

RESEARCH ARTICLE

In Vitro Detection of Acaricidal Resistance Status of *Rhipicephalus (Boophilus) microplus* against Commercial Preparation of Deltamethrin in Coastal Areas of South Gujarat, India

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ABSTRACT

A total of 2617 bovines (1682 cattle and 935 buffaloes) of coastal districts (Bharuch, Surat, Navsari and Valsad) of South Gujarat were screened for tick *Rhipicephalus (Boophilus) microplus*, the most economically important ectoparasite of bovines worldwide. Farmers chiefly relied on chemical acaricides (67.52%) to kill the ticks, and under chemical method 44.09, 24.17, 30.50 and 1.24 % animals had received cypermethrin, deltamethrin, ivermectin and fipronil, respectively, thus favouring the resistance. In adult immersion test (AIT), the median lethal concentration (LC₅₀) was 144.23, 93.97, 141.67 and 57.89 ppm for deltamethrin in Bharuch, Surat, Navsari and Valsad district, respectively. Resistance level was I in Valsad and II in other 3 districts for deltamethrin in AIT. In larval packet test (LPT), the median LC₅₀ was 297.29, 126.81, 127.83 and 93.92 ppm for deltamethrin in Bharuch, Surat, Navsari and Valsad district, respectively. Resistance level was III in Bharuch and II in other district in LPT.

Keywords: Acaricide, Deltamethrin, *Rhipicephalus (Boophilus) microplus*, Resistance, South Gujarat.

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INTRODUCTION

The tick causes economic loss due to lowered milk and meat yield, devaluation of leather, diseases transmission, additional hours of work required, additional facility costs, acaricides application and emergence of resistance against commonly used one (Estrada-Peña and Salman, 2013). *Rhipicephalus (Boophilus) microplus* is the most prevalent tick species found to infest bovine of South Gujarat, India (Ghosh *et al.*, 2006). Synthetic pyrethroids, deltamethrin and flumethrin are the predominant acaricides used to control ticks all over the world including India (Mathivathani *et al.*, 2011). There are reports regarding failure of the deltamethrin treatment world over against the ticks especially of *R. (B.) microplus* at the field level due to emergence of resistance (Andreotti *et al.*, 2011; Shakya *et al.*, 2020).

A lack of standardized techniques for diagnosing acaricide resistance appears to be the main difficulty in creating and maintaining a tick resistance monitoring system (FAO, 2004). Acaricide resistance can be detected by both *in vivo* and *in vitro* methods. The most widely used tests are bioassays, *in vitro* exposure of ticks (larvae or engorged females) to a single dose or to several increasing doses of an acaricidal compound (FAO, 2004). The commonly used bioassays for detection of acaricide resistance are larval packet test (LPT), larval immersion test (LIT) and adult immersion test (AIT) (Drummond *et al.*, 1973; FAO, 2004). Detection of acaricides resistance would provide immense help in formulation of different control strategies for management of acaricide resistance. It is quite demanding to assess the resistance status of commonly used deltamethrin from the coastal areas of South Gujarat, hence was studied.

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MATERIALS AND METHODS

Systematic survey on ixodid ticks of bovine was undertaken at four coastal districts of South Gujarat, India, *viz.*, Bharuch, Surat, Navsari and Valsad from June 2016 to May 2017. Systemic approach was made to collect owner's response pertaining to the question regarding method of tick control, name of chemical drugs, drug used on the advice of which person, frequency and effectiveness of drugs etc. The female engorged ticks were collected from the cracks and crevices of the animal sheds. For handling field ticks, the recommended method of FAO (2004) was followed. The ticks were identified by studying the standard morphological keys (Miranpuri, 1979).

Different concentrations of the commercial deltamethrin (Butox @1.25% EC) prepared in distilled water were used to conduct bioassays to know the status of acaricide resistance. The recommended dose was taken as 25 ppm for deltamethrin. The higher concentration of deltamethrin was used in bioassay, viz., 25, 50, 75, 100 and 125 ppm.

Adult Immersion Test (AIT)

The test was conducted according to Drummond *et al.* (1973) with some modifications. Ten randomly selected engorged female ticks were weighed and immersed for 2 minutes in 50 ml of prepared graded dose acaricides in glass beakers. As a control, 10 ticks of the same batch were treated similarly in distilled water for 2 minutes. Ticks were observed for their mortality on 14th day post-treatment (DPT) and laid egg masses were weighed. Dead ticks were confirmed by observing loss of motility and pedal reflex after exposing to light. The regression curve of mortality percentage was plotted against working concentrations of the acaricides in Microsoft Office Excel. The median lethal concentration 50% (LC₅₀) of deltamethrin for field tick isolates were calculated using the regression equation.

$$Y = mx + C$$

m = slope of the curve,

C = y intercept (correlation coefficient),

x = concentration of acaricide,

Y = probit value of mortality percentage

Resistance factors (RF) for field tick isolates were worked out by the ratio between LC₅₀ of field ticks and LC₅₀ of susceptible strain of *R. (B.) microplus* IVRI-I line (13.4 ppm LC₅₀ of deltamethrin). On the basis of RF, the resistance status in the field population of ticks was classified as susceptible (RF < 1.4), level I resistance (1.5 < RF < 5.0), level II resistance (5.1 < RF < 25.0), level III resistance (26.0 < RF < 40.0) and level IV resistance (RF > 41) (Kumar *et al.*, 2011).

Larval Packet Test (LPT)

Fifteen ticks were incubated at 28°C and 75-85% RH in labelled glass bottle covered with muslin cloth for oviposition. The eggs were further incubated at same condition in the BOD incubator for 18-25 days to hatch into larvae. The LPT was performed using 10-12 day old hungry larvae as recommended by FAO (2004) with some minor modifications. Approximately 0.6 ml of prepared acaricide solution was poured on Whatman filter paper number 1. After saturation of the compound the filter paper was dried in incubator at 37°C for 30 minutes. Treated and dried parallelogram of paper was folded in half forming equilateral triangular packet and sealed on the sides with adhesive tapes forming an open ended packet. After placing approximately 100 larvae with the help of paint brush, the open side of each packet was sealed with adhesive tape. The sealed packet was then placed in a desiccator having 50 ml of 10% KOH solution in the bottom and incubated in BOD. incubator at 28°C and

75-85% RH. After 24 hours, the packets were opened, and mortality percentage was calculated on the basis of live and dead larvae count. The regression curve of larvae mortality % was prepared in the same manner as in above test. Resistance factors (RF) for field tick's larvae isolates were worked out by the ratio between LC₅₀ of field tick's larvae and LC₅₀ of larvae of susceptible strain *R. (B.) microplus* IVRI-I line (111.8 ppm LC₅₀ of deltamethrin) (Castro-Janer *et al.*, 2009). On the basis of RF, the resistance level in the field population of tick's larvae was classified as described in AIT.

Statistical Analysis

The statistical analysis of the data was done using software IBM-SPSS 20 and Micro Soft Excel-2010. The differences in mean values of entomological data amongst the groups were analyzed by one way ANOVA.

RESULTS AND DISCUSSION

Animal owners' response (n=2617) to the questionnaire asked about the control measures adopted against the tick infestation in the area under study is illustrated in Table 1. Three major treatment approaches, mechanical by hand picking/ rearing poultry, chemical, and herbal/ no treatment were adopted by the animal owners in the studied areas to minimize the tick infestation of the animals. The farmers applied mechanical, chemical and herbal/ no treatment strategies on 21.40, 67.52 and 11.08% animals, respectively (Table 1).

Under chemical method adopted on 1767 animals, i.e., 67.52% animals exposed to chemical acaricides, 24.17, 44.09, 30.50 and 1.24 % animals had received deltamethrin, cypermethrin, ivermectin and fipronil, respectively (Table 1). Effective and non-effective treatment/ control strategies were observed on 50.25% and 49.75% bovines, respectively. As per recent reports, among all the methods, deltamethrin is commonly used as an acaricide by animal owners for tick control (Kumar *et al.*, 2019). Hlatshwayo and Mbatl (2005) observed that only few participants applied commercial acaricides (16%) on their animals. However, many farmers (70 %) perceived the use of commercial acaricides as beneficial, citing economical constraints as the main reason for their reduced level of participation.

Acaricide Resistance Status in *Rhipicephalus (Boophilus) microplus*

Adult Immersion Test (AIT) with Deltamethrin

The rate of mortality in the current study showed increasing trend with the increasing concentrations of the acaricide with decreasing amount of the egg mass (Table 2) and it varied significantly (p < 0.05) from the control group. In Navsari district, the Reproductive Index (RI) was 0.66±0.02 and 0.34±0.01 to 0.08±0.01 in the control and treated groups, respectively, with 48.25±1.93 to 88.60±1.33% of Inhibition of Oviposition (IO). The slope of the mortality curve was

Table 1: Animal owners response related to tick control measures adopted in south Gujarat

Question/ Information asked	No. (%)					
	Bharuch	Surat	Navsari	Valsad	Overall	
Mechanical	21 (3.63)	62 (7.10)	167 (25.93)	310 (59.50)	560 (21.40)	
Method of control	Chemical Deltamethrin	128 (22.15)	44 (5.03)	226 (35.10)	29 (5.57)	427 (16.32)
	Cypermethrin	176 (30.45)	362 (41.42)	167 (25.93)	74 (14.20)	779 (29.77)
	Ivermectin	159 (27.51)	320 (36.61)	25 (3.88)	35 (6.72)	539 (20.60)
	Fipronil	5 (0.86)	15 (1.72)	2 (0.31)	0 (0.00)	22 (0.84)
	Sub-total	468 (80.97)	741 (84.78)	420 (65.22)	138 (26.49)	1767 (67.52)
Herbal/ No treatment	89 (15.40)	71 (8.12)	57 (8.85)	73 (14.01)	290 (11.08)	
Total	578	874	644	521	2617	
Drug/ control measures advised by	Clinician	52 (9.00)	436 (49.89)	375 (58.23)	25 (4.80)	888 (33.93)
	Para-clinician	423 (73.18)	278 (31.81)	216 (33.54)	244 (46.83)	1161 (44.36)
	Self	103 (17.82)	160 (18.31)	53 (8.23)	252 (48.37)	568 (21.70)
Frequency of drug/ control measures used	Regular	327 (56.57)	623 (71.28)	438 (68.01)	259 (49.71)	1647 (62.93)
	Occasional	251 (43.43)	251 (28.72)	206 (31.99)	262 (50.29)	970 (37.07)
Effect of control measures adopted	Effective	320 (55.36)	378 (43.25)	297 (46.12)	320 (61.42)	1315 (50.25)
	Non-effective	258 (44.64)	496 (56.75)	347 (53.88)	201 (38.58)	1302 (49.75)

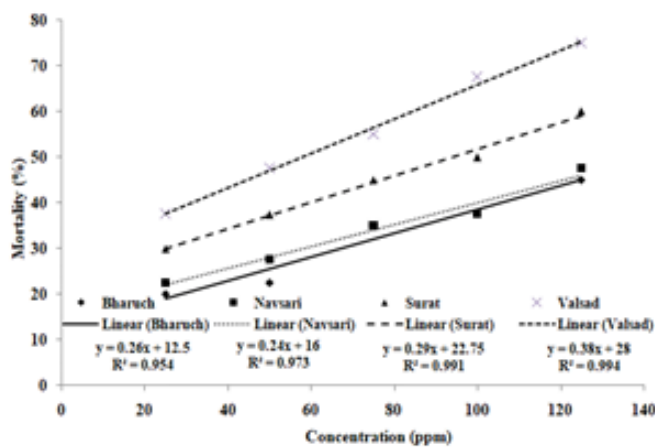


Fig. 1: Regression curve showing mortality of adult tick in AIT

0.24. The calculated median LC₅₀ was 141.67 ppm from the equation with 10.57 RF, thus indicated level II resistance in Navsari (Table 2; Fig. 1). The RI, slope of mortality curve, median LC₅₀ and RF value of other three districts were as mentioned in Table 2.

The present study noted level II resistance in Bharuch (RF=10.76) / Surat (RF=7.01)/ Navsari (RF=10.57) and level I in Valsad (RF=4.32) district (Table 2; Fig. 1). The targeted 4 districts are in close proximation and have almost same agro-climatic conditions, but this variation in the level of resistance can be due to variation in animal management practices, treatment and control strategy adopted by the local farmers. The farmers of Valsad relied mainly on the mechanical/ herbal method to minimize or destroy the ticks population present on their bovines. Such type of area-wise varying resistance level was observed by Sharma *et al.* (2012) who detected level I to level IV resistance in different areas for deltamethrin

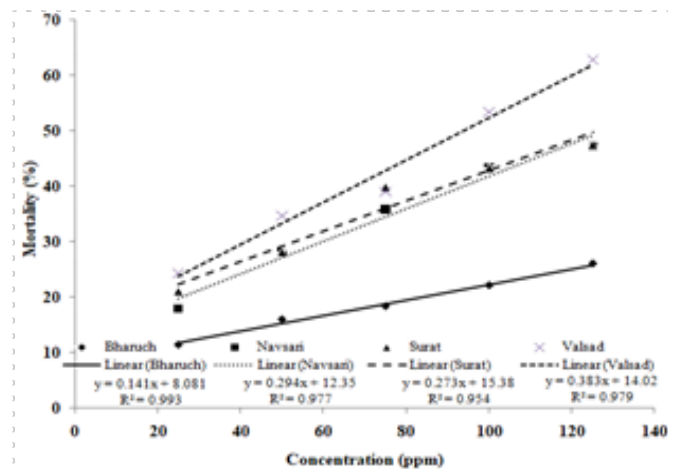


Fig. 2: Regression curve showing mortality of tick larva in LPT

against *R.(B.) microplus* in AIT. Mathivathani *et al.* (2011) found 64.72 % resistance to brown dog tick against deltamethrin in Chennai. Similarly, Kumar *et al.* (2017) detected resistance to deltamethrin at level I (RF=2.5-4.9) in 02 isolates, at level II in 03 isolates (RF=5.4-11.5) and level IV in 02 isolates (RF=48.1-95.7) of *R. (B.) microplus* in Andhra Pradesh. In the following year, the same author noted level II (RF=8.78) resistance to deltamethrin in Chittoor district (Kumar *et al.*, 2017). Shyma *et al.* (2015) observed level I resistance in the Banaskatha district of North Gujarat, India. Similarly, Godara *et al.* (2019) detected resistance to deltamethrin level I in two, level II in four, level III and Level IV in one isolate with resistant factor (RFs) 0.94 to 50.71 in North-Western Himalaya region.

Likewise, Castro-Janer *et al.* (2009) noted more sensitivity of AIT than LPT, with larger resistance factors. For mortality by AIT and LPT the discriminating concentrations estimated



Table 2: Adult immersion test (AIT) and Larval packet test (LPT) of ticks with deltamethrin

		AIT					LPT				
		Mean±SE					Mean±SE				
District	Concentration (ppm)	Tick weight (g) before treatment	Egg mass (g) on 14 th dpt	Reproductive index	% Inhibition of oviposition	Mortality on 14 th dpt	Slope, LC ₅₀ R ² , RF	Death	Mortality (%)	Slope, R ² , LC ₅₀ RF	
Bharuch	0	1.35±0.03	0.89 ^a ±0.01	0.66 ^e ±0.02	0.00 ^a ±0.00	0.00 ^a ±0.00	Slope=0.26, R ² =0.954	1.50 ^a ±0.50	1.43 ^a ±0.48	Slope=0.141 R ² =0.993	
	25	1.35±0.03	0.49 ^c ±0.01	0.36 ^d ±0.01	45.14 ^b ±2.11	2.00 ^b ±0.00	LC ₅₀ =144.23 RF=10.76	12.25 ^b ±0.63	11.56 ^b ±0.58	R ² =0.993 LC ₅₀ =297.29 RF=25.19	
	50	1.41±0.01	0.44 ^c ±0.03	0.31 ^c ±0.02	52.15 ^b ±3.75	2.25 ^b ±0.25		16.25 ^c ±0.48	15.29 ^c ±0.29		
	75	1.41±0.03	0.36 ^b ±0.01	0.26 ^b ±0.01	61.41 ^c ±2.03	3.50 ^c ±0.29		18.25 ^d ±0.48	17.63 ^d ±0.27		
	100	1.37±0.01	0.27 ^a ±0.02	0.20 ^a ±0.02	70.16 ^d ±3.26	3.75 ^c ±0.25	Level II resistance	23.00 ^e ±0.58	21.44 ^e ±0.40	Level III resistance	
	125	1.36±0.01	0.24 ^a ±0.02	0.18 ^a ±0.02	72.93 ^d ±3.60	4.50 ^d ±0.29		27.25 ^f ±0.48	25.65 ^f ±0.33		
Surat	F	1.57	149.31	129.61	93.24	53.31		290.85	431.51		
	P	0.219	0.00	0.00	0.00	0.00		0.00	0.00		
	0	1.19±0.02	0.78 ^a ±0.01	0.66 ^e ±0.01	0.00 ^a ±0.00	0.00 ^a ±0.00	Slope=0.29, R ² =0.991, LC ₅₀ =93.97, RF=7.01	1.50 ^a ±0.65	1.36 ^a ±0.58	Slope=0.273 R ² =0.954 LC ₅₀ =126.81 RF=10.74	
	25	1.18±0.01	0.39 ^d ±0.02	0.33 ^d ±0.02	49.71 ^b ±2.79	3.00 ^b ±0.00		23.00 ^b ±0.00	20.91 ^b ±0.00		
	50	1.20±0.01	0.31 ^c ±0.03	0.26 ^c ±0.02	60.14 ^c ±3.97	3.75 ^b ±0.25		32.00 ^c ±0.00	28.07 ^c ±0.00		
	75	1.18±0.02	0.27 ^b ±0.03	0.23 ^b ±0.02	64.72 ^{cd} ±3.84	4.50 ^{cd} ±0.29		43.00 ^d ±0.00	39.81 ^d ±0.00		
Navsari	100	1.21±0.02	0.22 ^{ab} ±0.02	0.18 ^{ab} ±0.02	72.37 ^{de} ±3.13	5.00 ^d ±0.41	Level II resistance	48.00 ^e ±0.00	43.24 ^e ±0.00	Level II resistance	
	125	1.22±0.02	0.18 ^a ±0.02	0.15 ^a ±0.02	77.97 ^f ±3.11	6.00 ^e ±0.41		58.00 ^f ±0.00	47.54 ^f ±0.00		
	F	1.04	109.66	99.89	83.09	54.60		5857.57	5350.73		
	P	0.427	0.00	0.00	0.00	0.00		0.00	0.00		
	0	1.17±0.02	0.77 ^a ±0.01	0.66 ^f ±0.02	0.00 ^a ±0.00	0.00 ^a ±0.00	Slope=0.24, R ² =0.973, LC ₅₀ =141.67 RF=10.57	0.75 ^a ±0.48	0.71 ^a ±0.45	Slope=0.294 R ² =0.977 LC ₅₀ =127.83 RF=10.83	
	25	1.16±0.02	0.40 ^e ±0.01	0.34 ^e ±0.01	48.25 ^b ±1.93	2.25 ^b ±0.25		19.00 ^b ±0.00	17.92 ^b ±0.00		
Valsad	50	1.18±0.01	0.29 ^d ±0.01	0.25 ^d ±0.01	62.83 ^c ±1.31	2.75 ^b ±0.25		32.00 ^c ±0.00	28.07 ^c ±0.00		
	75	1.16±0.02	0.24 ^c ±0.01	0.21 ^c ±0.01	68.12 ^d ±2.07	3.50 ^c ±0.29		43.00 ^d ±0.00	35.83 ^d ±0.00		
	100	1.19±0.02	0.17 ^b ±0.01	0.15 ^b ±0.01	78.06 ^e ±0.92	3.75 ^c ±0.25	Level II resistance	48.00 ^e ±0.00	43.24 ^e ±0.00	Level II resistance	
	125	1.20±0.02	0.09 ^a ±0.01	0.08 ^a ±0.01	88.60 ^f ±1.33	4.75 ^d ±0.25		58.00 ^f ±0.00	47.15 ^f ±0.00		
	F	0.76	559.26	315.56	477.99	48.00		11445.66	8955.78		
	P	0.589	0.00	0.00	0.00	0.00		0.00	0.00		
Valsad	0	1.28±0.02	0.82 ^f ±0.1±	0.64 ^f ±0.02	0.00 ^a ±0.00	0.00 ^a ±0.00	Slope=0.38, R ² =0.994, LC ₅₀ =57.89, RF=4.32	2.50 ^a ±0.29	2.35 ^a ±0.28	Slope=0.383 R ² =0.979 LC ₅₀ =93.92 RF=7.96	
	25	1.27±0.01	0.45 ^e ±0.01	0.35 ^e ±0.01	45.40 ^b ±1.79	3.75 ^b ±0.25		23.75 ^b ±0.48	24.05 ^b ±0.37		
	50	1.29±0.00	0.34 ^d ±0.01	0.27 ^d ±0.01	59.00 ^c ±1.32	4.75 ^c ±0.25		36.75 ^c ±0.48	34.42 ^c ±0.29		
	75	1.27±0.02	0.29 ^c ±0.01	0.23 ^c ±0.01	63.95 ^d ±1.47	5.50 ^d ±0.29		41.75 ^d ±0.48	38.75 ^d ±0.27		
	100	1.27±0.02	0.22 ^b ±0.01	0.18 ^b ±0.01	72.62 ^e ±0.91	6.75 ^e ±0.25	Level I resistance	55.75 ^e ±0.48	53.22 ^e ±0.21	Level II resistance	
	125	1.28±0.02	0.14 ^a ±0.01	0.11 ^a ±0.01	82.92 ^f ±1.02	7.50 ^f ±0.29		63.75 ^f ±0.48	62.66 ^f ±0.17		
	F	0.14	559.26	352.88	575.03	120.88		2396.47	6147.82		
	P	0.981	0.00	0.00	0.00	0.00		0.00	0.00		

Means with different superscript within column are statistically different. F=Frequency, p=Probability, 0 ppm=control, RF= resistance factor, R²=goodness of fit. Susceptible= RF<1.4; Level I= 1.5<RF<5; level II= 5.1<RF<25; level III= 26<RF<40; level IV= RF>41

by the Castro-Janer *et al.* (2009) were 4.98 ppm and 2365.8 ppm for Mozo strain.

Larval Packet Test (LPT) with Deltamethrin

The mortality percentage in the larvae of ticks gradually increased with increasing concentrations of the acaricide (Table 2). The level of resistance was III in Bharuch (RF=25.29) while II in the other districts (RF=7.96-10.83). The calculated median LC₅₀ of Bharuch, Surat, Navsari and Valsad district were 297.29, 126.81, 127.83 and 93.92 ppm, respectively (Table 2; Fig. 2). Likewise Mendes *et al.* (2007) in Brazil detected resistance level II and III for deltamethrin against *R. (B.) microplus* in LPT. Nandi *et al.* (2014) detected resistant level I (RR₅₀=1.71- 4.91) in 5 isolates and level II in Ludhiana isolate (RR₅₀= 5.59) of *R. (B.) microplus* against deltamethrin by LPT. Shyma *et al.* (2015) in the Banaskatha district of North Gujarat, India detected level II (RF>25) resistance against this pyrethroid. Kumar *et al.* (2019) detected LC₅₀ & LC₉₅ value of deltamethrin by LPT as 25.106 ppm and 328.031 ppm, respectively.

In conclusion, the area-wise acaricides resistance detection study is essentially required to develop long term control measures against the ticks. The present study involving coastal districts of south Gujarat observed varying level of resistance to deltamethrin. This information must be utilized while instituting treatment and control strategy against the bovine ticks in this area.

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