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

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ABSTRACT

A field study (February 2000 to August 2001) was conducted on communal and commercial farms in the Eastern Cape and North-West Provinces of South Africa to detect the levels of tick resistance to commonly used acaricides. The larvae obtained from engorged females of the one-host tick Boophilus decoloratus, the two-host tick Rhipicephalus evertsi evertsi and the three-host ticks Amblyomma hebraeum and Rhipicephalus appendiculatus were tested against various concentrations of amitraz, chlorfenvinphos and cypermethrin using the Shaw Larval Immersion Test method. Ticks from the communal farms showed higher levels of resistance to cypermethrin and some resistance to chlorfenvinphos whilst no resistance was detected against amitraz. However, ticks from commercial farms were equally resistant to amitraz, chlorfenvinphos and cypermethrin. The B. decoloratus populations tested were considerably more resistant to all the acaricides tested than the R. evertsi evertsi, A. hebraeum and R. appendiculatus populations. This supports the hypothesis that single-host ticks develop resistance faster than multi-host ticks. This trend was recorded on most of 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 areas. Possible factors which caused the resistance problems are discussed and acaricide management strategies recommended.

Keywords: Acaricide resistance, commercial farms, communal farms, single and multi-host ticks, South Africa, tick control

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). Because of the direct and indirect effects on their hosts, ticks are considered to be not only a significant threat to successful livestock production, but also seriously interfere with the economy of a country, especially in Africa (De Castro 1997). Ticks that are of major economic importance in South Africa include Amblyomma hebraeum, Boophilus decoloratus, Boophilus microplus, Rhipicephalus appendiculatus and Rhipicephalus evertsi evertsi (Walker
Acaricide resistance profiles of ticks in Eastern Cape and North-West Provinces of South Africa

1991). Historically these ticks have been controlled by the application of acaricides and this has led directly to the growing problem of tick resistance (Spickett 1998) that had previously been reported in the literature (Du Toit, Graf & Bekker 1941; Whitnall, Thorburn, McHardy, Whitehead & Meerholz 1952; Whitehead 1956; Baker, Miles, Robertson, Stanford & Taylor 1978; Taylor & Oberem 1995).

The main objective of the present study was to detect the levels of tick resistance to acaricides on selected communal and commercial farms in South Africa and to suggest possible acaricide management strategies that may increase the lifespan of the presently available acaricides. The tick species tested in this study were B. decoloratus (one-host tick), R. evertsi evertsi (two-host tick), and A. hebraeum and R. appendiculatus (three-host ticks).

MATERIALS AND METHODS

Study areas

The study was conducted (February 2000 to August 2001) at pre-selected communal farms/dip tanks \((n = 6)\) and commercial farms \((n = 6)\) in the North-West and Eastern Cape Provinces of South Africa.

The six communal farms/dip tanks all in the Eastern Cape were Mabeleni \((32\,^\circ\,58'\,S, 27\,^\circ\,35'\,E)\) and Mozana \((33\,^\circ\,14'\,S, 27\,^\circ\,28'\,E)\) in the East London district; Colleywable \((32\,^\circ\,01'\,S, 28\,^\circ\,34'\,E)\) and Cizele \((32\,^\circ\,02'\,S, 28\,^\circ\,30'\,E)\) in the Idutywa district and Ntubeni \((32\,^\circ\,19'\,S, 28\,^\circ\,48'\,E)\) and Ciko \((32\,^\circ\,15'\,S, 28\,^\circ\,35'\,E)\) in the Willowvale district.

Ticks were also collected from six commercial farms: Middelfontein \((25\,^\circ\,51'\,S, 27\,^\circ\,10'\,E)\) and Basfontein \((25\,^\circ\,54'\,S, 27\,^\circ\,09'\,E)\) in the Koster district and Woodstock \((25\,^\circ\,39'\,S, 26\,^\circ\,54'\,E)\) in the Swaruggens district all in the North-West Province, as well as at Brycedale \((30\,^\circ\,10'\,S, 27\,^\circ\,40'\,E)\), Sunny Grove \((33\,^\circ\,10'\,S, 27\,^\circ\,40'\,E)\) and Welgevind \((33\,^\circ\,04'\,S, 27\,^\circ\,46'\,E)\) in the East London district of the Eastern Cape Province.

Acaricide resistance testing procedures

Engorged female B. decoloratus, R. evertsi evertsi, A. hebraeum and R. appendiculatus were collected from the farms and incubated at 27°C and 80–90% relative humidity (RH) until they laid eggs that subsequently hatched into larvae. The larvae were then tested against various concentrations of amitraz 12.5 % m/v ("Triatix®"—Intervet South Africa Pty Ltd), chlorfenvinphos 30 % m/v ("Supona 30®"—Fort Dodge, Bayer Animal Health Pty Ltd) and cypermethrin 15 % m/v ("Curatik Dip®"—Fort Dodge, Bayer Animal Health Pty Ltd) using the "Shaw Larval Immersion Test" (SLIT). This assay was originally described by Shaw (1966) and later modified to include a longer holding period for the larval ticks after treatment (Shaw, Cook & Carson 1968). Mortality dose data were subjected to probit analysis using a BMDP statistical package (L.J Fourie, personal communication 2001). Factors of resistance (FOR) were calculated by comparing the response of larval ticks from the field which had been exposed to acaricides, with baseline data from susceptible laboratory strains of ticks, on the basis of their respective LC\(_{50}\) values. All the resistance testing was conducted at the Acaricide Resistance Testing Laboratory, Department of Zoology and Entomology, University of the Free State.

RESULTS

The LC\(_{50}\) values for amitraz, chlorfenvinphos and cypermethrin for the four tick species, as well as the FOR for each tick species and each acaricide tested are summarized in Tables 1 and 2. Susceptible reference strain values are included. The larvae obtained from engorged female ticks collected from the field were considered to be resistant when the FOR values were more than 100 for amitraz and cypermethrin or more than five for chlorfenvinphos. They were considered to be showing emerging resistance when the 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 based on the results of previous field trials (R.J. Taylor, personal communication 2001).

Boophilus 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 1). At Brycedale (Table 2) B. decoloratus was resistant to chlorfenvinphos and also showed emerging resistance to amitraz. At Sunny Grove (Table 2) resistance was detected against cypermethrin and emerging resistance to chlorfenvinphos with B. decoloratus. At Welgevind (Table 2) 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 2). At Basfontein (Table 2) a high
TABLE 1 Summary of tick resistance data collected from the communal farms/dip tanks \((n = 6)\) located in the Eastern Cape Province

<table>
<thead>
<tr>
<th>District</th>
<th>Dip tank</th>
<th>Tick species</th>
<th>Amitraz</th>
<th>Cypermethrin</th>
<th>Chlorfenvinphos</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>FOR</td>
<td>°R</td>
<td>FOR</td>
</tr>
<tr>
<td>East London</td>
<td>Mabeleni</td>
<td><em>B. decoloratus</em></td>
<td>0.005</td>
<td>S</td>
<td>280.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>A. hebraeum</em></td>
<td>0.260</td>
<td>S</td>
<td>1.000</td>
</tr>
<tr>
<td></td>
<td>Mozana</td>
<td><em>B. decoloratus</em></td>
<td>0.376</td>
<td>S</td>
<td>501.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>R. evertsi evertsi</em></td>
<td>0.0003</td>
<td>S</td>
<td>0.850</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>A. hebraeum</em></td>
<td>0.070</td>
<td>S</td>
<td>2.067</td>
</tr>
<tr>
<td>Idutywa</td>
<td>Colleywable</td>
<td><em>B. decoloratus</em></td>
<td>0.172</td>
<td>S</td>
<td>56.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>A. hebraeum</em></td>
<td>0.063</td>
<td>S</td>
<td>1.200</td>
</tr>
<tr>
<td></td>
<td>Cizele</td>
<td><em>B. decoloratus</em></td>
<td>0.189</td>
<td>S</td>
<td>90.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>A. hebraeum</em></td>
<td>1.300</td>
<td>S</td>
<td>7.333</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>R. appendiculatus</em></td>
<td>0.013</td>
<td>S</td>
<td>0.225</td>
</tr>
<tr>
<td>Willowvale</td>
<td>Ntubeni</td>
<td><em>B. decoloratus</em></td>
<td>0.016</td>
<td>S</td>
<td>5.65</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>R. evertsi evertsi</em></td>
<td>0.0003</td>
<td>S</td>
<td>0.325</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>A. hebraeum</em></td>
<td>0.43</td>
<td>S</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>R. appendiculatus</em></td>
<td>0.0014</td>
<td>S</td>
<td>0.190</td>
</tr>
<tr>
<td></td>
<td>Ciko</td>
<td><em>R. appendiculatus</em></td>
<td>0.011</td>
<td>S</td>
<td>0.030</td>
</tr>
</tbody>
</table>

FOR = Factor of resistance  
ER = Emerging resistance  
S = Susceptible  
°R = Degree of acaricide resistance

TABLE 2 Summary of tick resistance data collected from the commercial farms \((n = 6)\) in the Eastern Cape and North-West Provinces

<table>
<thead>
<tr>
<th>District</th>
<th>Farm</th>
<th>Tick species</th>
<th>Amitraz</th>
<th>Cypermethrin</th>
<th>Chlorfenvinphos</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>FOR</td>
<td>°R</td>
<td>FOR</td>
</tr>
<tr>
<td>East London (Eastern Cape Province)</td>
<td>Brycedale</td>
<td><em>B. decoloratus</em></td>
<td>77.75</td>
<td>ER</td>
<td>42.05</td>
</tr>
<tr>
<td></td>
<td>Sunny Grove</td>
<td><em>B. decoloratus</em></td>
<td>31.34</td>
<td>S</td>
<td>&gt; 300</td>
</tr>
<tr>
<td></td>
<td>Welgevind</td>
<td><em>B. decoloratus</em></td>
<td>0.97</td>
<td>S</td>
<td>12.03</td>
</tr>
<tr>
<td>Koster (North-West Province)</td>
<td>Middelfontein</td>
<td><em>B. decoloratus</em></td>
<td>311.05</td>
<td>R</td>
<td>212.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>R. evertsi evertsi</em></td>
<td>0.006</td>
<td>S</td>
<td>0.850</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>R. appendiculatus</em></td>
<td>IL</td>
<td>NC</td>
<td>0.950</td>
</tr>
<tr>
<td></td>
<td>Basfontein</td>
<td><em>B. decoloratus</em></td>
<td>&gt; 300</td>
<td>R</td>
<td>2.297</td>
</tr>
<tr>
<td>Swartruggens (North-West Province)</td>
<td>Woodstock</td>
<td><em>B. decoloratus</em></td>
<td>0.981</td>
<td>S</td>
<td>4.240</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>R. evertsi evertsi</em></td>
<td>0.0024</td>
<td>S</td>
<td>0.500</td>
</tr>
</tbody>
</table>

FOR = Factor of resistance  
R = Resistant  
ER = Emerging resistance  
S = Susceptible  
IL = Insufficient larvae  
NC = Not calculated  
°R = Degree of acaricide resistance
level of amitraz resistance was shown by B. decoloratus. Amblyomma hebraeum larvae were susceptible to both amitraz and cypermethrin and showed emerging resistance to chlorfenvinphos at Cizele farm (Table 1).

In summary, B. decoloratus displayed two amitraz, four cypermethrin and four chlorfenvinphos resistant strains and one, two and four emerging resistance strains to amitraz, cypermethrin and chlorfenvinphos respectively. The test results also revealed one case of a resistant strain of R. evertsi evertsi, and one emerging resistant strain of A. hebraeum to chlorfenvinphos. All the R. appendiculatus larvae tested were susceptible to the three acaricides.

DISCUSSION

Tick resistance to acaricides on the communal farms/dip tanks

The larval progeny of B. decoloratus collected at the communal farms/dip tanks demonstrated high levels of resistance to both chlorfenvinphos (one resistant and four emerging resistant farms) and cypermethrin (two resistant and two emerging resistant farms).

Boophilus decoloratus from the Mabeleni and Mozana dip tanks in the East London district showed considerable resistance to cypermethrin. In this area DDT was extensively used from 1949 to 1955 (Whitehead 1956). The possibility that previous DDT resistance may be associated with the current pyrethroid resistance has to be considered (Coetzee, Stanford & Davis 1987). Although cypermethrin resistance could have evolved through cross-resistance from DDT resistant strains of B. decoloratus at these communal dip tanks, high selection pressure could also have been responsible. Both dip tanks are close to the coast, where there are high humidity and high temperatures for most of the year, conditions that are ideal for tick populations to proliferate rapidly. Under such favourable climatic conditions ticks are able to thrive all year round and continuous 2-week interval dipping was necessary to control them.

This intensive dipping programme probably also contributed to an increased selection pressure for resistance. “Zeropar®” (Bayer), which is a mixture of alphamethrin and chlorfenvinphos, had been used at these dip tanks for nearly 6 years indicating that ticks in this area had been exposed to high levels of both pyrethroid and organophosphate (OP) active compounds.

It was clear that the B. decoloratus populations had developed significant resistance to all the currently used acaricides. The reasons for the more rapid selection for resistance in one-host ticks include a shorter generation time (Norval, Barrett, Perry & Mukhebi 1992) that leads to a quick succession of generations of ticks being exposed to the chemicals, resulting in the selective elimination of the majority of the susceptible individuals in the population (Matthewson & Baker 1975).

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 the adults detach (Walker 1991). As the life-cycle is so short this tick species is able to pass through two to four generations per year (Pegram, Perry, Mussisi & Mwanaumo 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 (I.G. Horak, personal communication 2001). The one-host life-cycle 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 attach to the time the engorged nymphs drop. The adult tick remains attached for 7 days and this tick has a continuous life-cycle 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, Keirans & Horak 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, Potgieter, Walker, De Vos & Boomker 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 (Loubser 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, Stoltz & Heyne. 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.

The larvae of *A. hebraeum* and *R. appendiculatus*, which are both three-host ticks, were susceptible to the acaricides tested. Only at Cizele dip tank did *A. hebraeum* show emerging resistance to chlorfenvinphos. Baker et al. (1978) previously reported that *A. hebraeum* was fully susceptible to chlorfenvinphos in dip tanks in the East London and Willowvale districts.

One of the most important factors, which affect 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. 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 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 (Solomon 1983). Each successive dipping would be a selective process that 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).

Breakdown in dipping efficacy is a common phenomenon in the Eastern Cape Province and it is often due not to acaricide failure to control ticks but rather to incorrect acaricide application (Shaw 1966). During this study acaricide failure at the Ntubenip tank in the Willowvale district resulted in inefficient tick control. The communal farmers complained that the ticks did not die after being dipped and concluded that the acaricide was not working. This coincided with the period during which heavy tick burdens of *B. decoloratus*, *R. evertsi evertsi*, *A. hebraeum* and *R. appendiculatus* were present 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 chlorfenvinphos was recorded in *B. decoloratus*. The dip tank manager at Ntubenip had not been replenishing the tank at the recommended concentration, which resulted in the poor tick control.

**Tick resistance to acaricides on the commercial farms**

The extent of resistance to chlorfenvinphos compared to either amitraz or cypermethrin was greater. This is surprising, as with the exception of Brycevale, OP acaricides had not been used for the past 10 years on any of the farms studied. As resistance to chlorfenvinphos was still detected, one can deduce that once resistance has become established in a tick population, the reversion of OP resistance in ticks back to susceptible 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 in use, 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 (Baker 1982; Whitehead 1959; Shaw et al. 1968).

Many commercial dairy farmers in the East London district use “Ektoban®” (Novartis), which is a mixture of cymiazol and cypermethrin. *Boophilus decoloratus* resistance to amitraz on these farms may have been as a result of cross-resistance with cymiazol, a chemical belonging to the same group as amitraz (Nolan 1981). Amitraz-resistant strains of *B. decoloratus* have previously been reported in South Africa (Taylor & Oberem 1995).

In summary there was much less resistance in the two- and three-host ticks when compared with that experienced by the one-host tick. This was probably due to the fact that there was much less contact with acaricides because they spend a greater proportion of their life-cycle off the host, have a broader
host range such as wild animals (Kunz & Kemp 1994) and longer life-cycle (Wharton & Roulston 1970). This would significantly reduce the selection pressure from acaricides on the multi-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 remove susceptible ticks, leaving only resistant males to mate with resistant females, leading to the proliferation of resistant ticks.

CONCLUSIONS AND RECOMMENDATIONS

Our results support the hypothesis that single-host ticks develop resistance faster than multi-host ticks. This trend was recorded on most of the farms where single- and multi-host ticks co-existed. It is concluded that the use of acaricides at high frequencies and high concentrations is one of the main causes of tick resistance in the study area.

Based on the findings during the study the following are recommended:

- On the communal farms where acaricide resistance was detected one should be careful not to change the acaricides as those used during the study period were still effectively controlling the multi-host ticks. The possible cross-resistance between DDT- and pyrethroid-resistant strains of ticks needs to be taken into consideration when planning any tick control programmes in the Eastern Cape Province.

- On the communal and commercial 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 the control of tick-borne diseases (TBDs) by way of 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 TBDs.

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 tick populations should be investigated.

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