Short communication: *In vitro* Evaluation of Resistance of *Rhipicephalus* (*Boophilus*) *microplus* against Three Widely Used Ixoidicides

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Abstract. *The deficiency of tick control causes large economic losses for the health of animals and the risk of transmission of zoonotic diseases. Their control is based on the use of acaricides such as organophosphates, synthetic pyrethroids, amidines and macrocyclic lactones; however, its inappropriate use has favoured the presence of populations resistant to the main families of acaricides. This study was conducted with the objective to evaluate the resistance of Rhipicephalus (Boophilus) microplus against three widely used ixoidicides in Nicaragua, namely coumaphos, amitraz and cypermethrin, applied in the concentrations indicated by the manufacturer. For this, the techniques of Adult Immersion Test (AIT) and Larval Package Test (LPT) were applied, for which ticks were collected from farms in Western Nicaragua. The results obtained through the AIT show that coumaphos has a higher percentage of oviposition inhibition of 86%, while cypermethrin only demonstrated an oviposition of 12.9%. Mortality in adults treated with coumaphos was 97.72%, and in those treated with amitraz it was 48.8%, while cypermethrin only provided a mortality of 14.71%. In this study, the Rhipicephalus (Boophilus) microplus ticks collected from cattle were determined to have elevated levels of resistance to cypermethrin but low resistance to coumaphos.*

Introduction

The common cattle tick *Rhipicephalus (Boophilus) microplus* is one of the most important tick species in the world, causing animal mortality and high costs related to the treatment and prevention of infectious diseases in cattle (Vargas-Cuy *et al.,* 2019). In Latin America, losses associated with the production of milk, meat and skins are reported (Bock *et al.*, 2004). During the parasitism stage in cattle, each adult tick can feed with 1 mL of blood, leading to a loss of 1 g of body weight and 8.9 mL of milk reduction (Narladkar, 2018). Ticks can cause indirect damage to cattle through the infectious agents they can transmit, primarily *Babesia bovis*, *B. bigemina* and *Anaplasma marginale*, during infestation, which can kill them (Rodriguez-Vivas *et al.*, 2018)

Synthetic acaricides are the primary method of tick control; however, the presence of ticks resistant to commercial acaricides is a major concern in areas with tropical and subtropical climates (Maya-Delgado *et al.*, 2020). Animals affected by *R. microplus* in Nicaragua are treated with knapsack sprays using pyrethroid-based acaricides and amitraz or with systemic treatments such as Fipronil and Ivermectin (Barrios *et al.*, 2022). The excessive use of conventional products and the lack of implementation of tick control strategies have promoted the development of drug resistance, a serious problem, since it leaves fewer options for its control (Canul-Ku *et al.*, 2012; Grace, 2015). If ticks are regularly exposed to chemical agents, they can develop resistance to the point that acaricides lose their effectiveness over time, and therefore the product rotation process can lead to the inability to use all active ingredients (Coles & Dryden, 2014). This study was conducted with the objective of evaluating the resistance of *Rhipicephalus (Boophilus) microplus* against three widely used ixoidicides, namely coumaphos, amitraz and cypermethrin, using the dose recommended by the manufacturing laboratory. These data will allow knowing the state of the resistance of ticks in bovines against the most widely used acaricides in Nicaragua.

Materials and methods

A cross-sectional study was carried out in a population of 471 cattle that belonged to 15 farms in western Nicaragua. From this population, a sample size of 60 cattle was calculated considering an accepted error of 10%, a level of confidence of 95% and an unknown prevalence (50%). The selection of each animal was carried out consecutively numbering the population and later a simple random sampling

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was carried out. From each selected animal, at least 5 engorged female ticks were collected.

Sample collection and transport

Engorged ticks of the species *Rhipicephalus (Boophilus) microplus* were collected between 6:00–7:00 am. The specimens were collected directly from the bovines. They were immediately placed in test tubes covered with moist cotton to provide oxygen. The samples were transported in a thermos to the parasitology laboratory of the Centro Veterinario de Diagnóstico e Investigación (CEVEDI).

Management of ticks in the laboratory

The engorged female ticks were separated to be observed with a stereoscope to carry out the taxonomic identification. Later they were washed and weighed and placed on a Petri dish. In addition, wet cotton was placed as a source of humidity, and oxygen, incubated in low light at 33°C. The ticks of the control group were incubated for 18 days until they began their oviposition (Figure 1). Once the eggs occurred, they were weighed and placed in test tubes for 21 more days until the day of hatching. The larvae obtained were placed in Petri dishes for 18 days until they reached the maturity required to apply the larval pack test (LPT).

Determination of efficacy in adults

It was carried out based on the Adult Immersion Test (AIT), using 5 repetitions per treatment in groups of 10 ticks per replica. The specimens were submerged for 3 minutes in the solutions of coumaphos (200 ppm), amitraz (200 ppm), cypermethrin (200 ppm) and water as control to each group containing 10 mL of the solutions prepared (Drummond *et al.*, 1973). They were subsequently dried and placed in Petri dishes (5.5 × 1.5 cm) to be incubated at a temperature of 27–28°C, and humidity of 70–80%. Mortality was recorded for 18 days based on three specific signs and characteristics: (i) increased darkness of the cuticle, (ii) leg without movement, when the ticks were inverted under a stereomicroscope and tested with a paintbrush and (iii) haemorrhagic skin lesions that were identified by observation under stereoscopes, as previously described (Pirali-Kheirabadi & Teixeira da Silva, 2011).

Fig. 1. Females of *R. microplus* in oviposition

Determination of efficacy in larvae

The previously described Larval Pack Test (LPT) was applied (Shaw, 1966). Solutions were prepared at the recommended concentration of 200 ppm for each acaricide; Whatman number 1 paper packets impregnated with the solutions and containing 90–100 larvae were used, and each packet was incubated at 33°C for 24 hours using 5 repetitions per group. Packages in the control group were impregnated only with distilled water. The mortality percentage was estimated by quantifying the dead larvae with the help of a stereoscope.

Statistical analysis

Percentages of mortality and oviposition in adults are described with means and their respective standard deviations (SDs). To compare the groups, the one way ANOVA F test was applied, and significant differences were considered when $p < 0.05$.

Results and discussion

Research on resistance has been scarce in Nicaragua; however, in other countries, it has been documented that the dosage and inadequate application of acaricides have increased resistance levels, mainly in cattle of small producers (Ravindran *et al.*, 2018).

In this study, significant differences (one way ANOVA, $p < 0.05$) were observed in the percentage of inhibition of oviposition in *R. microplus* when comparing the different acaricides most used in cattle farming in Nicaragua. It was found that coumaphos at a concentration of 200 ppm was the acaricide that provided a higher percentage of oviposition inhibition of 86% (SD = 8.40), although this is a lower result than that found by another investigation carried out in Mexico, in which they report a 100% inhibition of oviposition when the product is applied at a concentration of 50 ppm (Ravindran *et al.*, 2018). In the application of amitraz (200 ppm), an inhibition of 47.00% (SD = 7.60) was found, and the results were similar to those found in India, where they report inhibition of 78.35%; however, in that study, they applied a concentration of 1000 ppm (Dutta *et al.*, 2017). The inhibition of oviposition by cypermethrin was the lowest of the three acaricides studied with 12.90% (SD = 5.20), (Figure 2), a finding similar to that found in Colombia, where they found a low effectiveness of this pyrethroid for the control by *R. microplus* (Diaz-Rivera *et al.*, 2019).

The AIT revealed that mortality in adults treated with coumaphos was 97.72% (SD = 12.60), unlike

females treated with amitraz in which only a 48.8% mortality was observed, and in those treated with cypermethrin, the mortality was 14.71%. (SD = 2.80), (one way ANOVA, $p < 0.05$), (Figure 3).

The elevated level of resistance to cypermethrin in adult ticks is similar to that found in India, where the overall prevalence of pyrethroid-resistant *R. (B.) microplus* was found to be 66.6%. Cypermethrin resistance was detected in 16 areas and 96% of the *B. microplus* tick population was resistant to cypermethrin, this being the least effective product (Sharma *et al.*, 2012). The mechanism of action of pyrethroids is the modification of the sodium channels that are responsible for maintaining the nerve impulse and generate muscle paralysis (Stone *et al.*, 2014). These compounds cause membrane depolarization by slowing the gating kinetics of activation and inactivation of sodium channels, induction of a large, slow decaying tail current associated with membrane repolarization and in the delayed and prolonged opening of single sodium channels (Silver *et al.*, 2014). However, the low cost and high availability of this product in the region have increased its indiscriminate use, selective pressure and the population of *R. microplus* resistant to cypermethrin, in which it has been identified that the modification of the site acaricide binding occurs due to point mutations in domains II and III in the sodium channel-related gene (Diaz-Rivera *et al.*, 2019). However, studies have shown that pyrethroids can still show some efficacy when combined with other products, including a combination of cypermethrin, chlorpyrifos, and fenthion obtaining a 95% *in vitro* efficacy against *R. microplus* (Rodrigues *et al.*, 2018).

The LPT showed significant differences between

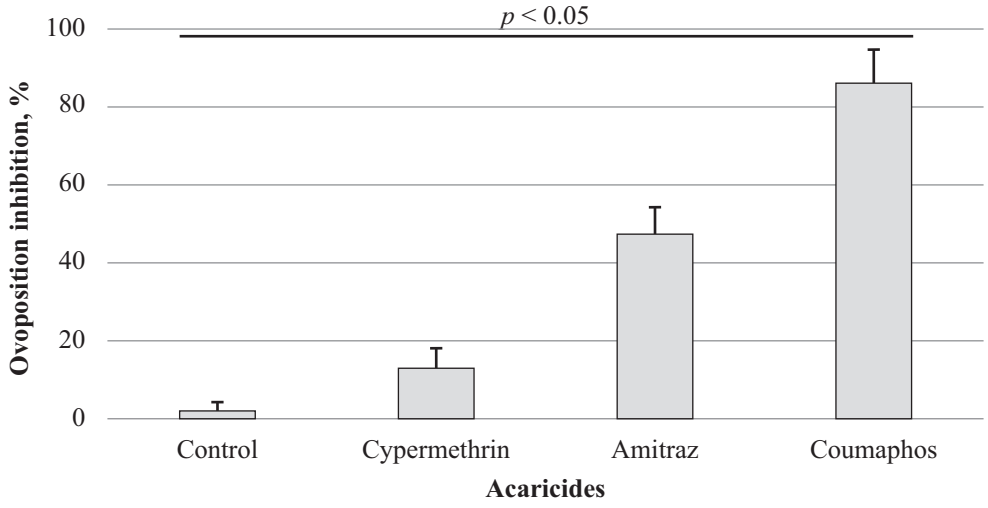

Fig. 2. Percentage of inhibition of oviposition in females of R. microplus treated with the different acaricides.
*: Significance value based on one way ANOVA test.
The upper horizontal bar represents the comparison between the four study groups.

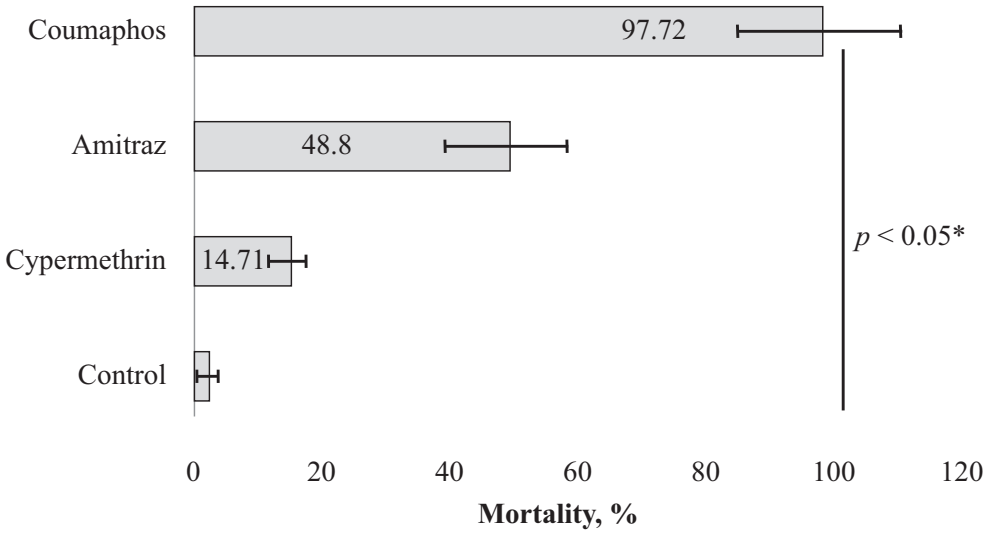

Fig. 3. Percentage of mortality in adult females of R. microplus treated with the different acaricides.
*: Significance value based on one way ANOVA test.
The vertical bar represents the comparison between the four study groups.

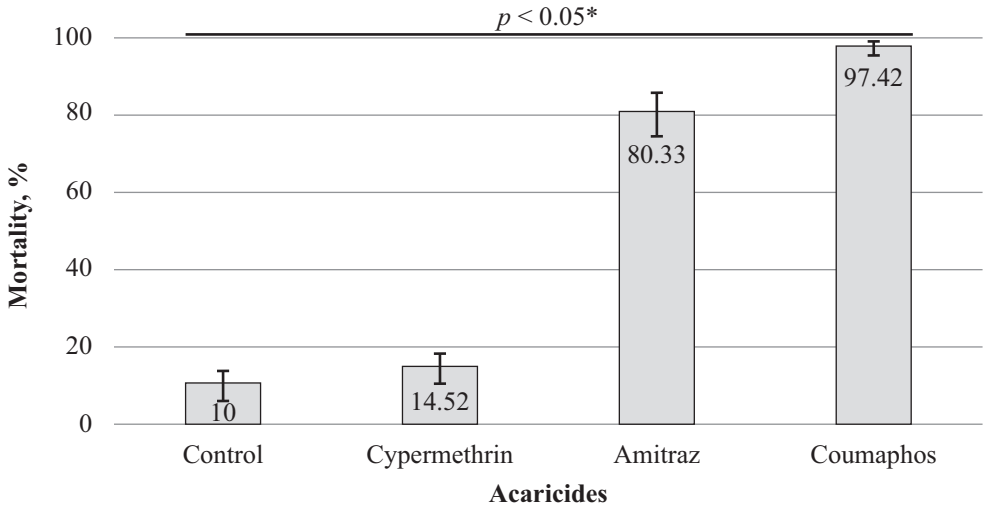

Fig. 4. Mortality percentage in R. microplus larvae treated with the different acaricides.
*: Significance value based on one way ANOVA test.
The vertical bar represents the comparison between the four study groups.

the acaricidal effectiveness (one way ANOVA, $p < 0.05$). It was found that coumaphos provides a percentage of mortality of 97.43% vs. only 14.52% observed in ticks treated with cypermethrin, with higher percentages of larval mortality in the packages impregnated with coumaphos with 97.43%, followed by amitraz with 80.33% (Figure 4). According to this study, amitraz is more effective against tick larvae than against adult ticks, so its use should be recommended during the period when tick larvae are most active in the cattle herd. The low mortality obtained with cypermethrin can be attributed to its low cost and indiscriminate use, which facilitated its rapid development in different parts of the Nicaraguan territory (Grace, 2015).

The resistance of ticks to acaricides is conferred mainly by two important mechanisms: increased activity of metabolic enzymes and genetic mutations that modify the sites of action (Rodrigues *et al.*, 2018). For example, single nucleotide polymorphism (SNP) in the first transmembrane region (TM1) of β-adrenergic-like (β-AL) has been identified in populations of amitraz-resistant *R. microplus* in Australia and indicating that this amino acid substitution could be a cause of resistance to amitraz (Corley *et al.*, 2013). However, in order to identify these mechanisms, molecular studies are required to determine the type of resistance that ticks are showing at the national level.

Conclusion

The most effective treatment against *Rhipicephalus (Boophilus) microplus* was coumaphos, with a higher control percentage compared with amitraz and cypermethrin. It was determined that coumaphos presented the highest mortality in adults, the highest percentage of inhibition of oviposition and the highest mortality in larvae.

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