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Short communication

Development of amitraz resistance in field populations of *Boophilus microplus* (Acari: Ixodidae) undergoing typical amitraz exposure in the Mexican tropics

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Abstract

The purpose of this study was to determine the effect of amitraz selection pressure on the development of resistance in field populations of *Boophilus microplus* in the Mexican tropics. Three farms (FA_1, FA_2 and FA_3) in Yucatan, Mexico, were selected in this study. Amitraz was applied as a whole body sprays to all cattle on each farm for tick control once monthly for 15 months. From each farm, 20–30 *B. microplus* engorged females were collected every 3 months. The modified larval immersion test was used to test susceptibility of *B. microplus* to amitraz. Larvae were exposed to serial dilutions of amitraz. Probit analysis was used to determine lethal dose at 50% mortality and associated 95% confidence limits. The resistance factors found in the three farms during the 15 months of amitraz selection pressure were FA_1 (1, 2, 4, 4 and 13), FA_2 (1, 6, 23, 21 and 22) and FA_3 (2, 13, 2, 6 and 6). It is concluded that amitraz selection pressure on field populations of *B. microplus* increased the resistance level in all populations studied in the Mexican tropics.

Keywords: Resistance; Acaricides; Amidine; Amitraz; Resistance ratio; *Boophilus microplus*; Mexican tropics

1. Introduction

Boophilus microplus (Canestrini) is an endemic cattle pest in tropical and subtropical regions of the world, causing major economic losses to cattle producers directly through feeding on parasitized cattle and indirectly by transmitting several disease-causing pathogens to the host (*Babesia bovis, Babesia bigemina* and *Anaplasma marginale*) (Solorio et al., 1999; Rodriguez-Vivas et al., 2004).

Acaricides have played a pivotal role in the control of *B. microplus*. However, as a consequence of extensive use of chemicals this tick specie has developed resistance to most of the major classes of acaricides in several countries. In Mexico, the first case of organophosphate (OP) resistance was detected in *B. microplus* ticks from a ranch near Tuxpan in the state of Veracruz in 1983 (Aguirre and Santamaría, 1986). Soon

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after, resistance to OPs became widespread in central and eastern Mexico. In 1986, synthetic pyrethroid (SP) acaricides were introduced into Mexico in order to alleviate OP resistance problems. Resistance to SPs was first detected in 1993 and soon became extensive (Fragoso et al., 1995). As a result resistance to both acaricide classes has developed in this cattle pest in at least 15 states of Mexico (Santamaría et al., 1999).

The formamidine, amitraz was introduced to control OP-resistant ticks at the same time the SPs were introduced in 1986, but its use was initially limited due to higher cost. The use of amitraz became more frequent after 1993 when SP-resistance started to reduce tick control efforts in Mexico. The first case of amitraz resistance was confirmed in 2001 from a farm near to Emiliano Zapata, Tabasco and is now widespread in the Mexican tropics (Soberanes et al., 2002). In southern Mexico, amitraz is the principal acaricide used to control ticks on cattle (Rodriguez-Vivas et al., 2006a).

The development of acaricide resistance in a tick population is dependent on the frequency of resistant individuals in the population and the intensity of chemical selection pressure (Kunz and Kemp, 1994). Although rapid onset and development of *B. microplus* resistance to SP, OP (Davey and George, 1998, Davey et al., 2003) and amitraz (Li et al., 2004) have been observed *in vitro* and *in vivo* (controlled laboratory trials); the frequency of acaricide resistance development on field populations under typical operational conditions has not been reported to our knowledge. The purpose of the present study was to determine the effect of amitraz selection pressure on the development of amitraz resistance in field populations of *B. microplus* in the Mexican tropics under typical tick control regimes and cattle production conditions.

2. Materials and methods

2.1. Study background

The study was conducted in Yucatan, Mexico, located between 19°30′ and 21°35′ North latitude and 90°24′ West longitude of the Greenwich meridian. Climate is generally sub-humid tropical with two seasons: rainy (June–October) and dry (November–May). The monthly maximum temperature varies from 35 °C to 40 °C (mean 26.6 °C). The relative humidity (RH) varies from 65% to 100% (mean 80%) and the annual rainfall varies from 415 mm to 1290 mm depending on the area (INEGI, 1996).

2.2. Production system and use of acaricides in Yucatan

The predominant livestock-production system is semi-intensive (beef farms), based mainly on year-round grazing on improved pastures, e.g., Guinea grass (*Panicum maximum*) and Star grass (*Cynodon plectostachyus*), with supplementary feeding during the dry season. The use of acaricides to control ticks is a common practice in Yucatan, Mexico (Solorio et al., 1999). Forty-one percent of the farms in the Yucatan state use amitraz to control ticks (Rodriguez-Vivas et al., 2006b), with 42% of those farms applying acaricides >12 times/year.

2.3. Study population

Based on the results of a survey of acaricide susceptibility in Yucatan, Mexico, by Rodriguez-Vivas et al. (2006a), three cattle farms were selected for this study (FA_1, FA_2 and FA_3). Each farm had a population of 80–200 *Bos indicus* and *Bos taurus* cross-bred cattle (approximately 60% cows, 4% bulls, 20% calves and 16% yearly stockers) utilizing a semi-intensive livestock-production system with stocking density of 0.60–0.62 animal unit/ha.

2.4. Acaricide management and sampling

All animals on each farm were treated with amitraz (Taktic® E.C. 12.5% [A.I.], Intervet, Mexico), as a whole body spray at the recommended dose using at least 4 l of total finished spray volume per animal. Treatments at the three farms were carried out monthly for a period of 15 months. During this period, five *B. microplus* generations were expected to develop on farms (Rodriguez-Vivas and Dominguez, 1998).

From each farm, 20–30 *B. microplus* engorged females were collected from at least 10 bovines at months 0, 6, 9, 12 and 15 of the experiment.

2.5. Dose–response bioassays

Engorged adult females were placed into small plastic boxes with air holes and transported to the parasitology laboratory at the College of Veterinary Sciences (FMVZ-UADY). Upon arrival, engorged adult females were placed on Petri dishes and incubated at laboratory conditions, at 27 ± 1.5 °C and a RH of 85–86% (Cen et al., 1998). After oviposition, eggs were transferred into 10-ml glass vials with a cotton cap. Eclosion of larvae occurred approximately 30 days after detachment and collection from engorged females. Live larvae of 7–14 days of age were used for bioassays.

The modified larval immersion test was used to test the susceptibility of *B. microplus* larvae to amitraz (Soberanes et al., 2002). Briefly, a commercial formulation of amitraz (Taktic® E.C., 12.5% [A.I.], Intervet, Mexico) was diluted in water (ranging from 0.0000125–0.0008%). Solutions, 10 ml each, were prepared in Petri dishes (15 mm in diameter), and then approximately 300–500 larvae were placed between two Whatman No. 1 papers and immersed to each one solution for 10 min. Three replicates of the acaricide dilution and a control (water) were used. Approximately 100 larvae from the treated and control solutions were transferred to clean filter paper packets, and kept for 72 h in an incubator (27 ± 1.5 °C, 80–90% relative humidity). After that the numbers of live and dead larvae were counted to calculate the percentage of larval mortality (Soberanes et al., 2002).

2.6. Data analysis

Probit analysis was performed on dose–response bioassay results from bioassays using Polo-PC (LeOra Software, 1987). This analysis provided an estimation of the 50% lethal concentration (LC_{50}) and associated 95% confidence limits (95% CL). Resistance ratios (RR) were calculated by dividing the LC_{50} value for the field population by the LC_{50} value for the reference susceptible strain (CENAPA strain). Differences between LC_{50} estimates of reference strain and field populations were designated as significant if their 95% confidence limits did not overlap. This procedure provided an estimate of the relative level of resistance in each population at different times of selection pressure. The CENAPA strain is a susceptible laboratory strain that was established at the National Center of Parasitology Laboratory, Jiutepec, Morelos, Mexico and it is used by the FAO as a tick reference susceptible strain for Latinoamerica.

3. Results

Resistance factors to amitraz as a result of selection pressure on the three field populations of *B. microplus* are shown in Table 1. At the beginning of the study, the 95% confidence limits of the LC_{50} estimates of the three studied populations overlapped with the 95% confidence limits of the susceptible reference strain. For that reason, at the beginning of the experiment, the three studied populations were considered as susceptible populations to amitraz.

Table 1
Resistance ratios to amitraz as a result of selection pressure on a field population of *Boophilus microplus* in the Mexican tropics

Identification	Slope (S.E.)	LC_{50} (×100)[a]	LC_{50} (95% CL)[b] (×100)	Resistance ratio
[c]CENAPA strain	1.82 (0.134)	0.001	0.0001–0.001	
Farm 1 (FA_2)				
07/03/2003	1.70 (0.189)	0.001	0.0001–0.002[d]	1.0
12/17/2003	1.56 (0.926)	0.002	0.001–0.003	2.0
04/12/2004	1.86 (0.090)	0.004	0.003–0.005	4.0[e]
08/22/2004	1.85 ((0.077)	0.004	0.003–0.006	4.0[e]
11/03/2004	1.41 (0.078)	0.013	0.002–0.027	13.0[e]
Farm 2 (FA_2)				
07/07/2003	0.93 (0.598)	0.001	0.000–0.002[d]	1.0
12/16/2003	1.05 (0.055)	0.006	0.004–0.009	6.0[e]
04/03/2004	1.23 (0.063)	0.023	0.016–0.035	23.0[e]
08/07/2004	0.77 (0.039)	0.021	0.012–0.042	21.0[e]
11/10/2004	1.26 (0.046)	0.022	0.014–0.036	22.0[e]
Farm 3 (FA_3)				
07/05/2003	2.48 (0.137)	0.002	0.001–0.003[d]	2.0
12/08/2003	1.10 (0.658)	0.013	0.010–0.024	13.0[e]
04/16/2004	1.14 (0.076)	0.002	0.001–0.003	2.0
08/06/2004	1.63 (0.069)	0.006	0.004–0.008	6.0[e]
11/06/2004	1.68 (0.61)	0.006	0.004–0.008	6.0[e]

[a] LC: lethal concentration.
[b] CL: confidence limits.
[c] *B. microplus* susceptible strain from CENAPA, Mexico.
[d] At the beginning of the study the 95% CL of the LC_{50} estimates overlapped with the 95% CL of the LC_{50} of the susceptible reference strain.
[e] Differences between LC_{50} estimates were designated as significant if their 95% confidence limits did not overlap.

After 15 amitraz treatments to the field *B. microplus* populations the FA_1, FA_2 and FA_3 reached a RR of 13.0, 22.0 and 6.0, respectively (Table 1) and the three populations were considered as resistant (the 95% confidence limits of the LC_{50} estimates of the three studied populations did not overlap with the 95% confidence limits of the susceptible reference strain).

The FA_2 and FA_3 populations responded quickly to amitraz selection pressure; however, the FA_1 required nine amitraz treatments to show some resistance level (Table 1).

4. Discussion

The selection with amitraz caused resistance in the field after 15 treatments. At the beginning of the study the 95% confidence limits of the LC_{50} estimates of the three studied populations (FA_1, FA_2 and FA_3) overlapped with the 95% confidence limits of the susceptible reference strain (CENAPA), populations were susceptible to amitraz. However, after 15 amitraz treatments the three populations were found to be resistance. Rodriguez-Vivas and Dominguez (1998) found in the Yucatan State, Mexico, that four *B. microplus* generations are produced in a year. For that reason, in the studied period approximately five tick generations were produced and the populations reached RRs of 13.0 and 22.0, respectively (Table 1).

The RR of the FA_1 and FA_2 field populations increased continuously in contrast with the FA_3 population, which in the third evaluation decreased their RR to 2.0 and subsequently increased upward to reach a RR of 6.0 (Table 1). The cause of this fluctuation is unknown, but it might be due to a refugia of untreated tick that allowed the immigration of susceptible individuals to dilute resistance allele (Georghiou and Taylor, 1977; Kunz and Kemp, 1994).

The increase of resistance found in the present study in the three field populations was in agreement with Li et al. (2004), who increased the RR of a *B. microplus* strain (Santa Luizia) using various concentrations of amitraz on larvae. The Santa Luizia strain responded quickly to selection, and the resistance factor was elevated from 13.3 in generation 1 to 154 in generation 6; although resistance decreased sharply without selection in the following generations (68.72) and at low dose pressure of amitraz (generation 9 = 50.7, generation 12 = 49.43). Because Li et al. (2004) used different concentration of amitraz and exposed larvae *in vitro*, our results may not be directly comparable with theirs.

In laboratory studies an untreated control group is required to compare its RR pattern with treated groups; however, in the Mexican field conditions it would be impropriated to leave an untreated group due to the high tick infestation level and risk of tick-borne disease transmissions (Solorio et al., 1999; Rodriguez-Vivas et al., 2004).

Although laboratory and controlled field studies are the best choice for measuring the degree of natural resistance of a tick population, they should always be confirmed by trials to ascertain the operational field efficacy of acaricide applications (Amaral, 1993). However, in field studies, environmental and biological factors play an important role in tick acaricide resistance. These factors include generation time, offspring per generation, mobility, migration, host range, fortuitous survival and refugia. A higher proportion of the population in refugia on the field slows the selection for resistance (Georghiou and Taylor, 1977).

The mode of action of amitraz, as well as the mechanism of *B. microplus* resistance to amitraz is not understood. Synergist bioassays on several amitraz-resistant strains from Mexico and one Brazilian strain of *B. microplus* indicated some involvement of esterase and glutathion-*S*-transferase (Li et al., 2004). However, at the present time, the major mechanism of resistance to amitraz is speculated to be insensitive target site, presumably the octopamine receptor (Li et al., 2004).

The rapid development of amitraz resistance in field populations of *B. microplus* in the Mexican tropics is alarming; particularly given the fact that *B. microplus* has the potential to develop high levels of resistance when selected *in vitro* and under field conditions (Soberanes et al., 2002; Li et al., 2004). However, to identify the wide-spread impact of amitraz selection pressure on field populations of *B. microplus*, and the economic impact on the Mexico's cattle industry, additional field studies are warranted.

It is concluded that typical amitraz selection pressure on field populations of *B. microplus* increased the resistance level in all populations studied in the Mexican tropics.

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References

Aguirre, E.J., Santamaría, V.M., 1986. Purificación y caracterización toxicológica de garrapatas *Boophilus microplus* resistentes a ixodicidas organofosforados y organoclorados. VII. Reunión Anual de la Asociación Mexicana de Parasitología Veterinaria A.C. Ciudad Victoria, Tamaulipas, México.

Amaral, N.K., 1993. Guidelines for the evaluation of ixodicides against the cattle tick *Boophilus microplus* (Canestrini 1887) (Acari: Ixodidae). Brazil J. Vet. Parasitol. 2, 144–151.

Cen, A.J., Rodriguez-Vivas, R.I., Domínguez, A.J.L., Wagner, G., 1998. Studies on the effect on infection by *Babesia* sp on oviposition of *Boophilus microplus* engorged females naturally infected in the Mexican tropics. Vet. Parasitol. 78, 253–257.

Davey, R.B., George, J.E., 1998. In vitro and in vivo evaluation of a strain of *Boophilus microplus* (Acari: Ixodidae) selected for resistance to permethrin. J. Med. Entomol. 35, 1013–1019.

Davey, R.B., George, J.E., Miller, R.J., 2003. Efficacy of various concentrations of coumaphos to control adult, nymphal, and larval stages of an organophosphate-resistant strain of *Boophilus microplus* on infested cattle. Am. J. Vet. Res. 64 (6), 684–689.

Fragoso, H., Soberanes, N., Ortiz, M., Santamaría, M., Ortiz, A., 1995. Epidemiología de la resistencia a ixodicidas piretroides en garrapatas *Boophilus microplus* en la Republica Mexicana. In: Rodríguez, S., Fragoso, H. (Eds.), Seminario Internacional de Parasitología Animal-Resistencia y Control en Garrapatas y Moscas de Importancia Veterinaria. Acapulco, Guerrero, México, pp. 45–57.

Georghiou, G.P., Taylor, C.E., 1977. Genetic and biological influences in the evolution of insecticide resistance. J. Econ. Entomol. 70, 253–264.

Instituto Nacional de Estadística, Geografía e Informática, INEGI. 1996. Climatología del estado de Yucatán. Anuario estadístico del Estado de Yucatán. INEGI. Gobierno del estado de Yucatán. pp. 3–21.

Kunz, S.E., Kemp, D.H., 1994. Insecticides and acaricides: resistance and environmental impacts. Rev. Sci. Tech. Off. Int. Epiz. 13, 1249–1286.

LeOra Software, 1987. A user's guide to probit or logit analysis. LeOra Software, Berkerley, CA.

Li, A.Y., Davey, R.B., Miller, R.J., George, J.E., 2004. Detection and characterization of Amitraz resistance in the southern cattle tick Boophilus microplus (Acari: Ixodidae). J. Med. Entomol. 41 (2), 193–200.

Rodriguez-Vivas, R.I., Dominguez, A.J.L., 1998. Grupos entomológicos de importancia veterinaria en Yucatán, México. Rev. Bioméd. 9, 26–37.

Rodriguez-Vivas, R.I., Mata, M.Y., Pérez, G.E., Wagner, W., 2004. The effect of management factors on the seroprevalence of *Anaplasma marginale* in *Bos indicus* cattle in the Mexican tropics. Trop. Animal Health Prod. 36, 135–143.

Rodriguez-Vivas, R.I., Rodriguez-Arevalo, F., Alonso-Díaz, M.A., Fragoso-Sanchez, H., Santamaria, V.M., Rosario-Cruz, R., 2006a. Amitraz resistance in *Boophilus microplus* ticks in cattle farms from the state of Yucatan Mexico: prevalence and potential risk factors. Prev. Vet. Med. 75, 280–286.

Rodriguez-Vivas, R.I., Alonso-Díaz, M.A., Rodriguez-Arevalo, F., Fragoso-Sanchez, H., Santamaria, V.M., Rosario-Cruz, R., 2006b. Prevalence and potential risk factors for organophosphate and pyrethroid resistance in *Boophilus microplus* ticks on cattle ranches from the State of Yucatan, Mexico. Vet. Parasitol. 136, 242–335.

Santamaría, V.M., Soberanes, C.N., Ortiz, N.A., Fragoso, S.H., Osorio, M.J., Martínez, I.F., Franco, B.L., Delabra, V.G., Quezada, D.R., Giles, H.I., Ortiz, E.M., 1999. Análisis de la situación actual mediante el monitoreo de susceptibilidad a ixodicidas en *Boophilus microplus* de 1993 a 1999 y medidas preventivas para retardar la resistencia al amitraz en México. In: In IV Seminario Internacional de Parasitología Animal: Control de la Resistencia en Garrapatas y Moscas de Importancia Veterinaria y Enfermedades que transmiten. de Octubre 20–22, 1999. Puerto Vallarta, Jalisco, México, pp. 103–117.

Soberanes, C.N., Santamaría, V.M., Fragoso, S.H., García, V.Z., 2002. Primer caso de resistencia al amitraz en la garrapata del ganado *Boophilus microplus* en México. Téc. Pec. Méx. 40, 81–92.

Solorio, R.J., Rodriguez-Vivas, V.R.I., Pérez, G.E., Wagner, G., 1999. Management factors associated with *Babesia bovis* seroprevalence in cattle from eastern Yucatan. Mexico. Prev. Vet. Med. 40, 261–269.