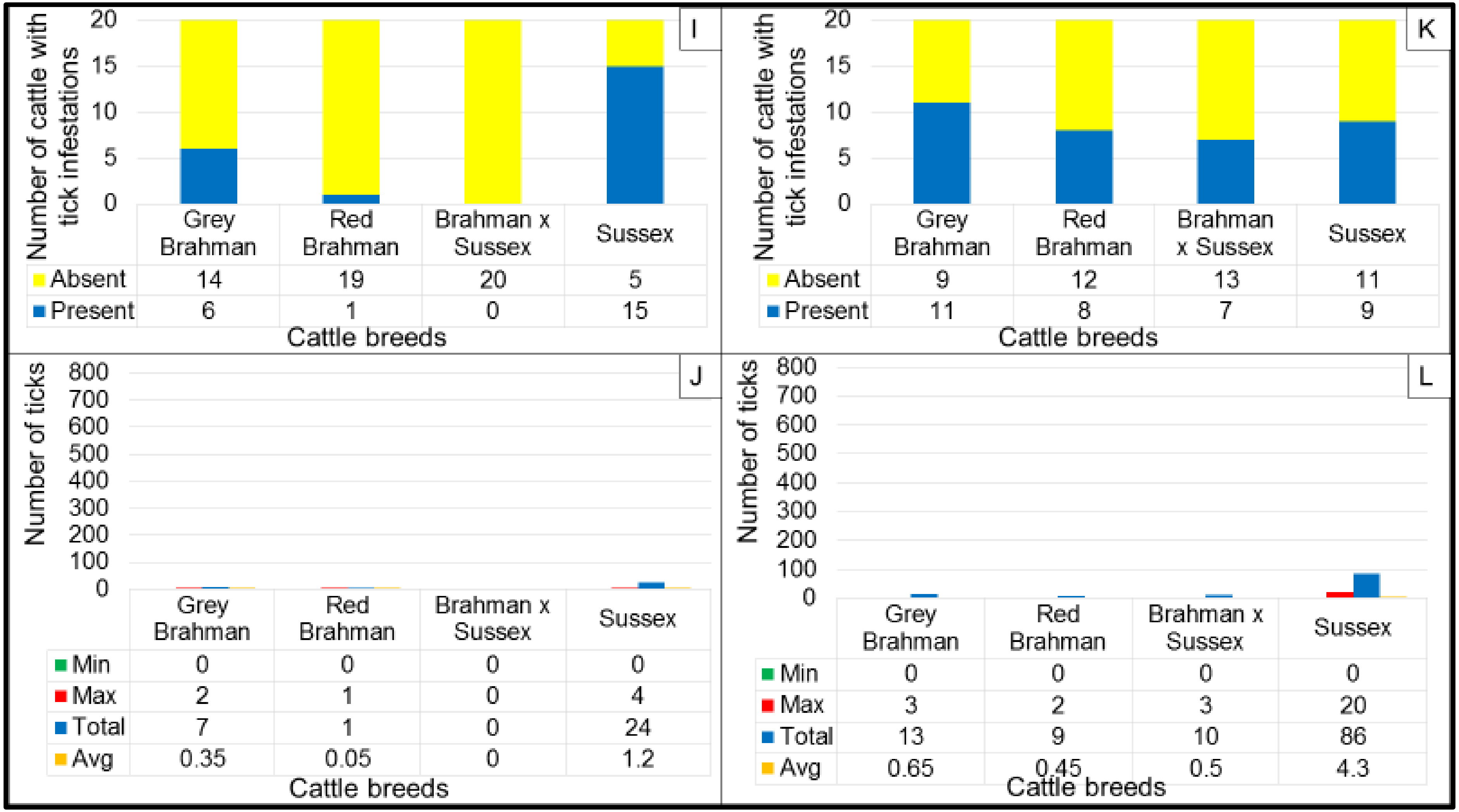


Figure 3.4 continued;

July 2014 **August 2014**



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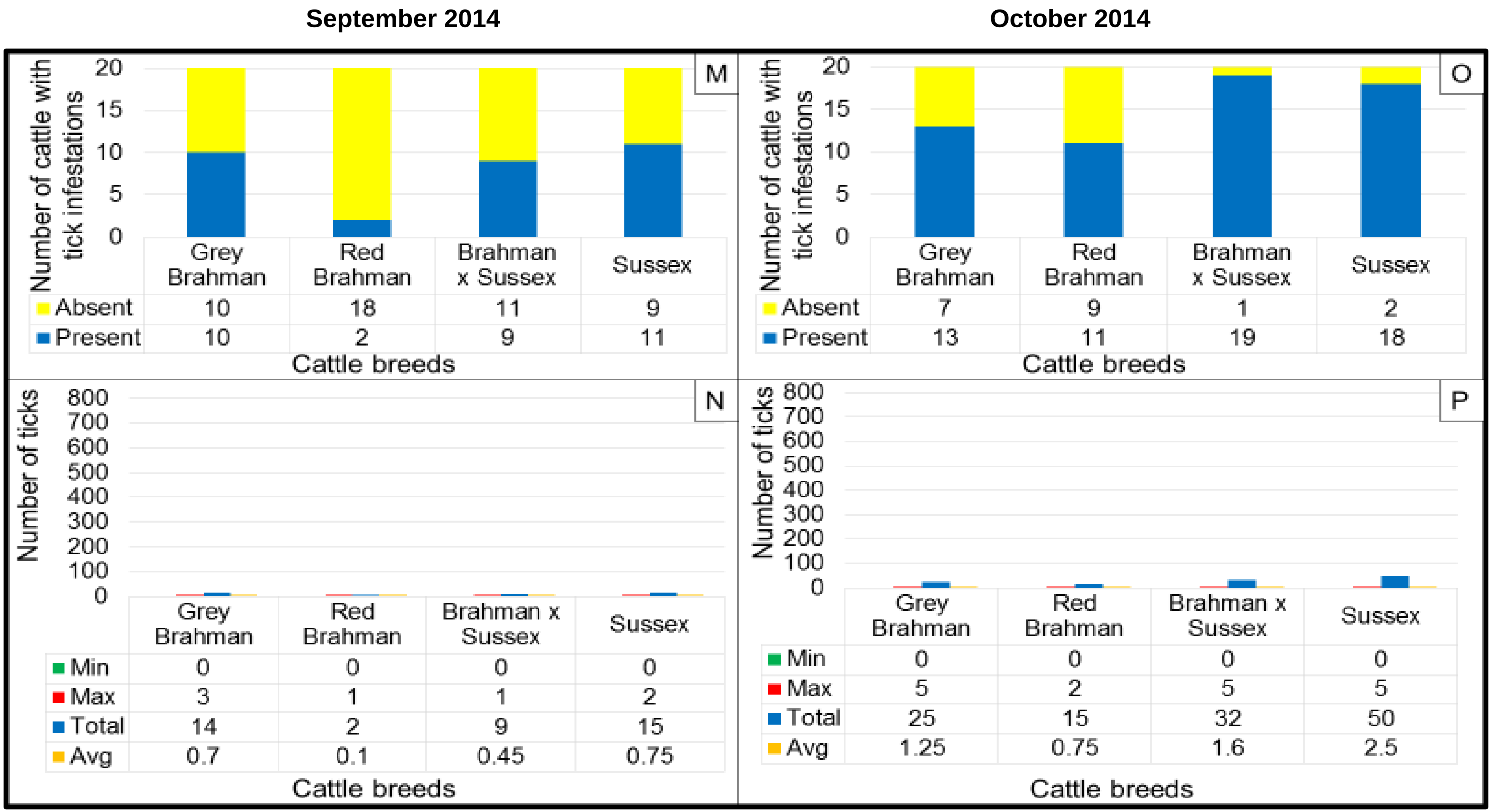

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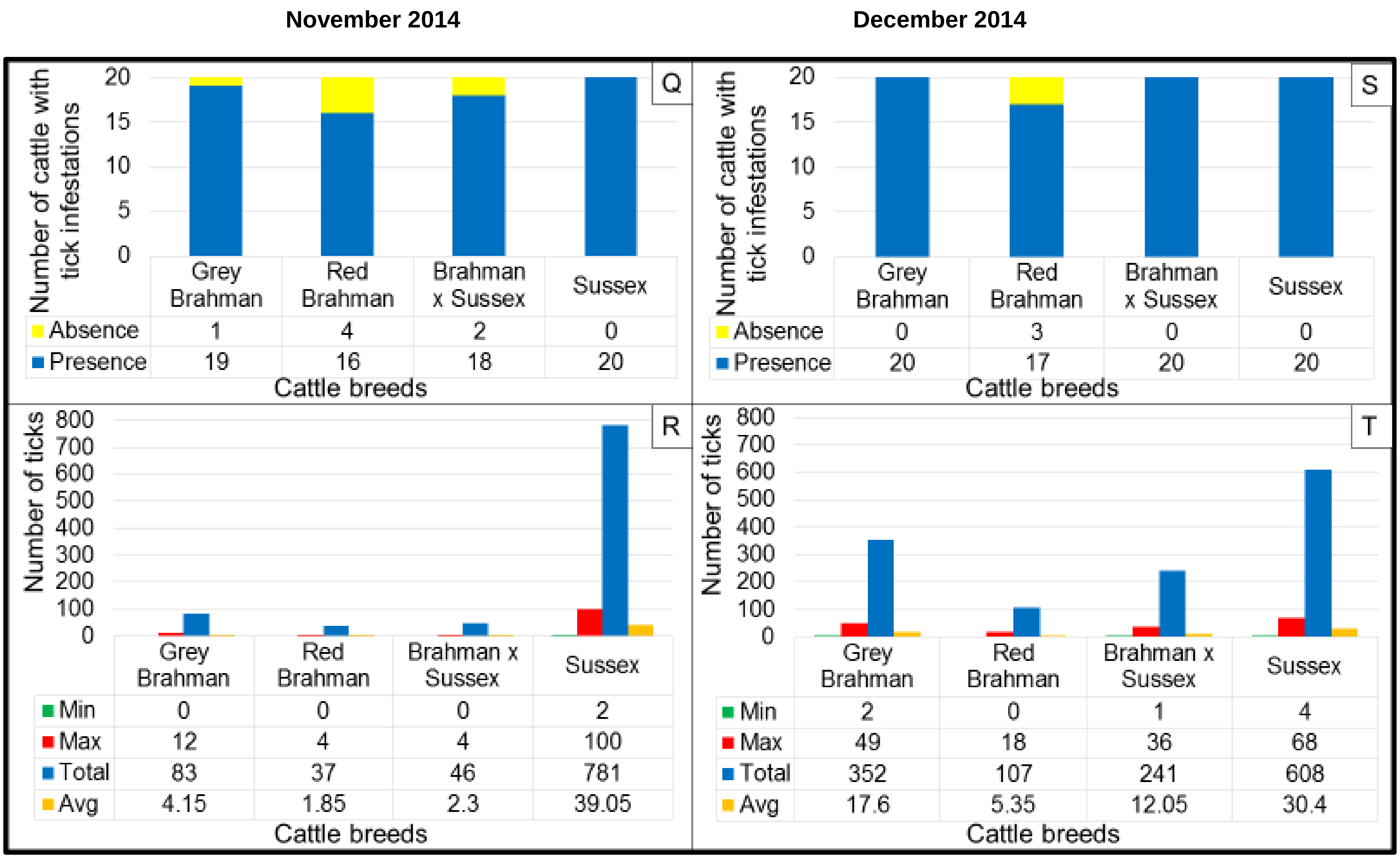

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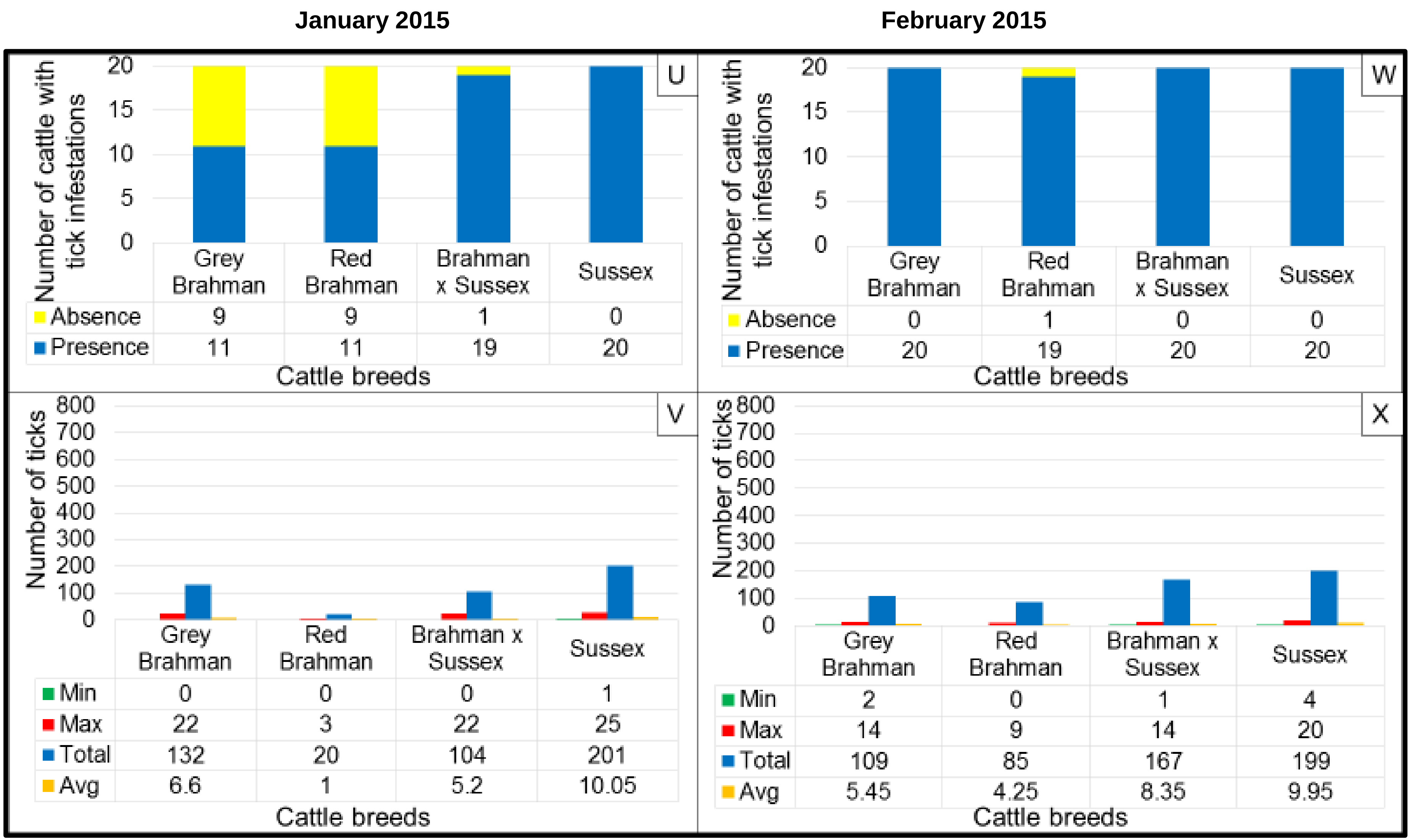

Figure 3.4 continued;

March 2015

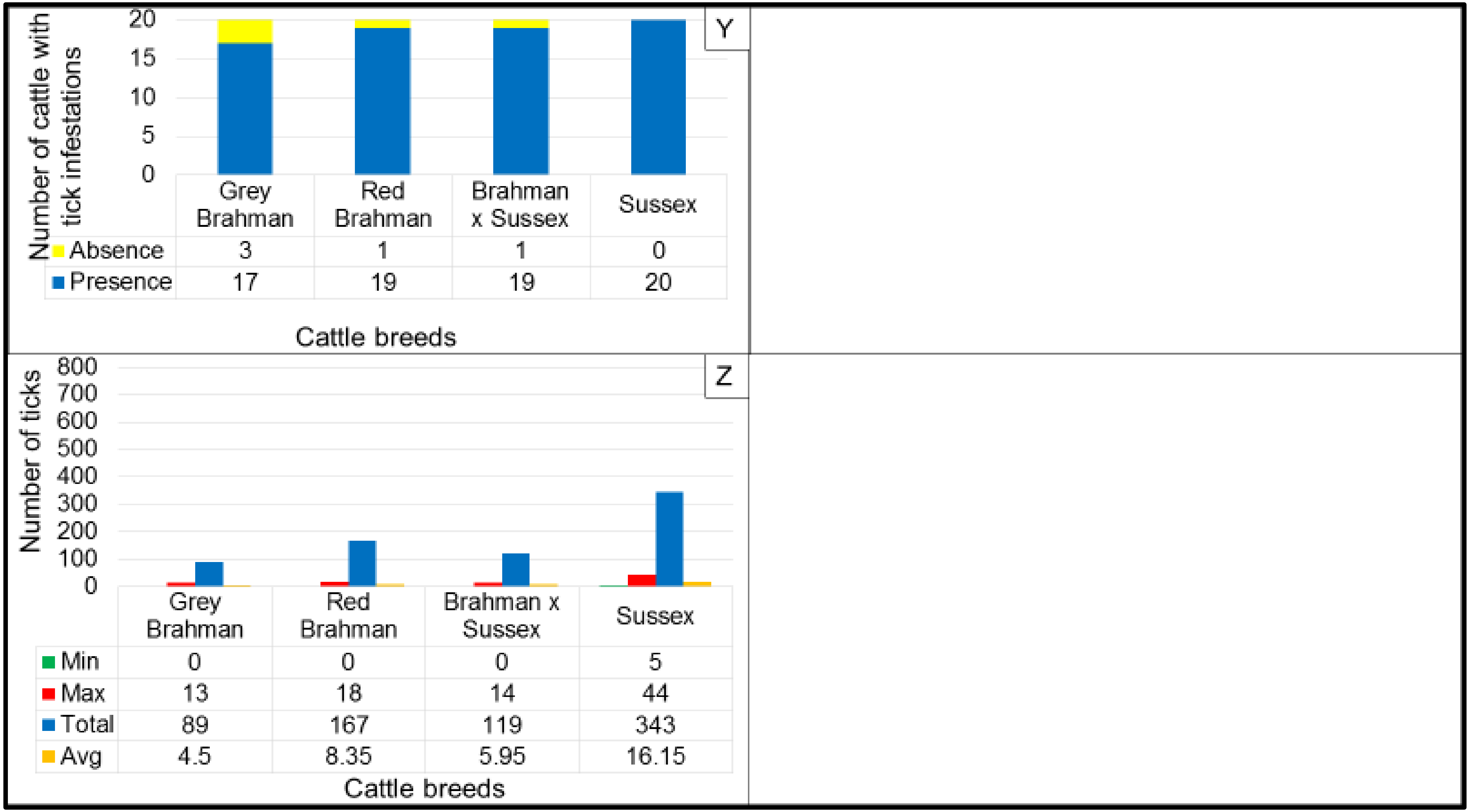

Figure 3.4 continued;

Table 3.2: When off-host were ticks sampled on the various farms with breed groups from March 2014 to March 2015.

Date	Farm	Tick count	Breed group	Time of day
21-Apr-14	Toggekry	2	Sussex group B	Morning
23-Apr-14	Blanquilla	1	Grey Brahman group B and Red Brahman group B	Late afternoon
24-Apr-14	Theron	2	Brahman x Sussex group A and B	Morning
04-Jul-14	Toggekry	12	Sussex group B	Morning
28-Oct-14	Welgegund	1	Sussex group B	Late afternoon
25-Nov-14	Tweeloop	2	Grey Brahman group B and Red Brahman group A and B	Morning

<u>May 2014</u>

After dipping during April, the number of cattle with tick infestations for each breed decreased in comparison to April, but all groups still had more than 30% of animals hosting ticks (Figure 3.4E). A total number of 27 ticks was collected from the Sussex breed still making them the group with the highest tick load compared to Grey Brahman; 6 ticks, Red Brahman; 9 ticks and Brahman x Sussex; 14 ticks (Figure 3.4F). Fluctuations in tick presence and abundance on the animals showed no significant difference from the previous month (p=1.00). No immature ticks were found in any of the camps by means of drag sampling. Production group one (Red Brahman group A, Brahman x Sussex group A and B and Sussex group A), were moved to a new camp on the farm Theron on 20 May 2014. Production group four (Grey Brahman group A), was also moved to a new camp on the farm Hamiltonrus. No animals required treatment for any health issues. Grazing provided sufficient feed, however, supplementary salt lick was also provided. All groups were found to be in optimal body condition. No dip or any other treatment was deemed necessary to be administered.

June 2014

With no dip being administered during May, the number of Sussex individuals infested with ticks increased from 60%-90% from the previous month (Figure 3.4G). Again, the Sussex groups carried the highest tick burden with 45 ticks compared with Grey Brahman; 7 ticks, Red Brahman; 8 ticks and Brahman x Sussex with 20 ticks (Figure 3.4H). The differences in tick load between the cattle groups is not significantly different (p=1.00). No immature ticks were recovered from the drag sampling in the camps and groups stayed in the same camps during this month (Annexure 3: Table 3.4). No health treatment was necessary, but supplementary salt lick was provided to all the groups. No differences were found in body condition scores, all animals being classified as optimum. No dip treatment was administered.

July 2014

Brahman x Sussex crossbred cattle had a 0% tick burden during July 2014 (Figure 3.4I), the Sussex group was the most affected with 75% of animals hosting a total of 24 ticks compared to Grey Brahman group with seven ticks, Red Brahman with one tick and Brahman x Sussex group with no ticks (Figure 3.4J). No significant inter group differences for tick load was recorded (p=1.00). Drag sampling produced 12 immature ticks collected from the vegetation of the camp on the farm Toggekry where production group two consisting of the Sussex group B were grazing. On 16 July 2014, production group one (Brahman x Sussex group A and B, Red Brahman group A and Sussex group A) were moved to a new grazing camp on the farm Border. No animals were treated for any ailments and only supplementary salt lick was provided to all the herds. No animals were under-or over weight, thus optimal body scores were recorded for all cattle groups. It was not deemed necessary to dip any of the cattle groups.

August 2014

In contrast with the previous month's findings the Grey Brahman group had the highest percentage of animals hosting ticks with 55%, compared to Red Brahman 40%, Brahman x Sussex 35% and Sussex 45% (Figure 3.4 K). However, Sussex cattle still had the highest on-host tick abundance with 86 ticks compared to Grey Brahman; 13 ticks, Red Brahman; nine ticks and Brahman x Sussex 10 ticks (Figure 3.4 L). No inter group differences were recorded for this month (p>0.05). No immature ticks were found on vegetation in any of camps through drag sampling and Production group three (Grey Brahman group A), was moved to a new camp on Hamiltonsrus on 12 August 2014. Production group two (Sussex group B), was moved to a new camp on farm Welgegund and Production group four (Grey Brahman group B and Red Brahman group B), was moved to a new camp on farm Tweeloop on 13 August 2014. No animals were found to being under-or over weight, thus optimal body scores were recorded for all cattle groups. None of the cattle groups were treated for any health issues other than tick infestation. All of the groups were treated for ectoparasite infestations with Ectoshield and Coopers tick grease between 27 to 29 August 2014.

<u>September 2014</u>

Tick hosting individuals increased within the Sussex cattle breed from 45%-55% from the previous month compared to Brahman 50%, Red Brahman 10% and Brahman x Sussex 45% (Figure 3.4M). Sussex cattle had a lower total on-host tick abundance than the previous month with a total of 15 ticks (Figure 3.4N). Other groups had the following tick abundances; Grey Brahman; 14 ticks, Red Brahman; two ticks and Brahman Sussex with a total of nine ticks. None of the differences were found to be significant ($p>0.05$). No immature ticks were collected from the vegetation in the camps via drag sampling. No groups were moved to new grazing camps during this time. Only one animal was considered to be under optimum weight out of all the test animals, belonging to the Brahman x Sussex group. Sussex animals were treated for mite infestations with the same products used during the routine dip treatment during inspection. A routine dip treatment with Ectoshield and Coopers tick grease was given to all of the groups during inspection at the end of the month (24 to 26 September 2014).

<u>October 2014</u>

During October, the number or animals hosting ticks in the Brahman x Sussex group were the highest with 95%, while 90% of the animals in the Sussex group, 65% in the Grey Brahman group and 55% in the Red Brahman group hosted ticks. The Sussex group, however, had the highest tick burden (Figure 3.4O) with a total tick load of 50, when compared to the Grey Brahman; 25 ticks, Red Brahman; 15 ticks and Brahman x Sussex with 32 ticks (Figure 3.4P). No significant differences were recorded between breed groups this month ($p>0.05$). Only one immature tick was sampled via the drag method on the vegetation of the farm Welgegund where production group two (Sussex group B) cattle grazed. Only production group four (Red Brahman group B and Grey Brahman group B) were moved to a new camp at Hamiltonsrus on 1 October 2014. Underweight cattle were identified within all the groups with a total of 30% of Grey Brahmans, 5% of Red Brahmans, 15% of Brahman x Sussex's and 5% of Sussex cattle affected. No individuals were found to be overweight. All cattle received salt lick to better maintain overall health. No animals were treated for specific ailments but all cattle groups were given Doraject + AD3E intestinal deworming and vitamin supplement as well as Coopers tick grease as immediate localised spot treatment for external parasites from 28 to 30 October 2014. This was done to help maintain general health of the animals.

<u>November 2014</u>

The effects of the various treatments in October should have been visible during this month, although the number of individuals with tick infestations continued to increase from the previous month. In the Sussex cattle group 100% of animals hosted ticks (Figure 3.4Q) followed by Grey Brahman with 95%, Brahman x Sussex with 90% and Red Brahman with 80%. The Sussex cattle group also exhibit a dramatic increase in tick load from 50 in October to 781 ticks sampled in November from the groups. The Red Brahman groups had the lowest on-host tick abundance with an increase from 15 to a total of 37 ticks (Figure 3.4R), while an increase of 25 to 83 was recorded for Grey Brahmans and 32 to 46 for the Brahman x Sussex cattle during the same period. For the first time, significant differences between on-host tick abundance for Sussex cattle, production group two and one, and the other cattle groups was recorded. The Sussex breed also had a much higher mean on-host tick abundance (Mean=39.05) than all three other breeds, Grey Brahman (Mean=4.15), Red Brahman (Mean=1.85) and

Brahman x Sussex (Mean=2.30). Two immature ticks were collected via drag sampling on the Farm Tweeloop where Production group 4 (Grey Brahman group B and Red Brahman group A and B) grazed. However, the total tick load on these groups remain significantly lower than that found on Sussex cattle. Sussex cattle group B, production group 2, was moved to a new camp on Welgegund on 1 November 2014 as well as the Red Brahman group B and Grey Brahman group B, that were moved from Tweeloop to a new camp on Blanquilla. One animal from both the Grey Brahman and Brahman x Sussex groups was found to be underweight according to the body condition scoring method used. All other animals were considered to have optimal body condition scores. No specific animals required any treatment for illnesses. All of the groups were dipped with Decatix 3 and Coopers tick grease for ectoparasites as part of the routine dipping treatment schedule from 25 to 28 November 2014.

<u>December 2014</u>

During December 2014, all the breeds had 100% tick infested individuals accept for the Red Brahman cattle with 85% animals hosting ticks (Figure 3.4S). In the groups with all animals infested with ticks, the Sussex group with 608 ticks recorded was the cattle breed with the highest tick load compared to Grey Brahmans; 352 ticks, and Brahman x Sussex with 241 ticks. The Red Brahman group had a total of 107 ticks (Figure 3.4T). There was a significant difference in the total on-host tick abundance recorded between the four cattle breeds (Annexure 3: Table 3.4). All on-host tick abundance mean counts differed from each other: Grey Brahman (Mean=17.60); Red Brahman (Mean=5.35); Brahman x Sussex (Mean=12.05); Sussex (Mean=30.40). No immature ticks were sampled in the off-host environment. No cattle groups were moved, but two animals from the Red Brahman group were found to be underweight, while all other animals were considered to have optimum body condition scores. No general health issues were reported and no animals were dipped as part of ectoparasite control.

<u>January 2015</u>

Sussex cattle had the most tick hosting individuals with a 100% compared to Grey Brahman with 55%, Red Brahman with 55% and Brahman x Sussex with 95% (Figure 3.5U). On-host tick abundance was noticeably lower than the previous month within the Sussex breed, and decreased from 608 in December 2014 to 201 ticks. In contrast, the Red Brahman group had the lowest on-host tick abundance of all the groups with a total of 20 ticks, Grey Brahman with 132 ticks and Brahman x Sussex with 104 ticks (Figure 3.5V). Significant differences occurred for on-host tick abundance between the Grey Brahman and Red Brahman groups with p=0.031 and between Red Brahman and Sussex groups with p=0.001, (Annexure 3: Table 3.4). Both the Grey Brahman (Mean=6.60) and Sussex (Mean=10.05) groups had higher mean total tick abundance scores than Red Brahman (Mean=1) and Brahman x Sussex (Mean=5.2) groups. No immature ticks were sampled in the off-host environment. Production group two (Sussex group B) was moved from Welgegund to Toggekry on 1 January 2015, where they were exposed to a new camp environment to graze in. Production group one (Red Brahman group A, Brahman x Sussex group A and B, and Sussex group A) was moved to a new camp on the same farm Theron. One animal from the Sussex group was found to be underweight and all other animals were considered to have optimum body condition scores. Some animals in the Grey Brahman herd had to be treated for lumpy skin disease (Poxviridae) with Lumpyvax and Finadyne. All animals were subject to the same dipping routine and were dipped during between 27 to 29 January 2015 with Decatix 3 and Coopers tick grease.

<u>February 2015</u>

There was an overall increase in the individual animals hosting ticks with all animals in all the groups hosting ticks except for the Red Brahman cattle groups where 95% of the animals hosted ticks (Figure 3.4W). This group also had lower numbers of on-host tick abundance with a total of 85 ticks compared to Sussex cattle with 199 ticks, Grey Brahman cattle with 109 ticks and Brahman x Sussex cattle with 167 ticks (Figure 3.4X). The only significant difference in mean total on-host tick counts was recorded between Red Brahman and Sussex groups with p=0.026 (Annexure 3: Table 3.4). The Sussex (Mean=9.95) group had a higher mean total tick abundance than the Grey Brahman (Mean=5.45), Red Brahman (Mean=4.25) and Brahman x Sussex (Mean=8.35) groups. No immature ticks were sampled via drag sampling from vegetation of camps where cattle grazed during February 2015. No groups were moved to new camps. All of the animals received salt licks as extra supplementation as the actual veld was at a very low level of quality and quantity. Body condition scoring indicated all animals to be ideal. Two of the Sussex cows received treatment for eye infections. One individual had nine ticks and the other had 15 ticks, compared to mean ticks per cow of 9.95. All of the groups were routinely dipped from 24 to 27 February 2015 with Ectoshield and Coopers tick grease.

<u>March 2015</u>

During the last month of sampling the number of individuals within each cattle group hosting ticks were still above 85% (Figure 3.4Y). Compared to each other, Grey Brahman cattle had 85%, Red Brahman cattle 95%, Brahman x Sussex 95% and Sussex cattle with 100% of individual animals hosting ticks. The number of tick infested individuals, however, decreased for both the Grey Brahman and Brahman x Sussex groups following the ectoparasite treatment in February. Figure 3.4Z indicates that Sussex cattle had the highest on-host tick abundance with 343 ticks followed by Red Brahman with 167 ticks, Brahman x Sussex cattle with 119 ticks and Grey Brahman with 89 ticks. A significant difference for on-host tick abundance between groups was again noted for this month between Sussex and Red Brahman (p=0.001), Sussex and Grey Brahman (p=0.001) and Sussex and Brahman x Sussex (p=0.001) groups (Annexure 3: Table 3.4). Descriptive analysis show that the Sussex (Mean=16.15) group had a higher mean tick abundance score than all three other selected breeds namely Grey Brahman (Mean=4.5); Red Brahman (Mean=8.35); Brahman x Sussex (Mean=5.95). No immature ticks were collected with drag sampling in any of the camps. Cattle grazed in the same camps as the previous month. Body condition scoring indicated all animals to be ideal. No general animal health issues were recorded for this month. All of the cattle groups were dipped with Ectoshield and Coopers tick grease from 24 to 28 March 2015.

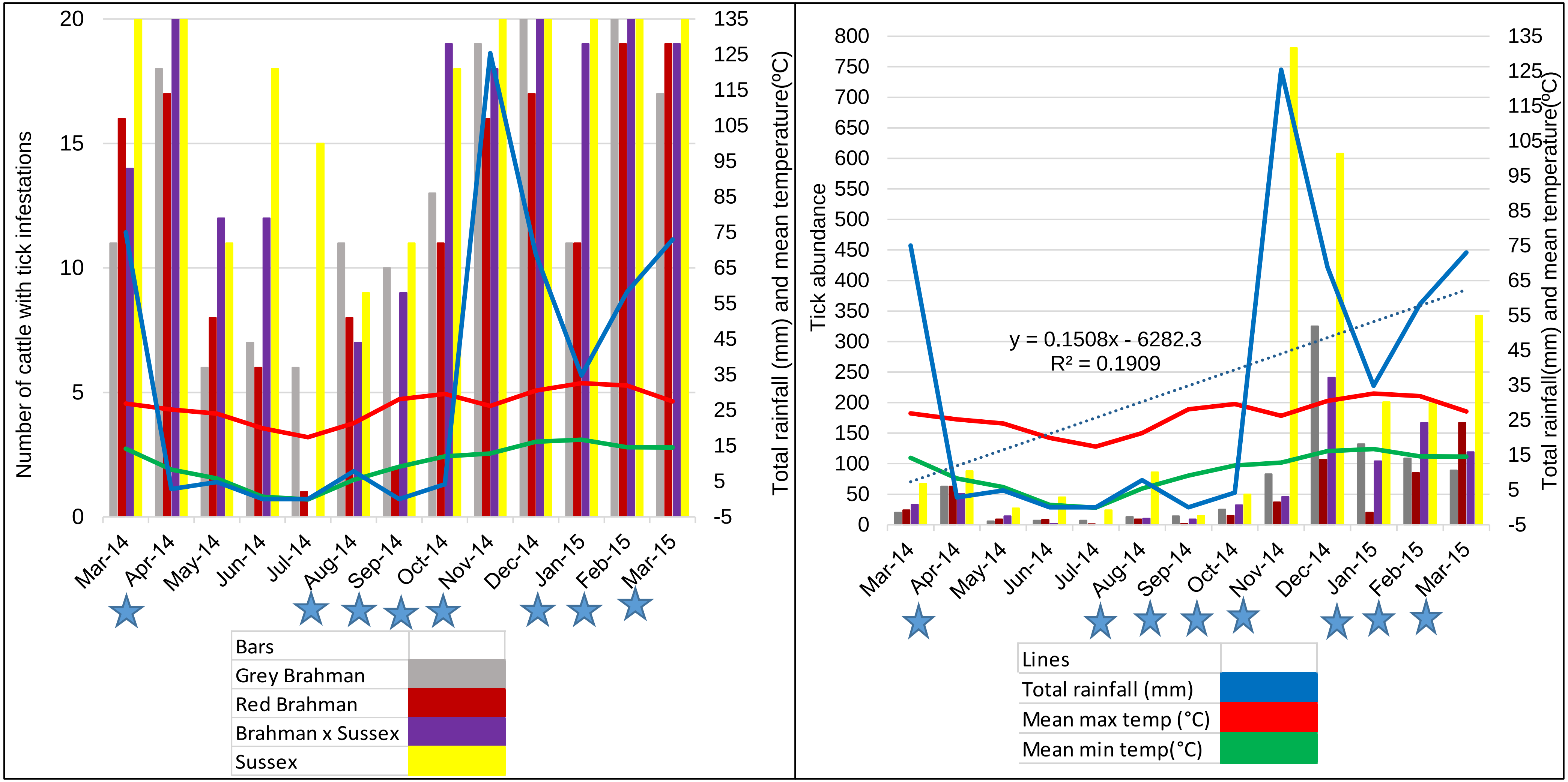

Figure 3. 5: Summary of on-host tick presence and abundance from March 2014 to March 2015 with mean maximum and minimum temperature (°C) and total rainfall (mm)with trend line. Months animals were treated for ectoparasites are indicated with a ★

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3.2.4 Mite occurrence

Only one recorded mite infestation on Sussex cattle (Figure 3.6) took place during the period of the study, in August 2014. The cattle handlers did state that more mite infestations usually occur throughout the year, especially on the Sussex cattle and particularly during warmer months of the year. No mites were found during inspection using a stereo microscope of the scraping samples. This indicates that the sampling method may not have been ideal. More scraping samples should be taken from one visible sight in future studies to ensure the presence of mites for identification.

Figure 3.6: Mite infestation on Sussex cattle during August 2014.

3.3 DISCUSSION

Differences in ectoparasite resistance between different *Bos indicus* and *Bos taurus* cattle breeds (Tanganyika shorthorn: Meru, Mbullu and Iringa red compared to Meru x Friesian and Iringa red x Friesian) were investigated by Wambura *et al*. (1998) in South Africa. The results of that study showed pure zebu (*B. indicus*) cattle had lower tick infestations when compared to zebu-taurine crosses in the same environment. Similar patterns of lower tick infestation in *B. indicus* cattle in relation to breed resistance between *B. indicus* and *B. taurus* cattle have been observed by Jonsson (2006) utilising Brahman-cross and Africaner-cross cattle, Ibelli *et al.* (2012) utilising Senepol x Nelore, Angus x Nelore and purebed Nelore cattle, Scholtz *et al*. (1991) and Spickett *et al.* (1989) both utilising Bonsmara, Nuguni and Hereford cattle. No studies have, however, been conducted on ectoparasite resistance of cattle breeds within the current study area (central Free State) where tick exposure is not as high as in some other provinces in South Africa (Hlatshwayo *et al.* 2001; Horak *et al.* 2015).

In this study Acari resistance of the three different cattle breeds (Brahman, Brahman x Sussex and Sussex) was analysed by recording tick and mite presence and abundance on the cattle groups. Both on-host and off-host environments were investigated to determine if the off-host abundance of ticks influenced the on-host tick presence.

In the current study at a locality in the Free State, 30 km west of Kroonstad (S27 39.089, E026 57.500) which has not previously mapped for tick presence, *H*. *rufipes* ticks were by far the most abundant tick species with 3797 individuals representing 79% of the total number of ticks collected over the 13 month study, followed by *R. evertsi evertsi* (596 individuals) that made up 12.3%, and *H. truncatum* (403 individuals) presenting 8.1% of the total tick count. The tick species with the lowest representation were *R. decoloratus* (33 individuals) representing 0.7% and *R. appendiculatus* (30 individuals) with 0.6% of the total number of ticks collected. However, Horak *et. al*. (2015), combining findings of several tick surveys in the Free State, described ticks that are well established and endemic to the Free State collected from cattle to be *R. appendiculatus*, and *R. decoloratus. Rhipicephalus microplus* also collected at a few localities in the eastern Free State during this survey, are considered to be non-endemic to the area.

Different areas of the animals from the different breeds were examined for tick infestations to determine preferred attachment sites for ticks on each breed. No ticks were found on the forequarter section of animals in the Grey- and Red Brahman groups for the entire test period. The Red Brahman group only exhibited ticks attached to two body locations, under the tail and the rear flank area, with the Grey Brahman group showing an additional attachment site on the udder. The Brahman cross Sussex group only had one site where ticks were collected from the forequarter and for the rest tick attachment areas were the same as for the Grey Brahmans. In the Sussex cattle group**,** ticks were collected from the tail head, under tail, rear flank, naval, fore flank, neck, shoulder and ears, making it the breed with the greatest variety of tick attachment sites.

Different tick species have different preferred attachment sites on their hosts of which *H. rufipes, R. evertsi evertsi* and *H. truncatum* usually prefer areas on the hindquarter for feeding while *R. appendiculatus* feeds around the head and ears and upper neck and *R. decoloratus* is found on various sites all over the body (Walker *et al.* 2003; De Menenghi *et al*. 2016; Nejash 2016). A gap in this study was that attachment sites were documented and then ticks from each animal were collected into one collection bottle which made it impossible to link specific tick species after identification to specific attachment sites. It can therefore only be concluded that ticks were collected from a wider variety of attachment sites on the Sussex breed compared to the other breeds and that the Brahman x Sussex breed correspond more to the Brahman than the Sussex breeds with regards to number of attachment sites and areas of attachment.

Hyalomma rufipes has a two-host life cycle that can take up to a year to complete. They are widely distributed throughout South Africa (Walker *et al.* 2003). In this study, they were sampled from all four breeds throughout the study except for the Brahman x Sussex group A and B and Sussex group A during July 2014 grazing on the farm Border, when very low numbers were also found on the other two breeds. This could have been due to the off-host environment on Border not having a high tick load, because this location consisted of residue maize production used for cattle feed after harvesting during winter when the natural fields had a low feeding capacity. Both male and female numbers of this species increased dramatically during November 2014 to March 2015 when higher rainfall and temperatures were experienced indicating it to have sufficient heat and draught tolerance in order to reproduce in the specific area (Socolovschi *et al*. 2009). Mattioli *et al.* (1997) also found *H. rufipes, H. truncatum* and *R. decoloratus* tick infestation acquired by host animals to peak during the rainy seasons and years as a reflection of climatic factors.

Rhipicephalus evertsi evertsi is also a two-host tick species that can complete more than one life-cycle in a year and can be found in desert, steppe, savanna and areas with temperate climatic conditions throughout South Africa (Walker *et al.* 2003). Representing 12.3% of Ixodid ticks sampled in total in this study, it was also found throughout the year on all the breeds except for May 2014 on the Grey and Red Brahman groups, July 2014 on the Red Brahman as well as the Brahman x Sussex group and during September 2014. *Rhiphicephalus evertsi evertsi* ticks were also commonly found on different cattle breeds investigated by Dreyer *et al*. (1998) in the eastern Free State. It was found that the two most abundant tick species; *H*. *rufipes* and *R*. *evertsi evertsi* occurred at all the test sites but with differences in abundances. Dreyer *et al*. (1998) also found these *Rhipicephalus* species to be present in most of the test areas during their studies in the eastern Free State and they were also described to be the widest distributed species belonging to the *Rhipicephalus* genus in Africa (Nyangiwe *et al.* 2007; Marufu *et al.* 2011).

Hyalomma truncatum, a two-host tick species, can take up to a year to complete one life cycle. Its presence in the on-host environment was also found to be higher during warmer months with adults more abundant during wet summer months and immature stages during dry autumn and spring months (Walker *et al*. 2003). It also generally occurs in areas with rainfall below 500mm per annuum, thus in areas more arid than the study area (Dreyer *et al.* 1998), such as deserts in Egypt, steppe and savanna areas throughout the Afrotropical zoogeographical regions (Walker *et al*. 2003). The summer peak of abundance for both *Hyalomma* species sampled that ranged over a few months from October 2014 to January 2015 in this study may indicate overlapping of more than one generation as also found in previous studies done for one to three host ticks found in the eastern Free State (Dreyer *et al*. 1998; Walker *et al.* 2003).

Rhipicephalus decoloratus is one-host tick species more commonly found in the eastern and northern parts of the Free State and usually during the summer and autumn to early winter months in temperate regions including savanna, grassland and wooded areas (Dreyer *et al*. 1998; Walker *et al.* 2003; Schroder *et al*. 2006). It can, however, be found west of Bloemfontein if the annual mean rainfall is at least 500mm (Horak *et al.* 2015). In comparison, the rainfall recorded for March 2014 to March 2015 was about 455mm in the current study area. Under favourable conditions they can complete more than one life cycle in a year (Walker *et al.* 2003). In this study, low numbers of female *R. decoloratus* were present during the winter (May 2014 to July 2014) but confined to the farm Theron, and mostly on the Sussex group (29 of 34 collected). In contrast to the current study the study by Dreyer *et al.* (1998) in the eastern Free State showed *R*. *decoloratus* to be more abundant than *H. rufipes* throughout the year.

The predominant winter occurrence in the current study raised the question of correct identification, as *Margaropus winthemi*, also a one host tick and usually occurring in the winter (Walker *et al*. 2003) can easily be mistaken for *R. decoloratus,* but this possibility was dismissed after a second look at the collection specimens (Both male and female *M. winthemi* have thicker legs than *Rhipicephalus* species and their legs have dense setae and the outer most segments have dark brown patches or rings).

Rhipicephalus appendiculatus is a three-host tick species that have a strictly seasonal, single generation in southern Africa (Walker *et al.* 2003). These ticks are commonly found in savanna and temperate climatic regions such as the North West, Gauteng, the coast of KwaZulu-Natal and the Eastern Cape, Mpumalanga, and the northern part of the Free State near Parys (Horak *et al.* 2015). In this study, they were mostly found on the Sussex cattle groups (26 of 28 collected) grazing on Border and Welgegund in August and November 2014. Three individuals were sampled during February 2015 from the Sussex group and one other individual was found on a Grey Brahman grazing on Blanquilla during March 2015, one on a Brahman x Sussex grazing on Border in August 2014. In a study done by Mooring *et al*. (1994) in Zimbabwe, *R. appendiculatus* was also found to be two to three times more numerous in the environment during higher rainfall periods.

Throughout the test period Sussex cattle had the highest tick loads of all the breeds with 2534 ticks compared to the Brahman (Grey; 919 & Red; 550 ticks) and Brahman x Sussex cattle groups (846 ticks).

Considering the individual tick species collected in this study (with the exception of one species), the Sussex breed group were the most preferred host. On a monthly basis significant differences were found in on-host tick abundance between the Sussex breed group and all the other breeds for November 2014, December and January 2015. The Sussex breed group also showed a significant difference compared to the Red Brahman and Brahman x Sussex groups for February and March 2015. Significant differences between the other breeds were only found for December between all the groups and January 2015 between the Grey Brahman and Red Brahman groups and Sussex and Red Brahman. This coincided with the warmer summer months where a higher tick count was found on all the groups in general especially after some scattered rainfall was recorded. The high tick loads on the Sussex group during the hottest months of November to March confirms the findings of Hansen (2004) that stress on cattle, suited for the particular environment, will be lower. The Brahman cattle breeds might therefore not be as affected by heat strain and environmental factors and could have a higher resistance tick burdens as confirmed by this study.

Tick abundance sampled in the off-host environment was less than expected but in line with immature behaviour in an off-host environment during low environmental humidity to preserve water balance (Walker *et al.* 2003). A correlation between off-host and on-host tick abundance was therefore not viable to obtain.

The presence and absence of free-living two-and three-host ticks in grazing camps (off-host environment), is influenced by variation in sward height and humidity. In turn vegetation quality and quantity is directly influenced by environmental conditions such as temperature and rainfall (Greenfield 2011). The drought experienced in South Africa during 2014 and 2015, might have played a major role in the absence of immature stages in the fields. When questing larvae experience severe dehydrating circumstances, they will move back down to ground level where the humidity is higher (Socolovschi *et al*. 2009; Schulz *et al*. 2014). Two and three host tick species remain inactive in the floor-covering vegetation if temperatures are too low or too high to maintain optimal body hydration. Unfed Ixodidae ticks need a relative humidity of 80% to survive, and this will determine the time spent questing for host animals to complete their lifecycles (Jongejan *et al*. 1994; Greenfield 2011).

It was found by Madder *et al.* (1999) that *R. appendiculatus* utilise a photoperiod induced behavioural diapause as survival method, where diapause will only be ended for further development to take place with the occurrence of an increase in more favourable environmental conditions with a higher ambient humidity (Mtambo *et al.* 2007). For this to happen adequate rain needs to fall and temperatures need to be moderate.

Many factors, such as seasonal occurrence of tick species, field conditions, temperature and rainfall, wind conditions as well as animal health, movement of cattle and tick control strategies may influence on-host and off-host tick presence and abundance (Ruiz-Fons *et al.* 2012). The results obtained in the current study strongly indicates that temperature and rainfall are great influencing factors that regulate seasonal activity of on-host and off-host ticks sampled in the test area. Rainfall and temperature influences the viability of the on-host and off-host microhabitat conditions, which directly influences questing success and survival of all stages of the Ixodidae tick lifecycle (Greenfield 2011; Schulz *et al.* 2014).

The overall tick presence and abundance for all the breeds peaked during the hottest months of the year, March 2014 to April 2014 and Nov 2014 to March 2015. Rainfall also peaked during these months indicating a positive correlation between on-host tick presence and rainfall by means of a trendline (y=0.1508-6282.3, R^2=0.1909) indicated in Figure 3.5.

Adults ticks of species collected in this study are described to be more abundant during the summer months with higher rainfall conditions than during the winter months with dry conditions (Walker *et al.* 2003). This finding was confirmed by both the number of males and females, which peaked from October 2014 to March 2014 and October 2014 to January 2015 respectively. The numbers of female ticks belonging to two-host tick species such as *H. rufipes, R. evertsi evertsi* and *H. truncatum*, sampled during this study period indicated that these species were successfully completing their lifecycles on the cattle and other host species in the specific environment (Nyangiwe *et al.* 2007; Horak *et al.* 2008). Numbers for one-and three-host tick species were considerably lower.

Dreyer *et al.* (1998) indicated low quality veld as an unsuitable off-host habitat, causing a lower prevalence of three-host tick species such as *R*. *appendiculatu*s. This is confirmed by the current study, where the only three host tick species, *R. appendiculatus* were found in very low numbers (total of 29) indicating that they were more susceptible to harsh microclimatic conditions, than one or two host tick species, during free-living life stages in the veld. They are also exposed to environmental conditions for longer periods of time during the different host seeking stages.

Seasonal rainfall and temperatures and the occurrence of wind can influence field conditions that in turn also influence on-host tick abundance dramatically. According to the producer, Mr. P. C. Esterhuyze, a mid-summer draught during December and January was not uncommon in this area and from his past experience the high temperatures and very low rainfall indicated a strong El-Niño system development. This caused cattle to be exposed to stressful environmental conditions during 2014 and 2015 ([1]Mr. P. C. Esterhuyze, personal communication; Du Preez 2014). Regular dust storms occurring during these summer months, directly influenced agricultural activities by causing air pollution, reduced solar radiation and damaged plant health that effects the ideal microhabitat conditions for ticks in grass coverage (Prakash *et al*. 2015; UNEP, WMO, UNCCD 2016). This could explain the absence of immature stages during drag sampling in the different camps.

It further might also have influenced the grazing quality of the natural veld camps in the study area during the entire test period. The decrease in veld quality and quantity due to a shortage of precipitation can also produce heat stress related symptoms in cattle (Scholtz *et al*. 2013). Cattle that exhibit reduced body condition as a result of poor vegetation quality, are more susceptible to ecto- and endoparasite infestation (Saymore *et al*. 2011). All cattle in the study were therefore given salt lick as supplementary feed.

Animal health issues such as diseases not noticed and treated might have a possible influence on cattle tick numbers as observed in the Eastern Cape by Nyamgiwe *et al*. (2011) where Nguni cattle were found to be less susceptible to tick infestation than Bonsmara cattle. Some of the Bonsmara's had died due to suspected *Babesia bovis* infection.

During October 2014, underweight individuals were recorded for all the breeds (30%, Grey Brahmans, 15% Brahman x Sussex, 5% for each of Red Brahman and Sussex groups) influencing the potential on-host tick infestation vulnerability. The influence of weight will be discussed in Chapter 5. The lower body condition scores could partly be due to reproductive activity as cows were either calving (September to November)

[1] Mr. P. C. Esterhuyze, Kroonstad, November 2014

or bulls were added to the herds for breeding (December to February). This activity takes place at an energy cost, thus causing some cows to have lower body scores. From October to December 2014 an increase in individuals hosting ticks, as well as number of ticks found on each breed were documented for all breeds. This occurred in spite of tick control treatments during September, October and November 2014. This is in agreement with studies that showed more individuals to become infested with higher numbers of parasites as their body scores lower due to harsh conditions, influencing total production potential including feed conversion, other physiological functions and general behaviour (Blackshaw *et al*.1994; Scholtz *et al.* 2013).

Other influential health related issues contributing to the difference in tick abundance between breeds may have been due to the Grey Brahman cattle group grazing in a camp with wetlands on Hamiltonsrus farm, thus exposing them to some breeding areas of biting midges (Ceratopogonidae). These health issues as well as the wetland location, with more ambient moisture present may also have contributed to a better supporting environment for free-living ticks when compared to the other grazing camps.

During February 2015, two individuals from the Sussex group were treated for eye infections. One hosted nine ticks and the other 15 ticks, with a group mean tick abundance of 9.95 per cow. The extra infection might have put more health related stress on these animals inceasing the likelyhood of a higher tick load but the proof of data for this is not sufficient. Previous studies indicate lower immunity linked to poor body condition as the physiological state of the animals are influenced by being exposed to internal and external stress factors (Saymore *et al*. 2011). In an attempt to cope with these challenges the majority of their energy is used to mobilise stress responsive reactions, thus affecting normal body functions like growth, immunity, reproduction and behaviour (Saymore *et al*. 2011).

Tick infestation on vegetation in different grazing camps can be positively correlated with tick infestation on various reservoir animals (stock and wild animals) (Smith *et al*. 1996). If groups of cattle with already established tick infestations enter a new camp, they will disperse ticks to this new area, just as smaller host animals such as birds will

(Smith *et al*. 1996). If the environmental conditions are favourable, ticks will be able to multiply successfully in the new environment (Schroder *et al*. 2006).

Brahman x Sussex group A and B were moved to a new grazing camp at the farm Border during July. This particular camp had very little natural veld as it was planted with maize the previous season and the refuse maize stalks were then utilized as winter feed, as is common practice in the Free State when the natural veld does not supply sufficient nutritional value (Gertenbach *et al.* 2004). As the new location can't be described as natural veld the animals normally graze on and the occurrence of any larger tick hosts in this camp was limited to winter months, tick distribution and survival were restricted to small host animals and birds (Hasle *et al*. 2009).

Rhipicephalus decoloratus ticks were only found on animals after they were moved to the second camp on Theron A, indicating that this is the only camp on the different farms where *R. decoloratus* can be found and that it was not yet established in other camps. Further collection on these farms need to be made over time to determine if *R. decoloratus* will indeed be established and distributed to other camps or fail too adapt and die out.

Host availability may play a significant role in on-host and off-host tick abundance and occurrence of different tick species in the different camps. A recent study in the Northern provinces of South Africa done by Hasle *et al*. (2009) identified guinea fowl and other birds to be important hosts for immature stages of various tick species and their associated tick-borne pathogens across large geographical distances. The various bird species moving through the test area may become heavily infested with immature ticks, dispersing them to surrounding camps as they travel (Smith *et al*. 1996; Hasle *et al*. 2009).

Other Acari attempted to be sampled included parasitic mites. Although one recorded mite infestation took place during the period of the study on Sussex group B, August 2014, the cattle handlers did state that more mite infestations are usually observed throughout the year, especially on the Sussex (*B. taurus*) cattle and particularly during warmer months. In contrast with this, other studies found that mites are usually transmitted during the winter when the animals are in close contact with each other

(Agumas *et al.* 2015). The regular checking of the cattle might have contributed to the low infestation transmission. It also might be possible that the micro habitat created with longer hair on the Sussex cattle is more favourable for the mites to survive during summer months.

3.4 CONCLUSION

The main objective of this chapter to explore crossbreeding as a measure to manage Acari infestation specifically comparing tick and mite resistance between Brahman, Sussex and Brahman x Sussex F1 crossbreds by determining Acari abundance and species composition for each breed was successfully accomplished.

A total of five Ixodidae species were collected from the animals over the 13 month study period including *H. rufipes*, by far the most abundant tick species (3797) followed by *R. evertsi evertsi (596)*, *H. truncatum* (393), *R. decoloratus* (29) and *R. appendiculatus* (30). All of these tick species except for *H. truncatum* showed a higher affinity for the Sussex breed than any of the other breeds.

Sussex cattle was the most tick and mite infested breed in this study and had the most recorded tick attachment sites. The group with the least ticks over the entire test period was the red Brahmans followed by Brahman x Sussex and Grey Brahmans. This proves that crossbreeding can be a means of controlling ticks and mites through breeding for resistance. No correlation was found between on-host and off-host ectoparasite diversity and abundance. The Grey Brahmans may have had more ticks than the crossbreds due to the fact that they grazed on a farm, Hamiltonsrus, were wild game also graze. The wild game animals might serve as a reservoir host for the different life stages of economically important ticks (Jongejan *et al.* 1994). Weather conditions, and general health issues of the cattle did have an influence on ectoparasite abundance. When the condition of microhabitats in the on-host or off-host environment deteriorates due to extreme weather fluctuations tick abundance in the veld as well on the animals' decreased.

As a result, the decision on how and whether to control ticks and tick-borne diseases should be made by considering various positive and negative outcomes for each method (Muhammad *et al*. 2008).

A programme for the control of *R. evertsi evertsi* ticks could be incorporated with one for the strategic control of *R. decoloratus* (one-host tick), where it could be considered that an acaricidal application in March would be very effective in reducing the numbers of *R. evertsi evertsi,* as this is the period with the highest *R. evertsi everstsi* burdens (February to May)

A tactical approach for the control of both *Hyalomma* species with local acaricidal applications during the long October to February peak would be practical and feasible. It is proposed that when producers observe burdens of more than 15 *Hyalomma* ticks in the perineal and inguinal areas of their cattle, the infestations should be treated with a local application of tick-grease.

The most effective control measures for ticks are focussed on parasitic ticks in the on-host environment and not yet for free-living ticks in the off-host environment (Schroder *et al.* 2006). An integrated approach should be tested where both cattle movement according to season and grazing availability should be done in sync with ectoparasite treatments according to each producer's available grazing pastures. Products aimed at controlling two and three host ticks should be focussed on rather than the application of one-host control products in the Kroonstad area.

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CHAPTER 4

DIPTERA FAMILY ABUNDANCE AND DIVERSITY

INTRODUCTION

Flies are considered to be of veterinary importance in three aspects namely pathogen transmission, the formation of cutaneous lesions and possible internal burrowing (Scholtz *et al.* 2008). Whether you are looking at intensive feedlot conditions or extensive beef production conditions, management procedures are influenced by the variety of potential dipteran disease vectors attracted to the cattle (Marchiori 2014). These veterinary important Diptera can either distribute disease among humans and production animals or be a nuisance to the animals influencing the profitability of beef production. The related stressed behaviour of the cattle can lead to a failure to reproduce effectively as well as a decrease in meat and milk production (Pruett *et al*. 2003; Davis *et al*. 2014). Primary and secondary effects are the same as described in Chapter 3 for tick infestation.

Brahman characteristics, including loose skin that quivers when the insects try to make contact with the animals hide, thicker skins, a sebum secretion that can possibly repel flying insects and more hair per square centimeter than described for breeds of *B. taurus* cattle (Bonsma 1980) was previously shown to withstand mosquito attacks better than Hereford cattle with regards to weight loss (Turner 1980).

The main objective in this chapter, was to explore crossbreeding as a measure to manage Diptera infestation by determining if there is any preference of dipteran species when the option for different cattle breeds are available. To be able to make valid assumptions it was also necessary to determine the diversity and abundance of veterinary important Diptera in the Kroonstad area. Gaining more information on potential Diptera vectors active throughout the year in this area, will enable the improvement of collection methods for future studies and this will again help with the implementation of management control strategies. Exploring the possibility of effectiveness of selecting cattle with lower observed fly load can prove to be valuable to obtain information on phenotypical traits (Pruett *et al.* 2003).

Specific objectives included:

- The comparison of Diptera abundance on the three selected cattle breeds to determine if crossbreeding can be a means of controlling flies through breed preference.

- Investigation of the association between on-host and off-host Diptera diversity and abundance.

- Determination of the influence of weather and seasonal conditions on Diptera abundance.

- Determination of arthropod orders and families present to determine their veterinary importance for the test area.

4.1 MATERIALS AND METHODS

The planned sampling methods where commercial baited fly traps (three litre REDTOP Flycatchers) were used, proofed to be inadequate for the purpose of this study and new methodology had to be developed to sample Diptera, in the vicinity of animals in the kraal, during the day. This included four REDTOP Flycatcher traps baited with urine, liver, feces and water (as a control) during inspection each month as described in Chapter 2. Validity of the adapted traps should be tested in future studies. A “controlled Diptera count study” on live animals was also done once during December 2014 and again during December 2015. Sticky fly tape (Victory’s FLY catcher) was used during this experiment to gain a better indication of the diversity of fly species active around the cattle during the day.

Fluorescent light collection traps were used to sample Diptera active during the night and found to be effective, thus no improvements were made. These traps were inspected for different Diptera families and other arthropods. These collection methods are described in Chapter 2.

4.2 RESULTS

4.2.1 Daytime collection

4.2.1.1 "Controlled fly count study"

4.2.1.1.1 Irritable behaviour

Diptera abundance around the cattle breeds as well as interaction with each of the cattle breeds was tested by means of a "Controlled fly count study". The behavioural traits observed to portray irritability included: forceful or light tail flicking, ear flaps, head shakes and skin twitching.

Sussex cattle showed the highest frequency of all-inclusive irritable behaviours monitored during both morning and afternoon observation sessions as well as the consecutive experimental days in December 2014 and December 2015 (Table 4.1). No significant difference was however observed between any of the groups during the 2014 collection (Annexure 4: Table 4.1). The differential behavioural counts are indicated in Table 4.1.

Observations during December 2015, however showed an all over higher frequency of irritability counts probably due to higher Diptera numbers present, caused by more favourable environmental conditions. When morning and afternoon counts were combined for both test days differences in irritable behaviour were found during December 2015 between the Sussex breed and all the other breeds as well as between the Red Brahman and the Grey Brahman groups and the Brahman x Sussex breed (Annexure 4: Table 4.1). More replications of this experiment should be done in future studies to validate preliminary statistical analysis (Annexure 4: Table 4.1).

Table 4.1: Differential agitation behaviour counts recorded for cattle in December 2014 and 2015.

December 2014													
Day 1 - Morning							Day 2- Morning						
	Forceful tail flick	Light tail flick	Ear flap	Head shake	Skin twitching	Total		Forceful tail flick	Light tail flick	Ear flap	Head shake	Skin twitching	Total
Grey Brahman	13	56	16	1	2	88	Grey Brahman	16	55	22	2	2	97
Red Brahman	19	40	25	4	5	93	Red Brahman	19	30	27	4	3	83
Brahman x Sussex	38	20	37	9	9	113	Brahman x Sussex	39	18	34	3	5	99
Sussex	55	26	44	5	5	135	Sussex	63	25	47	4	2	141
Day 1-Afternoon							Day 2-Afternoon						
	Forceful tail flick	Light tail flick	Ear flap	Head shake	Skin twitching	Total		Forceful tail flick	Light tail flick	Ear flap	Head shake	Skin twitching	Total
Grey Brahman	15	46	60	2	4	127	Grey Brahman	16	35	46	2	6	105
Red Brahman	5	23	14	5	2	49	Red Brahman	17	35	24	1	6	83
Brahman x Sussex	5	60	36	0	3	104	Brahman x Sussex	15	49	31	4	4	103
Sussex	75	30	14	5	3	127	Sussex	64	40	25	7	3	139
December 2015													
Day 1- Morning							Day 2- Morning						
	Forceful tail flick	Light tail flick	Ear flap	Head shake	Skin twitching	Total		Forceful tail flick	Light tail flick	Ear flap	Head shake	Skin twitching	Total
Grey Brahman	139	110	201	37	24	511	Grey Brahman	57	161	114	57	43	432
Red Brahman	41	66	118	3	10	238	Red Brahman	38	186	162	16	35	437
Brahman x Sussex	83	82	38	15	6	224	Brahman x Sussex	83	132	47	21	34	317
Sussex	68	233	125	53	46	525	Sussex	57	188	165	39	26	475
Day 1- Afternoon							Day 2 -Afternoon						
	Forceful tail flick	Light tail flick	Ear flap	Head shake	Skin twitching	Total		Forceful tail flick	Light tail flick	Ear flap	Head shake	Skin twitching	Total
Grey Brahman	154	127	107	44	20	452	Grey Brahman	78	200	63	47	27	415
Red Brahman	45	55	118	5	22	245	Red Brahman	79	215	55	23	32	404
Brahman x Sussex	58	109	51	10	12	240	Brahman x Sussex	98	172	51	48	40	409
Sussex	70	264	125	53	26	538	Sussex	84	203	125	50	27	489

4.2.1.1.2 Diptera presence

Total Diptera individuals counted landing on the individual cows from each breed during morning and afternoon observation sessions in December 2014 and again in December 2015 are indicated in Figure 4.1A and B. During the first and second day of observation in 2014, a difference was found for flies observed around the Sussex breed compared to all other groups. All Diptera presence values recorded in 2014 also differed from totals recorded in 2015 (Annexure 4: Table 4.2 and 4.3).

From the data recorded during the two days in December 2015 no significant differences could be calculated between any of the cattle groups in terms of Diptera presence or between observations day one and two ($p>0.05$). Although the data recorded was limited to the controlled Diptera count test, it was noticed that the most flies were observed around the Grey Brahman cow with a total of 1068 in the morning and around the Sussex cow in the afternoon, with 1161 on the first day. During the second day of observation in 2015 (Figure 4.1D) the most flies were observed around the Brahman x Sussex cow, with 1213 in the morning and around the Sussex cow in the afternoon with 1255.

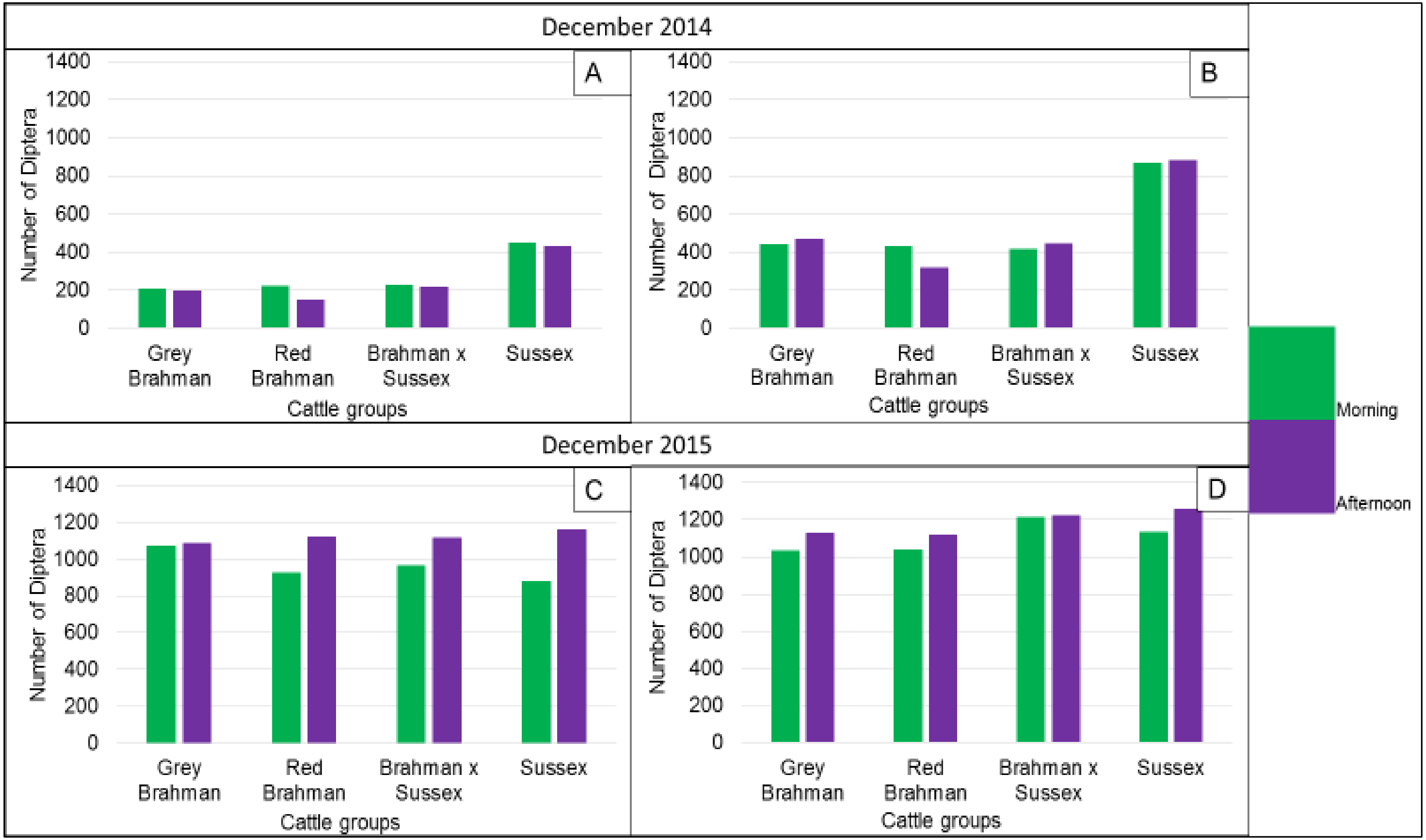

Figure 4.1: Number of Diptera observed landing on animals during December 2014 (A: day 1, B: day 2) and December 2015 (C: day 1, B: day 2).

4.2.1.2 Sticky fly traps

The four commercial sticky fly traps used to collect flies around the cattle (one placed in each holding pen for each breed representative) during the observation period in December 2015, indicated the dipteran families that were present during the day to be Muscidae, Drosophilidae, Miliciidae, Calliphoridae, Tabanidae, Piophilidae, Tachinidae, and Sacrophagidae. Species from the Muscidae family was the most dominant by far on both experimental days with a total of 400 for the first- and 389 for the second day Table 4.2. Traps were left in the pens from dusk until dawn each observation day.

Table 4.2 also indicate the large difference between the abundance in families sampled with the commercial sticky traps, with a significant difference recorded between the mean number of Muscidae and every other Diptera family sampled during the specific study ($p<0.05$).

Table 4.2: Descriptive statistics between Diptera families sampled with commercial sticky tape traps (Victory's FLY catcher) during two observation days in December 2014 and December 2015.

	N	Minimum	Maximum	Mean	Std. Deviation	Variance
Muscidae	8	90	106	98.63	5.502	30.268
Drosophilidae	8	1	6	3.50	1.604	2.571
Milichiidae	8	0	3	1.00	1.069	1.143
Calliphoridae	8	0	3	1.63	1.061	1.125
Tabanidae	8	0	2	1.13	.641	.411
Piophilidae	8	3	6	4.38	1.302	1.696
Sacrophagidae	8	0	2	.50	.756	.571
Ceratopogonidae	8	0	1	.13	.354	.125
Tachinidae	8	0	1	.13	.354	.125

N= Number of individuals

4.2.2 Night time collection

4.2.2.1 Arthropod orders sampled

As the traps were not designed to only sample certain species of arthropods, all of the organisms sampled using the flourescent light as attractant were identified and included in the results. The netting described in Chapter 2 around the trap kept large insects from entering the collection bottle, but smaller individuals from orders such as Coleoptera and Lepidoptera was able to enter through the netting. Data of two consecutive months was used as a representation of presence and abundance of arthropods for each season, sampled with two light traps for each production group for 13 months (Autumn: March and April 2014, Winter: June and July 2014, Spring: August 2014 and September 2014, Summer: January and February 2015). Although August is traditionally still part of winter, it was selected as part of “spring” in this study because higher temperatures already initiated an increase in abundance and diversity of arthropods compared to June 2014. The data gained from the traps gave insight about the abundance of orders and families of arthropods that are active throughout the year around the cattle groups at night, but offer no means of comparison between the cattle groups.

Figure 4.2 is a representation of arthropod presence and abundance during all seasons sampled.

During the autumn months of both March and April 2014, three orders in total were sampled; Coleoptera, Diptera and Lepidoptera. Diptera was the most abundant order during both months with 65% in March and 73% during April. Coleoptera represented a total of 18% during March and 14% during April 2014. During March, 17% and during April 2014,13% of the sample consisted of Lepidoptera.

During the winter (June and July 2014) the variety and abundance of arthropods sampled were low compared to the other seasons. During June, the same three orders was sampled as in autumn; Diptera with 48%, Coleoptera with 19% and Lepidoptera with 33%. However, during July the entire sample only consisted of Lepidoptera specimens.

Spring (August and September 2014), produced a larger variety of orders. Coleoptera individuals made up the larger part of the collection of August with 48% and September with 43%. The entire sample contained 45% dipteran specimens during August and 42% dipteran specimens during September. Lepidoptera made up 7% of the entire sample during August and 13% during September 2014. Other orders sampled during August and September included the following; Hymenoptera, Mantodea, Hemiptera and Neuroptera, however the number of individuals were less than 1% and not included on Figure 4.2. Acari made up 1 % of the total sample during September. The Acari found in the traps were identified as mites that could be parasites of the larger insects such as Coleoptera attracted by the fluorescent light.

In January 2015, the largest percentage (79%) of individuals sampled with the light trap belonged to the dipteran order. In contrast, the largest number of individuals in February belonged to the order Coleoptera with 52%, where they were the second most abundant during January with 13%. During February the sample mainly consisted of dipterans with 38%. Lepidoptera was the third most abundant order in both summer months with 7% in January and 5% in February. Other orders sampled during the summer included; Hymenoptera, Hemiptera and Acari. However, the number of individuals were too low (<1%) to be included in Figure 4.2.

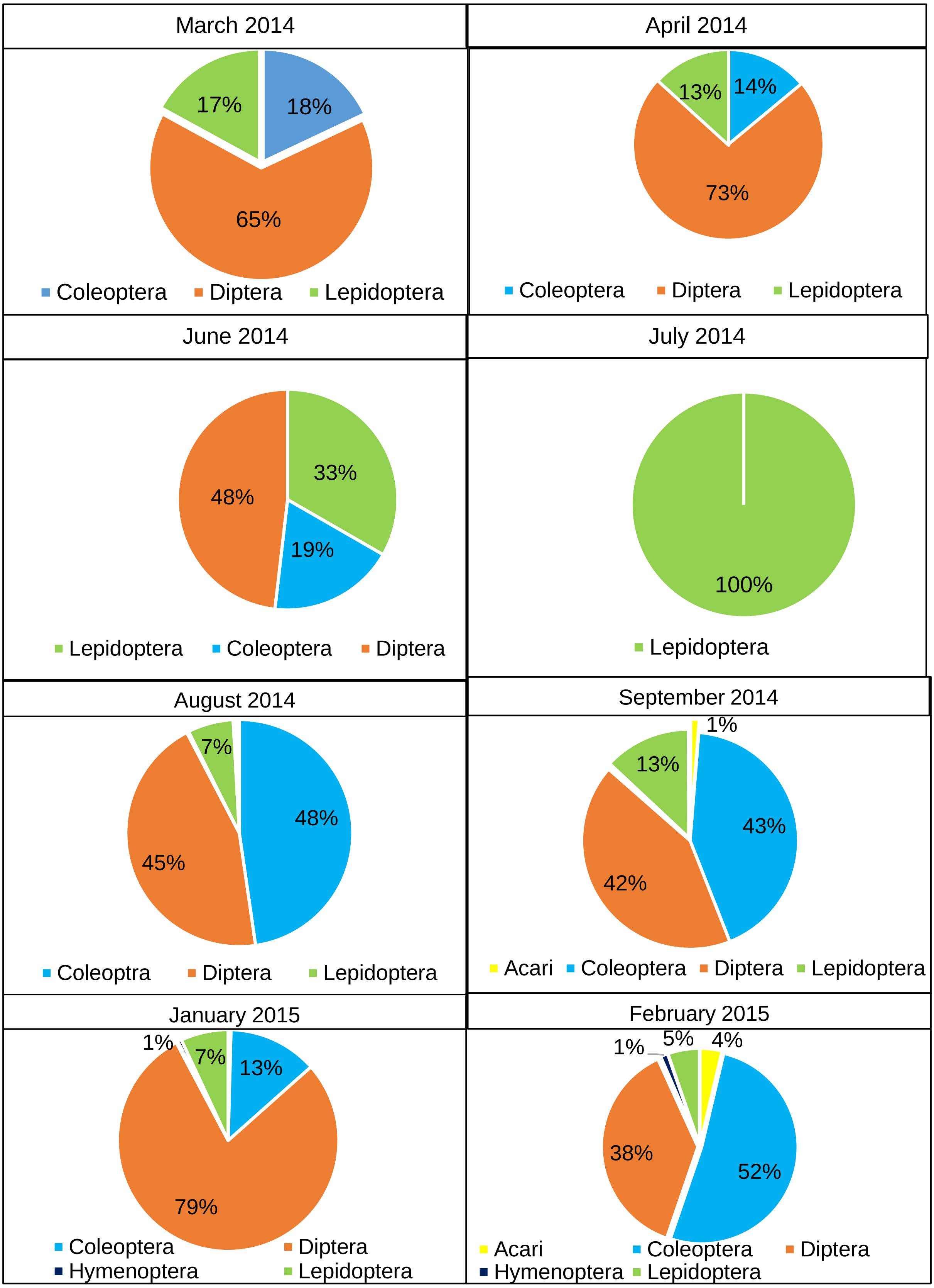

Figure 4.2: Orders of arthropods collected in fluorescent light traps during summer (January and February 2015).

4.2.2.2 Diptera family diversity and abundance

Of all the orders sampled with the fluorescent light method, only the Diptera were identified up to family level.

During the 13 month test period various Diptera families were collected and identified each month, sampled in the camps where the production groups grazed. For March and April, 12 Diptera families were identified of which only families with more than 10 individuals sampled were indicated in Figure 4.3. Three of the families sampled; Ceratopogonidae, Culicidae and Calliphoridae are considered to be of veterinary importance. Veterinary important individuals with the highest abundance belonged to the Ceratopogonidae family with 163 individuals collected in March and 290 individuals during April. Culicidae had a total of 35 individuals during March and 42 during April. With the Calliphoridae family represented by six individuals in March and two individuals during April. Other families sampled in the dipteran order were not considered to have significant veterinary importance in South Africa, including Pshycodidae, known to transfer human leishmaniasis in Namibia, but are not known to transfer any harmful pathogens in South Africa (Grové 1989). Other families with less than 10 individuals included Milichiidae, Mycetophillidae, Tachinidae and Piophilidae.

No Diptera families were sampled during July thus only the two families sampled during June are represented in Figure 4.3C and D, of which only the Culicidae family with a total of 11 individuals is considered to be of veterinary importance.

The most abundant veterinary important family sampled during August was Ceratopogonidae with 205 individuals and 233 individuals in September. Other veterinary important families sampled during spring included; Culicidae with 36 in August and 173 in September, Simuliidae with 18 in August and 21 in September; Muscidae with 12 in August and 27 in September. Other families with less than 10 individuals included Pshycodidae, Sciaridae, Mycetophilidae, Drosophiliidae and Phoridae.

The most abundant veterinary important family sampled in summer during January was Culicidae with 1123 individuals and 157 during February. Other veterinary important families sampled during summer included Ceratopogonidae with 344 in January and 40 in February, Simuliidae with 25 in January and 15 in February, Muscidae with 10 in January and 5 in February and lastly one individual from the Oestridae sampled during January 2015 and one individual from Calliphoridae sampled during February. Other families with less than 10 individuals included Scairidae, Phoridae, Mycetophilidae, Milichiidae, Dolichopodidae, Tipulidae and Tabanidae.

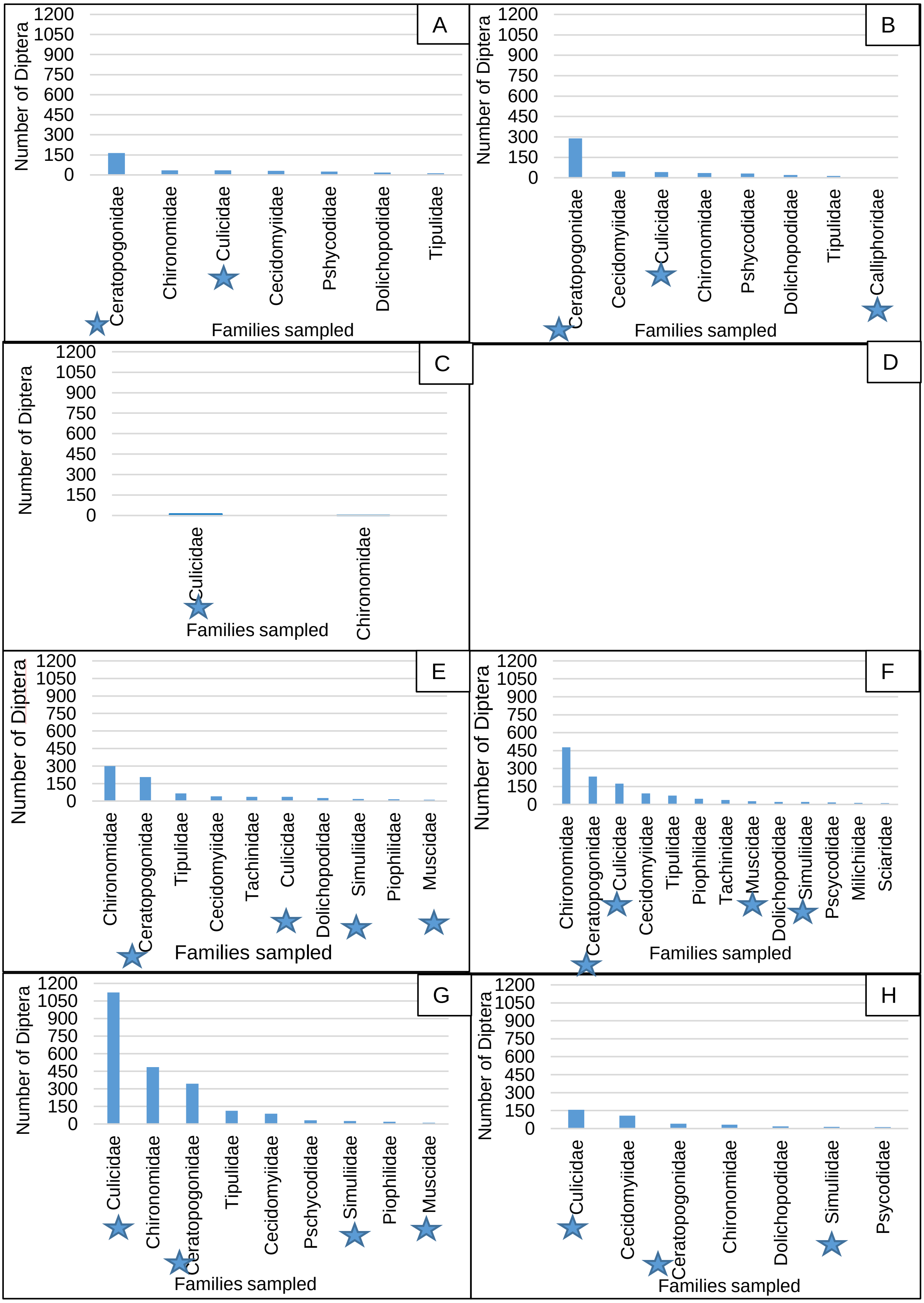

Figure 4. 3: Diptera family presence and abundance during A: March; B: April; C: June; D: July; E: August; F: September; G: January, H: February. The veterinary important families are indicated by a ★.

4.3 DISCUSSION

Diptera observed and collected during the day-time indicated a link in abundance to the environmental conditions and host animals. Previous studies (Castro *et al.* 2008; Parra *et al*. 2013) found that the reported number of flies active around cattle during the day, in particular horn flies, may vary between as well as within breeds. The suggestion that the variation in fly load may be heritable (Parra *et al*. 2013) may lead to the assumption that selecting for breeds of cattle, with a natural fly repelling nature, can be developed as an alternative to chemical control strategies.

Several pioneering studies also interpreted the variety of volatile substances that can allow flies to choose between suitable and non-suitable host animals, to be compounds associated with the preferred host animal. Substances such as ammonia (NH_3),CO_2 or 1-Octen-3-ol ($C_8H_{16}O$) that can be found in body liquids such as sweat, blood, urine and faeces of host animals (Mohr *et al*. 2010; Parra *et al*. 2013; Pickett *et al.* 2014). According to Pickens *et al*. (1992) a larger abundance of stable flies is usually caught within a diameter of 50m from either cattle or fly-breeding materials such as straw bedding. In this study both influential factors were present during the controlled fly count experiment causing the cattle to be exposed to a higher prevalence of veterinary important Diptera.

Woolley (2013) described cattle defensive behaviours against flies to range from tail flicking to leg stamping, head throwing, skin twitching and bunching behaviours. In the present study fly defensive behaviour intensity exhibited by individuals from each selected breed were recorded by observing the following behaviours; strong tail flick, weak tail flick, ear flap, head shake, and skin twitching. During December 2014 Sussex cattle showed a slightly higher frequency of irritable behaviours during both days of observation, however this was not found to be a significantly different between the breeds. The higher recorded attractiveness to the Sussex cattle can be ascribed to various factors such as differences in skin secretion, colour, morphological structure of ears and tail and size of breeds. Some of these specific morphological differences are discussed in more detail in Chapter 5.

Total number of Diptera counted on individual cows of each breed for December 2014 indicated a higher abundance of Diptera around the Sussex breed compared to the

Grey Brahman, Red Brahman, and Brahman x Sussex. The abundance and diversity of Diptera sampled can possibly be linked to the effects of the drought during 2014 and may indicate that throughout unfavourable conditions, when less flies are present and there is a choice of hosts, the flies might show a preference for the Sussex breed. During favourable conditions in December 2015, the effects of the drought were less than in 2014, and the combination of rain and high temperatures created a suitable environment for Diptera to survive and reproduce. This observation is supported by Kaufman *et al*. (1999) that found the abundance of Diptera active around cattle during the day to be correlated with suitable rainfall and temperature conditions. The more abundant presence of Diptera in the environment seems to eliminate the preference of the flies for any specific cattle breed, with all cattle breeds harbouring high fly loads.

The commercial sticky fly traps indicated Muscidae to be the most abundant veterinary important family to be present during the day time in 2015. This was also confirmed by Byford *et al.* (1992), where other Diptera familie namely Drosophilidae, Miliciidae, Calliphoridae, Tabanidae, Tachinidae, Piophilidae and Sacrophagidae were also present but at significant lower numbers than Muscidae. No significant difference in the number of Muscidae recorded could however be found between the different cattle breeds observed. Oberem *et al.* (2009) identified the species *Musca domestica*, *Stomoxys calcitrans, Fannia cannicularis* and *Musca xanthomelas* within the Muscidae family, that cause production losses, as common pests found around farmyards and in the veld.

Fluorescent light traps were used to collect insects, particularly Diptera, that include veterinary important families such as Culicidae and Ceratopogonidae, active at night. This particular trap design however, produced insufficient data to distinguish Diptera family preference between the selected cattle breeds, because some of the different breed groups grazed together in the same production groups. Therefore, traps placed in camps collected general arthropods around the cattle and was not breed specific. For future studies, a different approach would be suggested by designing traps to sample specific species or families of parasitic Diptera for example, for Culicidae, a CO_2 baited trap can be used Cooperband *et al.* (2008). The placement of the traps should also be considered carefully to initiate host finding activities. After being exposed to the relevant host habitat, mosquitoes locate and select hosts through a range of odor, visual, and thermal cues from the host animal. Different host odors, CO_2 production rate and levels of defensive behaviours may all contribute to differences in attractiveness of the different host species and their biting rate. The use of CO_2 may also be especially effective in attracting generalist feeders, such as individuals from the *Anopheles* genus, within an area that has a limited variety of hosts available (Cooperband *et al.* 2008).

The data gained from the light traps, gave an insight about the abundance of orders and families of arthropods that are active around the cattle groups. The three dominant orders observed to be present in all but one month of collection were Diptera, Coleoptera and Lepidoptera. Except for July 2014 Diptera was well represented during all the months, and its abundance ranged from 38% in February 2015 to 79% in January 2015. The absence of Diptera in the light traps during July 2014 can be attributed to very low winter temperatures, causing death or diapause of a variety of arthropods or a shift in flight activity (Irwin *et al.* 2001; Takken *et al*. 2008).

During autumn (March and April 2014), 12 Diptera families were identified from the night traps in total, of which Ceratopogonidae, Culicidae and Calliphoridae were considered to be of veterinary importance. The most individuals belonged to the Ceratopogonidae and Culicidae families during these two months. Previous studies in South Africa indicated that Ceratopogonidae midges can be active throughout the year, peaking in abundance during warmer and humid summer months and decreasing during colder months (Venter *et al*. 2014). In the current study their presence during autumn indicates the midge population in the test area could survive the maximum and minimum temperatures.

Members from the Calliphoridae family in the present study were the third most abundant veterinary important family identified during March and April. Calliphoridae, a family described to be more active during the day, was also sampled with the fluorescent light traps. This family is usually attracted by excrement, carrion, and exposed meat rather than a fluorescent light, thus this accidental collection is probably due to a practical error as the traps were placed in position and turned on just before sun set and removed as soon after sunrise. Some dipterans more active during the day such as calliphorids were therefore also sampled with these traps in those short periods of sunshine the traps were already switched on. Calliphorids were correspondingly also found in the day time collection traps.

Lower rainfall and temperatures during the winter months (June and July 2015), correlated with the very low abundance and diversity of dipteran species sampled at night during June 2014 with no dipterans being sampled in July 2014. The only veterinary important fly family sampled was Culicidae with a total of 11 individuals in June 2014, indicating that small populations of mosquitoes are able to overwinter in the test area. Although other studies indicated that ceratopogonids can be active throughout the year in some areas in South Africa (Venter *et al*. 1997; Meiswinkel *et al.* 2004), none were effectively sampled during the chosen winter months in the current study. The longevity of an adult ceratopogonid increases with a decrease in ambient temperatures, allowing the lifespan of ±20 days of an average female to be extended to up to 90 days (Venter *et al.* 2014). It should be recognized that as described by Venter *et al.* (2014) light traps can also be influenced by a great variety of environmental factors such as wind and frost that might lead to only a relatively small portion of active dipterans to be sampled. Flight activity of Ceratopogonidae and therefore the efficiency of the light traps is suppressed at temperatures below 10ºC (Venter *et al*. 2014).

During spring, increased ambient temperatures and rainfall indicating a shift in seasons, coincided with an increase in abundance and diversity of arthropods sampled with the fluorescent light traps. The entire sample contained 45% Diptera specimens during August 2014 and 42% dipteran specimens during September 2014. The most abundant veterinary important family sampled was Ceratopogonidae when more favourable environmental conditions and host availability was present. Other veterinary important families sampled during spring included Culicidae, Simuliidae and Muscidae.

As found in the current study, literature also indicates that Diptera, specifically Ceratopogonidae, abundance and species diversity in an area is significantly affected by environmental conditions that also determine the distribution of available soil moisture essential for midges and mosquitoes for larval development (Oberem *et al.* 2009). Thus, as temperatures continue to rise and scattered rainfall occurs during the selected summer months (January and February 2015), the abundance and diversity of insects sampled with the fluorescent light traps increased as expected. The dipteran order made up 79% of the total sample in January and 38% in February. The most abundant veterinary important family sampled in summer during January 2015 was Culicidae. Other veterinary important families sampled during summer included Ceratopogonidae, Simuliidae, Muscidae and lastly one individual from the Oestridae family was sampled during January.

4.4 CONCLUSION

Sussex cattle showed the highest frequency of all-inclusive irritable behaviours, but statistically significant differences in irritable behaviour were only found during December 2015 between the Sussex breed and all the other breeds. With regards to Diptera presence around the different breeds from observations in December 2014, more flies were observed around the Sussex breed compared to all other groups.

Total rainfall for each month also influenced host preference. Observations in December 2014 during the drought experienced in the study area, indicate a larger Diptera abundance on the Sussex breed compared to the other breeds. During the period of higher rainfall in 2015 when Diptera was more abundant, no specific host breed preference was found. This reinforces the practice of selecting cattle with lower ectoparasite loads to use for reproduction in commercial herds especially during times of drought.

A total of nine Diptera families were collected with sticky fly traps observed during day time in December 2015. Of these families, Muscidae were by far the most abundant with little or no specific preference for any of the cattle breeds.

Although the focus of this study was on Diptera collection, observations throughout the year by fluorescent light trapping revealed that species representing three main orders, Diptera, Coleoptera and Lepidoptera were the most abundant throughout the year except for July 2014 when only Lepidoptera were collected. On Diptera family level Ceratopogonidae were the most abundant during autumn and although no Diptera were collected in July 2014 a few specimens of the family Culicidae were collected during June 2014, spring was again dominated by the family

Ceratopogonidae and the summer months provided Culicidae as most abundant Diptera family of veterinary importance.

This study provided a greater overview of Diptera ectoparasites by determining the diversity and abundance of veterinary important Diptera in the Kroonstad region. Potentially economically important Diptera families have been identified throughout the sampling year.

Control strategies for ectoparasites on livestock should be revised as many arthropod populations are becoming more and more insecticide resistant (Parra *et al*. 2013). Integrated pest management should be applied to control pest dipteran pests associated with cattle in stable environments as well as in the fields. When Diptera populations are very high, water logged camps should be avoided to reduce the number of Diptera vector bites along with well managed chemical control methods that can include pyrethroids, amidines, organophosphates, macrocyclic lactones and growth regulators such as chitin synthesis inhibitors.

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CHAPTER 5

CHARACTERISTICS AND EFFECT ON ECTOPARASITE INFESTATION

INTRODUCTION

The largest bovine organ is the hide of the animal that can make up to 8% of total live mass (Hansen 2004). This organ is of significant importance acting as the barrier between the external environment and the animal, and is for this reason important for the assessment of adaptability of the animal to external factors (Bonsma 1983; Hansen 2004). Animals with thick, movable hides with high vascularity and smooth hair have been believed to be more repellent to ticks and insects and are thus more disease resistant than other animals (Personal communication- [1]Sytze Smith, Breed director at Brahman Breeders Association of South Africa). This chapter focusses on the structural differences of the hair and skin expected to be found between the Grey- and Red Brahman, Sussex and Brahman x Sussex cattle.

Variations in hair texture exists between species, between different areas of the body and even between individuals (De Marinis *et al*. 2006). The quantity and type of melanin available in cortical cells determine whether the hair will be black, brown or red (Rees 2003). Hair follicle density has previously been proved to be higher in *B. indicus* vs *B. taurus* cattle (Jian *et al.* 2014). Cattle breeds with high coat density and longer hair have lower thermal conduciveness than those with less dense, shorter hair because the air gets trapped between the hairs lowering heat loss efficiency (Maia *et al*. 2005).

The skin consists of two main layers; a superficial layer of stratified squamous epithelium known as surface epithelium/epidermis, and a layer of irregular dense connective tissue known as corium/dermis. Skin glands of bovids can be sub-classified as either being apocrine or merocrine glands. These sebaceous glands are located immediately under the surface of the epidermis, forming part of a superficial layer of glands occurring in the dermis (Jian *et al.* 2014). Apocrine glands have wide secretory tubules that open into the hair follicle. Sebum, is a sebaceous secretion of these glands, resulting from the partial destruction of glandular cells and consists of cellular debris and a lipoid mixture high in cholesterol (Ebling 1988). The greasiness

[1] Mr. Sytze Smith, Breed director at Brahman Breeders Association of South Africa, April 2014.

of the secretion of the sebaceous glands protects the surface area of the skin against a multitude of dangers such as drying out (Carter *et al*. 1954). Merocrine glands were identified as narrow tube-like structures coiled into a ball found closer to the inside margin of the epidermis than apocrine glands. The excretory duct passes between dermal papillae directly onto the skin surface, not into a hair follicle (Scrivener *et al.* 2002). Skin colour is determined by pigment presence within epidermis cells, causing the scattering of light particles. Predominant black and brown skin shades are caused by various melanin amounts, produced by specialised skin cells called melanocytes (Gates 1961). Smooth muscle can be identified in sheets, acting as hair follicle muscles (*arrectores pilorum*) alongside hair follicles (Cadar 2009).

Crossbreds between *B. indicus* and *B. taurus* cattle have already proven to be more efficient than purebred *B. taurus* breeds by maintaining effective heat regulation and increased production and reproduction qualities in more tropical climates (Jian *et al*. 2014). This is due to heat radiation that can have an influence on ectoparasite infestation. Animals that exhibit heat stress symptoms usually have a lower body score that can make them render them more susceptible to health issues such as parasite infestation (Bonsma 1983).

In order to achieve these positive hide qualities in a herd, breed standards serve as a guideline for breed improvement, but they are not always merely based on biological values, they are partly manmade strategies imperfect to scientific basis (Bonsma 1983). This is why many breed standards are now actively moving towards more functional ability selection, such as selecting animals with darker skin and more favourable coat characteristics for tropical or arid regions where heat stress is more prevalent (Bonsma 1983).

The main aim of this chapter, was to determine if there are differences in hair and skin characteristics, tail length, weight and rectal temperature between the different breeds (Grey Brahman, Red Brahman, Brahman x Sussex and Sussex) included in the study and to evaluate if these differences lead to an advantage concerning ectoparasite infestation.

To obtain this aim the higher ectoparasite loads found on the Sussex breed compared to the other breeds were compared with the following specific parameters of skin and hide characteristics:

- To describe and compare characteristics between the breeds and the potential influence of these characteristics on parasite load.

- To investigate if there is a difference in skin and hide characteristics recorded between the different cattle breeds.

- To determine if there is a relationship between mean body temperatures recorded for the different breeds and ectoparasite load.

- To determine if there is a relationship between breed weight and ectoparasite load.

- To determine if there is a difference in average tail length recorded between the breeds that might aid in fly repellence.

5.1 MATERIALS AND METHODS

Methods used to investigate these characteristics are extensively described in Chapter 2. Hair was sampled and skin thickness measurements were taken from 10 animals in each cattle breed on two occasions namely July 2014 and January 2015 to represent sampling from two different seasons. These collections were used for hair grading, colour and skin thickness tests. Once-off skin biopsies were taken by a qualified veterinarian during January 2015 to be used for all the skin histology tests. Tail length was measured on one occasion during March 2014. Rectal temperatures and bodyweight of 10 animals from each breed were recorded once during January 2015.

5.2 RESULTS

5.2.1 Body condition scoring

The months during which cattle with less than the optimum body scores (Table 2.2) were recorded are displayed in Table 5.1. These lower scores were recorded for Brahman x Sussex cattle in September 2014, for all groups during October 2014 with the Grey Brahman group consisting of 30% of the cattle in the group scored as thin, for Grey Brahman and Brahman x Sussex groups during November 2014, for Red Brahman cattle during December 2014 and for Sussex cattle during January 2015. In contrast, no cattle in any of the groups were scored as being overweight throughout the study.

Table 5.1: Months of test period (March 2014 to March 2015) when cattle breeds had less than optimal body scores.

September 2014				
	Grey Brahman	Red Brahman	Brahman x Sussex	Sussex
Thin	0	0	1	0
Optimum	20	20	19	20
Fat	0	0	0	0
October 2014				
Thin	6	1	3	1
Optimum	14	19	17	19
Fat	0	0	0	0
November 2014				
Thin	1	0	1	0
Optimum	19	20	19	20
Fat	0	0	0	0
December 2014				
Thin	0	2	0	0
Optimum	20	18	20	20
Fat	0	0	0	0
January 2015				
Thin	0	0	0	1
Optimum	20	20	20	19
Fat	0	0	0	0

5.2.2 Body weight

The mean body weight recorded (calculated from weighing 10 animals from each breed) for the different breeds are displayed in Figure 5.1, with Grey Brahman at 518kg, Red Brahman at 496.5kg, Brahman x Sussex at 625.4kg and Sussex at 648.3kg. The highest average weight was recorded for Sussex cattle, with Red Brahman cattle showing the lowest average weight. All cattle had an optimal body score of two when body weight was recorded. Differences in breed weight were calculated in mean weight (kg) to indicate the difference in on-host parasites due to breed as an affect and not the size or skin surface of the animals. Sussex cattle are generally bigger than the other selected breeds, but Brahman cattle have looser skin that can result in a larger skin surface.

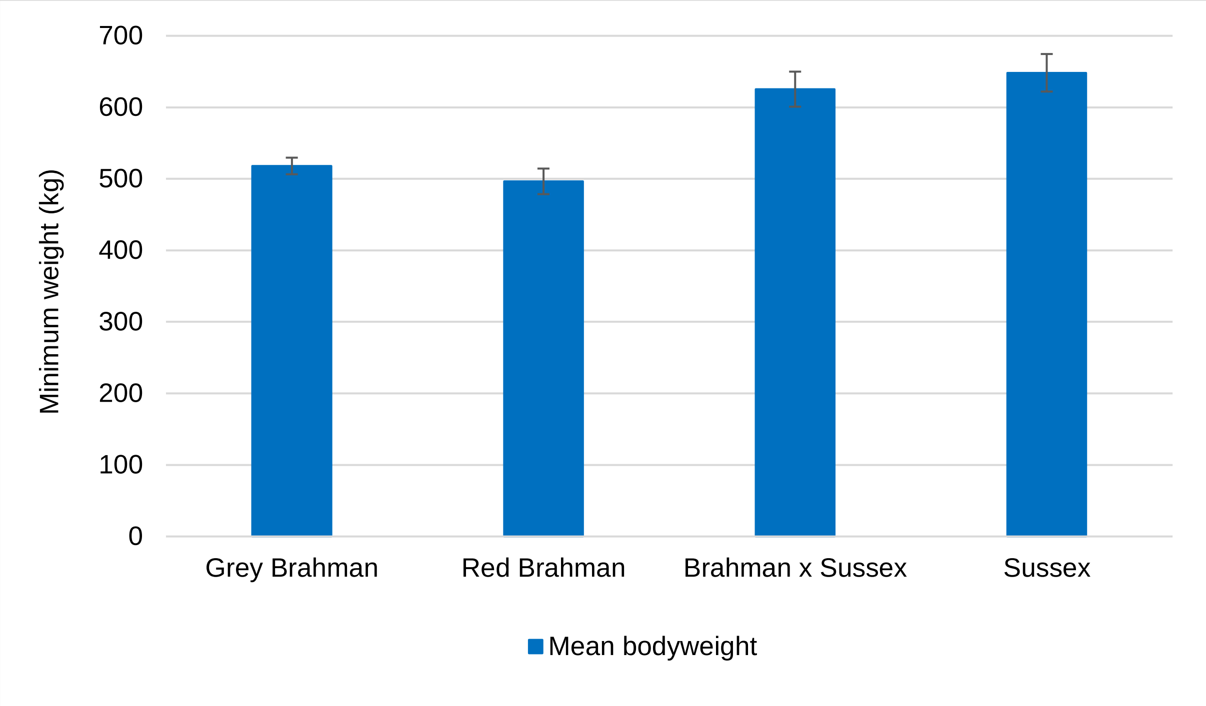

Figure 5.1: Mean body weight (kg) and standard error of the mean calculated for each cattle breed.

Significant differences ($p<0.05$) were found between the Grey Brahman and Brahman x Sussex breeds, Grey Brahman and Sussex breeds, Red Brahman and Brahman x Sussex, and Red Brahman and Sussex (Annexure 5: Table 5.1). When comparing mean body weight of the different breeds to the ectoparasite loads (ticks and flies) recorded on the animals (Chapter 3 and 4), it is apparent that allthough Sussex cattle are heavier they still had the most ticks per 1kg body mass with four ticks (Table 5.2). However, the total fly load observed around the animals calculated per 1kg body mass, showed no significant difference between the groups when average weight was taken into consideration with Brahman x Sussex cattle having the least flies present per 1kg at 11.1 flies (Table 5.2). No significant difference was for for ticks or flies per 1kg weight for breeds.

Table 5.2: Mean body weight recorded for each breed in relation to mean on-host tick and fly load.

Cattle breeds	Mean breed weight (kg)	Mean ticks per 1kg body weight	Mean flies per 1kg body weight
Grey Brahman	518	1.8	10.9
Red Brahman	496	1.1	10.7
Brahman x Sussex	625	1.4	9.3
Sussex	638.3	4	11.1
Standard deviation		1.3	0.8

5.2.3 Hair structure and characteristics

5.2.3.1 Hair cuticle roughness

5.2.3.1.1 Hair felting test

During the hair felting test (sampled from 10 animals from each breed) the Sussex cattle breed had the most individuals with hair that felted into a mass on both test occasions; 70% felting during July 2014 and 100% during January 2015 as indicated in Figure 5.2. Only 10% of the Grey Brahman breed had hair that felted when tested in July 2014 and none during January 2015. The Brahman x Sussex cross animals had 20% of animals whose hair felted in July 2014 and 30% in January 2015. On both occasions, no hair samples from the Red Brahman group showed any signs of felting.

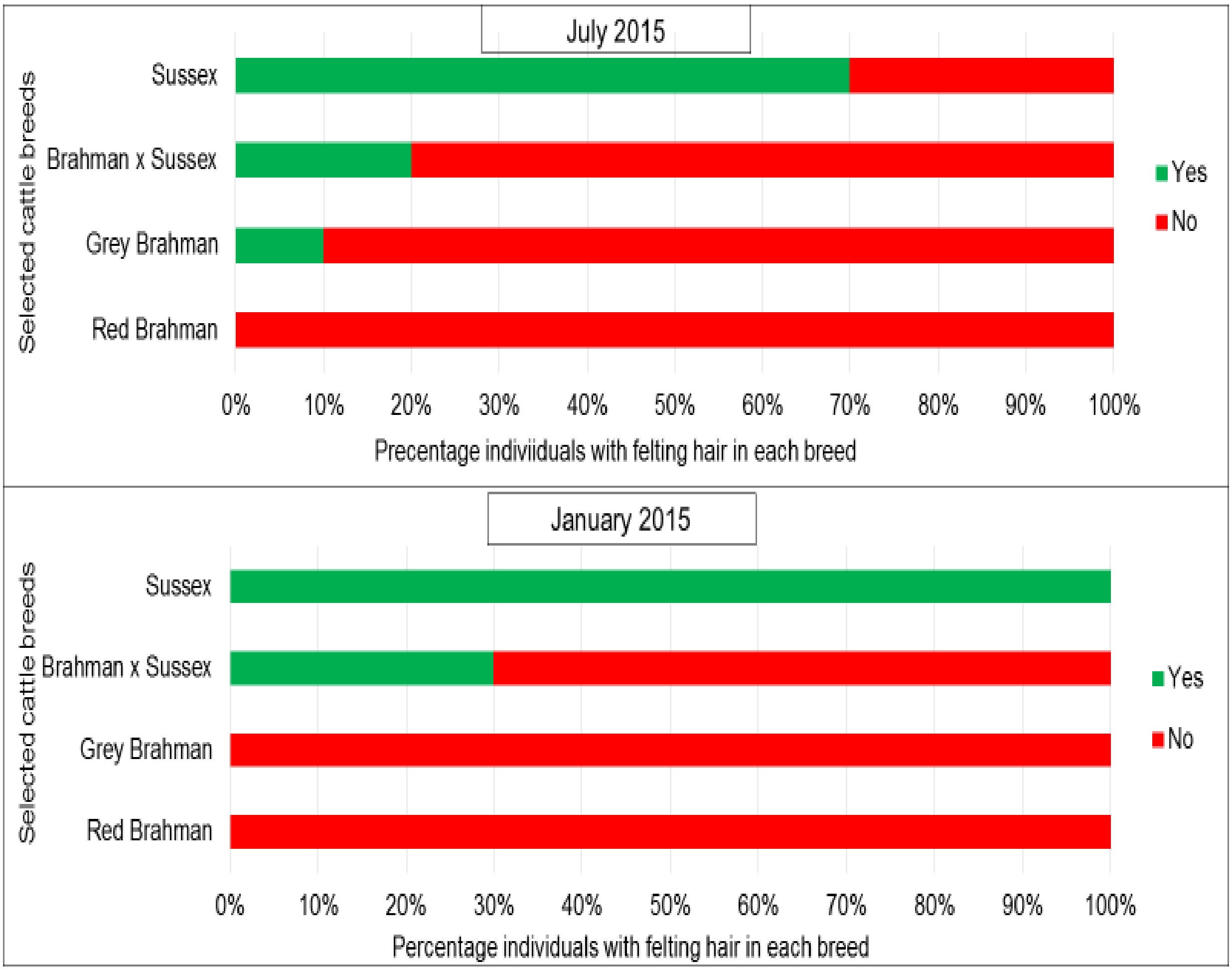

Figure 5.2: Percentage of individuals with felting hair in the different breeds in July 2014 and January 2015.

Significant differences in the number of animals with felting hair was found between the Sussex breed and every other breed with $p<0.05$ (Annexure 5: Table 5.2).

5.2.3.1.2 SEM comparison of hair structure

Figure 5.3 illustrates the wave-like patterns of hair cuticle scales from the different cattle breeds.

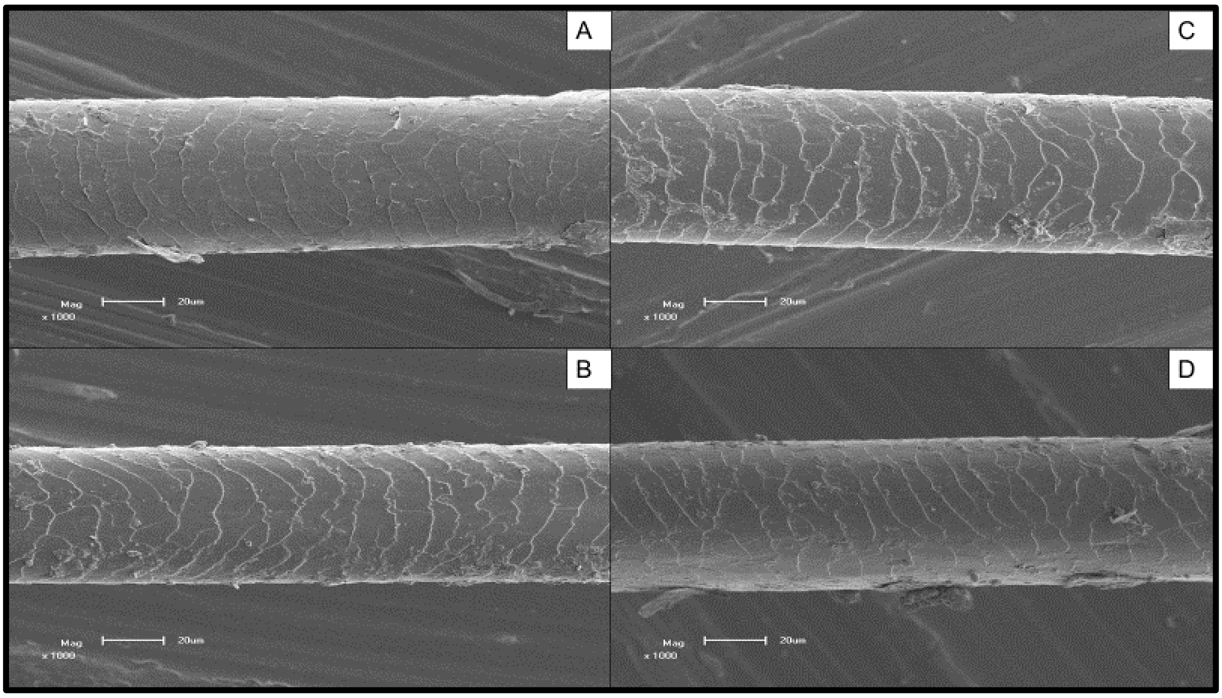

Figure 5.3: General hair structure of the hair cuticle of A: Grey Brahman, B: Red Brahman, C: Brahman x Sussex and D: Sussex cattle under the SEM at 1000 x magnification.

The mean, minimum and maximum hair thickness recorded for each breed are provided in Table 5.3. Sussex cattle had a maximum thickness of 133µm, Brahman x Sussex with 130µm, Red Brahman with 128µm and Grey Brahman with 114µm. The large difference between maximum and minimum measurements recorded for each individual breed, indicated that the hair samples were collected efficiently and included hair from both the under-and upper coat of cattle.

Table 5.3: Hair thickness in µm of the breeds, measured with the SEM.

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Grey Brahman	135	69.89	22.2978	1.9191	66.094	73.685	31.9	114
Red Brahman	135	69.798	29.1168	2.506	64.841	74.754	33.1	128
Brahman x Sussex	135	69.296	21.2514	1.829	65.679	72.914	35.9	130
Sussex	135	72.323	22.8253	1.9645	68.438	76.208	37.5	133

N=Number of hairs

None of the statistical analysis revealed a significant difference between the breeds (Annexure 5: Table 5.3 and 5.4).

5.2.3.1.3 Hair scale pattern

No visual differentiation of hair scale patterns could be made between the groups of cattle at 1000 x magnification (Figure 5.3), therefore the scale patterns were further investigated by measuring scale margin distances at 3000 x magnification (Figure 5.4). At this magnification, scales could be individually inspected. Scale margins were irregularly wavy with sharp edges visible for the Grey- and Red Brahman groups. Brahman x Sussex and Sussex scale margins may appear to be smoother but this could be due to the scales being damaged throughout the SEM preparation.

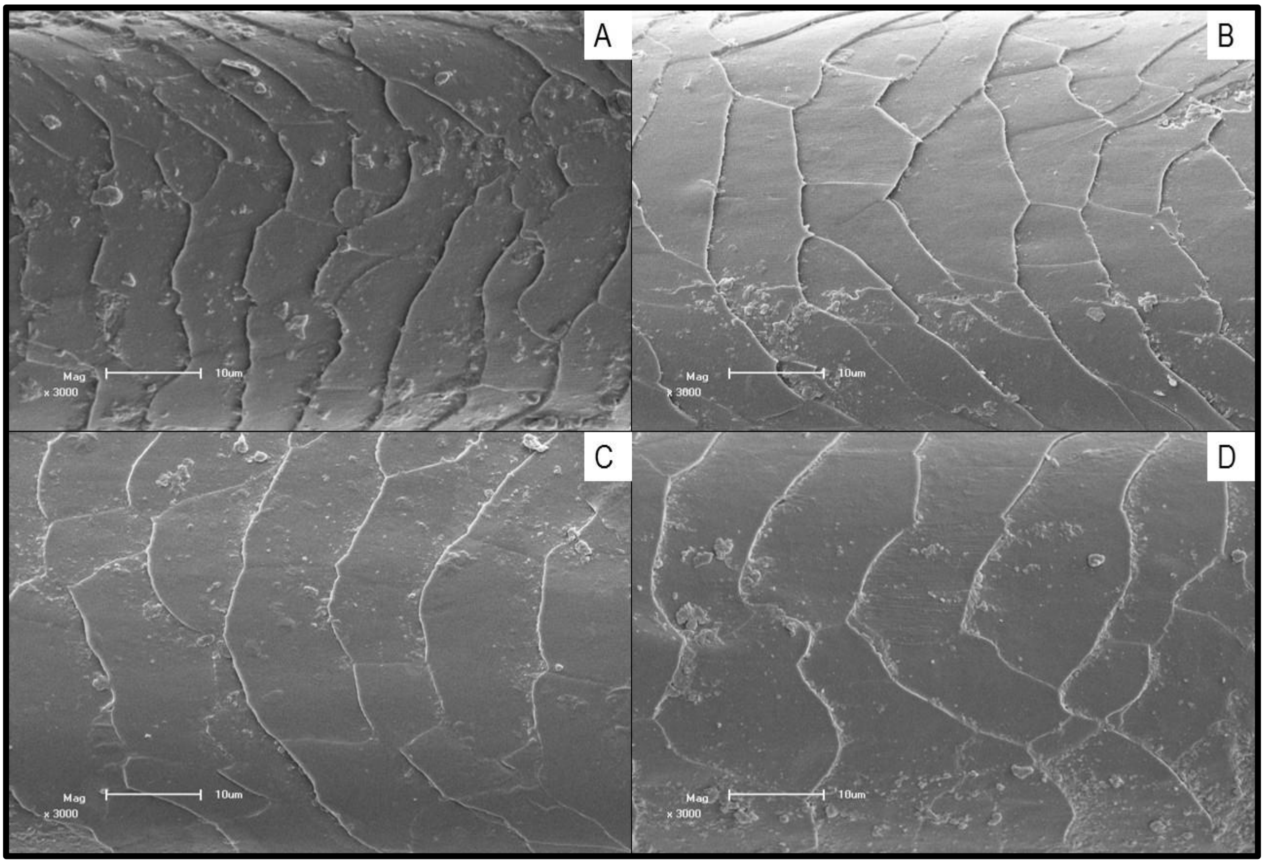

Figure 5.4: Hair scale cuticle pattern at 3000 x magnification under the SEM for cattle breeds; A: Grey Brahman, B: Red Brahman, C: Brahman x Sussex and D: Sussex.

Descriptive statistics that show the mean, minimum and maximum scale interval, distance measured between one scale margin to the next scale margin, are indicated in Table 5.4. Mean measurement between scale margins differed according to breed. Grey Brahman cattle had the smallest mean interval of 7.660µm compared to Red Brahman with 8.3050µm; Brahman x Sussex with 9.4761µm and Sussex cattle with 8.9824µm. The minimum and maximum measurements of a total of 160 taken for each breed varied considerably, indicating that a statistically dependable sample size was used that included a variety of hair samples for each breed. The Grey Brahman breed had a scale interval range from 1.27µm to 15.30µm, in relation to Red Brahman cattle with a range from 1.77µm to 15.30µm, Brahman x Sussex cattle from 3.54µm to 17.40µm and Sussex cattle from 3.75µm to 14.60µm.

Table 5.4: Descriptive statistics of mean scale intervals between cattle breeds.

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Grey Brahman	160	7.6670	2.36416	.18690	7.2979	8.0361	1.27	15.30
Red Brahman	160	8.3050	2.65699	.21005	7.8901	8.7199	1.77	15.30
Brahman x Sussex	160	9.4761	2.37300	.18760	9.1055	9.8466	3.54	17.40
Sussex	160	8.9824	2.49373	.19715	8.5931	9.3718	3.75	14.60

N=Number of scales

In the observed difference was of statistical significant difference in scale intervals between the breeds ($p<0.05$) at a 95% confidence interval (Annexure 5: Table 5.5). The difference in scale intervals were recorded between the following breeds; Grey Brahman compared to those recorded for Brahman x Sussex and Sussex groups with both p-values <0.05. The mean scale interval for Red Brahman cattle was also found to be statistically significantly different to the mean scale interval measured for the Brahman x Sussex cattle ($p<0.05$). This was done by comparing mean distance by using a Post-hoc test for both Tukey HSD and Games-Howell at a 95% confidence interval, as seen in Annexure 5: Table 5.6. Both tests are included to ensure reliability of the results obtained.

5.2.3.2 Hair colour

Twenty animals from each breed was examined to determine what the correct description would be for each breeds hair colour. All Sussex cattle had dark brown hair, Brahman x Sussex cattle had two individuals with red hair and 18 with brindle coloured hair, Red Brahman cattle all had red hair and Grey Brahman cattle had 3 individuals with white hair and 17 individuals with grey hair as indicated in Figure 5.5. Cattle with lighter coat colour and darker pigmented skin are better adapted to maintain general health and reproduce more effectively compared to darker coat colours and light skin pigmentation (Foster *et al*. 2009; Blaho *et al*. 2012). The advantage of the lighter coat colours is due to its lowered absorption of solar radiation, which results in reduced heat stress that can influence immunity and thus in effect parasite loads.

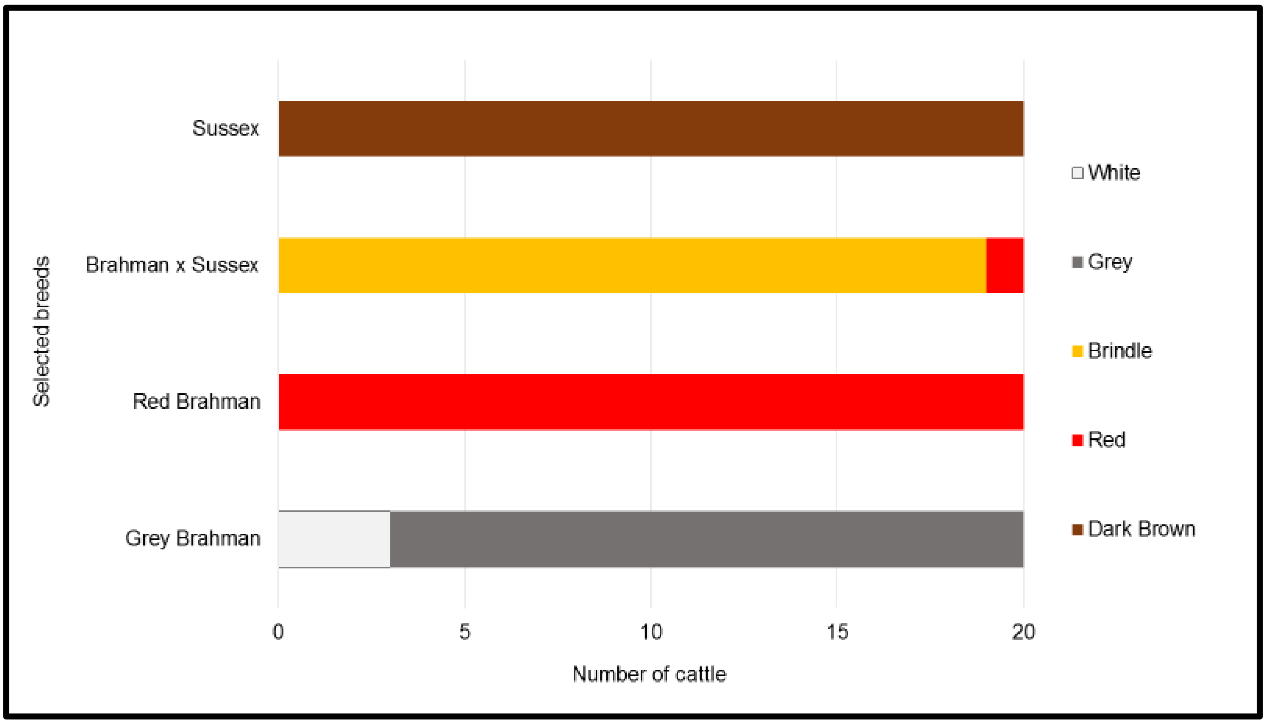

Figure 5.5: Hair colour chart for the different cattle breeds.

5.2.3.3 Hair density

Average hair density found for the different cattle groups (10 animals per group with one sample per animal analysed) as shown in Figure 5.6, indicate that Sussex cattle had the most hair in a 5mm x 5mm sample area with a mean density of 226.6, compared to Grey Brahman cattle with 192.1, Red Brahmans with 216.4 and Brahman x Sussex cattle with 190.2. All of the cattle groups had a minimum hair count of above 120 hairs per 5 mm^2 and a maximum count below 300. There was no significant difference recorded between cattle groups for mean hair count on the specified sample size (p>0.05 in both cases) (Annexure 5: Table 5.7).

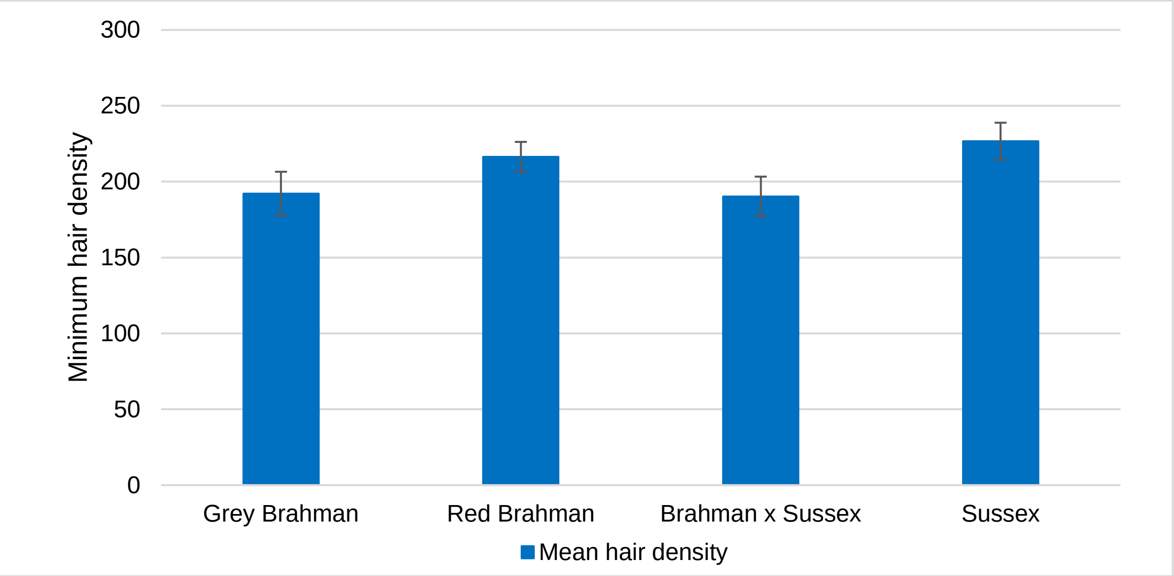

Figure 5.6: Hair density and standard error of the mean calculated per 5mm x 5mm sample for each cattle breed.

5.2.3.4 Hair length

Two types of hair coats were identified from each animal's skin biopsy sample and hair length (mm) was measured for the different cattle groups; the shorter inner coat and the longer outer coat. As seen in Figure 5.7, there was a wide variation in length between the short hair and long hair sampled (included hair from inner and outer coat). Grey Brahman cattle had a minimum length of 3mm and a maximum of 23mm; both Red Brahman cattle and Brahman x Sussex cattle had a minimum length of 2mm and maximum of 25mm; and Sussex cattle had a minimum hair length of 4mm and a maximum of 35mm.

Sussex cattle were found to have the longest average hair length with 17.37mm as shown in Figure 5.7 when compared to Grey Brahman cattle with an average of 10.96mm, Red Brahman cattle with an average of 11.39mm and Brahman x Sussex cattle with an average of 10.71mm.

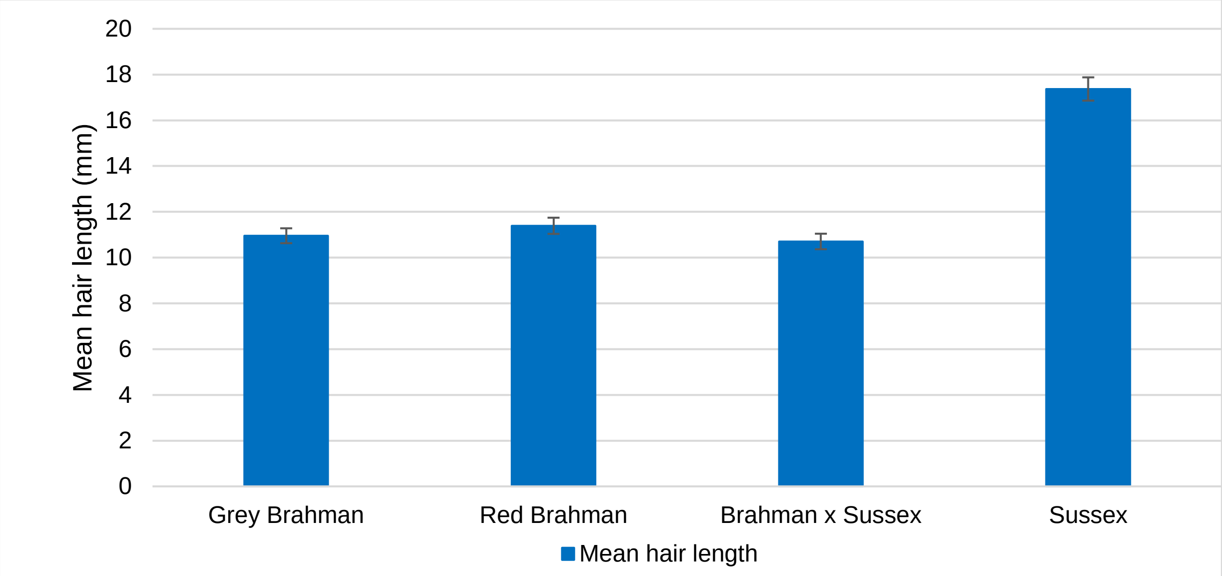

Figure 5.7: Mean hair length and standard error of the mean recorded in mm for the different cattle breeds.

There was a significant difference in average hair length between the breeds ($p<0.05$) (Annexure 5: Table 5.8). Significant differences were further analysed between various breeds by comparing group means by using a Post-hoc test for both Tukey HSD and Games-Howell tests at a 95% confidence interval (Annexure 5: Table 5.9). There was no significant difference found in hair length of Grey Brahman, Red Brahman and Brahman x Sussex groups. The hair length of the Sussex group ($p<0.05$) did, however, differ significantly from the other three groups.

5.2.4 Cellular layers of the skin

In Figure 5.8 a cross-section of the skin of a Red Brahman individual can be seen to indicate the different layers. Haematoxylin and Eosin was used for the staining process done by Idex Laboratories.

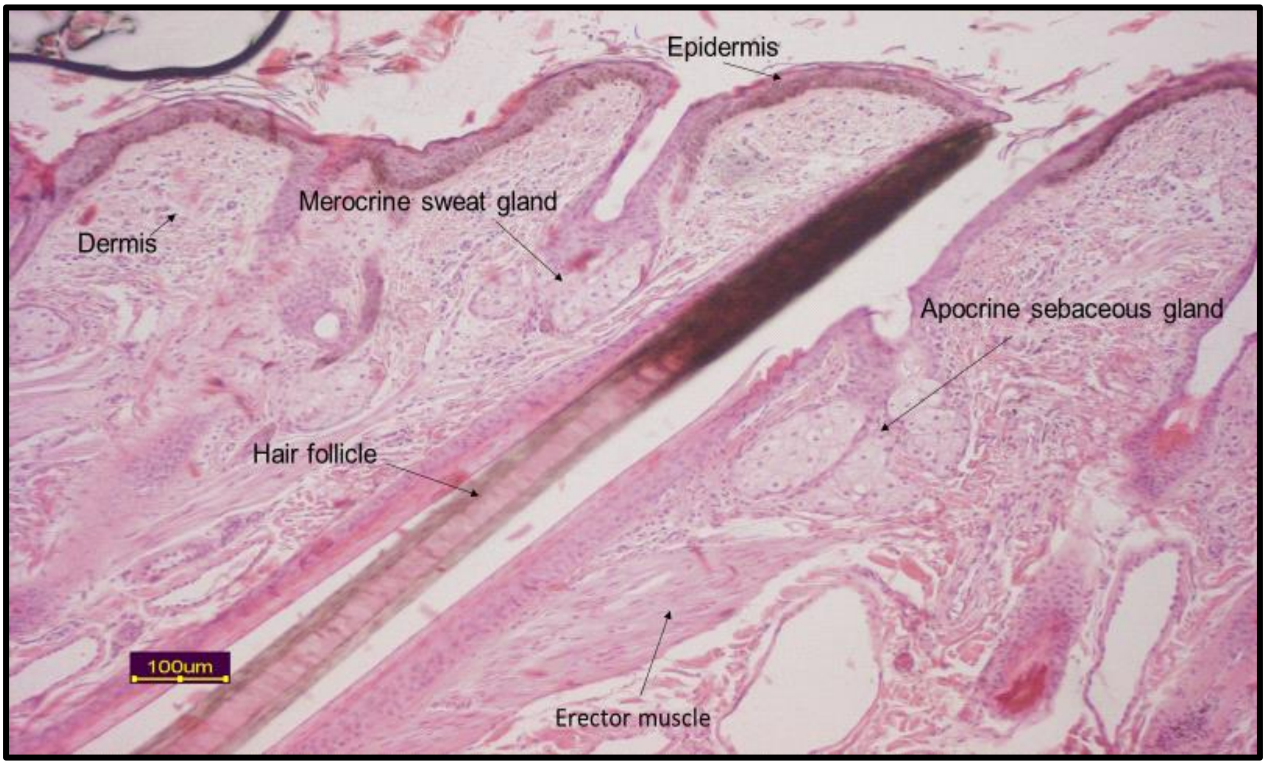

Figure 5.8: Cellular structures found in the epidermal and dermal skin layers of a Red Brahman cow.

5.2.4.1 Skin thickness

Figure 5.9 indicates that the Grey Brahman groups had the thickest skin measurements determined with callipers, with a mean of 1.25mm in July 2014 and 1.36mm in January 2015. In contrast, the Red Brahman breed had the thinnest skin measurement with a mean of 1.08mm in June 2015 and 1.05mm in January 2015 compared to intermediate groups Brahman x Sussex; 1.075mm mean July 2014, 1.235mm mean January 2015, and Sussex cattle groups with a mean of 1.2mm in July and 1.235mm during January. The twenty animals inspected for parasites for each breed was used to take skin measurements from (Grey Brahman, Red Brahman, Brahman x Sussex, Sussex).

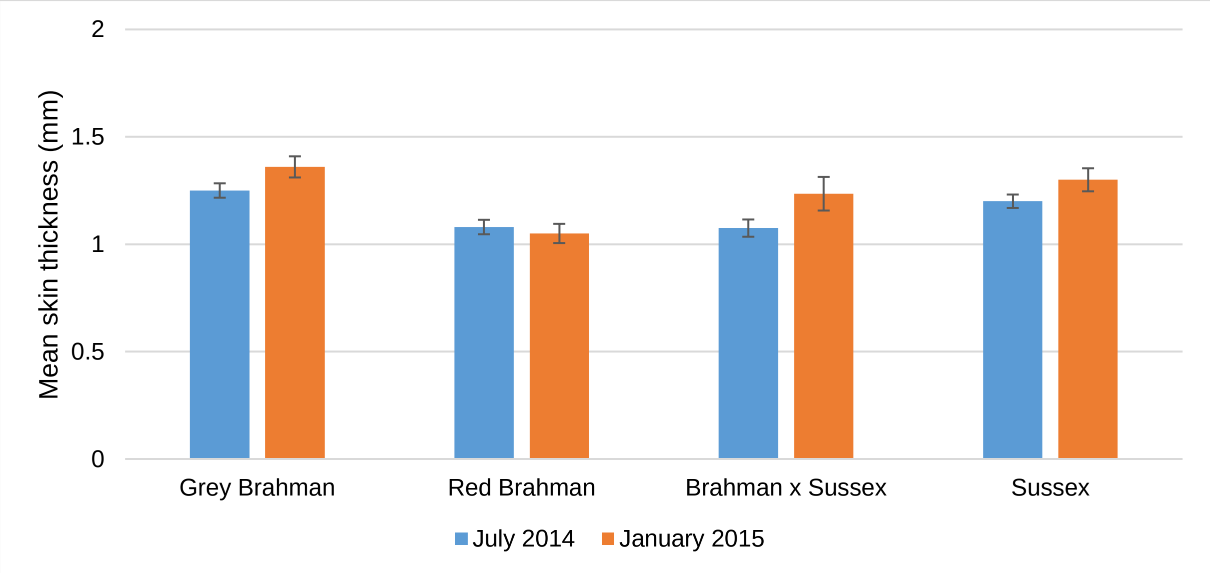

Figure 5.9: Comparative skin thickness measurements in mm and standard error the mean calculated for the different cattle breeds, once during winter (July 2014) and once during summer (January 2015).

There was a significant difference in average skin thickness between the breeds ($p<0.05$) for July 2014 and January 2015 (Annexure 5: Table 5.10). There was a significant difference between the Grey Brahman and Red Brahman ($p=0.005$), Grey Brahman and Brahman x Sussex ($p=0.004$), and Red Brahman and Sussex ($p=0.039$) groups for July 2014 (Annexure 5: Table 5.10). There were also significant differences found between the Grey Brahman and Red Brahman ($p=0.002$), and Red Brahman and Sussex ($p=0.015$) groups during January 2015 (Annexure 5: Table 5.10).

5.2.4.2 Skin glands

Differences between breed skin structure was only visually identified according to the following cellular structures; apocrine and merocrine gland shape and volume. Haematoxylin and Eosin was used for the staining process done by Idex Laboratories. A gap in this study was that the glands were only visually analysed as it was not possible to measure the skin glands volume and surface area accurately. In future studies it would be advantageous to do these measurements to gain a better idea how the glands differ between breeds.

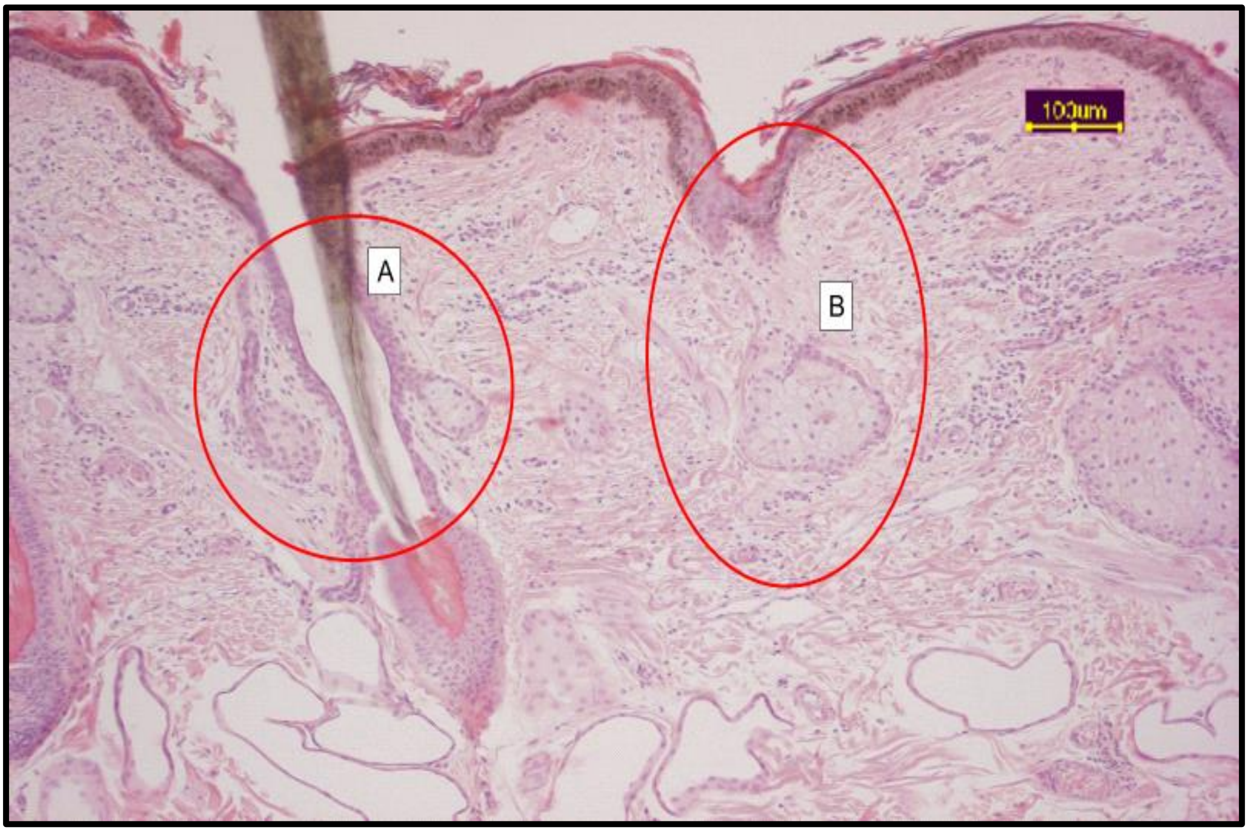

Figure 5.10: A: Apocrine and B: Merocrine sweat glands of a Brahman x Sussex cow.

5.2.4.2.1 Apocrine glands

The apocrine glands of Grey-and Red Brahman cattle, Figure 5.11A and B, were found to be "bag shaped" and visually bigger in volume when compared to the thinner less voluminous apocrine glands of Sussex cattle through cross-section slides (Figure 5.11D). Brahman x Sussex cattle and Brahman cattle had visually similar gland shape and sizes as seen in Figure 5.11C. These observations were made after examining ten slides for each breed. Haematoxylin and Eosin was used for the staining process done by Idex Laboratories.

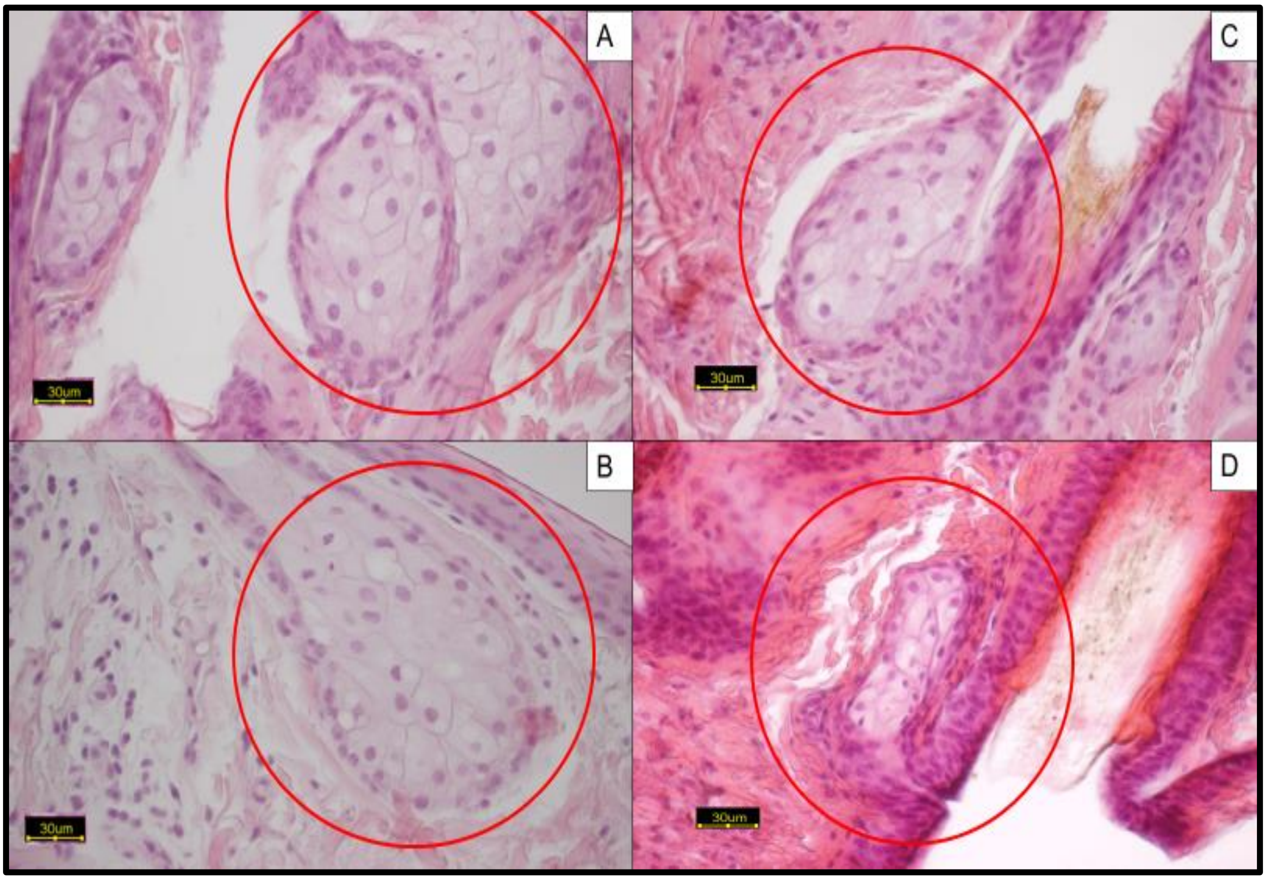

Figure 5.11: Apocrine sweat gland shape for A: Grey Brahman, B: Red Brahman, C: Brahman x Sussex and D: Sussex cattle.

5.2.4.2.2 Merocrine glands

These glands of Grey Brahman, Red Brahman and Brahman x Sussex cattle were found to be visually large in size and rounded in shape, Figure 5.5A, B and C. The merocrine glands of Sussex cattle appeared to be visually smaller and less rounded structures (Figure 5.12D). These observations were made after examining ten slides for each breed. Haematoxylin and Eosin was used for the staining process done by Idex Laboratories.

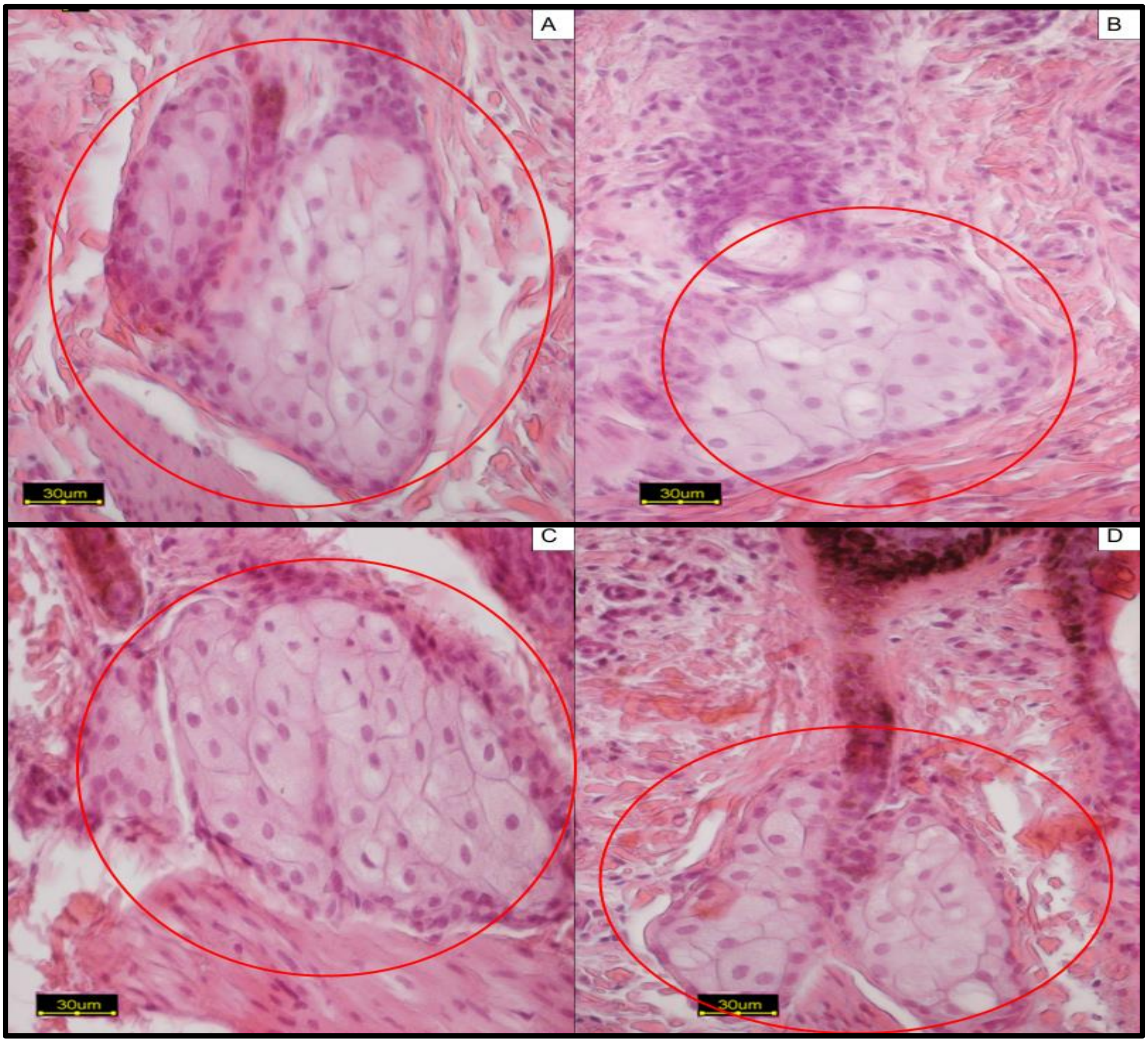

Figure 5.12: Merocrine sweat gland shape for A: Grey Brahman, B: Red Brahman, C: Brahman x Sussex and D: Sussex cattle.

5.2.4.3 Skin colour

Grey Brahman, Red Brahman and Brahman x Sussex cattle presented with dark skin tones during biopsy inspections. Sussex cattle, however presented with less pigmented skin than the other groups.

5.2.4.4 Muscles of the skin

As seen in Figure 5.13 smooth muscle were identified in sheets, acting as hair follicle muscles (*arrectores pilorum*) alongside hair follicles (Cadar 2009). No visual differences were found between the cattle groups. Haematoxylin and Eosin was used for the staining process done by Idex Laboratories.

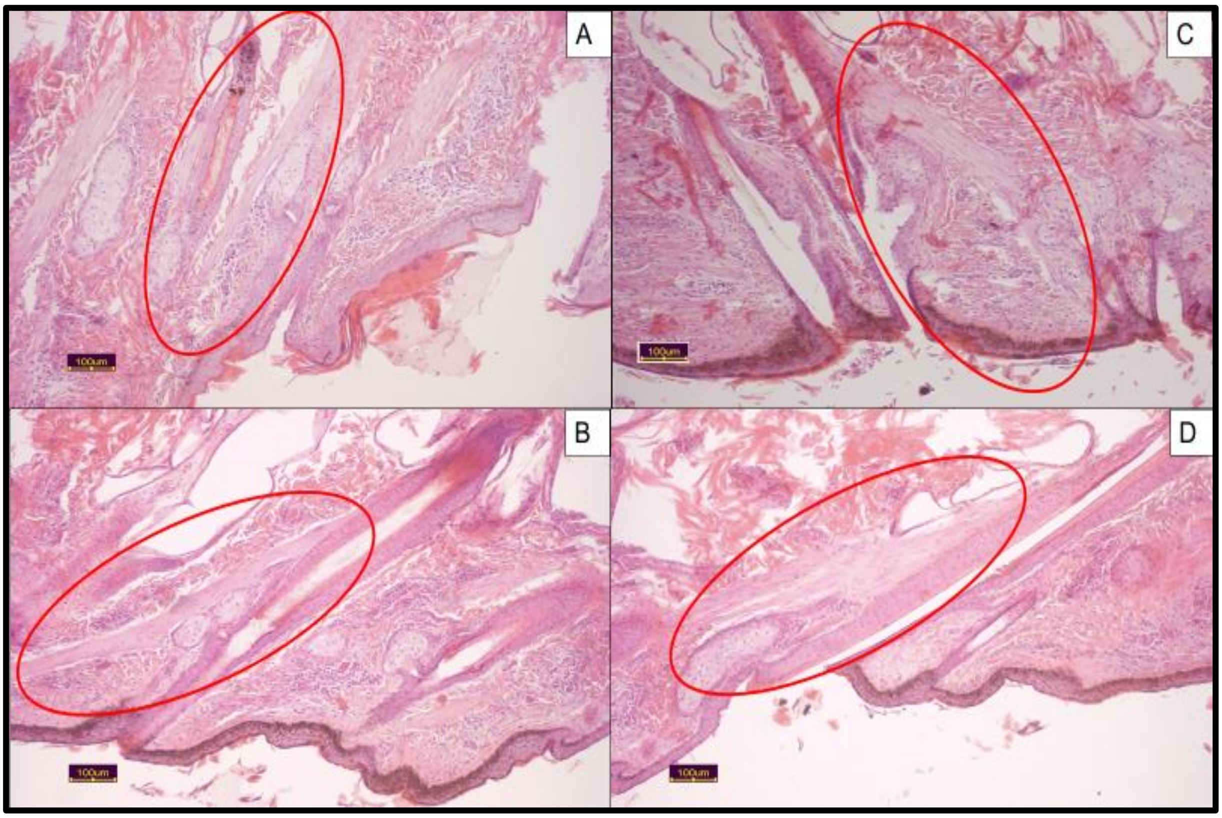

Figure 5.13: The erector muscle of hair follicles for A: Grey Brahman, B: Red Brahman, C: Brahman x Sussex and D: Sussex cattle.

5.2.5 Tail length

Figure 5.14 shows the average tail length recorded for animals of each breed, measured from the head of the tail to the end of the tail switch. Although a visual difference can be seen, a significant difference between breeds could not be proven. Grey Brahman cattle had an average tail length of 1.24m compared to Red Brahman cattle with 1.20m, Brahman x Sussex cattle with 1.27m and Sussex cattle with 1.19m. The same ten animals inspected for parasites for each breed group was used to take length measurements (Grey Brahman A=10, Grey Brahman B=10, Red Brahman A=10, Red Brahman B=10, Brahman x Sussex A=10, Brahman x Sussex B=10, Sussex A= 10, Sussex B=10).

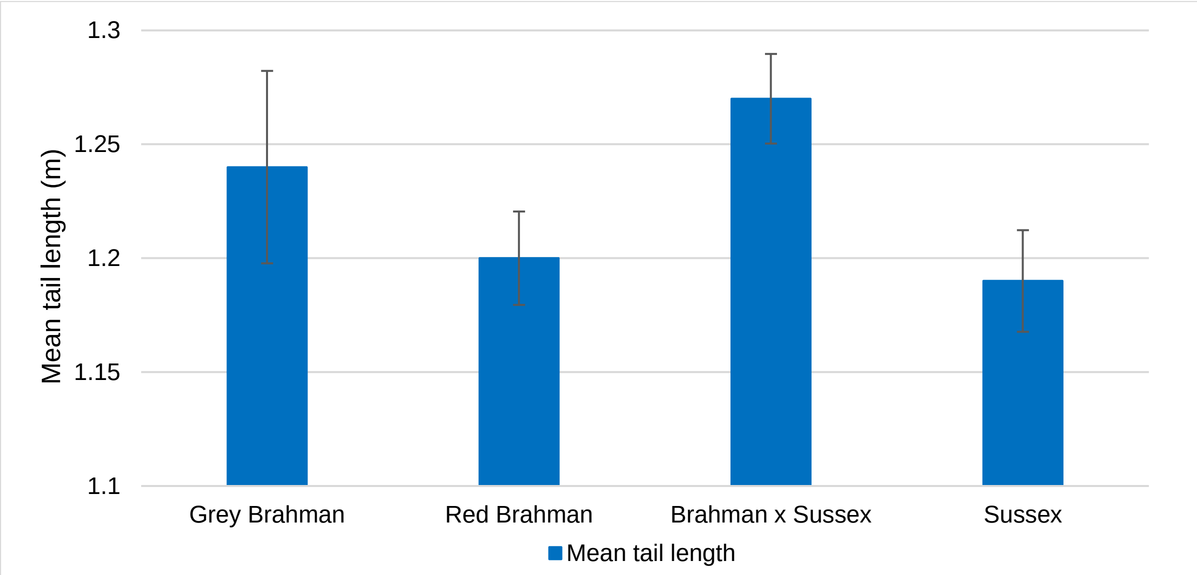

Figure 5.14: Mean tail length (meters) and standard error of the mean calculated for the different cattle breeds.

5.2.6 Rectal temperature

The average rectal temperature recorded for the different breeds are displayed in Figure 5.15. Sussex cattle had the highest average body temperature measurement of 40.26ºC, followed by Red Brahman cattle with 39.60ºC, Grey Brahman cattle with 39.49ºC and Brahman x Sussex cattle with 39.08ºC. All cattle had an optimal body score of 2 when rectal temperature was recorded. The producer stated that temperatures between 38.5°C and 39.5°C is normal, anything higher can indicate that the animals' body is under stress for example a pathogenic infestation.

Differences were found not to be significant.

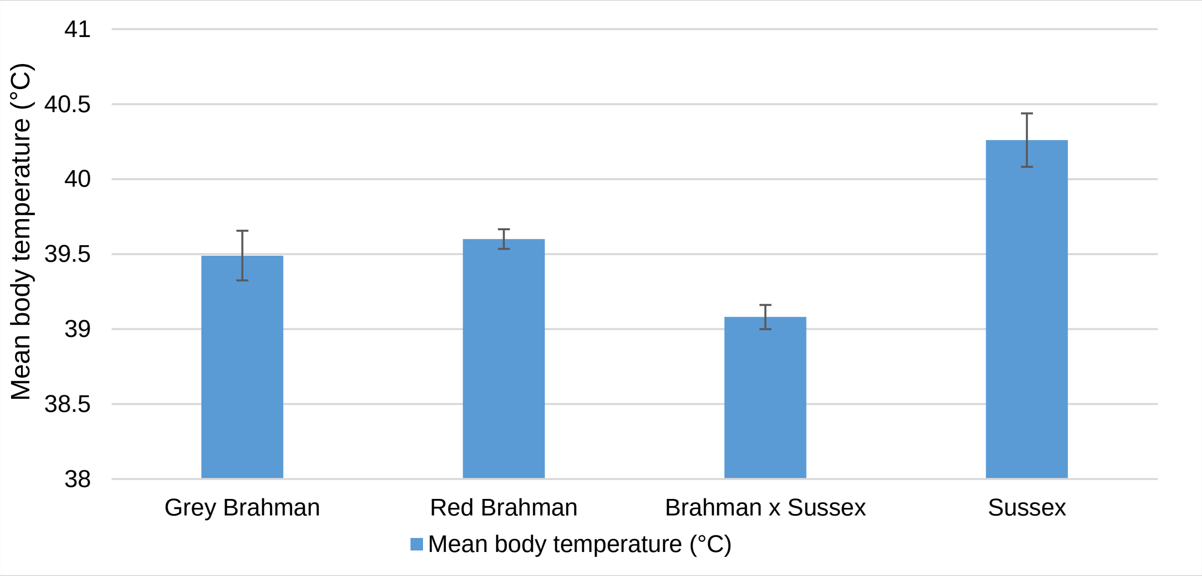

Figure 5.15: Mean rectal temperature (°C) and standard error of mean calculated for each cattle breed.

5.3 DISCUSSION

The hide/skin is the largest organ than can be identified of a bovine and can make out up to eight percent of total live mass. It acts as a barrier between the external environment and the animal, helps with heat regulation, sebum secretion and is for this reason important for the assessment of ectoparasite attractiveness for *B. indicus,* and *B. taurus* cattle (Bonsma 1983; Hansen 2004). In previous studies, it has been suggested that coat colour and thickness, as well as hide colour all play a role in heat tolerance and thus also ectoparasite load (Walker 1957). Immunity and behavioural aspects of cattle can also be influenced by their ability to thermoregulate (Hillman 2009).

In this study, it was found that Sussex cattle had the highest ectoparasite loads (ticks, mites and flies) during warmer and more humid months from March 2014 to March 2015). Different bovine characteristics were investigated to determine if any of them might contribute to differences in ectoparasite loads between the three cattle breeds.

Due to the perception that the physical condition of an animal has an influence on its ectoparasite infestation (Nyangiwe *et al*. 2011; Saymore *et al.* 2011), this aspect was investigated by examining each animal and assigning a corresponding body condition score every month during the study. All the cattle groups appeared to be in good physical condition throughout the test period, and underweight cattle were only identified within all the groups during the October 2014 observation week with a total of 30% of Grey Brahmans, 5% of Red Brahmans, 15% of Brahman x Sussex and 5% of Sussex cattle affected. Although all the cattle breeds had a higher tick load than recorded for the previous months, no statistical significant differences were recorded during this month between the different cattle breeds. Tick loads on individuals with lower body scores, in all the breed groups were also not significantly different from individuals with ideal body scores. This may be due to the low ectoparasite presence in the veld causing animal tick loads to be generally low on the animals and differences not to be as obvious as in areas where high tick loads are found on animals (Smith *et al*. 1996). The administered Doraject+AD3E (intestinal deworming and vitamin supplement) improved the general health of the animals to a point where only two individuals, one from the Grey Brahman group and one from the Brahman x Sussex group, was found to be underweight in November 2014, still with no implication on total tick load recorded on the animals.

Meltzer (1996) questioned the higher perceived ectoparasite loads between different breeds found previously and ascribed it to differences in size between *B. indicus* and *B. taurus* breeds. There was no difference in tick numbers when expressed as number of ticks per kg body weight between Brahman and Mashona heifers (Meltzer 1996). To test this theory in the current study, average weight of each breed was determined and compared to the ectoparasite loads. Results indicated that altough Sussex cattle were statistically significantly heavier (648.3kg) than all the other breeds (Grey Brahman at 518kg, Red Brahman at 496.5kg and Brahman x Sussex at 625.4kg), they still had the most ticks per 1kg body weight with 4 per kg compared to Grey Brahman (1.8 per kg), Red Brahman (1.1 per kg) and Brahman x Sussex (1.4 per kg).

The total fly load observed around the animals calculated per 1kg body weight, however showed no significant difference between the groups when the average weight was taken into consideration.

The various aspects of the coat assessment, showed Sussex cattle to have a more “coarse” hair type according to the felting test results found between the following breeds; Grey Brahman compared to the mean values recorded for Brahman x Sussex and Sussex groups, Red Brahman cattle compared to Brahman x Sussex cattle. Although this test indicated Sussex cattle to have a “rougher” hair structure, no significant differences between the mean hair thickness of the different breeds was found under the SEM microscope at 1000 x magnification. Hair density per 5mm x 5mm biopsies and inspection of the underlying dermal muscles also showed no significant differences between the breeds to account for differences in tick loads.

Several studies have previously indicated that under tropical conditions, animals with a lighter coat colour and darker pigmented skin, such as the Grey Brahman cattle, are better adapted to maintain general health and reproduce more effectively compared to darker coat colours and light skin pigmentation such as the Sussex groups (Foster *et al*. 2009; Blaho *et al*. 2012). Correspondingly, during this study the breed with the darkest coat, the Sussex cattle, were found to have the highest ectoparasite loads (ticks, mites and flies). The advantage of the lighter coat colours is due to its lowered absorption of solar radiation, which results in reduced heat stress that can influence immunity and thus in effect parasite loads.

Sussex cattle had the darkest coat colour, they also had lighter pigmented skin underneath their coats. The Sussex cattle had eye infections that were a direct result of light pigmented skin around the eyes of the animals. This can increase the susceptability to eye infections, lesions, cancer, general health and therefore also ectoparasite loads. Grey Brahman, Red Brahman and Brahman x Sussex cattle had darker pigmented skin than that of Sussex cattle, which has also been proven to be an influential variable in heat regulation. It has been reported in previous studies that dark skinned cattle have a higher evaporative heat loss and a greater number of sweat glands compared to lighter skinned cattle, thus being better adapted to semi-arid conditions (Jian *et al.* 2014) such as in South Africa. The skin thickness and skin colour contribution to thermoregulation will in turn influence the general health of the animal and as previously stated, animals that struggle to maintain general health in their environment, become more vulnerable to parasite infestations.

Horn fly attractiveness has also been reported to be associated with coat colour where greater numbers of flies were observed in cattle with darker rather than lighter coats (Blaho *et al*. 2012). This has been confirmed for Diptera attractiveness in the current study as well, where the total flies observed around Sussex cattle was more than that observed around the other breeds with lighter coat colours. The striped or brindle coat colour of Brahman x Sussex crossbred cattle might also be advantageous as it has been shown in previous studies that spots or patterns on a coat disrupts the intensity and/or angle of polarization of reflected light reducing the attractiveness to tabanid flies (Blaho *et al*. 2012). However, a study done by Doube (1984), indicated that coat colour did not contribute to any notable difference in attractiveness for the buffalo fly, *Haematobia irritans exigua*, thus it cannot be assumed that coat colour will significantly influence all fly species attractiveness to different breeds of cattle or coat colours.

Previous studies suggested that a thicker skin can play a role in heat tolerance and thus as a result also ectoparasite load (Walker 1957). Immunity and behavioural aspects regarding ectoparasite control such as self-grooming can also be influenced by the cow's ability to thermoregulate (Hillman 2009).

As correspondingly found during the current study, the shape of the skin glands where also found to differ between *B. indicus* and *B. taurus* breeds by Jian *et al*. (2014) and by Govindaiah *et al*. (1980). In the current study both the apocrine and merocrine glands of the Sussex breed differed from the other breeds corresponding to a higher tick load on the Sussex breed.

Tail length were also considered as influential factors concerning general ectoparasite load on the animals during this study. As cattle use their tails to swat away flies, average tail length should play a role in fly repelling behaviour efficiency. Although this might be true, the average tail length did not prove to be significantly different between the breeds and therefore could not be considered as a factor influencing differences in fly infestations between the different breeds.

It can thus be deducted that coat characteristics indicate how suitable the on-host parasitic microclimatic conditions can be for a certain breed and therefore influence ectoparasite loads on different breeds.

5.4 CONCLUSION

Various phenotypical breed characteristics such as skin thickness, coat type, -colour and length play a significant role in ectoparasite on-host survival and attractiveness. Sussex cattle have been found to have higher ectoparasite loads possibly caused by longer, denser, coarser, and darker coats than the other selected cattle groups.

From the results gained through this study it is evident that certain breed characteristics can have an influence on ectoparasite load. Sussex cattle were found to have coarser and longer coats, higher body temperatures, weight and the highest ectoparasite loads throughout the study. The Grey- and Red Brahman groups had the lowest parasite load accompanied by shorter and smoother coats helping them to regulate their body temperatures more effectively. Especially tick host resistance has been found to be a heritable trait as high as 82% in Brahman crossbred cattle (Piper *et al.* 2009). These previous studies confirm that as suggested by the results found in the current study that the resistance status of both *B. indicus* and *B. taurus* breeds can be improved by selection of ectoparasite resistance.

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CHAPTER 6

CONCLUSION

CONCLUSION AND RECOMMENDATIONS

Crossbreeding *Bos indicus* with *Bos taurus* cattle was explored as a measure to manage ectoparasite infestation specifically comparing tick, mite, fly and lice resistance between Brahman, Sussex and Brahman x Sussex crossbreds. The study area was located in the central Free State on different farms, not more than 15km apart, where cattle breeds were followed and ectoparasites collected on a monthly basis from March 2014 to March 2015. Ectoparasites were collected from both the on-host and off-host environment, to monitor their presence and abundance and to compare these results to possible ectoparasite repelling characteristics.

The main aim of the study was to determine if Brahman cattle have natural ectoparasite resistance due to certain breed characteristics when compared to other cattle breeds such as Sussex. This was done by comparing phenotypical traits such as skin characteristics, hair characteristics, tail length and body temperatures with ectoparasite loads. A further aim was to establish if the resistance qualities identified are preserved in the crossbred generations.

In Chapter 1 objectives were set to achieve the main aim of the study. All of the objectives were obtained to give an insight into the complex nature of ectoparasite resistance between *B. indicus* and *B. taurus* breeds in South Africa. However, it was found that some of the experimental design for data collection needed improvement. In Chapter 3 tick attachment sites were documented and ticks from each animal were collected into one collection bottle which made it impossible to link specific tick species after identification to specific attachment sites. In Chapter 4 more replications of the controlled fly count experiment should be done with more cows from each breed group for more accurate statistical analysis results. The effect the holding pen environment had on the experiment should also in future studies be taken into consideration for example, how far the animals are separated from each other, the effect of shadow in the pens and the influence of human presence around the cattle when observations are done. The suggested collection methods for sampling Diptera during the day should also be revised and improved upon. Weighing the animals once during every season to correlate ectoparasite loads with the weight of cattle during summer, autumn, winter and spring is also recommended as these results can then be compared to mean weight during various reproduction cycles of the cows. In future studies the apocrine and merocrine gland volume should be measured to obtain an indication of difference in volume size between the breeds.

Chapter 3 describes the total of five Ixodidae species that were collected from the animals over the 13 month study period. These included *Hyalomma rufipes*, by far the most abundant tick species (3797) followed by *R. evertsi evertsi* (596), *H. truncatum* (393), *R. decoloratus* (29) and *R. appendiculatus* (30). All of these tick species except for *H. truncatum* showed a higher affinity for the Sussex breed than any of the other breeds. This was reflected in the total number of ticks collected from Sussex cattle over the entire test period that was statistically significantly higher ($p<0.05$) than all the other breeds with 2534 ticks compared to the Brahman (Grey; 919 & Red; 550 ticks) and Brahman x Sussex cattle groups (846 ticks). The Sussex cattle groups also had the most tick infested individuals over the entire test period with 223 animals infested from a total of 240 individuals checked for ticks. The number of infested individuals from the Sussex groups did decrease during the colder months of May to Augustus 2014 with less than 45% of animals in the group infested with ticks. Seasonal factors like rainfall and temperature also had an influence on tick abundance with higher numbers found during the warmer months with higher rainfall figures.

Attachment areas for ticks were also investigated. These observations showed Sussex cattle to have at least nine areas of tick attachment with Red Brahman having two and Grey Brahman having three, for both breeds located on the hind quarters. The cross breed had four attachment sites corresponding with the three sites of the Grey Brahman breed on the hind quarter and one additional site on the fore quarter.

Chapter 4 describes the total number of Diptera species counted around individual cows of each breed during a drought spell in December 2014, compared to a higher rainfall period during December 2015. During December 2014 a significant higher

($p<0.05$) abundance of Diptera individuals were found around the Sussex breed compared to the other breeds. Rainfall however seemed to be a factor influencing host preference, for during December 2015, a significant higher number of Diptera species presence was observed overall when compared to December 2014 and no significant differences of Diptera abundance were then observed between any of the breeds during 2015. This indicated that throughout unfavourable conditions, when less flies are present and there is a choice of hosts available, Diptera species showed a preference for the Sussex breed. The more abundant presence of Diptera in the environment eliminates the preference of the flies for any specific cattle breed, with all of them having high fly loads.

A greater overview was gained on Diptera diversity and abundance as well as the presence of veterinary important Diptera ectoparasites in the Kroonstad region. A total of nine Diptera families found during the day were collected in December 2015. Of these families, Muscidae were by far the most abundant with little or no specific preference for any of the cattle breeds. When comparing irritable behaviours between the different breeds, Sussex cattle showed the highest frequency of irritable behaviour, but statistically significant differences were only found during December 2015 between the Sussex breed and all the other breeds. This reinforces the practice of selecting cattle with lower ectoparasite loads to use for reproduction in commercial herds especially during times of drought.

Although the focus of this study was on Diptera collection, observations throughout the year by fluorescent light trapping during the night, revealed that species represent three main orders. Diptera, Coleoptera and Lepidoptera were the most abundant specimens collected for all months observed except for July 2014 when only species from Lepidoptera were found for collection. On Diptera family level, Ceratopogonidae were the most abundant during the autumn months and although no Diptera families were collected for July 2014 a few specimens of the family Culicidae were collected during June 2014. Spring was again dominated by the family Ceratopogonidae and the summer months provided Culicidae as most abundant Diptera family of veterinary importance. This study provided a greater overview of Diptera ectoparasites by determining the diversity and abundance of veterinary important Diptera in the Kroonstad region. Potentially economically important Diptera families have been identified throughout the sampling year.

No association was found between on-host and off-host ectoparasite abundance for all ectoparasite species collected, but this needs to be investigated more intensively in future.

Various phenotypical breed characteristics such as skin thickness, coat type, -colour and length played a role in ectoparasite on-host survival and attractiveness. From the results gained it can be concluded that certain breed characteristics had an influence on ectoparasite load. Sussex cattle were found to have coarser and longer coats, higher body temperatures, weigh more and these characteristics were associated with the highest ectoparasite loads throughout the study compared to the other breeds. The Grey- and Red Brahman groups had the lowest parasite load associated with shorter and smoother coats, helping them to regulate their body temperatures more effectively.

Tail length and general health of the cattle had no effect on ectoparasite abundance. The few animals with lower than ideal body scores did not show higher ectoparasite, especially tick, abundance. This might be due to the low tick numbers generally found on the cattle in this region. When the condition of microhabitats in the on-host or off-host environment deteriorates due to extreme weather fluctuations tick abundance in the veld as well as on the animals' decreased.

In this study higher perceived ectoparasite loads between different breeds an not be ascribed to differences in size between *B. indicus* and *B. taurus* breeds. Average weight of each breed compared to the ectoparasite loads indicated that altough Sussex cattle were statistically significantly heavier, with a mean of 648.3kg, than the Grey Brahman at a mean of 518kg, Red Brahman at 496.5kg and Brahman x Sussex at 625.4kg, they still had the most ticks per kg body weight with 4 ticks per kg compared to Grey Brahman, 1.8 ticks per kg, Red Brahman; 1.1 ticks per kg and Brahman x Sussex; 1.4 per kg. The plus on the production side was that although the ectoparasite load on the crossbred group was significant lower, the mean weight was only 22.9kg lower than those of the Sussex group and a mean of 128kg higher than the Red Brahman group and 102kg higher than the Grey Brahman groups. This indicated that crossbreeding should be integrated into herd management plans as an effective measure in controlling ectoparasite loads on cattle in both intensive and extensive production systems. The heritability of tick host resistance indicated by the lower tick number per kg body weight of the cross breed compared to the Sussex breed is confirmed by the results found in the current study. It is also suggested that the resistance status of both *B. indicus* and *B. taurus* breeds can be improved by selection for ectoparasite resistance within the breeds

Control strategies of ectoparasites on livestock should be revised as many arthropod populations are becoming more and more insecticide resistant. Integrated pest management should be applied to control pest Diptera around cattle in a stabled environment as well as those in the fields. When Diptera populations are very high, water logged camps should be avoided to reduce the number of Diptera vector bites along with well managed chemical control methods that can include; pyrethroids, amidines, organophosphates, macrocyclic lactones and growth regulators such as chitin synthesis inhibitors.

ANNEXURE 1

OWNER CONSENT FORMS

Consent form from Mr. Bertiaan Luyt

OWNER CONSENT FORM

Permission to study use data in MSc obtained by Mr Bertiaan Luyt

I **Marilie Esterhuyze**, a masters student at the University of the Free State am currently doing a comparative study in ectoparasite tolerance between purebred Brahman, Sussex and Brahman crossbred cattle in the central Free State, South Africa towards the completion of a masters degree at the Department of Zoology & Entomology, Faculty of Science & Agriculture, University of the Free State.

From January 2015- December 2016 a student, Mr Bertiaan Luyt, as part of the practical requirements for completing his diploma in Agriculture at Potchefstroom College of Agriculture, conducted an evaluation of the different grass species found on the farms Blanquilla and Yvonne. Although the primary focus of his study was on these two farms, the vegetation type on all the farms included in the current study, is regarded as being visually homogeneous to the investigated farms and therefore regarded as being representative of the study area observed in the host parasite interactions and included in the current MSc study.

I **Bertiaan Luyt** hereby declare my willingness to partake in the study granting **Marilie Esterhuyze** permission to access and use data obtained during completing my Diploma in Agriculture and Potchefstoom College of Agriculture.

Name of person giving permission: Bertiaan Luyt

Signature: [signature]

Investigators signature: ______________________

Date: ______________________

Consent form from Mr. Pieter Esterhuyze

OWNER CONSENT FORM

Permission to utilise EFT Boerdery farms and cattle for research

I **Marilie Esterhuyze**, a master's student at the University of the Free State am currently doing a comparative study in ectoparasite tolerance between purebred Brahman, Sussex and Brahman crossbred cattle in the central Free State, South Africa towards the completion of a master's degree at the Department of Zoology & Entomology, Faculty of Science & Agriculture, University of the Free State.

I **Pieter Esterhuyze** hereby declare my willingness to partake in the study granting **Marilie Esterhuyze** permission to access farms, facilities on the farms and cattle for data collection during the required study period towards completion of her master's degree at the Department of Zoology & Entomology, Faculty of Science & Agriculture, University of the Free State.

Name of person giving permission: P.C. Esterhuyze

Signature: ______

Investigators signature: ______

Date: March 2014.

ANNEXURE 2

DATA RECORDING AND SAMPLE LABELLING

Date:

Group	
Animal tag	
Tail length	
Skin Thickness	
Sex	
Body condition score	
Weight	

Coordinates:

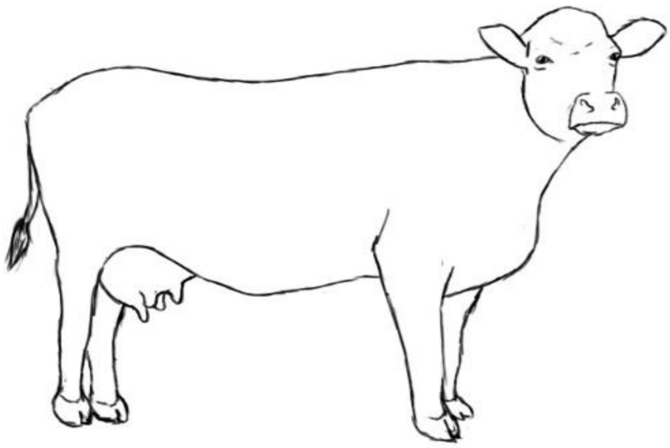
Weather conditions:

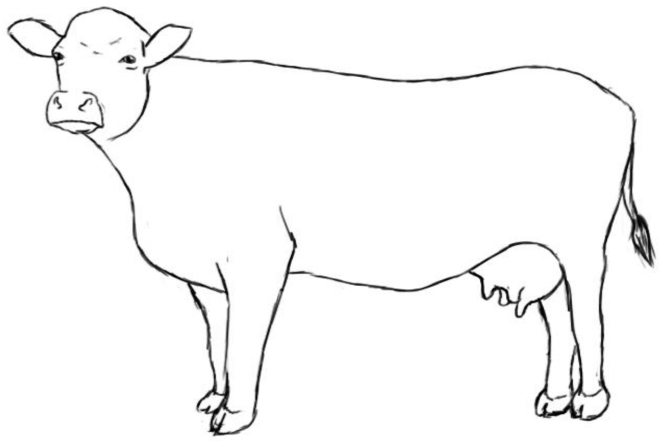
Tick attachment sites:

Mites: x Lice: △

Ticks: o

LEFT SIDE RIGHT SIDE





Labelling of ectoparasite, sebum skin and hair samples:

Group of cattle, Location
Date of sample, Animal ID

Example:

Sussex group B, Hamiltonsrus
14 February 2014, Sus1308

ANNEXURE 3

STATISTICAL ANALYSIS OF ABUNDANCE OF ACARI ON THE CATTLE BREEDS

Annexure Table 3.1: Statistical significant difference ($p<0.05$) calculated with Two-way ANOVA between breeds for on-host tick abundance from March 2014 to March 2015.

Breed		Mean difference	Standard error	Sig.
Grey Brahman	Red Brahman	5.523	11.256	0.624
	Brahman x Sussex	1.154	11.256	0.918
	Sussex	-24.846	11.256	0.028
Red Brahman	Grey Brahman	-5.523	11.256	0.624
	Brahman x Sussex	-4.369	11.256	0.698
	Sussex	-30.369	11.256	0.007
Brahman x Sussex	Grey Brahman	-1.154	11.256	0.918
	Red Brahman	4.369	11.256	0.698
	Sussex	-26	11.256	0.022
Sussex	Grey Brahman	24.846	11.256	0.028
	Red Brahman	30.369	11.256	0.007
	Brahman x Sussex	26	11.256	0.022

Annexure Table 3.2: Tests of between-subject effects as a dependent variable for tick abundance between the breeds.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	54844.134[a]	51	1075.375	26.979	0.000	0.582
Intercept	22589.816	1	22589.816	566.738	0.000	0.365
Selected breed	9118.841	3	3039.614	76.258	0.000	0.188
Date Num	24668.071	12	2055.673	51.573	0.000	0.385
Selected breed * Date Num	21057.221	36	584.923	14.675	0.000	0.348
Error	39381.050	988	39.859			
Total	116815.000	1040				
Corrected Total	94225.184	1039				
a. R Squared = 0.582 (Adjusted R Squared = 0.560)						

Annexure Table 3.3: Univariate test done for significant difference in tick abundance between sampling months as dependant variable.

Month		Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
March 2014	Contrast	65.650	3	21.883	0.549	0.649	0.002
	Error	39381.050	988	39.859			
April 2014	Contrast	35.650	3	11.883	0.298	0.827	0.001
	Error	39381.050	988	39.859			
May 2014	Contrast	12.900	3	4.300	0.108	0.956	0.000
	Error	39381.050	988	39.859			
June 2014	Contrast	44.637	3	14.879	0.373	0.772	0.001
	Error	39381.050	988	39.859			
July 2014	Contrast	15.450	3	5.150	0.129	0.943	0.000
	Error	39381.050	988	39.859			
August 2014	Contrast	213.250	3	71.083	1.783	0.149	0.005
	Error	39381.050	988	39.859			
September 2014	Contrast	3.650	3	1.217	0.031	0.993	0.000
	Error	39381.050	988	39.859			
October 2014	Contrast	32.650	3	10.883	0.273	0.845	0.001
	Error	39381.050	988	39.859			
November 2014	Contrast	19806.638	3	6602.213	165.638	0.000	0.335
	Error	39381.050	988	39.859			
December 2014	Contrast	6769.100	3	2256.367	56.608	0.000	0.147
	Error	39381.050	988	39.859			
January 2015	Contrast	832.800	3	277.600	6.964	0.000	0.021
	Error	39381.050	988	39.859			
February 2015	Contrast	406.738	3	135.579	3.401	0.017	0.010
	Error	39381.050	988	39.859			
March 2015	Contrast	1936.950	3	645.650	16.198	0.000	0.047
	Error	39381.050	988	39.859			

Annexure Table 3.4: Months' showing statistical significant differences ($p<0.05$) for multiple comparisons in on-host tick abundance between cattle breeds.

Month			Mean Difference (I-J)	Std. Error	Sig.[b]
November 2014	Grey Brahman	Red Brahman	2.300	1.996	1.000
		Brahman x Sussex	1.850	1.996	1.000
		Sussex	-34.900	1.996	0.000
	Red Brahman	Grey Brahman	-2.300	1.996	1.000
		Brahman x Sussex	-.450	1.996	1.000
		Sussex	-37.200	1.996	0.000
	Brahman x Sussex	Grey Brahman	-1.850	1.996	1.000
		Red Brahman	.450	1.996	1.000
		Sussex	-36.750	1.996	0.000
	Sussex	Grey Brahman	34.900	1.996	0.000
		Red Brahman	37.200	1.996	0.000
		Brahman x Sussex	36.750	1.996	0.000
December 2014	Grey Brahman	Red Brahman	12.250	1.996	0.000
		Brahman x Sussex	5.550	1.996	0.033
		Sussex	-12.800	1.996	0.000
	Red Brahman	Grey Brahman	-12.250	1.996	0.000
		Brahman x Sussex	-6.700	1.996	0.005
		Sussex	-25.050	1.996	0.000
	Brahman x Sussex	Grey Brahman	-5.550	1.996	0.033
		Red Brahman	6.700	1.996	0.005
		Sussex	-18.350	1.996	0.000
	Sussex	Grey Brahman	12.800	1.996	0.000
		Red Brahman	25.050	1.996	0.000
		Brahman x Sussex	18.350	1.996	0.000
January 2015	Grey Brahman	Red Brahman	5.600	1.996	0.031
		Brahman x Sussex	1.400	1.996	1.000
		Sussex	-3.400	1.996	0.533
	Red Brahman	Grey Brahman	-5.600	1.996	0.031
		Brahman x Sussex	-4.200	1.996	0.214
		Sussex	-9.000	1.996	.000
	Brahman x Sussex	Grey Brahman	-1.400	1.996	1.000
		Red Brahman	4.200	1.996	0.214
		Sussex	-4.800	1.996	0.098
	Sussex	Grey Brahman	3.400	1.996	0.533
		Red Brahman	9.000*	1.996	0.000
		Brahman x Sussex	4.800	1.996	0.098

b. Adjustment for multiple comparisons: Bonferroni.

Annexure Table 3.4 continued;

February 2015	Grey Brahman	Red Brahman	1.250	1.996	1.000
		Brahman x Sussex	-2.850	1.996	0.922
		Sussex	-4.450	1.996	0.156
	Red Brahman	Grey Brahman	-1.250	1.996	1.000
		Brahman x Sussex	-4.100	1.996	0.242
		Sussex	-5.700	1.996	0.026
	Brahman x Sussex	Grey Brahman	2.850	1.996	0.922
		Red Brahman	4.100	1.996	0.242
		Sussex	-1.600	1.996	1.000
	Sussex	Grey Brahman	4.450	1.996	0.156
		Red Brahman	5.700	1.996	0.026
		Brahman x Sussex	1.600	1.996	1.000
March 2015	Grey Brahman	Red Brahman	-3.900	1.996	0.306
		Brahman x Sussex	-1.500	1.996	1.000
		Sussex	-12.700*	1.996	0.000
	Red Brahman	Grey Brahman	3.900	1.996	0.306
		Brahman x Sussex	2.400	1.996	1.000
		Sussex	-8.800*	1.996	0.000
	Brahman x Sussex	Grey Brahman	1.500	1.996	1.000
		Red Brahman	-2.400	1.996	1.000
		Sussex	-11.200*	1.996	0.000
	Sussex	Grey Brahman	12.700*	1.996	0.000
		Red Brahman	8.800*	1.996	0.000
		Brahman x Sussex	11.200*	1.996	0.000

b. Adjustment for multiple comparisons: Bonferroni.

ANNEXURE 4

STATISTICAL ANALYSIS OF DIPTERA FAMILY ABUNDANCE AND DIVERSITY

Annexure Table 4.1: Statistical significant differences ($p<0.05$) between group means for irritable behaviour observed between the cattle breeds with Tukey HSD test for December 2014 and December 2015.

Dependent Variable				Mean Difference (I-J)	Std. Error	Sig.
Irritable behaviour 2014	Tukey HSD	Grey Brahman	Red Brahman	36.500	21.219	0.420
			Brahman x Sussex	-1.000	21.219	1.000
			Sussex	-23.500	21.219	0.705
		Red Brahman	Grey Brahman	-36.500	21.219	0.420
			Brahman x Sussex	-37.500	21.219	0.402
			Sussex	-60.000	21.219	0.146
		Brahman x Sussex	Grey Brahman	1.000	21.219	1.000
			Red Brahman	37.500	21.219	0.402
			Sussex	-22.500	21.219	0.729
		Sussex	Grey Brahman	23.500	21.219	0.705
			Red Brahman	60.000	21.219	0.146
			Brahman x Sussex	22.500	21.219	0.729
Irritable behaviour 2015	Tukey HSD	Grey Brahman	Red Brahman	18	3.240	0.017
			Brahman x Sussex	0.000	3.240	1.000
			Sussex	-39	3.240	0.001
		Red Brahman	Grey Brahman	-18	3.240	0.017
			Brahman x Sussex	-18	3.240	0.017
			Sussex	-57	3.240	0.000
		Brahman x Sussex	Grey Brahman	0.000	3.240	1.000
			Red Brahman	18	3.240	0.017
			Sussex	-39	3.240	0.001
		Sussex	Grey Brahman	39	3.240	0.001
			Red Brahman	57	3.240	0.000
			Brahman x Sussex	39	3.240	0.001

Annexure Table 4.2: Statistical significant difference in Diptera presence between cattle groups observed during the first and second day of sampling in 2014 with One-way ANOVA and Tukey HSD test.

Dependent Variable				Mean Difference (I-J)	Std. Error	Sig.
Total flies day one 2014	Tukey HSD	Grey Brahman	Red Brahman	18.500	27.141	0.899
			Brahman x Sussex	-18.000	27.141	0.906
			Sussex	-235	27.141	0.003
		Red Brahman	Grey Brahman	-18.500	27.141	0.899
			Brahman x Sussex	-36.500	27.141	0.586
			Sussex	253.500	27.141	0.003
		Brahman x Sussex	Grey Brahman	18.000	27.141	0.906
			Red Brahman	36.500	27.141	0.586
			Sussex	-217	27.141	0.005
		Sussex	Grey Brahman	235	27.141	0.003
			Red Brahman	253.5	27.141	0.003
			Brahman x Sussex	217	27.141	0.005
Total flies day two 2014	Tukey HSD	Grey Brahman	Red Brahman	80.000	41.547	0.346
			Brahman x Sussex	23.500	41.547	0.937
			Sussex	-423	41.547	0.002
		Red Brahman	Grey Brahman	-80.000	41.547	0.346
			Brahman x Sussex	-56.500	41.547	0.579
			Sussex	-503	41.547	0.001
		Brahman x Sussex	Grey Brahman	-23.500	41.547	0.937
			Red Brahman	56.500	41.547	0.579
			Sussex	-446.5	41.547	0.001
		Sussex	Grey Brahman	423	41.547	0.002
			Red Brahman	503	41.547	0.001
			Brahman x Sussex	446.5	41.547	0.001

Annexure Table 4.3: Statistical significant difference (p<0.05) in Diptera counts as observed between cattle breed groups and the specific dates during 2014 and 2015 when observations took place, with One-way ANOVA and Tukey HSD test.

Dependent Variable:	Total fly count				
(I) Breed and date			Mean Difference (I-J)	Std. Error	Sig.
Tukey HSD	Grey Brahman 2014	Red Brahman 2015	-720.750	101.524	0.000
		Brahman x Sussex 2015	-799.000	101.524	0.000
		Sussex 2015	-777.000	101.524	0.000
	Grey Brahman 2015	Grey Brahman 2014	750.000	101.524	0.000
		Red Brahman 2014	799.250	101.524	0.000
		Brahman x Sussex 2014	752.750	101.524	0.000
		Sussex 2014	421.000	101.524	0.007
	Red Brahman 2014	Grey Brahman 2015	-799.250	101.524	0.000
		Red Brahman 2015	-770.000	101.524	0.000
		Brahman x Sussex 2015	-848.250	101.524	0.000
		Sussex 2015	-826.250	101.524	0.000
	Red Brahman 2015	Grey Brahman 2014	720.750	101.524	0.000
		Red Brahman 2014	770.000	101.524	0.000
		Brahman x Sussex 2014	723.500	101.524	0.000
		Sussex 2014	391.750	101.524	0.015
	Brahman x Sussex 2014	Grey Brahman 2015	-752.750	101.524	0.000
		Red Brahman 2015	-723.500	101.524	0.000
		Brahman x Sussex 2015	-801.750	101.524	0.000
		Sussex 2015	-779.750	101.524	0.000
	Brahman x Sussex 2015	Grey Brahman 2014	799.000	101.524	0.000
		Red Brahman 2014	848.250	101.524	0.000
		Brahman x Sussex 2014	801.750	101.524	0.000
		Sussex 2014	470.000	101.524	0.002
	Sussex 2014	Grey Brahman 2015	-421.000	101.524	0.007
		Red Brahman 2014	378.250	101.524	0.020
		Red Brahman 2015	-391.750	101.524	0.015
		Brahman x Sussex 2015	-470.000	101.524	0.002
		Sussex 2015	-448.000	101.524	0.004
	Sussex 2015	Grey Brahman 2014	777.000	101.524	0.000
		Red Brahman 2014	826.250	101.524	0.000
		Brahman x Sussex 2014	779.750	101.524	0.000
		Sussex 2014	448.000	101.524	0.004

Annexure Table 4.3 continued;

Dependent Variable:	Total fly count				
(I) Breed and date			Mean Difference (I-J)	Std. Error	Sig.
Tukey HSD	Brahman x Sussex 2014	Grey Brahman 2015	-752.750	101.524	0.000
		Red Brahman 2015	-723.500	101.524	0.000
		Brahman x Sussex 2015	-801.750	101.524	0.000
		Sussex 2015	-779.750	101.524	0.000
	Brahman x Sussex 2015	Grey Brahman 2014	799.000	101.524	0.000
		Red Brahman 2014	848.250	101.524	0.000
		Brahman x Sussex 2014	801.750	101.524	0.000
		Sussex 2014	470.000	101.524	0.002
	Sussex 2014	Grey Brahman 2015	-421.000	101.524	0.007
		Red Brahman 2014	378.250	101.524	0.020
		Red Brahman 2015	-391.750	101.524	0.015
		Brahman x Sussex 2015	-470.000	101.524	0.002
		Sussex 2015	-448.000	101.524	0.004
	Sussex 2015	Grey Brahman 2014	777.000	101.524	0.000
		Red Brahman 2014	826.250	101.524	0.000
		Brahman x Sussex 2014	779.750	101.524	0.000
		Sussex 2014	448.000	101.524	0.004

ANNEXURE 5

STATISTICAL ANALYSIS OF BOVINE CHARACTERISTICS AND EFFECT ON ECTOPARASITE INFESTATION

Annexure Table 5.1: Statistical significant differences between breed mean weight comparisons with Tukey HSD and One-way ANOVA test.

(I) Breed			Mean Difference (I-J)	Std. Error	Sig.
Tukey HSD	Grey Brahman	Red Brahman	21.500	30.986	0.899
		Brahman x Sussex	-107.4	30.986	0.007
		Sussex	-130.3	30.986	0.001
	Red Brahman	Grey Brahman	-21.500	30.986	0.899
		Brahman x Sussex	-128.9	30.986	0.001
		Sussex	-151.8	30.986	0.000
	Brahman x Sussex	Grey Brahman	107.4	30.986	0.007
		Red Brahman	128.9	30.986	0.001
		Sussex	-22.900	30.986	0.881
	Sussex	Grey Brahman	130.3	30.986	0.001
		Red Brahman	151.8	30.986	0.000
		Brahman x Sussex	22.900	30.986	0.881

Annexure Table 5.2: Statistical significant differences in the number of animals with felting hair analyzed with a Tukey HSD and One-way ANOVA test.

(I) Breeds			Mean Difference (I-J)	Std. Error	Sig.
Tukey HSD	Grey Brahman	Red Brahman	0.500	1.173	0.971
		Brahman x Sussex	-2.000	1.173	0.426
		Sussex	-8.000	1.173	0.008
	Red Brahman	Grey Brahman	-.500	1.173	0.971
		Brahman x Sussex	-2.500	1.173	0.283
		Sussex	-8.500	1.173	0.007
	Brahman x Sussex	Grey Brahman	2.000	1.173	0.426
		Red Brahman	2.500	1.173	0.283
		Sussex	-6.000	1.173	0.023
	Sussex	Grey Brahman	8.000	1.173	0.008
		Red Brahman	8.500	1.173	0.007
		Brahman x Sussex	6.000	1.173	0.023

Annexure Table 5.3: Statistical significant differences in mean hair thicknesses of breeds analysed with a Welch test for equality and One-way ANOVA.

ANOVA					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	744.874	3	248.291	0.429	0.733
Within Groups	310558	536	579.399		
Total	311302.9	539			
Robust Tests of Equality of Means					
	Statistic[a]	df1	df2	Sig.	
Welch test of equality of means	0.48	3	296.277	0.696	

a. Asymptotically F distributed.

Annexure Table 5.4: Statistical significant difference tested by multiple comparisons between breeds for mean hair thickness with Tukey and Games Howell tests.

	(I) Breeds selected in study	(J) Breeds selected in study	Mean Difference (I-J)	Std. Error	Sig.
Tukey HSD	Grey Brahman	Red Brahman	0.0919	2.9298	1
		Brahman x Sussex	0.5933	2.9298	0.997
		Sussex	-2.4333	2.9298	0.84
	Red Brahman	Grey brahman	-0.0919	2.9298	1
		Brahman x Sussex	0.5015	2.9298	0.998
		Sussex	-2.5252	2.9298	0.824
	Brahman x Sussex	Grey Brahman	-0.5933	2.9298	0.997
		Red Brahman	-0.5015	2.9298	0.998
		Sussex	-3.0267	2.9298	0.73
	Sussex	Grey Brahman	2.4333	2.9298	0.84
		Red Brahman	2.5252	2.9298	0.824
		Brahman x Sussex	3.0267	2.9298	0.73
Games-Howell	Grey Brahman	Red Brahman	0.0919	3.1564	1
		Brahman x Sussex	0.5933	2.6511	0.996
		Sussex	-2.4333	2.7463	0.812
	Red Brahman	Grey Brahman	-0.0919	3.1564	1
		Brahman x Sussex	0.5015	3.1025	0.998
		Sussex	-2.5252	3.1842	0.858
	Brahman x Sussex	Grey Brahman	-0.5933	2.6511	0.996
		Red Brahman	-0.5015	3.1025	0.998
		Sussex	-3.0267	2.6841	0.673
	Sussex	Grey Brahman	2.4333	2.7463	0.812
		Red Brahman	2.5252	3.1842	0.858
		Brahman x Sussex	3.0267	2.6841	0.673

Annexure Table 5.5: Statistical significant difference and equality between mean scale intervals of cattle breeds with One-Way ANOVA and Welch test.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	299.364	3	99.788	16.293	0.000
Within Groups	3895.288	636	6.125		
Total	4194.652	639			
Robust Tests of Equality of Means					
	Statistic[a]	df1	df2	Sig.	
Welch	17.416	3	353.003	0.000	

a. Asymptotically F distributed.

Annexure Table 5.6: Between breed mean scale interval comparisons for statistical significant difference with Tukey HSD and Games Howell tests.

(I) Breeds			Mean Difference (I-J)	Std. Error	Sig.
Tukey HSD	Grey Brahman	Red Brahman	-0.63800	0.27669	0.098
		Brahman x Sussex	-1.80906	0.27669	0.000
		Sussex	-1.31544	0.27669	0.000
	Red Brahman	Grey Brahman	0.63800	0.27669	0.098
		Brahman x Sussex	-1.17106	0.27669	0.000
		Sussex	-0.67744	0.27669	0.069
	Brahman x Sussex	Grey Brahman	1.80906	0.27669	0.000
		Red Brahman	1.17106	0.27669	0.000
		Sussex	0.49363	0.27669	0.282
	Sussex	Grey Brahman	1.31544	0.27669	0.000
		Red Brahman	0.67744	0.27669	0.069
		Brahman x Sussex	-0.49363	0.27669	0.282
Games-Howell	Grey Brahman	Red Brahman	-0.63800	0.28117	0.108
		Brahman x Sussex	-1.80906	0.26482	0.000
		Sussex	-1.31544	0.27166	0.000
	Red Brahman	Grey Brahman	0.63800	0.28117	0.108
		Brahman x Sussex	-1.17106	0.28163	0.000
		Sussex	-0.67744	0.28808	0.089
	Brahman x Sussex	Grey Brahman	1.80906	0.26482	0.000
		Red Brahman	1.17106	0.28163	0.000
		Sussex	0.49363	0.27214	0.269
	Sussex	Grey Brahman	1.31544	0.27166	0.000
		Red Brahman	0.67744	0.28808	0.089
		Brahman x Sussex	-0.49363	0.27214	0.269

Annexure Table 5.7: One-way ANOVA and Welch test for equality and statistical significant difference between mean density of hair between cattle groups.

ANOVA					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	9749.475	3	3249.825	2.080	0.120
Within Groups	56235.300	36	1562.092		
Total	65984.775	39			
Robust Tests of Equality of Means					
	Statistic[a]	df1	df2	Sig.	
Welch	1.897	3	19.797	0.163	

a. Asymptotically F distributed.

Annexure Table 5.8: One-way ANOVA and Welch test for equality and statistical significant difference between mean difference in hair length (mm) between breeds.

ANOVA					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	6102.790	3	2034.263	67.228	0.000
Within Groups	24086.390	796	30.259		
Total	30189.180	799			
Robust Tests of Equality of Means					
	Statistic[a]	df1	df2	Sig.	
Welch	45.466	3	438.040	0.000	

a. Asymptotically F distributed.

Annexure Table 5.9: Between breed statistical significant difference in mean hair length with Tukey HSD and Games Howell tests.

(I) Cattle_breeds			Mean Difference (I-J)	Std. Error	Sig.
Tukey HSD	Grey Brahman	Red Brahman	-0.435	0.550	0.859
		Brahman x Sussex	0.250	0.550	0.969
		Sussex	-6.415	0.550	0.000
	Red Brahman	Grey Brahman	0.435	0.550	0.859
		Brahman x Sussex	0.685	0.550	0.598
		Sussex	-5.980	0.550	0.000
	Brahman x Sussex	Grey Brahman	-0.250	0.550	0.969
		Red Brahman	-0.685	0.550	0.598
		Sussex	-6.665	0.550	0.000
	Sussex	Grey Brahman	6.415	0.550	0.000
		Red Brahman	5.980	0.550	0.000
		Brahman x Sussex	6.665	0.550	0.000

Annexure Table 5.10: Statistical significant difference between breed mean skin thickness comparisons with Tukey HSD test for July 2014 and January 2015.

Dependent Variable				Mean Difference (I-J)	Std. Error	Sig.
Skin thickness July 2014	Tukey HSD	Grey Brahman	Red Brahman	0.17000	0.04954	0.005
			Brahman x Sussex	0.17500	0.04954	0.004
			Sussex	0.03500	0.04954	0.894
		Red Brahman	Grey Brahman	-0.17000	0.04954	0.005
			Brahman x Sussex	0.00500	0.04954	1.000
			Sussex	-0.13500	0.04954	0.039
		Brahman x Sussex	Grey Brahman	-0.17500	0.04954	0.004
			Red Brahman	-0.00500	0.04954	1.000
			Sussex	-0.14000	0.04954	0.030
		Sussex	Grey Brahman	-0.03500	0.04954	0.894
			Red Brahman	0.13500	0.04954	0.039
			Brahman x Sussex	0.14000	0.04954	0.030
Skin thickness January 2015	Tukey HSD	Grey Brahman	Red Brahman	0.31000	0.08114	0.002
			Brahman x Sussex	0.12500	0.08114	0.419
			Sussex	0.06000	0.08114	0.881
		Red Brahman	Grey Brahman	-0.31000	0.08114	0.002
			Brahman x Sussex	-0.18500	0.08114	0.112
			Sussex	-0.25000	0.08114	0.015
		Brahman x Sussex	Grey Brahman	-0.12500	0.08114	0.419
			Red Brahman	0.18500	0.08114	0.112
			Sussex	-0.06500	0.08114	0.854
		Sussex	Grey Brahman	-0.06000	0.08114	0.881
			Red Brahman	0.25000	0.08114	0.015
			Brahman x Sussex	0.06500	0.08114	0.854