Acaricide Resistance Status of the Rhipicephalus microplus in Brazil: A Literature Overview

Leandro de Oliveira Souza Higa¹, Marcos Valério Garcia¹, Jacqueline Cavalcante Barros¹, Wilson Werner Koller¹ and Renato Andreotti¹*

¹Animal Health, Empresa Brasileira de Pesquisa Agropecuária – Embrapa Beef Cattle, Campo Grande/MS, Brazil
²Post Graduate Program in Animal Science, Universidade Federal de Mato Grosso do Sul, Campo Grande/MS, Brazil

Abstract

Rhipicephalus microplus is a monoxenous species whose main hosts are bovines, leading to considerable losses in cattle production worldwide. In addition, this tick is the transmitter of pathogenic agents responsible for the bovine babesiosis and anaplasmosis (BBA) complex. These ectoparasites are still mainly controlled with chemical products. The constant exposure of ticks to acaricides, associated with the lack of adequate application procedures, accelerates the selection pressure of resistant individuals in the population, inevitably increasing resistance, as reported by many authors around the world. This article presents current information about the resistance situation of R. microplus in Brazil. We identify and discuss the main chemical bases being used as a means to guide new research.

Keywords: Boophilus; Acaricides; Bovine tick

Introduction

As the world’s second largest beef producer, Brazil is estimated to have a herd of 212.8 million bovines and export around 1.5 million tons of carcasses per year [1]. The Rhipicephalus microplus tick (Canestrini, 1888), previously known as Boophilus microplus, is the main tick found in Brazil that directly affects cattle, causing economic losses. According to Gomes [2], the economic losses caused by the Rhipicephalus microplus tick are due to a reduction in meat and milk production, as well as a devaluation of leather due to skin lesions caused by high infestations and the possible installation of myiasis. This tick species is responsible for the transmission of babesiosis (Babesia bovis and B. bigemina) and anaplasmosis (Anaplasma marginale), hemoparasites that characterize the disease named Bovine Babesiosis and Anaplasmosis (BBA) Complex. This tick also causes indirect economic harm due to the disease named Bovine Babesiosis and Anaplasmosis (BBA) Complex. This tick also causes indirect economic harm due to the disease named Bovine Babesiosis and Anaplasmosis (BBA) Complex. This tick also causes indirect economic harm due to the disease named Bovine Babesiosis and Anaplasmosis (BBA) Complex. This tick also causes indirect economic harm due to the disease named Bovine Babesiosis and Anaplasmosis (BBA) Complex.

The time or number of necessary applications it takes for a resistant allele to be established in the population and how long it takes for acaricides to lose their effectiveness depend on several variables. The most relevant of these are: the manner in which the resistant allele was inherited (dominant, co-dominant or recessive), the mutation frequency in the population before the first acaricide application, the number of individuals that for different reasons have not been in contact with the acaricide, and the acaricide application frequency together with the concentration dosage used [7]. Despite the difficulty in conducting such an evaluation, six applications are necessary every year, which may contribute to the emergence of resistant individuals [8].

One of the main problems currently facing the national cattle industry with regard to ectoparasite (mainly ticks) treatment and control is the lack of an official control policy managed by competent institutions. This leaves producers with the responsibility of selecting such control criteria. Generally, acaricides are not chosen based on their efficiency, their application is inadequate, and information about parasite regional epidemiology is neglected. The continuous use of products and their inadequate application throughout an extended period may promote population selection of acaricide resistant ticks, increasing the resistance problem [5].

It is clearly necessary to detect resistance early on and to use acaricides correctly, especially considering that it is becoming increasingly difficult to develop new molecules able to exert efficient control.

Resistance Development

Even though ways of developing tick resistance have yet to be elucidated, it is known that the constant and indiscriminate use of chemical products has led to the selection of mutations and the development of resistance in part of the tick population. This process is known as the establishment of the resistance allele [5]. This resistance may also occur naturally, with one in every one million individuals being born resistant [6].

After the resistant allele is established, selection pressure makes ticks carrying the resistance genes more representative in the overall population, which will in turn produce more resistant descendants [5].

As mentioned above, many types of chemical compounds are used to make acaricides, and most are based on organophosphate, pyrethroids, macrocyclic lactones, amitraz, fipronil or fluazuron [9]. Thus, the tick population may generate different forms of acquired resistance, which may vary depending on the selection pressure and give rise to different genetic characteristics in different degrees [10].

*Corresponding author: Renato Andreotti, Animal Health, Empresa Brasileira de Pesquisa Agropecuária – Embrapa Beef Cattle, Av. Rádio Maia nº 830, Zona Rural, CEP 79106-550, Campo Grande, MS, Brazil, Tel: +55 (67) 3368-2173; E-mail: renato.andreotti@embrapa.br

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Given these facts, the main resistance mechanisms may be divided into:

- Metabolic/Detoxification resistance: increase in the detoxification process and/or elimination of chemicals. This is usually due to increased expression or specificity of the enzyme responsible for drug metabolism [11]. Some insects respond to the presence of xenobiotic molecules by over-expressing the enzymes belonging to the cytochrome P450 complex [12]. Esterases (Est) and glutathione S-transferase are also part of the main protein families involved in the tick response [13].

- Resistance by action site insensitivity: modification in the nucleotide coding region may change the amino acids, which may in turn change the three-dimensional structure of the final protein. All these changes may interfere with the binding of molecules to the action site [14].

By studying the method of action of acaricides, it has been possible to explain the molecular actions that cause resistance, which includes increased expression of genes or of the activity of enzymes involved in xenobiotic/detox metabolism and mutations in neuroreceptors in acaricide-related neuromodulators, depending of the acaricide class (i.e, acaricide resistance in each class may have different causes). Xenobiotic/detox metabolism can also be associated with the cellular capacity to modulate drug molecules outside the cell. There are also acaricides that act on the nervous system of arthropods by affecting presynaptic axons, neurotransmitters or postsynaptic receptors, and by causing mutations in sodium channels (some acaricides use sodium channels to control the tick) [15-18].

Acaricide Resistance

Tick resistance to arsenicals, which were among the first pesticides to be developed, was first reported in 1936, in Australia, and then in 1938, in Africa [19]. In Brazil, as well as in other countries (e.g., Argentina and Australia), the resistance to the first acaricide compounds (arsenicals, organ chlorines, organophosphates and carbamates) developed quickly [20]. Resistance to arsenicals was first reported in Brazil in 1953 [21]. Figure 1 illustrates the resistance distribution in Brazil for six different acaricide classes as well as their respective associations (organophosphates and pyrethroids; organophosphates and amidines). In the first published report of resistance for six acaricide classes (organophosphates, synthetic pyrethroids, amidines, macrocyclic lactones, fipronil and fluazuron) conducted in Brazil (Rio Grande do Sul), Reck et al. [22] showed fluarazon resistance in the *R. microplus* tick population.

Here we analyzed 69 descriptions of acaricide resistance from different areas in Brazil found in 32 reports published as research articles or conference abstracts. A total of 44.93% of reports came from southern Brazil, possibly due to the high regional livestock activity associated with the intense use of acaricides for controlling *R. microplus* (Table 1).

New methodologies have been developed for resistance diagnosis, such as the larval immersion test [23] and larval tarsal test [24]. These tests were found to be efficient, and could be an option to those suggested by FAO (such as the adult immersion test and the larval packet test).

It is important to note that currently available tests are only useful to diagnose the problem once it is established, but they do not work to detect resistance before it occurs. Therefore, they are not useful for preventive measures against the resistance problem.

Organophosphates

Organophosphates first appeared in 1956 [20] to substitute the organ chlorines, which showed resistance to different tick species (*Rhipicephalus microplus, R. appendiculatus, R. decoloratus*) and high residual power in the environment [25].Organophosphates did not present residual power, suggesting a treatment interval of 21 days. Currently they can be found in association with pyrethroids or larval control products.

The class is characterized by its action mechanism, i.e., acetylcholinesterase (AChEs) enzyme inhibition [26]. Baffi et al. [27] also found a relation of these enzymes with organophosphates and pyrethroids. Temeyer [28] also verified that one of the three AChEs enzymes in the study were insensitive to phosphate compounds. The lack of this enzyme causes an increase in acetylcholine that becomes toxic for ticks, causing increased muscle contraction and paralysis.

Organophosphate resistance

Tick resistance to organophosphates is normally associated with a single semi-dominant gene; in other words, heterozygous individuals also present resistance, although to a lesser extent than homozygous individuals. The mechanism of resistance to organophosphates has yet to be entirely understood, but the involvement of a few processes is well-known, such as acetylcholinesterase insensitivity [29], the increase in esterase metabolism in the integument of the engorged resistant ticks, and the super expression of these enzymes in larvae [30].

In 1963, the herd in Rio Grande do Sul was mainly composed of European breeds, which are more susceptible to ticks. This explains the high tick infestations and their development of resistance to...

Table 1: Resistance to organophosphates (OP), pyrethroids, amidine (AM), macrocyclic lactones, fipronil and fluazuron classes; and associations between amidine and organophosphate (AM + OP), organophosphate and pyrethroid (OP + SP). This table shows 70 resistance descriptions found in 33 publications distributed in the Brazilian states. Resistance reports were found in RS (Rio Grande do Sul), SC (Santa Catarina), PR (Paraná), SP (São Paulo), RJ (Rio de Janeiro), MG (Minas Gerais), MS (Mato Grosso do Sul), GO (Goiás), RO (Rondônia), BA (Bahia), PE (Pernambuco), PB (Paraíba) and RN (Rio Grande do Norte).

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Organophosphates [31]. In 1970, most tick populations had already presented organophosphate acaricide resistance [20].

**Aminides (diaminicals)**

In the 1970’s, organophosphates were replaced by aminides, which have a residual power of 14 days, thus allowing for larger treatment intervals. Aminides are among the most popular acaricides for bovine tick control in Australia, Latin America and part of Africa. They present low toxicity for bovines and humans, and do not have a restriction period for slaughter [32].

In ticks, aminides are metabolized in a compound named N-2,4 – dimetilfenil N-metaformamidine, which strongly affects egg laying and is toxic to the tick’s different life phases, especially the larval phase.

This product has been linked to enzymes important for tick metabolism, such as mono oxidase [33]. Baxter and Barker [34] had no success to correlate the octopamine/tyramine receptor with amitraz *R. microplus* resistant population and Corley et al. [35] verified the increased frequency of the octopamine receptor gene (*RmβAOR*) in strains that went through selection pressure conditions with the use of amitraz, therefore correlating it to the resistance of this class.
Resistence to amidines

Evidence for amitraz resistance was reported in Rio Grande do Sul in 1994 [36] and later again in 1999 [37].

The highest resistance level to amitraz belongs to the Santa Luiza strain, which is 154 times more resistant than the R. microplus control strain [38].

Between 2005 and 2011, engorged female sensitivity and resistance to amitraz and cypermethrin was evaluated in Rio Grande do Sul. The number of ticks resistant to amitraz throughout the study period ranged from 48% to 85% of the total analyzed, while cypermethrin resistance ranged from 29% to 75%, and both compounds were considered ineffective in combating this ectoparasite [39].

In a recent study conducted in Rio Grande do Norte by Coelho et al. [40], 5% cypermethrin had superior average efficacy (95.1%) than 12.5% amitraz (84.6%). Mendes et al. [41] also found amitraz resistant populations in the states of São Paulo and Paraná, by using the Larvae Immersion Test.

Pyrethroids

Pyrethroids were created in 1977, and the development of organophosphate resistance led to a strong effort to implement their use. Two application methods were developed: pulverization, with a 21-day interval, and, "pour-on", with a residual period of 7 days.

Voltage-gated sodium channels are the target of pyrethroid activity and resistance development. Miller et al. [42], working with pyrethroid-resistant tick populations in Mexico, and He et al. [43], who used gene sequencing, identified the substitution of a specific amino acid in domain III (fenilamine for isoleucine) of the R. microplus sodium channel in these populations.

A PCR diagnostic test [44] was developed, which allowed the quick detection of this amino acid substitution in ticks, larvae and eggs. A large number of ticks were subjected to this methodology and this mechanism was confirmed in all samples studied in Mexico [45].

According to Lovis et al. [15], the L641 mutation in Domain II, of the sodium channels, responsible for the resistance of various pyrethroids, has a wide distribution. The authors found the presence of tick populations resistant to pyrethroids in Brazil, Argentina, Australia and South Africa. In this work, a mutation in Domain III has also been reported, but only in Mexico, what suggests a geographical separation for the occurrence of mutations in these two domains. Domingues et al. [46] also found in Brazil the C190A (Domain II) mutation in Minas Gerais.

Andreotti et al. [47] verified the absence of this sodium channel mutation in tick samples resistant to pyrethroids in Mato Grosso do Sul, Brazil. The domain III sodium channel mutation was also not detected in Brazilian or Australian tick populations [48]. However, Morgan et al. [49] and Jonsson et al. [50] reported nucleotide differences in the domain II region of the R. microplus sodium channel in pyrethroid-resistant populations in Australia. Lovis et al. [15] also found a specific mutation for flumethrin (G72V) only in Australia (the other countries in the study were Brazil, Argentina, Mexico and South Africa).

Using tick samples from the US and Mexico, Stone et al. [51] confirmed the presence of mutations not only in domain III (T2134A), but also in domain II (C190A). In addition, the mutation known as super-knockdown (M918T/T170C), previously found only in insects, was also identified.

Resistance to pyrethroids

In 1987, in vitro tests with engorged ticks were conducted in the state of São Paulo to evaluate the efficiency of six acaricides: thriclorfon + coumaphos, amitraz, decamethrin, fenvalerate and cypermethrin high-cis. The authors concluded that R. microplus is resistant to active principles of decamethrin and fenvalerate, both synthetic pyrethroids [52].

In in vitro tests conducted in 1988 in Rio de Janeiro, found that R. microplus sensitivity was lower for pyrethroid-based acaricides: cypermethrin, alfamethrin, and fenvalerale, among others [53].

In vitro tests conducted in the state of Pernambuco showed that the bovine tick was resistant to amidines, synthetic pyrethroids, cypermethrin and deltamethrin compounds [54].

In 2001, the effects of cypermethrin, deltamethrin and permethrin were evaluated on R. microplus tick larvae collected in Goiânia, in the state of Goiás. Permethrin at 2,500 ppm eliminated 100% of ticks, and was the only one that reached the recommended efficiency levels (>95%) established in Brazil [55]. Tick populations resistant to synthetic pyrethroids have also been identified in the states of São Paulo, Paraná, Mato Grosso do Sul and Minas Gerais [36].

In 2008, the Santa Luiza R. microplus strain from southern Brazil was found to be resistant to permethrin and amitraz [56]. Using bioassays, Mendes et al. [57] observed that tick populations from Rio Grande do Sul and Mato Grosso do Sul were resistant to cypermethrin, deltamethrin and flumethrin. This was the first report of resistance to flumethrin in Brazil.

In 2011 in Mato Grosso do Sul, Gomes et al. reported that R. microplus resistance to organophosphates, synthetic pyrethroids and amidines was mostly inefficient (<95%). Only two associations showed harmful effects on ticks: 60% DDVP + 20% chlorfenvinphos and 15% cypermethrin + 25% chlorpyriphos + 15% piperonyl butoxide + 1% citronella [58].

Phenylpyrazoles

The phenylpyrazoles were developed in the 1980s and made available on the market in the 1990s for use in agriculture and veterinary medicine [59,60]. Their application method is "pour-on", and they are prohibited for lactating animals.

Their mechanism of action is through neuronal modulation exerted by the fipronil molecule, causing GABA (γ-Amino butyric acid) receptor antagonism and blocking chloride channels [61], leading to hypereexcitation and death [59].

Originally, fipronil was used to control agricultural pests and other insects, and was later used for cattle ectoparasites. The use of products based on fipronil for agricultural use can interfere with R. microplus control and contribute to the process of compound resistance [62].

Resistance to phenylpyrazoles

The resistance of R. microplus to fipronil in field populations was first documented in Brazil and in Uruguay [62]. Between 2006 and 2009, in the city of Lages, in Santa Catarina, a study was conducted using 1% fipronil for 14 treatments. Using the stable test, it was found that after six treatments, effectiveness decreased from 100 to 79.3%, suggesting a partial resistance to the product [63].

Thiazolines

The formulation of thiazoline, the representative of the new
acaricide generation, contains pyrethroid. This acaricide may be applied either through pulverization or immersion. The waiting period for slaughter is three days and its use is allowed for lactating animals. To this date, there are no reports in the literature showing resistance to this active ingredient. However, Furlong et al. [64] analyzed various active ingredients and associations between 1997 and 2006 and verified that a product containing thiazole in association with one pyrethroid had an average efficiency of 61.2%.

**Macroyclic Lactones**

Macroyclic lactones first appeared in the early 1980s. These compounds are derived from products obtained from the fermentation of actinomycete fungi of the *Streptomyces* genus. Their residual power is stronger than that of pyrethroids and their parasiticide spectrum is broader. They are also efficient on different parasitic worms and *Dermatobia hominis* larvae.

According to Martin et al. [65], macroyclic lactones act by blocking the transmission of electrical activity in nerves and muscle cells, stimulating GABA release and binding in nerve endings. This causes an influx of chloride ions into the cell, causing a hyperpolarization and subsequent paralysis of the neuromuscular system [66].

**Resistance to macroyclic lactones**

The first report of macrocyclic lactone resistance in Brazil was in Rio Grande do Sul [67]. Employing the larvae immersion test (LIT), Klafke et al. [68] published the first report of resistance to ivermectin diagnosed in vitro. They also detected resistance to doramectin (avermectin) using the adult immersion test (AIT). Four years later, Klafke et al. [69] also reported resistance to ivermectin in larvae in the state of São Paulo using the larvae immersion test (LIT) and the packet larvae test (PLT).

**Fluazuron**

Fluazuron is a benzoyl phenyl urea derivative that regulates tick growth by inhibiting the incorporation of chitin in the cuticle [70]. Cruz et al. [71] suggest that fluazuron has harmful effects only on the hatchability of larvae from treated female ticks. They also analyzed the association between 3.0 g fluazuron and 0.5 g abamectin and concluded that the association with macrocyclic lactones had harmful effects against the reproductive parameters of *R. microplus* females.

**Resistance to fluazuron**

The first report of tick resistance to fluazuron in Brazil was reported by Reck et al. [22], who studied a tick population from Rio Grande do Sul. These authors observed resistance to all major classes of acaricides used in the country.

**Spinosad**

Spinosad, a recent chemical group generated from the fermentation of the *Saccharopolyspora spinosa* fungus (from the actinomycetes species), which is found naturally in soil, can be used in lactating cows [64]. This active ingredient was originally developed for insect control in agriculture, and it was the first representative of the natural products class (Naturallyte). This product has neuromuscular action, initially causing involuntary muscular contractions, followed by paralysis [72].

Spinosad is effective against *R. microplus* [73], *R. turanicus* and *Argas persicus* [74], and no resistance reports have been published. In *in vitro* tests, spinosad was also effective against *Amblyomma americanum* and *Dermacentor variabilis* [75]. Although it has not been widely used, in Brazil, spinosad has been shown to have an average effectiveness of 99.96% in *R. microplus* populations in farms in the state of Rondônia [76].

**How to Reduce Acaricide Resistance**

**Use of diagnostic tests**

To preserve acaricide efficacy, it is recommended the use in vitro bioassay tests to evaluate acaricide resistance before the application of the products, allowing the use of the most adequate chemical base. The tests normally used are: AIT (Drummond et al., 1973), LPT (FAO, 1984) and LIT (Shaw, 1966).

The AIT tests use engorged females, immersing them in flasks with the acaricides commercial dilutions. Posteriorly, the reproductive parameters and larvae hatching reveal the products' efficacy.

The LPT test uses larvae, which are left for a 24h period in filter paper packets embedded with different products. Posteriorly, the mortality rate is evaluated. The LIT test has a similar methodology to the AIT, testing larvae with acaricide dilutions. This test is important to diagnose the efficacy of pour on acaricides, such as macrocyclic lactones (Klafke et al., 2006).

**Combination acaricides and regular monitoring**

We can observe in the literature reported here in that most resistance cases are regarding products with just one chemical class. Therefore, combining chemical classes and monitoring resistance through diagnostic tests, in association with the correct acaricide application, are a means of increasing the durability of efficient products, diminishing the emergence of resistance.

**Conclusions**

Several recent publications have detailed the mechanisms of action and resistance to pyrethroids, including the different mutations that cause resistance in ticks. This type of work contributes to a better understanding of molecular diagnoses.

Active ingredients like flumethrin (pyrethroid) and fluazuron have also been recently added to the list of chemical groups to which ticks have developed resistance. There have also been several reports of resistance to fipronil and most macrocyclic lactones. This highlights the need to create tick control programs in Brazil, which require time and money.

There are differences in acaricide efficacy between *R. microplus* populations, both in Brazil and worldwide. These differences can be observed between neighboring properties, and are mostly due to the lack of standardization in the choice of control products and a failure to follow manufacturers’ recommendations. Therefore, the use of tests to evaluate tick resistance in each farm’s population becomes increasingly necessary, in order to obtain greater bovine tick control and prevent the emergence of increasingly resistant populations (including multiresistant ones).

**References**


28. Temeay KB, Pruett JH, Olafson PU (2010) Baculovirus expression, biochemical characterization and organophosphate sensitivity of rBmACHe, rBmACHeZ, and rBmACHe3 of Rhipicephalus (Boophilus) microplus. Vet Parasitol 172: 114-121.


