RESEARCH ARTICLE
Open Access

Journal of Veterinary and Animal Sciences
ISSN (Print): 0971-0701, (Online): 2582-0605

Deltamethrin resistance in *Rhipicephalus sanguineus* and *Rhipicephalus (Boophilus) microplus* tick population in Kerala

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Citation: Anand, A., Lakshmanan, B., Tambe, K.A., Joseph, S., Aravindakshan, T.V. and Jose, J. 2021.Deltamethrin resistance in *Rhipicephalussanguineus*and *Rhipicephalus (Boophilus) microplus*tick population in Kerala. *J. Vet. Anim. Sci.***52**(1): 19-25.

Received: 22.09.2020 *Accepted: 10.10.2020* *Published: 01.01.2021*

Abstract

The study evaluated deltamethrin resistance in Rhipicephalus sanguineus and Rhipicephalus (Boophilus) microplus tick populations of Kerala in India, using larval packet test (LPT). Dose response data were analysed by the probit method, the LC_{50} and LC_{95} of deltamethrin against ticks were determined by applying regression equation analysis to the probit-transformed data of mortality. In R. sanguineus, 50 per cent of isolates were found resistant at discriminating dose (600 ppm) by larval packet test. The p value obtained upon regression analysis was < 0.05 and was considered as significant. A majority of R. (B.) microplus were found to be susceptible to deltamethrin. However, these susceptible isolates survived doses which were twice the recommended doses (1.25 ppm – 100 ppm). The p value of isolates except isolate 1 and 5 were < 0.05 and statistically significant. The results highlight acaricide resistance to be one of the reasons for the alarming prevalence of tick-borne haemoparasites in Kerala and demand urgent interventions to ameliorate the resistance by alternate control strategies.

Keywords

Rhipicephalus sanguineus, Rhipicephalus (Boophilus) microplus, Larval Packet test

Ticks are considered harmful obligate blood-sucking ectoparasites of medical and veterinary importance, as they not only transmit economically important infections to animals but also play a major role in zoonotic pathogen transmission to humans (Balasubramanian *et al.,* 2019).

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In India, the warm humid climate favours the propagation and perpetuation of ticks (Haque *et al.,* 2014; Rani *et al.,* 2018).

Rhipicephalus (Boophilus) microplus, one host cattle tick, is the most prevalent tick causing considerable economic loss in dairy cattle worldwide (Guerrero *et al.,* 2001). They transmit rickettsial diseases such as ehrlichiosis and anaplasmosis and protozoal diseases such as babesiosis, besides inflicting direct effects on the animal *per se* through blood loss. *Rhipicephalus sanguineus*, a three-host brown dog tick, transmits several protozoan parasites like *Babesia canis, Ehrlichia canis* and *Leishmania infantum* (Dantas-Torres, 2008). Ticks and tick-borne diseases (TBDs) are a major constraint in the development and improvement of the livestock industry (Ahamed *et al.,* 2007). Chemical acaricides play a vital role in tick control. However, the indiscriminate and frequent use of acaricides has led to drug and multidrug resistance against almost all commercially accessible acaricides. Monitoring of ticks and drug efficacy are important to detect resistance at an early stage to help minimize the spread of resistance and to document the distribution pattern of acaricide resistance (Lovis *et al.,* 2011).

The common acaricides in use include synthetic pyrethroids, organophosphates, organochlorines, carbamates, amidines, macrocyclic lactones and formamidines. Synthetic pyrethroids (SP) are highly biodegradable and not very toxic to mammals and had been introduced in the 1960s and 1970s. Already in the late 1980s, resistance to synthetic pyrethroids (SP) had been reported in Brazil and Australia, and it was reported in Mexico in 1994. Nowadays SP resistance is extremely common and prevalent world over (Yessino *et al.,* 2016). In South India, deltamethrin resistance to cattle ticks, *R. (Boophilus) annulatus* and *R. (Boophilus) microplus* has been reported (Jyothimol *et al.,* 2014; Lenka *et al.,* 2016). While, in North India, acaricide resistance has been widespread against flumethrin, fipronil (Shyma *et al.,* 2015) and deltamethrin (Abdullah *et al.,* 2012; Shyma *et al.,* 2015) in different tick species infesting large ruminants. In India, there have not been many studies on drug resistance in *R. sanguineus*. Mathivathani *et al.* (2011) reported 64.72 per cent resistance against flumethrin and 59.35 per cent resistance against deltamethrin in *R. sanguineus* in Chennai by using adult immersion tests.

Among the various bioassay techniques developed for recognizing acaricide resistance in ticks, larval packet technique (LPT) is a reliable method (Stone and Haydock, 1962). The Food and Agriculture Organization of the United Nations (FAO) had adopted this method as the standard method for the detection and measurement of acaricide resistance (Morgan *et al.,* 2009). Singh *et al.* (2015) reported that the acaricide resistance frequency is higher in one host ticks, since a much larger portion of the entire population of such species are exposed to chemical encounter at any one time than multi host ticks.

In the light of high prevalence of tick-borne pathogens among domestic animals in Kerala and scanty reports of acaricide resistance in ticks, this study particularly focused upon the assessment of deltamethrin resistance in cattle and dog ticks by bioassay.

Materials and methods

Collection of ticks

Fully engorged adult female *R. sanguineus* and *R. (B.) microplus* ticks were collected from animals presented to veterinary hospitals, as well as from private kennels and farms in Thrissur district of Kerala. Kerala is a southern state of India, spanning an area of approximately 3,032 km^2, characterised with a tropical climate with an average annual temperature of 27.6°C and the relative humidity being generally over 70 per cent. The collected ticks were cleaned with distilled water, dried on an absorbent paper, identified by morphological keys (Sen and Fletcher, 1962) and sampled in separate bottles. Each bottle containing around three to five engorged female ticks were labelled and closed with a muslin cloth to maintain aeration and moisture. For egg laying and hatching, the bottles were placed in a desiccator maintained with a temperature of 28°C and relative humidity of 85 per cent. Nearly 2-10 days were

taken for egg laying (Fig. 1), while 15-21 days were required for the eggs to hatch into larvae. The hatched out tick larvae were maintained for 14-21 days in desiccators for performing larval packet test (LPT).

Fig. 1 Egg mass of *R. (B.) microplus*

Protocol of LPT

The LPT was performed as per the recommendations of FAO (2004) with minor modifications. Technical grade 100 per cent pure deltamethrin (PESTANAL, Sigma- Aldrich, USA) was used for the bioassay. Working concentrations of 240 ppm, 120 ppm, 60 ppm, 30 ppm of deltamethrin were prepared by serially diluting the stock concentration with distilled water. Bioassay was also performed with discriminating dose (DD) of deltamethrin (600 ppm). Rectangular packets (8 cm x 4.2 cm) were prepared using Whatman filter paper No.1. These packets were carefully impregnated with 500 µL of the above solutions and allowed to dry at room temperature. Distilled water was used as the control. Three replicates of each treatment were tested. Approximately, 100 numbers of 14-21 day old live larvae were deposited into each dried packet. The open end of these packets were sealed with bulldog clips and kept in desiccators at room temperature for 24 h. After the incubation period, the packets were opened and the number of dead and live larvae were counted manually to estimate the per cent mortality.

Statistical analysis

Dose response data were analysed by the probit method (Finney, 1952). The 50 per cent (LC_{50}) and 95 per cent (LC_{95}) lethal concentrations of deltamethrin against *R. sanguineus* and *R. (B.) microplus* were determined by applying regression equation analysis to the probit-transformed data of mortality with regression analysis. A value of $p < 0.05$ was considered as statistically significant.

Results and Discussion

The study was conducted to evaluate deltamethrin resistance in *R. sanguineus* and *R. (B.) microplus,* the most economically important ectoparasites of dogs and cattle, respectively. Per cent mortality of larvae was assessed at different deltamethrin concentrations. The isolates were considered resistant, if mortality of *R. sanguineus* and *R. (B.) microplus* treated with discriminating dose (DD: 600 ppm), were below 90 per cent.

Analysis of probit transformed mortality of *R. sanguineus* against log concentration of deltamethrin is given in Table 1. Isolate 5 and 6 of *R. sanguineus* showed 100 per cent mortality even at the lowest concentration of deltamethrin and hence LC_{50} and LC_{95} were not derived. Isolate 4 had the least LC_{50} (8.71 ppm) and LC_{95} (2089.29 ppm). Isolates 1, 2 and 3 were shown to be resistant at DD. Isolate 4, 5 and 6 were considered susceptible and mortality at DD was > 90 per cent. The LC_{95} of all the resistant isolates were higher than the recommended acaricidal dose of deltamethrin (1.25 ppm – 100 ppm). The *p* value obtained upon regression analysis with *R. sanguineus* isolates was < 0.05 and was considered significant.

The LC_{50} and LC_{95} values of different isolates of *R. (B.) microplus* (Table 2) showed that isolate 1 had the lowest LC_{50} (16.98 ppm) and LC_{95} (131.82 ppm), while the highest LC_{50} (223.87 ppm) and LC_{95} (602.56 ppm) were observed for isolate 5. Except for isolate 5, all other *R. (B.) microplus* isolates were found to be susceptible to deltamethrin. However, the LC_{50} and LC_{95} of all isolates were higher than the recommended dose. In susceptible isolates the least LC_{50} observed was 16.98 and LC_{95}

Table 1 Probit analysis of LPT with *R. sanguineus*

Tick	Slope ± SE	R^2 value	LC_{50} (in ppm)	LC_{95} (in ppm)	P value	Mortality % at DD
Isolate 1	0.6742 ± 0.0755	0.9648	107.15	26302.68	0.002	69.86%
Isolate 2	0.7624 ± 0.0615	0.9814	467.74	67608.29	0.001	54.80%
Isolate 3	0.4846 ± 0.0697	0.9430	26.30	67608.29	0.005	76.60%
Isolate 4	0.6955 ± 0.1548	0.8738	8.71	2089.29	0.019	91.02%
Isolate 5	Not calculated since 100 % mortality was observed with lowest concentration					100%
Isolate 6						100%

Table 2 Probit analysis of LPT with *R. (B.) microplus*

Tick	Slope ± SE	R^2 value	LC_{50} (in ppm)	LC_{95} (in ppm)	P value	Mortality % at DD
Isolate 1	1.8852 ±0.8352	0.6363	16.98	131.82	0.105	100 %
Isolate 2	2.0819 ± 0.2085	0.9716	51.29	309.03	0.002	99.11%
Isolate 3	2.0801 ± 0.5285	0.8418	26.92	165.95	0.028	98.69%
Isolate 4	1.6788 ± 0.4772	0.8095	24.55	229.09	0.037	98.28%
Isolate 5	3.8499 ± 1.4578	0.7054	223.87	602.56	0.075	81.58%
Isolate 6	5.4367 ± 1.1416	0.8862	151.36	301.99	0.016	100%

was 131.82. The *p* value of analysis with all *R. (B.) microplus* isolates except isolate 1 and 5 were less than 0.05 and statistically significant.

Resistance of ticks against acaricides could be monitored by using various bioassays, such as larval packet test (LPT), adult immersion test (AIT), larval tarsal test (LTT) and larval immersion test (LIT) (FAO, 2004). Acaricide resistance in ticks is an inherited phenomenon, defined as a reduction in susceptibility of ticks to the acaricide when it is used at the recommended dose (FAO, 2004). Fular *et al.* (2018) observed that the resistance level and mechanisms of resistance development varied in different parts of the world. Hence, regular monitoring of resistance is an inevitable control strategy in areas where tick and tick-borne parasites continue to pose a significant threat to livestock population.

Our study established that deltamethrin resistant phenotypes persisted among tick populations infesting cattle and dogs of Kerala. Bioassay using LPT revealed that at discriminating dose, 50 per cent of *R. sanguineus* were resistant to deltamethrin, while 83 per cent of *R. (B.) microplus* were susceptible. However, these susceptible isolates survived doses that were twice the recommended doses of 1.25 ppm to 100 ppm (Sandhu, 2006). Resistance status of *R. (B.) microplus* ticks against synthetic pyrethroids was globally studied using the FAO recommended larval packet test (Rosario-Cruz *et al.,* 2009; Abdullah *et al.,* 2012; Domingues *et al.,* 2012; Shyma *et al.,* 2013; Shyma *et al.,* 2015). Lovis *et al.* (2012) assigned phenotype resistance to tick population based on three criteria: Survival rate at DD, resistance ratio (RR) 50 and RR90. The population was designated resistant when their survival rate at DD was greater than 10 per cent or when the RR50 and RR90 were above four. In the present study, all *R. (B.) microplus* isolates, except isolate 5 were found susceptible, since mortality per cent was above 90 per cent at DD. The observed LC_{50} and LC_{95} of deltamethrin in susceptible tick population were much lower than that reported for resistant isolates (Shyma *et al.,* 2012; Aboelhadid *et al.,* 2018).

In Kerala, Jyothimol *et al.* (2014) had reported a low level of deltamethrin resistance in cattle ticks with LC_{50} and LC_{95} values being 2.15 ppm and 52.24 ppm, respectively in susceptible isolates of *R. (B.) microplus*, while those for *R. (B.) annulatus* were 2.11 ppm and 34.75 ppm, respectively. In Uttar Pradesh, a north Indian state, LPT to detect acaricide resistance revealed that the LC_{99} values of fenvalerate and fipronil were 2007.0 ppm and 4.8 ppm, respectively, while that of coumaphos

and malathion, that were highly toxic to larvae, yielded LC_{99} values as low as 28.4 ppm and 55.9 ppm, respectively (Kumar *et al.,* 2015). The LC_{50} and LC_{95} values of deltamethrin acaricide against susceptible lines of *Hyalomma anatolicum* in the Indian cattle population were 11.7 ppm and 34.9 ppm, respectively by LPT, as reported by Shyma *et al.* (2012). Shyma *et al.* (2015) reported LC_{50} and LC_{95} values of deltamethrin against resistant *R. (B.) microplus* isolates in North Gujarat to be 75.24 ppm and 367.74 ppm, respectively. Lenka *et al.* (2016) observed a wide variation (2.4 ppm-13.8 ppm) in the LC_{50} values in *R. (B.) microplus* isolate collected from South Indian states which was lower than that for North Indian isolates. Perusal of literature suggests that cattle ticks are resistant to almost all commercially available acaricides in India (Sharma *et al.,* 2012; Shyma *et al.,* 2013; Kumar *et al.,* 2014; Singh *et al.,* 2015; Lenka *et al.,* 2016). The global situation is not different. The resistant Ehanasia population of *R. annulatus* showed a high LC_{50} value of 100 ppm (Aboelhadid *et al.,* 2018) against deltamethrin. Domingues *et al.* (2012) reported resistance to cypermethrin by LPT, since resistance ratio (RR) between 16.0 to 25.0 and 85.7 per cent were resistant to chlorpyriphos because RR value ranged from 2.2 to 15.6. Santos *et al.* (2013) observed wide variation of LC_{50} values (264.9 µg/mL - 9923.9 µg/mL) in resistant field isolates in Brazil.

In South India, apart from the report of Mathivathani *et al.* (2011) in Tamil Nadu, deltamethrin resistance has not been studied in *R. sanguineus* isolates. The present study is the first report of acaricide resistance in dog ticks in Kerala state and it is identified that 50 per cent of *R. sanguineus* population in the state, selected for study, exhibited a mortality ranging from 53 to 77 per cent, at DD and were resistant to deltamethrin. The LC_{50} value of deltamethrin against susceptible *R. sanguineus* isolate was 8.71 ppm. Studies by Miller *et al.* (2002) revealed a resistance ratio of 7.3 against amitraz in a Panamanian strain of *R. sanguineus* by using LPT bioassay (Miller *et al.,* 2002).

The current investigation showed that deltamethrin resistance in Kerala is comparatively higher in the three host ticks (*R. sanguineus*) than among one host ticks (*R. (B.) microplus*). The high prevalence of tick-borne canine haemoparasites in this part of South India (Jain *et al.,* 2017; Wahlang *et al.,* 2019) despite the intensive use of deltamethrin, could be due to widespread drug resistance in the tick population. This necessitates studies on alternate control strategies, incessant monitoring and strategic application of other acaricides to control the tick population in this geographical area.

Acknowledgement

The authors gratefully acknowledge the technical support of Kerala Veterinary and Animal Sciences University, Pookode, Kerala, India for conducting the research.

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