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SHORT COMMUNICATION

Prevalence of Resistant Strains of *Rhipicephalus microplus* to Acaricides in Cattle Ranch in the Tropical Region of Tecpan of Galeana, Guerrero, Mexico

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ABSTRACT

Tick and tick borne diseases cause many problems to the cattle industry worldwide. The prevalence of resistant strains of *Rhipicephalus microplus* to different acaricides on cattle farms in the tropical region of Tecpan of Galeana, Guerrero, Mexico, and risk factors related to prevalence of resistant strains of *R. microplus*. Sixty one ranches infested were sampled; in each ranch were collected 30-50 fully-engorged female *R. microplus* ticks, of 10 cattle randomly selected, and evaluated in their progeny resistance to acaricides, using the larval packet test. The prevalence of resistant strains was total pyrethroids and amitraz. In organophosphorus 31.1, 48.3 and 82.2% of strains were resistant to clorpyriphos, coumaphos and diazinon, respectively. Risk factors favored (P<0.05) the development of resistant strains of acaricides. We concluded that the resistance of *R. microplus* to acaricides used to control a problem, and risk factors (livestock management) have accelerated the development of resistance.

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INTRODUCTION

There are approximately 899 known species of ticks (Barker and Murrell, 2004). About 10% of these species are established in Mexico. Of the identified 77 species of ticks, five genera of the Family *Argasidae* and seven in the *Ixodidae* have great importance for cattle (Akhtar *et al*., 2011). In Mexico and other tropical and subtropical regions, the most economically important ticks in cattle are *R. microplus* and *R. annulatus*, which are vector of *Babesia bigemina* and *Babesia bovis* (Rosario-Cruz *et al*., 2009). In areas with incidence of *Rhipicephalus* spp. actions are taken to control through strategic and selective treatments, depending on the time of the year, the parasite load per animal (infestation) and the percentage of animals infested by the ectoparasites (Irshad *et al*., 2010). The use of chemical control has exerted pressure in recent years for the selection of strains of *R. microplus* resistant (Rosario-Cruz *et al*., 2009). The objective of the study was to determine the prevalence of resistant strains of *R. microplus* to pyrethroids, amidines and organophosphorus in cattle ranches, using the larval packet technique and risk factors associated with the development of resistance in the tropical region of Tecpan of Galeana, Guerrero, Mexico.

MATERIALS AND METHODS

The municipality of Tecpan of Galeana is located 120 meters above the sea level, southwest of Chilpancingo, between parallels 17° 07' and 17° 42' north latitude and 100° 28' and 101° 06' west on Greenwich Meridian. Bordered on the north Coyuca Catalan and Ajuchitlán Progress on the east by Atoyac of Alvarez and Benito Juarez, on the south by the Pacific Ocean and west Petatlán.

The samples were obtained from the total population of cattle ranch (n=60) affiliated with the local livestock association Tecpan of Galeana, Guerrero, Mexico. Collection of 3-5 full-engorged females of *R. microplus* were made from 10 randomly selected cattle (Cabrera-Jimenez *et al*., 2008). These were placed in small bottles drilled in the top to allow air circulation and provided a humid environment (cotton swab moistened with water). The samples were transported to the Parasitology Laboratory of the National Center for Animal Health Verification (CENAPA) in Jiutepec, Mor. Mexico; where acaricides resistance work (samples in triplicate) by the larval packet test (LPT) developed by Stone and Haydock (1962) was performed. Engorged ticks were placed in petri dishes and incubated in obscurity at a temperature of

27±2°C and relative humidity (RH) of 80-90% to allow for oviposition. The eggs were placed in glass vials plugged with cotton under the same conditions of temperature and humidity to which the full ticks were to allow the eclosion of larvae.

The diagnosis of resistance was established by discriminating dose reference susceptible strains, which were to pyrethroids: cypermethrin (0.05%), deltamethrin (0.09%), flumethrin (0.01%); amidines: amitraz (0.0002%) (Stone and Haydock, 1962) and organophos-phorus: clorpyriphos (0.02%), coumaphos (0.02%), diazinon (0.08%) (Shaw, 1966). Readings were taken at 24 h after treatment and was seen as discriminating dose twice the concentration of acaricide that kills 99.9% of the population of susceptible ticks (Cabrera-Jimenez *et al*., 2008; Villarroel-Alvarez *et al*., 2006). Mortality percentage of larvae was calculated as:

Larval mortality (%) = Dead larvae/total larvae (100)

To relate the risk factors for ticks resistant, a survey of livestock farmer's included data on the frequency of dips for tick control, frequency of rotation chemical products, type and/or manner and/or dosage of the product, which produces cattle breeds and types of grasslands that are at his ranch. The prevalence was calculated using the following formula (Villarroel-Alvarez *et al*., 2006):

Prevalence % = No. of ranches with acaricides resistant ticks/No. total ranches sampled (100)

The significant influence of risk factors (independent variables) such as the rotation frequency of chemical formula used for tick control, product application form, the breed as a genetic factor and type of existing pastures on ranch about the prevalence of resistant ticks was analyzed by Chi square 2x2 contingency tables and the genetic factor of 3x2, with an alpha of P<0.05 (SAS, 2002).

RESULTS AND DISCUSSION

Total resistance was observed in ticks *R. microplus*, to amidines (amitraz, with larval mortality between 13.4-82.7%) and pyrethroids (flumethrin, deltamethrin and cypermethrin with larval mortality between 0-89.6, 7.8-91.8 and 11.5-91.3%, respectively). In organophosphorus, resistance was observed with 31.1, 48.3 and 82.2% to clorpyriphos, coumaphos and diazinon, with larval mortality between 36.7-100, 25.3-100 and 18.6-100%, respectively (Table 1). There are many records about the ability of parasites to develop resistance to chemicals products used for its control in ruminants (Alonso-Diaz *et al*., 2006). Rodriguez-Vivas *et al*. (2005) and Rosario-Cruz *et al*. (2009) reported resistant strains *R. microplus* to acaricides in Mexico.

Table 2 showed that on ranch that took between 3 and 4 years in rotating the acaricide used to control *R. microplus*, favored the highest prevalence (P=0.008) of resistant strains (49.1%), the practice of not rotate more frequently the product is due to the failure of technical assistance to livestock farmers, this favored the flourishing of resistant ticks (Rodriguez-Vivas *et al*., 2005). Alonso-Diaz *et al*. (2006) reported that the product rotation delays the development of resistance.

Livestock farmers who rotated the product between 3 and 4 years showed that the change of the product is due to the inefficiency observed for tick control. Villarroel-Alvarez *et al*. (2006) and George *et al*. (2004) reported that when the product fails to change, or can increase the concentration and frequency of the baths, practices that favoring the development of resistance in ticks.

On ranches acaricide products were tested by two ways of applying, i.e., the spray (52.5%) and pour-on (47.5%), of these, spray application favored (P=0.007) higher prevalence of strains of *R. microplus* resistant (Table 2). This could be due to that livestock farmers in preparing the solution presumptively dosed and used an average of 2 liters of solution prepared by spraying adult animal, an amount which according to George *et al*. (2004) does not guarantee deep moisturizing body surface of the animal as 4-8 liters are required solution per adult animal.

It has been observed that genetic composition of herds significantly (P<0.038) influenced the prevalence of resistant strains of *R. microplus*; as these were high in Holstein cattle ranches (37.7%) followed in Swiss cattle ranches and Swiss-Zebu hybrid ranches (Table 2). This indicates that European breeds are more susceptible to infestations by ectoparasites, which increases frequency of application of chemicals to control and therefore the selection pressure tick strains resistant to the formula (Rodriguez-Vivas *et al*., 2005; Alonso-Diaz *et al*., 2006).

We conclude that the resistance of *R. microplus* to acaricides is a problem that placed the chemical control method at a disadvantage to the evolution of ectoparasite; also risk factors related to the management & application of chemicals product and breeds that produced, has favored the development of resistance in ticks.

Table 1: Larval mortality and prevalence of strains of *R. microplus* resistance to acaricides used as chemical control in cattle ranches Tecpan of Galeana, Gro. Mexico.

Acaricide group	Active substance	Larval mortality ranges (%)	Ranches Positives No.		Negatives* No.	
			No.	%	No.	%
Organophosphorus	Clorpyriphos	36.7-100	19	31.1	42	68.8
	Coumaphos	25.3-100	29	48.3	31	55.6
	Diazinon	18.6-100	51	82.2	11	17.7
Pyrethroids	Flumethrin	0-89.6	61	100	0	0
	Deltamethrin	7.8-91.8	61	100	0	0
	Cypermethrin	11.5-91.3	61	100	0	0
Amidines	Amitraz	13.4-82.7	61	100	0	0

* Samples of ranches with 100% larval mortality, larval packet tests.

Table 2: Risk factors and *R. microplus* resistance to acaricides in cattle ranches in the municipality of Tecpan of Galeana, Gro. Mexico

Risk factors	Positive ranches		
Product rotation time*	No.	%	P value
1 to 2 years	25	40.9	0.008
3 to 4 years	30	49.1	
Application form			
Sprayed	29	47.5	0.007
Pour-on	18	29.5	
Breed			
Swiss-Zebu	12	19.6	0.038
Swiss	17	27.8	
Holstein	23	37.7	
Grassland type			
Temporal	14	22.9	0.741
Temporal and irrigation	35	57.3	

*Consecutive years of using the same product for tick control

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