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## Assessment of deltamethrin resistance in *Haemaphysalis* spp. on goats in Kerala by larval packet test

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**Abstract**

Tick- and tick-borne infections contribute to huge economic loss in livestock sector. The most practical strategy for tick control is chemotherapy, the efficacy of which has been challenged by the emergence of drug resistance. Acaricide resistance has been well studied in cattle ticks, but not in goat ticks. The status of deltamethrin resistance in *Haemaphysalis* spp. infesting goats of Thrissur and Palakkad districts was assessed by larval packet test using different concentrations of technical grade deltamethrin. In the present study, Ottapilavu, Mundupalam and Poomala isolates were found susceptible, while Vadakkanchery isolate was resistant. Log probit analysis was done to derive the LC<sub>50</sub> and LC<sub>90</sub> of resistant and susceptible isolates. *Haemaphysalis* spp. on goats are developing resistance to deltamethrin. Future investigations are to be conducted on molecular characterisation of resistance in these species of ticks and to detect the status of resistance to different group of acaricides throughout the state.

**Keywords:** Deltamethrin, *Haemaphysalis* spp., goats, acaricide resistance

**Introduction**

*Haemaphysalis* spp. is a widely distributed three host tick infesting goats possibly transmitting *Babesia motasi*, *B. ovis* and *Theileria ovis* infections in goats in India<sup>1</sup>. Apart from vector harm to livestock, the direct and indirect effect leads to heavy economic losses to farmers. Direct effects of heavy tick infestation leads to blood loss, tick worry, wound, hide damage and toxins, decreased milk production, reduced body weight. In India, the cost for controlling TTBDs in the dairy industry has been estimated at \$498.7 million per annum<sup>2</sup>. A very high prevalence (59.3%) of caprine theileriosis had been detected in Kerala which warrants immediate interventions<sup>3</sup>. Besides treatment of the disease, control of ticks also needs top priority. The most commonly followed technique to control tick infestation in India and in other countries, is the application of synthetic pyrethroids (deltamethrin and cypermethrin). Resistance to acaricides is a wide spread problem and is commonly detected by bioassays like adult immersion test and larval packet test<sup>4</sup>. Deltamethrin resistance in *Rhipicephalus (Boophilus) microplus* and *R. (B.) annulatus* has been reported from Kerala by larval packet test<sup>5</sup>. However, status of acaricidal resistance in multi host ticks from southern India is limited. Deltamethrin resistance has been reported in *Haemaphysalis* spp. from Karnataka<sup>6</sup>. No information is available on status of resistance among this species in Kerala. Hence, the present study was conducted to assess the status of acaricide resistance against deltamethrin in *Haemaphysalis* spp. infesting goats of Kerala and to provide a clear understanding of the high prevalence of tick infestation on goats in the state.

**Materials and Methods****Study area and tick rearing**

Fully engorged female ticks were collected from goats reared in different organised and unorganised farms in Ottapilavu, Poomala, Mundupalam and Vadakkanchery of Thrissur and Palakkad district of Kerala. After identification of ticks, they were kept individually at 28±1 °C and 85±5 per cent relative humidity (RH) in labelled glass vials for oviposition. The eggs laid were allowed to hatch to larvae under conditions of incubation.

### Larval packet test (LPT)

Deltamethrin (Technical grade 100% pure, Sigma Aldrich, USA) was diluted in methanol and used for performing larval packet test (LPT) as per the recommendations with minor modifications [4]. Briefly, 0.6 mL of different concentrations of deltamethrin (15, 30, 60, 120, 240, 600 ppm) in distilled water were used to impregnate filter paper packets, which were then dried, folded and sealed on one side with adhesive tapes, forming an open-ended triangular packet to place approximately 100 larvae. Then the open end of each packet was sealed and placed in a desiccator at  $28 \pm 1$  °C and  $85 \pm 5$  per cent RH. Each dose had three replicates and in control group distilled water was used. The packets were opened after 24 h and larval mortality was recorded.

### Statistical analysis

All the data were expressed as the Mean  $\pm$  SEM. Groups were compared by one-way ANOVA using SPSS software. Duncan's test was used for *post-hoc* analysis. A value of  $P < 0.05$  was considered as statistically significant. Dose response data were analysed by probit method [7] using IBM SPSS software.  $LC_{50}$  and  $LC_{90}$  values of deltamethrin and amitraz were determined by applying regression equation to the probit transformed data of mortality.

### Result and discussion

The ticks collected from goats were identified as *Haemaphysalis* spp. The per cent mortality of the larvae from different places, at different concentrations of deltamethrin was recorded (Tables 1, 2, 3, 4). Chi-square test was used for comparing the observed frequency of each dose with the frequency predicted by the probit function. Chi-square was found to be non significant indicating that the observed frequencies were in agreement with the expected frequency, and hence the predicted models were good fit. Comparison of mortality at different concentrations using the larvae from Ottapilavu tick isolates revealed that there was significant increase in the mortality at 15 ppm when compared with the control. There was no significant difference in the mortality between any other treatment groups. The mean mortality of Poomala tick isolates at 30 ppm was significantly low when compared to 600 ppm. There was no significant difference in mortality at 60 ppm, 120 ppm and at 240 ppm. The Mundupalam isolates of *Haemaphysalis* spp. revealed a high mortality even at the lowest dose of 30 ppm, though it was not significantly different from others. The Vadakkanchery isolates of *Haemaphysalis* spp. exhibited a significant difference in per cent mortality in the different test

concentrations. The mortality at 600 ppm, was cent per cent for all the isolates tested.

The proportion of ticks surviving a particular dose, designated as DD, could be considered as percentage resistance to the acaricide and it was reported to be 600 ppm for susceptible Yeerongpilly (Australia) strains of *R. (B.) microplus* [4]. However, DD must be established cautiously [8]. Published reports on the estimated DD of deltamethrin using IVRI susceptible lines of *R. (B.) microplus*, revealed lower values like 59.2 ppm [9] and 71 ppm [10]. Besides, the DD for susceptible lines of other ixodid tick species in India also did not show much variation. For *H. a. anatolicum*, the DD was 69.8 ppm [10] and for *Haemaphysalis* spp. it was 75 ppm<sup>6</sup>. A population could be designated resistant when the survival rate at the DD was over 10 per cent [11]. In the present study, Ottapilavu and Mundupalam isolates of *Haemaphysalis* showed more than 90 per cent mortality at concentrations from 30 ppm onwards and Poomala isolates showed 93.4 per cent mortality at 60 ppm itself. No known susceptible lines were available for *Haemaphysalis* spp. to compare the results of field isolates. Hence, with the available reports on DD with Indian susceptible lines of different tick species, these isolates could be considered susceptible for deltamethrin with LPT. However, one isolate namely, *Haemaphysalis* spp. from Vadakkanchery showed less than 50 per cent mortality even at 240 ppm and was considered resistant.

The lowest  $LC_{50}$  (0.996 ppm) and  $LC_{90}$  (6.028 ppm) were recorded for Ottapilavu isolates using deltamethrin against larvae of *Haemaphysalis* spp. The highest  $LC_{50}$  (80.09 ppm) and  $LC_{90}$  (270.61 ppm) of deltamethrin were observed for the resistant Vadakkanchery isolates. The  $LC_{50}$  and  $LC_{95}$  of IVRI-II line of susceptible *H. a. anatolicum* was found to be 11.7 ppm and 34.9 ppm, respectively [12]. In Kerala, LPT using reference susceptible lines of *R. (B.) annulatus* and *R. (B.) microplus* had revealed a low  $LC_{50}$  value of 2.11 ppm and 2.15 ppm, respectively, with deltamethrin [5, 13]. It was also observed that  $LC_{50}$  values of south Indian isolates were lower than that of north Indian isolates of the same tick species<sup>13</sup>. The field resistant *R. (B.) microplus* isolates showed a maximum  $LC_{50}$  of 13.8 ppm in their study. The LPT bioassay using *Haemaphysalis* spp. with deltamethrin in Karnataka revealed  $LC_{50}$  values ranging from 40.34 ppm to 44.36 ppm. However, for *R. sanguineus* tick population it was 62.13 ppm. These isolates were reported as deltamethrin resistant [6]. The  $LC_{50}$  of deltamethrin for resistant field isolates of *R. (B.) microplus* ticks in Andhra Pradesh was 40.05 ppm [14], which was lower than the  $LC_{50}$  observed for resistant *Haemaphysalis* spp. (80.09 ppm) in this study.

**Table 1:** Results of LPT with Ottapilavu isolates using deltamethrin

Sl. No	Concentration (ppm)	Percent mortality	Probit analysis				
		Mean $\pm$ SE	Slope $\pm$ SE	$\chi^2$ value	R <sup>2</sup> value	LC <sub>50</sub> (ppm)	LC <sub>90</sub> (ppm)
1	Control	6.6250 $\pm$ 2.4650 <sup>a</sup>	1.639 $\pm$ 1.048	0.263 <sup>ns</sup>	0.6817	0.996	6.028
2	15	97.725 $\pm$ 2.275 <sup>b</sup>					
3	30	98.860 $\pm$ 1.140 <sup>b</sup>					
4	60	100.0 <sup>b</sup>					
5	120	100.0 <sup>b</sup>					
6	240	100.0 <sup>b</sup>					
7	600	100.0 <sup>b</sup>					

Values with different superscripts indicate significant difference ( $p < 0.01$ )

**Table 2:** Results of LPT with Poomala isolates using deltamethrin

Sl. No.	Concentration (ppm)	Percent mortality	Probit analysis				
		Mean $\pm$ SE	Slope $\pm$ SE	$\chi^2$ value	R <sup>2</sup> value	LC <sub>50</sub> (ppm)	LC <sub>90</sub> (ppm)
1	Distilled water (control)	0.000 $\pm$ 0.000 <sup>a</sup>	1.795 $\pm$ 0.285	7.293 <sup>ns</sup>	0.8705	13.944	72.190
2	30	68.475 $\pm$ 18.475 <sup>b</sup>					
3	60	93.425 $\pm$ 0.325 <sup>bc</sup>					
4	120	94.950 $\pm$ 1.200 <sup>bc</sup>					
5	240	96.510 $\pm$ 3.490 <sup>bc</sup>					
6	600	100.0 <sup>c</sup>					

Values with different superscripts indicate significant difference ( $p < 0.01$ )

**Table 3:** Results of LPT with Mundupalam isolates using deltamethrin

Sl. No.	Concentration (ppm)	Percent mortality	Probit analysis				
		Mean $\pm$ SE	Slope $\pm$ SE	$\chi^2$ value	R <sup>2</sup> value	LC <sub>50</sub> (ppm)	LC <sub>90</sub> (ppm)
1	Distilled water (control)	0.0000 $\pm$ 0.000 <sup>a</sup>	1.722 $\pm$ 0.615	2.200 <sup>ns</sup>	0.734	4.10	22.74
2	30	94.160 $\pm$ 2.330 <sup>b</sup>					
3	60	96.070 $\pm$ 1.960 <sup>bc</sup>					
4	120	100.0 <sup>c</sup>					
5	240	100.0 <sup>c</sup>					
6	600	100.0 <sup>c</sup>					

Values with different superscripts indicate significant difference ( $p < 0.01$ )

**Table 4:** Results of LPT with Vadakkanchery isolates using deltamethrin

Sl. No.	Concentration (ppm)	Percent mortality	Probit analysis				
		Mean $\pm$ SE	Slope $\pm$ SE	$\chi^2$ value	R <sup>2</sup> value	LC <sub>50</sub> (ppm)	LC <sub>90</sub> (ppm)
1	Distilled water (control)	5.505 $\pm$ 0.745 <sup>a</sup>	1.301 $\pm$ 0.135	47.97 <sup>ns</sup>	0.627	80.09	270.61
2	15	19.99 $\pm$ 6.48 <sup>b</sup>					
3	30	25.17 $\pm$ 2.10 <sup>bc</sup>					
	60	32.465 $\pm$ 3.95 <sup>cd</sup>					
5	120	38.705 $\pm$ 0.975 <sup>de</sup>					
6	240	46.695 $\pm$ 5.025 <sup>e</sup>					
7	600	100.00 <sup>f</sup>					

Values with different superscripts indicate significant difference ( $p < 0.01$ )

Field isolates of ticks from southern India had reported low level of SP resistance in cattle, though studies have not yet been conducted to assess the status of acaricide resistance in goat ticks. The present study on *Haemaphysalis* spp. of ticks on goat revealed that resistance has been established in one of the isolates, though majority of the ticks studied remained susceptible to the drug. The extent of acaricides resistance in *Haemaphysalis* spp. on goats was lesser than in cattle ticks in the state. Incidence of acaricide resistance was observed to be high in one host ticks as a larger proportion of total tick population remained under chemical challenge at any one time than multi host tick [15]. However, the existence of deltamethrin resistance in goat ticks, though not wide spread, is a warning signal to use the drug more judiciously in field conditions. The wide variation in LC<sub>50</sub> of the reference lines reported by several authors suggests that a base line data of the respective tick species of a particular region need be developed to assess the resistance status in field conditions. Future studies should be directed to establish and evaluate susceptible reference *Haemaphysalis* spp. tick lines to study the drug resistance.

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