THE EXTENT OF ACARICIDE RESISTANCE IN THE EASTERN REGION OF THE EASTERN CAPE PROVINCE

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The extent of acaricide resistance in the eastern region of the Eastern Cape Province

by

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DEDICATION

I would like to dedicate this work to my father Budabuphangwa Ntondini for encouraging us, his children, to strive for more in life, to my mother, my children and all my family for their support.

DECLARATION

Apart from the assistance received from my supervisor, Prof Ivan Horak, who helped with the original identifications of some of the ticks that were collected and also with editing the manuscript, from Mr N. Nyangiwe, who collected the ticks for the acaricide resistance tests, from Mrs E. van Dalen of the Department of Zoology and Entomology of the University of the Free State, who taught me how to carry out the various tests for acaricide resistance and assisted with their interpretation, and from Dr R. Williams of The ARC-Onderstepoort Veterinary Institute, who constructed the maps, this dissertation represents the original work of the author.

I have adapted the distribution maps of four tick species from Mr N. Nyangiwe's MSc (Veterinary Science), dissertation (University of Pretoria), (with acknowledgement to him), so as to give the reader an idea of the overall distribution of these ticks. I contributed raw data towards the construction of these maps in that the identities of some of the ticks that I used in the acaricide resistance tests and the localities at which they were collected were used to complete the maps.

With the exception of the abovementioned adapted four maps no part of this dissertation has been presented for any other degree at any other university.

Candidate: .....Date: .....

SUMMARY

The extent of acaricide resistance in the eastern region of the Eastern Cape Province

by

Zoleka Ntondini

Supervisor: Professor I.G. Horak

The control of ticks, and to some extent tick-borne diseases, over much of South Africa is currently dependent on acaricides and will probably remain so for the foreseeable future. Resistance to these chemicals by ticks thus poses a major threat to the livestock industry especially as these chemicals constitute an ever-diminishing resource with fewer being discovered and the cost of their development becoming prohibitive.

In order to determine the extent of acaricide resistance in the eastern region of the Eastern Cape Province one-, two- and three-host ticks were collected from cattle at 58 dip-tanks over a period of 2 years. The one-host tick selected was *Rhipicephalus* (*Boophilus*) *microplus*, the two-host tick *Rhipicephalus evertsi evertsi* and the three-host tick *Rhipicephalus appendiculatus*. The ticks were tested for resistance to three compounds, namely amitraz, cypermethrin and chlorfenvinphos. The Shaw Larval Immersion Test detected emerging resistance to amitraz in the one-host tick *R.* (*Boophilus*) *microplus* at two dip-tanks and resistance at a third. It also revealed resistance in this tick to cypermethrin at one dip-tank, and emerging resistance to chlorfenvinphos at eight dip-tanks and resistance at two. The two-host tick *R. evertsi evertsi* was susceptible to amitraz and cypermethrin at all dip-tanks, but showed emerging resistance to chlorfenvinphos at seven dip-tanks and resistance at four. The three-host tick *R. appendiculatus* was susceptible to amitraz and chlorfenvinphos at all dip-tanks and demonstrated emerging resistance to cypermethrin at one. With the exception of *R.* (*Boophilus*) *microplus*, in which emerging resistance to amitraz was detected at one dip-tank by the Reproductive Estimate Test, all three tick species at all dip-tanks at which sufficient numbers of ticks had been collected, were susceptible to the three acaracides in both the Egg Laying Test and the Reproductive Estimate Test.

Thus despite its fairly long and widespread use in the eastern region of the Eastern Cape Province very little or no resistance to amitraz was detected in three tick species regularly encountered on cattle in this region, namely *R.* (*Boophilus*) *microplus*, *R. evertsi evertsi* and *R. appendiculatus*. On the other hand resistance to chlorfenvinphos was detected in both *R.* (*Boophilus*) *microplus* and *R. evertsi evertsi* at a number of dip-tanks even though it, or other organophosphorous-based compounds, had probably not been used for tick control in the region for a number of years.

The localities at which ticks were collected had already been mapped and the localities at which acaricide resistant ticks were encountered were mapped during this study. The three tick species that were targeted for acaricide resistance testing were widespread throughout the study region, but no pattern of geographic distribution for the acaricide resistant strains of these species that were detected, emerged.

The rapidity of selection for acaricide resistance appeared to be closely related to the life cycles of the three ticks and the number of days that they theoretically would spend annually on their preferred host animals. Thus a greater number of acaricide resistant strains were encountered amongst the one-host tick *R.* (*Boophilus*) *microplus* and the two-host tick *R. evertsi evertsi* than the three-host tick *R. appendiculatus*. The fist two ticks both complete more than one life cycle a year and hence spend a longer time on their cattle hosts than the three-host tick *R. appendiculatus*, which completes only one life cycle a year and in addition is a rapid feeder in all its stages of development.

To counter selection for acaricide resistance it is proposed that regular testing for resistance should be carried out, and that as soon as emerging resistance is detected in ticks on cattle at a particular dip-tank, that the acaricide in use at that dip-tank should be changed to a compound belonging to a completely different group of chemicals.

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ABBREVIATIONS

ECP – Eastern Cape Province

ELT – Egg Laying Test

RET – Reproductive Estimate Test

SLIT – Shaw Larval Immersion Test

ARC-OVI – Agricultural Research Council – Onderstepoort Veterinary Institute

FOR – Factor of Resistance

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It is true that many hands make light work.

1. INTRODUCTION

The present investigation is one of two studies conducted in the same region at the same time. The study reported here aimed at determining the geographic distribution of acaricide resistant ticks on cattle in the eastern region of the Eastern Cape Province (ECP), while the other study aimed at determining the species composition and geographic distribution of ticks infesting cattle, goats and dogs as well as that of free-living ticks present on the vegetation and in fowl houses in the same region. In the latter study an attempt was made to collect ticks from five cattle, five goats, five dogs, two fowl houses and from flannel strips dragged over the vegetation in the vicinity of each of 72 dip-tanks. During the collection of ticks from the cattle at some of the dip-tanks additional engorged female ticks were collected for the acaricide resistance study and these were dispatched from the collection localities to the laboratory for the acaricide resistance tests to be carried out.

The ECP is the second largest of South Africa's provinces. The area previously referred to as the Transkei is situated in the north eastern part of the Eastern Cape Province and has a 250km Indian Ocean coastline (Hoffman, Todd, Ntshona & Turner 1999). The province covers an area of about 17 million ha, and approximately 4 million ha has a long history of mixed farming by Africans with communal pastoralism as one of its key features. Most of this land is located in the eastern half of the province (Scogings & van Averbeke 1999). The various regions in the province differ to such an extent in relief, climate and vegetation that each region has its own character. In the west are the plains of the Great Karoo, in the north-east the Drakensberg Mountains, and the east has a coastline forming the wild coast.

The vegetation of South Africa includes nine biomes of which six, namely savanna, grassland, Nama-karoo, forest, fynbos and thicket are represented in the ECP (South African National Biodiversity Institute). This biodiversity favours the cultivation of a highly diverse range of agricultural products, from deciduous-, citrus- and subtropical

fruit to grain, cut flowers, livestock and game. The ECP has a dual agricultural economy, with both well-developed commercial farming and more subsistence-based production, mainly in the rural areas.

The north eastern region of the ECP, which was formerly known as the Transkei, in which this study was conducted, has good agricultural potential, and offers sufficient diversity of soil and climate to permit a variety of different forms of agriculture. The total area is over 4million ha in extent, of which more than half is devoted to grazing (Hoffman *et al*. 1999). Although a large proportion of land in the region is potentially suitable for agriculture, a variety of factors including poverty and insufficient rainfall, inhibit this potential. The climate of the region is unpredictable, with rain both in summer and winter, and the region is known for both floods and severe droughts.

From the earliest days, livestock has been an important factor in the lives and economy of rural people. Most rural people in South Africa were concentrated in the former independently governed states of which Transkei formed part. Livestock production is a major resource and component of agriculture production in these areas, because according to Bembridge 1987, 84% of the area can potentially be used only for grazing. Yet livestock contributes very little to the cash economy in terms of sales for slaughter to the market (Scogings & van Averbeke 1999). The rural nature of the north eastern regions of the ECP, the societal value placed on the actual possession of cattle and the communal grazing practiced in this region are partly responsible for this.

Nevertheless, livestock farming remains vitally important to the economy and development of the ECP. Kraaling at night for security reasons and dipping for the control of ticks and tick-borne diseases are the main management interventions made.

Since 1994, the government has been working to develop small-scale farming to boost job creation and economic development. According to Bembridge (1987), in terms of animal units, cattle, followed by sheep and goats, form the major proportion of

economically useful stock in the eastern region of the ECP. The cattle numbers in South Africa as at the end of February 2006 were estimated at approximately 14,094,000 by National Livestock Statistics, of which 22%, which is the highest percentage on a provincial basis, were present in the ECP. Unlike the commercial ranching areas of the drier Eastern Cape rangelands, where livestock numbers have declined, livestock numbers in communal areas have generally remained stable for the last 50-100 years (Scogings & van Averbeke 1999).

Within the livestock farming communities of the developing nations, the presence of ticks and the diseases that they transmit have been huge constraints to the intensification of livestock production. It is estimated that livestock form a 70% component of the livelihood of the world's poor (Peter, Van den Bossche, Penzhorn & Sharp 2005). It is envisaged by Peter *et al*. (2005) that in western consumer societies, per capita consumption of meat is expected to decline because of negative views about consumption of animal protein, environmental impacts and animal welfare. On the other hand malnutrition is rife in the developing world, and sources of protein in the form of meat and milk provide a means of alleviating this.

In South Africa inadequate data exist on direct economic losses that can be attributed to ticks and the diseases that they transmit (Peter *et al*. 2005). A year before the turn of the century it was estimated that tick-transmitted diseases in cattle cost South African stock farmers an estimated R400 million per year due to stock losses (Scogings & van Averbeke 1999). In an attempt to combat these losses the ECP spends on average an amount of R13 million a year on the acquisition of dipping compounds (Mrwebi, pers comm.). It is possible that the latter amount can be reduced by the practice of effective tick control strategies that will also ensure early detection of acaricide resistance in ticks at any specific time and place.

2. OVERVIEW

2.1 History of use of acaricides in the Eastern Cape Province

Dipping of cattle for the control of ticks and tick-borne diseases has been practiced in southern Africa for close on a century (Fletcher, 1984). The north eastern part of the ECP comprises the territory formerly known as the Transkei. This was a self- governance state in which dipping was a function monitored by the state, and dipping compounds were supplied free of charge to the inhabitants of the so-called homeland. A strict cattle dipping policy was enforced. All livestock owners were required to pay a certain amount as a livestock tax levy, and this money was used to subsidize the purchase of the dipping compounds (Gwababa, pers.comm).

The history of insecticide usage in the Transkei is fairly accurately known through records that have been kept. Arsenite of soda was the compound initially used in all dip-tanks in the region. This was later changed to an organophosphate compound known as Delnav, which was used until 1980. Around 1980 the amidine, amitraz, was introduced for the first time in the form of Triatix TR in all dip-tanks in the Transkei. In 1987 a new homeland government came into power and with it the livestock tax levy was stopped and the purchase of the dipping compounds became the sole responsibility of the state.

Dipping compounds were then purchased on tender. During this time new compounds came into being, including organophosphates, synthetic pyrethroids and combination drugs. At one stage all three compounds, i.e. amitraz, organophosphates and combination organophosphates and pyrethroids, were used in various regions of the Transkei with each compound being used exclusively in a particular area. Regular weekly dipping along the coast and fortnightly dipping inland were enforced in summer, with 28-day interval dipping applied along the coast and total suspension of dipping inland during winter.

There are only a few clearly-defined geographical areas in South Africa in which tick control measures are aimed only at single-host tick species, and treatment regimens that have been mainly designed for the control of multi-host ticks predominate (Baker 1982).

Thus the regular dipping required in most regions to control the multi-host tick species would favour the selection for resistance to the exclusively utilized compound or group of compounds at a particular locality. This would apply particularly to the one-host tick species *Rhipicephalus* (*Boophilus*) *decoloratus* and *Rhipicephalus* (*Boophilus*) *microplus*, which are widespread throughout the eastern regions of the ECP (Howell, Walker & Nevill 1978).

Currently the control of ticks and to some extent tick-borne diseases, in much of South Africa is dependent on the regular use of acaricides, and it will probably remain so for the foreseeable future. Selection for resistance to these chemicals by ticks thus poses a major threat to the livestock industry especially as these chemicals constitute an ever-diminishing resource with fewer being discovered and the cost of their development becoming prohibitive (Baker 1982). The purchase and use of dipping compounds in the Eastern Cape has not changed much. Dipping compounds are purchased by the state on tender for the communal farming communities. This unfortunately has maintained the situation of favouring the development of acaricide resistance, because the single dipping compound that is purchased on tender will be used virtually exclusively within a region. Consequently, there will be considerable pressure towards selection for resistance against the active ingredient of the particular product.

Furthermore, communal grazing, in which the movement of animals is the least restricted of all grazing systems, is practiced almost throughout the whole of the eastern part of the ECP. This also favours the spread of acaricide resistance as its spread is governed almost entirely by the movement of the host animals (Baker 1982). Intensive sampling during an assay of acaricide resistance in the Transkei, conducted over an 8-year period, revealed both the influence of the introduction of tick-infested cattle into this region from areas which had been subjected to intense acaricide usage, as well as the effect on the subsequent spread of this resistance by the practice of communal grazing on unfenced land (Baker 1982).

A number of studies have been conducted within South Africa to determine resistance of ticks to acaricides (Baker 1982). These studies suggest that single-host ticks develop resistance more rapidly than multi-host ticks (Mekonnen, Bryson, Fourie, Peter, Spickett, Taylor, Strydom & Horak 2002). Resistance to organophosphorus ixodicides in *R.* (*Boophilus*) *decoloratus* in South Africa was first recorded in 1967 by Shaw, Thompson and Baker (Baker, Miles, Robertson, Stanford and Taylor 1978). These one-host ticks have become resistant to many types of insecticides, and comparison of the resistance spectra of *R.* (*B.*) decoloratus and *R.* (*B.*) *microplus* in South Africa suggest marked similarities in their respective response to resistance selection (Nolan 1981). Some studies have, hower, shown that the susceptibility of *R.* (*B.*) *microplus* to some ixodicides was greater than that of *R.* (*B.*) *decoloratus* either where infestations of the two species occurred together or where each species occurred singly (Baker, Jordaan & Robertson 1981). Apparently the only recorded resistance among two-host ticks is that of *Rhipicephalus evertsi evertsi* to toxaphene in several regions of South Africa (Baker & Shaw 1965; Lourens & Tatchell 1979).

2.2 Economically important tick species

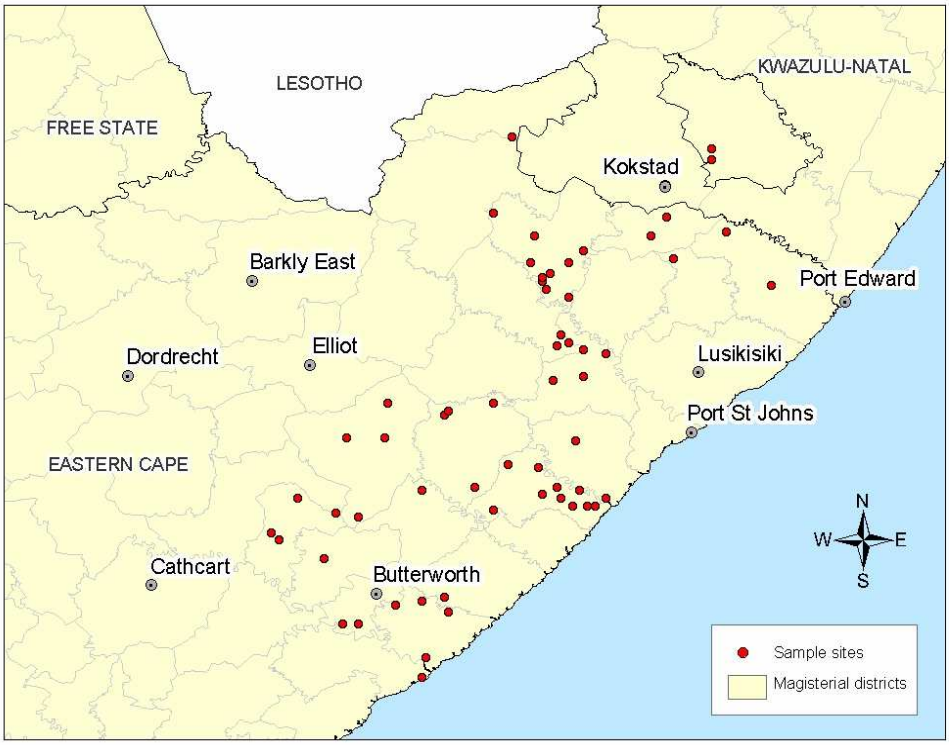
Ticks are without any doubt the most important external parasites of cattle in southern Africa (Howell *et al*. 1978). Besides economic loss caused by their blood sucking habits and wounds caused by their mouthparts, with resultant secondary abscesses, they also transmit economically important diseases (Norval & Horak 2004). Howell *et al.* (1978) have listed the species infesting domestic animals in South Africa, and have also mapped their geographic distributions.

The important species are *Amblyomma hebraeum*, which transmits *Ehrlichia ruminantium*, the cause of heartwater in cattle, sheep and goats (Norval & Horak 2004); *Rhiphicephalus* (*Boophilus*) species which transmit *Babesia bigemina* and *Babesia bovis* the cause of babesiosis in cattle and *Anaplasma marginale* the cause of anaplasmosis in cattle (Norval & Horak 2004); *Rhiphicephalus appendiculatus*, which transmits *Theileria parva* the cause of East Coast Fever and Corridor disease in bovines and *Rhiphicephalus*

evertsi evertsi, which transmits *Babesia bigemina* in bovines and *Babesia caballi* and *Theileria equi* the cause of piroplasmosis in horses (Norval & Horak 2004). Other important disease transmitting ticks include *Hyalomma truncatum*, which transmits a toxin causing sweating sickness in cattle, and *Ixodes ribicundus*, which transmits a toxin causing paralysis in sheep (Howell *et al*. 1978; Norval & Horak 2004), and *Haemaphysalis elliptica*, which transmits the virulent *Babesia canis rossi* causing babesiosis in domestic dogs (Lewis, Penzhorn, Lopez-Rebollar & De Waal 1996; Apanaskevich, Horak & Camicas 2007).

Nyangiwe (2007) has recently mapped the distribution of ticks infesting cattle, goats and domestic dogs in the eastern region of the ECP, and with the exception of *I. rubicundus* and *H. truncatum* he recovered all the abovementioned species. The species with the most widespread distributions that he recovered were *A. hebraeum*, *R.* (*Boophilus*) *microplus*, *R. appendiculatus* and *R. evertsi evertsi*. Engorged female ticks collected by Nyangiwe (2007) during his survey were used in the acaricide resistance tests described in this dissertation.

Figure 1: All dip-tanks at which animals were sampled for acaricide resistance testing, 2004/05



2.3 Tick species tested and acaricide compounds used

The three tick species selected for the acaricide resistance studies all belong to the family Ixodidae to which most ticks of veterinary importance belong. The tick species selected were *Rhiphicephalus* (*Boophilus*) species, *Rhiphicephalus evertsi evertsi* and *Rhiphicephalus appendiculatus* and were chosen because of their one, two and three-host life cycles respectively. Both *R.* (*Boophilus*) *microplus* and *R.* (*Boophilus*) *decoloratus* were intended to be used depending on the areas in which they were found occurring singly or together.

The three acaricides selected were firstly amitraz, which belongs in the formamidine group. It was developed in the early 1970s (Nolan 1981) and has now been established as a cattle tickicide in all major cattle tick areas of the world (Harrison 1981). The suggested action of this group of acaricides is the inhibition of the enzyme monoamine oxidase (Li, Davey, Miller & George 2004), which is responsible for degrading the neurotransmitters norepinephrine and serotonin. This results in accumulation of these compounds, which are known as biogenic amines. Affected ticks become quiescent and die (Ware & Whitacre 2004). This acaricide was chosen because of its long uninterrupted usage in certain regions of the ECP brought about by the procedure of putting acaricide acquisition out on tender.

The second acaricide selected was cypermethrin which belongs to the synthetic pyrethroids group of insecticides. The synthetic pyrethroids have an interesting evolution, which is conveniently divided into four generations, the first, second, third and fourth. Cypermethrin, the compound used in the study belongs in the fourth generation (Ware & Whitacre 2004). The pyrethroids have similar modes of action to each other, and this resembles that of DDT, and they are considered axonic poisons. They work by keeping sodium channels in neuronal membranes and neuromuscular junctions open thereby causing long trains of repetitive impulses leading to convulsion and ultimately death (Cremlyn 1991). They also affect calcium concentrations in nervous tissue by inhibiting an enzyme involved in calcium transport. This result in an increase of the amount of the neurotransmitter acetylcholine released at the junction (Al-Rajhi 1990). Cypermethrin was chosen because it represents a fourth generation synthetic pyrethroid and is thus one of the more recently developed acaricides.

The third compound selected was chlorfenvinphos, belonging to the organophosphate group of insecticides. These are chemical substances originally produced by the reaction of alcohols and phosphoric acid. They function as cholinesterase inhibitors, thereby affecting neuromuscular transmission. Their mechanism of action is irreversible inhibition of acetylcholinestarase. This enzyme is found in red blood cells and in

nicotinic and muscarinic receptors in nerves, muscle and the grey matter of the brain. They bind irreversibly, deactivating the esterase and resulting in an accumulation of acetylcholine at the endplate. The accumulation of acetylcholine at the neuromuscular junction causes persistent depolarization of skeletal muscles, paralysis and death (Senanayake & Karalliedde 1987). Chlorfenvinphos was chosen because of its widespread and long usage as an acaricide throughout South Africa and the many instances of tick resistance recorded against it.

3. GENERAL MATERIALS AND METHODS

3.1 Susceptible tick reference samples and their origins

In order to determine the response of larval ticks to acaricides, it was necessary to obtain standard populations of the four tick species against which field-collected samples of unknown acaricide tolerance could be compared. For this purpose it was preferable to obtain reference samples of ticks from areas where no acaricide had ever been used. However, because of the widespread use of acaricides in South Africa, this is not always possible.

A susceptible sample of *R.* (*Boophilus*) *microplus* was collected from cattle at the Sabié dip-tank, Moamba District, Maputo Province in Mozambique during April 2004. The use of chemical compounds for the control of ticks was last practiced in 2000 at this dip-tank. Engorged female ticks were imported into South Africa on a veterinary permit, identified to species and placed in separate vials in an acaridarium in which to lay eggs. The resultant larvae were fed on a calf, maintained under strict quarantine conditions at ClinVet International Laboratories outside Bloemfontein, Free State Province, South Africa. This calf was carefully monitored for tick-borne diseases in the event that any of the ticks might have been infected with organisms capable of causing a tick-borne disease. No disease was detected in the calf and the ticks were subsequently cycled on cattle.

An acaricide susceptible sample of *R.* (*Boophilus*) *decoloratus* was collected from cattle at a cattle-handling facility in Botshabelo in central Free State Province, South Africa. These ticks were also cycled on cattle at ClinVet Laboratories.

Unengorged male and female *R. evertsi evertsi* and *R. appendiculatus* were collected from an immobilized Burchell's zebra in the north eastern section of the Hluhluwe-iMfolozi Nature Reserve in Kwa-Zulu Natal Province, South Africa. These ticks were transported to the Onderstepoort Veterinary Institute, Onderstepoort, Gauteng Province, where they were fed on a susceptible yearling calf housed in quarantine facilities. The calf developed no tick-borne disease and the offspring of the ticks were subsequently transferred to ClinVet Laboratories where they were cycled on rabbits and cattle.

Ticks from these four collections were tested for susceptibility to the target acaricides and were used as susceptible reference species for calculating the Factor of Resistance (FOR) for the four tick species used in the survey.

Figure 2: Collection of live susceptible ticks from an immobilized zebra in Hluhluwe – iMfolozi Nature Reserve in KwaZulu – Natal

3.2 Collection of ticks for acaricide resistance testing

A list of all communal dip-tanks in the Transkei was obtained, and those at which more than 200 cattle were dipped were selected. A total of 1 057 such dip-tanks were identified and each of these was assigned a number. Using tables of random numbers 55 of these dip-tanks were identified at which ticks were to be collected at 50. The additional five tanks were to be used if it was impossible to reach one or more of the selected dip-tanks by means of a four-wheel drive vehicle, or if two of the 50 randomly selected dip-tanks were located too close to each other. Engorged females of three tick species, namely *R.* (*Boophilus*) *decoloratus*, *R. evertsi evertsi* and *R. appendiculatus* were to be collected from cattle at each of these dip-tanks as they represent the one, two and three-host tick species required for the study.

Sampling commenced in January 2004 and ceased at the end of May 2004, and recommenced in January 2005 and ceased at the end of May 2005. The reason for targeting these months was the greater likelihood of collecting adult engorged female ticks during the warmer months. Because insufficient numbers of ticks had been collected at some dip-tanks during 2004 additional collections were made during 2005.

Collection was done according to the predilection sites of the tick species, with *R.* (*Boophilus*) *decoloratus* collected mainly around the neck, dewlap and underside of the body (Baker & Ducasse 1967), *R. appendiculatus* collected on and around the ear area, and *R. evertsi evertsi* collected from under the tail (Baker & Ducasse 1967; Walker, Keirans & Horak 2000).

The collected ticks were stored in separate plastic containers with perforated lids, according to the site of the body from which they had been collected. The plastic holders contained soft tissue paper to restrict the ticks' movement and to supply protection, and

were kept in cooler boxes containing ice packs to keep them cool, and thus prevent oviposition before they reached the laboratory.

3.3 Identification and dispatch

The tick samples were transported to the Queenstown Provincial Veterinary Laboratory, Queenstown, ECP. Here thorough species identification under a stereoscopic microscope was done and the various tick species counted. It was soon realized that most if not all the *R*. (*Boophilus*) species collected were *R*. (*Boophilus*) *microplus* with very few *R*. (*Boophilus*) *decoloratus*. After identification and counting the ticks were immediately dispatched in cooler boxes to the Acaricide Resistance Testing Laboratory of the Department of Zoology and Entomology, University of the Free State, to be tested for acaricide resistance.

3.4 Test using unfed larvae

3.4.1 Shaw Larval Immersion Test (SLIT)

The Shaw Larval Immersion Test as developed by Shaw (1966) and later modified to ensure that the larvae were held for a longer period (Shaw, Cook & Carson 1968) was used to test the efficacy of the acaricides against unfed tick larvae. Engorged females of the different tick species were incubated at 27°C and >75%RH until they oviposited and the eggs had hatched. The larvae were then used for the SLIT. This entailed the aqueous dilution of the formulations of the three test compounds into eight dilutions including a control.

Figure 3: The sequence of dilutions used during the Shaw Larval Immersion Test

Tick larvae were scooped into sandwiches of folded circular filter papers and 10 ml of the required dilution of the acaricide was pipetted onto the filter paper sandwich containing tick larvae. The larval sandwich was left for 10 min and then the larvae were transferred into clean duplicate folded filter paper envelopes. This was done for each dilution and the envelopes were carefully secured to avoid escape of larvae. After 72 hours of incubation, the numbers of live and dead larvae were recorded and the percentage mortality for each concentration was calculated. Corrected mortality was calculated according to Abbott's formula i.e.

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

where % i = % mortality in concentration i

% c = % mortality in water control

CM % = corrected mortality

Corrected mortality was analysed using a log- probit programme to determine the LC50, LC99 and finally the Factor of Resistance (FOR) (Finney 1971; Raymond 1985).

Preference was given to conducting this test in the present investigation as it is regarded as more standardized and was the test used in the National Survey of acaricide resistance. Depending on availability of larvae, larvae were tested against all three compounds, namely amitraz (Triatix 125, Coopers Veterinary Products), cypermethrin (Curatik, Bayer Veterinary Health Division) and chlorfenvinphos (Disnis N.F., Bayer Veterinary Health Division) whenever possible.

Figure 4: Larval envelopes inside the incubator during the Shaw Larval Immersion Test

3.5 Tests using adult engorged female ticks

3.5.1 Egg Laying Test (ELT)

Engorged female ticks of almost uniform size and free of any visible abnormalities, were selected from the samples and separated into four groups each containing ten ticks. Three of these groups were used as treatment groups, and one group served as the untreated control. Each group was immersed for 10 minutes in the test acaricide at the

concentration recommended for use in the field. The ticks were then air-dried at room temperature for an hour, separated into groups of five ticks, weighed per group, pasted onto double-sided adhesive tape strips on glass test panels and incubated at 27°C and >75% RH.

The control group was immersed in distilled water for 10 minutes and thereafter the same procedure was followed as for the treated ticks. The control group was incubated separately from the treated groups to avoid contamination with acaricide. The ticks were then examined after seven days to ascertain the numbers that were still alive or were dead. They were re-examined after 21 days to weigh the eggs laid by each treatment group.

Of the 58 dip-tanks (9 of which were sampled twice) from which ticks were collected from cattle, about 29 yielded enough ticks to allow for the ELT to be done. Resistance was calculated using the following formula:

$$\%\text{Resistance} = \frac{\text{No of treated ticks laying eggs}}{\text{No of untreated ticks laying eggs}} \times 100$$

A value of above 80% indicates resistance, and between 50 and 80% emerging resistance. Anything below 50% indicates susceptibility.

3.5.2 Reproductive Estimate Test (RET)

The same initial procedures as in the Egg Laying test were followed and thereafter the weighed eggs were transferred into labelled flasks and were further incubated at the same temperature and relative humidity. They were then examined after 42 days and the numbers of larvae that had hatched were recorded.

In this test resistance was calculated using the following formula:

$$\%\ \text{Resistance} = \frac{\text{RE of acaricide- treated ticks}}{\text{RE of untreated ticks}} \times 100$$

RE = the estimated number of larvae that have hatched and are calculated using the following formula:

$$RE = \frac{m1 \times n \times h}{m2 \times s \times 4}$$

where m1 = mass of eggs per treatment group

m2 = mass of engorged female ticks per treatment group

n = number of ticks per group

h = hatchability of the eggs (scale of 0 to 4)

s = number of ticks surviving after 7 day incubation

3.6 Presentation of Data

The results of the Shaw Larval Immersion Test, the Egg Laying Test and the Reproduction Estimate Test are summarized separately for each species in the sections devoted to the individual tick species. The processed SLIT results obtained for each species at each separate dip-tank are also presented separately in each section devoted to the individual tick species. The raw data for the SLITs and for the ELTs and RETs for each dip-tank that was sampled for a particular tick species are presented separately for ticks collected during 2004 and 2005 and also separately for each tick species in the Annexure section of this dissertation.

3.7 Questionnaire Survey

During the survey on the geographic distribution of ticks conducted at the same time and in the same region as the acaricide resistance study a comprehensive questionnaire survey, most of which was unrelated to the present study, was conducted amongst the owners of the cattle. Some of the results of this questionnaire survey will be discussed under the General Discussion included towards the end of this dissertation.

4.1 ONE-HOST TICK SPECIES - *Rhiphicephalus* (*Boophilus*) species

4.1 Introduction

Members of this sub-genus are prevalent in many parts of the world, mainly in the tropics and subtropics (Estrada-Peña, Bouattour, Camicas, Guglielmone, Horak, Jongejan, Latif, Pegram & Walker 2006). Boophilids are one-host ticks and use cattle as their primary host. Besides cattle *R.* (*Boophilus*) *decoloratus* has been recorded in heavy infestations on horses, whilst infestations on other domestic animals appear to be less important (Walker 1991). It has also been collected in large numbers from zebras, kudus, nyalas, bushbuck and impalas (Horak 1998). *R.* (*Boophilus*) *microplus* is primarily a parasite of domestic cattle and has only occasionally been collected from sheep, goats and horses, and records of collection from wild animals are rare (Walker 1991). However, fairly large numbers of domestic goats in the eastern region of the ECP are infested with *R.* (*Boophilus*) *microplus* (Nyangiwe & Horak 2007).

Rhipicephalus (*Boophilus*) *decoloratus* is indigenous to the continent, whilst *R.* (*Boophilus*) *microplus* is of Asiatic origin and was probably introduced into South Africa and East Africa on cattle imported from Madagascar, where it had originally arrived with cattle from southern Asia (Hoogstraal 1956). The two ticks co-exist in some parts of South Africa, with *R.* (*Boophilus*) *decoloratus* being by far the more widely distributed of the two (Howell *et al.* 1978). *R.* (*Boophilus*) *microplus*, however, appears to be spreading and is now established in some regions in which only *R.* (*Boophilus*) *decoloratus* was previously recorded (Spickett & Malan 1978; Tonnensen, Penzhorn, Bryson, Stoltsz & Masibigiri 2003; Nyangiwe & Horak 2007).

Rhipicephalus (*Boophilus*) *decoloratus* and *R.* (*Boophilus*) *microplus* are the only *R.* (*Boophilus*) species found on cattle in South Africa (Howell *et al*. 1978; Walker 1991), and are both economically important as they transmit the pathogenic haemoparasites causing redwater (*Babesia* spp.), gallsickness (*Anaplasma marginale*) and spirochaetosis

(*Borrelia theileri*) to cattle (Norval & Horak 2004). *Babesia bovis* is transmitted by *R.* (*Boophilus*) *microplus*, its only known vector in southern Africa (De Vos, De Waal & Jackson 2004), while *Babesia bigemina* is transmitted by both *R.* (*Boophilus*) *microplus* and *R.* (*Boophilus*) *decoloratus* (De Vos *et al*. 2004).

These ticks are also important because they were the first ixodid ticks in which genetically inherited resistance to various acaricides was recorded. Initially resistance to arsenic was reported (Baker & Shaw 1965) and subsequently to organo-chlorines (Baker, Jordaan & Robertson 1979), and then carbamates and later organophosphates (Baker, Miles, Robertson, Stanford & Taylor 1978).Because of its presumed widespread distribution *R.* (*Boophilus*) *decoloratus* was selected as the species to be used in the current study.

4.2 Materials and methods

Engorged female ticks were collected mainly from the lower perineum, dewlap and underside of the body of cattle at various dip-tanks. These ticks were examined using a stereoscopic microscope to differentiate between *R.* (*Boophilus*) *decoloratus* and *R.* (*Boophilus*) *microplus*. They were stored in carefully labelled plastic containers with perforated lids, containing soft tissue paper to limit movement, and put on ice before dispatching to the laboratory for testing. On reaching the laboratory they were counted to ensure that a sufficient number of engorged female ticks were available for the Shaw Larval test as it was the test accorded priority status, and the remaining ticks were allocated to the Adult Immersion test. Thereafter the various tests to assess acaricide resistance were conducted as discussed in the General Materials and Methods.

4.3 Results

During the identification process it became apparent that contrary to what was expected *R.* (*Boophilus*) *microplus* and not *R.* (*Boophilus*) *decoloratus* was widely distributed in the study region. Thus although *R.* (*Boophilus*) *decoloratus* was originally selected as the one-host tick species to be studied, cattle at only two dip-tanks were found to simultaneously harbour both these boophilids. At all the other dip-tanks it appeared as if *R.* (*Boophilus*) *microplus* had displaced *R.* (*Boophilus*) *decoloratus*, and thus *R.* (*Boophilus*) *microplus* became the one-host tick species of choice for the study.

The dip-tanks at which Nyangiwe collected *R. (B) decoloratus* and *R. (B.) microplus* during 2004 and 2005 for his study on the geographic distribution of ticks in the eastern region of the Eastern Cape Province have been plotted in Fugures 5 and 6. It is obvious from these maps that the introduced *R. (B.) microplus* is now far more widespread in this region than the indigenous *R. (B.) decoloratus.*

Figure 5: Dip-tanks at which *Rhipicephalus* (*Boophilus*) *decoloratus* was collected during the survey conducted on the geographic distribution of ticks in the Eastern Cape Province (adapted from Nyangiwe 2007)

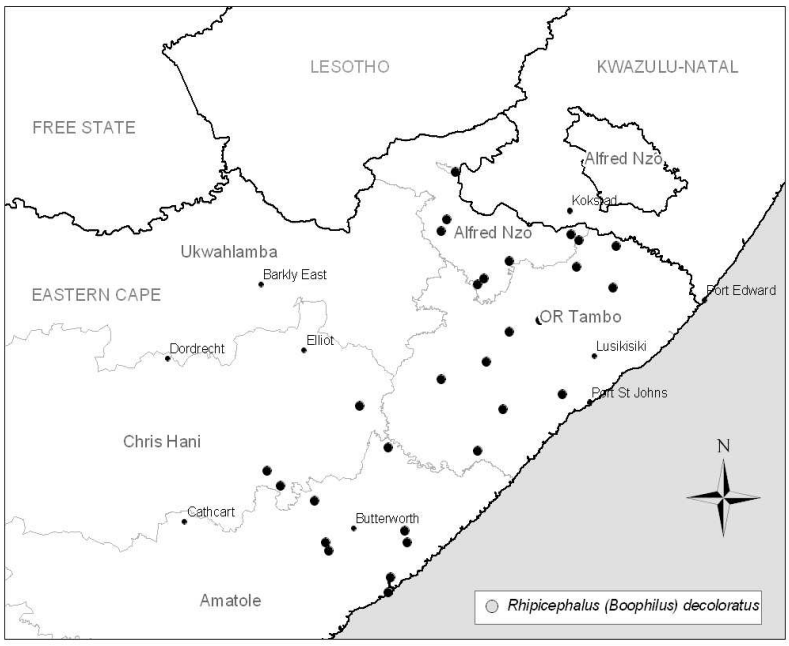
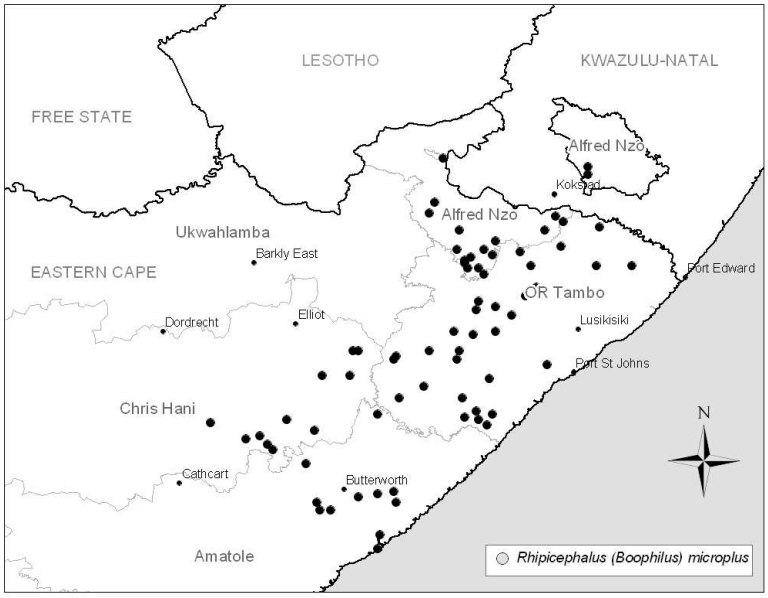


Figure 6: Dip-tanks at which *Rhipicephalus* (*Boophilus*) *microplus* was collected during the survey conducted on the geographic distribution of ticks in the Eastern Cape Province (adapted from Nyangiwe 2007)

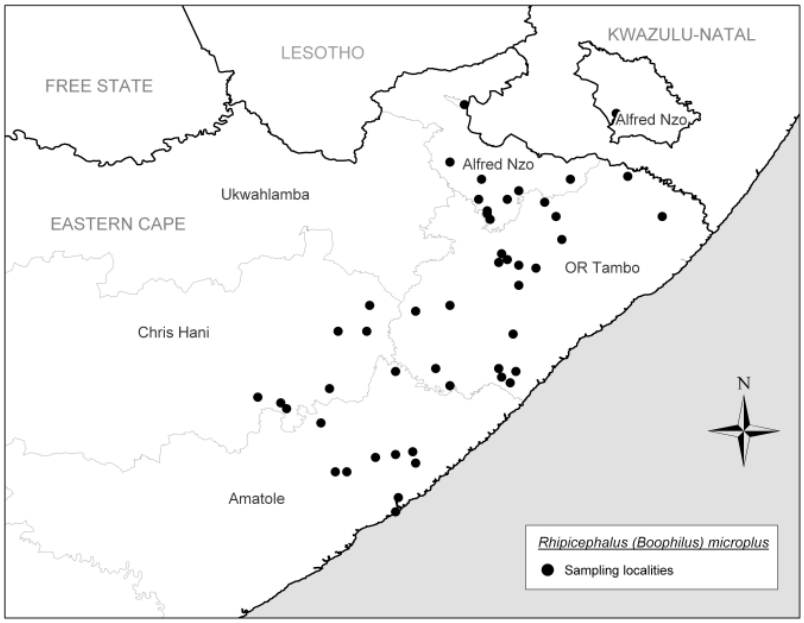


The dip-tanks at which *R. (B.) microplus* was collected for the present battery of acaricide resistance tests are plotted in Figure 7. The distribution of these dip-tanks as displayed in Figure 7 indicate that *R. (B.) microplus* was collected for acaricide resistance testing throughout the study region.

4.3.1 Shaw Larval Test

The summarized results of the SLIT on *R. (Boophilus) microplus* are presented in Table 1, and the results of the SLIT for the individual dip-tanks in Table 2. The dip-tanks at which *R. (B.) microplus* proved to be resistant to any of the test acaricides in various acaricide resistant tests conducted are presented in map format in Figures 8 and 9.

Figure 7: Diptanks in the eastern region of the Eastern Cape Province at which *Rhipicephalus (Boophilus) microplus* was collected for acaricide resistance testing during 2004 and 2005.



Of the 28 field isolates of *R.* (*Boophilus*) *microplus* collected during 2004, susceptibility of the larval offspring to amitraz was tested on 14 isolates, 12 on cypermethrin and eight isolates on chlorfenvinphos. One isolate showed emerging resistance to amitraz. Three showed emerging resistance to chlorfenvinphos, and another three, resistance to chlorfenvinphos. All isolates tested against cypermethrin showed high levels of susceptibility (Table 1).

SLITs were performed on *R. (Boophilus) microplus* collected at the Tutura dip-tank, Mnquma municipality, Amatole District during both 2004 and 2005 and the ticks proved to be susceptible on both occasions. The four localities at which *R. (Boophilus) microplus* displayed resistance or emerging resistance to amitraz and the 12 localities at which it displayed resistance or emerging resistance to chlorfenvinphos are spread throughout the survey region (Figures 8 and 9).

TABLE 1: Summary of Shaw Larval Immersion Test, adult Egg Laying Test and Reproductive Estimate Test for *Rhipicephalus* (*Boophilus*) *microplus*

Test and Acaricide	Number of tests	Number of dip-tanks at which there was:		
		Susceptibility	Emerging resistance	Resistance
Shaw Larval Immersion Test				
Amitraz	46	43	2	1
Cypermethrin	44	44	0	0
Chlorfenvinphos	37	25	9	3
Egg Laying Test				
Amitraz	40	40	0	0
Cypermethrin	38	37	1	0
Chlorfenvinphos	33	33	0	0
Reproductive Estimate Test				
Amitraz	40	39	1	0
Cypermethrin	38	38	0	0
Chlorfenvinphos	33	33	0	0

Of the 2005 collections, 40 field isolates were tested of which 32 isolates were tested against amitraz, 34 isolates against cypermethrin and 29 isolates against chlorfenvinphos. Results indicated emerging resistance to amitraz in one isolate and resistance to amitraz in another isolate and six isolates with emerging resistance to chlorfenvinphos. All the isolates tested against cypermethrin were susceptible as depicted in Table 2.

TABLE 2: The results of Shaw larval Immersion Tests conducted on *Rhipicephalus* (*Boophilus*) *microplus* at individual dip-tanks

Municipality, District and dip tank	*R. (Boophilus) microplus*		
	Amit	Cyper	Chlor
OLIVER TAMBO			
Nyandeni			
Luthubeni			
Zinkumbini	S	S	S
Ngqeleni	S	S	S
Nqakata	S	S	ER
King Sabata			
Mahibe 04	-	-	-
Mahibe 05	S	S	S
Mbozisa	-	-	-
Matanjana 04	-	-	-
Matanjana 05	S	S	S
Sitebe	S	S	-
Willo 04	-	-	-
Willo 05	S	S	S
Ntekelelo	-	-	-
Ndakana	-	-	-
Nengo	-	-	-
Qhinqolo	-	-	-
Lwandlana	-	-	-
Ngqanasiya	S	S	S
Mapuzi	-	-	-
Kwaaiman	S	S	S
Nzulwini	S	S	ER
Ingquza			
Tshali	-	-	-
Mbizana			
Tlamvukaz 04	-	-	-
Tlamvukaz 05	S	S	S
Mhlontlo			
Nohamba	S	S	S
Mdeni	ER	S	S
Mfanta	S	S	-
Bencusi	S	S	ER
Ngwemnyana	S	S	ER
Kwam	S	S	S
Nthabankulu			
Mgelekenqe	S	S	ER
Dumsi	S	S	S
Buwa	S	S	ER
Ndile	R	S	-
Mnceba	S	S	-
Mngcipongwa	S	S	S

Municipality, District and dip tank	*R. (Boophilus) microplus*		
	Amit	Cyper	Chlor
CHRIS HANI			
Engcobo			
All Saints	S	S	S
Ngxosi 04	-	-	-
Ngxosi 05	S	S	S
Upper Gqaga	S	S	S
Intsika Yethu			
Luthuli	-	-	-
Mhlahlane	-	-	-
Qutsa	S	-	-
Camama	S	-	-
AMATOLE			
Mnquma			
Dlepu	S	S	-
Kei Farm	S	S	ER
Mzantzi	S	S	R
Tutura 04	S	S	-
Tutura 05	S	S	S
Upper Ngculu	ER	S	R
Xaxashimba	S	S	-
Kabakazi	S	S	S
Nxaxho	S	S	S
Ngunduza	S	S	S
ALFRED NZO			
Umzinkulu			
Ironlotch	S	S	S
Magadla	S	S	ER
Esek	-	-	-
Glengary	S	S	R
Umzimvubu			
Kwa-Shushu	-	-	-
Elubacweni	S	S	S
Mvalweni	-	-	-
Mzinto 04	-	-	-
Mzinto 05	S	S	S
Ngwetsheni	S	S	ER
Nkweceni	S	S	S
Simkulu	S	S	S

-- = No test done

4.3.2 Adult Immersion Tests

In this test both the Egg Laying Test and the Reproductive Estimate Test were used to determine the extent of resistance. The summarized results of the ELT and the RET are listed in Table 1 above, while the individual dip-tank results of these tests obtained during 2004 and during 2005 are listed in Tables 9 and 10 in the annexures. The results from tick collections made during 2004 and 2005 indicate that with the exception of one tank at which there was emerging resistance to cypermethrin, the ticks were susceptible to all three compounds (Table 1, and Tables 9 and 10 in the Annexures).

Figure 8: Dip-tanks in the eastern region of the Eastern Cape Province at which *Rhipicephalus* (*Boophilus*) *microplus* demonstrated resistance to amitraz and cypermethrin

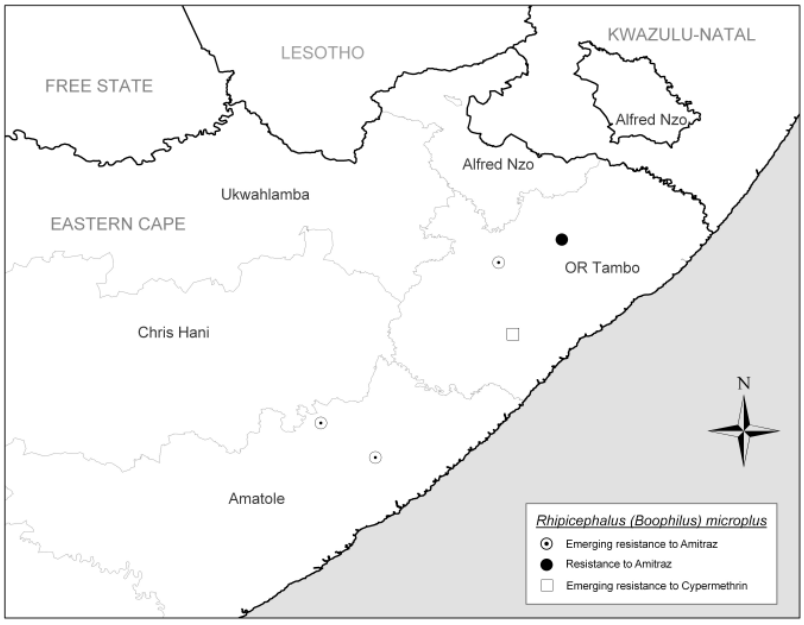
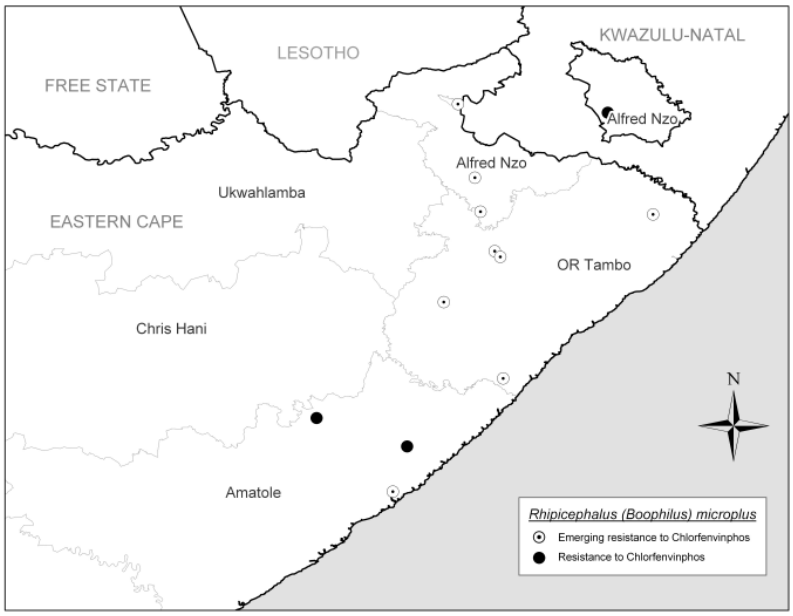


Figure 9: Dip-tanks in the eastern region of the Eastern Cape Province at which *Rhipicephalus* (*Boophilus*) *microplus* demonstrated resistance to chlorfenvinphos (Results of all tests combined)



4.4 Discussion

When comparing the work done by Mekonnen *et al*. (2002) with the present studies it is apparent that there is some similarity. Mekonnen *et al*. (2002) subjected ticks collected on three commercial farms in the East London area of the ECP and at some communal dip-tanks along the coastal areas of the ECP to acaricide resistance tests. They found emerging resistance on one farm to amitraz and resistance to chlorfenvinphos on all three farms. Strikingly they used *R*. (*Boophilus*) *decoloratus* as the one host tick of choice from all areas and make no mention of collecting *R*. (*Boophilus*) *microplus* on any of the commercial farms or at any of the dip-tanks. In the communal areas they detected no resistance at dip-tanks in East London, Idutywa and Willovale against amitraz, with emerging resistance and resistance at some dip-tanks in East London and Idutywa against cypermethrin, and emerging resistance and resistance against chlorfenvinphos picked up at some dip-tanks in East London, Idutywa and Willovale. Although the two studies used

different species of one host ticks, namely *R.* (*Boophilus*) *decoloratus* in Mekonnen *et al*'s (2002) tests and *R.* (*Boophilus*) *microplus* in the current tests, the results obtained for the Shaw Larval Tests concur. Both found very few dip-tanks at which there was resistance against amitraz, but emerging resistance against this compound was found in both studies.

Studies done by Baker *et al*. (1978, 1979) also yielded findings that were to some extent similar to the present results. *R.* (*Boophilus*) *decoloratus* was used in one of Baker's studies and considerable resistance was detected against the organophosphorus compounds. They concluded that organophosphorus resistance was significant in the region of the Transkei and probably South Africa and controlled use of compounds containing this group of chemicals was advised. In the other study, they used *R.* (*Boophilus*) *microplus* as test species and found that this tick had resistance to a range of compounds including toxaphane, lindane, dioxathion, but no resistance was shown to chlorpyrifos, bromophos ethyl, or the diamidine ixodicides including amitraz. The results of the current study thus confirm that other than emerging resistance of *R.* (*Boophilus*) *microplus* to the amidine group of compounds, there is no evidence of resistance by this tick to this group of compounds in the study area.

Nolan (1981) in his study on *R.* (*Boophilus*) *microplus* (then referred to as *Boophilus microplus*) in Australia, found that there was a strain of this tick showing resistance to the amidine group of compounds. He found that clenpyrin, which does not inhibit monoamine oxidase, was not affected by resistance, while amitraz, which is an inhibitor of this enzyme, was affected. He attributed the mechanism of resistance to be due to the mode of action of the compounds inside the ticks. He thus concluded that it appeared that the mechanism of resistance was more likely to be related to an alteration in the sensitivity of the target than to a change in the biochemistry of metabolism of the toxicant. This could possibly explain the differences in acaricide resistance picked up in the two boophilids in Mekonnen *et al*'s (2002) study and the current study. Solomon, Baker, Heyne & Van Kleef (1979) in a survey on the susceptibility of ticks, used the two

species of *R*. (*Boophilus*) as well as other tick species, and drew distribution curves based on the LC50 and LC99 values. They found that even though the two species were sampled at the same localities, a large proportion of *R*. (*Boophilus*) *decoloratus* occurred within the curve in values above field strength suggesting possible resistance, whilst the resistance spectrum of *R*. (*Boophilus*) *microplus* was not found outside the range of susceptibility portions of the population, suggesting that resistance was possibly confined to *R*. (*Boophilus*) *decoloratus*.

In 1982 resistance to organophosphorus-based acaricides was recorded in *R*. (*Boophilus*) *microplus* in at least 11 localities within the current survey area (Baker 1982). In the present study this tick was resistant to or displayed emerging resistance to chlorfenvinphos at 10 of the 36 localities at which it had been collected (Table 2). However, this chemical or other organophosphate-based acaricides, had probably not been used regularly or at all in the region during the previous 8 years. A similar finding was made by Mekonnen *et al*. (2003) on two commercial farms in the Eastern Cape Province, on which chlorfenvinphos had not been used for the past 10 years. However, when the *R*. (*Boophilus*) *decoloratus* on these two farms were tested for acaricide resistance they were resistant or displayed emerging resistance to chlorfenvinphos (Mekonnen *et al*. 2003). It would thus appear that once resistance to an organophosphate-based acaricide has become established in a population of ticks, the return to susceptibility is either very slow or does not take place (Stone 1972; Baker 1982).

Even when considerable ‘dilution’ of the original resistance has occurred because of the introduction of susceptible ticks into a region or a farm in or on which resistance was recorded accompanied by several years of non-use of a particular acaricide, it would seem that reselection for resistance is rapid once the compound is again applied (Baker 1982). The practice of communal farming prevalent in the study region, and the free movement of livestock that goes with it, would almost certainly ensure that there would be subsequent introductions of susceptible ticks into regions in which resistance had previously been detected.

The Shaw Larval Test, used in the present study, gives an indication of emerging resistance and thus can be a useful tool in tick control. There were dip-tanks at which the ticks showed either emerging resistance or resistance to amitraz and chlorfenvinphos, and therefore caution needs to be exercised at the affected dip-tanks with the use of a compound or closely related compounds against which ticks show resistance or emerging resistance.

The striking finding that *R.* (*Boophilus*) *microplus* is replacing *R.* (*Boophilus*) *decoloratus* in the eastern region of the Eastern Cape Province could be due to a number of reasons and may have significant consequences for the occurrence of redwater in this area. The distributions of tick species are not static and change in response to a range of factors, which include the movement of hosts, changes in local tick control procedures and selection for resistance to acaricides, as well as variations in seasonal rainfall (Tonnesen *et al*. 2004). The communal nature of the study area and lack of control over livestock movement because of the absence of proper fences also contribute to tick species dispersal (Baker 1982).

This increase in the geographic distribution of *R.* (*Boophilus*) *microplus* is not only of academic interest, but also has serious economic implications in the spread of diseases, particularly Asiatic redwater caused by *Babesia bovis* as it is its only vector. When a comparison is made between these two boophilids it is apparent that the total life cycle of *R.* (*Boophilus*) *microplus* is slightly shorter than that of *R.* (*Boophilus*) *decoloratus* in the laboratory (Arthur & Londt 1973; Londt & Arthur 1975), and that female *R.* (*Boophilus*) *microplus* produce slightly more eggs than do females of *R.* (*Boophilus*) *decoloratus* (Spickett & Malan 1978). Its spread may thus be enhanced by its slightly shorter life cycle and slightly higher egg production. Furthermore, cross-mating between the two species results in sterile eggs being produced (Spickett & Malan 1978).

The males of *R.* (*Boophilus*) *microplus* are sexually mature a few days earlier than those of *R.* (*Boophilus*) *decoloratus* (Londt & Arthur 1975), and in mixed infestations they

would thus have a greater chance of mating with females of their own species and also with those of *R.* (*Boophilus*) *decoloratus*. The cross-mated females would produce sterile eggs and *R.* (*Boophilus*) *microplus* would consequently make up an increasing percentage of future mixed populations. These may be some of the reasons that give this tick an advantage over *R.* (*Boophilus*) *decoloratus* and thus result in the increase in its geographic distribution.

5. TWO HOST TICK SPECIES - *Rhiphicephalus evertsi evertsi*

5.1 Introduction

The three major two-host tick species that infest cattle are the red-legged tick, *Rhipicephalus evertsi evertsi* and the bont-legged ticks, *Hyalomma rufipes* and *Hyalomma truncatum*. For the purpose of the present study *R. evertsi evertsi* was chosen as the most suitable of the two-host ticks, not only because of its widespread distribution within the study region (Fig. 10), but because its immature stages and its adults use cattle, sheep and goats as hosts. The adults of *H. rufipes* infest cattle, but its immature stages infest hares and birds (Walker 1991), while *H. truncatum* does not occur in the study region (Howell *et al.* 1978). Moreover, *R. evertsi evertsi* is believed to be the most widespread rhipicephalid in the Afrotropical region, and is present in the eastern half of Africa from South Africa in the south to eastern Sudan in the north (Walker, Keirans & Horak 2000). This species was named after Dr J.G. Everts, who collected the type specimens in the province then called the Transvaal in South Africa (Walker *et al.* 2000). The adults are medium-sized dark brown ticks with reddish-orange legs, from which their common name ‘red-legged tick’ is derived.

Rhipicephalus evertsi evertsi has a very wide host spectrum, with domestic horses and zebras probably its preferred hosts (Norval 1981), while domestic cattle, sheep and goats are also good hosts (Walker *et al.* 2000). Nyangiwe & Horak (2007) collected approximately as many adult *R. evertsi evertsi* from goats as from cattle in the present ECP survey region. Although *R. evertsi evertsi* is widely distributed and parasitizes a

variety of hosts, adult ticks are seldom abundant. Adults and immature ticks are present on host animals throughout the year, and the immatures are mainly found in the external ear canals and the adults in the peri-anal region under the tail (Baker & Ducasse 1967; Walker *et al*. 2000).

Rhipicephalus evertsi evertsi is a possible vector of *Babesia bigemina* to bovines and is a vector of *Babesia caballi* and *Theileria equi*, the cause of piroplasmosis in horses (Norval & Horak 2004). Engorging female ticks secrete a toxin, which can induce paralysis in lambs and calves (Norval & Horak 2004). This tick may also play a role in the transmission of the virus causing Crimean-Congo haemorrhagic fever in humans and of *Rickettsia conori*, the cause of tick-bite fever in humans (Walker *et al*. 2000).

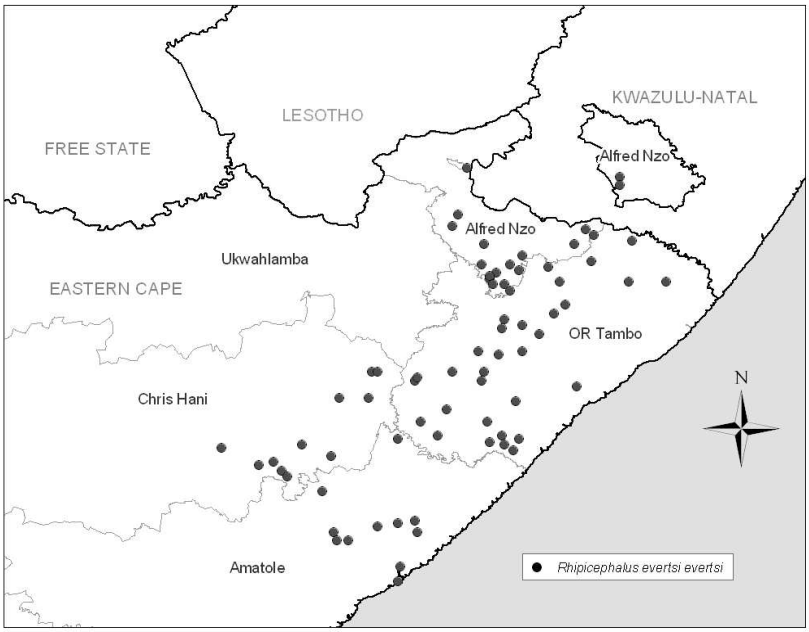
5.2 Materials and methods

Engorged female ticks were collected mainly from under the tail of cattle at various dip-tanks. These were identified using a stereoscopic microscope. They were stored in securely-labelled plastic containers with perforated lids, lined with soft tissue to limit movement, and put on ice before dispatching to the laboratory for testing. On reaching the laboratory they were counted to ensure that sufficient numbers of engorged female ticks were available for the Shaw Larval test as it was given priority amongst the tests, and the remaining ticks were used for the Adult Immersion test. The tests were carried out using the selected three chemical compounds as described in the General Materials and Methods.

5.3 Results

The dip-tanks at which Nyangiwe collected *R. evertsi evertsi* during 2004 and 2005 for his study on the geographic distribution of ticks in the eastern region of the Eastern Cape Province have been plotted in Figure 10. This map indicates that *R. evertsi evertsi* is widespread in this region.

Figure 10: Dip-tanks at which *Rhipicephalus evertsi evertsi* was collected during the survey conducted on the geographic distribution of ticks in the eastern region of the Eastern Cape Province (adapted from Nyangiwe 2007)



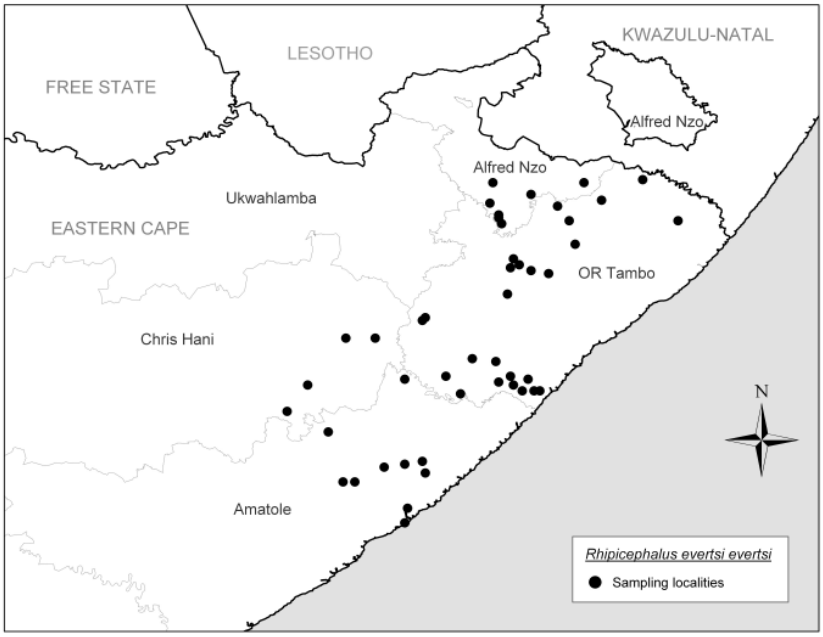
The dip-tanks at which *R. evertsi evertsi* was collected for the present battery of acaricide resistance tests are plotted in Figure 11. The distribution of these dip-tanks as displayed in Figure 11, indicate that *R. evertsi evertsi* was collected for acaricide resistance testing throughout the study locality.

5.3.1 The Shaw Larval Test

The summarized results of the SLITs on *R. evertsi evertsi* are presented in Table 3, and the results of the SLITs for the individual dip-tanks in Table 4. The dip-tanks at which *R. evertsi evertsi* proved to be resistant to any of the test acaricides in the various types of acaricide resistant tests conducted are presented in map format in Figure 12. The 11 localities at which *R. evertsi evertsi* displayed resistance or emerging resistance to chlorfenvinphos are spread throughout the survey region (Figure 12).

Of the collections made in 2004, 19 field isolates of *R. evertsi evertsi* were tested against amitraz, and all were susceptible to the test compound. Only 12 isolates were tested against cypermethrin and they were all susceptible, while of the four isolates tested against chlorfenvinphos, one showed resistance (Table 11 in Annexures).

Figure 11: Dip-tanks in the eastern region of the Eastern Cape Province at which *Rhipicephalus evertsi evertsi* was collected for acaricide resistance tesing during 2004 and 2005



For the 2005 collections, 32 isolates were tested against amitraz and cypermethrin and all showed susceptibility, and 31 isolates were tested against chlorfenvinphos, of which seven showed emerging resistance and three showed showed resistance to the compound (Table 12 in Annexures). A total of seven isolates of *R. evertsi evertsi* were thus developing resistance to chlorfenvinphos and four were already resistant (Tables 3 and 4).

Ticks collected at the Mahibe dip-tank, King Sabata Dalindyebo municipality, O.R. Tambo District during 2004 and 2005 proved to be susceptible on both occasions, while those collected at Matanjana dip-tank in the same district displayed resistance to

chlorfenvinphos in 2004, but were susceptible to this compound in 2005. Ticks collected at Ngxoki dip-tank, Engcobo municipality, Chris Hani District during 2004 were susceptible to all three acaricides in 2004, but displayed resistance to chlorfenvinphos during 2005.

TABLE 3: Summary of Shaw Larval Immersion Test, adult Egg Laying Test and Reproductive Estimate Test for *Rhipicephalus evertsi evertsi*

Test and Acaricide	**Number of tests**	**Number of dip-tanks at which there was:**		
		Susceptibility	**Emerging resistance**	**Resistance**
Shaw Larval Immersion Test				
Amitraz	51	51	0	0
Cypermethrin	44	44	0	0
Chlorfenvinphos	35	24	7	4
Egg Laying Test				
Amitraz	2	2	0	0
Cypermethrin	1	1	0	0
Chlorfenvinphos	0	0	0	0
Reproductive Estimate Test				
Amitraz	2	2	0	0
Cypermethrin	1	1	0	0
Chlorfenvinphos	0	0	0	0

TABLE 4: The results of Shaw larval Immersion Tests conducted on *Rhipicephalus evertsi evertsi* at individual dip-tanks

Municipality, District and dip tank	*R. evertsi evertsi*		
	Amit	Cyper	Chlor
OLIVER TAMBO			
Nyandeni			
Luthubeni	S	S	-
Zinkumbini	-	-	-
Ngqeleni	-	-	-
Nqakata	-	-	-
King Sabata			
Mahibe 04	S	S	-
Mahibe 05	S	S	S
Mbozisa	S	S	S
Matanjana 04	S	S	R
Matanjana 05	S	S	S
Sitebe	S	S	R
Willo 04	S	S	-
Willo 05	-	-	-
Ntekelelo	-	-	-
Ndakana	S	S	S
Nengo	S	S	S
Qhinqolo	S	S	S
Lwandlana	S	S	S
Ngoanasiya	S	S	S
Mapuzi	S	S	S
Kwaaiman	S	S	ER
Nzulwini	S	S	ER
Inguza			
Tshali	S	S	-
Mbizana			
Tlamvukaz 04	S	-	-
Tlamvukaz 05	S	S	S
Mhlontlo			
Nohamba	S	S	S
Mdeni	S	S	ER
Mfanta	S	S	S
Bencusi	S	S	-
Ngwemnyana	S	S	S
Kwam	S	S	S
Nthabankulu			
Mgelekenqe	S	S	S
Dumsi	S	S	S
Buwa	S	S	S
Ndile	S	S	ER
Mnceba	S	S	S
Mngciponow	S	S	ER

-- = No test done

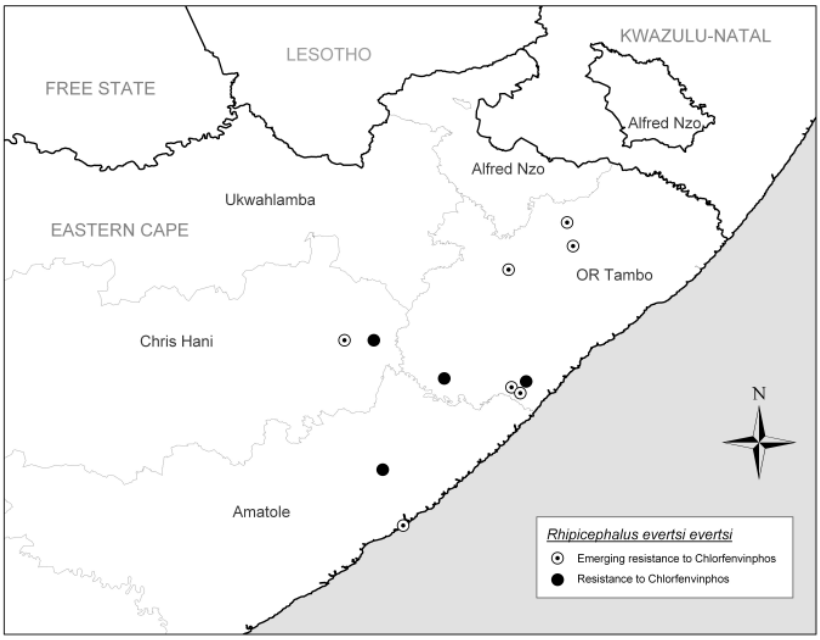
Municipality, District and dip tank	*R. evertsi evertsi*		
	Amit	Cyper	Chlor
CHRIS HANI			
Encobo			
All Saints	S	S	ER
Ngxosi 04	S	S	S
Ngxosi 05	S	S	R
Upper Gqaga	-	-	-
Intsika Yethu			
Luthuli	-	-	-
Mhlahlane	S	S	-
Qutsa	S	S	S
Camama			
AMATOLE			
Mnquma			
Dlepu	S	S	-
Kei Farm	S	-	-
Mzantzi	S	S	-
Tutura 04	-	-	-
Tutura 05	S	S	R
Upper Ngculu	S	-	-
Xaxashimba	S	S	-
Kabakazi	S	S	S
Nxaxho	S	S	ER
Ngunduza	S	S	S
ALFRED NZO			
Umzinkulu			
Ironlotch	-	-	-
Magadla	-	-	-
Esek	S	-	-
Glengary	-	-	-
Umzimvubu			
Kwa-Shushu	S	-	-
Elubacweni	-	-	-
Mvalweni	-	-	-
Mzinto 04	S	-	-
Mzinto 05	-	-	-
Ngwetsheni	S	-	-
Nkweceni	S	S	S
Simkulu	-	-	-

5.3.2 Adult Immersion Test

Enough ticks were only collected to be able to conduct two Egg laying Tests and two Reproductive Estimate Tests with amitraz, one of these tests with cypermethrin and none with chlorfenvinphos. The results indicate susceptibility to the two compounds tested (Tables 3, and Tables 13 and 14 in Annexures).

The dip-tanks at which *R. evertsi evertsi* demonstrated resistance to chlorfenvinphos have been mapped in Figure 12.

Figure 12: Dip-tanks in the eastern region of the Eastern Cape Province at which *Rhipicephalus evertsi evertsi* demonstrated resistance to chlorfenvinphos. (All tests combined)



5.4 Discussion

Even though *R. evertsi evertsi* adult ticks were not readily available in large numbers, prioritization of the Shaw Larval test and the use of larvae in this test ensured that results were procurable. Earlier resistance studies conducted on this tick showed that although it may be resistant to certain compounds, this resistance is not as pronounced as that of the one-host ticks (Matthewson & Baker 1975). In the present study this tick showed tolerance only to chlorfenvinphos. Even in dip-tanks that were sampled in both 2004 and 2005, tolerance showed by this tick was only to chlorfenvinphos.

The observation that there is some resistance to chlorfenvinphos may be due to the fact that organophosphates had previously been used extensively as acaricides in the study area for prolonged periods of time. As mentioned in the section devoted to the History of acaricide usage in the ECP, these compounds had been used as early as in the 1970's, and included a wide variety of these chemicals, amongst which were dioxathion, diazinon and chlorfenvinphos, and cross-resistance to the various compounds within this group occurred. As mentioned earlier for *R.* (*Boophilus*) species, it would seem that once resistance to the organophosphorus group of compounds becomes established in ticks this resistance may persist for several years, even in the absence of further use of these compounds.

Amongst some of the earliest studies on acaricide resistance in South Africa, Whitehead & Baker (1961) looked at resistance in *R. evertsi evertsi* sampled from a farm in the East London area of the ECP. The ticks there were resistant to toxaphene and BHC, but less so to DDT, sodium arsenite, dioxathion and carbamate. Mekonnen *et al.* (2002), in their study conducted more recently on farms in the East London area, found one case of a resistant strain of *R. evertsi evertsi* to chlorfenvinphos. This concurs with the results from the present study.

It is interesting that acaricide resistance in the two-host tick *R. evertsi evertsi* often only occurs after acaricide resistance in the one-host *R.* (*Boophilus*) spp. ticks is already

present on the same property. The reason for this probably has a lot to do with the life cycles of the ticks. Each parasitic life cycle of the one-host *R.* (*Boophilus*) spp. ticks takes approximately 21 days to complete on the host animal (Arthur & Londt 1973), while, depending on temperature, the off-host portions of the life cycle, namely ovipostion and larval hatching may require weeks or months to complete. Thus under favourable climatic conditions three or more life cycles may be completed annually and under less favourable conditions perhaps only one or two. This implies that if three life cycles are completed annually a tick and its offspring will spend a total of 63 days on a host in a single year. If this host is dipped in an acaricide at weekly intervals the tick and its offspring may theoretically be exposed to the same acaricide nine times in a single year, leading to severe selection pressure towards resistance.

The immature stages of the two-host tick *R. evertsi evertsi* take approximately 16 days to complete their life cycle on the first host (Rechav, Knight & Norval 1977), before detaching and moulting to adults, which will then later attach to the second host for approximately one week. This tick can also complete more than one life a year (Norval 1981), thus if it completes two life cycles in a year it and its offspring may also theoretically be exposed to the same acaricide nine times in a single year.

However, whereas within a farming environment *R.* (*Boophilus*) spp. are virtually exclusive parasites of cattle and only occasionally other domestic hosts (Nyangiwe & Horak 2007), both the adult and immature stages of *R. evertsi everstsi* parasitise cattle as well as other domestic animals (Walker *et al*. 2000; Nyangiwe & Horak 2007). It is not customary in South Africa to apply acaricides to sheep, goats or horses on a regular basis. Thus a particular strain of *R. evertsi evertsi* and its offspring will only be regularly exposed to acarides if it exclusively infests cattle, whereas if part of its life cycle is spent on other hosts it will not be under such intense selection pressure towards resistance.

6. THREE HOST TICK SPECIES- *Rhiphicephalus appendiculatus*

6.1 Introduction

Rhiphicephalus appendiculatus, commonly known as the brown ear tick because of its reddish-brown colour and its favourite site of attachment on the cattle ear pinna, is a three-host species. This tick is widely distributed throughout the eastern part of the African continent reaching its northern distribution limit in southern Sudan, Central African Republic and Democratic republic of Congo (Walker *et al*. 2000). In South Africa its distribution is confined mainly to the eastern parts of the country and it is particularly abundant in the coastal areas of the Eastern Cape Province (Baker & Shaw 1965; Rechav 1982; Fig. 13). This tick has an extremely wide host range amongst domestic and wild ruminants, of which cattle are undoubtedly one of the preferred hosts, and all stages of development of the tick readily feed on cattle (Howell *et al*. 1978; Walker *et al*. 2000). Other domestic animals that may be infested are sheep and particularly goats (Baker & Ducasse 1968; Nyangiwe & Horak 2007). The preferred wild hosts of all stages of development are African buffaloes and eland (Horak, Golezardy & Uys 2007), as well as various species of antelopes including waterbuck (Walker *et al*. 2000). Surprisingly many collections have been taken from domestic dogs as well as various species of wild carnivores (Horak, Braack, Fourie & Walker 2000; Horak, Emslie & Spicket 2001).

The importance of *R. appendiculatus* as a parasite rests on three aspects of its parasitism. Firstly it is the principal vector of *Theileria parva*, the causal organism of East Coast Fever in cattle and Corridor disease in buffaloes (Lawrence, Perry & Williamson 2004a, b). Secondly, if sufficiently large numbers of adult ticks attach they result in an immuno-suppressing toxicosis in cattle known as Tzaneen disease (Thomas & Neitz 1958). Thirdly the favoured attachment site of the adults on the ears of cattle can lead to invasion by the larvae of *Chrysomyia bezziana*, the Old worm screwworm fly, resulting in crumpling of the ear (Howell *et al*. 1978). It is also a carrier of *Rickettsia conori* causing tick-bite fever in humans (Walker *et al*. 2000).

6.2 Materials and methods

Engorged female ticks were collected mainly from the ears and around the ears of cattle at the selected dip-tanks. These ticks were identified using a stereoscopic microscope. They were stored in well-labelled plastic containers with perforated lids, containing soft tissue paper to limit movement, and put on ice before dispatching to the laboratory for testing. On reaching the laboratory they were counted to ensure that sufficient numbers of engorged female ticks were available for the Shaw Larval test as it was identified as the priority test, and the remaining ticks were used for the Adult Immersion test. The tests were carried out using the three selected chemical compounds as described in the General Materials and Methods.

6.3 Results

The dip-tanks at which Nyangiwe collected *R. appendiculatus* during 2004 and 2005 for his study on the geographic distribution of ticks in the eastern region of the Eastern Cape Province have been plotted in Figure 13. The map displayed in Figure 13 indicates that *R. appendiculatus* is widespread in the survey region.

6.3.1 Shaw Larval test

The summarized results of the SLITs on *R. appendiculatus* are presented in Table 5, and the results of the SLITs for the individual dip-tanks in Table 6. The dip-tanks at which *R. appendiculatus* proved to be resistant to any of the test acaricides in the various acaricide resistant tests conducted are presented in map format in Figure 15.

Of the collections made in 2004, 16 field isolates of *R. appendiculatus* were tested against amitraz, and all were susceptible to the test compound. Nine isolates were tested against cypermethrin and two against chlorfenvinphos and they were all susceptible (Table 15 in Annexures).

Figure 13: Dip-tanks at which *Rhipicephalus appendiculatus* was collected during the survey conducted on the geographic distribution of ticks in the Eastern Cape Province (adapted from Nyangiwe 2007)

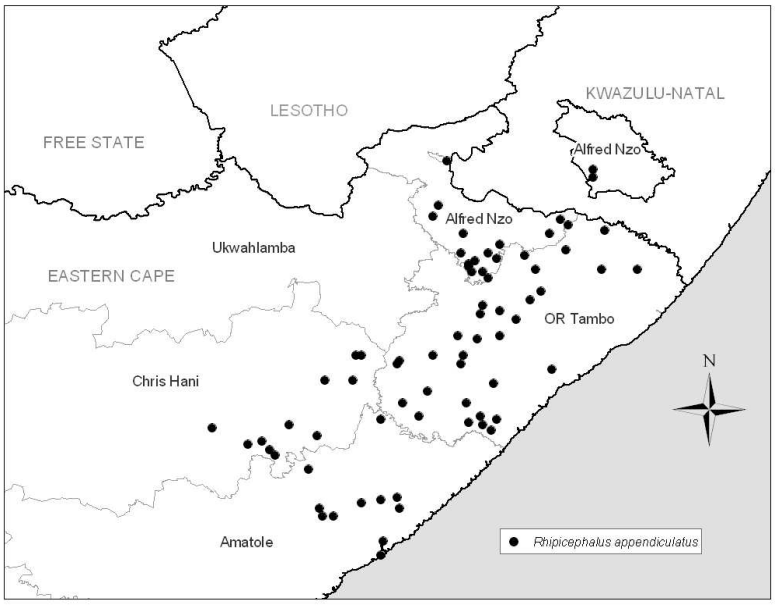
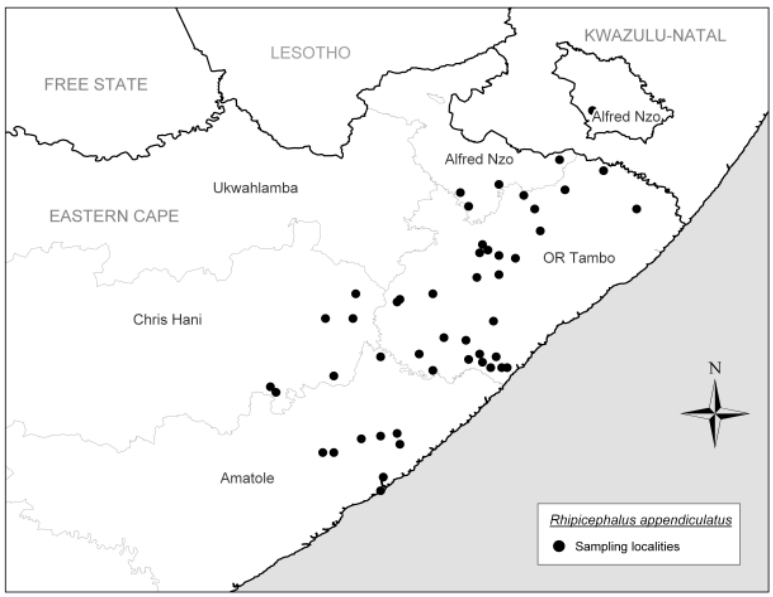


Figure14: Dip-tanks in the eastern region of the Eastern Cape Province at which *Rhipicephalus appendiculatus* was collected for acaricide resistance testing during 2004 and 2005



Of the 2005 collections, 34 isolates were tested against amitraz and 32 isolates against cypermethrin and 30 isolates were tested against chlorfenvinphos. One of the isolates displayed emerging resistance to cypermethrin, while all the others were susceptible to this chemical or to amitraz or chlorfenvinphos (Table 16 in Annexures). Thus of all the *R. appendiculatus* isolates subjected to the SLIT in 2004 and 2005 only one isolate displayed emerging resistance, and that to cypermethrin (Tables 5 and 6).

TABLE 5: Summary of Shaw Larval Immersion Test, adult Egg Laying Test and Reproductive Estimate Test for *Rhipicephalus appendiculatus*

Test and Acaricide	**Number of tests**	**Number of dip-tanks at which there was:**		
		Susceptibility	**Emerging resistance**	**Resistance**
Shaw Larval Immersion Test				
Amitraz	50	50	0	0
Cypermethrin	41	40	1	0
Chlorfenvinphos	32	32	0	0
Egg Laying Test				
Amitraz	31	31	0	0
Cypermethrin	26	25	1	0
Chlorfenvinphos	24	24	0	0
Reproductive Estimate Test				
Amitraz	31	31	0	0
Cypermethrin	26	25	1	0
Chlorfenvinphos	24	24	0	0

TABLE 6: The results of Shaw larval Immersion Tests conducted on *Rhipicephalus appendiculatus* at individual dip-tanks

Municipality, District and dip tank	*R. appendiculatus*		
	Amit	Cyper	Chlor
OLIVER TAMBO			
Nyandeni			
Luthubeni	S	S	S
Zinkumbini	S	S	S
Ngqeleni	S	S	S
Nqakata	S	S	S
King Sabata			
Mahibe 04	S	S	-
Mahibe 05	S	S	S
Mbozisa	S	S	S
Matanjana 04	S	-	-
Matanjana 05	S	S	S
Sitebe	S	S	S
Willo 04	S	S	-
Willo 05	S	S	S
Ntekelelo	S	S	S
Ndakana	S	S	S
Nengo	S	S	S
Qhinqolo	S	S	S
Lwandlana	-	-	-
Ngoanasiya	S	ER	S
Mapuzi	S	S	S
Kwaaiman	S	S	S
Nzulwini	S	S	S
Inguza			
Tshali	S	-	-
Mbizana			
Tlamvukaz 04	-	-	-
Tlamvukaz 05	-	-	-
Mhlontlo			
Nohamba	S	S	S
Mdeni	S	S	S
Mfanta	S	-	-
Bencusi	S	S	S
Ngwemnyana	S	S	S
Kwam	S	S	-
Nthabankulu			
Mgelekenqe	S	S	S
Dumsi	S	S	S
Buwa	S	-	S
Ndile	S	S	-
Mnceba	S	-	-
Mngciponow	S	S	S

-- = No test done

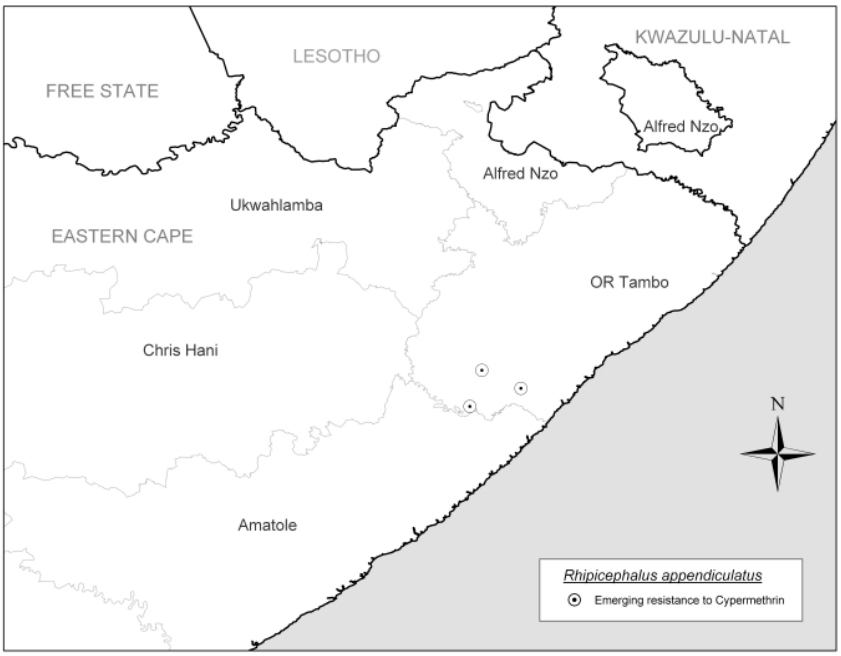
Municipality, District and dip tank	*R. appendiculatus*		
	Amit	Cyper	Chlor
CHRIS HANI			
Encobo			
All Saints	S	S	S
Ngxosi 04	S	-	-
Ngxosi 05	S	S	S
Upper Gqaga	S	-	-
Intsika Yethu			
Luthuli	S	S	S
Mhlahlane	-	-	-
Qutsa	S	S	S
Camama	-	-	-
AMATOLE			
Mnquma			
Dlepu	S	S	-
Kei Farm	S	S	-
Mzantzi	S	-	-
Tutura 04	-	-	-
Tutura 05	S	S	S
Upper Ngculu	-	-	-
Xaxashimba	S	S	-
Kabakazi	S	S	S
Nxaxho	S	S	S
Ngunduza	S	S	-
ALFRED NZO			
Umzinkulu			
Ironlotch	-	-	-
Magadla	-	-	-
Esek	-	-	-
Glengary	S	S	-
Umzimvubu			
Kwa-Shushu	-	-	-
Elubacweni	-	-	-
Mvalweni	S	-	-
Mzinto 04	S	S	-
Mzinto 05	-	-	-
Ngwetsheni	-	-	-
Nkweceni	-	-	-
Simkulu	-	-	-

Ticks were collected at the Mahibe, Matanjana and Willo dip-tanks, King Sabata district, O.R. Tambo Municipality during 2004 and 2005 and displayed susceptibility to all three acaricides on both occasions.

6.3.2 Adult Immersion test

In the Egg Laying Test and Reproductive Estimate Test all tick isolates were susceptible to amitraz and to chlorfenvinphos, but one isolate of *R. appendiculatus* displayed emerging resistance to cypermethrin in the ELT and another isolate displayed emerging resistance to this chemical in the RET (Table 5, and Tables 17 and 18 in the Annexures). The three localities at which *R. appendiculatus* displayed emerging resistance to cypermetrrin in the three types of acaricide resistance tests conducted, have been plotted in Figure 15. The three dip-tanks at which resistance or emerging resistance was detected were fairly close together in the south-eastern region of the OR Tambo District.

Figure 15: Dip-tanks in the eastern region of the Eastern Cape Province at which *Rhipicephalus appendiculatus* displayed emerging resistance to cypermethrin. (All tests combined)



Large numbers of adult engorged female *R. appendiculatus* were collected because of this tick's abundance in the survey region (Nyangiwe & Horak 2007; Fig. 10). Sufficient ticks for this test were collected at 27 dip-tanks. Ticks at only one of these 27 dip-tanks showed emerging resistance, and only to cypermethrin (Tables 11 and 12).

6.4 Discussion

It is clear from the results that, with a few exceptions, *R. appendiculatus* is still susceptible to all three test compounds at all the dip-tank localities at which it was tested. Although acaricide resistance has been reported in *R. appendiculatus* (Baker & Shaw 1965), this tick is theoretically less likely to be selected for acaricide resistance than the one-host *R.* (*Boophilus*) spp., or the two-host *R. evertsi evertsi*.

There are a number of possible reasons for this. Firstly, the majority of adult ticks attach to the ear pinnae and insufficient acaricide may be deposited on the ears either during plunge dipping or in the spray-race as cattle are inclined to hold their heads up during these procedures and thus tend to keep their ears from being properly wetted. Secondly, this is a three-host tick, and although all stages of development prefer to feed on ruminants they may not necessarily feed on cattle. Thus when cattle are dipped there may be several ticks on goats which are not dipped at the same locality (Nyangiwe & Horak 2007), and that are thus not exposed to acaricide. Thirdly, all stages feed very rapidly taking only 4 to 7 days to engorge (Branagan 1973, 1974), thus if an acaricide without a residual effect is applied some ticks may complete their engorgement and detach in the intervening intervals between acaricide applications. Fourthly, in South Africa and other southern African countries, *R. appendiculatus* completes only one life cycle a year (Norval, Walker & Colborne 1982). The adults are present in the late summer, the larvae in autumn and winter and the nymphs in winter and spring (Rechav 1982).

Thus theoretically if each developmental stage spends 6 days on a cattle host this will add up to a total of only 18 days in a year. Thus the possibility of a tick and its offspring being exposed to a particular acaricide might occur only twice in a year if animals are

treated at weekly intervals. With this reduced exposure to acaricides the possibility of *R. appendiculatus* in South Africa being selected for acaricide resistance is thus theoretically less likely than that for the two-host tick *R. evertsi evertsi* or the one-host *R.* (*Boophilus*) spp. ticks.

Further north in East Africa, closer to the Equator, *R. appendiculatus* can complete more than one life cycle in a year, and all stages of development may be on the same host at the same time (Kaiser, Sutherst, Bourne, Gorissen & Floyd 1988). Under these circumstances selection for acaricide resistance is much more likely to occur.

7. GENERAL DISCUSSION

In this study the SLIT was used to test the resistance of larvae to the various acaricides and the ELT and RET to test the resistance of adult ticks to these acaricides. In other countries the tests used on adult ticks are slightly different to the RET used in this study. Drummond, Gladney, Whetstone and Ernst (1971) and Drummond, Ernst, Trevino, Gladney & Graham (1973) describe estimated reproduction tests (ERT), which differ only slightly in the test parameters used for the RET described here.

At one of the dip-tanks, namely the Upper Ngculu tank in the Amatole District, *R.* (*Boophilus*) *microplus* displayed emerging resistance to amitraz and resistance to chlorfenvinphos in the SLIT, and hence a change to a synthetic pyrethroid would be advisable. In the SLITs performed on the ticks collected at the Mdeni dip-tank in the O. R. Tambo District, *R.* (*Boophilus*) *microplus* displayed emerging resistance to amitraz and *R. evertsi evertsi* to chlorfenvinphos. At the Ndile dip-tank in the same district municipality, *R.* (*Boophilus*) *microplus* displayed resistance to amitraz and *R. evertsi evertsi* emerging resistance to chlorfenvinphos in the SLIT. While at the Nzulwini dip-tank in the same municipality both *R.* (*Boophilus*) *microplus* and *R. evertsi evertsi* displayed emerging resistance to chlorfenvinphos in the SLIT. In the Amatole District

emerging resistance to amitraz was detected in *R.* (*Boophilus*) *microplus* and resistance to chlorfenvinphos in *R. evertsi evertsi* in the SLITs conducted at the Tutura dip-tank.

Although the results of the three tests used in the present study indicate that there is resistance or emerging resistance to the three acaricides used, these results cannot be directly extrapolated to field conditions. Under field conditions cattle may be infested with all stages of development of various tick species. When cattle are dipped the acaricide is allowed to dry on them, this differs substantially from the fixed 10 minute exposure to acaricide in the laboratory tests. Nevertheless, the tests do indicate that resistance is present.

Success stemming from the use of the products depends on the susceptibility of the tick to the acaricide used as well as its availability. The sale of acaricides accounts for a major portion of the veterinary market in many parts of the world. In South Africa total sales in the veterinary market were reported to be R 872 million for 2003 by Peter *et al.* (2005). Of this amount R 175 million (22%) were spent on products for the control of ectoparasites. In the ECP about R 13million is spent on acquisition of dipping compounds on tender for use only in the communal farming areas (Mrwebi *pers. comm.*). To ensure that this money is put to good use and is providing an effective service, constant surveillance for acaricide resistance may need to be instituted to ensure that decisions to change from one compound to another are well-informed.

Provided an inexpensive, reliable and speedy service for acaricide resistance testing was available, 6-monthly or annual resistance testing of the one-host ticks, either *R.* (*Boophilus*) *decoloratus* or *R.* (*Boophilus*) *microplus*, depending on which species is most prevalent at a particular locality would be ideal. It has been shown in several studies conducted in Africa that one host tick species develop resistance first, (Whitehead & Baker 1961; Baker & Shaw 1965; Baker *et al.* 1978). Therefore the use of *Rhiphicephalus* (*Boophilus*) species as target ticks for resistance testing would be the most appropriate. Testing of the two-host tick *R. evertsi evertsi* would have little practical importance because, with the exception of piroplasmosis of horses, this tick is not a major

vector of disease to cattle and whether it was resistant to acaricides or not would not be of much consequence. The comparative rarity with which resistance in three-host ticks is recorded also makes it less essential to test these ticks for acaricide resistance.

If such a test was available, it is suggested that as soon as emerging resistance to a particular compound is detected in the laboratory, the dipping compound used at the affected farm or locality should be replaced by one from a completely different group of acaricides. The latter compound should then be used continuously until emerging resistance to it appears before changing to a new compound. Should other external parasites such as flies or their larvae become a problem during the sustained use of any dipping compound, one or two targeted treatments with an acaricide with insecticidal properties could be introduced into the program.

In answer to the questionnaire survey conducted amongst stock owners at the various dip-tanks at which ticks were collected, the owners perceived disease protection as the main advantage to be gained from dipping.

Perceptions of cattle owners as to the advantages of dipping

Protection of		**Disease**	**Animals**	**Prevent trouble**
Teats	**Hides**	**protection**	**look clean**	**with Government**
0	5.8	98.5	24.6	21.7

This advantage could probably be more sustainably and especially cost-effectively attained under a system of endemic stability given the breeds of cattle involved, which tend to be tick-resistant, and the extensive range conditions involved, than under the current system of regular dipping. Endemic stability is defined as the state where the relationship between host, agent, vector and environment is such that clinical disease occurs rarely or not at all (Perry 1996, cited by De Vos, De Waal and Jackson 2004). It occurs when there is frequent transmission of the parasite and infection of all animals takes place during the period that young animals are protected by passively acquired and

non specific factors, and is often associated with poor or non-existent dipping programs on farms within regions endemic for tick-borne diseases (De Vos 1979).

The major tick-borne diseases that occur in the study area are heartwater, caused by *Ehrlichia ruminantium* and transmitted by *Amblyomma hebraeum*; redwater or babesiosis caused by *Babesia bigemina* transmitted by both *R.* (*B.*) *decoloratus* and *R.* (*B.*) *microplus* and *Babesia bovis* transmitted by *R.* (*B.*) *microplus*, and anaplasmosis caused by *Anaplasma marginale* transmitted by *R.* (*Boophilus*) species. Endemic stability to all these diseases has been mentioned in reviews by Allsopp, Bezuidenhout & Prozesky (2004), De Vos *et al.* (2004) and Potgieter & Stoltsz (2004). A marked reduction in the frequency of dipping in the study area could thus lead to endemic stability to the above mentioned diseases provided that young calves are exposed to ticks and the parasites that they transmit whilst still immune to infection. Such a program would be a benefit to the State because of a reduction in dipping costs and to the farmer because of the reduction in animal movement and disease control.

In order to make full use of such natural phenomena as endemic stability and acquired resistance to ticks, particularly in indigenous breeds, and in addition decrease the cost of dipping and the possibility of resistance to a particular acaricide, a program different to the one currently in use is suggested. This program will consist of a number of entities. In the first two years of its implementation all young calves must be clearly identified and immunised against the prevailing tick-borne diseases. These calves should for the rest of their lives be excluded from the current dipping program unless they develop very heavy tick burdens or are plagued by other external parasites. Should this occur a single treatment with an acaricide can be administered.

The older cattle can continue to be dipped under the current program until all of them have been replaced either through sale, consumption, disease or death. The calves born to the original, clearly marked and immunised calves need not be immunised, provided they are constantly exposed to natural tick infestation and hence tick borne diseases, as they should have acquired immunity to these diseases from their dams. This should in any

event occur under the drastically reduced dipping program suggested above. Thus, in the proposed program, dipping will probably only be necessary during the summer months and only on those occasions when tick burdens become excessive, particularly those due to *A. hebraeum.* The latter stipulation though, still requires that cattle are regularly inspected.

Particular note should be taken of the displacement of *R.* (*Boophilus*) *decoloratus* by *R.* (*Boophilus*) *microplus* and the consequences for the transmission of *Babesia bovis* infection by the latter tick, which is its only vector in South Africa. This may be particularly important where immunisation against bovine babesiosis is advised and practiced.

Other answers received were that despite all respondents using the dipping facilities, only half (51%) were satisfied with the present system, 49% citing perceived poor chemical efficacy and distance to dip-tanks as reasons. The majority of unsatisfied respondents (45%) sited poor chemical efficacy as a perceived problem, which was the case at 31 of the 72 dip-tanks surveyed in the geographical distribution of ticks study. As the acaricide resistance tests showed that there was little resistance to amitraz, one must assume that the dip-wash was not up to strength. A further consequence of this under-strength dipping is that the ticks had not been exposed to high-frequency use of full strength acaricide and hence the low incidence of selection for resistance. It must also be born in mind that the concentration of the amitraz used in dip-tanks is 1/3 of that used in spray races (Snyman *per comm.*). This may also contribute to the perception that the dip is not effective, at the same time exposing ticks to selection towards a low concentration of acaricide. Another reason for the dissatisfaction with the dipping results may be that because amitraz is bio-degradable and that some owners may have taken their animals for dipping several days after the dip-wash was replenished, and thus the strength of the compound would have been reduced to level at which it was no longer effective. Attempts should be made to include these 31 dip-tanks in future acaricide resistance testing to determine the cause of the cattle owners' dissatisfaction.

All respondents to the questionnaire stated that they grazed their cattle on communal land in summer as well as in winter, and that they all utilize the dipping system in summer in which 88% of cattle owners dip once every 2 weeks. Virtually all respondents (97%) also dip in winter at a frequency of once a month (91%). This situation highlights the heavy reliance on dipping as a means of tick control, promoting acaricide resistance through high frequency application, reducing natural herd immunity through lack of suitable tick challenge and necessitating high financial and infrastructural inputs from State authorities.

At a State Veterinary meeting held at Bhisho during May 2005 several of the ECP veterinarians and animal health technicians expressed concern at the extent of screw worm myiasis in cattle, particularly in the coastal regions where *A. hebraeum* was present. This concern had also been expressed by stockowners during tick collections made in the survey on the geographic distribution of ticks in the region. The exclusive use of amitraz, which does not control insects and their larvae, without making use of other compounds, that have both acaricidal and insecticidal properties, has probably contributed to this problem. Some form of chemical intervention seems advisable here.

Another interesting observation is the apparent persistence of resistant ticks to chlorfenvinphos even after many years since it was last used in the area, assuming that there was no further use after the last purchase made by the Department of Agriculture. The persistence of resistance to chlorfenvinphos is of concern should products belonging to this grouping be used on a large scale in the future. It would appear as if amitraz could be the only acaricide in which reversion to resistance has been sustained after use in the field. Organophosphates and pyrethroids do not appear to have reversion from resistance to susceptibility even after decades of not using the product in the field.

The fact that ticks and tick-borne diseases are considered a major problem in cattle, but less so in goats and sheep, and that only cattle are included in dipping or spraying programs, may have a significant impact to both tick control programs and possibly also on counteracting acaricide resistance. The role that goats, which are not dipped, play in

maintaining tick populations implies that unless they are included in tick control programmes, the long-term results may be unsatisfactory. In fact, during the geographic distribution of ticks survey conducted in the region Nyangiwe & Horak (2007) found that a greater number of goats than cattle were infested with *R. appendiculatus* and *R. evertsi evertsi*, and that they were also fairly good hosts of adult *A. hebraeum* and of *R.* (*Boophilus*) *microplus*. From another perspective, however, the fact that goats are currently not included in tick control programmes means that they could serve as reservoirs of untreated susceptible ticks and thus play a role in delaying selection for acaricide resistance.

8. RECOMMENDATIONS

As soon as emerging resistance to a particular compound is detected in the laboratory, the dipping compound used at the affected farm or locality should be replaced by one from a completely different group of acaricides. The latter compound should then be used continuously until emerging resistance to it appears before changing to a new compound. Should other external parasites such as flies or their larvae become a problem during the sustained use of any dipping compound, one or two targeted treatments with an acaricide with insecticidal properties could be introduced into the program.

In order to make full use of such natural phenomena as endemic stability and acquired resistance to ticks, particularly in indigenous breeds, and in addition decrease the cost of dipping and the possibility of resistance to a particular acaricide, a program different to the one currently in use has been proposed. This program will initially be based on the immunization of calves and increased dipping intervals and later on the acquisition of natural immunity and occasional summer dipping.

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