The distribution of *Rhipicephalus (Boophilus) microplus* and *Rhipicephalus (Boophilus) decoloratus* on a farm in the Eastern Cape Province, South Africa

By

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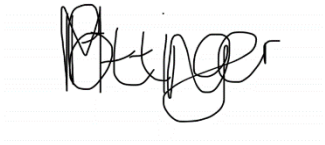
DECLARATION

I, Michelle Pottinger, declare that the Master's degree research dissertation that I herewith submit for the Master's degree qualification, MSc Zoology, at the University of the Free State is my independent work, and that I have not previously submitted it for a qualification at another institution of higher education.

Signed:  Date: 23-01-2019

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ABBREVIATIONS

AChE - Acetylcholinesterase

Bp – Base pair

ChCl3 – Chloroform

CTAB - Cetyltrimethylammonium Bromide

DNA – Deoxyribonucleic acid

EDTA – Ethylenediaminetetraacetic acid

EPNs - Entomopathogenic Nematodes

GABA - Gamma-aminobutyric acid

GPS – Global Positioning System

IAA - Isoamylalcohol

IGRs - Insect Growth Regulators

L – Litre

M/v – Mass/volume ratio

N/A – Non-Applicable

NaCl – Sodium Chloride

PCR - Polymerase Chain Reactions

Ppm – Parts per million

PRTF – Pesticide Resistance Testing Facility

R. (B.) dec - *Rhipicephalus (Boophilus) decoloratus*

R. (B.) mic - *Rhipicephalus (Boophilus) microplus*

RFLP - Restriction Fragment Length Polymorphisms

RH - Relative Humidity

RNase A – Ribonuclease A

SLIT – Shaw Larval Immersion Test

SOP – Standard Operating Procedure

TE – Tris-EDTA

TBE - Tris-borate-EDTA

UV- Ultra Violet

V/v – Volume/volume ratio

ETHICAL STATEMENT

The organisms which were tested on in this study, the adult ticks and larvae were removed from their natural environment, the cattle and vegetation. Ticks are ectoparasites and thus their removal did not have a negative impact on the ecosystem and was of an advantage to the cattle and the producer. This study was noninvasive and did not involve any physical harm to the cattle. The study collections were conducted during the usual farming management practices in order to avoid any additional stress being placed on the cattle. The producer and farm workers were present at the collections in order to create a familiar environment. Minimal contact was made with the cattle and collections occurred as quickly as possible. Any animal which exhibited excessive physical distress was released from the race and was not used in this study.

Ethical clearance was obtained from the Animal Research Ethics Committee at the University of the Free State. **Student project number: UFS-AED2017/0027.**

***See Appendix 1 for Ethical Clearance document and Appendix 2 for Producers consent form.**

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ABSTRACT

The Asiatic blue tick, *Rhipicephalus (Boophilus) microplus* is an invasive tick species which was introduced to South Africa in 1896. Reports dating back to the early 1900s state that the displacement of the African blue tick, *Rhipicephalus (Boophilus) decoloratus* had occurred within the Cape region. *Rhipicephalus (Boophilus) microplus* has the ability to adapt to new environments, is a vector of disease and has been reported to have developed resistance towards most available acaricides over a short period of time. The control of this species has become a major challenge to producers all over the world. The Eastern Cape Province accounts for the highest percentage of cattle production in South Africa. To date there is comprehensive data available on the tick distribution in South Africa however, many of the studies conducted in the Eastern Cape were completed on communal farms and the acaricide resistance status of these localities remains unknown as the majority of the studies have not included this aspect.

The aim of this study was to provide information regarding the blue cattle tick composition, distribution and acaricide resistance status of a commercial cattle farm near Grahamstown in the Eastern Cape. Engorged adult females were collected directly from the cattle and questing larvae were collected from the vegetation through drag sampling technique. All ticks and larvae were identified up to species level with the aid of morphological characteristics. Polymerase Chain Reactions-(PCR), was used to complement the morphological identification of the larvae as this life stage can be difficult to identify due to under developed features. A total of seven tick species; *Amblyomma hebraeum, Haemaphysalis elliptica, Hyalomma truncatum, Ixodes pilosus, Rhipicephalus (Boophilus) decoloratus, Rhipicephalus (Boophilus) microplus* and *Rhipicephalus evertsi evertsi,* were identified on the farm. *Rhipicephalus (Boophilus) decoloratus* was found to be the predominant tick and blue tick species on the farm while *R. (B.) microplus* was found to be present on the farm in various different camps over the study period, however, not in large numbers. The movement of a selected herd was tracked over a year period and provided a rough picture of how *R. (B.) microplus* was being spread over the farm. The relationship between temperature and humidity on the number of questing larvae collected was found to be inconclusive

presenting a weak correlation between both temperature and larvae collected as well as relative humidity and larval numbers questing.

The Shaw Larval Immersion test (SLIT), was conducted to establish resistance profiles for the various camps where tick collections were conducted. The chemicals which were tested included: Amitraz (Amidine), Chlrofenvinphos (Organophosphate) and Cypermethrin (Pyrethroid). Results were obtained for *R. (B.) decoloratus* as *R. (B.) microplus* was not collected in large enough numbers for testing. The results show that there is a definite shift towards the development and emergence of resistance on the farm towards Amidine based acaricides. Synthetic Pyrethroids and Organophosphates showed fewer extreme results. There was a definite variation between different camps on the farm. Multi-host tick resistance was also tested and it was found that both the three-host *Amblyomma hebraeum* and the two-host *Rhipicephalus evertsi evertsi* were susceptible to all the chemical groups tested.

The results of this study provide a foundation for tracking the invasion of *Rhipicephalus (Boophilus) microplus* as well as aiding the producer in the management of acaricide resistance on the farm.

Key words: Acaricide Resistance, Blue ticks, Cattle, Identification, Invasive, PCR, Questing larvae, Shaw Larval Immersion Test.

CHAPTER 1

General Introduction & Literature Review

Parasites have an effect on the regulation of animal populations within an ecosystem. Thus, parasites do have a role to play in nature, however, when there is a loss of zoonotic stability the effects can be detrimental to the host animals' health. The domestication of livestock and plants led to the establishment of agriculture in early civilizations. Cattle were domesticated approximately 9000 years ago and have since became an important source of protein for people all around the world (van As *et al*. 2012).

Ticks are ectoparasitic arthropods which affect the lives of humans, livestock and wildlife on a global scale. It has been estimated that over 80% of the world's cattle population is infested by ticks. Annually, ticks account for significant losses in animal productivity either via direct damage caused to the host, through attachment which result in blood loss, decrease in production and damage to the hide, in addition to acting as a vector for the transmission of potentially fatal pathogens (Madder *et al.* 2011; Guerrero *et al*. 2012; Manjunathachar *et al*. 2014; Yessinou *et al.* 2016). Production losses due to infestations can be immense and this has a negative impact on the economy of countries which are facing the challenge of tick control. At the same time the control of ticks to prevent the negative impacts as well as to prevent tick borne diseases is of the utmost importance (Lorusso *et al.* 2013; Manjunathachar *et al*. 2014).

The introduction of invasive species to a new region has a negative impact on the on the tick hosts in the specific area as the hosts do not have a natural immunity towards the invasive species and the diseases which they could transmit. New tick-borne diseases are also introduced into the area causing an increase in the financial cost for producers as they have to treat sick animals as well as to control ticks in general. It is clear that high tick burdens negatively impact the production of livestock and tick-borne diseases can also result in the loss of animals (Manjunathachar *et al*. 2014).

The most frequently used tick control method is the use of chemicals known as acaricides. The current acaricides available in South Africa consists of Amidines, Synthetic

Pyrethroids, Organophosphates, Macrocyclic Lactones and Insect Growth Regulators. Frequent use of any of these acaricides may lead to the development of resistance resulting in ineffective tick control. Resistance of *R. (B.) decoloratus* to acaricides has been a problem in South Africa for more than 70 years and Mekonnen *et al*. (2003), indicated that the development of tick resistance against most of these acaricides have been reported throughout South Africa. Knowledge of the resistance status of ticks against the acaricides used on a specific farm, as well as knowledge of the invasive species present, can influence treatment choices and prevent great production and financial losses (Yessinou *et al.* 2016).

In order to understand the impact of ticks and tick-borne diseases, the influence of invasive species and development of resistance to tick control, it is necessary to take a closer look at the biology and behaviour of ticks. The introduction and invasion of one-host blue tick species and control measures to prevent economical losses due to tick challenges, will also be addressed in this literature review.

1.1. Tick Classification

Guglielmone *et al.* (2010), stated that there are approximately 895 described hard and soft tick species in the world. Ticks belong to the phylum Arthropoda which also includes spiders, crustaceans, scorpions, insects and mites (Walker *et al.* 2003). This phylum contains many subphyla, with the subphyla Chelicerata, containing the class Arachnida that includes spiders, ticks and mites. All members of this class have jointed-appendages. Ticks and mites belong to the Order Acari with all tick species belonging to the suborder Ixodida.

Ticks are further divided into three families namely; Ixodidae; the hard ticks which have a hardened plate on the dorsal surface, known as a scutum or conscutum, Argasidae; the soft ticks which lack this hardened plate and Nuttalliellidae; which only comprises of one rare African species, *Nuttalliella namaqua.* This family shares characteristics of both the hard and soft ticks (Black & Piesmant 1994; Barker & Murrell 2004).

The cattle ticks under investigation in this study belong to the family Ixodidae. This family consists of two major phyletic lines, Prostriata which are represented by a single

subfamily Ixodinae and genus *Ixodes* and Metastriata which represent all of the other hard tick subfamilies and genera. The two blue tick species which are under investigation belong to the Metastriata phyletic line, forming part of the Rhipicephlinae subfamily which evolved primarily on mammals and falls within the *Rhipicephalus (Boophilus)* genus and subgenus (Black & Piesmant 1994; Murrell *et al.* 2001; Walker *et al.* 2003).

1.2. General Background

1.2.1. Life Cycles

The hard tick life cycle consists of four stages of which three, the larval, nymphal and adult stages needs to complete a blood meal on-host and egg production and emergence of larvae and nymphs can occur off-host in the physical environment. The different life stages of ticks have differences in their morphological structures. Larvae of all hard tick species have three pairs of legs in comparison to nymphs and adults having four pairs of legs, but both larvae and nymphs lack a genital aperture which is found in adults. Only in adult stages it is possible to distinguish between males and females; females have a scutum and an alloscutum whereas males have a conscutum which covers the entire dorsal surface as illustrated in Figure 1.1. In addition, males have plates on the ventral side, whereas these plates are absent in the females (Walker *et al*. 2003).

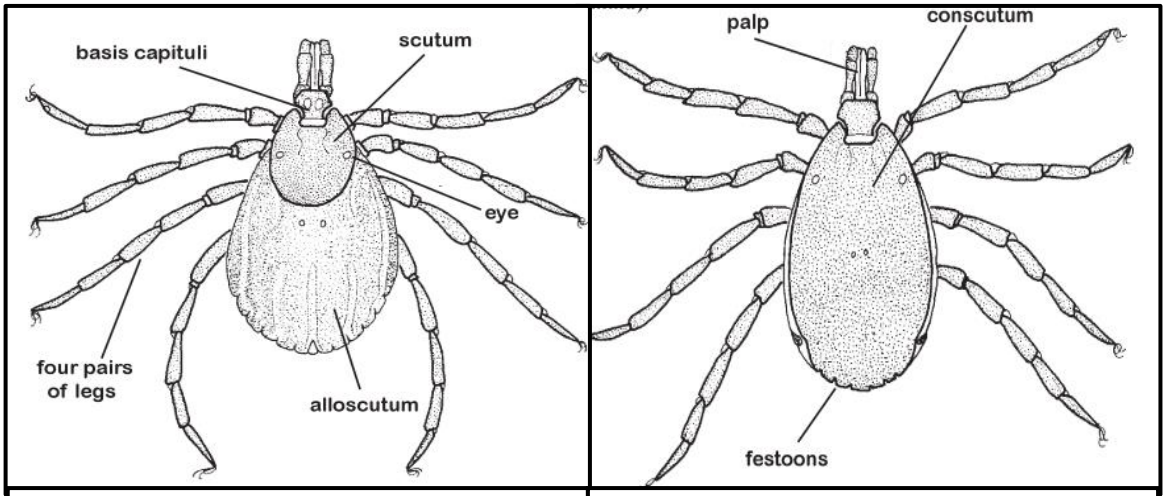

Figure 1.1: Dorsal view of a **(A)** Female and **(B)** Male hard tick. Source: Walker *et al*. (2003)

In order to be able to consume a full blood meal the body wall of the tick needs to expand, often by up to 100 times the original size. Males of many species need a blood meal to sexually mature in order to mate, however, they will also not expand to the same extent as the females. Once a full blood meal is taken the larva, nymph or adult tick will drop off the host and search for a suitable microenvironment to either moult or lay eggs and then die. In hard tick species, mating usually occurs on the host, with the exception of certain *Ixodes* species. Males attempt to mate with as many females as possible while feeding. The males will transfer a sperm sac to the female which will only mate once before fully engorging and dropping off from the host to find a suitable microenvironment to lay their eggs in. Hard tick females can lay between 2000-20000 eggs, depending on the species, in a single batch and will use the stored sperm to fertilize them (Walker *et al*. 2003).

Ixodid ticks display one-, two- or three-host life cycles. A three-host reproductive cycle is the most common and the longest life cycle, taking between 6 months to several years to complete. Each stage will feed on a host then detach, moult and seek out a new host. The preferred host for each life stage usually consists of different host species. Species such as *Amblyomma hebraeum* and *Ixodes pilosus* are examples of three-host tick species (Hoogstraal 1978; Walker *et al*. 2003). In two-host ticks such as *R. evertsi evertsi* and *H. truncatum* the larvae and nymphs will feed and moult on the same individual before detaching and moulting into an adult while in the physical environment. The adult will then need to seek a new host for its bloodmeal.

The tick species investigated in this study, *R. (B.) decoloratus* and *R. (B.) microplus* are one-host ticks of bovid species. The larval stage is the only free-living stage and can be found questing on vegetation in search of a host. Once a suitable host is located the larvae attaches and will remain on the host for the remainder of its life cycle. Each stage requires a blood meal in order to moult into the next form on the host. Once copulation has occurred the adult female will fully engorge and then drop off the host. Off host the female needs to find a suitable microenvironment to lay her eggs whereas the males will remain on the host in attempt to mate with multiple females (Walker *et al.* 2003). Oviposition occurs within 3-6 days after detachment from the host and the female will continue to lay eggs for up to 21 days. The one-host tick life cycle is much shorter than that of a two-host or three-host tick, resulting in two to

three generations that can be produced per year depending on climatic conditions and the availability of hosts.

Murrell *et al.* (2001), determined that the two-host life strategy evolved from a three-host life strategy, however the one-host life strategy may be a modification of the two-host life cycle instead of a product of the evolution of a three-host strategy. The selection pressure for the reduction of hosts in the two and one-host life strategies is far greater in species which are found on hosts that move around and cover large areas in comparison to species which are found on hosts that are nest dwellers.

1.2.2. Host Detection

Hard ticks acquire a suitable host in various ways; hunter ticks will actively move towards a host after receiving adequate stimuli such as high concentrations of carbon dioxide or odours produced by the host animal. Ambush ticks will quest in the tips of vegetation, waiting for a host to brush by, once a host comes into contact with the tick, it will crawl on to the host and seek a site for attachment (Walker *et al.* 2003). The two blue ticks under investigation typically locate their hosts through the ambush technique. Black & Piesmant (1994), stated that the adaption of host specificity is a result of parallel evolution between ticks and their hosts.

1.2.3. Feeding

The parasitic stages of hard ticks in contrast to other arthropods penetrate the skin of their host as they are obligately hematophagous (Black & Piesmant 1994). Ticks have specialized mouthparts for acquiring a blood meal, consisting of three parts; the hypostome, chelicerae and palps (Walker *et al.* 2003). The palps are used to locate a site for attachment while the chelicerae and hypostome penetrate the skin of the host and aid in acquiring a blood meal. The chelicerae have sharpened ends and movable rods which are used to create a feeding lesion by breaking the blood capillaries close to the surface of the skin. Blood and lymph are secreted into this lesion which is then fed on through the hypostome (Walker *et al*. 2003). A cement like saliva is secreted from the mouthparts, which ensures that the tick remains attached to the host until completely engorged.

According to Da Silva *et al*. (2013), the blood meal which is taken by each tick is the main cause of harm inflicted on the host. It was estimated that cattle will lose approximately 1g of body mass for every engorged female blue cattle tick that is attached to the host, with each female consuming a blood meal of approximately 2ml. In Australia *Rhipicephalus* (*Boophilus*) species was found to be responsible for a 0.6-1.5g reduction in the live weight gain for each tick that matures on the host (Peter *et al.* 2005). Cattle can lose more than 2kg of body mass over a three-week period of medium to high infestation. Peter *et al.* (2005), also found that *A. hebraeum* results in a loss of approximately 10 g in the live weight gain per tick. The degree of infestation will therefore determine to what extent the ticks have compromised the meat and milk production of the host.

1.2.4. Habitat and Distribution

Ticks are adapted to surviving in both the physical environment and on their preferred host, with both having its own set of challenges. In the physical environment, especially while moulting, they are at risk of freezing, drying out, starving and attack from both predators and pathogens. On the host there is the danger of removal from grooming, insufficient feeding due to immunity or the treatment with acaricides. Thus, the preference for specific hosts and environmental conditions, limits the distribution of the species (Walker *et al.* 2003).

Prolonged dry climatic conditions can have severe negative effects on the tick populations, particularly to those in the physical environment such as questing larvae. Many tick species are thus adapted to varied climatic conditions within their geographical range. In order to combat the dry season, various species will undergo diapause during these times as the reduction of their metabolic rate allows them to be able to survive until conditions become favorable once again (Walker *et al.* 2003).

Human activities play a great role in the geographic distribution of tick species, with the trade in livestock unintentionally introducing invasive tick species into new areas. Walker *et al*. (2003) stated that, although historic records of the distribution of certain tick species does exist, it is not always accurate due to miss-identification and the changes of species names.

1.2.5. Tick and Tick-borne Diseases

Ticks are vectors for disease and are thus of medical and veterinary importance. Manjunathachar *et al*. (2014), stated that ticks and tick-borne diseases are ranked fourth among the major infections of livestock. They can cause severe conditions which include paralysis, irritation, allergic reactions as well as damage to the hide and wounds that can lead to secondary bacterial infections. Many of the tick-borne diseases have a large impact in the livestock industry and once infected, an animal will remain a carrier for the rest of its life. According to Byaruhanga *et al.* (2016), over the past decade the number of cattle which have been exposed to tick-borne diseases has increased substantially and resulted in high mortalities and a reduction in herd sizes. There are several factors that encourage infections of tick-borne diseases in cattle herds, this includes; production systems, management practices, inadequate veterinary resources, lack of immunity within the herd and changes in rainfall patterns and climatic conditions (Manjunathachar *et al*. 2014; Byaruhanga *et al.* 2016).

Nyangiwe *et al.* (2011), stated that there are approximately 75 Ixodid tick species present in South Africa of which five are important vectors of disease for cattle. *A. hebraeum,* the South African bont tick, is a vector of *Ehrlichia ruminantium*, which causes heartwater; *R. appendiculatus,* the brown ear tick, a vector of *Theileria parva*, which causes East Coast fever and *R. evertsi evertsi,* the red-legged tick, a vector of *Anaplasma marginale* which causes gall sickness. The two focus species of this study *R. (B.) decoloratus* and *R. (B.) microplus*, both transmit *Babesia bigemina* a protozoan which causes bovine babesiosis, known as redwater in cattle. However, *R. (B.) microplus* transmits *B. bovis* which has a greater pathogenicity and acts over a shorter period of time in relation to *B. bigemina. R. (B.) decoloratus* also transmits *A. marginale,* and *Borrelia theileri*, which causes spirochaetosis in cattle, sheep, goats and horses (Walker et al. 2003).

1.3. Focus Species

The focus species of this study are the African blue tick, *R. (B.) decoloratus* and the Asiatic blue tick, *R. (B.) microplus.* Both are one-host cattle ticks which share similar morphological features, feeding sites on the host and have a similar preference for hosts and climatic conditions. *Rhipicephalus (Boophilus) microplus* produces approximately 500 more eggs and has a slightly shorter reproductive period in comparison to *R. (B.) decoloratus* (Tønnesen *et al*. 2004).

1.3.1. *Rhipicephalus (Boophilus) decoloratus* (Koch, 1844).

Rhipicephalus (Boophilus) decoloratus, also known as the African blue tick, due to the colour of the engorged female, has the widest distribution of one-host cattle ticks on the African continent south of the Sahara (Walker *et al*. 2003). It is found in regions with temperate climates, in savanna, grassland and woodland areas. The species is usually absent from drier areas such as Namibia, parts of South Africa such as the Northern Cape and Botswana. But recently, it was recovered in 10 localities in Northern Cape (Nyangiwe *et al.* 2017) and *R. (B.) decoloratus* was also found in all 18 surveyed localities in Namibia (Nyangiwe *et al.* 2013b) which now show its survival in areas which were previously too dry for the tick.

The most distinct characteristic of this species is that, it is the only one within the sub genus *Boophilus* which displays a 3+3 configuration of denticles on the hypostome. This species is mainly found on cattle, which are the maintenance host, however it can also be found on goats, sheep, horses and wild ungulates (Walker *et al.* 2003). The preferred feeding sites include the neck, dewlap, shoulder, belly, legs and back. *Rhipicephalus (Boophilus) decoloratus*, spend approximately three weeks on its host, starting with the larvae ascending onto the vegetation in search of a suitable host to complete their life cycle on. Females have been recorded laying between 1000-2500 eggs from 5-6 days up to 21 days after drop off from the host, from which larvae will then hatch approximately 3-6 weeks later depending on climatic conditions. The males will remain on the host and mate with as many females as possible. The entire life cycle can be completed in two months, this includes the non-parasitic and parasitic phase (Walker *et al*. 2003). Thus, it is possible for more than one life cycle to be completed within a year, depending on environmental conditions and availability of

hosts. The emergence of the larvae in Southern Africa is usually synchronised with the rise in temperature in spring, the larvae also occur during summer and in the cooler months of May and June.

1.3.2. *Rhipicephalus (Boophilus) microplus* (Canestrini, 1888).

The Asiatic blue cattle tick, *R. (B.) microplus* originated in South East Asia and was spread to many cattle producing areas such as South America, Southern Africa and Australia. The spread of this species is directly linked to the trade and transport of livestock around the globe. On the African continent this species has become well established in areas of South, East and West Africa (Walker *et al*. 2003). One of the main reasons why this species poses such a great threat to cattle producers is the fact that *R. (B.) microplus* not only transmits the protozoan, *B. bigemina*, it also transmits *B.bovis,* both causing a form of redwater with the. *B. bovis* strain (Asiatic Redwater) having a greater pathogenicity and acting over a shorter period of time in relation to the African form of redwater, *B. bigemina.*

Rhipicephalus (Boophilus) microplus is morphologically very similar to *R. (B.) decoloratus*. A noticeable difference is observed on the hypostome, with this species having a 4+4 configuration of denticles in comparison with the 3+3 configuration present on the hypostome of *R. (B.) decoloratus*. Cattle are the preferred host of this tick species, however, occasionally *R. (B.) microplus* can also be found on other livestock and wild ungulates. The sites of attachment include the shoulder, dewlap, flanks and belly. This species is also a one-host tick and has a slightly shorter reproductive cycle than *R. (B.) decoloratus*. *Rhipicephalus (Boophilus) microplus* females lay approximately 500 more eggs than *R. (B.) decoloratus*, the three life stages spend three weeks on the host and the egg laying can be completed in four weeks. Thus, this higher reproductive potential and shorter generation period allows this species to outcompete *R. (B.) decoloratus* in areas with favourable climatic conditions (Londt & Arthur 1975; Spickett & Malan 1978; Madder *et al.* 2011; Chevillon *et al*. 2013).

Cross mating between the two blue cattle tick species can occur, *R. (B.) microplus* males reach sexual maturity a few days prior to the *R. (B.) decoloratus* males and thus can mate with the available *R. (B.) decoloratus* females which then produce sterile

offspring. This could be another contributing factor in the increasing numbers of *R. (B.) microplus* in areas previously dominated by *R. (B.) decoloratus* according to Horak *et al.* (2009) and De Clercq *et al.* (2012). Following a study conducted at two communal areas in the Eastern Cape Province, Nyangiwe *et al.* 2013a found larvae exhibiting characteristics of both species from the vegetation. However, further research on the hybrid ticks needs to be conducted.

1.4. Introduction and Invasion of *Rhipicephalus (Boophilus) microplus*

According to Corson *et al*. (2003), European colonists successfully transported livestock to various parts of the world and were responsible for introducing eastern cattle tick species into tropical and sub-tropical regions of the Western hemisphere. The Asiatic blue tick, *R. (B.) microplus* has become widely distributed in various locations all over the globe. There are records of this tick species in Latin America and Mexico, Australia, Africa and Madagascar.

1.4.1. Factors Which Influence Distribution and Abundance

The two major influences on the distribution and abundance of tick species are the availability of preferred hosts as well as favourable climatic conditions. Humans have however, had an impact on the distribution of tick species due to the movement of livestock to various parts of the world and are responsible for introducing species into areas which they were not previously found in (Dantas-Torres 2015).

1.4.1.1. Climate Change

Tick distribution and abundance depends on various factors, the one which we have no control over is the changes in climatic conditions. According to Awa *et al.* (2015), rainfall and temperature have been found to be key climatic factors which influence the distribution of ticks, whereas humidity has proven to not have such a great effect. Dantas-Torres (2015), stated that climate change has resulted in warmer winters and extended autumn and spring seasons, this will continue to contribute to the expansion of the distribution range of tick species. Thus, previously unfavourable environments

are now able to support and sustain tick populations. Ticks spend most of their life cycle in the physical environment, as a result climatic conditions as well as host availability will affect their survival (Estrada-Peña *et al*. 2013; Biguezoton *et al*. 2016).

According to Estrada-Peña *et al.* (2013), the trends which have been forecasted for climate change will play an important role in the spread and changes in distribution of tick species in several regions and can result in the colonization of new territories, especially by *R. (B.) microplus* in Africa. However, predicting the future population distributions of tick's species based on current climate model predictions is not straightforward or completely accurate. These arthropods have complex life cycles and have various ecological needs which differ depending on the life stage. In the climate models conducted by De Clercq *et al.* (2015), it was found that *R. (B.) microplus* will spread in areas along coastlines, which are humid and warm as the survival of the eggs and larvae relies on humidity and temperature.

1.4.1.2. Uncontrolled Movement of Hosts

Other contributors to the rapid spread of ticks include the uncontrolled movement of host species and a lack of knowledge on the ecological plasticity of the ticks. Wild ungulates, such as buffalo, impala, kudu and bushbuck, can act as a place of refugia for certain tick species (Byaruhanga *et al.* 2016). Thus, the tick populations will be able to survive in the area where wild ungulates are present and will thrive once the preferred host returns (Tønnesen *et al*. 2004).

With the introduction to Benin it was initially hypothesised that *R. (B.) microplus* would have been limited to the localized areas where the imported cattle were kept and it would be possible to eradicate the species before it spreads. This however was not the case as this species has spread throughout West Africa and displaced local species in many locations in less than a decade. The most alarming part of the invasion is the fact that it has been predicted that the species has not yet reached its full climatic range and will continue to spread and displace local tick species. The limits remain unknown and although in this instance the uncontrolled movement of livestock is a key factor in the spread of ticks, the climatic conditions have also played a role that is not yet completely understood (Byaruhanga *et al.* 2016).

Species of ticks that currently occupy a large distribution can be considered to be called generalists and are more adapted to a wide variety of environments and climatic conditions. Thus, it can be said that these ticks have a great ecological or phenotypical plasticity which enables them to adapt to the different conditions. There has been limited research done on this aspect of tick species and this makes it difficult to predict potential areas for invasion and to determine possible barriers to the range.

1.4.1.3. Resource Limited Communal Farming

According to Katiyatiya *et al.* (2014), there are approximately 600 million farmers in communal areas that rely on livestock production as a means of supporting their livelihood in Africa. Thus, the animals need to be well adapted to thrive in a diverse array of environmental conditions in order to maximise production and profit. Communal areas are dominated by small scale, resource limited farmers. Indigenous breeds are the most suitable choice for these low grazing areas with Nguni being one of the best choices as it requires low maintenance and management (Nyangiwe *et al*. 2011). This breed does well in harsh conditions with limited grazing and water resources and is known for its smooth coat, thick skin and natural genetic immunity towards ticks and the diseases which they transmit (Marufu *et al*. 2011).

Lorusso *et al.* (2013), noted that farmers in the areas which they sampled in central Nigeria, did not use any form of chemical control. The farmers however relied on the removal of ticks by hand as well as grazing techniques which allow for natural spelling of the pasture. This is common in communal areas as farmers do not always have the means of purchasing acaricides.

According to Marufu *et al*. (2011), in rural areas ticks and tick-borne diseases are a great threat and challenge to the production of cattle. In many cases there is a lack of knowledge on the proper usage of chemicals for tick control and access to acaricides as well as poor animal health. Large tick infestations result in a loss of live weight gain and meat quality, loss in milk production, hide quality, fertility and in the case of disease even death. Countries such as Mali and Togo have experienced failures in acaricide treatment (Marufu *et al*. 2011). The rapid spread of the Asiatic blue tick in this part of Africa is largely aggravated by poor conditions and lack of resources needed for proper treatment of cattle with acaricides.

1.4.2. Distribution of *Rhipicephalus (Boophilus) microplus* on the African Continent

On the African continent, *Rhipicephalus (Boophilus) microplus* is found in areas along the eastern coastal belt and in regions in South Africa that experience summer rainfall. It occurs to be scattered in areas which experience savanna climates (Walker *et al*. 2003). This species has also been reported in Zimbabwe, Zambia, Mozambique, Swaziland, Madagascar and recently in Namibia and West Africa (Madder *et al.* 2011 Madder *et al.* 2012; De Clercq *et al.* 2012; Adakal *et al.* 2013; Nyangiwe *et al*. 2013a, 2013b; Biguezoton *et al*. 2016).

1.4.2.1. Africa

Over the past decade the most striking invasion and displacement by *Rhipicephalus (Boophilus) microplus* has occurred in West Africa at an extensive pace (Madder *et al*. 2011 & Madder *et al*. 2012). De Clercq *et al.* (2015), suspected that this invasion started in 2004, due to the importation of Girolando cattle into southern Benin from Brazil. In these countries, *R. (B.) microplus* has displaced various indigenous tick species including the African blue tick. The rising concern of this invasion is due to the fact that the Brazilian strain of *R. (B.) microplus* is not responding to acaricide treatment and as a result the ectoparasite numbers have increased rapidly (Adakal *et al.* 2013). De Clercq *et al*. (2012), predicted that the expansion of the range of *R. (B.) microplus* in Benin will continue northwards.

In a survey conducted in the Maputo Province of Mozambique, it was found that *R. (B.) microplus* was the only blue tick present on the cattle and goats which were sampled at 30 dip-tanks. Thus, Horak *et al.* (2009) concluded that complete displacement had occurred in this region.

Lorusso *et al.* (2013), could not indicate the presence of *Rhipicephalus (Boophilus) microplus* in Central Nigeria however, Eyo *et al*. (2014), found that *R. (B.) microplus* was the dominant species present in a study conducted in Eastern Nigeria a year later. In surveys done by Awa *et al.* (2015), in north eastern Uganda and Byaruhanga *et al.* (2016) in Cameroon, no indication of *R. (B.) microplus* presence was found which suggests that the range has not yet expanded east from West Africa.

This needs to be monitored as this region has favourable climatic conditions for the survival of the tick species. Estrada-Peña *et al.* (2013), stated that reproductive interference will not be enough to stop *R. (B.) microplus* from spreading into new areas, the cattle in these areas have no immunity towards the species and this allows the ticks to rapidly multiply.

1.4.2.2. South Africa

Studies on the distribution of tick species present in South Africa have been conducted for over a century and found to be dynamic with the constant movement of livestock by producers and changes in climatic conditions being major contributors to this phenomenon.

Rhipicephalus (Boophilus) microplus was introduced to South Africa by cattle imported from Madagascar in 1986 (Hoogstraal 1956). Reports dating back to the early 1900s showed that the displacement of the indigenous African blue tick, *R. (B.) decoloratus* began in the Cape Province (Nyangiwe *et al.* 2013a, 2013b). Howard (1908), was the first to report the presence of *R. (B.) microplus* in South Africa, around the southern areas of the Cape colony as well as around the town of King Williams town in the Eastern Cape. The displacement of *R. (B.) decoloratus* has been recorded in the Limpopo province by studies conducted by Tønnesen *et al.* (2004).

1.4.2.3. Eastern Cape

According to Nyangiwe *et al.* (2013a), in South Africa there are approximately 3.1 million beef cattle in the Eastern Cape, with communal farming that accounts for approximately 65% of it. Prior to the study conducted by Horak *et al*. (2009), the data that was available on the distribution of tick species in the Eastern Cape was collected more than 25 years ago by Baker (1982), who had created a distribution plot of various tick species as well as the acaricide resistance status at each location. *Rhipicephalus (Boophilus) microplus* seemed to be displacing *R. (B.) decoloratus* from the coast towards the inland regions. The distribution of *R. (B.) microplus* that was described by Howell in 1978 was discontinuous, its range extended from the southern regions of the Western Cape coast and adjacent inland areas to north eastern KZN with scattered

locations in the northern provinces. The results from the survey conducted by Horak *et al*. (2009), showed that the range of *R. (B.) microplus* has expanded to the point where this species had displaced *R. (B.) decoloratus* in areas in the eastern regions of the Eastern Cape. However, Nyangiwe *et al* (2011), found that in Dohne near the town of Stutterheim, the population of both *R. (B.) microplus* and *R. (B.) decoloratus* has been maintained for at least the past five years, although *R. (B.) microplus* was the dominant species present. Nyangiwe *et al*. (2013a), suggested that the displacement of the invasive species could also be due to acaricide resistant *R. (B.) microplus* populations in areas where the *R. (B.) decoloratus* populations are still susceptible.

1.5. Tick Control Measures

There are a variety of measures which have been developed in order to control tick loads on cattle. Each measure has both positive and negative aspects which need to be considered prior to usage.

1.5.1. Chemical Control and Acaricide Resistance

The most frequently used tick control measure is still the use of acaricides. The eradication of *R. (B.) microplus* with the use of acaricides was successful in the United States of America as well as certain areas in Argentina. However, the eradication of this blue tick in Australia and South Africa have been unsuccessful (Peter *et al.* 2005).

1.5.2. Current Acaricides Used in South Africa

In South Africa a large portion of the veterinary market is comprised of the sale of acaricides. In 2003, 22% of the total sales, approximately R175 million, comprised of ectoparasite acaricides, this would increase to 30% when the endectocides were included (Peter *et al.* 2005). To date, in South Africa there are more than 100 registered products for tick control. Five different chemical groups namely; Organophosphates, Amidines, Synthetic Pyrethroids, Macrocyclic Lactones and Fluazuron are used as active ingredients. These products often consist of a single chemical group or combinations of two or more

chemical groups. Each product contains the correct dilution and application concentration which needs to be administered as well as the required method of application. Application methods range from acaricides suitable for use in a plunge dip or spray race system to pour on and injectable treatments. To date in South Africa resistance has been reported towards all of the above-mentioned chemical groups with the exception of Fluazuron and Macrocyclic Lactones.

Organophosphates were one of the first chemical groups used in the control of ticks and include the following chemical classes: Chlorpyriphos, Chlorfenvinphos and Diazinon, to name a few. These compounds inhibit the release of cholinesterase, an enzyme which breaks down acetylcholinesterase (AChE). The neurotransmitters continue to send an electrical charge due to the increased level of AChE, the nervous system ultimately becomes overstimulated and this then leads to the death of the tick. The mechanism of resistance towards this chemical is primarily linked to target site insensitivity and various different point mutations have been found to cause this. In addition, oxidative metabolic activities also play a role in the development of resistance towards this chemical (Abbas *et al*. 2014). Acaricide formulations include; Coopers Supadip, Steladone 300 EC and Supona®**.**

Within the Amidine chemical group, Amitraz, a triazapentadiene compound is the most widely used for tick control. The mode of action of Amitraz results in toxic effects on octopamine's receptor. The mechanism for resistance is thought to be linked to an alteration in the target site caused by the substitution of two nucleotide base pairs in the octopamine receptor, however the exact cause is still unknown (Abbas *et al*. 2014). Acaricide formulations include: Eraditick Cattle Pour-on, Taktic® 25%, Delete®All and Milbitraz spray dip.

Pyrethroids are synthetically designed to be a model of Pyrethrin's which are a naturally occurring compound derived from the chrysanthemum family. Synthetic Pyrethroids are designed to exhibit a greater stability and longer lasting effect in comparison to their natural counterparts. Chemical classes available include; Cypermethrin, Deltamethrin and Flumethrin. The mode of action for both Pyrethrins and Pyrethroids are the same as they are both potent neurotoxins which act on the sodium channels. It affects the nerve membranes permeability of the sodium and potassium ion channels and results in nerve excitation. The resistance mechanism is

linked to a mutation which alters sodium channels to be less sensitive to pyrethroids. Oxidative metabolic resistance has also been reported to play a role in the development of resistance (Abbas *et al*. 2014; Yessinou *et al.* 2016). Acaricide formulations include: Bayticol, Drastic Deadline, Deltapor 10 Plus and Pro-Dip® Cyp 20 %**.**

Macrocyclic lactones include two chemical classes, avermectins and milbemycins. These compounds are naturally occurring fermentation products of *Streptomyces avermitilis* and *S. hygroscopicus* respectively. The mode of action of these compounds results in hyperpolarisation and paralysis of the neuromuscular systems due to an influx of chloride ions into cells. This occurs as the transmittance of electrical activity within the nerves and muscle cells are blocked due to the release of gamma-aminobutyric acid (GABA) which then binds to the nerve endings. The exact mechanism of resistance is still unknown; however, it has been hypothesised that resistance is due to the insensitivity of the target site of the glutamate gated chloride ion channels or GABA (Abbas *et al*. 2014). Acaricide formulations include: Ecomectin 1%, Ivermectin, Ivermax 1%, Virbamec LA® and Dectomax®.

Insect Growth Regulators (IGRs) have not yet been used to the same extent as other chemical groups available on the market. IGRs have been designed to mimic hormones and enzymes of arthropods which are linked to their growth and development and come in various forms, namely; juvenile hormone inhibitor and chitin synthesis inhibitors (McNair 2015). The juvenile hormone is responsible for the moulting between the different instars of the life cycles of ticks. Chitin synthesis inhibitors block the production of chitin which is a major component in the cuticle of arthropods.

While the other chemical groups offer a quick suppression of the tick population on their livestock, long term usage has led to the establishment of resistance. As a result, IGRs are often combined with another chemical groups in order to have a rapid suppression of the population while acting over a longer time period. One of the most frequently used products is Drastic Deadline Extreme which is a combination of Flumethrin 1% m/v, a synthetic pyrethroid and Fluazuron 2.5% m/v. Acatak is an acaricide formulation which only contains fluazuron and no other chemical group.

Studies conducted in the North West and Eastern Cape Provinces have shown that populations of *R. (B.) decoloratus* collected from communal dip tanks were either resistant or showed an establishment of resistance towards pyrethroids and organophosphates. On the commercial farms which were sampled resistance, or development of resistant populations towards both pyrethroids and organophosphates as well as amidines was found (Mekonnen *et al*. 2003).

The inconsistent use of chemical control results in the establishment of immunity within the tick species. Thus, in many parts of the world there have been reported cases of resistance to commonly used acaricides. As a result of this the control of one-host ticks has become increasingly difficult.

1.5.3. How Acaricide Resistance develops

Manjunathachar *et al*. (2014) stated that resistance can be caused by the inconsistent and incorrect dosage used as well as a high frequency of one chemical used over time on a specific farm

One-host ticks are exposed to acaricides at a greater frequency than two and three-host ticks due to their shorter reproductive period, are able to produce more generations per year and are therefore commonly used as indicator of resistance development. Resistance can be defined as the occurrence of individuals in a population that have the ability to tolerate doses of toxic substances that are lethal to the majority of the individuals in the population of the same species. Resistant genes naturally occur in every population at low frequencies (Manjunathachar *et al*. 2014; Yessinou *et al.* 2016).

There are several steps that occur before an acaricide can exert its toxicity. Once in contact with the arthropod it needs to enter the body, be converted in the active metabolism and transported to the action site. Each step that it goes through is controlled by one or more genes, any mechanism which alters one of the steps can lead to the formation of resistance (Yessinou *et al.* 2016). Resistance can therefore be a result of changes in one or more mechanisms; there can be a change in the excretion and absorbance of the acaricide, changes in metabolic pathways that allow for acaricide degradation or a modification of the target site. Metabolic resistance is

due to the increase in the enzymes activity that are responsible for acaricide detoxification. Changes to the target site are normally caused by point mutations, these structural changes then decrease the affinity of the acaricide. These mechanisms can also be responsible for causing cross resistance to acaricides which target the same sites. In most species point mutations are protein based and will retain the initial functions of the protein at a level which will ensure the ticks survival (Yessinou *et al.* 2016).

Resistance genes are naturally present within a tick population, at a low frequency. Resistance suspicions occur when there is a treatment failure in controlling tick infestations. Treatment failure can also occur when the incorrect application and concentration of an acaricide is used as well as due to faulty equipment, poor quality or expired chemicals and is not always a sign of resistance. The persistence of ticks after frequent and correctly prepared and applied treatments is however, a sign of tick acaricide resistance development.

1.5.4. The History of Acaricide Resistance

The control of ticks began in the late nineteenth century with the use of arsenic based compounds (Abbas *et al*. 2014). This was then followed by organochlorines, organophosphates, amidines, synthetic pyrethroids, phenylpyrazole, macrocyclic lactones and insect growth regulators.

In 1896 arsenic was first used for tick control by a farmer in Queensland, this practice soon spread over the rest of Australia as well as to the USA and South Africa (Abbas *et al*. 2014). In 1936, after 40 years of use the first cases of resistance were reported. In 1939 organochlorines were introduced to the market, this chemical had a longer residual activity, higher efficiency, lower toxicity and was much cheaper than arsenic. The first case of resistance was reported in 1952 in Brazil and a decade later in 1962 the chemical was banned due to its poor biodegradability and residue left in meat, milk and the environment and its affinity for lipids (Abbas *et al*. 2014; Yessinou *et al.* 2016).

In the mid-1950s organophosphates were used to control ticks as this compound was less stable and less persistent than organochlorines but it was found that certain organophosphates were in fact toxic to mammals. Resistance appeared in the mid-

1960s in Australia and as a result Amidines where then introduced to the market in the mid-1970s (Abbas *et al*. 2014). Resistance towards carbamates and organophosphates was reported by Shaw (1966), while resistance towards amidines was reported by Taylor and Oberem (1995) in South Africa. Today amitraz is the main active ingredient in this chemical class. In 2007, it was reported that amitraz was still one of the most popular acaricides in use, although Mexico, Australia, South Africa, South America and New Caledonia all have reported cases of resistance towards it (Abbas *et al*. 2014).

Synthetic pyrethroids were introduced in the 1970s following the build-up of resistance towards amidines. According to Yessinou *et al.* (2016), pyrethroids are currently the most used acaricide worldwide. However, resistance towards this acaricide has been reported by all the countries in which *R. (B.) microplus* is found. Resistance to this group was reported in South Africa by Coetzee *et al.* (1987). Pyrethroids are often selected for use due to the fact that this chemical class is a highly effective insecticide and acaricide, it is biodegradable, nontoxic to animals and people and there is no withholding period for milk and meat.

In 1981 macrocyclic lactones were introduced to the market, the chemical was divided into two categories; avermectin and milbemycin oxime. Both have a longer residual activity than pyrethroids and are active against a range of arthropods and nematodes, there is, however, a withholding period for milk and meat after treatment. In 2001 there were reports of resistance to avermectin in Brazil and in Mexico. Fipronil is the only phenylpyrazole in use and it has been in use since the mid-90s. It has a long residual activity and continues in the field for up to five weeks. Reports of resistance first appeared in 2007 in Uruguay and then in Brazil. In 1994 growth regulators were introduced as a new age form of chemical control, fluazuron was the first compound available on the market. There have however, already been a few reported cases of resistance towards IGRs (Yessinou *et al.* 2016). Reck *et al.* (2014), reported the first case of resistance towards fluazuron in a field population of *R. (B.) microplus.* It was found that to this strain of *R. (B.) microplus* known as the Jaguar strain is in fact also resistant to; cypermethrin, chlorpyriphos, amitraz, ivermectin and fipronil.

1.6. Alternative Control Methods

Alternative methods for the control of ticks which do not rely on chemical acaricides include the following; vaccinations, pasture management, artificial fertilizers, biological control, botanicals and genetically resistant hosts. These forms of tick control have gained popularity over the past decade as the development and emergence of resistance to most of the available acaricides as well as due to environmental harm and degradation (McNair 2015).

1.6.1. Vaccinations

Vaccinations are an important tool for enhancing cattle immunity towards tick-borne diseases. Currently Gavac, Tickgard and Tickgard plus, are available to build up the immunity towards redwater caused by *R. (B.) microplus*. McNair (2015), stated that this was the first ectoparasite vaccine to be commercially marketed. The vaccine is based on the concealed antigen Bm86 which was obtained from the mid gut of *R. (B.) microplus*. Currently there has been some success in the use of the vaccines as well as the combined use of vaccines and acaricides. However, it must be noted that the efficiency of the vaccine varies from area to area, depending on the strain which is present as the Bm86 antigen may vary depending on the geographical location of the strain. Thus, vaccines show promise for the control of ticks if specifically designed for the geographic location as well as if it is used in combination with other forms of control (Abbas *et al.* 2014).

1.6.2. Pasture Management

1.6.2.1. Rotational Grazing

Rotational grazing, involves the spelling of pastures for prolonged periods of time, this disrupts the life cycle of ticks as the host is removed from the environment and with no source of food many of the ticks will die off. It was found that a combination of acaricide use and rotational grazing is effective by reducing the tick load by up to 77-89% (Abbas *el al*. 2014). This does however require sufficient land but it a simple and effective technique to employ which if cost effective.

1.6.2.2. Pasture Burning

Pasture burning is a method which is employed by countries such as, Australia, South Africa, Zambia and the USA. The main purpose of pasture burning is to induce the emergence of new green grass which is far more palatable for cattle after a dry winter. This practise is also thought to kill off ticks present on the vegetation. It is not a full proof form of tick control as ticks will be able to recolonize the area again once the cattle return (Malan *et al.* 1997; Horak *et al.* 2006; Abbas *et al*. 2014).

1.6.2.3. Artificial Grazing and Use of Fertilizers

Pinto da Cunhaa *et al*. (2010) and Leal *et al*. (2017), both conducted studies which focused on the effects of the application of urea on pastures as a method of tick control. The results for both studies showed that there was a decrease in the number of *R. (B.) microplus* on the pasture due to an interference of the life cycle in both the laboratory and field settings.

Certain plant species in subtropical and tropical areas have been found to have an effect on controlling tick population by the entrapment of the larvae in a vicus fluid secreted by the plant. Toxic fumes are often also released and aid in killing the larvae. Examples of plant species which have been found to do this include; tropical legumes, *Stylosanthes spp*., *Brachiaria brizantha*, *Cissus adenocucaulis*, *Kigelia africana* and *Euphorbia hirta* (Manjunathachar *et al*. 2014).

1.6.3. Biological Control

Traditional biological control methods rely on strategies which includes the use of a natural enemy within the same area or imported from another area (Samish *et al*. 2004).

1.6.3.1. Bacteria

Ticks are known to contain a vast variety of endosymbiont bacteria, this includes, *Francisella*, *Rickettsi* and *Coxiella*. Many of the endosymbiont bacteria are essential for the survival of ticks, thus if there is a disruption in the balance of these endosymbionts it will result in the death of the tick. Under laboratory conditions it has

been found that *Cedecea lapagei* proved to be 100% effective in killing *R. (B.) microplus*. Due to the unique biology of various tick species, further research is needed on the potential use of bio-insecticides for the use of tick control (Samish *et al*. 2004).

1.6.3.2. Fungi

Over the past two decades the use of entomopathogenic fungi for the control of pests has grown in popularity (McNair 2015). In literature over 700 species of entomopathogenic fungi have been identified, however, only 10 species are currently being tested and developed for the control of ticks. Deuteromycetes, is a class of fungi which has shown great promise as it has the ability to penetrate the cuticle of arthropods irrespective of the life stage. The use of fungi has its drawbacks with one of the major problems being that fungi need high humidity to germinate. In addition, they often take a prolonged period to kill the host, are susceptible to ultra violet radiation, can target non-target hosts, is costly to produce and has a limited shelf life (Samish *et al*. 2004; Hedimbi *et al*. 2011).

1.6.3.3. Nematodes

Monteiro *et al.* (2014), stated that the use of entomopathogenic nematodes (EPNs), has gained interest as a form of biological control. EPNs are used to target the non-parasitic life stages of ticks, namely the engorged female which has dropped off the host and the eggs which are produced. In laboratory studies it was found that EPNs perform best in sandy soils as there is a greater aeration which allows for earlier detection of the ticks. EPNs belonging to the genus *Heterorhabditis* proved to be the most effective in the reduction of the egg mass laid and percentage of larvae which emerged.

Once in the environment there are a variety of factors which influence the efficiency of EPNs. Soils tend to have varied concentrations of components such as silt, lower levels of moisture and higher levels of UV radiation which can negatively affect the EPNs. The use of EPNs has a great potential as the target host, engorged ticks are found within the same environment as the nematodes, the ticks are immobile as

oviposition occurs and this allows for the mobile nematodes to find the ticks and the application of nematodes to the environment is simple (Samish *et al.* 2004).

1.6.3.4. Insects

Research has been conducted on various hymenopterous insects on the nymphal life stages of ticks. These insects lay their eggs in the ticks and the ticks are eaten alive as the larvae hatch from the eggs. Fire ants, *Pheidole megacephala* and parasitoids wasps, *Ixodiphagus* have been used in tick control (Manjunathachar *et al.* 2014).

Samish *et al*. (2004), mentioned that the chalcid wasp species belonging to the genus *Ixodiphagus*, has been used for the control of ticks. These wasps have been found to be generalists, parasitizing many different tick species. So far research has shown that this genus only paratises ticks and no other non-target species. Little is known of how the wasps will perform in a field environment and as a result further research is required before this method of tick control can be applied in the field.

1.6.3.5. Birds

It has been found that approximately 50 bird species feed on ticks. However, most of these species are generalists and do not solely feed on ticks (Samish *et al.* 2004).

Buphagus africanus and *Buphagus erythrorhynchus*, the yellow billed and red billed ox peckers are indigenous to Africa and are known to have a diet which exclusively consist of ecto-parasites and is largely made up of ticks. The reintroduction of these birds into areas where they were killed due to toxic acaricides has been on the rise over the past two decades, especially in areas which contain game. Ox peckers are visual predators and will tend to feed on the full engorged ticks before searching for the immature stages on the host (Samish *et al.* 2004).

In Africa it has been found that chickens are also natural predators for ticks. Chickens in contact with barns and kraals in which the cattle are kept in overnight, will feed on many of the ticks in these areas and reduce the tick load present on the cattle. Chickens are however not obligatory predators of ticks and the feeding on ticks depends largely on the availability of other food sources and the density of the tick population (Manjunathachar *et al.* 2014).

1.6.4. Botanicals

Benelli *et al.* (2017), stated that although a lot of research has been conducted on the use of plant extracts and oils to control tick populations, the majority of the research has had no success in the real world as many of the products require stabilization processes and there are problems with the extraction and production at a large scale. The use of nanoparticles in the fight against tick control has gained interest since 2011. To date there has been some success in the use of nanoparticles which are fabricated from plant extracts.

1.6.5. Genetically resistant hosts

An alternative control method which is cost effective and provides a long-term benefit to the producer is the use of cattle breeds that have a naturally immunity towards ticks. These breeds are indigenous to the area and will require a less frequent treatment regime, thus, money is saved in terms of the cost of the acaricides as well as the reduction in the rate of development of resistance. Cross breeding animals which have a higher natural immunity is a cost-effective long-term way of controlling ticks.

In a study conducted by Marufu *et al.* (2011), the relationship between the ticks counts and the coat characteristics of Nguni and Bonsmara cattle in South Africa was determined. The shorter and smoother surface provided by the coats of the Nguni breed in combination with the believe that smoother coats might secrete more sebum, seemed to deter the attachment of the ticks. The longer hair of the Bonsmara coat created a better microenvironment as it prevented the ticks from exposure to climatic conditions, grooming by the host and to predators such as birds. The results of this study coincide with the results of Nyangiwe *et al.* (2011), who found that Nguni animals proved to be less affected by ticks in the Eastern Cape.

Zebu species, in addition to their shorter hair and thicker skin, also have panniculus muscles which are well developed, have a high density of sweat glands, a very sensitive pilomotor nervous system, which results in the twitching of the skin with the slightest touch and erector pili muscles, which not only make the hair stand up but are also responsible for the secretion of sebum in the hair acting as a tick repellent (Manjunathachar *et al.* 2014).

1.7. Justification

The production of cattle and their products contribute to the economy of the country. Large scale losses in production due to heavy tick loads and tick-borne diseases can be catastrophic to the South African economy. The invasion of alien species such as *R. (B.) microplus* which are able to adapt to new environments, plays a role in the displacement of indigenous species and is a vector of diseases as well as its resistance to most of the available acaricides, poses an even greater threat to cattle farmers and the economy.

To date comprehensive research has been conducted on the distribution and composition of the two blue cattle tick species, *R. (B.) microplus* and *R. (B.) decoloratus* in the Eastern Cape Province. However, the majority of these studies do not include the acaricide resistance status of the tick species and were mostly conducted at communal farms where the distribution of tick species takes place over greater areas (Tønnesen *et al.* 2004, Horak *et al.* 2009, Nyangiwe *et al.* 2013a**,** 2013b).

A previous initial discovery of the invasive Asiatic blue tick, *R. (B.) microplus* on a farm in the Eastern Cape Province resulted in this study to monitor the invasion of *R. (B.) microplus* and the possible displacement of the African Blue tick, *R. (B.) decoloratus* on this farm. This combined with tracking chemical tick control measures to establish possible tick resistance development in different camps on the farm and its possible influence on the distribution of the invasive tick species, presented an opportunity to investigate displacement and resistance development on a commercial farm.

The aim of this study was to investigate the invasion of the Asiatic Blue tick, *R. (B.) microplus* and the possible displacement of the African Blue tick, *R. (B.) decoloratus* on a commercial cattle farm in the Eastern Cape Province over a period of 18 months. During this period, development of acaricide resistance in different camps was also monitored and its possible influence on the distribution of the invasive tick species, *R. (B.) microplus*.

1.8. Main Objectives:

- To determine the composition and distribution of the two blue tick species infesting cattle and questing larvae from vegetation.
- To determine if *R. (B.) microplus* numbers have increased on this farm since initial detection in 2014.
- To establish the relationship between humidity and temperature on the collection of questing larvae.
- To conduct Polymerase Chain Reactions, (PCR), for identification of the larvae to complement the morphological identification.
- To establish resistance profiles for the blue ticks for each camp where collections were conducted.

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CHAPTER 2

Study Location

2.1. Introduction

Displacement of *R. (B.) decoloratus* by *R. (B.) microplus* on cattle on commercial farms in the Eastern Cape have not been investigated extensively. An initial discovery of the invasive Asiatic blue tick, *R. (B.) microplus* in 2014 on a commercial farm, located in the Eastern Cape Province, resulted in this study to monitor the possible invasion of *R. (B.) microplus* as well as development of tick resistance on this farm over a period of 18 months. This chapter describes the area where the collection of live engorged adult tick as well as questing larvae were conducted, the vegetation type, the rainfall and temperature patterns, a description of the host animals and husbandry practices on the farm and the laboratory setting where the laboratory component of the methodology was completed. Four fieldwork collection trips, each a week long occurred from 21-24 March 2016, 24-26 April, 20-23 November 2017 and 9- 11 April 2018. Since the presence and distribution of the invasive species were investigated, time span rather than seasonal abundance were taken into an account and one summer period was added just for additional field data puposes.

2.2. Farm Location

Collections were conducted on an extensive commercial beef producer called Claypits C8 & C9. The farm is located approximately 40km to the west of Makhanda, formerly known as Grahamstown and is situated in Coombs which forms part of the Ndlambe district of the Eastern Cape. The farm consists of three separate properties which in total covers an area of approximately 2500ha. For this study only two of the properties namely the main farm and the land above the tennis courts were included as indicated in Figure 2.1. GPS coordinates: [33°19'25.5"S; 26°51'17.7"E].

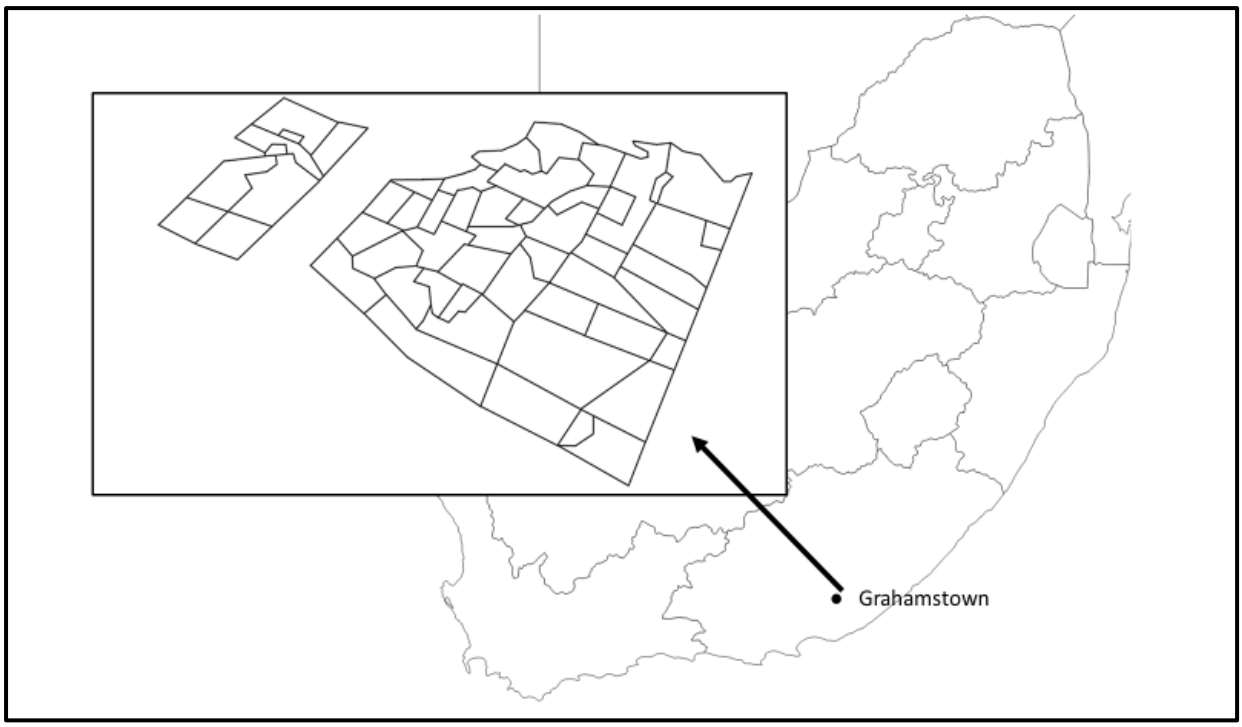

Figure 2.1: The location of the farm, relative to the town of Grahamstown.

2.2.1. Camps

The farm has been divided into 53 camps of which six are exclusively used as sheep pastures while the remaining 47 are utilized by cattle. The use of camps is determined by food and water availability and to a far lesser extent the tick burden. The camps are illustrated in Figure 2.2, the names which correspond to the numbers on the map are represented in Table 2.1. Of the 53 camps, 40 were included in the study over the study period, either for the collection of live engorged adults from host animals grazing in the camps, the collection of larvae from vegetation found in the camps, or both. In addition, the resistant profiles of tick larvae reared from engorged females collected from cattle hosts utilising certain camps where live collections occurred, was determined. The 13 camps coloured in red in Figure 2.2 and Table 2.1 were not included in the study as the producer did not use these camps for the grazing of cattle over the study period.

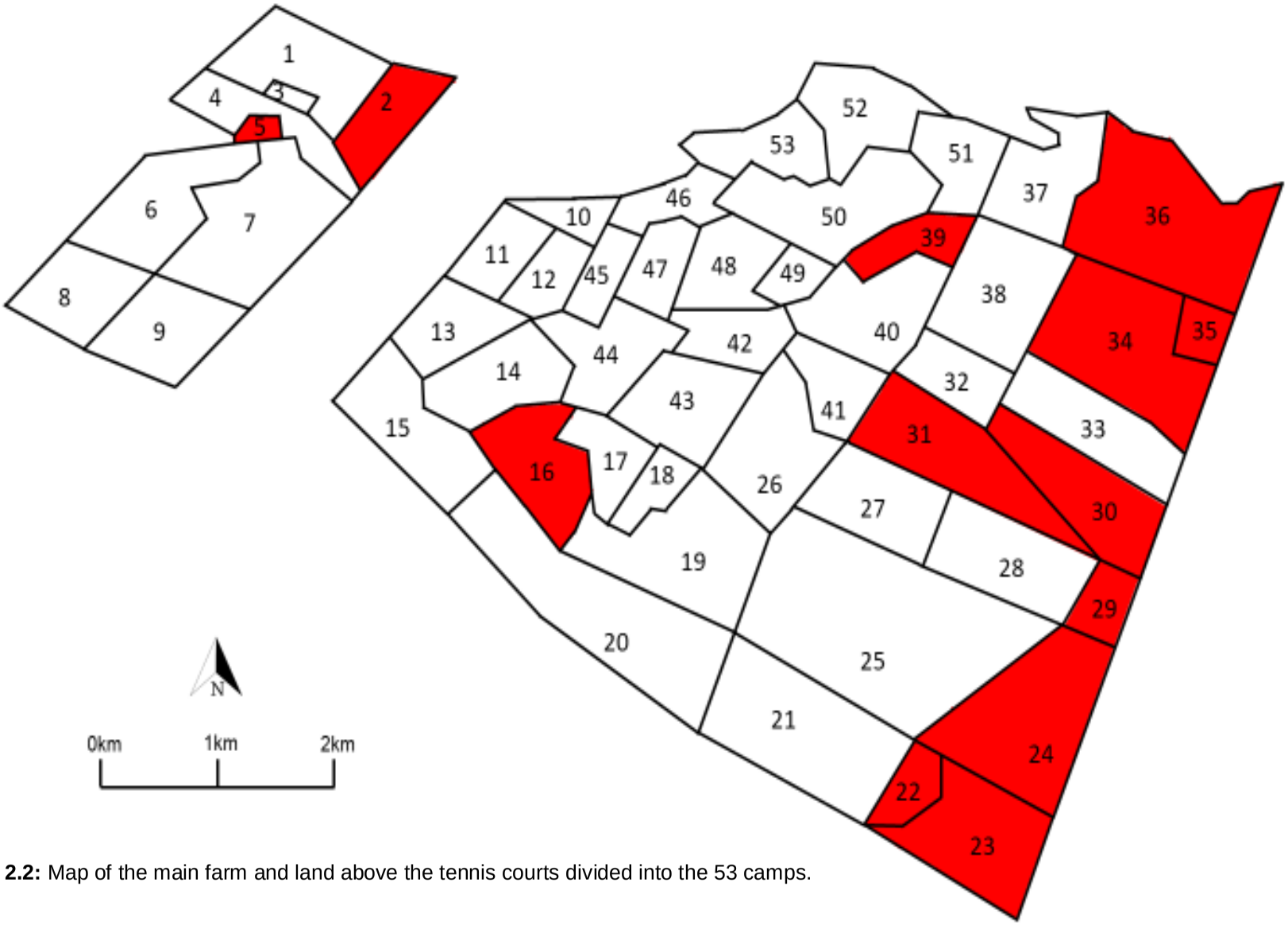

Figure 2.2: Map of the main farm and land above the tennis courts divided into the 53 camps.

Table 2.1: List of names of each Camp represented in Figure 2.2.

1. Morne Fir	28. Jackal Ridge
2. Gavin Huts	29. Small Ridge
3. Spray Race	30. Old Pineapple Lands
4. Vlei and Amos	31. Barbers Kloof
5. Anti Fusi	32. Koekweni
6. Gum Tree Dam	33. Berts
7. Fish Dam	34. Black Forest
8. Arthurs Reservoir	35. Black Forest Garden
9. Gavin Hill	36. Sheep
10. Red Grass	37. Krantz
11. Lolweni	38. Stokweni
12. Church Pregnant	39. School Fir Tree
13. Church	40. School
14. Quarry	41. Barbers Orchard
15. Bushalt	42. Dads House
16. Horse	43. November
17. Milk Cow	44. Sheds
18. Milk Cow Pregnant	45. Mesnge
19. New Windmill	46. Hiltons
20. Tembisile Dam	47. Dougs House
21. Blind Rise	48. Barbers Dam
22. Kap Sheep	49. School Pregnant
23. Kap Windmill	50. Lands and Old Oranges
24. Green Shed	51. Kens
25. Guava	52. Gaalboom
26. Singeni	53. Hiltons
27. Fir Tree Ridge	

Some camps like Lands and Old Oranges camp were not only used for grazing activities but also for the production of sorghum with Quarry camp containing an active quarry as illustrated in Figure 2.3. All camps either had a natural water source or water was pumped from underground sources to feed it through a pipeline into dams from where it was distributed to water toughs in camps where natural water sources were absent as illustrated in Figure 2.4.

A: Lands and Old Oranges Camp	B: Quarry Camp

Figure 2.3: Camps which are not only used for grazing activities. **(A)** Lands and Old Oranges camp containing fields used for the plantation of sorghum. **(B)** Quarry camp contains a quarry used by the producer. (Source: Author unpublished 2018).

A: Barbers Dam Camp	B: Bushalt Camp

Figure 2.4: Camps on the farm either contain natural waters sources as seen in **(A)** Barbers Dam camp while others require water to be pumped to the surface like in **(B)** Bushalt camp. (Source: Author unpublished 2018).

2.2.2. Vegetation type

The vegetation found in the study site, can be described as Albany coastal thicket according to Cowling (1983). Palmer (2004), described the Albany coastal belt to comprise of short grassland and bushy clumps close to the coast. This zone is bordered by subtropical forest, savanna and Nama-karoo. The Albany thicket is rich in vast variety of species which have not all been documented; however, it is estimated that there are 2400 vascular plants, of which 200 are considered to be endemic.

The vegetation within the camps varied in relation to the amount of open grazing land and dense bushy shrubs and covered both sour and sweet veld. The lower grounds consisted of sweet veld which was made up of dense grass and bushes with a few open patches of land as illustrated in Figure 2.5. The higher grounds consisted of sour veld which had more open areas with a few trees and shrubs, as can be seen in Figure 2.6.

A: Kens Camp	B: Morne Fir Camp

Figure 2.5: Dense sweet veld found in the lower lying camps on the farm such as in **(A)** Kens and **(B)** Morne Fir camps. (Source: Author unpublished 2018).

A: Gumtree Dam Camp	B: Gavin Hill Camp

Figure 2.6: Open sour veld found on the higher areas of the farm such as **(A)** Gumtree Dam and **(B)** Gavin Hill camps. (Source: Author unpublished 2018).

2.2.3. Temperature & Rainfall

The climate in Makhanda has been described as warm and temperate. This area experiences a significant annual rainfall with rain experienced even in the driest months. On average 680mm of rainfall is experienced annually with peaks occurring during March-April and October-November. The least amount of rainfall is experienced in July. On average the annual temperature is 16.8 °C, with February being the warmest and July being the coldest months of the year. With average midday temperatures ranging from 26.8°C in February and 18.9°C in July (Palmer 2004).

2.2.4. Host Animals & Husbandry

There were approximately 345 South Devon cattle on the farm with bulls presenting as large and muscular with a smoother hair coat and cows with a more feminine appearance and a thick woolly hair coat as indicated in Figure 2.7. This breed is known for having a docile temperament, a high fertility rate and are good milk producers. The bulls are often separated from the herds depending on the season and were kept together or individually in their own camp. Calves were kept with their mothers until

they were weaned at eight months old, when they were weighed and the heavy females were kept on the farm while lower weight females and bulls were sold. No new females were brought onto the farm and new bulls were introduced every few years. The herd was divided into smaller groups namely; the milk cows, dry cows, cows and calves, staff cattle and cattle that were for sale.

A: Bull	B: Cow

Figure 2.7: Appearance of South Devon cattle found on the farm, **(A)** bulls are large and muscular, **(B)** cows have a more feminine appearance with thick woolly hair. (Source: Author unpublished 2018).

Cattle were not kraaled over night as the producer practises extensive farming, allowing the cattle to graze freely within the camps, 24 hours a day. Herds were moved between camps in a rotational grazing manner which the producer keeps track of in a data book. The herds were checked on at least once a week to ensure that all members were accounted for, to check the tick load and to look for any individuals which were displaying signs of distress to be diagnosed and treated. Cattle were treated with acaricides every fortnight or when emergency dipping was needed due to heavy tick infestations. Calves were treated with acaricides from the age of one month old and all calves were vaccinated for tick-borne diseases such as redwater, heartwater and gall sickness as well as for anthrax poisoning. The producer also had approximately 260 sheep on the farm and wildlife such as impala, warthog, bushbuck, kudu, duiker, rabbits, jackal, lynx and tortoises moved freely over the farm.

2.3. Laboratory work

All laboratory procedures were performed at the Pesticide Resistance Testing Facility (PRTF), located in the Department of Zoology and Entomology at the University of the Free State in Bloemfontein, South Africa. The facility has specialised equipment and separate rooms to allow for the incubation and rearing of ticks, the testing of resistance towards the selected acaricides and the storage and preparation of dip chemicals used for testing without cross contamination to a laboratory equipped for molecular procedures. The room allocated for tick rearing has a climate control system which kept the room at ±28°C with a relative humidity of ± 75%, maintained in incubation containers through saturated NaCl solutions that fill 20% of each container which was kept closed by a lid. This allowed for the optimal conditions for oviposition and emergence of larvae to take place. The room was also used for tick preparation and identification before sample incubation takes place. A second room, equipped for housing of the acaricides, was used to make the dip dilutions, in a fridge at ±4^{0}C. This room was also equipped with a fume hood for safe dilution of the dip samples prepared for acaricides resistance testing. The third room, was used for the exposure of tick collections to acaricide dilutions during the performance of the acaricide resistance testing. A separate laboratory, fully equipped for the extraction, amplification and running of electrophoresis gels for DNA identification was available for molecular procedures.

2.3.1. Laboratory Safety & Waste Disposal

In the laboratory, the minimum protective clothing worn at all times was a laboratory coat and latex gloves. Appropriate safety measures were taken while working with chemicals, toxic substances and products as indicated on chemical containers. All the chemicals required for the study were stored in the appropriate manner according to the Material Safety Data Sheet, in access restricted rooms, with adequate ventilation. The preparation of all chemical solutions was done in a fume hood and were disposed of in the appropriated waste collection bottles. The waste disposal throughout the study was handled according to the procedure stipulated in the appropriate SOPs of the PRTF. Biological and chemical waste was disposed of separately and removed from the PRTF by an external accredited waste disposal company.

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CHAPTER 3

Distribution and Composition of Tick Species

3.1. Introduction

In the 1970s the number of cattle in South Africa has been estimated to be around 14 million with almost 80% contributing to beef production while the remaining 20% represent dairy production. Approximately 80% of the land in the country was suitable for the extensive grazing of livestock (DAFF 2013). This number has greatly decreased over the years due to the expansion of human settlements, industrial activities such as mining, the use for crops and forestry and conservation areas. Approximately 70% of agricultural land is utilized for the production of livestock and wildlife. Intensive farming practices have increased over the past decade due to the decrease in availability of water. It has been estimated that approximately 75% of cattle spend more time in feed lots than ranging freely over grazing land in South Africa. The production of livestock is critical to ensure food security in South Africa. The Eastern Cape contains the highest concentration of livestock in the country. Livestock products produced in the country over 2006-2010, accounted for 47%, R50 586 million of the agricultural sectors revenue (DAFF 2010). Livestock are not only used for the production of food and financial benefit, they are also valuable for the production of manure, use as transport, hard labour and for enhancing the cultural status (Avenue *et al.* 2013).

The distribution of tick species is dependent on biotic factors such as host availability and vegetation structure, and abiotic factors such as temperature, rainfall, humidity and the photoperiod. Cumming (2002), determined for African tick species that although the vegetation within the physical environment does play a small role in the distribution of tick species, climatic conditions such as temperature and rainfall play a far greater role in the distribution and range expansion of ticks. These factors tend to have a greater impact in areas where there is a constant supply of host animals such as in Benin where the movement of livestock occurs throughout the country with over 2 million cattle being moved over the course of a year (Biguezoton *et al.* 2016). This practice has had an impact on the spread of *R. (B.) microplus* across the country. Adinci *et al.* (2018), conducted a study on the impact of the movement of cattle on the

tick infestation and found that the tick load present on the cattle before and after movement to new grazing lands showed a decrease in number which is a result of the detachment of adults along the way. Larvae can survive for up to five months while waiting for a favourable host to pass by. The establishment of *R. (B.) microplus* in the new locations will however, depend on both abiotic and biotic factors.

Estrada-Peña (2003), created a climate model which predicted the effect of a 1°C and 2°C increase and decrease in temperature on specific tick species in South Africa. The results showed that a decrease in temperature will have a positive effect on the range expansion of *H. truncatum*. While an increase of 1°C will have a small positive impact on the range expansion of *R. (B.) decoloratus* and *A. hebraeum*. A 2°C increase will however have a negative effect on all three species. This correlates with findings of Dantas-torres (2015), predicting that in some instances an increase in temperature of 2°C can result in a decrease in the range of *R. (B.) decoloratus*, *A. hebraeum*, *H. truncatum* and *R. appendiculatus* in South Africa.

Warmer winters and extended spring and autumn season will potentially result in the range expansion of various tick species. The rise in temperatures and changes in the rainfall pattern are resulting in the alteration of habitats and thus areas that were previously unsuitable are now favourable. Host animals in these areas have not been exposed to the species or the diseases that they transmit and as a result the consequences of the lack of immunity can be deadly (Estrada-Peña 2003). The prediction of the range expansion is however a complex process, due to complex interactions occurring between all of the ecological processes and interactions surrounding the life cycle of tick species (Dantas-Torres 2015).

Range expansion and introduction of tick species into areas where they were not found previously, often has a negative impact on the hosts present in that region. The invasion of *R. (B.) microplus* has occurred in many tropical and subtropical countries worldwide. The most striking and recent invasion occurred in West Africa, over the past decade where *R. (B.) microplus* has displaced indigenous species in parts of Benin, Burkina Faso, Togo, Mali as well as the Ivory Coast (Madder *et al.* 2010, 2012; de Clercq *et al.* 2012; Adakal *et al.* 2013; Biguezoton *et al.* 2016). The full range of the species has not yet been reached and it is predicted that *R. (B.) microplus* will continue

to expand within these countries and could potentially spread to neighbouring countries.

The mapping of the distribution of both *R. (B.) microplus* and *R. (B.) decoloratus* in South Africa began in the late 19th century (Howell *et al*. 1978). *Rhipicephalus (Boophilus) microplus* was found in patches along the coast while *R. (B.) decoloratus* was present throughout most of the eastern region of the Eastern Cape. Three years later a more extensive mapping of the distribution of *R. (B.) microplus* was conducted by Baker *et al.* (1981) and Baker (1982) within the eastern region of this province and the results supported the findings by Howell *et al.* (1978). Over two decades later in 2004 and 2005 Horak *et al*. (2009), conducted collections in this area and found that the distribution of the species had in fact reversed with *R. (B.) microplus* now being the dominant species present. From this study, it was found that *R. (B.) microplus* displaced the indigenous tick, *R. (B.) decoloratus* in the north-eastern regions of the Eastern Cape Province. Nyangiwe *et al.* (2011) conducted a study on in the eastern region of the province on an experimental farm at the Dohne research station. In this area it was found that both *R. (B.) decoloratus* and *R. (B.) microplus* were present in the same area together and although *R. (B.) microplus* was the dominant species in the location, over the 5-year period the ratio between the blue ticks did not change significantly.

This study therefore focussed on the composition and distribution of *R. (B.) decoloratus* and *R. (B.) microplus* on Claypits, a commercial farm, near Grahamstown in the Eastern Cape Province to investigate the current state of blue tick species distribution on this farm over an 18-month period. Live adult ticks were collected directly off the cattle and larvae was collected via drag sampling over vegetation in different camps on the farm. All adult ticks and immatures were identified up to species level with the exception of blue ticks which did not have mouthparts or were damaged, these were identified up to genus level.

The following objectives were covered in this chapter:

- To determine the composition and distribution of the two blue tick species and questing larvae on vegetation.
- To determine the number of different tick species present on the farm
- To determine if the *R. (B.) microplus* numbers have increased since the species was first found on the farm in 2014.
- To track the movement and spread of *R. (B.) microplus* on the farm by following a selected herd over the period of a year.
- To establish a relationship between humidity and temperature on the collection of questing larvae.

3.2. Methods & Materials

3.2.1. Sample Collections

Samples were collected during three separate fieldwork trips which occurred from the; 24th - 26th April 2017, 20th – 23rd November 2017 and 9th – 11th April 2018. During each field trip, adult ticks on the host and questing larvae on the vegetation were collected. The producer also conducted additional collections of adult ticks from a selected herd prior to each treatment with acaricides. Live adult tick samples were additionally sent in to the facility via a courier service for identification and testing of ticks when large infestations were encountered.

3.2.1.1. On-host: Adult Ticks

The cattle used with in this study were of good health and body condition according to the producer. The age and sex of the animals were not of concern for this study as the presence and not abundance of the invasive species was investigated. Calves were excluded from the collections to prevent them from being exposed to unnecessary stress while in the crush. The collections were conducted in accordance to the usual management practices of the farm in order to avoid placing additional stress on the animals. The producer and his workers were present and aided in the collections in order for it to be completed in the least amount of time possible and provide a familiar environment for the cattle to further lessen stress levels.

Engorged adult female ticks were collected directly from at least ten cattle from each herd while in a race prior to treatment with acaricides. The cattle were selected based on the degree of infestation, whereby those infested with the greatest number of tick's in each herd were selected for collection. During the collection the entire animals' body, from head to tail, was checked for ticks by means of palpation. The sites labelled in Figure 3.1 bellow illustrate the areas where the majority of the ticks were found.

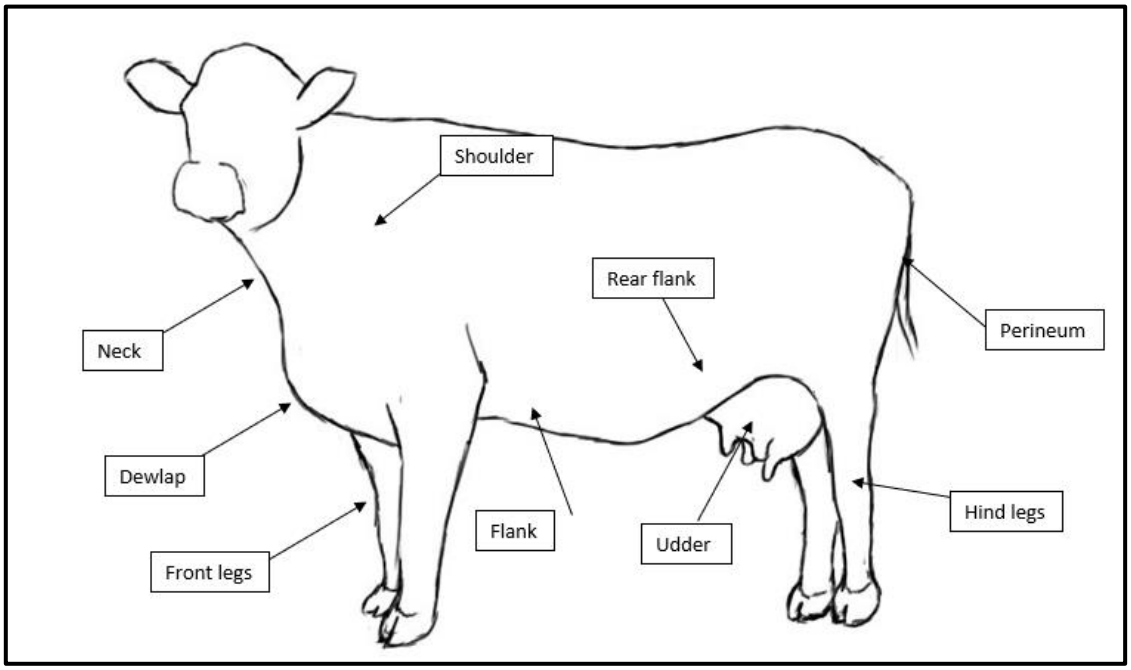
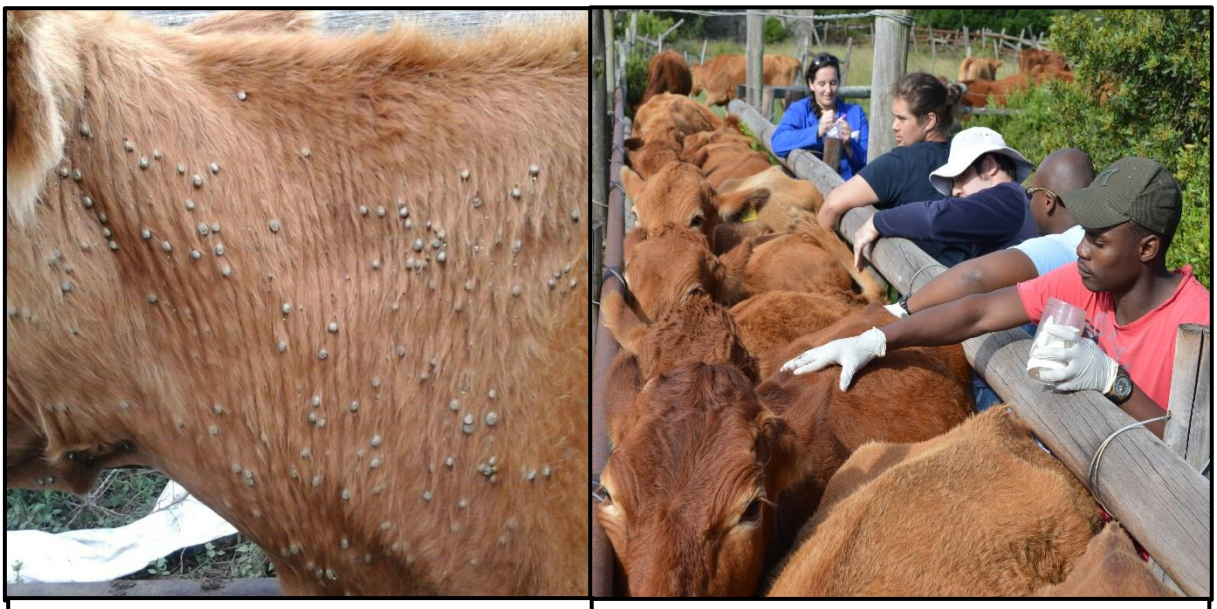


Figure 3.1: The location on the cattle of which collections of adult ticks occurred. (Source: Sonenshine & Roe 2014).

The ticks were collected from the following regions of the cattle, namely the neck, flank, dewlap, perineum and between the hind legs seen in Figure 3.1. This was done by the use of a large blunt tipped pincette or by hand, while wearing latex gloves as seen in Figure 3.2. Blue ticks were the focus of the collection however; other tick species were also collected for further identification. All adult ticks, collected from each cattle group, were placed into one plastic collection bottle with small air holes punched into the top of the bottle and lid. Each collection bottle contained paper towelling and a piece of fine mesh placed over the open end, under the lid in order to prevent any of the smaller ticks from escaping. Each collection bottle was labelled clearly with the camp reference number, the name of the camp which the cattle were in prior to the collection, the collection date and name of the collector.



A: A large infestation of blue ticks on cow's neck/dewlap region.	B: The collection of adult ticks from cattle while in a race.

Figure 3.2: The high tick burden present on cattle found on Claypits seen in **A,** are collected by students seen in **B**. (Source: Author Unpublished 2018).

All live ticks collected were transported in an incubation container, which contained a NaCl solution in order to maintain a higher humidity in the container from the farm to the PRTF. A raised glass plate was inserted into the incubation container to keep the live ticks from coming into contact with the NaCl solution. The humidity and temperature inside the container were monitored from the time the ticks are collected till the point when they reached the testing facility and were placed into the incubation room. In the facility, the temperature and humidity are routinely kept constant at ±28°C and >70% Relative Humidity (RH) respectively.

3.2.1.2. Selected Herd

One of the cattle herds, consisting of the cows and calves, was selected to be followed from April 2017 to April 2018. This herd was chosen with aid from the producer as the herd needed to remain fairly stable over the period of the study. The producer conducted the tick collections from the herd prior to normal acaricide treatments as scheduled by his treatment program, approximately every second to third week depending on the season and tick load. After collection and treatment this herd was either moved back to the same camp or moved to a new camp depending on water and food availability.

These collections were placed into pre-labelled and prepared glass collection bottles containing 70% ethanol, which were provided to the producer at the start of the study. The camp name, collection date and note of the degree of infestation was included on the label. These collection bottles were then collected from the producer during the field visits to the farm and identification of the ticks in the collection were done upon return to the laboratory.

When sudden large infestations occurred on any of the herds on the farm, the producer sent live adult tick collections via a courier service to the facility for identification and acaricide resistance testing. These samples were sent in a plastic collection bottle in a sealed zip lock bag within a sealed box filled with polystyrene packing material or in a sealed envelope.

3.2.1.3. Off-host: Collection of Larvae

Larvae were collected from the vegetation during the three field visits by means of tick drags in the camps inhabited by the cattle herds three weeks prior to collection of engorged ticks. Additional tick drags were conducted in camps which were regularly used by the producer or were considered to be 'problem' camps in terms of high tick infestation. The vegetation present at the drag sites was photographed and recorded.

The drags consisted of ten 1m x 10cm weighted flannel strips connected to a 120cm broomstick with Velcro strips, the broomstick having a rope attached at either end. The flannel strips were dragged over the vegetation in each camp at least 20m apart from each other for 150m at a time as seen in Figure 3.3 A. Four drags were performed in different areas of each camp, based on the watering and resting points within it. The larvae stuck to the drag strips were removed by means of a fine-tipped pincette as seen in Figure 3.3 B and stored in 70% ethanol in internally labelled plastic vials for later identification and counting.

A: Tick drag being performed over the vegetation in the camp.	B: A single larvae stuck to the flannel strip.

Figure 3.3: Tick drags conducted on the vegetation within a camp on the farm seen in A to pick up larvae as seen in B which are removed with a pincette and placed into ethanol tubes. (Source: Author Unpublished 2018).

Drags were not performed over grass with heavy dew early in the morning or immediately after rain, as this would wet the flannel strips and decrease their efficacy for picking up questing ticks. The temperature and humidity were measured and recorded by means of a temperature and humidity meter at the start and end of the collection. Other climatic conditions such as the wind strength and cloud cover observed and determined by the collectors, were also recorded. The elevation and GPS co-ordinates of each camp were acquired from Google Earth once the farm and camps were mapped out electronically.

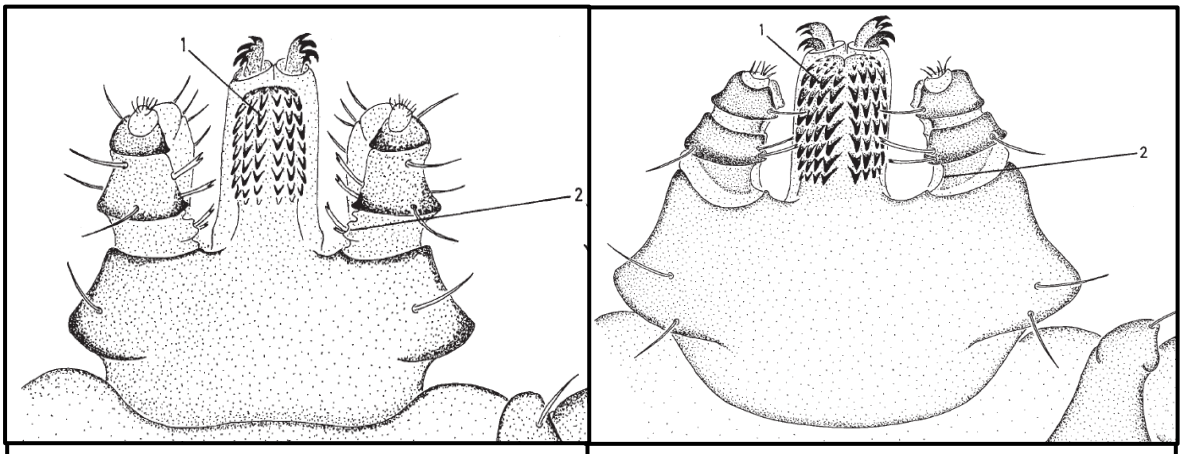
3.2.2. Morphological Identification

A Nikon dissection microscope, model SMZ645, was used for morphological identification. All adult ticks and larvae were identified up to species level under a magnification of 5X with the aid of an auxiliary objective of 2X.

3.2.2.1. Adults

All the adult ticks collected were identified by using morphological descriptions as documented by Walker *et al.* (2003). The main characteristics used to differentiate

between the two blue tick species were mostly based on the dentition. *Rhipicephalus (Boophilus) decoloratus* has 3+3 configuration while *R. (B.) microplus* has a 4+4 configuration of denticles on the hypostome as illustrated in Figure 3.4.A and 3.4.B respectively. *R. (B.) decoloratus* has a protuberance with pectinate setae on the internal margin of article 1 on the palps. Whereas the internal margin of article 1 of *R. (B.) microplus* is concave, short and lacks a protuberance.



A: *R. (B.) decoloratus*. B: *R. (B.) microplus*.

Figure 3.4: (A) Mouth parts of *Rhipicephalus (Boophilus) decoloratus*, 1.) 3+3 rows of denticles on the hypostome, 2.) The internal margin of article 1 of the palps has a protuberance with pectinate setae. **(B)** Mouth parts of *Rhipicephalus (Boophilus) microplus,* 1.) 4+4 the rows of denticles on the hypostome, 2. The internal margin of article 1 is concave, short and lacks a protuberance (Source: Walker *et al*. 2003).

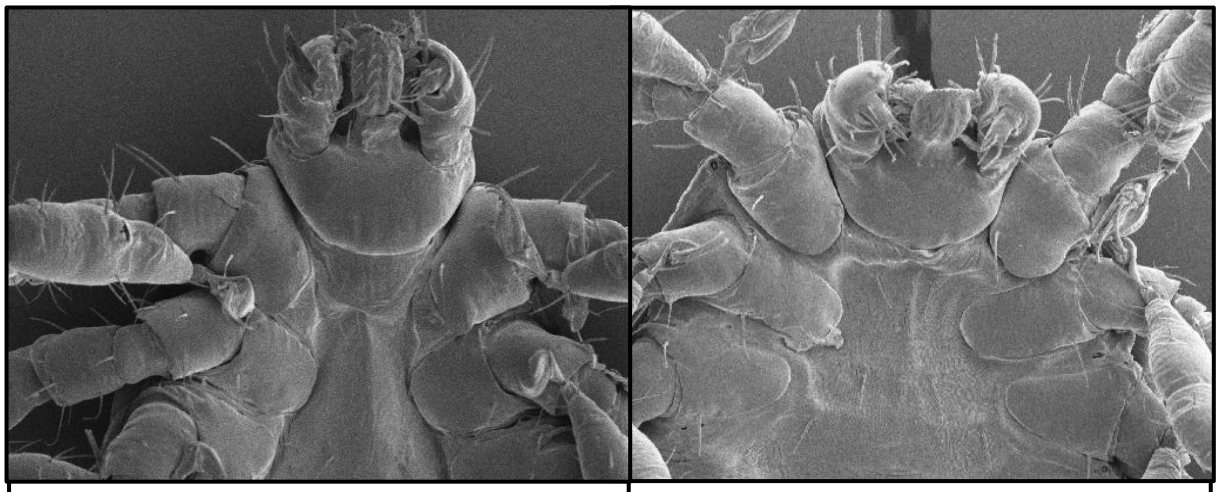
At least twenty fully engorged *R. (B.) decoloratus* females were identified, allocated for acaricide resistance testing and placed into conical vials for incubation. The remaining ticks were identified, counted, separated at species level and stored in 70% ethanol in plastic tubes with internal labels.

In some cases, the ticks still had skin or hair of the host stuck to the mouthparts. This was removed carefully with a fine tipped pincette, however, in a few cases the removal was not successful and the entire mouth part was removed. Blue ticks with no mouth parts could thus only be identified up to genus level. Other ticks which were collected were also identified up to species level using descriptions made by Walker *et al.* (2003). In cases when sufficient numbers of other tick species, namely, *A. hebraeum*

and *R. evertsi evertsi* were collected, they were also placed in conical vials for incubation and acaricide resistance testing.

3.2.2.2. Larvae

The identification of the larvae was completed via the use of identification keys that were described by Gothe (1967) and additional morphological features which were described by Berry (2017). Both species are broad and oval in shape with 2+2 configuration of teeth on the hypostome. *Rhipicephalus (Boophilus) microplus* larvae have a smooth scutum which lacks setae and extends over approximately two thirds of the dorsal surface. On the dorsal side *R. (B.) microplus* has a short internal spur on coxa I while the remaining coxae contain no spurs as illustrated in Figure 3.5(B.) *R. (B.) decoloratus* larvae have a scutum that is broader than long, it contains fine setae and is finely punctuated and lacks a short internal spur on coxae I as illustrated in Figure 3.5(B.) (Berry 2017).



A: *R. (B.) microplus*.	B: *R. (B.) decoloratus*.

Figure 3.5: (A) *Rhipicephalus (Boophilus) microplus* larvae with a short internal spur present on coxae 1. **(B)** *Rhipicephalus (Boophilus) decoloratus* larvae, no spur present on coxae 1. Source: 3.5 **A**. (A. Maris Unpublished 2016), 3.5 **B**. (Author Unpublished 2016).

All larvae were identified up to species level and the number of each species collected in each camp was counted. Larvae of the same species from each camp was stored together in 70% ethanol in plastic vials with an internal label containing the reference number, collection date and species.

3.2.3 Environmental Parameters

Relative humidity (RH) temperature (T) were recorded with a hand held meter and the time of collection was recorded at the beginning and end of each larval collection within the different camps. This information along with the name of the camp and the date was recorded in a data book.

3.2.4 Data Analysis

The percentage of abundance of each species identified was determined in the overall sample set collected. The mean, variance, standard deviation and standard error was determined for the *R. (B.) microplus* and *R. (B.) decoloratus* samples.

A liner correlation coefficient was used to determine the correlations between the number of larvae collected and the temperature and relative humidity recorded at the collection. Values close to 1.0 indicate a strong liner relationship while values close to -1.0 indicate a strong negative relationship. A hypothesis test using the Z-test was performed to determine if the null hypothesis which states that there is relationship between the number of larvae collected and the temperature and relative humidity during the collection, or if the alternative hypothesis that there is not relationship is true.

3.3. Results

3.3.1. Overall Composition of All Tick Species Collected

Seven species of tick were found on the farm over the 18-month study period as seen in Table 3.1 below.

Table 3.1: All tick species collected on Claypits over the study period, presented from most to least abundant

Species	Male	Female	Nymph	Larvae	Total	%
Rhipicephalus (Boophilus) decoloratus	618	5294	0	8954	14866	89.45
Amblyomma hebraeum	43	45	10	994	1092	6.57
Rhipicephalus evertsi evertsi	51	148	6	85	290	1.74
Rhipicephalus (Boophilus)	0	240	0	0	240	1.22
Rhipicephalus (Boophilus) microplus	7	112	0	19	138	0.84
Ixodes pilosus	3	17	0	0	20	0.12
Hyalomma truncatum	0	7	0	0	7	0.04
Haemaphysalis elliptica	0	0	0	3	3	0.02

Of these species collected 89.45% of the total collection of both adult ticks and larvae comprised of *R. (B.*) *decoloratus*. *Amblyomma hebraeum* at 6.57% was the second most predominant species identified, followed by *R. evertsi evertsi* at 1.74%. Blue ticks that were unable to be identified to species level due to damage to the mouthparts and other morphological features, made out 1.22% of the collection and thus these ticks were grouped in the genus *Rhipicephalus (Boophilus)*. *Rhipicephalus (Boophilus) microplus* only consisted of 0.84% of the total collection. This species was then followed by *I. pilosus*, *H. truncatum and H. elliptica* which comprised of 0.12%, 0.04% and 0.02% of the total collection, respectively.

3.3.2. *Rhipicephalus (Boophilus) decoloratus* Collection

A breakdown of *R. (B.) decoloratus* specimens collected in all of the camps sampled on the farm during the four fieldwork trips can be seen in Table 3.2. The highest total number of adult *R. (B.) decoloratus* ticks, 784, were collected in April 2018 while the highest total number of *R. (B.) decoloratus* larvae, 3077, were collected in November 2017. Of all the camps in which collections were made over the study period, Milk Cow camp was the only camp from which both adults and larvae were collected during each field trip. The camp in which the highest number of adult *R. (B.) decoloratus* ticks was collected was Guava camp with 528 adults found on the hosts during the April 2017 collection. New Windmill camp delivered the highest number of larvae during the April 2018 collection with a total of 1237 *R. (B.) decoloratus* larvae identified. The standard deviation value of 623.7782 and standard error value of 115.8327 indicate that the data points are spread out around the mean of 442.1724.

Table 3.2: The total abundance of *Rhipicephalus (Boophilus) decoloratus* specimens collected during the field collections.

	March 2016		April 2017		November 2017		April 2018		Total	Total	**Total**
Camp	Adults	Larvae	Adults	Larvae	Adults	Larvae	Adults	Larvae	Aduts	Larave	**Specimens**
Milk Cow	63	249	105	582	91	314	273	580	532	1725	**2257**
Church	94	118	*	89	*	92	*	60	94	359	**453**
Arthors Reservour	180	5	*	*	*	*	217	55	397	60	**457**
Fish Dam	76	85	*	*	*	*	*	114	76	199	**275**
Church Pregnant	122	30	*	19	*	4	*	*	122	53	**175**
Bushalt	80	*	328	33	90	97	*	*	498	130	**628**
Gavin Hill	104	*	*	*	422	4	*	40	526	44	**570**
Barbers Dam	*	32	*	1	*	8	140	34	140	75	**215**
Barbers Orchard	*	93	*	*	*	*	*	*	0	93	**93**
Vlei & Amos	*	64	*	*	*	*	*	*	0	64	**64**
Gaalboom	*	8	20	2	*	69	*	9	20	88	**108**
New Windmill	*	768	*	*	*	354	*	1237	0	2359	**2359**
Quarry	*	144	163	1	*	14	*	11	163	170	**333**
Stokweni	*	3	*	*	15	170	*	*	15	173	**188**
Morne Fir	*	35	*	*	1	22	*	*	1	57	**58**
Furtree Ridge	*	1	*	*	*	*	*	*	0	1	**1**
Krans	*	1	*	*	*	*	*	*	0	1	**1**
Kens	*	2	*	*	*	*	*	6	0	8	**8**
Guava	*	*	528	5	*	803	*	209	528	1017	**1545**
Sheds	*	*	*	*	164	51	*	7	164	58	**222**
Lolweni	*	*	*	*	55	13	*	36	55	49	**104**
School Pregnant	*	*	*	*	*	15	*	94	0	109	**109**
Singeni	*	*	*	*	*	27	*	*	0	27	**27**
November	*	*	*	*	*	180	*	208	0	388	**388**
Tembisile Dam	*	*	*	*	*	894	*	336	0	1230	**1230**
Msenge & Red Grass	*	*	*	*	*	260	*	*	0	260	**260**
Lands & Old Oranges	*	*	*	*	*	*	427	38	427	38	**465**
Gumtree Dam	*	*	*	*	*	*	*	36	0	36	**36**
Milk Cow Pregnant	*	*	*	*	*	*	*	194	0	194	**194**
Total Number	656	1389	1039	150	747	3077	784	2724	3226	7340	**12823**

* indicate camps where no collection was performed during that specific fieldtrip as they were not in use by the producer for the grazing of cattle.

3.3.3. *Rhipicephalus (Boophilus) microplus* Distribution

Figure 3.6 A-E illustrates the camps on the farm in which *R. (B.) microplus* was collected indicted in red. The "♀ & ♂" symbols represent the female and male adults collected while the "♀/♂" represents the larvae collected. The "*" represent the other camps on the farm from which ticks or larvae were collected for that specific collection period but only *R. (B.) decoloratus* were found.

The initial discovery of *R. (B.) microplus* on the farm occurred in 2014 in Krantz camp as indicated in Figure 3.6A. Five *R. (B.) microplus* females and a single *R. (B.) microplus* male were found during this collection. No *R. (B.) microplus* was found in the other five camps in which collections were conducted. No collections were conducted on the farm in 2015.

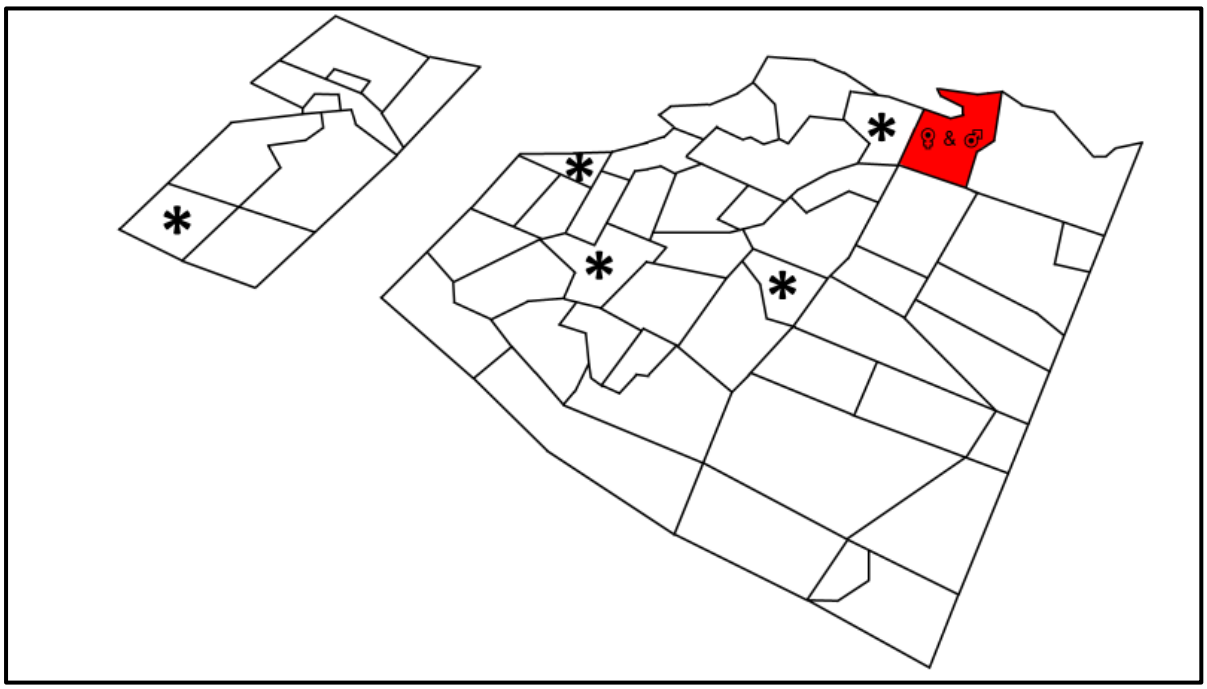


Figure 3.6A: The distribution of *Rhipicephalus (Boophilus) microplus* identified on Claypits in 2014.

During the March 2016 collection, *R. (B.) microplus* females were found in the Church camp on the main property as well as in Gavin Hill, Arthurs reservoir and Fish dam camps on the smaller property while larvae were identified in the Vlei & Amos camp as seen in Figure 3.6B. No other *R. (B.) microplus* specimens were found in 13 of the other camps also sampled during this collection period. Overall a total of 10 adult female *R. (B.) microplus* specimens and a single *R. (B.) microplus* specimen were collected.

In 2017 *R. (B.) microplus* specimens were collected in April and November. During the collection conducted in April *R. (B.) microplus* was collected and identified in seven of the nine camps which were sampled seen in Figure 3.6C. No *R. (B.) microplus* specimens were collected in the Barbers Dam and School Pregnant camps. In the Church and Church Pregnant only *R. (B.) microplus* larvae were found while in Gaalboom camp only a single adult female *R. (B.) microplus* specimen was collected. Two adult females and two *R. (B.) microplus* larvae were collected in the Quarry camp, while both adult males and females as well as larvae were collected in the Guava, Milk Cow and Bushalt camps. A total of 32 adult ticks and 14 larvae specimens were collected in April 2017.

Seen in Figure 3.6D *R. (B.) microplus* was collected from seven of the 20 camps sampled in November 2017. A total of 26 adults and seven larval *R. (B.) microplus* specimens were collected from the following camps, larvae was only collected from the Morne Fir and November camps while only adult females were collected from Lolweni and Sheds camps. Nineteen adult ticks comprising of both males and females were collected from Gavin hill camp while adult female ticks and larvae were collected from the Bushalt and Stokweni camps. Collection in April 2018 produced *R. (B.) microplus* in four of the 19 camps which were sampled as indicted in Figure 3.6E. A single larva was collected and identified in Milk cow pregnant camp. Adult females were collected and identified in Barbers dam, Lands and Old oranges and Arthurs reservoir camps. During this collection a total of 12 adult females and a single larva were collected and identified.

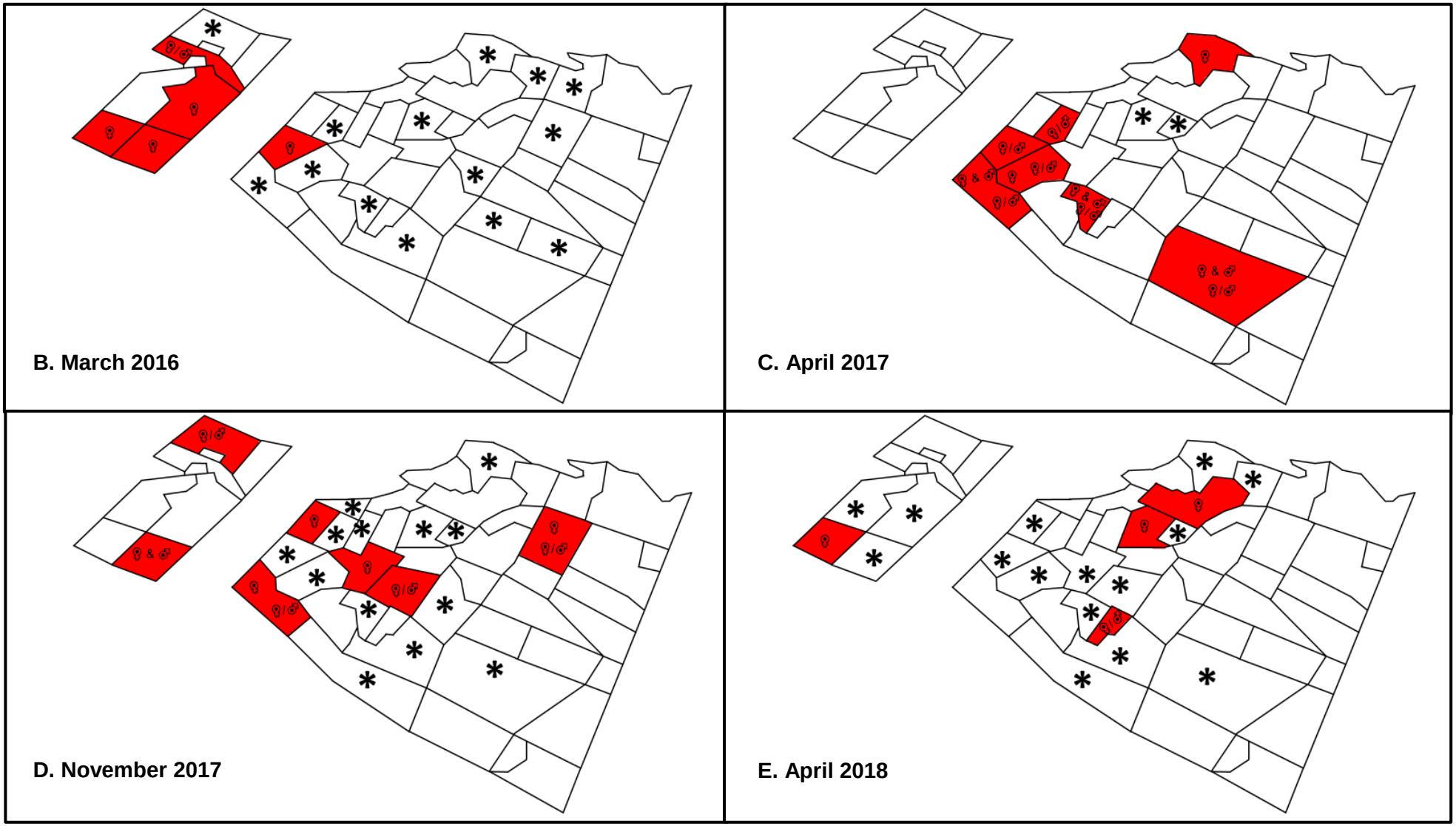


Figure 3.6 B-E: Camps on the farm in which *Rhipicephalus (Boophilus) microplus* was collected during March 2016 **(B),** April 2017 **(C),** November 2017 **(D)** and April 2018 **(E)** collection periods are indicted in red.

A summary of the camps from which *R. (B.) microplus* was collected as well as the number collected in each camp over the course of the study period is seen in Table 3.3. *R. (B.) microplus* was collected from Church, Gavin Hill, Arthurs Reservoir and Bushalt camps in three of the four collection. In the remaining camps *R. (B.) microplus* was only found during one of the collection periods. The standard deviation value of 5.9379 and standard error value of 1.3622 indicates that the data points lie closely around the mean of 5.4211.

Table 3.3: The total abundance of *Rhipicephalus (Boophilus) microplus* specimens collected in selected camps during field collections.

	March 2016		**April 2017**		**November 2017**		**April 2018**		Total		Total
Camp	Adults	Larvae	Adults	Larvae	Adults	Larvae	Adults	Larvae	Adults	Larave	Specimens
Church	2	*	2	*	*	*	*	*	4	0	4
Gavin Hill	2	*	*	*	18	1	*	*	20	1	21
Arthors Reservour	2	*	*	*	*	*	3	*	5	0	5
Vlei & Amos	1	*	*	*	*	*	*	*	1	0	1
Fish Dam	4	*	*	*	*	*	*	*	4	0	4
Gaalboom	*	*	1	*	*	*	*	*	1	0	1
Guava	*	*	15	1	*	*	*	*	15	1	16
Quarry	*	*	2	2	*	*	*	*	2	2	4
Milk Cow	*	*	3	6	*	*	*	*	3	6	9
Bushalt	*	*	11	2	1	2	*	*	12	4	16
Church Pregnant	*	*	1	*	*	*	*	*	1	0	1
Sheds	*	*	*	*	5	*	*	*	5	0	5
Lolweni	*	*	*	*	1	*	*	*	1	0	1
Stokweni	*	*	*	*	1	2	*	*	1	2	3
November	*	*	*	*	*	1	*	*	0	1	1
Morne Fir	*	*	*	*	*	1	*	*	0	1	1
Lands & Old Oranges	*	*	*	*	*	*	6	*	6	0	6
Barbers Dam	*	*	*	*	*	*	3	*	3	0	3
Milk Cow Pregnat	*	*	*	*	*	*	*	1	0	1	1
Total Number	11	0	35	11	26	7	12	1	84	19	103

* indicate camps where no collection was performed during that specific fieldtrip as they were not in use by the producer for the grazing of cattle.

3.3.4. Population Trend of *Rhipicephalus (Boophilus) microplus* 2016-2018.

Figure 3.7, illustrates the total number of *R. (B.) microplus* collected and identified during each field collection visit to the farm as well as the additional collections conducted by the producer over the course of the study period which is indicated in green. The breakdown of the producer's collections will be covered in the section which follows.

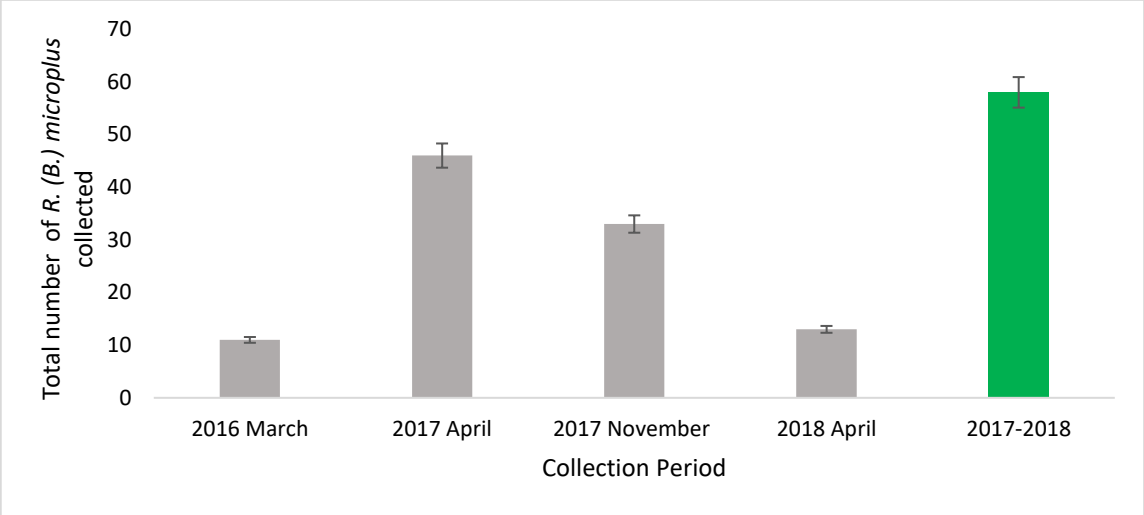


Figure 3.7: The total number of *Rhipicephalus (Boophilus) microplus* collected over the study period on Claypits, also indicating standard error.

During the 2016 collection which occurred in March, 11 *R. (B.) microplus* specimens were identified as seen in Figure 3.7. Of the 2368 specimens that were identified in 2016 *R. (B.) microplus* only represented 0.46% of the total blue tick collection seen in Table 3.4. The highest percentage, 2.42%, of *R. (B.) microplus* was collected from Fish Dam camp this was followed by 1.89% and 1.54% collected in Gavin Hill and the Vlei & Amos camps respectively. While *R. (B.) microplus* comprised of 1.07% and 0.93% of the collections obtained in Arthurs Reservoir and Church camps.

During the April 2017 collection a total of 46 *R. (B.) microplus* specimens were identified seen in Figure 3.7. This represented 2.39% of the total blue tick and larvae collection for this period seen in Table 3.4. The highest percentage, 5% of *R. (B.) microplus* was collected in the Church Pregnant camp this was followed by Gaalboom and Bushalt, in which 4.35% and 3.48% of the collection was *R. (B.) microplus*. Guava,

Quarry, Church and Milk Cow camps had the following *R. (B.) microplus* percentages; 2.91%, 2.35%, 2.20% and 1.29%.

The November collection yielded a total 33 specimens identified as *R. (B.) microplus,* 0.77% of the total blue tick collection seen in Figure 3.7 and Table 3.4. The highest percentages of *R. (B.) microplus* collected were found in Gavin Hill, 4.27%, Morne Fir, 4.17% and Sheds, 2.27%. Stokweni, Bushalt and Lolweni produced similar results with 1.60%, 1.58% and 1.45% of the collections being that of *R. (B.) microplus*. The collection in November only comprised of 0.55% of this species.

In 2018 0.3% of the total blue tick collection represented *R. (B.) microplus* with a total of 13 specimens being identified seen in Figure 3.7 and Table 3.4. This species was only identified in four of the camps sampled namely; Barbers Dam, 1.69%; Lands & Old Oranges, 1.27%; Arthurs Reservoir, 1.09% and Milk Cow Pregnant 0.51%.

Over the study period from March 2016 to April 2018, a total of 12823 *R. (B.) decoloratus* and 103 *R. (B.) microplus* specimens were collected and identified during the field trip visits. Thus, of the total blue ticks collected 0.80% of the total collection was represented by *R. (B.) microplus* seen in Table 3.4. The greatest percentage, 3.55% of this species was collected in Gavin Hill camp over the study period. This was then followed by Bushalt, 2.48% and Sheds 2.2% of the total collection. The following camps each represented the following portions of the total collection; Morne Fir, 1.69%; Stockweni, 1.57%; Vlei & Amos, 1.54%; Fish Dam, 1.43%; Barbers Dam, 1.38%; Lands & Old Oranges, 1.27%, Quarry, 1.19%; Arthurs Reservoir 1.08% and Guava 1.02%. Milk Cow, Church, Church Pregnant, Gaalboom, Lolweni, November and Milk Cow Pregnant all had values of below 1%.

Table 3.4: The percentage of *Rhipicephalus (Boophilus) microplus* collected in each camp during the field collection trips.

	March 2016			April 2017			November 2017			April 2018			March 2016 - April 2018		
Camp	***R. (B.) dec***	***R. (B.) mic***	***% R. (B) mic***	***R. (B.) dec***	***R. (B.) mic***	***% R. (B) mic***	***R. (B.) dec***	***R. (B.) mic***	***% R. (B) mic***	***R. (B.) dec***	***R. (B.) mic***	***% R. (B) mic***	***R. (B.) dec***	***R. (B.) mic***	***% R. (B) mic***
Milk Cow	312	0	0	687	9	1,29	405	0	0	853	0	0	2257	9	0,40
Church	212	2	0,93	89	2	2,20	92	0	0	60	0	0	453	4	0,88
Arthurs Reservoir	185	2	1,07	N/A	N/A	N/A	N/A	N/A	N/A	272	3	1,09	457	5	1,08
Fish Dam	161	4	2,42	N/A	N/A	N/A	N/A	N/A	N/A	114	0	0	275	4	1,43
Church Pregnant	152	0	0	19	1	5,00	4	0	0	N/A	N/A	N/A	175	1	0,57
Bushalt	80	0	0	361	13	3,48	187	3	1,58	N/A	N/A	N/A	628	16	2,48
Gavin Hill	104	2	1,89	N/A	N/A	N/A	426	19	4,27	40	0	0	570	21	3,55
Barbers Dam	32	0	0,00	1	0	0	8	0	0	174	3	1,69	215	3	1,38
Barbers Orchard	93	0	0,00	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	93	0	0
Vlei & Amos	64	1	1,54	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	64	1	1,54
Gaalboom	8	0	0	22	1	4,35	69	0	0	9	0	0	108	1	0,92
New Windmill	768	0	0	N/A	N/A	N/A	354	0	0	1237	0	0	2359	0	0
Quarry	144	0	0	164	4	2,38	14	0	0	11	0	0	333	4	1,19
Stokweni	3	0	0	N/A	N/A	N/A	185	3	1,60	N/A	N/A	N/A	188	3	1,57
Morne Fir	35	0	0	N/A	N/A	N/A	23	1	4,17	N/A	N/A	N/A	58	1	1,69
Furtree Ridge	1	0	0	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	1	0	0
Krans	1	0	0	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	1	0	0
Kens	2	0	0	N/A	N/A	N/A	N/A	N/A	N/A	6	0	0	8	0	0
Guava	N/A	N/A	N/A	533	16	2,91	803	0	0	209	0	0	1545	16	1,02
Sheds	N/A	N/A	N/A	N/A	N/A	N/A	215	5	2,27	7	0	0	222	5	2,20
Lolweni	N/A	N/A	N/A	N/A	N/A	N/A	68	1	1,45	36	0	0	104	1	0,95
School Pregnant	N/A	N/A	N/A	N/A	N/A	N/A	15	0	0	94	0	0	109	0	0
Singeni	N/A	N/A	N/A	N/A	N/A	N/A	27	0	0	N/A	N/A	N/A	27	0	0
November	N/A	N/A	N/A	N/A	N/A	N/A	180	1	0,55	208	0	0	388	1	0,26
Tembisile Dam	N/A	N/A	N/A	N/A	N/A	N/A	894	0	0	336	0	0	1230	0	0
Msenge & Red Grass	N/A	N/A	N/A	N/A	N/A	N/A	260	0	0	N/A	N/A	N/A	260	0	0
Lands & Old Oranges	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	465	6	1,27	465	6	1,27
Gumtree Dam	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	36	0	0	36	0	0
Milk Cow Pregnant	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	194	1	0,51	194	1	0,51
Total Number	**2357**	**11**	**0,46**	**1876**	**46**	**2,39**	**4229**	**33**	**0,77**	**4361**	**13**	**0,30**	**12823**	**103**	**0,80**

*N/A = indicate camps where no collection was performed during that specific fieldtrip, *R. (B.) dec = Rhipicephalus (Boophilus) decoloratus, R. (B.) mic = Rhipicephalus (Boophilus) microplus.*

3.3.4.1. Movement of Selected Herd

The three map diagrams indicated in Figure 3.8A-C, show the movement of the selected herd (Cows and Calves herd) as it was moved around the farm from camp to camp starting on the 24th April 2017 in Guava camp until the 9th April 2018 in Barbers Dam camp. The red colour indicates the camps in which *R. (B.) microplus* was collected in and the total number of the blue ticks identified is represented in Table 3.5. The cattle were moved according to decisions made by the producer based on water and food availability.

Over this period the Gaalboom camp was used the most, a total of seven times, Kens was used four times, New Windmill, School Pregnant, Lands and Old Oranges and Barbers Dam camps were used three times and the remaining camps were either used twice or once. It is important to note that, four collections were not taken by the producer over the year period due to low numbers of tick.

No samples were collected when the cattle were classified as “clean” as there were not many blue ticks present on the cattle seen in Table 3.5. Initially *R. (B.) microplus* was absent in Koekweni, Berts, Below Dougs house and Hiltons, these camps were not used again during the year as illustrated in Figure 3.8A.

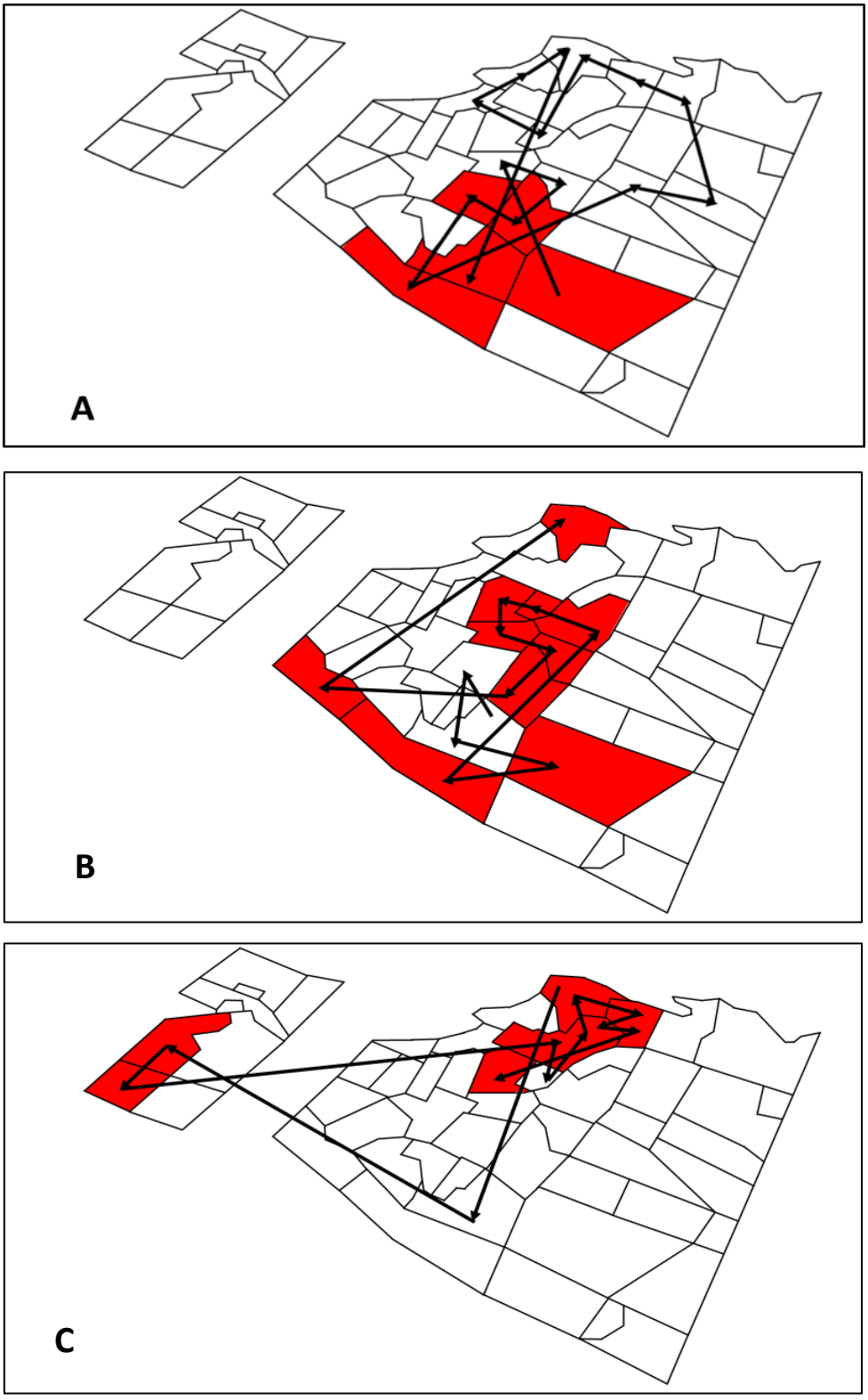


Figure 3.8: The movement of the Cows and Calves herd: **(A)** starting in the Guava camp on the 24th April 2017 and ending on the 15th August 2017 in New Windmill camp; **(B)** starting in the New Windmill camp on 15th August 2017 and ending on the 1st December 2017 in Gaalboom camp and **(C)** starting in the Gaalboom camp on 1st December 2017 and ending on the 9th April 2018 in Barbers Dam camp. Red indicated the camps where *Rhipicephalus (Boophilus) microplus* was found.

The total blue tick collections of the camps which the cattle were in as well as the total percentage of *R. (B.) microplus* in each collection is seen in Table 3.5. A total of 58 *R. (B.) microplus* was identified which represented 2.69% of the total collection.

Table 3.5: The schedule followed by the producer for the selected herd which was tracked from April 2017- April 2018 with the number of blue ticks collected.

Date In	Date Out	Camp	*R. (B.) microplus*	*R. (B.) decoloratus*	*R. (B.) microplus%*
2017/04/11	2017/04/24	Guava	16	127	11,19
2017/04/24	2017/05/09	Dads House + Barbers Orchard	0	23	0,00
2017/05/09	2017/05/23	Singeni + November	5	124	3,88
2017/05/23	2017/06/07	Tembisile Dam	3	69	4,17
2017/06/07	2017/06/19	Berts + Koekweni	0	98	0,00
2017/06/19	2017/07/07	Krantz+ Kens+ Gaalboom +School Pregnant	No Sample	No Sample	N/A
2017/07/07	2017/07/27	Hiltons + Below Dougs + Gaalboom	0	32	0,00
2017/07/27	2017/08/15	New Windmill + November	2	63	3,08
2017/08/15	2017/08/31	Guava	5	76	6,17
2017/08/31	2017/09/13	Tembisile Dam	4	65	5,80
2017/09/13	2017/09/28	School + School Pregnant	1	135	0,74
2017/09/28	2017/10/11	Barbers Dam + Dads House	1	26	3,70
2017/10/11	2017/10/25	Barbers Orchard	1	9	10,00
2017/10/25	2017/11/08	Singini	1	29	3,33
2017/11/08	2017/11/21	Bushalt	3	187	1,58
2017/11/21	2017/12/01	Gaalboom	1	107	0,93
2017/12/01	2017/12/11	New Windmill	No Sample	No Sample	N/A
2017/12/11	2018/01/12	Gumtree Dam + Arthurs Reservoir	1	106	0,93
2018/01/12	2018/01/22	Old Oranges + Lands	2	194	1,02
2018/01/22	2018/02/06	School Pregnant	No Sample	No Sample	N/A
2018/02/06	2018/02/22	Old Oranges + Lands + Gaalboom	3	132	2,22
2018/02/22	2018/03/06	Gaalboom + Kens	0	154	0,00
2018/03/06	2018/03/09	Gaalboom + Kens	5	231	2,12
2018/03/09	2018/03/22	Old Oranges + Lands + Kens	No Sample	No Sample	N/A
2018/02/16	2018/04/16	Barbers Dam + Old Oranges + Lands	4	109	3,54
		Total	**58**	**2096**	**2,69**

3.3.5. Effect of Temperature and Humidity on Larval Collection

Figure 3.9 shows the comparison between the total number of larvae collected in relation to the relative humidity and temperature recorded during the collections. Samples Res 16/20 (New Windmill), 17/04 (Milk Cow), 17/36 (Guava) and 17/37 (Tembisile Dam), the total larvae collected ranged from 600-1000 while the relative humidity ranged between 20-40%. This is a low humidity at which many larvae should not be active. When the humidity ranged between 95-70%, the following samples showed a rise in number of the larvae collected; Res 16/04 (Milk Cow), 16/05 (Church),18/02 (Milk Cow), 18/15 (Fish Dam), 18/21 (Milk Cow Pregnant), 18/22 (New Windmill), 18/23 (November), 18/28 (Tembisile Dam), 18/29 (Guava).

The lowest temperature during which collections were conducted at was 15°C, this occurred on two occasions and sample Res 16/04 (Milk Cow) contained 255 larvae whereas sample Res 18/25 (School Pregnant) contained 94 larvae. The highest temperature during the collections was 35 °C when in sample Res 16/12 (Morne Fir), 47 larvae were collected. While at 34°C, sample Res 16/10 (Jackals ridge) contained 2 larvae and at 33°C Res 16/09 (Fir Tree Ridge) three larvae were collected. Large peaks in the larvae collections occurred in samples Res 16/20 (New Windmill) at 32.5°C, 17/04 (Milk Cow) at 24 °C, 17/36 (Guava) at 23°C, 17/37 (Tembisile Dam) at 18.5°C, 18/02 (Milk Cow) at 27°C and 18/22 (New Windmill) at 26.5°C.

A correlation between the number of larvae collected and the relative humidity and temperature was determined in two separate scatter plots seen in Figure 3.10. The correlation coefficient for the temperature was -0.092, indicating that there was a very weak negative correlation between the temperature at the time of the collection and the total number of larvae collected. The relative humidity had a value of 0.061 which indicated a very weak positive correlation between the number of larvae collected and the relative humidity at the time of collection. P-values of 0.0005 and 0.0001 for the relationship between the number of larvae collected and the temperature and relative humidity, respectively were calculated. The null hypothesis was rejected due to a statistically significant difference

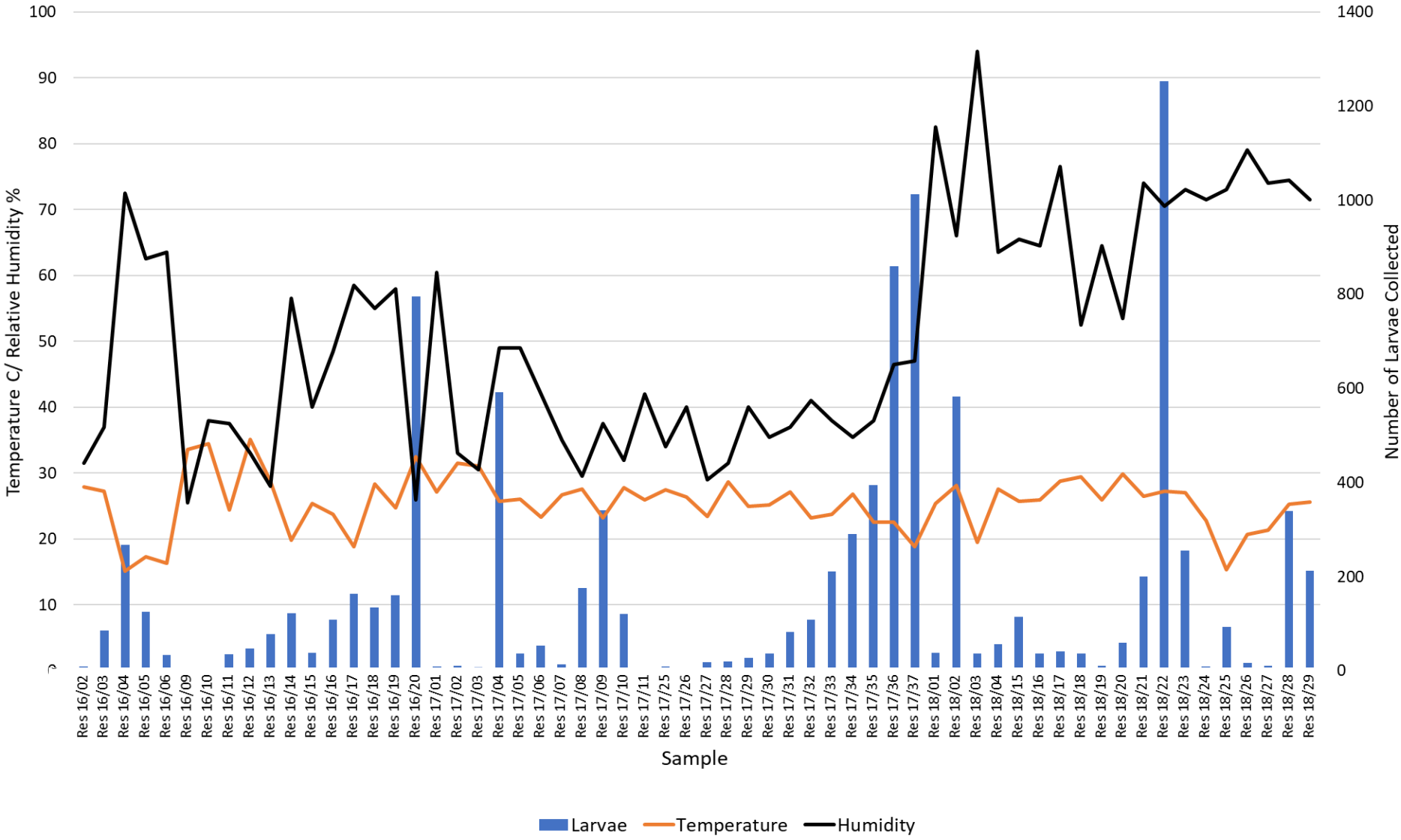


Figure 3.9: A comparison of the total larvae collected in relation to the relative humidity and temperature at the time of the collection.

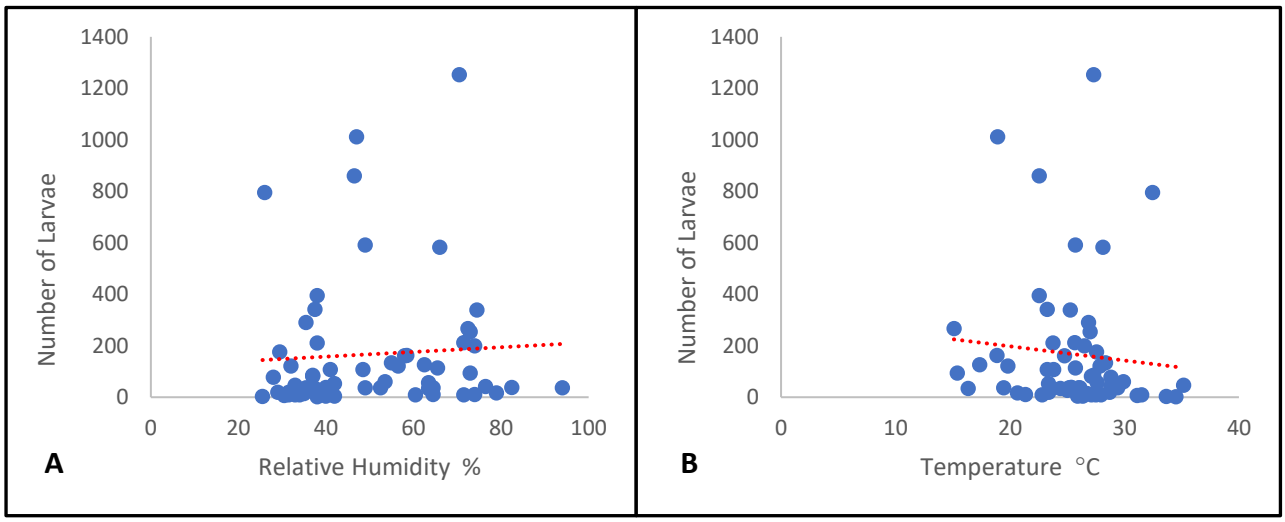


Figure 3.10: The linear correlation between the **(A)** relative humidity and **(B)** temperature to the number of larvae collected.

Table 3.6: Statistical analysis of the relationship between the temperature and relative humidity to the number of larvae collected.

	Temperature	**Relative humidity**
Correlation coefficient	-0.092	0.061
Z-value	-3,4875	-4,2527
p-value	0.0005	0.0001

3.4. Discussion

A thorough knowledge of tick species found in a specific area is of the utmost importance to be able to prepare for possible disease outbreaks and employ efficient control measures. In this study the two blue tick species were the focus species of the study, but other tick species which were present on the cattle were also collected and identified to determine the range of tick species present on the farm.

Amblyomma hebraeum, R. evertsi evertsi, I. pilosus, *H. truncatum* and *H. elliptica* comprised of the five other tick species collected and were present in 6.57%, 1.74%, 0.12%, 0.04% and 0.02% of the total number of ticks collected, respectively. These species were present in far lower numbers on the cattle and on the vegetation in relation to *R. (B.) decoloratus* with 89.45% although the Asiatic blue tick, *R. (B.) microplus* only comprised of 0.84% of the total numbers. The remaining 1.22%, only

identified as *Rhipicephalus (Boophilus)* species due to a lack of mouthparts to enable further identification, most probably was also part of *R. (B.) decoloratus* if the large number of *R. (B.) decoloratus* compared to *R. (B.) microplus* is taken into account. In a survey done by Horak *et al.* (2009), 11 Ixodid species were found in the eastern region of the Eastern Cape province of which *R. appendiculatus* was the most dominant species found on cattle followed by *R. evertsi evertsi, R. (B.) microplus, A. hebraeum*, and *R. (B.) decoloratus* in order of abundance*. Haemaphysalis elliptica* and *I. pilosus* were also collected in their study but not from cattle and they did not record any collections of *H. truncatum*. On cattle from the current study no *R. appendiculatus* was found in contrast with the survey conducted by Horak *et al*. (2009).

Yawa *et al.* (2018) determined that the vegetation of an area has an impact on the distribution of free-living ticks. During their study 10 different species were collected and identified; *R. (B.) decoloratus* (32.5%), *R. evertsi evertsi* (18.8%), *R. appendiculatus* (17.3%), *A. hebraeum* (16.3%), *R. simus* (7.7%), *I. pilosus* (3.8%), *H. rufipes* (3.5%), *R. follis* (0.08%), *H. elliptica* (0.04%) and *H. silacea* (0.02%). *Amblyomma hebraeum* and *R. evertsi evertsi* were find in high numbers in the Kowie thicket during summer, while *R. (B.) decoloratus* was the most abundant in the Bisho Thorn veld during summer and *R. (B.) microplus* was absent in this study area.

In the current study *A. hebraeum* (6.57%) was the second most predominant species identified on the farm and present in greater numbers than those of *R. (B.) microplus.* Known as the Southern African Bont tick this species is usually found in areas within the southern eastern regions of the African continent and along the eastern coastal belt of South Africa and it occurs in a variety of climatic conditions (Walker *et al.* 2003). It is a three-host species with adults found on large herbivores such as cattle, eland, buffalo, giraffe and rhinoceroses. They can also be found on sheep and goats. Immatures are sometimes found on the same host as the adults but are also known to feed on smaller antelope species, scrub hares and even tortoises.

Rhipicephalus evertsi evertsi (1.74%), the red legged tick, was the third most predominate tick species present on the farm, just ahead in numbers of *R. (B.) microplus*. This species is a two-host tick which is widely distributed over the African continent south of the Sahara and in South Arica in a variety of climatic conditions with

the exception of the Northern Cape Province. Adults are found on livestock such as cattle, sheep and donkeys, while immatures can be found on scrub hares and smaller antelope species (Walker *et al.* 2003).

Ixodes pilosus (0.12%), the sour-veld tick occurs in areas along the southern and eastern coastal belt of South Africa, particularly in areas which have sour-veld. This three-host species can be found on cattle, dogs, sheep, goats and wild ungulates (Walker *et al.* 2003).

Hyalomma truncatum (0.04%), is a two-host tick which is endemic to the Afrotropical geographical region and is found widespread over the African continent south of the Sahara. In South Africa this species is found throughout the country with the exception of southern parts of KwaZulu Natal, south- eastern Mpumalanga and Gauteng and the eastern half of the Cape and Free State provinces. Adults are found on large domestic herbivores such as cattle, horses, goats and sheep as well as wild herbivores. The immatures are found on rodents and hares and will also attach to humans (Walker *et al.* 2003).

Haemaphysalis elliptica (0.02%), the Southern African yellow dog tick was present in the lowest numbers during the study. This species is found to be wide spread over the African continent south of the Sahara. In South Africa it is found in the eastern part of the country all the way from the city of East London, through KwaZulu Natal up into Zimbabwe. It is also present in Limpopo, Mpumalanga, Gauteng, North West and north-eastern Free State. *H. elliptica* is found mostly on dogs and wild carnivores such as jackal, wild cats and wild dogs. Immature stages may be found on rodents or on the same host as adults. However, there have been many records of this species being found on cattle and livestock as a result of a close association between the main hosts and livestock (Walker *et al.* 2003).

Due to the location of the farm in the Southern parts of the Eastern Cape as well as the presence of hosts such as cattle, sheep, kudu, impala, wild hares, tortoises, dogs, rodents and jackal on the farm, all of the other tick species found on the farm have the ability to thrive and are also indigenous to the area. *Rhipicephalus (Boophilus) microplus* is the only invasive species found during this study.

Blue tick composition from collections on Claypits showed both *R. (B.) decoloratus* and *R. (B.) microplus* to be present over the course of the study period. *Rhipicephalus (Boophilus) decoloratus* was the dominant blue tick species and accounted for 14866, (89.45%) of the total ticks identified over the total collection period. This result was expected as the producer has reported many cases of large infestations of blue ticks on his cattle. *Rhipicephalus (Boophilus) microplus* accounted for only 138, 0.84% of the total ticks identified

In order to establish displacement of an indigenous species, the number and distribution of this species first needed to be established. *Rhipicephalus (Boophilus) decoloratus* specimens were collected in every single camp sampled throughout the study. The total number of adult specimens collected during one fieldtrip, ranged from a minimum of 55 from Lolweni during November 2017 to a maximum of 528 collected in Guava during the April 2017 collections. The highest overall adult collection occurred in April 2017 with 1039 *R. (B.) decoloratus* adults being identified. One of three outliers not included in this minimum and maximum numbers, are adult collection data from Gaalboom in April 2017 which only produced 20 specimens. Two weeks prior to the fieldtrip in April 2017 the farmer had to do an emergency treatment with acaricides due to heavy tick loads. A long lasting injectable, Ivermax Gold, was used on the cattle and as result this prevented many ticks from attaching to the cattle. Stokweni produced 15 adult specimens in November 2017 but was rested from August 2016 up until mid-October 2017, causing the number of larvae on the vegetation to decrease due to the lack of suitable hosts. When the producer then used the camp again, low adult tick loads were observed. Morne Fir was used for the grazing of three bulls at the time of the collection in November 2017. The limited number of hosts to sample from, resulted in the low number of specimens collected.

According to Walker *et al.* (2003), *R. (B.) decoloratus* is found all year round with larval peaks in early spring as the temperatures begin to rise and the rainfall returns. Nyangiwe *et al.* (2011) and Horak *et al.* (2016), also found that *R. (B.) decoloratus* showed a rise in the number of questing larvae in spring months from September to November which was accompanied by a rise in adult numbers to ensure maintenance of the population year-round in the Amatole district of the Eastern Cape and in the

Kruger National Park, respectively. This corresponds to the highest number of larvae, 3077, collected in November 2017, in the current study however the highest number of larvae, collected in a single camp was 1237 specimens found in the New Windmill camp, occurred in April 2018. Thus, although there was a reported seasonal peak in spring which was seen in the larval data for November 2017, this species can be found all year round as seen in the result from the New Windmill camp. Spickett *et al* (2011) found high numbers of *R. (B.) decoloratus* during December and January and again from March to July also indicating larval peaks during November and March/April in the North West province.

Marufu *et al.* (2010) found that higher tick loads were found in the dry- hot season compared to the dry-cold season with high humidity and temperature increasing the rate at which larvae emerge in the Chris Hani district in the Eastern Cape. It was reported that *R. (B.) microplus* feeds more successfully on cattle than *R. (B.) decoloratus*, it was also noted that greater numbers of *R. (B.) microplus* females complete their feeding cycle and mature than *R. (B.) decoloratus*. When *R. (B.) microplus* was present cattle seemed to have an increased immunity towards *R. (B.) decoloratus* which resulted in smaller blood meals and fewer eggs produced. In warm, wet areas *R. (B.) microplus* has a 3.5 times greater reproductive potential, however, in colder, dryer areas with wild ungulate hosts available *R. (B.) decoloratus* will potentially thrive.

As seen many climatic factors can influence the number of ticks found in field collections. In this study conducted near Makhanda, drags were performed mostly in late Spring or Autumn, camps that could be considered as outliers, where less than 10 larvae were found from drags, were Stokweni, Arthurs Reservoir, Gaalboom, Firtree ridge, Krantz and Kens observed during March 2016; Barbers dam, Gaalboom, Quarry and Guava in April 2017; Church Pregnant, Gavin Hill and Barbers Dam in November 2017 and Gaalboom, Kens, and Sheds in April 2018. The collection of larvae can be affected by more factors than the number of engorged adults on the host. Within a camp the distribution of the larvae is unknown, drags are usually conducted in areas which contain visual signs of cattle presence, such as dung or hoof prints. In some camps this is not always clear and drags are conducted at the discretion of the person doing the drag. Thus, it is possible to miss an area where a

lot of the larvae are present. Camps which have been spelled for prolonged periods also have a lower number of larvae due to lack of hosts.

Horak *et al.* (2016) stated that in their study in the Kruger National Park, the fluctuation in the number of larvae collected was influenced by the availability of hosts and climatic conditions as the parasitic phases are temperature dependant, with lower temperatures that prolong oviposition and emergence. Phalatsi *et al*. (2004), noted that the preoviposition, oviposition and the incubation period of the eggs produced where shortened with an increase in temperature, while humidity was found to have a negligible effect. *Rhipicephalus (Boophilus) decoloratus* larvae survived for 10-35 weeks after hatching and their survival was inversely dependant on the accumulation of temperature. Larvae that hatched in December or January survived for the shorted time period, while larvae that hatched at the end of April prior to the onset of the cool temperatures survived for the longest time period.

The vegetation in terms of the amount of shaded areas and suitable microenvironments available as well as climatic conditions such as rain, wind, temperature and humidity can have an effect on the number of larvae questing on the vegetation.

The collection of larvae occurred from 10am until as late as 5pm, with no collections occurring before or after this time. A wide variety of weather conditions were experienced during the collection of larvae from very hot and humid conditions to cold, windy and even at times misty rain. Scatterplots showed a weak correlation between the number of larvae collected and the temperature or humidity recorded. The p-values proved that the there was a great statistical difference, rejecting the null hypothesis that stated that there was a relationship between the two climatic variables and the number of larvae collected. There were a few cases where increase in humidity and decrease in temperature resulted in an increase in number of larvae collected, while a decrease in humidity and increase in temperature resulted in a decrease in the number of larvae collected.

Milk Cow camp was sampled during every single trip with both adult ticks and larvae being collected in large numbers. This camp was not rested once over the study period

and was in fact already in use prior to the start of the study. In addition to this there are many trees and bushy areas within the camp which can provide a perfect microenvironment for engorged adult females to lay their eggs. The high abundance of *R. (B.) decoloratus* in this camp could be linked to the frequency at which this camp has been used for the grazing of cattle. More collections from cattle and in the field of the other camps however needs to be conducted to statistically prove this statement. This camp is the only camp on the farm which is in constant use with a small herd comprising of the cows which are milked, their young calves and a single bull. Due to the constant host availability, the number of ticks increase as the life cycle can be completed at a quicker rate due to less time spent questing on the vegetation, waiting for a host.

The constant use of a camp can therefore be one of the key factors in influencing tick load and resting a camp removes a source of food i.e. the host. As a result, many larvae die out as they are unable to find a food source. Phalatsi *et al*. (2004), found that the maximum survival period for larvae exposed to the elements was 84 days. Although wild ungulates are also found on Claypits farm, the majority in terms of number of hosts available within a specific camp will be greatly reduced. This was also proved, on Claypits when during the November 2017 field trip, the producer noted that he had not used a specific camp, Stokweni for grazing from August 2016 to September 2017, he had then placed cattle in the camp and was pleasantly surprised by the low tick burden.

A comparison of the number of larvae collected in the Milk Cow camp to a camp such as Gavin Hill, which is also used for grazing on a regular basis, showed a major difference. Although larvae were found in the Gavin Hill camp on more than one occasion the total was far less (34 in 2018), than that of the 583 larvae found in Milk Cow camp in 2018. Besides the fact that is camp is not used year-round without resting, the vegetation is vastly different. This camp is mostly covered in open grassland with some trees and shrubs along the fence line. The type of vegetation present in the camps where the larval collections occurred therefore can also have an influence on the number of ticks present in the area. The farm covers both sweet veld with sparse vegetation and open grassland and sour veld with denser, taller vegetation. The tick loads on cattle in sweet and sour range lands in communal areas

of the Chris Hani district in the Eastern Cape was studied by Marufu *et al.* (2010). *Rhipicephalus (Boophilus) decoloratus* were found on both sweet and sour veld. In the dry hot season cattle grazing in the sweet range land had a lower tick burden than those found in the sour range land. In Zambia, Zieger *et al*. (1998), found that *R. (B.) decoloratus* larvae were found in greater numbers in areas with covered vegetation than in open grassland. Phalatsi *et al.* (2004), found that *R. (B.) decoloratus* larvae preferred to quest on taller substrates which links to the preferred host of larger bovids, this increases their chances of coming into contact with the chest or abdomen of the animals.

The question of how *R. (B.) microplus* found its way onto this farm remains unanswered. There are a few possible ways in which this could have occurred. The only new cattle brought onto the property are bulls, however, this does not happen frequently. Wild ungulates such as kudu, impala and reebok roam freely over the farm land and surrounding areas. Although it is rare, there have been records of *R. (B.) microplus* being found on these hosts (Walker *et al*. 2003). The possibility which carries the most weight is the fact that the cattle are driven through the area between the main farm and the separate piece of land. This land belongs to another cattle producer who had lost cattle due to Asiatic red water, only transmitted by *R. (B.) microplus* according to literature (Walker *et al*. 2003). Thus, the producer's cattle could have initially acquired *R. (B.) microplus* in this way. A final possibility for the introduction would be via humans. This species does not attach to humans however, it is possible that the larvae could have been on clothing or equipment that may have come into contact with *R. (B.) microplus* in another location.

Tick collections began on Claypits in 2008 on a year to two yearly bases. No *R. (B.) microplus* specimens were collected or identified until 2014 when five adult females and a single adult male were collected from cattle on the 10th of December (EMS van Dalen – Unpublished data). This herd had initially been in Gaalboom camp and had been transferred to Krantz and Kens camps where the collections occurred. These three camps lie next to each other on the Northern edge of the main farm. Tick collections were also conducted in Barbers Orchard, Sheds, Red Grass and Arthurs Reservoir during the 2014 collection however, no *R. (B.) microplus* specimens were found in any of these camps. Over the course of the current collections *R. (B.)*

microplus has been identified in Gaalboom in April 2017 (0.93% of total blue tick count) and in Gaalboom + Kens (2.12% of total tick count) as part of the farmer's collections in the beginning of March 2018.

Tønnesen *et al*. (2004), stated that displacement can occur within 4-10 tick generations and thus it is possible for complete displacement to occur within 1-3 years. This statement was supported from the results of a study conducted in the Eastern Cape by Nyangiwe *et al*. (2013b), who found *R. (B.) microplus* in high numbers at two communal farms where ten years prior to this study *R. (B.) decoloratus* was the only blue tick species found at both study sites.

Although *Rhipicephalus (Boophilus) microplus* was found in Gaalboom and Kens since its initial introduction in 2014, collections conducted within the camps in 2016, November 2017 and 2018 only contained *R. (B.) decoloratus* and no *R. (B.) microplus* specimens. It has been four years since the initial discovery of *R. (B.) microplus* however, from the data it shows that although the species are still be present in this area the number have not drastically increased and there are no signs of displacement as the African blue tick has proved to be dominating this area.

Of the 19 camps in which *R. (B.) microplus* specimens were collected, this species was only found on more than one occasion in four of the camps. In Arthurs Reservoir *R. (B.) microplus* was collected during the March 2016 and April 2018 collections, while the species was collected from Gavin Hill camp in 2016 and November 2017. *R. (B.) microplus* was found in both Church and Bushalt camps during two consecutive collections, 2016 and April 2017 in Church and April 2017 and November 2017 in Bushalt. From this it is clear that the species has not become well established in any of the camps which were sampled. This is further supported by looking at the percentage of the collection which *R. (B.) microplus* contributed to. The Church Pregnant camp in April 2017 obtained the highest result of 5% for a single collection but ending up at 0.57% overall for all collections. When looking at the result for the entire collection period Gavin Hill had the greatest collection percentage of 3.55% overall followed by Bushalt with 2.48%. These two camps were also the only camps where *R. (B.) microplus* were found during two out of four collections and need to be investigated further in follow up surveys.

The movement of *R. (B.) microplus* on the farm from the initial collection site can occur due to the frequent movement of cattle on the farm. The Cows and Calves herd was therefore tracked over the course of a year to determine that if *R. (B.) microplus* specimens *were* present in a camp, the movement of the herd would aid in the spread of the species. The parasitic stage on the cattle will move with the host and can drop off in a different camp. This can give *R. (B.) microplus* the opportunity to become established and thrive all over the farm. The producer moves the cattle around to different camps when food and water availability becomes scarce, this usually occurs after acaricide treatment approximately every fortnight. Acaricide treatment should prevent the distribution of ticks from one camp to another but with the presence of resistance as discussed in Chapter 5, distribution of *R. (B.) microplus* can still take place. There are four other herds of cattle present on the farm and these herds will also contribute to the movement of the species across the farm.

Over the year period Gaalboom and Kens were used the most for grazing but the results showed that these camps may not have favourable conditions for *R. (B.) microplus* to become established as this species was not found during three of the seven and one of four collections in this area on the farm. The collection from Guava camp showed the highest number of *R. (B.) microplus* for these collections with 11.19% during an April 2017 collection and 6.17% during August 2017. This together with Barbers Orchard that showed a 10% *R. (B.) microplus* presence during October 2017, might be the camps that needs to be followed up during future surveys. Overall throughout all of the remaining collections only very low numbers of *R. (B.) microplus* was identified again contradicting displacement of the African Blue tick. It was not possible to make a concreate conclusion from the movement results, another two or three years of data as well as a possibility of tracking all the herds on the farm would prove how *R. (B.) microplus* is being spread on the farm.

In South Africa many studies have been conducted on the distribution and displacement of indigenous species by *R. (B.) microplus*. An extensive study was conducted by Nyangiwe *et al*. (2017), with collections being made in the Northern Cape, Free State, Western and Eastern Cape provinces. Out of the 80 sample locations *R. (B.) microplus* was present at 64 of these areas while *R. (B.) decoloratus*

was found at 47 of these locations. Overall *R. (B.) microplus* accounted for 71.8% of the total collection while *R. (B.) decoloratus* accounted for 28.2% of the collection. *R. (B.) microplus* was found in all four provinces and is now found to be widespread throughout the coastal areas of the Eastern Cape as well as in the western areas of the Western Cape and north-eastern area of the Northern Cape. *R. (B.) microplus* was only present in one of the locations in the Free State. *R. (B.) microplus* was first recorded in King Williams town by Howard (1908). and since then became established in the Eastern Cape, KwaZulu Natal, Mpumalanga, Limpopo, Gauteng and North west provinces on grassland and savanna type vegetation. In the eastern region of the Eastern Cape during winter *R. (B.) decoloratus* larvae become inactive while *R. (B.) microplus* are still active (Nyangiwe *et al*. 2017). This is another contributing factor to why *R. (B.) microplus* would be able to complete more life cycles per year.

In the Soutpansberg region of the Limpopo province Tønnesen *et al*. (2004), found that of the blue ticks collected in this area, 6.6% were *R. (B.) decoloratus* while the majority, 93.4% were identified to be *R. (B.) microplus*. Prior to this study *R. (B.) microplus* had not been previously found in this area although in 1980-1985 farmers experienced outbreaks of Asiatic red water, which indicates that *R. (B.) microplus* must have been present in the area during that time period even though it was not recorded. A similar result occurred in a study conducted by Nyangiwe *et al*. (2013b), on communal grazing sites in the Eastern Cape near East London. It was found that *R. (B.) microplus* was high in numbers at both sites with an even higher number of *R. (B.) microplus* being collected during the second year of sampling.

Horak *et al.* (2009), documented that *R. (B.) microplus* was the dominate species found in the eastern regions of the province which were previously dominated by *R. (B.) microplus.* Complete or partial displacement had occurred in this area. In comparison to these studies, the current study on a farm located more to the western regions of the Eastern Cape Province, displacement of the indigenous species does not seem to be occurring yet. A possible reason for this could be linked to the fact that Claypits is a commercial beef producer who has the funds and access to adequate forms of chemical control. During the study conducted by Tønnesen *et al*. (2004), it was noted that at the communal dip tanks in the Soutpansberg region of the Limpopo, *R. (B.) microplus* was the dominate species present whereas on the commercial farms

R. (B.) decoloratus was the dominant tick species present. Almost complete displacement was observed at the communal dip tanks. He found that displacement progressed at a slower pace on the commercial farms however a greater number of *R. (B.) microplus* collected at the end of the two-year survey was reported. Thus, if the farmer continues to use acaricides at the correct dosage and monitors acaricide resistance on the farm it could be a possible method of slowing down the establishment of *R. (B.) microplus* on the farm.

Rhipicephalus (Boophilus) microplus is not displacing *Rhipicephalus (Boophilus) decoloratus* over the entire country like it has in the Eastern Cape. Horak *et al*. (2015), conducted a study on the distribution of tick species in the Free State. *R. (B.) decoloratus* was found in the centre and eastern regions while *R. (B.) microplus* was only found at a location in the eastern part of the province with a total of 10 specimens being collected. Nyangiwe *et al*. (2017), speculated that *R. (B.) microplus* is mostly found in the eastern and northern Grassland and Savanna regions of the country. To prove this statement ticks were collected from locations in the Eastern, Northern and Western Cape. *R. (B.) microplus* was found in 51 of 53 of the Albany thicket locations sampled in the Eastern Cape with *R. (B.) microplus* larvae accounting for six times the *R. (B.) decoloratus* larvae collected in the western locations sampled in this province. Possible hybrid larvae were identified at 20 of the locations sampled in the Eastern Cape. In the Northern Cape *R. (B.) microplus* was found at eight of the 18 locations while *R. (B.) decoloratus* was found at 10 of the locations sampled with both species co-existing in the north-eastern region of this province. This was the first study to identify *R. (B.) microplus* in the Northern Cape which suggests that the introduction may have been recent. In the Western cape *R. (B.) microplus* was found at four of the locations while *R. (B.) decoloratus* was found at three of the locations. In the western cape it was suggested that *R. (B.) microplus* has a patchier distribution due the predominate shrub vegetation in relation to grassland and savanna.

In the current study a factor that could have played a role in the lack of displacement of *R. (B.) decoloratus* by *R. (B.) microplus*, is that South Africa is currently facing a drought that has been on-going for four years. Although daily temperatures and rainfall figures were not collected during this study this could have contributed to making the environment less attractive for *R. (B.) microplus* to thrive.

3.5. Conclusion

From all the data which has been collected on the farm over the past three years and even prior to this study it is possible to conclude that displacement of *R. (B.) decoloratus* by *R. (B.) microplus,* has not yet occurred on the farm. It would appear that the occurrence of *R. (B.) microplus* on the farm can still be considered a recent introduction and therefore the species is still only occurring in certain parts and not over the entire farm since it was initially identified. Therefore, these results are in disagreement with other studies in particular the findings from Limpopo and Eastern Cape Provinces. Many factors can cause the establishment and rate of the displacement, with one of the major influencing factors being the management practices of the farm. The fact that the farm is a commercial farm, a closed farming system is in use and the producer has a greater control over the movement and acaricide treatment of the herds, may be part of the reason for the possible delay in displacement. Within the context of this study, further research is required to determine whether the invasive tick is establishing itself or not under the reported farming conditions.

3.6. Recommendations

It is recommended that epidemiological survey to be taken seriously especially during this climate change period where species are invading new territories. This include, among other things, grazing strategies (taking into account drought impact) to lower tick infestation on and off-host. This will assist to reduce mortalities related to ticks and tick-borne disease and it is also advisable to do awareness campaigns about this serious pest, *R. (B.) microplus* which transmits more virulent strain than the indigenous tick, *R. (B.) decoloratus*.

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CHAPTER 4

Genetic Identification of Larvae

4.1. Introduction

Morphological identification has been used since scientists began to identify and classify organisms. Over the years, keys have been drawn up to aid scientists in the identification of various organisms based on morphological features which are unique to the specific species. This form of identification is more practical and can be done in the field in a shorter amount of time. However, morphological identification does have many drawbacks including the misidentification of organisms. Immature life stages and damaged organisms may be missing key morphological traits and closely related species can be difficult to distinguish.

In our study, the focus blue ticks we have chosen to name *R. (B.) decoloratus* and *R. (B.) microplus,* were originally known as *Boophilus decoloratus* and *Boophilus microplus*, respectively. However, morphological and molecular evidence synonymised them with *Rhipicephalus* genus (Beati & Keirans 2001; Murrell, Campbell & Barker 2000), thus they were included in a valid tick names by Horak *et al.* (2002) as *R. (B.) decoloratus* and *R. (B.) microplus*. Unlike Guglielmone *et al.* (2010) who omitted the subgenus, we chose to include the subgenus in our nomenclature in the current study. To avoid confusion with name changes, *Boophilus* was reassigned as a subgenus (Ali *et al*. 2016).

The need for molecular identification to complement morphological identification was studied by Berry (2017), in order to differentiate between *R. (B.) microplus* and *R. australis*. When looking at the morphological characteristics alone it is impossible to differentiate between the two species, causing *R. australis* to be placed under *R. (B.) microplus* throughout the past two decades as it was thought to be the same species. With the aid of molecular techniques, it was found that the they are in fact two distinct species. Morphological differences were found to be too variable and thus an unbiased identification is not always possible. However, with the aid of molecular analysis it is possible to make a positive distinction between the two species.

Lempereur *et al.* (2010), were the first to develop and validate a PCR-RFLP test which can be used for the identification of *R. (Boophilus)* ticks. The ticks which were used in the study all originated on the African content, thus, this test showed consistent results between different countries in Africa. Further research needs to be conducted to see if this test would also work on the Australian and Latin American strains of *R. (B.) microplus,* as there have been reports that the strains are genetically different on the three continents.

In the current study the identification of *R. (B.) decoloratus* and *R. (B.) microplus* larvae was initially done using morphological character traits described by Arthur & Londt (1973) and Londt & Arthur (1975). The identification was based on the presence of a spur on coxa 1 as well as the shape of the scutum. It was often difficult to determine if the spur was present or not due to the small size of the larvae. In order to confirm that the identification of immature stages was done correctly, a sample of the total larval collection were also identified by making use of molecular identification techniques.

The objective which was determined in this chapter was as follows:

- To conduct Polymerase Chain Reactions, (PCR) and Restriction Fragment Length Polymorphisms, for identification of the larvae to complement and confirm the morphological identification.

4.2. Methods & Materials

4.2.1. DNA Extraction

An adaption of a cetyltrimethylammonium bromide (CTAB) DNA extraction method supplied by the department of Plant Sciences at the University of the Free State was used for the extraction of DNA from the larvae collected and identified via morphological characteristics.

Approximately 70 larvae, morphologically identified as *R. (B.) decoloratus,* were place into pre-labelled 2ml Eppendorf tubes in triplicate to be crushed in 50ul 10% CTAB

buffer for DNA extraction. The same was done for larvae morphologically identified as *R. (B.) microplus* and a mixture of 70 *R. (B.) decoloratus* and 70 *R. (B.) microplus*.

The tubes were filled to 500 µl of 10% CTAB buffer and the samples were incubated at 55˚C for 5 hours. The content of each tube was extracted once with 500 µl chloroform (ChCl3) / isoamylalcohol (IAA) (24:1 v/v) and centrifuged at 12 000 g for 10 min at 4ºC. DNA was precipitated from the aqueous phase with 500 µl isopropanol. The sample tubes were incubated at room temperature for 20 min and centrifuged at 12 000 g for 5 min at 4ºC. All the liquid was drawn off using the water-pump and the precipitate was washed at room temperature by adding 500 µl ice-cold 70% (v/v) ethanol. The sample tubes were once again incubated at room temperature for 20 min and centrifuged at 12 000 g for 5 min at 4ºC. All of the liquid was drawn off and the pellet was left to air dry for 1 hour at room temperature. Once dry, the pellet was resuspended in 50 µl TE buffer overnight at 4ºC.

The following morning 2 µl RNase A (10 mg/ml) was added to each sample and incubated at 37ºC for 1 – 2 hours. The DNA was extracted with 20 µl 7.5 M ammonium acetate and 200 µl ChCl3 / IAA (24:1) and tubes were placed into the centrifuge at 12 000 g for 10 min at 4ºC. DNA was precipitated from the aqueous phase at -20ºC with 500 µl ice-cold 100% ethanol overnight.

The next morning tubes were centrifuged for 15 min at 12 000 g at 4ºC and the liquid was drawn off. The pellet was washed twice with 500 µl ice-cold 70% (v/v) ethanol by centrifuging at 12 000 g for 10 min at 4ºC each time. All liquid was drawn off and the pellet was air dried. The pellet was then dissolved in 50 µl TE at 37ºC for 2 hours. The purity and concentration of DNA extracted from the larvae was determined using a Thermo Fisher Scientific NanoDrop 2000/2000c prior to the amplification step.

Table 4.1: Solutions used in the extraction of DNA during the CTAB method.

1 M Tris pH 8.0	
Tris	60.5g
Water	500ml
0.5 M EDTA pH 8.0	
EDTA-Na.2H2O	93g
Water	500ml
5 M NaCl	
NaCl	146.1g
Water	500ml
10% CTAB	
CTAB	10g
Water	100ml
CTAB extraction buffer	
1 M Tris-Cl (100 mM)	2ml
0.5 M EDTA (20 mM)	0.8ml
5 M NaCl (1.4 M)	5.6ml
10% CTAB (2%)	4ml
β-mercapto-ethanol (0.2%)	1ml
PVP (1%)	0.2g
Water	7.56ml
$ChCl_3$ / IAA	
ChCl3 (24 parts)	96ml
Isoamylalcohol	4ml
70% Ethanol	
Ethanol	70ml
Water	100ml
TE	
1 M Tris-Cl (10 mM)	1ml
0.5 M EDTA (1 mM)	0.2ml
Water	100ml
7 M Ammonium acetate	
Ammonium acetate	57.8g
Water	100ml

4.2.2. PCR – Polymerase Chain Reaction

The KAPA2G Robust Hot-Start PCR Kit, sourced from Merck South Africa, was used to amplify the DNA extracted from the larvae.

4.2.2.1. Preparation of Master Mix

All the reagents were completely thawed and properly mixed before beginning with the preparation of the master mix. The reaction mixture contained the components at the specified volume represented in Table 4.2, seen below.

Primers used; BOOPHITS2 (Forward) – GCC-GTC-GAC-TCG-TTT-TGA and BOOPHITS2 (Reverse) – TCC-GAA-CAG-TTG-CGT-GAT-AAA, were obtained from Sigma-Aldrich now known as Merck.

Table 4.2: Components used to make up master mix.

Component	Volume (µL)
PCR-Grade Water	Up to 25 µl
5X KAPA2G Buffer B	5.0 µl
5X KAPA Enhancer 1	5.0 µl
10 mM KAPA dNTP Mix	0.5 µl
10 µM Forward Primer	1.25 µl
10 µM Reverse Primer	1.25 µl
Template DNA	1-100 µl
5 U/µL KAPA2G Robust HotStart DNA Polymerase	0.1 µl

4.2.2.2. Set Up of Individual Reactions

The following volumes of master mix containing the primers were added to each sample DNA template in individual PCR tubes as represented in Table 4.3, and centrifuged briefly.

Table 4.3: The volume of components added to each sample of extracted DNA.

Sample	DNA Template (µL)	Master Mix (µL)
Decoloratus 1	0.9	24.1
Decoloratus 2	9.1	15.9
Decoloratus 3	11.1	13.6
Microplus 1	10.1	14.9
Microplus 2	11.5	13.5
Microplus 3	11.5	13.5
X-Combination 1	11.4	13.6
X-Combination 2	11.5	13.5
X-Combination 3	10.6	14.4

4.2.2.3. Polymerase Chain Reaction

The cycling protocol used to run the PCR through the denaturation, annealing and extension steps is represented in Table 4.4.

Table 4.4: The PCR cycling protocol.

Step	Temperature	Duration	Cycles
Initial Denaturation	95°C	3 min	1
Denaturation	95°C	15 sec	
Annealing	60°C	15 sec	40
Extension	72°C	45 sec/kb	
Final Extension	72°C	1min/kb	1

4.2.3. Restriction Enzymes

Restriction enzymes were used to digest the PCR product. This was done by mixing 4µl amplified DNA and Msp1 enzyme (6 U) to total volume of 15µl. The DNA was left to digest at 37°C overnight.

4.2.4. Gel Electrophoresis

4.2.4.1. Preparation of 2% Agarose gel

To prepare a 2% agarose gel, 105ml TBE Buffer (Tris-borate-EDTA) and 2.10g agarose powder was added into an erlenmeyer flask and boiled in a microwave until all of the agarose was dissolved. Distilled water was added to compensate for the evaporation by filling the erlenmeyer flask back up to the original mark. Before the gel was poured into the 15x15 cm tray it was cooled to about 55°C. Ethidium bromide was added to obtain a 0.5ug/ml concentration and the mixture was swirled gently until it was well mixed.

4.2.4.2. Preparation of Gel Tray

Rubber stoppers were placed at either end of the gel casting tray and it was placed on a level surface with a black background. The comb was adjusted so that it rested level on the casting try. The agarose gel was poured onto the casting tray, all of the bubbles were removed in the gel by sliding a spatula along the bottom of the tray. The comb was inserted and the gel was left to set for an hour, once set the rubber stoppers and comb were removed carefully in order to avoid damaging the gel. The plastic tray containing the gel was then placed into the electrophoresis tank with the wells closest to the negative electrode while being submerged in 1x TBE. DNA is negatively charged and will thus run from the negative to the positive electrode.

4.2.4.3. Running of the Gel

To each 10 µL sample, 5 µl of loading dye; bromophenol blue was added. While 10 µl of the loading dye was added to the 50 base pair DNA ladder (N3236). All samples and the 50-1350 base pair sized ladder were briefly centrifuged to concentrate the DNA at the bottom of the tube. 15 µl of each sample and the ladder were then carefully added into predetermined wells in the gel. Once all of the wells were filled the cover was placed on the tray and the power supply was turned on. The gel was run at 80mA for 3 and a half hours, the position of the DNA was monitored throughout this time period, by removing the gel and observing the position of the DNA bands in a Vilber

Lourmat UV trans-illuminator. Once the DNA had migrated a sufficient distance the power was turned off and the safety cover was removed.

4.2.5. Observation of Bands

The DNA was visualised on a UV transilluminator and photographed by the camera attached to the transilluminator. The surface of the UV lamp was cleaned with a paper towel and the gel was disposed of into a biological waste container for used gels. A programme called E-CAPT version 14.1 was used to ensure that the bands which were photographed were the bands of the sample which was tested and not from other sources such as contamination which may be present on the gel.

4.3. Results

The results from the PCR as seen in Figure 4.1 and Table 4.5 confirm the morphological identification of the larvae as *R. (B.) decoloratus* and *R. (B.) microplus*. The sample mixture of *R. (B.) decoloratus* and *R. (B.) microplus*, clearly showed restriction fragments of both species. The sample displayed restriction fragments which were unique to each, *R. (B.) microplus*; 450bp and 250bp, *R. (B.) decoloratus*; 350bp, 150bp and 50bp and both *R. (B.) microplus* and *R. (B.) decoloratus* shared; 100bp.

The lanes which were empty did not contain a high enough concentration of DNA and as a result did not show up on the gel. The DNA may have been lost during the amplification and/or gel electrophoresis steps.

Lanes 4, 13 and 14 seen in Figure 4.1 contained the remains of the ladder and samples from the previous run. The previous run was used to determine the timing of how long it took the ladder and samples to split without smudging or running off of the gel.

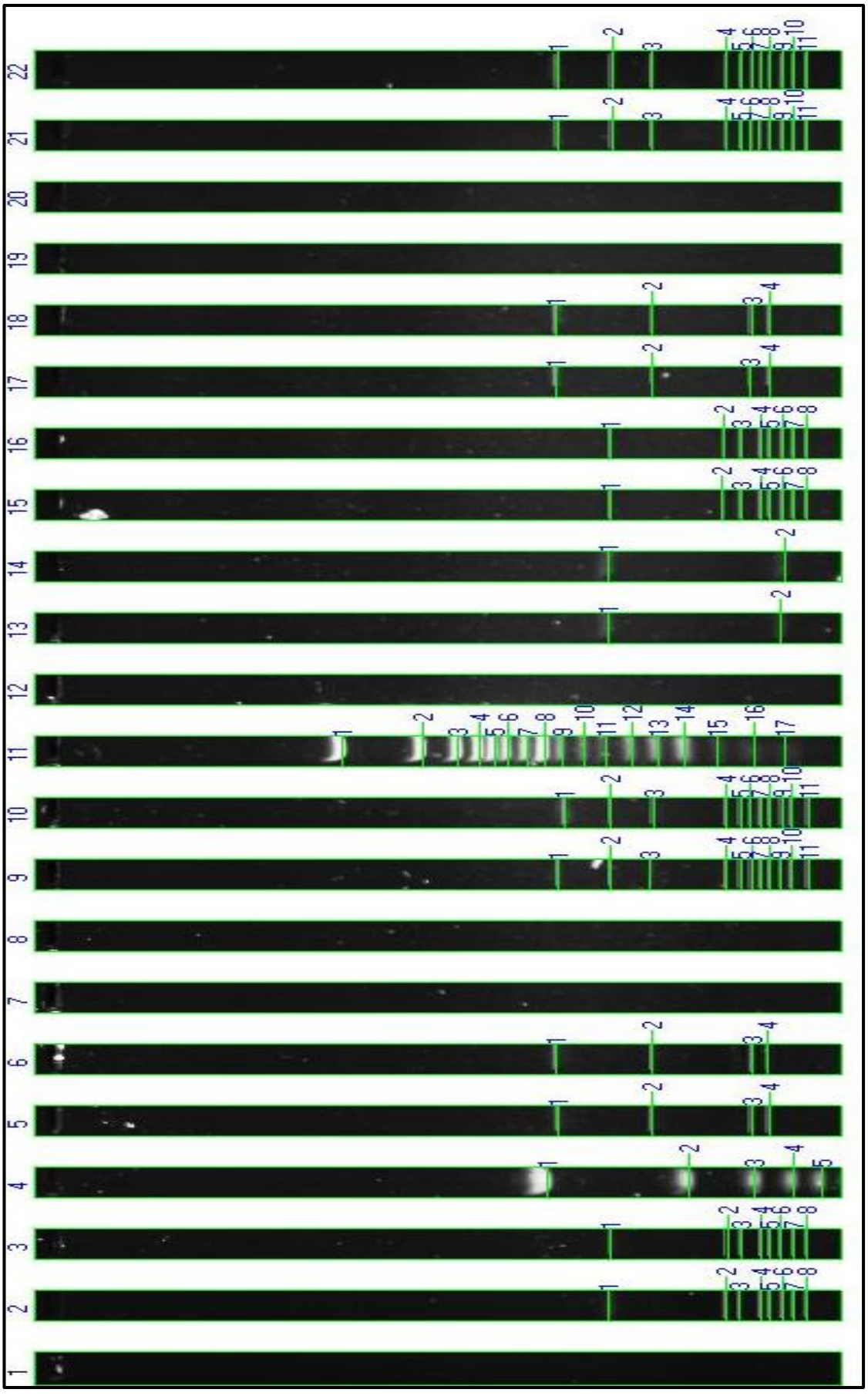

Figure 4.1: DNA bands observed and marked.

Table 4.5: Table of the base pairs identified from the bands seen in Figure 4.1.

Lane	Base Pairs	Identification
1	-	Empty
2	1-350bp, 2-150bp, 4-100bp, 7-50bp	*R. (B.) decoloratus* 2a
3	1-350bp, 2-150bp, 4-100bp, 7-50bp	*R. (B.) decoloratus* 3a
4	-	Old Ladder
5	1-450bp, 2-250bp, 3- 100bp	*R. (B.) microplus 2a*
6	1-450bp, 2-250bp, 3- 100bp	*R. (B.) microplus 3a*
7	-	Empty
8	-	Empty
9	1-450bp, 2-350bp, 3-250bp, 4-150bp, 6-100bp, 10-50bp	Mixed sample 2a
10	1-450bp, 2-350bp, 3-250bp, 4-150bp, 6-100bp, 10-50bp	Mixed sample 3a
11	See Figure 4.1.	Ladder
12	-	Empty
13	-	Old samples
14	-	Old samples
15	1-350bp, 2-150bp, 4-100bp, 7-50bp	*R. (B.) decoloratus* 2b
16	1-350bp, 2-150bp, 4-100bp, 7-50bp	*R. (B.) decoloratus* 3b
1	1-450bp, 2-250bp, 3- 100bp	*R. (B.) microplus* 1b
18	1-450bp, 2-250bp, 3- 100bp	*R. (B.) microplus* 2b
19	-	Empty
20	-	Empty
21	1-450bp, 2-350bp, 3-250bp, 4-150bp, 6-100bp, 10-50bp	Mixed sample 2b
22	1-450bp, 2-350bp, 3-250bp, 4-150bp, 6-100bp, 10-50bp	Mixed sample 3b

4.4. Discussion

Morphological identification of *R. (B.) decoloratus* and *R. (B.) microplus* larvae in this study was successfully confirmed with the use of PCR and restriction fragments caused by the enzyme Msp1 making the use of morphological identification still valid for distinguishing between closely related species. The results observed in this study were similar to those found by Lempereur *et al.* (2010). Kamani *et al.* (2017), conducted a similar study using both morphological and molecular identification of *R. (B.) microplus* in Nigeria. Molecular identification of *Amblyomma*, *Hyalomma*, *Rhipicephalus* and *R. (Boophilus)* was done by a method which targets the ITS-2

region to confirm the morphological identification. DNA in their study was extracted by using the Ilustra tissue Kit.

Nyangiwe *et al*. (2017), reported possible hybrid larvae at 20 locations sampled during a study conducted in the Eastern Cape Province. They also previously found larvae which displayed morphological characteristics of both blue tick species (Nyangiwe *et al*. 2013). During this study *R. (B.) microplus* males were found mating with *R. (B.) decoloratus* females on 17 occasions, however no cases of *R. (B.) decoloratus* males mating with *R. (B.) microplus* females were recorded. Molecular identification techniques can prove to be valuable in such instances to identify a possible hybrid of the two species in order to establish for certain if a hybrid species does exist.

4.5. Conclusion

Morphological identification of especially larvae needs expertise and someone not familiar with and practised in the identification of these small individuals might make a wrong identification, making molecular investigation a more reliable tool although molecular expertise will be required. The use of molecular techniques for the identification of ticks should be considered especially in areas where closely related species are found. The results showed that it will also be possible to identify both species if present in a mixed sample although this data will not be quantitative as only the presence or absence will be determined. Molecular identification has proved to be a vital tool in the use for identification of species and will continue to be essential in studies which have a taxonomic component.

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CHAPTER 5

Acaricide Resistance Profiles

5.1. Introduction

The domestication of cattle caused an ongoing battle in the control of ticks. Over the span of a century various dip remedies or acaricides, consisting of different chemical groups have been produced. Cattle producers want a quick and effective solution to tick control and have thus relied heavily on the use of chemical control in the past (Abbas *et al.* 2014). Acaricides offer a short-term suppression of the tick population, but long-term usage results in the development of resistance due to miss-use or over use of the products. Resistance can be defined as the ability of a population to survive and reproduce despite the administration of a chemical which is applied within the recommended dosage range or higher. It can be categized into three types; acquired resistance, cross resistance and multi resistance.

Acquired resistance develops when there is a loss in sensitivity towards the mode of action of the acaricide over time due to heritable traits passed from the female to the offspring (Chapman 1997; Meyer *et al.* 2012; Abbas *et al.* 2014). Selection pressures favour the ticks which have resistant genes thus when an acaricide is used for a prolonged period of time the susceptible ticks will be killed, leaving the resistant members of the population to thrive. There is a well-defined link between the degree of resistance and the concentration of the chemical being used (Mitchell 1996).

Cross resistance occurs when two acaricides with a similar mode of action are used, causing resistance to develop towards both acaricides. An example of this occurred between organophosphates and carbamate based acaricides (Li *et al.* 2005; Abbas *et al.* 2014). Both act by inhibiting the acetylcholinesterase (AChE) enzyme, disrupting the functioning of the tick's nervous system. The mechanism of resistance for both chemical groups, is the insensitivity of the AChE enzyme (Dawker *et al.* 2013; Abbas *et al.* 2014). Before the rotation or switching of acaricides it is important to ensure that the mode of action is not similar in order to avoid the development of cross resistance.

Multi resistance occurs when acaricides with different modes of action are no longer effective. The mechanisms for this type of resistance are thought to be both target site and metabolic mutations and is the worst type of resistance as it limits the usage of different acaricides. This type of resistance has been reported against the strains of *R. (B.) microplus* present in the southern parts of Mexico showing resistance towards six different chemical groups (Foil *et al.* 2004; Abbas *et al.* 2014).

In order for resistance to develop it needs to be established within the genetic make-up of the population. The genes which encode for the development of resistance are present at very low levels within the alleles. As the selection pressure grows there will be an increase in the frequency of these genes and over time this creates a resistant population. The length of time that it takes for a resistant population to emerge differs between populations as it is also dependent on factors which include; the dominance of the resistant alleles, genetic diversity within the population, the fitness of the resistant ticks, the frequency and concentration of the acaricide treatment and the percentage of the population in refugia (Mulchandani *et al*. 1998; Abbas *et al.* 2014). At the point where the acaricide treatment fails, the resistant genes will then be at a high frequency throughout the entire population.

In this chapter resistance profiles for three different chemical groups, Amidines, Synthetic Pyrethroids and Organophosphates were investigated for various camps on the farm to obtain the following objectives:

- To determine resistance development and compile the resistance profile for the blue tick populations towards Amidines in each camp where collections were conducted.
- To determine an overall acaricide resistance status of the farm towards three different chemical groups.
- To determine if multi host tick species show resistance to the acaricide used by the producer.
- To provide recommendations for future treatment plans which the producer could implement.

5.2. Materials & Methods

5.2.1. Treatment Strategies Used by the Producer

A combination of Milbatraz and Drastic Deadline Extreme has been used since September 2007 to dip cattle on the farm. Ivermax has only been used for the past six years as an emergency treatment when outbreaks of heavy tick burdens occur. Calves are treated with acaricides from the age of one month old.

5.2.1.1. Milbatraz

The producer treats his cattle every fortnight since September 2007 with a Bayer product called Milbatraz, containing 12.5% m/v Amitraz that can also control ticks, lice and mites on cattle. Approximately 26 dip treatments, either via the spray race or by hand spraying are done per year. These treatments are usually conducted over a period of three days in order to dip all the herds present on the farm. It is for external usage only and animals should not be killed within seven days of treatment. This dip should not be mixed with other acaricides and needs to be prepared freshly before each treatment. The manufacturer recommends that one part of Milbatraz is mixed with 500 parts water. This chemical is unfortunately toxic to bees and fish.

5.2.1.2. Drastic Deadline Extreme

Drastic Deadline Extreme is a pour-on acaricide which is used by the producer twice a year, usually at the end of November and again in March. This acaricide is an endoparasite and blue tick development inhibitor for use on cattle. It contains both an insect growth regulator; Fluazuron 2.5% m/v and a synthetic pyrethroid; Flumethrin 1.0% m/v. The synthetic pyrethroid offers rapid elimination of the ticks on the cattle while the growth regulators offer a long-term suppression of the population by inhibiting the development and egg production of the ticks which it is applied to. Due to the dual action it can be used on blue tick populations which are resistant to amidines, synthetic pyrethroids and organophosphates. This acaricide also controls tsetes flies and red lice on cattle, should not be diluted and the recommended application is 1ml per 10kg. This acaricide is also toxic to bees, fish and certain aquatic

invertebrates. The residual activity of this acaricide is estimated to last for up to 12 weeks after application.

5.2.1.3. Ivermax

Ivermax is used when heavy tick loads occur, usually only to the animals which show the greatest degree of infestation within the herd, this is only done three to four times per year. The producer uses Ivermax 1%, a macrocyclic lactone, applied in the form of an injection at 1ml per 50kg. In addition to ticks this product controls gastrointestinal roundworms, lungworms, grubs, sucking lice and mange mites. This product is derived from avermectains which was isolated from the fermentation of *Streptomyces avermitilis*. The effects persist for up to 28 days after application. This product is toxic to fish and certain aquatic species.

5.2.2. Application Methods

The application methods used on the farm include both hand spraying and the use of a spray race. Plunge dipping stopped over a decade ago, once the spray race was constructed. Drastic Deadline Extreme is a pour-on acaricide and is not diluted before use. Ivermax is an injectable, the required amount is drawn up in a syringe and injected into the rump of the animal.

5.2.2.1. Spray Race

The dip remedy, Milbatraz is not completely soluble in water, thus prior to starting the motor for the spray race the producer uses a large stick to stir up the dip solution already in the sump tank, in addition he measures the amount of liquid in the tank with the stick. After consulting his ‘dip book’ which contains recordings of all the measurements of the dip tank prior and after treating, it is possible to tell if more water has entered the system i.e. the level has increased or if water has left the system via evaporation. Based on this, the amount of Milbatraz and that needs to be added is calculated and then added. Once again, the mixture is stirred thoroughly to ensure that the dip particles are suspended evenly throughout the liquid. This is very important

as the tank contains dirt and dung particles that entered during previous dipping sessions, these particles are heavy and sink to the bottom often taking the chemical particles with them.

The location of the spray race was selected as it was not close to a water source and was not at risk of being flooded when heavy rain occurred. All nozzles are checked prior to dipping to ensure that none are blocked. The floor of the spray race, footbath as well as the walk ways leading to and from the race are cobbled in order for the hooves to spread out. This allows for the removal of mud and dung and the hooves are exposed to the acaricide on the floor as well as the fluid dripping off the animals. The hooves are the preferred site of attachment for immatures. The footbath at the entrance of the spray race is divided into two sections, the first section becomes full of mud and dung while the second section will allow for the acaricide to come into contact with the hooves. The cobbled walk way at the exit of the spray race is long and elevated so that any liquid flows back down towards the sump tank. Sieves are positioned at the entrance of the sump tank to separate the dung and larger particles from entering the tank. A limit of 500 animals per dip session is never surpassed as fresh dip needs to be added once this limit has been reached.

5.2.2.2. Hand Spraying

Hand spraying involves the use of a high-pressure hand pump connected to a car battery. This method is used to treat smaller herds (5-40) of cattle located in other areas of the farm. This avoids the trouble of herding the cattle to the spray race and saves time and labour. The producer makes up 25 litres of dip before each treatment in a large plastic bottle. No old dip is used and no run off is collected. The cattle are herded into a race and each are separated by wooden poles so the animals can be sprayed individually to ensure that the entire animal is exposed to the acaricide.

5.2.2.3. Pour-on

Drastic Deadline Extreme were administered twice a year by pouring the specified volume, calculated according to the weight of the animal, from the top of the neck to the tail base. Cattle are also herded into a race for individual treatment.

5.2.2.4. Injectable

Ivermax is injected into the rump of the animal by the use of a specialised syringe and the weight of the animal also needs to be established for correct dosage. A new needle is applied for each animal that is injected. The cattle are also treated while within a race in order to limit movement while the injection occurs. This treatment was only performed to selected individuals which have a high tick load. Approximately two to three times a year the producer also treated the entire herd.

5.2.3. Tick Collections

Ticks were collected from cattle grazing on different camps on the farm as described in Chapter 3 (3.2.1.1). Twenty live adult female *R. (B.) decoloratus* from each collection allocated for acaricide resistance testing were placed in incubation containers in the incubation room of the PRTF which was kept at a constant temperature of ±28°C and >70% relative humidity. The live ticks were checked daily until the onset of oviposition. Approximately +35 days after the first appearance of eggs, the ticks were again observed daily to establish a hatch date, which was considered to be the day when approximately 70% of the larvae have hatched. Resistance testing was then conducted 15 -21 days after the determined hatch date.

5.2.4. Acaricide Preparation

The following three dip formulations were used for resistance testing, each representing a different chemical class namely; Amidines (Triatix), Synthetic pyrethroids (Pro-dip) and Organophosphates (Supadip). The details of these dip formulations are represented in Table 5.1.

Table 5.1: Chemicals used for resistance testing

Name	**TRIATIX®125**	**PRO-DIP™CYP 20%**	**COOPERS SUPADIP**
Batch	3098	W1284	N/A
Active Ingredient	Amitraz 12.5% m/v	Cypermethrin 20% m/v	Chlorofenvinphos 30% m/v
Expiry Date	08/2018	12/2018	08/2018
Packaging	500ml bottle	1000ml bottle	500ml bottle

The acaricide concentrations which the ticks are exposed to in the Shaw Larval Immersion Test (SLIT) include the recommended field concentrations of each acaricide as prepared from a 1% stock solution diluted from each acaricide remedy as indicated in Table 5.2. The concentrations used were the following; Amitraz 0.025ppm, Cypermethrin 0.015 and 0.03ppm and Chlorofenvinphos 0.03 and 0.05ppm. The various concentrations were prepared by the use of double distilled water, which was also used as the control. Ten ml of each test concentration and 10ml of distilled water were placed into prelabelled test tubes which were used in the next step of testing. Thorough mixing was done throughout the preparation to ensure a uniform acaricide solution at every step.

Table 5.2: Dilution tables for resistance testing

SERIAL DILUTIONS MADE FROM A 12.5% (M/V) AMITRAZ SOLUTION (TRIATIX)				
DILUTION #	**Concentration (% m/v)**	**Dip**	**2X Distilled water**	**Total**
3	Control	-	10ml	10ml
2	0.02	2.5ml of Stock 1	97.5ml	100ml
STOCK 1	1%	4ml Amitraz solution	46ml	50ml
SERIAL DILUTIONS MADE FROM A 20% (M/V) CYPERMETHRIN SOLUTION (PRO-DIP)				
4	Control	-	10ml	10ml
3	0.015	1.5ml of Stock 1	98.5ml	100ml
2	0.03	3ml of 1% Stock 1	97ml	100ml
STOCK 1	1%	2.5ml Cypermethrin solution	47ml	50ml
SERIAL DILUTIONS MADE FROM A 30% (M/V) CHLORFENVINPHOS SOLUTION (SUPADIP)				
4	Control	-	10ml	10ml
3	0.05	5ml of Stock 1	95ml	100ml
2	0.03	3ml of Stock 1	97ml	100ml
STOCK 1	1%	1.67ml Chlorofenvinphos solution	48.33ml	50ml

5.2.5. Shaw Larval Immersion Test

A modification of the method described by Shaw (1966), known as the Shaw larval immersion test (SLIT) was used to expose larvae collected from different camps of the farm over the study period, to the field concentration of each acaricide. Efficacy was then determined by the % larvae killed by exposure. A mortality percentage of above 80% was considered to be effective, a mortality percentage lower than 80% but greater than 50% was considered to show the development of resistance while a mortality percentage of below 50% was considered to be resistant towards the acaricide tested.

5.2.5.1. Set-Up

The set up for the test can be seen in Figure 5.1. Double sided tape was placed around the edges of the glass plate and the stainless-steel tray, in order to trap any larvae that may escape.

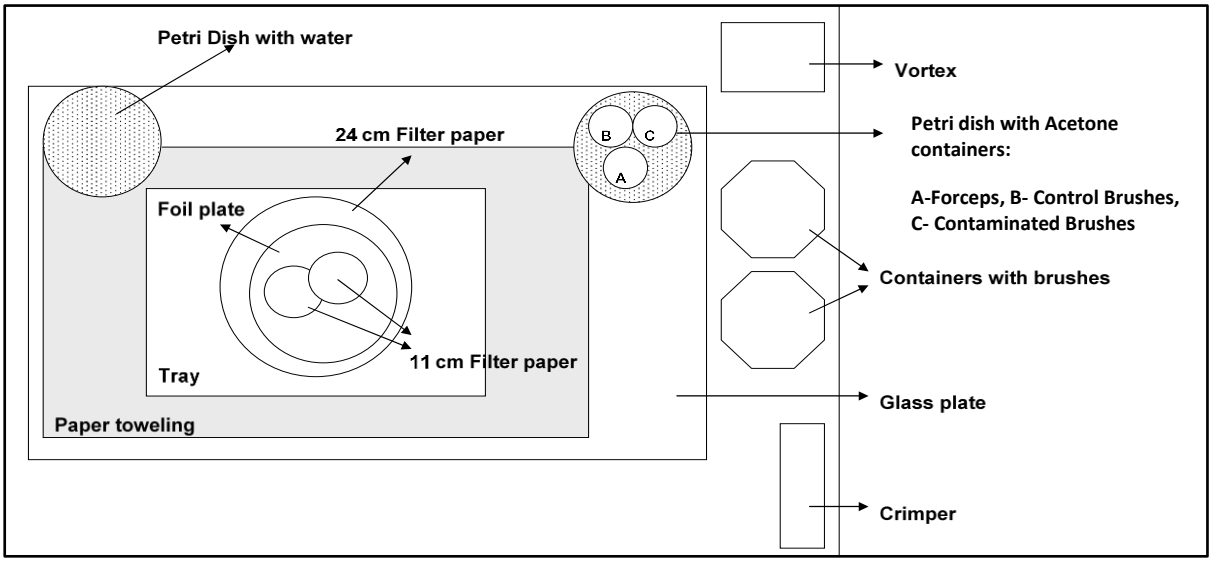

Figure 5.1: The set-up of the SLIT. Source: PTRF M01 – Shaw Larval Immersion Test, Standard Operating Procedure.

5.2.5.2. Exposure to Acaricides

The conical vial containing the larval sample to be tested was placed in a petri dish filled with water. A round filter paper, 24cm in diameter, was placed in the stainless-steel tray to soak up any liquid or droplets that might spill during the execution of the test. A foil plate that contained two circular filter papers with a diameter of 11cm each, was then placed on the 24cm filter paper. The test was started when the cotton stopper was removed from the vial with forceps and placed to the side of the 11cm filter papers in the pie plate. A demarcated control brush (not previously in contact with any acaricides) was used to push some ticks from the neck of the flash on to the brush. The larvae were then brushed onto the one filter paper and covered by the second filter paper. The cotton stopper was placed back on the vial and the test tube containing the control was vortexed for 10 seconds before being poured in a 'zig-zag' pattern over the filter paper sandwich. The moment that the liquid was poured on the filter paper a timer was started. This process was repeated at 60 second intervals for each concentration which was tested each time using a new uncontaminated brush for the transfer of the larvae to the filter paper.

Once all the concentrations had been tested the larvae which had escaped onto the cotton stopper were removed with masking tape and the vial was placed back into the demarcated incubation box in the incubation room.

5.2.5.3. Post Exposure Treatment

A new square piece of foil was placed on the metal tray with a new 24cm filter paper on top of it. Exactly 10 minutes after exposure, starting with the control, the filter paper sandwich was picked up from the plate with forceps, some of the liquid was allowed to drain off and was placed on one corner of the 24cm filter paper. The foil plate was discarded. The filter paper sandwich was then pulled apart by the forceps and placed on a dry part of the 24cm filter paper, so that excess liquid could be drawn up.

Using a designated paint brush approximately 70-100 larvae were bushed into a pre-labelled filter paper envelope, the edges were crimped and masking tape was applied to further prevent larvae from escaping. The two envelopes for each chemical were

clipped together with a bull dog clip and incubated for 72 hours at a temperature and humidity of ±28°C and >70%, respectively. This process was repeated for each of the concentrations which were tested with a new piece of foil and 24cm filter paper for each test. The metal tray was also wiped down with acetone in between each chemical concentration. The control and chemical envelopes were kept in separate incubation boxes in the incubation room.

5.2.5.4. Mortality Counts

After 72 hours the filter paper envelopes were removed from the incubation container, the number of live and dead larvae were counted and documented. Starting with the controls the masking tape was removed from the edges and the envelope was pulled open. The live larvae, which moved around were squashed with a prodder and were counted as they were squashed. This was done on a 24cm filter paper on a large glass plate that had double sided tape placed around the edges to catch any larvae that tried to escape during the counting process.

The total of the live larvae was recorded on a corner of the envelope. The envelope was turned over and gently tapped so that all the dead larvae fall onto the 24cm filter paper below. The dead larvae were counted and recorded underneath the live larvae number. This process was done for each filter paper envelope, the glass surface was cleaned with acetone in between chemical concentrations and a new 24cm filter paper was used for each chemical concertation.

The mortality percentage could be determined from the number of live and dead larvae which was counted. The results from water control, not allowed to have a mortality of more than 10%, were used to calculate corrected mortalities by using Abbots formula as follows: Where %i = % mortality in concentration i, %c = % mortality in water control, CM % = corrected mortality.

$$CM\% = \frac{\%i - \%c}{100 - \%c} \times \frac{100}{1}$$

5.3. Results

The degree of resistance against the field concentration of each acaricide were evaluated as indicated in Table 5.3 and in addition specific colours were used to represent each range.

Table 5.3: The resistance ranges used in the representation of the resistance results.

Mortality Count Percentage Range	Result	Colour
100-80%	Susceptible	Green
80%<%<50%	Development of resistance	Yellow/Orange
50-0%	Resistant	Red

5.3.1. One-host Tick Resistance

Resistance testing could only be performed on *R. (B.) decoloratus* as the sample sizes of *R. (B.) microplus* were too small for testing.

5.3.1.1. Amidine Resistance

The producer is currently using an acaricide which is amidine based. The *R. (B.) decoloratus* populations on this farm showed a definitive shift towards the development of resistance to this chemical as indicated in Figure 5.2. Of the samples which were tested overall, only 12% fell within the susceptible range. The remaining 88% comprised of the emergence of resistance at 67% and resistance at 21%.

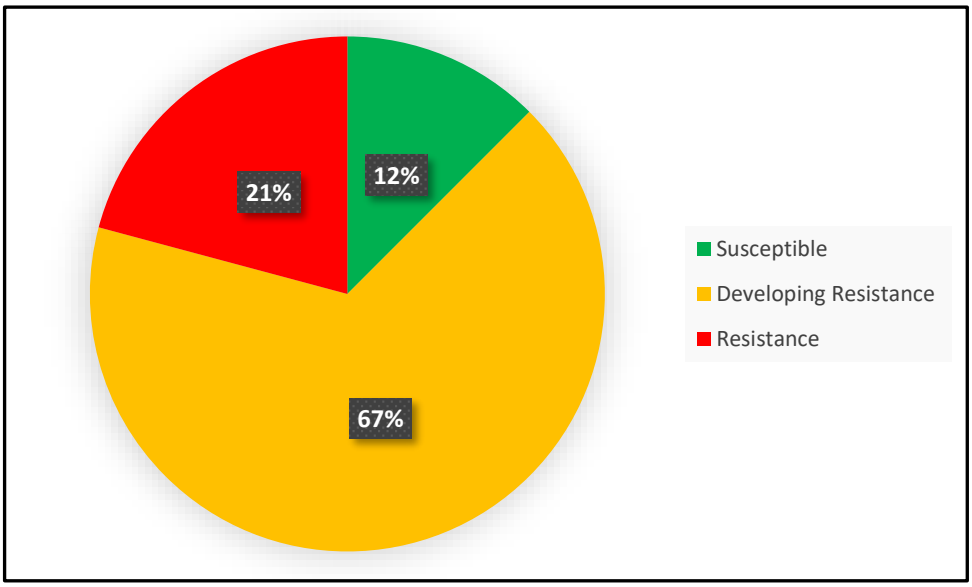

Figure 5.2: The resistance status of Amidine at a concentration of 250ppm.

In March 2016 ticks collected from two of the seven camps, Milk cow and Fish Dam, were susceptible to amidines with mortality counts of 85.7% and 83.9%, respectively as can be seen in Figure 5.3A. The remaining five camps all displayed development towards resistance with the following mortality counts: Church Pregnant 79.9%, Arthurs Reservoir 78.3%, Bushalt 77.6%, Gavin Hill 75.3% and Church 69.4%. Church had the lowest mortality count during this year.

Figure 5.3B. illustrates the resistance results for populations collected in April 2017, the results showed a definite shift towards the development and emergence of resistance to amidines on the farm. Of the five camps which were tested Guava had the lowest mortality count of 36.1% which indicated that this camp is resistant to Amitraz. Bushalt, 68.4%; Quarry, 68.3%; Milk Cow, 66.1% and Gaalboom, 62.8%, all had mortality percentages within the range of development of resistance.

The resistance status of the ticks collected in November 2017 is seen in Figure 5.3C. Of all the camps tested Gavin Hill and Sheds both obtained results which fell in the resistant range with values of 49.8% and 45.5%. Bushalt, 60.3%; Milk cow, 55.3%; Lolweni, 55.3% and Stockweni 51.8% all had mortality percentages that fell within the range of development of resistance.

The results from Figure 5.3D. again shows a definite trend towards resistance and the emergence of resistance on the farm. Both Milk Cow and Gavin Hill camps had a further decrease in mortality counts from 60.7% and 49.8% in 2017 to 48.8% and 40.4% in 2018 respectively putting them both in the resistant range. Arthers reservoir, 79.3%, Lands and Old oranges, 76.6%, Barbers and lands, 64.8% and Barbers Dam, 58.8% fell within the development of resistance range. A sample from Barbers dam and Lands and Old oranges (Barbers and lands) was a representation of both of these camps as the cattle were allowed to freely roam between the two camps.

Milk Cow camp was the only camp on the farm from which adult collections were conducted during every field work trip. The mortality count of the camp initially fell within the susceptible range with a mortality percentage of 85.7% in 2016, while in 2018 this result had decreased to a value of 48.8% which falls within the resistant range.

Gavin Hill camp was tested three times, thus once a year, this camp showed a decrease in the mortality percentage from 75.3% in 2016, 49.8% in November 2017 to 40.4% in 2018. This camp went from the development of resistance to the emergence of resistance.

Collections from Bushalt occurred in 2016 and in both April and November 2017. The results showed a steady decline by having mortality percentages of 77.6%, 68.4% and 60.3%. Although all of the values fall within the development of resistance range the results show a definite decline towards the emergence of resistance.

A summary of the results of the Amidine resistance can be seen in Table 5.4.

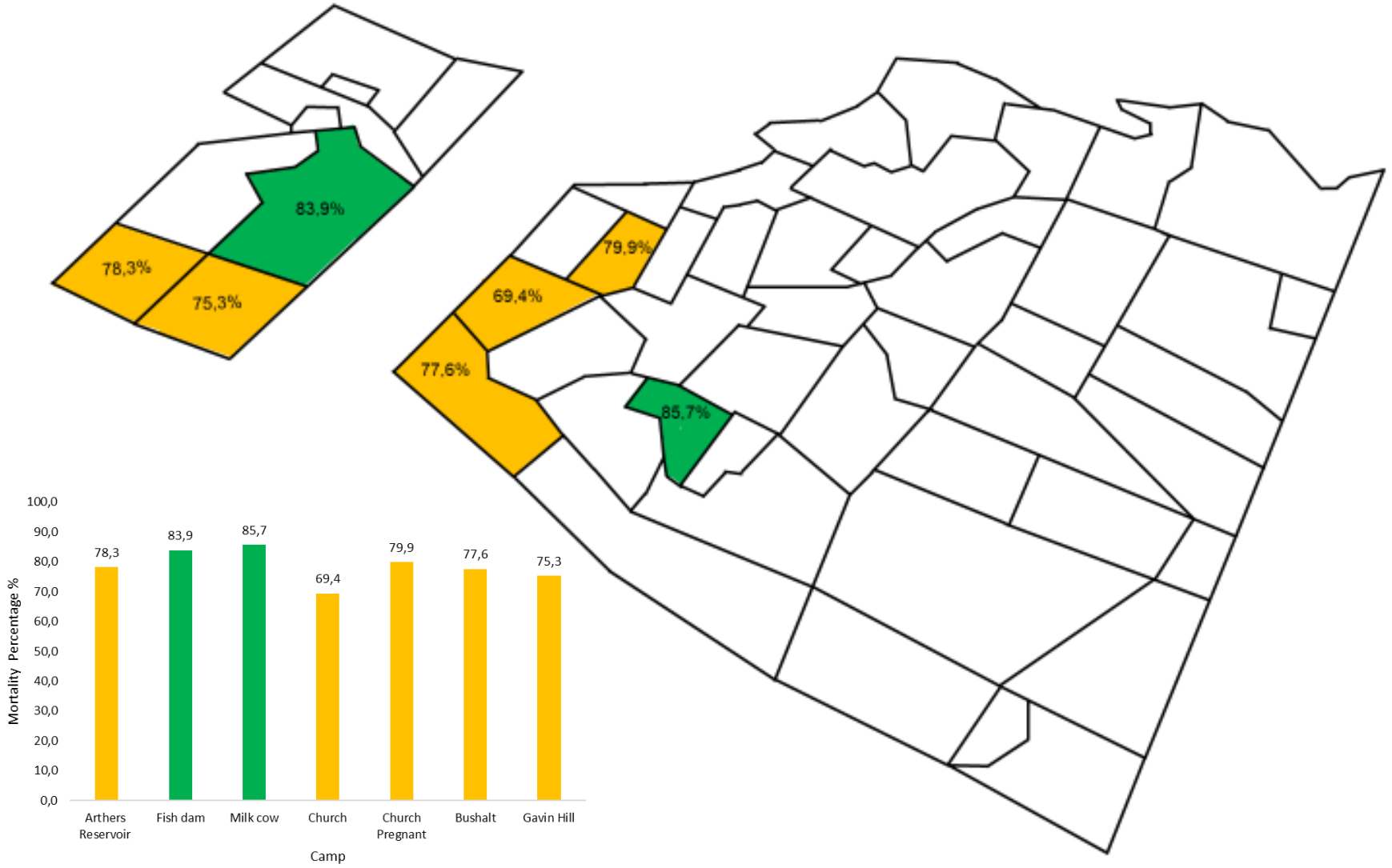

Figure 5.3A: The resistance profiles towards amidines in various camps on the farm in which blue ticks were collected in 2016.

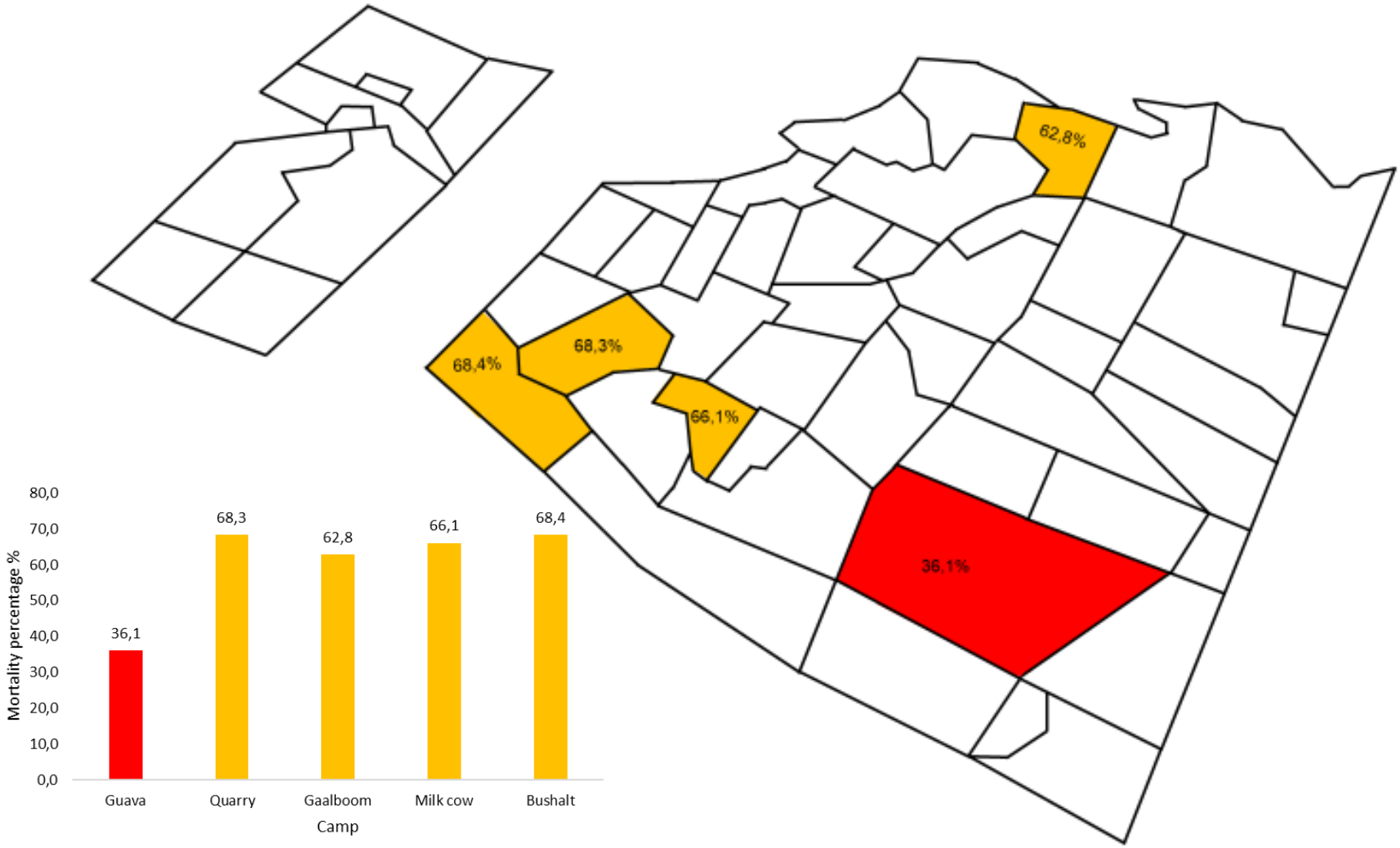

Figure 5.3B: The resistance profiles towards amidines in various camps on the farm in which blue ticks were collected in April 2017.

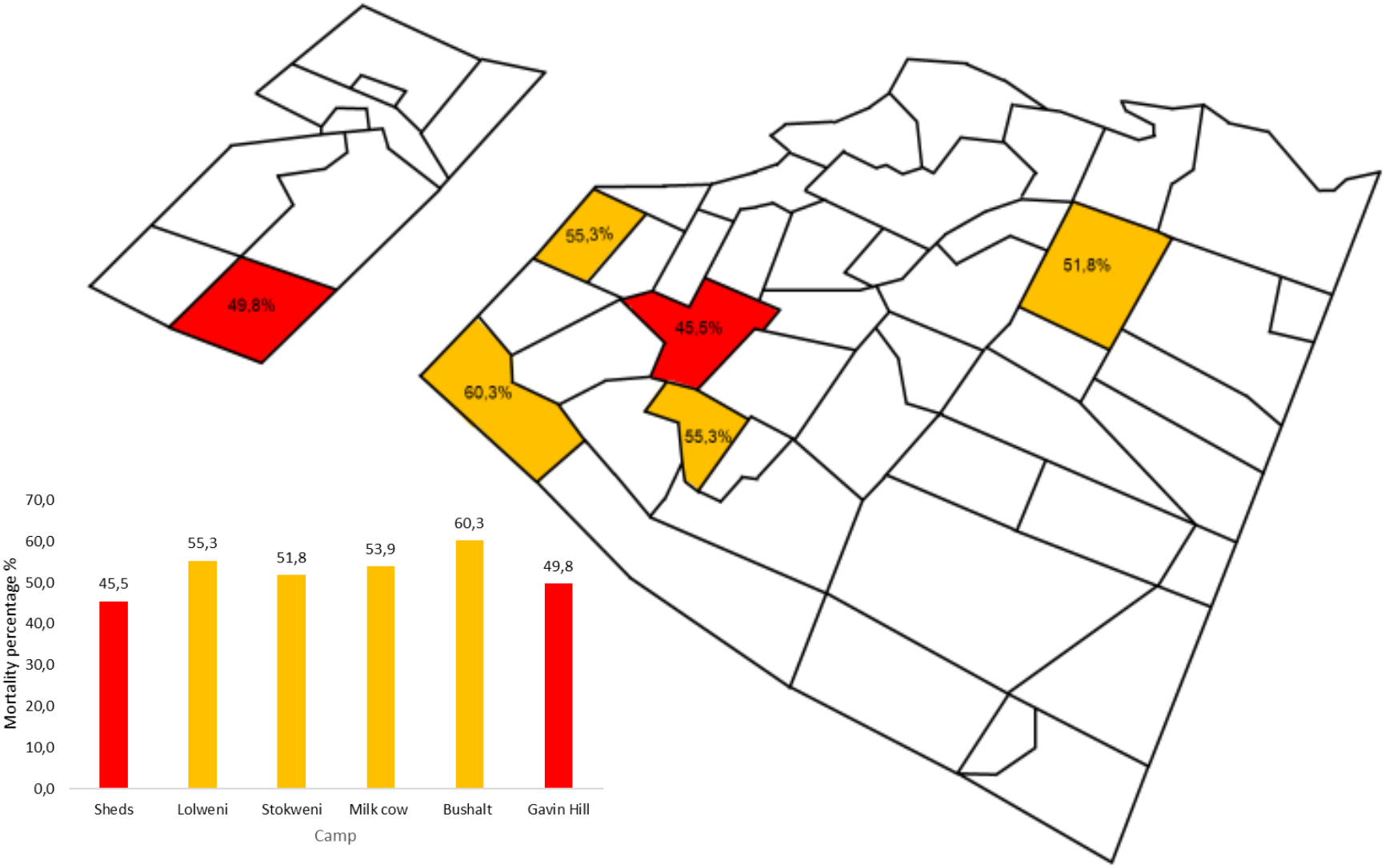

Figure 5.3C: The resistance profiles towards amidines in various camps on the farm in which blue ticks were collected in November 2017.

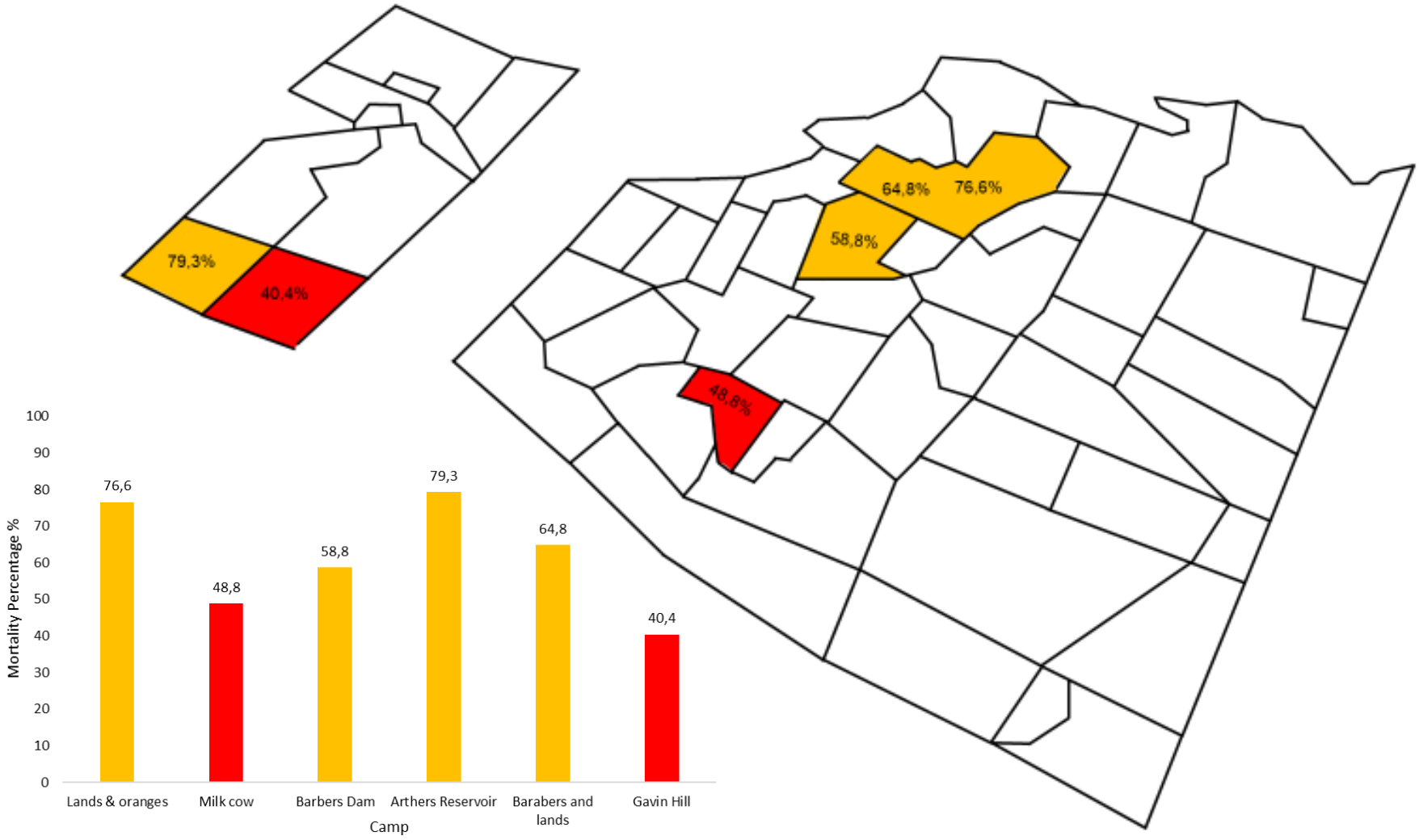

Figure 5.3D: The resistance profiles towards amidines in various camps on the farm in which blue ticks were collected in 2018.

Table 5.4: A summary of the Amidine results over the study period.

Camp	March 2016	April 2017	November 2017	April 2018
Milk Cow	85.7	66.1	55.3	48.8
Bushalt	77.6	68.4	60.3	-
Gavin Hill	75.3	-	49.8	40.4
Fish Dam	83.9	-	-	-
Church Pregnant	79.9	-	-	-
Arthurs Reservoir	78.3	-	-	79.3
Church	69.4	-	-	-
Guava	-	36.1	-	-
Quarry	-	68.3	-	-
Gaalboom	-	62.8	-	-
Sheds	-	-	45.5	-
Lolweni	-	-	55.3	-
Stokweni	-	-	51.8	-
Lands & Old Oranges	-	-	-	76.6
Barbers & Lands	-	-	-	64.8
Barbers Dam	-	-	-	58.8

5.3.1.2. Pyrethroid & Organophosphate Resistance

There is a definite resistance development towards pyrethroids at a concentration of 150ppm as seen in Figure 5.4A. The greatest proportion, 77% of samples displayed and emergence of resistance. Only 9% of the samples fell within the susceptible range and 14% were considered to be resistant towards this chemical.

Pyrethroids at a concentration of 300ppm proved to be effective for the control of the tick population present on the farm. Figure 5.4B shows that 77% of the samples fell within the susceptible range and 23% showed the emergence of resistance. Not a single sample tested showed signs of resistance towards this chemical at this concentration.

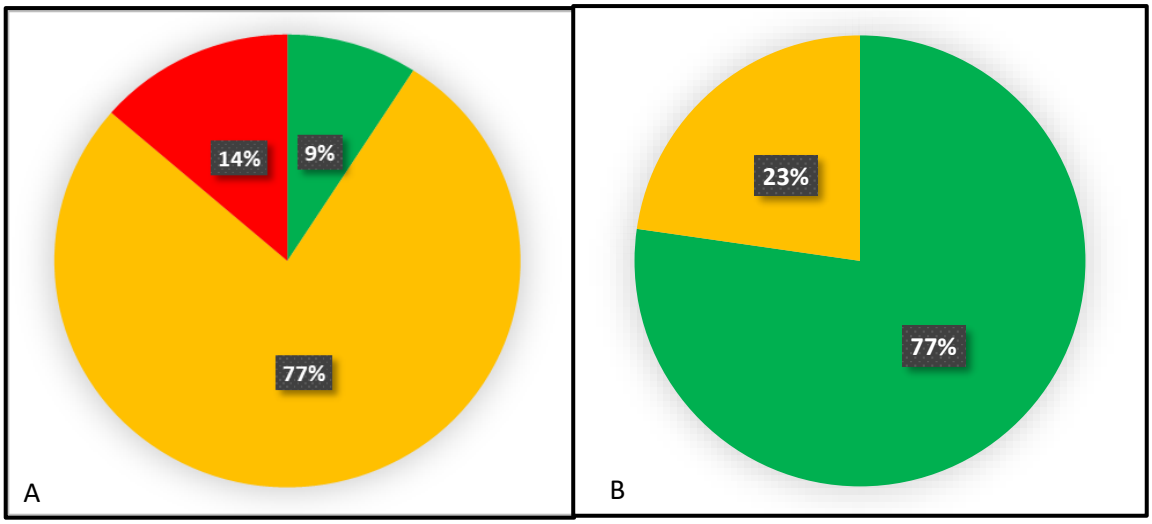

Figure 5.4: **(A)** The resistance status of pyrethroids at a concentration of 150ppm. **(B)** The resistance status of pyrethroids at a concentration of 300ppm.

Organophosphates at a concentration of 300ppm show a fairly constant set of results as the resistance ranges do not differ by extreme amounts as seen in Figure 5.5A Emerging resistance was detected in 45% of the samples tested, 32% of the samples were considered to be susceptible and 23% of the samples tested proved to be resistant.

The results of organophosphates at a concentration of 500ppm showed effective control of more than half, 54%, of the samples tested, falling within the susceptible range as seen in Figure 5.5B. The remaining samples consisted of 32% displaying the emergence of resistance and 14% displaying resistance.

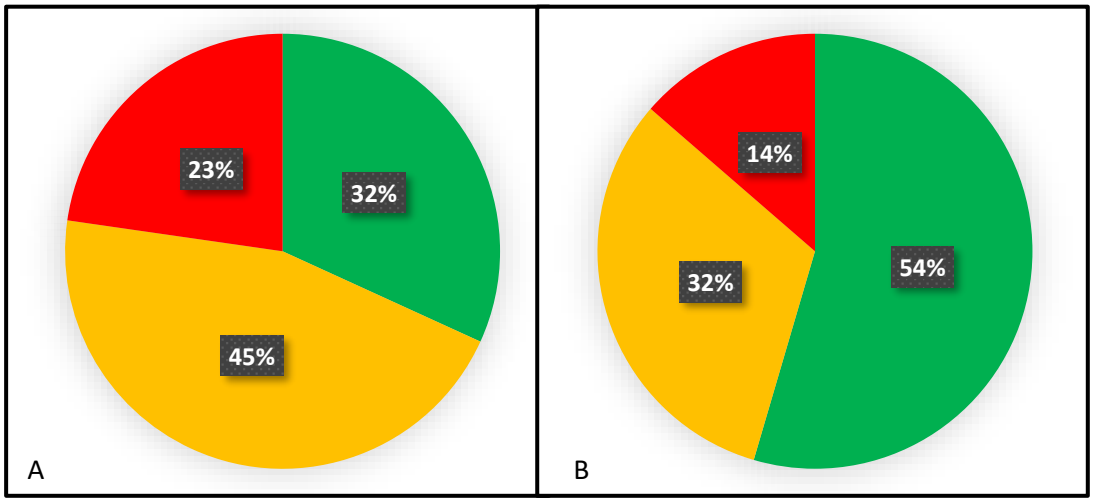

Figure 5.5: (A) The resistance status of organophosphates at a concentration of 300ppm. **(B)** The resistance status of organophosphates at a concentration of 500ppm.

5.3.2. Multi Host Ticks

Multi host ticks were collected form cattle in camps that produced enough ticks of a specific species to be able to do resistance testing on them.

5.3.2.1. *Amblyomma hebraeum* Resistance Status

The resistance status of various population samples of *A. hebraeum,* a three-host tick, collected on the farm over the study period is seen in Figure 5.6. The sample collected from the herd that was present in Arthers Reservoir camp displayed 100% mortality to all the acaricides and concentrations tested with the exception of amidine which had a mortality of 99.6%. The sample collected from the herd in Milk Cow camp showed a more varied result, with 100% mortality occurring towards amidine and the 300ppm concentration of pyrethroids. The lowest mortality percentage of 91.9% occurred towards the 300ppm concentration of organophosphates. The 150ppm pyrethroid and 500ppm organophosphate resulted in mortality percentages of 98.5% and 99.5%, respectively as indicated in Figure 5.6. These results can however be interpreted as susceptibility of *A. hebraeum* to all acaricides tested.

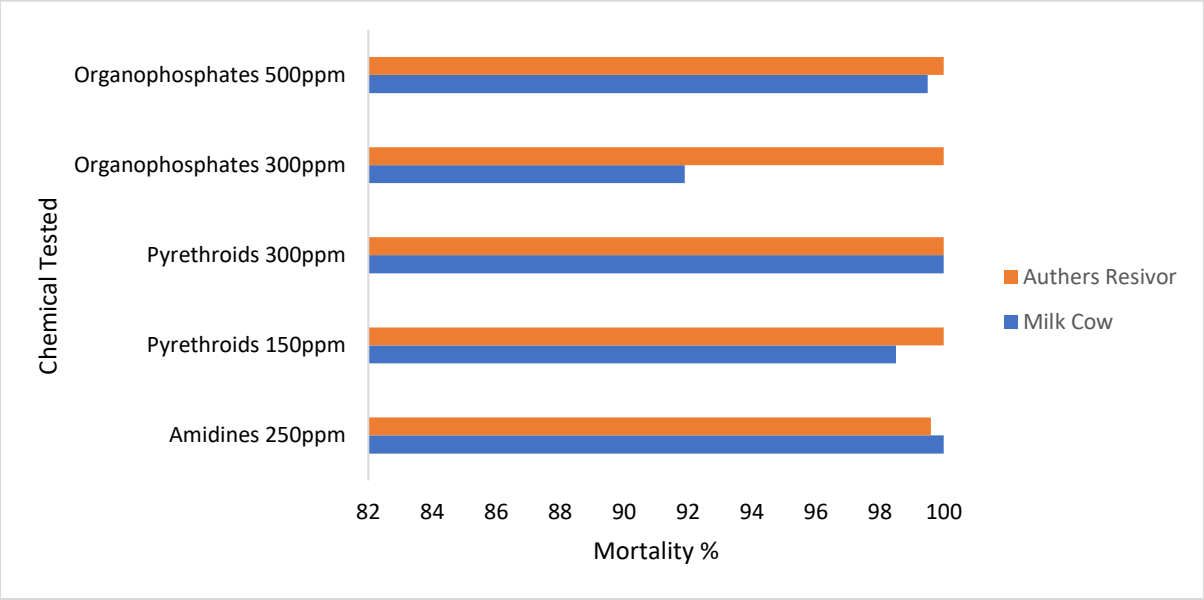

Figure 5.6: The mortality percentage of *Amblyomma hebraeum* samples which were collected in two different camps on the farm.

5.3.2.2. *Rhipicephalus evertsi evertsi* Resistance Status

The mortality percentages acquired from the resistance testing of *R. evertsi evertsi,* a two-host tick, for all populations fell within the range of being classified as susceptible as illustrated in Figure 5.7. Out of all the camps tested, Milk Cow camp was the only camp which did not have a 100% mortality value towards any of the chemical groups that were tested with the lowest mortality percentage of 88.8% obtained for the 150ppm concentration of the pyrethroid. On the other end of the scale, the sample collected from the Bushalt camp showed a 100% mortality towards all the chemical groups tested. The samples collected form Lolweni and Gavin Hill camp showed lower mortality values towards 300ppm organophosphates of 96.5% and 98.3% respectively and towards the 150ppm pyrethroid of 89.7% and 98.8% respectively.

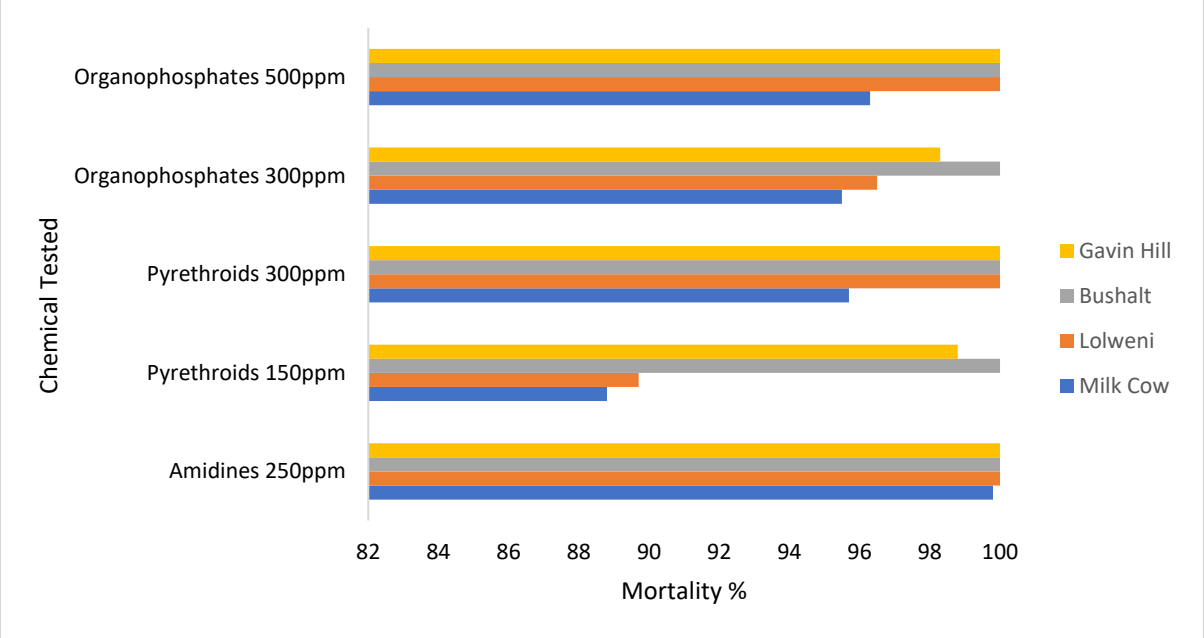

Figure 5.7: The mortality percentage of *Rhipicephalus evertsi evertsi* samples which were collected in four different camps on the farm.

5.4. Discussion

Rhipicephalus (Boophilus) microplus has developed resistance towards most available acaricides on a global scale. Although the displacement of *R. (B.) decoloratus* by *R. (B.) microplus* in South Africa was reported for different areas in South Africa (Tønnesen *et al*. 2004, Horak *et al.* 2009, Nyangiwe *et al*. 2013, Nyangiwe *et al*. 2017), no information is available to our knowledge of the resistance status of *R. (B.) microplus* in South Africa. Resistance information on the endemic *R. (B.) decoloratus* is mostly outdated and a more current profile needs to be investigated.

Resistance studies conducted in South Africa and the rest of the world did either investigated resistance of ticks collected from selected animals on a specific commercial farm or communal area (Mekonnen *et al.* (2002, 2003), Mendes *et al.* (2011), Brito *et al.* (2011), Lovis *et al.* (2013), Adehan *et al.* 2016). In addition to this *R. (B.) microplus* is the focus species of the majority of these studies, particularly in the most recent ones. In this study not enough, engorged female *R. (B.) microplus* specimens could be found in one camp to be able to determine its resistance status. This might be an indication of possible susceptibility of this species to the current acaricides used on this farm and successful control thereof.

One of the unique attributes of this study is that the resistance profiles of various camps on an individual farm were tested and the results have shown that there is in fact a variation of the percentage resistant individuals present in different camps on the same farm.

The main acaricide used for tick control for the past 10 years were Milbatraz, an amidine based acaricide. Amidines are a very important chemical group as it has a low toxicity towards animals such as mammals, birds and reptiles. Once this chemical has entered the tick it acts on the octopamine receptor, decreasing the number of neurons which are excited and results in uncoordinated motor activity. Exposure to amidines leads to paralysis and death. Resistance mechanisms towards amidines include an increase in metabolic activity, in which the tick produces metabolizing enzymes which are able to detoxify the toxin at a quicker rate before it reaches the target site (Aguilar *et al*. 2018).

Testing done from 2016 to 2018 showed a definite shift towards the development of emergence of resistant and resistant tick populations on the farm. It is interesting to note that although all the samples were taken from cattle present on the same farm, sharing the same land, the results varied from camp to camp. Each camp which was sampled for live adult ticks proved to have its own unique resistance profile. Thus, it was possible to identify “problem” camps based on the lower mortality counts instead of only having an overall resistance status for Amitraz on the farm based on collections from one herd representing the resistance status of the farm as is the practice currently.

Ticks used in resistance testing were collected once from cattle in 12 camps, during the three-year study period. The only two camps of these 12 that could be considered to still have a majority of susceptible individuals with regards to amidines, were Fish dam and Church Pregnant with 83.9% and 79.9% mortality counts, respectively. A possible reason for this could be due to the low number of ticks which were collected in these camps. Eight of these camps showed tick populations in which emergence of resistance were observed that ranged from 76.6% mortality counts in Lands and Old Oranges camp to 51.8% in Stokweni and a whole range in between consisting of Church (69.4%), Quarry (68.3%), Barbers and Lands (64.8%), Gaalboom (62.8%), Barbers Dam (58.8%) and Lolweni (55.3%) A further two of these camps, Guava and Sheds were classified as resistant with mortality counts of 36.1% and 45.5%, respectively.

Blue ticks from Guava camp had the lowest mortality count of all the camps tested, although ticks from this camp were only tested once (April 2016). Over the course of late 2016 and early 2017 a bull died due to an extremely high tick burden in this camp. An astonishing 803 *R. (B.) decoloratus* larvae were identified from tick drags performed in the camp during November 2017. This number was remarkably higher than the six *R. (B.) decoloratus* larvae collected in the camp in April earlier the same year while, 209 *R. (B.) decoloratus* larvae was collected in Guava camp in April 2018. The producer had only used the camp, once for two weeks since the collection in November 2017. The drop in the number of larvae collected could be linked to multiple factors which include the lack of hosts resulted in larvae starving and dying, tick drags being conducted in areas which did not contain the greatest number of ticks and seasonality.

A possible explanation for the resistant population found in Sheds camp, also only tested once in April 2017, is that it is located right next to the crush used for treatment of most of the cattle groups on the farm. This cause cattle from different groups and different camps to be herded to this camp on a two to three weekly basis. During this process the cattle sometimes spend a whole day in this camp both before and after treatment that can cause resistant individuals to drop off and produce resistant progeny even though the camp is not frequently used for grazing.

There are a few possible factors which play a role in the variation between the camps of which one is the frequency of use. A more frequently used camp provides a more constant availability of hosts, a higher level of exposure to acaricides and therefore a more resistant tick population can develop. The producer tends to favour certain camps due to the water sources, size of the camp and food availability. Examples of such camps is Barbers Dam, with a tick population showing a 58.8% mortality count, which has a large dam providing a constant source of water while a camp like November requires water to be pumped to the water trough. Guava camp, with a tick population showing resistance at a 36.1% mortality count is very large in size and has a greater area for grazing in comparison to Koekweni, thus, cattle can be kept longer in Guava. Ticks from both November and Koekweni were never tested for resistance due to the absence of cattle in those camps during field collections.

Another factor that can influence between camp resistance variation is the movement pattern of the cattle, this can cause resistant ticks from one camp to be moved to a more susceptible camp via the host and the initiation or establishment of resistance in a new camp. The uncontrolled movement of wild ungulate on the farm aid in the movement of ticks to different camps on the farm. Camps with large water sources could potentially attract the ungulates for longer periods of time, allowing a larger number of tick to either detach or attach to the animals.

The treatment application method can also play a role. Different acaricides application methods, each have their own advantages as well as disadvantages. The producer uses a spray race to treat the large herds, with the advantage that the dip is evenly suspended in the fluid and injuries are rare. Disadvantages include; the animals are not always completely covered in the chemical, as some run through the race and often many enter at once, thus covering parts of other animals. The concentration of

the acaricide decreases as the cattle pass through the race with the first group being treated with the full strength of the acaricide. The acaricide clings to the coat of the animals and lowers the run off and this causes the last cattle to be treated with a lower concentration of the acaricide. The under dosage of cattle can lead to a far quicker rate in the development of resistance as the ticks are not exposed to a lethal dosage as found in a study by Muyobela *et al.* (2015), in Zambia where cattle were only treated with 3L of diluted acaricide instead of the recommended 10L. In the current study the producer hand sprays the smaller herds, with the advantage of the dip prepared freshly to a more accurate concentration. The entire body of the animal can be treated with special attention placed on preferred attachment sites. The disadvantages include; it takes longer and can only be done on a small number of cattle. The excess dip enters the environment directly and build up in the soil of the race. Another factor that can play to the advantage of susceptibility being retained, is the presence of wild life that freely roams around the farm and could act as refugia for susceptible ticks. It can however also be responsible for the movement of resistant ticks to other areas on the farm.

The other camps from which ticks were tested more than once, showed a steady increase in resistance development from 2016 – 2018 with Arthurs reservoir at 78.3% during 2016 and 79.3% during 2018, as the only exception. This could be due to a small error in the resistance testing process or the collection of engorged females which contained few resistant individuals.

Milk cow camp was sampled during each fieldwork trip and was in constant use for grazing of a cattle group throughout this period. The tick population from this camp showed a steady trend from susceptibility to the emergence of resistance and finally the development of resistance with mortality counts steadily dropping from 85.7% in 2016 to 66.1% in April 2017, 55.3% in November 2017 and ending up at 48.8% in 2018. Milk Cow camp has been constantly used for many years so it was expected that of all the camps on the farm the highest resistance towards Milbatraz should be present in this camp.

Ticks from Gavin Hill presenting with a decrease of mortality count from 75.3% in 2016 to 49.8% in November 2017 to 40.4% in April 2018 and from Bushalt with a decrease of 77.6% in 2016 to 68.4 % in April 2016 to 60.3% in November. Both showed a steady

increase of resistant populations with time. Gavin Hill was also used by the producer quite frequently throughout the study period as it is a large camp and has a sufficient water source.

Overall the results from the resistance towards Amitraz on the farm showed that there is a shift towards resistance development on this farm and that the camps Milk Cow, Gavin Hill, Guava and Sheds can be considered to be problem camps with regards to amidine resistance on the farm.

Pyrethroids were used for tick control on the farm prior to the use of Amitraz. Pyrethroids can enter the tick through the cuticle, it spreads throughout the body of the tick and will result in a loss of movement which will ultimately lead to death. The mode of action of pyrethroids has been described to be neurophysiologic. It prolongs the closing of the sodium channels which results in the depolarization of the membrane potential and this then blocks the nerve impulses from being transmitted. Mechanisms for pyrethroid resistance fall into two categories; the increase in metabolic activity and alteration of the sodium channel due to a mutation (Aguilar *et al*. 2018).

Currently the producer is using Drastic Deadline Extreme which is a pyrethroid and insect growth regulator combination. Fourie *et al.* (2013), conducted a study on the efficacy of Drastic Deadline Extreme towards *R. (B.) microplus* and *R. (B.) decoloratus* at the Dohne Research institute in the Eastern Cape Province by cattle treatments with Drastic deadline Extreme on days 7, 63,126 and 189 days after the cattle group was introduced onto the camp. Their results showed a decrease in the monthly tick counts on the cattle followed by a decrease in the number of larvae present on the vegetation in the camp. Although there might be a presence of resistant individuals towards the pyrethroid at 150ppm, the concentration of flumethrin in Drastic Deadline Extreme, in the current study, the producer should keep on using the product as the insect growth regulator component will decrease the tick population in the camps in the long run.

Prior to the use of Milbatraz, the producer used an organophosphate based acaricide for tick control until total resistance to Organophosphates were experienced (EMS van Dalen, unpublished data). This has no longer been used on the farm for 12 years and the current results of the overall resistance profile of blue ticks to Organophosphates can be an indication of the reversion of tick populations on the farm back to

susceptibility to Organophosphates. Ticks exposed to 150ppm and 300ppm Organophosphate solutions showed 32% and 54% of the populations tested to be susceptible and only 23% and 14% to still be resistant, respectively. Thus, it is probable that during the past 12 years, the genes responsible for the resistance towards organophosphates have decrease in frequency within the tick population due to no selection pressure. This hypothesis should be tested over the next couple of years, to see if the populations will revert back to being susceptible to this chemical group.

5.4.1. Comparison of the Two and Three-host Tick Resistance Status

The results which were observed for *A. hebraeum* and *R. evertsi evertsi* proved that these species were completely susceptible to all the chemical groups tested at the variation of concentrations. These results compliment the findings of Mekonnen *et al.* (2002, 2003) for *A. hebraeum* and *R. evertsi evertsi* collected from selected commercial farms in the Eastern Cape and North West provinces. One-host ticks are estimated to spend between 42-63 days per year on the host and depending on the frequency of the treatment regimen the ticks can be exposed to acaricides around 9 times per year if the cattle are dipped on a weekly basis (Phalatsi *et al.* 2004). *R. evertsi evertsi* spend approximately 42 days and *A. hebraeum* 21 days on the cattle and are exposed for a far lower frequency of treatments per year (Mekonnen *et al.* 2002, 2003). *A. hebraeum* and *R. evertsi evertsi* are not exposed to the acaricide used to treat the cattle at the same frequency as one-host ticks (Mekonnen *et al.* 2002, 2003).

Amblyomma hebraeum adults are found on larger ungulates and are thus exposed to the acaricide while attached to the cattle. The immatures; nymphal and larval stages however, can also be found on smaller antelope, scrub hares and tortoises which are not treated with acaricides (Walker *et al*. 2003). In a similar manner *R. evertsi evertsi* adults are found on large ungulates while the immatures can be found on the same host or on scrub hares and smaller antelope species (Walker *et al*. 2003). Thus, for both species the adults and a portion of the immatures will be exposed to acaricides while attached to the cattle. In turn the susceptible individuals will be killed off while the next generation of immatures are thriving on the smaller hosts. Due to the fact that

these two species are found on a variety of host species, the length of their life cycle is increased as more time is needed to seek out a new host once moulting has occurred. Prolonged periods can occur where no suitable hosts are available or when environmental conditions are unfavourable. During this time these species can go into a state of diapause and remain dormant for months until conditions become favourable once again (Walker *et al*. 2003).

One-host tick species can complete multiple life cycles within a single year due to the fact that only one-host is needed so only the larvae have to spend time questing on vegetation until a host passes by. Once on the host there is a constant food source and suitable microenvironment within the fur of the animal. There is however the danger of being removed via grooming or being killed by the treatment with acaricides.

An *A. hebraeum* and *R. evertsi evertsi* sample were collected and tested from the Milk Cow camp. As mentioned, this camp has been constantly used until the end of the April 2018 collection. Thus, it is possible to hypothesise that these two species present in the camp will be exposed to the acaricide at a slightly higher frequency then those present in other camps on the farm which are used in a rotational method such as Bushalt, Lolweni, Gavin Hill and Arthurs Reservoir camps which both displayed an almost 100% mortality. This then resulted in the varied results which were observed for the Milk Cow camp in comparison to the other camps.

Although the majority of the motility values obtained were 100%, a variation was also observed in the samples tested. All of the samples are still susceptible to the use of all the chemicals tested and can still be controlled by the use of Milbatraz. Thus, it is possible to state that although Milbatraz is no longer as effective in the control of the blue ticks, it is still effective in the control of *A. hebraeum* and *R. evertsi evertsi*.

On Claypits there is a definite shift towards the development and emergence of resistance towards Amidines and Pyrethroids on the farm. While Organophosphates results have shown that the ticks are reverting back to being susceptible towards this chemical group. The two and three-host ticks tested proved that the resistance of these ticks has not yet occurred due to the length of their life cycles and the amount of time which the adult stage spends on the host.

This study presented the situation on a commercial farm. It is important to note that although many studies have been conducted on commercial farms, a greater proportion have been conducted on communal farms. The stage of the development and emergence of resistance which is obtained on communal farms is often far more advanced due to the miss use of chemicals either via under or over dosage, the use of expired chemicals as well as the mixing of homebrew solutions. Commercial farmers have access to the latest technology, latest acaricide formulations and are able to have a relatively high production rate, generally producing synthetic or cross breeds. Emerging farmers and communal farmers do not have access to latest technologies and expensive new acaricide products and have a far lower production output. The knowledge basis of communal farmers on chemical in the Eastern Cape was gathered and analysed by Masika *et al*. (1997). It was found that 98% of farmers interviewed in the central Eastern Cape did participate in communal dipping funded by the government. Many of the farmers also used their own additional treatment in the form of motor oil, household disinfectant, removal by hand and pour on acaricides. Prolonged use of motor oil could result in lead poisoning in the cattle and leaving residues in the meat and milk. Many use mixtures of pour on flumethrin with paraffin and oils, this could result in an under dosage or the deactivation of the chemical. A similar finding was presented by Yessinou *et al.* (2017), who conducted their study in Benin in West Africa. There is a lack of general know how and knowledge on adequate tick control methods amongst communal farmers.

5.5. Conclusion

There is no single solution for the control of ticks. Many treatment regimens and eradication programs fail due to the inadequate knowledge of the ecology of ticks. Changes in the distribution of the tick species due to changes in climatic conditions or host availability and the inability to remove all ticks and larvae from the environment, as well as wildlife that can act as a place of refugia for many species, can aid in their re-infestation. In addition to this the head strong attitude of producers often makes it difficult to implement these programs as well as to ensure the success. Strategies for the future of tick control need to be suited towards the individual needs of each farming

sector, with plans which are designed for both communal and commercial producers, as the management practices do differ.

In order to attempt to prolong the use or acaricides, operational factors need to be addressed and monitored. The frequency and dilution of applications needs to be done according to the manufacture's recommendations. The rotation of acaricides needs to be done by using acaricides with different modes of action to reduce the resistance selection pressures as well as preventing cross resistance. Vaccinations, botanical products, biological control, environmental management such as rotational grazing and pasture burning and the selection of resistant hosts are some of the alternative forms of control which can also be employed to complement chemical control.

5.6. Recommendations

It is recommended that the producer continues using his current treatment regime until the acaricides are no longer effective at all. It is advised that the producer should send in a live tick samples at least once a year in order for the PRTF to keep track of the resistance status of the farm. A way in which the producer can prolong the use of his current acaricide would be to continue to practice pasture spelling on a larger scale.

The producer could potentially look into cross breeding his South Devon cows and heifers with cattle indigenous to the country. Indigenous breeds tend to have a far lower tick load in comparison to exotic breeds like the South Devon breed. Thus, the number of treatments with the acaricide can potentially be decrease and as a result, the long-term resistance should develop at a slower rate.

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APPENDIX 1

UNIVERSITY OF THE FREE STATE
UNIVERSITEIT VAN DIE VRYSTAAT
YUNIVESITHI YA FREISTATA

UFS·UV
HEALTH SCIENCES
GESONDHEIDSWETENSKAPPE

Animal Research Ethics

Miss M Pottinger
97 Villa Bain
Henrietta Grove
Langenhoven Park
Bloemfontein
9300

Dear Miss Michelle Pottinger

Student Project Number: UFS-AED2017/0027

Project Title: The distribution of Rhipicephalus (Boophilus) microplus and Rhipicephalus (Boophilus) decoloratus on a farm in the Eastern Cape Province, South Africa.

Department: Zoology and Entomology (Bloemfontein Campus)
You are hereby kindly informed that, at the meeting held on 31-Aug-2017, the Interfaculty Animal Ethics Committee approved the above project.

Kindly take note of the following:
1. A progress report with regard to the above study has to be submitted Annually and on completion of the project. Reports are submitted by logging in to RIMS and completing the report as described in SOP AEC007: Submission of Protocols, Modifications, Amendments, Reports and Reporting of Adverse Events which is available on the UFS intranet.

2. Researchers that plan to make use of the Animal Experimentation Unit must ensure to request and receive a quotation from the Head, Mr. Seb Lamprecht.

3. Fifty (50%) of the quoted amount is payable when you receive the letter of approval.

Yours Sincerely

Derek Litthauer
2017.08.31
20:54:46 +02'00'

Prof. Derek Litthauer Chair: Animal Research Ethics Committee

APPENDIX 2

UNIVERSITY OF THE FREE STATE
UNIVERSITEIT VAN DIE VRYSTAAT
YUNIVESITHI YA FREISTATA

UFS·UV
NATURAL AND AGRICULTURAL SCIENCES
NATUUR- EN LANDBOUWETENSKAPPE

ZOOLOGY & ENTOMOLOGY

Consent for fieldwork collections

To whom it may concern:

I, Michelle Pottinger, a Masters student, conducting a study, titled; The distribution of Rhipicephalus (Boophilus) microplus & R.(B.) decoloratus on a farm in the Eastern Cape Province, South Africa.

as a requirement towards the completion of this degree at the department of Zoology and Entomology, Faculty of Natural and Agricultural Sciences, University of the Free State, Bloemfontein needs to collect the following specimens:

1. Adult ticks
2. Larvae
3. Dip
4.

from the farm/farms Gaypits

during the following study periods 1 Jan 2018 - 31 Dec 2018

Student: Signature: _____ Date: 23/04/17

I G. Dixon,

owner of the farm/s, Gaypits

hereby declare my willingness to partake in this study and give my consent by granting Michelle Pottinger permission to access the farm, farm facilities and animals on the farm for specimen collection during the required study period as indicated above.

Producer: Signature: _____ Date: 23-04-17