

Factors associated with the efficiency of acaricides on different populations of *Rhipicephalus microplus*

Fatores associados à eficiência de acaricidas sobre diferentes populações de *Rhipicephalus microplus*

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ABSTRACT

Tick infestation causes major problems in cattle. Tick parasitism accounts for significant economic losses in many beef and dairy herds in the vast majority of the states in the Brazilian territory, including the Santa Catarina state in the southern region of the country. Tick resistance to several active principles occurs due to a number of factors, including the indiscriminate, injudicious and inadequate use of tick insecticides. Considering the great importance of fighting tick infestations in cattle, we evaluated the efficiency of 8 different topical active principles against the ixodid tick *R. microplus* and identified the main factors that contribute to the development of ticks resistant to acaricides in the farms and bovine herds studied. For such purpose, *R. microplus* females were collected in 39 farms located in different municipalities of the western region of Santa Catarina state in southern Brazil. At the time of sample collection, information about herd management, history of acaricide used in the herd, number of annual applications of these pesticides, frequency of acaricide rotation, and frequency of technical monitoring for strategic tick control were retrieved. We collected this data to determine contributing factors to the development of resistant *R. microplus* populations. For the sensitivity profile of these ticks to a number of different acaricides tested, 10 engorged females for each principle and a control were used. The results of our study show that most associations between pyrethroids and organophosphates had an efficiency between 96.6% and 100% in the control of *R. microplus*, except for the combination of alphacypermethrin 15%, ethion 16%, and chlorpyrifos 8.5% that had 93.4% of efficiency. Among the chemicals not associated with any other type of compound, amitraz 12.5% was effective in 98.3% of the cases. In contrast, cypermethrin 15% had an efficiency in only 25.8% of the farms/herds analyzed. Based on the results of the present study, we may infer that the methods of control and management used by producers may be related to the low indexes of resistance to topical acaricides in the different populations of ticks in the study area.

Keywords: Tick. *In vitro*. Acaricides. Control. Resistance.

RESUMO

A infestação por carrapatos em bovinos gera grandes problemas, sendo esse parasito responsável por elevados prejuízos econômicos em diversos rebanhos de produção de corte e, também, de leite na maioria das regiões brasileiras, inclusive em Santa Catarina. A resistência dos carrapatos frente aos diversos princípios ativos pode ser decorrente de fatores como o uso indiscriminado de carrapaticidas, bem como a inadequada forma de aplicação dos mesmos. Tendo em vista a grande importância em combater essas infestações, buscou-se avaliar a eficiência de oito diferentes princípios ativos de contato contra o carrapato *Rhipicephalus microplus*, bem como, identificar os principais fatores que possam contribuir com a seleção de carrapatos resistentes nas propriedades avaliadas. Para isso, fêmeas de *R. microplus* foram coletadas em 39 propriedades de diferentes municípios do Oeste de Santa Catarina. No momento da coleta também foram obtidas informações referentes ao sistema de manejo dos animais, histórico dos carrapaticidas utilizados, número de aplicações anuais, frequência de rodízios de acaricidas e, ainda, frequência de acompanhamento técnico para controle estratégico, com a finalidade de evidenciar os diferentes fatores que possam estar contribuindo para o surgimento de eventual resistência das populações do *R. microplus*. A avaliação de suscetibilidade foi realizada através da técnica de biocarrapaticidograma, utilizando 10 teleóginas ingurgitadas para cada princípio e mais o controle. Dentre os principais resultados obtidos, a maioria das associações entre piretróides e organofosforados demonstraram ter eficiência entre 96,6% e 100%, com exceção da associação de Alfacipermetrina 15%, Ethion 16% e Clorpirifós 8,5% com 93,4% de eficiência. Dentre as

bases químicas sem nenhum tipo de associação, o Amitraz 12,5% demonstrou ser eficaz em 98,3%, em contrapartida a Cipermetrina 15% teve eficiência em apenas 25,8% das propriedades analisadas. A partir dos resultados obtidos, pode-se afirmar que as formas de controle e manejo adotados pelos produtores podem estar relacionadas com os baixos índices de resistência aos carrapaticidas de contato, nas diferentes populações de carrapato na região do estudo.

Palavras-chave: Carrapato. *In vitro*. Acaricidas. Controle. Resistência.

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Introduction

The tick *Rhipicephalus microplus* is an ectoparasite that belongs to the family Ixodidae. It originated in the Asian continent from where it has spread to several regions around the globe, including Brazil (Willadsen & Jongejan, 1999). In Brazil, cattle farming represents a major and profitable activity. In 2016, the country held the position as the world's largest beef exporter and the second largest commercial herd with 218.23 million heads of cattle, only behind the United States (IBGE, 2016). According to Grisi et al. (2014), the economic impact caused by the bovine tick totals up to US\$ 3.24 billion per year. The losses associated with tick parasitism that are experienced by producers may be directly or indirectly related to the cattle herds and include the transmission of pathogens that cause cattle tick fever, reduction of the value of the hides due to myiasis, decrease in the production of meat and milk, and increase in production costs due to the expenses involved in measures implemented for the control of tick infestations (Jonsson, 2006; Rocha et al., 2011; Reck et al., 2014).

Chemical control has been the method most frequently used over the years to eliminate this ectoparasite from farms and animal hosts. However, tick resistance to acaricides has been noted during the last couple of decades as producers often carry out such control measures injudiciously, without the assistance of a technician or veterinarian, and without taking the epidemiology of the disease and ectoparasite

into account (Brito et al., 2006; Rocha et al., 2011). As a result, the probability of ticks developing resistance against acaricides has become progressively more common and the pace at which it occurs is increasing rapidly (Gomes et al., 2011). The resistance of *R. microplus* is easily noted in several regions of the country, and this factor has been determinant in several treatment protocol failures (Camillo et al., 2009). A number of studies have shown that the bovine tick has been developing genetic mutations, including mechanisms to reduce the absorbance of the chemical compound used. Moreover, physiological mutations occur with regard to the storage and excretion of these substances to create several strategies when exposed to different chemical substances to survive and thrive in such environment (Veríssimo, 2015).

Due to the development of resistance of *R. microplus* to the different acaricides available in the market, researchers have been searching for new associations between organophosphates and pyrethroids to develop products that are efficient against different strains of the bovine tick. Thus, the present study aimed to evaluate the current scenario of the effectiveness of topical acaricides in cattle farms of the western region of Santa Catarina state in southern Brazil, to establish the current scenario of tick resistance in the area, and also to determine the main factors related to its development and occurrence of *R. microplus* populations in the premises evaluated.

Materials and Methods

Area delimitation

Our survey started in May 2017 and was completed in December 2018 and was exclusively focused on the western region of Santa Catarina state in southern Brazil. Sample collection was carried out in 39 livestock beef or dairy cattle farms from 13 different municipalities in the region, aiming to obtain the largest possible variety of different populations of the ixodid tick *R. microplus*. The following are the municipalities and respective number of farms in each of these municipalities where ticks were sampled: Abelardo Luz (3 farms), Waters of Chapecó (3 farms), Aguas Frias (2 farms), Coronel Freitas (2 farms), Ipuaçú (4 farms), Marema (2 farms), Nova Itaberaba (2 farms), Ouro Verde (2 farms), Palma Sola (11 farms), Passos Maia (1 farm), São Carlos (1 farm), Xanxerê (3 farms), and Xavantina (3 farms).

Collection of teleoginae and information retrieval from farms studied

For each *in vitro* test, 100 to 150 engorged females of *R. microplus* were sampled. Tick samples were collected manually from naturally infested cattle from premises that were treated with topical acaricides 25 days ago or more, or 45 days ago or more if the treatment was performed with systemic products. Ticks were gently sampled to prevent any mechanical injury to the reproductive system of the female tick and avoid any interference with egg laying. At the time of each sample collection, a form was filled out with information regarding the number of animals on the farm, the management system of the herd, annual tick treatment history, last active principle used for tick control, history of tick resistance, and rotation in the use of active principles in tick control. The data collected were statistically analyzed and compared with the results obtained in the *in vitro* tests to obtain the main factors influencing the development of tick resistance to acaricides. After sample collection, the teleoginae were submitted to the Laboratory of Veterinary Parasitology at the Western Santa Catarina University located in the municipality of Xanxerê, SC, southern Brazil, where the *in vitro* tests were carried out with a maximum interval of 48 h between sampling and testing. During this interval, the engorged females were stored at a temperature between 4 °C and 5 °C.

Acaricides

In this study, acaricides used in the *in vitro* tests were selected according to the largest number of farms in the study area that use these pesticides; eight different topical acaricides were tested, including six associations between pyrethroids and organophosphates and two formulations without any association. The following were the acaricides selected in the present study: amitraz 12.5%, alfacipermetrin 5% + ethion 16% + chlorpyrifos 8.5%, cypermethrin 15%, cypermethrin 15% + chlorpyrifos 25% + piperonyl butoxide 1%, cypermethrin 15% + chlorpyrifos 25% + citronella 1%, chlorpyrifos 30% + cypermethrin 15% + fenthion 15%, chlorpyrifos 50% + cypermethrin hi-cis 6%, and dichlorvos 60% + chlorpyrifos 20%.

In vitro assay

In our study, tests were carried out according to the protocol and guidelines provided by Drummond et al. (1973), known as immersion or sensitivity profile tests. In order to perform the *in vitro* assay, 100 engorged females were selected, washed in running tap water, and weighed on a precision scale, respectively. Then, groups containing

10 engorged females were formed with a maximum variation of 0.5 g per group. Thus, eight groups representing each acaricide and two control groups were selected. Emulsion immersion of the engorged females was performed immediately on a standard dilution of 20 mL of water, in which the concentration of each acaricide used followed the manufacturers' instructions. The control groups were immersed in distilled water. The immersion lasted 5 min. During this timeframe, samples were shaken at least three times. After the immersion, the teleoginae were dried with filter paper wrapped by PVC arches and then packed in petri dishes. Storage was carried out in incubator under previously controlled conditions at 28 °C and 80% relative humidity for a period of 14 days for egg laying.

After the oviposition, eggs were weighed and packed in test tubes capped with cotton to provide good air circulation. Subsequently, these specimens were returned to the greenhouse for an additional 28 days for larval hatching. At the end of this period, tubes were evaluated, and we estimated the percentage of larval hatching and, therefore, obtained the reproductive efficiency. In order to calculate the efficiency of the acaricide, we used the formulas developed by Drummond et al. (1973).

In order for the acaricide to be considered efficient, the parameters approved by the Ministry of Agriculture to license an active principle were used. Therefore, only products that reached efficiency levels above 95% were considered effective in controlling the bovine tick in a cattle farm (Brasil, 1997).

Statistical analysis

Data were subjected to analysis of variance using the GLM procedure of SAS statistical software (Cody, 2018) with a significance level of 5%. In case of significant difference, the averages were compared using the Tukey test.

Results and Discussion

The results of the present study demonstrate that tick resistance to topical acaricides is present in the different cattle farms of the area studied. However, the efficiency indexes found by farm are considered satisfactory since 6 of 8 acaricides tested *in vitro* had average efficiency that was above the minimum limit of 95% (see Table 1). For greater reliability of the results, all the controls had oviposition and larval hatching patterns according to the usual cycle of *R. microplus*.

The acaricide D (25.8%) presented lower average efficiency, and the G (93.4%), although high, on the 95% recommended by MAPA (Brasil, 1997), can be considered

Table 1 – Efficiency index of acaricides in farms located in the western region of Santa Catarina state in southern Brazil.

Variables	Treatments*							
	A	B	C	D	E	F	G	H
Number of farms	35	37	29	32	35	28	31	33
Minimal efficiency (%)	48	100	0	0	89	92	16	64
Maximum efficiency (%)	100	100	100	100	100	100	100	100
Average efficiency (%)	97.5 ^a	100 ^a	96.6 ^a	25.8 ^b	99.4 ^a	99.6 ^a	93.4 ^a	98.3 ^a
Default error	0.02	0.0	0.03	0.06	0.003	0.003	0.03	0.01
IC (95%)	94.1-100	100-100	89.5-100	12.9-38-7	98.7-100	98.9-100	87.5-99,3	95.9-100

*p<0.001; A: cypermethrin 15%, chlorpyrifos 25%, citronella 1%; B: chlorpyrifos 30%, cypermethrin 15%, fenthion 15%; C: cypermethrin 15%, chlorpyrifos 25%, piperonyl butoxide 1%; D: cypermethrin 15%; E: dichlorvos 60%, chlorpyrifos 20%; F: chlorpyrifos 50%, cypermethrin hi-cis 6%; G: alphacypermethrin 5%, ethion 16%, chlorpyrifos 8.5%; H: amitraz 12.5%; Distinct letters (a/b) in the column indicate statistical differences.

that there are populations of ticks resistant to these two products. The resistance indexes of the chemical compound cypermethrin 15% (acaricide D) observed in this study was 93.75% (30/32 farms). This is in agreement with the results obtained by Santos & Vogel (2012) in a study in which the authors evaluated 347 farms in Rio Grande do Sul state in southern Brazil. Similar findings are reported by Fernández-Salas et al. (2012) who evaluated 53 farms in Mexico and found cypermethrin-resistant ticks in 90.6% of these farms. The high resistance indexes to cypermethrin is due to the fact that this active principle is also used for the control of flies and has been associated with different formulations, including organophosphates, which facilitates the selection pressure on the tick population. Chemical compound B was 100% efficient on the 37 farms where it was tested. This may be explained by the fact that this is a relatively new product in the market and, therefore, has been used in the strategic control in tick populations with high indices of resistance since it has not been used indiscriminately to date (Reginato et al., 2017). The other acaricides evaluated in our study presented satisfactory and average efficiency (> 95%) ranging from 96.6% to 99.6%.

Our results show that the current scenario of resistance in different bovine tick populations in the western region of the Brazilian state of Santa Catarina has been changing over the years. These indices differ from the indices found in the same area by Pazinato et al. (2014), when these authors analyzed 31 farms where 6 different active principles were used. The main difference was the efficiency of 87.94% of the acaricide amitraz, which was lower than the efficiency observed in the present study (98.30%). The explanation for the current high efficacy index of amitraz may be related to the resistance reversal, which may occur in drugs from the amidine group. Studies have shown that after this chemical compound is not on tick populations for 15 to 20 generations, these ticks may become susceptible again to this group of acaricides (Furlong et al., 2007). In Mato Grosso do Sul state in central-west Brazil, Gomes et al. (2011) evaluated

12 different active principles. These authors observed that, regardless of the chemical group, the active principles without any type of association did not reach efficiency above 65% in all the assessment tests, including amitraz that, in our study, only 6.06% (6/33) the farms presented tick population resistance.

The results of our study show that most associations between pyrethroids and organophosphates had an average efficiency between 96.6% and 100% in the control of *R. microplus*, except for the combination of alphacypermethrin 15%, ethion 16%, and chlorpyrifos 8.5% that had 93.4% of efficiency. Similarly, in study of Gomes et al., 2011, the association of cypermethrin 15% + chlorpyrifos 25% + piperonyl butoxide 15% and citronellal 1%, achieved an average efficiency of 100% in all 14 farms where the chemical compound was tested. Moreover, these authors explained that this product was recently launched into the market at the time and it might be used in the strategic control of ticks in farms to delay the emergence of resistant tick populations in the future. In the Rio Grande do Sul state in southern Brazil, *in vitro* tests were conducted using nine different active principles, three of which had no association with other drugs and six chemicals that had at least two associations with other compounds. The chemical compounds with isolated active principles did not achieve superior efficiency (66.17%). In contrast, among the associations tested, only two formulations obtained an average efficiency above 95%, demonstrating a scenario of marked tick resistance to acaricides in this particular area (Machado et al., 2012). In the same Brazilian state, Reginato et al. (2017) evaluated a total of 54 farms where 4 different chemical compounds were used. Only the association between cypermethrin 15% + chlorpyrifos 30% + fenthion 15%, which was assessed in 51 properties, had a mean of 98.76% efficacy. Our results, where the overall mean of the same association reached 100% efficacy in the 37 analyzed properties, are in agreement with their results.

Table 2 – Creation system of animals and average of annual acaricide treatments in different farms in the western region of Santa Catarina state in southern Brazil.

Management		Frequency	Average Annual Treatment
Creation system	Semi-intensive	38.46% (15/39)	3.66
	Extensive	61.54% (24/39)	4.91
Rodizio of acaricides	Frequently	53.85% (21/39)	-
	Do not do	46.15% (18/39)	-

There are numerous causes and factors that contribute to the increase of resistance of *R. microplus* to topical acaricides. According to Rocha et al. (2011), management systems of animals in different farms, as well as the number of annual acaricide treatments, are considered important factors that will influence the final efficiency of these drugs. Among the 39 farms evaluated in the present study, most of 61.54% (24/39) use an extensive animal management system. In these farms, we noted that the producers carried out an average of 4.91 acaricides treatments per year. In contrast, 38.46% (15/39) farms use a semi-intensive management system and treatment mean was 3.66 treatments per year (see Table 2). This difference in the number of treatments according to the production system used in one particular farm may be explained by the greater challenge that cattle from the extensive system is subjected to daily, as 95% of the *R. microplus* population is present in the pastures where these animals graze. However, cattle from a semi-intensive production system are exposed to a less intense challenge since, in these farms, the animals are separated by pickets; therefore, theoretically, tick control becomes easier to perform (Rocha et al., 2011). Due to this difference in the number of annual treatments performed in the different management systems used in each farm, we suggest that the risk of ticks developing resistance in farms in which extensive management is used is higher, as there is a need to carry out more treatments for the control of *R. microplus*.

With regard to the correlation between the management system used on each farm and the resistance of the different *R. microplus* populations to acaricides, it is important to point out that producers lack information on tick control. Thus, drug efficacy is compromised and, as a result, tick control is not satisfactory in the field. It should be emphasized that each acaricide should be used according to the manufacturer's recommendations, including doses, proportion, dilution, amount of syrup per animal, and interval between applications. If these recommendations are not followed correctly, this may lead to suboptimal efficiency of the product. Therefore, technical assistance to eradicate ticks should be considered to ensure adequate strategic control and reduce the possibility of resistance development in tick populations (Campos & Oliveira, 2005; Amaral et al., 2011). In the present study, we observed that

100% of the farms carried out the sensitivity profile test for the first time in order to assess the susceptibility of ticks to a number of acaricides. Based on this information, we may infer that the treatments carried out on each of the farms were done subjectively, thus explaining the use of the chemical compound cypermethrin 15%. *In vitro* assays showed that cypermethrin 15% had an average efficiency of only 25.8%. Another important factor related to the resistance of ticks to acaricides is the rotation of active principles in the farms. In this survey, 53.85% of the producers reported that they carry out a rotation in the use of chemical compounds for tick control, whereas 46.15% said they do not (Table 2). According to Furlong & Sales (2007), when the acaricide rotation scheme is not adequately followed, tick resistance to acaricide will occur secondarily, since tick populations will gradually come into contact with all the chemical compounds used in a particular farm. Therefore, a product should only be replaced with a different one when it is shown one to be no longer efficient to fight off the different populations of *R. microplus* based on the results provided by the sensitivity profile assay.

Regarding the efficiency indexes of the active principles tested in the present study, the history of the products used by the producers should be considered. In this case, 30.76% reported using products that included chemical compounds such as avermectins and fluzuron in its composition for *R. microplus* eradication, since it is easy to use and due to its duration of action. However, such chemicals were not assessed by *in vitro* assays, suggesting that the wide use of these active principles may be related to the low resistance index to topical acaricides in this study, since the use of the latter has decreased considerably in region.

Conclusion

Based on the findings of the present study, we conclude that the topical acaricides in the western region of Santa Catarina, southern Brazil, show satisfactory efficiency indices considering what is recommended in the current legislation. Best results were achieved when we used the associations of pyrethroids and organophosphates, thus showing the efficacy of the acaricides among different strains of *R. microplus*. These results may be related to the method of control and management used by producers in the study

area. Veterinarians and technicians should provide producers with the guidelines on the strategic control of the tick in combination with adequate management and handling/use of acaricides, considering the monitoring/surveillance of the efficiency of the chemical compounds used, ideal time of application of drug and dilutions as well, since tick resistance occurs due to inadequate control of acaricides. This is an important alternative to minimize the factors that may predispose the occurrence of resistance among the different populations of *R. microplus*, thus enabling maintenance of the effectiveness of topical acaricides along successive generations of the bovine tick.

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Conflict of Interest

The authors state they have no conflicts of interest to declare.

Ethics Statement

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