

Reproductive variations of pyrethroid resistant and susceptible populations of *Rhipicephalus (Boophilus) microplus* from different regions of Uttarakhand

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ABSTRACT

A study was conducted to compare the reproductive profile of deltamethrin susceptible and resistant populations of *Rhipicephalus (Boophilus) microplus* collected from Himalyan and sub-Himalyan regions of Uttarakhand state to assess their implications in developing control strategies. The *in-vitro* bioassays revealed that ticks from Kashipur were 34-times more resistant to deltamethrin; ticks from Nagla and Dehradun were more than 5-times resistant whereas ticks from Pantnagar, Almora and New Tehri had resistance factor of 4.71, 3.21 and 2.04, respectively, when compared to the susceptible tick population from Pithoragarh. The mean pre-oviposition period ranged from 3.0 ± 0.39 (Kashipur) to 7.08 ± 0.2 days (New Tehri). The female ticks of Nagla laid eggs for the longest period 12.17 ± 0.42 days. No significant difference was observed in mean daily incubation period of different populations of *R. microplus*. The mean daily percentage hatch was higher for eggs laid on first seven days and declined thereafter. The index of egg laying was comparable for Pithoragarh (0.2262 ± 0.001) and New Tehri (0.226 ± 0.004) populations and lowest for Kashipur (0.206 ± 0.005). The study revealed that reduction in susceptibility to deltamethrin did not affect the reproductive potential of the resistant population of ticks. Thus number of applications along with the correct dosage of the drug is important in formulating management strategies against ticks.

Key words: Deltamethrin, Reproductive biology, *Rhipicephalus (Boophilus) microplus*, Ticks

Ticks are widely distributed in both tropical and sub-tropical countries (De Castro 1997) and account for heavy production losses (Holdsworth *et al.* 2006, Rajput *et al.* 2006). Heavy infestation of one host cattle tick *Rhipicephalus (Boophilus) microplus* produces anaemia, reduced growth rates and milk production, anorexia, hide damage, toxicosis and even mortalities. It transmits babesiosis and anaplasmosis and is responsible for causing systemic alterations of bovine haemostasis (Berger *et al.* 2009). The cost of ticks and tick-borne diseases in India has been estimated as Rs 2000 crore annually (Minjauw and McLeod 2003). The most common approach for tick control is the use of acaricides. However, indiscriminate and frequent use of acaricides, under dosing and use of poor-quality chemicals has resulted in development of acaricide resistance (Chen *et al.* 2009).

The objective of the present study was to compare the variations in reproductive biology of different populations of *R. microplus* exhibiting variations in susceptibility to deltamethrin and their implications in developing control strategies.

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MATERIALS AND METHODS

Rhipicephalus microplus ticks were collected from different geographical locations from Himalyan and sub-Himalyan regions of Uttarakhand state. *R. microplus* collected from Kashipur (550 m above mean sea-level) and Pithoragarh (1 958 m above mean sea-level) are being maintained as reference deltamethrin 'resistant' and 'susceptible' strains, respectively, since year 2006 in our laboratory. Besides these, *R. microplus* were also collected from Nagla (243.8 m above mean sea level), Pantnagar (243.8 m above mean sea level) and Dehradun (700 m above mean sea-level) located in the foothills of Himalyas with a sub-tropical climate, and from Almora (1 638 m above mean sea level), and New Tehri (1650 m above mean sea-level) situated in the high altitude Himalyan region of Uttarakhand state with a temperate climate.

About 25–50 engorged females were collected from 10–15 cattle each in an area and they were weighed and those having similar weights were selected (Davey *et al.* 1980) and were kept in vials covered with muslin cloth at 85–92% relative humidity and $27 \pm 1^\circ\text{C}$ temperature in B.O.D. incubator for egg laying and hatching. About 3000, larvae of 7–14 days age were used to infest the crossbred cattle calf

(2 for each population). The ticks on the animal were monitored daily for engorgement. Twenty-five engorged female ticks that detached on day 22 of engorgement were randomly selected for evaluation of reproductive profile. Female ticks were examined daily until oviposition began to determine the pre-oviposition period. To reduce errors in larval age estimates, eggs deposited during every 24 hours period were carefully removed and placed in glass vial (mouth of the vial being closed by cotton stopper). The egg masses were weighed and counted. Both, the female and the eggs were replaced in the incubator for oviposition and hatchability respectively. The eggs thus obtained were examined daily until the first hatch commenced to determine the incubation period. The larvae and unhatched eggs were carefully counted to determine the percentage egg hatch. The hatched larvae were kept at 85–92% relative humidity and $27\pm1^\circ\text{C}$ temperature in B.O.D. incubator and were used for running *in-vitro* bioassays (Larval Packet Test). The resistance factor (RF) was calculated for each population of ticks to determine the status of resistance by dividing the LC_{50} of larvae of each population by the LC_{50} of susceptible tick population. The index of egg laying was calculated by the following formula (Sabatini *et al.* 2001)

$$\text{Index of egg laying} = \frac{\text{Weight of egg mass (mg)}}{\text{Weight of engorged tick (mg)}} \times 100$$

Data were analysed by GLM and univariate analysis of variance using GraphPad Prism Software (Version 4, 2003).

RESULTS AND DISCUSSION

The *in-vitro* laboratory larval packet assays revealed that Kashipur ticks were 34 times more resistant to deltamethrin; Nagla and Dehradun ticks were more than 5 times resistant whereas Pantnagar, Almora and New Tehri cattle ticks had resistance factor of 4.71, 3.21 and 2.04, respectively, when compared to the susceptible Pithoragarh tick population.

The results of variations in reproductive profile of different populations of *R. microplus* exhibited variations in susceptibility to deltamethrin (Table 1). The mean pre-

oviposition period ranged from 3.0 ± 0.39 days (Kashipur) to 7.08 ± 0.23 days (New Tehri). The mean oviposition period varied from 8.83 ± 0.44 days (Kashipur) to 12.17 ± 0.42 days (Nagla). The mean incubation period was the longest (22.7 ± 0.4 days) for Kashipur population and the minimum (20.55 ± 0.1 days) for New Tehri population. No significant difference was observed in mean daily incubation period of different populations of *R. microplus*. The eggs laid towards the end of oviposition showed longer incubation periods for all tick populations. Significant differences ($P<0.05$) were observed in incubation periods for eggs laid on different days of different tick populations.

The number of eggs laid by the female ticks of Kashipur (3365.0 ± 86.72), Nagla (3354.8 ± 115.0) and Dehradun (3377.8 ± 84.8) populations was low as compared to susceptible Pithoragarh (3537.5 ± 54.12) and New Tehri (3543.42 ± 49.5) population. The per cent hatch of the resistant Kashipur (86.75 ± 2.5) population was also low as compared to Pithoragarh (89.13 ± 1.4) population. However, the differences were insignificant. More than 50% hatching occurred in eggs laid for the first 8 days. The per cent hatch reduced for eggs laid in subsequent days of oviposition. No hatching took place in eggs laid on last days of oviposition in Kashipur (13th day) and Dehradun (16th day) tick populations. The mean daily percentage hatch was significantly higher ($P<0.05$) for eggs laid on first 7 days and declined thereafter. Significant differences ($P<0.05$) were observed in index of egg laying of different populations of *R. microplus*. The index of egg laying was comparable for Pithoragarh (0.2262 ± 0.001) and New Tehri (0.226 ± 0.004) populations and lowest for Kashipur (0.206 ± 0.005).

The observed results of reproductive profile of the susceptible tick population are in consonance with earlier studies (Davey *et al.* 1980, Sinha *et al.* 1982, Davey and George, 1998). Davey *et al.* (2006) observed that the permethrin susceptible ticks had a pre-oviposition period, oviposition period, incubation period, number of eggs laid and % hatch of 3.0, 12.1, 22.5, 3670 and 78.1 as compared to 3.0, 9.9, 23.0, 3344 and 82.2 for permethrin resistant

Table 1. Biological variations (mean $\pm$ SE) in different populations of *Rhipicephalus (Boophilus) microplus* collected from Uttarakhand state

Tick population	Engorgement weight (mg)	Preoviposition period (days)	Oviposition period (days)	No. of eggs oviposited	Incubation period (days)	Egg mass (g)	Hatching (%)	Index of egg laying
Almora	350.25 ± 4.70^a	3.42 ± 0.15^a	10.25 ± 0.35^a	3542.8 ± 50.10^a	20.73 ± 0.15^a	0.0784 ± 0.002^a	88.78 ± 0.71^a	0.224 ± 0.002^b
Pithoragarh	347.58 ± 5.16^a	3.83 ± 0.24^a	10.16 ± 0.52^a	3537.5 ± 54.12^a	20.95 ± 0.15^a	0.0784 ± 0.001^a	89.13 ± 1.42^a	0.226 ± 0.001^a
Kashipur	346.58 ± 2.75^a	3.00 ± 0.39^a	8.83 ± 0.44^b	3365.0 ± 86.72^a	22.7 ± 0.49^a	0.0714 ± 0.002^a	86.75 ± 2.52^a	0.206 ± 0.005^b
Pantnagar	345.30 ± 5.63^a	3.58 ± 0.45^a	11.58 ± 0.26^a	3458.6 ± 92.65^a	21.05 ± 0.10^a	0.0766 ± 0.002^a	88.7 ± 0.99^a	0.222 ± 0.003^b
Nagla	350.08 ± 4.55^a	3.33 ± 0.19^a	12.17 ± 0.42^a	3354.8 ± 115.0^a	21.71 ± 0.17^a	0.0747 ± 0.002^a	88.2 ± 1.12^a	0.213 ± 0.005^b
New Tehri	348.25 ± 4.90^a	7.08 ± 0.23^b	10.08 ± 0.31^a	3543.42 ± 49.5^a	20.55 ± 0.16^a	0.0787 ± 0.001^a	89.02 ± 1.10^a	0.226 ± 0.004^a
Dehradun	349.8 ± 5.10^a	5.08 ± 0.83^b	11.67 ± 0.71^a	3377.8 ± 84.8^a	21.74 ± 0.25^a	0.0752 ± 0.002^a	88.93 ± 1.15^a	0.215 ± 0.003^b

Means in columns with different superscripts differ significantly ($P<0.05$).

R. microplus. However, in the present study, the per cent hatch of the resistant population was slightly lower as compared to the susceptible ticks. Singh *et al.* (2009) also observed no differences in per cent hatch of eggs laid by treated and control ticks following adult immersion test. Short pre-oviposition period, longer oviposition period and lower mean per cent hatch does not put resistant ticks to disadvantage as these biological differences are very small to lower their chances of survival.

In the presence of acaricides, the susceptible tick population would be wiped out and whatever small number of resistant individuals is present, they would escape the toxic effect of the drug. In the absence of acaricide selection pressure, the resistant population of arthropods reduces (Roush and Daly 1990) and the susceptible tick population flourishes. In other words, it implies that continued application of acaricide is critical in development of acaricide resistance as number of exposures increase and hence selection pressure. Therefore, the number of applications along with the correct dosage of the drug is important in formulating management strategies against ticks.

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