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Research Article

SUSCEPTIBILITY OF *RHIPICEPHALUS SANGUINEUS SENSULATO* (S.L.) LATREILLE (ACARI: IXODIDAE) TO CYPERMETHRIN, IVERMECTIN, AND AMITRAZ

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ABSTRACT: Chemical acaricides, *viz.* amitraz, cypermethrin, and ivermectin have been in use for control of ectoparasites (particularly ticks) in domestic animals over the past two decades. The present study aimed to appraise the acaricidal effects of ivermectin (IVM), amitraz, and cypermethrin against brown dog tick *Rhipicephalus sanguineus sensulato* (s.l.), by *in-vitro* bioassay based susceptibility tests, *viz.* larval immersion test and larval packet test. Lethal concentrations (LC50 and LC95) values of acaricides were ascertained by applying regression equation analysis to the probit transformed data of mortality and resistance factors (RF) were determined. LC50 values of 11.39, 22.51, and 10.75 ppm, and LC95 values of 57.06, 78.74, and 50.28 ppm were estimated for amitraz, cypermethrin, and ivermectin, respectively. The susceptible status against amitraz and ivermectin whereas, the resistance of level I against cypermethrin was recorded in the study. The present study is the only report of resistance to cypermethrin in *R. sanguineus* (s.l.), ticks from this region.

Keywords: Acaricides, Brown dog tick, Susceptibility.

INTRODUCTION

The brown dog tick, *Rhipicephalus sanguineus sensulato* (s.l.), (Latreille, 1806; Acari: Ixodidae) has domestic dogs as the main host but also infests a variety of other domestic and wild hosts, including wild cats, rodents, birds and humans [1]. They are responsible for the transmission of various important canine pathogens, including *Babesia vogeli*, *Anaplasma platys*, and *Ehrlichia canis* [2] along with many zoonotic disease agents, such as *Coxiella burnetii*, *Rickettsia conorii*, and *Rickettsia rickettsii* [3]. The dogs are commonly found to be infested with ectoparasites like fleas and ticks [4]. These ectoparasites cause direct and indirect harm such as irritation, skin inflammation, pruritus, self-wounding, disturbance, and allergic responses [5]. Ticks and tick-borne diseases are a major constraint for optimum health and vigor in animals. There are different measures for controlling tick infestations in dogs and the use of chemical agents is the most common and widely used strategy. Presently, several commercially available chemical acari-cides, *viz.* synthetic pyrethroids, macrocyclic lactones [6, 7], organophosphates, formamidines [8], sarolaner [9], etc. are being used for tick control in dogs. However, there is no data available on the *in vitro* efficacy of these commonly used acaricides against *R. sanguineus* (s.l.), in India. This led to an attempt to screen representatives of the three most commonly used acaricide classes, *viz.*, amitraz (formamidine), cypermethrin (synthetic pyrethroid), and ivermectin (macrocyclic lactone) for their acaricidal activity and resistance status against *R. sanguineus* (s.l.).

MATERIALS AND METHODS

Collection and identification of ticks

The dropped-off engorged female ticks were collected from the clinics, kennels/plucked whereas,

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the male ticks were collected by plucking from the skin of pet/ stray dogs for identification (ken-nel/household/ dogs, n=32; clinics, n=18) in and around Palampur, Himachal Pradesh located in the northwestern Himalayas. Adult ticks were identified as *R. sanguineus* (s.l.) under a stereomicroscope, according to identification keys as per Estrada-Pena *et al.* [11]. In the laboratory, the preparation of ticks for the bioassay was performed according to the protocol described by FAO [12]. Briefly, the ticks (n=16) were washed in water, thoroughly dried using paper towels, and were kept for oviposition in petri dishes Thereafter, they were incubated at 85 ± 5% relative humidity (RH) and 28 ± 1 °C temperature. The laid eggs (oviposition after 14-18 days) were transferred into glass vials, covered with muslin cloth (to allow air and humidity passage). These were then incubated for hatching at the above-mentioned conditions. The 14-21 days live larvae were then used for the larval bioassays [12].

Larval packet test

Technical grade cypermethrin (AccuStandard® Inc. U.S.A) was utilized to prepare the stock solutions of 10,000 ppm in methanol and subsequent working concentrations (25, 50, 100, 200, 400, and 800 ppm) were prepared in distilled water from the stock solutions. Formulated amitraz (Taktic® 12.5% EC, MSD Animal Health, India) was used for the preparation of different concentrations of amitraz (7.8, 15.6, 31.25, 62.5, 125, 250 and 500 ppm) in distilled water. The modified larval packet test was conducted [13]. Briefly, to an 8 x 9 cm filter paper (Whatman No. 1, Whatman, Buckinghamshire, United Kingdom) a volume of 0.7 mL of each dilution was applied. The filter papers were dried for 30 minutes in an incubator with a temperature of 37 °C, folded in half, and sealed on the sides with adhesive tapes, forming an open-ended packet. Afterward, putting approximately 100 larvae inside these packets, the top of each packet was sealed with adhesive tape and these packets containing larvae were incubated at 28±1 ℃ and 85±5% RH for 24 h. The control group was treated with distilled water. The packets were opened and the numbers of live and dead larvae were counted. The larvae that moved their legs but did not walk were counted as dead. For each concentration of acaricides, the test was conducted in triplicate.

Larval immersion test (LIT)

To prepare a 1% stock solution, technical grade ivermectin (primarily ivermectin B1a, Sigma-Aldrich, USA) was diluted in absolute ethanol. 1% ethanol-Triton X-100 solution (ethanol with Triton X-100 at 2%, diluted at 1% in distilled water) was used as diluents for the preparation of various working concentrations. The working concentrations of 10, 20, 40, 80, 160, and 320 ppm were prepared by two-fold serial dilutions of the primary stock solution. The LIT was performed as per some previous works [14, 15]. Briefly, 0.5 ml of each immersion solution was transferred into 2 ml microcentrifuge tubes. Approximately 150 larvae were added to each one. After the addition of larvae, the tube was closed and shaken vigorously for a few seconds and then gently for 10 minutes to ensure the immersion of larvae. After opening the tubes, the larvae were transferred with a brush to a paper filter for drying. The paper filter was folded in half diagonally twice and sealed on one side with adhesive tapes, forming an open-ended triangular packet. Approximately, 100 dried larvae were transferred to these filter papers. The open end of each packet was sealed with adhesive tape. It was then placed in an incubator maintained at 28 ± 1 °C and 85 ± 5% RH. The control group was treated with 1% Eth-TX. The packets were opened after 24 hours. The numbers of live and dead larvae were counted. For each concentration of ivermectin, the test was conducted in triplicate.

Statistical analysis and calculation of resistance status

Dose-response data was analyzed using GraphPad Prism 4 software by probit method [16]. The lethal concentrations for 95% (LC95) and 50% (LC50), their confidence limits (CL 95% values) of all three acaricides (amitraz, cypermethrin, and ivermectin) against *R. sanguineus* (s.l.) were determined. The regression equation analysis was applied to the probit-transformed data of mortality. The resistance factors (RF) for different isolates were computed by the quotient between LC50 of field isolates and LC50 of the susceptible isolate [9]. The resistance status was categorized as susceptible (RF < 1.5), level I (1.5 < RF < 5), level II (5 < RF < 25), level III (25 < RF < 40), and level IV (RF > 40) based on RF [17]. The LC50 values of amitraz (5.0 ppm) and cypermethrin (46.0 ppm) for the susceptible strain of *R. sanguineus* (s.l.) described by Rodriguez Vivas *et al.* [9] were used. Further, LC50 of 8.71 ppm reported for ivermectin susceptible strain [18] was employed for the calculation of the RF against ivermectin.

RESULTS AND DISCUSSION

The values of LC50, LC95, slope, RF, and RL values of amitraz, cypermethrin, and ivermectin against *R. sanguineus* (s.l) are presented in Table 1. For all acaricides, a concentration-dependent mortality response was observed. The regression plots of mortality of tick larvae against log concentrations of amitraz, cypermethrin, and ivermectin were plotted by GraphPad Prism 4 software (Fig. 1-3). The statistical model was a good fit as it was indicated by the values of the coefficient of determination (R^2) for the larval bioassays, which ranged from 0.81 to 0.95. Lower values of slope of mortality were recorded in all bioassays demonstrating heterogenicity in tick populations. The LC50 values of 11.39, 22.51, and 10.75 ppm, and LC95 values of 57.06, 78.74, and 50.28 ppm were estimated for amitraz, cypermethrin, and ivermectin, respectively. Results indicate the susceptible status of *R. sanguineus* (s.l) against amitraz and ivermectin whereas, level I (91.5<RF<5) resistance status was recorded against cypermethrin.

Table 1. Slope, % mortality,, LC_{50}, LC_{95}, R^2 and Resistance factor (RF) for *R. sanguineus* (s.l.) against various acaricides by larval bioassay.

Treatment	Conc. (ppm)	(%) Mortality (Mean ± SE)	LC_{50} (ppm) 95% CL	LC_{95} (ppm) 95% CL	Slope ± SE	R^2	RF (RL)
	7.8	53.96±0.67	11.39	57.06	2.34 ± 0.66	0.81	0.25 (S)
	15.6	57.63±2.45	(11.13-11.65)	(54.46-59.78)	(0.24 to 4.45)		
	31.25	67.55±0.59					
Amitraz	62.5	90.17±1.84					
	125	100					
	250	100					
	500	100					
	25	65.29±0.19	22.51	78.74	3.02 ± 0.51	0.95	4.5 (I)
	50	77.94±0.93	(22.12-22.91)	(75.97-81.62)	(0.83 to 5.2)		
Cyper-	100	96.01±0.81					
methrin	200	100					
	400	100					
	800	100					
Ivermectin	10	61.17±2.19	10.75	50.28	2.45 ± 0.50	0.89	1.23 (S)
	20	71.95±2.01	(10.52-10.99)	(48.08-52.57)	(0.84 to 4.05)		
	40	75.56±0.75					
	80	98.73±0.66					
	160	100					
	320	100					

[CL: Confidence limit; R2: Goodness of fit , co-efficient of determination; RL: S (susceptible) = RF < 1.5; level I = 1.5 < RF < 5].

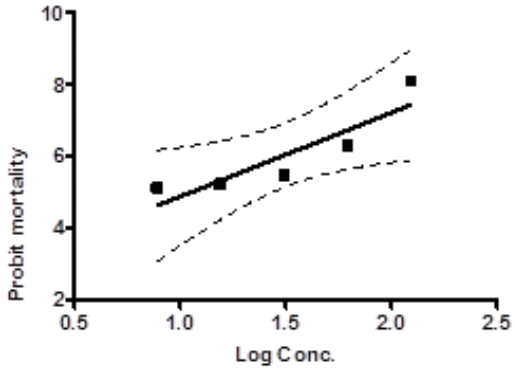

Fig. 1. Dose mortality curve of *R. sanguineus* (s.l.) against amitraz by Larval packet test.

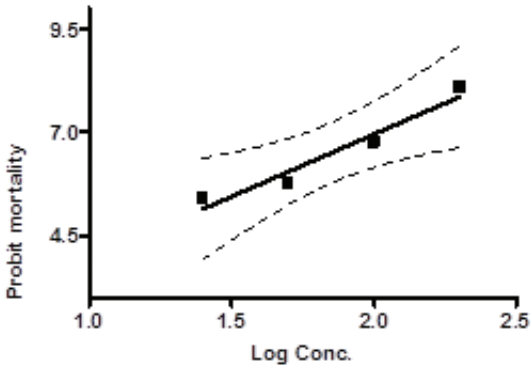

Fig. 2. Dose mortality curve of *R. sanguineus* (s.l.) against cypermethrin by Larval packet test.

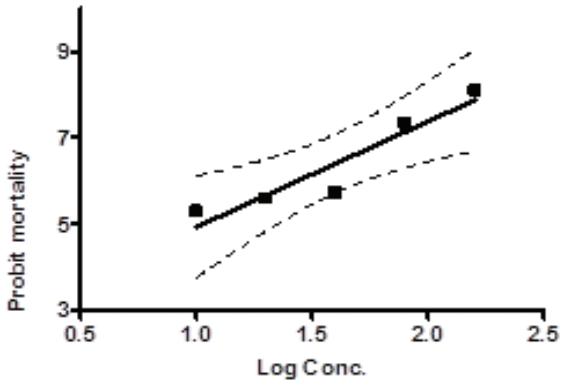

Fig. 3. Dose mortality curve of *R. sanguineus* (s.l.) against ivermectin by Larval immersion test.

Infestations of multi-host tick, *R. sanguineus* (s.l) are difficult to control as the free-living stage can survive long periods without a host hiding in cracks and crevices or within walls [1]. In recent years, *R. sanguineus* (s.l) has shown resistance to various commonly used acaricide groups, *viz.* organophosphates, synthetic pyrethroids [7], macrocyclic lactones, and formamidines [18] from different geographical locations worldwide. The results of the current study reveal low levels of resistance against cypermethrin, one of the most commonly used synthetic pyrethroid acaricides in the region. The indiscriminate and extended use of cypermethrin for tick control has led to several reports of resistance development in cattle ticks, *R. microplus* from the region [17,19,20]. Similarly, from the southern state of India, recent reports of development of resistance (level I) against cypermethrin in *Rhipicephlus (Boophilus) annulatus* and *Haemaphys alisbispinosa* is available [21]. However, there are no reports of cypermethrin resistance in *R. sanguineus* (s.l) and the present study seems to be the only report from the region whereas, susceptibility against amitraz and ivermectin recorded correlates with the less wide usage of these acaricides for tick control. Likewise, Bicalho *et al.* [22] reported high efficacy for these compounds against *R. sanguineus* in the region of Belo Horizonte, Minas Gerais State, whereas, some degree of tolerance to deltamethrin and cypermethrin was recorded. Further studies to elucidate the mechanism and inheritance of resistance in *R. sanguineus* (s.l) populations are required to be conducted for validation.

CONCLUSION

Results of larval packet tests and larval immersion assay showed susceptibility of *Rhipicephalus sanguineus sensulato* (s.l.) for amitraz and ivermectin. Level I resistance of brown dog tick was observed against cypermethrin.

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