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Short communication

Prevalence and potential risk factors for amitraz resistance in *Boophilus microplus* ticks in cattle farms in the State of Yucatan, Mexico

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Abstract

Southern cattle tick, *Boophilus microplus*, control in the Mexican tropics is based on the application by spray, dip or pour-on of acaricides. In Mexico the first report of ticks resistant to amitraz was reported in 2002. Amitraz resistance affecting the cattle industry of Yucatan State, Mexico, has not been investigated. A cross-sectional study was carried out in order to investigate the possible association between management risk factors and the prevalence of *B. microplus* ticks resistant to amitraz in Yucatan, Mexico. Ninety-eight cattle farms were randomly selected and a sample of 20–30 engorged *B. microplus* females was collected from at least 10 cattle from each farm. Collected ticks were taken to the laboratory where the larval progeny were obtained. The modified larval immersion test was used to detect ticks resistant to amitraz. A discriminating dose of 0.0002% amitraz was used. A questionnaire was applied on each collaborating farm. For the statistical analysis

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a 2 × 2 contingency table was used to identify potential risk factors. The prevalence of farms with *B. microplus* resistant to amitraz was 19.40%. However, even though tick resistance to amitraz is a present problem there was no evidence that it was associated with the risk factors analyzed in this survey.

Keywords: Resistance; Acaricides; Amitraz; Prevalence; *Boophilus microplus*; Cattle; Mexico

1. Introduction

The southern cattle tick, *Boophilus microplus,* is a one host parasite and is one of the most widely distributed species of Ixodidae, and economically, the most important. The tick is responsible for severe economic losses caused by blood loss, injection of toxins and tick borne diseases (Solorio et al., 1999). Chemical acaricides have played an important role in the control of this economically damaging pest. However, as a consequence of extensive use of acaricides, *B. microplus* developed resistance to major classes of acaricides in several countries (Kunz and Kemp, 1994). Ticks resistance is define as the development of an ability in a strain of some ticks to tolerate doses if a toxicant that would prove lethal to a majority of individuals in a normal population of the same specie (Kunz and Kemp, 1994).

In Mexico, resistance to organophosphate (OP) acaricides first developed in the 1980s, and resistance to pyrethroids (P) subsequently developed in the 1990s (Santamaría et al., 1999). Amitraz, along with P was introduced to control OP-resistant ticks in Mexico in 1996 (Soberanes et al., 2002). Amitraz is a formamidine acaricide that has been used effectively in the control of ticks on cattle; however, resistance to amitraz in *B. microplus* has been reported in many countries such as Australia (Kunz and Kemp, 1994), Colombia (Benavides et al., 2000), South Africa (Strydom and Peter, 1999), and Brazil (Furlong, 1999). The first case of amitraz resistance in *B. microplus* from Mexico was confirmed in a strain collected in 2001 from a ranch in the state of Tabasco (Soberanes et al., 2002). However, amitraz resistance in *B. microplus* on cattle in the State of Yucatan, Mexico, has not been investigated. For that reason, a cross-sectional study was carried out in order to investigate the possible association between management risk factors and prevalence of *B. microplus* resistant to amitraz in Yucatan, Mexico.

2. Materials and methods

2.1. Study area

The present survey was carried out in the State of Yucatan, located in the Southeast of Mexico. The climate of the State is sub-humid tropical with a summer rainy season. The monthly maximum temperature varies from 35 to 40 °C and the mean temperature is 26.6 °C. The relative humidity (RH) varies from 65 to 95% (mean 80%) and the annual rainfall is 950 mm (INEGI, 1994). According to the soil type, vegetation and

agricultural development, the State of Yucatan is classified into three zones: the sisal zone (centre), the agricultural (southern) and the livestock zone (eastern) (INEGI, 1994).

The predominant livestock-production system in the State is semi-intensive (beef farms), mainly based on a 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 acaricide control of ticks is a common practice in most of the farms (INEGI, 1994).

2.2. Study population

A cross-sectional study was carried out. A simple random sampling model (Dean et al., 1994) was used to determine the number of farms to be sampled. The sample size (98) was calculated, using an expected prevalence of 50% (the tick resistance to amitraz in the State of Yucatan was unknown), farm census of 4629 individual entities, a confidence level of 95% and an error of 10%. From a list provided by the Yucatan Cattleman's Association 98 farms were randomly selected. Each farm was visited 1–2 times to collect engorged females *B. microplus*, which led to the exclusion of nine farms that were not possible to collect engorged females. Nine other randomly selected farms replaced them. A questionnaire on general management of farms was administered to all farmers or managers. In each farm, a sample of 20–30 engorged female *B. microplus* was collected from at least 10 heads of cattle and transported to the Parasitology Laboratory of the College of Veterinary Sciences (UADY).

2.3. Laboratory analysis

Up on arrival, engorged females were placed into Petri dishes and incubated in darkness at $27 \pm 1.5\ ^{\circ}C$ and 85–86% relative humidity (Cen et al., 1998). The eggs laid were transferred into two 10 ml glass tubes with a cotton cap, to allow larvae eclosion. Larvae of 7–14 days of age were used (Kemp et al., 1998).

The modified larval immersion test was used to test the susceptibility of *B. microplus* larvae to amitraz (FAO, 1984). A discriminating dose of amitraz 0.0002% (Taktic® 12.5%, Intervet, Mexico) in an emulsifiable concentrate formulation was used (Soberanes et al., 2002). The discriminating dose was calculated by doubling the mean lethal dose 99.9% derived from the series of tests conducted with a susceptible strain (Kemp et al., 1998). Two replicates of the acaricide and a control were used. The treated larvae were exposed 72 h to the acaricide and the numbers of live and dead larvae were counted to calculate the percentage of larval mortality. If one or more larvae were found alive, the strain was considered as resistance.

2.4. Statistical analysis

Prevalence of cattle farms with *B. microplus* ticks resistant to amitraz was calculated in the studied population. The exposure variables considered to be possible risk factors for amitraz resistance are showed in Table 1.

Table 1
Exposure variables considered to be possible risk factors of *Boophilus microplus* resistance to amitraz in the State of Yucatan, Mexico

Variable	Description	Term
Stocking density	Number of cattle on ranch per hectare	$\leq$1 animal/ha, >1 animal/ha
Recommended dose	Recommended dose of acaricide applied	Yes, no
Dipping interval	Number of acaricide treatments during the last 2 years	$\leq$12 times/year, >12 times/year
Type of dipping	Type of acaricide applications during the last 2 years	Immersion, aspersion
Acaricide rotation	Use of acaricide rotation	Yes, no
Amitraz use	Use of amitraz treatments during the last 2 years	Yes, no
Another control program	Use of another tick control program	Yes, no
Use of fly control	Use of acaricide to control fly	Yes, no
Adjacent to another farm	Farm adjacent to another farm with a tick control program	Yes, no

Univariate analysis (x^2) to identify risk factors was performed using 2×2 contingency tables. P-value <0.05 were considered statistically significant.

3. Results

3.1. Questionnaire survey

Ninety-eight cattle farms in the Yucatan State were studied. The questionnaire survey in all farms described the tick resistance management practices that farmers use with their animals in Yucatan, Mexico. Almost 30% (29/98) of the farms had $\leq$50 animals, 27.6% (27/98) had 51–100 animals, 21.4% (21/98) had 101–200 animals and 21.4% (21/98) had >200 animals. In relation with stocking density, 73.5% (72/98) of the farms had $\leq$1 animal/ha and 26.5% (26/98) had >1 animal/ha. A large proportion of farms (79.6%, 78/98) used crossbreed with *Bos indicus*.

All farms used acaricides to control ticks. Only 32.7% (32/98) of the farms used technical supervision (veterinarian or biologist) for acaricide use. The majority of the farms (72.4%, 71/98) used the recommended dose by the manufacturer. In relation with Interval of acaricide application, 41.8% (41/98) of the farms applied acaricides >12 times/year and 58.2% (57/98) applied $\leq$12 times/year. The farms (74.5% (73/98)) used aspersion (hand spraying), 18.4% (18/98) immersion and 7.1% (7/98) pour on.

Acaricide rotation in the last 2 years was performed in the majority of the farms (70.4%, 69/98). The reasons of acaricide rotation were: to prevent tick resistance (21.4%, 21/98), marketing opportunities (24.5%, 24/98) and low efficacy of acaricides (54.1%, 53/98). Only 21.4% (21/98) of the farms used another control measure to control ticks; from this, 76.5% (75/98) of the farms used macrocyclic lactones. The acaricides used to control ticks in the farms were: amidines 40.8% (40/98), OP 29.6% (29/98), SP 21.4% (21/98), Organochlorines OC 4.1% (4/98), mixture of OP and SP 3.1% (3/98), and phenylpyrazoles 1.0 (1/98)%.

Table 2
Cattle farm distribution with *Boophilus microplus* resistance to amitraz in the State of Yucatan, Mexico

Zone	Number of municipalities	Number of tested farms	Farms with *B. microplus* resistant to amitraz	Frequency of farms with *B. microplus* resistant to amitraz (%)
Centre	11	22	5	22.2
Eastern	10	65	13	20.0
Southern	8	11	1	9.0
Total	29	98	19	19.4

3.2. Prevalence and risk factors

Farms in three zones and 29 municipalities were sampled and the cattle farm distribution with *B. microplus* resistant to amitraz in the State of Yucatan, Mexico is shown in Table 2.

Prevalence of farms with *B. microplus* resistant to amitraz was 19/98 (19.4%, confidence interval 12.8–26.0%).

The mortality of the *B. microplus* resistance strains tested by the modified larval immersion test is showed in Table 3.

None of the nine risk factors analyzed had $p < 0.05$.

4. Discussion

The present study is the first in Mexico to detect prevalence and potential risk factors associated with amitraz resistance in *B. microplus* using a simple random sampling technique. In Mexico, the presence of resistance to acaricides has had a period of approximately 8 years for the different families of acaricides. At the beginning of 1993, amitraz started to be used intensively in the country due to the presence and dissemination of *B. microplus* resistant to pyrethroids. Amitraz was then used as an alternative for tick control (Soberanes et al., 2002). In Mexico, from 1994 to 2000 the use of amitraz in the cattle industry has increased around 1000% (Fragoso and Soberanes, 2001). The prevalence found in the present study (19.4%) is still low; this finding concurs with Jonsson et al. (2000) who reported a low prevalence (around 10%) in dairy farms in spite of the heavy use of amitraz in

Table 3
Larval mortality of 98 *Boophilus microplus* tick strains tested by the modified larval immersion test (discriminating dose of amitraz 0.0002%), in cattle farms from the State of Yucatan, Mexico

No. of farms	Mortality[a]	Frequency (%)
79	100% (susceptible strains)	80.61
9	96–99% (resistance strains)	9.18
8	70–95% (resistance strains)	8.16
2	<70% (resistance strains)	2.04

[a] If one or more larvae were found alive in the modified larval immersion test, the strain was considered as resistance.

Queensland, Australia. The low prevalence might be explained by a semidominant mode of inheritance or lower fitness in ticks resistant to amitraz (Kemp et al., 2003). The mode of action and the mechanism of resistance to amitraz is not well known (Kemp et al., 1999). Synergist bioassays on several amitraz-resistant strains from Mexico and one Brazilian strain of *B. microplus* indicated some involvement of esterase and glutathion-*S*-transferese (Li et al., 2004). However, the major mechanism of resistance to amitraz is speculated to be insensitive target site, presumably the octopamine receptor (Li, 2004).

The level of amitraz resistance detected in the larval immersion test in the 19 *B. microplus* resistance strains (Table 3) may suggest that the frequency of gene(s) that confer amitraz resistance was low and may exist as heterozygotes (Li et al., 2004) in most field populations in Yucatan, Mexico. Further studies to know the inheritance of amitraz resistance of *B. microplus* strains in the State of Yucatan, Mexico are needed.

Factors that are associated with increased probability of *B. microplus* resistance to amitraz have been identified in Australia (Jonsson et al., 2000). These factors that were associated with the prevalence of resistance included region, frequency and method of amitraz application. At present, no management risk factors for amitraz resistance in *B. microplus* were identified in Yucatan, Mexico; this might be due to the reduced number of cases of *B. microplus* resistant to amitraz in farms from Yucatan, Mexico and/or the semidominant mode of inheritance of amitraz resistance. However, the intensive use of amitraz (40.8% of the farms), high interval of acaricide application (41.8% of the farms applied acaricides >12 times/year) and method of application (74.5% of the farms used hand spraying) in farms from the State of Yucatan, Mexico might increase the prevalence and these and other risk factors might influence the appearance of amitraz resistance and could be identified within the next fewer years. The possibilities of using mixtures of acaricides with biocontrol agents, such as co-formulation of tick vaccines and injectable acaricides or *Metarhizium* fungal spores with acaricides to prevent the spread of *B. microplus* resistant to amitraz on cattle farms in Southeast Mexico need to be studied. It is concluded that *B. microplus* resistance to amitraz in cattle farms from the State of Yucatan, Mexico; even though no risk factors were associated with the phenomenon, it is a present problem.

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