The use of a pour-on and spray dip containing Amitraz to control ticks (Acari: Ixodidae) on cattle

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ABSTRACT

Knockdown and persistence efficacies of a pour-on containing Amitraz 1 % and Cypermethrin 1 % and a spray dip containing 12.5 % Amitraz were compared. Knock-down and persistence efficacies of the Amitraz spray dip against *Rhipicephalus (Boophilus) decoloratus* and *Amblyomma hebraeum* were significantly higher for the duration of the trial than those of the pour-on. In the case of *Rhipicephalus appediculatus* and *Rhipicephalus evertsi evertsi*, efficacy was significantly higher on Days 2 and 5. Resistance testing prior to the start of the trial indicated that *R. (B.) decoloratus* was resistant to both Cypermethrin and Amitraz, yet in the case of the spray dip excellent efficacy results were obtained.It is thought that the cattle's scruffy winter coat may have hindered the spread of the pour-on, but that the thorough wetting and especially the higher concentration of active ingredient applied v*ia* the spray dip allowed this formulation to be effective. These results show that under certain conditions a spray dip containing 12.5 % Amitraz may be more effective than a pour-on containing 1 % Amitraz and 1 % Cypermethrin, despite apparent *in vitro* resistance.

Key words: acaricides, amitraz, cattle, formamidines, pour-on, pyrethroids, spray dip, ticks.

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INTRODUCTION

Ticks and the tick-borne diseases are major causes of economic losses in livestock. While little exact data existed, 25 years ago in South Africa it was estimated that losses associated with tick-borne diseases in cattle amounted to R70–200 million per annum[1]. In Australia, it has been estimated that *Rhipicephalus* (*Boophilus*) *microplus* costs the livestock industry more than 100 million Australian dollars per year[4].

One of the most efficient means of containing tick-borne diseases and also preventing the loss in live mass gain, milk yield and hide damage due to tick infestation is through the judicious use of acaricides. In South Africa, ticks were first controlled using arsenic. Subsequently numerous compounds have been registered and currently in there are 104 acaricides comprising 5 chemical groups and 22 different active ingredients registered for use[4]. While dipping and spraying of cattle have traditionally been the mainstay of tick control, other methods such as pour-on or patch treatment and the use of injectable endectocides, have been developed.

Pour-on formulations developed during the late 1970s and early 1980s have the combined advantage of being easy to use, the correct volume of active ingredient can be applied to the cattle, there is no need to construct expensive plunge dips, they are more environmentally friendly because dipwash is not discarded, and in most cases they have a long residual action.

Initially the pour-on formulations contained pyrethroids as the active ingredient. However, with the development of acaricide resistance during the past 10 years (1995–2005), other actives such as the formamidines have been added either singly or in combination with pyrethroids. Formamidines, more specifically Amitraz, control both the single and multi-host ticks and several agricultural pests. The formamidines work by interacting with the octopamine receptors of the central nervous system as well by inhibition of the monoamine oxidases[3].

Reports originating in the field over recent years have suggested that, while the pour-ons containing Amitraz were effective for most of the year, there were certain periods (March to June) when their efficacy, especially against *Rhipicephalus* (*Boophilus*) spp., was extremely poor. This study was undertaken in order to investigate this notion, and also to compare the efficacy of a pour-on containing a formamidine in combination with a pyrethroid with that of a spray dip containing only a formamidine.

MATERIALS AND METHODS

The study site was situated in the Berlin area of the Eastern Cape Province, approximately 60 km from the coast, in a high tick challenge environment. Grazing on the 1200 ha farm consisted of veld grass, with *Themeda triandra* being the predominant species. The farm is divided into 6 camps.

For the past 5 years all cattle were dipped at 2–3-week intervals in the summer and 4–5-week intervals in the winter with an acaricide containing Amitraz. In May and June 2004, 18 Bonsmara and Bonsmara crossbreed cattle, of both sexes, between 2 and 4 years of age, were selected for study and individually identified using ear tags.

Pre-treatment tick counts were conducted on the animals on Day 0. The animals were then ranked in descending order according to the number of *R. (B.) decoloratus* engorging females (>5 mm) counted on each animal. After ranking, the animals were blocked into 6 replicates of 3 animals each and allocated to 1 of the 3 groups at random by random allocation of numbers .

Rhipicephalus (*Boophilus*) spp. ticks were collected at the start of the trial and tested for resistance to acaricides at the South African Bureau of Standards using the standard Shaw Larval Packet Test

Details of treatment and tick counts are shown in Table 1.

Both formulations were administered topically on Day 0 and again on Day 7. Animals in group T1 were spray-dipped using a motorised pump equipped with a spray lance and a nozzle which operated

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Table 1: **Study design.**

Group (n = 6)	Product	Dose rate	Treatment route	Treatment day	Tick count days
C1 (control)	Untreated	N/A	N/A	N/A	0, 2, 4, 7, 10, 14
T1	Amigard* (Amitraz 12.5 % spray dip)	1 ℓ/500 ℓ water	Topical (spray)	1, 7	0, 2, 4, 7, 10, 14
T2	Amipor** (Amitraz 1 %, Cypermethrin 1 % and Piperonyl butoxide 5 % m/v pour on)	1 mℓ/10 kg body weight	Topical (pour-on)	1, 7	0, 2, 4, 7, 10, 14

*Amigard (G3512) Argos Veterinary Science (Pty) Ltd.
**Amipor (G2058) Argos Veterinary Science (Pty) Ltd.

Table 2: **Knock-down and persistence efficacy of 2 acaricide formulations against *Rhipicephalus* (*Boophilus*) *decoloratus*.**

Group	Knock-down efficacy (%)		Persistence efficacy (%) [H-T formula]			
	Day 2	Day 4	Day 4	Day 7	Day 10	Day 14
C1	–160.9[a]	–806.1[a]	0[a]	0[a]	0[a]	0[a]
T2	–338.3[a]	–871.2[a]	–7.2[a]	–29.8[a]	–51.6[a]	–22.6[a]
T1	83.4[b]	75.8[b]	97.3[b]	96.1[b]	96.2[b]	99.5[b]

[a,b,c]Values in columns with no superscripts in common differ significantly ($P < 0.05$).

at approximately 770 kPa (100 psi). A flow meter was used to fill a 200 ℓ capacity container with 120 ℓ of water. The spray wash was prepared by adding 240 mℓ of Amigard to 120 ℓ of water in the container, which was then circulated through the pump for approximately 1 minute. Cattle were treated with the wash using the pump and spray lance starting from the ventral aspects of the body and working upwards until the animal was completely wet. Approximately 20 ℓ of spray wash was used per animal. Animals in group T2 was treated with Amipor applied to the midline of the cattle from the withers to the base of the tail with 60 mℓ capacity syringes. The animals were all weighed on Day 0 in order to calculate the dose to be used.

Cattle were restrained by means of a neck clamp in the cattle race during the counting of ticks. Ticks were counted on Days 2, 4, 7, 10, and 14 at their predilection sites and differentiated as follows:

A. hebraeum. Predilection sites: brisket, axillae, ventral body aspects, crutch and under tail. Differentiation: males (M), flat (unengorged) females (f), semi-engorged females (1/2 F) and engorged females (F)

R. appendiculatus. Predilection site: ears. Differentiation: males and flat (unengorged) females (f), semi-engorged females (1/2 F) and engorged females (F).

R. evertsi evertsi. Predilection site: under tail and around anus. Differentiation: males and flat (unengorged) females (f), semi-engorged females (1/2 F) and engorged females (F).

R. (B). decoloratus. Predilection site: entire body. Differentiation: engorged females > 5 mm.

Tick counts from the individual animals were, pooled and the total tick counts per group for each tick species obtained.

Efficacy was assessed in terms of knock-down efficacy and persistence efficacy. Knockdown efficacy was calculated on Days 2 and 4 as follows

$$\text{Knock-down efficacy} = 100 \times \frac{(n_0 - n)}{n_0},$$

where n_0 is the number of ticks in the treated group before treatment and n is the number of ticks in the same group after treatment.

Persistence efficacy was calculated on Days 4, 7, 10 and 14 using the Henderson-Tilton formula[2]:

$$\text{Persistence efficacy} = 100 \times \left[1 - \frac{(T_a \times C_b)}{T_b \times C_a)}\right],$$

where T_a is the number of ticks counted in the treated group after treatment, T_b is the number of ticks counted in the treatment group before treatment, C_a is the number counted in the control group after treatment and C_b is the number counted in the control group before treatment.

For each period and for each tick species, knock-down efficacy (Days 2 and 4) or persistence efficacy (Days 4, 7, 10 and 14) was compared between groups using a 2-tailed Fisher's exact test. In the few instances in which post-treatment counts exceeded pre-treatment counts, Fisher's exact test could not be used, and therefore analysis of variance was applied using efficacies calculated for each individual animal. Statistical analyses were done using NCSS 2004 statistical software (NCSS, Kaysville, UT) and a public domain statistical calculator, EpiCalc 2000 (http://www.brixtonhealth.com.epicalc.html). The significance level (alpha) was set at 0.05 for all analyses.

RESULTS

On Day 0 the cattle carried the following tick species: *R. (B.) decoloratus* (moderate to high infestation), *R. appendiculatus* (very low infestation), *R. evertsi evertsi* (moderate infestation) and *A. hebraeum* (moderate infestation).

The knock-down and persistence efficacies against *R. (B).decoloratus* are shown in Table 2. Knock-down and persistence efficacies were significantly higher for group T1 for the duration of the trial and were never below 75 %.

For *A. hebraeum* (Table 3) the knock-down and persistence efficacies were

Table 3: **Knock-down and persistence efficacy of 2 acaricide formulations against *Amblyomma hebraeum*.**

Group	Knockdown efficacy (%)		Persistence efficacy (%) [H-T formula]			
	Day 2	Day 4	Day 4	Day 7	Day 10	Day 14
C1	16.0[a]	40.0[a]	0[a]	0[a]	0[a]	0[a]
T2	45.0[b]	70.0[a]	50.0[a]	91.1[b]	4.8[a]	30.6[a]
T1	100.0[c]	100.0[b]	100.0[b]	96.4[b]	100.0[b]	100.0[b]

[a,b,c]Values in columns with no superscripts in common differ significantly ($P < 0.05$).

Table 4: **Knock-down and persistence efficacy of 2 acaricide formulations against *Rhipicephalus appendiculatus.***

Group	Knockdown efficacy (%)		Persistence efficacy (%) [H-T formula]			
	Day 2	Day 4	Day 4	Day 7	Day 10	Day 14
C1	–38.2[a]	–2.9[a]	0[a]	0[a]	0[a]	0
T2	56.4[b]	87.2[b]	87.5[b]	100.0[b]	100.0[b]	–
T1	100.0[c]	100.0[c]	100.0[c]	100.0[b]	100.0[b]	–

[a,b,c]Values in columns with no superscripts in common differ significantly ($P < 0.05$).

Table 5: **Knock-down and persistence efficacy of 2 acaricide formulations against *Rhipicephalus evertsi evertsi.***

Group	Knockdown efficacy (%)		Persistence efficacy (%) [H-T formula]			
	Day 2	Day 4	Day 4	Day 7	Day 10	Day 14
C1	–1.7[a]	18.3[a]	0[a]	0[a]	0[a]	0[a]
T2	67.4[b]	87.0[b]	84.0[b]	97.9[b]	100.0[b]	100.0[b]
T1	100.0[c]	100.0[c]	100.0[c]	100.0[b]	100.0[b]	100.0[b]

[a,b,c]Values in columns with no superscripts in common differ significantly ($P < 0.05$).

significantly higher for group T1 and were never less than 95 %. For group T2 the highest efficacy was, achieved on Day 7, after which it then dropped rapidly.

The efficacy for group T1 against *R. appendiculatus* was significantly higher than that for group T2 on Days 2 and 4, after which there was no significant difference in persistence efficacies between the 2 groups (Table 4)

A similar pattern was seen for *R. evertsi evertsi* (Table 5).

The results of the acaricide resistance testing are shown in Table 6.

Table 6: **Acaricide resistance determination (Larval Packet Tests).**

Chemical mortality	Concentration (ppm)	% Corrected
Amitraz	250	13.88
Chlorfenvinphos	500	99.59
Cypermethrin	150	34.70

Legend: ppm–parts per million.
Interpretation of mortality results: >90 % regarded as effective; ≥80 % to <90 %: effective with reservations; ≥50 % to <80 %: indications of developing resistance; ≥0 % to <50 %: indications of resistance.

DISCUSSION

The knock-down and persistence efficacies for group T1 were superior to those for T2 for all tick species tested, with the exception of *R. appendiculatus* and *R. evertsi evertsi* from Day 7 onwards, when there was no significant difference in control between the 2 groups.

Differences in knock-down and persistence efficacy between groups T1 and T2 were most marked for *R.(B.) decoloratus,* where at all post-treatment counts, tick numbers in the T2 group were higher than in the control group. The T1 group, however, showed good control, and persistence control was never less than 96 %. These differences in efficacy may be related to a number of factors. The study took place in May and June, when the *R. (B). decoloratus* challenge is high. At this time of year, cattle in the southern hemisphere are also starting to grow a longer winter coat and the hair is often matted (Fig. 1).

The ability of the pour-on to move easily and rapidly over the animals may therefore be hindered (Fig. 2).

Spraying of the animals, particularly where care is taken to ensure that they are thoroughly wetted, would therefore ensure delivery of product to the entire animal at the correct dosage (Fig. 3).

Results of the *in vitro* study suggested that the *R. (B.)* spp. ticks encountered were resistant to both Amitraz and Cypermethrin (Table 6). This indicated that both products should not have performed well against *R. (B.) decoloratus.* However, this was not the case for the animals in group T1, which achieved excellent efficacy against this species. The difference between the laboratory and field results is most likely related to the amount of active ingredient present after the animals have been treated. In the dipped animals approximately 20 ℓ of dipwash was used to thoroughly wet each animal (Fig. 3). After, standing approximately 3 ℓ of dipwash remains on the dipped animals. At a dilution rate of of 2 mℓ/ℓ i.e 250 mg Amitraz/ℓ of dipwash the average amount of Amitraz left on the cattle would be 750 mg. In the case of

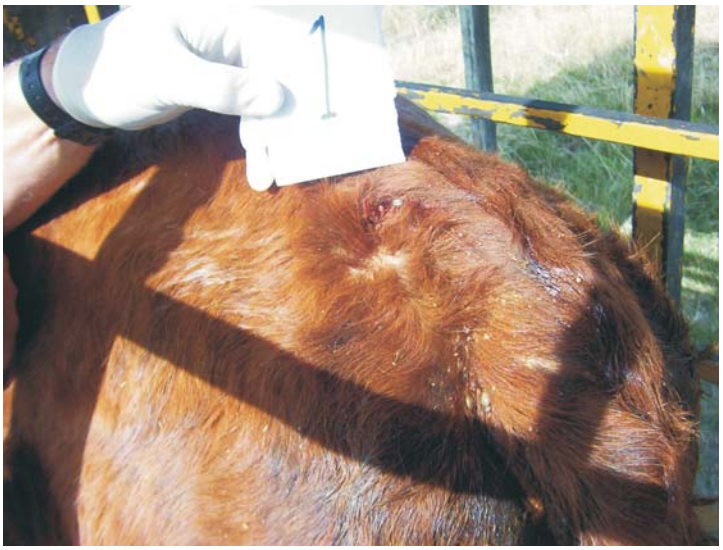

Fig 1: **Prior to treatment the scruffiness and matting of the hair coat is evident.**

Fig 2: **Two days after treatment it is evident that the pour on has not spread much further than the neck region. The coat is very matted and scurfy.**

Fig 3: **One of the animals that had been sprayed, showing thorough wetting and coverage.**

cattle treated with the pour-on approximately 50 mℓ of product was applied to each animal. This translates to an amount of 500 mg of Amitraz on each animal. The increased concentration of Amitraz as well as better overall delivery to the animal is the most probable reason for the improved efficacy.

The combination pour-on used to treat group T2 performed poorly in the control of *A. hebraeum*, with good persistence control obtained only on Day 7. By contrast, good persistence efficacy against *R. appendiculatus* and *R. evertsi evertsi* was achieved beyond Day 7. In Fig. 3 it can be seen that there was extremely poor initial spread of the pour-on. This is the most likely reason for the poor knock-down control achieved by this formulation.

These results show that under the conditions encountered in this study, a spray-dip containing 12.5 % Amitraz may be more effective than a pour-on containing 1 % Amitraz and 1 % Cypermethrin, despite apparent *in vitro* resistance.

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