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The prevalence of pyrethroid resistance phenotype and genotype in *Rhipicephalus* (*Boophilus*) *microplus* in Yucatan, Mexico

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ABSTRACT

A field survey was conducted to evaluate susceptibility of *Rhipicephalus* (*Boophilus*) *microplus* to cypermethrin on 49 farms in three areas of Yucatan, Mexico. The modified larval packet test was used to evaluate larval mortality at different cypermethrin concentrations. Dose–mortality regressions, lethal concentrations (LC_{50}–LC_{99}), confidence intervals and slope were estimated by probit analysis. Phenotype was defined as susceptible, tolerant or resistant when the resistance factor (RF) derived from both LC_{50} and LC_{99} determinations were <3, 3–5 or >5, respectively. An allele specific PCR (AS-PCR) was used to determine the frequency of a sodium channel mutation (F1550I, Phe → Ile) associated with pyrethroid resistance. Overall, 26.5%, 40.8% and 32.6% of tick populations were susceptible, tolerant and resistant to cypermethrin, respectively. A substantial inter-population variation in the level of cypermethrin response was evident (resistance factors ranged from 0.3 to 2599 and from 0.7 to >5000 when were indicated by the LC_{50} and LC_{99}, respectively). The F1550I mutation (R allele) in *R. microplus* was present in all studied areas. The increasing presence of the R allele correlated well with increased levels of response indicated by both the LC_{50} ($r^2 = 0.659$, $p = 0.001$) and LC_{99} ($r^2 = 0.688$, $p = 0.001$) to cypermethrin. These results indicated that the F1550I mutation is a major common mechanism responsible for pyrethroid resistance in field populations of *R. microplus* ticks in the Mexican tropics. Both bioassay and AS-PCR showed that the prevalence of cypermethrin-resistant/tolerant *R. microplus* is high in Yucatan, Mexico and the relationship between the RF and the frequency of the R allele supports the role of F1550I as one of the most important mechanisms conferring pyrethroid resistance in these *R. microplus* populations.

1. Introduction

Rhipicephalus (*Boophilus*) *microplus* (Canestrini) is a haematophagous arachnid (Acari: Ixodidae) and is a major cattle pest in tropical and subtropical agrosystems of the world. This tick species causes significant economic damage to cattle production by both direct infestation of cattle and diseases it transmits (*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 this tick species, however, as a consequence of extensive exposures to acaricides, *R. microplus* has developed resistance to all major classes of acaricides (Jonsson et al., 2000; Rodriguez-Vivas et al., 2006b; Chevillon et al., 2007).

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In Mexico, resistance to pyrethroids was first detected in 1993 by discriminating dose bioassays (Fragoso et al., 1995). The *R. microplus* isolates were identified as co-resistant to pyrethroids and organophosphates and showed a wide geographical distribution in livestock regions (Ortiz et al., 1995). The bioassay was used to determine the prevalence of farms with pyrethroid resistant *R. microplus* in the states of Yucatan, Quintana Roo, Tabasco, and Chiapas as 66%, 95%, 94%, and 90.8%, respectively (Rodriguez-Vivas et al., 2006a, 2007). These results indicate the general problem of pyrethroid resistance in southern Mexico; however, they do not provide insight into the level of resistance in those populations. To establish the levels of phenotypic resistance of *R. microplus* populations to acaricides, the resistance factor (RF) (Beugnet and Chardonnet, 1995; Bianchi et al., 2003) is typically determined and interpreted. The RF_{50} is an indicator of the phenotypic response (i.e. larval mortality) in half of the population exposed to an acaricide, in comparison to reference isolate values. Nevertheless, the RF_{50} does not explain population behavior. To more objectively evaluate the behavior of the majority of a tick population to an acaricide, the use of other LCs (i.e. LC_{90}, LC_{95}, or LC_{99}) (Cameron et al., 1995; Miller et al., 2007), as well as the slope on probit analysis (Robertson and Preisler, 1992) are recommended. In the present study, we added a new alternative to analyze RFs by using two RF determinations (judged by $LC_{50} + LC_{99}$).

At the molecular level, resistance to pyrethroids was first observed in a DDT-resistant strain of housefly, *Musca domestica*, and termed knockdown resistance or *kdr* (Milani, 1954). The molecular basis for *kdr* resistance has been investigated in many arthropods including ticks (Jamroz et al., 2000). He et al. (1999) determined that a single mutation (F1550I, Phe → Ile amino acid substitution) in the S6 transmembrane segment of the domain III in the sodium channel is associated with pyrethroid resistance in *R. microplus*. Subsequently, Guerrero et al. (2001) developed an allele-specific polymerase chain reaction (AS-PCR) assay to genotype *R. microplus* at this locus and Rosario-Cruz et al. (2005) identified target site insensitivity in six pyrethroid-resistance populations of *R. microplus* from Mexico. The resistant isolates were found to possess high frequencies of the mutated sodium channel allele. In a recent study conducted in Yucatan, Mexico Rodriguez-Vivas et al. (2011) measured changes in resistance phenotype and genotype in the presence or absence of pyrethroid selection pressure on field populations of *R. microplus* and they found a clear correlation between the phenotype and genotype in three of four treated farms confirming that the F1550I mutation is a major cause of pyrethroid resistance in Mexico. Besides these findings, little is known about this correlation in large field populations of Mexico.

Due to the increasing occurrence of acaricide control failures in Mexican cattle farms and the ecological factors in the Yucatan state, which are so favorable to successful tick infestation of cattle, we conducted a cross sectional study to determine the prevalence of pyrethroid resistance, defined by both phenotypic and genotypic criteria, in field populations of *R. microplus* in Yucatan, Mexico.

2. Materials and methods

2.1. Study area and sampling size

The study was carried out on farms in three areas of Yucatan, Mexico. Panaba and Tizimin areas specialize in beef cattle production and Sucila in dairy cattle production (INEGI, 2002). The state is located between 19°30′ and 21°35′ N latitude and 90° 24′ W longitude. The climate of the state is sub-humid tropical with a summer rainy season. The monthly mean temperature is 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. There are two different seasons: rainy (June–October) and dry (November–May) (INEGI, 2002). According to the type of soil, vegetation and agricultural development, the state of Yucatan is classified into three zones: the sisal zone (center-north), the agricultural zone (southern) and livestock zone (eastern). The state has 4629 cattle farms comprised of 624,488 head of cattle (INEGI, 2002). Sixty-five percent of the cattle population in the state of Yucatan is concentrated in the livestock zone, where the present study was carried out (Tizimin, Panaba and Sucila areas) (INEGI, 2002). 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 common practice in Yucatan, Mexico (Solorio et al., 1999). Twenty one percent of the farms in Yucatan state use pyrethroids to control ticks and 42% of all farms applied acaricides >12 times/year (Rodriguez-Vivas et al., 2006a).

It was determined that samples from 49 of the 4629 farms would be necessary to accurately determine the prevalence of pyrethroid resistance in the state of Yucatan. The sample size of 49 was based on calculations from data obtained in a limited bioassay study conducted by Rodriguez-Vivas et al. (2005), which indicated an expected frequency of resistance of 77%; confidence intervals (CI) = 90 ± 10%. Farms were randomly selected from a list provided by the Cattlemen's Association of the State of Yucatan. To collect ticks, each farm was visited once or twice depending on the availability of engorged females.

2.2. Tick collection and production of R. microplus larvae

A sample of 30–50 engorged female *R. microplus* ticks was collected from at least 10 animals on each farm and transported to the parasitology laboratory at the Campus de Ciencias Biológicas y Agropecuarias of the Universidad Autónoma de Yucatán (CCBA-UADY). Engorged adult females were placed in a Petri dish and incubated at 27 ± 1.5 °C and a RH of 85–86% (Cen et al., 1998). After oviposition (14–18 d), pooled eggs from all females were randomly divided into two 3 ml glass vials and plugged with a cotton cap. At 7–14 d after larval eclosion (ca. 37–44 d after collection of engorged females) one vial of larvae was used for bioassays (Kemp et al., 1998) and the other was frozen at −70 °C for DNA isolation and PCR.