RESEARCH ARTICLE

Acaricide Resistance in *Rhipicephalus sanguineus* Infested Dogs in Agartala, Tripura

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Abstract

Development of acaricide resistance in tick population is a major concern in livestock as well as in pet animals. Reduced responsiveness has been observed against commonly used acaricides in *Rhipicephalus sanguineus* tick infested dogs in the Agartala city of North-eastern part of India. Two acaricides Deltamethrin and Fipronil were chosen for acaricidal resistance study using Adult Immersion Test (AIT) and Larval Packet Test (LPT). AIT study revealed the lowest and highest mortality of adult ticks at 25 ppm and 200 ppm concentration of Deltamethrin, respectively. Similarly for fipnonil, lowest and highest mortality was recorded at 25 ppm and 100 ppm, respectively. With increasing the concentration of both deltamethrin and fipronil, the reproductive index of ticks was found to be decreased with increased inhibition of oviposition. In LPT study, lowest mortality of *R. sanguineus* larvae was recorded at 25 ppm of deltamethrin and highest mortality at 200 ppm. For fipronil, lowest and highest mortality was recorded at 25 ppm and at 100 ppm, respectively. The study revealed that both the acaricides produced lower mortality of ticks at their market recommended doses. However, fipronil was found to be more effective than that of deltamethrin. The observed resistance warrants immediate measures to prevent further development of acaricide resistance in tick population in dogs in the study area.

Key words: Acaricide resistance, Agartala, Deltamethrin, Dogs, Fipronil, *Rhipicephalus sanguineus*.

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Introduction

Ticks are one of the most important ectoparasites of dogs. It also ranked second only to mosquitoes as vector of many human infectious diseases worldwide (Estrada-pena and Jongejan, 1999). Ticks are responsible for causing distress to their hosts in the form of itching, skin lesions, blood loss and tick-induced paralysis or may also act as vector for various pathogens. *Rhipicephalus sanguineus* commonly known as the brown dog tick is the most widespread tick in the world (Dantas-Torres, 2008). *R. sanguineus* act as a vector for various tick-borne parasites such as *Babesia canis*, *B. vogeli*, *B.gibsoni*, *Hepatozoon canis* and *Ehrlichia canis* that affect dogs in different regions of the world (Shaw *et al*., 2001). *R. Sanguineus* ticks complete more than 95% of its lifecycle in the environment and dog serves as the principal disseminating agent of it (Needham and Teel, 1991). Controlling *R. sanguineus* infestations by using different acaricides is difficult as the free-living stages of these tick hides in cracks and crevices or within walls (Miller *et al*., 2001).

For controlling tick infestations in dogs, strategies like managemental and environmental controls and application of chemical acaricides to the dogs are followed worldwide. Environmental control measures are often complex and have varying success rates, whereas nowadays different chemical acaricides such as synthetic pyrethroid, amidine and phenyl pyrazole are widely used for tick control. However, indiscriminate use of these acaricides results in the development of resistance among the prevalent tick species in that area. Resistance in ticks including *R. sanguineus* can be diagnosed by surveying tick populations using bioassays, *viz.,* larval packet test (LPT), larval immersion test (LIT) and adult immersion test (AIT) (Rodriguez-Vivas *et al*., 2017; Shyma *et al*., 2019). According to Burridge *et al*. (2004), fipronil was the most effective acaricide for controlling *R. sanguineus* when compared with carbaryl, cyfluthrin, chlorpyrifos, permethrin, pyrethrins, amitraz, and selamectin. Nowadays Cypermethrin and Fipronil are rampantly used in dogs for controlling ticks

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in different parts of India. Studies have been carried out to determine the status of acaricide resistance in *R. sanguineus* against deltamethrin, cypermethrin and ivermectin in India (Mathivathani *et al*. 2011; Shyma *et al*. 2019; Sunkara *et al*., 2022). Though many reports from pet owners/pet attendants about repeated failure of acaricides in controlling ticks in dogs were encountered, no systematic study on the status of acaricide resistance in ticks of dogs has been carried out in Agartala. Therefore, the present study was undertaken to evaluate the status of resistance against the two regularly used acaricides compound, *viz*., Deltamethrin and Fipronilin *R. sanguineus* ticks of dogs of Agartala.

Materials and Methods

Collection and Maintenance of Ticks

For the present study, engorged female ticks (Fig. 1) were collected from naturally infested dogs from Veterinary Clinical Complex, College of Veterinary Sciences and Animal Husbandry, R.K. Nagar, West Tripura; different veterinary hospitals, veterinary dispensaries, stray dogs of NGOs and private clinics etc. in and around Agartala city (India) without damaging their mouth parts. The laboratory work was conducted in the Department of Veterinary Parasitology of the College from March 2022 to February 2023. After collection, ticks were put into clean glass vials covered with muslin cloth to allow exchange of sufficient air and moisture. They were washed, dried and identified through microscopy.

Fig.1: Engorged *Rhipicephalus sanguineus* female ticks

Bioassays

In vitro testing of acaricide resistance was done by two bioassays, *i.e*. Adult Immersion Test (AIT) and Larval Packet Test (LPT). Deltamethrin and fipronil were chosen for determination of acaricidal resistance. Dilutions were prepared in distilled water for deltamethrin and in acetone for fipronil.

Adult Immersion Test was conducted as per the protocol described by Shyma *et al*. (2015). The index of egg laying and percentage inhibition of fecundity was calculated using the formulae given by Drummond *et al*. (1973). Larval Packet Test was conducted on 12 to 14 days old larvae as per the protocol described by Shyma *et al*. (2015). The packets were opened after 24 h under light, both the living and the dead larvae were counted with help of a brush.

Statistical Analysis: The LC_{50} and LC_{95} concentrations of deltamethrin and fipronil against *R. sanguineus* were calculated by probit analysis.

Results and Discussion

Adult Immersion Test

Results of Adult Immersion Test showed that the lowest mortality (33.33±0.002%) of adult *Rhipicephalus sanguineus* ticks was observed at 25 ppm of deltamethrin, while highest mortality (86.66±0.002%) was exhibited at 200 ppm (Table 1). Similarly, in case of fipronil, lowest mortality (53.33±0.003%) of adult ticks was recorded at 25 ppm, while higher mortality (93.33±0.001%) was observed at 100 ppm (Table 2). With increasing the concentration of both deltamethrin and fipronil, the reproductive index (RI) was found to be decreased with increased inhibition of oviposition (IO) in adult ticks (Table 1, 2). Shyma *et al*. (2015) in North Gujarat, India did AIT in *Rhipicephalus (Boophilus) microplus* tick against deltamethrin, flumethrin, and fipronil and also found that with increasing drug concentration, the RI was found to be decreased and IO was increased. Literature on the effect of chemical acaricides on RI and IO of the canine soft ticks was meager; however, Yadav *et al.* (2024) recorded comparable trends on bovine *Rhipicephalus microplus* ticks for *in-vitro* RI and IO, and their larval mortality with the use of polyherbal aqueous formulation. The probit model parameter estimate test of significance and estimated LC_{50} and LC_{95} of the present study are presented in Table 3 and Table 4 for deltamethrin and fipronil, respectively. The findings revealed that in case

Table 1: AIT results of deltamethrin against fully engorged adult *R. sanguineus* ticks in and around Agartala, Tripura

Group	Deltamethrin concentration (ppm)	Average tick weight per replicate ± SE (gm)	Mean egg mass ±SE (gm)	Reproductive Index (RI) ±SE	Percent Inhibition (IO%) ±SE	Mean tick mortality (%) ±SE
1	25	0.55±0.002	0.08±0.001	0.145±0.00	82.42±0.00	33.33±0.002
2	50	0.54±0.001	0.06±0.00	0.111±0.002	86.55±0.001	46.66±0.001
3	100	0.55±0.002	0.04±0.002	0.072±0.001	91.27±0.002	66.66±0.001
4	200	0.56±0.004	0.02±0.001	0.035±0.001	95.76±0.001	86.66±0.002
	Control (Distilled water)	0.57±0.002	0.47±0.004	0.825±0.002	0.00±0.00	0.00±0.00

of deltamethrin, LC_{50} was 47.801 ppm with 95% confidence interval (21.691-75.412) and LC_{95} was 406.477 ppm with 95% confidence interval (185.990-7333.130), whereas in case of fipronil the LC_{50} was 23.972 ppm with 95% confidence interval (3.397-36.687) and LC_{95} was 128.473 ppm with 95% confidence interval (77.115-1864.299). The effect of concentration of deltamethrin and fipronil was found non-significant. Earlier, Eiden *et al.* (2015) in USA observed 100 % mortality with fipronil at the discriminating concentration with LD_{50} ranged from 0.023-0.024%. Sudha Rani *et al.* (2018) in Karnataka found the LC_{50} for deltamethrin against *R. sanguineus* tick as 62.13 ppm. They observed increased resistance of ticks when exposed to deltamethrin followed by cypermethrin.

Larval Packet Test

Larval packet test was conducted with different concentration of deltamethrin in *R. sanguineus* tick larvae which showed lowest mortality (40.16±0.45%) at 25 ppm, while the highest mortality (87.16±0.12%) was observed at 200 ppm. Similarly, in case of fipronil lowest mortality (50.33±0.52%) of larvae was recorded at 25 ppm, while highest mortality (99.66±0.17%) was exhibited at 100 ppm (Table 5). The probit model parameter estimate test of significance revealed that in the case of deltamethrin, the LC_{50} was 37.482 ppm with 95% confidence interval (33.764-41.108) and LC_{95} was 430.579 ppm with 95% confidence interval (351.415-552.221) (Table 6), whereas in case of fipronil (Table 7) the LC_{50} was 27.099 ppm with 95% confidence interval (21.349-31.845) and LC_{95} was 88.915 ppm with 95% confidence interval (73.156-121.075). It shows that there was no significant impact of concentrations of deltamethrin on *R. sanguineus* larvae. However, there was highly significant impact of concentrations of fipronil against *R. sanguineus* larvae ($p<0.01$). Earlier, Pradeep *et al.* (2010) observed 30 % resistance for deltamethrin against dog tick *R. sanguineus* in Bangalore. Shyma *et al.* (2019) in Deesa, Gujarat did LPT in *R. sanguineus* and found the LC_{50} and LC_{90} for deltamethrin as 9.59 ppm and 75.87 ppm, respectively, and stated that deltamethrin produced a lower level of mortality (72%) at its market recommended dose. Tian *et al.* (2023) observed the LD_{50} value for fipronil from 0.019-0.049% in Florida and California, and concluded that fipronil resistance was not detected which suggests this acaricide may provide suitable tick control. Similarly, in the present study, we also found fipronil to be more effective in comparison to deltamethrin. The rampant and indiscriminate use of these acaricides has caused reduction in their efficacy on ticks as observed by the pet owners and pet practitioners in the study area.

Table 2: AIT results of fipronil against fully engorged adult *R. sanguineus* tick in and around Agartala, Tripura

Group	Fipronil concentration (ppm)	Average tick weight per replicate ±SE(gm)	Mean egg mass ±SE (gm)	Reproductive Index(RI) ±SE	Percent Inhibition (IO%) ±SE	Mean tick mortality (%) ±SE
1	25	0.55±0.001	0.07±0.004	0.127±0.004	82.46±0.001	53.33±0.003
2	50	0.54±0.005	0.04±0.001	0.074±0.001	89.78±0.005	73.33±0.002
3	75	0.56±0.004	0.01±0.00	0.017±0.004	97.65±0.002	86.66±0.002
4	100	0.58±0.002	0.006±0.004	0.010±0.001	98.62±0.001	93.33±0.001
	Control (Distilled water)	0.58±0.004	0.42±0.001	0.724±0.002	00±0.00	0.00±0.00

Table 3: Probit analysis in fully engorged adult *R. sanguineus* tick determined by AIT with deltamethrin

Coefficient	Estimate ±SE	95% CI	L-R χ^2	*P*-Value	Goodness of Fit χ^2 (2)	*P*-Value
Intercept	-2.972±0.999	-3.970 to -1.973	-2.976	0.003	3.149	0.978^{NS}
Conc-ppm	1.769±0.544	0.703 to 2.836	3.251	0.001		
LC_{50}	47.801	21.691-75.412				
LC_{95}	406.477	185.990-7333.130				

NS: Non-significant ($p>0.05$)

Table 4: Probit analysis in fully engorged adult *R. sanguineus* tick determined by AIT with fipronil

Coefficient	Estimate ±SE	95%CI	L-R χ^2	*P*-Value	Goodness of Fitχ^2 (2)	*P*-Value
Intercept	-3.113± 1.413	-4.526 to -1.699	-2.202	0.028	3.149	0.978^{NS}
Conc-ppm	2.256± 0.833	0.623 to 3.889	2.708	0.007		
LC_{50}	23.972	3.397 to 36.687				
LC_{95}	128.473	77.115 to 1864.299				

NS: Non-significant ($p>0.05$)

Table 5: Larval packet test with the larvae of *R.sanguineus* for deltamethrin and fipronil

Total no. of alive larvae taken	Deltamethrin conc. (ppm)	Mean ±SE of larvae died after 24 h (%)	Fipronil conc. (ppm)	Mean ±SE of larvae died after 24 h (%)
200	25	40.16±0.45	25	50.33±0.52
200	50	56.00±0.21	50	71.33±0.46
200	100	75.16±0.56	75	92.31±0.14
200	200	87.16±0.12	100	99.66±0.17
200	Control (DW)	0.00±0.16	Control (DW)	0.00±0.18

Table 6: Probit mortality in larvae of *R. Sanguineus* determined by LPT with deltamethrin

Coefficient	Estimate ±SE	95% CI	L-Rχ2	*P*-Value	Goodness of Fit χ2 (2)	*P*-Value
Intercept	-2.442± 0.156	-2.598 to -2.285	-15.624	0.000	8.664	0.564^{NS}
Conc-ppm	1.551± 0.086	1.384 to 1.719	18.120	0.000		
LC50	37.482	33.764 to 41.108				
LC95	430.579	351.415 to 552.221				

NS: Non-significant (p>0.05)

Table 7: Probit mortality in larvae of *R. sanguineus* determined by LPT with fipronil

Coefficient	Estimate ±SE	95% CI	L-Rχ2	*P*-Value	Goodness of Fit χ2 (2)	*P*-Value
Intercept	-4.568 ±0.246	-4.813 to-4.322	18.576	0.000	68.942	0.000^{*}
Conc-ppm	3.188 ± 0.149	2.896 to 3.479	21.464	0.000		
LC50	27.099	21.349 to 31.845				
LC95	88.915	73.156 to 121.075				

*Highly significant (p<0.01)

Our study seems to be the first systematic report on acaricide resistance in *R. Sanguineus* ticks of dogs in Agartala. *R. Sanguineus* is the most prevalent tick in dogs in India which imposes serious threat to the dog population. Thus, use of acaricides is the most common means to control the tick infestation and prevention of tick borne diseases in dogs. However, the results obtained in AIT and LPT in the present study for both deltamethrin and fipronil showed lower mortality of ticks at their market recommended doses which clearly suggests the possible development of resistance in tick against these drugs as opined earlier by Becker *et al.* (2019). Until the tick population is controlled, the control of haemoparasitic infection is not possible. Therefore, the various stakeholders such as pet owners, veterinarians, animal husbandry authorities etc. should jointly identify mitigation strategies to overcome the developing acaricide resistance in ticks of dogs in Agartala through close collaboration with the animal health research institutions of the State.

Conclusion

Present study presents the first systematic report on the status of resistance in *R. Sanguineus* ticks of dogs against two very commonly used acaricides in Agartala. The study concludes that the use of deltamethrin and fipronil produces lower mortality of *R. sanguineus* ticks at their market recommended doses which is suggestive of probable development of some degree of resistance in ticks against these drugs. Therefore, following appropriate treatment regime along with awareness to the pet owners is utmost required regarding use of acaricides for controlling tick infestation. Further, the molecular bases of such resistance and state-wide epidemiological studies are necessary together with an all-round approach for containment and eradication of acaricide resistant ticks in Agartala as well as in the State as a whole.

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