The effects of a pour-on formulation of fluazuron 2.5% and flumethrin 1% on parasitic and free-living populations of *Rhipicephalus decoloratus* and *Rhipicephalus microplus* on and off bovine (Bonsmara breed) hosts

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Abstract

The present study demonstrated the efficacy of a pour-on formulation of fluazuron 2.5% and flumethrin 1% (Drastic Deadline eXtreme®) against *Rhipicephalus decoloratus* and *Rhipicephalus microplus* on cattle on pasture previously grazed by experimentally infested animals. Six tick-free cattle were placed on the pasture and treated 7 days later (Day 0) with the pour-on. They were re-treated on Days 63, 126 and 189 and monthly tick counts were done. Mean numbers of adult *R. decoloratus/microplus* decreased from 53 and 14 on Days 56 and 112 respectively to 2 or less on all other occasions including Day 254. Compared to the numbers of *R. decoloratus/microplus* larvae collected from vegetation in the previous year, larval numbers declined by 40.7% on Day 28, and thereafter reduction remained between 84% and 100%. Pairs of tracer calves placed on the pasture for 7 days each month were then held in pens and adult ticks that detached collected. Reduction in the numbers of *R. decoloratus* collected from tracer animals was 75% on Day 56 and remained above 93% except for Day 224 when it temporarily decreased to 78.5%.

Reduction in the numbers of *R. microplus* was 97.5 % on Day 28 and remained above 98% until the conclusion of the study on Day 254. Treatment with the pour-on formulation of fluazuron and flumethrin resulted in a marked decrease in the numbers of *R. decoloratus/microplus* on treated cattle followed by a reduction in the numbers of larvae questing on the vegetation and ticks picked up by tracer calves.

Keywords efficacy, pour-on, fluazuron, flumethrin, *Rhipicephalus decoloratus*, *Rhipicephalus microplus*

Introduction

The intensive use of acaricides by stock farmers has led to ever-increasing reports of acaricide resistance in several tick species around the world. In South Africa resistance to DDT was first reported by Whitehead (1956), BHC by Whitnall et al. (1952), the carbamates and organophospates by Shaw (1966) and by Shaw et al. (1967), the synthetic pyrethroids by Coetzee et al. (1987), and the amidines by Taylor and Oberem (1995). Resistance to the effects of these chemicals is not confined to the one-host ticks *Rhipicephalus decoloratus* and *Rhipicephalus microplus*, but several multi-host tick species are now also displaying resistance (cf George et al. 2004). In the interim macrocyclic lactones and insect growth regulators as well as combinations of chemicals in the abovementioned groups have become available for the control of ticks.

The extent of the problem has been demonstrated in acaracide resistance studies conducted in the Eastern Cape and North West Provinces of South Africa (Mekonnen et al. 2002, 2003). These studies revealed that some populations of the one-host tick *R. decoloratus* collected on communal farms in the Eastern Cape Province were resistant or were developing resistance to a synthetic pyrethroid and an organophoshate, while some populations of *R. decoloratus*, collected on commercial farms in both provinces, were resistant or exhibited emerging resistance to both these chemicals as well as to an amidine.

Compounding the problem of acaricide resistance is the rapid spread of the introduced Pantropical blue tick, *R. microplus*, often superseding or even completely displacing the indigenous African species, *R. decoloratus*. Tønnesen et al. (2004) have recorded the displacement of *R. decoloratus* by *R. microplus* in Limpopo Province in the north of South Africa. While Horak et al. (2009), in a survey conducted at 72 dip-tanks in

the Eastern Cape Province, noted that *R. microplus* was now the predominant species. Complete displacement appears to have occurred in Maputo Province, in southern Mozambique, where a survey conducted at 30 dip-tanks revealed that only *R. microplus* and no *R. decoloratus* was present on cattle and goats (Horak et al. 2009). Encroachment at the expense of *R. decoloratus* has also been reported in Zimbabwe, the Eastern Province of Zambia and in Tanzania (Mason and Norval 1980; Berkvens et al. 1998; Lynen et al. 2008). The first introduction of *R. microplus* into West Africa and its subsequent spread have recently been documented (Madder et al. 2007, 2011, 2012).

Whereas *R. decoloratus* is the vector of *Babesia bigemina*, the cause of African redwater in cattle, *R. microplus* is the vector of *B. bigemina* and *Babesia bovis*, the latter organism being responsible for a more aggressive form of redwater (De Vos et al. 2004). Furthermore, the slightly shorter life cycle of *R. microplus* (Arthur and Londt 1973; Londt and Arthur 1975), its greater fecundity (Spickett and Malan 1978), and propensity to feed almost exclusively on cattle (Mason and Norval 1980), predispose it to being selected for acaricide resistance more rapidly than *R. decoloratus.*

In response to these challenges a novel combination of the acarine growth regular fluazuron 2.5% and the synthetic pyrethroid flumethrin 1% (Drastic Deadline eXtreme®), in a formulation suitable for application as a pour-on has been developed. The fluazuron component of the pour-on interferes with chitin synthesis in acarines thus inhibiting larvae from moulting into nymphs and nymphs into adults (Bull et al. 1996). Inhibition of these processes is particularly noticeable in one-host ticks such as *R. decoloratus* and *R. microplus* in which moulting takes place on the host animal. Fluazuron also inhibits the hatching of eggs (George et al. 2004) and has been found to cause histopathological changes in the midgut *of Rhipicephalus sanguineus* (De Oliveira et al. 2013). Its efficacy when administered as a pour-on persists for approximately 12 weeks (Bull et al. 1996). The flumethrin component of the pour-on has virtually no knock-down effect on tick larvae, however , at the same time one of the flumethrin isomers (*trans* –Z II) was extraordinarily toxic to larvae, being some fifty times more toxic than *cis-* cypermethrin and deltamethrin (Schnitzerling et al. 1989). The European ticks exposed to flumethrin (Bayticol® Pour on) treated hairs collected within first week after treatment of cattle and sheep died with two hours of exposure, whereas the exposure time to kill increased between 5 to 12 when the ticks

were exposed to hairs collected after three weeks of treatment. (Mehlhorn et al, 2011) It is, however, an effective but does not cause detachment of exposed ticks, and consequently affected non-viable ticks may still be present on cattle for some days after treatment (Hopkins et al. 1985). Efficacy on cattle persists for two to three weeks (Sosa 1985). Flumethrin also inhibits oviposition by female ticks and if oviposition does take place the eggs are not viable (Stendel 1985).

The aim of this study was to determine the efficacy of the fluazuron 2.5%/flumethrin 1% pour-on against *R. decoloratus* and *R. microplus* on cattle that were continuously exposed to infestation with these ticks. A second objective was to determine the effect of treating these cattle on the subsequent level of pasture contamination, as measured by drag-sampling the vegetation for questing ticks, and exposing successive sets of tracer calves on the pasture grazed by the treated cattle.

Materials and methods

The Döhne Agricultural Development Institute is an experimental farm located in the Amahlathi District, Eastern Cape Province, South Africa (Figure 1). The Campagna Production System (32°29'S, 32°28'E) consists of natural grazing in a sweetveld region adjacent to Döhne, and is managed by the Institute. The pasture utilized in the study consisted of a fenced camp approximately 7 ha in size within the Campagna Production System. The vegetation in the camp is characterized by an open, treed savanna with a moderate shrub and grass cover and is classified as Eastern Province Thornveld (Acocks 1988). The grass cover within the camp consists of *Andropogon appendiculatus, Eragrostis curvula, Eragrostis plana, Sporobolus africanus, Themeda triandra* and *Heteropogon contortus* and no irrigation is applied. Potable water is available *ad libitum*.

During November 2010, six young Bonsmara cattle were placed in the experimental camp and on three occasions, one week apart, were each infested with 10, 000 larvae of *R. decoloratus* (Fig. 2) and 10, 000 larvae of *R. microplus* (Fig. 3). The animals remained in the camp for 14 months, and during this time questing ticks were collected from the vegetation each month by drag-sampling (Spickett et al. 1992). The cattle were removed during December 2011, and played no further role in the current study. The 26 cattle used in the present investigation were all over 6 months of age, of mixed sex and tick-free. Six were Bonsmara (a local breed), and the remainder
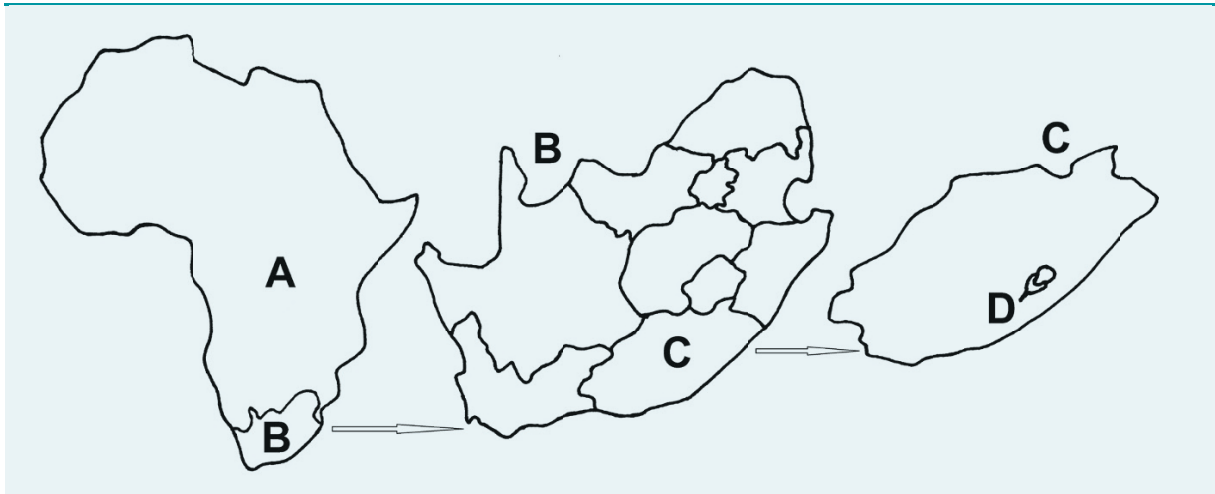
Fig. 1 Illustration showing the locality of the study site, which was located within the continent of Africa (A), country of South Africa (B), Eastern Cape province (C). The position of Döhne (D) is indicated by a pin, coordinates 32°31′S, 27°28′E

Fig. 2 Adult specimen of *Rhipicephalus decoloratus*
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Fig. 3 Adult specimen of *Rhipicephalus microplus*
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Friesians. They were all de-wormed and vaccinated with frozen African redwater and anaplasmosis vaccine prior to introduction to the experimental pasture.

Towards the end of January 2012, the 6 Bonsmara cattle were placed in the camp to acclimatize for 7 days before treatment with the fluazuron 2.5% and flumethrin 1% combination in a pour-on formulation on Day 0. Additional treatments were applied on Days 63, 126 and 189. The dosage level was 1ml of pour-on per 10 kg bodyweight and the volume applied was based on the mean body weight of the 6 cattle at each treatment interval. The cattle were restrained in a crush and neck clamp before treatment, and the pour-on was administered by means of a suitably-sized syringe along the back of the animals, from the base of the neck to the base of the tail. These cattle also served to seed the pastures with engorged female ticks that may have survived or escaped treatment towards the end of the 63 day intervals at which treatment was administered.

Whole body *in situ* tick counts were conducted on the 6 treated animals on Day 28 and thereafter at approximately 28 day intervals. Considering the difficulty with which the immature stages of ticks are found, only adult engorged and semi-engorged ticks were counted, but not removed. Ticks were detected by palpation and by direct observation following parting of the hair. Areas examined, not necessarily in this order, were 1) the head, ears and neck, 2) fore legs and shoulders, including around the hooves, 3) the dorsal strip from the shoulders to the base of the tail, 4) the flanks, not including the shoulders, 5) ventrally from brisket to inner thighs, 6) the outer surface of the hind legs including around the hooves, and 7) the peri-anal region, tail and tail-brush. The ticks detected in this way were identified to genus level and counted and arithmetic means calculated.

The remaining 20 cattle were divided into 10 groups of two. These animals served as tracers, and the first pair was placed in the camp from Day-7 to Day-1. The following pair was placed in the camp on Day 28 and thereafter consecutive pairs were introduced into the experimental camp at 28 intervals from March until October 2012. After each pair of tracers had spent 7 days in the camp they were removed and within 24 h placed in roofed pens, specifically designed for the collection of detached adult ticks. No contact between animals in the pens was possible. The penned the cattle stood on metal grids above collection trays from which detached ticks could be collected. Each pair of animals spent from 25 to 31 days in the pens in order to ensure that all ticks had sufficient time to detach. While in the pens the tracer cattle were fed

commercially available dry pellets and dried alfalfa hay and water was available *ad libitum*. Trained personnel, under the supervision of a veterinarian, were responsible for the health care of the study animals and all animals were observed daily. All engorged female ticks that dropped from each pair of animals in the pens were collected, identified and counted.

Ticks, questing from the vegetation of the experimental camp, were collected by means of 10 weighted flannel strips (100 cm x 10 cm) attached with Velcro tape adjacent to each other on a 120-cm-long wooden spar. An operator dragged this spar over the vegetation for a distance of 100 m by means of a twine harness attached to its ends (Spickett et al. 1992). Drags were repeated 10 times at approximately 50 meter intervals in the experimental pasture. After each drag, the ticks on the flannel strips were collected by means of fine-tipped forceps and stored in 70% ethanol in internally labelled glass vials. Drag-sampling of the vegetation was performed on Day-1 and thereafter at approximately 28 day intervals until the conclusion of the study. Since historical values for the numbers of larvae questing from the vegetation dating back to the time when the original experimentally infested cattle grazed the camp, comparisons between the arithmetic means of the present tick counts and the historical counts could be made.

The species names of some of the ticks that were collected have been combined for various reasons. The ticks that detached from the tracer cattle and had been collected from the pans beneath their pens could readily be identified as either *R. decoloratus* or *R. microplus* on their morphological features. This was not possible for the body counts on the treated cattle, during which the ticks were not removed, and these ticks have been designated *R. decoloratus/microplus*. A similar problem exists with the differentiation of the questing larvae of these two species, where several individuals not conforming to the accepted morphology of either species were encountered. Consequently these larvae have also been designated *R. decoloratus/microplus*. Similarly the adults of *Rhipicephalus follis* and *Rhipicephalus simus*, which by preference attach in the tail brush, could not be differentiated during the body counts and have been designated *R. follis/simus*. Although one could argue that *Hyalomma rufipes* cannot be distinguished from *Hyalomma truncatum* during a body count, a previous survey indicated that only *H. rufipes* is present on Campagna (Nyangiwe et al. 2011).

Minimum and maximum temperatures were recorded on a daily basis in the experimental pasture and within the holding pen environment using minimum and maximum thermometers.

Results

On Day 28 after treatment, ticks, potentially belonging to eight species, were counted on the treated cattle. However, without removal of these ticks it was impossible to differentiate between *R. decoloratus* and *R. microplus* and between *R. follis* and *R. simus,* and they have been designated *R. decoloratus/microplus* and *R. follis/simus* as mentioned earlier. Trends in the mean numbers of *R. decoloratus/microplus* and *Rhipicephalus appendiculatus* counted on the treated animals are graphically illustrated in Figure 4. A peak in the mean numbers of *R. decoloratus/microplus* was noted on Day 56 after initial treatment and a lesser peak on Day 112. Re-treatment on Day 126 resulted in a significant decrease in numbers, which never recovered thereafter. The mean numbers of *R. appendiculatus* counted on the treated cattle progressively decreased from 71 to zero by Days 169 and 196, and then increased from a single tick on Day 224 to 12 at the conclusion the study. Excluding *Ixodes pilosus*, of which only one female tick was counted, trends in the mean numbers of the lesser tick species are graphically depicted in Figure 5. No *Rhipicephalus evertsi evertsi, R. follis/simus* or *H. rufipes* were counted on the treated cattle on Day 84 (re-treatment on Day 63) and Day 140 (re-treatment on Day 129). No *R. evertsi evertsi* at all were encountered after Day 112, but ticks in the *R. follis/simus* grouping reappeared on Day 169 and numbers increased thereafter. A small number of *H. rufipes* were present on Day 254 (65 days after the last re-treatment).

The mean numbers of questing *R. decoloratus/microplus* larvae collected from the vegetation in the experimental camp during 2011 and 2012 and the reduction in numbers between the 2 years are graphically illustrated in Figure 6. With the exception of Day 28 after application of the pour-on, when reduction in larval numbers was 40.7%, it remained above 84% and reached 100% on 2 occasions. On Day 254, 65 days after the last pour-on application larval numbers were still 84.5% below those of the previous year.

Six tick species were recovered from the tracer cattle held in the collection pens. Prior to the first application of the pour-on, the mean numbers of engorged female *R.*
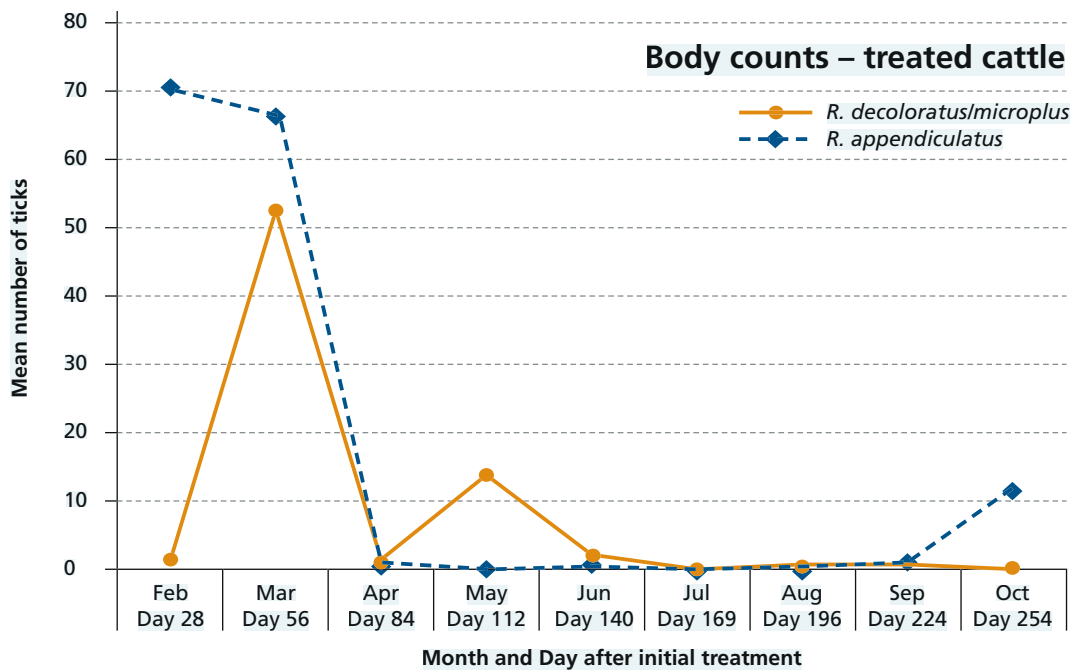
Fig. 4 Mean numbers of adult *Rhipicephalus decoloratus* and/or *R. microplus* and *R. appendiculatus* counted on cattle treated with a pour-on formulation of fluazuron 2.5 % and flumethrin 1 %
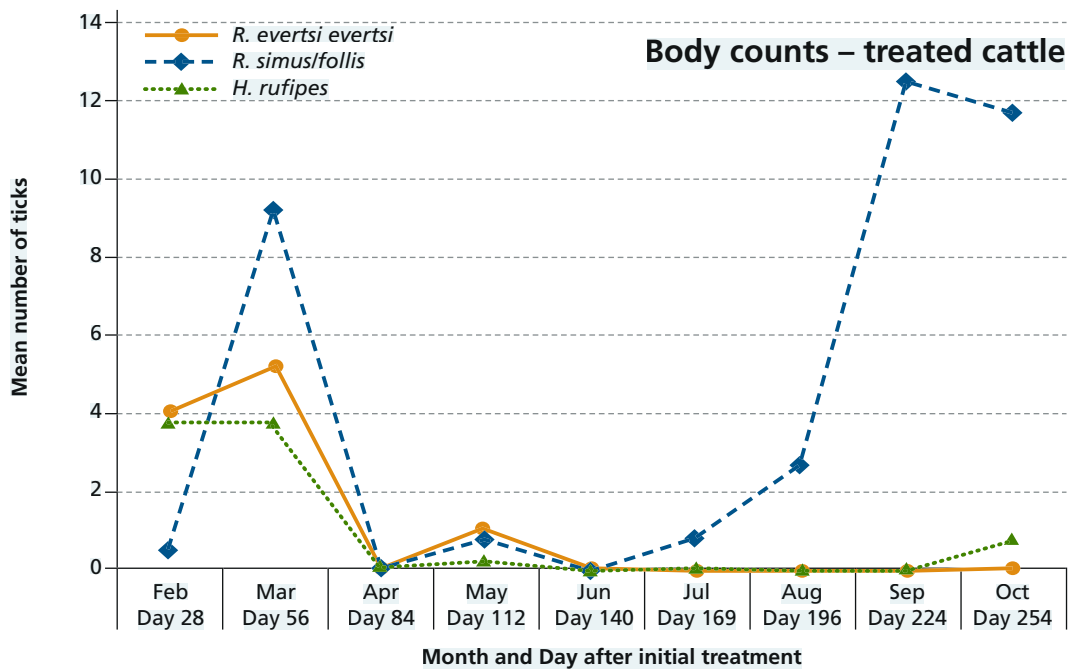

Fig. 5 Mean numbers of adult *Rhipicephalus evertsi evertsi*, *R. follis* and/or *R. simus* and *Hyalomma rufipes* counted on cattle treated with a pour-on formulation of fluazuron 2.5 % and flumethrin 1 %
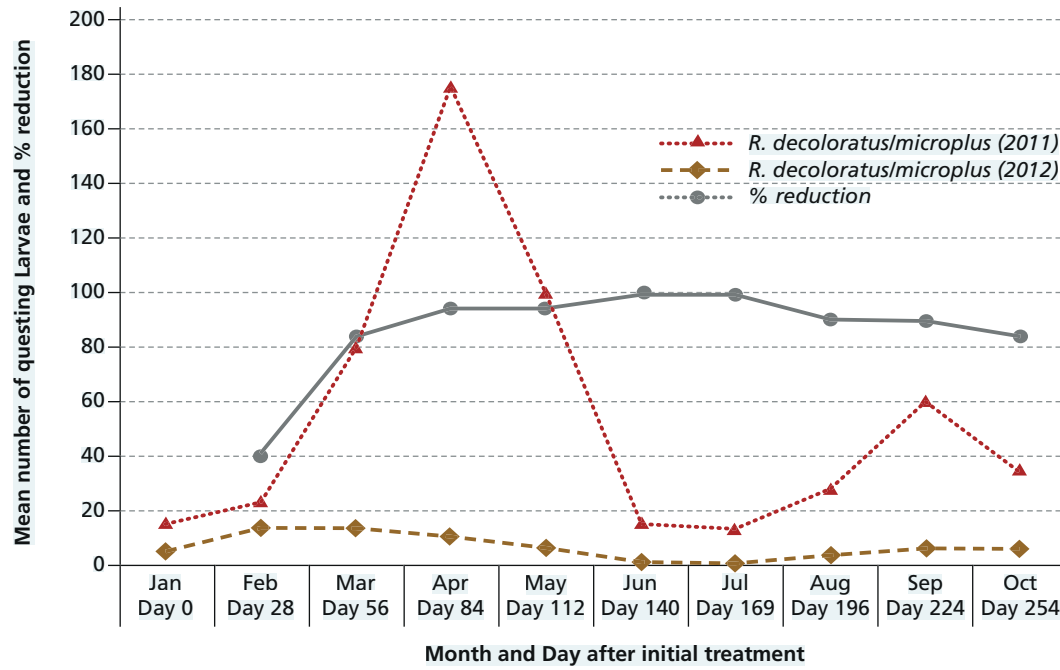
Fig. 6 Reduction (%) in the numbers of *Rhipicephalus decoloratus* and/or *R. microplus* larvae on the vegetation of a camp grazed by cattle treated with a pour-on formulation of fluazuron 2.5 % and flumethrin 1 %
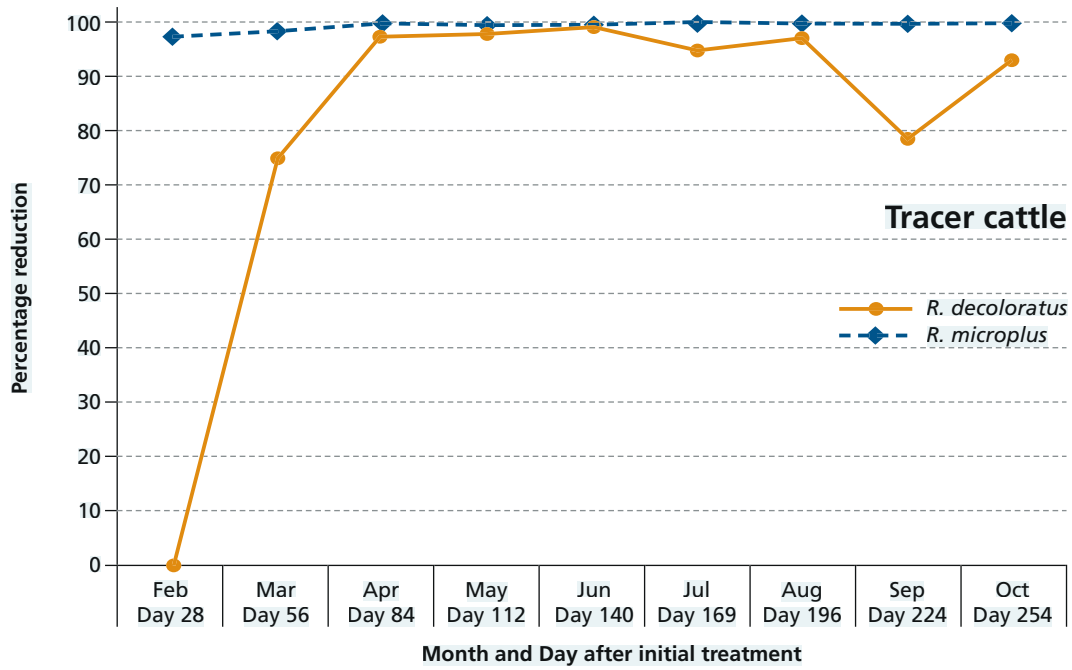
Fig. 7 Reduction (%) in the numbers of adult *Rhipicephalus decoloratus* and *R. microplus* collected from pairs of tracer cattle exposed for 7 days in a camp grazed by cattle treated with a pour-on formulation of fluazuron 2.5 % and flumethrin 1 %

Fig. 8 Reduction (%) in the numbers of adult *Rhipicephalus appendiculatus* and *R. evertsi evertsi* collected from pairs of tracer cattle exposed for 7 days in a camp grazed by cattle treated with a pour-on formulation of fluazuron 2.5 % and flumethrin 1 %
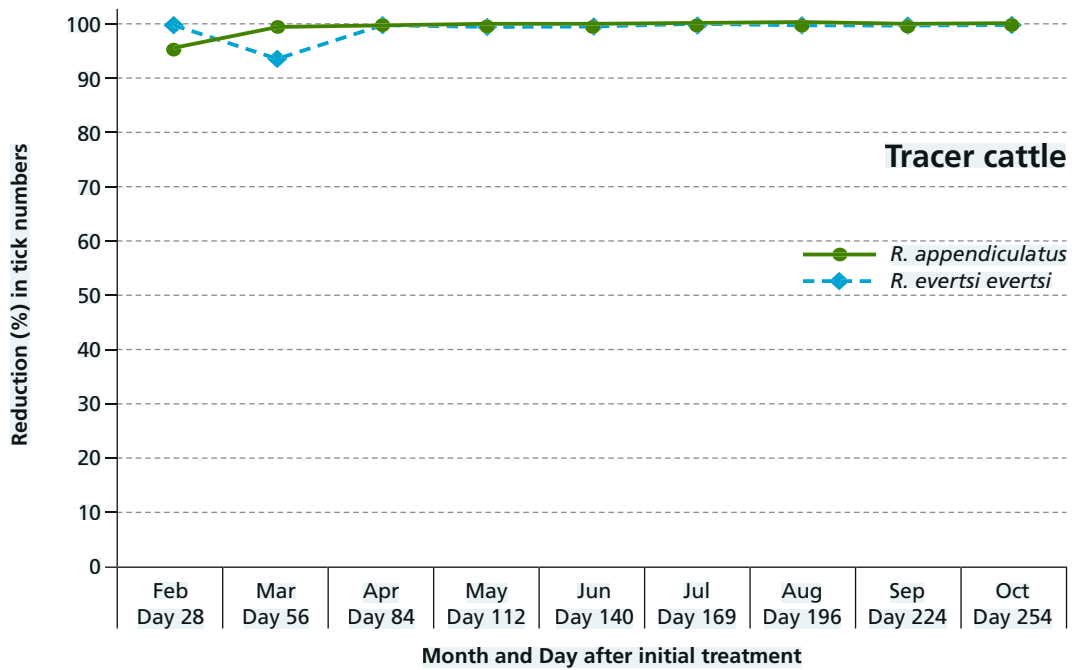
decoloratus and *R. microplus* detaching from the tracer animals were 282 and 3 293 respectively. This implied that very large numbers of larvae of both species were questing for hosts on the vegetation of the experimental camp. The percentage reduction in the numbers of *R. decoloratus* and *R. microplus* collected from the tracer cattle is graphically illustrated in Figure 7, while the percentage reduction in the numbers of adult *R. appendiculatus* and *R. evertsi evertsi* collected from these animals is graphically depicted in Figure 8. Too few *Haemaphysalis silacea* and *I. pilosus* were collected from the tracer cattle to warrant graphic representation.

There was no reduction in the mean number of adult *R. decoloratus* collected from the tracer cattle 28 days after administration of the pour-on. However, the reduction in numbers had reached 75% by Day 56, and with the exception of Day 224, when it declined to 78.5%, remained above 93% for the remainder of the study. The reduction in the numbers of *R. microplus* collected from the tracer cattle exceeded 97% on all occasions. The numbers of *R. appendiculatus* collected from the tracer cattle was reduced by 95.8% on day 28 and reduction exceeded 99% on all other occasions. With the exception of Day 56 when the numbers of *R. evertsi evertsi* were reduced by 93.7% all other values were 100%.

Ambient temperatures recorded in the experimental pasture during the study ranged from -3.1ºC to 38.9ºC. Ambient temperatures recorded at ClinVet, where the cattle were confined in tick-collection pens ranged from 0.6ºC to 35.8ºC.

Discussion

The initial treatment of the six cattle that remained on the pasture throughout the study (and thus also served as seeders of the pasture), would appear to have had an immediate effect on the numbers of *R. decoloratus/microplus* on these animals. This was evident from the small number of ticks they harboured on Day 28 after treatment. Considering the very large numbers of *R. decoloratus* (282 ticks) and *R. microplus* (3 293 ticks) picked up by the tracer cattle during the 7 days they spent on the pasture during January this effect was even more impressive.

Treatment of the six cattle also had a marked knock-on effect on the numbers of *R. decoloratus/microplus* larvae collected from the pasture by comparison with those collected in the previous year (2011). Furthermore, the numbers of questing larvae collected from the pasture during 2011 peaked in April with a lesser peak in

September. The larvae questing for hosts during these peak times would most probably have result in noticeable peaks in the number of engorged female ticks on hosts approximately one month later, namely May and October. In the present study, however, not only were the numbers of questing larvae markedly reduced, but no peaks in abundance were observed. However, small increases in the numbers of adult ticks counted on the treated cattle were observed in March and May, while a small increase in the numbers of *R. decoloratus* collected from the tracer cattle was observed in September. Other than this, the numbers of *R. decoloratus/microplus* counted on the treated cattle and collected from the tracer animals were significantly reduced. The reduction in the off-host questing tick population was thus also clearly reflected in a marked decrease in the numbers of engorged ticks collected from successive sets of tracer cattle.

This was the exact result that was anticipated for the fluazuron/flumethrin pour-on formulation. The fluazuron component had no immediate acaricidal effect on the parasitic population of *R. decoloratus* or *R. microplus,* but it would have inhibited larvae from moulting to nymphs, and nymphs to adults and thus resulted in a reduction in the number of adult ticks present on the cattle 14 days or more after treatment. Female *R. decoloratus* and *R. microplus* already present on the cattle at the time of treatment would not have been affected by fluazuron and would have engorged and detached as usual and laid eggs. However, the hatching of larvae from these eggs would have been inhibited by the minute amount of fluazuron ingested by female ticks while engorging. This in turn would lead to a reduction in the numbers of free-living larvae on the pasture. The flumethrin component of the pour-on would not only have had a direct acaricidal effect on the parasitic ticks on the cattle, but it would have inhibited the production of viable eggs by female ticks that had been exposed to doses that were sublethal (Hopkins et al. 1985; Stendel 1985). Consequently the flumethrin component like the fluazuron component would also have reduced the numbers of free-living larvae on the pasture. The reduction in the number of *R. decoloratus* and *R. microplus* larvae questing for hosts from the pasture is clearly reflected in the decreasing numbers of ticks collected from consecutive pairs of tracer calves. As the efficacy of both compounds persists for some time (Sosa 1985; Bull et al. 1996), application of the pour-on at two monthly intervals is thus entirely adequate for the control of *R. decoloratus* and *R. microplus* on cattle continuously exposed to infestation.

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Similar results have been recorded on mixed wildlife and cattle farms in a Valley Bushveld region of the Eastern Cape Province and in the Central Province, Zambia. Cattle on the Valley Bushveld farm were treated monthly with an acaricide, while Angora goats on the same farm were also treated, but less frequently. This resulted in significant reductions in the numbers of *Amblyomma hebraeum* on greater kudus (*Tragelaphus strepsiceros*), scrub hares (*Lepus saxatilis*), helmeted guineafowls (*Numida meleagris*) and the vegetation on the farm compared to the numbers on the same animal species and on the vegetation in a wildlife reserve which shared a common boundary of approximately 11 km with the farm (Horak and Knight 1986; Petney and Horak 1987). In Zambia regular acaricidal treatment of cattle resulted in a significant reduction in the numbers *R. appendiculatus* and *R. evertsi evertsi* on impalas (*Aepyceros melampus*) and the vegetation on the same farm as the treated cattle when compared to impalas and the vegetation on a neighbouring game farm on which there was only wildlife and no chemical control was practiced (Zieger et al. 1998).

Judging by the reduction in numbers of *R. appendiculatus* and *R. follis/simus* adults counted on the bodies of the treated cattle, treatment success of 100% during certain months seemed to have been achieved. However, these ticks have peaks of seasonal abundance during the summer months and hence reduction in numbers during the cooler months of the year is a natural phenomenon attested to by the reappearance of *R. follis/simus* in August and *R. appendiculatus* in September on the treated animals. Oddly very few adult *R. appendiculatus* and no *R. follis*, *R. simus* or *H. rufipes* were picked up by the tracer cattle during their 7-day exposures in the experimental camp.

Conclusion

Two-monthly treatment of cattle with a combination of fluazuron 2.5% and flumethrin 1% in a pour-on formulation resulted in a significant decrease in the numbers of *R. decoloratus* and *R. microplus* on treated animals. This in turn lead to in a decrease in the numbers of larvae questing for hosts from the vegetation, which in turn resulted in decreased numbers of *R. decoloratus* and *R. microplus* collected from tracer cattle exposed to the same infested pasture.

Acknowledgements and Compliance statement

References

Acocks JPH, (1988) Veld types of South Africa. Mem Bot Surv S Afr No 28. Government printer, Pretoria.

Arthur DR, Londt JGH (1973) The parasitic life cycle of *Boophilus decoloratus* (Koch, 1844). J Ent Soc S Afr 36:87-116

Berkvens DL, Geysen DM, Chaka G, Madder M, Brandt JRA (1998) A survey of the ixodid ticks parasitising cattle in The Eastern Province of Zambia. Med Vet Entomol 12:234-420

Bull MS, Swindale S, Overend D, Hess EA (1996) Suppression of *Boophilus microplus* populations with fluazuron – an acarine growth regulator. Aust Vet J 74:468-470

Coetzee BB, Stanford GD, Davis DAT (1987) Resistance by the blue tick (*Boophilus decoloratus*) to the synthetic pyrethroid, fenvalerate. Onderstepoort J Vet Res 54:83-86

De Oliveira PR, Calligaris IB, Roma GC, Bechara GH, Camargo-Mathias MI (2013) Fluazuron-induced morphophysiological changes in the cuticle formation and

midgut of *Rhipicephalus sanguineus* Latreille, 1806 (Acari: Ixodidae) nymphs. Parasitol Res 112:45-58

De Vos AJ, De Waal DT, Jackson LA (2004) Bovine babesiosis. In: Infectious diseases of livestock. Editors JAW Coetzer and RC Tustin. Oxford University Press: Cape Town, pp 406-424

George JE, Pound JM, Davey RB (2004) Chemical control of ticks on cattle and the resistance of these parasites to acaricides. Parasitology 129:S353-S366.

Hopkins TJ, Woodley IR, Blackwell R (1985) The safety and efficacy of flumethrin pour-on used to control *Boophilus microplus* on cattle in Australia. Vet Med Rev 2:112-125

Horak IG, Knight MM (1986) A comparison of the tick burdens of wild animals in a nature reserve and on an adjacent farm where tick control is practiced. Jl S Afr Vet Ass 57:199-203

Horak IG, Nyangiwe N, De Matos C, Neves L (2009) Species composition and geographic distribution of ticks infesting cattle, goats and dogs in a temperate and a subtropical coastal region of south-eastern Africa. Onderstepoort J Vet Res 76:263-278

Londt JGH, Arthur DR (1975) The structure and parasitic life cycle of *Boophilus microplus* (Canestrini, 1888) in South Africa (Acarina: Ixodidae). J Ent Soc S Afr 38:321-340

Lynen G, Zeman P, Bakuname C, Di Giulio G, Mtui P, Sanka P, Jongejan F (2008) Shifts in the distributional ranges of Boophilus ticks in Tanzania: evidence that a parapatric boundary between *Boophilus microplus* and *B. decoloratus* follows climate gradients. Exp Appl Acarol 44:147-164

Madder M, Thys E, Geysen D, Baudoux C, Horak I (2007) *Boophilus microplus* ticks found in West Africa. Exp Appl Acarol 43:233-234

Madder M, Thys E, Achi L, Touré A, De Deken R (2011) *Rhipicephalus* (*Boophilus*) *microplus*: a most successful invasive tick species in West-Africa. Exp Appl Acarol 53:139-145

Madder M, Adehan S, De Deken R, Adehan R, Lokossou R (2012) New foci of *Rhipicephalus microplus* in West Africa. Exp Appl Acarol 56:385-390

Mason CA, Norval RAI (1980) The ticks of Zimbabwe. I. The genus *Boophilus*. Zimbabwe Vet J 11:36-43

Mehlhorn H, Schumacher B, Jatzlau A, Abdel-Ghaffer F, Al-Rasheid KAS, Bhushan C (2012) The effects of flumethrin (Bayticol® Pour –on) on European ticks exposed to treated hairs of cattle and sheep. Parasitol Res 110: 2181-2186

Mekonnen S, Bryson NR, Fourie LJ, Peter RJ, Spickett AM, Taylor RJ, Strydom T, Horak IG (2002) Acaricide resistance profiles of single- and multi-host ticks from selected commercial and communal farming areas in the Eastern Cape and North-West provinces of South Africa. Onderstepoort J Vet Res 69:99-105

Mekonnen S, Bryson NR, Fourie LJ, Peter RJ, Spickett AM, Taylor RJ, Strydom T, Kemp DH, Horak IG (2003) A comparison of three methods to detect acaricide resistance in *Boophilus decoloratus* on dairy farms in the Eastern Cape Province, South Africa. Jl S Afr Vet Ass 74:1-44

Nyangiwe N, Goni S, Hervé-Claude LP, Ruddat I, Horak IG (2011) Ticks on pastures and on two breeds of cattle in the Eastern Cape province, South Africa. Onderstepoort J Vet Res 78(1), Art. #320, 9 pages. doi:10.4102/ojvr.v78i1.320

Petney TN, Horak IG (1987) The effect of dipping on parasitic and free-living populations of *Amblyomma hebraeum* on a farm and on an adjacent nature reserve. Onderstepoort J Vet Res 54:529-533

Schnitzerling HJ, Nolan J, Hughes S (1989) Toxicology and metabolism of isomers of flumethrin in larvae of pyrethroid-susceptible and resistant strains of the cattle tick *Boophilus microplus* (Acari: Ixodidae). Exp Appl Acarol 6:47-54

Shaw RD (1966) Culture of an organophosphorous-resistant strain of *Boophilus microplus* (Can.) and an assessment of its resistance spectrum. Bull Ent Res 56:389-409

Shaw RD, Thompson G, Baker JAF (1967) Resistance to cholinesterase-inhibitors in the blue tick, *Boophilus decoloratus*, in South Africa. Vet Rec 81:548

Sosa E (1985) Evaluation of the efficacacy and residual effect of flumethrin pour-on against *Boophilus microplus* in cattle in Uruguay. Vet Med Rev 2:126-131

Spickett AM, Malan JR (1978) Genetic incompatability between *Boophilus decoloratus* (Koch, 1844) and *Boophilus microplus* (Canestrini, 1888) and hybrid sterility of Australian and South African *Boophilus microplus* (Acarina: Ixodidae). Onderstepoort J Vet Res 45:149-153

Spickett AM, Horak IG, Van Niekerk A, Braack LEO (1992) The effect of veld-burning on the seasonal abundance of free-living ixodid ticks as determined by drag-sampling. Onderstepoort J Vet Res 59:285-292

Stendel W (1985) Experimental studies on the tickicidal effect of Bayticol pour-on. Vet Med Rev 2:99-111

Taylor RJ, Oberem P (1995) Some characteristics of an amitraz resistant strain of *Boophilus decoloratus* originating from South Africa. In: Coons L, Rothschild M (eds). Proceedings of the Second International Conference on Tick-borne Pathogens at the Host-Vector Interface, 28 August – 1 September 1995, Kruger National Park, South Africa: 62

Tønnesen MH, Penzhorn BL, Bryson NR, Stoltsz WH, Masibigiri T (2004) Displacement of *Boophilus decoloratus* by *Boophilus microplus* in the Soutpansberg region, Limpopo Province, South Africa. Exp Appl Acarol 32:199-208

Whitehead GB (1956) DDT resistance in the Blue tick, *Boophilus decoloratus* (Koch). Jl S Afr Vet Med Ass 27:117

Whitnall ABM, Thorburn JA, McHardy WM, Whitehead GB, Meerholz FA (1952) A BHC resistant tick. Bull Ent Res 43:51

Zieger U, Horak IG, Cauldwell AE, Uys A, Bothma J Du P (1998) The effect of chemical tick control on cattle on free-living ixodid ticks and on ticks parasitic on sympatric impala in the Central Province, Zambia. S Afr J Wildl Res 28:10-15

These tables have been supplanted by figures and are not to be published, but we have left them here for reference

Table A. Mean numbers of ticks counted on the bodies of six cattle treated with a fluazuron 2.5%/flumethrin 1% pour-on on Days 0, +63, +126 and + 189

Tick species		+28	+56	+84	+112	+140	+169	+196	+224	+254
Rhipicephalus decoloratus/microplus		1.8	52.8	1	14.2	2	0.5	0.5	0.3	0.5
Rhipicephalus appendiculatus		70.7	66.8	0.8	0.2	0.7	0.0	0.0	1.0	11.8
Rrhipicephalus evertsi evertsi		4	5.2	0	1	0	0	0	0	0
Rhipicephalus follis/simus		0.5	9.2	0	0.8	0	0.8	2.7	12.5	11.7
Hyalomma rufipes		3.8	3.8	0	0.2	0	0	0	0	0.8
Ixodes pilosus		0	0.2	0	0	0	0	0	0	0

The mean numbers of questing *Rhipicephalus decoloratus/microplus* larvae collected from the vegetation in the experimental camp during 2011 and 2012 and the reduction in numbers between the 2 years are summarized in Table B. With the exception of Day 28 after application of the pour-on, when reduction in larval numbers was 40.7%, it remained above 84% and reached 100% on 2 occasions. On Day 254, 65 days after the last pour-on application larval numbers were still 84.5% lower than in the previous year.

Table B. Reduction (%) in the mean numbers of *Rhipicephalus decoloratus/microplus* larvae collected from the vegetation during 2012 compared to the previous year

Tick species and year/ Day and month	Day 0	28	56	84	112	140	169	196	224	254
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct
R. decoloratus/microplus (2011)	13.5*	21.4	79.3	174.9	99	14.4	11.8	25.4	59.1	33.5
R. decoloratus/microplus (2012)	3.7*	12.7	12.1	9.8	5.4	0.0	0.0	2.4	5.7	5.2
Reduction (%)		40.7	84.7	94.4	94.5	100	100	90.6	90.4	84.5

* Pre-treatment larval counts

Table C Mean number of engorged female ticks collected from successive pairs of tracer cattle each exposed for 7 days on natural pasture

Tick species	Month during which tracers were exposed and mean numbers of ticks collected									
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct
Rhipicephalus decoloratus	282	534	70	7	5	0	15	8	60.5	19
Rhipicephalus microplus	3293	82.5	52	5	12.5	0	6	0.5	1	0.5
Rhipicephalus appendiculatus	168	7	1.5	0	0	0	0	0	0	1.5
Rhipicephalus evertsi evertsi	8	0	0.5	0	0	0	0	0	0	0
Haemaphysalis silacea	2	0	1	0	0	0.5	0	0	0	0
Ixodes pilosus	2	0	0	0	0	0	0	0	0	0

Table D Percentage reduction in the mean number of ticks collected from pairs of tracer calves after 7 days of exposure on the experimental pasture

Tick species	Month during which ticks were collected from tracers and % reduction								
	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct
Rhipicephalus decoloratus	0	75	97.5	98.2	100	94.7	97.2	78.5	93.5
Rhipicephalus microplus	97.5	98.4	99.8	99.6	100	99.8	99.9	99.9	99.9
Rhipicephalus appendiculatus	95.8	99.1	100	100	100	100	100	100	99.1
Rhipicephalus evertsi evertsi	100	93.7	100	100	100	100	100	100	100
Haemaphysalis silacea	100	50	100	100	75	100	100	100	100
Ixodes pilosus	100	100	100	100	100	100	100	100	100