Veterinary Parasitology 207 (2015) 309–317

Contents lists available at ScienceDirect

Veterinary Parasitology

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

Susceptibility of *Rhipicephalus* (*Boophilus*) *microplus* to ivermectin (200, 500 and 630 μg/kg) in field studies in Brazil

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ARTICLE INFO

Article history:
Received 31 July 2014
Received in revised form
10 December 2014
Accepted 12 December 2014

Keywords:
Avermectins
Cattle
Resistance
Ticks

ABSTRACT

The present study aimed to determine the susceptibility of 17 *Rhipicephalus* (*Boophilus*) *microplus* populations, originating in the Southeast and Southern regions of Brazil, to different ivermectin concentrations (200, 500 and 630 μg/kg), administered through subcutaneous or topical (pour-on) routes. *R.* (*B.*) *microplus* populations from the states of Minas Gerais (seven populations), São Paulo (seven populations) and Paraná (three populations) were chosen for the tests. The selected cattle were allocated to treatment groups on day 0, and block formation was based on the arithmetic mean of female ticks (4.5–8.0 mm long) counted on three consecutive days (−3, −2 and −1). To evaluate the therapeutic and residual efficacies of these formulations, tick counts (females ranging from 4.5 to 8.0 mm long) were performed on days 3, 7 and 14 post-treatment, and continued on a weekly basis thereafter until the end of each experiment. The results obtained throughout this study, utilizing field efficacy studies, allowed us to conclude that the resistance of *R.* (*B.*) *microplus* against 200 and 500 μg/kg ivermectin is widely disseminated because all tick populations that had contact with these specific concentrations were diagnosed as resistant. However, it is possible to infer that *R.* (*B.*) *microplus* resistance against 630 μg/kg ivermectin was also widespread, diagnosed at six of ten analyzed properties. Resistance of these ectoparasites to 630 μg/kg ivermectin is most likely emerging in three other populations of *R.* (*B.*) *microplus*. Strategies of resistance management need to be quickly determined to keep the selection pressure at a minimum level in Brazil.

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http://dx.doi.org/10.1016/j.vetpar.2014.12.012
0304-4017/

1. Introduction

The southern cattle tick, *Rhipicephalus* (*Boophilus*) *microplus*, is the most important ectoparasite of cattle. This tick is a vector for various diseases and, moreover, poses as a major obstacle to animal productivity in tropical and subtropical areas of the world where the species is endemic (FAO, 2004).

Current methods for controlling cattle ticks rely primarily on the utilization of chemical compounds, with very distinct criteria (Klafke et al., 2006; Lopes et al., 2013). One example is ivermectin, which belongs to a chemical group known as the macrocyclic lactones, which emerged after 1979 and has a wide spectrum of activity. A distinctive feature of macrocyclic lactones is their continued presence in animals for long periods of time, due to their high affinity for adipose tissue (Lopes et al., 2009).

Controlling this ectoparasite is a key factor in improving production, but the efforts to combat it on most cattle farms are performed incorrectly, characterized by excessive and disordered use of therapeutic approaches. This, in turn, increases production costs and accelerates the selection of parasite resistance, which represents a very significant, if not the main, economic issue in animal production (Fiel et al., 2001).

Considering that *R.* (*B.*) *microplus* resistance to macrocyclic lactones, especially to ivermectin, is rapidly increasing in some areas (Castro-Janer et al., 2011), it is essential to comprehend the scope of the phenomenon in a specific region, to evaluate its spatial distribution (Fernández-Salas et al., 2012). Without this information, creation of efficient strategies to handle resistance in the field is impossible. It is essential for developing a progressive and more rational chemical control strategy against this ectoparasite in cattle (Lopes et al., 2014a).

The present study aimed to determine the susceptibility of 17 *R.* (*B.*) *microplus* populations, originating in the Southeast and Southern regions of Brazil, to different ivermectin concentrations (200, 500 or 630 μg/kg), administered subcutaneously or topically (pour-on), in *in vivo* field studies.

2. Materials and methods

2.1. Study locations, division of groups, dose determination/procedures and tick counts

Between March 2013 and February 2014, experiments were conducted in different rural properties of Brazil. A total of 17 *R.* (*B.*) *microplus* populations were evaluated for their susceptibility to ivermectin. Seven tick populations were selected from the state of São Paulo, seven from the state of Minas Gerais and the remaining three tick populations were obtained from the state of Paraná. The number of experimental groups utilized in each rural property (tick population), as well as the choice of tested products, were defined by considering the availability of naturally infested animals in each location, as well as the structure of each farm, because the experimental animals had to be separated into different paddocks when using pour-on formulations. The chemical compounds used at each property, as well as the history of avermectin use for each herd during the previous five years, are described in Table 1.

For each experiment, 20–30 mixed breed cattle (*Bos taurus* × *Bos indicus*) naturally infested by *R.* (*B.*) *microplus*, with ages ranging from 18 to 36 months, were utilized. The total number of experimental animals was 360. In all studies, only clinically healthy cattle with a good nutritional condition were selected. Cattle chosen for all studies had not been medicated for a period of at least 120 days before the beginning of each experiment. All animals were maintained on a grazing regimen, following that already in force on each rural property.

Cattle were allocated to treatment groups on day 0, being randomly designated to treatments according to a randomized complete block design. Block formation was based on arithmetic means of female ticks (measuring 4.5–8.0 mm) counted on three consecutive days (−3, −2 and −1), as recommended by Wharton and Utech (1970). In each experiment, animals were divided into 10 blocks containing two or three cattle, and these were randomly placed in one of the treatment groups inside each block. All experimental animals were weighed on day −1 for each experiment to determine the appropriate dosage of medication. The number of cattle per group used in all experiments (10 animals per group) was determined in accordance with recommendations described by the Brazilian Ministry of Agriculture, Livestock and Food Supply (Ministério da Agricultura, Pecuária e Abastecimento – MAPA), Ordinance N° 48 (Brazil, 1997).

Acaricidal compounds used for treatment of the experimental cattle were commercial products available in the Brazilian veterinary market that contained ivermectin in different concentrations and had different recommended routes of administration: 0.5% (500 μg/kg) pour-on ivermectin (Ivomec® Pour-on – Merial Saúde Animal); 1% (200 μg/kg) injectable ivermectin (Ivomec Injetável® – Merial Saúde Animal) and 3.15% (630 μg/kg) injectable ivermectin (Ivomec Gold® – Merial Saúde Animal). All products were stored and used according to the label specifications. In each experiment, the cattle were individually weighed one day prior to treatment to calculate the correct dosage. Moreover, the body weight scales were tested and verified for accuracy. Each treatment was performed individually in cattle, and the administration of the products was always performed by a veterinarian.

To ensure dosing techniques were conducted using similar standards, in each experiment the volumes administered were calculated using the measured individual weight of each animal before treatment and, if necessary, in 1 mL were rounded down. Example: an animal of 176 kg would receive 17.6 mL using a pour-on product, therefore received 17.5 mL, and an animal of 176 kg would receive 3.52 mL using an injectable product, therefore received 3.5 mL. The animals were appropriately restrained in a chute during the treatment.

According to Klafke et al. (2012), there is little information available about ivermectin resistance, therefore this active ingredient was chosen for the efficacy studies. In addition, Brazil possesses more than 70 registered commercial brands of ivermectin for cattle, a factor which