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ORIGINAL PAPER

Control Strategies for the Tick *Rhipicephalus Microplus* (Canestrini, 1888) on Cattle: Economic Evaluation and Report of a Multidrug-Resistant Strain

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Abstract

Purpose The aim of this study was to evaluate four strategies for application of acaricides to control the tick *Rhipicephalus microplus* among infested cattle, and to show which of these has the best cost–benefit ratio.

Methods For this, 72 cattle were selected and divided into four groups: Group 1 (G1): fipronil, pour-on; Group 2 (G2): fluazuron, pour-on; Group 3 (G3): moxidectin, injectable; and Group 4 (G4): chlorpyrifos 30 g, cypermethrin 15 g and fenthion 15 g, spraying (atomizing chamber). Every seven days, the numbers of semi-engorged females were counted and laboratory tests were conducted using different commercial technical-grade products for resistance monitoring.

Results G4 showed the best percentage reduction, with the highest rate on the seventh day post-treatment (DPT) (83.23%). G3 was the second best strategy, with a percentage of inverse reduction such that the best results were on the 28th DPT (82.85%), while G1 and G2 reached their best results on the 21st DPT (32.63% and 2.79%).

Conclusion It was noteworthy that the formulation used in G4 was the only one that was efficient for strategic control and that, based on the economic analysis, was shown to be economically viable over the medium term due to the need for investment. The presence of a multidrug-resistant strain in the state of Mato Grosso do Sul in vitro, for the chemical bases amitraz, cypermethrin and cypermethrin + DDVP, is reported here for the first time.

Keywords Cost–benefit · Acaricide · Cattle Tick · Aspersion · Efficiency

Introduction

The cattle tick *Rhipicephalus microplus* (Canestrini, 1888) is considered to be one of the biggest obstacles to cattle rearing worldwide, causing losses relating to production, reproduction and animal health. In Brazil alone, presence of this tick generates expenditure of 3.24 billion dollars annually [1].

Several strategies are used to control this ectoparasite in Brazil, although it is still predominantly done using acaricides, through various commercial products available on the Brazilian market. Acaricides thus constitute an important health management tool, with different mechanisms of action described for each chemical family. Pyrethroids (sodium channel modulators), organophosphates (acetylcholinesterase inhibitors), amidine (action in the octopamine channel), macrocyclic lactones (activating action in the chlorine channel via GABA and/or glutamate), phenylpyrazole (blocking action in the chlorine channel) and fluazuron (growth regulator) can be considered to be the main acaricide classes available nowadays [2–10]. However, their use may result in emergence of tick populations that are resistant to these chemical bases in this country [11, 12].

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Nonetheless, even with large differences between drug administration routes, which each have advantages and disadvantages [13], depending on the type of rearing (farm animals), few reports are found in the literature regarding the cost–benefit relationship of each control strategy.

Pour-on acaricides are preferred on many farms because they are easy to apply to animals. However, according to data available in the literature, there are differences in acaricide efficacy for the same or similar chemical formula, regarding its tick control results, depending on the method of application. The spray method is considered more appropriate [14–16].

This study presents an evaluation of different strategies for controlling the tick *Rhipicephalus microplus* among naturally infested cattle, encompassing the economic aspects of these strategies, and also describes the presence of a multidrug-resistant strain.

Materials and Methods

Selection of Animals, Place and Date of Study and Acaricides

Seventy-two mixed-breed (Brangus) 14-month-old bulls that were naturally infested with *R. microplus* ticks were selected. These belonged to the Sanyo Farm, which is located in the municipality of Agua Clara, Mato Grosso do Sul, Brazil (latitude 20°46′24" S; longitude 52°32′24" W), at an altitude of 309 m. The climate is characterized as humid tropical, with a dry season of one to three months and average temperature above 18 °C in all months of the year [17]. The present study was conducted from April 2 to May 18, 2018. All the procedures performed using animals followed the norms published by the Conselho Nacional de Controle de Experimentação Animal (National Animal Experimentation Control Board, CONCEA), and the project was approved by the Comissão de Ética no Uso de Animais (Ethics Committee for the Use of Animals, CEUA) of Embrapa Beef Cattle, protocol numbers 01/2016.

Acaricide Selection

To choose the acaricide to be used, as well as to analyze the resistance profile in the local tick population, engorged females ticks (±450) were collected from the herd before the period of this experiment, for bioassays [18] and adult immersion tests (AITs) to be performed. For this purpose, three groups of ten engorged female ticks (triplicates/30 ticks) per acaricide were used for each of the twelve products tested, totaling 430 engorged female ticks. However, according to Holdsworth *et al*. [19] these tests can be used as preliminary guidance prior to dose determination or confirmatory studies. The following acaricides were used: cypermethrin 15 g + chlorpyrifos 30 g + fenthion 15 g; cypermethrin 15 g + chlorpyrifos 25 g + PBO 15 g + citronella 1 g; cypermethrin 15 g + chlorpyrifos 25 g + PBO 1 mL; cypermethrin 5 g + DDVP 60 g; amitraz 12.5 g; cypermethrin 15 g; dichlorvos 60 g + chlorpyrifos 20 g; cypermethrin 20 g + chlorpyrifos 50 g; and cypermethrin 6 g + chlorpyrifos 50 g. In addition, the following technical-grade products were used: fipronil 1% (technical grade 91.9%), fluazuron 2.5% (technical grade 99.43%) and doramectin 1% (technical grade 98.41%). These were donated by Dexter Latina Chemicals Ltda. (bioassays based on the protocols described by [20, 21], respectively). A control group was also set up.

After immersion of the engorged females in the respective acaricide treatments mentioned above, the ticks were allocated to Petri dishes for incubation and evaluation of reproductive parameters (weight of the engorged female, weight of the eggs and hatchability rate). Through this, values needed for calculating acaricide efficiency based on the following formula were obtained:

$$\text{Reproductive efficiency (RE)} = \frac{\text{total egg}}{\text{engorged female weight}} \times \text{hatchability} \times 20{,}000\,*$$

$$\text{Control percentage} = \frac{\text{RE(control group} - \text{RE(treated group)}}{\text{RE(control group)}} \times 100$$

* 20,000 larvae correspond to 1.0 g of eggs of *R. microplus* [22]

Experimental Design

The cattle were divided into four experimental groups, according to the average counts of semi-engorged ticks of length 4.5 and 8.0 mm [23] that were present over the entire body surface of the animals, obtained on days -2 and -1. Only cattle with counts higher than 40 ticks on the entire body surface and with good nutritional and health status were included in the study. The four cattle with the highest counts were destined to replication number 1, the following four to replication number 2 and so on, to form 18 replications. The animals of each replication were randomly included in each of the experimental groups, as described in Table 1.

On day zero, the animals in groups 1, 2 and 3 received treatment in accordance with the routes and doses for those treatments that their manufacturers recommended. For the cattle belonging to group 4, in addition to the manufacturer's recommendations, an atomizing chamber (Coimma®, Dracena SP) was used, and 310 L of acaricide solution was prepared for treating all the animals in that group. After treatment, the groups remained together in the same 48-ha

Table 1 Distribution of experimental groups

Group	Number of Cattle	Treatment	Administration Route	Dose/Dilution
1	18	Fipronil 1%	Topical (Pour-on)	1 mL/10 kg
2	18	Fluazuron 2.5%	Topical (Pour-on)	1 mL/10 kg
3	18	Moxidectin 10%	Injectable (Subcutaneous)	1 mL/100 kg
4	18	Chlorpyrifos 30 g, Cypermethrin 15 g and Fenthion 15 g	Topical (Spray)	25 mL/20 L (dilution)

paddock under continuous grazing system with *Brachiaria* spp., thus sharing the same infestation conditions, that is, the pressure conditions of the pasture infested by tick larvae. The animals were left to graze on the pasture and also received mineral supplementation ad libitum in a trough, along with a water supply. Tick counts were performed on the 2nd day, 7th day and every seven days thereafter until the 35th day post-treatment (DPT). The percentage reduction in the average number of ticks was calculated in accordance with the following formula:

$$Percentage\ reduction = \left(\frac{\begin{matrix} Mean\ count \\ before\ the\ treatment \end{matrix} - \begin{matrix} Mean\ count \\ after\ the\ treatment \end{matrix}}{\begin{matrix} Mean\ count \\ before\ the\ treatment \end{matrix}} \right) \times 100$$

In vitro Efficacy of "Initial Acaricide Solution" and "Final Acaricide Solution"

At the end of the second-day count (from ticks on cattle), a total of 40 engorged females from animals that were treated at the beginning of the acaricide bath and 40 from animals treated at its end were collected from the cattle to perform *in vitro* adult immersion tests (AITs).

For this, engorged females were collected directly from the abovementioned animals. These were then sent under constant refrigeration to Embrapa's tick biology laboratory, where they were washed, dried on filter paper, separated into groups and weighed for AITs to be performed. After immersion in the respective acaricide solutions, the specimens were packed into Petri dishes and placed in a biological oxygen demand (BOD) incubator at a temperature of 27 °C and 80% humidity. After a 48-h period, the effect of the acaricidal broth on the engorged females was observed, i.e. their survival and mortality was observed. After oviposition took place among the engorged females that had survived, the egg masses were separated and placed in tubes for subsequent hatching and the percentage hatching was estimated.

The aim of these tests was to evaluate the acaricide solution that was used in the atomizing chamber, as described by Drummond *et al*. [18], with adaptations. During the spraying process, the atomizing chamber has a system for reusing the acaricide solution. Through this, the initially clean solution that is applied to the animals gradually becomes a dirty solution. In this bioassay, the efficacies of the clean solution (immediately after dilution of the acaricide) and the dirty solution (immediately after passage of the last animal in the treated batch) were evaluated. It is important to highlight that the methodology of this bioassay enabled evaluation of two groups, i.e. live ticks (engorged females that performed oviposition) and dead ticks (without oviposition), and used an efficacy formula based on mortality.

Statistical Analysis

For the statistical analysis, the means of the counts of semi-engorged females in the four groups and the percentage reductions were compared using the Kruskal–Wallis test and the BioEstat 5.3 statistical software.

Cost–Benefit Calculation

The acaricides used to treat these cattle were pour-on fipronil 1% (TopLine®; Boehringer Ingelheim), pour-on fluazuron 2.5% (Forbox; Biogénesis Bagó); injectable moxidectin 10% (Onyx®; Zoetis); and a spray consisting of chlorpyrifos 30 g, cypermethrin 15 g and fenthion 15 g (Colosso FC 30; Ourofino). These were all purchased at the same establishment, for the amounts of US$ 88.70, 86.00, 193.00 and 45.70, respectively. These amounts in US$ were obtained through conversion of the amounts in reais, at the exchange rate at the time of the experiment, of R$ 3.72 = US$ 1.00 [24].

Table 2 Previous *in vitro* tests on different commercial and technical-grade acaricides on a strain of *R. microplus* using the adult immersion technique

Formulation	Weight of Engorged Females (g)	Egg Mass Weight (g)	Hatchability (%)	Efficiency (%)
Cypermethrin 15 g + Chlorpyrifos 30 g + Fenthion 15 g	2.55	0	0	100
Cypermethrin 15 g + Chlorpyrifos 25 g + PBO 15 g + Citronella 1 g	2.5	0	0	100
Cypermethrin 15 g + Chlorpyrifos 25 g + PBO 1 mL	2.52	0	0	100
Cypermethrin 5 g + DDVP 60 g	2.45	0.35	90	64.24
Amitraz 12.5 g	2.6	1.16	95	0
Cypermethrin 15 g	2.55	0.91	90	10.66
Dichlorvos 60 g + Chlorpyrifos 20 g	2.54	0.07	1	99.92
Cypermethrin 20 g + Chlorpyrifos 50 g	2.54	0.38	2	99.17
Cypermethrin 6 g + Chlorpyrifos 50 g	2.43	0	0	100
Doramectin 1%	2.38	0	0	100
Fipronil 1%	2.28	0	0	100
Fluazuron 2.5%	2.15	0	0	100
Control	2.47	0.96	92.5	–

Table 3 Means counts of semi-engorged ticks and percentage reductions in the treated groups during the experimental period

Study Day	Experimental Group /Mean Number of Ticks				Percent Reduction (%)			
	G1 Fipronil pour-on	G2 Fluazuron pour-on	G3 Moxidectin injectable	G4 Chlorpyrifos, Cypermethrin and Fenthion spray	G1	G2	G3	G4
0 (-2 and -1)	65.47	64.81	65.44	65.25	–	–	–	–
2	60.33	73.06	51.72	13.56	7.85	0.00	20.97	79.23
7	59.28	84.67	61.28	10.94	9.46	0.00	6.37	83.23
14	64.81	89.63	69.87	15.59	1.01	0.00	0.00	76.11
21	44.11	63.00	63.56	11.00	32.63	2.79	2.89	83.14
28	72.22	65.67	11.22	30.17	0.00	0.00	82.85	53.77
35	92.00	100.50	36.33	75.56	0.00	0.00	44.48	0.00
Overall Mean	65.46[a]	77.33[a]	51.34[a,b]	31.72[b]	8.49[a,b1]	0.47[a1]	26.26[a,b1]	62.58[b1]

[*]Differences in statistical analyses between the tick counts ([a,b]) and percentage reduction values ([a,b1])

The same physical installations were used for management of all the treatment groups. However, for group 4, there was the addition of an atomizer chamber, which represented an investment expenditure of US$ 4,838.70. All other expenses relating to treatment or management of the animals were excluded from the calculations because they were the same for all groups.

The benefit obtained through the treatment was taken to be the percentage reduction in the number of ticks attained through use of each of the products and the persistence of this reduction over the experimental period (35 days).

Table 4 Expenditure on tick control according to each experimental group

Product	Infrastructure Cost (US$)	Product Dose per Animal (mL)*	Product Dose Cost Per Animal (US$)	Cost Per mL (US$)
Fipronil	–	21.00	0.37	0.018
Fluazuron	–	21.00	0.36	0.016
Moxidectin	–	2.10	1.62	0.771
Spray Chamber	4,838.70	6.25	0.28	0.045

*Dose calculated based on the mean weight of all experimental animals

Table 5 Financial return of the investment for acquisition of a spray chamber when using the strategy of five annual treatments

Product	Dose Cost Per Animal (U$)	Susceptible Herd (n)	Annual Amount Spent on Drugs with the Strategy of 5 Treatments Per Year (U$)		Savings When Using Spraying (U$)	Return on Investment for Spray Chamber Purchase (Years)
			Product	Spraying		
Fipronil	0.36 (22.22%)	4000	7,200.00	5,600.00	1.600,00	3.01
Fluazuron	0.37 (24.32%)	4000	7,400.00	5,600.00	1.800,00	2.68
Moxidectin	1.61 (82.61%)	4000	32,200.00	5,600.00	26.600,00	0.18

Data compared with the dosage of the contact acaricide used in the spray chamber

Results

The bioassays performed with different contact acaricides demonstrated that six out of the nine commercial products tested showed adequate efficacy (> 95%). Five of these belonged to the group of acaricides with formulae based on pyrethroids and organophosphates (association), as shown in Table 2.

Table 3 shows the results regarding the average numbers of ticks counted during the experimental period. There was a significant difference ($p < 0.05$) between the group treated with the veterinary spray (31.72) and the groups treated with fipronil (65.46), fluazuron (77.33) and moxidectin (51.34). The treatment by means of aspersion also showed a positive result regarding the percentage reduction of ticks, with the highest rate on the 7th DPT (83.23%), and ending the study on the 35th DPT without any further effect. Injectable moxidectin was the second best strategy, with a percentage of inverse reduction such that worse rates were presented in the first weeks and better results on the last dates, with 82.85% on the 28th DPT, which was the highest percentage obtained. The moxidectin group ended the study with 44.48%. On the other hand, the two pour-on treatments, fipronil and fluazuron, presented their highest percentage reductions on the 21st DPT (32.63% and 2.79% respectively), and did not reach satisfactory percentages in any of the counts.

The *in vitro* immersion test on adult ticks with the acaricide used in the aspersion reached maximum efficacy (100%) with the clean solution, while the dirty solution that remained after the passage of the animals presented lower percentage efficacy (90%). These *in vitro* efficacy figures are much more satisfactory than those obtained in the field, in which the maximum reached was 83.23% on the 7th DPT.

The cost analysis on the treatments, presented in Table 4, showed that the dosage cost of the product used in the atomizing chamber had the best cost–benefit relationship. This represented significant savings, compared with the other treatments. Moxidectin, on the other hand, had the highest cost: almost five times more expensive than the other acaricides.

Table 5 shows a comparison of the cost of the aspersion treatment and the return on investment for installation of the atomizing chamber, in relation to the other treatment strategies, with regard to treating a herd of 4000 animals. This treatment showed a faster financial return in comparison with moxidectin (0.18 years) because this was the most costly strategy, followed by fluazuron (2.68 years) and fipronil (3.01 years).

Discussion

During the period of the experiment, none of the cattle died; nor did they present any clinical symptoms of any disease or any intoxication resulting from the treatments. Regarding the results shown in Table 2, it can be seen that the contact acaricide product cypermethrin 15 g + chlorpyrifos 30 g + fenthion 15 g reached 100% efficacy in *in vitro* tests. It was noteworthy that acaricides of formulations based on cypermethrin and amitraz did not present adequate *in vitro* efficacy, and neither did the acaricide product of composition cypermethrin 5 g + DDVP 60 g, which was the only one among the associations that showed any indications of established resistance. The results from this study corroborate the findings of Higa *et al*. [25] and Valsoni *et al*. [26], who also found populations of *R. microplus* in the state of Mato Grosso do Sul that were resistant to pyrethroids, starches and some associations.

From Table 2, it can be noted that the technical-grade products of doramectin, fipronil and fluazuron were used on concentration curves to ascertain the situation of these chemical bases in the tick population. Efficacy of 100% was presented in *in vitro* tests, compared with the commercially available products.

In a study conducted by Gupta and Gupta [27], a comparison between the efficacy of technical-grade fipronil and a commercial formulation, through *in vitro* adult immersion tests revealed an agreement between the efficacies found (difference between efficacies < 8%). However, the present study used these acaricides at technical grade for *in vitro* tests and subsequently applied the products in the field. As mentioned for the contact acaricide, the efficacy of the acaricide applied in the field is susceptible to abiotic factors such as ultraviolet light, temperature and humidity, rain and possibly the application route (pour on or injectable), which may contribute to divergence between the results [10, 16, 28].

Regarding the field results that are presented in Table 3, an intermediate percentage reduction among the group of animals treated with moxidectin can be seen (10%), reaching values below 90% in all counts. In a study conducted by Cruz *et al*. [29], the efficacy of different concentrations of ivermectin (0.5, 1.0 and 3.15%) was studied in the states of Minas Gerais and São Paulo, also under field conditions. Only the acaricide administered at the concentration of 3.15% reached 90% field efficacy in the experiment, and the presence of strains resistant to this and other concentrations of the drug on different farms was described. According to their data, the efficacy of ivermectin reached its peak between the 14th and the 21st day, i.e. similar to what was found in the present study, where the percentage reduction in tick concentration was higher only on the 28th day (82.85%). These findings suggest that a population resistant to ivermectin was present, thus corroborating other reports of resistant strains that have been made through use of *in vitro* techniques in Mato Grosso do Sul [26] and in other regions of the country [30–32].

Another important finding from the present study was the low percentage reductions in the numbers of ticks present on the animals treated with fipronil and fluazuron. Valsoni *et al*. [26] had already described the presence of a resistant strain in Mato Grosso do Sul, but using laboratory tests. The percentage reduction in tick numbers achieved through using fluazuron was less than 3%, throughout the study period, which corroborated the findings of Reck *et al*. [21] in this state, who also described resistance to this chemical base, observed through field tests. This was also corroborated by the in vitro data produced by Valsoni *et al*. [26].

The results shown in Table 3 show that the injectable and pour-on treatments were generally inefficient in controlling ticks, compared with the aspersion treatment. These results are concordant with those of Higa *et al*. [16] and Camillo *et al*. [33], who studied strategies for acaricide applications (spraying and pour-on) and reported that the sprayed treatment attained greater efficacy. However, the present results did not corroborate the findings of Campos Jr. and Oliveira [34], who reported that pour-on fipronil achieved 90% efficacy in the microregion of Ilheus, state of Bahia. It should be noted that the parasite cycle of *R. microplus* has an average duration of 21 days, which is a period equivalent to the effectiveness of the atomizing chamber. This makes it clear why this treatment is efficient for strategic control of the parasite [35].

These results express the importance of monitoring the efficacy of acaricides used on farms. Application of pour-on products ends up becoming more usual because this is easier to do than conventional spraying (i.e. without using the chamber). Use of pour-on products is also easier than using injectable drugs such as 10% moxidectin, since the product needs to be administered behind the animal's ear.

The increase in the number of ticks during the study period suggests that the products were under constant challenge. The results from the *in vitro* tests showed that the tick reduction rates from use of these products were excellent (100 and 90%). However, the rates found in the field were lower, such that the maximum efficacy was 83.23%. This corroborates the findings from studies conducted by Rodrigues *et al*. [15] and Correa *et al*. [36]. It should also be borne in mind that the minimum effectiveness required by the Ministry of Agriculture, Livestock and Supply is 95% [37].

The chamber forms a facilitating tool for application of contact acaricides. The investment required for its acquisition is offset by the way in which it provides greater ease of application in herds with larger numbers of animals, given that there is no need to weigh the animals, unlike in other treatment methods. Moreover, in the present study, it gave rise to better efficacy in applying the formulation containing chlorpyrifos 30 g, cypermethrin 15 g and fenthion 15 g, thereby demonstrating a better cost–benefit ratio [11, 38, 39]. However, correct maintenance and care with regard to replacement and refilling of the solution are required [11], along with measures to avoid the appearance of resistance, which is essential for this method to be satisfactory on farms over the long term.

It has been estimated that in Brazil alone, the expenditure generated for tick control and the losses caused by this tick is more than US$ 3.24 billion per year [1]. Hence, it is important to calculate the cost–benefit relationships of the available control strategies.

Regardless of the efficacy of the products used, the results presented in Tables 4 and 5 show that in addition to being more efficient in controlling the parasite, the atomizing chamber presented lower cost of treatment than the other drugs. Nonetheless, it needs to be considered that installation of this chamber requires an investment of US$ 4,438. Unfortunately, an investment of this magnitude would not be compatible for small producers, since it would take a long time to achieve a financial return because of the small herd sizes.

However, for a herd of around 4000 cattle that presents susceptibility to the tick (such as the herd of the farm of this study), and with the strategy proposed by Furlong *et al*. [11] and Bonatte Junior *et al*. [40] (i.e. use of four to five treatments per year in Brazil), use of the chamber as a replacement for pour-on treatments would result in savings of approximately 20%. Thus, the investment would be recovered over a period of three years. Furthermore, in comparison with moxidectin, the financial return would be achieved from time of the first treatment performed on the entire herd. This benefit is independent of the fact that the other treatment options presented here were not effective for use in strategic control.

Presence of these ticks directly affects the economic and productive performance of production systems. Thus, according to Calvano *et al*. [41], an increase in the technological level applied in conjunction with correct adoption of tick control will promote increased production such that the full genetic potential of these animals can be expressed.

However, even with the advantages presented by the treatment using the atomizing chamber, the inefficacy presented by the other formulations shows that there is a need to conduct new studies on farms with sensitive tick populations, to compare the persistence of efficacy and consequently the intervals between treatments. Furthermore, a prolonged study aimed at observing the number of annual treatments and evaluating pasture infestation levels in strategies with different drugs could demonstrate the effect of control also in relation to decontamination of pastures, considering that 95% of the tick population is in the pasture [42]. In the light of these results, there is an urgent need for new investigations in this area of the cattle production chain, and for the information from such investigations to be made available to all parties with an interest in this sector.

Conclusion

The aspersion treatment using a combination of acaricides presented the best result regarding efficiency of tick control among the groups evaluated. This result was the only one that can be recommended for use in strategic tick control among cattle.

The presence of a multidrug-resistant strain in the state of Mato Grosso do Sul, detected *in vitro* in relation to the chemical bases amitraz, cypermethrin and cypermethrin + DDVP, is reported here for the first time.

Treatments with pour-on fipronil, pour-on fluazuron and injectable Moxidectin did not present satisfactory efficiency in the field evaluations.

The economic analysis on use of an atomizing chamber showed that this provides a viable economic return.

Declarations

Conflict of Interest There were no conflicts of interest that might have biased the work reported in this paper.

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