Acaricide resistance profiles of single and multi-host ticks in commercial and communal farming areas in the Eastern Cape and North-West Provinces of South Africa

by

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DEDICATION

To my wife Yessem Bereded, my children Samson Sileshi and Meron Sileshi for their continued support.

To my parents who understood the importance of education.

DECLARATION

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

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SUMMARY

Acaricide resistance profiles of single and multi-host ticks in commercial and communal farming areas in the Eastern Cape and North-West Provinces of South Africa

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Tick resistance to acaricides is an increasing problem in South Africa and poses a real economic threat to the livestock and veterinary pharmaceutical industries. New acaricides are extremely expensive to develop so the present acaricides should be seen as an ever-diminishing resource, which should be protected by all means possible.

The main objective of the study was to detect the levels of tick resistance to acaricides at selected commercial and communal farms in South Africa. Also to compare the *in vitro* adult and larval test methods and to investigate acaricide management strategies which may increase the lifespan of the presently used acaricides.

To meet these objectives a field survey (February 2000 to August 2001) was carried out at selected communal and commercial farms in the Eastern Cape and

Northwest Provinces of South Africa to monitor levels of field tick resistance to acaricides. The larvae were originally obtained from engorged female *A. hebraeum*, *B. decoloratus*, *R. appendiculatus* and *R. evertsi evertsi*. The larvae were tested against different concentrations of amitraz, chlorfenvinphos and cypermethrin using the Shaw Larval Immersion Test (SLIT). Mortality dose data were subjected to probit analysis using a BMDP statistical package. Factors of resistance (FOR) were calculated by comparing the larval response of ticks from the field, which had been exposed to acaricides, with baseline data from very susceptible laboratory strains of ticks, on the basis of the LC_{50} values.

On the communal farms high levels of tick resistance were detected to cypermethrin as well as partial resistance to chlorfenvinphos whilst no resistance was detected against amitraz. On the commercial farms, however, ticks were equally resistant to amitraz, cypermethrin and chlorfenvinphos. The populations of *B. decoloratus* on these farms had developed higher levels of resistance to the test acaricides than the equivalent *R. evertsi evertsi, R. appendiculatus* and *A. hebraeum* populations. Higher levels of tick resistance to amitraz was observed on commercial farms than on communal farms, however, there was no significant differences in tick resistance to chlorfenvinphos and cypermethrin at both the commercial and communal farms. It was surmised that inappropriate use of acaricides might have resulted in higher tick resistance to the currently available acaricides on the commercial as well as the communal farms. Correct acaricide usage may solve this problem to a limited extent.

Comparative *in vitro* tests were also carried out on the larvae and adults of *B. decoloratus* to determine the susceptibility of this tick to different concentrations of the currently used acaricides, (amitraz, chlorfenvinphos and cypermethrin) at three commercial dairy farms, (“Brycedale”, “Sunny Grove” and “Welgevind”) near East London in the Eastern Cape Province of South Africa.

Resistance of field strains of *B. decoloratus* were determined using the SLIT and the Adult Immersion Test (AIT) as the latter test took into account factors such as oviposition assessment and reproductive ability. At "Brycedale", resistance to amitraz and chlorfenvinphos was detected with the AIT method. Emerging resistance to amitraz and resistance to chlorfenvinphos were also detected at "Brycedale" with the SLIT method. At "Sunny Grove" resistance was detected to cypermethrin and at "Welgevind" resistance was detected to chlorfenvinphos with the SLIT whilst no resistance was detected using AIT. It would appear that the *B. decoloratus* populations tested on these dairy farms were more resistant to chlorfenvinphos than to amitraz or cypermethrin.

Variable results were obtained using the SLIT, the Reproductive Estimate Test (RET) and the Egg laying Test (ELT). Nearly 50% of the dairy farms sampled showed resistance to chlorfenvinphos and the majority had susceptible *B. decoloratus* populations to both amitraz and cypermethrin.

"Brycedale" had a serious resistance problem whilst "Sunny Grove" and "Welgevind" dairies had much less resistance problems. At "Brycedale", the SLIT, RET and ELT methods all recorded resistance to amitraz and chlorfenvinphos whilst cypermethrin resistance was also detected with the ELT. At "Sunny Grove", the SLIT detected emerging resistance to chlorfenvinphos and resistance to cypermethrin while the other two test methods were negative. At "Welgevind" the SLIT detected resistance to chlorfenvinphos and the ELT resistance to cypermethrin whilst the RET did not detect any resistance at "Welgevind".

In general there was a good correlation between the RET and the ELT whilst in many cases there was poor correlation between the SLIT and the two AIT methods (RET and ELT).

From this study it would appear that the ELT was a good method to detect resistance within seven days, as opposed to the 42 days required for the RET and the 60 days for the SLIT. The ELT and the RET could possibly be used as screening methods to detect acaricide resistance on farms whilst the SLIT would remain the test of choice for National surveys. In addition the ELT is less costly and does not require sophisticated equipment for field testing for resistance, compared with other *in vitro* test methods. This method, however, still needs to be validated and standardized for use in South Africa and the rest of Africa where tick control is important.

SAMEVATTING

Weerstandigheid van bosluise teen akarasiedes neem toe in Suid-Afrika en bedreig beide die vee en farmaseutiese industrie. Die ontwikkeling van nuwe akarasiedes is duur, as sulks moet die akarasiedes wat huidiglik in gebruik is bewaar word en as ‘n nie-hernubare bron beskou word.

Die doelstellings van hierdie studie was om die vlakke van weerstandigheid van geselekteerde bosluisspesies teen akarasiedes in bepaalde kommunale en kommersiële plase in Suid-Afrika te bepaal. Die volwassene en larvale onderdompeling *in vitro* toetse is met mekaar vergelyk en bestuurspraktyke wat die langlewenheid van akarasiedes, wat huidiglik in gebruik is kan verleng, word voorgestel.

Ten einde hierdie doelstellings te verwesenlik is ‘n veld opname onderneem (Februarie 2000 tot Augustus 2001) op geselekteerde kommersiële en kommunale plase in die Oos Kaap en Noordwes Provinsies van Suid-Afrika. Larwes vir die weerstandigheids toetse is bekom van volgesuigde *A. hebraeum*, *B. decoloratus*, *Rhipicephalus appendiculatus* en *R. evertsi evertsi* bosluise. Die oorlewing van larwes by verskillende konsentrasies van amitraz, chlorfenvinfos en siepermetrien is met behulp van die “Shaw Larval Immersion Test” (SLIT) getoets. Mortaliteit dosis data is aan analises (probit) onderwerp ten einde die konsentrasie waar 50% van die larwes vrek (LC_{50}) te bepaal. Die faktor van weerstandigheid (FOR) is bereken deur die LC50 waardes van vatbare stamme met die toets stamme te vergelyk.

Op die kommunale plase is hoë vlakke van weerstandigheid teen siepermetrien en gedeeltelike weerstandigheid teen chlorfenvinfos aangeteken. Geen weerstandigheid teen amitraz is waargeneem nie. Op die kommersiële plase was

die bosluise gelykwaardig weerstandig teen amitraz, siepermetrien en chlorfenvinfos. Die populasies *B. decoloratus* het hoër vlakke van weerstandigheid teen die toets akarasiedes getoon vergeleke met *R. evertsi evertsi*, *R. appendiculatus* en *A. hebraeum* populasies op dieselfde plase. Daar word vermoed dat die onoordeelkundige gebruik van akarasiedes op beide kommunale en kommersiële plase tot die hoë vlakke van weerstandigheid kon gelei het.

Vergelykende *in vitro* toetse is ook met die larwes en volwassenes van *B. decoloratus* uitgevoer ten einde hul vatbaarheid vir amitraz, chlorfenvinfos en siepermetrien te bepaal. Bosluise is op drie kommersiële plase (Brycedale, Sunny Grove en Welgevind) in die Oos Londen omgewing versamel.

Die larwes en volwassenes is onderwerp aan SLIT en AIT (volgesuigde wyfie onderdompeling) toetse. Laasgenoemde toets sluit ook bepalings op die eierlegging en reproduktiewe vermoëns van die wyfies in. Op Brycedale is weerstandigheid teen amitraz en chlorfenvinfos met die AIT metode waargeneem. Met die SLIT metode is opkomende weerstandigheid teen amitraz en weerstandigheid teen chlorfenvinfos waargeneem. Op Sunny Grove is weerstandigheid teen siepermetrien en op Welgevind weerstandigheid teen chlorfenvinfos met die SLIT metode waargeneem. Geen weerstandigheid kon met die AIT metode waargeneem word nie. Wisselvallige resultate is verkry met die SLIT, die reproduktiewe skattings toets (RET) en die eierleggings toets (ELT). Ongeveer 50% van die *B. decoloratus* populasies wat op melkboerderye versamel is, was weerstandig teen chlorfenvinfos. Die oorgrootte meerderheid van hierdie populasies was vatbaar vir beide amitraz en siepermetrien.

Brycedale het ‘n ernstige weerstandigheid probleem getoon, terwyl die Sunny Grove en Welgevind plase minder van ‘n probleem getoon het. Op Brycedale het die SLIT, RET en ELT metodes weerstandigheid teen amitraz en chlorfenvinfos

aangetoon, terwyl die ELT ook op weerstandigheid teen siepermetrien gedui het. Op Sunny Grove het die SLIT metode op opkomende weerstandigheid teen siepermetrien gedui. Die ander twee toetsmetodes kon dit nie bevestig nie. Op Welgevind het die SLIT metode weerstandigheid teen chlorfenvinfos aangedui en die ELT metode weerstandigheid teen siepermetrien. Geen weerstandigheid met die RET metode kon op Welgevind aangedui word nie.

Oor die algemeen was daar ‘n goeie verband tussen die RET en ELT metodes, maar in baie gevalle ‘n swak verband tussen die SLIT en twee AIT metodes (RET en ELT).

Hierdie studie het aangedui dat die ELT metode sensitief is om weerstandigheid binne ses ure, vergeleke met die 42 dae vir die RET en 60 dae vir die SLIT, aan te dui. Die ELT metode is voorts ook goedkoper en benodig nie gesofistikeerde toerusting nie. Hierdie metode moet egter nog eers gevalideer en gestandariseer word vir gebruik in Suid-Afrika en ander lande in Afrika, waar bosluisbeheer van groot belang is.

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ABBREVIATIONS

&	and
AH	Animal Health
AIT	Adult Immersion Test
BHC	Benzene hexachloride
CM	Corrected mortality
CO_2	Carbon dioxide
DD	Discriminating dose
DDT	Dichlorodiphenyitrichloroethane
ELT	Egg Laying Test
FAO	Food and Agriculture Organization
Fig	Figure
FOR	Factor of resistance
g	Gram
ID	Identification
ie	that is
LC_{50}	Lethal concentration which kills 50% of the population
LPT	Larval Packet Test
m	meter
mg	milligram(s)
ml	millilitre
o/o	Percentage
%R	Percentage resistance
^{O}C	Degrees Celsius
OP	Organophosphate
Pers. comm.	Personal communication

RET	Reproductive estimate test
RH	Relative humidity
RSA	Republic of South Africa
SABS	South African Bureau of Standards
SLIT	Shaw Larval Immersion Test
Spp	Species
TBDs	Tick-borne diseases
USD	United States of America, dollar
w/v	weight/volume
WHO	World Health Organization

CHAPTER I: GENERAL INTRODUCTION

Ixodid ticks are one of the most economically important external parasites of livestock in the tropical and sub-tropical parts of the world (Bram, 1983). Heavy infestation can cause loss of blood, reduce the rates of live-weight gain and lower milk yield, whilst the long-mouthed ticks downgrade the quality of the hides (De Castro, 1997). It is estimated that 80% of the world's 1214 million cattle suffer to some extent from the deleterious effects caused by ticks (McCosker, 1979). They also transmit a number of important tick-borne diseases TBDs such as heartwater (*Cowdria ruminantium*), bovine babesiosis (*Babesia bovis* and *B. bigemina*), anaplasmosis (*Anaplasma marginale*) and theileriosis (*Theileria parva* complex, comprising *T. parva parva*, *T. parva bovis* and *T. parva lawrencei*) (Norval, 1994). Some ixodid ticks also produce toxins, which cause paralysis in sheep and sweating sickness in calves (Jongejan & Uilenberg, 1994).

Because of the direct and indirect effects on their hosts, ticks are considered to be not only a serious threat to successful livestock production but also seriously interfere with the economy of a country, especially in Africa. From a medical viewpoint, hard ticks are vectors of typhus such as Rocky Mountain spotted fever (*Rickettsia rickettsi*) and tick-bite fever (*R. conori*). In addition, they can spread Q-fever (*Coxiella burneti*) and many other arboviruses, including tick borne encephalitis, Colorado tick-fever and Crimean-Congo haemorrhagic fever. They also transmit tularaemia (*Francisella tularensis*) and cause tick paralysis in man (Service, 1996). The increase in interactions between humans, wild hosts and domestic animals has led to the emergence of infectious diseases in regions where they have not previously been encountered (Campos-Pereira Szabo, Bechara, Matushima, Duarte, Rechav, Fielden, & Keirans, 2000).

Ticks undergo a one-, two- or three-host life-cycle (Teel, 1985) and one-host ticks spend all three stages of their development on one host. In the case of two-host ticks, the larvae develop into nymphae on the first host, then drop to the ground where they moult into adults, which then seek a second host. In three-host ticks each of the larval, nymphal and adult stages feeds and engorges on a separate host before moulting to the next stage (Jongejan & Uilenberg, 1994).

The number of hosts, which a tick species requires to complete its life cycle, also affects its ability to transmit disease. If an infection is present in, or is acquired by, one developmental stage, and is transmitted by the same stage, the method of transmission is known as intra-stadial. An example of this form of disease transmission is when an infected adult male tick leaves a host and finds another to which it transmits the disease (Horak, Stoltsz & Heyne, 2000).

Research on South African ticks commenced nearly 200 years ago and since then more than 80 tick species have been documented (Walker, 1991). The ecology and detailed taxonomic descriptions (Walker, 1991) and other biological factors of most of the South African ticks (Theiler, 1962) are well documented.

Throughout most of the twentieth century, tick infestations on cattle were mainly controlled by chemicals, administered by plunge dipping, spraying in spray races or by hand-spraying (Dipeolu & Ndungu, 1991), and more recently, in the form of pour-ons, spot-ons, parenteral injections and intra-ruminal boluses.

Arsenic was first used for tick control in 1893 in South Africa (Bekker, 1960) and this inorganic compound was followed by a range of organic acaricides which included the organochlorines, organophosphates, carbamates, amidines and the pyrethroids. The progression through the different acaricide classes was driven

primarily by economic demand but always tempered by the development of acaricide resistance to these chemicals. Marketing pressures and other factors, such as a growing environmental awareness of the harmful effects caused by a build-up of residues, also contributed to the development of safer acaricides (Norval, Barrett, Perry & Mukhebi, 1992).

Tick resistance to acaricides used for tick control is an increasing problem in South Africa. The first published report on the development of tick resistance was to arsenic (Du Toit, Graf, & Bekker 1941). The resistance was detected in *B. decoloratus* after controlled field trials (Du Toit *et al*., 1941) and laboratory tests (Whitnall & Bradford, 1947; Whitehead, 1959) confirmed the resistance. Later, resistance to arsenic was also detected in *R. evertsi evertsi* (Whitehead & Baker, 1961; Matthewson & Baker, 1975), *Rhipicephalus appendiculatus* (Baker & Shaw, 1965), *A. hebraeum* (Matthewson & Baker, 1975) and *Boophilus microplus* (Baker, Jordan & Robertson, 1979).

Resistance to gamma benzene hexachloride (BHC) was also reported in *B. decoloratus* (Whitnall, Thorburn, Mchardy, Whitehead & Meerholz, 1952; Whitehead, 1959) and later in *R. evertsi evertsi* (Whitehead & Baker, 1961; Solomon, Baker, Heyne & Van Kleef, 1979) and *R. appendiculatus* (Baker & Shaw, 1965). In 1956 pyrethrum resistance was recorded in *B. decoloratus* (Whitehead, 1956) and in 1959 Dichlorodiphenyitrichloroethane (DDT) resistance in *B. decoloratus* was also published (Whitehead, 1959). DDT resistance was also reported in *B. microplus* (Baker *et al*., 1979) and in *A. hebraeum* (Matthewson & Baker, 1975).

B. decoloratus resistance to toxaphene (Whitnall *et al*., 1952) and dieldrin (Fiedler, 1952) were first reported in 1952. *R. evertsi evertsi* (Whitehead, 1959), *R. appendiculatus* (Whitehead & Baker, 1961; Baker & Shaw, 1965) and *A.*

hebraeum (Baker, Jordaan, & Robertson, 1981) also all showed resistance to toxaphene.

The widespread field use of the organophosphate (OP) acaricides resulted in *Boophilus decoloratus* ticks becoming resistant (Shaw, Thompson & Baker, 1967; Malan, 1973; Baker *et al*., 1978), and this was later followed by *R. appendiculatus* and *R. evertsi evertsi* also becoming resistant to the OP acaricides (Solomon *et al*., 1979). Similarly, resistance by *Boophilus* ticks inevitably followed the prolonged use of the pyrethroids (Coetzee, Stanford & Davis, 1987a). More recently amitraz resistance in *A. hebraeum, B. decoloratus* and *Boophilus microplus* has also been confirmed in South Africa (Taylor, pers. Comm., 2001).

Tick resistance to these chemicals poses a real economic threat to the livestock and veterinary pharmaceutical industries as they are an ever-diminishing resource, which has to be protected (Baker, 1982) as the cost of developing a new acaricide is estimated at US$ 230 million per compound (De Alva, 1995). The world wide annual economic losses caused by ticks and TBDs have been estimated to be more than US$ 7.0 billion (McCosker, 1979) and it is believed that Africa's share is greater than that of all the other continents combined (De Waal, 1996). In Africa tick control by acaricides cost US$ 720 million in 1989, whilst the corresponding figure for South Africa in 1994 was close to US$ 28 million (De Waal, 1996). When the economic costs of resistance and the development of new acaricides are considered it is clear that it is advantageous to prolong the use of available acaricides (Sutherst & Commins, 1979).

The escalation of acaricide resistance in ticks has encouraged the establishment of different acaricide resistance testing methods in different Acaricide Resistance Testing Laboratories. The laboratory methods usually involve either larval or adult ticks (Solomon, 1983) and include the Shaw Larval Immersion Test (SLIT) or the

Larval Packet Test (LPT), both of which are based on the testing of larvae. The Adult Immersion Test (AIT) however, uses field-collected adult ticks. The SLIT and the Food and Agriculture Organization (FAO)-adapted LPT are the most important larval acaricide resistance testing methods currently being used worldwide (Stendel, 1980). The SLIT is a timed immersion of unfed larvae in an aqueous test wash followed by a holding period in a clean environment and finally counting the numbers of live and dead larvae. Up to 1973 this was the only method used in Africa for detecting acaricide resistance in ticks (Lourens & Shaw, 1975) and was developed by Shaw (1966) in the late sixties.

The principle of the FAO-adapted LPT involves exposing unfed larvae to paper treated acaricide in oil for the whole period of the test which usually takes about 24 hours, followed by mortality counts. This method was developed by Stone & Haydock (1962) in Australia and adapted for use on African ticks by Tatchell (1973).

The main disadvantage of these specific tests (SLIT and LPT) is that they require at least 6 weeks for the LPT and more than 60 days for the SLIT to get results. Both of these tests also require trained technical assistance, as well as a range of expensive equipment, and susceptible reference strains.

The RET and ELT both use engorged adult female ticks. The RET detects tick resistance within 42 days and the ELT detects it within one week and the test can be performed by less experienced personnel using less expensive equipment. The *in vivo* stall test is, however, still the most conclusive indicator of acaricide efficacy and is used to confirm resistance if it is present (Nolan, Roulston, & Wharton, 1977). The disadvantage of this method is its expense (Stendel, 1980).

Acaricide resistance is more widespread and diverse in one-host ticks (Nolan, 1990) and one of the main reasons for the more rapid selection for resistance in one-host ticks is their short generation time (Norval *et al*., 1992). Resistance to acaricides is usually slower to develop in the two- and three-host ticks, where longer generation times with less acaricidal exposure of the immature tick stages mean that only some of the stages of the ticks are exposed to the acaricide (Matthewson & Baker, 1975). The presence of alternative hosts (Kunz & Kemp, 1994) and the presence of these ticks on wild animals ensure that untreated susceptible ticks (Nolan, 1990) help to reduce the selection pressure. Due to differences in the biology of the one- and multi-host tick species the selection pressures for the development of resistance to acaricides will also be different.

It is hypothesized that the rate of development of resistance would be quicker in the single-host ticks compared with multi-host ticks. Also the detection of resistance at an early stage, combined with an understanding of the factors which enhance its development, could result in the formulation of better resistance management strategies.

The main objectives of the project were:

- To compare acaricide resistance profiles of one- and multi-host ticks on cattle in the Eastern Cape and Northwest Provinces of South Africa.
- To compare the *in vitro* adult and larval test methods used to detect resistance in *B. decoloratus* populations.
- To recommend acaricide management strategies for those farms where acaricide resistance are experienced.

CHAPTER II. LITERATURE REVIEW

2.1 Historical record of the ticks

Ticks are obligate, non-permanent ectoparasites of terrestrial vertebrates (Sonenshine, 1991) and are exclusively hematophagous in all-feeding stages of their life cycle and have considerable medical and veterinary importance (Walker, 1991). It has been postulated that ticks evolved as obligate parasites of reptiles in the late paleozoic or early mesozoic era (Hoogstraal, 1976).

Ticks belong to the phylum Arthropoda, class Arachnida, order Acari, suborder Ixodida and include three important families namely the Argasidae, the Nuttalliellidae and the Ixodidae (Klompen, Black, Keirans & Oliver, 1996). There are nearly 800 tick species of which 150 belong to the family Argasidae and 650 to the family Ixodidae and only one to the family Nuttalliellidae (Hoogstraal, 1976).

Although ticks have been known since biblical times, it was not until the second half of the 19th century, when the world cattle population increased rapidly to feed the expanding human population that the importance of the diseases which they transmit and their serious debilitating effect on cattle became apparent (Solomon, 1983). The first overall review of southern African ticks was published as early as 1908 by C.W. Howard (Walker, 1991) and the geographical distribution of many of these ticks has already been documented (Theiler, 1962). To date more than 80 tick species have been recorded in South Africa (Walker, 1991) and about 20 of them regularly infest domestic livestock (Baker, 1982). Ticks, which are of major economic importance in South Africa, include *A. hebraeum*, *B. decoloratus*, *Boophilus microplus*, *R. appendiculatus* and *R. evertsi evertsi*.

2.2 Description of the four different tick species used for resistance testing

2.2.1 *Amblyomma hebraeum* (bont tick)

A. hebraeum is a three-host tick (Lounsbury, 1899) with one generation per year (Rechav, 1982) where the larvae, nymphae and adults feed on separate hosts (Theiler, 1943). Cattle are the preferred host of the adult ticks (Horak, 1982) but they also feed on a wide range of other species including sheep, goats, horses, donkeys and pigs (Theiler, 1962). The immature stages feed on a wide range of hosts including birds and small and large mammals (Horak, Potgieter, Walker, De Vos & Boomker, 1983). On domestic livestock the adults are usually found on the underside of the body (Howell, Walker & Neville, 1978) where they attach in clusters to the groin, axillae, dewlap, belly, perineum and the perianal regions of cattle (Petney, Horak & Rechav, 1987), as well as the feet of sheep and goats (Horak *et al*., 2000). Nymphae attach around the feet (Baker & Ducasse, 1967) whilst the larvae are commonly found on the head, face, dewlap, neck, feet and legs (Petney *et al*., 1987).

A. hebraeum is an exclusively southern African tick and it is commonly called the southern African bont tick (Walker & Olwage, 1987). In South Africa it occurs in the warm, moist coastal areas of the Eastern Cape, KwaZulu-Natal, Mpumalanga and Gauteng (Horak *et al*., 2000). It is also found in southern Zimbabwe, eastern Botswana, southern Mozambique and Swaziland (Walker & Olwage, 1987). Vegetation is the most important limiting factor (Norval, 1977) and this tick is more often associated with wooded habitats and does not commonly occur in open and treeless areas (Norval, 1983).

A. hebraeum is more prevalent in grassy areas where trees or bushes give shade to the free-living stages. In southern Africa, this tick has been recorded from the low altitude coastal regions and the higher altitudes of the highveld (Theiler, 1948). *A.*

hebraeum requires between 300 – 800 mm of rainfall and the pattern of seasonal occurrence is dependent on climate and varies considerably throughout the distributional range of the tick (Paine, 1982). In general, however, adults tend to be most numerous during the wet season, and larvae and nymphae during the dry season, but all stages can also be found on hosts throughout the year (Horak, 1982). In the coastal areas of the Eastern Cape Province of South Africa adults are present on cattle throughout the year with peak abundance in February and March (Rechav, 1982) whilst nymphal peak activity is from September until December and larval peak activity from February to June (Norval, 1977). In KwaZulu-Natal the adult ticks increase from September and then decline in January with nymphal peak numbers occurring between May and September and larval peaks between February to May (Baker & Ducasse, 1967).

A. hebraeum is an important vector of *Cowdria ruminantium* and is also known as the "heartwater tick" (Jongejan & Uilenberg, 1994). The adult ticks commonly attach in clumps, which leads to abscesses, and suppuration lesions and myiasis. Attachment of the adult ticks to the teats is a frequent cause of intramammary infection and subsequent loss of one or more udder quarters (Baker *et al.* 1977). In the Eastern Cape Province of South Africa, it is also one of the most serious pests of livestock and wildlife (Theiler, 1948). The prevalence of foot abscesses in Angora and Boer goat flocks in the bushveld of the Eastern Cape was also significantly related to the seasonal abundance of adult and nymphal *A. hebraeum* (MacIvor & Horak, 1987).

A. hebraeum adults have a long feeding period and on cattle good control of this tick can be achieved by treatment of cattle at weekly to fortnightly intervals with conventional acaricides (Norval, 1994).

2.2.2 *Boophilus decoloratus* (blue tick)

B. decoloratus is a one-host tick (Walker, 1991), which is indigenous to Africa (Wedderburn, Jagger, Mccartan & Hunter, 1991) and presumably evolved as a parasite of ungulates in East Africa and may have found its way south with the migration of indigenous tribes and their livestock (Henning, 1956). Cattle are its main domestic hosts although heavy infestation may also occur on horses (Hoogstraal, 1976) and wild ungulates (Theiler, 1962). Other domestic animals appear to be much less important as hosts (Baker & Ducasse, 1967).

Baker & Ducasse (1967) reported that *B. decoloratus* showed no marked preference for any particular attachment site and it is known that *B. decoloratus* completes the parasitic phase of its life cycle with in three weeks on the same host. The short life cycle allows the tick to pass through several generations in one year (Norval, 1994). In the Eastern Cape Province of South Africa, *B. decoloratus* was present on cattle throughout the year, although it did not show any seasonal pattern of occurrence; it was more abundant during autumn and spring (Rechav, 1982). In Zambia, the blue tick is common from March to July and again in October to November (MacLeod, 1970) and it appears to have two to four generations per year (Pegram, Perry, Mussisi & Mwanaumo, 1986). Rainfall plays an important role in limiting the distribution of this species and the annual rainfall level for *B. decoloratus* to survive is 375 mm (Theiler, 1949). Temperature and altitude are also one of the main factors regulating the seasonal patterns and distribution of the blue tick (Rechav, 1982) which is usually absent from areas below 600 m in Zimbabwe (Lawrence & Norval, 1979).

B. decoloratus transmits *Babesia bigemina*, which causes redwater in cattle (Heyne, 1986). As *B. decoloratus* is a one-host tick it can be effectively controlled by three-weekly acaricide treatment of cattle. Zebu cattle develop a considerably

better host resistance to this tick than European breed cattle and require fewer acaricide treatments (Norval, 1994).

2.2.3 *Rhipicephalus appendiculatus* (brown ear tick)

This tick species is a three-host tick (Lounsbury, 1904) with cattle the preferred domestic hosts of all stages of development (Yeoman & Walker, 1967) however, sheep, goats, horses, donkeys and mules are also parasitized to lesser extent (Norval *et al*., 1992). African buffalo (*Syncerus caffer*), eland (*Taurotragus oryx*), and waterbuck (*Kobus ellipsiprymnus*) are also among the preferred wild hosts of all stages of this tick (Horak, Spickett, Braack. & Penzhorn, 1993). Adults attach to the ears of their hosts (Norval, 1994), whilst the nymphae and larvae are commonly found on the ears, head, legs and feet (Baker & Ducasse, 1967).

R. appendiculatus is confined to the eastern, central and southern regions of Africa (Norval *et al*., 1992). In South Africa it is present in the Northern, Northwest, Gauteng, and Mpumalanga provinces as well as along the east coast of KwaZulu-Natal and the coastal regions of the Eastern Cape Province (Horak *et al*., 2000). The peak adult activity occurs from mid-November until the end of March with nymphae present from April to September and larvae from February until the end of June (Baker & Ducasse, 1967).

Free-living *R. appendiculatus* survive best in savannah woodland with good vegetation cover (Norval, 1994) but do not occur in open grassland (Theiler, 1962) or deep forests (Norval *et al*., 1992). This tick also disappears when overgrazing and environmental degradation occurs, as the microhabitat conditions in these unshaded habitats are generally unsuitable for the survival of the free-living stages (Short, Floyd, Norval & Sutherst, 1989). *R. appendiculatus* adults are regulated by the combined influence of humidity, temperature and day length and it is found at

altitudes ranging from sea level to 2000 m above sea level but is more prevalent in areas where the rainfall ranges from 500 mm to 2000 mm annually (Walker, Keirans & Horak, 2000).

Rhipicephalus appendiculatus is the main vector of *Thileria parva parva*, the causative organism of East Coast fever in cattle (Norval, 1994) and is also an efficient vector of *T. parva lawrencei* transmitted from African buffalo to cattle, causing corridor disease (Norval *et al*., 1992) as well as *T. parva bovis* (January disease) of cattle (Fivaz, Norval, & Lawrence, 1989). Exotic (*Bos taurus*) cattle suffer serious production losses whilst Zebu (*Bos indicus*) cattle become fairly resistant to the tick and require fewer acaricide treatments. In the absence of theileriosis, adequate control of adults can usually be achieved by localized application of acaricides to the ears (Norval, 1994).

2.2.4 *Rhipicephalus evertsi evertsi* (red-legged tick)

This tick has a two-host life cycle in which the larva and nymph share the same host (Norval, 1994), adults feed on cattle, sheep, goats, horses, zebras and elands (Horak *et al*., 1983). The normal predilection site for the larvae of *R. evertsi evertsi* is deep in the ear canal whilst the adults are found almost exclusively in the perineal region of cattle (Londt, Horak, & De Villiers, 1979; Dreyer, 1997).

Rhipicephalus evertsi evertsi is known as the “red-legged tick” (Walker, 1991) and is common in South Africa. In the Eastern Cape Province, *R. evertsi evertsi* was found throughout the year but was active mainly during summer with two peaks of abundance, one in September/October and the other in April/May (Rechav, 1982). This tick is more abundant in open areas than in the bush habitats (Rechav, 1982) and was encountered in large numbers in the lowveld areas of KwaZulu-Natal at altitudes below 4500 feet (Baker & Ducasse, 1967). The critical level for *R. evertsi*

evertsi to survive is 250 mm where it can maintain itself in grassy areas if the rainfall is above 250 mm (Theiler, 1950).

R. evertsi evertsi is one of the most important vectors of *Babesia equi* in horses and heavy infestations of the immature stages can result in ear damage (Horak *et al*., 2000). The engorging females sometimes transmit a toxin, which causes paralysis in sheep known as "spring lamb paralysis" (Hamel & Gothe, 1978). The occurrence and severity of this paralysis is dependent upon the number of engorging female ticks infesting the lambs (Gothe & Bezuidenhout, 1986).

2.3 Tick control in South Africa

The application of acaricides by dipping or spraying was introduced into southern Africa in the latter part of the 19th century to control East Coast fever (Norval *et al*., 1992) and this form of tick control was then used for most of the twentieth century and remained the most important methods for tick control throughout the world (Dipeolu & Ndungu, 1991). More recently with the rapid increase in the cost of labour and materials, acaricide usage has become less economically acceptable (McCosker, 1979). Acaricide usage has also lead to the growing problem of tick resistance to the acaricides. This has stimulated research into other more innovative methods of tick control, which includes vaccines against ticks, slow release acaricide devices and the topical application of pour-on acaricides (Norval *et al*., 1992).

In South Africa, chemical tick control started in 1893 when effective arsenic acaricides became available for use as plunge dips (Bekker, 1960). In 1938 *B. decoloratus* developed resistance to arsenic (Du Toit *et al.*, 1941). The use of arsenicals continued until the introduction of gamma BHC (Whitnall & Bradford, 1947). After only 18 months of field-use, gamma BHC resistance in *B. decoloratus*

had increased to such an extent that control was no longer evident (Whitnall *et al*., 1952). In the 1940's DDT became available providing effective field control of ticks, however, resistance to DDT was reported within five years of its field use (Whitehead, 1956). Toxicity, environmental awareness and other factors led to arsenic, DDT and gamma BHC being removed from the acaricide market.

In the early 1960's Diazinon was registered as one of the first organophosphate (OP) acaricides (Sykes, pers. comm.,, 2001). Later other OP acaricides were introduced providing a large number of acaricides, which allowed the stock farmer to continue with the tick control, until resistance to this acaricide group was also detected (Shaw *et al*., 1967). In the early 1970's "Triatix®"(Intervet) was the first amidine to be registered for tick control and in the 1980's flumethrin and permethrin, both pyrethroids, were also registered for tick control (Sykes, pers. comm., 2001)

2.4 Acaricides used in South Africa

The history of the synthetic acaricides is nearly 100 years old and most of these are organics such as the chlorinated hydrocarbons, the carbamates, the organophosphates, the pyrethroids, the amidines and the macrocyclic lactones (Davies, 1988). In South Africa the acaricides used for tick control have to be marketed for use only after extensive tests to ensure the safety and efficacy according to Act 36 of 1947 (Appendix 8) (Cotton, pers. comm., 2001), some of these products are listed in Appendix 1.

2.4.1 Arsenical compounds

Arsenic is the only inorganic acaricide (Solomon, 1983) and water-soluble sodium arsenite and was first used for tick control (Soll, 1989). Arsenic was initially widely used for tick control in South Africa (Spickett, 1998), but today it is no longer in use due to environmental toxicity and tick resistance. Resistant ticks increase the levels of the sulfhydryl containing compounds such as glutathion and cystein-cystine and this is the main mechanism of resistance (Solomon, 1983). The geographical distribution of arsenic resistant tick strains in South Africa has been mapped by Baker (1982).

2.4.2 Chlorinated hydrocarbons

The chlorinated hydrocarbon (organochlorine) compounds are synthetic acaricides which contain carbon, chlorine, hydrogen and sometimes oxygen and include DDT, gamma BHC, lindane, toxaphene, and others (Oudejans, 1991). They are very persistent acaricides and the mode of action is by interfering with the nerve conduction of ticks (Solomon, 1983) by affecting the ion channels, most notably the sodium ion channels (Adams, 1995). These products have mostly been withdrawn from the market because of their high toxicity and long lifespan which endangers the environment (Spickett, 1998). The geographical distribution of the chlorinated hydrocarbon resistant tick strains in South Africa has also been mapped by Baker (1982).

2.4.3 Organophosphates

The organophosphorous insecticides are esters of phosphoric acid (Oudejans, 1991). In South Africa the active chemicals of this group are commonly registered for cattle tick control and include chlorfenvinphos, diazinon and dichlorphos and many others (Swan, 2001). Organophosphates are toxic to birds and inhibit or

suppress the enzyme acetyl-cholinesterase, which is responsible for the breakdown of acetylcholine (Wharton & Roulston, 1970). This enzyme has an important role in the synaptic transmission of nerve impulse in ticks. Most of the organophosphate acaricides used for tick control are phosphorothionates, which are converted by the tick into the "phosphate" or the "oxon" active ingredient. These "oxons" are much more toxic to the tick than the original chemical so in a sense the ticks help to bring about their own destruction (Roulston, 1980). Large numbers of organophosphates are currently used to control ticks in South Africa and they are often used in combination, with other groups of acaricides, principally the pyrethroids (Swan, 2001). The geographical distribution of organophosphate resistant tick strains in South Africa has also been mapped by Baker (1982).

2.4.4 Carbamates

The carbamates are esters of carbamic acid (Oudejans, 1991) and closely resemble the organophosphates in their biological activity as they also inhibit the enzyme acetylcholinesterase, which is required for the termination of nerve impulses at the synaptic level. Carbaryl and propoxur are registered carbamates for use in South Africa (Spickett, 1998).

2.4.5 The amidine group (diamidines, formamidines or "tick detaching agents")

The amidine acaricides act by inhibiting the enzyme monoamine oxidase which is responsible for the metabolism of neurotransmitter amines present in the nervous system of susceptible ticks and mites (Atkinson, Binnington & Roulston 1974). Amitraz and cymiazol are registered formamidines used for tick control in South Africa (Swan, 2001). Amitraz was first developed in the early 1970's and has now become established as an important acaricide in all major tropical cattle production areas of the world (Harrison, 1981). The first reported field trials with amitraz were

successfully carried out in South Africa and because the primary activity was to force the ticks to detach they were referred to as “tick detaching acaricides” (Taylor, pers. comm., 2001).

The mode of action probably involves an interaction with octopamine receptors in the tick nervous system, causing an increase in nervous activity (Tomlin, 1994). Within 30-60 minutes of being treated with amitraz the ticks become agitated and detach their mouthparts and move rapidly over the animal in a disoriented way (Thullner, Kemp, Mckenna & Willadsen, 2000). Detached ticks may move on to another animal but they will not re-attach and feed (Harrison, 1981). Amidines act as detaching agents and do not cause mortality directly (Solomon, 1983). Amitraz mode of action may also involve octopamine receptor interaction (Kemp, pers. comm., 2001). Fly control in the cyclic amidines group is not good (Spickett, 1998).

The amidines are the most biodegradable group and they are the least harmful to the environment. Tests have shown that cattle, sheep and goats dipped or sprayed with amitraz do not have any residues of the compound in their milk (Harrison, 1981).

2.4.6 The pyrethroid group

The pyrethroids have played an important role in the control of ectoparasites of cattle and they have been used successfully for cattle tick control (Wilkins & Badenhorst, 1984). Members of this group registered for tick control in South Africa, include cypermethrin, flumethrin, deltamethrin, cyfluthrin, cyhalothrin and alphamethrin (Swan, 2001). They are toxic to fish and other aquatic organisms. Some of the active ingredients of the pyrethroids are used in combination with other acaricides, principally organophosphates (Peter, pers. comm., 2001). This

group of acaricide is known for its stability in the field and their good fly control. The principal mode of action of the pyrethroids is by interference with nerve conduction (Solomon, 1983) and decreased target sensitivity is the predominant pyrethroid resistance mechanism in ticks (Nolan, Wilson, Green & Bird, 1989).

2.4.7 Other acaricides

The macrolactones which are currently available to kill ticks, act systemically against single host ticks (Swan, 2001). The best known avermectin is ivermectin, which is a fermentation product derived from the actinomycete, *Streptomyces avermitillis* (Burg, Miller, Baker, Birnbaum, Currie, Hartman, Kong, Monaghan, Olson, Putter, Tunac, Wallick, Stapley, Oiwa & Omura, 1979). Ivermectin affects neural transmission, which is mediated by gamma aminobutyric acid and causes the death of certain parasitic nematodes and arthropods (Kass, Wang, Walrand & Stretton, 1980). In ticks, ivermectin inhibits female engorgement by reducing the body weight of females, leading to a reduction in the weight of eggs and decreased progeny (Wilkins, Conroy, Ho, O'shanny & Capizzi, 1981). Ivermectin when administered at the recommended dose significantly reduced the numbers of engorged female ticks on cattle in South Africa (Schroeder, Swan, Soll & Hotson, 1985).

2.5 The history of acaricide resistance in ticks in South Africa

Tick resistance to acaricide chemicals is not a new phenomenon in South Africa where records of resistance to arsenicals, organochlorines, organophosphates, carbamates, amidines and pyrethroids have already been documented (Baker, 1982). Resistance has mainly been identified in *B. decoloratus* but has also been recorded in the *R. evertsi evertsi, R. appendiculatus* and *A. hebraeum* (Solomon, 1983).

The first practical field chemical tick control programme was with the use of arsenic in the form of sodium arsenate, which was introduced for the control of cattle ticks in South Africa in 1893 (Bekker, 1960) and it was used for 50 years as the only effective acaricide available to the cattle owners at that time (Matthewson & Baker, 1975). Gamma benzene hexachloride (gamma BHC) was first introduced after resistance to arsenic had become an economic problem. However, resistance to this chemical soon followed some 18 months after it was introduced for tick control (Whitnall *et al.*, 1952).

Dichlorodiphenyltrichloroethane (DDT) was made available commercially for tick control after the failure of gamma BHC, but five years after its introduction resistance was reported in *B. decoloratus* (Whitehead, 1956). Other chlorinated hydrocarbons such as toxaphene and dieldrin were also released for tick control, however, resistance was soon reported (Whitnall *et al.*, 1952). It was also confirmed that ticks, which were resistant to gamma BHC, were also resistant to toxaphene, dieldrin and aldrin and this illustrated cross-resistance between these chemicals (Roulston, 1980).

2.5.1 Acaricide resistance in one-host ticks

Boophilus decoloratus ticks are important not only as vectors of various pathogens but also because they quickly developed resistance to a wide range of acaricides (Walker, 1991). The development of resistance in this species was usually the main reason for the introduction of new acaricides (Tatchell, 1986).

On the basis of controlled field trials, an arsenic resistant strain of *B. decoloratus* was first reported in the East London district of South Africa in 1939 (Du Toit *et al*., 1941) and this was later confirmed in the laboratory (Whitnall & Bradford,

1947). Gamma BHC resistant strains of *B. decoloratus* were later reported (Whitnall *et al*., 1952; Whitehead, 1959) as well as DDT (Whitehead, 1956), toxaphene (Whitnall *et al*., 1952; Baker *et al*., 1981) and dieldrin resistant strains (Fiedler, 1952). Later resistance to the newer organic acaricides, was confirmed in the organophosphates (Baker, Miles, Robertson, Stanford & Taylor, 1978), the pyrethroids (Coetzee *et al*., 1987a) and the amidines (Taylor & Oberem, 1995). *B. decoloratus* was the first tick species to develop resistance to a range of acaricides used in South Africa (Whitehead & Baker, 1961) and Table 1 illustrates recorded ixodid tick resistance in one-, two-, and three-host ticks in South Africa.

Table 1. Summary of the published records of acaricide resistance in ixodid ticks in South Africa

Tick species	**Active ingredient**	**Published resistance (author/s and date published)**
Single-host ticks		
B. decoloratus	Sodium arsenate	Du Toit *et al. (*1941) Whitnall & Bradford (1947) Whitehead (1959)
	DDT	Whitehead(1956) Whitehead (1959)
	Gamma BHC	Whitnall *et al.*(1952) Whitehead (1959)
	Toxaphene	Whitnall *et al.*(1952) Baker *et al.* (1981)
	Dieldrin	Fiedler (1952)
	Chlorfenvinphos, Dioxathion and Quintiofos	Baker *et al.* (1978)
	Carbaryl	Shaw *et al.* (1967)
	Amitraz	Taylor & Oberem (1995)
	Fenvalerate	Coetzee *et al.*(1987a)
Boophilus microplus	Sodium arsenate	Baker *et al.* (1979)
	DDT	Baker *et al.* (1979)
Multi-host ticks		
R. evertsi evertsi (Two-host)	Sodium arsenate	Whitehead & Baker (1961) Matthewson & Baker (1975)
	Toxaphene	Whitehead (1959)
		Whitehead & Baker (1961)
	Gamma BHC	Whitehead & Baker (1961)
R. appendiculatus (Three-host)	Sodium arsenate	Baker & Shaw (1965) Matthewson & Baker (1975)
	Gamma BHC	Baker & Shaw (1965)
	Toxaphene	Whitehead & Baker (1961) Baker & Shaw (1965)
	Lindane	Baker & Shaw (1965)
	Dioxathion	Solomon et al. (1979)
A. hebraeum (Three-host)	Sodium arsenate	Matthewson & Baker (1975)
	DDT	Matthewson & Baker (1975)
	Lindane	Matthewson & Baker (1975)
	Toxaphene	Baker *et al.* (1977)
	Dioxathion, Chlorfenvinphos, Quintiofos and Bromophosethyl	Baker *et al.* (1978)

2.5.2 Acaricide resistance in multi-host ticks

Resistance by the two-host tick *R. evertsi evertsi* to arsenic (Whitehead & Baker, 1961; Matthewson & Baker, 1975), toxaphene (Whitehead, 1959; Whitehead & Baker, 1961) and gamma BHC (Whitehead & Baker, 1961) has already been described (Table 1).

A. hebraeum has shown resistance to arsenic, gamma BHC, DDT (Matthewson & Baker, 1975), toxaphene (Baker, *et al*., 1977) dioxathion, chlorfenvinphos, quintiofos and bromophos-ethyl (Baker *et al*., 1978) (Table 1).

R. appendiculatus was reported to be resistant to arsenic (Baker & Shaw, 1965; Matthewson & Baker, 1975), gamma BHC (Baker & Shaw, 1965), toxaphene (Whitehead & Baker, 1961; Baker & Shaw, 1965) and dioxathion (Solomon *et al*., 1979) (Table 1).

2.6 Development of acaricide resistance and possible resistance mechanisms in ixodid ticks

The World Health Organization (WHO) Committee on Insecticide Resistance (1957) defined resistance as “the development of an ability in a strain of insects or other arthropods to tolerate doses of toxicants, which would prove lethal to the majority of individuals in a normal population of the same species”. This ability is inherited and occurs through the selective effect of chemicals which affect selection of pre-existing resistance genes, which are present at very low levels in a population (Brown, 1976).

Resistance is the inevitable consequence of the use of acaricides (Sutherst & Comins, 1979; Nolan, 1990) and the history of acaricide resistance in ticks in

South Africa certainly supports the latter statement (Du Toit *et al.*, 1941). Tick resistance can exist in the absence of chemical pressure (Stone, 1962) and this suggests that resistant genes pre-exist in a population and can be selected for by exposure to insecticides (Nolan *et al.*, 1977).

Acaricide resistance is the phenotypic expression of an evolutionary process accelerated by chemical selection and often involves inherited characters (Nolan & Roulston, 1979). The process occurs primarily through the selective effect of chemicals favouring pre-existing resistant mutants, which are already present in field populations of ticks at low frequencies (Stone, 1972). Acaricides do not kill all the ticks on the host and those which survive may develop resistance (Sutherst & Comins, 1979) and the risk of this happening increases if the population of susceptible ticks is completely eliminated by the over use of acaricides (Spickett, pers. comm., 2001).

Nolan (1985) indicated that any chemical used for the control of arthropods, by interfering with some biochemical or physiological system to produce its lethal effect, must pass through several obstacles before reaching its target as an active toxicant. Any change in the nature of these barriers, or extent of their activity, can also lower the effective concentration of the toxic compound (Nolan, 1985). This change may occur through a spontaneous chance mutation, occurring either before or during the use of a certain pesticides, by producing a few heterozygote individuals with this beneficial characteristic (Nolan, 1985).

The rate at which resistance develops is influenced by the following single or multiple factors:

- The degree of dominance of the resistant alleles (Stone, 1972). The more abundant the number of initially resistant individuals, the faster the development of resistance.

- The strength of the acaricide used coupled with the frequency of acaricide application (Sutherst & Comins, 1979). A very effective acaricide applied frequently will result in the rapid elimination of a higher proportion of the susceptible ticks. This will result in higher selection rates, and as a consequence a higher incidence of inter-breeding between resistant members giving rise to genetically resistant offspring (Sutherst & Comins, 1979).
- The duration of the life cycle of the ticks (Spickett, 1998). The shorter the life cycle of ticks, the faster will be the development of acaricide resistance. A quick succession of generations of these ticks will be exposed to the chemicals resulting in the selective elimination of a large number of the susceptible individuals in that population.
- Persistence of the pesticide (residual activity) or previous use of similar pesticides in the population may also influence the rate of the development of resistance (Whitehead & Baker, 1961).
- High gene frequency for resistance in a population of ticks will result in an increased selection pressure (Stone, 1968).
- The dose level at which the acaricide was applied (Riddles & Nolan, 1986). A high dose rate of acaricide application will eventually select for the most dangerous of several alterative resistance mechanisms or “supergenes”. Alternatively it is believed that when a chemical is used at a very low dose rate there is low selection pressure as the chemical is less effective and more members of the population survive (Spickett, 1998).

Tick resistance to acaricides is the result of the genetic selection of individuals in a population through the action of the acaricide which either kills or affects the reproduction of the more susceptible ticks (Stone, 1972). In order for the less susceptible ticks to survive and form the nucleus of resistant strains of ticks, the mechanism by which resistance is conferred, must be inheritable, and must be passed on from one generation to the next (Stone, 1972).

Tick resistance to acaricides is due to a range of different parameters including increased detoxification by metabolic breakdown of the toxicant and reduced sensitivity to the toxicant by the target system (Stone, 1972). Increased detoxification has been shown to be responsible for tick resistance to arsenicals and pyrethroids (Roulston, Schunter & Schnitzerling, 1966). Interference with nerve conduction is the main mode of action of the pyrethroids as acaricides (Solomon, 1983) and biochemical findings indicate that decreased target sensitivity is the predominant pyrethroid resistance mechanism in ticks (Nolan *et al*., 1989). The resistance mechanism to organophosphates is usually by changing the target enzyme, acetylcholinesterase (Nolan & Schnitzerling, 1986) which is essential for correct nerve function (Roulston *et al*., 1966).

Tick resistance has a genetic basis and mutation or sometimes amplification of structural genes occur. The appearance of resistance is quicker if the genes are dominant or slower if they are recessive (Tellier, Steffan & Buhlmann, 1991). Resistance can also arise from different types of mutations possibly affecting the same gene (Riddles & Nolan, 1986).

The survival of resistant ticks following acaricide treatments usually involves two modes of selection, which occur at very different rates (Sutherst & Comins, 1979). The first phase is the rapid selection of a partially dominant resistance allele caused by the preferential survival of heterozygous individuals and the second phase the much slower selection, which occurs with recessive alleles (Sutherst & Comins, 1979). It is assumed that the heterozygote selection process generally predominates. In the initial stage the allele is at such a low frequency that there is no detectable reduction in acaricide effectiveness and homozygotes are too rare to have any effect on the selection rate (Sutherst & Comins, 1979). During this phase the dispersal of resistance allele to neighbouring farms occurs unnoticed. In the

final phase the resistant allele is sufficiently common to reduce acaricide effectiveness noticeably (Sutherst & Comins, 1979). In this phase, homozygote selection is important but, because of the very high selection rate, this phase is of extremely short duration and due to the previous dispersal of resistance alleles, the acaricide quickly loses favour throughout the region (Sutherst & Comins, 1979).

2.7 Strategies for the management of acaricide resistance

Acaricide resistance strategies are often employed as countermeasures to overcome tick resistance when it has been detected in a population of ticks (Nolan, 1990). These tactics are designed to delay or, if possible, avoid the development of resistance to a new acaricides (Sutherst & Comins, 1979).

Many different resistance management strategies have been proposed but only a few are viable (Roush, 1993). Some of these involve reducing the dipping frequency in order to minimize the period of selection by retaining acaricide susceptible ticks within a tick population (Spickett, 1998) and avoiding high dosing rates (Roush, 1993).

The detection, monitoring and risk assessment of tick resistance are important requirements for developing successful management strategies (Chapman, 1992). If tick resistance can be identified with reliable resistance test methods, the preferred option would then be to use an alternative acaricide (Nolan, 1990). However, if there are not many suitable alternatives to the current acaricides then other options for managing the resistance problem such as the use of increased concentrations of the active or the addition of synergists should be considered (Nolan, 1990). Another strategy would be to delay the development of resistance by using a single acaricide for as long as possible, until laboratory results highlight

the development of resistance, then one has to change to a another acaricide group (Lourens & Tatchell, 1979).

Sound ecologically based treatment schedules are also needed to maximize the efficacy of the acaricides, whilst reducing the number of applications (Nolan, 1990). The impact of these strategies, in delaying resistance, will be enhanced if these chemical strategies are integrated with non-chemical control measures such as the use of tick resistant Zebu-type cattle (Sutherst & Comins, 1979). One way to minimize the residual selection in the treatment of ticks is to reduce the interval between treatments and maintain the concentration of the active chemical at a high level (Nolan, 1990).

CHAPTER III: ACARICIDE RESISTANCE PROFILES OF ONE- AND MULTI-HOST TICKS

3.1 Introduction

The blue tick *B. decoloratus* has developed resistance to a variety of chemicals used to control it at many different localities in southern Africa (Baker, 1982). In South Africa published reports indicate a wide distribution of *B. decoloratus* resistance all over the country (Spickett, pers. comm,, 2001). The acaricide resistance testing protocol most commonly used in South Africa to detect acaricide resistance is the "Shaw Larval Immersion Test" (SLIT). In this study we also used the SLIT originally described by Shaw (1966) and later modified to include a longer holding period for larval ticks after treatment (Shaw, Cook & Carson, 1968).

3.2 Materials and methods

3.2.1 Study areas

The study was conducted at selected communal (n = 6) and commercial farms (n = 6) in the North-West and Eastern Cape Provinces of South Africa (Fig.1).

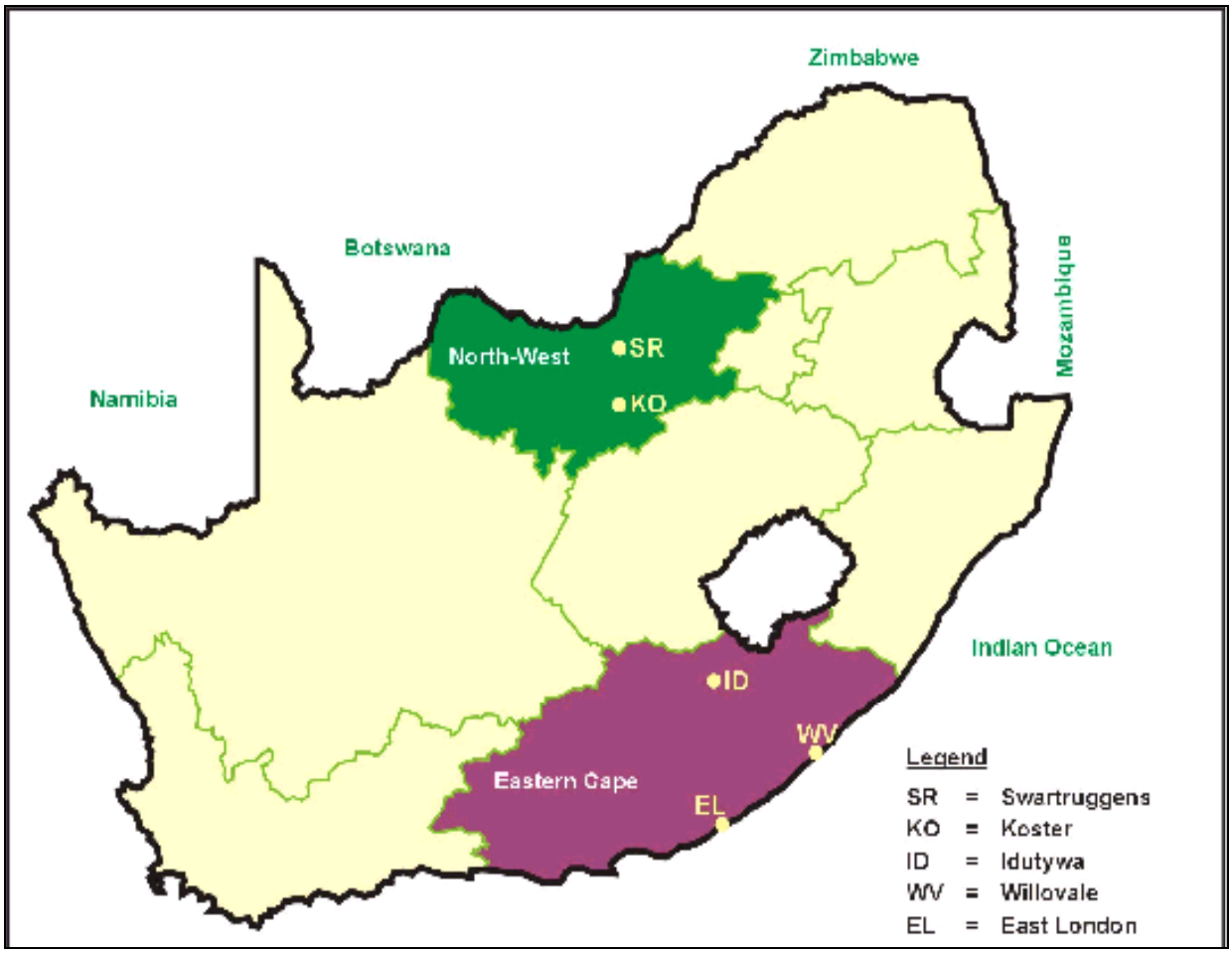


Fig. 1. Map of South Africa illustrating the location of the North-West and Eastern Cape Provinces where the tick collection occurred

3.2.1.1 Communal farms/dip tanks

Ticks were collected from six pre-selected communal grazing areas from three districts in the Eastern Cape Province of South Africa. The ticks were then used for acaricide resistance testing.

Fig. 2. A communal dip tank in the Eastern Cape Province of South Africa

The six communal farms/dip tanks were in the:

East London district;

- Mabeleni (32^{o} 58’ South and 27^{o} 35’ East) a communal grazing area/dip tank,
- Mozana (33^{o} 14’ South and 27^{o} 28’ East) a communal grazing area/dip tank.

Idutywa district;

- Colleywable (32^{o} 01’ South and 28^{o} 34’ East) a communal grazing area/dip tank,
- Cizele (32^{o} 02’ South and 28^{o} 30’ East) a communal grazing area/dip tank.

Willowvale district;

- Ntubeni (32^{o} 19’ South and 28^{o} 48’ East) a communal grazing area/dip tank,
- Ciko (32^{o} 15’ South and 28^{o} 35’ East) a communal grazing area/dip tank.

In these rural communities, cattle play a vital economic, social and cultural role by supplying meat, milk, draught power, cash sales, cultural ceremonies as well as

manure for the kraals. The economic levels of cattle production in these communal farming areas was very low and this manifested in a poor calving rate and high mortality from parasitic diseases, especially TBDs. The cattle kept on these small-scale, farming systems, were kraaled at night to facilitate the evening milking of the cows and to limit stock theft.

Dipping of cattle fortnightly in summer and monthly in winter had historically been used to control ticks. A dip tank manager controlled each dip tank and the cattle owners' sons usually mustered the cattle. Tick infested cattle were common at all of the communal grazing areas visited during this project. The extensive tick burdens on the communal farms had also created a significant problem for neighbouring commercial farmers where TBDs had increased significantly. Historically, government had funded the dipping of communal cattle. A wide variety of dipping products were currently used which included "Zeropar®" (Bayer AH) a combination of 30% chlorfenvinphos an organophosphate acaricide and 3 % alphamethrin a pyrethroid acaricide. "Zeropar®" has been used at the communal dip tanks for almost a decade in the Eastern Cape Province of South Africa. Prior to this, amitraz 12.5% ("Triatix®"-Intervet) was commonly used at most of the communal dip tanks.

State monitoring of ticks and TBDs of communal cattle in the Eastern Cape has been poor over the past two decades due to financial restraints (Amaral, pers. comm.,, 2001). Mortality in the cattle due to heartwater and other common TBDs was common, especially in the coastal valley bushveld areas (Amaral, pers. comm.,, 2001). The numbers of cattle dipped at each dip tank varied from tank to tank with an average of 1000-2000 cattle per tank. The majority of the cattle were Nguni or Nguni/Brahaman crosses.

3.2.1.2 Commercial farms

Ticks were collected from six commercial farms (three in the North-West Province and three in the Eastern Cape Province).

The Koster district (North-West Province)

- "Middelfontein" (25^{o} 51' South and 27^{o} 10' East) a commercial farm,
- "Basfontein" (25^{o} 54' South and 27^{o} 09' East) a commercial farm.

The Swartruggens district (North-West Province)

- "Woodstock" (25^{o} 39' South and 26^{o} 54' East) a commercial farm.

The East London district (Eastern Cape Province)

- "Brycedale" (30^{o} 10' South and 27^{o} 40' East) a commercial dairy
- "Sunny Grove" (33^{o} 10' South and 27^{o} 40' East) a commercial dairy
- "Welgevind" (33^{o} 04' South and 27^{o} 46' East) a commercial dairy.

The commercial farms were all well managed and the farmers used different formulations of acaricides, application methods and application intervals for tick control. "Triatix®"-Intervet (amitraz 12.5%) and "Ektoban®"-Novartis (cymiazole 175g/l and cypermethrin 25g/*l*) were mostly used to control the ticks.

The cattle breeds on the farms were mostly exotic-crosses with Simmental breeds mixed with locally developed Bonsmara (Afrikaner x Shorthorn). Each farm had between 100 - 500 head of cattle and some of the farmers exported cattle to neighbouring countries as well as embryos to Canada, Brazil, Australia and Argentina.

3.2.2 Tick collection in the study areas (communal and commercial farms)

Cattle, which were mustered prior to dipping in the communal areas or examined in a crush at the commercial farms, were carefully examined to identify the different tick species present. Only fully engorged adult female *A. hebraeum*, *B. decoloratus*, *R. appendiculatus* and *R. evertsi evertsi* were then removed manually before the cattle were treated with an acaricide. Ticks were collected from each farm during the peak occurrence of the adult ticks in summer (January - April 2001). One-host ticks were collected from any part of the body of the animal whilst multi-host species were collected from their specific predilection sites.

Fig. 3 Tick collection in progress at a dip tank at a communal grazing area in the Eastern Cape Province of South Africa.

The ticks were then stored in small plastic containers with perforated lids and placed between layers of paper to restrict movement and to absorb any excess moisture. Data, including the tick species, date of collection, farm name, and code number, were all recorded on each container. The plastic containers were protected from excessive heat or direct sunlight and were then transferred to the "Acaricide Resistance Testing Laboratory" (ARTL) of the Department of Zoology and Entomology at the University of the Free State. After identification with a stereoscopic microscope the ticks were washed on a sieve using clean tap water and all damaged and undersized ticks were discarded. Ticks were then air-dried in absorbent paper, placed in a glass flask and incubated.

3.2.3 Tick rearing

All the engorged female ticks collected in the field were incubated at 27^{o}C and 80-90 % R.H. in a glass jar incubator, in a temperature controlled-environment room equipped with a humidifier and a fan heater. The ticks were maintained under these conditions until egg-laying and larval hatching were completed. The egg laying period required by the *Amblyomma* tick species was approximately seven weeks, whilst the *Rhipicephalus* species took four weeks and the *Boophilus* species, three weeks. The hatching period of the *Amblyomma*, *Rhipicephalus* and *Boophilus* species was about three weeks (Strydom, per. comm, 2001).

3.2.4 Acaricides used in the study

The acaricides used during this study were chosen because they were currently widely used in South Africa and were commercially available. All were registered according to Act 36 of 1947 (Appendix 8) for the control of ticks (Swan, 2001). The acaricides tested were:

1. Amitraz 12.5 % m/v ("Triatix®" - Intervet South Africa Pty. Ltd.). Registration number G845 (Act 36/1947), South Africa. Chemical name of amitraz is N-methyl-N'-2,4-xylyl-N-(N-2,4-xylylformimidoyl)-formamidine and the empirical formula is $C_{19}H_{23}N_3$.

Amitraz is the most widely used acaricide in the diamidine group in South Africa (Taylor, pers. comm., 2000). The mode of action of amitraz is by affecting the tick nervous system by causing an increase in nervous activity (Tomlin, 1994), afterwards it is rapidly biodegraded.

2. Chlorfenvinphos 30 % m/v ("Supona 30®" - Fort Dodge, Bayer Animal Health Pty Ltd.). Registration number G1284 (Act 36/1947), South Africa. The chemical name of chlorfenvinphos is phosphoric acid 2-chloro-1-(2,4-dicholorophenyl)-ethenyl diethyl ester and the empirical formula is $C_{12}H_{14}Cl_3O_4P$.

Chlorfenvinphos is an organophosphate acaricide and it acts principally by binding and inhibiting acetylcholinesterase (ACHE), an enzyme widely distributed in nerves and muscles. Its function is to regulate neurotransmission at synapses by destroying the neurotransmitter acetylcholine (ACH) (Tomlin, 1994).

3. Cypermethrin 15 % m/v ("Curatik Dip®" - Fort Dodge, Bayer Animal Health Pty Ltd.). Registration number G505 (Act 36/1947), South Africa. Chemical name of cypermethrin is 3-(2,2-dicholoroethenyl)-2,2-dimethylcyclopropane-carboxylic acid cyano (3-phenoxyphenyl)-methylester and the empirical formula is $C_{22}H_{19}Cl_2NO_3$.

Cypermethrin is one of the pyrethroid acaricides currently in use in South Africa with good residual activity (Peter, pers. comm.,, 2001).

3.2.5 Acaricide resistance testing procedures

Prior to testing the larvae with the acaricides, filter paper envelopes were prepared and marked, noting the acaricide concentration, tick species, code and testing date. In addition, serial dilutions of the emulsified commercial acaricides (amitraz 12.5 % or chlorfenvinphos 30 % or cypermethrin 15 %) were prepared for each sample using seven different concentrations for each chemical.

Fig.4 The SLIT Laboratory work in progress at the ARTL in the Department of Zoology and Entomology, University of the Free State (Acknowledgement, Professor L.J. Fourie).

Batches of larvae, between 14 - 21 days old, were dipped in the different acaricide preparations according to the published protocol of acaricide resistance testing (Shaw, 1966) which is described in detail in the Appendix 2. The larvae were packeted and the test was read according to the protocol outlined in Appendix 2.

3.2.6 Data analysis

All relevant data were captured on to a specially prepared data captured form and computerised for statistical analysis. With the larval test, mortality dose data were subjected to probit analysis using the BMDP statistical package at the University of the Free State.

Responses of field ticks exposed to acaricides were compared with baseline data obtained from susceptible strains on the basis of the LC_{50} value (an estimate of the acaricide concentration which will kill 50 % of the population). A "Factor of Resistance" (FOR) was calculated by dividing the LC_{50} value obtained with a field strain to that of a susceptible reference strain. The degree of resistance is the number of times the LC_{50} value of a field strain exceeded that of the susceptible tick strain (Wilson, 1980).

B. decoloratus susceptible reference strains were obtained from the University of the Free State (Fourie, pers. comm.,, 2001). *A. hebraeum*, *R. appendiculatus* and *R. evertsi evertsi* susceptible reference strains were obtained from Dr R.J Taylor. Corrections for control mortality were also made using the formula below (Abbott, 1925):

$$\text{Corrected mortality (\%)} = \frac{\text{(\% test mortality - \% control mortality)}}{\text{100 - \% control mortality}} \times 100$$

3.3 Results

The LC_{50} values of amitraz, chlorfenvinphos and cypermethrin tested against the four different tick species, as well as factors of resistance (FOR) for each tick species, and each acaricide tested are illustrated in Tables 2-5. Susceptible reference strain values were included as controls. The larvae obtained from engorged female ticks collected from the field were considered to be resistant when these FOR values were more than 100 for amitraz and cypermethrin or more than five for chlorfenvinphos. They were considered emerging resistant when FOR values were between 50 and 100 for amitraz and cypermethrin and between 2.5 and five for chlorfenvinphos. The cut-off points for the tests were determined based on previous field trials (Taylor, pers. comm., 2001).

A. hebraeum larvae were susceptible to both amitraz and cypermethrin, however, there was partial resistance to chlorfenvinphos (Table 2). Two amitraz, four cypermethrin and three chlorfenvinphos resistant strains of *B. decoloratus* were detected (Table 3). One, two and four emerging resistant strains of *B. decoloratus* were detected to amitraz, cypermethrin and chlorfenvinphos, respectively (Table 3). All *R. appendiculatus* larvae tested were susceptible to the three acaricides used (Table 4) and only one chlorfenvinphos resistant strain of *R. evertsi evertsi* was found (Table 5).

B. decoloratus larvae from the Mabeleni and Mozana dip tanks, in the East London district, showed considerable resistance to cypermethrin as well as partial resistance to chlorfenvinphos (Table 6). At “Brycedale” (Table 7) *B. decoloratus* was resistant to chlorfenvinphos and also showed emerging resistance to amitraz. At “Sunny Grove” (Table 7) resistance was detected against cypermethrin and emerging resistance to chlorfenvinphos. At “Welgevind” (Table 7) the *B. decoloratus* population was resistant to chlorfenvinphos. At “Middelfontein” a

commercial farm in the Koster district of the North-West Province *B. decoloratus* was resistant to both amitraz and cypermethrin, whilst *R. evertsi evertsi* was resistant to chlorfenvinphos (Table 7). At "Basfontein" (Table 7) a high level of amitraz resistance was shown in *B. decoloratus*.

Appendices 4 – 7 summarize the percentage corrected mortality (% CM) of the different concentrations of acaricide tested against the different ticks species. The %CM is calculated from the dead/alive tick count. The counts show a good kill in relation to increases in concentration except where there is a resistant strain.

Table 2. *In vitro* larval bioassay: Results of the susceptibility of *A. hebraeum* larvae to amitraz, cypermethrin and chlorfenvinphos

Farm name	*A. heb.* strain	Active compound								
		Amitraz			Cypermethrin			Chlorfenvinphos		
		LC_{50}	FOR	Comments	LC_{50}	FOR	Comments	LC_{50}	FOR	Comments
	Ref Strain	0.0001	NC		0.000015	NC		0.00052	NC	
Mabeleni	S-3	0.000026	0.260	S	0.000015	1.000	S	0.00024	0.462	S
Mozana	S-6	0.000007	0.070	S	0.000031	2.067	S	0.00064	1.231	S
ColleyWable	S-7	0.0000063	0.063	S	0.000018	1.200	S	0.00026	0.500	S
Cizele	S-11	0.00013	1.300	S	0.00011	7.333	S	0.0013	2.500	ER
Ntubeni	S-12	0.000043	0.430	S	0.000036	2.4	S	0.00022	0.423	S

Key

R = Resistant
ER = Emerging resistance
S = Susceptible
FOR = Factor of resistance
NC = Not calculated
A. heb. = *A. hebraeum*

	Amitraz FOR	Cypermethrin FOR	Chlorfenvinphos FOR
R =	> 100	> 100	> 5
ER =	50-100	50-100	2.5-5
S =	< 50	< 50	< 2.5

Table 3. *In vitro* larval bioassay: Results of the susceptibility of *B. decoloratus* larvae to amitraz, cypermethrin and chlorfenvinphos

Farm name	*B. dec.* strain	Active compound								
		Amitraz			Cypermethrin			Chlorfenvinphos		
		LC_{50}	FOR	Comments	LC_{50}	FOR	Comments	LC_{50}	FOR	Comments
	Ref Strain	0.000042	NC		0.000057	NC		0.00041	NC	
Mabeleni	S-1	0.0000002	0.005	S	0.016	280.9187	R	0.0015	3.511	ER
Mozana	S-4	0.000016	0.376	S	0.028	501.7667	R	0.0012	2.976	ER
Colleywable	S-8-1	0.0000072	0.172	S	0.0032	56.53710	ER	0.00036	0.872	S
Cizele	S-10	0.0000079	0.189	S	0.0051	90.10600	ER.	0.0025	6.053	R
Ntubeni	S-15	0.00000068	0.016	S	0.00032	5.653710	S	0.0013	3.148	ER.
Basfontein	S-19	0.097	2326	R	0.00013	2.296819	S	0.00044	1.065	S
Woodstock	S-20	0.000041	0.981	S	0.00024	4.240282	S	0.00032	0.775	S
Middelfontein	S-23	0.013	311.01	R	0.012	212.0141	R	0.0009	2.179	S
Brycedale	S-100	0.0033	77.75	ER	0.0024	42.049	S	0.0026	6.199	R
Sunny Grove	S-101	0.0013	31.34	S	2.0E+01	>200	R	0.0020	4.860	ER
Welgevind	S-102	0.000041	0.971	S	0.00068	12.032	S	0.0024	5.920	R

R = Resistant
ER = Emerging resistance
S = Susceptible
FOR = Factor of resistance
NC = Not calculated
B. dec. = *B. decoloratus*

	Amitraz FOR	Cypermethrin FOR	Chlorfenvinphos FOR
R =	> 100	> 100	> 5
ER =	50-100	50-100	2.5-5
S =	< 50	< 50	< 2.5

Table 4. *In vitro* larval bioassay: Results of the susceptibility of *R. appendiculatus* larvae to amitraz, cypermethrin and chlorfenvinphos

Farm name	*R. app* strain	Active compound								
		Amitraz			Cypermethrin			Chlorfenvinphos		
		LC_{50}	FOR	Comments	LC_{50}	FOR	Comments	LC_{50}	FOR	Comments
	Ref Strain	0.00005	NC		0.0002	NC		0.0006	NC	
Ciko	S-18	0.0000056	0.11	S	0.00006	0.3	S	0.00031	0.517	S
Cizele	S-9	0.00000064	0.013	S	0.000045	0.225	S	0.00038	0.633	S
Ntubeni	S-16	0.0000069	0.14	S	0.000038	0.19	S	0.00017	0.283	S
Middelfontein	S-26	IL	NC		0.00019	0.95	S	0.0007	1.167	S

R = Resistant
ER = Emerging resistance
S = Susceptible
FOR = Factor of resistance
IL = Insufficient larvae
NC = Not calculated
R. app = *R. appendiculatus*

	Amitraz FOR	Cypermethrin FOR	Chlorfenvinphos FOR
R =	> 100	> 100	> 5
ER =	50-100	50-100	2.5-5
S =	< 50	< 50	< 2.5

Table 5. *In vitro* larval bioassay: Results of the susceptibility of *R. evertsi evertsi* larvae to amitraz, cypermethrin and chlorfenvinphos

Farm name	*R. e. evertsi* strain	Active compound								
		Amitraz			Cypermethrin			Chlorfenvinphos		
		LC_{50}	FOR	Comments	LC_{50}	FOR	Comments	LC_{50}	FOR	Comments
	Ref Strain	0.00005	NC		0.00002	NC		0.0005	NC	
Mozana	S-5	$1.5x10^{-9}$	0.00003	S	0.000017	0.850	S	0.000066	0.132	S
Ntubeni	S-17	$1.3x10^{-9}$	0.000026	S	0.0000065	0.325	S	0.00024	0.480	S
Woodstock	S-21	$1.2x10^{-7}$	0.0024	S	0.00001	0.500	S	0.00015	0.300	S
Middelfontein	S-24	$4.0x10^{-7}$	0.008	S	0.000017	0.850	S	0.0029	5.800	R

R = Resistant
ER = Emerging resistance
S = Susceptible
FOR = Factor of resistance
NC = Not calculated
R. e. evertsi= R. evertsi evertsi

	Amitraz FOR	Cypermethrin FOR	Chlorfenvinphos FOR
R =	> 100	> 100	> 5
ER =	50-100	50-100	2.5-5
S =	< 50	< 50	< 2.5

Table 6. Summary of the tick resistance data collected from the communal farms/dip tanks (n = 6) in the Eastern Cape Province

District	Dip tank	Tick species	Amitraz		Cypermethrin		Chlorfenvinphos	
			FOR	Comment	FOR	Comment	FOR	Comment
East London	Mabeleni	*A. hebraeum*	0.260	S	1.000	S	0.462	S
		B. decoloratus	0.005	S	280.9	R	3.5	ER
	Mozana	*A. hebraeum*	0.070	S	2.067	S	1.231	S
		B. decoloratus	0.376	S	501.8	R	3.0	ER
		R. evertsi evertsi	0.00003	S	0.850	S	0.132	S
Idutywa	Colleywable	*A. hebraeum*	0.063	S	1.200	S	0.500	S
		B. decoloratus	0.172	S	56.5	ER	0.872	S
	Cizele	*A. hebraeum*	1.300	S	7.333	S	2.50	ER
		B. decoloratus	0.189	S	90.1	ER	6.053	R
		R. appendiculatus	0.013	S	0.225	S	0.633	S
Willowvale	Ntubeni	*A. hebraeum*	0.43	S	2.4	S	0.423	S
		B. decoloratus	0.016	S	5.65	S	3.14	ER
		R. appendiculatus	0.0014	S	0.190	S	0.283	S
		R. evertsi evertsi	0.00003	S	0.325	S	0.480	S
	Ciko	*R. appendiculatus*	0.011	S	0.030	S	0.517	S

R = Resistant
ER = Emerging resistance
S = Susceptible
FOR = Factor of resistance

Table 7. Summary of the tick resistance data collected from the commercial farms (n = 6) in the Eastern Cape and North-West Provinces

District	Farm name	Tick species	Amitraz		Cypermethrin		Chlorfenvinphos	
			FOR	Comment	FOR	Comment	FOR	Comment
East London (Eastern Cape Province)	Brycedale	*B. decoloratus*	77.75	ER	42.05	S	6.20	R
	Sunny Grove	*B. decoloratus*	31.34	S	3508	R	4.86	ER
	Welgevind	*B. decoloratus*	0.97	S	12.03	S	5.92	R
Koster (North West Province)	Middelfontein	*B. decoloratus*	311.005	R	212.01	R	2.179	S
		R. appendiculatus	IL	NC	0.950	S	1.167	S
		R. evertsi evertsi	0.008	S	0.850	S	5.800	R
	Basfontein	*B. decoloratus*	>200	R	2.297	S	1.065	S
Swartruggens (North West Province)	Woodstock	*B. decoloratus*	0.981	S	4.240	S	0.775	S
		R. evertsi evertsi	0.0024	S	0.500	S	0.300	S

R = Resistant
S = Susceptible
FOR = Factor of resistance
IL = Insufficient larvae
NC = Not calculated

3.4 Discussion

3.4.1 Tick resistance to acaricides on the communal farms/dip tanks

Laboratory results obtained from the larval progeny of *B. decoloratus* collected at the communal farms/dip tanks (Table 6) demonstrated high levels of resistance to both cypermethrin (two resistant and two emerging resistant farms), and chlorfenvinphos (one resistant and four emerging resistant farms), and no resistance to amitraz (all susceptible). *B. decoloratus* from the Mabeleni and Mozana dip tanks in the East London district showed considerable resistance to cypermethrin.

Resistance in ticks to pyrethroids was reported after only 18 months of use in the field (Coetzee *et al*., 1987b) and in Australia cross-resistance between DDT and the pyrethroids has been reported (Nolan *et al*., 1977; Nolan, 1981). In South Africa Coetzee *et al*. (1987b) observed the association in *B. decoloratus* between fenvalerate resistance, which is a pyrethroid, and DDT resistance. In East London, DDT was extensively used from 1949 to 1955 (Whitehead, 1956), so the possibility that the previous DDT resistance may be associated with the current outbreak of pyrethroid resistance has to be considered. There are, however no grounds to imply that the cypermethrin resistance evolved from DDT resistant strains of *B. decoloratus* at the Mabeleni and Mozana communal dip tanks.

One of the most important factors, which affects the efficacy of an acaricide, is the use of an acaricide at the incorrect concentration and this is one of the prime causes of tick control failure at communal dipping tanks (Jonsson, 1997). This was recorded at the Mozana dip tank near East London, where there was no dip tank manager and farmers were themselves responsible for the replenishment of acaricides in the dip (Magadla, pers. comm., 2001). The farmers believed that they

needed to increase the concentration of the acaricides in the dip tank during the peak tick season to control the excessive tick burdens infesting their cattle. This type of increased dip concentration would have undoubtedly have lead to a higher selection pressure for tick resistance (Spickett, 1998) as the high acaricide concentration would effectively kill all susceptible ticks leaving only a residue of highly resistant individuals in the population (Spickett, 1998). Each successive dipping would be a selective process, which would concentrate the genes responsible for the resistance and eventually the majority of the ticks in the population would be resistant to the acaricide being applied against them (Whitehead & Baker, 1961).

At both the Mabeleni and Mozana dip tanks high levels of tick resistance to cypermethrin were observed. These two dip tanks were both close to the coast, where there was high humidity and high temperatures for most of the year, which were ideal conditions for tick population to rapidly expand. Under such favourable climatic conditions the ticks were able to thrive all year round and two-week interval dipping all year round was necessary to control them. This intensive dipping programme probably also contributed to an increased selection pressure for resistance and "Zeropar®", which is a mixture of alphamethrin and chlorfenvinphos, had been used at these dip tanks for nearly 6 years (Magadla, pers. comm.,, 2001). The ticks in this area had, therefore, been exposed to high levels of both pyrethroid and organophosphate dips in the field. The *R. evertsi evertsi* which were also collected from the "Mozana" and "Ntubeni" dip tanks were found to be susceptible to all acaricides tested (Table 6).

Engorged female *A. hebraeum* were collected from the "Mabeleni", "Mozana", "Colleywable", "Cizele" and "Ntubeni" dip tanks and *R. appendiculatus*, were collected from the "Cizele", "Ntubeni" and "Ciko" dip tanks. The *A. hebraeum* and *R. appendiculatus*, which are both three-host ticks, were all susceptible to the

acaricides tested. Only at "Cizele" dip tank did *A. hebraeum* show emerging resistance to chlorfenvinphos (Table 6). Baker *et al*. (1978) previously reported that *A. hebraeum* was fully susceptible to chlorfenvinphos in dip tanks in the East London and Willowvale districts.

Breakdown in dipping is a common phenomenon in the Eastern Cape and it is often due, not to acaricide failure to control ticks, but rather to incorrect acaricide application (Shaw, 1966). Even during this study acaricide failure at the "Ntubeni" dip tank in the Willovale district (Table 6) resulted in loss of tick control. The communal farmers complained that the ticks did not die after being dipped and they concluded that the acaricide was not working. This coincided with heavy tick burdens of *A. hebraeum*, *B. decoloratus*, *R. appendiculatus* and *R. evertsi evertsi* on the cattle. *In vitro* laboratory tests, however, indicated that all the ticks at this dip tank were susceptible to the test acaricides, although emerging resistance to chlorfenvinvos was recorded in *B. decoloratus* (Table 6). The dip tank manager at "Ntubeni" had not been replenishing the tank at the recommended concentration, which resulted in poor tick control.

3.4.2 Tick resistance to the acaricides on the commercial farms

The *B. decoloratus* population on the commercial farms was quite resistant to chlorfenvinphos and moderately resistant to amitraz and cypermethrin (Table 7). Preliminary results from the "National Tick Resistance Survey" (NTRS), however, indicated that *B. decoloratus* was very resistant to cypermethrin, partially resistant to chlorfenvinphos and more susceptible to amitraz (Fourie, pers. comm.,2001). The resistance status of ticks on a particular farm will, however, depend on the historical acaricide use pattern.

At "Middelfontein", *B. decoloratus* demonstrated multiple resistance to both amitraz and cypermethrin (Table 7). The farmers in this area of the North-West Province of South Africa were at that time using both amitraz and cypermethrin which they mixed with citric or olive oil as a pour-on, a practice not recommended by the manufacturers.

At "Basfontein", *B. decoloratus* was resistant to amitraz, however, on this particular farm, only four concentrations were used per test as opposed to the standard seven. This was because of an insufficient number of larvae and it was suggested that the strain be re-cycled on a calf and the sample re-tested.

Many commercial dairy farmers in the East London district use "Ektoban®", which is a mixture of cymiazol and cypermethrin. *B. decoloratus* resistance to amitraz on these farms may have been as a result of cross-resistance with cymiazol, an active ingredient belonging to the same chemical group as amitraz. In Australia, Nolan (1981) reported high levels of *B. microplus* resistance to amitraz with cross-resistance to a closely related amidine, cymiazol.

In South Africa, amitraz-resistant strains have been reported in *B. decoloratus* (Taylor & Oberem, 1995) and more recently in *A. hebraeum* and *B. microplus* (Taylor, pers. comm., 2001). In our study, only *B. decoloratus* was collected for resistance testing, as *B. microplus* was not found during the study period. Chlorfenvinphos resistant *B. decoloratus* was found on two of the three of the dairies in the study (Table 7).

3.4.3 Differences in selection pressure in one- and multi-host ticks

It was clear from the results that the *B. decoloratus* populations had developed significant resistance to all the currently used acaricides. There was much less resistance in the *A. hebraeum*, *R. appendiculatus* and *R. evertsi evertsi* populations due to the fact that there was much less contact with acaricides which had decreased the selection pressure for resistance.

The reasons for the more rapid selection for resistance in one-host ticks are numerous and include a shorter generation time (Norval *et al*., 1992) which allows more frequent exposure to the acaricides (Wharton & Roulston, 1970). The shorter life cycle also leads to a quick succession of generations of ticks being exposed to the chemicals, resulting in the selective elimination of the majority of ticks which were the susceptible individuals in the population (Matthewson & Baker, 1975). *B. decoloratus* appears to have as many as four generations per year (Pegram *et al*., 1986), whilst the three-host ticks have a single generation which may extend over one, two or even three years (Wharton & Roulston, 1970).

Other factors which can delay the development of resistance is the availability of alternative hosts (Kunz & Kemp, 1994). Two and three host ticks spent a greater length of their life cycle off the host and have a broader host range, such as wild animal hosts (Nolan, 1990) which would help to reduce the selection pressure from

acaricides on the multi-host ticks compared with that experienced by the one-host ticks. The extended period of use of acaricides coupled with the high frequency of acaricide application may also have increased the resistance problems on these farms. Solomon (1983) reported that high dipping frequencies removes susceptible ticks, leaving only resistant males to mate with resistant females, leading to the proliferation of resistant ticks.

Boophilus decoloratus is a one-host tick and all stages occur mainly on cattle at the same time and takes three weeks from the time the larvae attach to when the adults detache (Walker 1991). As the lifecycle is so short this tick species is able to pass through two to four generations per year (Pegram *et al*., 1986) which means the ticks are on the host for 42-63 days each year and can potentially be exposed nine times during the year if weekly dipping is practiced (Horak, pers. comm., 2001). The one-host lifecycle of this tick also means that all three parasitic stages of development are exposed at each dipping hence increasing the likelihood of selection for resistance.

Rhipicephalus evertsi evertsi is a two-host tick (Theiler, 1943). It takes 14-18 days from the time the larvae attaches to the time the engorged nymphs drop. The adult tick remains attached for seven days and this tick has a continuous lifecycle in warmer months and can probably complete two or more life cycles in a year and consequently it is on the host for 42 days or more during the year (Walker *et al*., 2000). Larvae and nymphs may share the same host as the adults (Norval, 1994) that feed on cattle, sheep, goats, horses, zebras and eland (Horak *et al*., 1983) and consequently may not be exposed to acaricide at each dipping. If, however, only cattle are present at a locality, all developmental stages could be exposed to an acaricide applied at weekly intervals on six occasions during a year and hence the pressure to select for resistance would be high.

Amblyomma hebraeum is a three-host tick (Lounsbury, 1899) with one generation per year (Rechav, 1982) in which the larvae, nymphs and adults feed on separate hosts (Theiler, 1943). Cattle are the preferred host of the adult ticks (Horak, 1982) but they also feed on a wide rage of other species including sheep, goats, horses and donkeys (Theiler, 1962). The immature stages feed on a wide range of hosts including birds and small and large mammals and consequently might not be exposed to acaricides at all (Horak *et al*., 2000). The larvae and nymphs of this tick species spend one week each on an animal while females spend one to two weeks and males one to ten weeks on an animal. The total time spent per annum on hosts is approximately 21 days or more.

Rhipicephalus appendiculatus is a three-host tick (Walker *et al.*, 2000) with cattle the preferred domestic hosts of all stages of development (Yeoman & Walker 1967), however, sheep, goats, horses, donkeys and mules are also parasitized to a lesser extent and the larvae and nymphs can utilize a number of host species (Norval *et al*. 1992). Total time on hosts is 15-21 days in a year and consequently there are only five days a year within a particular tick's life cycle that the adults can potentially be exposed to acaricides on the host, hence the low level of resistance in this tick species.

In conclusion this study supports the hypothesis that single-host ticks develop resistance faster than multi-host ticks.

CHAPTER IV. A COMPARISON OF THE *IN VITRO* LARVAL AND ADULT BIOASSAY METHODS TO DETERMINE ACARICIDE RESISTANCE AT THREE COMMERCIAL DAIRIES ALL WITH PREVIOUSLY REPORTED TICK CONTROL PROBLEMS

4.1 Introduction

A number of laboratory and field test methods have been developed for the detection of resistance of ticks to acaricides. These techniques usually involve the use of either larval or adult ticks (Solomon, 1983). The acaricide resistance tests used in this study were the Shaw Larval Immersion Test (SLIT) and the Adult Immersion Test (AIT) which include both the Reproductive Estimate Test (RET) and the Egg Laying Test (ELT).

- The SLIT is based on using the larval stage of ticks to detect resistance. This method was first described by Shaw (1966) and subsequently modified to include a longer holding period for larval ticks after treatment (Shaw *et al*., 1968).

- The RET uses field collected engorged female ticks, which are then tested directly in the laboratory for resistance (Drummond, Ernest, Trevino, Gladney & Graham, 1973). The RET was first described by Drummond *et al*. (1973) who used it for evaluating the efficacy of new acaricides, as well as acaricide resistance testing and toxicity studies (Whitnall & Bradford, 1947; Stone & Webber, 1960). Engorged adult female ticks are sometimes preferred to the larvae for testing for acaricide resistance because of their greater tolerance to chemicals (Graham & Drummond, 1964). With multi-host ticks, when it is possible to obtain sufficient adult ticks, at the same stage of engorgement, then the bioassay on adult ticks has certain advantages. The test can be used directly on the adult ticks as well as the quicker response obtained when screening for tick resistance (Solomon, 1983).

One disadvantage of this test is that it is difficult to obtain enough ticks and difficult to assess the mortality in the relatively immobile engorged female ticks (Spickett, pers. comm., 2001). To overcome this, Drummond, Ernest, Trevino, Gladney. & Graham (1973) suggested that a ratio of reproductive efficiency be used as an assay criterion. This method is being assessed for resistance testing at several laboratories in the world but has never been standardised (Nari, pers. comm.,2001).

- The ELT is an assessment of oviposition and is the same as the initial stage

of the RET. The ELT is based on a comparison of the number of eggs laid by engorged treated field ticks compared with engorged control female ticks. The method is similar to the "Adult Immersion Test-Discriminating Dose" (AIT-DD) which is in the process of development by the "Food and Agriculture Organization" (FAO) of the United Nations (Nari, pers. comm., 2001).

4.2 Materials and methods

4.2.1 Study areas

The study was conducted at "Brycedale" (30^{o} 10' South and 27^{o} 40' East), "Sunny Grove" (33^{o} 10' South and 27^{o} 40' East) and "Welgevind" (33^{o} 04' South and 27^{o} 46' East) all dairy farms in the East London district in the Eastern Cape Province of South Africa. Ektoban®, which is a mixture of cymiazol and cypermethrin was being used by all of the farmers at the time of tick collection, however, tick control failure had been reported on most of these dairies.

Adult and larval resistance tests were then carried out to investigate the breakdown in tick control. Engorged female *B. decoloratus* were collected from each dairy from the 23rd to the 25th of April, 2001 and comparative laboratory tests on the larval progeny and the adults of *B. decoloratus* were undertaken. A comparison of

the susceptibility of *B. decoloratus* to the different acaricides was done by using the SLIT, RET and ELT.

4.2.2 Acaricide resistance testing procedures

The commercial acaricides used to test the susceptibility of *B. decoloratus* in the three tests were the same as that described in section 3.2.4.

The SLIT was conducted at the Acaricide Resistance Laboratory (ARL) of the Department of Zoology and Entomology at the University of the Free State. The method used was originally described by Shaw (1966) and a detailed protocol of the procedure is given in section 3.2.5 and in Appendix 2. The data was analysed as described in section 3.2.6.

For the RET, engorged female *B. decoloratus* of uniform size and free from visible abnormalities were collected from the "Brycedale", "Sunny Grove" and "Welgevind" dairy farms and were tested by measuring the RET. The laboratory protocol which was first described by Drummond *et al*. (1973) had been slightly modified by the South African Bureau of Standards (SABS) in East London, South Africa (Strydom, pers. comm., 2001) and is outlined in the Appendix 3. Some of the laboratory equipment needed for the test are shown in Fig. 5.

Briefly field collected engorged female ticks were washed in water and air dried (Stone, 1957) and divided into groups according to size. Groups of ten ticks were weighed and randomly allocated into two and four replicates for each treatment and control group. Concentrations of the acaricide used were the recommended field concentrations, i.e. 0.025%, 0.05% and 0.015% for amitraz, chlorfenvinphos and cypermethrin respectively.

Fig. 5 Illustration of some of the Laboratory equipment needed to run the Adult Immersion Test (AIT) at the SABS laboratory in East London (Acknowledgements, Dr C. de Bruin/Dr T. Strydom)

The treatment groups were immersed in field concentrations of the three test acaricides and the control group was immersed in water. They were then incubated at 27^{o}C and 80 – 90% R.H. At the end of the hatching period, which normally takes 42 days for *B. decoloratus*, the RE was calculated by estimating the number of larvae which had hatched using a numerical scale of 0 to 4 as follows:

Scale	% hatch
0	0
1	<25
2	25 – 50
3	50 – 75
4	75 - 100

The RE for each treatment group was calculated as follows:

$$RE = \frac{m_1 \times n \times h}{m_2 \times s \times 4}$$

m_1 = mass of eggs per treatment group (mg)

m_2 = mass of engorged female ticks per treatment group (mg)

n = number of ticks per treatment group

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

s = number of female ticks alive after seven days of incubation.

The percentage Reproductive Estimate (%RE) was calculated by dividing the RE of female ticks treated with test acaricide by the RE of untreated (control) female ticks times one hundred:

$$\%RE = \frac{\text{RE of female ticks treated with test acaricide}}{\text{RE of untreated (control) female ticks}} \times 100$$

In this study the tick population was considered resistant if the %RE was greater than 80 % and susceptible if it was less than 80 %.

In the ELT, fully engorged female *B. decoloratus* ticks of uniform size and free from any visible abnormalities were collected from “Brycedale”, “Sunny Grove” and “Welgevind” all commercial dairies in the East London district in the Eastern Cape Province of South Africa. The ticks were initially washed in distilled water and air dried at room temperature. They were then divided into groups (ten ticks per group) according to size with ten ticks randomly allocated to two or four replicates for each treatment and control group. Concentrations of acaricide were 0.025%, 0.05% and 0.015% for amitraz, chlorfenvinphos and cypermethrin respectively. The AIT-DD being tested by FAO uses a Discriminating Dose (DD) of acaricide and this has only been worked out for *B. microplus* (Nari, pers. comm.,

2001). The initial procedures for this test were the same as that already described for the AIT (see Appendix 3).

After seven days of incubation at 27°C and 80 – 90% R.H. the number of engorged female *B. decoloratus*, which had laid eggs, was assessed. With the ELT engorged female *B. decoloratus* which have been immersed in water (control ticks) (Fig. 9A) should lay a normal quantity of eggs within seven days of being in an incubator. Ticks, which have been treated with an acaricide, and still lay as many eggs as the control ticks, are considered to be resistant (Fig. 9B). Those, which were treated with acaricide but do not lay eggs, are considered susceptible (Fig. 9C) (Kemp, pers. comm., 2001). In this study, the ticks were considered resistant if the percentage resistance (%R) was greater than 80% on the seventh day after incubation. They were considered susceptible if they lay less than 80% when compared with the eggs laid by the control. The results of the ELT are summarized in Table 10.

The percentage resistance (%R) obtained with the different test acaricides was calculated by dividing the number of treated engorged female ticks laying eggs (egg laying response) with the number of untreated (water control) ticks laying eggs multiplied by a hundred (Table 10).

$$\%R = \frac{\text{No. of treated engorged female ticks laying eggs}}{\text{No. of untreated (water control) engorged female ticks laying eggs}} \times 100$$

4.3 Results

Shaw Larval Immersion test

The results of the SLIT used to determine the susceptibility of the *B. decoloratus* larvae, from the three commercial dairy farms, to amitraz, chlorfenvinphos and cypermethrin are summarized in Table 8.

The *B. decoloratus* population from Brycedale, Sunny Grove and Welgevind commercial dairies all showed resistance to the test acaricides. For amitraz there was no resistance at Sunny Grove and Welgevind and only emerging resistance at Brycedale. For chlorfenvinphos there was emerging resistance at Sunny Grove and full resistance at Brycedale and Welgevind, which resulted in the known control problems. For cypermethrin there was no resistance at Brycedale and Welgevind but a high FOR was recorded at Sunny Grove. Probit graphs illustrating examples of susceptible and resistant strains of *B. decoloratus* are shown in Figs. 6 to 8.

Table 8. *In vitro* bioassay: Results on the susceptibility of *B. decoloratus* larvae from three commercial dairy farms in the East London district, Eastern Cape Province to amitraz, chlorfenvinphos and cypermethrin.

Dairy farm	Active compound								
	Amitraz			Chlorfenvinphos			Cypermethrin		
	LC_{50}	FOR	Comments	LC_{50}	FOR	Comments	LC_{50}	FOR	Comments
Ref. strain	$4.2x10^{-5}$			$4.1x10^{-4}$			$5.7x10^{-5}$		
Brycedale	$3.3x10^{-3}$	77.751	ER	$2.6x10^{-3}$	6.199	R	$2.4x10^{-3}$	42.049	S
Sunny Grove	$1.3x10^{-3}$	31.340	S	$2.0x10^{-3}$	4.860	ER	$2.0x10^{1}$	>200	R
Welgevind	$4.1x10^{-5}$	0.971	S	$2.4x10^{-3}$	5.920	R	$6.8x10^{-4}$	12.032	S

Key

R = Resistant

ER = Emerging resistance

S = Susceptible

FOR = Factor of resistance

LC_{50} is percent concentrations

	Amitraz FOR	Cypermethrin FOR	Chlorfenvinphos FOR
R =	> 100	> 100	> 5
ER =	50-100	50-100	2.5-5
S =	< 50	< 50	< 2.5

FIGs 6-7-8

Reproductive Estimate Test

The mean engorgement weights of engorged female ticks (m_2), the total mass of eggs (m_1), the hatchability (h) of the eggs to larvae, the RE, as well as the %RE of the *B. decoloratus* immersed at recommended field concentrations of acaricides are illustrated in Table 9.

At "Brycedale" farm *B. decoloratus* was resistant to amitraz (Table 9) (%RE = 84.83), and chlorfenvinphos (%RE = 80.34). Poor percentage control at "Brycedale" against all three acaricides supported the field observations where heavy *B. decoloratus* burdens were difficult to control. No resistance was detected at either "Sunny Grove" or "Welgevind dairy farms and this also supports the field observations.

The mean weight of the egg masses produced by the water treated control females (0.109g) was almost 2.2 times greater than the egg masses produced by the acaricide treated females (0.049g) (Table 9). Similarly the hatchability capacity (h) of eggs in the water treated control females was higher than the hatching capacity of eggs obtained from treated females (Table 9). In addition it was observed that the RE of all water treated females was greater than those of the acaricide treated females. There was, however, no real difference between the mean weight of the female ticks allocated per treatment group (0.215g) and mean weight of female ticks allocated per control group (0.218g) (Table 9).

Table 9 Percentage resistance, percentage reproductive estimate, reproductive estimate and percent control of female *B. decoloratus* from three commercial dairies after treatment with different acaricides (amitraz, chlorfenvinphos and cypermethrin) compared with a water control.

Commercial dairy farms	Acaricide tested	No. of ticks immersed & incubated	Survivors after 7 days	*Mass of females (mg)	+Mass of eggs (mg)	h	RE	%RE	^{o}R
"Brycedale"	Amitraz	20	20	3.790	1.503	4.00	0.397	84.83	R
	Chlorfenvinphos	20	20	3.771	1.620	3.50	0.376	80.34	R
	Cypermethrin	20	18	3.822	1.406	3.00	0.307	65.60	S
	Control	20	19	3.791	1.687	4.00	0.468		
"Sunny Grove"	Amitraz	20	20	4.573	1.154	2.00	0.126	25.45	S
	Chlorfenvinphos	20	20	4.446	0.003	0.00	0.000	0.000	S
	Cypermethrin	20	20	4.397	1.629	3.00	0.278	56.16	S
	Control	20	20	4.543	2.250	4.00	0.495		
"Welgevind"	Amitraz	40	40	8.623	0.015	0.00	0.000	0.00	S
	Chlorfenvinphos	40	37	8.546	0.721	1.75	0.040	7.46	S
	Cypermethrin	40	40	8.496	3.545	3.00	0.313	58.40	S
	Control	40	39	8.670	4.527	4.00	0.536		

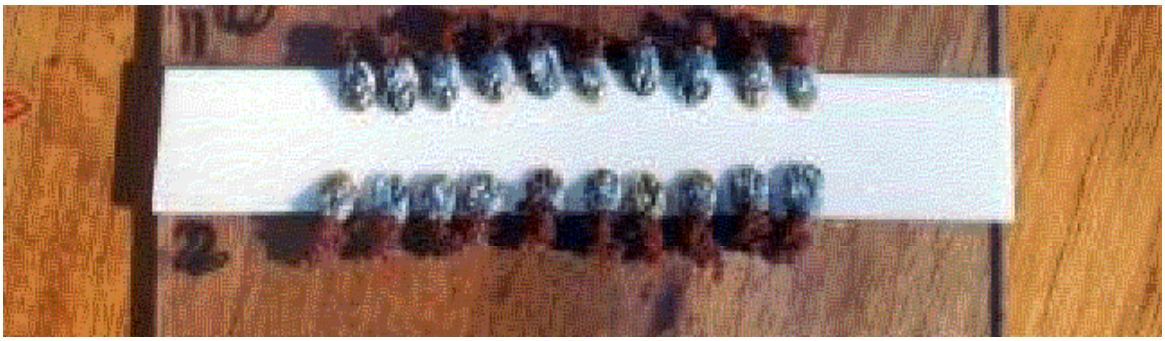
* = Engorgement weight of treated female ticks
\+ = Total mass of eggs per treatment group
h = Hatchability estimate (scale from 0 – 4)
RE = Reproductive Estimate
%RE = Percentage reproductive estimate
^{o}R= Degree of resistance
B. decoloratus resistant if %RE is greater than 80%
B. decoloratus susceptible if %RE is less than 80%

Egg Laying Test

At the "Brycedale" dairy farm, acaricide treated female *B. decoloratus* were resistant to amitraz (84.2%) and chlorfenvinphos (94.7%) but susceptible to cypermethrin (73.7%) (Table 10). At "Sunny Grove" the population of *B. decoloratus* were susceptible to amitraz, chlorfenvinphos and cypermethrin. At "Welgevind", resistance was only detected to cypermethrin (82.1%), whilst amitraz and chlorfenvinphos still successfully controlled *B. decoloratus*.

A

B



C

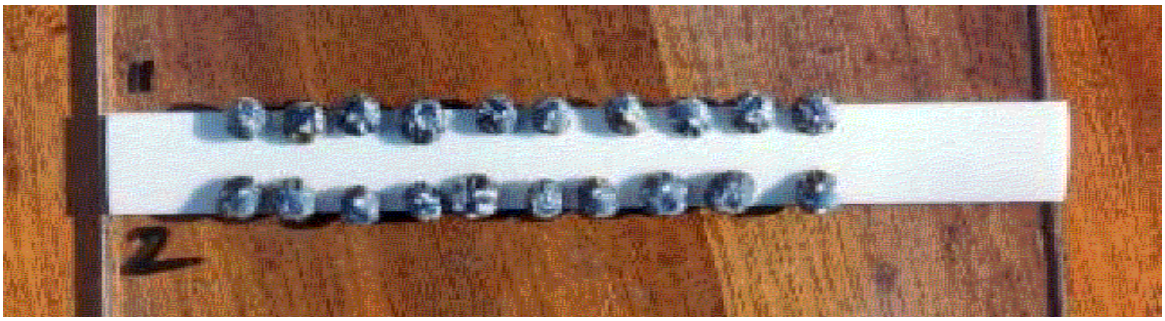


Fig. 9A) Egg-laying response of engorged adult female *B. decoloratus* after immersion in water (control group) after seven days of incubation

B) Egg-laying response of resistant engorged adult female *B. decoloratus* after immersion in test acaricide (treatment group), after seven days of incubation.

C) Egg-laying response of susceptible engorged adult female *B. decoloratus* after immersion in test acaricide (treatment group),after seven days of incubation

Table 10. Egg-laying response of engorged adult female *B. decoloratus* after immersion in three different acaricide groups and then incubated for seven days.

Dairy farms	Acaricide	A	B	C	%R	^{o}R
Brycedale	Amitraz (250 ppm)	20	20	16	84.2	R
	Chlorfenvinphos (500 ppm)	20	20	18	94.7	R
	Cypermethrin (150 ppm)	20	18	14	73.7	S
	D = Control	**20**	**19**	**19**		
Sunny Grove	Amitraz (250 ppm)	20	20	7	38.8	S
	Chlorfenvinphos (500 ppm)	20	20	1	5.6	S
	Cypermethrin (150 ppm)	20	20	13	72.2	S
	D = Control	**20**	**20**	**18**		
Welgevind	Amitraz (250 ppm)	40	40	0	0.0	S
	Chlorfenvinphos (500 ppm)	40	37	5	12.8	S
	Cypermethrin (150 ppm)	40	40	32	82.1	R
	D = Control	**40**	**39**	**39**		

ppm = Parts per million

A = Number of engorged female *B. decoloratus* treated with acaricide (treatment group) or treated with water (control group (D)).

B = Number of female ticks alive after seven days of incubation

C = Number of females laying eggs after seven days of incubation

%R = Percentage resistance

$$\%R = \frac{\text{No. of treated ticks laying eggs (C)}}{\text{No of untreated (control) laying eggs (D)}} \times 100$$

^{o}R= Degree of resistance

Resistant if %R is over 80%.

4.4 Discussion

The resistance of *B. decoloratus* ticks on the three farms, to chlorfenvinphos was greater than of either amitraz or cypermethrin. This is surprising, as with the exception of "Brycedale" organophosphate (OP) acaricides had not been used for the past ten years, on any of the farms studied. As resistance to chlorfenvinphos was still detected, one can deduce that once OP resistance has become established in a tick population reversion back to susceptibility is either very slow or does not occur (Stone, 1972).

Other OP acaricides, which had been used earlier, may have produced the cross-resistance to the OP compounds as resistance to one member of a group of chemically similar acaricides can result in a degree of resistance to other members of the same group (Whitehead, 1959; Shaw *et al*., 1967, Baker, 1982). Cross-resistance to *B. decoloratus* within groups of chemically related acaricides has been previously documented in the area (Baker *et al*., 1978).

4.5 A comparison of the Shaw Larval Immersion Test (SLIT), the Reproductive Estimate Test (RET) and the Egg Laying Test (ELT)

The susceptibility of *B. decoloratus* to various acaricides was determined using the SLIT (Shaw, 1966), the RET and the ELT (Drummond *et al*., 1973). The period required to detect resistance in *B. decoloratus* was seven, 42 and 60 days of incubation using the ELT, RET and SLIT, respectively. The ELT was far quicker than either the RET and SLIT.

Table 11. Comparison of the results of the three tests used on the different farms

Farms	Tick species	Test methods								
		SLIT			RET			ELT		
		A	Ch	Cy	A	Ch	Cy	A	Ch	Cy
Brycedale	*B. decoloratus*	ER	R	S	R	R	S	R	R	S
Sunny Grove	*B. decoloratus*	S	ER	R	S	S	S	S	S	S
Welgevind	*B. decoloratus*	S	R	S	S	S	S	S	S	R

SLIT : Shaw Larval Immersion Test
RET : Reproductive Estimate Test
ELT : Egg Laying Test
A : Amitraz
Ch : Chlorfenvinphos
Cy : Cypermethrin
S : Susceptible
ER : Emerging resistance
R : Resistance

At the "Brycedale" dairy *B. decoloratus* showed resistance to amitraz, and chlorfenvinphos when tested with the ELT and RET. However, with the SLIT, the "Brycedale" *B. decoloratus* population was only shown to be resistant to chlorfenvinphos, with emerging resistance to amitraz (Table 11). At "Sunny Grove" the *B. decoloratus* population was susceptible to amitraz and chlorfenvinphos with the RET and the ELT with susceptible to cypermethrin (%RE=72%) whilst the SLIT showed resistance to cypermethrin and emerging resistance to chlorfenvinphos (Table 11). However, on this farm chlorfenvinphos had never been used for tick control. At "Welgevind" the ELT method detected cypermethrin resistant *B. decoloratus*, whilst with the SLIT, resistance to chlorfenvinphos was detected (Table 11).

Resistance to cypermethrin was detected on two of the farms using the ELT whilst it was only detected on one farm using the SLIT. "Ektoban", a mixture of cymiazol and cypermethrin, has been used for tick control for nearly ten years on these farms

and the farmers reported that it no longer controlled the ticks. This would indicate that the tick resistance was to one or both of the actives and was probably due to resistance to cypermethrin. Coetzee *et al*. (1987a) demonstrated that the development of resistance to one of the pyrethroids took 18 months after intensive application as an acaricide.

It would appear from our study that the field population of *B. decolotatus* were more resistant to chlorfenvinphos and only moderately resistant to cypermethrin and less resistant to amitraz. There was also good agreement between the high field burdens of *B. decoloratus* ticks observed on the cattle at "Brycedale" dairy and the test results.

In summary amitraz resistance detected at "Brycedale" using the RET and the ELT with an indication of emerging resistance using the SLIT method. Chlorfenvinphos resistance was detected at "Brycedale" using the SLIT, the RET and the ELT and at "Welgevind" using the SLIT. Cypermethrin resistance was detected at "Welgevind" with the ELT and at "Sunny Grove" with the SLIT. While there was general agreement in results from SLIT, RET and ELT, some refinement of the techniques and further sampling is needed for direct comparison of the three tests. In addition the ELT and the RET need to be refined by using the Adult Immersion Test-Discriminating Dose (AIT-DD) test method.

CHAPTER V. GENERAL DISCUSSION

5.1 Resistance status of ticks collected from the communal and commercial farms in South Africa.

The *in vitro* larval tests indicated that the *B. decoloratus* population in the study areas had developed a high degree of resistance to both cypermethrin and chlorfenvinphos. The levels of resistance were greater than that seen in *A. hebraeum*, *R. appendiculatus* and *Rhipicephalus evertsi evertsi*. Previous studies (Purchase, 1955; Whitehead & Baker, 1961; Nolan, 1990; Kunz & Kemp, 1994) support this finding as the development of resistance in one-host ticks is usually faster than in two-and three-host ticks. The rate of development of tick resistance against acaricides is also linked to the degree of dominance of the resistant alleles, the frequency of acaricide application and the strength of the acaricide used (Whitehead & Baker, 1961; Stone, 1972).

Our results from the commercial farms are similar to that of the "National Tick Resistance Survey" as both surveys demonstrated high *B. decoloratus* resistance to pyrethroids (Fourie, pers. comm., 2001). The pyrethroid acaricides have a long residual activity (Adams, 1995) and are consequently very effective in controlling ticks when compared with amitraz or chlorfenvinphos. The pyrethroids rapidly eliminate all susceptible members of the target tick population, resulting in a higher incidence of inter-breeding between resistant members. This soon leads to a higher proportion of genetically resistant offspring and an overall increase in resistance in the population (Solomon, 1983).

Multiple resistance to more than one chemical group of acaricides was reported at some of the commercial farms. At "Brycedale" multiple resistance to all three actives tested was extremely worrying, as dipping was no longer controlling the *B.*

decoloratus population. At "Middelfontein" *B. decoloratus* was resistant to both amitraz and cypermethrin. In the "National Tick Resistance Survey" multiple resistance by *B. decoloratus* to chlorfenvinphos and cypermethrin (Fourie, pers. comm., 2001) was reported from the same farm. One must be careful not to change the dipping programme from cypermethrin to another pyrethroid as the use of an alternative pyrethroids might accelerate the selection process, leading to an even more severe pyrethroid resistance problem (Beugnet & Chardonnet, 1995).

In the study areas, few *A. hebraeum* and *R. evertsi evertsi* were collected, however, and *R. appendiculatus* was more numerous in the communal farming areas, probably as a result of more scrub and bush in these areas which protects the free-living immatures. Zebu type cattle were common in the traditional grazing areas and as they are more tick resistant (Baker & Ducasse, 1967) this would have lead to decreased tick burdens. In Uganda, however, Kaiser, Sutherst, & Bourne (1982) found that indigenous Zebu cattle carried heavier *R. appendiculatus* burdens and they concluded that the resistance to this species was not as strong as with other tick species.

Previous DDT-resistance in the field tick population may have resulted in the pyrethroid resistance in East London. This link between the use of the pyrethroid acaricides for tick control and cross-resistance to DDT (Nolan *et al.*, 1989) shows that once ticks have acquired resistance to an acaricide then the ability is retained long after the acaricide has been replaced (Roulston, 1980).

5.2 Comparison of the different resistance testing methods used during this study (SLIT, RET and ELT)

An increased awareness of acaricide resistance has led to the multiplication of a range of bioassay methods used at different laboratories. The *in vitro* methods include the "gauze-bag" technique (Graham & Drummond, 1964), the "teabag" method (Fiedler, 1968) and the "pipette" bioassay method (Kigaye & Matthysse, 1973). At present, however, the most widely used bioassay methods are the AIT (Drummond *et al*., 1973), the SLIT (Shaw, 1966) and the LPT (Stone & Haydock, 1962). During this study we used the SLIT and ELT and the RET methods which are both part of the AIT. All these test methods have their advantages and disadvantages.

The SLIT has the advantage that it uses unfed larvae, which are more easily standardized, and the mortality of the larvae can be recorded easily (Wharton & Roulston, 1970). The larvae are also treated identically which leads to more statistically credible results (Lourens & Shaw, 1975). One disadvantage of this method however is that it magnifies the factors of resistance (FOR) (Lourens & Shaw, 1975). In addition, the exposure of tick larvae for ten minutes in an emulsion of a commercial acaricide is not a satisfactory imitation of the field situation (Lourens & Shaw, 1975).

The ELT and the RET use commercial acaricides at the recommended field concentration to immerse the female ticks (Drummond *et al*., 1973). The big advantage of both the ELT and the RET over the SLIT is that the adult tests can be interpreted earlier, i.e. seven days with the ELT and 42 days with the RET. One disadvantage of both the ELT and RET is that sufficient numbers of fully engorged female ticks are sometimes not available to do the test. Another disadvantage of the ELT and RET is that female ticks may have already started to lay eggs before

they reach the laboratory. The adult tests are not yet standardized so any comparison of results from different laboratories would be difficult (Kemp, pers. comm., 2001). It is also not clear how much of the percentage resistance (%R) in this study was due to loss of control through acaricide resistance in the field. In the present study, however, if the %R was above 80% then resistance was considered to be present.

Although the three test methods could not be compared statistically, the ELT and the RET in most cases showed similar acaricide resistance results which differed from the SLIT. Stendel (1980) also noted that the AIT mimicked the field conditions better than the SLIT and he also noted that there was poor correlation between the larval and adult test results. Malan (1973) and Nari (1981) also reported that data from engorged female ticks did not coincide with similar data from the larval tests.

The acaricide resistance tests differ from screening tests, which are undertaken to select new acaricides because with the former the acaricide is used as a standard to check any changes in the ticks, whilst in the screening tests, the ticks are used to evaluate the chemicals. Naturally there would be advantages in having the same test methods for screening acaricides and detecting acaricide resistance (Busvine, 1977).

5.3 Present tick control programmes in the study area.

Ticks were controlled by fortnightly dipping at the communal dip tanks and weekly or fortnightly dipping on the commercial farms. One consequence of the intensive chemical control approach has been the development of acaricide resistance, as well as the loss of immunity to ticks and TBDs by the hosts. Integrated tick management programmes need to be supported by host resistance studies, chemical control programmes, vaccination of hosts as well as a cost/benefit analysis of the acaricidal programme (Jongejan and Uilenberg, 1994).

An integrated holistic approach to tick management has to be based on all the natural constraints to the ticks and the TBDs. This should include studies on the life cycle and estimates of the economic damage caused, as well as the costs of any control measures which need to be implemented (Tatchell, 1984). Pegram, Hargreaves & Berkvens (1995) suggested a tactical approach for the control of *B. decoloratus* which would allow farmers to monitor the *B. decoloratus* burdens themselves and to treat accordingly. Lighter tick burdens could be ignored allowing a constant tick challenge, which would only need to be controlled periodically. Accurate information on the tick ecology, such as geographical distribution, seasonal variations and preferential attachment sites are all required, together with data on the prevalence of the TBDs at both traditional and commercial livestock production systems in an area (Pegram *et al.*, 1986).

In our study low numbers of *B. decoloratus* ticks were observed on the cattle kept at the communal farms/dip tanks, however, at the commercial farms where more exotic and crossbred cattle were kept, higher numbers of blue ticks were seen.

Kaiser, Sutherst & Bourne (1982), Pegram *et al*. (1986) and Tatchell & Easton (1986) reported that the numbers of *B. decoloratus* on undipped indigenous cattle

was usually too low to cause any significant production losses. De Vos & Potgieter (1983) also concluded that from an epidemiological point of view it was better to tolerate *B. decoloratus* infestations on cattle, as they would ultimately lead to an increased endemic stability to *B. bigemina* and reduce the risk of mortality from redwater. De Vos & Potgieter (1983) also concluded that, unless regular dipping is necessary to limit the direct damage done by ticks, then the control of ticks is not justified economically as a means of minimising the risk of redwater outbreaks.

CHAPTER VI. CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

Larvae were obtained from engorged adult female *A. hebraeum, B. decoloratus, R. appendiculatus* and *R. evertsi evertsi,* from six communal and six commercial farms in five districts of the Eastern Cape and North-West Provinces of South Africa. The larvae were then tested for resistance against the three most important acaricide groups used in these areas, namely, the formamidines (amitraz), the organophosphates (chlorfenvinphos) and the pyrethroids (cypermethrin).

Our results support the hypothesis that single-host ticks develop resistance much faster than multi-host ticks. This trend was recorded on all the farms where single- and multi-host ticks co-existed. It was concluded that the use of acaricides at high frequencies and high concentrations was one of the main causes of tick resistance in the study area. The high dipping frequency and over use of the acaricides removed all the susceptible ticks from the population leaving only resistant males to mate with resistant females, leading to a population of homogenous resistant ticks.

The efficacy of the different acaricide groups in controlling the tick populations at the communal and commercial farms also varied considerably. On the communal farms amitraz was highly effective in controlling both single- and multi-host ticks, whilst cypermethrin and chlorfenvinphos still controlled most of the multi-host ticks.

The populations of *B. decoloratus* on the majority of the commercial farms were equally resistant to amitraz, cypermethrin and chlorfenvinphos. The *A. hebraeum, R. appendiculatus* and *R. evertsi evertsi* populations on the commercial farms were, however, still susceptible to the acaricides used. Resistant *B. decoloratus*

ticks were, however, common on both the communal and the commercial farms. Cypermethrin and chlorfenvinphos were, however, still effective in controlling multi-host ticks. In general, higher level of tick resistance to amitraz was observed on commercial farms than on the communal farms.

A comparison of the three *in vitro* acaricide resistance testing methods (SLIT, RET and ELT) was done on the *B. decoloratus* populations from three dairies with known acaricide resistance problems. The ELT was able to give results after seven days and it was suggested that it could be used as a screen for acaricide resistance. The ELT illustrated resistance at "Brycedale" dairy to amitraz and chlorfenvinphos. The RET, which detects resistance within 42 days, also detected severe acaricide resistance at the "Brycedale" dairy, but not at the other two dairies. The SLIT, which detects resistance after 60 days found OP resistance as well as emerging resistance to amitraz at "Brycedale" and pyrethroid resistance on "Sunny Grove". The SLIT also indicated that the tick population at "Brycedale" was susceptible to cypermethrin. From the above findings it was clear that there was good agreement between the ELT and the RET but poor correlation between the SLIT and the two other tests (ELT and RET).

The different acaricide resistance testing methods such as the SLIT of the "Acaricide Resistance Testing Laboratory" at the Department of Zoology and Entomology, University of the Free State and the ELT and the RET at the SABS in East London were both routinely used during the two year training period. This acquired technology transfer will assist the Government of Ethiopia to also establish a Acaricide Resistance Testing Laboratory in Ethiopia.

The data generated from the study should benefit planning strategies to develop resistance management programmes in South Africa and Ethiopia as well as any

other African countries which need to start acaricide resistance testing programmes.

Presently, in both communal and commercial farming sectors, cattle were kept free of ticks by the regular application of acaricides, which is usually done irrespective of tick burdens. To delay acaricide resistance, this approach needs to be re-evaluated and a shift towards less intensive and more tactical tick control needs to be implemented. For example, strategic tick control with the application of acaricides only at critical times of the year and the use of locally adapted Zebu cattle should help to maintain endemic stability to the TBDs.

6.2 Recommendations

Based on the findings during the study the following recommendations should be implemented:

At "Brycedale" where tick resistance to all three tested acaricides was recorded a new vigorous tick control programme for the dairy has to be considered. This should include:

- Regular ELT tests to monitor the resistance. One needs to repeat the ELT on a regular basis to determine which acaricide group is the most effective. Then use this acaricide sparingly on a threshold or strategic programme.
- Use other acaricide groups with different mechanisms, e.g. growth regulators or systemics.
- Reduce the frequency of acaricide application as this is one of the main causes of resistance (strategic or threshold dipping).
- Use zero grazing or other pasture management techniques where the cattle would not readily encounter ticks.

- Try alternative cash crops for a few years to determine whether the ticks can be starved out.
- Use dairy breeds which are more resistant to ticks.
- Vaccination against the important TBDs which would allow tick burdens on the dairy cows while preventing disease transmission.

At “Sunny Grove” and “Welgevind” where only tick resistance to cypermethrin was detected it is recommended that the farmers should change to amitraz, which is still effective against *Boophilus*. One could also try other acaricide groups, with no history of resistance against them at these dairies.

At the communal farms where acaricide resistance was also detected one should be careful not to change the acaricides as the presently used acaricides were still effectively controlling the multi-host ticks such as *A. hebraeum*. In addition the heavy burden of resistant *B. decoloratus* should increase the levels of endemic stability to *B. bigemina.* The evident cross-resistance between the DDT-resistant and the pyrethroid-resistant strains of ticks need to be taken into consideration when planning any tick control programmes in the Eastern Cape Province.

On the commercial and the communal farms an integrated approach to tick control should be implemented as soon as possible, with emphasis on tick resistant cattle, strategic chemical control of ticks and TBD vaccination of the hosts. The integrated tick control programme should keep the tick burdens at levels where they have no economic effects on production but are high enough to maintain endemic stability to the TBD.

The early detection of acaricide resistance is not presently possible with the current bioassay techniques as they are not sufficiently sensitive to detect low frequencies of resistant individuals in a population. Therefore the development of a rapid,

inexpensive bioassay method, which would give a quick and accurate indication of the presence of resistance in the tick populations should be investigated.

The ELT results from this study were encouraging as they were obtained within a week. In addition, the ELT was less expensive and did not require sophisticated equipment for testing. It should, however, be emphasized that the test has not yet been standardized. Further research on the ELT and the RET should be done urgently so that they can be standardized for future tick resistance studies. The use of SLIT should still be recommended for all National Tick Resistance Surveys.

The establishment of an Acaricide Resistance Testing Laboratory in Ethiopia is imperative as there is a growing problem of tick resistance to the currently used acaricides in that country. This would be essential to safeguard the few effective acaricides we have left and to utilize them as wisely as possible.

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VIII. APPENDICES

Appendix 1. Some of the acaricides registered in South Africa for the control of ticks on cattle (Swan, 2001).

1.Organophosphates

Trade name	Active ingredient	Registration number
Daz-dust, Milborrow (Bayer AH), powder	Diazinon 2 % m/m	G421
Disnis NF dip, Milborrow (Bayer AH), liquid	Chlorfenvinphos 9 % m/v	G1015
Karbadip spray, Milborrow (Bayer AH), powder	Carbaryl 50 % m/m	G1291
Notix, Fort Dodge (Intervet SA), dip	Chlorfenvinphos 30 % m/v	G2506
Steladone, Novartis AH, liquid	Chlorfenvinphos 300g/l	G1328
Supona 30 cattle dip, Fort Dodge (Bayer AH), liquid	Chlorfenvinphos 30 % m/v	G1284
Supona aerosol, Fort Dodge (Bayer AH), aerosol	Chlorfenvinphos 0.5 % m/v, dichlorphos 0.83 %, gentian violet 0.1 % m/v	G411
Tick dressing “S” Milborrow (Bayer AH), grease	Chlorfenvinphos 0.3 % m/m	G434

2. Pyrethrins and synthetic pyrethrorids

Trade name	Active ingredient	Registration number
Agricura tick grease, Agricura (Bayer AH), grease	Cypermethrin 0.025 % m/v	G1104
Bacdip plus (Bayer AH), aerosol	Flumethrin 0.2 % m/v	G50
Bayticol (Bayer AH), liquid	Flumethrin 2 % m/v	G489
Blitzdip, Milborrow (Bayer AH), pour-on	Cypermethrin 1 % m/v	G1049
Clout, Intervet SA, pour-on	Deltamethrin 1 % m/v	G1447
Crede-ecto-cymethrin, experto vet, liquid	Cypermethrin 20 % m/v	G2527
Curatix dip, Fort Dodge (Bayer AH), liquid	Cypermethrin 15 % m/v	G505
Cylence, Bayer AH, liquid	Cyfluthrin 1 % m/v	G1725

Decatix 3, Intervet SA, liquid, dip, spray	Deltamethrin 2.5 % m/v	G1348
Deltab, Intervet. Tablets for spraying/dipping	Deltamethrin 25% m/m	G2517
Back-pack tablets for spraying	Deltamethrin 25% m/m	G2518
Drastic deadline, Bayer AH, pour-on solution	Flumethrin 1 % m/v	G723
Elantik, Elanco, pour-on dip.	Zeta- cypermethrin	G2675
NCD CYP 20%, Logos Agvet, liquid	Cypermethrin 20 % m/v	G2312
Grenade cattle dip and spray, Intervet, SA, liquid	Cyhalothrin 5 % m/v	G1029
Paracide, Pfizer AH, liquid	Alphamethrin 7 % m/v	G791
Prodip CYP 20 %, Logos Agvet, liquid	Cypermethrin 20 % m/v	G2311
Stopatik, Logos Agvet, liquid	Cypermethrin 2 % m/v	G1431
UL-TRI-PAR, Milborrow (Bayer AH), spray	Cypermethrin 0.5 % m/v	G1740

3. Formamidines

Trade name	Active ingredient	Registration number
Amidip 200, Logos Agvet, EC	Amitraz 20 % m/v	G2601
Crede-ecto-imitraz, Experto vet, spray	Amitraz 25 % m/v	G2528
Ecotraz 250, Eco AH, liquid	Amitraz 25 % m/v	G1999
Milbitraz LS, Milborrow (Bayer AH), wet/powder	Amitraz 23.75 % m/v	G2385
Milbitraz spray dip, Milborrow (Bayer AH). Liquid concentrate	Amitraz 12. 75 % m/v	G2084
Triatix, Intervet, cattle spray	Amitraz 12.5 % m/v	G845
LS Dip	Amitraz 23.75 % m/v	G846
Wettable powder cattle spray	Amitraz 23.75 % m/v	G850

4. Combinations

Trade name	Active ingredient	Registration number
Amipor, Logos Agvet , pour-on	Amitraz 1% m/v, cypermethrin m/v, piperonyl butoxide 5 % m/v	G2058
Amispray, Logos Agvet, liquid	Cypermethrin 7 g, Amitraz 25 g/ml	G2551
Crede-ecto-cymatraz, experto vet, liquid	Cypermethrin 7% m/v, Amitraz 25% m/v	G2529
Crede-ecto-tracypor, experto vet, liquid	Cypermethrin 15g, Amitraz 17.5 g/l	G2668
Ectoline, Bayer AH, pour-on	Flumethrin 0.5 % m/v, cyfluthrin 0.5 % m/v	G2002
Ektoban, Novartis AH, liquid	Cymiazol 175 g, cypermethrin (high-cis) 25 g/l	G598
Milborrow tick and maggot oil plus, Milborrow (Bayer AH), oil	Chlorfenvinphos 1 % m/v, cypermethrin 0.1 % m/v, pine oil 4 % m/m	G1494
Paragon, Fort Dodge (Intervet, SA), liquid	Chlorfenvinphos 30 % m/v, esfenvalerate 2.2 % m/v	G2278
Pouracide-NF, Pfizer AH, pour-on	Alphamethrin 0.5 % m/m, cypermethrin 1% m/m, tetrachlorumphos 2 % m/m, piperomyl butoxide 7.5 % m/m	G971
Sumiplus, Fort Dodge (Bayer AH), concentrate	Chlorfenvinphos 30 % m/v, esfenvalerate 2.2 % m/v	G1181
Tickgard, Fort Dodge (Pfizer AH), dip	Chlorfenvinphos 30 % m/v, alphamethrin 3 % m/v	G1486
Zeropar, Fort Dodge (Bayer AH), concentrate	Chlorfenvinphos 30 % m/v, alphamethrin 3 % m/v	G1152

Appendix 2. Acaricide resistance testing procedures

The original reference of the Acaricide Resistance Testing Procedure was from Shaw (1966) and slightly modified at the “Acaricide Resistance Laboratory”, at the University of the Free State (Fourie, pers. comm., 2001).

1. Dipping of the larvae in the different acaricide concentrations

Dipping commenced with a water control, then the weakest acaricide concentration was placed on the magnetic stirrer, which was switched on. With a fine brush, about 200 larvae were picked up from the inside neck of the flask and placed on the filter paper. The bung was pushed back into the neck of the flask with the forceps, and the flask containing larvae was placed back in the petri dish. The brush was stroked backwards along the filter paper to brush the larvae off the bristles; the tip of the brush was stored in uncontaminated acetone tube B. Ten mls of water was transferred with a pipette whilst the stopwatch was switched on and 5 ml of water squirted in a zigzag pattern over the larvae on the filter paper. Another sheet of 11 cm paper was then placed over the ticks, and the remaining five ml of water squirted over the top of the "sandwich".

A pipette filled with the weakest acaricide concentration was used to squirt five ml of acaricide mixture over the larvae, which were on the filter paper. The remaining five ml was pipetted on the top of the “sandwich” after placing another sheet of 11 cm filter paper over the larvae. This procedure was then repeated for each of the seven concentrations used.

2. Method for packetting of the larvae

Ten minutes after larval exposure to the acaricide, the filter paper "sandwich" (water control) was picked up with forceps, gently pressed dry then placed on a piece of a 24 cm filter paper. The "sandwich" was opened with the forceps and each half placed on the dry portion of paper. The forceps were then rinsed in acetone tube A and the first filter paper envelope opened. Whilst holding it open with the left hand, a number five paintbrush was used to pick up and push the larvae through. For the water control an uncontaminated brush was used. Seventy to 100 larvae were stroked as close to the centre of the open envelope as possible. The same procedure was then followed with the replicate envelope. The paintbrush was then placed in acetone tube A and the envelopes sealed with a crimper. After cleaning the tray the brushes were rinsed in acetone.

To packet the other larvae the same procedures were then repeated but this time using a number six brush which was used to pick up the ticks. Once all the different acaricide concentrations were completed, the plates of envelopes were placed in an incubator maintained at 27^{o}C and 80-90 R.H.

3. Reading the test

After 72 hours of acaricide exposure, the envelopes were removed from the incubator starting with the water control, the first envelope was opened and was placed on a sheet of paper and examined under a low power on the microscope. By using a prodder, live ticks, which moved, were squashed as they were counted. The envelope was then turned over onto a clean sheet of paper and gently shaken. All dead ticks were then counted. The number of dead, live, and the total ticks present, were recorded. Only larvae capable of walking were classified as alive and after gentle stimulation with a brush or by breathing CO_2 with doubtful cases. All other

larvae, including those, which move their appendages but did not walk, were recorded as dead. This procedure was repeated with all the envelopes, stacking the completed ones in order of concentration of the acaricide.

Appendix 3. Laboratory methodology for the Reproductive Estimate Test (RET) (Adult Immersion Test).

This test procedure was originally described by Drummond *et al*. (1973) and slightly modified by SABS in East London (Strydom, pers. comm., 2000)

Ticks in each treatment group were weighed and this was recorded. Water was used as a control and the field recommended acaricide concentrations were prepared for each treatment group. Ticks in the control group were transferred to a glass jar (200 ml) and adequately covered with water.

The above mentioned procedure was repeated with acaricide for the ticks in each of the treatment groups, making sure to shake each solution before immersion. Each consecutive treatment group was then immersed at exactly 30 second intervals and a stopwatch was used to ensure proper timing. During the remainder of the ten minutes period, the glass jar containing the ticks and the acaricides were gently agitated several times.

Ten minutes after immersion of the ticks in the control group, the liquid was decanted off, and the ticks were inverted onto a clean, dry piece of filter paper in an aluminium foil dish. All the ticks were placed on their ventral sides, each on a dry portion of the filter paper. The above procedure was then repeated with the ticks in the treated group at exactly 30 seconds intervals. They were then air dried at room temperature for an hour. The ticks were then pasted onto double-sided adhesive strips on glass test panels with their ventral sides facing upwards, keeping their capitula clear of the tape. The glass panels were placed in the incubator maintained at 27 ^{0}C and 80-90 % R. H.

The ticks were examined after seven days and the number of dead ticks i.e. those which had turned black were recorded and this was not seen as mortality due to the acaricide but rather natural die-off. The ticks were re-examined after three weeks at the end of egg-laying period. The eggs laid in each treatment group were then weighed and transferred to labelled flasks and later incubated at 27 ^{o}C and 80-90 % R. H. The results were then recorded on relevant forms. The ELT method is the same as the RET to egg laying stage at seven days.

Appendix 4. Corrected mortality data from the larvae of *A. hebraeum* exposed to different concentrations of amitraz, cypermethrin and chlorfenvinphos

Farm/dip tank	*A. heb.* strain	Amitraz		Cypermethrin		Chlorfenvinphos	
Mabeleni	S-3	Conc.	% CM	Conc.	% CM	Conc.	% CM
		0.000006	34.41	0.00002	48.28	0.00013	20.24
		0.000032	62.64	0.0001	76.10	0.0004	80.96
		0.00016	63.94	0.0005	91.49	0.0012	91.21
		0.0008	56.78	0.002	97.43	0.003	97.48
		0.004	55.28	0.01	98.67	0.01	98.15
		0.02	65.52	0.05	100.00	0.03	100.00
		0.1	96.98	0.2	100.00	0.1	100.00
Mozana	S-6	0.000006	31.83	0.00002	29.26	0.00013	23.76
		0.000032	79.15	0.0001	79.96	0.0004	24.53
		0.00016	86.10	0.0005	95.40	0.0012	54.78
		0.0008	89.35	0.002	97.86	0.003	87.08
		0.004	92.22	0.01	98.40	0.01	98.57
		0.02	93.82	0.05	100.00	0.03	100.00
		0.1	97.96	0.2	100.00	0.1	100.00
Colleywable	S-7	0.000006	34.81	0.00002	42.68	0.00013	40.98
		0.000032	87.62	0.0001	77.39	0.0004	26.60
		0.00016	93.00	0.0005	94.48	0.0012	97.87
		0.0008	96.58	0.002	97.92	0.003	98.20
		0.004	98.35	0.01	99.13	0.01	99.12
		0.02	100.00	0.05	100.00	0.03	100.00
		0.1	100.00	0.2	100.00	0.1	100.00
Cizele	S-11	0.000006	3.80	0.00002	4.37	0.00013	5.67
		0.000032	29.19	0.0001	55.45	0.0004	18.57
		0.00016	57.50	0.0005	86.74	0.0012	50.01
		0.0008	85.32	0.002	94.49	0.003	74.04
		0.004	88.61	0.01	100.00	0.01	81.79
		0.02	90.48	0.05	100.00	0.03	95.98
		0.1	100.00	0.2	100.00	0.1	100.00
Ntubeni	S-12	0.000006	12.83	0.00002	24.66	0.00013	19.53
		0.000032	35.31	0.0001	77.90	0.0004	83.01
		0.00016	80.15	0.0005	93.15	0.0012	91.86
		0.0008	93.44	0.002	97.11	0.003	96.72
		0.004	96.94	0.01	98.40	0.01	100.00
		0.02	99.32	0.05	100.00	0.03	100.00
		0.1	100.00	0.2	100.00	0.1	100.00

Conc. = Concentrations
%CM = Percentage corrected mortality

Appendix 5. Corrected mortality data from the larvae of *B. decoloratus* exposed to different concentrations of amitraz, cypermethrin and chlorfenvinphos

Farm/dip tank	*B. dec.* strain	Amitraz		Cypermethrin		Chlorfenvinphos	
Mabeleni	S-1	Conc.	% CM	Conc.	% CM	Conc.	% CM
		0.000006	75.65	0.00002	8.15	0.00013	17.00
		0.000032	91.93	0.0001	9.65	0.0004	19.31
		0.00016	96.90	0.0005	14.30	0.0012	35.63
		0.0008	98.85	0.002	26.20	0.003	67.33
		0.004	100.00	0.01	29.63	0.01	79.64
		0.02	100.00	0.05	47.63	0.03	91.48
		0.1	100.00	0.2	98.21	0.1	100.00
Mozana	S-4	0.000006	29.61	0.00002	5.67	0.00013	5.67
		0.000032	59.95	0.0001	34.36	0.0004	34.36
		0.00016	81.88	0.0005	43.15	0.0012	43.15
		0.0008	100.00	0.002	72.45	0.003	72.45
		0.004	100.00	0.01	74.99	0.01	74.99
		0.02	100.00	0.05	95.58	0.03	95.58
		0.1	100.00	0.2	100	0.1	100.00
Colleywable	S-8-1	0.000006	44.50	0.00002	24.89	0.00013	39.45
		0.000032	62.94	0.0001	27.25	0.0004	47.14
		0.00016	95.60	0.0005	20.04	0.0012	54.13
		0.0008	98.18	0.002	31.60	0.003	79.46
		0.004	100.00	0.01	41.79	0.01	91.92
		0.02	100.00	0.05	58.32	0.03	91.49
		0.1	100.00	0.2	100.00	0.1	99.10
Cizele	S-10	0.000006	41.68	0.00002	10.19	0.00013	12.02
		0.000032	64.35	0.0001	15.78	0.0004	10.55
		0.00016	86.58	0.0005	22.37	0.0012	15.32
		0.0008	91.81	0.002	20.04	0.003	48.43
		0.004	95.51	0.01	80.82	0.01	88.54
		0.02	100.00	0.05	52.49	0.03	92.83
		0.1	100.00	0.2	100.00	0.1	89.53
Ntubeni	S-15	0.000006	65.51	0.00002	47.29	0.00013	21.93
		0.000032	90.48	0.0001	37.80	0.0004	37.41
		0.00016	97.46	0.0005	38.63	0.0012	24.35
		0.0008	97.67	0.002	36.98	0.003	47.30
		0.004	99.24	0.01	70.72	0.01	94.58
		0.02	100.00	0.05	94.53	0.03	98.46
		0.1	100.00	0.2	100.00	0.1	100.00
Basfontein	S-19	0.000006	12.96	0.00002	11.61	0.00013	5.59
		0.00016	19.82	0.0005	79.76	0.0012	86.62
		0.004	21.73	0.01	97.43	0.01	93.37
		0.1	29.32	0.2	99.13	0.1	94.41

Woodstock	S-20	0.000006	26.77	0.00002	6.77	0.00013	21.02
		0.000032	48.77	0.0001	17.22	0.0004	45.82
		0.00016	70.03	0.0005	71.32	0.0012	93.85
		0.0008	75.53	0.002	91.96	0.003	96.49
		0.004	55.07	0.01	97.32	0.01	98.04
		0.02	90.68	0.05	98.69	0.03	99.34
		0.1	89.32	0.2	100.00	0.1	100.00
Middelfontein	S-23	0.000006	7.65	0.00002	9.58	0.00013	2.09
		0.000032	22.51	0.0001	3.87	0.0004	12.70
		0.00016	33.45	0.0005	6.04	0.0012	61.41
		0.0008	55.04	0.002	5.95	0.003	97.35
		0.004	60.26	0.01	9.75	0.01	98.27
		0.02	44.82	0.05	77.43	0.03	100.00
		0.1	44.83	0.2	99.29	0.1	100.00
Brycedale	S-100	0.000006	9.66	0.00002	14.88	0.00013	9.86
		0.000032	20.50	0.0001	22.25	0.0004	7.62
		0.00016	34.70	0.0005	25.02	0.0012	15.32
		0.0008	46.57	0.002	56.18	0.003	23.14
		0.004	38.13	0.01	64.11	0.01	92.38
		0.02	61.90	0.05	73.50	0.03	100.00
		0.1	65.82	0.2	78.83	0.1	100.00
Sunny Grove	S-101	0.000006	10.36	0.00002	18.88	0.00013	11.70
		0.000032	23.97	0.0001	20.55	0.0004	22.30
		0.00016	36.55	0.0005	32.90	0.0012	22.76
		0.0008	59.07	0.002	22.12	0.003	33.02
		0.004	55.90	0.01	23.61	0.01	95.44
		0.02	63.74	0.05	34.98	0.03	100.00
		0.1	68.41	0.2	45.13	0.1	100.00
Welgevind	S-102	0.000006	9.95	0.00002	24.58	0.00013	15.17
		0.000032	36.51	0.0001	16.25	0.0004	14.37
		0.00016	81.30	0.0005	31.07	0.0012	14.02
		0.0008	95.59	0.002	46.42	0.003	31.45
		0.004	100.00	0.01	94.70	0.01	100.00
		0.02	100.00	0.05	98.73	0.03	100.00
		0.1	100.00	0.2	100.00	0.1	100.00

B. dec. = *B. decoloratus*
Conc. = Concentrations
%CM = Percentage corrected mortality

Appendix 6. Corrected mortality data from the larvae of *R. appendiculatus* exposed to different concentrations of amitraz, cypermethrin and chlorfenvinphos

Farm/dip tank	R.ap strain	Amitraz		Cypermethrin		Chlorfenvinphos	
		Conc.	% CM	Conc.	% CM	Conc.	% CM
Cizele	S-9	0.000006	65.07	0.00002	25.80	0.00013	13.52
		0.000032	84.17	0.0001	49.69	0.0004	52.01
		0.00016	89.36	0.0005	92.74	0.0012	78.47
		0.0008	96.18	0.002	97.94	0.003	96.47
		0.004	99.11	0.01	98.76	0.01	99.38
		0.02	100.00	0.05	100.00	0.03	100.00
		0.1	100.00	0.2	100.00	0.1	100.00
Ntubeni	S-16	0.000006	37.82	0.00002	25.55	0.00013	40.88
		0.000032	79.12	0.0001	77.99	0.0004	87.28
		0.00016	93.81	0.0005	85.19	0.0012	89.11
		0.0008	97.90	0.002	95.15	0.003	96.64
		0.004	99.41	0.01	98.53	0.01	100.00
		0.02	100.00	0.05	100.00	0.03	100.00
		0.1	100.00	0.2	100.00	0.1	100.00
Ciko	S-18	0.000006	46.24	0.00002	22.24	0.00013	30.66
		0.000032	74.44	0.0001	60.96	0.0004	43.31
		0.00016	85.43	0.0005	92.24	0.0012	91.50
		0.0008	97.58	0.002	98.08	0.003	95.15
		0.004	100.00	0.01	99.14	0.01	100.00
		0.02	100.00	0.05	100.00	0.03	100.00
		0.1	100.00	0.2	100.00	0.1	100.00
Middelfontein	S-26	0.000006	NC	0.00002	5.65	0.00013	12.86
		0.000032	NC	0.0001	27.45	0.0004	26.41
		0.00016	NC	0.0005	73.05	0.0012	56.45
		0.0008	NC	0.002	96.82	0.003	92.08
		0.004	NC	0.01	98.47	0.01	97.16
		0.02	NC	0.05	98.26	0.03	100.00
		0.1	NC	0.2	100.00	0.1	100.00

R. ap. = *R. appendiculatus*
Conc. = Concentrations
%CM = Percentage corrected mortality
NC = Not calculated

Appendix 7. Corrected mortality data from the larvae of *R. evertsi evertsi* exposed to different concentrations of amitraz, cypermethrin and chlorfenvinphos

Farm/dip tank	*R.e.e* strain	Amitraz		Cypermethrin		Chlorfenvinphos	
Mozana	S-5	Conc.	% CM	Conc.	% CM	Conc.	% CM
		0.000006	90.40	0.00002	55.54	0.00013	62.82
		0.000032	91.34	0.0001	65.73	0.0004	68.05
		0.00016	94.95	0.0005	83.83	0.0012	88.11
		0.0008	96.97	0.002	94.10	0.003	94.49
		0.004	100.00	0.01	100.00	0.01	100.00
		0.02	100.00	0.05	100.00	0.03	100.00
		0.1	100.00	0.2	100.00	0.1	100.00
Ntubeni	S-17	0.000006	89.60	0.00002	59.44	0.00013	24.12
		0.000032	93.07	0.0001	80.47	0.0004	64.29
		0.00016	98.71	0.0005	97.03	0.0012	95.07
		0.0008	99.39	0.002	98.88	0.003	97.77
		0.004	100.00	0.01	100.00	0.01	98.71
		0.02	100.00	0.05	100.00	0.03	100.00
		0.1	100.00	0.2	100.00	0.1	100.00
Woodstock	S-21	0.000006	77.81	0.00002	50.93	0.00013	40.83
		0.000032	94.03	0.0001	87.81	0.0004	71.46
		0.00016	97.11	0.0005	97.55	0.0012	88.03
		0.0008	98.73	0.002	98.73	0.003	97.36
		0.004	100.00	0.01	100.00	0.01	99.27
		0.02	100.00	0.05	100.00	0.03	100.00
		0.1	100.00	0.2	100.00	0.1	100.00
Middelfontein	S-24	0.000006	75.61	0.00002	40.42	0.00013	0.13
		0.000032	73.79	0.0001	80.28	0.0004	13.43
		0.00016	88.23	0.0005	95.26	0.0012	26.91
		0.0008	96.23	0.002	97.34	0.003	49.18
		0.004	98.66	0.01	98.47	0.01	64.23
		0.02	100.00	0.05	100.00	0.03	92.63
		0.1	100.00	0.2	100.00	0.1	100.00

R.e.e = *R. evertsi evertsi*
Conc. = Concentrations
%CM = Percentage corrected mortality

Appendix 8. Act 36 of 1947: Fertilizers, farm feeds, agricultural remedies and stock remedies Act (Cotton, pers. comm., 2001)

All stock remedies are controlled through this Act and these are marketed, registered and administered according to the Fertilizers, Farm Feeds, Agricultural Remedies and Stock Remedies Act (Act 36 of 1947).

One of the remedies considered for registration is the "dips and ecto-parasite control remedies". Some of the procedures needed before the stock remedy is registered are outlined below:

- A remedy is not registered if the effectiveness is not fully proved.
- Both the safety of the animal being treated, as well as the humans who may consume animal products and who applies the remedy must be guaranteed
- Stock remedies can be easily be identified by the phrase "for animal use only" or external use as well as "Registration Number G…; Act 36/1947 in both official languages, at the top of the label. The label of each remedy is carefully controlled to ensure that it is accurate.

IX. PERSONAL COMMUNICATIONS

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