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Kumar SS
Department of Veterinary Parasitology, College of Veterinary Science, Sri Venkateswara Veterinary University, Tirupati, Andhra Pradesh, India

Rayulu VC
Department of Veterinary Parasitology, College of Veterinary Science, Sri Venkateswara Veterinary University, Tirupati, Andhra Pradesh, India

Rao KS
Department of Veterinary Parasitology, College of Veterinary Science, Sri Venkateswara Veterinary University, Tirupati, Andhra Pradesh, India

Kumar NV
Department of Veterinary Parasitology, College of Veterinary Science, Sri Venkateswara Veterinary University, Tirupati, Andhra Pradesh, India

Correspondence
Kumar SS
Department of Veterinary Parasitology, College of Veterinary Science, Sri Venkateswara Veterinary University, Tirupati, Andhra Pradesh, India

Acaricidal resistance in *Rhipicephalus* (*Boophilus*) *Microplus* ticks infesting cattle of Andhra Pradesh

Kumar SS, Rayulu VC, Rao KS and Kumar NV

Abstract
The present study was conducted during the period from January to July, 2015 to determine the status of resistance in *Rhipicephalus* (*Boophilus*) *microplus* ticks infesting cattle in different parts of Andhra Pradesh to commonly used acaricides *viz.*, deltamethrin, cypermethrin and amitraz. Larval Packet Test (LPT) bioassay revealed the highest LC_{50} values for both deltamethrin and cypermethrin against *R. (B.) microplus* ticks collected from Chittoor (40.05 ppm and 88.68 ppm) followed by Prakasam (12.59 ppm and 36.58 ppm) and YSR Kadapa (8.10 ppm and 30.82 ppm), respectively. The tick populations of Chittoor showed significantly ($P<0.05$) higher resistance to deltamethrin (RF=8.78; RL=II) and cypermethrin (RF=7.04; RL=II) followed by Prakasam (RF=2.76; RL=I); (RF=2.90; RL=I) and YSR Kadapa (RF=1.78; RL=I); (RF=2.45; RL=I), respectively. The tick populations in Anantapur, Krishna and Vizianagaram were found to be susceptible (RL S = RF<1.1) to synthetic pyrethroids. Modified LPT indicated that the *R. (B.) microplus* ticks collected from different parts of Andhra Pradesh were totally susceptible to amitraz. LC_{50} values for various tested populations of *R. (B.) microplus* for amitraz were recorded in between 76.02 and 84.94 ppm against 73.97 ppm of reference population.

Keywords: Acaricides, Resistance Factor (RF), Resistance Level (RL), *Rhipicephalus (Boophilus) microplus*

1. Introduction
Cattle rearing being supplementary to agriculture has been a part of social and cultural heritage of Indian civilization. Cattle have been the source of small, marginal and landless farmers, a majority of whom live below the poverty line [1]. Ticks and tick borne diseases (TTBDs) are widely distributed, particularly in tropical and sub-tropical countries and cause major economic losses to livestock sector [2]. Ticks are the most harmful blood suckers and it has been estimated that 80% of the world's cattle population is exposed to tick infestation [2]. *Rhipicephalus (Boophilus) microplus* a one host tick of cattle is one of the most widely distributed tick species in India, where the warm and humid climate favours its perpetuation and propagation [3]. The negative economic impact of *R. (B.) microplus* to cattle is due to direct and indirect effects [4]. Ticks cause huge economic losses through blood loss, dermatitis, general stress, decrease in production, host immune suppression and reduction in live weight. Tick bite marks diminish the value of skins and hides upto 20-30 percent and the wounds left by tick bites predispose the animal to screw-worm attack [5]. *Rhipicephalus (Boophilus) microplus* transmits pathogens that cause babesiosis and anaplasmosis in cattle [6].
Use of acaricides is the most common tick control method adopted by the cattle owners in India. Wide spread and indiscriminate use of acaricidal compounds has led to the development of resistance in ticks [7, 8]. In addition, continued use of acaricides for long periods exerts selection pressure on the ticks resulting in resistance development [9]. Recently, there are several reports on emergence of acaricidal resistance in *R. (B.) microplus* in certain states of India including Punjab [10, 11], Uttarakhand [3, 12], Karnataka [13], Haryana [14], Kerala [8, 15], Gujarat [16] and Bihar [17]. Periodic monitoring of the ticks for development of resistance against commonly used acaricides in the particular region is essential to recommend the appropriate acaricide for effective control of the ticks and tick borne diseases. Hence the present study was undertaken to detect the status of resistance in *R.* (*B.*) *microplus* against commonly used acaricides in cattle of Andhra Pradesh.

2. Materials and Methods

2.1 Reference susceptible tick population

Engorged female *R.* (*B.*) *microplus* ticks were collected from naturally infested cattle from Mundala and Poleru remote areas of Konchili mandal, Srikakulam district of Andhra Pradesh where the ticks had never exposed earlier for any acaricidal treatment and they were susceptible to the recommended concentrations of deltamethrin, cypermethrin and amitraz. These ticks were used as the standard to assess the susceptibility/resistance status of the ticks collected in the present study.

2.2 Collection of field ticks:

Engorged female tick samples of *R.* (*B.*) *microplus* were collected from certain places of Andhra Pradesh *viz.*, Krishna, Prakasam, Vizianagaram, Anantapur, Chittoor and YSR Kadapa during the study period from January to July, 2015. These ticks were placed in petridishes and incubated in Biological Oxygen Demand (BOD) incubator at 85-95% RH (relative humidity) with 27±1 ºC and were examined daily until oviposition begins. The eggs thus obtained were separated and allowed to hatch in glass tubes closed with cotton plugs and kept at optimal conditions. Hatched out larvae/seed ticks were maintained for 14-21 days in BOD incubator at 27±1 ºC and 85-95% RH. The larvae aged between 14 to 21 days were subjected for larval packet test.

2.3 Larval Packet Test (LPT):

Detection of resistance in *R. (B.) microplus* against deltamethrin (Butox®, 1.25%) and cypermethrin (Dermeez®, 10%) was carried out by LPT as per the methodology suggested by FAO [2]. Against amitraz (Extick 12.5%), modified LPT as described by FAO [2] and Miller *et al*. [18] was adopted. The methodology of modified LPT was similar to LPT except the usage of formulated amitraz and nylon fabric in place of technical amitraz and filter paper, respectively. Filter paper packets were prepared and impregnated with different concentrations of deltamethrin (Butox®, 1.25%), cypermethrin (Dermeez®, 10%) and amitraz (Extick®, 12.5%). One part of olive oil and two parts of trichloroethylene was used as diluents. The concentrations of deltamethrin, cypermethrin and amitraz ranged from 3.125 to 800 ppm, 6.25 to 3200 ppm and 62.5 to 500 ppm respectively, were used in LPT. Control papers were treated with diluents only. Approximately, 100 larvae were transferred into acaricide impregnated filter paper packets before finally sealing the packet with another clamp at the top. Sealed packets were maintained at 27±1 ºC with 85-95% RH for 24 hours in BOD incubator. After 24 hrs the packets were observed for live and dead counts to obtain the larval mortality data. Each concentration of acaricides was tested in duplicate and the average mortality was scored.

2.4 Resistance characterization

Larvae of ticks collected from different places were tested with different concentrations of deltamethrin, cypermethrin and amitraz. Resistance factor (RF) was obtained by comparing the LC_{50} of the field ticks with the LC_{50} of the reference susceptible ticks. The resistance level (RL) in the field population of ticks was classified as susceptible (RF≤1.4), level I (RF=1.5-5), level II (RF=5.1-25), level III (RF=25.1-40) and level IV (RF>40) [19].

2.5 Acaricides in practice to control tick infestation in Andhra Pradesh

Different acaricides used for control of ticks and their mode of application at various places of the study were collected and presented in Table 1. The data from the questionnaire revealed that deltamethrin and cypermethrin were the most frequently used acaricides. Use of amitraz for tick control has also been reported at few places. The frequency of acaricide application was mostly dependent on the appearance of tick infestations on the animals.

Table 1: Data on different acaricides in practice to control tick infestation in cattle and buffaloes in Andhra Pradesh

S. No.	Place	Commonly used acaricides	Mode of application	Strategy		Frequency	Rotation of acaricides
				Specific time intervals	Irregular intervals (When ticks seen)	No. of acaricide applications during the preceding year	Yes (Y) or No (N)
1.	Anantapur	Deltamethrin, Cypermethrin	Spray	--------	Acaricides were used when ever ticks were seen on animal body	1-4 times	N
2.	Chittoor	Deltamethrin, Cypermethrin, Amitraz	Spray	--------	Acaricides were used when ever ticks were seen on animal body	>8 times	Y
3.	YSR Kadapa	Deltamethrin, Cypermethrin	Spray	--------	Acaricides were used when ever ticks were seen on animal body	4-5 times	N
4.	Krishna	Deltamethrin, Cypermethrin, Ivermectin	Spray, Injectable	--------	Acaricides were used when ever ticks were seen on animal body	1-3 times	Y
5.	Prakasam	Deltamethrin, Cypermethrin, Amitraz, Ivermectin	Spray, Injectable	--------	Acaricides were used when ever ticks were seen on animal body	6-8 times	Y
6.	Vizianagaram	Deltamethrin, Cypermethrin	Spray	--------	Acaricides were used when ever ticks were seen on animal body	1-4 times	N

2.6 Statistical Analysis

Mortality counts of the larvae after deltamethrin, cypermethrin and amitraz treatments were subjected to probit analysis for calculating LC_{50} (lethal concentration to 50% of larvae) and LC_{99} (lethal concentration to 99% of larvae) values along with their respective 95% fiducial confidence intervals.IBM Statistical Package for Social Sciences (IBM SPSS 20.0 V, Illinois, Chicago) was used for the analysis. The level of significance was set at 5%.

2. Results and Discussion

The LC_{50} and LC_{99} values with 95% confidence intervals of deltamethrin, cypermethrin and amitraz were determined against reference susceptible *R. (B.) microplus* ticks and are presented in Table 2, 3 and 4, respectively. The LC_{50} values for reference tick population against deltamethrin and cypermethrin were 4.56 ppm and 12.60 ppm, respectively. When compared with the reference susceptible ticks, LPT revealed the highest LC_{50} values for both deltamethrin (40.05 ppm) and cypermethrin (88.68 ppm) against *R. (B.) microplus* ticks collected from Chittoor (RL = II) followed by Prakasam (12.59 ppm and 36.58 ppm) (RL = I) and YSR Kadapa (8.10 ppm and 30.82 ppm) (RL = I), respectively. The tick populations in Anantapur (5.02 ppm and 13.85 ppm), Krishna (4.78 ppm and 13.06 ppm) and Vizianagaram (4.87 ppm and 13.30 ppm) were found to be susceptible (RL = S) to deltamethrin and cypermethrin, respectively. Modified LPT indicated that the *R. (B.) microplus* ticks collected from different parts of Andhra Pradesh were totally susceptible to amitraz. LC_{50} values for various tested populations of *R. (B.) microplus* for amitraz were recorded in between 76.02 and 84.94 ppm against 73.97 ppm of reference population.

Table 2: Lethal concentrations of Deltamethrin on larvae of various populations of *R. (B.) microplus* by LPT

Tick population	LC_{50}	LC_{99}	χ 2	Slope	RF	RL
Reference Population	4.56[a] (3.61-5.47)	41.97[a] (28.66-76.48)	18.69*	2.413 ± 0.164	-	-
Anantapur	5.02[a] (4.05-5.99)	42.70[a] (29.33-76.76)	19.76*	2.503 ± 0.163	1.10	S
Chittoor	40.05[d] (33.29-48.16)	1695.31[b] (1083.45-2983.62)	35.68*	1.430 ± 0.056	8.78	II
YSR Kadapa	8.10[b] (6.26-10.09)	664.13[b] (400.94-1299.03)	23.82*	1.216 ± 0.065	1.78	I
Krishna	4.78[a] (3.76-5.77)	42.76[a] (28.73-81.10)	21.39*	2.444 ± 0.163	1.05	S
Prakasam	12.59[c] (10.13-15.32)	857.41[b] (524.80-1627.99)	24.81*	1.269 ± 0.063	2.76	I
Vizianagaram	4.87[a] (3.97-5.75)	44.84[a] (31.37-76.73)	16.23*	2.413 ± 0.159	1.07	S

Probit analysis using IBM SPSS 20.0 V
Values are estimate of lethal concentration (ppm) with 95% Fiducial confidence intervals
Values with different superscripts are significantly different (P< 0.05); * indicates P<0.05
RF = Resistance Factor; RL = Resistance Level; Susceptible (S) = RF<1.4; Level I= 1.5<RF<5;
Level II= 5.1<RF<25; Level III= 25.1<RF<40; Level IV= RF>40

Table 3: Lethal concentrations of Cypermethrin on larvae of various populations of *R. (B.) microplus* by LPT

Tick population	LC_{50}	LC_{99}	χ 2	Slope	RF	RL
Reference Population	12.60[a] (10.15-15.13)	214.52[a] (143.04-381.65)	20.357*	1.890 ± 0.111	-	-
Anantapur	13.85[a] (11.22-16.59)	241.02[a] (159.88-430.70)	20.671*	1.875 ± 0.108	1.10	S
Chittoor	88.68[c] (72.63-107.96)	6098.20[c] (3767.80-11147.87)	41.051*	1.266 ± 0.048	7.04	II
YSR Kadapa	30.82[b] (25.94-36.27)	1050.25[b] (708.75-1727.66)	23.759*	1.518 ± 0.068	2.45	I
Krishna	13.06[a] (10.48-15.73)	224.15[a] (147.73-407.35)	21.561*	1.884 ± 0.110	1.04	S
Prakasam	36.58[b] (30.76-43.17)	1362.17[b] (904.90-2286.05)	24.341*	1.481 ± 0.066	2.90	I
Vizianagaram	13.30[a] (10.64-16.06)	235.56[a] (154.07-433.49)	21.979*	1.864 ± 0.108	1.05	S

Probit analysis using IBM SPSS 20.0 V
Values are estimate of lethal concentration (ppm) with 95% Fiducial confidence intervals
Values with different superscripts are significantly different (P< 0.05); * indicates P<0.05
RF = Resistance Factor; RL = Resistance Level; Susceptible (S) = RF<1.4; Level I= 1.5<RF<5;
Level II= 5.1<RF<25; Level III= 25.1<RF<40; Level IV= RF>40

Table 4: Lethal concentrations of Amitraz on larvae of various populations of *R. (B.) microplus* by modified LPT

Tick population	LC_{50}	LC_{99}	χ 2	Slope	RF	RL
Reference Population	73.97[a] (60.06-86.19)	333.61[a] (244.71-577.50)	13.67*	3.556 ± 0.287	-	-
Anantapur	80.31[a] (66.80-92.79)	365.54[a] (270.59-606.60)	13.01*	3.535 ± 0.269	1.08	S
Chittoor	83.08[a] (70.14-95.31)	361.61[a] (270.84-582.71)	12.62*	3.642 ± 0.271	1.12	S
YSR Kadapa	76.02[a] (62.44-88.19)	348.53[a] (257.12-588.12)	12.98*	3.518 ± 0.278	1.03	S
Krishna	78.12[a] (64.26-90.70)	352.16[a] (258.85-600.15)	13.76*	3.557 ± 0.276	1.06	S
Prakasam	84.94[a] (71.78-97.49)	375.42[a] (280.66-604.94)	12.71*	3.605 ± 0.264	1.15	S
Vizianagaram	79.14[a] (65.64-91.51)	333.75[a] (246.99-563.91)	14.15*	3.722 ± 0.288	1.07	S

Probit analysis using IBM SPSS 20.0 V
Values are estimate of lethal concentration (ppm) with 95% Fiducial confidence intervals
Values with different superscripts are significantly different (P< 0.05); * indicates P<0.05
RF = Resistance Factor; RL = Resistance Level; Susceptible (S) = RF<1.4; Level I= 1.5<RF<5;
Level II= 5.1<RF<25; Level III= 25.1<RF<40; Level IV= RF>40

Table 5: Status of resistance in *R. (B.) microplus* against different acaricides in certain places of Andhra Pradesh

Acaricide	Place / District						
	Anantapur	**Chittoor**	**YSR Kadapa**	**Krishna**	**Prakasam**	**Srikakulam**	**Vizianagaram**
Cypermethrin	S	R**	R*	S	R*	S	S
Deltamethrin	S	R**	R*	S	R*	S	S
Amitraz	S	S	S	S	S	S	S

S - Susceptible R* - Level I resistance (RL I) R** - Level II resistance (RL II)

Results of LPT revealed the development of various levels of resistance against both deltamethrin and cypermethrin in tick populations of *R. (B.) microplus* collected from Chittoor (RL II), Prakasam (RL I) and YSR Kadapa (RL I), while the tick populations collected from Anantapur, Vizianagaram and Krishna were susceptible to synthetic pyrethroids (Table 5).

Variations in synthetic pyrethroid susceptibility in ticks have been reported from other parts of India also. Ghosh *et al*. [17] reported the development of resistance in the tick populations of various places of Bihar against deltamethrin and cypermethrin. Sharma *et al*. [20] observed variations in development of deltamethrin (RL I to IV) and cypermethrin resistance (RL I and II) in a cross sectional study on *R. (B.) microplus* ticks collected from six agro-climatic regions of India. Similarly, Shyma *et al*. [14] detected cypermethrin resistance (RL I) in Fatehabad district of Haryana and Jyotimol *et al*. [15] observed the development of low level (RL I) deltamethrin resistance in *R. (B.) microplus* in Thumburmuzhi and Vithura regions of Kerala. The variations in the development of resistance in different tick populations to both the synthetic pyrethroids tested might be due to the repeated usage of same acaricide routinely, inadequate dose, usage of substandard acaricide, improper application strategy of acaricide etc., in addition to the existing host fauna and local environmental conditions suitable for tick survival. Development of acaricidal resistance also varies with strain specific difference and genetic tolerance of ticks [21]. The questionnaire designed in the present study also indicated that the frequency of application of acaricides is high in Chittoor (>8 times) followed by Prakasam (6-8 times) and YSR Kadapa (4-5 times) compared to other places of Andhra Pradesh. Spraying of animals, frequently (>5-6 per annum) with acaricide was considered as the main risk factor for development of resistance [22].

No significant difference was found in the resistance pattern of *R. (B.) microplus* larvae against amitraz among all places of the study. The LC_{50} value of amitraz for reference tick population was 73.97 ppm. Whereas LC_{50} values for various populations of *R. (B.) microplus* tested with modified LPT were recorded in between 76.02 to 84.94 ppm. The modified LPT result clearly indicated that the *R. (B.) microplus* larvae from all places of the study in Andhra Pradesh were susceptible to amitraz. The main reason for not development of amitraz resistance might be due to the less exposure of *R. (B.) microplus* ticks to amitraz comparatively to synthetic pyrethroids *viz.* deltamethrin and cypermethrin. The data collected through a questionnaire developed in the present study also revealed that there is no usage of amitraz compounds for tick control till date in certain places like Anantapur, YSR Kadapa, Krishna and Vizianagaram. However, usage of amitraz is recently started in Chittoor and Prakasam districts as an alternate to synthetic pyrethroid resistant ticks. There are few reports on development of amitraz resistance in *R. (B.) microplus* infesting Indian livestock. Amitraz resistance in *R. (B.) microplus* was recently reported from Punjab [11] and Gujarat [16].

Use of chemical acaricides is the mainstay of tick control in India. Synthetic pyrethroids such as deltamethrin and cypermethrin have been widely used. However, continuous use of acaricides exerts selection pressure on the ticks, which may result in the development of resistance in the tick populations. Therefore judicial use of available acaricides and preserving their efficacy with continuous monitoring on development of acaricidal resistance is very much warranted. The non-acaricidal methods like host resistance to ticks, pasture spelling, immunization, hand picking, brushing etc. are generally uneconomical and impractical for many reasons in a country like India [21]. Therefore, use of acaricides is the only option available with the farmers, which they are practicing. It is generally believed that the acaricides are potent if applied at the correct concentration and interval of time and can be considered as acaricides of choice for the control of ticks and tick-borne diseases [23].

3. Conclusion

The study indicates that synthetic pyrethroids should be used judiciously and there should be frequent monitoring of efficacy of these acaricides in order to avoid development of resistance in tick populations. This will ensure the better utilization of currently available acaricides. The LC_{50} values obtained during the present investigations can be taken as base-line values for monitoring the development of acaricide resistance in *R. (B.) microplus*.

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