Comparison of 3 tests to detect acaricide resistance in *Boophilus decoloratus* on dairy farms in the Eastern Cape Province, South Africa

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ABSTRACT

The susceptibility of the larval offspring of engorged female *Boophilus decoloratus,* and of the engorged females, collected from cattle on the dairy farms Brycedale, Sunny Grove and Welgevind in the Eastern Cape Province, South Africa, was tested against the acaricides amitraz, chlorfenvinphos and cypermethrin. Resistance was determined by means of the Shaw Larval Immersion Test (SLIT) for larvae and the Reproductive Estimate Test (RET) and Egg Laying Test (ELT) for adults. At Brycedale the tests all indicated resistance to chlorfenvinphos, and RET and ELT indicated resistance to amitraz and emerging resistance to cypermethrin. At Sunny Grove, *B. decoloratus* was resistant to cypermethrin using SLIT and exhibited emerging resistance to chlorfenvinphos with SLIT and to cypermethrin with both RET and ELT. At Welgevind, resistance was recorded against chlorfenvinphos (SLIT) and against cypermethrin (ELT), and emerging resistance against permethrin (RET). The results obtained with RET and ELT were generally comparable, but often differed from those obtained with SLIT. Resistance could be detected within 7 days with ELT compared to 42 days with RET and 60 days with SLIT.

Key words: acaricide resistance tests, *Boophilus decoloratus,* dairy cattle, South Africa.

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INTRODUCTION

The resistance of *Boophilus decoloratus* to acaricides has been a problem on farms in the Eastern Cape Province of South Africa for more than 60 years[16]. The occurrence of resistance in the closely related and more widely distributed *Boophilus microplus* has resulted in the development of a number of laboratory and field tests, entailing the use of either larvae or adult ticks, to detect this phenomenon[9]. The present study describes the evaluation of 3 of these tests in detecting acaricide resistance in *B. decoloratus*. The tests used were the Shaw Larval Immersion Test (SLIT) for tick larvae, and the Reproductive Estimate Test (RET) and the Egg Laying Test (ELT) for adult ticks.

A slight modification of SLIT, first described by Shaw[7], was used. In it the larvae were incubated for 72 h after treatment before the test was read[8]. In RET, engorged female ticks are exposed to acaricide and their subsequent production of larvae is used as a measure of resistance[3]. This test was originally used to evaluate the efficacy of new acaricides[3], as well as in acaricide resistance testing[13]. ELT is based on a comparison of the number of eggs laid by treated and untreated engorged female ticks. These tests were applied to ticks collected from cattle on dairy farms that had reported the failure of tick control regimes.

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MATERIALS AND METHODS

Study localities

The studies were conducted from April to August 2001 on the farms Brycedale (30°10′S, 27°40′E), Sunny Grove (33°10′S, 27°40′E) and Welgevind (33°04′S, 27°46′E) in the East London district of the Eastern Cape Province, South Africa.

Test methods

Three bio-assays of acaricide resistance, namely SLIT, RET and ELT, were compared during the study, and the resistance of *Boophilus decoloratus* to 3 commercially available acaricides was assessed. The active ingredients of these acaricides were amitraz (Triatix, Intervet SA), chlorfenvinphos (Supona, Bayer Animal Health, Fort Dodge) and cypermethrin (Curatik Dip, Bayer Animal Health, Fort Dodge). The recommended field concentrations (amitraz 0.025 %; chlorfenvinphos 0.05 %; cypermethrin 0.015 %) were used in RET and ELT.

SLIT: the test used was that originally described by Shaw[7] and later modified to increase the period of larval incubation after treatment to 72 h before the test was read[8]. Empirical studies have shown that a factor of resistance (FOR) of >100 for both amitraz and cypermethrin and >5 for chlorfenvinphos indicate resistance. FOR values between 50 and 100 for both amitraz and cypermethrin and values between 2.5 and 5 for chlorfenvinphos are regarded as indicating emerging resistance (R J Taylor, unpubl. data, 2001). The susceptible Botshabelo reference strain of *B. decoloratus* was used to calculate the FOR values and the results could be read after 60 days.

RET: engorged female *B. decoloratus* of uniform size and free of visible abnormalities were collected from cattle on the 3 farms. The ticks were washed in water, air-dried[11], and grouped according to size. Groups of 10 ticks were weighed and randomly allocated to 2 or 4 replicates for each treatment and the control group. The treatment groups were immersed in the recommended concentrations of the acaricides and the control group was immersed in sterile water. The test and control groups were incubated at 27 °C and 80–90 % relative humidity (RH), to permit oviposition and egg hatching[3].

At the completion of hatching, which, for *B. decoloratus,* usually takes 42 days,

Table 1: **The susceptibility of *Boophilus decoloratus* larvae to acaricides on dairy farms in the Eastern Cape Province.**

Farm	Acaricide								
	Amitraz			Chlorfenvinphos			Cypermethrin		
	LC_{50}	FOR	Status	LC_{50}	FOR	Status	LC_{50}	FOR	Status
Reference strain	4.2×10^{-5}			4.1×10^{-4}			5.7×10^{-5}		
Brycedale	3.3×10^{-3}	77.751	ER	2.6×10^{-3}	6.199	R	2.4×10^{-3}	42.049	S
Sunny Grove	1.3×10^{-3}	31.340	S	2.0×10^{-3}	4.860	ER	2.0×10^{-1}	>200	R
Welgevind	4.1×10^{-5}	0.971	S	2.4×10^{-3}	5.920	R	6.8×10^{-4}	12.032	S

LC_{50} = acaricide concentration that kills 50% of ticks; FOR = factor of resistance.
S = susceptible; ER = emerging resistance; R = resistant.

the Reproductive Estimate (RE) was calculated using the following formula to estimate the number of larvae that had hatched:

$$RE = m_1 \times n \times h \quad m_2 \times s \times 4,$$

where 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: 0, zero hatching; 1, <25 %; 2, 25–50 %; 3, 50–75 %; 4, 75–100 %); and s = number of female ticks surviving after 7 days of incubation.

The %RE for female ticks was calculated as follows:

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

An RE value of >80 % indicates resistance, and we consider RE values between 50 and 80 % as indicative of emerging resistance.

ELT: the collection and incubation of engorged female *B. decoloratus* was similar to that described for RET, but the number of ticks that had laid eggs was assessed on the 7th day of incubation at 27 °C and 80–90 % RH (Kemp, pers. comm., 2001).

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

A value of >80 % indicates resistance, and between 50 and 80 % emerging resistance.

RESULTS

SLIT

The larval offspring of *B. decoloratus* females collected on the farms Brycedale, Sunny Grove and Welgevind showed resistance to 1 or more of the test acaricides (Table 1). At Brycedale, emerging resistance to amitraz was recorded, while at Sunny Grove and Welgevind, larvae were susceptible to this chemical. At both Brycedale and Welgevind, resistance to chlorfenvinphos was present, while at Sunny Grove emerging resistance was recorded. At Brycedale and Welgevind the ticks were susceptible to cypermethrin, but at Sunny Grove they were highly resistant.

RET

On Brycedale, *B. decoloratus* was resistant to amitraz and chlorfenvinphos, whereas on both Sunny Grove and Welgevind it was susceptible to both these acaricides (Table 2).

ELT

Female *B. decoloratus* on Brycedale were resistant to amitraz and chlorfenvinphos and showed emerging resistance to cypermethrin (Table 3). At Sunny Grove this tick was susceptible to amitraz and chlorfenvinphos, but displayed an emerging resistance to cypermethrin. At Welgevind, resistance only to cypermethrin was detected.

DISCUSSION

The results obtained with SLIT cannot be compared directly with those obtained with either RET or ELT as the first test is based on assessing the resistance of tick larvae to acaricides, whereas the last 2 are based on assessments engorged female ticks. The pattern of resistance recorded on the 3 farms confirms these differences in that results obtained with SLIT often differed from those obtained with RET and ELT (Table 4).

Organophosphate (OP) acaricides had not been used for the past 10 years on either Sunny Grove or Welgevind (Amaral, pers. comm., 2001). As SLIT detected emerging resistance or resistance to

Table 2: **Reproductive estimate for engorged female *Boophilus decoloratus* after immersion in acaricide and 7 days' incubation.**

Farm	Acaricide	No. of female ticks		Total mass (mg)		Hatching estimate (1–4) (h)	Reproductive estimate (RE)	% Reproductive estimate (%RE)	Resistance status
		Immersed (n)	Alive after 7 days (s)	Eggs (m_1)	Ticks (m_2)				
Brycedale	Amitraz	20	20	1503	3790	4.00	0.397	84.83	R
	Chlorfenvinphos	20	20	1620	3771	3.50	0.376	80.34	R
	Cypermethrin	20	18	1406	3822	3.00	0.307	65.60	ER
	Control	20	19	1687	3791	4.00	0.468		
Sunny Grove	Amitraz	20	20	1154	4573	2.00	0.126	25.45	S
	Chlorfenvinphos	20	20	3	4446	0.00	0.000	0.00	S
	Cypermethrin	20	20	1629	4397	3.00	0.278	56.16	ER
	Control	20	20	2250	4543	4.00	0.495		
Welgevind	Amitraz	40	40	15	8623	0.00	0.000	0.00	S
	Chlorfenvinphos	40	37	721	8546	1.75	0.040	7.46	S
	Cypermethrin	40	40	3545	8496	3.00	0.313	58.40	ER
	Control	40	39	4527	8670	4.00	0.536		

S = susceptible; ER = emerging resistance; R = resistant.

Table 3: **Ovipositing response of engorged adult female *Boophilus decoloratus* after immersion in acaricides and 7 days' incubation.**

Farm	Acaricide	No. of female ticks			Resistance	
		Immersed and incubated	After 7 days		Percentage	Status
			Alive	Ovipositing		
Brycedale	Amitraz	20	20	16	84.2	R
	Chlorfenvinphos	20	20	18	94.7	R
	Cypermethrin	20	18	14	73.7	ER
	Water-treated control	20	19	19		
Sunny Grove	Amitraz	20	20	7	38.8	S
	Chlorfenvinphos	20	20	1	5.6	S
	Cypermethrin	20	20	13	72.2	ER
	Water-treated control	20	20	18		
Welgevind	Amitraz	40	40	0	0.0	S
	Chlorfenvinphos	40	37	5	12.8	S
	Cypermethrin	40	40	32	82.1	R
	Water-treated control	40	39	39		

S = susceptible; ER = emerging resistance; R = resistant.

Table 4: **Comparison of the results of 3 methods to determine the acaricide resistance status of *Boophilus decoloratus.***

Farm	Test	Acaricide and resistance status		
		Amitraz	Chlorfenvinphos	Cypermethrin
Brycedale	Larval immersion	ER	R	S
	Reproductive estimate	R	R	ER
	Egg-laying	R	R	ER
Sunny Grove	Larval immersion	S	ER	R
	Reproductive estimate	S	S	ER
	Egg-laying	S	S	ER
Welgevind	Larval immersion	S	R	S
	Reproductive estimate	S	S	ER
	Egg-laying	S	S	R

S = susceptible; ER = emerging resistance; R = resistant.

chlorfenvinphos on both farms more than 10 years after the last use of an OP acaricide it would appear that once OP resistance has become established in a tick population its reversion to susceptibility is either very slow or does not occur[12]. The OP resistance on these farms could have originally been induced by acaricides containing OPs other than chlorfenvinphos, as resistance to 1 member of a group of chemically related acaricides can result in a degree of resistance to other members of the same group or a closely related group[1,8,15]. Furthermore cross-resistance between chemically related acaricides has previously been documented for *B. decoloratus* within the region of the present study[2].

'Ektoban' (Bayer Animal Health), which is a mixture of cypermethrin and cymiazol, had been used for tick control for nearly 10 years on both Sunny Grove and Welgevind and the owners reported that it no longer controlled ticks. Resistance to cypermethrin was detected on Sunny Grove with SLIT and on Welgevind with ELT. The high burdens of *B. decoloratus* observed on the cattle at Brycedale were in agreement with the results obtained from the acaricide resistance tests conducted in the laboratory.

Each of the 3 tests has certain practical advantages. SLIT uses unfed larvae, which are more easily standardised than adult ticks, and the mortality of the larvae can be recorded easily[14]. The larvae are also treated identically, leading to statistically more credible results[4]. A disadvantage of this method, however, is that the exposure of larvae to an emulsion of a commercial acaricide for 10 minutes is not a satisfactory imitation of the field situation[4]. Both RET and ELT require that engorged female ticks be immersed in commercial acaricides at the recommended field concentration[3]. The advantage of these tests is that they can be interpreted earlier than SLIT, which requires 60 days, namely within 42 days for RET and within 7 days for ELT. A disadvantage is that sufficient fully engorged female ticks are not always readily available for the tests[6].

Although the results of the 3 test methods could not be compared statistically, RET and ELT in most cases showed similar results and these often differed from those obtain by SLIT (Table 4). In previous studies a poor correlation between larval and adult test results has also been observed[5] and it has been stated that the Adult Immersion Test (AIT) reflected field conditions better than SLIT[10].

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