Veterinary Parasitology 189 (2012) 327–332

Contents lists available at SciVerse ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

Survey of pyrethroid and organophosphate resistance in Brazilian field populations of *Rhipicephalus* (*Boophilus*) *microplus*: Detection of C190A mutation in domain II of the *para*-type sodium channel gene

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ARTICLE INFO

Article history:
Received 17 September 2011
Received in revised form 17 April 2012
Accepted 20 April 2012

Keywords:
Cattle tick
Cypermethrin
Chlorpyriphos
Larval packet test
Target site insensitivity
Allele-specific PCR

ABSTRACT

The cattle tick *Rhipicephalus* (*Boophilus*) *microplus* causes expressive damage to livestock in Brazil and other countries. Its control is becoming more difficult due to the development of resistance in populations. Early detection of resistance can help in developing effective control strategies. This study evaluated the susceptibility of *R. microplus* to cypermethrin and chlorpyriphos and was the first attempt to identify the mechanism of resistance (target site insensitivity) in cattle tick populations from Minas Gerais state (Southeastern Brazil). Engorged female ticks were collected from 10 ranches within the state of Minas Gerais, and susceptibility was evaluated with the larval packet test (LPT) using technical grade cypermethrin and chlorpyriphos. It was possible to analyze LPT results of seven populations. Target site insensitivity was investigated in all 10 isolates by using molecular approaches for detection of the T2134A substitution within the domain III S6 segment and the C190A in the domain II S4-5 linker from the *para*-type sodium channel gene. LPT showed that all seven populations were resistant to cypermethrin with resistance ratio (RR) ranging from 16.0 to 25.0 and 85.7% were resistant to chlorpyriphos (RR = 2.2–15.6). Although the T2134A mutation was not detected, the C190A mutation was highly prevalent, being present in 82–100% of the alleles sampled in field populations. A significant correlation was found between the LC50 values for cypermethrin and the frequency of the C190A mutation suggesting that it might be responsible for the phenotypic resistance detected.

1. Introduction

The cattle tick *Rhipicephalus* (*Boophilus*) *microplus* (Canestrini, 1887) (Acari: Ixodidae) is one of the most important parasites of cattle in tropical and subtropical countries. In Brazil, it is responsible for annual losses of about U$2 billion due to mortality, decrease in both milk production and weight gain, deteriorating effects on leather quality, costs for acaricide drugs and transmission of cattle fever disease agents (Grisi et al., 2002).

The control of *R. microplus* mainly relies on the use of chemical products mostly without following any

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0304-4017/
http://dx.doi.org/10.1016/j.vetpar.2012.04.031

technical criteria (leading to an excessive number of applications and too low volume of product per animal) which contributes to accelerating the development of resistance to acaricides (Alonso-Díaz et al., 2006; Mendes et al., 2007, 2011). In Brazil, the first record of cattle tick resistance to organophosphates and pyrethroids was in the 1970s and 1980s, respectively (Arteche, 1972; Leite, 1991). Resistance persisted and now it is found throughout the country (Alonso-Díaz et al., 2006; Andreotti et al., 2011; Mendes et al., 2011).

Pyrethroids exert a neurotoxic effect on arthropods by binding to the sodium channels and prolonging the opening of these transmembrane proteins and inhibiting the deactivation and stabilizing the open configuration of the channel (Dong, 2007). Structural changes in sodium channels due to mutations may decrease the interaction between pyrethroids and its target site, and thus reduce the sensitivity of arthropods to these acaricides (Dong, 2007). Three mutations in the sodium channel have been associated with resistance to pyrethroids in *R. microplus* populations (He et al., 1999; Chen et al., 2009; Morgan et al., 2009; Jonsson et al., 2010; Guerrero et al., 2012).

He et al. (1999) identified a point mutation in the S6 segment of domain III of the *para*-type sodium channel of Mexican strains of *R. microplus* resistant to permethrin. This mutation involves the substitution of a thymine by an adenine (T2134A), resulting in the replacement of a phenylalanine by an isoleucine at susceptible and resistant individuals, respectively.

The mutation described by Morgan et al. (2009) is located at domain II S4-5 linker of the *para*-sodium channel gene and it is a substitution of a cytosine in the susceptible strain to an adenine in the resistant strain (C190A). This substitution led to a leucine to isoleucine replacement that was correlated to pyrethroid resistance (Morgan et al., 2009). Jonsson et al. (2010) reported another substitution in tick populations from Australia: G214T in the domain II S4-S5 linker, which is a glycine to valine change that is associated with resistance to the pyrethroid flumethrin only.

Both detection of the levels of acaricide resistance and understanding the mechanism of resistance in *R. microplus* are important to the development of an effective tick control program. A rational use of pesticides will help to delay the development of resistance and reduce pesticide contamination of the environment as well as chemical residues in meat and milk. This study aimed at evaluating (i) the susceptibility of Brazilian field populations of *R. microplus* to the synthetic pyrethroid cypermethrin and the organophosphate chlorpyriphos and (ii) the role of target site insensitivity mediated by T2134A and C190A substitutions.

2. Materials and methods

2.1. Tick collection

In April 2010, 100 engorged females of *R. microplus* were collected from 10 cattle ranches in the 'Triângulo Mineiro' and 'Alto Paranaíba' regions within the state of Minas Gerais in Southeastern Brazil. The state has the highest milk production in the country and is a leading producer of beef cattle (Pesquisa, 2009). After collection, ticks were stored in plastic containers and sent by post to the Laboratory of Parasitic Diseases, School of Veterinary Medicine, Federal University of Minas Gerais, Belo Horizonte.

2.2. Larval packet test

The bioassay, larval packet test (LPT) (Stone and Haydock, 1962), recommended by FAO (2004), was conducted to detect resistance to cypermethrin and chlorpyriphos.

Ticks were washed with distilled water, dried on absorbent paper and divided into Petri dishes, 30 ticks per plate (separated according to the ranch). These dishes were kept in an incubator at $28 \pm 1.5\ ^{\circ}C$ and approximately 85% relative humidity and 14 days later, eggs were collected and transferred to glass tubes sealed with hydrophobic cotton to allow larval hatching. Egg masses from many female ticks from the same farm were mixed before hatching so that larvae used in these experiments were not all siblings.

Technical grade cypermethrin (93.59% purity) (Allvet®, Londrina, Brazil) was serially diluted in a mixture of trichloroethylene (Synth, Diadema, Brazil) and olive oil (Sigma–Aldrich, São Paulo, Brazil) (2:1, v/v), resulting in different concentrations (in % of active ingredient): 5, 4, 2.4, 2.04, 1.632, 0.979, 0.588, 0.353, 0.212, 0.127 for field populations and 0.1, 0.06, 0.022, 0.013, 0.008, 0.005, 0.003, 0.002 for the *R. microplus* 'Porto Alegre' strain. This strain has been maintained at the Instituto Biológico de São Paulo without contact to acaricides and is considered susceptible.

Filter papers (Whatman n° 1) measuring 8.5 cm × 7.5 cm were impregnated with 0.67 ml of each cypermethrin concentration, including the negative control (only the mixture of trichloroethylene and olive oil). Two papers were used per concentration. Approximately 100 larvae, aged between 14 and 21 days, were added to each of these papers which were folded and sealed with bulldog clips on the sides and top. Papers were stored in the incubator under the conditions described above and larvae mortality was assessed after 24 h of exposure. Larvae unable to move were considered dead.

The same dilution and larvae exposure procedures were performed with chlorpyriphos (97.43% purity) (Ourofino, Cravinhos, Brazil). In this case the concentrations used were (in % of active ingredient): 0.128, 0.064, 0.032, 0.016, 0.008, 0.004, 0.002, 0.001, 0.0005, and 0.00025 for both field populations and 'Porto Alegre' strain.

Mortality data were analyzed by POLO-PC (Leora Software, 1987) in order to obtain the lethal concentration for 50% of the population (LC_{50}) with a 95% confidence interval (CI 95%). The resistance ratio (RR) was calculated by dividing the LC_{50} obtained from the field populations by the LC_{50} obtained from the 'Porto Alegre' susceptible reference strain. Differences in LC_{50} were considered significant when their 95% fiducial limits did not overlap. Tests showing mortality rates between 5% and 10% in the control group were submitted to Abbott's formula (Abbott, 1925).