Ticks and Tick-borne Diseases xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Ticks and Tick-borne Diseases

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

Multiple resistance to acaricides in field populations of *Rhipicephalus microplus* from Rio Grande do Sul state, Southern Brazil

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ARTICLE INFO

Article history:
Received 9 May 2016
Received in revised form 2 September 2016
Accepted 29 September 2016
Available online xxx

Keywords:
Rhipicephalus microplus
Multiple resistance
Control
Acaricides
Brazil

ABSTRACT

Acaricide resistance is a major obstacle to the control of *Rhipicephalus microplus*. Historically, the indiscriminate use of chemical compounds has contributed to the selection of populations resistant to different classes of acaricides. Therefore, multiple acaricide resistance is an important threat to the chemical control of the cattle tick. To investigate the occurrence and extent of multiple resistance to acaricides in Southern Brazil we performed larval tests with cypermethrin, chlorpyriphos, amitraz, fipronil and ivermectin on 104 cattle tick field samples from different ranches in Rio Grande do Sul, between the years 2013 and 2015. Adult immersion tests with a commercial formulation mixture of chlorpyriphos and cypermethrin were performed on 75 samples. Four levels of resistance were established according to the mortality of larvae: Level I: mortality between 82% and 95%; Level II: mortality between 57% and 82%; Level III: mortality between 25% and 57%; and Level IV: mortality lower than 25%. Resistance to cypermethrin was detected in 98.08% of the samples evaluated, mostly at resistance level IV. The frequency of samples resistant to amitraz, chlorpyriphos, ivermectin and fipronil was 76.92%, 60.58%, 60.58% and 53.85% respectively. Multiple resistance to three or more compounds was found in 78.85% of the samples. The results obtained in this study are alarming and reveal a new scenario for the challenge of tick control using chemicals. This is an issue of high importance to cattle production systems where this tick is responsible for a high economic impact.

1. Introduction

The southern cattle tick (*Rhipicephalus microplus*) is the ectoparasite that causes the highest costs and losses to the Brazilian cattle industry (Grisi et al., 2014). Tick control is performed almost exclusively by application of chemical acaricides. However, a major concern that arises with the use of chemicals is the selection of resistant strains (Kunz and Kemp, 1994). Drug resistance can be defined as a heritable reduction in the susceptibility of a parasite population to the action of a drug (Devaney, 2013). The result of this process is a reduction in treatment efficacy and, consequently, an increase in the costs of control. The increase in animal production indexes in Brazil over the last three decades was the result of increased investment in the primary industry sector, with an increasing number of cattle herds and increased spending on supplies. The sale of antiparasitic drugs increased by 28.3% between 2008 and 2013 (USD 2.58 billion in that period) (SINDAN, 2014), which reflects the use of these drugs in Brazilian cattle. The frequent and indiscriminate use of acaricides caused the evolution of resistant populations to almost all available classes of tick control in Brazil. In several regions, tick populations are frequently found to be resistant to synthetic pyrethroids (SP) and organophosphates (OP) (Mendes et al., 2011), amitraz (Lovis et al., 2013), macrocyclic lactones (ML) (Klafke et al., 2012), fipronil (Castro-Janer et al., 2010) and recently to fluazuron (Reck et al., 2014). The situation, therefore, is critical.

The most problematic consequence of the development of acaricide resistance is the selection of tick populations with multiple

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http://dx.doi.org/10.1016/j.ttbdis.2016.09.019
1877-959X/

drug resistance. As in bacteria, which have developed multiple resistance to antimicrobial drugs (Woodford et al., 2011), this phenomenon has developed across arthropod pest species. There are reports of multiple resistance to insecticides in flies (Liu and Yue, 2000), moths (Pu et al., 2010), cockroaches (Gondhalekar and Scharf, 2012) and mosquitoes. In the cattle tick, field isolates and laboratory strains with multiple resistance to acaricides (MRA) have been reported in Colombia (Benavides et al., 2000), Mexico (Fernández-Salas et al., 2012a,b) and Brazil (Mendes et al., 2011; Lovis et al., 2013; Reck et al., 2014).

In this study we conducted bioassays in tick populations from the state of Rio Grande do Sul (RS) (Southern Brazil), in order to evaluate for resistance to the main classes of chemicals used for the control of *R. microplus* in Brazil: cypermethrin, chlorpyriphos, a mixture of cypermethrin with chlorpyriphos, amitraz, fipronil and ivermectin.

In a scenario where the development of new chemicals to control ticks seems unlikely, it is necessary to determine strategies to delay the development of resistance to available drugs. The use of diagnostic techniques to accurately and reliably determine the existence of MRA tick populations is critical to the design of strategies for control programs and resistance management.

2. Material and methods

2.1. Ticks

The strains of *R. microplus* used were: 1) Mozo, susceptible reference strain maintained without any contact with acaricides; 2) Jaguar, one colony of ticks isolated from a field population collected in February 2011 in the municipality of Eldorado do Sul-RS, is resistant to SP, OP, amitraz, fipronil, ivermectin, moxidectin, abamectin and fluazuron (Pohl et al., 2012; Reck et al., 2014); 3) JUA, one colony of ticks isolated from a field population collected in February 2009 in the municipality of Jacareí, southeast Brazil (Pohl et al., 2011) and selected for macrocyclic lactone resistance for several generations by infesting cattle injected with ivermectin at the label rate (200 μg/kg). The colonies of ticks were maintained in cattle in the Isolation Unit of the Laboratory of Parasitology of Instituto de Pesquisas Veterinárias Desidério Finamor (LPA-IPVDF), Eldorado do Sul, RS as described previously (Reck et al., 2009). The handling of host animals strictly followed the recommendations of the Institutional Committee of Ethics in Animal Experimentation (CEUA-IPVDF; study number 07/2012).

Rhipicephalus microplus field samples were obtained between December 2012 and December 2015. Engorged females from 104 different ranches located in the state of Rio Grande do Sul, Brazil (Supplementary Table 1) were sent to the LPA-IPVDF as part of the institutional acaricide resistance surveillance program, which is based on the execution of acaricide sensitivity tests with adults (adult immersion test – AIT), confirmation of resistance with larval tests (larval packet test – LPT, and larval immersion test – LIT) and feedback from the ranchers/veterinarians with information generated by the bioassays to assist their strategy of chemical control of ticks.

2.2. Preparation of ticks

Handling procedures of ticks were conducted as recommended by the FAO (2004). Ticks from susceptible (Mozo) and resistant colonies (Jaguar and JUA) were collected after natural detachment from hosts. Field populations were received at the laboratory inside plastic bottles. After reception, they were washed with water and dried with paper towels. From each sample, thirty engorged females were incubated in an environmental chamber at 27 ± 1 °C and 80–90% relative humidity for 14 days to allow egg laying. Eggs were mixed thoroughly and incubated in glass vials (5 mL) closed with cotton plugs to allow the passage of air and moisture for another 14 days under the same conditions to permit larvae hatching. Larvae 14–21 days of age were used in larval tests. When available, twenty engorged females were used in adult immersion tests.

2.3. Chemicals

The bioassays were performed with technical grade acaricides: cypermethrin, chlorpyriphos, ivermectin and fipronil (Sigma Chemical Co., St. Louis, MO, USA). Larval tests with amitraz were performed with a commercial formulation at 12.5% (Triatox®, MSD Saúde Animal, São Paulo, Brazil). The adult immersion test was performed with a mixture of 15% cypermethrin with 25% chlorpyriphos (Colosso® – Ouro Fino Saúde Animal Ltda, Ribeirão Preto, Brazil).

2.4. Larval tests with discriminating doses (DD)

The use of a DD for each acaricide is a way to substantially reduce the amount of work required to determine resistance (FAO, 2004). The DD of chlorpyriphos and cypermethrin were 1% and 0.2%, respectively (FAO, 2004), and were tested with a larval packet test (LPT). For the commercial formulation of amitraz, 0.03% of active ingredient was used with a modified LPT as suggested by Miller et al. (2002). Fipronil tests were conducted with the concentration determined by Castro-Janer et al. (2009) (0.0008%). Ivermectin tests were performed with 0.01% of active ingredient (Klafke et al., 2012). Fipronil and ivermectin resistance was evaluated with larval immersion tests.

For validation of the DDs, full dose response bioassays with increasing concentrations of each active ingredient (including the DDs) were conducted with susceptible (Mozo) and resistant reference strains (Jaguar; Juarez).

2.4.1. Larval packet test (LPT)

A LPT-DD was performed as recommended by the FAO. Initially, acaricides were diluted in a mixture containing two parts of trichloroethylene (Synth, Diadema, Brazil) and one part commercial olive oil (TCE-OO) in order to prepare the impregnation solutions. A volume of 0.67 mL of each acaricide solution was used to impregnate a piece of quantitative filter paper (85 mm × 75 mm – Whatman No. 1, Whatman Inc., Maldstone, England) or nylon fabric in the case of amitraz (85 mm × 75 mm – Type 2320, Cerex Advanced Fabrics, Pensacola, FL). The material was left to dry for 2 h inside a fume hood to allow for trichloroethylene evaporation. After drying, packets of the same acaricide were wrapped in aluminium foil and maintained at −20 °C until the moment of use.

On the day of testing, filter papers were taken from the freezer, folded in the middle and sealed on both sides with metal clips to form the packets. Approximately 100 larvae were transferred to each packet using a flat No. 2 paintbrush. The packets were sealed with a third clip on top and incubated at 27 ± 1 °C and 80–90% relative humidity. Control groups were exposed to filter papers impregnated with acaricide-free TCE-OO. After 24 h, larvae mortality was determined by counting total dead and living individuals. Larvae that were paralysed or moving only their appendices without the ability to walk were considered dead. Three packets impregnated with each acaricide as well as controls were prepared for each tick sample.

2.4.2. Larval immersion tests (LIT)

The LIT with fipronil and ivermectin were performed as described by Castro-Janer et al. (2009) and Klafke et al. (2012), respectively, with some modifications. For fipronil, a stock solution

Please cite this article in press as: Klafke, G., et al., Multiple resistance to acaricides in field populations of *Rhipicephalus microplus* from Rio Grande do Sul state, Southern Brazil. Ticks Tick-borne Dis. (2016), http://dx.doi.org/10.1016/j.ttbdis.2016.09.019