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Endectocide activity of a pour-on formulation containing 1.5 per cent ivermectin +0.5 per cent abamectin in cattle

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ABSTRACT

The present work aimed to evaluate, through ten different studies, the therapeutic efficacy of a new pour-on formulation, containing 1.5 per cent ivermectin +0.5 per cent abamectin, against parasites of cattle. Results obtained on trials against *Rhipicephalus (Boophilus) microplus* showed that the pour-on combination of 1.5 per cent ivermectin +0.5 per cent abamectin obtained superior efficacy indexes against this ectoparasite, when compared with formulations containing 0.5 per cent ivermectin, 1 per cent ivermectin and the combination of 1 per cent abamectin +20 per cent levamisole. The results of efficacy of the ivermectin+abamectin and the 0.5 per cent ivermectin against *Haematobia irritans* were similar. Against *Cochliomyia hominivorax* larvae, all pour-on formulations tested (1.5 per cent ivermectin +0.5 per cent abamectin, 0.5 per cent ivermectin and 0.5 per cent abamectin), as well as 1 per cent doramectin administered subcutaneously, were considered ineffective. Cattle medicated with 1.5 per cent ivermectin +0.5 per cent abamectin, pour-on, remained free from parasitism by *Dermatobia hominis* larvae during 42 days (96 per cent efficacy), while values superior to 90 per cent were obtained by 0.5 per cent ivermectin (92 per cent) and 0.5 per cent abamectin (93 per cent) until the 42nd and 35th days post treatment, respectively. Against *Haemonchus placei* and *Oesophagostomum radiatum*, the pour-on of ivermectin+abamectin showed better efficacy than the 0.5 per cent ivermectin and 0.5 per cent abamectin. As to *Cooperia punctata*, there was no difference regarding efficacy results obtained by the avermectins combination and abamectin. The pour-on combination of 1.5 per cent ivermectin +0.5 per cent abamectin obtained high efficacy against *R. (B.) microplus*, *D. hominis* and some species of cattle gastrointestinal helminths when compared with formulations of 0.5 per cent ivermectin and 0.5 per cent abamectin administered through the same route.

INTRODUCTION

Infections by endoparasites and infestations by ectoparasites represent a serious problem in cattle, accounting for large economic losses. One factor that increases such losses is related to the occurrence of resistance from parasites against antiparasitic drugs. However, despite this situation, the control of cattle parasites still relies heavily on the use of chemicals (Souza and others 2008).

Macrocyclic lactones (MLs) are the most commonly used products to control parasites in cattle. In 1981, the first 1 per cent injectable ivermectin for cattle was released in the veterinary market (Lopes and others 2014b). In the 1990s, an injectable long-action formulation containing 3.15 per cent ivermectin was introduced to the market because of the presence of parasite strains resistant to avermectins (Carvalho and others 1998).

The use of pour-on formulations is increasing (Lopes and others 2014a) due to easier application and lower costs without the use of syringes and needles, as well as the smaller risk of pathogen transmission from animal to animal, usually caused when using the same needle for several animals (Hooke and others 1997). Such facts significantly increased the use of this administration route in some countries since its discovery in the 1990s. While the use of ML pour-ons may be a factor in the selection for resistance, it is unlikely to be a major one (Bartley and others 2012). Recent studies report the ineffectiveness of 500 µg/kg ivermectin, administered as pour-on route, against helminths of cattle (Bartley and others 2012, Lopes and others 2014a). The increasing population of chemical-resistant parasites, combined with the lack of discovery of new molecules with

positive cost benefit, makes the search for alternatives in chemical parasite control necessary. One option is complementing the endectocide activity of avermectins with the same mode of action, through the combination of different compounds (Borges and others 2008). Because of this, the present work aimed to evaluate the therapeutic efficacy of a new pour-on formulation, containing 1.5 per cent ivermectin +0.5 per cent abamectin (MSD Animal Health, Brazil), against *Rhipicephalus (Boophilus) microplus*, *Haematobia irritans*, *Cochliomyia hominivorax* larvae, *Dermatobia hominis* larvae and gastrointestinal nematodes parasitising cattle.

MATERIALS AND METHODS

Ten experiments were conducted in order to evaluate the endectocide efficacy of a pour-on formulation containing the combination of 1.5 per cent ivermectin +0.5 per cent abamectin. Number of groups, as well as treatments, administration routes and dosages used in each experiment, is described in Table 1. In each experiment, animals that received injectable formulations remained in the same paddocks as animals in the control groups while each group treated with a pour-on formulation was placed in an exclusive paddock during the whole experimental period. Therapeutic efficacy of each formulation against each parasite was calculated based on arithmetic means.

The animals were weighed individually one day before treatment in order to calculate the correct dosage. All the clinical procedures were performed according to the 'Good Clinical Practice' guide (VICH GL9 2000, http://vich.eudra.org/pdf/2000/GL09_st7.pdf).

Efficacy against natural infestations of *R. (B.) microplus*

Field studies were performed on rural properties on the cities of São João da Boa Vista/São Paulo (SP), São José do Rio Pardo/SP and Gastão Vidigal/SP, Brazil. Crossbreed zebuine animals were selected by means of counts of semiengorged *R. (B.) microplus* females (4.5–8 mm in length), present on the left side of each animal (Wharton and others 1970) on three consecutive days (days −3, −2 and −1). Later, these cattle were ranked according to mean numbers of ticks, and distributed through blocks comprising four to five groups of 10 animals each, followed by randomly allocated treatments. To evaluate the therapeutic and residual efficacies, new counts of *R. (B.) microplus* females were performed on post-treatment days (PTDs) 3, 7, 14, and weekly until the end of each experiment. Efficacy percentages were calculated according to Holdsworth and others (2006), based on tick survival.

Efficacy against artificial infestations of *R. (B.) microplus*

Twenty-four Holstein breed cattle were selected, with ages ranging between 6 and 10 months, weighing between 120 and 210 kg. After selection, the animals were housed in Cattle Sector II of CPPAR (Animal Health Research Center/UNESP Jaboticabal), where they underwent a 40-day period of adaptation, in individual pens with suspended floors.

Each artificial infestation was performed with 5000 *R. (B.) microplus* (Field strain) larvae, with a mean age of 14 days, applied along the dorsal line of each animal three times a week in the 24 days before treatment (D-24 to D-1), totalling 11 infestations. After the final infestation (24 h before treatment), the animals were randomised to treatments according to a completely randomised block design. The block formation was based on the arithmetic mean of fully engorged *R. (B.) microplus* females counted on three consecutive days (−3, −2, −1). Animals were separated into six blocks of four animals each, and, inside each block, animals were randomly placed in one of the treatments.

Engorged female ticks that naturally detached from the animals were counted daily, from day 1 until day 56 post treatment. During this period, all cattle were infested with approximately 5000 viable and unfed larvae twice a week (every Tuesday and Thursday of each week), as recommended by Holdsworth and others (2006). Acaricidal efficacy of tested formulations was calculated using counts of engorged female ticks detached from the animals of each group, with collected data grouped into 7-day intervals.

Efficacy against natural infestations of *H. irritans*

These field studies were performed on rural properties located in the cities of União de Minas/Minas Gerais (MG) and Formiga/MG, Brazil. Animals were selected by means of counts of *H. irritans* present on the whole body surface of each animal on two consecutive days (days −2 and −1). These animals were ranked according to the mean number of flies and assigned in blocks into three groups of 15 animals each, followed by random allocation to treatment. To evaluate therapeutic and residual efficacies, new counts of *H. irritans* were performed on PTDs 3, 7, 14, and weekly until the end of each experiment. These counts were performed by two researchers, situated at both sides of the animal; always between 07.00 and 10.00. Efficacy percentages were calculated based on fly survival.

Efficacy against natural infestations of *C. hominivorax* larvae

The field studies were performed on rural properties situated in União de Minas/MG and Formiga/MG, Brazil. Bulls were ranked according to their bodyweight obtained on day −2, and assigned in blocks into five groups of 10 animals each, followed by randomly allocated treatments. On day 0 (treatment day), each animal received 10 ml of 2 per cent Xylocaine for local anaesthesia, administered subcutaneously, in the distal region of the scrotum. After an incision in the scrotum, bulls were neutered using a scalpel. Animals were treated at the same time as they were neutered. Afterwards, all cattle were released on their respective paddocks in

TABLE 1: Experimental design used in all trials

Parasite	Experiment number–type	Local (city/state)	Number of animals per group	Treatments
Rhipicephalus (Boophilus) microplus	1. Natural infestation	São João da Boa Vista/São Paulo	10	T01: control T02: ivermectin 1.5% (1.5 mg/kg)+abamectin 0.5%* (0.5 mg/kg), pour-on (1 ml/10 kg) T03: ivermectin 1%† (0.2 mg/kg), injectable (1 ml/50 kg)
	2. Natural infestation	São José do Rio Pardo/São Paulo	10	T01: control T02: ivermectin 1.5% (1.5 mg/kg)+abamectin 0.5%* (0.5 mg/kg), pour-on (1 ml/10 kg) T03: ivermectin 0.5%‡ (0.5 mg/kg), pour-on (1 ml/10 kg) T04: ivermectin 1%† (0.2 mg/kg), injectable (1 ml/50 kg)
	3. Natural infestation	Gastão Vidigal/São Paulo	10	T01: control T02: ivermectin 1.5% (1.5 mg/kg)+abamectin 0.5%* (0.5 mg/kg), pour-on (1 ml/10 kg) T03: fipronil 1%§ (1 mg/kg), pour-on (1 ml/10 kg) T04: fluazuron 2.5%¶ (2.5 mg/kg), pour-on (1 ml/10 kg) T05: abamectin 1% (1 mg/kg)+levamisole 20%** (20 mg/kg), pour-on (1 ml/10 kg)
	4. Artificial infestation	CPPAR-UNESP, Jaboticabal/São Paulo	6	T01: control T02: ivermectin 1.5% (1.5 mg/kg)+abamectin 0.5%* (0.5 mg/kg), pour-on (1 ml/10 kg) T03: abamectin 0.5%†† (0.5 mg/kg), pour-on (1 ml/10 kg) T04: ivermectin 0.5%‡ (0.5 mg/kg), pour-on (1 ml/10 kg)
Haematobia irritans	5. Natural infestation	União de Minas/Minas Gerais	15	T01: control T02: ivermectin 1.5% (1.5 mg/kg)+abamectin 0.5%* (0.5 mg/kg), pour-on (1 ml/10 kg) T03: ivermectin 0.5%‡ (0.5 mg/kg), pour-on (1 ml/10 kg)
	6. Natural infestation	Formiga/Minas Gerais	15	T01: control T02: ivermectin 1.5% (1.5 mg/kg)+abamectin 0.5%* (0.5 mg/kg), pour-on (1 ml/10 kg) T03: ivermectin 0.5%‡ (0.5 mg/kg), pour-on (1 ml/10 kg)
Cochliomyia hominivorax	7. Natural infestation	Formiga/Minas Gerais	10	T01: control T02: ivermectin 1.5% (1.5 mg/kg)+abamectin 0.5%* (0.5 mg/kg), pour-on (1 ml/10 kg) T03: abamectin 0.5%†† (0.5 mg/kg), pour-on (1 ml/10 kg) T04: ivermectin 0.5%‡ (0.5 mg/kg), pour-on (1 ml/10 kg) T05: doramectin 1%‡‡ (0.2 mg/kg), injectable (1 ml/50 kg)

Continued

TABLE 1: Continued

Parasite	Experiment number–type	Local (city/state)	Number of animals per group	Treatments
	8. Natural infestation	União de Minas/Minas Gerais	10	T01: control T02: ivermectin 1.5% (1.5 mg/kg)+abamectin 0.5%* (0.5 mg/kg), pour-on (1 ml/10 kg) T03: abamectin 0.5%†† (0.5 mg/kg), pour-on (1 ml/10 kg) T04: ivermectin 0.5%‡ (0.5 mg/kg), pour-on (1 ml/10 kg) T05: doramectin 1%‡‡ (0.2 mg/kg), injectable (1 ml/50 kg)
Dermatobia hominis larvae	9. Natural infestation	Formiga/Minas Gerais	10	T01: control T02: ivermectin 1.5% (1.5 mg/kg)+abamectin 0.5%* (0.5 mg/kg), pour-on (1 ml/10 kg) T03: ivermectin 0.5%‡ (0.5 mg/kg), pour-on (1 ml/10 kg) T04: abamectin 0.5%†† (0.5 mg/kg), pour-on (1 ml/10 kg)
Helminth	10. Natural infestation	Formiga/Minas Gerais	6	T01: control T02: ivermectin 1.5% (1.5 mg/kg)+abamectin 0.5%* (0.5 mg/kg), pour-on (1 ml/10 kg) T03: ivermectin 0.5%‡ (0.5 mg/kg), pour-on (1 ml/10 kg) T04: abamectin 0.5%†† (0.5 mg/kg), pour-on (1 ml/10 kg)

*MSD Animal Health, Brazil
†Ivomec injectable: Merial Animal Health
‡Ivomec pour-on: Merial Animal Health
§Top Line: Merial Animal Health
¶Acatak: Novartis Animal Health
**Exodus pour-on: Pearson
††Abamectina pour-on: Alvet Animal Health
‡‡Dectomax injectable: Zoetis Animal Health

order for the natural infestation to occur. Scrotum lesions of all animals were examined three, five and seven days post treatment (DPT) to determinate preventive efficacy of formulations in question, according to the methodology adopted by Martins and others (2009).

Efficacy against natural infestations of *D. hominis* larvae

Crossbred cattle, naturally infested with *D. hominis* larvae, were selected by means of two parasite counts (days −2 and −1) from a herd of cattle aged 15 months on a rural property situated in the municipality of Formiga/MG, Brazil. Quantification of larva nodules present throughout the entire body of the animals was performed by light compression (visual and tactile inspection) to certify whether these nodules contained live larvae. From the mean larvae counts, 40 animals were randomised into four uniform groups. Subsequent larvae counts, which were required for therapeutic and residual evaluation of the used compounds, were performed on PTDs 7, 14, 28, 35 and 49. Based on quantifications obtained from each animal, arithmetic means and efficacy percentages were calculated.

Anthelmintic efficacy

Twenty-four male calves, aged between 7 and 11 months, originating from a herd situated in Formiga/MG, Brazil, were selected by individual counts of eggs per gram (EPG) of faeces, using saturated sucrose or salt solution (Gordon and Whitlock 1939). Based on the mean of three consecutive EPG counts (days −3, −2 and −1), the animals were divided into four groups. On the 7th DPT, all the animals underwent postmortem examination based on previous studies (Silva 2008) where a peak plasma concentration was observed around this date (Tmax). Collection, counting and specific identification of parasites within each organ were carried out according to methodologies described by Levine (1968) and Ueno and Gonçalves (1998). Therapeutic efficacy was calculated for each nematode species using arithmetic means, according to the recommendations of Dobson and others (2009) and Vercruysse and others (2011).

Data analysis

In the anthelmintic evaluation, statistical analysis was performed using a generalised linear mixed model with fixed treatment and random block effects and a block-treatment interaction (SAS 1996). In evaluations against ectoparasites, a split plot in time analysis was used. Data obtained at each experimental date were log(x+1) transformed in the experiments using cattle naturally infested by *R. (B.) microplus, H. irritans, C. hominivorax* larvae and *D. hominis* larvae. Regarding artificial tick infestations (stall test), data were grouped in intervals of seven consecutive days. Analyses were performed using the mean values of data grouped in these intervals through application of the F test and compared using the Tukey test (SAS 1996). All hypotheses were tested at a 0.05 level of significance.

RESULTS

Table 2 presents arithmetic means of *R. (B.) microplus* female (4.5–8.0 mm in length) counts, present on the left side of naturally parasitised cattle, together with the efficacy percentages and results of data analysis. On natural infestation experiments, the 1.5 per cent ivermectin +0.5 per cent abamectin combination reached acaricidal efficacy against *R. (B.) microplus* superior to 95 per cent between the 3rd and 21st DPT or between the 3rd and 28th DPT, depending on the experiment. Regarding 0.5 per cent ivermectin, 1 per cent ivermectin and the 1 per cent abamectin +20 per cent levamisole, these formulations, in all trials, showed efficacy values inferior to the ivermectin+abamectin combination, administered as a pour-on, with the sole exception of the 28th DPT on experiment 1. However, there is no statistical difference between the combination of ivermectin+abamectin and 1 per cent ivermectin. Compounds containing 1 per cent fipronil and 2.5 per cent fluazuron, used in experiment 3, demonstrated better efficacy indexes during the whole study when compared with the pour-on combination of ivermectin +abamectin. However, it is important to reinforce that there was no statistical difference in average tick counts from these three groups when comparing them with the average number of ticks present in the group that received 1.5 per cent ivermectin +0.5 per cent abamectin (Table 2).

Results of therapeutic efficacy evaluation against *R. (B.) microplus* in experimentally infested cattle, as well as the results of data analysis are presented in Table 3. Based on such information, it was possible to verify that the 1.5 per cent ivermectin +0.5 per cent abamectin combination reached efficacy indexes superior to 95 per cent from the 8th to the 42nd DPT, while remaining formulations (0.5 per cent ivermectin and 0.5 per cent abamectin, both administered pour-on) did not reach 90 per cent efficacy during the whole study. Results of the statistical analysis, regarding the counts of fully engorged female ticks detached from the animals, reinforce such inference (Table 3).

Analysing the results from experiments 5 and 6, conducted with *H. irritans* (Table 4), the authors are able to verify that the 1.5 per cent ivermectin +0.5 per cent abamectin formulation reached elevated efficacy values (>98 per cent) between 3rd and 14th DPT. There is no statistical difference (P>0.05) between the groups treated during the whole studies (Table 4).

Regarding studies of preventive efficacy conducted against *C. hominivorax* larvae in naturally infested animals after orchiectomy (Table 5), it is possible to conclude that none of the used formulations (1.5 per cent ivermectin +0.5 per cent abamectin pour-on, 0.5 per cent abamectin pour-on, 0.5 per cent ivermectin pour-on and 1 per cent injectable doramectin) demonstrates satisfactory preventive efficacy against such parasite throughout all experimental period. Still, regarding trials conducted against *C. hominivorax*

TABLE 2: Mean number of *Rhipicephalus (Boophilus) microplus* in cattle naturally infested, belonging to control and treated groups; efficacy and results of variance analysis

Day of study	Experiment (local)	Mean value* of female ticks (4.5–8.0 mm in length) count on cattle/experimental groups				Efficacy (%)		
		T01: control	T02: 1.5% ivermectin + 0.5% abamectin†	T03: 0.5% ivermectin‡	T04: 1% ivermectin‡	T02	T03	T04
0	1. São João da Boa Vista/São	20.3^{a}	19.5^{a}	20.2^{a}	20.5^{a}	–	–	–
3	Paulo	20.2^{a}	0.8^{b}	2.2^{b}	2.0^{b}	**95**	**89**	**90**
7		26.2^{a}	0.2^{c}	1.9^{b}	1.9^{b}	**99**	**92**	**93**
14		31.7^{a}	0.5^{b}	2.1^{b}	2.1^{b}	**98**	**93**	**93**
21		22.5^{a}	0.4^{c}	5.6^{b}	1.9^{bc}	**98**	**75**	**91**
28		25.8^{a}	14.2^{ab}	11.9^{ab}	6.6^{b}	**42**	**53**	**74**
0	2. São José do Rio Pardo/São	20.3^{a}	20.7^{a}	20.6^{a}	20.3^{a}	–	–	–
3	Paulo	20.2^{a}	1.0^{c}	5.3^{b}	5.1^{b}	**95**	**74**	**74**
7		23.9^{a}	0.5^{c}	3.3^{b}	2.1^{bc}	**98**	**86**	**91**
14		25.1^{a}	0.1^{c}	4.4^{b}	1.9^{b}	**99**	**83**	**92**
21		21.9^{a}	0.2^{c}	4.4^{b}	0.8^{c}	**99**	**80**	**96**
28		22.5^{a}	1.5^{b}	11.9^{a}	3.9^{b}	**93**	**48**	**83**
35		20.7^{a}	11.9^{a}	17.7^{a}	7.9^{a}	**43**	**15**	**62**

Day of study	Experiment (local)	T01: control	T02: 1.5% ivermectin +0.5% abamectin†	T03: fipronil 1%‡	T04: 2.5% fluazuron‡	T05: 1% abamectin+20% levamisole ‡	T02	T03	T04	T05
0	3. Gastão	53.1^{a}	53.0^{a}	52.9^{a}	53.0^{a}	53.0^{a}	–	–	–	–
3	Vidigal/São	58.9^{a}	1.6^{b}	1.1^{b}	3.2^{b}	1.9^{b}	**97**	**98**	**94**	**97**
7	Paulo	50.7^{a}	0.8^{b}	0.4^{b}	1.3^{b}	1.2^{b}	**98**	**99**	**97**	**97**
14		37.1^{a}	1.8^{bc}	0.6^{c}	0.7^{c}	3.7^{b}	**95**	**98**	**98**	**90**
21		36.3^{a}	0.7^{b}	0.1^{b}	0.2^{b}	1.4^{b}	**97**	**99**	**99**	**96**
28		42.2^{a}	1.7^{bc}	0.3^{c}	0.5^{c}	2.4^{b}	**95**	**99**	**99**	**94**
35		25.0^{a}	7.0^{b}	1.8^{d}	2.5^{cd}	5.6^{bc}	**71**	**92**	**90**	**77**
42		27.3^{a}	10.9^{b}	5.5^{b}	7.9^{b}	9.3^{b}	**60**	**80**	**71**	**66**
49		26.7^{a}	13.0^{ab}	7.4^{b}	10.3^{b}	11.5^{b}	**51**	**72**	**61**	**57**

Experiments 1, 2 and 3
*Means followed by the same letter in the line do not differ (P>0.05)
† MSD Animal Health, Brazil
‡Commercial formulation purchased from the local market
SP, São Paulo

larvae, despite the fact that 1.5 per cent ivermectin +0.5 per cent abamectin and 1 per cent doramectin presented a progressive reduction in the number of live myiasis in experiment 7, the elevated efficacy indexes observed on the last experimental date (7th DPT) can be attributed to the spontaneous cure process of myiasis, since only one lesion in one of the animal from the control group was still infested by *C. hominivorax* larvae at this point.

Table 6 presents efficacy percentages and mean number of *D. hominis* larvae in cattle for treated and control groups. Analysing results described in this table, it can be verified that 1.5 per cent ivermectin +0.5 per cent abamectin and 0.5 per cent ivermectin formulations reached efficacy indexes superior to 90 per cent against *D. hominis* larvae from the 7th to the 42nd DPT, while the 0.5 per cent abamectin compound reached such indexes between the 7th and 35th DPT. Parasite counts in treated groups were statistically inferior (P≤0.05) than those from the control group, between the 3rd and 42nd DPT. However, there was no significant statistical difference in average *D. hominis* larvae counts between treated groups during the whole experiment.

Arithmetic means of counts of each nematode species recovered from the postmortem examination and efficacy percentages of evaluated formulations are shown in Table 7. In a general way, against gastrointestinal nematodes, 1.5 per cent ivermectin +0.5 per cent abamectin and 0.5 per cent abamectin formulations presented better anthelmintic effects when compared with the 0.5 per cent ivermectin formulation. Results from statistical analysis reinforce such conclusion (Table 7). For *Haemonchus placei*, the 1.5 per cent ivermectin +0.5 per cent abamectin combination reached a 92 per cent efficacy, while compounds containing 0.5 per cent ivermectin and 0.5 per cent abamectin obtained 0 per cent and 66 per cent efficacies, respectively. Anthelmintic efficacies of 1.5 per cent ivermectin +0.5 per cent abamectin against *H. similis, Cooperia punctata* and *Oesophagostomum*

TABLE 3: Average number of engorged *Rhipicephalus (Boophilus) microplus* females naturally detached from control and treated animals and percentages (arithmetic means)

Day of study	Experiment (local)	Mean value* of engorged females detached from cattle/experimental groups				Efficacy (%)		
		T01: control (saline solution)	T02: 1.5% ivermectin +0.5% abamectin†	T03: 0.5% abamectin‡	T04: 0.5% ivermectin‡	T02	T03	T04
0	4. CPPAR/UNESP,	69.7^{A}	69.8^{A}	69.3^{A}	69.2^{A}	–	–	–
1–7	Jaboticabal/São	64.8^{A}	22.9^{B}	17.4^{B}	25.5^{B}	64	73	60
8–14	Paulo	70.3^{A}	0.6^{C}	8.1^{B}	8.8^{B}	99	88	87
15–21		115.5^{A}	0.4^{C}	28.1^{B}	11.6^{B}	99	75	89
22–28		123.4^{A}	0.1^{C}	37.3^{B}	27.8^{B}	99	69	77
29–35		159.1^{A}	0.3^{C}	102.2^{AB}	48.2^{B}	99	35	69
36–42		120.6^{A}	1.8^{C}	131.6^{A}	54.7^{B}	98	0	54
43–49		92.9^{A}	8.0^{B}	–	–	91	–	–
50–56		52.2^{A}	22.2^{B}	–	–	57	–	–

Experiment 4
*Means followed by the same letter in the line do not differ (P>0.05)
†MSD Animal Health, Brazil
‡Commercial formulation purchased from the local market

radiatum were of 100 per cent, 96 per cent and 100 per cent, respectively, while 0.5 per cent abamectin reached 100 per cent, 97 per cent and 99 per cent, respectively; 0.5 per cent ivermectin obtained efficacies of 99 per cent, 0 per cent and 94 per cent against all three aforementioned helminth species.

For *C. pectinata, C. spatulata, Trichostrongylus axei, T. colubriformis, Trichuris discolor, Capillaria bovis* and *Bunostomum phlebotomum*, the small number of animals in control group (less than two animals) infected by such species made it impossible to draw conclusions on efficacy indexes obtained and, consequently, impossible to statistically analyse the average counts of these helminth species between treated and control groups.

DISCUSSION

Results obtained on studies with *R. (B.) microplus* revealed that the pour-on combination of 1.5 per cent ivermectin +0.5 per cent abamectin showed better efficacy indexes against this ectoparasite when compared with formulations containing 0.5 per cent ivermectin, 1 per cent ivermectin and the 1 per cent abamectin+20 per cent levamisole. Using the same tick strain, Lopes and others (2013a, b, c) diagnosed *R. (B.) microplus* resistance to 0.5 per cent abamectin, using a stall test for the evaluation. In field trials, it was possible to observe elevated acaricidal efficacy for the pour-on combination of ivermectin+abamectin up to the 3rd DPT. On the other hand, the same formulation, when evaluated

TABLE 4: Average number of *Haematobia irritans* from control and treated groups; efficacy and results of variance analysis

Day of study	Experiment (local)	Experimental groups/mean* number of *H. irritans*			Efficacy (%)	
		T01: control	T02: 1.5% ivermectin +0.5% abamectin†	T03: 0.5% ivermectin‡	T02	T03
0	5. Formiga/Minas Gerais	80.7^{a}	80.5^{a}	79.1^{a}	–	–
3		79.3^{a}	0.1^{b}	0.1^{b}	**99**	**99**
7		94.1^{a}	1.4^{b}	1.6^{b}	**98**	**98**
14		113.4^{a}	0.4^{c}	8.9^{b}	**99**	**92**
21		28.0^{a}	3.3^{c}	7.5^{b}	**88**	**73**
28		45.9^{a}	15.8^{b}	25.7^{ab}	**65**	**44**
0	6. União de Minas/Minas Gerais	126.6^{a}	125.7^{a}	127.2^{a}	–	–
3		91.7^{a}	0.9^{b}	0.8^{b}	**98**	**98**
7		85.4^{a}	0.5^{b}	0.7^{b}	**99**	**99**
14		56.1^{a}	0.4^{b}	1.0^{b}	**99**	**97**
21		32.8^{a}	2.5^{b}	2.0^{b}	**91**	**90**
28		31.4^{a}	4.9^{b}	9.6^{b}	**82**	**64**
35		34.8^{a}	21.3^{a}	29.7^{a}	**37**	**0**

Experiments 5 and 6
*Means followed by the same letter in the line do not differ (P>0.05)
†MSD Animal Health, Brazil
‡Commercial formulation purchased from the local market

TABLE 5: Total active myiasis in cattle from control and treated groups; efficacy and results of variance analysis

Day of study	Experiment (local)	Total* active myiasis					Efficacy (%)			
		T01: control	T02: 1.5% ivermectin +0.5% abamectin†	T03: 0.5% abamectin‡	T04: 0.5% ivermectin‡	T05: 1% doramectin‡	T02	T03	T04	T05
0	7. Formiga/Minas Gerais	7^{A}	7^{A}	7^{A}	7^{A}	7^{A}	–	–	–	–
1		7^{A}	7^{A}	7^{A}	7^{A}	7^{A}	0	0	0	0
2		7^{A}	6^{A}	7^{A}	7^{A}	7^{A}	14	0	0	0
3		7^{A}	5^{A}	7^{A}	7^{A}	6^{A}	28	0	0	14
4		4^{A}	2^{A}	5^{A}	6^{A}	2^{A}	50	0	0	50
5		2^{AB}	2^{AB}	2^{AB}	6^{A}	0^{B}	0	0	0	100
6		1^{A}	1^{A}	2^{A}	3^{A}	0^{A}	0	0	0	100
7		1^{A}	0^{A}	1^{A}	1^{A}	0^{A}	100	0	0	100
3	8. União de Minas/Minas Gerais	1^{A}	0^{A}	0^{A}	2^{A}	0^{A}	100	100	0	100
5		7^{AB}	5^{BC}	2^{C}	10^{A}	5^{BC}	28	71	0	28
7		9^{A}	10^{A}	7^{A}	9^{A}	5^{A}	0	22	0	44

Experiments 7 and 8
*Total followed by the same letter in the line does not differ (P>0.05)
†MSD Animal Health, Brazil
‡Commercial formulation purchased from the local market

against *R. (B.) microplus* parasitising experimentally infested cattle, reached efficacy values superior to 98 per cent after the 8th DPT. Delay in the action against this tick species was observed, for MLs, by Gonzales and others (1993) and Davey and George (2002). Borges and others (2008) evaluating the injectable combination of 2.25 per cent ivermectin+1.25 per cent abamectin, and, through a stall test, verified efficacy values greater than 99 per cent after the 5th DPT. These authors state that this could be a consequence of the ingestion of low concentrations of the active principle from cattle blood by adult ticks in the initial post-treatment period, due to the pharmacokinetic profile of MLs. Other aspect that should be reinforced is the action period of 1.5 per cent ivermectin +0.5 per cent abamectin in different trials conducted against *R. (B.) microplus* (natural and artificial infestations). Such formulation presented anti-*R. (B.) microplus* activity of 21–28 days and 49 days for studies with natural and artificial infestations, respectively. This difference can be justified by the pour-on administration of such product. Animals treated and maintained in the stall test did not suffer interference of rain or direct sunlight, unlike those animals treated with the same compound and maintained in field conditions.

Against *H. irritans*, the pour-on ivermectin+abamectin combination was compared only with 0.5 per cent ivermectin, and, in this case, both formulations presented elevated efficacy (>90 per cent) for very similar periods of time (14–21 days) against this ectoparasite. On the other hand, against *C. hominivorax* larvae, all pour-on formulations tested (1.5 per cent ivermectin +0.5 per cent abamectin, 0.5 per cent ivermectin and 0.5 per cent abamectin), as well as 1 per cent doramectin administered subcutaneously, were considered ineffective, in two different experiments, when administered preventively against larvae of this ectoparasite. Similar results, with ivermectin and abamectin (administered through pour-on or injectable routes), were obtained by Lopes

TABLE 6: Average number of *Dermatobia hominis* larvae from control and treated cattle; efficacy and results of variance analysis

Day of study	Experiment (local)	Experimental group/mean* number of *D. hominis* larvae				Efficacy (%)		
		T01: control	T02: 1.5% ivermectin +0.5% abamectin†	T03: 0.5% ivermectin‡	T04: 0.5% abamectin‡	T02	T03	T04
0	9. Formiga/Minas Gerais	24.8^{a}	17.9^{a}	18.0^{a}	18.0^{a}	–	–	–
7		28.6^{a}	0.2^{b}	0.8^{b}	0.4^{b}	99	98	97
14		25.2^{a}	0.2^{b}	2.3^{b}	0.6^{b}	99	97	90
21		24.9^{a}	0.1^{b}	0.6^{b}	0.4^{b}	99	98	97
28		14.9^{a}	0.1^{b}	0.4^{b}	0.4^{b}	99	97	97
35		6.2^{a}	0.3^{b}	0.4^{b}	0.8^{b}	94	93	98
42		6.3^{a}	0.2^{b}	1.4^{b}	0.5^{b}	96	91	77
49		10.0^{a}	4.7^{a}	6.3^{a}	6.9^{a}	52	31	37

Experiment 9
*Means followed by the same letter in the line do not differ (P>0.05)
†MSD Animal Health, Brazil
‡Commercial formulation purchased from the local market

TABLE 7: Mean counts of helminth species collected from cattle belonging to control and treated groups; efficacy and results of variance analysis

		Experimental groups/mean* number of helminth species				Efficacy (%)		
Helminth species	Experiment (local)	T01: control	T02: 1.5% ivermectin+ 0.5% abamectin†	T03: 0.5% ivermectin‡	T04: 0.5% abamectin‡	T02	T03	T04
Haemonchus placei	10. Formiga/Minas Gerais	2377.5^{AB}	184.9^{C}	4359.5^{A}	801.2^{B}	**92**	**0**	**66**
H. similis		222.8^{A}	0.0^{B}	0.9^{B}	0.0^{B}	**100**	**99**	**100**
Cooperia punctata		5499.7^{A}	232.7^{B}	5573.7^{A}	170.9^{B}	**96**	**0**	**97**
Trichostrongylus axei		8.6^{A}	0.0^{B}	0.1^{B}	0.0^{B}	**100**	**99**	**100**
Oesophagostomum radiatum		251.4^{A}	0.0^{C}	14.4^{B}	1.4^{C}	**100**	**94**	**99**

Experiment 10
*Means followed by the same letter in the line do not differ (P>0.05)
†MSD Animal Health, Brazil
‡Commercial formulation purchased from the local market

and others (2013a, b, c). Published works that describe pharmacokinetic aspects of formulations (pour-on and/or injectable) can elucidate the possible ineffectiveness on these cases. According to Toutain and others (1997), since not all parasites feed on blood plasma, the distribution of drugs for different compartments (lymph, skin secretions, abomasum secretions, among others) occur in different concentrations than those found in plasma, what could partially justify the lack of correlation between pharmacokinetic values observed by a formulation and the inefficacy of such drug against determinate ectoparasites that do not feed on plasma. Other aspect these authors take into account is that there is no complete definition if a formulation should reach a determinate concentration to start exerting an antiparasitic activity, or if the period (time) of exposition from a parasite to the active principle is more important for a formulation to express its antiparasitic action. In either case, this is the first report of 1 per cent doramectin (administered subcutaneously) inefficacy against *C. hominivorax* larvae.

Animals medicated with 1.5 per cent ivermectin +0.5 per cent abamectin, pour-on, remained free of parasitism by *D. hominis* larvae for a period of 42 days (96 per cent efficacy), while efficacy values superior to 90 per cent were obtained by 0.5 per cent ivermectin (92 per cent) and by 0.5 per cent abamectin (93 per cent), until the 42nd and 35th DPT, respectively. This protection period found for pour-on formulations is similar to those obtained by 1 per cent ivermectin and 1 per cent abamectin, both administered subcutaneously, but is inferior to the period of protection found by Borges and others (2008) for 1 per cent doramectin or high-concentration avermectins (>3.15 per cent), administered subcutaneously, which was superior to 100 days. Regarding protection periods of chemical compounds against parasites, the aforementioned authors evaluated the endectocide activity of an injectable formulation containing 2.25 per cent ivermectin+1.25 per cent abamectin against *R. (B.) microplus* and *Dermatobia hominis* larvae and found, in an overall way, longer protection periods against these ectoparasites than the present study observed for the pour-on combination of 1.5 per cent ivermectin +0.5 per cent abamectin. A possible explanation for such difference on the protection period of both ivermectin+abamectin combinations is related to administration routes, since, according to Laffont and others (2003), topical administration does not guarantee a controlled drug delivery in cattle.

Regarding results obtained on the anthelmintic trial, it was possible to verify that the pour-on combination of ivermectin+abamectin obtained better efficacy indexes than the conventional 0.5 per cent ivermectin and 0.5 per cent abamectin formulations for *H. placei* and *O. radiatum*. Against *C. punctata*, there was no difference regarding efficacy values obtained by the avermectins combination and abamectin.

In accordance with Vercruysse and others (2001), a nematode strain is considered resistant when the efficacy of a given formulation, calculated using its geometric means, is less than 90 per cent. Furthermore, the VICH's (International Cooperation on Harmonization of Technical Requirements for Registration of Veterinary Medicinal Products) (VICH, Guideline 12, 2001) GL12 (Anthelmintics: Cattle) highlights the importance of considering the significant difference between treatment and control groups at 5 per cent probability level. Recent studies have indicated that arithmetic means should be used to diagnose whether a helminth strain is resistant to a given formulation (Dobson and others 2009, Vercruysse and others 2011). The WAAVP (World Combination for the Advancement of Veterinary Parasitology) guidelines for assessing efficacy of anthelmintic formulations prepared for ruminants and horses (Geary and others 2012) report that resistance can be attributed to a population of helminths showing a substantial reduction in efficacy (≤80 per cent) when treated with an anthelmintic that had previously demonstrated efficacy levels above 95 per cent against that species. Based on aforementioned criteria, it is possible to affirm that *H. placei* and *C. punctata*

populations are resistant to 0.5 per cent ivermectin administered as a pour-on. Studies to determine the therapeutic doses of ivermectin, doramectin and moxidectin for cattle indicate the *Cooperia* species may be dose-limiting for moxidectin and some of the avermectins (Vercruysse and Rew 2002). Thus, lower efficacy for ivermectin could be expected against this genus. Reports of *Cooperia* resistant to ivermectin, administered as a pour-on or injectable formulation (Lopes and others 2009, 2013a, Edmonds and others 2010; Bartley and others 2012, De Graef and others 2012), reinforce the inference previously described.

H. similis and *O. radiatum* were considered sensitive to all three tested formulations (1.5 per cent ivermectin +0.5 per cent abamectin, 0.5 per cent ivermectin and 0.5 per cent abamectin) on this anthelmintic trial.

Overall, ivermectin presented better efficacy results against *R. (B.) microplus* and *D. hominis* larvae than abamectin. On the other hand, abamectin showed to be more effective against helminths than ivermectin. Relates of Egerton and others (1974) and Shoop and Soll (2002) can justify this data since, according to these researchers, abamectin is more active against nematodes (Egerton and others 1979) and slightly less efficient against arthropods (Shoop and Soll 2002) when compared with ivermectin.

The higher effectiveness obtained by topical association of ivermectin+abamectin, when compared with other topical formulations used in this study, can be justified by the fact that this new formulation (ivermectin +abamectin) contains ivermectin at a threefold higher concentration than the other formulations used in this study. Moreover, in some cases, the greater effectiveness was due to the additional presence of abamectin 0.5 per cent in the new formulation. Finally, there may be some synergistic interaction between the two active ingredients present in this formulation; however, further studies should be carried out to confirm this hypothesis.

Based on results obtained throughout the present studies, the pour-on combination of 1.5 per cent ivermectin +0.5 per cent abamectin obtained superior efficacy against *R. (B.) microplus*, *D. hominis* and some species of cattle gastrointestinal nematodes, when compared with formulations of 0.5 per cent ivermectin and 0.5 per cent abamectin, administered through the same route. Similar results were also obtained by the injectable combination of 2.25 per cent ivermectin +1.25 per cent abamectin (Borges and others 2008). Such results demonstrate the benefits of associating these avermectins for use on cattle, independent of the administration route used.

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