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

Dharmesh C Patel¹*, Jayesh B Solanki², Niranjan Kumar³

**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 (Ghos 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 acaridical 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.

**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.

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**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.

\[ Y = mx + C \]

\[ m= \text{slope of the curve}, \]
\[ C= \text{y intercept (correlation coefficient)}, \]
\[ x= \text{concentration of acaricide}, \]
\[ Y= \text{probit value of mortality percentage} \]

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 described in AIT. Resistance factors (RF) for field tick larvae isolates were worked out by the ratio between LC50 of field ticks and LC50 of susceptible strain of *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 Mbati (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.

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**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...
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The calculated median LC$_{50}$ 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 LC$_{50}$ 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 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

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

| Question/Information asked | Bharuch | Surat | Navsari | Valsad | Overall |
|----------------------------|---------|-------|---------|--------|---------|
| Method of control          |         |       |         |        |         |
| Mechanical                 | 21 (3.63) | 62 (7.10) | 167 (25.93) | 310 (59.50) | 560 (21.40) |
| Chemical                   | 128 (22.15) | 44 (5.03) | 226 (35.10) | 29 (5.57) | 427 (16.32) |
| Deltamethrin               | 176 (30.45) | 362 (41.42) | 167 (25.93) | 74 (14.20) | 779 (29.77) |
| Cypermethrin               | 159 (27.51) | 320 (36.61) | 25 (3.88) | 35 (6.72) | 539 (20.60) |
| Ivermectin                 | 5 (0.86) | 15 (1.72) | 2 (0.31) | 0 (0.00) | 22 (0.84) |
| Fipronil                   |         |       |         |        |         |
| 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 | Para-clinician | Self |         |
|-----------------------------------|------------|----------------|------|---------|
| Regular                           | 52 (9.00) | 436 (49.89) | 375 (58.23) | 25 (4.80) | 888 (33.93) |
| Occasional                        | 423 (73.18) | 278 (31.81) | 216 (33.54) | 244 (46.83) | 1161 (44.36) |
| Total                              | 475 (81.28) | 714 (83.70) | 591 (92.57) | 269 (50.67) | 2539 (96.16) |

| Frequency of drug/ control measures used | Regular | Occasional |         |
|------------------------------------------|---------|------------|---------|
| Effective                                | 320 (55.36) | 297 (46.12) | 320 (61.42) |
| Non-effective                            | 258 (44.64) | 496 (53.88) | 201 (38.58) |
| Total                                    | 578 | 644 | 521 |

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

Fig. 2: Regression curve showing mortality of tick larva in LPT
Table 2: Adult immersion test (AIT) and Larval packet test (LPT) of ticks with deltamethrin

| District | Concentration (ppm) | Mean±SE | LPT Mean±SE |
|----------|---------------------|---------|-------------|
| Bharuch  | 0                   | 1.35±0.03 | 1.50±0.50 |
|          | 25                  | 1.35±0.03 | 1.25±0.63 |
|          | 50                  | 1.41±0.01 | 1.16±0.55 |
|          | 75                  | 1.41±0.03 | 1.25±0.63 |
|          | 100                 | 1.37±0.01 | 1.25±0.63 |
|          | 125                 | 1.36±0.01 | 1.25±0.63 |
| F        | 1.50±0.00           | 1.25±0.63 |
| P        | 0.219               | 0.00     | 0.00        |
| Surat    | 0                   | 1.19±0.02 | 1.50±0.65 |
|          | 25                  | 1.18±0.01 | 2.30±0.69 |
|          | 50                  | 1.20±0.01 | 3.20±0.69 |
|          | 75                  | 1.18±0.02 | 4.30±0.69 |
|          | 100                 | 1.21±0.02 | 4.30±0.69 |
|          | 125                 | 1.22±0.02 | 4.30±0.69 |
| F        | 1.04                | 83.09    | 48.00±0.00 |
| P        | 0.427               | 99.89    | 5857.57    |
| Navsari  | 0                   | 1.17±0.02 | 0.75±0.48 |
|          | 25                  | 1.16±0.02 | 19.00±0.00 |
|          | 50                  | 1.18±0.01 | 3.20±0.00 |
|          | 75                  | 1.16±0.02 | 4.30±0.00 |
|          | 100                 | 1.19±0.02 | 4.30±0.00 |
|          | 125                 | 1.20±0.02 | 4.30±0.00 |
| F        | 0.76                | 477.99   | 11445.66   |
| P        | 0.589               | 48.00    | 8955.78    |
| Valsad   | 0                   | 1.28±0.02 | 2.50±0.29 |
|          | 25                  | 1.27±0.01 | 23.75±0.48 |
|          | 50                  | 1.29±0.00 | 36.75±0.48 |
|          | 75                  | 1.27±0.02 | 41.75±0.48 |
|          | 100                 | 1.27±0.02 | 55.75±0.48 |
|          | 125                 | 1.28±0.02 | 63.75±0.48 |
| F        | 0.14                | 575.03   | 2396.47    |
| P        | 0.981               | 120.88   | 6147.82    |

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

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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=796-10.83). The calculated median LC$_{50}$ 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 \textit{R. (B.) microplus} in LPT. Nandi et al. (2014) detected resistant level I (RR$_{50}$=1.71- 4.91) in 5 isolates and level II in Ludhiana isolate (RR$_{50}$= 5.59) of \textit{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 LC$_{50}$ & LC$_{95}$ 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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