ACARICIDE RESISTANCE IN *RHIPICEPHALUS (BOOPHILUS) MICROPLUS* TICKS AGAINST DELTAMETHRIN AND AMITRAZ IN HARYANA

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SUMMARY

Rhipicephalus (Boophilus) microplus, a one host tick, has been reported to have developed resistance to all major classes of acaricides, including synthetic pyrethroids and formamidines. Fully engorged female *R. (B.) microplus* ticks were collected from various Gaushalas (cow shelters) located in district Hisar, Haryana. The ticks were subjected to adult immersion test with a discriminating dose against deltamethrin (1.25%) and amitraz (12.5% EC). Prevalence of resistance was determined based on the number of ticks that laid eggs or which died before laying eggs. Prevalence of resistance against deltamethrin ranged from 46.6% to 76.6%, and against amitraz 10% to 23.3% depending on the location. It seemed that a long time exposure to synthetic pyrethroids and comparatively less exposure to amitraz resulted into this type of prevalence pattern.

Keywords: Acaricide resistance, Deltamethrin, Amitraz, Rhipicephalus(Boophilus) microplus

Ticks and tick-borne diseases (TTBDS) cause an estimated economic loss of US \$13-14 billion to cattle producers globally (de Castro, 1997). In India, cost of controlling TTBD's is estimated to be as \$498.7 million annually (Minjauw and McLeod, 2003). A widespread resistance to the organophosphate compound, diazinion and synthetic pyrethroids, deltamethrin and cypermethrin, has been experimentally validated in Indian isolates of R.(B.) microplus collected from six agro-climatic regions of the country (Kumar et al., 2011; Sharma et al., 2012). Furthermore, Shyma et al. (2015) reported resistance against deltamethrin, flumethrin, and fipronil in R. (B.) microplus ticks collected from North Gujarat. Gaur et al. (2016) assessed acaricide resistance status of R. (B.) microplus and H. anatolicum ticks against deltamethrin and diazinon in Hisar (Haryana) and its adjoining district Churu (Rajasthan) using adult immersion test (AIT) and larval packet test (LPT). The first case of amitraz resistance in India was detected in R. (B.) microplus ticks from Banaskantha district, Gujarat (Singh et al., 2013) that was followed by reports from other parts of India (Kumar et al., 2014; Singh et al., 2014). Since there appears to be no published report on amitraz resistance from Haryana, the present study was designed to assess the resistance status against amitraz among R. (B.)microplus ticks collected from Haryana. This was compared with more widely used acaricide, deltamethrin.

Adult engorged female ticks dropped from the animals were collected from various Gaushalas around Hisar located at Aryanagar, Shahpur, Dhiranwas, Hansi, Gurana and Datta. The ticks were identified and freshly dropped females which had not started egg laying were separated out, washed and placed individually in labeled vials, closed with muslin cloth to allow air and moisture exchange. These ticks were placed in desiccators at $85\pm5\%$ relative humidity maintained by 10% KOH and maintained at 10°C in BOD incubator for a few hours till the experiment was conducted (Gaur *et al.*, 2016).

The discriminating dose (DD) worked out by the Indian Veterinary Research Institute on IVRI-1 line of *R*. (*B*.)

microplus ticks was used in current study. For deltamethrin DD was 59.2ppm (Shyma *et al.*, 2013) and for amitraz DD was 487.7ppm (Kumar *et al.*, 2014). Dilutions of acaricides were freshly prepared according to the recommended DD by using commercial formulation of deltamethrin (1.25%) and amitraz (12.5% EC). Distilled water was taken as control.

Adult Immersion Test with a discriminating dose (AITDD) was conducted as per the method of FAO (2004) with minor modifications. Test and control groups consisted of thirty healthy, engorged adult female ticks. Each group consisted of three replicates of ten ticks each. Ticks of test group were dipped in 20 ml of acaricide dilutions for 10 min at room temperature with gentle and intermittent shaking. Acaricide solution was poured off and the ticks were dried gently on filter paper. Control group was treated with distilled water. Post treatment, ticks were stuck onto doublesided sticky tape in petri dishes, with ventral side up. These were placed in desiccators with 85% relative humidity and incubated at 28°C for 7 days. After 7 days of incubation, number of ticks that oviposited was counted. Percentage resistance was calculated as per the formula, R(%) = (Nt/Nw) \times 100. Here R (%) refers to percent resistance, Nt refers to number of treated ticks laying eggs and Nw refers to number of untreated ticks laying eggs.

The results of the AITDD are shown in Table 1. The prevalence of resistance against deltamethrin was highest (76.6%) in the ticks collected from Gurana while it was lowest (46.6%) in the ticks collected from Dhiranwas. Development of such high degree of resistance may be due to indiscriminate use of deltamethrin in the area. In case of Amitraz, the prevalence of resistance was highest (23.3%) in the ticks collected from Gurana and lowest (6.6%) in the ticks collected from Hansi and Dhiranwas. Thus, the prevalence of resistance against amitraz was found to be quite low in comparison with deltamethrin, which may be due to less exposure of the ticks to amitraz.

Gaur *et al.* (2016) assessed acaricide resistance status of *R. (B.) microplus* and *H. anatolicum* ticks against deltamethrin and diazinon in Hisar (Haryana) and its adjoining district of Churu (Rajasthan) using AIT and LPT.

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Table 1
Prevalence of resistance in Rhipicephalus (Boophilus.)
microplus ticks against deltamethrin and amitraz

Location	Acaricide	No. of ticks		Resistance (%)
		Treated	Laid eggs	
1. Aryanagar	Deltamethrin	30	18	60.0
	Amitraz	30	3	10.0
	Control	30	30	0.0
2. Shahpur	Deltamethrin	30	20	66.6
	Amitraz	30	4	13.3
	Control	30	30	0.0
3. Dhiranwas	Deltamethrin	30	14	46.6
	Amitraz	30	2	6.6
	Control	30	30	0.0
4. Hansi	Deltamethrin	30	22	73.3
	Amitraz	30	2	6.6
	Control	30	30	0.0
5. Gurana	Deltamethrin	30	23	76.6
	Amitraz	30	7	23.3
	Control	30	30	0.0
6. Datta	Deltamethrin	30	21	70.0
	Amitraz	30	6	20.0
	Control	30	30	0.0

The adult immersion test with a discriminating dose (AIT-DD) is more simple and quick assay than classical AIT (Drummond, 1973). The objective of determining the country specific discriminating concentration is to study the acaricide resistance status of field ticks. Commercial formulations are readily available to farmer and their inefficacy is the major resistance development parameter opted at the field level. One of the major advantages of using AITDD is that, it is not necessary to weigh the eggs or to estimate percent hatch, which allows the test to be completed in 7 days rather than 4 to 5 weeks (FAO, 2004). Thus, this test can be used as a quick and quantitative estimation of resistance against any acaricide. In this study, ticks (R. *microplus*) were collected and subjected to AIT-DD against deltamethrin and cypermethrin. The results revealed a high degree of resistance against deltamethrin (96.67%) and cypermethrin (93.33%) indicating need for adoption of alternative tick control strategy (Singh et al., 2010).

The use of a DD for each acaricide is a means of substantially reducing the amount of work needed to determine whether resistance is present or not. It is necessary to perform complete dosage mortality test at the first discovery of resistance to an acaricide in the field. This can be used as a diagnostic tool for determining if failure of tick control by a compound is due to resistance, for estimating the proportion of a tick population that is resistant and for surveys to estimate the extent of distribution of resistance to an acaricides (Kumar *et al.*, 2011). The percentage of ticks surviving treatment at DD can be taken as the percentage resistance to an acaricide. Care should be taken when determining what will be selected as the critical DD.

It is important to assess the efficacy of acaricides in a particular geographical area as resistance status varies in different areas. AITDD helps in selection of an effective acaricide for a particular area within a reasonable time and it is easy to perform. This study clearly points out a high prevalence of acaricide resistance against deltamethrin as compare to amitraz in Haryana. However, judicious use of both the acaricides is warranted.

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