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Original

In vitro evaluation of five ixodicides against *Rhipicephalus microplus* in Catacamas, Olancho, Honduras

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ABSTRACT

Objective. The objective of the present investigation was to evaluate the in vitro efficacy against *Rhipicephalus microplus* of five commercial products used in the site with the highest production of cattle in Honduras. **Materials and methods.** Ticks were collected in four cattle farms in Catacamas (14° 50′ N, 85° 53′ S), considering a quantity of 300 bovines, obtaining approximately 1.000 teleogynes of *R. microplus* to form a control group with distilled water and five treatment groups, each group included two replicates with 50 individuals (n = 100). The adult immersion method was performed with each of the five commercial products and the following rates were estimated: mortality, oviposition, hatching, reproductive efficiency and product efficacy. **Results.** Four of the commercial products with the following active ingredients (Product 1: cypermethrin, chlorpyrifos, piperonyl butoxide. Product 2: cypermethrin, ethion, piperonyl butoxide. Product 3: amitraz. Product 4: coumaphos), showed an efficacy greater than 90%. On the other hand, a product (Product 5: cypermethrin), presented an efficiency of 68%. This product of the pyrethroid family shows efficacy problems with a mortality of 33% and a reproductive rate of 30%. **Conclusions.** One of the products evaluated (cypermethrin) showed low in vitro efficacy against *R. microplus* in Catacamas, Honduras. It is necessary to modify the use of cypermethrin in this locality, preventing possible problems of resistance to ixodicides.

Keywords: Acaricides; amidines; effectiveness; ticks; organophosphates; parasitosis; teleogynous (*Source: DeCS*).

RESUMEN

Objetivo. El objetivo de la presente investigación fue evaluar la eficacia *in vitro* contra *Rhipicephalus microplus* de cinco productos comerciales empleados en el sitio con mayor producción de bovinos en Honduras. **Materiales y métodos.** Se colectaron garrapatas en cuatro fincas ganaderas en Catacamas (14°50′ N, 85°53′ S), considerando una cantidad de 300 bovinos, obteniendo aproximadamente 1000 teleóginas de *R. microplus* para conformar un grupo control con agua

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destilada y cinco grupos tratamiento, cada grupo incluyó dos réplicas con 50 individuos (n=100). Se realizó el método de inmersión de adultas con cada uno de los cinco productos comerciales y se estimaron las siguientes tasas: mortalidad, ovoposición, eclosión, eficiencia reproductiva y eficacia del producto. **Resultados.** Cuatro de los productos comerciales con los siguientes ingredientes activos (Producto 1: cipermetrina, clorpirifós, butóxido de piperonilo. Producto 2: cipermetrina, ethión, butóxido de piperonilo. Producto 3: amitraz. Producto 4: coumafós), mostraron una eficacia superior al 90%. Por otra parte, un producto (Producto 5: cipermetrina), presentó una eficacia del 68%. Este producto de la familia de los piretroides muestra problemas de eficacia con una mortalidad del 33% y una tasa reproductiva del 30%. **Conclusiones.** Uno de los productos evaluados (cipermetrina) presentó una baja eficacia *in vitro* contra *R. microplus* en Catacamas, Honduras. Es necesario modificar el uso de la cipermetrina en esta localidad previniendo posibles problemas de resistencia a ixodicidas.

Palabras clave: Acaricidas; amidinas; eficacia; garrapatas; organofosforados; parasitosis; teleóginas (*Fuente: Decs*).

INTRODUCTION

Ticks are ectoparasites that affect wildlife, domestic animals, and people, creating health problems worldwide (1). These parasites can transmit a variety of microorganisms such as viruses, bacteria, protozoa, and helminths to vertebrates (2). For beef and dairy cattle, tick infestations are [Page 3 of 16:] an important cause of loss of productivity, due to the increased risk of morbidity and mortality of the animals (3).

In the tropics, *Rhipicephalus microplus* is considered one of the most relevant ectoparasites in bovine production (4,5) and it is estimated that 80% of bovids worldwide would be exposed to infestation by this parasite (6). Infestations of *R. microplus* can decrease feed consumption, reduce feed conversion, cause damage to the skin of cattle, reduce milk production, transmit diseases, and cause expenses in ixodicidal chemical products or veterinary costs for treatment of transmitted diseases (*Babesia bigemia, B. bovis, Anaplasma marginale*) (7,8). A tropical country that has managed to estimate these costs (Mexico), reports approximately USD 573.61 million linked to the treatment of *R. microplus* (9).

The commonly used method for the control of *R. microplus* in developing countries is the use of ixodicidal chemical products, with the intention of causing the death of most of the individuals and thus interrupting the biological cycle of this parasite (1). However, reducing tick control with the application of chemicals can become ineffective, due to the phenomenon of resistance to antiparasitics (10). Of a total of 77 countries that are attached to the World Organization for Animal Health (OIE) [for its original acronym in Spanish], 55% have reported problems of resistance to external ruminant parasites, among which is *R. microplus*.

In a study, the presence of *R. microplus* was demonstrated in four Central American countries, including Honduras (4,11,12,13), where resistance of 66% to deltamethrin, 5562% to flumethrin, and 58 % to cyfluthrin has been reported. However, one of the widely used pyrethroids in Honduras: cypermethrin, has not been evaluated.

In Honduras, the municipality of Catacamas (department of Olancho) is the site with the highest production of beef and dairy cattle. Like other countries in the tropics, this cattle herd maintains recurrent infestations by *R. microplus*, causing the frequent use of ixodicidal chemicals as the main control strategy. In addition, in Honduras as it is a developing country, it is possible that control activities are carried out without the supervision of the veterinarian, in the absence of drug rotation, possibly omitting the doses and frequency recommended by the manufacturer according to the concentration of the products. (14). This would promote the development of antiparasitic resistance of local populations of *R. microplus* to chemical products, especially those commonly used.

Due to the recurrence of infestations by *R. microplus* in Catacamas, Honduras, it is necessary to study the efficacy of commercial products used as the main control strategy. The objective of this research is to evaluate the *in vitro* efficacy of five different commercial products in the recommended doses, considering for the first time the evaluation of cypermethrin, due to its frequent use in control strategies and presenting it in the formula of three of the five products evaluated.

MATERIALS AND METHODS

Place of Study. The municipality of Catacamas (Olancho, Honduras) brings together most of the Honduran cattle herd (14°50′ N, 85°53′ S). Catacamas has an annual temperature range between 25ºC and 35ºC and an average relative humidity of 92%. Livestock production is characterized by an extensive dualpurpose system (meat and milk), which is constantly expanding, causing logging, and burning of forests, mainly those close to the Tawahka Biological Reserve.

Collection and Morphological Identification. Approximately 1000 teleogines of *R. microplus* were collected, directly from bovines of four cattle farms, during November 2019 and January 2020. The owners of these farms verbally reported problems to control infestations of *R. microplus*. For the collection, tweezers, latex gloves, and paper bags of wood pulp with a vent were used. The bags were identified with the date and site of the collection and were later transported to the Parasitology Laboratory of the Universidad Nacional de Agricultura [National University of Agriculture] in Catacamas, Honduras.

For the typification of *R. microplus*, the genus and species of tick were identified with the help of an electric light stereoscope (brand Omax, model AC100); observing morphological structures such as: palps, shield, capitulum and hypostome for their typification as *R. microplus* (5,15,16). Teleogynes with lesions on the palps, lack of locomotion, and with morphological characteristics different from *R. microplus* were ruled out.

To purchase the ixodicides, commercial overthecounter products were purchased in Catacamas, with the following characteristics: Product 1 [TexVet Max® brand (spray), cypermethrin 15 g, chlorpyrifos 25 g, piperonyl Butoxide 1 ml]. Product 2 [brand Ultrametrin® (spray), cypermethrin 10 g, ethion 40 g, piperonyl butoxide 10 g]. Product 3: [Fulminado® brand (spray), amitraz 208 g] Product 4 [Page 5 of 16:] [Asuntol® brand (spray), coumaphos 200 g]. Product 5 [Paredon® brand (spray), cypermethrin 150 mg]. All products recommended at a dilution of 1 ml of solution in 1000 ml of water.

Groups y Replicas. According to 5 commercial products to be evaluated, 6 study groups were formed (including a control group). Each group was made up of 50 teleogines and one reply (n=100). Ticks were washed with distilled water to remove residues, dried with absorbent paper, and weighed on an analytical balance with a precision of 0.0001 g to form the groups with similarity in weight.

The adult immersion technique (24) was carried out, which consists of immersing for 3 minutes in 20 ml of solution with the recommended dose for each of the replicas of the commercial products using distilled water as a vehicle in beaker bottles with a capacity of 500 ml. The dose of the products administered was in accordance with the descriptions of each of the manufacturers (4,17,18). The excess commercial product was absorbed with cellulose paper. The individuals of the six groups were observed up to 30 days at ambient temperature and humidity, with a photoperiod of 12 light hours, estimating the mortality rate, the oviposition rate, the hatching rate, the reproductive efficiency, and the efficacy of the commercial products as proposed by Drummond et al., 1973 (4,19,20). For this study, rate is understood as the relationship between the number of individuals who present an expected condition (for example, death), divided into the total number of individuals evaluated. The groups were placed and observed in transparent polyethylene containers (round container, with a capacity of 250 ml) at room temperature and humidity for 30 days for evaluation (17).

Mortality Rate. Mortality rate for this study was defined as the number of individuals killed in a given period (21,22). Teleogynes with color change, lack of bowel movements and absence of locomotion were considered dead during the days of observation (17). The mortality rate was estimated with the count of dead teleogines per group, divided by the total number of teleogines per group, multiplied by 100 (23). The formula used to calculate the mortality rate was:

$$Mortality\ rate = \frac{\sum dead\ ticks}{\sum \text{Total ticks}} \times 100$$

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Oviposition Rate. Oviposition is defined as the process of laying eggs from the female's body (24, 22). Each group was observed until the presence of eggs was identified, which were transferred to [Page 6 of 16:] other transparent polyethylene containers at a temperature of 26ºC and relative humidity of 70%, according to their group of origin. The oviposition rate was estimated with the weight of the eggs divided by the weight of the teleogines, multiplied by one hundred (17). The formula used to calculate the oviposition rate was:

$$Oviposition\ rate\ = \frac{Weight\ of\ the\ eggs}{\text{Weight of teleogines}} \times 100$$

Hatching Rate. Hatching is the time when the larvae begin to break free from the egg once they have reached their development (some eggs do not hatch). The hatching rate was carried out by means of visual appreciation by stereoscope, following the technique described by Araque et al (16) with three readings from three different people. The result of this technique estimates a categorical hatching percentage of 0, 25, 50, 75 and 100%.

Reproductive Efficiency. Reproductive efficiency was defined as the ability of an engorged tick to convert part of its initial weight into viable larvae (19,25). It was estimated with the weight of the eggs per group, multiplied by the hatching percentage, divided with the weight of the teleogines per group (18,25,26). The formula used was:

$$Reproductive\ efficiency\ = \frac{\text{Weight of the eggs} \times (\%\ \text{hatching})}{\text{Weight of teleogines}}$$

Product Efficacy. In this study, it was understood that the efficacy of commercial products is the maximum in vitro effect that a product causes on the reproductive efficiency of teleogines (26). The efficacy threshold for evaluating each of the commercial products in this study was ≥90%, being the minimum level internationally accepted (18,27). To estimate the effectiveness of each product, the following calculation was applied:

$$Product\ efficacy\ = \frac{\text{ER control group} - \text{ER treated group}}{\text{ER control group}} \times 100$$

Statistic Analysis. The central tendency and the assumption of normality of the weight of teleogines and eggs were explored with a ShapiroWilk test. A descriptive analysis was performed to explore possible [Page 7 of 16:] variation, overscattered values, and homoscedasticity of the data. An Analysis of Variance (ANOVA) [for its acronym in Spanish] with Welch correction (which considers the heteroskedasticity of the data) was applied to identify the existence of the effect of commercial products on the data of the weight of oviposited eggs for each group (the null hypothesis would be equal weight given the lack of the effect of ixocidicides). Subsequently, a *PostHoc* test with the Bonferroni correction was applied to identify the products causing the differences according to each of the estimated data.

RESULTS

The weight in grams of the ticks of the six groups evaluated at the beginning of the experiment presented an arithmetic mean of 10.67 g, a median of 10.76 g and a standard deviation of 0.27 g; complying with the assumption of normality (ShapiroWilk: W=0.92167, pvalue=0.3001) and the absence of overscattered values. On the other hand, the ANOVA analysis with Welch correction proposes a significant difference in the weight in grams of the oviposited eggs between the groups due to the application of the products (F=1544.1, num df=5, denom df = 2.3596, pvalue=0.0002). The results of the *PostHoc* test with the Bonferroni correction are presented in Table 1. However, the group that offers the greatest differences is product 5 (cypermethrin) with respect to the other products applied.

The results of the mortality rate, the oviposition rate, the hatching rate, the reproductive efficiency, and the efficacy of the product are presented in table 2. In general terms, the mortality rate presented a range between 33 to 94%, the oviposition rate presented a range between 0 to 95%, the hatching rate presented a range between 0 to 100%, the reproductive rate presented a range between 0 to 95%, and the efficacy of the products presented a range between 68 to 95%. Four of the commercial products obtained an efficacy greater than 90%, while only one product (5: cypermethrin) had an efficacy of 68%.

Table 1. PostHoc test with Bonferroni correction to compare the differences in weight in grams of the oviposited eggs between the six groups.

	Control Group	**Product 4: coumaphos**	**Product 5: cypermethrin**	**Product 3: amitraz.**	**Product 1: cypermethrin, chlorpyrifos, piperonyl butoxide.**
Product 4: Coumaphos	1.10×10^{-06}	-	-	-	-
Product 5: Cypermethrin	6.20×10^{-06}	0.0034	-	-	-
Product 3: Amitraz.	8.60×10^{-07}	1	0.0015	-	-
Product 1: cypermethrin, chlorpyrifos, [Page 8 of 16:] Piperonyl butoxide.	7.70×10^{-07}	1	0.001	1	-
Product 2: cypermethrin, ethion, piperonyl butoxide.	8.70×10^{-07}	1	0.0015	1	1

Table 2. Mortality rate, oviposition rate, hatching rate, reproductive efficiency, and in vitro efficacy of 5 commercial products against R. microplus.

Treatment	**Mortality rate %**	**Oviposition rate %**	**Hatching rate %**	**Reproductive efficiency %**	**Product efficacy %**
Control	4	95	100	95	
Product 1: cypermethrin, chlorpyrifos, piperonyl butoxide. (combination of Pyrethroid, organophosphate and pesticide synergist)	94	0	0	0	95
Product 2: Cypermethrin, ethion, piperonyl butoxide. (combination of Pyrethroid, organophosphate and [Page 9 of 16:] pesticide synergist)	65	2	25	1	94
Product 3: Amitraz. (amide)	68	2	25	1	93
Product 4: Coumaphos (organophosphate)	93	5	25	1	92
Product 5: Cypermethrin (Synthetic pyrethroid)	33	30	75	30	68

DISCUSSION

The results of this study suggest that four out of five commercial products evaluated are effective *in vitro* for the control of *Rhipicephalus microplus* in Catacamas, Olancho, Honduras. Consequently, cattle producers in this region have efficient chemical tools to control this ectoparasite. Furthermore, the efficacy of cypermethrin observed in this study (68%) suggests the need for regulation in the sale, distribution and use of this active principle in the cattle herd of Catacamas, Honduras. The foregoing should be taken with caution because this study is based on the doses recommended by the manufacturers of the products, and it is necessary to continue with more indepth analyzes that contemplate experimental designs with discriminant doses for each of the chemical molecules.

The efficacy difficulties of cypermethrin against *R. microplus* in Catacamas, Honduras, could be explained due to the wide use of this molecule for the control of ticks that affect cattle. It should be noted that, of the five commercial products evaluated, three contain cypermethrin (Products 1, 2 and 5, Table 2). This implies an increase in the frequency of exposure and variation in the administered doses of cypermethrin (the products have different concentrations), modifying the ability of *R. microplus* to tolerate the recommended doses. Therefore, it is necessary to consider a regularization of the use of cypermethrin and its combination with other chemical molecules in commercial products in Catacamas, Honduras.

The resistance of the ectoparasite *R. microplus* to different formulations of ixodicides can vary, depending on the use by veterinarians and farmers. For example, the national [Page 10 of 16:] tick eradication campaign in Mexico (19741984) caused resistance to antiparasitics due to the abuse of coumaphos, chlorpyrifos and ethion (3). This led to the use of synthetic pyrethroids (for example, cypermethrin) from 1986 as an alternative tool to counteract the resistance problems caused by the abuse of these organophosphates. The above is an example of how resistance can vary according to active ingredients widely applied in a geographic region. However, the resistance problem in Mexico is the opposite of that observed in Catacamas, Honduras, where cypermethrin shows a low efficacy of 68%, and products with coumaphos and ethion present an efficacy of more than 90%. It is necessary to advise livestock users of ixodicides in Catacamas, Honduras, about the effect of antiparasitic practices, avoiding the abuse of the same molecules, and promoting the rotation of products and active principles. In the case of veterinarians, it is necessary to continue the evaluations of efficacy in more places in Honduras and at different times of the year. On the other hand, the implementation of an integrated parasite control is necessary since the rotation and combination of chemical molecules is not enough to control tick infestations. The therapeutic reduction by producers to a single control method (especially chemical control) is not very sustainable and profitable in the long term in cattle farming (27,28). For example, a resistance study of *R. microplus* in Colombia suggests a great variation in efficacy between farms that rotate the combination of chlorpyrifos with cypermethrin (2293%), compared to the organophosphate ethion (99100%) (30). In addition, in other sites in Colombia, a resistance of 44% is reported by organophosphate products used for the control of *R. microplus* (17, 30). Therefore, it is necessary to understand that there are adaptation mechanisms of *R. microplus* that make them resistant to chemical products with humanmediated variations. In addition, ticks, like other arthropods, present population variations in space and time, these being unknown for the place of this study and that could contribute to establishing effective control plans (for example, the months of the year with the highest abundances can be determined, suggesting increasing control efforts at that time).

In the case of Catacamas, Honduras, it is located in a geographical area surrounded by state natural reserves, with little specialized livestock, low technology and low productivity. This bovine production system is characterized by the occupation of large areas of land, keeping the cattle in sites that adjoin or occupy native forest, which can serve as a favorable ecological niche [Page 11 of 16:] for *R. microplus* (temperatures, humidity, vegetation, and susceptible animals). However, for the development of an integral control of parasites it is necessary to stimulate applied research, validated in the bovine system of Catacamas, Honduras. This implies the participation of producers, veterinarians and academia in research and training programs for the control of *R. microplus*: epidemiological studies that suggest the ideal time to use chemical control; management, rest, alternation, and rotation of grazing to reduce the number of larvae; and silvopastoral systems. In addition, the above is complemented by increasing the immunity of bovines, supplementing nutritionally with minerals, nonprotein nitrogen and protein of high biological value, genetic selection of animals, adequate prophylactic programs, confinement of pregnant and lactating animals and biological control of ticks with native predatory species (27).

It is necessary to mention that two of the products of this study that showed high efficacy are combinations of organophosphates, pyrethroids and synergistic pesticides. The combination and synergy of different active ingredients would explain the efficacy greater than 90%, despite the resistance of *R. microplus* to the pyrethroid called cypermethrin (23,31). However, synergy with pyrethroids should be considered with reserve since the efficacy difficulties towards this family of products would be perpetuated at the site of this study. Therefore, it is recommended to apply the precautionary principle, suspending the use of pyrethroids in Catacamas, Honduras, due to the low efficacy reported in countries close to Central America and the smuggling of cattle with neighboring countries, which can favor the crossborder exchange of cattle with high tick infestation (30,31,32,33,34).

This study suggests the efficacy of four commercial products *in vitro*, and problems of efficacy of a product for the control of *R. microplus* in Catacamas, Honduras. Because the study site is the area with the highest bovine production in this country, it is prudent to modify the practices regarding the use of ixodicidal chemicals, reducing the possibility of causing future efficacy problems for other active principles. This makes it necessary to carry out *in vivo* studies of the four products with high efficiency (Table 2) in cattle farms. In addition, it is important to explore the efficacy of these products in other species of ticks present, such as the genus *Amblyomma* (35). It is also necessary to study the practices of farmers and veterinarians regarding the use of commercial products for the control of ticks in relation to the abundances in the pastures and the levels of infestation during the [Page 12 of 16:] year. This information should be complemented with a comprehensive parasite control program, led by academia and farmers' associations.

Conflicts of Interest

The authors affirm that they have no conflicts of interest regarding this research.

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