



Original Articles

Resistance of *Rhipicephalus (Boophilus) microplus* (Canestrini, 1888) to acaricides used in dairy cattle of Teixeiraópolis, Rondônia, Brazil

Resistência de *Rhipicephalus (Boophilus) microplus* (Canestrini, 1888) aos acaricidas utilizados em gado leiteiro de Teixeiraópolis, Rondônia, Brazil

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ABSTRACT

Rhipicephalus microplus is one of the main ectoparasites of dairy cattle in Brazil, whose control is mainly based on the use of chemicals. However, the low variety of chemicals available, coupled with the indiscriminate use of these substances, has led to increased resistance for this tick. The objective of this study was to evaluate the resistance profile of the tick *Rhipicephalus microplus* to different acaricidal products used on dairy cows in Teixeiraópolis-RO, using the Adult Immersion Test (TIA) or biocarrapaticidogram. Data was collected from nine farms by performing nine *in vitro* evaluation tests on engorged *R. microplus* females, using six different tick repelling treatments available on the market. Replicates of each treatment with 20 teleogins were performed for each treatment and a control group in distilled water was used for positive and negative controls. The efficiency amplitudes of each group and the average effectiveness of the acaricidal compounds were evaluated and compared by the Kruskal-Wallis test with a significance level of 95%. Statistical analyses were performed in the Action Stat statistical program. The tick repellents tested presented the following effectiveness averages: 100% (Chlorpyrifos at 50% + Cypermethrin at 6%); 99.99% (15% Cypermethrin + Chlorpyrifos at 25% + Citronellal at 1%); 77.63% (Cypermethrin at 20% + Chlorpyrifos at 50%); 100% (15% Cypermethrin + Chlorpyrifos at 25% + Piperonyl butoxide at 1%) -; 21.68% (Amitraz to 12.5%) -; 42.17% (Deltamethrin at 5%). Products with combinations of active ingredients were the most effective, and thus considered the best alternative for tick control.

RESUMO

Rhipicephalus (Boophilus) microplus (CANESTRINI, 1888) é um dos principais ectoparasitos na bovinocultura leiteira do Brasil. O controle baseia-se principalmente no uso de produtos químicos. No entanto, a baixa diversidade de produtos químicos disponíveis, aliada ao uso indiscriminado dessas substâncias, têm levado ao aumento da resistência deste carrapato. Neste estudo, objetivou-se avaliar por meio de coleta de dados o perfil de resistência do carrapato *Rhipicephalus microplus* a diferentes produtos acaricidas utilizados em vacas leiteiras do município de Teixeiraópolis-RO, por meio do Teste de Imersão de Adultos (TIA) ou biocarrapaticidograma. Foram coletados dados de nove propriedades realizando nove testes de avaliação *in vitro* de fêmeas ingurgitadas de *R. microplus*, utilizando seis diferentes tratamentos de carrapaticidas disponíveis no mercado, realizando uma réplica para cada tratamento onde foram consideradas 20 teleóginas para cada tratamento, foi utilizado um grupo controle em água destilada para controles de positivos e negativos. Foram avaliadas as amplitudes da eficiência de cada grupo e as médias da eficácia dos compostos acaricidas, as quais foram comparadas pelo teste Kruskal-Wallis com nível de

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significância de 95%. As análises estatísticas foram realizadas com o auxílio do programa estatístico *Action Stat*. Os carrapaticidas testados apresentaram as seguintes médias de eficácia: 100% (Clorpirifós a 50% + Cipermetrina a 6%); 99,99% (Cipermetrina a 15% + Clorpirifós a 25% + Citronelal a 1%); 77,63% (Cipermetrina a 20% + Clorpirifós a 50%); 100% (Cipermetrina a 15% + Clorpirifós a 25% + Butóxido de piperonila a 1%) -; 21,68% (Amitraz a 12,5%) -; 42,17% (Deltametrina a 5%). Os produtos com associações de princípios ativos foram os mais eficazes, sendo assim, considerados como melhor alternativa de controle carrapaticida.

INTRODUCTION

Brazil is one of the leading producers of milk in the world, with a herd of 17 million dairy cows in 2017 and a production of more than 30 million tons of milk per year (IBGE, 2017). The dairy sector moves the economy of small cities, helping distribute income and directly and indirectly generating jobs. With the estimated population growth and increased consumption of milk "per capita", milk production is estimated to reach ~48 billion liters in 2026 (SILVEIRA NETO et al., 2017).

However, one of the major problems milk producers face are tick infestations of their herds, leading to direct and indirect losses for producers. According to an estimate by the Food and Agriculture Organization of the United Nations, approximately 150 million farms are involved in milk production worldwide. This activity is characteristic of developing countries and family farming, due to the rapid return that milk production provides to rural families (FAO, 2016). Among the milk producing countries, Brazil ranks 3rd in the world, after the United States and India (FAO, 2019).

In Rondônia state, the municipality of Teixeiraópolis (study area herein) contains ~9,400 dairy cows distributed among approximately 540 dairy farms, which represents about 1.62% of the annual milk production in Rondônia (IBGE, 2017). However, dairy farmers in this region have suffered from infestations of the tick *Rhipicephalus (Boophilus) microplus*, which have damaged animal health and production efficiency.

R. microplus is a tick species that belongs to the phylum Arthropoda, Class Arachnida, subclass Acari, order Parasitiformes, suborder Metastigmatase and family Ixodidae (BARROS-BATTESTI et al., 2006). The species main hosts are bovids, however, can be found on other domestic and wild hosts (MONTEIRO, 2016). Morphologically, this subgenus is characterized by having the base of gnathosoma hexagonal with rostrum and palps short, flattened, wrinkled laterally and dorsally, dorsal shield without ornamentation, without marginal festoons and generally with caudal extension (DANTAS et al., 2017). The body is oval and wide in the front and presents grayish-blue brown and white coloration on its front and sides (TAYLOR et al., 2017).

Rhipicephalus microplus is an ectoparasite that causes significant economic losses to the dairy cattle industry in Brazil. For dairy cows, intense infestations can lead to weight loss, reduced milk production, and anemia,

mainly due to irritation caused by tick bites (GRISI, 2002; RODRIGUEZ-VIVAS et al., 2017; GARCIA et al., 2019). Approximately 80% of Brazilian farms suffer from infestations that, along with the aforementioned problems, lead to cow death, mainly from bovine parasitic sadness (GRISI, 2002). In addition, *R. microplus* infestations increase labor and medicine expenses (GRISI, 2002; DANTAS et al., 2017). In 2014, these losses totaled 3 billion dollars in Brazil alone (GRISI et al., 2014;).

In 2009, studies evaluated the economic impact of ectoparasites in South America, indicating losses of 2.5 million cattle in Brazil, which represents losses of 75 million kg of meat, 1.5 billion liters of milk, along with \$8.6 million in secondary damage and \$25 million in chemical acaricides to combat ticks (RODRIGUEZ-VIVAS et al., 2017).

To mediate damages caused by ticks, producers try to control infestations with tick repellent products. However, the indiscriminate use and incorrect dosages of these products can decrease their effectiveness and lead to tick populations that are resistant to the active ingredients used in such products (PEREIRA; LABRUNA, 2008; PEDRASSANI ; REISDORFER, 2015; GARCIA et al., 2019). In addition to the costs of applying acaricides, these products can leave residues in meat and milk, which forces producers to discard milk production during the grace period of these chemicals, as well as contaminate the environment due to misuse of these substances (GOMES 2011; GARCIA et al., 2019).

The grace period described in instructions of tick repellents is the period when the active ingredient is excreted into milk or meat. However, even if milk producers adopt the recommended discard period for the product, studies about how these products act in healthy animals have revealed that the excretion speed of residues in milk or meat can vary in sick individuals or even between healthy individuals. The economic impact of such grace period is high for producers, as they must stop selling their products (TORRES, 2007). Main determinants of risk perception in rural dairy farmers derive from the lack of technical guidance, which such little guidance linked to traders. Thus, the invisibility of risks associated with handling veterinary pesticides, increases the exposure of dairy farm workers to these chemical agents and can lead to serious health problems. Such invisibility of risks also leads farmers to neglect the grace period between the application of veterinary pesticides in cattle and milk collection for human

consumption; thus, risking the health of milk and meat consumers (SILVA et al., 2012).

To determine the grace period of acaricides, as well as the contamination risks, it is necessary to test the resistance profile of ticks to these substances. For this, the biocarrapaticodogram test is recommended to measure the resistance of teleogins to acaricide products (CAMILO et al., 2009; SILVEIRA NETO et al., 2017). Thus, in order to reduce the damage caused by these ectoparasites, this study aimed to evaluate the resistance profile of the tick *Rhipicephalus microplus* to different acaricide products used on dairy cows in the municipality of Teixeiraópolis-RO.

MATERIAL AND METHODS

Data collection

The work was carried out by collecting information from the database of the Veterinary Parasitology Laboratory at the the São Lucas University Center, Ji-Paraná-RO, where the analysis requests containing the results and active ingredients used by milk producers were obtained. Data was selected from 9 farms in the municipality of Teixeiraópolis-RO (latitude 10°55'03" south and longitude 62°14'58" west, altitude of 260 meters), since this municipality presents potential dairy production in the central region of Rondônia state. Due to the fact that this study involves data collection and focuses on a species that is not a chordate, it was not necessary to obtain permission from the Ethics Committee on Animal Use (CEUA).

Sampling

At each farm, engorged teleogins were collected from animals that had not been treated with tick repellent at least 21 days prior. The collected teleogins were transported in closed isothermal boxes at 10°C to the Parasitology Laboratory of the Veterinary Hospital at the São Lucas University Center, Ji-Paraná-RO. Specimens were washed under running water, dried with paper towels and separated according to their vigor, weight and motility, and then distributed in Petri dishes to form homogeneous groups of 20 teleogins.

Adult Immersion Test/Biocarrapaticodogram

To evaluate the resistance of adult ticks to acaricides, we used the Adult Immersion Test (AIT) method (DRUMOND et al., 1973), known as biocarrapaticodogram, which determines the efficiency of acaricides. The AIT was performed on groups of 20 teleogins per treatment and in a control group with distilled water, in which negative or positive controls were verified.

The tests were performed in replicate for each treatment using 20 teleogins with average individual weights ranging from 0.160 to 0.540 grams in each replica. The results were expressed by the average values obtained in

each battery. Thus, the egg laying of teleogins and the hatchability index of these eggs were used as parameters. The teleogin groups were washed in a sieve with running water, dried with soft paper towels, and weighed on an analytical scale (accuracy of 0.0001g). Then, ticks were immersed in treatments containing dosages recommended by the manufacturer and controls for 5 minutes.

After immersion, teleogins were dried with paper towels to prevent opportunistic proliferation, placed in Petri dishes identified with the active ingredient used and kept in a greenhouse at a temperature of $\pm 27^{\circ}\text{C}$ and $\text{RH} > 80\%$ for 15 days. Biological parameters were evaluated as described by Drumond et al (1973), using the following indicators: weight of teleogins, weight of the eggs and hatching rate. Then, the reproductive efficiency (RE) was calculated as follows: total egg weight x hatching percentage x 2000 (estimated number of larvae per gram of eggs) and divided by the total weight of the teleogins. Afterwards, egg laying was evaluated and eggs were weighed and placed in screened containers and placed in an oven for 15 days under the previously described conditions.

The larvae were identified in a freezer and, along with the unviable eggs, were transferred to vials with 70% alcohol. To calculate the hatching rate, the average percentage of three samples containing 200 larvae and/or eggs was considered (SOARES; MONTEIRO, 2011). The reading to determine the hatching percentages was performed with a stereoscope microscope. Products with results equal to or greater than 95% were considered effective, according to the current standard (BRASIL, 1997). From the RE, the effectiveness of the tested products (EP) was calculated as follows: RE of the control group - RE of the treated group x 100 divided by the RE of the control group (SOARES; MONTEIRO, 2011).

Treatments

Treatment 1. Class: Organophosphate + Pyrethroid; Active ingredient and concentration: Chlorpyrifos 50% + Cypermethrin 6%.

Treatment 2. Class: Organophosphate + Pyrethroid; Active ingredient and concentration: Cypermethrin 15% + Chlorpyrifos 25% + Citronellal 1%.

Treatment 3. Class: Organophosphate + Pyrethroid; Active ingredient and concentration: Cypermethrin 20% + Chlorpyrifos 50%.

Treatment 4. Class: Organophosphate + Pyrethroid; Active ingredient and concentration: Cypermethrin 15% + Chlorpyrifos 25% + Piperonyl butoxide 1%.

Treatment 5. Class: Formamidine; Active ingredient and concentration: Amitraz 12.5%.

Treatment 6. Class: Pyrethroid; Active ingredient and concentration: Deltamethrin 5%.

Statistical analysis

The efficiency amplitudes of each group and the average effectiveness of the acaricide compounds were evaluated, which were compared by the Kruskal-Wallis test with a significance level of 95%. Statistical analyses were performed in the Action Stat statistical program.

RESULTS AND DISCUSSION

Based on the evaluation of tick resistance in cattle and the variety of commercial formulations available in the region, all farms had at least two formulations that presented effectiveness below the minimum value (i.e., 95%) stipulated by the Ministry of Agriculture (BRASIL, 1997) (Table 1).

Table 1 – Formulations that presented average effectiveness <95% at each farm in Teixeiraópolis-RO.

Farm	Number of formulations tested	Formulations below 95% (Chemical Principle)
1	6	Amitraz 12.5% and Deltamethrin 5%
2	6	Amitraz 12.5% and Deltamethrin 5%
3	6	Amitraz 12.5% and Deltamethrin 5%
4	6	Cypermethrin 20% + Chlorpyrifos 50%
5	6	Cypermethrin 20% + Chlorpyrifos 50%, Amitraz 12.5% and Deltamethrin 5%
6	6	Amitraz 12.5% and Deltamethrin 5%
7	6	Amitraz 12.5% and Deltamethrin 5%
8	6	Amitraz 12.5% and Deltamethrin 5%
9	6	Amitraz 12.5% and Deltamethrin 5%

The results in Table 1 show that 33.33% of the farms presented resistance to 50% of the formulations tested, indicating a problem for milk producers. Considering that there are few commercial formulations available, there may be problems regarding the effectiveness of these products in the future. Gomes et al. (2011) and Soares and Monteiro (2011) conducted studies in Mato Grosso do Sul state and found that there was efficient resistance to at least one product tested in all the properties they visited.

Table 2 shows the last products used at the farms studied, with most acaricide formulations containing organophosphate and pyrethroids. Such results corroborate with Silva (2010) and Dantas et al. (2017), who reported that these two compounds are often used in combination because the action of pyrethroids is enhanced when associated with organophosphates.

Table 2 – Tick repellent products used by dairy farmers in the municipality of Teixeiraópolis-RO.

Farms	Products used	Chemical group
1	Cypermethrin 15% + Chlorpyrifos 25% + Citronellal 1%	Pyrethroid + Organophosphate + Repellent
2	Chlorpyrifos 30% + Cirpremethrin 15% + Fenthion 15	Organophosphate + Pyrethroid + Organophosphate
3	Amitraz 12,5%	Formamidine
4	Chlorpyrifos 50% + Cypermethrin 6%	Pyrethroid + Organophosphate
5	Amitraz 12,5%	Formamidine
6	Chlorpyrifos 50% + Cypermethrin 6%	Pyrethroid + Organophosphate
7	Cypermethrin 15% + Chlorpyrifos 25% + Citronellal 1%	Pyrethroid + Organophosphate + Repellent
8	Cypermethrin 15% + Chlorpyrifos 25% + Citronellal 1%	Pyrethroid + Organophosphate + Repellent
9	Cypermethrin 15% + Chlorpyrifos 25% + Citronellal 1%	Pyrethroid + Organophosphate + Repellent

Among the six formulations tested, 50% presented average effectiveness in *in vitro* tests higher than 95% at all farms (Table 3). Products containing Chlorpyrifos 50% + Cypermethrin 6% and Cypermethrin 15% + Chlorpyrifos 25% + Piperonyl Butoxide 1%, had 100 %

average effectiveness and the product containing Cypermethrin 15% + Chlorpyrifos 25% + Citronellal 1% presented average effectiveness of 99.99%, all of which were satisfactory regarding efficiency.

Table 3 – Effectiveness of acaricidal products in immersion tests of the bovine tick *Rhipicephalus microplus*, in Teixeiraópolis-RO.

Formulation and Active Ingredients		Effectiveness (%)	
Formulation	Active ingredients	Range	Average
1	Chlorpyrifos 50% + Cypermethrin 6%	-	100a
2	Cypermethrin 15% + Chlorpyrifos 25% + Citronellal 1%	99.1 – 100	99.99a
3	Cypermethrin 20% + Chlorpyrifos 50%	4.02 – 100	77.63c
4	Cypermethrin 15% + Chlorpyrifos 25% + Piperonyl butoxide 1%	-	100a
5	Amitraz 12,5%	11 – 43.32	21.68b
6	Deltamethrin 5%	12.08 - 83.46	42.17b

Means followed by the same letters showed no difference by Kruskal-Wallis test ($p < 0.05$).

The formulation containing Cypermethrin 20% + Chlorpyrifos 50% showed average tick repellent effectiveness of 77.63%, which is below the recommended level (95%). Nevertheless, this formulation showed 100% efficacy at some farms.

The formulation containing Amitraz 12.5% presented the lowest average tick repelling effectiveness (21.68%), with efficiency amplitude ranging from 11.00 to 43.32% (Table 3). Despite the low efficacy of this product, it is still administered at some farms in the municipality of Teixeiraópolis-RO (COELHO et al., 2015). The formulation containing Deltamethrin 5% presented average effectiveness of 42.17%, with an amplitude ranging from 12.08 to 83.43% (Table 3). The average tick repelling effectiveness of this product is statistically comparable to the average of the product containing Amitraz, which is considered ineffective according to legislation.

When administering only one formulation (formamidine or pyrethroid), the results of tick repelling effectiveness were not satisfactory, regardless of the chemical group applied (Table 3). Even considering combinations of chemical groups, half of the formulations tested did not express significant results, which shows serious tick resistance in the region of Teixeiraópolis-RO. In their study about tick resistance in Mato Grosso do Sul state, Gomes et al. (2011) also found similar results, where 58.3% of the formulations containing only one active ingredient showed resistance of ticks on cattle.

Deltamethrin presented an average efficacy of 42.17% (Table 3), with results similar to those found by Campos Junior and Oliveira (2005), Spagnol et al. (2010), Vita et al. (2012) and Abbas et al. (2014), who obtained efficiencies of 33.90, 74.58 and 65.4%, respectively. The low effectiveness of Deltamethrin was also observed in Minas Gerais state in studies conducted by Spagnol et al. (2010) and Gomes et al. (2011), where test averages were below what was allowed in the legislation, thus corroborating the results obtained herein. However, the results obtained by Campos Júnior and Oliveira (2005) and Eckstein et al. (2016) revealed that 40% of the tests containing this formulation were ineffective, presenting 100% inefficiency for Deltamethrin, which differs from the results obtained herein.

It is noteworthy that 50% of the formulations presented inefficient tick repellence, especially Amitraz, which showed 100% ineffectiveness (Table 3). Low effectiveness results were also found by Vargas et al. (2003) and Silveira Neto et al. (2017), who observed inefficiencies of over 50%. The ineffectiveness of the tests differed from the results obtained by Pedrassani and Reisdorfer (2015), Eckstein et al. (2016) and Gomes et al. (2011), who obtained favorable results for the product tested.

The associated formulations expressed better effectiveness indices (Table 3). Such results are similar to those obtained by Koler et al. (2009), Abbas et al. (2014), Higa et al. (2016), Dantas et al. (2017) and Silveira Neto et al. (2017), who obtained more effective combinations in their studies due to the fact that associated products reacted together with different action mechanisms, allowing different tick repellent compounds to interact.

Regarding combinations, Cypermethrin 20% + Chlorpyrifos 50% was less effective, with an average of 77.63% (Table 3). This percentage is similar to the results found by Silveira Neto et al. (2017), who obtained more than 70% effectiveness for this formula. Although products containing combinations are considered to be the best in terms of their effectiveness herein, it should be noted that a product containing combinations expressed tick repelling effectiveness of only 4.02% (Table 3), indicating that there are tick populations resistant to these formulations. Therefore, it is essential that these products be administered with caution to prevent these resistant populations from proliferating.

Muniz et al. (2020) verified the effectiveness of *Metarhizium anisopliae* in oil/water emulsion against the tick *Rhipicephalus microplus* under hot and dry conditions and demonstrated efficient tick control through fungal conidia. According to Beys-da-Silva et al. (2020), biological controls have been the main alternatives for controlling ticks, especially in recent decades. As tick repellent, the most studied biocontrol agent is *Metarhizium anisopliae*, which is pathogenic for arthropods. Therefore, by reducing the environmental impact, persistence of chemical agents and resistance

developed, these new strategies have been studied to control the bovine tick.

The resistance of acaricidal products has been described in all regions of the country due to failures in their handling at farms, i.e., systematic application of a single product and excessive number of annual applications (BARROS, 2005; FURLONG, 2007; FARIAS et al., 2008). Studies conducted by these authors revealed that there is a high deficiency regarding access to technical information about the correct administration of these products. There is also clear resistance or negligence regarding the application of technical recommendations, such as those described by Furlong et al. (2003). Active ingredients such as Amitraz and Deltamethrin proved to be ineffective at all farms. However, it is necessary to consider the facility in which producers acquire these products, that is, they are relatively cheap chemicals, which increases their use in routine treatments on a farm.

In a study in Mexico, Oliveira et al. (2020) recorded resistance in *Amblyomma mixtum* and *Rhipicephalus microplus* to pyrethroid and amidein families since effectiveness did not exceed 40%. These authors found that the effectiveness in immature stages in *Rhipicephalus microplus* was 93.3% for the amidine family and 26.2% for the pyrethroid family.

Many producers make dangerous mistakes when choosing and administering tick repelling insecticides, as they follow unreliable indications (usually based on the experience of other producers) regarding the effectiveness of products, and each farm presents tick populations with different resistance profiles. Since producers frequently apply acaricides inappropriately and fail to manage them, they contribute to the selection pressure of ticks, which favors resistance.

CONCLUSIONS

Products containing a single active ingredient in their formulation did not express tick repelling effectiveness. However, acaricide formulations with combinations were effective, so it is recommended to combine active ingredients as an alternative to control ticks in dairy cattle. The fact that all the studied farms presented ticks resistant to at least two acaricide formulations, reveals the need to perform tick sensitivity tests to determine which tick repellent products should be administered. The Adult Immersion Test (AIT) or biocarrapacidogram is indicated for this purpose because it proved to be an effective, easy and low-cost test.

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