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Acaricide resistance status of *Rhipicephalus (Boophilus) microplus* and *Hyalomma anatolicum* collected from Haryana

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ABSTRACT

Rhipicephalus (Boophilus) microplus and *Hyalomma anatolicum* are the commonest tick species in India infesting cattle and buffaloes and cause significant economic losses to dairy and leather industries by adversely affecting the milk production and quality of hides. Tick control strategy involves mainly the use of synthetic acaricides which results into development of acaricide resistance. In the present study, the tick samples were collected from organized and unorganized farms of Hisar (HSR) and Fatehabad (FTH) districts of Haryana state to determine the status of development of acaricide resistance. The discriminating doses (DD) of deltamethrin, cypermethrin and diazinon were established against reference susceptible IVRI-I (national registration no. NBAII/IVRI/BM/1/1998) and IVRI-II (national registration no. NBAII/IVRI/HA/1/1998) lines of *Rhipicephalus (Boophilus) microplus* and *Hyalomma anatolicum*, respectively, using adult immersion and larval packet tests. The results indicated that FTH isolates of *R (B) microplus* and *H anatolicum* developed resistance against all the 3 acaricides while the HSR isolates of both the tick species are slowly developing resistance. The data on field status of acaricide resistance in two sampled districts of Haryana will be helpful to adopt suitable strategy to overcome the process of development of resistance in ticks.

Key words: Acaricide, *Hyalomma anatolicum*, Resistance factor, *Rhipicephalus (Boophilus) microplus*

Introduction of exotic blood in Indian cattle has led to increased tick menace in intensive farming. Use of chemical acaricides is the most common tick control method adopted by the cattle owners and these chemicals are available in different trade names over-the-counter throughout the country. However, continuous and indiscriminate use of acaricides led to the appearance of the resistant tick and thus making tick control more complicated, (FAO 2004). In a recent study, large scale resistance to OP compound diazinon and synthetic pyrethroids, deltamethrin and cypermethrin was experimentally validated in Indian isolates of *Rhipicephalus (Boophilus) microplus* collected from 6 agro-climatic regions of the country (Sachin Kumar *et al.* 2011, Sharma *et al*. 2012a). They have reported a resistant factor in the range of 3.6–66.0, 2.0–95.7 and 2.06–9.9, respectively, against diazinon, deltamethrin and cypermethrin, respectively. The present paper was aimed at monitoring the status of resistance, if any, in 2 common cattle ticks *R.(B.) microplus* and *Hyalomma anatolicum* collected from Haryana state.

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MATERIALS AND METHODS

Reference tick lines: The reference susceptible IVRI-I (national registration no. NBAII/IVRI/BM/1/1998) and IVRI-II (NBAII/IVRI/1/1998) tick lines of *R. (B.) microplus* and *H. anatolicum* were used as the standard to determine discriminating doses for characterization of acaricide resistance in field ticks. These tick lines were established and successfully maintained in the Entomology Laboratory of Indian Veterinary Research Institute, Izatnagar. The biological parameters were continuously monitored and susceptibility status of the colonies was established by periodic testing against several organophosphates, organochlorines, synthetic pyrethroids and formamidine compounds in *in vitro* independent bioassays. The genetic homogeneity of the tick lines was established by analysis of the 16srDNA gene sequences (accession nos. GU222462, GU323287, GU323288, HM176656, HM176657, HM176658) (Rinesh Kumar *et al.* 2011).

Determination of discriminating doses

Chemical acaricides: For generating discriminating doses

(DDs) of deltamethrin, cypermethrin and diazinon, technical grade (100% pure) acaricides were used for preparation of stock solutions of diazinon in methanol (50 000 ppm), deltamethrin in acetone (10 000 ppm) and cypermethrin in methanol (10 000 ppm). For the experimental bioassay, different concentrations of the acaricides were prepared in distilled water from the stock solutions and tested against *R. (B.) microplus* and *H. anatolicum.*

Adult immersion test (AIT): Adult immersion test was conducted according to Drummond *et al*. (1973) and Benavides *et al*. (1999) with minor modifications for the generation of discriminating doses of the acaricides. Different concentrations of acaricides were prepared in distilled water from their stock solutions to conduct dose-response bioassay. The pre weighed engorged females of susceptible IVRI-I line of *R.(B.) microplus* were subjected to AIT for 2 min and then soaked on tissue paper before transferring into the petri-dishes. After 24 h, ticks were transferred to the glass tubes covered with muslin cloth and were kept in desiccator placed in BOD incubator maintained at 28 ± 1°C and 85 ± 5% RH. The mortality of ticks was recorded by observing loss of motility and pedal reflex after exposing to light. The control group was treated in respective solvents as their content in different concentrations of acaricide was equal to or less than 10% level. The safety level of methanol against susceptible IVRI-I line *R. (B.) microplus* has previously been evaluated (Sharma *et al*. 2012b). Each concentration was replicated 10 times and 5 adults were used per replication (n=10×5). The following parameters were compared:

(a) Mortality: Data were recorded up to 14 days post treatment (dpt) when normal ticks complete egg laying.
(b) The egg masses laid by the live ticks were recorded.
(c) Reproductive index (RI) = egg weight (EW)/ engorged female weight (IFW)
(d) Percentage inhibition of oviposition

$$(\text{IO\%}) = \frac{\text{IO\%} = \text{RI control} \times \text{RI treated}}{\text{RI control}} \times 100$$

Larval packet test (LPT): The LPT was conducted as per FAO (1971) with minor modifications. The Whatman filter paper No.1 was cut in parallelogram shape (5.5 cm × 5 cm) and were impregnated with 0.5–0.6 ml of different concentrations of deltamethrin, cypermethrin and diazinon with the help of pipette and dried for at least 30 min in incubator at 37°C. Treated and dried parallelograms of paper were folded in half forming equilateral triangular packets and sealed on the sides with adhesive tapes forming an open-ended packet. After insertion of approximately 150 larvae, the open side of each packet was sealed with adhesive tape and the packets were placed in a desiccators kept in BOD incubator maintained at 28 ± 1°C and 85 ± 5% RH. The packets were removed after 24h, and larval mortality was calculated. The dose–response data of the individual acaricide against the reference susceptible ticks (IVRI-I or IVRI-II lines) were analyzed. The mortality data of the ticks were probit transformed and plotted against the log values of the acaricide concentrations. The LC_{50} and LC_{95} values of diazinon, deltamethrin and cypermethrin were determined by applying regression equation analysis (Finney 1962) using GraphPad Prism version 4.00 Software. For characterization of field ticks, discriminating dose (DD) was determined as $2 \times LC_{95}$ of IVRI-I or IVRI-II lines (Jonsson *et al*. 2007).

Collection of field ticks and characterization: Two steps stratified sampling method was adopted to collect engorged females of *R. (B.) microplus* and *H. anatolicum* from the animal sheds during July and August 2011. After initial processing at collection stations the ticks were transported to the Entomology Laboratory, IVRI, Izatnagar, and maintained at 28± 1°C and 85±5% RH in the BOD incubator. As per the collected sample size the bioassay was selected. In the present case LPT was adopted for monitoring of resistance. Three to five adults were pooled together and were maintained as per Ghosh and Azhahianambi (2007). The larvae hatched in different tubes were pooled separately for each isolate and 2 week-old larvae were used in LPT as mentioned above using different DDs of the acaricides (2 ×, 4×, 6×, 8×) and their LC_{50} values were determined. Resistance factors (RF) for field isolates were worked out by the quiescent between LC_{50} of field isolates and LC_{50} of reference susceptible lines (IVRI-I and IVRI-II) (Castro-Janer *et al.* 2009). On the basis of RF, the resistance status of the field isolates was classified as susceptible ($RF \leq 1.4$), level I resistance ($1.5 < RF < 5.0$), level II resistance ($5.1 < RF < 25.0$), level III resistance ($25.1 < RF < 40$) and level IV resistance ($RF > 40.1$) (Sharma *et al.* 2011a).

RESULTS AND DISCUSSION

The LC_{50} and LC_{95} values with 95% confidence limit of deltamethrin, cypermethrin and diazinon were determined against reference IVRI-I and IVRI-II lines of *R. (B.) microplus* and *H. anatolicum*, respectively, and are presented in Table 1. The discriminating doses (DD) play an important role in the monitoring of acaricide resistance in field ticks and were established using the technical grade acaricides against reference homogenous susceptible tick lines of *R. (B.) microplus* and *H. anatolicum* because the commercial products are prepared from many proprietary ingredients/ solvents and it is difficult to assess the response due to active ingredients (Shaw 1966).

In the present study, the DDs of deltamethrin, cypermethrin and diazinon were calculated as 71.0, 701.4 and 1273.2 ppm using LPT while 59.2, 698.2 and 1270.4 ppm using AIT, respectively, for IVRI-I line. For IVRI-II line the DDs were 69.8, 701.8 and 1300.0, ppm for deltamethrin, cypermethrin and diazinon, respectively, using LPT. The DDs obtained using LPT was adopted for the

Table 1. LC_{50} and LC_{95} with 95% confidence limit (CL) of diazinon, deltamethrin and cypermethrin

Reference tick lines (Test used)	Diazinon		Deltamethrin		Cypermethrin	
	LC_{50} (95% CL)	LC_{95} (95% CL)	LC_{50} (95% CL)	LC_{95} (95% CL)	LC_{50} (95% CL)	LC_{95} (95% CL)
IVRI-I (AIT)	372.0 (341.3–405.3)	635.2 (582.7–692.4)	13.4 (12.4–14.5)	29.6 (27.7–31.7)	138.5 (134.5–142.6)	349.1 (323.2–377.0)
IVRI- I (LPT)	499.7 (497.7–501.7)	636.6 (630.9–642.3)	11.8 (11.5–12.1)	35.5 (34.1–36.9)	242.4 (240.0–244.8)	350.7 (347.2–354.2)
IVRI- II (LPT)	246.1 (243.7–248.6)	650.0 (639.21–665.0)	11.7 (11.5–11.9)	34.9 (34.2–35.6)	245.91 (245.2–246.6)	350.9 (347.4–354.4)

IVRI-I, *Rhipicephalus (Boophilus) microplus*, IVRI II, *Hyalomma anatolicum.*

Table 2. Acaricide resistance data of *R (B) microplus* and *H. a. anatolicum* collected from Haryana using larval packet test (LPT)

Tick species	Tickisolates	Deltamethrin			Cypermethrin			Diazinon		
		Slope ±SE	RF	**RL	Slope ±SE	RF	**RL	Slope ±SE	RF	**RL
Rhipicephalus (Boophilus) microplus	*IVRI-I	3.42±0.50	1.0	S	10.23±2.47	1.0	S	15.61±1.41	1.0	S
	HSR	2.36±0.22	4.5	I	2.06±0.88	0.98	S	6.06±1.15	4.2	I
	FTH	4.10±1.5	10.8	II	3.46±0.33	3.9	I	1.16±0.65	12.0	II
Hyalomma anatolicum	*IVRI-II	3.45±0.30	1.0	S	10.63±2.02	1.0	S	3.87±0.80	1.0	S
	HSR	2.29±0.35	1.05	S	1.41±0.26	0.18	S	2.50±0.38	1.82	I
	FTH	3.31±1.23	4.65	I	30.51±1.6	2.25	I	3.81±1.37	4.46	I

*Reference susceptible lines, RF, resistance factor, S, susceptible, **RL, resistance level.
Susceptible = RF up to 1.4, Level I = 1.5<RF<5, level II= 5.1<RF<25, level III= 26<RF<40, level IV= RF>41.

characterization of field ticks because the availability of adult female ticks was not sufficient to conduct AIT.

There are low variation in the LC_{50} and LC_{95} values of the acaricides irrespective of the assay used and high slope values in the regression analysis of the reference susceptible line in comparison to the field isolates which established the homogeneity in the IVRI-I and IVRI-II lines (Table 2). A number of workers have reported wide variations in the LC_{50} and LC_{95} values of these acaricides against reference susceptible tick lines of *R.(B.) microplus* maintained in their laboratories depending on the types of bioassay adopted (Aguirre *et al*. 2000, FAO 2004, Robertson *et al*. 2006, Jonsson *et al*. 2007, Castro-Janer *et al*. 2009, Roma *et al.* 2009). The wide variation in the LC_{50} values in cited literature strongly suggested that the generation of base line data of representative tick species of a particular country before working out the resistance status in field ticks is essential.

The data revealed (Table 2) that ticks in the areas have developed resistance to 3 commonly used acaricides. The FTH isolates of *R. (B.) microplus* and *H. anatolicum* were found resistant to all the 3 acaricides tested while the HSR isolates developed comparatively less resistance to the tested acaricides. The slope of mortality was steeper in reference susceptible lines in comparison to resistant ticks which inferred that for each unit increase in concentration of the acaricide there is a greater mortality amongst the ticks of IVRI-I line. The HSR isolate of *R. (B.) microplus* developed resistance to deltamethrin (RF=4.5) and diazinon (RF=4.2) at level I while susceptible to cypermethrin (Table 2). On the contrary, FTH isolate of *R. (B.) microplus* showed higher level of resistance (level II) to deltamethrin (RF=10.8) and diazinon (RF= 12.0) and low level of resistance (level I) to cypermethrin (RF=3.9). However, HSR isolate of *H. anatolicum* was susceptible to pyrethroids whereas slightly resistant (level I) to OP (diazinon) while FTH isolate of *H. anatolicum* showed low level of resistance (level I) to all the 3 tested acaricides. Earlier, Sangwan *et al.* (1993) reported the susceptibility of these isolates to commonly used acaricides. Recently, the acaricide resistance status in ticks collected from different parts of the country has been reported (Sachin Kumar *et al.* 2011, Sharma *et al*. 2012a, Shyma *et al*. 2012).

The present investigation showed that the level of acaricide resistance is comparatively higher in the one host ticks, *R. (B). microplus* than in the three host tick, *H. anatolicum* which corroborates the observations made on global basis (Wharton and Roulston 1970). Millar (1988) described pyrethroids resistance in terms of family resistance in as much as the ticks resistant to one pyrethroid compound cannot be controlled by another compound for a long time. Although at present the cypermethrin resistance in field isolates is relatively less pronounced in Indian ticks, it is not unlikely that higher resistance to this compound will be acquired by the ticks in near future. If compared to multi-host ticks, a much larger fraction of the total population of one host ticks remains under chemical challenge because of their shorter life cycle and host specificity to domestic cattle. However, off the host ticks of the genus *Hyalomma* may come in contact with environmental residues of the insecticides increasing the selection pressure. The detection of acaricide resistance in *Hyalomma* ticks is of particular importance in planning the control programme because of the continuous introduction of these ticks from Rajasthan to Haryana through the migrating cattle herds and the involvement of these ticks in transmission of *Theileria annulata* infection. Existence of high acaricidal resistance in *R. (B.) microplus* populations has also been documented in Rajasthan (Sharma *et al*. 2012a) and introduction of these resistant ticks to Haryana is possible. The results signify that there is a need for continuous monitoring of acaricide resistance in field situation for strategic application of available acaricides and for maintaining the life span of the product.

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REFERENCES

Aguirre D H, Vinabal A E, Salatin A O, Cafrune M M, Volpogni M M, Mangold A J and Guglielmone A A. 2000. Susceptibility to two pyrethroids in *Boophilus microplus* (Acari: Ixodidae) populations of north-west Argentina. Preliminary results. *Veterinary Parasitology* **88**: 329–34.

Benavides O E, Romero N A, Rodriguez J L and Silva Z J. 1999. Evidencia preliminar de la aparicion de resistenciaa lactonas macrociclicas en cepas de garrapata *Boophilus microplus* en Colombia. *Memorias IV Seminarios Internacional de Parasitologia*. Puerto Vallarta, Jalisco., Mexico, 20–22 October 1999, 266–264.

Castro-Janer E, Rifran L, Piaggio J, Gil A, Miller R J and Schumaker T T S. 2009. *In vitro* tests to establish LC_{50} and discriminating concentrations for fipronil against *Rhipicephalus (Boophilus) microplus* (Acari: Ixodidae) and their characterization. *Veterinary Parasitology* **162**: 120–28.

Drummond R O, Ernst S E, Trevino J L, Gladney W J and Graham O H. 1973. *Boophilus annulatus* and *Boophilus microplus*: laboratory test of insecticides. *Journal of Economic Entomolology* **66**: 130–33.

FAO. 1971. Recommended methods for the detection and measurement of resistance of agricultural pests to pesticides – tentative method for larvae of cattle ticks, *Boophilus microplus* spp. FAO method No. 7. *FAO Plant Protection Bulletin* **19**: 15–18.

FAO. 2004. *Resistance Management and Integrated Parasite Control in Ruminants. Guidelines*. Animal Production and Health Division, FAO, pp. 25–77.

Finney D J. 1962. *Probit Analysis – A Statistical Treatment of the Response Curve*. Cambridge University Press, Cambridge, pp. 1–318.

Ghosh S and Azhahianambi P. 2007. Laboratory rearing of *Theileria annulata* free *Hyalomma anatolicum anatolicum* ticks. *Experimental and Applied Acarology* **43**: 137–46.

Jonsson N N, Miller R J and Robertson J L. 2007. Critical evaluation of the modified-adult immersion test with discriminating dose bioassay for *Boophilus microplus* using American and Australian isolates. *Veterinary Parasitology* **146**: 307–15.

Millar T A. 1988. Mechanism of resistance to pyrethroid insecticides. *Parasitology Today* **4** (7): S8–S12.

Rinesh Kumar, Paul S, Kumar S, Sharma A K, Gupta S, Rawat A K S, Chaudhuri P, Ray D D and Ghosh S. 2011. Nucleotide specific changes in the hypervariable region of 16S rDNA gene as possible marker to differentiate the tick genera. *Indian Journal of Animal Sciences* **81** (12): 1204–07.

Robertson J L, Russell R M, Preisler H K and Savin N E. 2006. *Pesticide Bioassays with Arthropods*. 2nd edn. CRC Press, Boca Raton, FL, USA.

Roma G C, de Oliveira P R, Pizano M A and Mathias M I C. 2009. Determination of LC_{50} of permethrin acaricide in semi-engorged females of the tick *Rhipicephalus sanguineus* (Latreille, 1806) (Acari: Ixodidae). *Experimental Parasitology* **123** (3): 269–72.

Sachin Kumar, Paul S, Sharma A K, Kumar R, Tewari S S, Chaudhuri P, Ray D D, Rawat A K S and Ghosh S. 2011. Diazinon resistant status in *Rhipicephalus (Boophilus) microplus* collected from different agro-climatic zones of India. *Veterinary Parasitology* **181**: 274–81.

Sangwan A K, Chhabra M B and Singh S. 1993. Acaricide resistance status of common livestock ticks of Haryana. *Indian Veterinary Journal* **70**: 20– 24.

Sharma A K, Kumar R, Kumar S, Nagar G, Singh N K, Rawat S S, Dhakad M L, Rawat A K S, Ray D D and Ghosh S. 2012a. Deltamethrin and cypermethrin resistance status of *Rhipicephalus (Boophilus) microplus* collected from six agro-climatic regions of India. *Veterinary Parasitology* **188**: 337–45.

Sharma A K, Kumar S, Tiwari S S, Srivastava S, Kumar R, Ray D D, Chaudhuri P, Rawat A K S and Ghosh S. 2012b. Comparative acaricidal properties of different solvents and surfactants on *Rhipicephalus (Boophilus) microplus* (Acari: Ixodidae). *Indian Journal of Animal Sciences* **82**(2): 154–58.

Shaw R D. 1966. Culture of an organophosphorus resistant strain of *Boophilus microplus* (Canestrini) and assessment of its resistance spectrum. *Bulletin of Entomological Research* **56**(4): 398–405.

Shyma K P, Kumar S, Sharma A K, Ray D D and Ghosh S. 2012. Acaricide resistance status in Indian isolates of *Hyalomma anatolicum anatolicum. Experimental and Applied Acarology.* 58(4): 471–81.

Wharton R H and Roulston W J. 1970. Resistance of ticks to chemicals. *Annual Review of Entomology* **15**: 381–403.