



Revista MVZ Córdoba
ISSN: 0122-0268
ISSN: 1909-0544
revistamvz@gmail.com
Universidad de Córdoba
Colombia

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Revista MVZ Córdoba, vol. 22, 2017

Universidad de Córdoba, Colombia

Disponible en: <http://www.redalyc.org/articulo.oa?id=69353273015>

Effect of the use of fluazuron for control of *Rhipicephalus (Boophilus) microplus* in cattle

Efecto del uso de fluazurón para control de *Rhipicephalus (Boophilus) microplus* en bovinos

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Redalyc: <http://www.redalyc.org/articulo.oa?id=69353273015>

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Recepción: 12 Septiembre 2016

Aprobación: 16 Enero 2017

RESUMEN:

Objetivo. Demostrar el efecto poblacional sobre la garrapata del uso de fluazurón en ganado en pastoreo. **Materiales y métodos.** Estudio de caso realizado en Fusagasugá (Cundinamarca, Colombia); se conformaron tres grupos de 25 novillas cada uno, G1= dos aplicaciones de Fluazurón separadas 60 días, G2= fluazurón sólo el día 0 del estudio y G3= tratado por aspersión con Clorpirifos. Se hizo periódicamente recuento de teleoginas en cinco bovinos de cada grupo y se determinó el grado de contaminación de praderas con larvas de garrapata, mediante rastreo. **Resultados.** Los niveles de infestación entre los grupos no fueron homogéneos, y los potreros también demostraron diversos niveles de infestación larvaria. El compuesto fue útil para proteger a los animales de la infestación por garrapatas por más de ocho semanas. Dada la temperatura del suelo en la región, el período adulto larva se cumple entre 50 y 65 días. **Conclusiones.** El efecto del tratamiento del ganado con fluazurón sobre la contaminación larvaria de las praderas, sólo se reflejará casi dos meses después del tratamiento. El diseño de esquemas estratégicos debe considerar este componente, de la temperatura del suelo.

PALABRAS CLAVE: Acaricida, Garrapatas, Inhibidores de síntesis de quitina, Manejo Integrado de Plagas, Parasitología.

ABSTRACT:

NOTAS DE AUTOR

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Objective. To demonstrate the effect on the tick population of the use of fluazuron in grazing cattle. **Materials and methods.** Case study performed in Fusagasuga (Cundinamarca, Colombia); three groups of 25 heifers each were formed, G1= two applications of Fluazuron separated 60 days, G2= fluazuron applied only on study day 0 and G3= treated with a spray of chlorpyrifos. Periodically tick count was done in five animals of each group and the degree of contamination of pastures with tick larvae was determined by dragging. **Results.** Infestation levels between the groups were not homogeneous, and the paddocks also showed different levels of larval infestation. The compound was useful for protecting animals from tick infestation for more than eight weeks. Given the soil temperature in the region, the adult larva period is fulfilled between 50 and 65 days. **Conclusions.** The effect of treatment of cattle with fluazuron on larval contamination of pastures will only be reflected almost two months after treatment. Strategic design schemes must consider this component, of the soil temperature

KEYWORDS: Acaricide, Chitin synthesis inhibitors, Integrated Pest Management, Parasitology, ticks, acaricide.

INTRODUCTION

The cattle tick *Rhipicephalus* (*Boophilus*) *microplus* is an ectoparasite of bovine cattle endemic up to the American tropics, distributed in Colombia to about 2800 m.a.s.l. (1). The persistent presence of ticks on farms has led to the intensive use of various types of medicines (ecto-parasiticides), in an attempt by producers to achieve control. Most experts agree that the use of compounds has led to the development of various levels of resistance to most compounds that have been introduced into the market for tick control (2,3,4,5). In Colombia, multi-resistant tick strains have been described for various types of molecules, specifically organophosphates, synthetic pyrethroids and amitraz, as is the case with the Montecitos strain, isolated in the Department of Santander (6).

An alternative for tick control in situations where resistance to various types of molecules occurs, is the use of compounds with different modes of action, such as chitin inhibitors (7,8,9). Chitin is a complex amino polysaccharide and an important component of the arthropod cuticle. During each molt it has to be formed again by polymerization of individual sugar molecules (7).

Fluazuron (Acatak®, Elanco Animal Health) is a benzophenyl urea that has shown to be effective against ticks, particularly against the common cattle tick, *R. microplus* (8,10). A characteristic of this type of compound is that it acts on different phases of the life cycle of the arthropod, when its immature stage is exposed to the compound, it is not able to complete the ecdysis and in consequence, it dies during the molting process. Benzophenylureas also have transovarial effects; When female ticks produce eggs in which the compound has been incorporated into the nutrients of the egg, the development of the vitellum normally occurs, but the larvae that develop are unable to hatch (7).

Because there is not extensive information on the population effects of the use of this type of compounds for tick control in cattle in tropical conditions, the objective was to establish, through tick count in animals and pastures, the effect of fluid applications in cattle over the tick population dynamics.

MATERIALS AND METHODS

Experimental design, location and animals. This research is a case study, based on observations on groups of animals, where each group grazed in an independent paddock (11). The objective of the study was to show the effect, on tick populations in paddocks and animals, of one or two applications of fluazuron on animals. The research was carried out at a commercial farm located in Fusagasuga (Cundinamarca, Colombia), at 1726 m.a.s.l., with an average temperature of 20°C, where *Bos taurus* x *Bos indicus* crossbred heifers are kept. The company is dedicated to the production of embryos and manages various groups of fast growing heifers (Figure 1), which leads to an ideal weight (weight over 300 kg) before being synchronized and implanted.



Figure 1. Breed make-up (*Bos taurus* x *Bos indicus*)
Of the heifers used in the study.

In the recent past, the farm had several problems related to tick control in these animals, which involved frequent use of tick-killing agent baths with various products; The farmer mentioned that tick problems fluctuated in intensity in relation to the time of year and the pattern of grassland use, sometimes requiring treatments every three or four weeks. The farm paddocks are constituted by native grass and various levels of coverage with African Bermuda grass (*Cynodon nlemfluensis*).

Three groups of 25 heifers each were formed, grazing in different pastures, using two alternating areas for each group. The groups were randomly assigned to one of three tick-pesticide treatments as follows: G1=two separate Fluzuron applications 60 days, G2=Fluzuron application only on day 0 of the study, and G3=spray-treated cattle with Chlorpyrifos EC24%® (the last product to be applied with relative success on the farm). One week before the study (day -7), the animals were weighed and a general observation was made on the level of tick infestation in the animals that until this point, they were all in the same paddock. The animals were randomly assigned to the groups, ensuring that all groups had a similar weight and breed makeup (11).

To develop the research, six pasture sites of similar size were assigned, so that each group grazed in two paddocks alternately, depending on the fodder availability in each paddock. In conjunction with the farm staff, the physical location of the paddocks was established, with the use of landmarks, the farm was located with the help of Google Maps® in order to design the larval sampling schemes in the prairies (Figure 2). Group 1 was assigned to prairies 1A and 1B, group 2 to prairies 2A and 2B and group 3 to prairies 3A and 3B. Each paddock was enclosed by an electric fence.

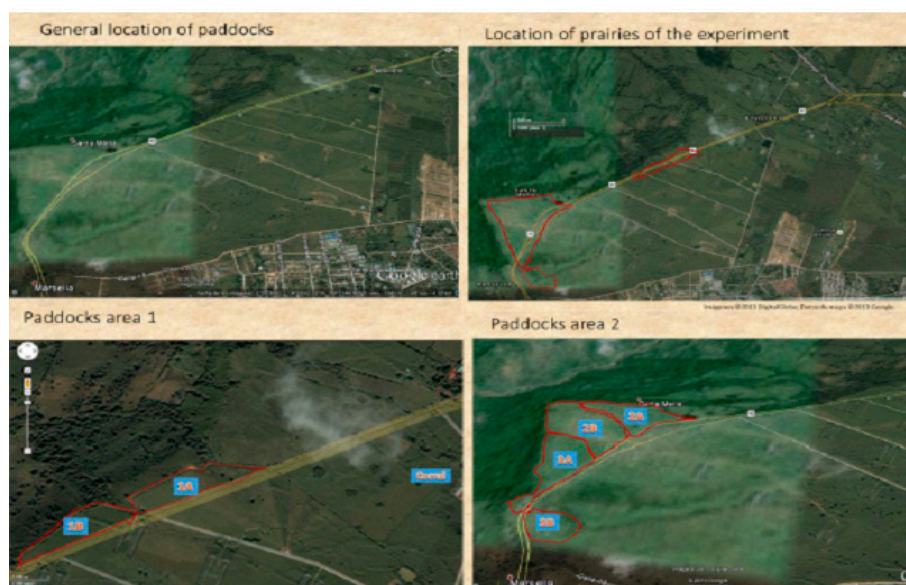


Figure 2. Map preparation of the experimental prairies with the help of Google Maps® to determine areas and transects in which the estimation of the population of larvae in the prairies was carried out.

Tick Control Treatments. Day 0 of the experiment was December 20, 2013. At that time, groups 1 and 2 received a pour-on application of Fluazuron (Acatak®, Elanco Animal Health) at the dose of 2.5 mg / Kg, which is equivalent to administering 10 ml of solution (25 g / liter) per 100 kg of animal weight. Group 1 received a second application of Fluazuron on February 18 (60 days after the first application). As for the control group that received spray treatment with Chlorpyrifos EC24%® at an effective concentration of 2.4 g of active compound per liter of spray, the animals were treated according to the level of infestation observed and received treatments on December 20, January 17, March 18 and April 23.

Tick Population. To determine the density of adult ticks in the animals of each group, a complete count of standard ticks was performed on five pre-selected animals of each group, using the Wharton & Utech method (12) which counts ticks of size greater than 4, 5 mm long on the side of the animal. These are the engorged females that will fall in the following 24-hours, thus establishing the daily tick density of the animal (12,13). Additionally, an observation and / or scraping was performed on the neck side to determine the occurrence of immature tick stages, which was recorded as absence, or subjective intensity of the presence of immature tick stages (14).

The degree of contamination of each of the prairies with tick larvae was done using the flag-tracking method (15,16) in which 1m x 1m white cloths were adhered to a wooden frame or cardboard, to allow vertical contact with the grass tips (Figure 3). This larva tracking method is based on counting the number of larvae captured on the cloth after the operator crosses a transect. For erect growth pastures, it is better than the fabric tracking method (15,17). A transect is defined as a path of 50 to 70 meters long, which is traversed by a person who carries the cloth with which the larvae are collected in the prairies. After completing the route, the operator counts the number of larvae that adhered to the fabric (16). Twelve transects were examined in each paddock per sampling day, randomly attempting to cover all sections of the area. The altitude of the pasture and the meteorological conditions prevailing on the day of each evaluation were recorded.



Figure 3. Use of the flag tracking method to determine the level of contamination of grass with tick larvae. Each operator walks a path of 50-70 m (transect) and determines the number of larvae caught in the fabric. On each evaluation date twelve random transects were established in each paddock.

Biotic parameters of ticks in the laboratory. In order to determine the development times of the non-parasitic phases according to temperature, teleogines were collected and taken to the laboratory, where they were kept in an incubator at 23°C, to allow oviposition and larval hatching, thus calculating the duration of the adult-larva period (PAL), according to what several authors have described (11,18)

Statistical analysis. A descriptive data analysis was performed, based on the preparation of fluctuation curves over time for tick counts on animals and larvae found in the prairies. Individual tick counts were transformed on a logarithmic scale to determine geometric averages (12,17).

RESULTS

The research took place for 18 weeks in the first semester of the year, evaluating in a consecutive way levels of infestation of the animals and degree of contamination of the prairies with tick larvae. The parasite density observed over time in all three groups is shown in figure 4. On day -7 of the experiment, when animals were assigned to groups, levels of infestation were moderate (2-3 ticks per animal) but similar in all three groups.

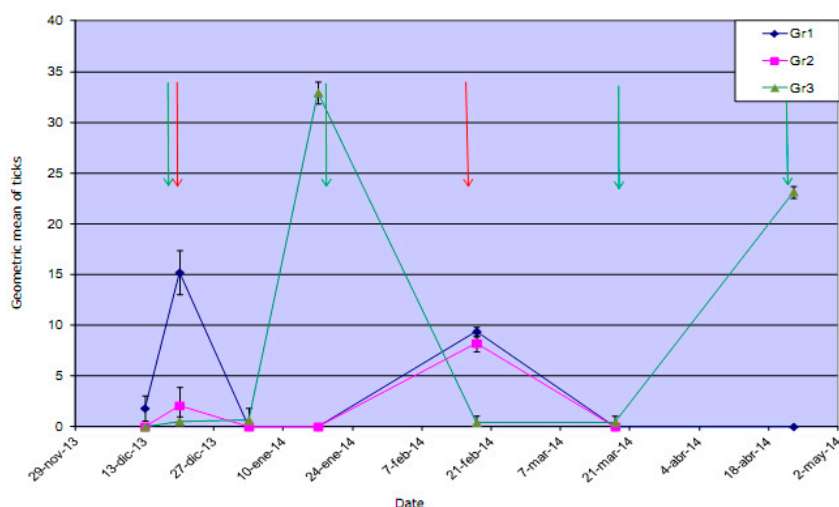


Figure 4. Geometric average of standard ticks (*Rhipicephalus microplus*) found in five animals from each experimental group. Vertical arrows indicate product application dates. Groups 1 and 2 received a Fluzuron application (red) and group 1 received a second application on February 18. Group 3 (green arrow) received application of Chlorpyrifos on indicated dates.

On the day of application of the first treatment the tick counts in group 1 were significantly higher; After application of the first treatment in all three groups, the levels of tick infestation in the animals decreased drastically; however, the levels of infestation in group 3 (treated with chlorpyrifos) were found to be high at four weeks after beginning the study. This can be explained by the relatively high levels of larval contamination in the paddocks.

The results of the larval tracking test are presented in Figure 5, where a log with the total larvae recovered in the twelve transects is shown for each paddock on each evaluation date. Paddocks 1A and 1B presented higher levels of contamination with larvae, particularly in the first weeks of follow up. Figure 6 shows two situations observed during the development of the traces; the first, when low numbers of ticks are caught and the second, when tick larvae catches are plentiful. Careful adult tick counts in five animals from each group adequately showed the levels of infestation with adult ticks. However, the recommended counting method for this type of evaluation (12) should be considered only for adult ticks larger than 4.5 mm, corresponding to larvae that adhered to the animal three weeks prior. At times, the infestation levels of immature animals (larvae, nymphs, young adults) were marked, but this was not shown in adult tick counts.

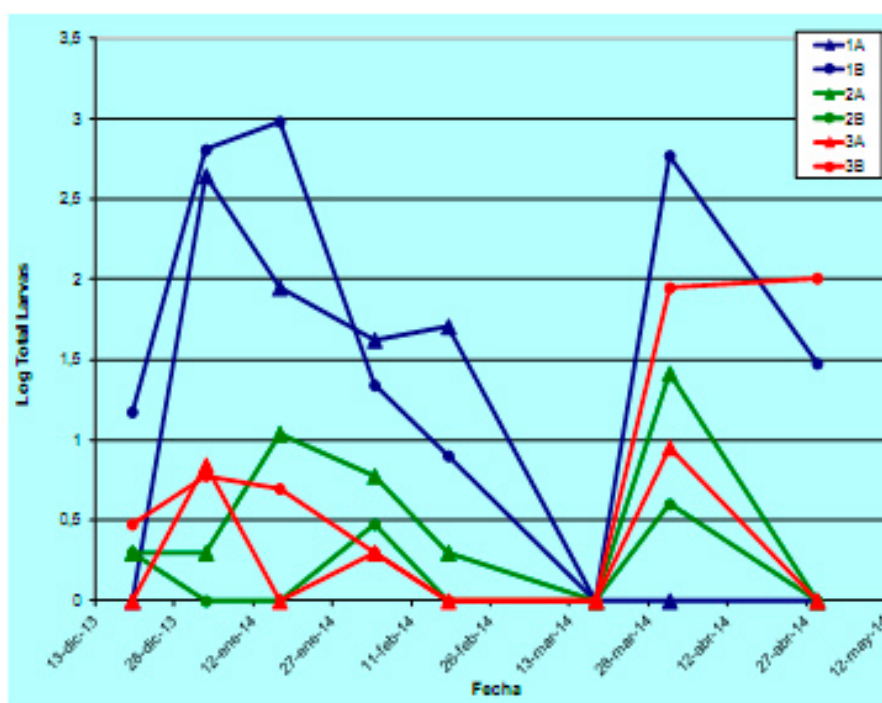


Figure 5. Contamination levels of prairie grass with tick larvae in the paddocks used by each experimental group. The nomenclature of the pastures is explained in figure 2.



Figure 6. Evidence of various levels of tick contamination in pastures. On the left, a single tick larva is observed among several adhered seeds. On the right, a group of more than 100 larvae.

Figure 7. illustrates this condition. This corresponds to the populations of larvae that adhered to the animals about two weeks prior. Under this type of circumstance generally the cattle raiser treats his animals. An additional indicator of the degree of infestation of the animals is then required. If left untreated, they will become adult ticks a week later. Table 1 shows the counting dates in which infestations by nymphs were detected in the animals monitored.



Figure 7. Nymphs and young tick adults (size <4.5 mm) in the jowl of a bovine of the group treated with Chlorpyrifos. This parasite size is not considered in the counts.

Table 1. Number of animals in each group (out of a total of five) in which moderate to high infestations were detected with nymphs of *Rhipicephalus microplus* during each of the evaluations in pen.

Count date	Gr1	Gr2	Gr3
13-Dec-13	4	0	0
20-Dec-13	0	0	0
3-Jan-14	0	0	5
17-Jan-14	2	0	5
18-Feb-14	5	2	0
18-Mar-14	0	0	3
23-Apr-14	0		5

DISCUSSION

It is noteworthy, from the results observed, that the dynamics of the larval population in the paddocks is affected by ecological components of the prevailing climate in the region and the microclimate of each of the prairies (19), and that its explanation not only depends on the degree of contamination from the animals that grazed there a few weeks earlier.

Fluazuron treatment provided protection for the animals for at least seven weeks, despite the high levels of contamination with pasture larvae, evidenced in the evaluations during December and January, mainly pastures 1A and 1B (Figure 5).

Because of logistics aspects of field work development, tick counts on animals were only carried out on pre-established dates. During the evaluations of weeks 2 and 4 post-treatment, no ticks were found in cattle in groups 1 and 2, while in group 3 the geometric average was 33 ticks per animal.

The following count was done one month later, on week 9 post-treatment, when a minimum number of ticks (geometric mean less than 10 teleogines per animal) was found in animals of groups 1 and 2. At that time, the second application of Fluazuron in group 1 was held, and tick counts returned to zero again, both in group 1 and in group 2 until the end of the study. Group 2 was only followed until week 13 of the study. For group 1 and group 3, tick counts were performed until week 18.

Due to the use of the prairies prior to the beginning of the study, in the first weeks of follow-up, several levels of larval infestation were evidenced in the paddocks, implying that the tick challenge of the groups was possibly not homogeneous; Which would have required the artificial infestation of prairies, with a known number of engorged ticks, as has been done in other investigations or formal efficacy tests (11, 17). This was not applicable in this research carried out under commercial terms. At least for the first six weeks of the study, the levels of grassland infestation can only be explained by the degree of contamination they suffered in previous weeks and by the favorability of each paddock to accommodate non-parasitic stages of the tick (16,19).

To improve the understanding of the population effect of the Fluazuron treatment on larval populations in the prairies in relation to the development time of the non-parasitic phases under the temperature conditions in the study area (average 20°C) a laboratory simulation was done, determining the PAL of engorged ticks collected on the study farm, kept in an incubator at 23°C (Figure 8). At this temperature the PAL was 65 days, which contrasts what was found under the conditions of the Colombian flat altillanura (18) where the PAL fluctuated between 27 and 32 days at an incubation temperature of 30°C.



Figure 8. Determination of the duration of oviposition and of the Adult-Larva period of teleogines of *Rhipicephalus microplus* kept at 23°C in the laboratory.

This finding indicates that, under the conditions of Fusagasuga, the adult tick populations observed on the animals (expressed by the count of teleogines larger than 4.5 mm) will only be reflected by an increase in the contamination of the prairies with larvae between 9 and 10 weeks later, if the ticks find adequate conditions for the development of their non-parasitic phases in the prairie. The difference in the periods of larval development according to soil temperature and its effect on the dynamics of tick larvae in the prairies is a fundamental component that must be considered in the design of integrated tick control schemes (20), particularly those based on grassland management (21).

In relation to the levels of larvae infestation in the prairies shown in Figure 5, the levels of infestation observed until mid-February and early March correspond to populations of adult ticks that contaminated the paddocks prior to the beginning of this study and partially, the increase in larval populations in mid-March, possibly corresponds to the contamination of the prairies made by the animals the week prior to the beginning of the study, when the infested animals were placed in their respective prairies. It is noteworthy that in paddock 1A, where the animals of group 1 treated with Fluazuron were located, the late March peak was not observed.

On the other hand, the teleogine counts on the animals could, in turn, reflect the larval populations that existed in the prairies three weeks earlier (12,13), thus allowing an approach to the protective effect of the treatment. In the first week of January, abundant populations of larvae were found, particularly in paddocks 1A and 1B, indicating that two or three weeks later, high counts of teleogines were observed in the animals. This was only observed in group 3, indicating that the other two groups showed protection provided by the treatment (8).

In conclusion, the use of the product was shown to protect animals from tick infestation for a period of seven to eight weeks. To ensure complete sterilization of ticks in the paddocks, treatment would be required every six weeks. Given the temperature of the soil in temperate regions such as Fusagasuga, the Adult-Larva period of the ticks is between 50 and 65 days, an aspect proven in the laboratory. This implies that the effect of treatment with Fluazuron on grassland contamination will only be shown almost two months after

treatment. The design of strategic schemes for tick control should consider the soil temperature component, to be able to program treatments properly and manage the animals in the paddocks.

CONFLICT OF INTERESTS.

The authors of this manuscript declare that there is no conflict of interest that jeopardizes the validity of the investigation.

ACKNOWLEDGMENT

To San Gabriel cattle ranch for allowing the development of this study in the company's facilities. To the field staff of the company, especially Mr. Genaro Vásquez and Mr. Pascual Alarcón. We recognize the priceless support provided by colleagues Sandra Pinzón Riveros and Jorge Riveros Roza for the mobilization of field activities.

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