

## Efficacy of Three Ectoparasiticides vs *Rhipicephalus microplus*

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### ABSTRACT

The effect of three ectoparasiticides —cipermetrin, amitraz, and Ivermectrin— on *Rhipicephalus microplus* was evaluated. Ingurgitated female ticks were taken out of cattle at a cattle genetic research farm. Once the female ticks lay their eggs, they were centrifuged at room temperature. After egg hatching, a hundred larvae 14-21 days old, were sampled on 2 x 2 cm filter paper strips upon the larvoscopic examination using impregnated filter paper. Four ectoparasiticide concentrations with three replicas each were evaluated. Every ectoparasiticide was tested for its effect on each sample in a 1 ml dose and increasing concentrations of 0.05 %, 0.125 %, 0.250 %, and 0.5 %; whereas the control group was administered castor oil. The larvae under medication in the trials remained 24 hr. in the dark at room temperature, followed by a living-and-dead specimen counting. The data were processed by the statistical package SPSS (version 15.0). Acarid likelihood mortality percentage associated with ectoparasiticide kind and concentration variables was estimated by the Probit Regression technique. Treatment was less effective for cipermetrin (37.1 %), rather than amitraz and Ivermectrin (89.9 % and 73.3 %, respectively) at similar concentrations. Further evaluations of ectoparasiticide efficacy for cattle under ranching conditions and the implementation of techniques to diagnose resistance to acaricides are recommended.

**Key Words:** acaricide, ectoparasites, *Rhipicephalus microplus*

### INTRODUCTION

Ticks are parasites found across the tropics, with a great capacity for adaptation and propagation (Estrada and Venzal, 2006). Great losses are produced every year by ticks, causing skin damage, blood loss, toxic effects, milk production and offspring decline and cost increase. Ticks also transmit etiological agents like viruses, bacteria and protozoa (Guglielmone *et al.*, 2007).

*Boophylus microplus* is the most frequent tick species observed in cattle raising (Rodríguez, 2005) worldwide (Taylor, Coop and Wall, 2007). Recently, it was located within the *Rhipicephalus* genre, due to phylogenie. It is now called *Rhipicephalus microplus* (Murrel, Campbell and Braker, 2000).

Guglielmone and Signorini (1995) have reported that dry areas with annual temperatures above 15 °C (typical in Cuba) are more prone to develop ticks.

Organophosphate chemical acaricides (amidines, phenylpirazolones and ectocides, known as macrocyclic lactones) are mostly used to attack *R. microplus* (Rodríguez *et al.*, 2010). Ojeda *et al.*, 2011 remark that the irrational use of these compounds has induced resistance in many regions of the world, thus increasing costs to develop new acaricides and posing limitations as to knowledge

and competence to produce new drugs. The integrated control method has gained strength against *R. microplus*, which includes immunization with GAVAC and acaricide immersions. However, there are reports on the existence of these acarids even when control methods are applied. Guglielmone *et al.* (2006) have stated that knowing the resistance-susceptibility condition of *R. microplus* populations in a given area is essential to implement steps to improve the use of ectoparasiticides. The aim of this research was to evaluate the efficacy of three ectoparasiticides on *Rhipicephalus microplus*.

### MATERIALS AND METHODS

Ingurgitated *Rhipicephalus microplus* females from unit 24 of Turiguano Genetic Research Farm were used in the investigation. The fresh eggs were put in centrifuge vials plugged with gauze at room temperature.

The larval test was used with impregnated paper to expose the ticks (Chagas 2008). After hatching, samples of 100 larvae each (14/21 days old) were put on 2 x 2 filter paper.

Four different acaricide concentrations with three replicas each were evaluated. Every sample and replica was treated with 1 ml of increasing concentrations of the ectoparasiticides (Ticomin 100 ec, Supatraz 125 and Labiomec<sup>®</sup>) 0.05; 0.125;

0.250 and 0.5 %, a control group was treated with castor oil. The larvae were kept in the dark for 24 h at room temperature. The live and dead specimens were counted. The larvae moving their tips, but unable to locomote were considered dead as well.

#### *Statistical analysis*

SPSS software (v 15.0), 2006, was used to process data. The likely acarid mortality percent was estimated in relation the ectoparasiticide and concentration variables were estimated by Probit.

#### *Criteria for success*

Ectoparasiticides with at least 50 % mortality with the 0.05 % concentration were considered efficient.

## RESULTS AND DISCUSSION

Using the reported procedure probable *Rhipicephalus larvae* mortality was achieved when they were exposed to different ectoparasiticide concentrations evaluated (Table 1). According to the results, cipermetrin treatment (Ticomin) showed the lowest mortality, probably due to inadequate use of Piretroid in production units. The best results were achieved with amitraz (Supratraz), (89.9 %), whereas efficacy of 73.3 % was accomplished with ivermectin (Labiomec). The control group behaved within the range set up by Chagas (2008).

Locomotive activity is blocked by piretroids (Ticomin), or by excitability, movement incoordination, irritability, paralysis, lethargy and death of insects, acarids and ticks (Rodríguez, 2005). Though widely accepted for its efficacy, lasting residue action and little toxicity to man and cattle, the application of cipermetrin does leave resistant populations of ticks. So, other active principles like Formamidines and Avermectins are necessary (Guglielmone *et al.* 2007).

Amitraz is a powerful insecticide/acaricide which inhibits mono-oxidase, an  $\alpha_2$ -adrenergic antagonist and inhibitor of prostaglandin synthesis (Gupta, 2007). The advantage of ivermectin is that application is parenteral and its antiparasitic scope is wide and powerful in low doses administered to arthropods and nematodes, acting on mature and immature stages, as in hypo biotic larvae. Ivermectin binding to receptors increases membrane permeability to chlorine, muscle/neuron cell membrane hyperbolization occurs, thus affecting the parasite's feeding capacity and its ability to

stay in the loci due to flaccid capacity (Martins, Robertson and Wolstenholme, 2002).

In Labiomec (Cuba) ivermectin is often used against nematodes and myiasis, but the procedure may select resistant populations of parasites, which are not the target in the treatment, as may occur in the case of ticks. Martins and Furlong (2001), and Anziani *et al.* (2004) alerted on the development of *R. microplus* populations; as well as other intestinal parasites (*Haemonchus* and *Cooperia*) avermectin-resistant in the south of Brasil and Argentina, respectively. Castro *et al.* (2010) reported incipient resistant to ivermectin by populations of this genre in Uruguay. Consequently, Gugliamone *et al.* (2007) suggest these drugs should be used with caution. In southeast Mexico, Rodríguez *et al.* (2007) studied field populations of *R. microplus* and found that most ranches had ticks with multiple resistant to ixodicides: organophosphates, amitraz and, especially, synthetic piretroids. However, the efficacy of products used for immersion is dependent, among other factors, on ectoparasiticide absorption, and it will be higher if larger contact surface is used, usually blocked by hair, blots or filth (Heine, Krieger, Dumont y Hellmann, 2005). Generalization of integrated control with the application of vaccines may delay acaride resistance development, provide immunogenic administration is effective (Rodríguez, 2005).

Gavac is used in Cuba for the control of *R. microplus* ticks in bovine. Every vaccine dose contains 2 ml of Gavac, with 100 micrograms of the antigen (Vargas *et al.*, 2005a), though a new formulation was later introduced with more efficacy than (Valdés *et al.*, 2005; Vargas *et al.*, 2005b).

So far, there are no reports on the existence of ixodicides by the genre studied in Cuba; however, efficacy is low. This may be caused by inadequate dilutions. The traditional methods for tick chemical control demand formulations that can be diluted in water and can be administered in immersion or showers (George *et al.*, 2004). Moreover, in areas with great tick infestation a relation between *Rhipicephalus* y *Amblyomma* is present, thus complicating controls. Chemical susceptibility and application schedules should be different in each case (Españe, Lines and Demedio, 1996). In the case of *Amblyomma*, other control methods are to be implemented. Immunization with Gavac is not achieved in this case and immersion and

showering frequencies should be different, as it is a tick carried by three hosts, and weekly baths are not required.

Currently, it is clear that the only efficient control method to decelerate growth of chemical resistant populations (Ortiz and Franco, 2005).

Economically, ectoparasite control is critical. Rodríguez (2005) states that weight loss in bovine infected by *Rhipicephalus*, accounts for 0,26 kg/tick/year, or losses of thousands of millions of USD to the world cattle raising business.

Additionally, Jonsson (2006) demonstrated that along with cutting down feed consumption, some changes were observed in hemoglobin, white corpuscles, cholesterol, albumin, globulin, amylase, alkaline phosphatase, and hepatotoxic compound secretion.

It is also critical to keep acaricides viable in order to decrease production costs and overdose, which may lead to resistance (FAO, 2003).

## CONCLUSIONS

Amitraz was effective in all the doses applied, followed by ivermectin, and finally cipermetrin; probably due to drug resistance.

## RECOMMENDATIONS

Efficacy evaluation is to be used widely, and new techniques for acaricide resistance diagnosis should be introduced.

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Received: 20-9-2012

Accepted: 21-10-2012

**Table 1. Probable mortality estimation in *Rhipicephalus* larvae, depending on the acaricide and concentration values.**

Acaricide	Concentration (%)	Probability (%)
Control	-	1.2
	0.05	7.1
	0.125	13.8
	0.250	23.9
	0.5	37.1
cipermetrin	0.05	55.4
	0.125	69.7
	0.250	81.5
	0.5	89.9
amitraz	0.05	30.1
	0.125	44.4
	0.250	59.5
	0.500	73.3