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CONTROL OF INDUCED INFESTATIONS OF ADULT *AMBLYOMMA HEBRAEUM* WITH SUSTAINED RELEASE IVERMECTIN

M. D. SOLL[1], I. H. CARMICHAEL[1] and SHEILA J. GROSS[2]

ABSTRACT

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The efficacy of ivermectin, administered in a sustained release formulation by intraruminal pumps at approximate daily dose rates of 20, 40 and 60 μg/kg, was evaluated in 16 cattle against induced infestations of 3 strains of adult *Amblyomma hebraeum*. Engorged female ticks were mass-measured and incubated, and reproductive data recorded. There was an increase in mortality of male and female ticks compared to that of controls with increasing daily dose of ivermectin, and a decrease in the number of ticks engorging. Ticks fed on ivermectin-treated cattle had a smaller mass when engorged and laid smaller egg masses, both absolutely and as a proportion of engorged mass. Index of reproduction was reduced 100 % at 60 μg/kg/day, > 99 % at 40 μg/kg/day and 96 % at 20 μg/kg/day. Differences occurred between the 3 strains of *A. hebraeum* used in the study, especially with regard to engorged mass and reproductive variables.

Practical implications of the application of sustained release ivermectin for the control of *A. hebraeum*, specifically with reference to heartwater (*Cowdria ruminantium*), are discussed.

INTRODUCTION

The administration of systemically active compounds as a sustained release injection, bolus or implant would be expected to give better long-term control of parasites than that obtained from single oral or parenteral treatment. Drummond, Whetstone, Ernst & Gladney (1972) found that a number of insecticides, administered daily in the feed of cattle, controlled ticks, but that this method of treatment was difficult to implement practically.

Ivermectin is active against a wide range of internal and external parasites, including some tick species, at extremely low dosages (Campbell & Benz, 1984). The dose required is low enough to make it an excellent candidate for systemic tick control by administration via a controlled release system.

Minimum effective daily dosages of ivermectin for several species of ticks, including *Amblyomma americanum*, *A. cajennense*, *A. maculatum*, *Dermacentor andersoni*, *D. variabilis* and *Rhipicephalus sanguineus*, as well as the single host ticks *D. albipictus* and *Boophilus microplus*, have already been determined in the laboratory (Drummond, Whetstone & Miller, 1981; Lancaster, Kilgore & Simco, 1982; Nolan, Schnitzerling & Bird, 1981).

Under field conditions in South Africa (Schröder, Swan, Soll & Hotson, 1985) and Zambia (Pegram & Lemche, 1985), multiple injections of ivermectin at intervals of 1 or 2 weeks reduced numbers of *Boophilus decoloratus*, *Hyalomma truncatum*, *Rhipicephalus appendiculatus*, *Amblyomma hebraeum* and *Amblyomma variegatum* present on naturally-infested cattle.

Ivermectin administered by means of sustained release ear implants in cattle gave 70 % control of *A. cajennense* and 85 % control of *A. americanum* over a 7-week period (Miller, Drummond & Oehler, 1983).

A specially weighted osmotic pump for the administration of ivermectin to cattle at a controlled zero order rate was subsequently developed (Pope, Wilkinson, Egerton & Conroy, 1985). A trial was conducted at the MSD Research Centre, Hennops River, to evaluate the efficacy of ivermectin administered by this system at approximate dosage rates of 20, 40 and 60 μg/kg/day against induced infestations of *Amblyomma hebraeum* on cattle.

[1] MSD Research Centre, Private Bag 3, Halfway House 1685, Republic of South Africa

[2] Merck & Co. Inc., Rahway, New Jersey, USA

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MATERIALS AND METHODS

Sixteen Friesland and Friesland Cross steers, with a body mass of approximately 200 kg, were ranked by mass within breed and allocated to replicates of 4 animals each. Animals within a replicate were randomly allocated to one of the following treatment groups: untreated control; 1 ALZET* 2ML4 mini osmotic pump filled to release ivermectin at 4 mg/day, equivalent to approximately 20 μg/kg/day; 2 ALZET 2ML4 pumps (approximately 40 μg/kg/day) or 3 ALZET 2ML4 pumps (approximately 60 μg/kg/day).

Cattle were housed either in specially designed cattle crates with anti-grooming stanchions or tethered in stalls to prevent grooming or interference with tick infestations.

Four areas (2 on each side of the midline) on the backs of the animals were shaved. Specially designed linen and leather bags were glued to these areas using contact adhesive. The 'free' end of the bag was fitted with a 'Velcro†' strip which facilitated daily opening and closing of the bag for inspections. Bags were attached at least 1 day before infestation with ticks to allow fumes from the contact adhesive to dissipate. Before infestation, ticks were assessed for mobility to ensure that all were viable.

Twenty adult male *A. hebraeum* were placed in each of the 2 bags on the left of each animal on Day 2. Twenty adult female *A. hebraeum* were placed in each of these bags on Day 7. This ensured sufficient time for male attachment and pheromone production prior to the placing of females.

This process was repeated for the other 2 bags with the same numbers of males and females being placed on Days 7 and 12 respectively. A total of 320 ticks of each sex was thus used for each of the 4 treatment groups.

Male and female *A. hebraeum* of 3 different strains were used: an Onderstepoort 'heartwater free' strain, a South African Bureau of Standards (SABS) organophosphate sensitive strain and an SABS organophosphate resistant strain. Equal allocations of these 3 strains were made to bags within replicates to ensure that comparisons among treatment groups would be balanced for tick strain.

The number of ticks that failed to attach or those that fed and dropped was recorded for each bag daily. Live, unattached ticks were left in the bags and dead and engorged ticks were removed. On Day 32, all unattached ticks were removed, sexed, and assessed for viability

* Trademark of ALZA Corp., USA

† Trademark of Velcro, South Africa

and stage of engorgement. All fully engorged female ticks were removed daily, mass-measured, placed in individual mass-measured glass vials and incubated at approximately 28 °C and 86 % relative humidity. Thereafter, the dates of onset of egg laying, completion of egg laying and onset of egg hatching were recorded.

Spent, female ticks were removed from the vial approximately 46 days after the onset of egg laying, and the vial and eggs mass-measured to enable calculation of the mass of eggs laid.

For each animal, where possible, a sample of eggs from 2 ticks placed on Day 7 and from 2 ticks placed on Day 12 was mass-measured and the number of eggs counted to enable estimation of the number of eggs/g.

Each vial was examined approximately 9 weeks after completion of egg laying and the percentage hatch of eggs visually estimated.

Evaluation of data

The proportion of dead males, dead females and engorged females recovered was calculated for each bag on each animal and transformed, using the double arcsine procedure (Miller, 1978).

The mean retransformed proportion was multiplied by the number placed to calculate the mean number of each type recovered.

The egg mass:tick mass ratio and proportion hatch were transformed using the angular transformation (Snedecor & Cochran, 1980).

Egg size (eggs/mg) was estimated for up to 4 ticks for each animal, if available. These values were averaged for each animal and multiplied by the mass of eggs laid to estimate the total number of eggs laid by each tick.

The index of reproduction (IR) was calculated for each tick by multiplying number of eggs laid by proportion hatch. The number of eggs and IR were transformed to the natural logarithm of (value + 1).

Three time intervals were calculated for each tick that engorged and was incubated: days to engorgement, days from engorgement to onset of egg laying, and days from onset of egg laying to onset of hatching. If a tick laid no eggs or if the eggs did not hatch, the interval value was assumed to be missing. Ticks that did not lay eggs were given a zero value for mass of eggs, number of eggs and IR and a missing value for per cent hatch. Ticks that did not produce hatched eggs were assigned an IR of zero regardless of the reason (i.e., died, did not engorge, did not lay eggs, eggs did not hatch).

The means of the transformed and untransformed data were calculated for each bag on each animal; the mean for all the bags on each animal was then calculated and finally the mean for all the animals in each treatment regimen. Appropriate back transformations were applied to the treatment means.

Three animals were found to have drug remaining in the boluses when the cattle were slaughtered on Day 42. The data for these 3 animals were transformed and summarized as described above, but were not included in the treatment means. The data were not statistically analysed because there were too few replicates remaining for meaningful analysis after excluding the 3 animals.

RESULTS

Efficacy of sustained-released ivermectin against induced *A. hebraeum* infestation is summarized in Table 1.

There was an increase in mortality of both male and female ticks compared to controls with increasing daily dose of ivermectin, and a decrease in the number of ticks engorging. For the ticks that engorged and were incubated, the time required for full engorgement increased with increasing dose of ivermectin. Ticks fed on ivermectin-treated cattle had a lower mass when engorged and laid smaller egg masses, both absolutely and as a proportion of engorged mass. The eggs tended to be smaller and not to hatch as well. No ticks fed on cattle treated at 60 μg/kg/day were able to reproduce successfully; although a few ticks engorged and laid eggs, none of these eggs hatched. The index of reproduction was reduced 96 % at 20 μg/kg/day, > 99 % at 40 μg/kg/day and 100 % at 60 μg/kg/day.

The 3 strains of ticks differed for some of the variables examined. The data for female ticks of each strain that fed on control cattle are summarized in Table 2.

TABLE 1 Efficacy of ivermectin against induced infestations of *Amblyomma hebraeum*

Variable	Ivermectin, μg/kg/day			
	Control	20	40	60
Animals	4	4	2	3
Bags/animal	4	4	4	4
Ticks/bag				
Males	20	20	20	20
Females	20	20	20	20
Dead ticks recovered[1]				
Males	0,1	0,5	9,3	12,6
Females	0,2	0,4	1,7	3,1
Engorged females recovered[1]	19,8	15,0	0,5	0,1
Days to engorgement[2]	9,4	14,6	19,0	17,7
Days from engorgement to onset of egg laying[2]	10,1	12,0	12,2	11,0
Days from onset of egg laying to onset of hatch[2]	65,1	66,3	57,5	—
Mass of engorged females[2], g	3,08	2,04	1,39	1,27
Mass of egg mass[2], g	1,70	0,87	0,40	0,15
Egg mass:tick mass[3], %	54,8	41,3	25,0	11,3
Egg size[2], eggs/mg	11,3	11,6	16,4	22,0
Eggs laid[4]	17 504	7 503	4 763	2 518
Eggs hatched[3], %	95,3	83,5	1,4	0
Index of reproduction[5]	7 976	318	0,1	0
Percentage control	—	96%	>99%	100%

[1] Retransformed mean of radians, multiplied by number placed; the proportion was transformed using the double arcsine procedure

[2] Arithmetic mean

[3] Retransformed mean of radians; the proportion was transformed using the arcsine-square root procedure

[4] Geometric mean; the number of eggs was transformed to ln (number + 1)

[5] Geometric mean; the sum of ln (index + 1) for each female tick in a bag was divided by the number initially placed

TABLE 2 Comparison of 3 strains of *Amblyomma hebraeum* on untreated cattle

Variable	Onderstepoort	SABS resistant	SABS sensitive
No. of ticks placed	100	120	100
Engorged females recovered	92	120	98
Days to engorgement[1]	10,2	9,0	9,2
Days from engorgement to onset of egg laying	11,3	9,4	9,7
Days from onset of egg laying to onset of hatch[1]	65,5	64,8	64,9
Mass of engorged female[1], g	2,44	3,41	3,31
Mass of egg mass[1], g	1,30	1,92	1,84
Egg mass:tick mass[2], %	52,3	56,4	55,3
Egg size[1], eggs/mg	10,9	11,7	11,2
Eggs laid[3]	13 337	20 504	18 921
Eggs hatched[2], %	93,7	97,1	93,8
Index of reproduction[4]	7 500	14 530	10 140
Adjusted index[5]	3 673	14 530	8 432

[1] Arithmetic mean

[2] Retransformed mean of radians; the proportion was transformed using the arcsine-square root procedure

[3] Geometric mean; the number of eggs was transformed to ln (number + 1)

[4] Geometric mean; index for each engorged female recovered was transformed to ln (index + 1)

[5] The sum of ln (index + 1) for each engorged female recovered was divided by the number of female ticks placed and then retransformed

Generally, the SABS organophosphate-susceptible and resistant strains were similar. These ticks engorged more rapidly and started laying sooner than the Onderstepoort strain. On average, their masses were approximately 1 g greater when engorged and they produced more eggs. The adjusted index of reproduction was greatest for the SABS organophosphate-resistant strain, 42 % less for the sensitive strain and 75 % less for the Onderstepoort strain.

Discussion

Differences in fecundity and engorged mass between ticks of different origin used in this trial may be attributed to strain differences. However, photoperiod and temperature have been shown to influence significantly the mass of engorged females, feeding time and potential number of offspring produced by *A. americanum* ticks held under laboratory conditions (Barnard, Morrison & Popham, 1985). It is possible that the ticks from the 2 sources of supply used in this trial had been subjected to differing environmental conditions at the laboratories of origin; if so this may account for the differences seen between the 'strains'. These 'strain' differences are not expected to have influenced the results of the trial, as equal allocations of ticks of similar strain were made to bags within replicates.

Although ivermectin at daily dosages of approximately 40 μg/kg and greater caused increased mortality of both male and female *A. hebraeum* (Table 1), the most significant measure of control is that evaluated by calculation of the reproductive potential of the female tick.

Only 0,5 % of female ticks placed on cattle receiving 60 μg/kg/d completed engorgement. These ticks were small and laid very few eggs, which did not hatch. The reproductive index of ticks in this group was thus 0. Small (2,5 %) numbers of females engorged on animals receiving 40 μg/kg/d but the reproductive index was still reduced by > 99 % relative to untreated controls. Approximately 75 % of female ticks placed on animals treated at 20 μg/kg/d engorged. These ticks, however, were smaller and laid fewer eggs which did not hatch as well as those of ticks from control animals. The reduction in index of reproduction for this group was 96 %.

These data support the findings of Drummond *et al.* (1981) who reported that female ticks engorging on ivermectin-treated animals take longer to engorge, are usually lighter and lay smaller egg masses than females engorging on untreated animals. In the present study ticks placed on treated animals usually attached normally, but engorged partially only. They remained attached and partially engorged for long periods. Sometimes they detached and wandered around in the bags before dying.

Amblyomma hebraeum is one of 11 species of *Amblyomma* capable of transmitting heartwater (*Cowdria ruminantium*) (Uilenberg, 1983). The commonest mode of transmission is transstadial, although transovarial transmission has recently been demonstrated (Bezuidenhout & Jacobsz, 1986).

Conventional methods of tick control (eg. dipping) rely on direct contact of the chemical with the tick. For ivermectin to be effective, however, the tick must attach and at least partially engorge to ingest sufficient ivermectin to bring about paralysis. Transmission of *Cowdria ruminantium* occurs while feeding. Injection of semi- and fully engorged *A. hebraeum* ticks with ivermectin has been shown to be detrimental to salivary gland function (Kaufman, Ungarian & Noga, 1986). It is not known whether the feeding period of *Amblyomma* on treated animals would be sufficient to allow transmission of heartwater to susceptible animals.

The maintenance of enzootic stability of heartwater in endemic areas requires exposure of animals to feeding *Amblyomma*.

It is easy to control *A. hebraeum* by short interval dipping where alternative game hosts are not abundant (Norval, 1981). However, once control breaks down, the distribution of *A. hebraeum* may expand rapidly as it has in Zimbabwe (Norval, 1983a) with accompanying heartwater outbreaks (Norval, 1981, 1983b).

In South Africa, adult *A. hebraeum* reach peak numbers in the summer months (Baker & Ducasse, 1967; Londt, Horak & De Villiers, 1979; Schröder, 1980).

Utilizing available technology (Pope *et al.*, 1985) it is possible to provide sustained blood ivermectin levels to cattle for a period of months. Administration shortly before the onset of peak *A. hebraeum* activity in October in the northern Transvaal, for instance, could result in a substantially reduced reproductive potential of females during the tick season.

Because ticks must attach and engorge to some extent, the "knockdown effect" seen with some dips may not be apparent with sustained low blood ivermectin levels, and the effect of treatment may only be seen later in the season or the following season as a result of the extremely low reproductive potential of *Amblyomma* feeding on the treated animals.

The effect of continuous ivermectin exposure on immature ticks has not been determined. According to Nolan (1983) *Boophilus microplus* frequently survive to the young adult stage before succumbing to ivermectin. Soll (unpublished observations, 1986) observed that *A. hebraeum* nymphs engorged on both untreated control animals and animals treated with ivermectin at approximately 40 μg/kg/d but that many of those engorging on treated animals did not moult successfully. Treatment of cattle under field conditions may not only prevent engorgement of adult *A. hebraeum*, but also limit the number of nymphs moulting to adults which could infest animals later in the season. Larvae parasitize a wide range of alternative hosts and their specific control is probably not as critical as that of the other 2 developmental stages.

The efficacy of continuous administration of ivermectin in a sustained release formulation against *A. hebraeum* may facilitate control or even elimination of the tick from certain areas and may thereby alter the epizootiology of heartwater in the cattle population in those areas. This concept needs to be tested in the field with a system designed to provide ivermectin release over an extended period.

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