Short communication

First report of the cattle tick *Rhipicephalus microplus* resistant to ivermectin in Mexico

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1. Introduction

The southern tick *Rhipicephalus microplus* is the main ectoparasite of cattle in tropical and subtropical countries of Australia, Africa and America (Rodriguez-Vivas et al., 2005; Jonsson and Hope, 2007). Chemicals, including organophosphates (OP), synthetic pyrethroids (SP), amitraz (Am) and macrocyclic lactones (MLs) play a major role in controlling *Rhipicephalus* (Aguilar-Tipacamu and Rodriguez-Vivas, 2003; George et al., 2004). The intensive use of these chemicals has led to the development of resistant tick populations in tropical and subtropical countries (George et al., 2004; Rodriguez-Vivas et al., 2006a,b; Rodriguez-Vivas et al., 2007).

In Mexico, *R. microplus* has developed resistance to all main classes of acaricides in past decades owing to intensive use of chemical acaricides (Rodriguez-Vivas et al., 2007). Resistance to OP acaricides first developed in the 1980s in Mexico, and resistance to SP emerged in the 1990s. Amitraz was introduced along with SP to control OP-resistant ticks in 1986. Initially, Am was not widely used, owing to its higher cost, but its use became more prevalent and intensive after SP resistance was discovered in 1993 (Fragoso et al., 1995). The first case of Am resistance in *R. microplus* from Mexico was confirmed in 2001 at a ranch in the state of Tabasco (Soberanes et al., 2002). Recently, Miller et al. (2008) reported the first case...
of *R. microplus* resistant to fipronil in Northern States of Mexico. Presently, many tick populations are resistant to multiple classes of acaricides in Mexico (Rodríguez-Vivas et al., 2007).

In the last 8–10 years, MLs (ivermectin, doramectin and moxidectin) have been used to control both gastrointestinal nematodes (GIN) and cattle ticks in Mexico (Aguilar-Tipacamu and Rodríguez-Vivas, 2003; Rodríguez-Vivas et al., 2006a). Resistance to MLs has been widely documented in several species of GIN (Fiel et al., 2001; Geary, 2005; Demeler et al., 2009). However, tick resistance to MLs has been rarely documented. The first reports of tick resistance to MLs were made in Brazil. Martins and Furlong (2001) reported cross-resistant *R. microplus* to doramectin, ivermectin and moxidectin by in vivo test, and recently, Klafke et al. (2006) reported *R. microplus* resistant to ivermectin in the State of Sao Paulo, Brazil.

In Mexico, *R. microplus* populations, resistant to MLs have not been documented; however, under field conditions ivermectin treatment failures have been reported by veterinarians and farmers. The main objective in this study is to report for the first time the presence of *R. microplus* populations resistant to ivermectin in Mexico.

2. Materials and methods

2.1. Study background

The study was carried out in the tropical region of Mexico (Yucatan State). The state is located between 19°30' and 21°35' north latitude and 90°24' west longitude of the Greenwich meridian. The climate of the state is subhumid tropical with a summer rainy season. The monthly maximum temperature varies from 35°C to 40°C (mean 26.6°C). The relative humidity (R.H.) varies from 65 to 100% (mean 80%) and the annual rainfall varies from 415 mm to 1290 mm depending on the area. There are two different seasons: rainy (June–October) and dry (November–May) (INEGI, 2002). The state has a cattle farm population of 4629 with 624,488 head of cattle. The predominant livestock–production system is semi-intensive (beef farms), based mainly on year-round grazing on improved pastures i.e. Guinea grass (*Panicum maximum*) and Star grass (*Cynodon nlemfuensis*), with supplementary feeding during the dry season. The use of acaricides to control ticks is a common practice in Yucatan, Mexico (Rodriguez-Vivas et al., 2006a), and acaricide resistance in the *R. microplus* population to OP, SP and Am has been reported (Rodriguez-Vivas et al., 2006a,b; Rodriguez-Vivas et al., 2007). Macrocyclic lactones (ivermectin, doramectin and moxidectin) are the main drugs used to control GIN and the main alternative to control cattle ticks in Yucatan, Mexico (Rodriguez-Vivas et al., 2006a).

2.2. Tick population and sample collection

During the year 2008, under field conditions, ivermectin treatment failures have been reported by veterinarians and farmers in Yucatan, Mexico. In order to determine the susceptibility of field populations of *R. microplus* to ivermectin, the following three cattle farms were studied: Lechería de la Universidad Autónoma de Yucatan (LUADY), San Pedro Navajuelas (SPN) and San Fernando (SFDO). In the last 5 years, in the SFDO and SPN, ivermectin has been routinely used to control GIN and ticks (2–3 times per year), and in LUADY ivermectin was used only in special cases when a high GIN (egg output) was observed.

In each farm two tick samples were taken at different times. Dates for tick collection in each farm were as follows: SFDO population (19/09/2008 and 31/12/2008), SPN population (15/09/2008 and 11/12/2008) and LUADY population (15/09/2008 and 10/02/2009).

A sample of 30–50 engorged adult female *R. microplus* was collected from at least 10 animals on each farm. Engorged adult females were placed into small plastic boxes with air holes and transported to the Parasitology Laboratory at the Campus de Ciencias Biológicas y Agropecuarias of the Universidad Autónoma de Yucatán. Upon arrival, engorged adult females were placed in Petri dishes and incubated at 27±1.5°C and a R.H. of 85–86% (Cen et al., 1998). After oviposition (14–18 days), eggs were transferred into two 3 mL glass vials and plugged with a cotton cap. Eclosion of larvae occurred approximately 30 days after collection of engorged females. Live larvae of 14–21 days of age were used for bioassays.

2.3. Bioassays

The larval immersion test (LIT) was used to test the resistance to ivermectin of collected *R. microplus*. Technical grade ivermectin (ivermectin B1 mainly, Sigma–Aldrich, USA) was diluted at 1% in absolute ethanol (ivermectin stock solution). Also, a previous ethanol solution with Triton X–100 at 2% was diluted at 1% in distilled water (Eth-TX 1%). Then, the ivermectin stock solution was diluted at 0.01% (top dose immersion solution) in Eth-TX 1%. Finally, concentrations of ivermectin in immersion solutions were obtained performing 30% serial dilutions starting from 0.01% (100 ppm). Concentration (%) of immersion solutions was as follows: 0.01, 0.007, 0.0049, 0.00343, 0.0024, 0.00168, 0.00117, 0.00082, 0.00057 and 0.00028 and Eth-TX 1% was used as the control solution.

Immersion solutions, 0.5 mL each, were transferred into 1.5 mL microcentrifuge tubes (three repetitions for each solution) and approximately 300 larvae were added to each one. The larvae were immersed for 10 min. Then, the tubes were opened and the larvae were transferred with a paintbrush to a paper filter. After drying, about 100 larvae were transferred to a paper filter (850 mm × 750 mm) that was folded and closed with “bulldog” clips forming a packet. The packets were incubated at 27–28°C and 80–90% relative humidity for 24 h, when the mortality was determined. Only larvae capable of locomotion were considered alive.

The mortality data were submitted to probit analysis and a chi-square test was used to test the hypothesis of parallelism and equality (p = 0.05) with POLO PLUS software (LeOra software, 2003) to estimate the lethal concentrations (LC) for 50 and 99% with their respective confidence limits of 95% (CI95%). Resistance ratios (RR) were calculated in relation to Deucht reference strain
3. Results

The LC estimate of ivermectin to kill 50 and 99% of the three R. microplus field populations together with their respective CL95% and slopes are shown in Table 1. SFDO, SPN and LUADY tick populations had significantly higher LC50/LC99 estimates than the reference susceptible Deutch strain (Table 1 and Fig. 1). Also, there was significant difference between LC50/LC99 from SFDO, SPN and LUADY tick populations, which indicates not only the presence of resistant populations, but also different levels of resistance to ivermectin in the field populations studied. There was no difference observed at the LC50 and LC99 estimates at the different collection dates from any of the three populations studied (Table 1). The hypothesis of parallelism and equality between regression lines for Deutch susceptible reference and the three studied field populations was rejected ($p < 0.05$).

4. Discussion

The development of acaricide resistance in a tick population is dependent on the frequency of occurrence of resistant individuals in the population and the intensity of chemical selection pressure (Kunz and Kemp, 1994). Resistance is defined as the ability of individuals in a population to survive doses of a toxicant that, in a typical population of the same species, would prove lethal to most individuals (FAO, 2004). In recent years, a greater reliance

Table 1

Lethal concentration (50 and 99%) estimates for ivermectin with their respective confidence limits at 95%, slopes and resistance ratio of field populations of *Rhipicephalus microplus* from Yucatan, Mexico, using the larval immersion test.

<table>
<thead>
<tr>
<th>Farm</th>
<th>Slope ± S.E.</th>
<th>LC50 CL95%</th>
<th>LC99 CL95%</th>
<th>RR50 CL95%</th>
<th>RR99 CL95%</th>
</tr>
</thead>
<tbody>
<tr>
<td>LUADYa</td>
<td>3.82 ± 0.12</td>
<td>0.00114</td>
<td>0.00104–0.00126</td>
<td>0.0046</td>
<td>0.0038–0.0060</td>
</tr>
<tr>
<td>LUADYb</td>
<td>3.83 ± 0.10</td>
<td>0.00129</td>
<td>0.00117–0.00141</td>
<td>0.0052</td>
<td>0.0043–0.0066</td>
</tr>
<tr>
<td>SPNa</td>
<td>2.72 ± 0.07</td>
<td>0.00199</td>
<td>0.00181–0.00218</td>
<td>0.0142</td>
<td>0.0115–0.0184</td>
</tr>
<tr>
<td>SPNb</td>
<td>2.40 ± 0.06</td>
<td>0.00206</td>
<td>0.00186–0.00229</td>
<td>0.0192</td>
<td>0.0148–0.0266</td>
</tr>
<tr>
<td>SFDOa</td>
<td>1.67 ± 0.06</td>
<td>0.00338</td>
<td>0.00320–0.00475</td>
<td>0.0941</td>
<td>0.00516–0.2238</td>
</tr>
<tr>
<td>SFDOb</td>
<td>1.54 ± 0.06</td>
<td>0.00481</td>
<td>0.00355–0.00719</td>
<td>0.1526</td>
<td>0.0608–0.7630</td>
</tr>
<tr>
<td>DEUTCHc</td>
<td>4.72 ± 0.07</td>
<td>0.00056</td>
<td>0.00052–0.00060</td>
<td>0.0017</td>
<td>0.0015–0.0021</td>
</tr>
</tbody>
</table>

LC: lethal concentration, CL: confidence limits, RR: resistance ratio (LC from the population studied divided by the LC from the susceptible reference strain), NA: not applicable.

* Data obtained from the first samples.

b Data obtained from the second samples.

c Susceptible reference strain from USDA, Cattle Tick Fever Research Laboratory, Edinburg, TX, USA.
on tick control in Mexico using injectable or pour-on MLs compounds has occurred (Rodríguez-Vivas et al., 2010). Ivermectin, doramectin and moxidectin at 0.2–0.5 mg/kg BW have been shown to be effective to control immature and adult stages of R. microplus (Aguilar-Tipacamu and Rodríguez-Vivas, 2003). Recently, parenteral long-acting MLs formulations (ivermectin 0.63–0.80 mg/kg BW, moxidectin 1 mg/kg BW) have been developed which are not only effective against existing parasitic infestations (endo and ectoparasites) of cattle but also provide protection against reinfection for a period of time beyond that of standard formulations. Dupuy et al. (2007) studied the plasma kinetics disposition of moxidectin following a long-acting formulation (1 mg/kg BW) and found high persistence concentration of moxidectin and the time that the plasma and hair concentrations exceeded 2 ng/mL or 2 ng/g (level considered as effective to control ticks) was 120 and 100 days, respectively. In Mexico, long-acting MLs showed efficacy of >95% to control field populations of R. microplus with persistence efficacy up to 70 days posttreatment (Arieta-Román et al., 2008). The high persistence concentration of MLs in cattle will mean that any resistant or partially resistant ticks will be selectively accumulated before blood levels of moxidectin have fallen to levels that permit infestation with sensitive ticks. R. microplus has a relatively short life cycle (four tick generations per year) and high reproductive potential in Yucatan, Mexico (Cen et al., 1998; Rodríguez-Vivas and Dominguez, 1998), as a result this species is frequently exposed to MLs used to control them and other endoparasites on cattle. In the present study we identified for the first time in Mexico a R. microplus field-population (SFDO) that was highly resistant to ivermectin.

The LC50/LC99 estimates of the three field populations were different, showing the SPN and SFDO tick populations with the higher resistance ratio compared to the Deutch and LUADY (Table 1 and Fig. 1). The systematic use of ivermectin to control ticks and GIN in the last 5 years in both farms, may have contributed to the selection of resistance to ivermectin in these populations of R. microplus. A special case is the LUADY tick population, with RR of only 2.04 and 2.39 (different LC50/LC99 compared with the value of the susceptible Deutch strain) and most likely due to the low frequency of ivermectin treatment at the farm. Further studies are needed to confirm if this RR is sufficient to lead to control failure in the field and consider this tick population as resistant.

The LC50 (0.00056%, CL95%: 0.00052–0.00060) of the Deutch strain used as susceptible in the present study is slightly higher than those reported by Klafke et al. (2006) (0.00042, CL95%: 0.00039–0.00045) for Porto Alegre strain in Brazil. Nonetheless, higher RR50 values were obtained in SFDO tick population (6.84 and 8.59) and similar to SPN tick population (3.55 and 3.68) to those found by Klafke et al. (2006) for Barra Alegre field tick population (3.78) in relation to Porto Alegre. Additional information obtained from this study is that the SFDO tick population exhibited the lowest slope in both samples (1.67–1.54, Table 1). This result is in agreement with the statement of Robertson and Preisler (1992) who argued that data heterogeneity as well as low-slope linked with both high-LC50 and high-LC99 values suggest that many individuals have the resistant-type allele. Theoretically a susceptible strain composed of totally susceptible individuals will produce the highest slope for a regression line of dose–response data. With selective pressure from exposure to insecticides, a population will become heterozygous for resistant genotypes and as the frequency of resistant genotypes increases, the slope of the regression line will drop off and the line will shift to the right (Robertson and Preisler, 1992). After further selection, the resistant alleles will become fixed in the population. Heterogeneity is once again reduced and the slope increases. However, the data shift to the right (higher LC estimates) remains.

The susceptibility of R. microplus to ivermectin measured by the LC50/LC99 did not differ in the three field populations when bioassays were carried out twice at different times of collection (Table 1). The purpose to test the same tick population in two different times (and probably in different tick generations) was to evaluate the variability of R. microplus resistance to ivermectin and to evaluate this new technique for wider use in Mexico. Although, LIT is not yet recommended by FAO, Sabatini et al. (2001) concluded that LIT was approximately 400 times more sensitive than the Larval Packet Test to diagnose R. microplus resistant to MLs.

In Mexico, no registered cases of resistance of ticks to MLs have been reported. The present study demonstrates for the first time three R. microplus populations resistant to ivermectin exist in Mexico. In the short term milbemycins (moxidectin) may provide therapeutic and prophylactic cover against ivermectin-resistant R. microplus in Yucatan, Mexico, as described in nematodes (Bartley et al., 2006), but if used exclusively, because they have similar modes of action to ivermectin (Taylor, 2001), resistance will also evolve to moxidectin. The use of MLs to control ticks and GIN in cattle can be expected to continue to increase dramatically in Yucatan, Mexico, due to convenience (wide spectrum activity against endo- and ectoparasites), increasingly affordable prices (especially generic versions of MLs) and lack of effective chemical alternatives. Further research is needed to evaluate the frequency, spatial distribution and risk factors associated to R. microplus resistance to ivermectin in the Mexican tropics.

In conclusion, we report for the first time field populations of R. microplus resistant to ivermectin in Mexico.

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References


