



First report of fluazuron resistance in *Rhipicephalus microplus*: A field tick population resistant to six classes of acaricides



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ABSTRACT

The control of the cattle tick *Rhipicephalus microplus* is based mainly on the use of chemical acaricides, which has contributed to the emerging problem of selection of resistant tick populations. Currently, there are six main classes of acaricides commercially available in Brazil to control cattle ticks, with fluazuron, a tick growth regulator with acaricidal properties, being the only active ingredient with no previous reports of resistance. Ticks (designated the Jaguar strain) were collected in a beef cattle ranch located at Rio Grande do Sul state, Southern Brazil, after a complaint of fluazuron treatment failure. To characterize the resistance of this strain against acaricides, larval tests were performed and showed that the Jaguar strain was resistant to all of the drugs tested: cypermethrin (resistance ratio, RR = 31.242), chlorpyrifos (RR = 103.926), fipronil (RR = 4.441), amitraz (RR = 11.907) and ivermectin (3.081). A field trial was conducted to evaluate the efficacy of fluazuron treatment in heifers that had been experimentally infested with the Jaguar or a susceptible strain. Between 14 and 28 days after treatment, the average efficacy in cattle experimentally infested with the susceptible strain was 96%, while for the Jaguar strain the efficacy was zero. Additionally, the Jaguar strain response to fluazuron was evaluated *in vitro* using a modified adult immersion test (AIT) and the artificial feeding assay (AFA). With the AIT, 50 ppm of fluazuron inhibited 99% of larvae hatching in the susceptible strain (POA) and less than 50% in the Jaguar strain. Results of the AFA showed a larval hatching rate of 67% at 2.5 ppm of fluazuron with the Jaguar strain; conversely, only 3% of larvae of the susceptible strain hatched at the same fluazuron concentration. The results showed here demonstrated the first case of fluazuron resistance in *R. microplus* and the first tick population resistant to six classes of acaricides in Brazil.

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1. Introduction

The cattle tick *Rhipicephalus microplus* is a major cause of concern for cattle breeding in the tropical and subtropical world, particularly in Latin America. Its parasitism reduces weight gain, causes anaemia, increases the risk for myiasis occurrence, and can also result in the transmission of *Babesia bovis*, *B. bigemina* and *Anaplasma marginale*, which are the causative agents of cattle tick fever (Corrier et al., 1979; Nari, 1995; Reck et al., 2013). Currently, tick control is mainly, if not exclusively, based on the use of chemical acaricides (Kunz and Kemp, 1994).

The control of cattle ticks with chemical compounds started at the end of the nineteenth century with the use of arsenic (Angus, 1996). The use of this compound was followed by the use of organochlorines (OC) in the 1940s, and a few years later, resistance to this class was reported. Subsequently, these were replaced by organophosphates in the 1950s (George, 2000). According to the Brazilian Compendia of Veterinary Products (SINDAN, 2013), there are currently six main classes of chemical acaricides marketed for tick control in Brazil: (i) organophosphates (OP), (ii) formamidines (amitraz), (iii) synthetic pyrethroids (SP), (iv) macrocyclic lactones (ML), (v) phenylpyrazoles (fipronil), and (vi) benzoylphenyl ureas (fluazuron). The large scale and frequent use of acaricides aiming to control ticks on cattle has favoured the selection of tick populations that are resistant to several active ingredients (AI) of commercial acaricides. In recent years, there have been an increasing number of products in the Brazilian market that are mixtures of two or more AIs (SINDAN, 2013).

Reports of resistance against the main classes of acaricides, particularly for OP, SP, and amitraz, have been published in the regions in which the cattle tick is found (Alonso-Díaz et al., 2006; Soberanes-Céspedes et al., 2002; Miller et al., 2005). As a result, the use of ML, fipronil, fluazuron, and mixtures of SP and OP has become common. The first report of OP resistance was registered in Australia (Shaw and Malcolm, 1964), and eight years later in Brazil (Arteche, 1972). Amitraz started to be used in 1975 in Australia and in 1977 in Brazil, and acaricide resistance to this chemical class was reported in Australia by 1981 (Nolan, 1981). Twelve years later, amitraz resistance was documented in Brazil (Gloria et al., 1993). SPs were introduced in the early 1980s, and resistance was simultaneously detected by 1989 in both Australia and in the state of Rio Grande do Sul (RS), Brazil (Nolan et al., 1989; Laranja et al., 1989). MLs became commercially available in 1981 and resistance in Brazil was first reported in 2001, once again in the state of RS (Martins and Furlong, 2001). Fipronil has been on the market since 1996, and within a decade, between 2004 and 2006, the first report of *in vitro* resistance was published in RS, Brazil (Martins et al., 2006). One year later, fipronil resistance was confirmed in a stall test in Uruguay (Cuore et al., 2007). Fluazuron is a benzoylphenylurea derivative that regulates tick growth by inhibiting chitin incorporation into the tick's cuticle, acts systemically, and has acaricidal properties; this was released in the market in 1994, and to date had been considered the only AI without any evidence of resistance, despite the remarkable increase in its use to control cattle

tick-resistant populations, particularly in Southern Brazil (Kemp et al., 1990; Graf et al., 1994). This study aimed to describe the first report of fluazuron resistance in *R. microplus*, and also report the first tick strain that was multi-resistant to six classes of acaricides.

2. Materials and methods

2.1. Tick strains

2.1.1. Jaguar strain

Ticks were collected from beef cattle at a ranch located in the municipality of Eldorado do Sul, RS, Southern Brazil. This farm only breeds *Bos taurus taurus* cattle, and apart from raising beef cattle, it breeds bulls for sale. Additionally, it has an intense animal trade activity with other ranches, exchanging cattle with several other properties in RS and others states of Brazil. Since 2010, there has been a suspicion of fluazuron treatment failure as reported by property staff. In the past years, cattle have been heavily infested with ticks at this location, particularly during the late summer and mid-autumn (February to May). Tick control had been based on fluazuron treatments (Acatak® Pour On, Novartis Saúde Animal, Barueri, SP, Brazil), pour-on application and dipping vats with a mixture of chlorpyrifos and cypermethrin (Colosso® Pour On and Colosso® Pulverização, Ourofino Saúde Animal, Cravinhos, SP, Brazil), and the periodic use of long-acting formulations of avermectins. This property had a history of a high level of tick resistance to cypermethrin, deltamethrin, amitraz, mixture of cypermethrin + chlorpyrifos, and mixture of cypermethrin + ethion, according to data from previous adult immersion tests (AIT) conducted at IPVDF (data not shown). On February 11th, 2011, ticks from this ranch were sampled to establish a laboratory colony. At that time, the frequency of treatment with fluazuron was shorter than every six weeks, and the frequency of treatments using mixtures of organophosphate and pyrethroid was less than every three weeks. Even with these practices to attempt control, the majority of cattle was observed to be highly infested with all stages of *R. microplus*. Some bulls had more than 700 adult ticks. Ticks referred to as "Jaguar R" were obtained by the selection of ticks which survived the first AIT bioassays, as described below.

2.1.2. São Gabriel (SG) strain

Ticks were obtained from cattle at FEPAGRO Experimental Station of São Gabriel, municipality of São Gabriel, RS, Brazil (30°20' S, 54°15' W). This experimental station has a closed herd, *i.e.* it was maintained without the introduction of cattle from other farms for more than 10 years. The tick population from this herd has a history of resistance against synthetic pyrethroids, amitraz and macrocyclic lactones (Martins and Furlong, 2001); however, it was never exposed to fluazuron, so it was considered a susceptible field strain for this study.

2.1.3. Porto Alegre (POA) strain

These ticks were collected from a ranch along the border of Brazil and Uruguay in 1992. It has been kept at Porto Alegre municipality, RS, Brazil, in the facilities of

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