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Resistance to cypermethrin and amitraz in *Rhipicephalus (Boophilus) microplus* on the Santa Catarina Plateau, Brazil

Situação da resistência do *Rhipicephalus* (*Boophilus*) *microplus* à cipermetrina e amitraz no Planalto Catarinense, Brasil

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Abstract

With the objective of evaluating *Rhipicephalus* (*Boophilus*) *microplus* resistance to cipermetrina and amitraz, were collected engorged tick females from cattle on 20 farms on the Santa Catarina Plateau, in southern Brazil, between January of 2004 and May of 2006. Were also received 20 groups of engorged *R.* (*B.*) *microplus* females, collected by cattle farmers requesting acaricidal efficacy (AE) testing. Were performed *in vitro* tests, which consisted of immersing engorged females in cypermethrin (0.015%) and in amitraz (0.025%). An AE ≥95% was considered indicative of effectiveness. Of the 20 collected groups, 18 (90%) showed cypermethrin resistance and 1 (5%) showed amitraz resistance. Of the 20 received groups, 19 (95%) showed cypermethrin resistance and 2 (10%) showed amitraz resistance. The AE of cypermethrin was found to be ≥95%, 85-94%, and 55-64%, respectively, in 4 (57.1%), 2 (28.6%), and 1 (14.3%) of 7 reference groups, collected in the 1997-2001 period. The AE of amitraz was ≥95% in all of those groups. Among the groups of specimens received for analysis in that period, the AE of amitraz was ≥95% in 6 (85.71%) and 75-84% in 1 (14.28%). *R.* (*B.*) *microplus* resistance to acaricides is increasing on the Santa Catarina Plateau.

Keywords: *Rhipicephalus* (*Boophilus*) *microplus*, cattle tick, acaricide resistance.

Resumo

Com os objetivos de avaliar a resistência do *Rhipicephalus* (*Boophilus*) *microplus* à cipermetrina e ao amitraz no Planalto Catarinense, no período de janeiro de 2004 a maio de 2006, foram coletadas teleóginas em bovinos de 20 propriedades onde os produtores autorizaram a coleta (amostras por conveniência) e recebidas teleóginas de mais 20 propriedades com objetivo de diagnóstico de eficácia. Os testes *in vitro* foram realizados por imersão de teleóginas em cipermetrina (0,015%) e amitraz (0,025%). Considerando a eficácia igual ou superior a 95%, das 20 propriedades amostradas, 18 (90%) apresentaram resistência à cipermetrina e uma (5%) ao amitraz. Das 20 propriedades com teleóginas para diagnóstico, 19 (95%) apresentaram resistência à cipermetrina e duas (10%) ao amitraz. No período de agosto de 1997 a dezembro de 2001, das sete amostras coletadas, em quatro (57,1%) a eficácia da cipermetrina foi ≥95%, em duas (28,6%) entre 85% e 94% e em uma, (14,3%) entre 55% e 64%. A eficácia do amitraz foi ≥95%, em teleóginas de todas as propriedades. Nas amostras para diagnóstico, seis (85,71%) apresentaram eficácia para o amitraz ≥95% e uma (14,28%) entre 75% e 84%. A resistência desse ixodídeo aos carrapaticidas testados é crescente no Planalto Catarinense.

Palavras-chave: *Rhipicephalus* (*Boophilus*) *microplus,* carrapato dos bovinos, resistência a acaricidas.

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Introduction

Rhipicephalus (*Boophilus*) *microplus*, commonly known as the southern cattle tick, is one of the major ectoparasites of cattle and is considered one of the major obstacles to livestock production in Brazil. The losses are principally due to blood loss, which inhibits weight gain, as well as to the transmission of *Anaplasma* spp. and *Babesia* spp. Grisi et al. (2002) reported that, in Brazil, *R.* (*B.*) *microplus* and the pathogens it transmits are responsible for annual losses of approximately two billion dollars.

Currently, *R.* (*B.*) *microplus* is controlled primarily through the use of chemicals (acaricides), which are often used inappropriately, leaving residues in milk and meat, as well as causing damage to the environment and increasing the likelihood of selection (higher numbers of acaricide-resistant ticks). Klafke (2008) stated that resistance can be defined as a change in the frequency of genes in a given population, caused by artificial selection. This change can be detected by a significant increase in the number of individuals for which the lethal dose is higher than is that for most of the individuals of the same species.

Over the course of a decade, Farias et al. (2008) studied the susceptibility/resistance of the cattle tick, as well as the dynamics of use of the various groups of commercially available acaricides, in southern Rio Grande do Sul. The authors noted that the products that were most commonly used during the first three-year period were pyrethroid-based acaricides, which resulted in the selection of pyrethroid-resistant populations. In the third three-year period, amitraz-based products dominated the market, leading to the selection of amitraz-resistant populations. In the first three-year period, all of the tick populations analyzed were sensitive to amitraz (efficacy rate = 95%), whereas at the end of the study (i.e., in the third three-year period) such efficacy was detected in only 79% of the populations studied.

On the Santa Catarina Plateau, located in southern Brazil, Souza et al. (1984) noted an oviposition inhibition rate of less than 74% for all organophosphorus acaricides tested. The results of that study suggest that resistant strains of *R.* (*B.*) *microplus* were present on two of the three cattle farms studied.

The response of the various tick strains to the various acaricides on the market can be determined by in vivo tests (by dipping the parasitized animals) or by in vitro tests (primarily with engorged female ticks under laboratory conditions). According to Amaral (1993), engorged females and unfed larvae are two *R.* (*B.*) *microplus* instars used in in vitro tests of acaricides intended for use in cattle. The most common test consists of immersing engorged female ticks in liquid acaricide. The test can also be performed with larvae, which can be either immersed in liquid acaricide or placed in envelopes impregnated with it. In vitro tests with engorged females constitute a safe and practical method for the identification of acaricide resistance. The objective of the present study was to evaluate and compare *R.* (*B.*) *microplus* specimens collected from the Santa Catarina Plateau, in terms of their resistance to cypermethrin and amitraz.

Material and Methods

Between January of 2004 and May of 2006, were manually collected engorged *R.* (*B.*) *microplus* females directly from naturally infested cattle of various breeds and bloodlines on 20 Santa Catarina Plateau farms whose owners did not know whether acaricides had been used on their farms and allowed us to collect specimens (convenience sample). In addition, were received 20 groups of engorged *R.* (*B.*) *microplus* females, collected by cattle farmers requesting tests of acaricidal efficacy (AE). In cases in which the number of engorged females collected on the farms was insufficient to perform the immersion test, the strain was reproduced by infesting stalled cattle, the immersion test being subsequently performed. The engorged females collected on each farm were placed in 500-mL glass flasks with perforated lids to allow aeration. The flasks were labeled and transported to the Laboratory of Parasitology and Parasitic Diseases of the Santa Catarina State University Center for Agricultural and Veterinary Sciences, located in the city of Lages, Brazil.

In vitro tests, which consisted of immersing the engorged females in liquid acaricide less than 24 hours after collection, were performed in accordance with the method proposed by Drummond et al. (1973). The engorged females that showed the most vitality were selected and weighed, being subsequently divided into three groups of 10 specimens each, all of approximately the same weight. Each group was immersed for 5 minutes in cypermethrin (0.015%), amitraz (0.025%), or distilled water (control). After immersion, the engorged females were dried on absorbent paper, placed in Petri dishes, properly identified, and maintained in a biological oxygen demand chamber that was climate-controlled (temperature, 27 ± 1 °C; relative humidity, 80 ± 10%), in constant darkness, for 18 days. At the end of that period, were used an analytical scale (precision, 0.001 g) to weigh the eggs laid by each group of engorged females. The eggs were subsequently placed in test tubes, which were stored in a climate-controlled environment (under the same conditions as those described above) in order to determine the hatching rates. Were also analyzed the results of the immersion tests performed at the Laboratory of Parasitology and Parasitic Diseases of the Santa Catarina State University Center for Agricultural and Veterinary Sciences between August of 1997 and December of 2001 and involving the use of amitraz and cypermethrin in specimens collected or received for AE analysis.

Were calculated the reproduction rate by applying the following Equation 1:

$$RR = \frac{\left(\begin{array}{l}\text{Egg mass weight (g)} \\ \times\ \text{hatching rate (\%)}\end{array}\right)}{\text{Engorged female weight (g)}} \times 20{,}000 \qquad (1)$$

where *RR* is the reproduction rate. Were calculated AE by applying the following Equation 2:

$$AE(\%) = \frac{(RR_{control} - RR_{study})}{RR_{control}} \times 100 \qquad (2)$$

Acaricides with an AE of at least 95% were classified as effective. This is in accordance with the current legislation for registering acaricides with the Brazilian National Ministry of Agriculture (BRASIL, 1990).

Results and Discussion

Table 1 shows the AE of the cypermethrin- and amitraz-based acaricides tested on the engorged females collected from cattle on 20 Santa Catarina Plateau farms and on the 20 groups of specimens sent to us by farmers in the same region. Among the 20 groups of engorged *R.* (*B.*) *microplus* females collected from the farms, the AE of cypermethrin was found to be ≥95%, 85-94%, and <74%, respectively, in 2 (10%), 5 (25%), and 13 (65%). These results suggest that cypermethrin-resistant ticks are present on 90% of the farms. They are also in agreement with the results obtained by Mendes et al. (2001), who found the efficacy of pyrethroid-based acaricides to be <50% in various regions of the state of São Paulo, also located in southern Brazil. In the present study, the AE of amitraz was found to be ≥95% and 85-94%, respectively, in 19 (95%) and 1 (5%) of the 20 groups of engorged *R.* (*B.*) *microplus* females collected from the farms on the Santa Catarina Plateau. These results are similar to those obtained by Heimerdinger et al. (2006) in the city of Santa Maria, also located in southern Brazil (in the state of Rio Grande do Sul). Those authors investigated the efficacy of amitraz in controlling *R.* (*B.*) *microplus* in naturally infested Holstein cattle and found a mean AE of 97.93%. However, our results differ from those obtained by Campos Júnior and Oliveira (2005), who analyzed *R.* (*B.*) *microplus* specimens collected from cattle on 30 farms in the city of Ilhéus, located in northern Brazil, and found that the AE of amitraz was within the legally acceptable range in only 6.7% of the specimens. This difference is principally due to the frequency of previous use of the acaricide, its management, and the favorability index for the spread of *R.* (*B.*) *microplus* on those farms.

The AE of cypermethrin was found to be ≥95%, 85-94%, and <74%, respectively, in 1 (5%), 2 (10%), and 17 (85%) of the 20 groups of engorged *R.* (*B.*) *microplus* females received for AE analysis. These results suggest that cypermethrin-resistant ticks are present on 95% of the farms. The AE of amitraz was found to be ≥95% in 18 (90%) of the 20 groups of specimens received for AE analysis. The results were suggestive of amitraz resistance (AE <55%) in only 2 (10%) of the groups.

The AE of cypermethrin was found to be <55% in 9 (45%) of the 20 groups of specimens collected and in 14 (70%) of those received for AE analysis. The in vitro tests with amitraz revealed that AE was <95% in only 1 (5%) of the 20 groups of specimens collected and in 2 (10%) of those received for AE analysis.

In general, cattle farmers request AE tests late, after the AE has diminished to approximately half or less. These results confirm the need for the early identification of acaricide resistance, because AE monitoring facilitates *R.* (*B.*) *microplus* control.

Table 2 shows the AE of cypermethrin and amitraz in the engorged *R.* (*B.*) *microplus* females collected between August of 1997 and December of 2001, as well as the AE of amitraz in the specimens received for AE analysis in the same period. The AE of cypermethrin was found to be ≥95%, 85-94%, and 55-64%, respectively, in 4 (57.1%), 2 (28.6%), and 1 (14.3%)

Table 1. Efficacy of cypermethrin (0.015%) and amitraz (0.025%), as determined by in vitro tests of engorged *Rhipicephalus* (*Boophilus*) *microplus* females collected from cattle on farms between January of 2004 and May of 2006. Santa Catarina Plateau, Brazil.

% AE	Specimens collected by researchers		Specimens received from farmers	
	Cypermethrin	Amitraz	Cypermethrin	Amitraz
	n* (%)	n* (%)	n* (%)	n* (%)
95-100	2 (10.0)	19 (95.0)	1 (5.0)	18 (90.0)
85-94	5 (25.0)	1 (5.0)	2 (10.0)	0 (0.0)
75-84	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
65-74	1 (5.0)	0 (0.0)	2 (10.0)	0 (0.0)
55-64	3 (15.0)	0 (0.0)	1 (5.0)	0 (0.0)
< 55	9 (45.0)	0 (0.0)	14 (70.0)	2 (10.0)
Total	20 (100)	20 (100)	20 (100)	20 (100)

*number of groups (farms); AE, acaricidal efficacy.

Table 2. Efficacy of cypermethrin (0.015%) and amitraz (0.025%), as determined by in vitro tests of engorged *Rhipicephalus* (*Boophilus*) *microplus* females collected from cattle on farms between August of 1997 and December of 2001. Santa Catarina Plateau, Brazil.

% AE	Specimens collected by researchers		Specimens received from farmers
	Cypermethrin	Amitraz	Amitraz
	n* (%)	n* (%)	n* (%)
95-100	4 (57.1)	16 (100)	6 (85.7)
85-94	2 (28.6)	0 (0.0)	0 (0.0)
75-84	0 (0.0)	0 (0.0)	1 (14.3)
65-74	0 (0.0)	0 (0.0)	0 (0.0)
55-64	1 (14.3)	0 (0.0)	0 (0.0)
< 55	0 (0.0)	0 (0.0)	0 (0.0)
Total	7 (100)	16 (100)	7 (100)

*number of groups (farms); AE, acaricidal efficacy.

of the 7 groups of specimens collected in that period. The AE of amitraz was ≥95% in all of the specimens collected in that period. Among the 7 groups of specimens received for analysis in that same period, the AE of amitraz was ≥95% in 6 (85.71%) and 75-84% in 1 (14.28%).

Our results show that the number of farms on which there were cypermethrin-resistant and amitraz-resistant ticks increased over time. This is in agreement with the findings of Farias et al. (2008) for farms in the state of Rio Grande do Sul.

Conclusions

Cypermethrin-resistant and amitraz-resistant strains of *R.* (*B.*) *microplus* are on the rise on the Santa Catarina Plateau. There is a delay on the part of cattle farmers in requesting tests to identify acaricide-resistant *R.* (*B.*) *microplus*.

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