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In vitro evaluation of effectiveness of synthetic pyrethroids against brown dog tick

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ABSTRACT

Rhipicephalus sanguineus, the brown dog tick, is one of the most cosmopolitan ixodid ticks of veterinary and public health importance. The domestic dog is the main host of *R. sanguineus*, but occasionally, can infest a wide range of domestic and wild hosts, including humans. It causes debilitating effects due to blood losses in affected animals and also transmits several pathogens such as *Ehrlichia canis*, *Babesia canis*, *Haemobartonella canis* and *Hepatozoon canis*. Engorged female *R. sanguineus* kept for oviposition. Hatched larvae of *Rhipicephalus sanguineus* were subjected to larval packet test (LPT) to know its efficacy against deltamethrin and cypermethrin. The LC_{50} and LC_{95} concentrations were calculated by plotting regression curve of mortality against different concentrations of acaricides. A dose dependent increase in larval mortality was observed for both cypermethrin and deltamethrin. LC_{50} and LC_{95} for both deltamethrin and cypermethrin are calculated to be 9.59, 75.87 and 7.08, 219.85, respectively. The results indicated that *R. sanguineus* ticks were susceptible for cypermethrin, having high percentage mortality at the recommended concentration (200 ppm). However, deltamethrin produced a lower level of mortality at its market recommended dose (25 ppm).

Key words: Cypermethrin, Deltamethrin, *Rhipicephalus sanguineus.*

INTRODUCTION

The brown tick, *Rhipicephalus sanguineus* is the most common tick infesting dogs.These ticks are of veterinary importance, as it produces debilitating effects due to blood losses in affected animals and also transmits several pathogens such as *Ehrlichia canis*, *Babesia canis*, *Haemobartonella canis* and *Hepatozoon canis* (Borges *et al*., 2007). *Rhipicephalus sanguineus* infestations are difficult to control as the tick life cycle requires access to three hosts, but each free-living stage of tick is capable of surviving for long periods in the environment. Moreover, free-living *R. sanguineus* hides in structural cracks and crevices or within walls, making it difficult to control infestations by treating premises using acaricides (Miller *et al.,* 2001). Numerous studies are currently under way to find an effective control strategy that would minimize the damage caused by these parasites. Nowadays the most efficient method to control tick populations is by using chemical compounds, such as synthetic pyrethroid that acts in the nervous system of the ticks (Mencke *et al.,* 2003). The commercially available synthetic pyrethroids, deltamethrin and cypermethrin, are the two predominant acaricides used for tick control in dogs. Although pet owners have reported treatment inefficiency of these chemicals in field conditions, limited data is available on resistance in dog tick to these chemicals. Keeping in view of the requirement of preserving the life span of costly acaricides and expenses involved in generation and marketing of new group of chemicals for the control of economically important tick species, it is of outmost importance to check the efficacy status of *R. sanguineus* for the suitable implementation of tick control measures. The present study was aimed to generate data on pyrethroid (SP) efficacy (deltamethrin and cypermethrin) against *R. sanguineus* collected from Banaskantha district of North Gujarat.

Collection of *Rhipicephalus sanguineus* ticks: *Rhipicephalus sanguineus* was collected from the dogs that are presented to Teaching Veterinary Clinical Complex, Deesa, without damaging their mouth parts. The history of acaricde application was collected based on questionnaire survey. Ticks were then put into clean vials covered with muslin cloth to allow air and moisture exchange and brought to department of Veterinary Parasitology, College of Veterinary Science and Animal Husbandry, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar.

Laboratory maintenance: Ticks brought to the laboratory were washed, dried and were then identified (Sen and Fletcher, 1962 and Geevarghese, 2000). Five adult engorged ticks were placed in labeled glass vials, which were then placed individually in desiccator) in BOD incubator at 28±1°C and 85±5% relative humidity for oviposition. A

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period of about 13-15 days was required for *Rhipicephalus sanguineus* for laying of eggs. The eggs were allowed to hatch to larvae for 28-35 days under similar conditions of incubation. The 12-14 day old larvae were then used for the *in vitro* assay.

Acaricides: The commercial products, namely 1.25% EC deltamethrin and 10% EC cypermethrin at the concentrations of 12.5, 25, 50, 100, and 200 ppm and 100, 200, 400, 800 and 1600 ppm respectively were prepared in distilled water from the stock solution.

Larval Packet Test (LPT): The LPT was conducted as per the guidelines of FAO (1971) with minor modifications. Briefly, 0.6 ml of different concentration of acaricides in distilled water were used to impregnate 3.75 cm by 8.5 cm filter paper rectangles (Whatman filter paper no. 1, W & R Balston Limited). The acaricide dissolved filter paper was then dried by keeping it for 30 min in incubator at 37°C. The rectangles were then folded in half and sealed on the sides with adhesive tapes forming an open-ended packet to place tick larvae. Approximately 100-150 larvae were inserted with the help of fine paint brush and the packet was sealed with adhesive tape. These packets were then placed in a desiccator in BOD incubator maintained at 28±1°C and 85±5 % RH. The packets were then removed after 24 h and live and dead larvae were counted for mortality calculation. Control packets impregnated with diluents only were also prepared for each series of concentration to be tested.

Statistical analysis: LC_{50} and LC_{95} concentrations of deltamethrin and cypermethrin against *Rhipicephalus sanguineus* were calculated by plotting regression curve of mortality (probit) against values of acaricide concentrations (log) (Finney, 1971).

The ticks collected were identified as *Rhipicephalus sanguineus*. Graphs were plotted between log concentrations of acaricides and probit mortality for determination of LC_{50}, LC_{95}, slope and coefficient of determinations (R^2) values (Fig 1). For the determination of acaricidal activity of these drugs, percentage mortality of ticks was compared with the recommended dose of market formulated drug. Data on the effects of different concentrations of deltamethrin and cypermethrin on the larvae of *Rhipicephalus sanguineus* are presented in Table 1. The mean mortality of larvae treated with various concentrations of deltamethrin ranges from 68% to approximately complete mortality. Insignificant mortality (0.80±0.37) was recorded in larvae of control group treated with distilled water.

Synthetic pyrethroids (SP) are the most preferred acaricide to control ticks in pet animals in Gujarat. These chemicals have been used for years, without the proper

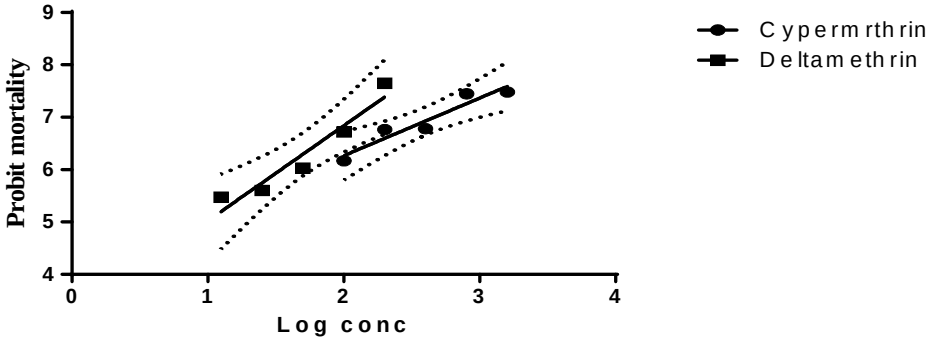

Fig 1: Dose dependent mortality curve of *Rhipicephalus sanguineus* against deltamethrin and cypermethrin.

Table 1: Response of *Rhipicephalus sanguineus* against various acaricides by LPT.

Acaricide	Conc. (ppm)	Mortality (%) (mean ± SE)	Slope	R^2	LC_{50} (ppm)	LC_{95}(ppm)
Deltamethrin	12.5	68.21	1.816 ± 0.3042	0.9224	9.59	75.87
	25.0	72.62				
	50.0	84.75				
	100.0	95.70				
	200.0	99.60				
Cypermethrin	100	87.83	1.100 ± 0.	0.9107	7.08	219.85
	200	96.12				
	400	96.26				
	800	99.28				
	1600	99.34				
Control		0.8±0.374				

R^2- Goodness of fit.

dosage. The experimental data on status of acaricide resistance in brown dog ticks from Gujarat is scanty or unavailable. The aim of the present study was to determine the quantum of acaricidal resistance in different field isolates of *R. sanguineus*in North Gujarat against commonly used SP compounds, deltamethrin and cypermethrin.

Results clearly depicts that *R. sanguineus* ticks were susceptible for cypermethrin having high percentage mortality at the recommended as well as higher concentrations, hence it is effective for these ticks at its market formulated dose rate whereas, deltamethrin produced a lower level of mortality (72%) at its market recommended dose. A dose dependent increase in larval mortality was observed for both drugs. However, deltamethrin could produce 99.6% mortality at the highest concentration tested (200ppm). Mathivathani *et al.* (2011) reported resistance in *R. sanguineus* ticks against deltamethrin and flumethrin, collected at random from dogs of Chennai by Adult immersion test. Reports of resistance against deltamethrin are available against brown dog ticks from various parts of world (Jonsson *et al.*, 2000; Ducornez *et al.*, 2005). Resistance has been reported to be more prevalent in one host tick like *Rhipicephalus* (*Boophilus) microplus* owing to the more frequent exposure to chemical challenge (Sharma *et al*., 2012; Kumar *et al*., 2013). However, reports of development of resistance in multi host tick like *R. sanguineus* are limited (Coles and Dryden, 2014; Evora *et al*., 2015; Rodriguez-vivas *et al*., 2017).

The present study revealed an emerging problem of resistance in *R. sanguineus* of North Gujarat. Greater detail exploring biochemical base as well as physiological process of detoxification in ticks population may be an area of interest for future research. An integrated control system should be initiated, involving veterinary officers, pet owners, for judicious use of chemical acaricides for tick control which will help mitigate the impact of these ticks in animal and human health.

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