Original Article

Detection of Deltamethrin Resistance in Cattle Tick, *Rhipicephalus microplus* Collected in Western Haryana State of India

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Abstract

Background: Out of 931 species of ticks, *Rhipicephalus microplus* is the most widely studied tick species due to its pivotal role in transmission of babesiosis and anaplasmosis, resulting in huge economic loss in cattle and buffalo's industry. Chemical control using deltamethrin forms the mainstay of tick control strategy because of high potency and low toxicity. However, inadvertent use of deltamethrin has led to the development of deltamethrin resistance in field ticks

Methods: The engorged female ticks were collected in a plastic container covered with a cotton plug from 10 places (Jarwa, Barwa, Khajakhera, Banisi, Nakipur, Dhangar, Badopal, Shivalya Dharamshala, Siwani and Meham) of five districts of western Haryana. The Larval Packet Test (LPT) was conducted for the characterization of resistance in field tick.

Results: In the present study, ticks were collected from 10 places from 5 districts of western Haryana and evaluated against deltamethrin using larval packed test. The LC_{50} values (confidence interval) of ticks larvae against deltamethrin collected from Nakipur, Dhangar, Barwa, Badopal, Shivalya Dharamshala, Siwani, Jarwa, Khajakhera, Meham and Banisi are 73.6 (67.2–81.9), 61.2 (61.6–98.5), 52.7 (14.4–101.0), 140.0 (86.7–448.6), 65.8 (37.1–95.2), 232.1 (201.0–304.7), 3.72 (0.20–9.87), 21.3 (12.0–31.6), 107.6 (96.8–127.6), 54.2 (43.4–58.4) ppm, respectively. The resistance factor ranges from 0.31 to 11.86, indicating variable resistance among field isolates.

Conclusion: Data generated on deltamethrin resistance status in R. microplus from Haryana, India can be used as an indicator for the management of the species in the state.

Keywords: Acaricide resistance; *Rhipicephalus microplus*; Deltamethrin; Haryana

Introduction

Ticks (Acari: Ixodida) are becoming increasingly relevant as a sanitary problem for human, domestic, and wild animals worldwide. Ticks belong to the phylum Arthropoda, class Arachnida, subclass Acari, superorder Parasitiformes, order Ixodida and superfamily Ixodoidea. Further superfamily Ixodoidea contains the 3 families, such as Ixodidae, Argasidae, and Nuttalliellidae. Out of 931 species of ticks, there are 722 species of Ixodidae which are known and classified till now (1). Among the Ixodidae, *Rhipicephalus microplus* has been reported as the most economically most important and widely distributed ecto-parasites (2, 3). *Rhipicephalus microplus* is a voracious blood feeder and plays a pivotal role in transmission of babesiosis and anaplasmosis, resulting in huge economic loss in cattle and buffalo's industry (4, 5). To control ticks, the use of

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chemical acaricides like synthetic pyrethroids, is the predominant approach implemented in the field (6). Amongst synthetic pyrethroids, Deltamethrin is most widely used acaricide in India because of its high potency and ease of availability under field condition. *Rhipicephalus microplus* being a one host tick species is more prone to selection against deltamethrin due to frequent exposure or under-dosing of drugs. It leads to the emergence of deltamethrin resistance in field ticks (7, 8, 9). Inadvertent use of deltamethrin and heavy tick infestation was reported from several organised cattle farms of western Haryana. Acaricide resistance in the ticks can be noticed and diagnosed at the farmer level based upon the persistence of ticks on animals beside the application of acaricides. However, to facilitate global monitoring of status of acaricide resistance and providing a firm basis for comparison of test results, standardized diagnostic methods are adopted. Therefore, FAO has recommended the use of the Larval Packet Test (LPT) for field investigations of acaricide resistance. In the present study, status of deltamethrin resistance in five districts of western Haryana was evaluated using larval packet test.

Materials and Methods

Collection of ticks

The present study was conducted during the period from April to August, 2019 to determine the status of deltamethrin resistance in *R. microplus* ticks of Western Haryana. Adult fully engorged dropped down female ticks were collected in a clean plastic container covered with a cotton plug from ten places (Jarwa, Barwa, Khajakhera, Banisi, Nakipur, Dhangar, Badopal, Shivalya Dharamshala, Siwani and Meham) of five districts of western Haryana. Ticks collected were immediately transported to the laboratory (25±2 ºC and 75% relative humidity) at the Department of Veterinary Parasitology, College of Veterinary Science, Lala Lajpat Rai University of Veterinary and Animal Sciences for Larval Packet Test (LPT). Identification of ticks was carried out using available standard literature (10, 11). For Larvae, two adult female ticks of each isolate were kept in each well cleaned and labelled glass vials, covered with muslin cloth and then, placed in desiccators at 28±1 °C and 85±5% relative humidity for 1–2 months. Ticks started laying eggs by 3–4 days of incubation and continued to lay eggs for the next 8–10 days. Then the adult ticks were removed and eggs were left for hatching in the vials. After that, the larvae were allowed to mature for 8–10 days. Around 10–14 days old larvae were used for Larval Packet Test. The sample collection places was represented in Fig. 1.

Acaricides

For the LPT, technical grade pure deltamethrin (Sigma-aldrich, USA) was

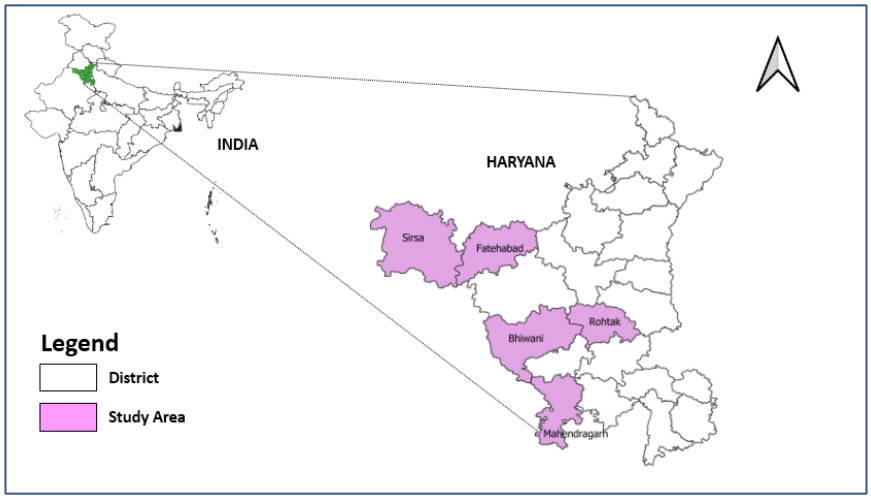

Fig. 1. Illustration of the sampling sites in Haryana, India, 2019

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used to prepare 5,000 ppm stock solution by diluting in acetone. Then, the different working concentrations of deltamethrin were prepared in distilled water from the stock solution and tested against larvae of *R. microplus* ticks. Distilled water was used to treat the control packets.

Larval Packet Test (LPT)

The LPT was conducted according to FAO (12) guidelines**.** Briefly, 0.6ml of the working solutions of different concentrations of deltamethrin (12.5, 25, 50, 100, 200 ppm) were used to impregnate 3.75 cm by 8.5 cm filter paper rectangles (541 Whatman filter paper) and were kept for 30 minutes in the BOD incubator to dry the filter paper. Then the rectangles were folded in half and sealed on the sides with adhesive tapes, forming an open-ended packet for placing the ticks' larvae. Control packets were also prepared by impregnating with the distilled water only. Approximately 150 larvae (10 to 14 days old) were put inside the packets using a fine brush and sealed with adhesive tape. These packets were then kept in a desiccator separately to avoid contamination, which were then placed in a BOD incubator maintained at 28±1 °C and 85±5% RH. The test was performed in triplicate for each concentration of each acaricide. After 24 h of incubation, larval mortality was calculated. Larvae capable of walking were considered alive, whereas those which moved their appendages but did not walk were counted as dead.

Statistical analysis

Regression analysis of LPT was done by probit method (13) using GraphPad Prism-5 statistical software. For calculating Resistance factor (RF) of ticks, LC_{50} of Indian Veterinary Research Institute (IVRI-I) *R. microplus*) against deltamethrin (11.8ppm) is used as country specific reference susceptible tick line (7). Resistant factor (RF) for ticks was calculated as the coefficient of LC_{50} of field ticks and LC_{50} of reference susceptible IVRI line I tick (14).

Resistant factor (RF) = LC_{50} of field ticks /LC_{50} of reference susceptible IVRI line I ticks. On the basis of RF, the resistance status in the field ticks population were classified as Susceptible (RF< 1.4), level I resistance (1.5< RF< 10.0), level II resistance (10.1<RF<25.0), level III resistance (25.1<RF<40), level IV resistance (RF>41) (15).

Results

On the basis of the dose response data, the mortality slopes, LC_{50}, LC_{95}, 95% confidence limit, Resistance factor (RF) and Resistance level (RL) of field ticks were determined (Table 1 and Fig. 2). Data generated showed

Table 1. Mortality slope, $LC_{50,}$ LC_{95} value with 95% CI and RF values of larvae of *Rhipicephalus microplus* against Deltamethrin collected from different places of Haryana, India, 2019

Places	District	Slope±SE	R^2	LC_{50} (ppm) (95% CI)	LC_{95} (ppm) (95% CI)	RF[#]	RL
NAKIPUR	BHIWANI	2.262±0.1285	0.993	73.6 (67.2–81.9)	383.1 (321.6–504.0)	6.23	II
BARWA		3.794±0.8104	0.915	52.7 (14.4–101.0)	141.2 (103.5–475.5)	4.46	I
SIWANI		1.436±0.1234	0.985	232.1 (201.0–304.7)	3130.0 (1610.2–7862.7)	19.66	II
DHANGAR	FATEHABAD	2.115±0.806	0.801	61.2 (61.6–98.5)	284.6 (249.5–319.4)	5.18	II
BADOPAL		2.625±0.392	0.957	140.0 (86.7–448.6)	580.7 (277.5–1088.2)	11.86	II
SHIVALYA DHARAMSHALA		2.005±0.525	0.879	65.8 (37.1–95.2)	424.5 (286.8–476.7)	5.57	II
JARWA	MAHENDRAGARH	0.970±0.0349	0.997	3.72 (0.20–9.87)	176.2 (125.0–378.2)	0.31	S
KHAJAKHERA	SIRSA	1.809±0.1446	0.987	21.3 (12.0–31.6)	168.6 (141.0–291.0)	1.80	I
MEHAM	ROHTAK	1.286±0.0355	0.991	107.6 (96.8–127.6)	1971.1 (1131.5–3515.8)	9.11	II
BAINSI		1.823±0.2202	0.971	54.2 (43.4–58.4)	421.8 (313.7–597.2)	4.59	I

[#]RF Resistance factor; RL Resistance level; S susceptible; Susceptible = RF<1.4; Level I= 1.5< RF< 5; level II= 5.1< RF< 25; level III= 26< RF< 40; level IV= RF>40.0

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that the LC_{50} values (confidence interval) of ticks larvae against Deltamethrin collected from Nakipur, Dhangar, Barwa, Badopal, Shivalya Dharamshala, Siwani, Jarwa, Khajakhera, Meham and Banisi are 73.6 (67.2–81.9), 61.2 (61.6–98.5), 52.7 (14.4–101.0), 140.0 (86.7–448.6), 65.8 (37.1–95.2), 232.1 (201.0–304.7), 3.72 (0.20–

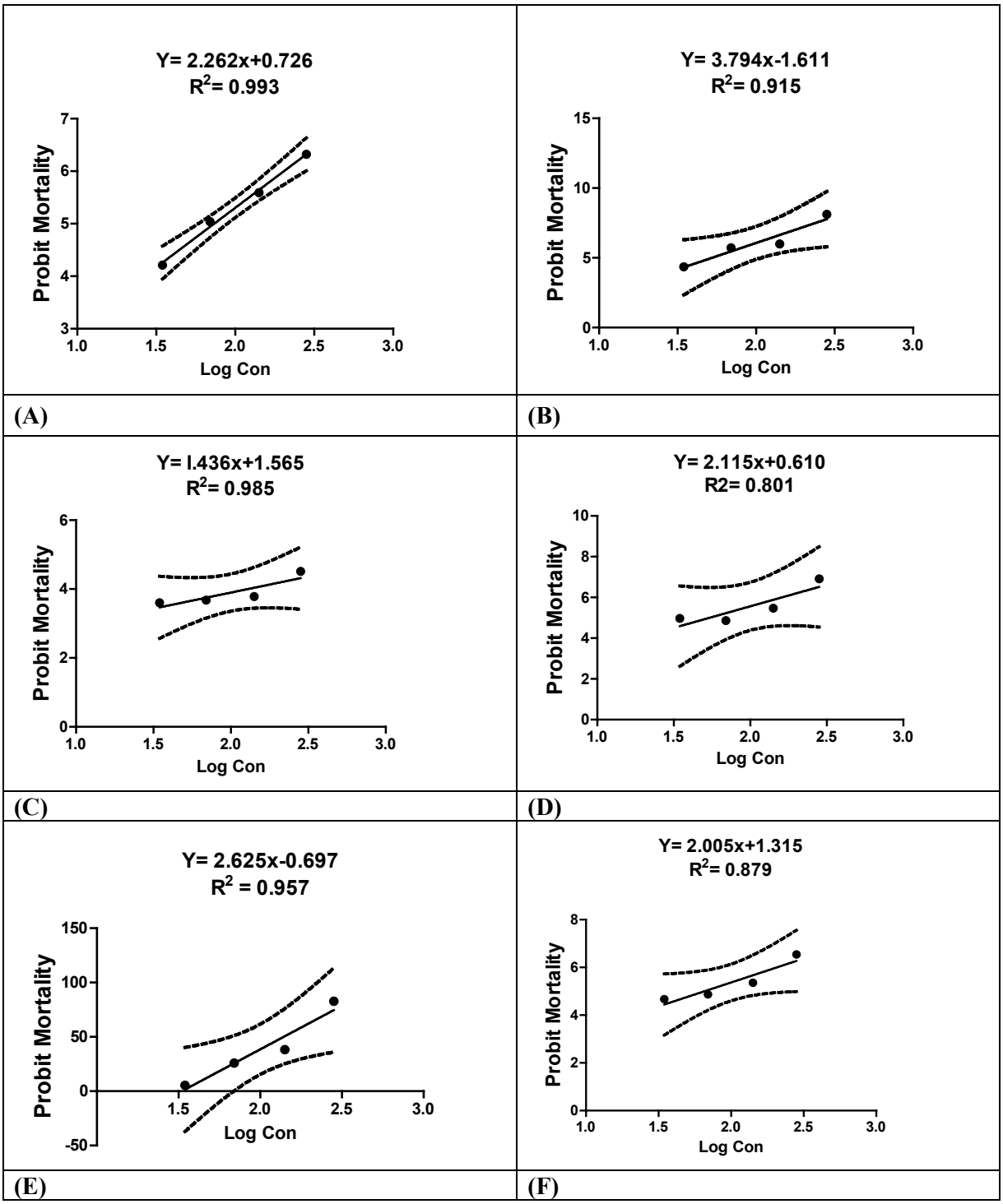

Fig. 2. Individual regression curve showing probit mortality in LPT against log concentration of deltamethrin in *Rhipicephalus microplus* ticks collected from different locations of Haryana, India, 2019 (A-J)

(A=Nakipur, B=Barwa, C=Siwani, D=Dhangar, E=Badopal, F=Shivalya Dharamshala, G=Jarwa, H=Khajakhera I=Meham, J=Bainsi)

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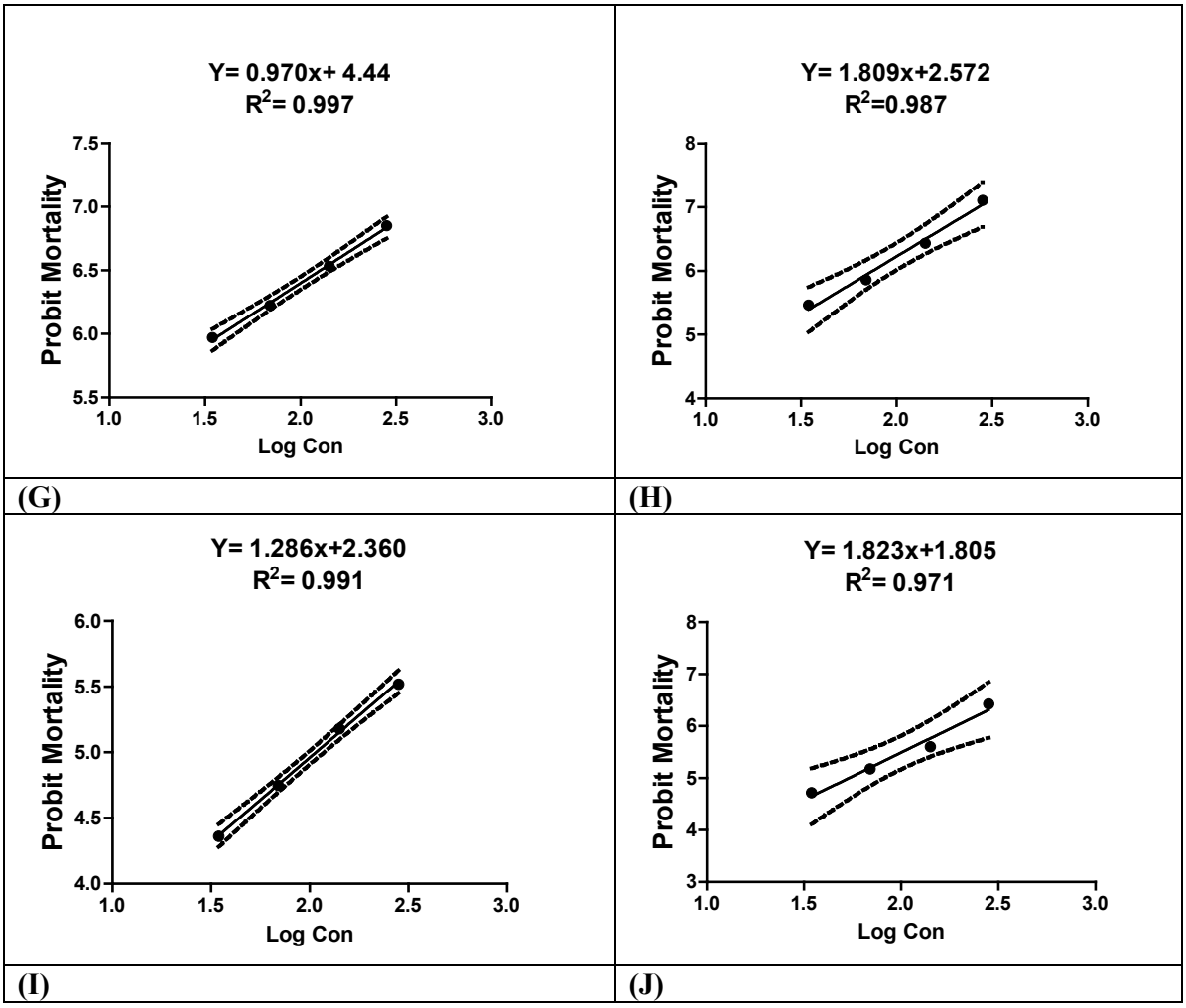

continued Fig. 2. Individual regression curve showing probit mortality in LPT against log concentration of deltamethrin in *Rhipicephalus microplus* ticks collected from different locations of Haryana, India, 2019 (A-J)
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9.87), 21.3 (12.0–31.6), 107.6 (96.8–127.6), 54.2 (43.4–58.4) ppm, respectively (Table 1). However, resistance factor ranges from 0.31 to 19.66, indicating variable resistance status among the field isolates. Only Jarwa (Mahendragarh) isolate was found susceptible, three other field isolates (Barwa, Khajakhera and Banisi) showed low levels (level I) of resistance, whereas, six isolates (Nakipur, Dhangar, Badopal, Shivalya Dharamshala, Siwani and Meham) showed moderate resistance (level II) to deltamethrin. Slope value ranges from 0.970±0.0349 to 3.794±0.8104 which indicates heterogeneity of field tick population characterized by a flatter curve, therefore leading too

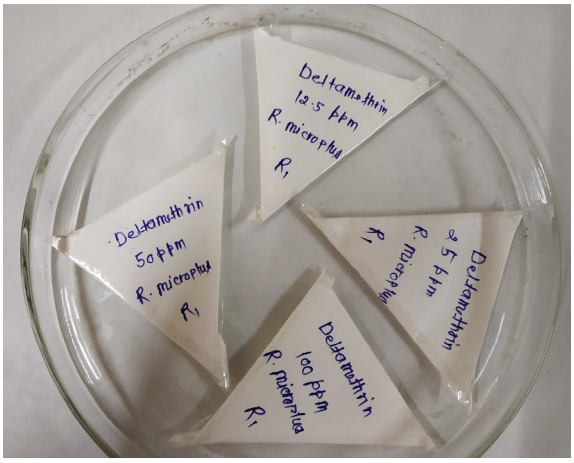

Fig. 3. The Larval Packet Test (LPT) bioassay of deltamethrin against filed collected *Rhipicephalus microplus*, Haryana, India, January, 2019

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much greater value of LC_{95} in comparison to LC_{50} values. The goodness of fit (R^2) value of the isolate ranges from 0.801 to 0.997 indicating a good fit of observed data in statistical model. In 9 out of 10 isolates, LC_{50} estimatewere 1.80–19.66 times higher than the reference susceptible IVRI-I tick line. The LPT bioassay was showed in Fig. 3.

Discussion

Synthetic pyrethroids and organophosphates are most frequently used acaricides in India followed by formamidines and macrocyclic lactones (16). In most of the northern Indian states, acaricidal resistance is a rampant problem due to widespread and indiscriminate usages of chemical acaricides (16, 17, 18, 7, 19). Reports of deltamethrin resistance in *R. microplus* ticks are also available from various parts of North India (7, 17, 24-27). In a study at Central India (Jabalpur, Madhya Pradesh), deltamethrin treated animals showed re-infestation with ticks by the 14th day of treatment, which provides indication of the reduced efficacy of deltamethrin in tick control in animals and animal sheds (23). Later, high resistance ratio (RF= 22.35 to 34.64) of field tick against deltamethrin was also recorded from Mhow districts in Madhya Pradesh (28). Several tick isolates from Western India (Gujarat) were also found resistant (RF= 2.52-22.59) against deltamethrin (29). There are scarce reports on the emergence of deltamethrin resistance from the Southern part of India (30, 31). In a recent study from South India, researchers have recorded a positive correlation (R=0.66) between the tick burden on household cattle and resistance factor and suggested that the deltamethrin resistance was one of the contributing factors for persisting tick load on cattle (31). Recently, deltamethrin resistance (RF=3.3-21.8) has also been recorded from the Assam state of Eastern India (32). Earlier from different parts of Punjab, low to severe (level I to IV) deltamethrin resistance has reported (20) while in the present study, using LPT only a low level (level I-II) of resistance (RF=1.8–19.66) was observed in ticks collected from selected organized cattle farms of Western Haryana. In a similar LPT-based study (21) from Haryana, the Level II resistance was recorded from all isolates of Fatehabad which corroborate with findings of the present manuscript. Some resistance workers screened *R. microplus* of Haryana and reported the emergence of level I (RF=3.5) deltamethrin resistance from Hisar district of Haryana (22). Previously, there were few adult immersion tests with discriminating doses (AIT-DD) based on deltamethrin resistance reports from Haryana (8). In this study, larval packet test is preferred as it is considered as a more sensitive tool for detection and monitoring of acaricide resistance in ticks (15).

In the present report, several organized farms of Western Haryana are screened for development of deltamethrin resistance in ticks using larval packet test which could be conducted with fewer numbers of ticks (12). Characterization of the deltamethrin resistance in field ticks of Western Haryana indicated that out of 10 isolates, resistance factor of 9 isolates was higher than the reference susceptible IVRI-I tick line. On comparing the resistance factor of different isolates, it could be concluded that deltamethrin resistance may not yet prevail in the Mahendragarh District of Haryana and still the compound is effective for the control of field ticks. Other isolates have shown low to moderate (Level I-II) resistance which showed that the situation is still not grave and could be controlled by stringent alternative tick control strategies with emphasis on integrated tick management approaches.

Conclusion

Resistance monitoring is a continuous process to provide an accurate pattern of resistance in different time intervals. The present study indicated the clue of resistance pattern to synthetic acaricides that may be helpful to formulate a suitable strategy for tick management in Haryana state of India.

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The authors declared that there was no conflict of interest among them.

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