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SENSITIVITY OF *BOOPHILUS MICROPLUS* (ACARI: IXODIDAE) TO PYRETHROIDS AND ORGANOPHOSPHATE IN FARMS IN THE VALE DO PARAÍBA REGION, SÃO PAULO, BRAZIL

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ABSTRACT

A study to evaluate the resistance of *B. microplus* to the active principles cypermethrin, deltamethrin and chlorpyriphos was performed together with the survey of the tick control practices used in 12 farms located in Vale do Paraíba Region, São Paulo, Brazil. Results obtained by the larval packet test (LPT) technique, adopted by FAO, showed established resistance to pyrethroids (cypermethrin 16.7% sensitive, 8.3% resistant level I and 75% resistant level II; deltamethrin 25% / sensitive, 33.3% resistant level II and 41.7% resistant level III) and emerging resistance to organophosphate compounds (chlorpiriphos 58.3% sensitive, 33.3% resistant level I and 8.4% resistant level II). The inquiry applied to the farmers showed that the combination of organophosphates and pyrethroids compounds products is the most commonly tick-killing compounds used in the region, followed by amitraz and macrocyclic lactones.

KEY WORDS: *Boophilus microplus*, resistance, pyrethroids, organophosphate.

RESUMO

SENSIBILIDADE DO *BOOPHILUS MICROPLUS* (ACARI: IXODIDAE) AOS PIRETRÓIDES E ORGANOFOSFORADOS EM FAZENDAS LOCALIZADAS NA REGIÃO DO VALE DO PARAÍBA, SÃO PAULO, BRASIL. Foi realizada pesquisa da resistência do *Boophilus microplus* aos princípios ativos cipermetrina, deltametrina e clorpirifós e, concomitantemente, feito o levantamento sobre as práticas adotadas no controle do carrapato em 12 propriedades localizadas na região do Vale do Paraíba, São Paulo, Brasil. Os resultados obtidos por meio da técnica LPT (Larval Packet Test), segundo a classificação adotada, mostraram que, para a cipermetrina, 16.7% das fazendas apresentaram populações classificadas como sensíveis; 8.3% com resistência nível I e 75% com resistência nível II. Para a deltametrina, 25% sensíveis; 33.3% com resistência nível II e 41.7% com resistência nível III. Para o organofosforado clorpirifós 58.3% mostraram-se sensíveis; 33.3% com resistência nível I e 8.4% com resistência nível II. Os questionários aplicados aos produtores revelaram que os produtos à base da associação de organofosforados com piretróides são os mais usados na região, seguidos do amitraz e lactonas macrocíclicas.

PALAVRAS-CHAVE: *Boophilus microplus*, resistência, piretróides, organofosforados.

INTRODUCTION

Parasitic infestation by ticks causes clinical and subclinical syndromes that impair the development of cattle herds, mainly in dairy production with substantial economics losses. The knowledgement on management practices; including the methods used on the tick control in the Vale do Paraíba Region is important for the implementation of parasitary control programs.

Resistance of *Boophilus microplus* to pyrethroids and organophosphate has been reported in several countries (Beugnet; Chardonnet, 1995; Romero *et al.*, 1997; Romero *et al.*, 1998; Davey; George, 1998; Baxter; Backer, 1999; Crampton *et al.*, 1999; Miller-Robert *et al.*, 1999; Jamroz *et al.*, 2000; Benavides; Romero, 2000; Guerrero *et al.*, 2001; Bianchi *et al.*, 2003). There are few reports on the resistance of ticks by means of the larval packet test (Mendes; Veríssimo, 1999; Vieira-Bressan *et al.*, 1999; Mendes *et al.*, 2001) in the Vale do Paraíba Region, Brazil.

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The objective of the present study was to evaluate the sensitivity of *B. microplus* field strains collected from farms located in the Vale do Paraíba Region for two pyrethroids and one organophosphate compound, as well as to access the tick-control methods most commonly employed in these farms.

MATERIAL AND METHODS

The study was performed in 12 farms located in the Vale do Paraíba Region (22º 55' 26" S – 45º 27'42"W) during two years. Information on tick control methods was gathered by a survey of the tick control practices adopted in twelve farms. The method used was the Larval Packet Test (STONE; HAYDOCK, 1962).

The following active principles (concentration given in parentheses) were tested: cypermethrin (0.8%, 0.4%, 0.25%, 0.1%, 0.05% and 0.0125%), deltamethrin (0.8%, 0.4%, 0.2%, 0.1%, 0.05%, 0.025%, 0.00625%) and chlorpyriphos (0.4%, 0.2%, 0.1%, 0.05%, 0.025% and 0.0125%). The Mozo strain was used as the standard susceptible to access resistance. Females of the tick *B. microplus*, collected in 12 farms were kept in a chamber at 27° C and relative humidity over 80%. After 14 days, eggs were collected and transferred to a conic tube (5 mL) with a cotton lid to allow air and moisture exchange. After another 2 weeks the eggs were hatched and after a further 2 weeks the larvae were ready for testing. Samples with approximately 100 larvae aging 14 to 21 days old were removed from the tube with the aid of a brush and placed in filter paper impregnated with the compound to be studied. After 24 hours, alive and dead larvae were counted. In order to calculate lethal concentrations 50% and 99% (LC_{50} and LC_{99}) was used the probit analysis in Polo-PC software. Data were discarded when mortality rates in the control group were over 10%. Tests showing mortality rates in the control group between 5% to 10% were submitted to Abbott's formula (ABBOTT, 1925). The Resistance Factor (RF) was obtained by the quotient between LC_{50} of the unknown individual progenies and LC_{50} obtained for the Mozo strain.

Table 1 - Level of resistance classification used in the present study for pyrethroids and organophosphate compounds.

Classification	Pyrethroids RF	Organophosphate RF
Sensitive	≤ 2.4	< 1.4
Resistant level I	2.5 – 5.4	1.5 – 4.4
Resistant level II	5.5 – 50	4.5 -50
Resistant level III	>50	> 50

The system for the classification of resistance for pyrethroids and organophosphates compounds was based on the model presented by BIANCHI et *al.*, (2003), with some changes in the values that determine tick sensitivity of ticks and in the expressions used by the authors to classify samples as sensitive or resistant. Two classifications were considered because there was a greater degree of variation in the magnitude of the RF for pyrethroids and organophosphates compounds, (Table 1) (BARROS, personal communication, 2004).

RESULTS

Inquiry of Tick Control Practices

From the 12 analyzed farms 72.7% were dedicated to milk production and 27.3% to both beef and dairy production. All of the had mixed breed cattle and performed acaricide treatments. The results for the products that had been used in the last five years and that were being used at the moment of the questionnaire were respectively, amitraz (38.1% and 37.5%), association of organophosphate and pyrethroid (23.8% and 43.7%), macrocyclic lactone (14.3% and 18.8%). Regarding to application methods spray was the most common for tick control (67%) followed by the injectable route (33%).

Resistance trials

Cipermethrin

Most of the tick populations (69.2%) were resistant level II for cypermethrin, with values ranging from 12.2 to 38.6. Resistant level I was only found in one farm (8.3%) and 16.7% were classified as sensitive. Farm 10 ticks showed to be more sensitive than Mozo strain

Deltamethrin

Most of the samples analyzed for deltamethrin showed to be resistant level III (41.7%) and resistant level II (33.3%), the remaining were sensitive (25%). Farm 10 ticks showed to be sensitive than Mozo strain.

Chlorpyriphos

Most of the tick population showed to be sensitive to chlorpyriphos (58.3%), whereas the remaining was resistant level I (33.3%) and level II (8.4%). It was observed that farm 10 presented a RF similar to that of the Mozo strain, with LC_{50} equal to 0.016%.

Table 2 - Values for lethal concentrations 50% (LC_{50}) and 99% (LC_{99}) and their respective 95% confidence limits for 12 *Boophilus microplus* population tested against the pyrethroid cypermethrin.

Strain (Farms)	$LC_{50\ (\%)}$	Confidence limit 95%	$LC_{99\ (\%)}$	Confidence limit 95%	RF
Mozo*	0.0123	0.0059 – 0.0193	1.41	0.6997 – 4.5206	
1	0.317	0.1157 – 3.6938	1.944	0.5909 – 25489.4	25.7
2	0.475	0.4104 – 0.5662	2.432	1.6921 – 4.2215	38.6
3	0.425	0.2803 – 0.8336	2.348	1.0759 – 23.6177	34.5
4	0.225	0.1199 – 0.5304	1.552	0.6177 – 32.8560	18.2
5	0.452	0.1651 – 120.5383	8.855	1.1657 – 3.2510	36.7
6	0.348	0.2427 – 0.5249	1.423	0.8225 – 5.2731	28.3
7	0.268	0.0791 – 28.7434	3.425	0.6446 – 1.8225	21.8
8	0.0058	0.0003 – 0.0145	0.088	0.0434 – 0.4860	0.5
9	0.0339	0.0187 – 0.0527	3.589	1.4549 – 16.7159	2.7
10	< 0.0123	-	<1.4	-	<1
11	0.466	0.1375 – 2646.42	30.451	2.1360 – 1.4522	37.8
12	0.15	7.026517E-02 – 0.5295205	3.11	0.7569834 – 212.1577	12.2

RF: Resistance Factor
*Reference sensitive strain

Table 3 - Values of lethal concentrations 50% (LC_{50}) and 99% (LC_{99}) and their respective 95% confidence limits for 12 *Boophilus microplus* population tested against the pyrethroid deltamethrin.

Strain (Farms)	$LC_{50\ (\%)}$	Confidence limit 95%	$LC_{99\ (\%)}$	Confidence limit 95%	RF
Mozo*	0.00232	0.0007 – 0.0046	0.78	0.4143 – 2.2923	
1	0.0256	0.0242 – 0.0526	0.511	0.3436 – 0.8762	11.0
2	0.174	0.0610 – 0.6256	1.341	0.4466 – 368.5854	75
3	0.156	0.0708 – 0.3829	1.541	0.4006 – 1.0891	67.2
4	0.086	0.0507 – 0.1318	0.721	0.3785 – 2.7836	37.0
5	0.271	0.1648 – 0.5460	2.945	1.1277 – 32.7913	116.8
6	1.183	0.1595 – 0.2098	0.653	0.5128 – 0.9289	509.9
7	0.0698	0.00059 – 1.3371	0.344	0.0977 – 4.9163	30.1
8	0.00089	0.000043 – 0.002816	0.108	0.0527 – 0.5502	0.4
9	0.0028	0.0008 – 0.0059	0.707	0.3558 – 2.1847	1.2
10	<0.002	-	<0,78	-	<1
11	0.225	0.1197 – 0.3903	2,160	0,9669 – 15,8392	97.0
12	0.0137	0.0049 – 0.0259	0,3202	0,1240 – 3,2817	6.0

RF: Resistance Factor.
*Reference sensitive strain.

Table 4 - Values of lethal concentrations 50% (LC_{50}) and 99% (LC_{99}) and their respective 95% confidence limits for 12 *Boophilus microplus* populations tested against the organophosphorous compound chlorpyriphos.

Strain (Farms)	$LC_{50\ (\%)}$	Confidence limit 95%	$LC_{99\ (\%)}$	Confidence limit 95%	RF
Mozo*	0.0141	0.0015 – 0.0222	0.0311	0.0197 – 0.0724	
1	0.041	0.0327 – 0.0199	0.724	0.4574 – 1.4114	2.9
2	0.0074	0.0051 – 0.0098	0.534	0.3350 – 1.0399	0.5
3	0.084	0.0744 – 0.0969	3.47	2.2098 – 6.1834	5.9
4	0.041	0.0325 – 0.0506	13.02	5.1277 – 51.9154	2.9
5	0.039	0.0236 – 0.0594	1.40	0.5403 – 10.7399	2.7
6	0.0096	0.0036 – 0.0166	2.82	0.9894 – 21.8052	0.7
7	0.0126	0.0085 – 0.0166	0.152	0.1000 – 0.3090	0.8
8	0.0125	0.0091 – 0.0155	0.102	0.0740 – 0.1723	0.9
9	0.0077	0.00000073 – 0.0151	0.0244	0.0100 – 0.3027	0.5
10	0.016	0.0122– 0.0195	0.076	0.0559 – 0.1299	1.1
11	0.042	0.0351– 0.0513	1.45	0.7938 – 3.4444	3.0
12	0.0031	0.00018 – 0.0083	0.435	0.1728 – 6.2412	0.2

RF: Resistance Factor
*Reference sensitive strain.

DISCUSSION

The results for cypermethrin (Table 2) are in accordance with the findings of MANGOLD et al. (2001) using Argentinean strain (13.5); of VIEIRA-BRESSAN et al., (1999) using samples from Caçapava (12.29); of MENDES et al., (2001) for the Mancilha strain (8.68); of MENDES; VERÍSSIMO (1999) for the strains Lorena (9.82) and Nova Odessa (8.89). FRAGOSO, et al., (2004) reported that the Mora strain showed a resistance factor equal to 118.7, considered to be resistant level III according to the standard adopted in this study. MENDES & VERÍSSIMO (1999) classified sensitive strains in tick samples collected in Colina (1.27) and Mogi das Cruzes (1.02).

The results for deltamethrin are similar to results reported by BEUGNET; CHARDONNET (1995) and BIANCHI et al. (2003), who found RF classified as resistant level II and resistant level III in tick samples collected in New Caledonia farms. Tick population in farm 6 showed a RF equal to 509.9 (Table 3). FRAGOSO et al. (2004), in a similar study, found a RF to deltamethrin equal to 104 for Mora strain. These authors also reported the occurrence of strains resistant to deltamethrin with RF over 300. Ticks resistant level II to deltamethrin were also reported by VIEIRA-BRESSAN et al. (1999) and MENDES et al. (2001) with the same RF (11) in samples collected in the Vale do Paraíba region. The sensitivity to deltamethrin observed in this study shows that the RF is much greater for this acaricide than for cypermethrin. The high frequency of populations classified as sensitive (16.7%) can possibly be due to the fact that the use of pyrethroids have been abandoned, altering thus the action mechanism related to changes in sodium channels. According to FOIL et al. (2004) high resistance to pyrethroids can be obtained with just one site of mutation in sodium channels. Ticks sensitivity profile to organophosphates compounds could be emerging. According to FRAGOSO et al. (2004), resistance to organophosphates compounds develops in approximately 7-8 years after their use. Most of the population analyzed was sensitive to chlorpyriphos (Table 4), similar to what was observed by MENDES et al. (2001) for Mancilha strain. Resistance level II strains observed in this study showed similar characteristics to those of strains Tully, Ingham, Biarra and Mackay from Australia. The resistance to organophosphates compounds had been reported, in Brazil, by SHAW et al., 1968; AMARAL et al. (1974) and PATARROYO; COSTA (1980). These authors had used the larval immersion test.

The absence of reports on the resistance to amitraz may be due to the late development of methods to measure resistance, as well as the slow characteristic of this product in the development of resistance (FRAGOSO et al., 2004). Resistance to organophosphate had been reported since 1963 (LEAL et al., 2003), when the use of pyrethroids and formamidines became popular. In the nineties, resistance to pyrethroids and amitraz was first reported (FAO, 2004), and from the year 2000 on, there were reports on the resistance to macrocyclic lactones (MARTINS; FURLONG, 2001; BENAVIDES; ROMERO, 2000). A return to the use of organophosphate and their associations may be observed, and the reemergence of resistance to these products for sure will occur.

Results regarding the acaricide methods application were similar to those obtained by ROCHA (1995). The misuse of spray application leads to tick resistance (BIANCHI et al., 2003). The use of macrocyclic lactones generic compounds injectable for tick control increased from 14.3% to 18.8% due to affordable prices on cattle market The combination between pyrethroids and organophosphates compounds increased from 23.8% to 43.7%. The high frequency of tick samples classified as sensitive may be due to the use of amitraz that eliminated the tick population resistant to pyrethroids.

Based on the obtained data an alert on good practices aiming tick control should be recommended in order to identify and monitor resistance, minimizing the use of acaricides as well as mistakes in the products choice.

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