Acaricide resistance patterns in one-host *Rhipicephalus* spp. at communal dip tanks and neighbouring commercial farms in the KwaZulu-Natal Midlands

By

Dr Caryn Shacklock

Submitted in partial fulfilment of the requirements for the degree of

Master of Science (Tropical Animal Health)

in the Department of Veterinary Tropical Diseases
Faculty of Veterinary Science, University of Pretoria

Study Leaders
Dr Hein Stoltsz
Dr Robin Taylor

30 October 2019

Declaration

I hereby declare that this dissertation, which I hereby submit for the Master of Science degree in the Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, to be my own work and has not been previously submitted by me for degree purposes at another tertiary institution.

C L Shacklock

30 October 2019

Acknowledgements

I would like to acknowledge Afrivet Business Management for the generous sponsorship of my studies and, in particular, Dr Oberem, Dr Du Preez and Dr Van Rensburg. Thank you for your support and assistance.

Thank you to the Belgian Directorate-General for Development Co-operation Framework Agreement (FA4 DGD-ITM 2017-2021) awarded to the Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria for the bursary to fund the research and travel.

I gratefully acknowledge the University of Pretoria, my supervisor, Dr Hein Stoltsz and academic and admin staff – particularly, Ms Rina Serfontein. Thank you for your assistance and guidance.

Dr Robin Taylor. Thank you for your wonderful mentorship and guidance over the past two years. I have learnt an enormous amount from you and appreciate all your time and effort.

Ms Selina Maphalala. I couldn't have done this without you! Thank you for your capable assistance.

Thank you to my family for your sacrifices while I added one more ball for us to juggle. Love you all.

Table of contents

List of figures

List of tables

Summary

Acaricide resistance patterns in one-host *Rhipicephalus* spp. at communal dip tanks and neighbouring commercial farms in the KwaZulu-Natal Midlands

Student: **Dr Caryn Shacklock**

Study Leaders: **Dr Hein Stoltsz**
Dr Robin Taylor

Degree**:** **Master of Science (Tropical Animal Health)**

Department: **Veterinary Tropical Diseases**

This project was conducted in order to ascertain the presence or absence of acaricide resistance in ticks in an area of KwaZulu-Natal (KZN) where tick-borne diseases pose a real and dire threat to communal and commercial livestock. The results of this study will assist farmers and state veterinarians in their tick control strategies and aid in the battle against stock losses due to ticks and tick-borne diseases.

The aim of the project was to collect one-host *Rhipicephalus* spp. (blue ticks) from cattle presented at communal dip tanks and from cattle on commercial dairy and/or beef farms to test for the presence of acaricide resistance.

The ticks were identified as either *R. microplus* or *R. decoloratus*, then the engorged female ticks were incubated and the hatched larvae subjected to the Shaw Larval Immersion test (SLIT). The Shaw Larval Immersion test was developed in 1966 by RD Shaw (Shaw, 1966) to determine the spectrum of acaricide resistance in tick populations.

The three acaricides selected for the laboratory bio-assay are included in the classes of topical acaracides most frequently used in KZN, namely amidines, organophosphors and pyrethroids.

Both tick species were present in the study area and two commercial farms showed a mixed population of both tick species.

All fifteen populations of ticks tested in this study showed resistance to at least one class of acaricide, and four of the 15 (26%) showed resistance to two classes of acaricides. 80% of the tick samples tested was resistant to cypermethrin, a synthetic pyrethroid.

It can be concluded from this study that:

1. acaricide resistance is present in one-host *Rhipicephalus* spp. in the KwaZulu-Natal Midlands and this poses a real and significant threat to tick control efforts in this region of KwaZulu-Natal,
2. resistance to pyrethroids is developing at a faster rate than other acaricides and,
3. both blue tick species were identified in the study area however only one or the other species was represented at almost all of the 15 sites sampled. The exceptions were two commercial farms, where both *R. decoloratus* and *R. microplus* were identified in a mixed population.

1. Background and general introduction

There are currently at least 867 known species of ticks in the world (Jongejan and Uilenberg, 2004; Walker, 2003). Ticks are vectors for more infectious diseases than any other arthropod currently known to man (Jongejan and Uilenberg, 2004), and their impact on livestock health and production in Africa is significant. Ticks belong to the phylum Arthropoda. They are invertebrate arachnids, forming part of the order Acari and the suborder Ixodida. Two families of ticks fall under this sub-order; Argasidae (soft ticks) and Ixodidae (hard ticks).

The hard tick species that were investigated during this study are the blue ticks of cattle, *Rhipicephalus (Boophilus) decoloratus* the native African blue tick, and *Rhipicelphalus (Boophilus) microplus* the exotic Asiatic blue tick. Some controversy has arisen over the nomenclature of these two tick species in recent years and the genus, *Boophilus,* has become a sub-genus of the genus, *Rhipicephalus* (Barker and Murrell, 2003). Both *Boophilus microplus* and *Boophilus decoloratus* were described by scientists in the late 1800, and by 1965 there were five *Boophilus* tick species on record (Murrell and Barker, 2003). However, as a result of DNA sequence studies conducted by Murrell et al. in 2000, evidence was found that showed that the *Rhipicephalus* genus was paraphyletic, with the *Boophilus* genus sharing close genetic ancestry with *Rhipicephalus* (Murrell and Barker, 2003). The Oxford English Dictionary defines a group of organisms as paraphyletic when a 'group of organisms (is) descended from a common evolutionary ancestor or ancestral group, but not including all the descendant groups'. Consequently, Horak et al. included the updated nomenclature in the reference of valid tick names in 2002 and this was validated by others in later years (Horak et al., 2018). In a global list of valid tick names published by Guglielmone et al. in 2010, the subgenus *Boophilus* is omitted entirely from the nomenclature of the two blue ticks (Guglielmone et al., 2010) and so, for the purposes of this paper, the names *R. decoloratus* and *R. microplus* will be used.

Resistance to an acaricide in a population of arthropods can be defined as the development of an ability to survive a dose of acarcide that would usually prove fatal to the majority of another population of the same species, through a process of genetic adaptation (Mota-Sanchez et al., 2002). There is growing concern in South Africa and many other parts of the

world over the development of resistance to chemical tick control measures (George et al., 2008) and the study area of the KwaZulu-Natal (KZN) Midlands is no exception.

The state-owned dip tanks that are strategically placed throughout the province of KwaZulu-Natal (KZN) provide an ideal sampling setting for this study of acaricide resistance in blue tick populations. In a survey conducted by Hesterberg et al. in 2007, 77% of communal livestock owners in KZN reported that ticks were a major cause of concern (Hesterberg et al., 2007). Damage to teats and hides, and tick-borne diseases were cited as the main reasons for employing tick control measures - most of which are provided by the regional provincial state veterinary services. Various dip tanks in and around the Umgeni district municipality were visited throughout the course of the study and engorged female ticks collected from as many cattle as possible at each site.

The acaricide provided by the state to the various communal livestock owners in KZN is obtained through a provincial tender process and is distributed by the regional state vet officers at dip tanks and cattle holding areas. The product that is currently in use is Afrivet Decatix 3®, which contains 2.5% deltamethrin and can be applied as a spray or in a plunge dip tank. This product has been in use in the majority of the communal areas visited during this study for the past four to five years. It is speculated that, given the duration of use, there will likely be significant pyrethroid resistance in these tick populations.

Commercial farmers in the same area employ very different dipping strategies such as spray races and pour-on products, and use a much wider range of dips than the communal herds. Some private farmers admit to not using acaricides at all, but prefer to make use of Babesiosis blood vaccines as part of their disease control strategy rather than specific tick control (anecdotal reports). Tick-borne diseases, in particular Asiatic redwater caused by *Babesia bovis*, result in major economic and livestock losses for farmers in this area (Terkawi et al., 2011). It can be speculated that, because many commercial (especially dairy) farmers in this area farm with or rear exotic breeds such as Holstein and Jersey cattle, the susceptibility of these herds to ticks and tick-borne diseases is higher than many indigenous cattle breeds that have a degree of innate resistance to ticks (Rechav and Kostrzewski, 1991).

The differences in farming strategies, cattle breeds and tick control methods between communal and commercial herds may have an effect on the resistance profiles of the tick populations, and part of the purpose of this study is to examine this and draw comparisons between the two types of herds.

According to a census conducted by the regional State Veterinary Office in 2017, the communal cattle population size in Umgeni district is currently at approximately 4,000 head of cattle; with representation at each of the eight dip tanks in this district ranging from 80 to 1,100 head (source: Pietermaritzburg State Veterinary Office, 2018). In reality, however, many of the Umgeni district dip tanks are no longer in use, and at many of those where cattle are still presenting for 'dipping', hand spraying is being used to apply the acaricide. As a result of this, the study area had to be expanded to include the district municipalities surrounding Umgeni (see table and map below) to make up the required sample size.

The samples were all tested against three commercially-available topical acaricidal chemicals that represent the three classes of acricides, namely amitraz (amidine), cypermethrin (pyrethroid) and chlorfenvinphos (organophosphors).

All but one of the state-run dip tanks made use of a deltamethrin product that was used either in a dip tank or hand spray knapsack. Brandvlei dip tank in the Mooi River area had not been able to dip for a few months due to structural damage to the dip tank, so the local animal health technician had injected the cattle with a long-acting macrocyclic lactone.

The commercial farmers made use of a wide range of products from all the groups of acaricides, as well as various different methods of application ranging from the use of injectable macrocyclic lactones, to dip tanks and spray races. All but one farmer made use of combination topical products that contain more than one acaricidal chemical.

It is speculated that the long-term and frequently injudicious use of chemical acaricides in this area has led to significant acaricide resistance in the resident tick populations. This study was undertaken to ascertain the extent to which resistance occurs in the KZN Midlands, and to investigate whether the type of production systems or tick control method used by the various livestock owners in the study area will have an effect on the resistance profiles.

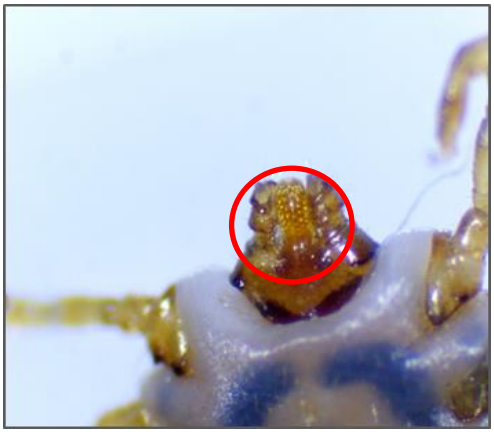
2. Literature review

2.1 Ticks

Rhipicephalus microplus and *R. decoloratus* are both one-host ticks whose primary maintenance hosts are cattle, although *R. decoloratus* is more of a 'generalist' tick and can be found on horses, donkeys, wild ungulates, small stock and even birds and rodents (Horak et al., 2018). The preferred areas of attachment for both species include the dorsum, upper limb, dewlap, flank and belly of the bovine (Walker, 2003). These two tick species are almost impossible to differentiate macroscopically, particularly the engorged females; however, there are distinct morphological differences that can be identified microscopically and there are differences in the life cycles and habitats of the two species (Horak et al., 2018).

2.1.1 *Rhipicephalus decoloratus*

Both blue tick species have short mouthparts, an inornate scutum and well-developed anal plates. *Rhipicephalus decoloratus* differs from *R. microplus* and the other species in the (previous) *Boophilus* sub-genus as it has a two column hypostome with rows of three denticles on each column (3 + 3 dentition), while the other species have rows of four denticles on each column of the hypostome (4 + 4 dentition). It can also be identified by an extra protuberance on the palps, and the size and shape of the adanal plates (the medial spur of which is longer than the lateral) (Walker, 2003).



a

b

Figure 1 (a) *Rhipicephalus decoloratus* mouthparts and (b) adanal plates

Rhipicephalus decoloratus is widely distributed throughout temperate, savanna grasslands of Africa, usually where the annual rainfall is in excess of 500 mm (Horak et al., 2018). The southern and eastern coastal parts of the country and neighbouring interior, parts of the Free State and the Northern provinces of South Africa, as well as the study area of KwaZulu-Natal, provide natural habitat for this species (Baker, 1989). The north-western parts of South Africa are more arid and thus this species of tick is not prevalent in these areas.

Figure 2 Geographical distribution of *Rhicephalus decoloratus* in Africa (www.AfriVIP.org)

After engorging on the host, African blue tick females detach and lay up to 2,500 eggs. Larvae emerge from the eggs 3-6 weeks later and climb up vegetation and onto a host animal. The three stages of the blue tick's life cycle (larva, nymph and adult) all develop on the host animal over the course of approximately three weeks, and the entire life cycle can take up to two months to complete (Walker, 2003). These ticks are active all year round if the climate is warm enough, although a seasonal pattern is noted with large numbers of larvae hatching in the spring and summer months (www.afrivip.org).

The African blue tick is a vector of *Babesia bigemina* (African redwater), *Anaplasma marginale* (gallsickness), and *Borrelia theileri* (spirochetosis) (De la Fuente et al., 2008) in South Africa, and heavy infestations can cause painful lesions and can even have a negative impact on the hide quality of an animal at slaughter (Moyo and Masika, 2009).

2.1.2 *Rhipicephalus microplus*

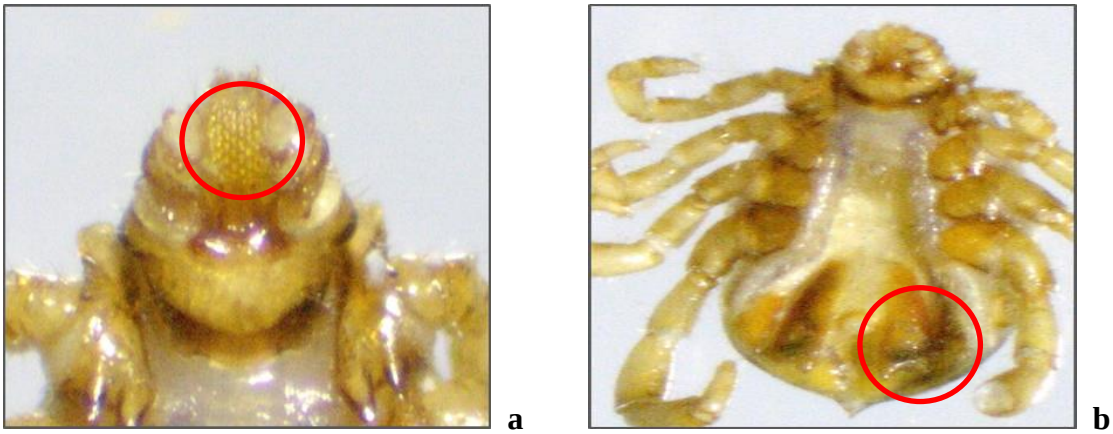
This tick is reported to have originated in Asia and was brought to Africa with the movement of cattle via Madagascar (Estrada-Peña et al., 2006). Over the past few decades, throughout South Africa, *R. microplus* has steadily encroached and taken over much of the areas previously occupied by *R. decoloratus* (Nyangiwe et al., 2013), and it can now be found in the southern and eastern coastal regions of the Eastern Cape and KwaZulu-Natal, and the North-eastern regions of the Western Cape province (Nyangiwe et al., 2017).

Figure 3 Geographical distribution of *Rhicephalus microplus* in Africa (www.AfriVIP.org)

The reason for this range expansion and for the apparent displacement of *R. decoloratus* by *R. microplus* has been postulated to be related to the following factors:

- The life cycle of *R. microplus* is slightly shorter in duration that that of *R. decoloratus*, so Asiatic blue tick females are ready for mating sooner that their African counterparts (Walker, 2003).
- The females of *R. microplus* produce a higher number of eggs than *R. decoloratus* (Walker, 2003).
- *Rhipicephalus microplus* males reach sexual maturity a few days before the males of *R. decoloratus* and, although there is a strong preference for mating with females of the same species, cross mating between the two species can occur. This hybridization, however, results in the production of sterile progeny (Nyangiwe et al., 2013).

Rhipicephalus microplus has the typical 4 + 4 columns of denticles on the hypostome, and is one of the features used to differentiate it from *R. decoloratus* (Walker, 2003). The size and shape of the adanal plates also differ from those of the African blue tick with the lateral and medial spurs being of a similar length.



a **b**

Figure 4 (a) *Rhipicephalus microplus* mouthparts and (b) adanal plates

The Asiatic blue tick transmits the same diseases as its African counterpart, however, it also is a vector of *Babesia bovis*, which produces a more pathogenic form of babesiosis than *B. bigemina* (De Vos, 1979). In addition to anaemia, hypotension and other circulatory disorders, *B. bovis* infections can result in cerebral babesiosis and 'respiratory distress syndrome' (Bock et al., 2004). The acute form of the disease can result in a higher mortality rate than *B. bigemina*.

Tick transmitted diseases are a major cause of economic concern for communal and commercial farmers alike and their global impact is estimated to cost the cattle industry billions of dollars (Jongejan and Uilenberg, 2004). Mortalities, production loss, veterinary treatments and tick control costs all contribute towards making these diseases potentially crippling for farmers, especially in the face of growing acaricide resistance in tick populations around the country and indeed globally (George et al., 2004).

Several mechanisms of acaricide resistance, such as target site mutations, have developed in blue tick populations over the past few decades and many populations show resistance to more than one class of acaricide (Foil et al., 2004).

A number of tests have been developed to screen tick populations for acaricide resistance including the Shaw Larval Immersion Test (SLIT), the FAO Larval Packet Test (LPT), and adult immersion tests [including the Reproduction Estimate Test (RET) and the Egg laying Test (ELT)] (Mekonnen et al., 2003). The SLIT was used in the present study. This test was developed by RD Shaw in 1966 (Shaw, 1966) and uses hatched larvae immersed in serial dilutions of acaricide to assess resistance of the tick population or strain to the particular active ingredient (Shaw et al., 1968). In more recent years, molecular detection tests like the polymerase chain reaction (PCR) assay have been developed to identify the mutated genes responsible for the development of resistance (Morgan et al., 2009). This will have a significant impact on the diagnosis of acaricide resistance in ticks as this test is far more rapid than the lengthy bio-assays such as the SLIT.

2.2 Acaricides and tick control

Indigenous African cattle breeds have thrived for many years in a challenging environment where ticks and tick-borne diseases pose a significant threat to livestock (Spickett et al., 1989). The introduction of exotic breeds of cattle to the continent and their subsequent vulnerability to this threat has highlighted the need for adequate tick control strategies for both communal and commercial farmers (Rechav and Kostrzewski, 1991). Strategic grazing, tick vaccines, selective cattle breeding for innate tick resistance and parasite predators such as oxpeckers and guinea fowl are all strategies that can assist in tick control (De Deken et al., 2014). However, chemical acaricide use is still one of the primary means of tick control in many areas and incorrect application and over-use of certain products has led to the development of resistance in one-host tick populations (Mekonnen et al., 2002).

The first dipping compound to be used for tick control in cattle was Arsenic, which was introduced in the early 1900 (George et al., 2008). Historically, the use of arsenic in plunge dips was revolutionary in the success of tick control strategies (George et al., 2004), however, resistance to the arsenical products developed after a number of years and forced product developers to search for alternatives (George et al., 2008).

Figure 5 Examples of arsenical dips from the 1950s

The subsequent evolution of acaricides globally has been driven by the development of resistance in tick populations to many of the active ingredients in use. There are four classes of acaricides that are most commonly used for tick control in South Africa today. They are the organophosphors, amidines, pyrethroids (natural and synthetic) and the macrocyclic lactones. Three chemicals representing the first three classes of acaricides mentioned above were used in this resistance study, and will be described in more detail in this chapter.

An interesting development in the efforts to find alternative methods of ectoparasite control is the discovery of entomogenous fungi or myco-acaricides. It has been well documented that a number of fungi, such as *Beauveria bassiana*, are effective ectoparasiticides that are capable of killing various life stages of ticks (Kaaya and Hassan, 2000), (Polar et al., 2008). The commercialisation of these fungi for use by livestock owners has not yet been realised in South Africa, however, there is a movement in the veterinary pharmaceutical industry towards 'green' alternatives to chemicals in order to lessen the environmental impact and slow down the rate of resistance development. A number of plant-based acaricides and repellents have also been documented (Benelli et al., 2016).

Three chemicals were selected for this resistance study to represent the most frequently used classes of chemical acaricides in South Africa.

2.2.1 Chlorfenvinphos

Chlorfenvinphos is an organophosphorous compound. The organophosphorous group of chemicals provides an efficient means of tick control in livestock through the inactivation of the enzyme, acetylcholinesterase, which results in hyper-stimulation of the central nervous system (CNS) (Stone, 1968). This leads to the eventual paralysis and death of the parasite (Danbirni et al., 2012).

This chemical can be extremely toxic to vertebrates (Taylor, 2001), with a minimum toxic oral dose for cattle of 22 mg/kg reported by the MSD Veterinary Manual.

Young calves appear to be more susceptible to toxicity through topical application than adult cattle (https://www.msdvetmanual.com/toxicology/insecticide-and-acaricide-organic-toxicity/organophosphates-toxicity). However, it is rapidly metabolised and safe to use at the manufacturers' recommended dosage. Chlorfenvinphos is available as a spray, pour-on or plunge dip formulation, and also in combination with other acaricidal chemicals.

2.2.2 Amitraz

Amitraz forms part of the amidine group of ectoparasiticides and is highly effective against single and multi-host ticks (Davey et al., 1984). The target site for this chemical in the tick is the octopamine receptors and this agonistic interference, as well as inhibition of the enzyme monoamine oxidase, leads to CNS hyper-excitability and death (Taylor, 2001). Amitraz can be applied as a pour-on or spray as well as in a plunge dip and in combination with other chemicals. Amitraz requires careful stabilisation in a dip tank with calcium hydroxide (Taylor, 2001) in order to maintain an alkaline pH. The toxicity in cattle is relatively low, however, amitraz is extremely toxic to horses (www.afrivip.org).

2.2.3 Pyrethroids

The pyrethroid group of pesticides is divided into natural and synthetic pyrethroids. These chemicals act by disrupting the sodium channels and prolonging the flow of sodium into and out of the cells, thereby causing neural excitation (Taylor, 2001). Synthetic pyrethroids such as cypermethrin and deltamethrin are fast acting with a very low toxicity and a significant

degree of fly control which makes them an attractive choice for farmers. However, the rate of development of resistance to pyrethroids is rapid compared to other classes of chemicals (George et al., 2004). Pyrethroids are often used in combination with other products such as piperonyl butoxide (PBO) which has a synergistic effect on pyrethroids and enhances its acaricidal ability (Taylor, 2001).

The sale and use of pesticides in South Africa is regulated by the Fertilizers, Farm Feeds, Agricultural Remedies and Stock Remedies Act, Act 36 of 1947 (Fertilizers). Products for use on animals against external parasites are required to be registered under this Act and to meet certain safety and efficacy standards.

2.2.4 Acaricide resistance

Resistance to ectoparasiticides develops in a population through the genetic selection of ticks with a pre-existing inherent ability to withstand a dose of acaricide. The subsequent reproduction of this sub-population of 'resistant' ticks, which is subjected to selection pressure through the use of acaricides, results in eventual genetic adaptation and the spread of resistance (Rosario-Cruz et al., 2009). Acquired resistance can be described as a decreasing sensitivity to acaricidal chemicals that progresses with time, and is inherited by future generations of ticks (Abbas et al., 2014). Genetic selection results in an increasingly resistant population over time.

Resistance can be expressed phenotypically and/or genotypically (Guerrero et al., 2014). The resistance phenotype is the biological expression of susceptibility or resistance to a chemical and this is what has been assessed by the Shaw larval bio-assay (Rodriguez-Vivas et al., 2018). A livestock owner will be able to observe the resistance phenotype of the tick population in his or her cattle herd by the effectiveness of the chemical tick control methods in use.

The resistance genotype refers to the molecular biology of the tick and is the result of genetic mutations that have resulted in a potential phenotypic expression of resistance (Rodriguez-Vivas et al., 2018). Without genetic sequencing it is impossible to relate the phenotypic expression of resistance to a particular genotype and multiple genetic variations can result in the same biological expression of resistance (Guerrero et al., 2014). The development of

molecular assays to identify the genes responsible for acaricide resistance in ticks is a big step forward for the livestock health industry.

There are other factors that influence the development of acaricide resistance in a population of ticks besides genetics. The chemical nature and bio-kinetics of the selected acaricide, as well as the concentration and frequency of application, can impact the development of resistance (Abbas et al., 2014). Under-strength dosing, irregular application intervals and the use of the same chemical for an extended period of time have all been found to exacerbate the rate at which resistance can develop (Jonsson et al., 2000) (Abbas et al., 2014).

The biology of the parasite, and even the host, can also influence the development of resistance (Abbas et al., 2014). An example of this is the fact that blue ticks are single host ticks that spend their entire life cycle on the host (Horak et al., 2018). They are generally exposed to more frequent doses of acaricides than ticks that spend part of their life cycle off the host (in refugia), and are therefore more likely to develop resistance to acaricidal chemicals (Rodriguez-Vivas et al., 2018).

Scientists and researchers have been studying the phenomenon of resistance for more than half a century (Wharton and Roulston, 1970). Reports of acaricide failure in South African blue tick populations date back as far as 1941 (Du Toit and Graf, 1941), when tick control efforts in cattle herds in the Eastern Cape were thwarted by apparent resistance to sodium arsenite.

Acaricide resistance can develop in individual ticks in a population through one of several mechanisms, such as:

1. mutation of the target site of the acaricide within the tick;
2. a reduction in the ability of the chemical to penetrate the outer layer of the tick's body, or
3. an alteration to the way in which the chemical is metabolised or sequestered by the parasite (Guerrero et al., 2012).

Resistance to pyrethroids, for example, occurs at the target site, where amino acids substitutions or nucleotide mutations affect the sodium channels of cells so that the acaricide becomes ineffective (Guerrero et al., 2012).

3. Hypothesis and aim

H_1: Acaricide resistance exists in one-host *Rhipicephalus* spp. (blue ticks) sampled from communal dip tanks and commercial cattle farms in the rural districts of KZN.

The aim of the study was to investigate the spectrum of acaricide resistance in one-host *Rhipicephalus* spp. (blue ticks) collected from cattle at communal dip tanks and commercial herds, using the Shaw Larval Immersion Test (SLIT) and to draw comparisons between their respective resistance profiles in relation to different tick management strategies that may have been employed.

4. Materials and methods

4.1 Study area

The Umgeni district municipality falls within the province of KwaZulu-Natal. It covers 1,520 km^2 and, according to municipal census data, has a current population of around 100,000 residents (Stats SA 2011 census). The landscape of KwaZulu-Natal is mainly savanna bushlands, grasslands, and forest areas, and most of the province experiences cool, dry winters and warm, wet summers (Fairbanks and Benn, 2000). The regional state vet office records approximately 4,000 communal cattle in the area (from source). There are eight state owned communal dip tanks in the district, although not all of them are in use, and many communal cattle owners have resorted to hand-spraying their cattle instead of plunge dipping. Animal health care is performed primarily by state veterinary services in the area and, according to a survey conducted by Hesterberg et al., less than 20% of the communal cattle owners interviewed by the researchers consulted a private veterinarian (Hesterberg et al., 2007). The role of the dip tanks in the area, therefore, play a far more important role than just tick control, and the demise of some of the region's dip tank structure has a far-reaching impact on animal health in general.

Cattle ownership in the area is a complex issue with many non-agricultural factors playing a role. Cattle are vital to many traditions and cultural practices in the area, with *Lobola* (bride price) that is usually paid in head of cattle from the groom to the bride's family, being a classic example (Alcock and Hornby, 2004). However, many communities in the study area

have limited resources with animal health being given a low priority, and the need for effective primary animal health care interventions is paramount (Ndoro et al., 2014).

4.2 Study design and sampling

A cross-sectional survey of the acaricide resistance in the blue tick population in and around the Umgeni district municipality was conducted.

Between twenty-five and forty engorged female blue ticks were randomly collected from cattle presented for routine dipping at communal dip tanks and on commercial farms. The ticks had to be a minimum of 4 mm long to be ready for egg laying and thus be viable for the study [per laboratory standard operating procedure (SOP)]. The progeny of 25-30 female ticks made up one sample that was tested in the laboratory. The total number of ticks collected from communal dip tanks was approximately 400 and a similar number was also collected from commercial herds for testing.

As per laboratory and field SOPs (see attached), the ticks were carefully removed, either digitally or with blunt-ended narrow forceps, from the body of healthy cattle standing in the crush before dipping; and placed into clean plastic bottles with aerated lids. Tissue paper was placed between the ticks and the lid of the bottle before closing. The bottles were transported back to the laboratory immediately after collection in a lockable purpose-designed ‘toolbox’.


a

b

c

Figure 6 **(**a) Tick collection, (b) tick collection bottles, and (c) tick transportation

4.3 Study population

The Umgeni municipality of KZN was identified as the study area due to its rural setting and the high number of communal and commercial cattle herds present in and around this municipal district. The Afrivet Tick Laboratory, where the testing was carried out, is located in the Umgeni region, and the author has a good working relationship with the state veterinarian and animal health technicians who manage the communal dip tanks in this area. Owner consent was obtained through the regional state veterinarian and a state vet office representative was present at sampling. None of the communal farmers whose animals were sampled was concerned by the process and, in fact, most livestock owners were interested in and grateful for the information transferred to them during these visits.

The commercial farmers were also asked for consent to sample ticks from their animals and were provided with the tick resistance data for their own farms after testing in order for them to use this information to assist in product selection for tick control. Some farmers collected the samples themselves and submitted them to the laboratory for testing.

The communal dip tanks and the commercial farms that were sampled are listed in the tables below, as well as their respective GPS co-ordinates and acaricides of choice. In order to maintain confidentiality, the commercial farms are listed as Farm 1-8 rather than by the farm name. The state-owned dip tanks are allocated a number and also listed by name.

a

b

Figure 7 (a) State owned communal dip tank in KwaZulu-Natal, and (b) Hand spraying communal cattle

Table 1 Communal dip tanks sampled

Dip tank	Municipality	GPS co-ordinates		Acaricide	Method of application
		South	East		
1. Egamalethu	Umgeni	S29.16366	E30.07203	Deltamethrin	Hand Spray
2. Mpophomeni	Umgeni	S29.33429	E30.10573	Deltamethrin	Hand Spray
3. Mpolweni	Umgeni	S29.42196	E30.48977	Deltamethrin	Hand Spray
4. French East	Umvoti	S29.15338	E30.47070	Deltamethrin	Dip tank
5. Nkosana	Okhahlamba	S28.79037	E29.16400	Deltamethrin	Dip tank
6. Mhlota	Okhahlamba	S28.84775	E29.22669	Deltamethrin	Dip tank
7. Brandvlei	Mooi Mpofana	S29.08210	E30.20170	Deltamethrin + Ivermectin	Pour on and injectable
8. Bellview	Mooi Mpofana	S29.18072	E30.30328	Deltamethrin	Dip tank

Table 2 Commercial farms sampled

Farms	Municipality	GPS co-ordinates		Acaricide	Method of application
		South	East		
Farm 1	Dr N D Zuma	S29.83055	E29.6457	Chlorfenvinphos	Dip tank
Farm 2	Umgeni	S29.33439	E30.3	Amitraz	Spray race
Farm 3	Umgeni	S29.29003	E30.13520	Chlorfenvinphos + Flumethrin	Hand spray
Farm 4	Okhahlamba	S28.61731	E29.4320	Chlorfenvinphos + Ivermectin + home remedies	Pour on
Farm 5	Umgeni	S29.34594	E30.22583	Chlorfenvinphos + Cypermethrin	Spray race
Farm 6	Harry Gwala	S30.2449	E30.17466	Amitraz	Spray race
Farm 7	Msundusi	S29.80136	E30.34146	Amitraz + Cypermethrin	Pour on
Farm 8	Umgeni	S29.3963	E30.165	Ivermectin	Injectable

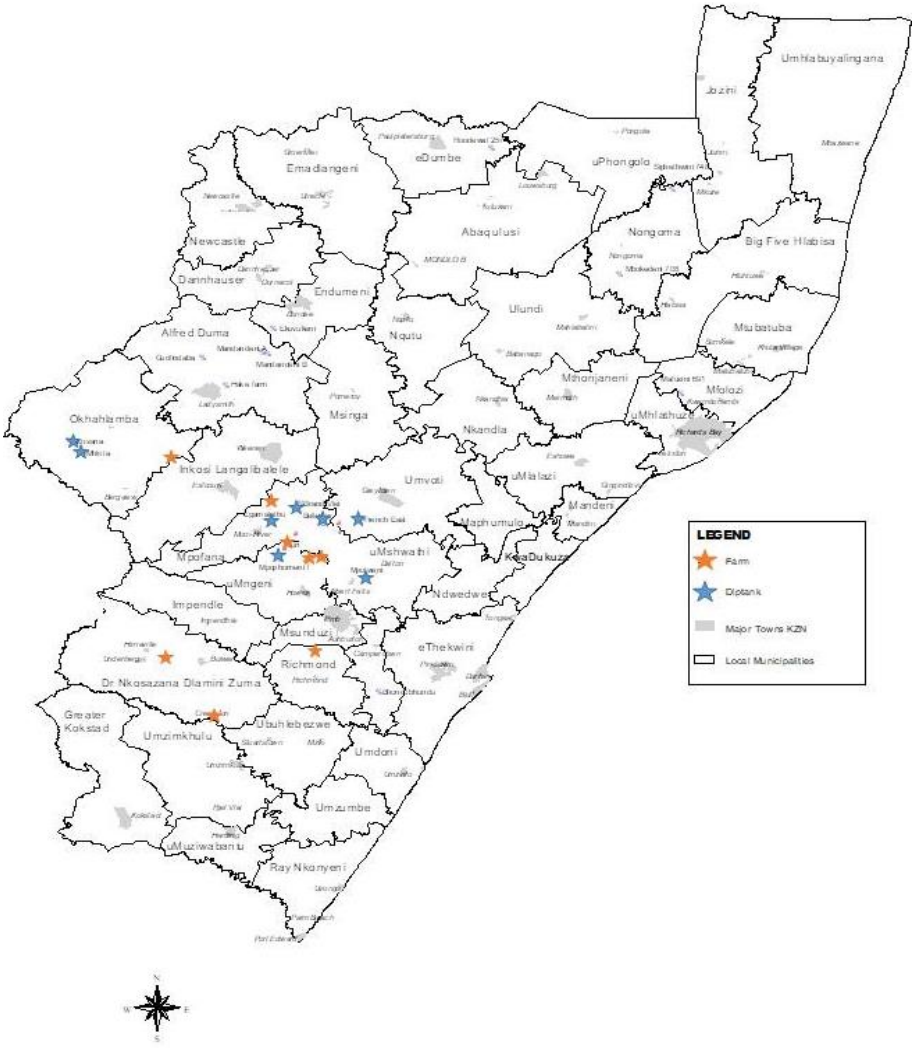


Figure 8 Map of state-owned dip tanks and commercial farms in KwaZulu-Natal. Distribution of dip tanks and farms sampled

4.4 Laboratory procedure

After collection and transportation to the laboratory, the ticks were sorted and male ticks as well as non-study species that may have been accidentally collected were removed. The ticks were identified by the author to species level using a Nikon light microscope at 10 x magnification and the viable engorged female ticks were placed in a single Erlenmeyer flask (per each sample site), stoppered with cotton wool and gauze and placed in an incubator. The ticks from farm 1 were not identified to species level due to a technical fault in the laboratory on the day. The ticks were incubated at a humidity of 80-85% and a temperature of 25-27 °C for approximately 40 days for ovipositioning to take place. The

eggs were allowed to hatch and the larvae left to mature for 15-21 days. The tests were conducted on day 18 post-hatching or as close to day 18 as logistics allowed. As per laboratory SOP, biosafety measures were employed to ensure the safety of personnel working with the ticks and to ensure the containment of ticks within the laboratory work space (see attached SOP).


a

b


c

Figure 9 (a) Sorting of ticks, (b) identification of species, and (c) incubation in Erlenmeyer flasks

The Shaw Larval Immersion Test (SLIT) (Shaw, 1966)

Equipment and materials

- Magnetic stirrer
- Incubator: maintained at a temperature of 27 ± 1 °C and at a relative humidity of 85 ± 5%
- Paper crimper
- Stainless steel tray of dimensions approximately 330 mm x 450 mm x 60 mm
- Stopwatch
- Aluminium foil dishes
- Camel-hair brushes
- Erlenmeyer flask of 100 ml capacity
- Stainless steel spatula
- Filter paper of diameter 150 mm - Whatmann No 1
- Forceps
- Pipettes, graduated

- Scissors
- Paper towels
- White paper sheets or another white surface
- Metal paper clips, plastic coated
- Acetone of analytical reagent grade or other organic solvent suitable for cleaning purposes
- Test chemicals

Table 3 Test chemicals used in SLIT

Product + Act 36 registration No.	Active ingredient	Concentration	Batch No. + Expiry
Afrivet Triatix 125 *G3189*	Amitraz	12.5%	5363 03/2020
Virbac Pro-Dip™ Cyp *G23311*	Cypermethrin (Mendes et al., 2011)	20%	C1221 09/2023
Coopers Supadip *G3349*	Chlorfenvinphos	30%	0418002A 04/2020

Cypermethrin was used in the laboratory bio-assay for this trial even though most of the dip tanks made use of a deltamethrin product for tick control. It is important to note, however, that there is well documented research to support the theory of cross resistance between the pyrethroid products (Mendes et al., 2011) and cypermethrin resistance in the laboratory will indicate deltamethrin resistance in the field.

The Shaw Larval Immersion test (SLIT) can be conducted as either a 'long-range' test, where the test chemical is diluted through a series of seven concentrations and the tick larvae are exposed to all seven dilutions, or a 'short range' test where the chemical is diluted to a single discriminating concentration (DC). These concentrations are determined by the concentrations used in the field for adequate tick control, and the known LC_{50} and LC_{99} of susceptible strains of ticks and have been formalised into a laboratory SOP, based on a SABS-approved test procedure. Analysis of a long-range test produces a 'factor of resistance' while the corrected mortality (CM) of a DC test is used to determine the resistance status of the population being tested.

SLIT procedure (see attached SOP)

- Serial dilutions of each of the three test chemicals are prepared in clean plastic containers. There are seven dilutions for each chemical (see table below). If there are insufficient larvae for a full test then a single discriminating concentration (DC) is made up for each test chemical.
- Approximated 200 larvae are brushed onto a clean, dry filter paper contained in an aluminium foil 'pie dish'.
- 10 ml of test chemical is then pipetted or syringed onto the larvae before covering them with a second dry filter paper.
- A 10-minute exposure time is allowed before brushing the larvae into dry filter paper envelopes and the envelopes are crimped closed. This process is repeated for each of the seven dilutions of the three different acaricides and then the envelopes are incubated for 72 hrs. A water control sample is also used.
- After the 72 hrs incubation period, the filter paper packets are removed from the incubator and the larvae are emptied onto a sheet of clean white paper for counting.
- The numbers of live and dead larvae are counted for each concentration as well as the water sample - with the live larvae being squash as they are counted, and the corrected mortality is established using Abbott's formula.

Table 4 Serial dilutions and discriminating concentrations used in SLIT

Dilution (%)	Amount of dip (ml)	Amount of water (ml)	Total vol. (ml)
Amitraz 12.5% (amidines) serial dilutions			
0.000006	18	78	96
0.000032	20	80	100
0.00016	20	80	100
0.0008	20	80	100
0.004	20	80	100
0.02	20	80	100
0.1	10	90	100
1% (master solution)	**4**	**46**	**50**
Cypermethrin 20% (pyrethroids) serial dilutions			
0.00002	10	40	50
0.0001	10	40	50
0.0005	10	30	40
0.002	10	40	50
0.01	20	80	100
0.05	20	60	80
0.2	10	40	50
1% (master solution)	**2**	**38**	**40**
Chlorfenvinphos (organophosphors) serial dilutions			
0.00013	30	60	90
0.0004	30	60	90
0.0012	40	60	100
0.003	30	70	100
0.01	30	60	90
0.03	30	70	100
0.1	10	90	100
1% (master solution)	**2**	**58**	**60**

Discriminating concentrations (DC)

Amitraz	0.001%
Cypermethrin	0.002%
Chlorfenvinphos	0.003%

Figure 10 Shaw larval immersion test procedure

4.5 Data management and analysis

The corrected mortality (CM) of the larvae is calculated using Abbott's Formula (Fleming and Retnakaran, 1985). This calculation factors in the mortality experienced in the control (water) group that has not been exposed to acaricides, and gives a truer reflection of the larval mortality from the acaricide exposure.

$$\text{Abbott corrected mortality} = \frac{\text{\% mortality in treatment - \% mortality in control}}{\text{100 - \% mortality in control x 100}}$$

The concentration versus CM is plotted on log-probit paper to determine the LC_{50} and LC_{99} at each concentration (Stephan, 1977; Miller and Tainter, 1944). The lethal concentration 50 (LC_{50}) is the concentration or dose of acaricide that will kill 50% of the population in the sample population. Similarly, the LC_{99} is that dose which will kill almost all (99%) of the larvae exposed to the acaricide.

The **factor of resistance** in relation to the LC_{50} of each acaricide is then determined using the LC_{50} of a known susceptible strain of blue ticks. In this case, the LC_{50} from previously tested strains of blue ticks from an area where no acaricides have been used was used as the reference point (source: Dr R J Taylor). The factor of resistance refers to the number of times

the LC_{50} of the tested larvae exceeds the LC_{50} of the known susceptible strain, and is targeted towards a phenotypic expression of resistance in the field rather than in the laboratory.

Table 5 LC_{50} and LC_{99} of susceptible tick strain

Tick species	Acaracide	Test	LC_{50}%	LC_{99}%
Rhipicephalus spp.	Cypermethrin	Shaw LIT	0.00002	0.0002
	Chlorfenvinphos	Shaw LIT	0.00063	0.0018
	Amitraz	Shaw LIT	0.00001	0.0002

A cut off point for the factor of resistance is set for each chemical to determine whether the ticks are either resistant or susceptible to the acaricide (see table). The organophosphors show resistance in the field at a much lower point than the other two classes of acaricide, hence the significantly lower factor of resistance.

Table 6 Factor of resistance-, susceptible or resistant scale

Acaracide	Scale	Status
Amitraz	< 100	Susceptible
	> 100	Resistant
Cypermethrin	< 100	Susceptible
	> 100	Resistant
Chlorfenvinphos	1-2	Susceptible
	3-5	Incipient
	6-10	Resistant
	11-20	Massively resistant

When a test was conducted using only a single discriminating concentration (DC) a cut-off point of a corrected mortality (CM) of 50% was used to determine the factor of resistance.

5. Results and discussion

The ticks collected from the eight state-run communal dip tank herds all produced enough eggs and viable larvae for testing. Two samples produced enough larvae for long range tests, while two samples were subjected to long range tests for one chemical and a DC test for the remaining two chemicals. Four samples only produced enough larvae to test a DC for each chemical.

The samples from the commercial farms yielded smaller populations of viable larvae and the ticks from one farm (Farm 8) failed to lay viable eggs. The farm owner reported the use of a macrocyclic lactone product in the cattle a week before tick collection so, even though there were live, engorged ticks visible on the cattle, the effect was on the reproductive performance of the ticks. The ticks collected on the seven remaining farms produced sufficient viable larvae for testing. Of the seven farm samples, one full long-range test was conducted, three samples were subjected to DC tests for all three chemicals, and three samples were subjected to a long-range test of seven concentrations for one acaricide and DC tests for the other two chemicals. This is summarised in the table below.

Table 7 Types of SLIT tests conducted on samples

Sample site	Long-range test	Short test (DC)	Long-range + DC
Dip tank samples	2 samples	4 samples	2 samples
Farm samples	1 sample	3 samples	3 samples

5.1 Tick species distribution

On arrival at the laboratory, a representative number of ticks from each sample population was examined under a light microscope to ascertain which blue tick species is present at the sample site. The findings are listed in the results table below, and the distribution of the two species is show in Map 2.

Rhipicephalus decoloratus and *Rhipicephalus microplus* were both identified during this study and there appears to be an even distribution of the two species throughout the study area.

- *Rhipicephalus decoloratus* was found at four dip tanks and three farms
- *Rhipicephalus microplus* was identified at four dip tanks and two farms
- Both species were found as mixed populations on two of the commercial farms
- The ticks from one commercial farm were not identified to species level due to a technical error in the laboratory.

Multiple studies conducted in various regions of South Africa indicate that *R. microplus* is expanding its range and in many areas is displacing *R. decoloratus* entirely (Nyangiwe et al., 2017). In a study very similar to this one, conducted in the Bushbuck Ridge region of South Africa in 2012, Malan et al. found that only *R. microplus* was identified in 12 communal dip tank herds and *R. decoloratus* was totally absent from the region (Malan, 2016). Detailed information regarding the distribution of these two tick species in the province of KwaZulu-Natal is difficult to find, however an in-house survey of data collected at the Afrivet Laboratory over three years, revealed that the co-existence of both species is still evident in the interior of KZN (author's own data). The coastal and northern regions appear to be inhabited mainly by *R. microplus* (see Map 3 below) however, further studies and investigations are required in order to plot and monitor the dynamics of this distribution.

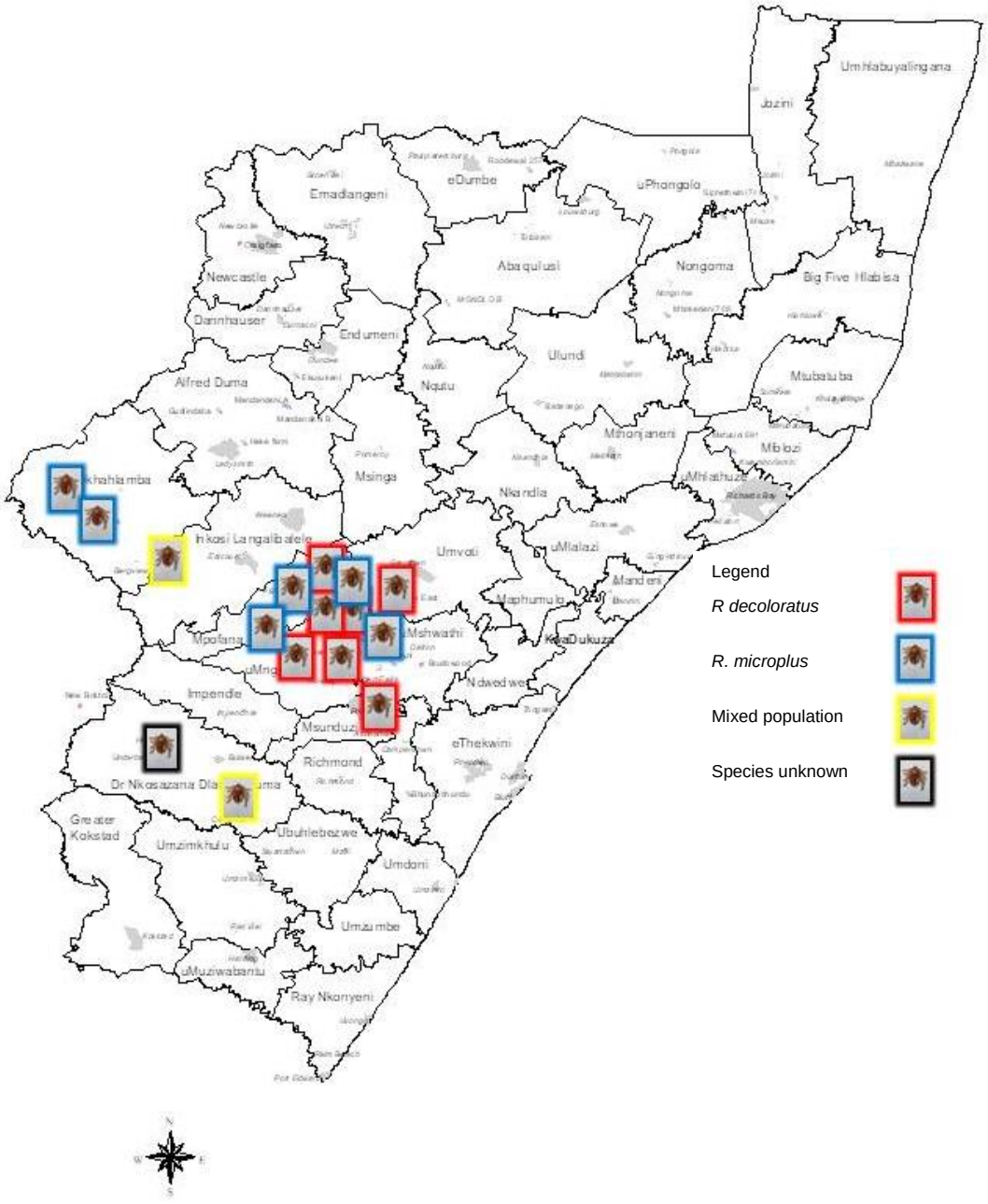


Figure 11 Geographical distribution of blue tick species in study area

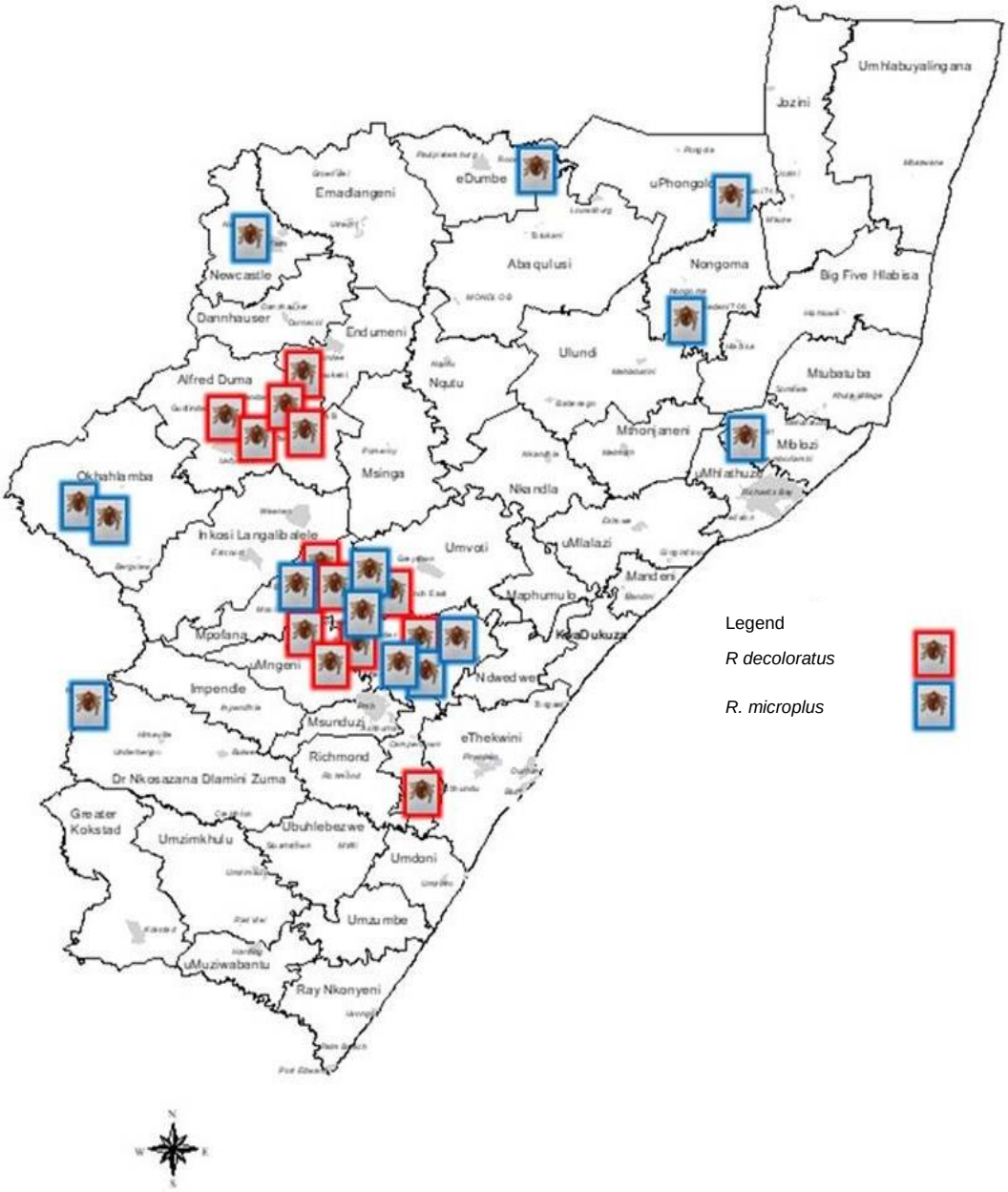


Figure 12 Geographical distribution of blue ticks species in KwaZulu-Natal

(Afrivet Laboratory Services)

5.1 Resistance profiles

Table 8 Summary of resistance profiles of communal dip tanks

Dip tank	Tick sp.	Resistance		
		Amitraz	**Cypermethrin**	**Chlorfenvinphos**
Dip tank 1	*R. microplus*	R	R	S
Dip tank 2	*R. decoloratus*	S	R	S
Dip tank 3	*R. decoloratus*	S	R	S
Dip tank 4	*R. decoloratus*	R	R	S
Dip tank 5	*R. decoloratus*	S	R	S
Dip tank 6	*R. microplus*	S	R	S
Dip tank 7	*R. microplus*	R	S	S
Dip tank 8	*R. microplus*	R	S	S
Total		**4/8**	**6/8**	**0/8**

R = Resistant; S = Susceptible

Table 9 Summary of resistance profiles of commercial farms

Farm	Tick sp.	Resistance		
		Amitraz	**Cypermethrin**	**Chlorfenvinphos**
Farm 1	*Rhipicephalus* spp.	R	S	S
Farm 2	*R. decoloratus*	R	R	S
Farm 3	*R. microplus*	S	R	S
Farm 4	*R. microplus* + *R. decoloratus*	S	R	R
Farm 5	*R. decoloratus*	S	R	S
Farm 6	*R. microplus* + *R. decoloratus*	S	R	S
Farm 7	*R. microplus*	S	R	S
Total		**2/7**	**6/7**	**1/7**

R = Resistant; S = Susceptible

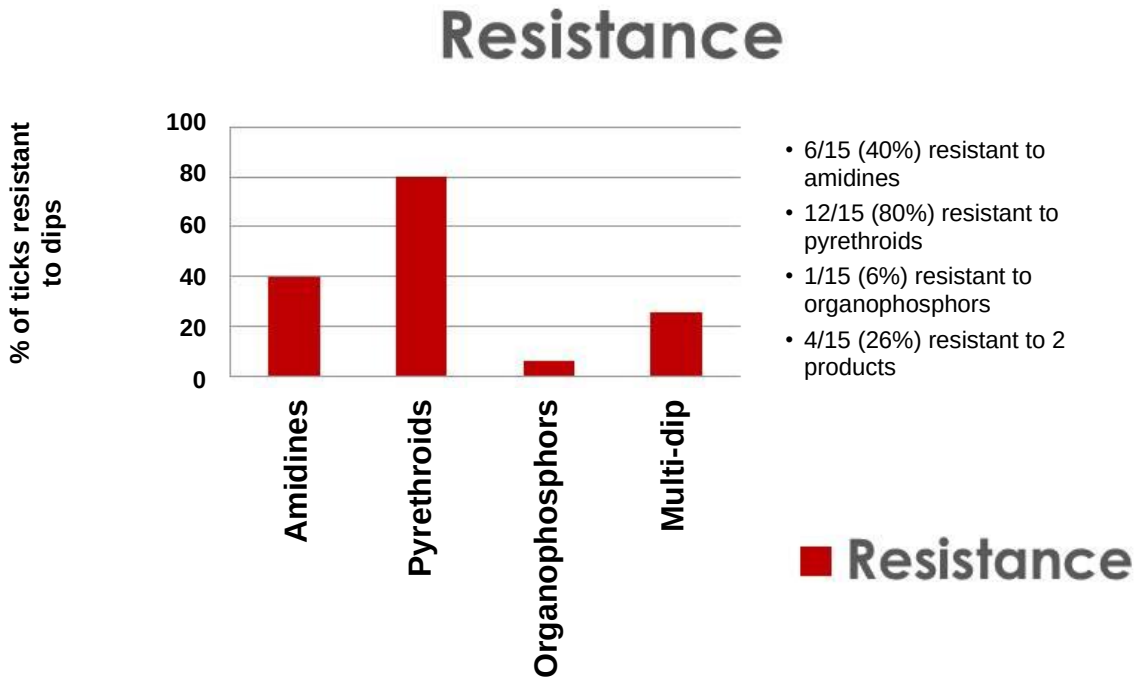


Figure 13 Acaricide resistance in blue ticks in KwaZulu-Natal Midlands

From the data presented above, it is clear that acaricide resistance is a present and current threat to tick control efforts in both communal and commercial herds. At face value, it appears that there is no significant difference in resistance profiles between the two groups of sample populations.

- Both communal and commercial herds show a significant degree of resistance to pyrethroid dips with a resistance prevalence of 75% and 85% respectively. This is in line with research that suggests that pyrethroid resistance develops at a more rapid rate than many of the other chemicals used for tick control (Morgan et al., 2009), and there is no discrimination between the two sample groups in this regard.
- Both sample groups show a smaller percentage of the tested populations with resistance to amidines. However, the communal herds have a higher prevalence of amitraz resistance, with 50% of the samples showing resistance compared to 28.5% of the commercial farm samples. The livestock owners at state-managed dip tanks made use of an amitraz dip for many years prior to changing over to deltamethrin a few years ago, so this may explain the development of amidine resistance in many of these tick populations.

- Organophosphor resistance is almost non-existent in both groups of tick populations, with only a single commercial farm sample showing resistance in the laboratory. This may be due to the lack of use of organophosphorous products in recent years. The toxicity of organophosphors to animals and the environment is a factor which plays a role in this, as well as the milk and meat withdrawal periods which are prohibitive to beef and dairy farmers.
- The commercial farmers use a varied selection of mostly combination products for tick control in their herds. It is unknown to the author how long the products have been in use by each farmer, so a historical picture of the resistance profiles is difficult to paint. However, it is clear from the data that pyrethroid products have been used in recent years by the majority of the famers, the result of which is the significant resistance shown by the tick populations to cypermethrin.
- There does not appear to be a significant correlation between the tick species identified at a site and the resistance profile.

The raw data of corrected mortalities and factors of resistance for the tests conducted on these samples are attached as Annexure 3. A summary of results is tabulated below.

Table 10 Shaw Larval Immersion Test (SLIT) results: Factor of resistance (FoR) and Corrected mortality (CM) of tick strains

Dip tank/Farm	Acaricide	Factor of resistance (FoR)	Resistant (R)/ Susceptible (S)
Egamalethu	Amitraz	LC_{50} = 0.0018% FoR = 180	R
	Cypermethrin	LC_{50} = 0.0027% FoR = 135	R
	Chlorfenvinphos	LC_{50} = 0.00045% FoR = 1.22	S
Mpophomeni	Amitraz	LC_{50} = 0.00025% FoR = 25	S
	Cypermethrin	DC CM = 10%	R
	Chlorfenvinphos	DC CM = 95%	S
Bellvue	Amitraz	LC_{50} = 0.00048% FoR = 48	S
	Cypermethrin	LC_{50} = 0.005% FoR = 250	R
	Chlorfenvinphos	LC_{50} = 0.0012% FoR = 1.9	S
Brand Vlei	Amitraz	DC CM = 36%	R
	Cypermethrin	DC CM = -5%	R
	Chlorfenvinphos	DC CM = 65%	S
French East	Amitraz	DC CM = 57%	S
	Cypermethrin	DC CM = 1%	R
	Chlorfenvinphos	DC CM = 98%	S
Mpolweni	Amitraz	DC CM = 93%	S
	Cypermethrin	DC CM = 30%	R
	Chlorfenvinphos	DC CM = 99%	S
Nkosana	Amitraz	DC CM = 30%	R
	Cypermethrin	DC CM = 72%	S
	Chlorfenvinphos	DC CM = 93%	S
Mhlota	Amitraz	LC_{50} = 0.00032% FoR = 100	R
	Cypermethrin	DC CM = 99%	S
	Chlorfenvinphos	DC CM = 100%	S
Farm 1	Amitraz	DC CM = 27%	R
	Cypermethrin	DC CM = 94%	S
	Chlorfenvinphos	DC CM = 100%	S
Farm 2	Amitraz	LC_{50} = 0.0036% FoR = 360	R
	Cypermethrin	DC CM = 17%	R
	Chlorfenvinphos	DC CM = 100%	S
Farm 3	Amitraz	LC_{50} = 0.0004% FoR = 40	S
	Cypermethrin	LC_{50} = 0.0074% FoR = 370	R
	Chlorfenvinphos	LC_{50} = 0.0012% FoR = 1.9	S
Farm 4	Amitraz	DC CM = 83%	S
	Cypermethrin	DC CM = 10%	R
	Chlorfenvinphos	DC CM = 45%	R
Farm 5	Amitraz	DC CM = 98%	S
	Cypermethrin	LC_{50} = 1% FoR = 5000	R
	Chlorfenvinphos	DC CM = 70%	S
Farm 6	Amitraz	LC_{50} = 0.0004% FoR = 40	S
	Cypermethrin	DC CM = 9%	R
	Chlorfenvinphos	DC CM = 100%	S
Farm 7	Amitraz	DC CM = 70%	S
	Cypermethrin	DC CM = 3%	R
	Chlorfenvinphos	DC CM = 77%	S
Farm 8	Did not test		

Key: DC = Discrimination concentration
LC_{50} = Lethal concentration 50
CM = Corrected mortality
e-R = Emerging resistance

An integrated tick management system is a holistic approach to tick control in the face of growing acaricide resistance. There are many strategies that can be employed along with chemical acaricides that will reduce the impact of tick-borne diseases, improve tick control, and also slow down the rate of the development of resistance in a tick population (Pegram et al., 1993).

With regards to acaricide use, some recommended strategies to alleviate resistance are listed below:

- Reduce the frequency of acaricide application through the use of vaccination and biological alternatives (Rodriguez-Vivas et al., 2018)
- Make use of synergized products, for example piperonyl butoxide with pyrethroid products, to improve the efficacy of the acaricide (Rodriguez-Vivas et al., 2018).
- Make use of combination products, or rotate the chemical class of acaricide within a season (Abbas et al., 2014).
- Ongoing monitoring of the resistance profile of the population on a farm through phenotypic assessment of the acaricide efficacy or bio-assay is advised (Abbas et al., 2014).

Other strategies to employ in an integrated tick management program can include:

- Environmental management interventions such as pasture burning and rotational grazing (www.afrivip.org)
- The selection of cattle with innate immunity to ticks and tick-borne diseases (De Castro and Newson, 1993).
- The introduction of parasite predators such as oxpecker into a herd of cattle (Mwangi et al., 1991).
- The use of vaccination against ticks and tick-borne diseases (Pegram et al., 1993).

The agricultural and veterinary industries have long relied on the use of chemical ectoparasiticides to aid in the control of ticks and tick-borne diseases. However, chemical control on its own has had little success in eradicating external parasites from South African cattle herds and, with the presented evidence of advancing resistance to acaricides in many tick populations in KwaZulu-Natal, an integrated, more holistic approach to tick control is called for. The judicious and strategic use of chemical ectoparasiticides is a vital link in an

integrated approach to tick control and frequent monitoring of tick populations for resistance to acaricides will empower livestock owners in their choice of products. Perhaps though, a move towards non-chemical alternatives to conventional dips, such as myco-acaricides or plant-derived repellents, needs to be explored by the veterinary pharmaceutical industry in anticipation of this trend of resistance continuing. Vaccines against ticks and tick-borne diseases offer an immunological alternative to chemical control in herds with high burdens of resistant ticks, however the innate immunity shown by indigenous cattle breeds in South Africa towards ticks and the diseases they transmit, is by far the most valuable tool at our disposal. Strategic dipping that allows for the development of endemic stability of tick-borne disease in a cattle herd is the goal. Endemic stability refers to the balanced state in a herd with a low incidence of clinical disease, despite a high number of infected vectors present (Coleman et al., 2001). The careful timing of an animal's first exposure to acaricides plus the judicious use thereafter will facilitate this strategy by allowing for the development of immunity towards the diseases to which they have been exposed at an early age.

6. Conclusions

It can be concluded from the results reported above, that significant acaricide resistance has developed in both communal and commercial herds in the KwaZulu-Natal Midlands. The majority of tick populations that were tested during this study showed resistance to pyrethroids particularly. This can be explained by the frequent use of pyrethroid chemicals by both groups of farmers, either on its own or in combination with other product(s), and the reported fact that resistance to pyrethroids develops faster in ticks than the other classes of acarcidal chemicals.

It can also be concluded that both species of blue tick, viz *R. decoloratus* and *R. microplus*, occur in similar population numbers in this area, although the reported displacement of *R. decoloratus* by *R. microplus* may result in a change in this species distribution in the near future. The species of blue tick present at a sample site did not appear to have an impact on the resistance profile of that population.

Regardless of the farming systems, the scale of commercialisation or the tick control methods employed by livestock owners in the KwaZulu-Natal Midlands, acaricide resistance is a reality that needs to be addressed by all role-players, in order to limit the potential devastation that ticks and tick-borne diseases can cause.

References

ABBAS, R.Z., ZAMAN, M.A., COLWELL, D.D., GILLEARD, J. & IQBAL, Z. 2014. Acaricide resistance in cattle ticks and approaches to its management: the state of play. *Veterinary Parasitology*, 203, 6-20.

ALCOCK, R. & HORNBY, D. 2004. Traditional land matters: A look into land administration in tribal areas in KwaZulu-Natal, Legal Entity Assessment Project Pietermaritzburg.

BAKER, G. 1989. The seasonal tick populations on traditional and commercial cattle grazed at four altitudes in Natal. *Journal of the South African Veterinary Association*, 60, 95-101.

BARKER, S.C. & MURRELL, A. 2003. Phylogeny, evolution and historical zoogeography of ticks: a review of recent progress. *Ticks and Tick-borne Pathogens.* Springer.

BENELLI, G., PAVELA, R., CANALE, A. & MEHLHORN, H. 2016. Tick repellents and acaricides of botanical origin: a green roadmap to control tick-borne diseases? *Parasitology Research*, 115, 2545-2560.

BOCK, R., JACKSON, L., DE VOS, A. & JORGENSEN, W. 2004. Babesiosis of cattle. *Parasitology*, 129, S247-S269.

COLEMAN, P., PERRY, B.D. & WOOLHOUSE, M. 2001. Endemic stability – a veterinary idea applied to human public health. *The Lancet*, 357, 1284-1286.

Danbirni, S., Natala, A.J., Ahembe, T.R., Gberindyer, F.A., Danbirni, S. & Ubani, E.O.A. 2012. Acaricidal efficacy of three organophosphates on different stages of *Amblyomma variegatum*. *International Journal of Animal and Veterinary Advances*, 4(2): 76-79.

DAVEY, R., AHRENS, E. & GEORGE, J. 1984. Efficacy of sprays of amitraz against *Boophilus* ticks on cattle. *Preventive Veterinary Medicine,* 2, 691-698.

DE CASTRO, J. & NEWSON, R. 1993. Host resistance in cattle tick control. *Parasitology Today,* 9, 13-17.

DE DEKEN, R., HORAK, I., MADDER, M. & STOLTSZ, H. 2014. Tick control. *Biology of Ticks*, 409-444.

DE LA FUENTE, J., ESTRADA-PENA, A., VENZAL, J.M., KOCAN, K.M. & SONENSHINE, D.E. 2008. Overview: ticks as vectors of pathogens that cause disease in humans and animals. *Frontiers in Bioscience,* 13, 6938-6946.

DE VOS, A. 1979. Epidemiology and control of bovine babesiosis in South Africa. *Journal of the South African Veterinary Association,* 50, 357-362.

DU TOIT, P. & GRAF, R. 1941. Resistance to arsenic as displayed by the single host Blue Tick *Boophilus decoloratus* (Koch)* in a localised area of the Union of South Africa: preliminary report. *Journal of the South African Veterinary Association,* 12, 50-58.

ESTRADA-PEÑA, A., BOUATTOUR, A., CAMICAS, J.-L., GUGLIELMONE, A., HORAK, I., JONGEJAN, F., LATIF, A., PEGRAM, R. & WALKER, A. 2006. The known distribution and ecological preferences of the tick subgenus Boophilus (Acari: Ixodidae) in Africa and Latin America. *Experimental and Applied Acarology,* 38, 219-235.

FAIRBANKS, D.H. & BENN, G.A. 2000. Identifying regional landscapes for conservation planning: a case study from KwaZulu-Natal, South Africa. *Landscape and Urban Planning,* 50, 237-257.

FERTILIZERS, F.F. Agricultural remedies and stock remedies act (Act No. 36 of 1947). *South Africa, Available from: Government Gazette,[statute on the internet]. c2005 [cited 2005 Feb 19]*.

FLEMING, R. & RETNAKARAN, A. 1985. Evaluating single treatment data using Abbott's formula with reference to insecticides. *Journal of Economic Entomology,* 78, 1179-1181.

FOIL, L., COLEMAN, P., EISLER, M., FRAGOSO-SANCHEZ, H., GARCIA-VAZQUEZ, Z., GUERRERO, F., JONSSON, N., LANGSTAFF, I., LI, A. & MACHILA, N. 2004. Factors that influence the prevalence of acaricide resistance and tick-borne diseases. *Veterinary Parasitology,* 125, 163-181.

GEORGE, J., POUND, J. & DAVEY, R. 2004. Chemical control of ticks on cattle and the resistance of these parasites to acaricides. *Parasitology,* 129, S353-S366.

GEORGE, J., POUND, J. & DAVEY, R. 2008. Acaricides for controlling ticks on cattle and the problem of acaricide resistance. *Ticks: Biology, Disease and Control*. Cambridge University Press Cambridge.

GUERRERO, F., PÉREZ DE LEÓN, A.A., RODRIGUEZ-VIVAS, R.I., JONSSON, N., MILLER, R.J. & ANDREOTTI, R. 2014. Acaricide research and development, resistance and resistance monitoring. *Biology of Ticks,* 2, 353-381.

GUERRERO, F.D., LOVIS, L. & MARTINS, J.R. 2012. Acaricide resistance mechanisms in *Rhipicephalus* (*Boophilus*) *microplus*. *Revista Brasileira de Parasitologia Veterinária,* 21, 1-6.

GUGLIELMONE, A.A., ROBBINS, R.G., APANASKEVICH, D.A., PETNEY, T.N., ESTRADA-PEÑA, A., HORAK, I.G., SHAO, R. & BARKER, S.C. 2010. The Argasidae, Ixodidae and Nuttalliellidae (Acari: Ixodida) of the world: a list of valid species names. *Zootaxa,* 2528, 1-28.

HESTERBERG, U., BAGNALL, R., PERRETT, K., HORNER, R. & GUMMOW, B. 2007. A questionnaire survey of perceptions and preventive measures related to animal health amongst cattle owners of rural communities in KwaZulu-Natal, South Africa. *Journal of the South African Veterinary Association,* 78, 205-208.

HORAK, I.G., HEYNE, H., WILLIAMS, R., GALLIVAN, G.J., SPICKETT, A.M., BEZUIDENHOUT, J.D. & ESTRADA-PEÑA, A. 2018. The Ixodid Ticks (Acari: Ixodidae) of Southern Africa, Springer.

JONGEJAN, F. & UILENBERG, G. 2004. The global importance of ticks. *Parasitology,* 129, S3-S14.

JONSSON, N., MAYER, D. & GREEN, P. 2000. Possible risk factors on Queensland dairy farms for acaricide resistance in cattle tick (*Boophilus microplus*). *Veterinary Parasitology,* 88, 79-92.

KAAYA, G.P. & HASSAN, S. 2000. Entomogenous fungi as promising biopesticides for tick control. *Experimental and Applied Acarology,* 24, 913-926.

MALAN, R.C. 2016. Acaricide resistance in *Rhipicephalus* (*Boophilus*) species at a communal dipping system in the Mnisi community Mpumalanga Province. University of Pretoria.

MEKONNEN, S., BRYSON, N., FOURIE, L. & PETER, R. 2002. Acaricide resistance profiles of single-and multi-host ticks from communal and commercial farming areas in the Eastern Cape and North-West Provinces of South Africa. *The Onderstepoort Journal of Veterinary Research,* 69, 99.

MEKONNEN, S., BRYSON, N.R., FOURIE, L.J., PETER, R.J., SPICKETT, A.M., TAYLOR, R.J., STRYDOM, T., KEMP, D.H. & HORAK, I.G. 2003. Comparison of 3 tests to detect acaricide resistance in *Boophilus decoloratus* on dairy farms in the Eastern Cape Province, South Africa. *Journal of the South African Veterinary Association,* 74, 41-44.

MENDES, M., LIMA, C., NOGUEIRA, A., YOSHIHARA, E., CHIEBAO, D., GABRIEL, F., UENO, T., NAMINDOME, A. & KLAFKE, G. 2011. Resistance to cypermethrin, deltamethrin and chlorpyriphos in populations of *Rhipicephalus* (*Boophilus*) *microplus* (Acari: Ixodidae) from small farms of the State of São Paulo, Brazil. *Veterinary Parasitology,* 178, 383-388.

MILLER, L.C. & TAINTER, M. 1944. Estimation of the ED50 and its error by means of logarithmic-probit graph paper. *Proceedings of the society for Experimental Biology and Medicine,* 57, 261-264.

MORGAN, J.A., CORLEY, S.W., JACKSON, L.A., LEW-TABOR, A.E., MOOLHUIJZEN, P.M. & JONSSON, N.N. 2009. Identification of a mutation in the para-sodium channel gene of the cattle tick *Rhipicephalus* (*Boophilus*) *microplus* associated with resistance to synthetic pyrethroid acaricides. *International Journal for Parasitology,* 39, 775-779.

MOTA-SANCHEZ, D., BILLS, P.S. & WHALON, M.E. 2002. Arthropod resistance to pesticides: status and overview. *Pesticides in Agriculture and the Environment,* 241-272.

MOYO, B. & MASIKA, P. 2009. Tick control methods used by resource-limited farmers and the effect of ticks on cattle in rural areas of the Eastern Cape Province, South Africa. *Tropical Animal Health and Production,* 41, 517-523.

MURRELL, A. & BARKER, S.C. 2003. Synonymy of *Boophilus* Curtice, 1891 with *Rhipicephalus* Koch, 1844 (Acari: Ixodidae). *Systematic Parasitology,* 56, 169-172.

MWANGI, E.N., DIPEOLU, O.O., NEWSON, R.M., KAAYA, G.P. & HASSAN, S.M. 1991. Predators, parasitoids and pathogens of ticks: a review. *Biocontrol Science and Technology,* 1, 147-156.

NDORO, J., MUDHARA, M. & CHIMONYO, M. 2014. Livestock extension programmes participation and impact on smallholder cattle productivity in KwaZulu-Natal: A propensity score matching approach. *South African Journal of Agricultural Extension,* 42, 62-80.

NYANGIWE, N., HARRISON, A. & HORAK, I.G. 2013. Displacement of *Rhipicephalus decoloratus* by *Rhipicephalus microplus* (Acari: Ixodidae) in the Eastern Cape Province, South Africa. *Experimental and Applied Acarology,* 61, 371-382.

NYANGIWE, N., HORAK, I.G., VAN DER MESCHT, L. & MATTHEE, S. 2017. Range expansion of the economically important Asiatic blue tick, *Rhipicephalus microplus*, in South Africa. *Journal of the South African Veterinary Association,* 88.

PEGRAM, R., TATCHELL, R., DE CASTRO, J., CHIZYUKA, H., CREEK, M., MCCOSKER, P., MORAN, M. & NIGARURA, G. 1993. Tick control: new concepts. *World Animal Review,* 74, 2-11.

POLAR, P., MOORE, D., KAIRO, M.T. & RAMSUBHAG, A. 2008. Topically applied myco-acaricides for the control of cattle ticks: overcoming the challenges. *Experimental and Applied Acarology,* 46, 119-148.

RECHAV, Y. & KOSTRZEWSKI, M. 1991. The relative resistance of six cattle breeds to the tick *Boophilus decoloratus* in South Africa.

RODRIGUEZ-VIVAS, R.I., JONSSON, N.N. & BHUSHAN, C. 2018. Strategies for the control of *Rhipicephalus microplus* ticks in a world of conventional acaricide and macrocyclic lactone resistance. *Parasitology Research,* 117, 3-29.

ROSARIO-CRUZ, R., ALMAZAN, C., MILLER, R.J., DOMINGUEZ-GARCIA, D.I., HERNANDEZ-ORTIZ, R. & DE LA FUENTE, J. 2009. Genetic basis and impact of tick acaricide resistance. *Frontiers in Bioscience,* 14, 2657-2665.

SHAW, R. 1966. Culture of an organophosphorus-resistant strain of *Boophilus microplus* (Can.) and an assessment of its resistance spectrum. *Bulletin of Entomological Research,* 56, 389-405.

SHAW, R., COOK, M. & CARSON JR, R. 1968. Developments in the resistance status of the southern cattle tick to organophosphorus and carbamate insecticides. *Journal of Economic Entomology,* 61, 1590-1594.

SPICKETT, A.M., DE KLERK, D., ENSLIN, C. & SCHOLTZ, M. 1989. Resistance of Nguni, Bonsmara and Hereford cattle to ticks in a bushveld region of South Africa. *Onderstepoort Journal of Veterinary Research,* 56, 245-250.

STEPHAN, C.E. 1977. Methods for calculating an LC 50. *Aquatic toxicology and hazard evaluation.* ASTM International.

STONE, B. 1968. Brain cholinesterase activity and its inheritance in cattle tick (*Boophilus microplus*) strains resistant and susceptible to organophosphorus acaricides. *Australian Journal of Biological Sciences,* 21, 321-330.

TAYLOR, M.A. 2001. Recent developments in ectoparasiticides. *Veterinary Journal,* 161, 253-268.

TERKAWI, M.A., THEKISOE, O.M., KATSANDE, C., LATIF, A.A., MANS, B.J., MATTHEE, O., MKIZE, N., MABOGOANE, N., MARAIS, F. & YOKOYAMA, N. 2011. Serological survey of *Babesia bovis* and *Babesia bigemina* in cattle in South Africa. *Veterinary Parasitology,* 182, 337-342.

WALKER, A.R. 2003. Ticks of domestic animals in Africa: a guide to identification of species, Bioscience Reports Edinburgh.

WHARTON, R. & ROULSTON, W. 1970. Resistance of ticks to chemicals. *Annual Review of Entomology,* 15, 381-404.

Annexure 1
Shaw larval immersion test SOP

SA BUREAU OF STANDARDS - STANDARD METHODS

Veterinary pesticides: The efficacy of chemicals against tick larvae

SECTION 1 OBJECTIVE

1.1 To determine the efficacy of a chemical compound (at various concentrations) against tick larvae and to compare under laboratory conditions the efficacy to that of a known effective concentration of a reference formulation.

EQUIPMENT AND MATERIALS

2.1 **Magnetic stirrer**

2.2 **Incubators:** Two incubators maintained at a temperature of 27 ± 1 °C and at a relative humidity of 85 ± 5%

2.3 **Paper crimper**

2.4 **Stainless steel tray** of dimensions approximately 330 mm x 450 mm x 60 mm

2.5 **Stopwatch**

2.6 **Aluminium foil dishes**

2.6.1 Large aluminium foil dishes (1) of bottom diameter approximately 160 mm and of depth approximately 18 mm

2.6.2 Small aluminium foil dishes (2) of bottom diameter approximately 70 mm and of depth approximately 65 mm

2.7 **Camel-hair brushes**

2.8 **Erlenmeyer flask** of 100 ml capacity and provided with foam plastic stopper and, around the neck of the flask, a collar fashioned from a length of masking tape folded lengthwise with one half of the adhesive side facing outwards to serve as a trap for escaping tick larvae.

2.9 **Stainless steel spatula** with a collar of masking tape fashioned as in 2.8 above

2.10 **Filter paper** of diameter 150 mm and made up as follows:

2.10.1 Unfolded filter papers

2.10.2 Filter papers that have been folded into envelopes with the smooth side on the outside as shown in Figure 1 and suitably marked.

2.11 **Forceps**

2.12 **Pipettes:** graduated

2.12.1 Use pipettes of 10 ml capacity and fitted with suitable filler bulbs for delivery of the control solution, the reference chemical solution and the test chemical solutions; or

2.12.2 A 10 ml transfer pipetting system with sufficient tips for delivering the control solution, the reference chemical solution and the test chemical solutions.

2.13 **Masking tape**, of width 15-25 mm

2.14 **Scissors**

2.15 **Paper towels**

2.16 **White paper sheets** or other white surface

2.17 **Metal paper clips:** plastic coated

2.18 **Acetone** of analytical reagent grade or other organic solvent suitable for cleaning purposes.

2.19 **Reference chemical:** A reference chemical that is preferably registered as a stock remedy under the Fertilizers, Farm Feeds, Agricultural Remedies and Stock Remedies Act, 1947 (Act No. 36 of 1947), the active ingredient of which preferably belongs to the same chemical group as or has a mode of action similar to that of the test chemical.

2.20 **Test chemical:** A sufficient quantity of the test chemical to provide at least 10 ml of each of the dilutions referred to in 4.1.4

- Alcan Style 203 has been found suitable
- Alcan Style 508 has been found suitable
- Whatman No. 1 filter paper has been found suitable

SECTION 3 TEST TICKS

3.1 Healthy, normal ixodid tick larvae of the required species and strain, that were hatched in the Erlenmeyer flask (see 2.8) 2-4 weeks before the test.

SECTION 4 PROCEDURE

4.1 Preparation of equipment and materials

4.1.1 **Lay out of equipment:** Lay out the equipment as suggested in Appendix A

4.1.2 **Control solution:** As the control solution, use the solvent (or mixture of solvents) that is recommended by the sponsor for the dilution of the test chemical.

4.1.3 **Reference chemical:** Using a suitable solvent, dilute the reference chemical to 25% or less of the recommended concentration, or to a concentration that has in a similar test been found to be less than 100% effective but more than 50% effective against the same tick species. Pour dilution into a stoppered measuring cylinder and mix well.

4.1.4 **Test chemical:** Prepare a series of six dilutions (X, X/2, X/4, X/8, etc.) of the test chemical in measuring cylinders and shake well. If the material is solid, dissolve it in acetone or another soluble organic solvent and dilute water with it; if it is a liquid, dilute with the organic solvent or with water, or both.

4.2 Exposure

4.2.1 Application of materials

A. Place a large aluminium foil dish containing an unfolded filter paper onto a clean paper towel in the stainless-steel tray.

B. While stirring the control solution in the small aluminium foil dish on the magnetic stirrer (using a paper clip as the stirrer vane), draw a 10 ml aliquot of sample into a clean pipette.

C. Using the spatula, draw between 400 and 500 tick larvae onto the filter paper in the large aluminium foil dish.

D. Start the stopwatch and immediately squirt approximately 5 ml of the control solution in a zig-zag pattern over the tick larvae.

E. Place and unfolded filter paper over the tick larvae and squirt the rest of the control solution in the pipette in a zig-zag pattern over it. Put the aluminium foil dish containing the filter papers with larvae aside for 10 min.

F. Repeat the procedure described in A-E above once more using the same solution. Ensure that the application of the control solution to the two sets of tick larvae is completed within 1 min.

G. Using (instead of the control solution) the reference chemical followed by the series of four dilutions of the test chemical in ascending order of concentration, repeat the procedure described in A-F above, ensuring that the exposure time per treatment is 10 min.

NOTE:

1. An exposure time of 10 min per treatment can easily be achieved if each exposure period is started exactly on the minute (use the stopwatch to ensure proper timing).

2. Take extreme care when tick larvae are handled; when the flak of tick larvae is opened, place the plastic foam stopper on the filter paper on which the larvae will be placed (see C above) and mop up stray larvae with masking tape during any free time.

4.2.2 Transfer of tick larvae

H. Exactly 10 min after the application, use the forceps to place the first two filter papers containing the tick larvae treated with the control solution (see 4.2.1 D and E) onto clean paper towel in the stainless steel tray. Discard the foil dish.

I. Using the forceps, lift the top filter paper and place it on a dry portion of the paper towel, with the tick larvae uppermost. Move the other filter paper containing larvae to a dry portion of the paper towel. Gently press down the two pieces of filter paper in order to dry them.

J. By means of pushing a clean camel-hair brush forward through the tick larvae on the filter papers, transfer between 70 and 100 larvae to the centre of an opened filter paper envelope. Discard unused larvae.

NOTE: clean the forceps and camel-hair brush with acetone kept in glass containers

A. Repeat the procedure given in A-C above, using the replicate filter papers containing the tick larvae treated with the control solution.

B. Refold the envelopes and seal the open edges with the paper crimper. Place both envelopes in a clean large aluminium foil dish.

NOTE: A transfer time of 10 min per treatment can easily be achieved if each transfer procedure is started exactly on the minute

C. So repeat the procedure given in A-E above, using the tick larvae treated with the series of four dilutions of the test chemical (in ascending order of concentration) that the entire transfer procedure is completed in 10 min.

D. Incubate the larvae treated with the control solution in a separate incubator from the larvae treated with the reference and the test chemicals.

4.3 Mortality counts

4.3.1 After 72 hrs remove all the envelopes from the incubators.

4.3.2 Using the scissors, cut off the crimped edge of the first envelope containing the larvae treated with the control solution.

4.3.3 Place the unfolded envelope on a clean white surface.

4.3.4 Count the live larvae by collecting them on a piece of masking tape, and then count the dead larvae.

4.3.5 Record the number of live and dead larvae.

4.3.6 Repeat the above procedure, using the second envelope containing larvae treated with the control solution.

4.3.7 Repeat the procedure given in 4.3.2-4.3.5 above first with the larvae treated with the reference chemical and then with the larvae treated with the series of four dilutions of the test chemical in ascending order of concentration.

NOTE: Use a form such as that shown in Figure 2 for keeping record of the test.

SECTION 5: CALCULATION

5.1 Combine the mortality figures for each pair of replicates and calculate the percentage mortality for the control solution, for the reference chemical and for each of the of the four concentrations of the test chemical.

5.2 If the mortality of the tick larvae treated with the control solution is 10% or less, the percentage mortality of the larvae treated with the reference chemical and that of the larvae treated with the four dilutions of the test chemical is corrected as follows, using Abbott's formula:

Corrected mortality, % (CM %) = $\frac{Pi-Pc}{100-Pc} \times 100$

where Pi = mortality obtained using reference chemical or test chemical (%)
Pc = mortality obtained using control solution (%)

NOTE: If the mortality of the larvae treated with the control solution exceeds 10%, or if the mortality exceeds 20% when all the reference and test chemical mortalities are 100%, discard the results and repeat the test.

5.3 Using the concentration and CM % data, plot a graph on log-probability paper and determine the lethal concentration value required by the sponsor.

Annexure 2
Tick containment SOP

PURPOSE

Acaricide resistance testing in the laboratory requires the use of the in vivo larval bioassay Shaw Larval immersion test as described by Shaw (1966). The larvae are obtained from engorged female ticks collected from the field from areas suspected of field acaricide resistance. The engorged females, their eggs and the hatched larvae are required to be maintained at optimum temperature and humidity factors in a sealed incubator in the laboratory, until the time of testing.

The incubation of these live larvae for the purpose of acaricide resistance testing carry a minimal biological risk for the personnel inside and outside the tick-handling facility. The main risk to laboratory personnel relates to the ticks' obligate haematophagy, ability to crawl under the protective equipment and personnel clothing, remain hidden and/or attached to the host as well as survive on or under furniture for long periods of time.

Therefore protocols need to be in place to safely work with ticks and their larvae in the laboratory environment, to minimize the risk of staff being bitten as well as to prevent the contamination of the environment with resistant ticks.

The purpose of this standard operating procedure is to describe the basic principles of containment and management of ticks that are applicable to, and implemented at, the Afrivet Tick Unit. This SOP contains institutional policies, general information about laboratory operations and references to applicable international and national regulations and guidelines that protect the safety and security of laboratory personnel and the surrounding environment.

This standard operating procedure document is part of a larger library of manuals, SOPs, and associated job aides that will assist in implementing an overarching laboratory quality management system. These additional documents cover topics such as diagnostic procedures, and equipment, supplies, facility, information and quality management.

It is the policy of the Afrivet Tick Unit to provide a safe and secure work environment. By following the guidelines and recommendations herein, the safety of the work environment should be improved by minimizing and/or eliminating, where possible, biological hazards in this facility and ensuring that operations with live tick specimens are conducted in a safe, secure and reliable manner. These policies are applicable to all laboratory managers, investigators, and technicians conduct or are engaged in laboratory work.

SCOPE

This document describes standard operating procedures pertaining to the containment and management of the tick samples (both adult and larval stages) at the Afrivet Tick Unit. This includes step-by-step directions and best practices for implementing.

The scope of the Afrivet Tick Unit tick containment and management program is to set requirements necessary to control risks associated with the handling, storage and disposal of tick samples (both adult and larval stages). The standard operating procedure described herein will enable the Afrivet Tick Unit to:

- Establish and maintain a management system to control or minimize risk to acceptable levels in relation to personnel as well as the environment which could be directly or indirectly exposed to live ticks.
- Provide assurance that the requirements are in place and implemented effectively.
- Provide a framework for training and raising awareness of laboratory biosafety and biosecurity guidelines and best practices for personnel.

The management system approach enables the Afrivet Tick Unit to effectively identify, monitor and control the laboratory biosafety and biosecurity aspects of its activities.

This manual serves to demonstrate that the Afrivet Tick Unit recognizes the documents listed below as informative references and seeks compliance through risk-based, sustainable approaches:

- OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2018

DEFINITIONS

- **Acaricide resistance** – the ability in a strain of ticks to tolerate doses of acaricides that would prove lethal to the majority of individuals in a normal population of the same species
- **Risk Group 2** – (moderate individual risk, low community risk) as defined by the WHO, an invertebrate that can cause human or animal disease, but is unlikely to be a serious hazard to laboratory workers, the community, livestock or the environment. Laboratory exposures may cause infection, but effective treatment and preventative measures are available and the risk of spread of infection is limited.

RESPONSIBILITIES

- **Dr Caryn Shacklock** – Veterinarian, laboratory manager
- **Selina Maphalala** – laboratory technical assistant

PROCEDURE

Identification of tick samples

Ticks are identified according to their morphological characteristics and species are differentiated, if necessary, by examining the mouthparts under a microscope.

Tick handling

Unfed nymphs and adults are handled by the hind leg with fine forceps and minimal pressure. Engorged ticks of all life cycle stages are best handled with blunt-end forceps, as to prevent rupturing.

It is recommended to use magnifying loupes when counting or handling ticks.

Personal protective equipment

Personnel working in the Afrivet Tick Unit tick testing suite are required to don a white laboratory coat when handling tick samples to allow easy visualization if a tick crawls on the suit while working. The sleeves of the coat must be sealed with a tight-fitting elastic band and disposable latex gloves worn up over the ends of the sleeves. Long gumboots are worn in the laboratory, with sticky double-sided tape around the tops of the boots, as well as around the wrists, to act as barrier to the ticks, particularly the larvae.

Labelling of tick samples

Label information includes:

- genus and species
- date of sample received
- Predicted hatch date
- Actual hatch date
- Laboratory number allocated
- Isolate name given to the sample, usually based on the farm/dip tank from which they were collected

We have found that labels hand-written in pencil on adhesive white stickers, which do not fade, smudge, collect mould, or wrinkle easily under humid conditions, work best.

Safeguarding tick samples

Flasks of adult ticks are held in flat dishes of soapy water whilst in the incubator to ensure that any larvae that escape past the cotton wool stopper, remain contained within the incubator. A water trap is placed at the bottom of the incubator, which assists in the maintenance of humidity within the incubator, and that will also catch any ticks or larvae that may have escaped from the flask and fallen through the grid bars of the shelves. There is a double sided sticky tape barrier around the perimeter of the incubator door.

When a flask of ticks is removed from the incubator to be worked with on the adjacent counter, every care must be taken to ensure that the flask is not dropped. Only one flask is to be handled at a time, and it should be carried by the neck with one hand, and the other hand placed underneath for support. If a flask of ticks is dropped, the area is to be quickly contained by spraying methylated spirits or acetone liberally on the ticks and surrounding areas. The ticks and broken glass are swept up using a dedicated dustpan and brush into a container, soaked in acetone or spirits and the container sealed tightly immediately. The dustpan and brush must then also be submerged in acetone to kill any ticks that remain on them.

Adult and larval stages of ticks are worked with on a white counter so that they are easily visible. All work is conducted on a stainless steel tray that is bordered with sticky double-sided tape that is applied freshly before each work session. The tape acts as a barrier trap for any larvae that may crawl out of the work area. The counter top is also bordered by the same sticky trap.

Any small larvae that are seen to be crawling on the counter top are picked up with the sticky side of a piece of masking tape, soaked in acetone and disposed of in a sealable bin.

Petroleum jelly or tick grease must be applied to door frames to trap any escaping ticks.

Tick storage

Engorged female ticks may be housed in sterile clear glass Ehrlenmeyer flasks. Flasks are plugged securely with a bung made of cotton wool wrapped paper towel. This serves as secure tick barrier while allowing sufficient air exchange for the engorged female ticks. These females are contained for a minimum period of 21 days [for 1-host *Rhipicephalus* species] and longer (for *Amblyomma hebraeum*) until larvae hatch and are at a suitable age for testing (normally 15-21 days).

After identification, and labelling, these flasks are placed in a sealed incubator. The most critical parameter in maintaining a laboratory colony is relative humidity. Most Ixodid colonies require a relative humidity of between 80 and 95%, depending upon species. Maintenance of incubation parameters are discussed under the relevant standard operating procedure.

The incubator is housed in a separate room to the office and HPLC room.

Sample waste

A contract is set up with ClinX, a local medical and biological waste remove company.

They provide and collect bins and drums for the laboratory's used chemicals and other biological waste.

REFERENCES

Thangamani, S., & Bente, D. (2014). Establishing protocols for tick containment at Biosafety Level 4. *Pathogens and Disease*, *71*(2), 280-283. http://doi.org/10.1111/2049-632X.12187

Annexure 3
Animal- and research ethic approval certificates

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Animal Ethics Committee

PROJECT TITLE	**Acaricide resistance in *Rhipicephalus* spp. Ticks at rural dip tanks and commercial farms in and around the Umgeni district of KZN**
PROJECT NUMBER	**V052-18**
RESEARCHER/PRINCIPAL INVESTIGATOR	**Dr. C Shackleck**

STUDENT NUMBER (where applicable)	**U_97019926**
DISSERTATION/THESIS SUBMITTED FOR	**MSc**

ANIMAL SPESIES/SAMPLES	**Bovine**
NUMBER OF ANIMALS	**20-35 ticks (is regarded as 1 sample) from 8-10 dip tanks**
Approval period to use animals for research/testing purposes	**October 2018 – October 2019**
SUPERVISOR	**Dr. H Stoltz**

KINDLY NOTE:

Should there be a change in the species or number of animal/s required, or the experimental procedure/s - please submit an amendment form to the UP Animal Ethics Committee for approval before commencing with the experiment

APPROVED	Date 19 October 2018
CHAIRMAN: UP Animal Ethics Committee	Signature

S4285-15

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Animal Ethics Committee

Extension No. 1

PROJECT TITLE	**Acaricide resistance in *Rhipicephalus* spp. Ticks at rural dip tanks and commercial farms in and around the Umgeni district of KZN**
PROJECT NUMBER	**V052-18**
RESEARCHER/PRINCIPAL INVESTIGATOR	**Dr. C Shacklock**

STUDENT NUMBER (where applicable)	**U_97019926**
DISSERTATION/THESIS SUBMITTED FOR	**MSc**

ANIMAL SPESIES/SAMPLES	**Ticks (Bovine)**
NUMBER OF ANIMALS	**350 ticks collected from 5 dip tanks; 2 commercial farms sampled**
Approval period to use animals for research/testing purposes	**March 2019 – March 2020**
SUPERVISOR	**Dr. H Stoltz**

KINDLY NOTE:

Should there be a change in the species or number of animal/s required, or the experimental procedure/s - please submit an amendment form to the UP Animal Ethics Committee for approval before commencing with the experiment

APPROVED	Date 5 March 2019
CHAIRMAN: UP Animal Ethics Committee	Signature

S4285-15

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Research Ethics Committee

PROJECT TITLE	Acaricide resistance in single-host *Rhipicephalus spp.* Blue ticks at rural dip tanks and commercial farms in and around the Umgeni district of KZN.
PROJECT NUMBER	REC035-18
RESEARCHER/PRINCIPAL INVESTIGATOR	Caryn Shacklock

DISSERTATION/THESIS SUBMITTED FOR	MSc

SUPERVISOR	Hein Stoltsz

APPROVED	Date 14 March 2019
CHAIRMAN: UP Research Ethics Committee	Signature A.M. Duncan