ACARICIDE RESISTANCE IN *RHIPICEPHALUS (BOOPHILUS)* SPECIES AT A COMMUNAL DIPPING SYSTEM IN THE MNISI COMMUNITY, MPUMALANGA PROVINCE

By

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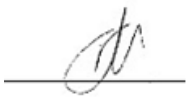
DECLARATION

Apart from the assistance received, which has been reported in the Acknowledgements, and in appropriate places in the text, this dissertation is the original work of the author. The investigations in this dissertation have not been presented for any other degree at any other University



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SUMMARY

Acaricide Resistance in *Rhipicephalus (Boophilus)* species at a communal dipping system in the Mnisi Community, Mpumalanga Province

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A study was conducted (November 2012) on the communal dipping system in Mnisi, Mpumalanga Province of South Africa to detect levels of blue tick resistance to commonly used acaricides. The larvae obtained from engorged females of the one host tick *Rhipicephalus (B). microplus* from twelve communal dipping areas were tested against various concentrations of amitraz, chlorfenvinphos and cypermethrin using the Shaw Larval Immersion Test method. Only *R. (B). microplus* ticks were identified from all sample areas, indicating a displacement of the indigenous *R. (B). decoloratus* tick in this area. Resistance testing using the Shaw Larval Immersion Test showed that no resistance to chlorfenvinphos was detected at any of the dip tanks, which was in keeping with the absence of known use of this product in the area. An important finding was the rapid development of resistance to the pyrethroids, which had only been in use for four months prior to conducting the study. Only one area (Hlalakane) yielded a *R(B).spp* population that was wholly susceptible to all three compounds. Resistance to amitraz was variable, with half (six out of 12) of the dip tanks comprising susceptible *R(B).spp* populations and two dip tanks with emerging resistance to amitraz.

Possible risk factors which caused the resistance problems are discussed and acaricide management strategies recommended.

Keywords: Acaricide resistance, blue ticks, Shaw Larval Immersion Test

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ABBREVIATIONS

&	and
CM	Corrected mortality
CNS	Central nervous system
DC	Discriminating concentration
FAO	Food and Agricultural Organisation
Fig.	Figure
FMD	Foot-and-mouth disease
FOR	Factor of resistance
g	gram
ha	hectares
i.e.	That is
LC_{50}	Lethal concentration which kills 50 % of the population
LC_{99}	Lethal concentration which kills 99 % of the population
LPT	Larval Packet Test
m	meter
mg	milligram(s)
ml	millilitre(s)
MCP	Mnisi Community Programme
MTA	Mnisi Traditional Authority
no.	Number
%	percentage
^0C	Degrees Celsius
OP	Organophosphate
Pers. comm.	Personal communication
PHD	Predicted hatch date

R	Rand
R. (B.)	*Rhipicephalus (Boophilus)*
RH	Relative humidity
SA	South Africa
SLIT	Shaw Larval Immersion Test
Spp	Species
TBDs	Tick-borne diseases

CHAPTER I: GENERAL INTRODUCTION

Ticks are obligate haematophagous ectoparasites of animals, both domestic and wild, and humans and have a widespread geographic distribution (Kiss & Cadar, D. & Spinu, M. 2012). Globally, ticks transmit a greater selection of pathogenic organisms such as protozoans, rickettsiae, spirochaetes and viruses, than any other arthropod vector group. They are considered to be among the most important vectors of pathogens affecting livestock, humans and companion animals, second only to mosquitoes (Kiss & Cadar, D. & Spinu, M. 2012, Jongejan & Uilenberg 2004). Economic losses are great, not only through mortalities and loss of production caused by tick-borne diseases (TBDs), but through the direct effects of high tick burdens (Rajput *et al.* 2006). The blood-sucking feeding habits of ticks, particularly in high burdens, causes reduction in live weight and anaemia, while the bites from the ticks cause damage to hides and teats, ultimately resulting in secondary production losses (Rajput *et al.* 2006). The control of ticks has, therefore, become of great importance due to their effects on livestock profitability and the health status of the animal (Taylor 2001).

One tick in particular, *Rhipicephalus (Boophilus) microplus* has garnered its globally infamous reputation as one of the most important disease vectors of cattle through its extensive geographical distribution and disease transmission abilities (Madder *et al.* 2011). Although not endemic to South Africa (SA), it has become well established since its introduction from Southern Asia via Madagascar over 100 years ago (Horak *et al.* 2015).

A second ixodid tick species of economic importance in South Africa, is the indigenous *Rhipicephalus (Boophilus) decoloratus* (Tonnesen *et al.* 2004). Both ticks are responsible for transmission of the diseases babesiosis and anaplasmosis in cattle. In 1980, economic losses inflicted by babesiosis and anaplasmosis amounted to cost SA between R 70 and R 200 million per year (Regassa, Penzhorn & Bryson 2003). Both species, for the purposes of this thesis, are referred to by the common name blue ticks.

While the only known vector of *Babesia bovis* in SA is *R. (B). microplus*, *B. bigemina* is transmitted by both species of blue tick, as is the pathogen causing anaplasmosis (*Anaplasma marginale*) (Tonnesen *et al.* 2004). Bovine babesiosis is widely distributed in South Africa (Regassa *et al.* 2003),

but the distribution of *B. bovis* and *B. bigemina* is invariably linked to that of their main vectors (De Vos 1979). The distribution of *B. bovis* has been limited by the high rainfall and humidity requirements of its vector, *R. (B). microplus* (De Vos 1979). Thus it has been confined to areas of the Eastern Cape, KwaZulu-Natal and eastern parts of Mpumalanga and Limpopo provinces (Regassa *et al.* 2003). The multi-vector transmissible *B. bigemina* is found throughout most parts of the country, only being absent from the drier parts of the Western and Northern Cape, Limpopo and Western Free State (Regassa *et al.* 2003). That being said, the distributions of tick species are not static, however (Tonnesen *et al.* 2004). The displacement of *R. (B). decoloratus* by *R. (B). microplus* in various parts of South Africa, is a prime example of how distribution of tick species shift in response to seasonal rainfall variations, host movement as well as changes in local tick control strategies and selection for acaricide resistance (Tonnesen *et al.* 2004). Furthermore, the invasive success of *R. (B.) microplus* is also a factor of species interaction and high adaptivity of the species to hosts and climate. The recent recording of *R. (B). microplus* in Namibia (Nyangiew, Matthee & Horak, I. & Matthee, S. 2013) and West Africa (Madder *et al.* 2011) serve as prime examples of its invasive skills.

Acaricides, in spite of some well-publicized drawbacks, still form the basis of tick control (De Castro 1997, R. J. Peter *et al.* 2005). This is partly attributed to their efficacy in reducing tick burdens in the long term as well as initially clearing the animal of ticks. However, acaricide resistance proves to be a major obstacle to the short and long term efficacy of acaricides and the subsequent future of chemical control. It has even been suggested by De Castro (1997) that acaricide management should be equivalent to that of a finite natural resource - such is the influence of acaricide resistance and has become a balancing act between maintaining susceptibility and controlling parasitoses (Sangster 2001, Graf *et al.* 2004).

It is hypothesized that acaricide resistance in the one-host ticks of the *R. (Boophilus*) genus in the communal dipping system of the Mnisi community, Mpumulanga, is present. When the resistance status is known, the effective acaricide can be selected, which will reduce further development of resistance and increase the cost benefit of tick control.

The main objective of this study was to determine the extent and distribution of *R. (Boophilus*) *species* resistance against selected acaricides in the Mnisi area. Secondly, it was hoped that the study would be able to compare resistance status between the *R (B).species*

CHAPTER II: LITERATURE REVIEW

2.1 Introduction to the *Rhipicephalus (Boophilus)* species of South Africa

Ticks belong to the phylum, Arthropoda and constitute the largest collection of organisms in the order, Acarina (Rajput *et al.* 2006). They are divided into two families: the soft bodied ticks (Argasidae) and hard bodied ticks (Ixodidae) (Sonenshine, Nicholson & Lane 2002). About 80 % of all the ticks of veterinary importance fall into the Ixodidae family (Holdsworth *et al.* 2006), including the *R.(B).spp* (subfamily Rhipicephalinae) (Sonenshine *et al.* 2002).

The *Rhipicephalus* (*B*). spp have been involved in a nomenclature controversy of late (Horak 2009). Originally grouped under the genus *Boophilus*, these ticks underwent a reclassification into the genus *Rhipicephalus*. While published as *Rhipicephalus microplus* and *R. decoloratus* in 2010 in a list of valid tick names (Guglielmone *et al.* 2010)blue tick species are referred to throughout this paper as *Rhipicephalus (Boophilus) microplus* and *R. (B.) decoloratus*.

Descriptions of ticks found in South Africa were first described in the literature over 200 years ago, with the first overall review of southern African ticks published in 1908 (J. B. Walker 1991). The African blue tick, *R. (B.) decoloratus,* is one of four economically important tick species indigenous to South Africa (Horak *et al.* 2015). Some of its notoriety has been lost since the introduction of the Asiatic blue tick, *R. (B.) microplus*, into the country from Madagascar about a century ago (Horak *et al.* 2015). The first reports of the displacement of *R. (B.) decoloratus* by *R. (B.) microplus* came in the early 1900s (Tonnesen *et al.* 2004). Tonnesen (2004) briefly reviews current theory behind the success the Asiatic blue tick has had in displacing its endemic counterpart. General views of the advantages that *R. (B.) microplus* has, include (Tonnesen *et al.* 2004, Estrada-Pena, A. & Salman, M. 2013):

- Shorter life cycle (approximately 1 week shorter than the *R (B).decoloratus* (Madder & Horak, I. & Stoltz, H.)
- While there is a tendency for assortative mating – i.e. each species has a preference for mating with its own species, they will also mate with females of other species. The male
- *R. (B).microplus* ticks reach sexual maturity a few days earlier than male *R.(B).decoloratus* ticks, allowing them, in mixed infestations, a greater chance of mating with females of their own species as well as excess males mating with female *R.(B).decoloratus* ticks. Since cross-mated females produce eggs that are sterile, it goes to say that *R. (B). microplus* would therefore

comprise an increasing portion of mixed populations of these two ticks (Horak, Nyangiwe & De Matos, C. & Neves, L. 2009). In a recent study, however, Horak *et al (2013)* do propose the possibility that hybridisation may occur,

- Female *R. (B). microplus* produce slightly more eggs than their African counterpart (Horak *et al.* 2009)
- Successful feeding on cattle - Where both species are present, there is a tendency for cattle to develop resistance to *R. (B.) decoloratus,* leading to reduction in engorgement weight of the *R. (B.) decoloratus,*and, thus, fewer eggs and ultimately an overall decrease in population

It can also not be ruled out that, certainly in the case of West Africa, that the more chemically susceptible local species could have been eradicated chemically, rather than being displaced biologically (Madder *et al.* 2011). While there appears to be a zone of reproductive interference where both ticks occur, under favourable climatic conditions, it does little to hinder the invasion of *R. (B). microplus (Tonnesen et al. 2004, Estrada-Pena, A. & Salman, M. 2013).* In fact, in humid and tropical forest habitats, the displacement of *R. (B). decoloratus* by *R. (B). microplus* is total (Madder *et al.* 2011). Data obtained in Tanzania shows that the critical factor in the encroachment of *R. (B.) microplus* into *R. (B.) decoloratus* territory, is associated with the 58 mm isohyet and the 22 – 23 ^{o} C isotherm (Estrada-Pena, A. & Salman, M. 2013).

The life cycles of Ixodidae ticks are grouped into three basic categories i.e. the three-host life cycle (e.g. *Amblyomma* spp.), two-host life cycles (e.g. *Hyalomma* spp.) and the single-host life cycle (*Boophilus* spp.) (Holdsworth *et al.* 2006). *R. (B).spp* are currently classified into the genus *Rhipicephalus*. However, as ticks formerly classified as genus *Boophilus*, both blue tick species are one-host parasites of ungulates (Sonenshine *et al.* 2002).

2.1 Description of tick species

2.1.1 *Ripicephalus (Boophilus) decoloratus* – African blue tick

When Howell *et al* (1978) mapped the distribution of these two ticks in South Africa, R. *(B.) decoloratus* was widespread, with *R. (B.) microplus* restricted to coastal pockets (Howell & Walker, J.B. & Neville,

E.M. 1978). How things have changed! Indeed, the present distribution pattern of the two ticks has been reversed (Horak *et al.* 2009).

The habitat requirements of the *R. (B.) decoloratus* are warmth and moisture, and, apart from where it has been displaced, its distribution pattern follows the wetter regions of South Africa i.e. along the coastal regions of the Western and Eastern Cape, throughout Kwazulu – Natal, Mpumalanga, Limpopo, North West Provinces and the eastern Free State (Madder & Horak, I. & Stoltz, H.). It is absent from drier areas that receive less than 380 mm per annum (Madder & Horak, I. & Stoltz, H.). *R. (B.) decoloratus* can survive in areas where there is a maximum of 90 days of frost spread over a period of 150 days a year (A. Estrada-Pena *et al.* 2006)

The entire life cycle of the *R. (B). decoloratus* can take up to 2 months to be completed (Madder & Horak, I. & Stoltz, H.) i.e. this species is able to pass through two to four generations annually (Mekonnen *et al.* 2002) Engorged females lay between 1 000 to 2 500 eggs (Madder & Horak, I. & Stoltz, H.).

Preferred hosts of the *R. (B.) decoloratus* include cattle, impalas, eland, nyala, zebras and horses (Madder & Horak, I. & Stoltz, H.).

2.1.2 *Rhipicephalus (Boophilus) microplus* – Pantropical / Asiatic blue tick

The distribution of the *R. (B). microplus* in South Africa has broadened greatly since its introduction into the country. It extends along the southern and eastern coasts of the Western and Eastern Cape Provinces as well as Kwazulu-Natal (Madder & Horak, I. & Stoltz, H.). *R. (B) microplus,* has also crept in to the interior of the country, with small pockets found in the provinc.es of Mpumalanga and Limpopo. Although African populations of *R. (B). microplus* have requirements of high total rainfall, they can support long periods of dryness in winter (A. Estrada-Pena *et al.* 2006).

The life cycle of is similar to that of the *R. (B.) decoloratus,* but takes approximately one week less to complete (Madder & Horak, I. & Stoltz, H.). Females of this species lay over 500 more eggs than *R. (B). decoloratus.*

Cattle are the most efficient hosts of *R. (B). microplus*, with goats playing a small, but significant role (Nyangiew *et al.* 2013).

2.2 Tick control

2.2.1 Introduction

Tick control programmes are usually initiated for the ultimate goal of tick-borne diseases control and to increase productivity or to prevent the formation of lesions (Norval 1989). Tick control comprises the control of tick-borne pathogens through a reduction in the number of ticks infesting the host (Estrada-Pena, A. & Salman, M. 2013). This can be achieved by employing variety of methods, namely (De Deken, Horak & Madder, M. & Stoltsz, H)

- Tick vaccines
- Biological control
    - Predators, pathogens, parasites
    - Host's resistance
    - Pasture spelling
    - Habitat modification
- Chemical control (through the application of acaricides)

As acaricide application has been the mainstay of tick control programmes since 1909 (J. G. Walker & Klein, E.Y. & Levi, S.A. 2014, Moyo, B. & Masika, P.J. 2009) and forms the basis of this thesis, the importance of the use of chemical control in tick control programmes are elaborated upon.

2.2.2 Chemical tick control

Acaricides are those chemicals used to control tick infestations and tick-borne diseases (Kemp 1994). Because of their effectiveness, they have become the most widely used method of tick-born disease control in livestock (Norval 1989, J. G. Walker & Klein, E.Y. & Levi, S.A. 2014). However, despite this, the cost effectiveness and socio-economic and environmental desirability (e.g. milk and meat residues) of intensive acaricidal treatments have, for some time, been subject to much debate (Moyo, B. & Masika, P.J. 2009, Spickett, A.M. & Fivaz, B.H. 1992). The development of acaricide resistance has furthermore deemed their use unsustainable (J. G. Walker & Klein, E.Y. & Levi, S.A. 2014).

The use of acaricides essentially changes tick population ecology through the reduction of the effective carrying capacity of ticks in the environment in which acaricides are used (J. G. Walker & Klein, E.Y. & Levi, S.A. 2014). Current chemical tick control strategies apply the use of acaricides either strategically, opportunistically or selectively (De Deken *et al.*).

Selective control, according to De Deken et al (date unknown), entails the application of acaricide at strategic times throughout the year so as to control season peaks in tick abundance. Its focus is to decrease adult tick numbers to levels where economic damage is lower than the cost of control. In areas where animals are considered to have a high level of immunity to ticks, or where favourable climatic conditions temporarily increase tick numbers, opportunistic control can initiated. That is to say, only when the tick burden is considered to be greater than the economic threshold at various points in time. Selective control sees farmers treating only those animals that have tick burdens that are economically unviable. It is should be conducted in conjunction with a culling programme to reduce numbers of those individual cattle that consistently harbour high tick numbers, ensuring a herd with a high tick immunity. This method is also thought to be advantageous in small herds to reduce treatment costs and delay the onset of acaricide resistance (De Deken *et al*)

Acaricide efficacy is a factor of the degree of toxicity of a chemical as well as the quality, quantity and degree of dispersal of active ingredient (George, Pound & Davey 2004), which is considerably influenced by the chosen method of application i.e. contact delivery systems or systemic delivery systems. Traditional methods of acaricide application have been by direct application (contact) to the skin of cattle by either dipping or spraying (J. G. Walker & Klein, E.Y. & Levi, S.A. 2014, R. Peter *et al.* 2006). As is the want of human nature, the need for cheaper, easier alternatives have been sought. Not only have application methods been improved upon for ease of use in the form of pour-on or patch treatments and injectable (systemic) formulations (R. Peter *et al.* 2006), but advances have been made in prolonging the period of efficacy and increasing the spectrum of chemical activity (Taylor 2001). It is not the aim of this paper to review all the known acaricide groups. Rather those chemical groups relevant to the study are highlighted, namely the amidines, pyrethroids and organophosphates.

All the important acaricide chemical groups of our time were the product of the insecticide development industry, including the organochlorines (DDT), organophosphates, amidines and pyrethroids (Graf *et al.* 2004). The burgeoning market was born out of necessity due to the development and spread of

acaricide resistance. The use of DDT in the field of tick control began in the 1940's as a response to the development of resistance to the arsenic. By the 1960's, DDT resistance lead to the introduction of organophosphate use in tick control, which was followed ten years later by the use of amidines. It is interesting to note that, while amidine resistance was first seen in the late 1970's, the spread and establishment thereof, was hindered by the introduction of pyrethroids into the market (Graf *et al.* 2004). This could either be as a result of the pyrethroids controlling the amitraz-resistant ticks or the cessation of use of amidine-based products, or a combination of the two.

The amidines were developed in response to resistance development to the organophosphates (De Deken *et al.* , Li *et al.* 2004). Amidines, of which amitraz is the main member, are available in dip, spray or pour-on formulations for the control of single- and multi-host tick species (Taylor 2001, R. Peter *et al.* 2006). In dip tanks, amitraz had to be stabilised by the addition of calcium hydroxide and maintained by standard replenishment methods (Taylor 2001, De Deken *et al*, R. Peter *et al.* 2006). An alternative method has since been developed through the use of total replenishment formulations whereby the dip tank is replenished with the full concentration of amitraz at weekly intervals prior to use (R. Peter *et al.* 2006).

The amidines control both the single and multi-host ticks (R. Peter *et al.* 2006). Amitraz acts on the octopamine receptor sites of the central nervous system of the ticks as well as by inhibition of the monoamine oxidases. This results in neuronal hyperexcitability and death. (Taylor 2001, R. Peter *et al.* 2006). Furthermore, amitraz has expellant action against attached ticks (George *et al.* 1998), causing ticks to detach within 30 minutes to eight hours (De Deken *et al.*). The ovacide activity, long-term persistence and relative cost-effectiveness of the chemical (De Deken *et al.*) have all contributed to the popularity of amitraz as a go-to acaricide.

Organophosphates (OP) e.g. chlorfenvinphos, are also available in pour-on, spray or dip formulations. This group of acaricide acts by blocking the action of the enzyme acetylcholinesterase (AchE) by binding with the enzyme, thereby preventing the degradation of acetylcholine (Ach). Ach thus accumulates at the post-synaptic membranes, resulting in neuromuscular paralysis (Taylor 2001, De Deken *et al.*). The need for bioactivation, as well as CNS penetration, the OPs do not have a rapid action like that of the pyrethroids (De Deken *et al.*).

Synthetic pyrethroids formulations e.g. cypermethrin are also applied directly to livestock via pour-on, spot-on, spray or dip (Taylor 2001, De Deken *et al.*). While this group is highly effective against organophosphate-resistant ticks, they do have some drawbacks, notably their relatively high cost and *R. (B).spp.,*rapid development of resistance to this chemical group (De Deken *et al.*).

Synthetic pyrethroids work through the interference of sodium channels of the parasite nerve axons causing a delay in repolarisation and, ultimately, paralysis. The lethal activity of pyrethroids is attributed to the action of the chemical on both peripheral and central neurones, while the knock-down effect is suspected to be produced by the peripheral neurones only (Taylor 2001).

These three acaricide groups, the amidines, organophosphates and pyrethroids, especially designed for dips, sprays and pour-ons, constitute a large portion of the acaricides used in South Africa as part of tick control strategies.

2.2.3 Tick control in South Africa

Politics, policy and economics have had a major influence on tick control strategies employed in South Africa, and inadvertently, the tick resistance status in the country. The first example of political influence was the enforcement of a compulsory tick control programme by the Veterinary Service of the Department of Agriculture of South Africa in response to the introduction of East Coast fever in the early 20th century (Hlatshwayo & Mbati 2005). Here, acaricide use in the form of dipping, proved to be the most effective and efficient method (Masika, Sonandi & van Averbeke 1997) and firmly established itself as the most important player in tick control strategies used in the country. The economic importance of the acaricide market in South Africa is clearly indicated in the 2004 paper by Peter and colleagues, estimating a total value in sales of R 175 million. At the time, 104 products were registered for tick control, comprising of five chemical groups with 22 different active ingredients (R. J. Peter *et al.* 2005).

Briefly, dipping policy in South Africa can be divided into two groups: the commercial farming areas and the communal farming areas (Kemp 1994). Communal farming areas are defined as those areas in which cattle belonging to multiple farmers graze unfenced communal land (Ntondini, Dalen & Horak 2008). Unless in strictly regulated areas, or in the event of an ECF outbreak, currently control of dipping policy in the commercial farming is not so easily regulated: farmers themselves are responsible for

instituting their own tick control strategies. Farmers, whether they be commercial or communal, are, however, under obligation of the Animal Diseases and Parasites Act (Act 13 of 1956) not allowed to move tick-infested cattle. Resource-poor stock farmers, on the other hand, are particularly susceptible to changes in legislature and the state of economy. Low incomes, the high cost of veterinary medicines and private services, as well as limited education in animal health care make subsistence farmers highly dependent on state veterinary services (Jenjezwa, V.R. & Seethal, C.E.P 2014).

Since the eradication of East Coast Fever between 1954 and 1960, tick control in communal farming areas (especially in the former homelands) (Masika *et al.* 1997) has been, state-run, provided for under the Animal Diseases and Parasites Act (Act 13 of 1956) (Hlatshwayo & Mbati 2005, Eastern Cape Department of Rural Development and Agrarian Reform 2013). While management of dipping services has changed hands a few times over the years (Hlatshwayo & Mbati 2005), the responsibility of tick control has never formally been in the hands of the communal farmers themselves. Recently, however, policy is once again changing, with the trend towards the dip tanks becoming the responsibility of the community as well as the state (Kemp 1994, Eastern Cape Department of Rural Development and Agrarian Reform 2013). The regulatory framework governing tick control strategies are listed in Appendix 6. Role players in new dipping policies (e.g. as instituted by the Eastern Cape Province), will include (Eastern Cape Department of Rural Development and Agrarian Reform 2013):

- The Department of Agriculture
- Local municipality
- Traditional leadership
- State veterinarian
- Animal health technicians (AHTs)
- Community animal health workers (CAHWs)
- Dipping committees
- Cattle owners

These dipping policies aim to:

- Ensure that the provincial herd belonging to communal farmers is protected from TBDs and tick damage
- Control the regulation of dipping regimes

- Assist with the traceability of livestock by insisting on precise record keeping of stock numbers (births, deaths, sales etc.)
- Minimise disease spread through the standardisation of cattle movement

While tick control strategies differ throughout the farming sectors in South Africa, farmers in both the communal and commercial sectors do have one thing in common: the advancing threat of acaricide resistance.

2.3 Acaricide Resistance

2.3.1 Introduction

The Food and Agricultural Organisation (FAO) defines tick resistance as the significant increase in the number of individuals within a tick population that can tolerate doses of drug or drugs that have proved to be lethal for most individual in the same species (FAO, Working group on parasite Resistance 2004). Acaricide resistance, sometimes against multiple classes of acaricides, is a serious growing problem globally, particularly in the species *R. (B). microplus* (Kiss & Cadar, D. & Spinu, M. 2012). Many countries (e.g. Mexico) have reported the development of resistance of the Pantropical blue tick to all major classes of acaricide (Li *et al.* 2004, Perez-Cogolla, Rodriguez-Vivas & Ramirez-Cruz, G.T. & Miller, R.J. 2010, Perez-Cogolla, Rodriguez-Vivas & Ramirez-Cruz, G.T. & Rosado-Aguilar, J.A. 2010), and which has serious repercussions on the future of tick control strategies (Li *et al.* 2004). Resistance has led to instability and increased costs in areas where it exists in *R(B.)spp.,*populations (Rajput *et al.* 2006). Now, it is almost taken for granted that resistance must be expected in these tick within five to 10 years after the introduction of any novel type of acaricide, unless there is a change in control practices (Rajput *et al.* 2006). Currently, the level of resistance in a tick population is determined by means of bioassay techniques (Andreotti *et al.* 2011).

2.3.2 Factors influencing the establishment of acaricide resistance

The development of tick resistant populations is a complex matter. Resistance begins to develop with the selection of individuals with pre-disposed resistance genes (usually heterozygotes). (Mitchell 1996). Once the number of herterozygous ticks passes a critical threshold, one begins to see the emergence

of fully resistant ticks in the population. The rate of selection for resistance is determined by the selection pressure (Mitchell 1996). Resistance emergence is usually attributed to some, or all of the following factors: related to acaricide use (application methods, treatment intervals, acaricide concentration, mixtures or rotation of acaricides (Kemp 1994)), ecological niches and the genus of ticks involved (Fernandez-Salas & Rodriguez-Vivas, R.I. & Alonso-Diaz, M.A. 2012), frequency of resistance alleles, dominance and other factors (Kemp 1994).

Resistance is often first reported when chemical treatment fails to control parasitism (Rajput *et al.* 2006, Shaw 1966). Other factors that influence product efficacy include degree of toxicity of the chemical, quality, quantity and degree of dispersal of the active ingredient (George *et al.* 2004). Usual methods for application of acaricides (dipping, spray-race, hand-spraying) are theoretically equally efficient under ideal conditions (George 2000). However, ideal conditions almost never exist. Poor equipment maintenance and / or use thereof not only is a waste of the user's money, but can also increase the rate of selection for resistance. Problems encountered with the above application methods that lead to ineffective tick control (and possibly contributing to tick resistance) include: incorrect product dilution, stripping of active ingredient, improper replenishment, inadequate mixing before use and inadequate wetting of animals with the product (George 2000). It is important that therapeutic inefficacy is not mistaken for acaricide resistance and vice versa. Failure to control ticks is frequently as a result of inadequate treatment and many reports of resistance are baseless (Rajput *et al.* 2006). .

Frequent use of the same chemical product over an extended period of time is one of the main factors in the emergence of resistance (Frisch 1999). It has been reported that the application of a chemical product more than six time a year can contribute to the development of resistant populations (Fernandez-Salas & Rodriguez-Vivas, R.I. & Alonso-Diaz, M.A. 2012, N. N. Jonsson & Mayer, D.G. & Green, P.E. 2000). This allows for resistant individuals to be selected by enhancing the frequency of a resistant gene (Klafke *et al.* 2006). This statement holds particularly true for the one-host ticks *Rhipicephalus (B). decoloratus* and *R. (B). microplus* – the short generation time means greater acaricidal exposure (Graf *et al.* 2004, Mekonnen *et al.* 2002).

2.3.3 Acaricide resistance testing methods

Confirmation of field resistace resistance must be made by laboratory tests (Rajput *et al.* 2006) and correlated against known management practice (Mitchell 1996).

Shaw developed the larval immersion test in 1966 with the advantage that the larvae are immersed in a solution or suspension of the acaricide in order to increase toxicity (Sabatini *et al.* 2001). The Shaw Larval Immersion Test (SLIT) was used as the standard test procedure during the National Acaricide Resistance Survey (Ntondini *et al.* 2008). Thus, in order for this survey to yield data analogous to that of the National Survey, the SLIT is to be used in the proposed survey. The SLIT uses unfed larvae. Standardisation of unfed larvae is more easily achieved than adult ticks and the mortality of the larvae can be recorded easily. Because the larvae are treated the same, the results are more credible statistically (Mekonnen *et al.* 2003).

2.3.4 Acaricide resistance management

Sangster (2001) highlights three major components to the evolution of acaricide resistance. First, resistance has to be *established*. This is considered to largely be a random event over which we have not control as it is influenced by population size and diversity as well as rate of mutation of resistant genes. Where we do have influence, however, is in the *development* of resistance, particularly through the use of acaricides (i.e. the selective agent). This process results in the dispersal of the resistant genes through the wider population. Finally, the *emergence* of resistance occurs when fit populations of resistant tick survive under continuous selection pressures. Emerging resistance then warrants further management options for tick control.

Early detection of resistance is essential in order to avoid further selection of resistant ticks using the same active ingredient and to delay the spread of resistance (Fernandez-Salas & Rodriguez-Vivas, R.I. & Alonso-Diaz, M.A. 2012, Klafke *et al.* 2006). It is, however important to remember that resistance detection is only a part of resistance management (Sabatini *et al.* 2001)

George, Pound and Davey (2004) recommend further steps once it has been established that a specific acaricide is the cause of treatment failure and the use of sensitive reliable diagnostic methods are an integral part of the process. They deem it essential to 1) determine which acaricide is a suitable

alternative, 2) investigate the epidemiology of resistance, 3) develop control strategies so that the rate of selection of resistant genotypes is minimized and finally, 4) develop new acaricides.

The extreme cost involved severely hampers the development of new acaricides (Mekonnen *et al.* 2002) and further highlights the importance of judicial use of present acaricides in order to preserve their efficacy. Monitoring resistance not only includes the detection of resistance, it also insists that detection of resistance occurs while it is at a low frequency (Sangster 2001) before the acaricide loses effectiveness completely.

Treatment failure as a result of resistance has added importance because the lack of tick control implies increased difficulty in the control of tick-borne diseases (Sangster 2001). Aside from tick-borne diseases, severe economic losses can also be indirectly caused by tick worry, blood loss and damage to hides (Jongejan & Uilenberg 2004, Holdsworth *et al.* 2006, Sabatini *et al.* 2001). The level of tick control and corresponding herd immunity can be also affected by acaricide resistance (Foil *et al.* 2004).

Strategies of resistance management should be targeted to keep the selection pressure at a minimum level, while still achieving an appropriate control of the tick (Perez-Cogolla, Rodriguez-Vivas & Ramirez-Cruz, G.T. & Rosado-Aguilar, J.A. 2010). Currently, there are no guidelines to produce a recommendation to the farmer after resistance is detected (Estrada-Pena, A. & Salman, M. 2013).

Mitchell (1996) posed the question: can strategic acaricide use can slow the onset of resistance i.e. slow down the rate of selection or reduce the selection pressure. He highlights two controllable factors when using acaricides – ensuring the correct target concentration on the animal, and the frequency of application.

Three main strategies should be considered when attempting to delay the onset of field resistance

1) Monitoring

2) Rotation of acaricides

3) The use of combination products

The use of combination products is a double-edged sword. On one hand, they potentiate each other against target species of tick and if they are used at high enough concentrations, they could delay the onset of resistance. For example, a product containing deltamethrin and amitraz. However, a product

containing cymiazole and cypermethrin has poor effect against multihost ticks. Secondly, if resistance is present to one of the chemicals, it is unlikely that the second chemical will control the tick population on its own, resulting in simultaneous development to two chemicals. Thus the use of combination products should be instituted with caution as well as being fully informed of all the variables affecting the tick control programme.

Models in the field of resistance management have suggested a few strategies to aid in resistance management (Sangster 2001), including:

- Dipping on a three-weekly basis during the tick season
- The use of an early season dip
- Using mixtures of acaricides
- Employing pasture spelling to reduce exposure of cattle to overwintering tick larvae

However, are these methods feasible in the communal grazing areas of South Africa such as Mnisi? Pasture spelling becomes very difficult, if not impossible, under communal grazing systems. The presence of three-host tick species in the area complicates matters even further. Where multi-host species are present, one needs to treat weekly at concentrations relevant to South Africa in order to control these ticks effectively thereby immediately negating the suggestion for dipping on a three-weekly basis. It appears that the utopic idea of the acaricide resistant management strategies employed in other countries are not feasible for application in South Africa.

2.3.5 Acaricide resistance in the South African context

Since the first recorded acaricide resistance by *Rhipicephalus (Boophilus) microplus* in 1979 (Baker & Jordaan, J.O. & Robertson, W.D. 1979), tick resistance to acaricides has become an increasing problem in South Africa and poses a real economic threat to the livestock and veterinary pharmaceutical industries (Mekonnen *et al.* 2002). Indeed, resistance studies done on communal dip tanks in 2002 in the Eastern Cape showed that *R. (B). decoloratus* populations had developed significant resistance to the three main chemical actives used in the country at the time i.e. the amidines, pyrethroids and organophosphates (Mekonnen *et al.* 2002).

Sodium arsenite was the first effective acaricide used in South Africa (Whitehead 1973). It had a successful fifty year run until 1938, when resistance to arsenic was detected in the R.(B).spp., then known as *Boophilus decoloratus,* (Whitehead 1973). Arsenic then went on to be replaced by benzene hexachloride (BHC), only for an alternative to be sought 18 months later, in the form of DDT. By that stage, populations of arsenic-BHC-resistant blue ticks became well established along South Africa's east coast. DDT was effective in controlling resistance in these populations for five years before resistance to the chemical reared its ugly head (Whitehead 1973). From these humble beginnings, the South African acaricide market has grown to incorporate at least 104 registered acaricides comprising five chemical groups and 22 different active ingredients (R. Peter *et al.* 2006), and so has, unfortunately, the development of resistance.

South Africa offers an interesting perspective on acaricide resistance development, in particular reference to communal farming. One study attributed the spread acaricide resistance in South to any one, or combination of, at least 3 factors (Ntondini *et al.* 2008):

Continuous, regular use of compounds belonging to the same chemical group, with no simultaneous resistance monitoring programmes in place (Ntondini *et al.* 2008). This situation is suspected to have largely arisen from the purchase of dipping compounds on tender in the communal farming areas (Mekonnen *et al.* 2002).

The communal grazing system itself, whereby animals that may act as hosts to acaricide-resistant ticks, distribute these ticks within the communal grazing area. Due to uncontrolled movement of cattle, communal dipping offers little or no biosecurity measures to minimize the adverse impacts of acaricide resistance ticks, should it emerge (George *et al.* 2004). It also has to be noted, however, that the distribution of resistant ticks can also have a diluting effect, lowering the resistance in certain areas and lowering the gene frequency of genes coding for resistance (Madder, pers. comm.). The purchase of animals by communal farmers from commercial farms that have been under intense and regular acaricide-based tick control regimes may also spread acaricide-resistant tick populations throughout the communal grazing areas.

Perceived state-provided acaricide resistance and the desirability for total tick control have lead communal farmers to seek alternate forms of tick control, including, unsolicited use of acaricides, old engine oil, household disinfectants like Jeyes fluid (which can lead to problems of toxicity) and manual

removal of ticks (Moyo, B. & Masika, P.J. 2009). The unsolicited use has also been suspected of accelerating the development of resistance (Kemp 1994). Other poor management practices that not only enhance the emergence of resistance, but also lead to decreased efficacy of the acaricides, are often evident at these communal dip tanks. Two of the most important of these are the use of acaricides at the incorrect concentrations and incorrect acaricide application (Mekonnen *et al.* 2002). One study of cattle tick control practices and producer attitudes towards tick control in the Eastern Cape (Spickett, A.M. & Fivaz, B.H. 1992) found that method of acaricide application influenced resistance (hand-spraying had a higher incidence of resistance compared to that of plunge dipping and pour-ons). They also found that cost, irrespective of efficacy, was a major factor in type of chemical used.

Despite the above studies, there is still very little reliable information of the prevalence of tick resistance, with particular reference to amitraz, in southern Africa (N. N. Jonsson & Hope 2007). A major consequence of the lack of adequate information is the inability to determine suitable alternative acaricides and strategies for implementation of an integrated tick control programme (George *et al.* 2004). For this reason, any opportunity to determine prevalence of resistance and factors determining resistance is encouraged and thus forms the basis for this study. Tick control strategies at the communal dip tanks are being aimed at reducing dipping frequency to extend the life of acaricides and to increase the level of endemic stability in the national herd (Kemp 1994).

2.4 Tick control in the Mnisi Community Programme

Mnisi is a rural community in the Bushbuckridge Municipal Area, Mpumulanga Province, South Africa. Over two thirds of the land shares it's boundaries with the Kruger National Park (KNP), Manyaletti, Handover and Sabi private parks (Fig. 1) (Pretoria 2015b). The Mnisi Community Programme (MCP) covers a land area of 30,000 ha and a human population of over 40 000. Domestic livestock include 14,400 head of cattle owned by 1,300 farmers (Lazarus 2014). Because the main agricultural activity is cattle farming, an important wildlife-livestock interface is created in this area. The importance of this interface is highlighted further by the fact that foot-and-mouth disease (FMD) and Corridor disease are endemic in many of the species of abundant wildlife present. This situation is made all the more vulnerable by the high host densities and the sub-tropical climate – rendering conditions suitable for

many tick species to prosper. Among these ticks are known vectors of the important tick-borne diseases: Corridor disease, redwater, heartwater and anaplasmosis (Pretoria 2015b).

The Mnisi community is largely composed of subsistence farmers and is a working example illustrating the influence that polices, politics and economics have on tick control programmes. These farmers are heavily reliant on government's support structures for help, particularly the veterinary authorities. Furthermore, all the villages and communal land fall under the authority of the Mnisi Traditional Authority (MTA). This body consists of a structure of representatives from the village-indunas, including the Chief himself. The MTA is recognized by government and governs together with the local municipal and provincial authorities (Pretoria 2015b).

Even with the presence of game fences along the boundaries, frequent contact between wildlife and livestock (e.g. after flooding, washed fenced or close contact of wildlife and livestock at fences) compelled further control measures to be established in the form of control zones between conservation areas and livestock areas, of which the MCP is one. This Here, comprehensive disease surveillance measures have been instituted – mainly in the form of community cattle dip tanks throughout the region (Pretoria 2015b). Communal compulsory dipping is practiced on a weekly basis along with inspections as part of the FMD prevention programme. Free dip is supplied by the government as an incentive to farmers to attend the weekly inspections (Lazarus 2014). This has further encouraged a close association between animal heath technicians and local farmers.

According to an unpublished study conducted in 2012, 90.2 % of the farmers made use of the state's plunge dip facilities to control ectoparasites on their cattle (Simela 2012). The other 9.8% made use of other control methods, including hand spraying or pour-ons, This figure includes the two "dip tank" where, as there is no plunge dip facility, pour-ons are made available. A whopping 78 % of these farmers used these additional methods in addition to the plunge dipping provided. Thus, in despite frequent dipping, this preliminary study on animal health and socio-economics showed that ticks and tick-borne diseases were still the main concern for farmers (Madder, per. comm.). Historically, amitraz was used in the area. Since July 2012, however, changes have been implemented in acaricide use, possibly as a result of reduced acaricidal effect after dipping (Madder, per. comm.). Furthermore, because there is a large variation in dipping facilities available to the communities of the area, different control strategies and acaricide application methods have had to be implemented. These different

control strategies and the acaricidal products used could be responsible for different degrees of resistance, if found, and especially resistance to different active components of acaricides. A detailed study taking into account these variables could explain the observed resistance profiles.

Although the government provides free dipping services, it has been found that farmers are still spending a considerable amount of money on acaricides. This could be highly indicative of the degree of resistance that exists in the tick populations at Mnisi. As such, it was important that this observation be verified and formally recorded and the reasons for the suspected acaricide resistance illuminated.

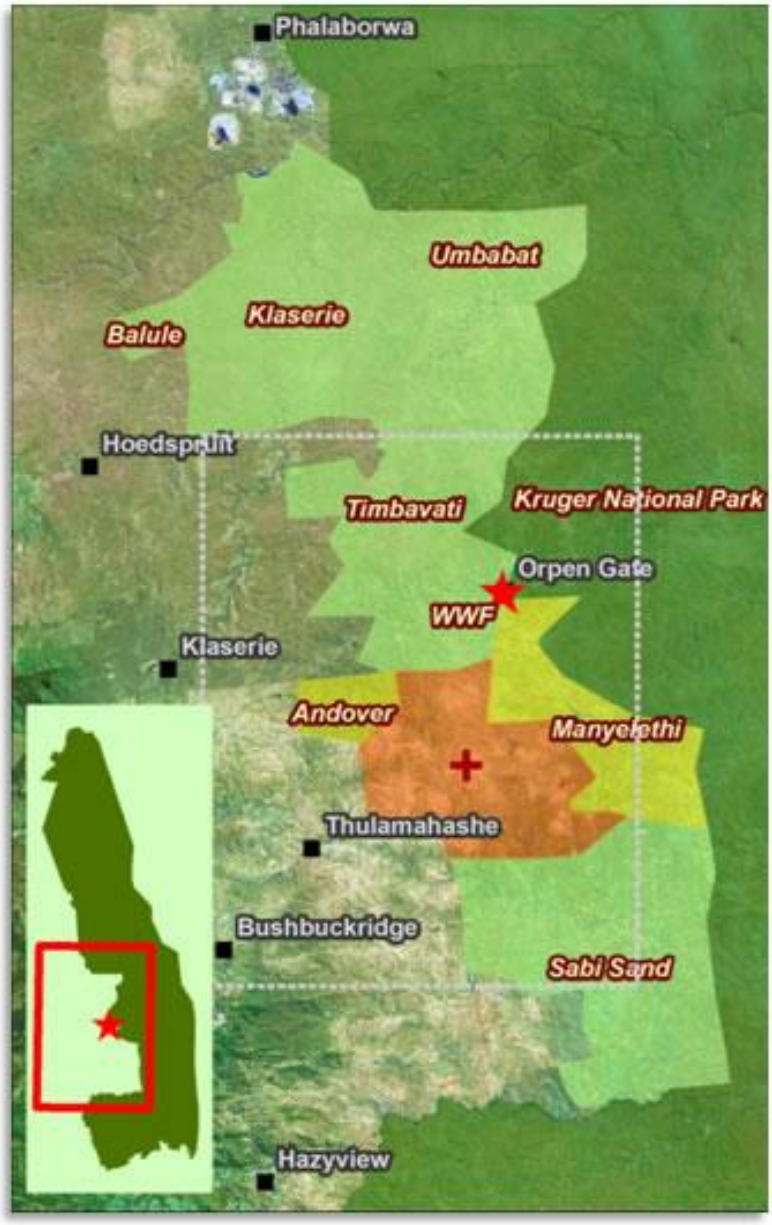

Fig. 1. Map indicating the Mnisi community in relation to the Kruger National Park and adjacent game reserves. (Pretoria 2015b)

CHAPTER III: METHODS AND MATERIALS

3.1 Study area

The Mnisi community (Fig. 1) is divided into three wards, namely, Bushbuckridge-1, Bushbuckridge-2 and Bushbuckridge-3 (Lazarus 2014). Each ward has an animal health technician (AHT) that supervises activities (dipping and inspection) at these communal dip tanks. A total of fifteen dip tanks are located throughout in this area (Fig. 2), with five dip tanks allocated per ward.

The Mnisi populous comprises 1 311 cattle owners, with an estimated 14398 head of cattle between them (Simela 2012). Thirteen of the fifteen dip tanks are plunge dips, while the remaining two, “Hlalakane” and “Welverdiend B” are crush pens (Simela 2012). Dipping and inspection of cattle occurs on a weekly basis.

3.2 Study design

At least fifty to sixty *R. (B).* spp. collected from each dip tank were to be collected, identified and separated into each of the two *R. (B).* spp. Each species would then be pooled and termed an isolate. (If both species were to be present at a specified tank, two isolates would then be represented.) Each isolate would be allocated the same unique number as the dip tank from which it originates, followed by the species name.

All fifteen dip tanks of the Mnisi area were to be included in the survey. However, due to time limitations, only twelve dip tank were sampled.

Females from one isolate (dip tank) and one species were to be pooled and kept in an Erlenmeyer flask in an incubator for egg laying and hatching of larvae. It was highly recommended that each isolate consisted of 20 to 25 fully engorged females. This was done to ensure that each isolate yielded enough progeny to conduct a long range (LR) SLIT using seven concentrations (Taylor, per. comm.).

Larvae were tested 18 to 21 days from hatching date. The date of hatching was determined to be when approximately 75% of larvae had hatched. A geometric series of dilutions (Appendix 2)was be prepared to give seven concentrations of the chosen acaricide. However, in those cases where insufficient larvae were available for LR testing, larvae would then be exposed to one discriminating concentration (DC)

per active compound. The DC is based on the premise that once a dosage mortality line for a susceptible has been well established, it can be used to determine the concentration required to ensure 100 percent mortality of the larval population in question (FAO, Working group on parasite Resistance 2004). If a significant amount of larvae survive in a sample of larvae is treated with this discriminating concentration, it is indicative of resistance to the chemical being tested. Fifty percent or more of ticks surviving treatment at the DC can be considered resistant. Recommended DC's for the three chemical actives tested are provided in Appendix 2 (Taylor, per. comm.).

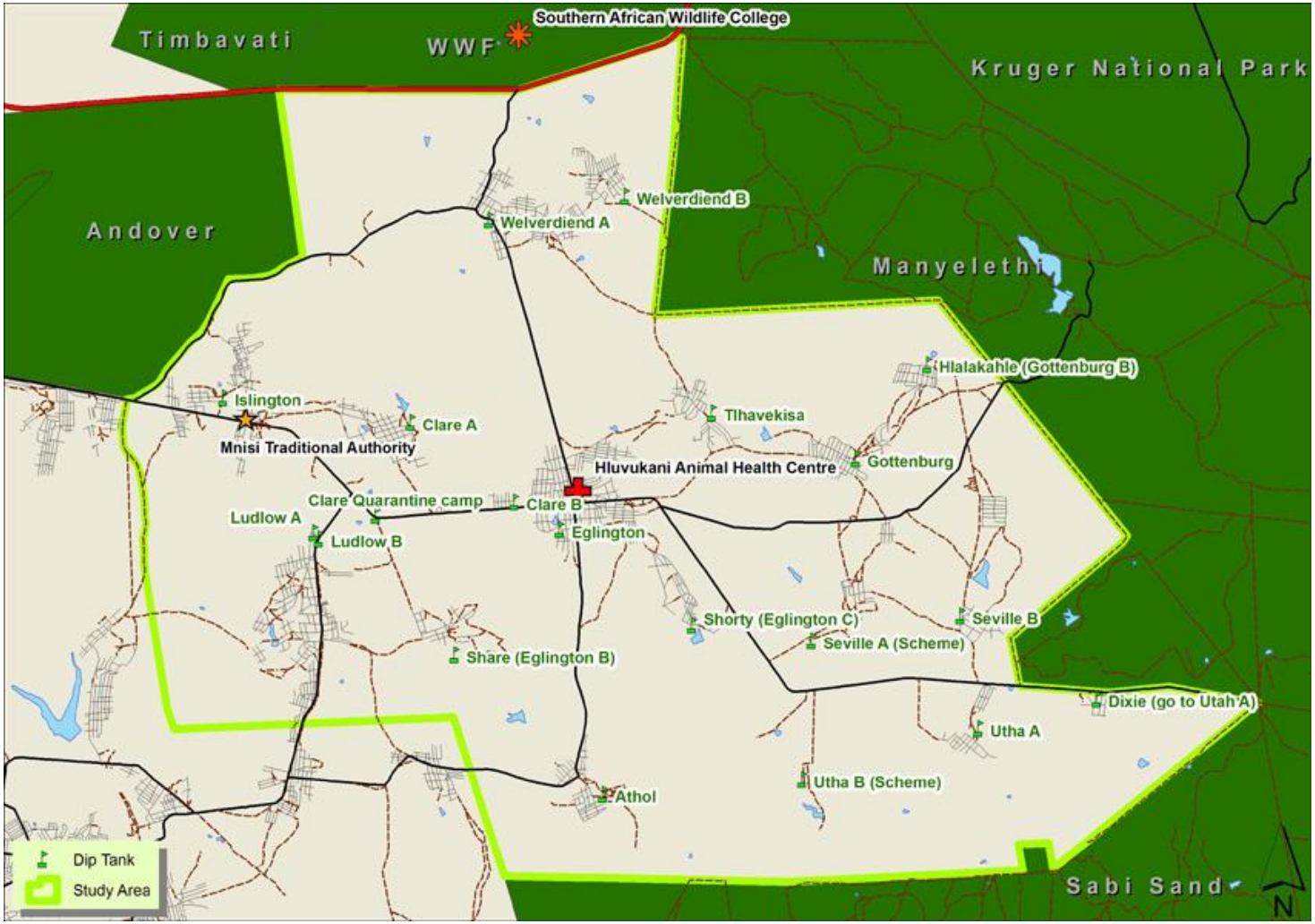

Figure 2. Of dip tanks (Pretoria 2015a)

Table 1 GPS co-ordinates of dip tanks sampled and unique number allocation

Dip tank	GPS Coordinates		Dip tank no. allocated	Acaricide and mode of application	Active
Hlalakahle	**-24,617222**	**31,433889**	**1**	**Pour on**	**Pyrethroid**
Gottenburg	**-24,638333**	**31,415833**	**2**	**Plunge dip**	**Pyrethroid**
Eglington	**-24,653611**	**31,342222**	**3**	**Plunge dip**	**Pyrethroid**
Share	**-24,681667**	**31,315833**	**4**	**Plunge dip**	**Pyrethroid**
Utha A	**-24,699722**	**31,445556**	**5**	**Plunge dip**	**Pyrethroid**
Utha scheme	**-24,710833**	**31,4011667**	**6**	**Plunge dip**	**Pyrethroid**
Islington	**-24,622778**	**31,259167**	**7**	**Plunge dip**	**Pyrethroid**
Welverdiend A	**-24,583333**	**31,325556**	**8**	**Plunge dip**	**Pyrethroid**
Seville B	**-24,674167**	**31,441389**	**9**	**Plunge dip**	**Pyrethroid**
Shorty	**-24,675556**	**31,374722**	**10**	**Plunge dip**	**Pyrethroid**
Welverdiend B	**-24,578333**	**31,359444**	**11**	**Pour-on**	**Pyrethroid**
Seville A	**-24,679444**	**31,404444**	**12**	**Plunge dip**	**Pyrethroid**

3.3 Tick collection in the study area

Fifty five to sixty fully engorged blue ticks *(R. (Boophilus) spp.)* were manually removed from as many different animals per dip tank as possible. Collection took place before the cattle were dipped, sprayed or treated with an acaricide. Only engorged females greater than 4mm were collected as this is the minimum size of engorged female correlated with ability to lay eggs (Taylor, per. comm.).

Ticks collected were stored in plastic containers with perforated lids, placing the ticks between layers of paper towel in order restrict movement and to absorb excess moisture. Containers were kept away from excessive heat or direct sunlight. Data, including date of collection and tank name, were recorded on each container. Samples were then returned immediately to the Hans Hoheisen Wildlife Research Station. Ticks were differentiated into the relevant species i.e. *R. (B). decoloratus* and *R. (B). microplus*,

through the variation of the denticles on the hypostome, using a light microscope. Those ticks that could not be identified, were damaged or undersized, were discarded. The remaining ticks were placed in an Erlenmeyer flask and incubated as described below.

3.4 Tick incubation

All the engorged female ticks collected in the field were incubated at 25 ^{0}C and an R.H. of > 75 % in glass Erlenmeyer flasks. The ticks were monitored and maintained in this condition until egg laying and larval hatching were completed. Each flask was labelled with the predicted hatch date (PHD), estimated to be forty days from collection date (Taylor, per. comm.). The actual hatching date (AHD) was determined to be the date when approximately 75 % of the eggs had hatched, and recorded.

3.5 Acaricides used in the study

The acaricides used during this study were chosen because they were currently widely used in South Africa and were commercially available. All were registered according to Act 36 of 1947 for the control of tick

The following acaricide groups were presented in the bioassays:

- Organophosphates - Chlorfenvinphos 30 % m/v
  Cooper's Supadip
  Reg. no. G3349 Act 36/1947

- Amidines - Amitraz 12.5 % m/v
  Triatix ® 125
  Reg. no. G3189 Act 36/1947

- Pyrethroid - Cypermethrin 20 % m/v

Pro-Dip™ Cyp 20 %

Reg. no. G23311 Act 36/1947

3.6 The Shaw Larval Immersion Test

Procedures for the resistance test were conducted in accordance with those followed by Dr R J Taylor (per. comm.) for the "Shaw Larval Test". For a more detailed explanation of the test, refer to Appendix 3.

Approximately 200 larvae were transferred with a soft brush to a filter paper placed upon a disposable aluminium plate. Five ml of clean water or diluted acaricide (starting with the lowest concentration) was poured onto the ticks on the filter paper; and then a second filter paper was placed on top of the first filter paper containing the ticks; and the remaining five ml of water or acaricide was poured onto the filter paper sandwich. These were then set aside for exactly ten minutes each. After the elapsed ten minutes, the filter paper "sandwich" was opened and the papers placed on dry paper towel to absorb excess moisture. One hundred larvae (estimated) were then transferred, with a clean brush, into each of two replicates of a dry, pre -folded, conical filter paper envelope which were then sealed off with a paper crimper. The dilution was marked onto each of the two envelopes as well as other details including, date of test, isolate number and acaricide concentration.

Envelopes were stored in the incubator at 25 ^{0}C and RH >75% (saturated NaCl solution), stacked in sequence on racks in such a way that they did not make contact. Mortality rates were determined 72 hours later, starting with the water control and lowest concentration of acaricide. Data collected was registered for a probit analysis (Appendix 4).

3.7 Data analysis

All relevant data were captured on to a specially prepared data capture form on EXCEL and computerised for analysis. For the SLIT, corrected mortality dose data (using Abbott's formula) were plotted on probit analyses paper.

The LC_{50} and LC_{99} values were then read off this graph and compared to that of the known susceptible reference strain (Appendix 1) and resistance factors calculated. Responses of field ticks exposed to acaricides were compared with baseline data obtained from susceptible strain on the basis of the LC_{50} value (an estimate of the acaricide concentration which will kill 50 % of the population). A factor of resistance (FOR) was calculated by divided the LC_{50} value obtained with a field strain to that of a known susceptible reference strain. The degree of resistance is the number of times the LC50 value of a field strain exceeds that of the susceptible tick strain

$$\text{CM \%} = \frac{\%\, \boldsymbol{i} - \%\, \boldsymbol{c}}{\mathbf{100} - \%\, \boldsymbol{c}} \times \frac{\mathbf{100}}{\mathbf{1}}$$

where **% *i*** **=** **% mortality in concentration *i***

% c **=** **% mortality in water control**

CM % **=** **corrected mortality**

CHAPTER IV RESULTS

Only *R. (B). microplus* ticks were identified from all twelve dip tanks, thus no comparative resistance studies could be conducted between *R. (B). microplus* and *R. (B). decoloratus*. The LC_{50} values of amitraz, cypermethrin and chlorfenvinphos tested against each tick isolate as well as factors of resistance (FOR) for eight of the tick strains and each acaricide tested are illustrated in Table 2. The remaining four tick isolates did not have enough larvae to conduct a long range resistance test (i.e. against seven different concentrations) and were thus tested using discriminating concentrations, the results of which are shown in Table 3. A summary of the results of the SLIT used to determine the susceptibility of the *R. (B). microplus* data from the twelve Mnisi dip tanks tested, to amitraz, cypermethrin and chlorfenvinphos are summarised in Table 4.

Susceptible reference strain values for *R. (B). decoloratus* (as used by RJT Labs, Taylor, pers, comm., 2012) were included as controls. The larvae obtained from engorged females collected from the field were considered to be resistant when these FOR values were 100 or more for amitraz and cypermethrin or more than five for chlorfenvinphos. Tick were considered to be developing an emerging resistance when FOR values were between 50 and 99 for amitraz and cypermethrin and between 2.5 and five for chlorfenvinphos. The cut off points for the tests were determined based on previous field trials (Taylor, pers. Comm.). For tests using discriminating concentrations, larvae were considered to be resistant if the percentage mortality was less than fifty percent (Taylor, pers. comm.).

R. (B.) microplus larvae from all dip tanks tested were found to be completely susceptible to chlorfenvinphos (Table 4). Eleven of the twelve strains of ticks were found to be resistant to cypermethrin, only larvae from “Hlalakane” proved to be completely susceptible (Table 4). In larvae from “Eglington” on the other hand, emerging resistance to cypermethrin was detected. Amitraz resistance was identified at the following dip tanks (Table 4): “Eglington”, “Utha A”, “ Seville A”, “Utha scheme”, “Seville B” and “Welverdiend B”. At both “Islingron” and “Shorty” the R. (B). microplus population was found to have emerging resistance to amitraz. “Hlalakane” was the only dip tank shown to have a *R. (B). microplus* population that was wholly susceptible to all three chemical actives. Five out of twelve dip tanks (41 %) had multi-resistant populations to both amitraz and cypermethrin.

Appendix 4 summarise the percentage corrected mortality (% CM) of the different concentrations of acaricide tested against the different *R. (B). microplus* strains. The % CM is calculated from the

dead/alive tick count. On a whole, the trend of the count shows a good kill in relative to increases in concentration except where there is a resistant strain.

Table 2. *In vitro* larval bioassay: Results of the susceptibility of *R. (B). microplus* to amitraz, cypermethrin and chlorfenvinphos

		Active Compound								
		Amitraz			Cypermethrin			Chlorfenvinphos		
Dip tank	**strain**	**LC_{50}**	**FOR**	**comments**	**LC_{50}**	**FOR**	**Comments**	**LC_{50}**	**FOR**	**Comments**
Hlalakane	1	0.0001	10	S	0.0005	25	S	0.000135	0.21	S
Gottenburg	2	0.00028	28	S	0.011	550	R	0.00025	0.4	S
Eglington	3	0.001	100	R	0.001	50	ER		See Table	
Share	4	0.00012	12	S	0.007	350	R	0.00025	0.4	S
Utha A	5	0.001	100	R	0.014	700	R	0.0003	0.48	S
Islingron	7	0.006	60	ER	0.0056	280	R	0.00031	0.49	S
Shorty	10	0.0009	90	ER	0.045	2250	R	0.00051	0.81	S
Seville A	12	0.0015	150	R	0.036	1800	R	0.0006	0.95	S

Key			Amitraz	Cypermethrin	Chlorfenvinphos
R	= Resistant		FOR	FOR	FOR
S	= Susceptible	R =	> 100	> 100	> 5
ER	= Emerging Resistance	ER =	50 – 99	50 – 99	2.5 - 5
FOR	= Factor of Resistance	S =	< 50	< 50	< 2.5

Table 3. *In vitro* larval bioassay: Results of the susceptibility of *R. (B). microplus* to discriminating concentrations of amitraz, cypermethrin and chlorfenvinphos

		Active Compound					
		Amitraz		Cypermethrin		Chlorfenvinphos	
Dip tank	**strain**	**% M**	**comments**	**% M**	**Comments**	**%M**	**Comments**
Eglngton	3					100	S
Utha Scheme	6	29	R	33	R	100	S
Welverdiend A	8	67	S	47	R	100	S
Seville B	9	0	R	0	R	100	S
Welverdiend B	11	43	R	25	R	100	S

Key				Amitraz	Cypermethrin	Chlorfenvinphos
R	= Resistant			%M	%M	%M
S	= Susceptible	R	=	< 50 %	< 50 %	< 50 %
ER	= Emerging Resistance					
%M	= Percentage Mortality	S	=	> 50 %	> 50 %	> 50 %

Table 4: Summary of the susceptibility of *R. (B.) microplus* larvae to amitraz, cypermethrin and chlorfenvinphos

		Active compound		
		Amitraz	**Cypermethrin**	**Chlorfenvinphos**
Dip tank	**strain**	**Resistance status**	**Resistance Status**	**Resistance Status**
Hlalakane	1	S	S	S
Gottenburg	2	S	R	S
Eglington	3	R	ER	S
Share	4	S	R	S
Utha A	5	R	R	S
Utha Scheme	6	R	R	S
Islingron	7	ER	R	S
Welverdiend A	8	S	R	S
Seville B	9	R	R	S
Shorty	10	ER	R	S
Welverdiend B	11	R	R	S
Seville A	12	R	R	S

Key

R = Resistant

S = Susceptible

ER = Emerging Resistance

CHAPTER V. DISCUSSION

All of the literature supports the displacement of *R. (B). decoloratus* by *R. (B). microplus* where both species occur together (Tonnesen *et al.* 2004) and it appears that Mnisi is no exception. In this light, the fact that 100 % of ticks were collected were identified as *R. (B). microplus* should come as no surprise. What is does, do, however, is pose the question of how this tick was first introduced into this area? Mnisi, theoretically, should have strict control on the movement of cattle in and out of the area as it is in a FMD controlled zone, as well as sharing two thirds of its borders with game reserves. It has already been stated that cattle are the preferred hosts of *R. (B). microplus* while the *R.(B).decoloratus* is found on a variety of antelope species. Should the level of control of the movement of cattle in this area be further investigated? It would seem that it should be the case.

However the R.(B).microplus did find its way behind the red line, the fact remains that it is ever-present, and appears to have displaced the R.(B).decoloratus This finding also appears to support the theory that a zone of reproductive interference does little to prevent the spread of *R. (B). microplus* where climatic conditions are favourable, the cattle have little or no resistance to *R. (B). microplus* and the movement of cattle is a common occurrence (Estrada-Pena, A. & Salman, M. 2013). As very little is known about what drives the rapid adaptation if *R. (B). microplus* to environments beyond its physiological limits, let alone adaptation to prevailing regional climatic conditions (Estrada-Pena, A. & Salman, M. 2013), more research in the physiology of these ticks needs to be conducted in order to understand what general mechanisms regulate this adaptive behaviour (Estrada-Pena, A. & Salman, M. 2013). This is one of the important factors that need to be considered when implementing tick control strategies.

R. (B). microplus has developed resistance to all three of the major classes of acaricide in many countries (Li *et al.* 2004), including South Africa (Kemp 1994). . The findings of this study show that resistance to two of the classes i.e. the amidines and the pyrethroids, is present at the majority of the dip tanks in the Mnisi area. The absence of chlorphenvinphos-resistant R.(B).spp. populations in the area (Table 4) correlates with the lack of reported use of the product in recent years. Amitraz was the main chemical used over a number of years. It was decided in July 2012, four months before this resistance survey was conducted, to change all the dip tanks over to acaricides from the pyrethroid group (Stoltz, per. com.). Thus it was anticipated that there would be evidence of amitraz resistance

development at some, if not all the dip tanks tested. However, as pyrethroids were only in use for a period of four months, it was unclear to what extent pyrethroid resistance had developed. A recent survey in the area hinted at the presence of resistance due to farmers being dissatisfied with the level of tick control, resulting in their purchasing of alternate compounds.

According to Kemp (1994), amitraz resistance is not wide spread in South Africa. At Mnisi, amitraz resistance was present at 50 % of the dip tanks, with emerging resistance evident at 16 %. Given that amitraz had been in use in the area for many years, this result, if not even a higher prevalence, was to be expected. So why was there not more widespread amitraz resistance? One suggestion put forward is that the population of amitraz resistant ticks could have declined. This supposition is based on the observations that, following the regular use of a pyrethroid on amitraz resistant ticks, it has been found that the resistance factor to amitraz declines after about two years to a level where amitraz could again be used (Taylor, pers. Comm,). This, however, is unlikely given the fact that pyrethroids have only officially been in use for four months prior to the study. Thus one has to start looking at the demographics of each dip tank more closely. Are they closed systems i.e. are there hindrances (geographic or otherwise) that limit the movement of cattle at these dip tanks, thus preventing the transference of resistant populations? Do the tick populations in these areas not possess the genetic potential to develop resistance? Unfortunately more studies would need to be conducted in order to answer these questions more specifically. Whatever the reason, the lower than expected amitraz finding confirms the findings of Kemp (1994). Furthermore, in a survey conducted in South Africa mapping resistance to the three major actives used here, amitraz resistance occurred at a fairly low frequency suggesting that, in any geographical region, not all farms develop amitraz resistance at the same time (Mekonnen *et al.* 2002).

What was not anticipated was the extremely high percentage of pyrethroid resistance (83 %), given that this group only replaced the use of amidines four months earlier. It is highly unlikely that this could be a long enough exposure period in order to reveal a pyrethroid resistance. A more likely scenario would be that over the years, the farmers have resorted to the use of other acaricidal treatment, which included the pyrethroids. The movement of cattle from areas where pyrethroid resistance is prevalent, in to the Mnisi district, would also result in the impression that pyrethroid resistance had developed over the short time period of four months. Again, one has to bear in mind how the movement regulations of cattle in this area are actually enforced. A third, less obvious, reason for the perceived sudden development

of pyrethroid resistance is if there had been a previous history of DDT exposure and resistance in the area. There has been documented cross-resistance between DDT and pyrethroids (Taylor, pers. Comm.).

The resistance profile found at Mnisi shows no evidence of a particular pattern and no comment can be made on the delivery system of the active ingredients as there were too few samples to give a conclusive answer. The most concerning finding is the detection of resistance to multiple active ingredients. Multi-resistance (to the amidines and pyrethroids) was found at half of the dip tanks, while no resistance to the organophosphates was detected at all. So where to from here?

Mnisi has many complicating variables when suggesting a tick control programme (e.g the presence of economically important multihost tick such as *Amblyomma hebraeum*). For the short term, where multi-resistant blue ticks exist, it is strongly encouraged to reduce this challenge by changing to use of organophosphates at these dip tanks together with strategic use of insect growth regulators and a macrocyclic lactone, e.g. ivermectin. Tanks that present with a single resistance profile should be changed on to a dip that shows no resistance. All cattle farmers using the communal dipping system should also be discouraged from using alternate compounds. The object of all of the above is geared towards the control and management of resistance. In order to achieve this, other management measures should also be initiated to limit the spread of resistance as well as limit the development of cross-resistance. Management and maintenance of dip tanks and dipwash are also imperative to the success of the programme as are education and communication. Aside from resistance management, any dipping programme must further ensure that it is limiting the outbreak of tick borne diseases as well as tick control.

CHAPTER VI. CONCLUSION

The early emergence of resistance in *R. (B). microplus* has put tick control at the forefront of thinking about resistance and integrated tick control management (Sangster 2001). This study of the resistance profile of the communal dipping system at Mnisi is no exception. It is imperative, that when implementing a tick control system, all factors need to be taken into account. Acaricide resistance management should be considered as important in the control of the ticks themselves and the diseases they spread. South African farming systems, in particular the communal grazing and dipping areas such as Mnisi, have many variables that influence the tick control programmes. These should not be viewed in isolation of each other, but rather take into account how the one influences the next in order for a fully integrated tick control management system to be employed and that continued monitoring of these systems takes place in order to manage acaricide resistance in this country.

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VII. APPENDICES

Appendix 1. LC_{50} and LC_{99} values of a known susceptible *R. (B.) decoloratus.* To the three major chemical actives: amitraz, deltamethrin and chlorfenvinphos (Taylor, pers. comm., 2012)

Active compound	LC_{50}	LC_{99}
Amitraz	**0.00001**	**0.0002**
Deltamethrin	**0.00002**	**0.0002**
Chlorfenvinphos	**0.00063**	**0.0018**

R. (B) microplus.= Ripicephalus (Boophilus) microplus
LC_{50} = Lethal concentration which kills 50 % of the population
LC_{99} = Lethal concentration which kills 99 % of the population

Appendix 2. Range of concentrations used for all three chemical actives (Taylor, pers. comm., 2012)

Active compound	Range of Conc. Used in LR SLIT	Discriminating Concentration
Amitraz	0.000006 0.000032 0.00016 0.0008 0.004 0.02 0.1	**0.001**
Cypermethrin	0.00002 0.0001 0.0005 0.002 0.01 0.05 0.2	**0.002**
Chlorfenvinphos	0.00013 0.0004 0.0012 0.003 0.01 0.03 0.1	**0.003**

Conc. = Concentration
LR = Long Range
SLIT = Shaw Larval Immersion Test

Appendix 3. The Shaw Larval Immersion Test,

As carried out in Dr R.J. Taylor's Laboratory, East London, South Africa

Making up the concentrations

1. Acaricide and range to be used
2. Calculate the dilutions
3. Using a felt-tipped pen, label the 500 ml plastic dishes with the acaricide and concentration they will contain.
4. Place the dishes in a line in order of increasing concentration
5. Using a pair of forceps, place a magnetic stirring bar in the bottom of each dish.
6. Starting with the master solution, measure out the appropriate amounts of water for each dish.
7. Measure the required amount of concentrate into the Master solution and place on stirrer. Stir well.
8. Using the master solution, measure the required amount of concentration into each dish.

Conducting the test

1. Place a few sheets of paper towel on a tray. This is to soak up any drops of water or liquid which may fall
2. On this, place an aluminium foil plate and in the plate place 1 sheet of 12.5 cm filter paper
3. Place the weakest dip concentration on the stirrer and switch it on
4. Draw up 10 ml of the dip wash with the syringe and lay it on the side of the tray. Place the second concentration on the stirrer.
5. Pick up the flask of ticks from the petri dish, dry the bottom on the paper towel, and, using the forceps, remove the cotton wool plug from the flask and place it on the filter paper in the plate

6. Take a no. 5 brush and push some ticks from the neck of the flask onto the bristles (when picking up ticks with the brush, **always push** the brush forwards into the ticks. When brushing ticks off the bristles, stroke the brush backwards on the filter paper.)

7. Place the brushful of ticks on the filter paper and push the plug back into the neck of the flask with the forceps and place the flask back in the petri dish.

8. Rinse the forceps in acetone tube A and lay them back between the bundles of brushes.

9. Stroke between 400 and 500 of the larvae onto the filter paper and plunge the head of the brush into acetone tube B. Leave it there for the moment.

10. Pick up the syringe with 10 ml of water in the right hand, and at the same time as you start the stopwatch, start to squirt the 10 mL of water in a zig-zag pattern over the ticks on the filter paper.

11. Place another sheet of 11 cm paper over the ticks, and squirt another 10 mL on the top of the "sandwich".

12. Lift the aluminium foil plate and place it on the counter to your right

13. Repeat movements 2,5,6,7,8 and 9

14. Pick up the syringe and when the stopwatch reaches exactly 60 seconds, squirt 10 Ml of the dip wash from the pipette onto the ticks

15. Repeat movement 11

16. Lift the aluminium plate and place it next to the one on your right.

17. Fill the pipette with dip wash from the bowl on the stirrer and place the pipette on the side of the tray once more.

18. Take the aluminium dish off the stirrer and place the next one there

19. Rinse the used brush from tube B in tube A then set it aside.

20. Movements 2,5,6,7,8,9,11-19 may be repeated until all the concentrations have been used

21. Stop the stirrer.

Packeting of larvae

1. After all the concentrations have been used, wash the pipette with water, acetone and water again and place it out of the way, ready to use in the next test
2. Wash all the used no. 5 brushes in acetone and dry them. Make sure that all the larvae have been removed by the washing. The easiest way to clean them is to place them in a glass beaker, heads down. Squirt acetone over the bristles and shake the brushes in the acetone. Fold a sheet of paper towel in half, and holding the brushes at right angles to the paper, rub the bristles over the towel until no more larvae fall out.
3. Place the brushes back in place ready for use again.
4. Using the paper towel, wipe down the tray, squashing any stray larvae and mopping up drops of liquid. Throw the paper away
5. Take the ticks out of the petri dish and wipe any stray larvae from the flask and plug. They can now be taken back to the incubator. Remove the petri dish and paper towel.
6. Place the filter paper envelopes on top of the pile of 11 cm filter papers
7. Place two fresh sheets of paper towel on the tray and bring the first aluminium plate i.e. the one which was dipped first, onto the tray next to the paper.
8. Pick up the forceps.
9. After exactly 10 minutes, as the second hand reaches 60 seconds, pick up the filter paper “sandwich” with the forceps and place it on one section of the paper towel.
10. Throw the aluminium foil plate away.
11. Open the “sandwich” with the forceps and place each half, tick side up, on a dry portion of paper.
12. Press the papers down gently with the tip of the forceps to dry them.
13. Rinse the forceps in acetone tube A.

14. Pick up the first filter paper envelope and open it. Holding it open with your left hand, pick up a no. 5 paintbrush (N.B. this is the water control, so use an uncontaminated brush) and push the brush through the larvae.
15. Stroke about 100 larvae as close to the centre of the open envelope as possible.
16. Put down the envelope and do the same with the replicate envelope.
17. Place the paintbrush in the acetone tube A (contaminated brushes are placed in tube C).
18. Fold up the envelope you are holding, place it with the open edges next to the cog of the crimper.
19. Turn the crimper handle and allow the open edges to run between the cogs, sealing them.
20. Label each packet with the date, species, strain number and active ingredient.
21. Sealed packets containing the larvae are placed in a vertical position on a metal rack.
22. Fold in all the edges of the 24 cm papers, use the bundle to mop the tray, then throw the papers away.
23. Rinse the brush in tube A.
24. Place two fresh sheets of 24 cm paper on the tray, together with the next aluminium foil plate.
25. Wait until the stopwatch reaches 60 seconds once more and repeat steps 9 -22, this time using a no. 6 (contaminated) brush to pick up the ticks.
26. Once all concentrations have been done, the stopwatch is stopped.
27. Wash the brushes first in tube C then in tube A.
28. Place the metal rack containing the sealed envelopes in the incubator.
29. Spray the waste bucket with acetone to kill the ticks.

Reading the test

1. After 72 hours the envelopes are removed from the incubator.
2. Starting with the water control, the first envelope is opened by cutting off the crimped edge.
3. Open the envelope and place it on a sheet of paper, and using the prodder, squash all live ticks i.e. the ones which run around, counting them as you do so.
4. Write the total of live ticks on one edge of the paper.
5. Turn the envelope over a clean sheet of paper and shake it gently. All the dead larvae will fall off and these can now be counted.
6. Write the total on the right of the live total. Add the two together and write the grand total on the left. Reading from the left, you now have **Total, Alive, Dead**
7. Repeat the procedure with all the envelopes, stacking the completed ones in order of concentration.

These figures are entered in to an EXCEL spread sheet and a percentage of mortality for each concentration is calculated. In cases where the percentage mortality (%M) of the water control is less than 10 %, the % mortality for the concentrations is corrected by that figure and the graph plotted using these figures. If the water control is greater than 10 % the test is discarded and repeated again.

8. Calculate corrected mortality according to Abbott's formula

i.e. CM % = $\frac{\%\, i - \%\, c}{100 - \%\, c}$ x $\frac{100}{1}$

where % *i* = % mortality in concentration *i*

% c = % mortality in water control

CM % = corrected mortality

9. Plot concentration and CM % on log-probit paper and determine the LC_{50} and LC_{99}

Appendix 4. Corrected mortality data from the larvae of R. (B.) microplus exposed to different concentrations of amitraz, cypermethrin and chlorfenvinphos

Dip tank	R.(B).mic. strain	Amitraz		Cypermethrin		Chlorfenvinphos	
Hlalakahle	1	Conc.	% CM		% CM	Conc.	% CM
		0.000006	46.40	0.00002	22.57	0.00013	49.89
		0.000032	22.85	0.0001	49.84	0.0004	85.04
		0.00016	66.84	0.0005	39.10	0.0012	100.00
		0.0008	22.76	0.002	59.43	0.003	100.00
		0.004	100.00	0.01	85.65	0.01	100.00
		0.02	100.00	0.05	100.00	0.03	100.00
		0.1	100.00	0.2	100.00	0.1	100.00
Gottenburg	2						
		0.000006	-5.32	0.00002	8.92	0.00013	21.72
		0.000032	20.82	0.0001	-1.06	0.0004	42.04
		0.00016	39.25	0.0005	7.26	0.0012	97.59
		0.0008	18.97	0.002	12.29	0.003	100.00
		0.004	100.00	0.01	41.58	0.01	100.00
		0.02	100.00	0.05	72.82	0.03	100.00
		0.1	100.00	0.2	97.42	0.1	100.00
Eglington	3						
		0.000006	-13.27	0.00002	3.40		
		0.000032	-9.35	0.0001	-2.09		
		0.00016	3.59	0.0005	19.55		
		0.0008	33.15	0.002	44.28		
		0.004	93.49	0.01	100.00		
		0.02	100.00	0.05	96.91		
		0.1	100.00	0.2	100.00		
Share	4						
		0.000006	18.83	0.00002	19.00	0.00013	20.16
		0.000032	-3.29	0.0001	6.23	0.0004	58.99
		0.00016	9.85	0.0005	10.35	0.0012	100.00
		0.0008	91.22	0.002	15.05	0.003	100.00
		0.004	100.00	0.01	21.39	0.01	100.00
		0.02	100.00	0.05	56.38	0.03	100.00
		0.1	99.27	0.2	99.45	0.1	100.00
Utha A	5						
		0.000006	-11.74	0.00002	-3.32	0.00013	9.67
		0.000032	-5.94	0.0001	-7.57	0.0004	42.16
		0.00016	24.38	0.0005	-5.13	0.0012	99.09
		0.0008	17.80	0.002	-10.81	0.003	100.00
		0.004	95.56	0.01	29.37	0.01	100.00
		0.02	98.95	0.05	92.93	0.03	100.00
		0.1	99.30	0.2	100.00	0.1	
Islingron	7						
		0.000006	2.68	0.00002	3.91	0.00013	25.55
		0.000032	1.41	0.0001	20.70	0.0004	44.05
		0.00016	13.25	0.0005	7.61	0.0012	96.61
		0.0008	17.89	0.002	7.36	0.003	100.00
		0.004	81.87	0.01	28.40	0.01	100.00
		0.02	96.89	0.05	80.48	0.03	100.00
		0.1	97.37	0.2	100.00	0.1	100.00

Shorty	10						
		0.000006	-7.85	0.00002	-13.47	0.00013	1.21
		0.000032	-.058	0.0001	-17.91	0.0004	25.88
		0.00016	1.27	0.0005	-10.24	0.0012	96.54
		0.0008	38.71	0.002	-5.74	0.003	100.00
		0.004	96.76	0.01	16.09	0.01	100.00
		0.02	99.12	0.05	30.51	0.03	99.52
		0.1	100.00	0.2	94.67	0.1	100.00
Seville A	12						
		0.000006	2.46	0.00002	-9.98	0.00013	-0.05
		0.000032	-1.41	0.0001	-4.36	0.0004	12.49
		0.00016	4.23	0.0005	3.86	0.0012	92.64
		0.0008	2.51	0.002	6.76	0.003	100.00
		0.004	84.44	0.01	11.20	0.01	100.00
		0.02	97.62	0.05	27.64	0.03	100.00
		0.1	100.00	0.2	91.91	0.1	100.00

R. (B) mic. = *Rhipicephalus (Boophilus) microplus*
Conc. = Concentrations
%CM = Percentage corrected mortality

Appendix 5. Percentage mortality from the larvae of R. (B.) microplus exposed to discriminating concentrations of amitraz, cypermethrin and chlorfenvinphos

Dip tank	R. (B). microplus strain	Amitraz		Cypermethrin		Chlorfenvinphos	
Utha Scheme	6	Conc. 0.001	% M 44	Conc. 0.002	% M 46	Conc. 0.003	% M 100.00
Welverdiend A	8	0.001	67	0.002	47	0.003	100
Seville B	9	0.001	38	0.002	36	0.003	100
Welverdiend B	11	0.001		0.002		0.003	100
Eglington	3					0.003	100

R. (B) mic. = *Rhipicephalus (Boophilus) microplus*
Conc. = Concentrations
%M = Percentage mortality

Appendix 6. List of regulatory framework providing for tick control strategies in South Africa (Jenjezwa, V.R. & Seethal, C.E.P 2014, Eastern Cape Department of Rural Development and Agrarian Reform 2013)

- Agricultural Development Act, Act 8 of 1999
- Animal Health Act, Act 7 of 2002 3.3. Government Priorities, 2009 – 2014
- Meat Safety Act, Act 40 of 2000
- National Environmental Management act, Act 107 of 1998
- Strategic Plan for the Department of Agriculture and Rural Development, 2010–2015
- Veterinarian and Para-veterinarian professions Act, Act 19 of 1982
- Animal Identification Act, Act 6 of 2002
- Animal Diseases and Parasites Act (Act 13 of 1956)

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Animal Ethics Committee

PROJECT TITLE	**Acaricide resistance in Ripicephalus (Boophilus) species in a communal dipping system in the Mnisi community, Mpumalanga province**
PROJECT NUMBER	**V061**
RESEARCHER/PRINCIPAL INVESTIGATOR	**Dr. R Malan**

STUDENT NUMBER (where applicable)	**99065810**
DISSERTATION/THESIS SUBMITTED FOR	**MSc**

ANIMAL SPECIES	***Bovine***	
NUMBER OF ANIMALS	**500**	
Approval period to use animals for research/testing purposes		**November 2012-November 2013**
SUPERVISOR	**Prof. Maxime Madder**	

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 **29 May 2013**
CHAIRMAN: UP Animal Ethics Committee	Signature