Medicinal chemistry

de Oliveira Souza Higa et al.. Med chem 2015, 5:7
DOI: 10.4172/2161-0444.1000281

Review Article Open Access

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

Leandro de Oliveira Souza Higa[2], Marcos Valério Garcia[1], Jacqueline Cavalcante Barros[1], Wilson Werner Koller[1] and Renato Andreotti[1*]

[1]Animal Health, Empresa Brasileira de Pesquisa Agropecuária – Embrapa Beef Cattle, Campo Grande/MS, Brazil
[2]Post 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 additional hours of work required, additional facility costs, acaricide acquisition and equipment used for its application [2]. Recent studies estimate that *R. microplus* parasitism causes in livestock potential losses around US$ 3.24 billion in Brazil per year [3].

It is widely known that acaricides have been used as the main way of controlling this ectoparasite since the 1895, by using arsenicals [ARTECHE *apud* 4]. According to Furlong and Prata [5], these chemical compounds include organophosphates, amidines, pyrethroids, phenylpyrazoles, cymiazole, naturalyte (contact acaricides), macrocyclic lactones, phenylpyrazoles (systemic), and more recently, fluazuron.

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].

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].

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

Received June 19, 2015; **Accepted** July 17, 2015; **Published** July 21, 2015

Citation: de Oliveira Souza Higa L, Garcia MV, Barros JC, Koller WW, Andreotti R (2015) Acaricide Resistance Status of the *Rhipicephalus microplus* in Brazil: A Literature Overview. Med chem 5: 326-333. doi: 10.4172/2161-0444.1000281

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.

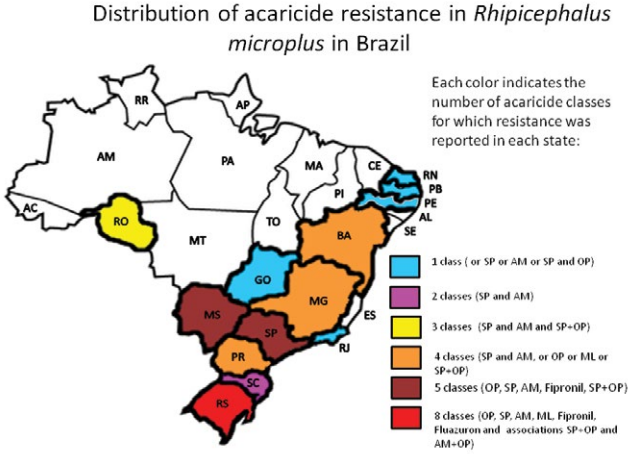

Figure 1: accumulated resistance to organophosphates (OP), syntethic pyrethroids (SP), amidine (AM), macrocyclic lactones (ML), fipronil and fluazuron. The associations between organophosphates and pyrethroids (OP+SP) and amidines and organophosphates (AM+OP) were also considered as classes. The number of resistance classes evaluated are shown with a color code indicating at least one resistance report, and each report is related to a class. We included a total of 70 descriptions found in 33 publications, representing a 'sampling' of the current resistance situation in Brazil.
Sources: Andreotti et al. [47]; Brito et al. [76]; Camillo et al. [77]; Campos Junior and Oliveira [78]; Castro-Janer et al. [62]; Coelho et al. [40]; Domingues et al. [46]; Farias et al. [79]; Fernandes [55]; Flausino et al. [54]; Furlong [37]; Gomes et al. [58]; Klafke et al. [68]; Koller et al. [80]; Laranja et al. [81]; Leite [53]; Lopes et al. [82]; Machado et al. [83]; Martins [84]; Martins et al. [85]; Martins et al. [67]; Mendes et al. [41]; Mendes et al. [8]; Mendes et al. [86]; Pereira [87]; Raynal et al. [88], Reck et al. [22]; Santana et al. [89]; Santos et al. [90]; Silva et al. [91]; Silva et al. [92]; Souza et al. [93]; Veiga et al. [94].

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 larvae 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

States	Organophosphates	Pyrethroids	Aminidine	Macrocyclic Lactones	Fipronil	Fluazuron	SP + OP	AM + OP
RS	(Wharton; Roulston, 1977)[31] (Martins, 1995) [84] (Farias et al, 2008) [79] (Reck et al., 2014) [22]	(Laranja et al, 1989) [81] (Martins, 1995) (Martins et al., 1992) [84] (Farias et al, 2008) [79] (Santos et al., 2008) [90] (Camillo et al., 2009) [77] (Mendes et al., 2013) [41] (Reck et al., 2014) [22] (Machado et al., 2014) [83]	(Farias et al., 2008) [79] (Santos et al., 2008) [90] (Camillo et al., 2009) [77] (Reck et al., 2014) [22] (Machado et al., 2014) [83]	(Martin; Furlong, 2001) [67] (Reck et al., 2014)[22]	(Castro-Janer et al., 2010) [62] (Reck et al., 2014)[22]	(Reck et al., 2014)[22]	(Farias et al., 2008) [79] (Camillo et al., 2009) [77] (Machado et al., 2014)[83]	Machado et al., 2014) [83]
SC		(Veiga et al., 2012) [94]	(Veiga et al., 2012) [94]					
PR		(Souza et al., 2003) [93]	(Souza et al., 2003) [93]				(Souza et al., 2003) [93]	
SP	(Mendes et al, 2001) [8] (Mendes et al., 2011) [8]	(Mendes et al., 2001) [8] (Pereira, 2006) [87] (Mendes et al., 2011) [8]	(Pereira, 2006) [87]	(Klafke et al., 2006) [68]	(Castro-Janer et al., 2010) [62]		(Pereira, 2006) [87] (Mendes et al., 2011) [8]	
RJ		(Leite, 1988) [53] (Flausino et al., 1995)[54]						
ES								
MG	(Domingues et al., 2012) [46]	(Domingues et al., 2012) [46]	(Furlong, 1999) [37]	(Lopes et al., 2014) [82]				
MS	(Koller et al., 2009) [80] (Gomes et al., 2011) [58]	(Koller et al., 2009) [80] (Mendes et al., 2013) [41] (Gomes et al., 2011) [58]	(Koller et al., 2009) [80] (Gomes et al., 2011) [58]		(Castro-Janer et al., 2010) [62]		(Gomes et al., 2011) [58] (Andreotti et al., 2011) [47]	
MT								
GO		(Fernandes, 2001) [55] (Silva et al. 2000) [91]						
RO		(Brito et al., 2011) [76]	(Brito et al., 2011) [76]				(Brito et al., 2011) [76]	
AC								
AM								
PA								
RR								
AP								
TO								
BA	(Raynal et al., 2013) [88]	(Campos Junior; Oliveira, 2005) [78] (Raynal et al., 2013) [88]	(Campos Junior; Oliveira, 2005) [78]				(Campos Junior; Oliveira, 2005) [78] (Raynal et al., 2013) [88]	
PI								
MA								
CE								
SE								
AL								
PE							(Santana et al., 2013) [89]	
PB		(Silva et al., 2005) [92]						
RN			(Coelho et al., 2013) [40]					

Table 1: Resistance to organophosphates (OP), pyrethroids, amidine (AM), macrocyclic lactones, fipronil and fluazuron classes; and associations between aminidine 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) e RN (Rio Grande do Norte).

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

Amidines (diaminicals)

In the 1970's, organophosphates were replaced by amidines, which have a residual power of 14 days, thus allowing for larger treatment intervals. Amidines 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, amidines are metabolized in a compound named N-2,4 – dimetilfenil N-metilformamidine, 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.

Resistance 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] 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 (fenilanine 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 L64I 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: thrichlorfon + coumaphos, amitraz, decamethrin, fenvalerate and cypermethrin high-cis. The authors concluded that *R. microplus* is resistant to active principles of decametrina 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 fenvalerate, 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 chlorine channels [61], leading to hyperexcitation 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 thiazoline in association with one pyrethroid had an average efficiency of 61.2%.

Macrocyclic Lactones

Macrocyclic 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], macrocyclic 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 macrocyclic 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 fluarazon 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 fluarazon

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 (Naturalyte). 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 test uses 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 (Klakfe 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 multi-resistant ones).

References

1. ANUALPEC (2014) Anuário brasileiro da pecuária.
2. Gomes A (1998) O carrapato-do-boi Boophilus microplus: ciclo, biologia, epidemiologia, patogenia e controle. In: Kessler RH, Schenk, MA. M. (Ed.). Carrapato, tristeza parasitária e tripanossomose dos bovinos.
3. Grisi L, Leite RC2, Martins JR3, Barros AT4, Andreotti R4, et al. (2014) Reassessment of the potential economic impact of cattle parasites in Brazil. Rev Bras Parasitol Vet 23: 150-156.
4. Merlini LS, Yamamura MH (1998) Estudo in vitro da resistência de Boophilus

microplus a carrapaticidas na pecuária leiteira do norte do estado do Paraná. Semina: Ci. Agrárias 19: 38-44.

5. Furlong J, Prata JRS (2000) Resistência dos carrapatos aos carrapaticidas. Embrapa Gado de Leite.
6. Roush RT1 (1993) Occurrence, genetics and management of insecticide resistance. Parasitol Today 9: 174-179.
7. Food and Agriculture Organization – FAO (2003) Producción y Sanidad Animal. In: Food and Agriculture Organization – FAO. Resistencia a los antiparasitarios: Estado actual com énfasis en América Latina. 157a ed. Roma: FAO.
8. Mendes MC, Lima CK, Nogueira AH, Yoshihara E, Chiebao DP, et al. (2011) Resistance to cypermethrin, deltamethrin and chlorpyriphos in populations of Rhipicephalus (Boophilus) microplus (Acari: Ixodidae) from small farms of the State of SÃ£o Paulo, Brazil. Vet Parasitol 178: 383-388.
9. SINDAN (Sindicato Nacional da Indústria de Produtos para Saúde Animal), Compêndio de Produtos Veterinários SINDAN 2014.
10. Freitas DRJ, de Polh PC, Vaz Junior I da S (2005) Caracterização da resistência para acaricidas no carrapato Boophilus microplus. Act Scien Vet 33: 109-17.
11. Li X, Schuler MA, Berenbaum MR (2007) Molecular mechanisms of metabolic resistance to synthetic and natural xenobiotics. Annu Rev Entomol 52: 231-253.
12. Hemingway J, Field L, Vontas J (2002) An overview of insecticide resistance. Science 298: 96-97.
13. Ranson H, Claudianos C, Ortelli F, Abgrall C, Hemingway J, et al. (2002) Evolution of supergene families associated with insecticide resistance. Science 298: 179-181.
14. Pereira MC, Labruna MB, Szabó MP, Klafke GM (2008). Rhipicephalus (Boophilus) microplus (biologia, controle e resistência). (1stedn), MedVet Livros, São Paulo, Brazil.
15. Lovis L, Guerrero FD, Miller RJ, Bodine DM, Betschart B, et al. (2012) Distribution patterns of three sodium channel mutations associated with pyrethroid resistance in Rhipicephalus (Boophilus) microplus populations from North and South America, South Africa and Australia. Int J Parasitol 2: 216 - 224.
16. Martin T, Ochou OG, Vaissayre M, Fournier D (2003) Oxidases responsible for resistance to pyrethroids sensitize Helicoverpa armigera (HÃ¼bner) to triazophos in West Africa. Insect Biochem Mol Biol 33: 883-887.
17. Oakeshott JG, Home I, Sutherland TD, Russell RJ (2003) The genomics of insecticide resistance. Genome Biol 4: 202.
18. Rufingier C, Pasteur N, Lagnel J, Martin C, Navajas M (1999) Mechanisms of insecticide resistance in the aphid Nasonovia ribisnigri (Mosley) (Homoptera: Aphididae) from France. Insect Biochem Mol Biol 29: 385-391.
19. Wharton RH, Norris KR (1980) Control of parasitic arthropods. Vet Parasitol 6: 135-64.
20. Wharton RH (1974) Ticks with special emphasis on Boophilus microplus. Control of Arthropods of Med and Vet Imp 35-52 (in press).
21. Freire JJ (1953) Arseno e cloro-resistência e emprego do Tiofosfato de Dietilparanitrofenila (parathion) na luta anticarrapato Boophilus microplus (Canestrini, 1888). Bol Dir Prod Anim 9: 3-31.
22. Reck J, Klafke GM2, Webster A, Dall'Agnol B, Scheffer R, et al. (2014) First report of fluazuron resistance in Rhipicephalus microplus: a field tick population resistant to six classes of acaricides. Vet Parasitol 201: 128-136.
23. Klafke GM, Castro-Janer E, Mendes MC, Namindome A, Schumaker TT (2012) Applicability of in vitro bioassays for the diagnosis of ivermectin resistance in Rhipicephalus microplus (Acari: Ixodidae). Vet Parasitol 184: 212-220.
24. Lovis L, Mendes MC, Perret JL, Martins JR, Bouvier J, et al. (2013) Use of the Larval Tarsal Test to determine acaricide resistance in Rhipicephalus (Boophilus) microplus Brazilian field populations. Vet Parasitol 191: 323-331.
25. George JE, Pound JM, Davey RB (2008) Acaricides for controlling ticks on cattle and the problem of acaricide resistance: 415-416. Ticks: biology, disease and control. (1stedn) Bowman AS, Nuttall PA, editors.
26. Guerrero FD, de León AAP, Rodriguez-Vivaz, Jonsson N, Miller RJ, et al. (2014) Acaricide research and development, resistance, and resistance monitoring. Biology of Ticks. (2nd ed.) Edited by Sonenshine DE, Roe RM, Oxford University Press: 353 - 374.
27. Baffi MA, de Souza GRL, de Sousa CS, Ceron CR, Bonetti, AM. (2008) Esterase enzymes involved in pyrethroid and organophosphate resistance in a Brazilian population of Riphicephallus (Boophilus) microplus (Acari, Ixodidae). Mol Biochem Parasit 160: 70-73.
28. Temeyer KB, Pruett JH, Olafson PU (2010) Baculovirus expression, biochemical characterization and organophosphate sensitivity of rBmAChE, rBmAChE2, and rBmAChE3 of Rhipicephalus (Boophilus) microplus. Vet Parasitol 172: 114-121.
29. Foil LD, Coleman P, Eisler M, Fragoso-Sanchez H, Garcia-Vazquez Z, et al. (2004) Factors that influence the prevalence of acaricide resistance and tick-borne diseases. Vet Parasitol 125: 163-181.
30. Villarino MA, Waghela SD, Wagner GG (2001) Histochemical localization of esterases in the integument of the female Boophilus microplus (Acari: Ixodidae) tick. J Med Entomol 38: 780-782.
31. Wharton RH, Roulston WJ (1977) Acaricide resistance in Boophilus microplus in Austrália. In: Workshop on hemoparasites (Anaplasmosis and Babesiosis). Cent Inter Agr Trop 2: 73-92.
32. Jonsson NN, Hope M (2007) Progress in the epidemiology and diagnosis of amitraz resistance in the cattle tick Boophilus microplus. Vet Parasitol 146: 193-198.
33. Holden JS, Hadfield JR (1975) Chlorodimeform and its effect on monoamine oxidase activity in the cattle tick, Boophilus microplus. Experientia 31: 1015-1017.
34. Baxter GD, Barker SC (1999) Isolation of a cDNA for an octopamine-like, G-protein coupled receptor from the cattle tick, Boophilus microplus. Insect Biochem Mol Biol 29: 461-467.
35. Corley SW, Jonsson NN, Piper EK, Cutulle C, Stear MJ, et al. (2013) Mutation in the RmÎ²AOR gene is associated with amitraz resistance in the cattle tick Rhipicephalus microplus. Proc Natl Acad Sci U S A 110: 16772-16777.
36. Nolan J (FAO/UN 1994) Acaricide resistence in the cattle tick Boophilus microplus. Report of Workshop Leader, 21-25
37. Furlong J (1999) IV Seminario internacional de parasitologia animal - Control de la resistancia en garrapatas y moscas de importancia veterinaria y enfermededades que transmiten. Diagnosis of the susceptibility of the cattle tick, Boophilus microplus, to acaracides, Minas Gerais, Brazil 4: 41–6.
38. Miller RJ, Davey RB, George JE (2002) Modification of the food and agriculture organization larval packet test to measure amitraz-susceptibility against ixodidae. J Med Entomol 39: 645-651.
39. Santos FCC, Vogel FSL. (2012) Resistência do carrapato Rhipicephalus (Boophilus) microplus frente ao amitraz e cipermetrina em rebanhos bovinos no Rio Grande do Sul de 2005 a 2011. Rev Port Ciên Vet 107: 121-124.
40. Coelho WAC, Pereira JS, Fonseca ZAAS, Andre WPP, Bessa EM, et al. (2013) Resistência de Rhipicephalus (Boophilus) microplus frente à cipermetrina e amitraz em bovinos leiteiros no nordeste do Brasil. Act Vet Bras 7: 229-232.
41. Mendes EC, Mendes MC, Sato ME (2013) Diagnosis of amitraz resistance in Brazilian populations of Rhipicephalus (Boophilus) microplus (Acari: Ixodidae) with larval immersion test. Exp Appl Acarol 61: 357-369.
42. Miller RJ, Davey RB, George JE (1999) Characterization of pyrethroid resistance and susceptibility to coumaphos in Mexican Boophilus microplus (Acari: Ixodidae). J Med Entomol 36: 533-538.
43. He H, Chen A, Davey RB, Ivie GW, Wagner GG, George JE (1999) Sequence analysis of the knockdown resistance-homologous regions of the para-type sodium channel gene from pyrethroid-resistance Boophilus microplus (Acari: Ixodidae). J of Med Entomol 36(5): 539 – 543.
44. Guerrero FD, Davey RB, Miller RJ (2001) Use of an allele-specific polymerase chain reaction assay to genotype pyrethroid resistant strains of Boophilus microplus (Acari: Ixodidae). J Med Entomol 38: 44-50.
45. Rosario-Cruz R, Guerrero FD, Miller RJ, Rodriguez-Vivas RI, Tijerina M, et al. (2009) Molecular survey of pyrethroid resistance mechanisms in Mexican field populations of Rhipicephalus (Boophilus) microplus. Parasitol Res 105: 1145-1153.
46. Nogueira Domingues L, dos Santos Alves Figueiredo Brasil B, Passos de Paiva Bello AC, Pinto da Cunha A, Thadeu Medeiros de Barros A, et al. (2012) 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. Vet Parasitol 189: 327-332.
47. Andreotti R, Guerrero FD, Soares MA, Barros JC, Miller RJ, et al. (2011)

Acaricide resistance of Rhipicephalus (Boophilus) microplus in State of Mato Grosso do Sul, Brazil. Rev Bras Parasitol Vet 20: 127-133.

48. Guerrero FD, Lovis L, Martins JR (2012) Acaricide resistance mechanisms in Rhipicephalus (Boophilus) microplus. Rev Bras Parasitol Vet 21: 1-6.

49. Morgan JA, Corley SW, Jackson LA, Lew-Tabor AE, Moolhuijzen PM, et al. (2009) Identification of a mutation in the para-sodium channel gene of the cattle tick Rhipicephalus (Boophilus) microplus associated with resistance to synthetic pyrethroid acaricides. Int J Parasitol 39: 775-779.

50. Jonsson NN, CutullÃ¨ C, Corley SW, Seddon JM (2010) Identification of a mutation in the para-sodium channel gene of the cattle tick Rhipicephalus microplus associated with resistance to flumethrin but not to cypermethrin. Int J Parasitol 40: 1659-1664.

51. Stone NE, Olafson PU, Davey RB, Buckmeier G, Bodine D, et al. (2014) Multiple mutations in the para-sodium channel gene are associated with pyrethroid resistance in Rhipicephalus microplus from the United States and Mexico. Parasit Vectors 7: 456.

52. Pereira MC, Lucas R (1987) Estudos in vitro da eficiência de carrapaticidas linhagem de Boophilus microplus, proveniente de Jacarei, SP, Brasil. Rev Facul Med Vet Zoot 24: 7-11.

53. Leite RC (1988) Boophilus microplus (Canestrini, 1887) susceptibilidade, uso atual e retrospectivo de carrapaticidas em propriedades das regiões fisiográficas da Baixada do Grande Rio e Rio de Janeiro: uma abordagem epidemiológica. PhD thesis. Rio de Janeiro: UFRRJ Universidade Federal Rural do Rio de Janeiro.

54. Flausino JRN, Gomes CCG, Grisi L (1995) Avaliação da resistência do carrapato Boophilus microplus ao amitraz e a piretróides, no município de Seropédica, Rio de Janeiro. Rev Bras Parasitol Vet 4 Suppl: 45.

55. Fernandes FF (2001) Efeitos toxicológicos e resistência a piretroides em Boophilus microplus de Goiás. Arq Bras Med Vet Zootec 53: 538-43.

56. Li AY, Davey RB, Miller RJ, Guerrero FD, George JE (2008) Genetics and mechanisms of permethrin resistance in the Santa Luiza strain of Boophilus microplus (Acari: Ixodidae). J Med Entomol 45: 427-438.

57. Mendes MC, Duarte FC, Martins JR, Klafke GM, Fiorini LC, et al. (2013) Characterization of the pyrethroid resistance profile of Rhipicephalus (Boophilus) microplus populations from the states of Rio Grande do Sul and Mato Grosso do Sul, Brazil. Rev Bras Parasitol Vet 22: 379-384.

58. Gomes A, Koller WW, Barros ATM. (2011) Suscetibilidade de Rhipicephalus (Boophilus) microplus a carrapaticidas em Mato Grosso do Sul, Brasil. Cien Rural 41: 1447-1452.

59. Tanner PA, Meo NJ, Sparer D, Romano MN, Keister DM, et al. (1997) Advances in the treatment of heartworm, fleas and ticks. Can Pract 22: 40-47.

60. Chandler GT, Cary TL, Volz DC, Walse SS, Ferry JL, et al. (2004) Fipronil effects on estuarine copepod (Amphiascus tenuiremis) development, fertility, and reproduction: a rapid life-cycle assay in 96-well microplate format. Environ Toxicol Chem 23: 117-124.

61. Rauh JJ, Lummis SC, Sattelle DB (1990) Pharmacological and biochemical properties of insect GABA receptors. Trends Pharmacol Sci 11: 325-329.

62. Castro-Janer EA (2010) Resistência de Rhipicephalus (Boophilus) microplus Acari:Ixodidae) a fipronil: padronização de bioensaios in vitro, detecção de resistência em populações de campo e avaliação sobre resistência cruzada com outras drogas. PhD thesis. Universidade de São Paulo, Instituto de Ciências Biomédicas da Universidade de São Paulo.

63. Souza AP, Paim F, Belllato V, Sartor AA, Moura AB, et al. (2014) Avaliação da eficácia do fipronil em Rhipicephalus (Boophilus) microplus em tratamentos consecutivos. Arq Bras Med Vet Zootec 66: 55-60.

64. Furlong J, Prata MCA, Martins JR. (2007) O carrapato dos bovinos e a resistência: temos o que comemorar? A Hora Vet 159: 26-32.

65. Martin RJ, Buxton SK, Neveu C, Charvet CL, Robertson AP (2012) Emodepside and SL0-1 potassium channels: a review. Exp Parasitol 132: 40-46.

66. Bloomquist JR1 (1993) Toxicology, mode of action and target site-mediated resistance to insecticides acting on chloride channels. Comp Biochem Physiol C 106: 301-314.

67. Martins JR, Furlong J (2001) Avermectin resistance of the cattle tick Boophilus microplus in Brazil. Vet Rec 149: 64.

68. Klafke GM, Sabatini GA, de Albuquerque TA, Martins JR, Kemp DH, et al. (2006) Larval immersion tests with ivermectin in populations of the cattle tick Rhipicephalus (Boophilus) microplus (Acari: Ixodidae) from State of Sao Paulo, Brazil. Vet Parasitol 142: 386-390.

69. Klafke GM. (2011) Diagnóstico e mecanismos da resistência a ivermectina em Rhipicephalus (Boophilus) microplus (Acari: Ixodidae). PhD thesis. Universidade de São Paulo, Instituto de Ciências Biomédicas.

70. Kemp DH, Dunster S, Binnington KC, Bird PE, Nolan J (1990) Mode of action of CGA 157419 on the cattle tick Boophilus microplus. Bull Soc Fr Parasitol 8: 100-48.

71. Cruz BC, Teixeira WF, Maciel WG, Felippelli G, FÃ¡vero FC, et al. (2014) Effects of fluazuron (2.5 mg/kg) and a combination of fluazuron (3.0 mg/kg) + abamectin (0.5 mg/kg) on the reproductive parameters of a field population of Rhipicephalus (Boophilus) microplus on experimentally infested cattle. Res Vet Sci 97: 80-84.

72. Salgado VL (1998) Studies on the Mode of action of Spinosad: Insects Symptoms and Physiological Correlates. Pest Biochem Phy 60: 91-102.

73. Jonsson NN, Miller RJ, Kemp DH, Knowles A, Ardila AE, et al. (2010) Rotation of treatments between spinosad and amitraz for the control of Rhipicephalus (Boophilus) microplus populations with amitraz resistance. Vet Parasitol 169: 157-164.

74. Cetin H, Cilek JE, Oz E, Aydin L, Deveci O, et al. (2009) Comparative efficacy of spinosad with conventional acaricides against hard and soft tick populations from Antalya, Turkey. Vet Parasitol 163: 101-104.

75. Miller RJ, White WH, Davey RB, George JE, De Leon AP (2011) Efficacy of Spinosad Against Acaricide Resistant and Susceptible Rhipicephalus (Boophilus) microplus and Acaricide Susceptible Amblyomma americanum and Dermacentor variabilis. J Med Entomol 48: 358-365.

76. Brito LG, Barbieri FS, Rocha RB, Oliveira MC, Ribeiro ES (2011) Evaluation of the Efficacy of Acaricides Used to Control the Cattle Tick, Rhipicephalus microplus, in Dairy Herds Raised in the Brazilian Southwestern Amazon. Vet Med Int 2011: 806093.

77. Camillo G, Vogell FF, Sangionil LA, Cadore GC (2009) Ferrari R. Eficiência in vitro de acaricidas sobre carrapatos de bovinos no Estado do Rio Grande do Sul, Brasil. Cien Rural 39: 490-95.

78. Campos Júnior DA, Oliveira PR (2005) Avaliação in vitro da eficácia de acaricidas sobre Boophilus microplus (Canestrini, 1887) (Acari: Ixodidae) de bovinos no município de Ilhéus, Bahia, Brasil. Cien Rural 35: 1386-92.

79. Farias NA, Ruas JL, Santos TRB (2008) Análise da eficácia de acaricidas sobre o carrapato Boophilus microplus, durante a última década, na região sul do Rio Grande do Sul. Cien Rural 38: 1700-1704.

80. Koller WW, Gomes A, Barros ATM (2009) Diagnóstico da resistência do carrapato-do-boi a carrapaticidas em Mato Grosso do Sul. Embrapa Gado de Corte. ISSN 1983-9715

81. Laranja RS, Martins JR, Cereser VH, Corrêa BL, Ferraz C (1989) Seminário Brasileiro De Parasitologia Veterinária 6 - 1989. Identificação de uma estirpe de Boophilus microplus resistente a carrapaticidas piretróides, no Estado do Rio Grande do Sul, Bagé.

82. Lopes WD, Cruz BC2, Teixeira WF2, Felippelli G2, Maciel WG2, et al. (2014) Efficacy of fipronil (1.0 mg/kg) against Rhipicephalus (Boophilus) microplus strains resistant to ivermectin (0.63 mg/kg). Prev Vet Med 115: 88-93.

83. Machado FA, Pivoto FL2, Ferreira MS, Gregorio Fde V3, Vogel FS, et al. (2014) Rhipicephalus (Boophilus) microplus in the western-central region of Rio Grande do Sul, Brazil: multiresistant tick. Rev Bras Parasitol Vet 23: 337-342.

84. Martins JR (1995) A situation report on resistance to acaricides by the cattle tick Boophilus microplus in the state of Rio Grande do Sul, Southern Brazil. Seminário Internacional De Parasitologia Animal Acapulco, México. In:. INIFAP.

85. Martins JR, Correa BL, Maia JZ (1992) Resistência de carrapatos a carrapaticida Piretroide no Rio Grande do Sul, Gramado, Rio Grande do Sul: p. 46. Congresso Estadual de Medicina Veterinária, 11.

86. Mendes MC, Silva MX, Bracco JE. (2001) Teste bioquímico para determinar a resistência de duas cepas do carrapato Boophilus microplus (Canestrini, 1887) Biochemical test to determine the resistance of two strains of the tick Boophilus microplus (Canestrini, 1887). Rev Bra de Parasitol Vet 10: 61-65.

87. Pereira JR1 (2006) [In Vitro efficacy of comercial formulations of ixocidides in engorged female of Boophilus microplus collected of dairy cattle at ParaÃba Valey in the state of SÃ£o Paulo]. Rev Bras Parasitol Vet 15: 45-48.

88. Raynal JT, Silva AA, Sousa Tde J, Bahiense TC, Meyer R, et al. (2013)

Acaricides efficiency on Rhipicephalus (Boophilus) microplus from Bahia state North-Central region. Rev Bras Parasitol Vet 22: 71-77.

89. de Santana BB, Ramos RA, Santana Mde A, Alves LC, de Carvalho GA (2013) Susceptibility of Rhipicephalus (Boophilus) microplus (Acari: Ixodidae) to pyrethroids and their associations in Pernambuco, Brazil. Rev Bras Parasitol Vet 22: 276-280.

90. Santos TRB dos, Farias NA da R, Cunha Filho NA, Vaz Junior I da S (2008) Uso de acaricidas em Rhipicephalus (B.) microplus de duas regiões fisiográficas do Rio Grande do Sul. Acta Sci Vet, 36: 25-30.

91. Silva MCL, Sobrinho RN, Linhares GFC (2000) Avaliação in vitro da eficácia do clorfenvinfós e da cialotrina sobre o Boophilus microplus, colhidos em bovinos da bacia leiteira da microrregião de Goiânia–Goiás. Cien Anim Bras 2: 143-148.

92. Silva WW, Athayde ACR, Araújo GMB, Santos VD dos, Silva Neto AB da (2005) Resistência de fêmeas ingurgitadas de Boophilus microplus e Riphicephalus sanguineus (ACARI: IXODIDAE) a carrapaticidas no semi-árido paraibano: efeito da cipermetrina e do amitraz. Agropec. Cien no Semi-árido 1: 59-62.

93. Souza AP, Sartor AM, Bellato V, Perussolo S (2003) Eficácia de carrapaticidas em rebanhos de bovinos leiteiros de municípios da região centro sul do Paraná. Rev de Cien Agrovet 2:131-135.

94. Veiga LP, Souza AP, Bellato V, Sartor AA, Nunes AP, et al. (2012) Resistance to cypermethrin and amitraz in Rhipicephalus (Boophilus) microplus on the Santa Catarina Plateau, Brazil. Rev Bras Parasitol Vet 21: 133-136.