In Vitro Detection of Acaricidal Resistance of Cattle Tick against Commercial Preparation of Deltamethrin and Cypermethrin in Three Villages of Udaipur District (Rajasthan)

ABSTRACT

Acaricidal resistance studies were carried out Department of Veterinary Parasitology in Udaipur to detect resistance status against commonly used commercial acaricides, 1.25% Butox® (Deltamethrin) and 10% w/v DERMEEZ® (Cypermethrin) against ticks collected from three villages (Vallabhnagar, Ranchorpura and Kikawas) of Udaipur, Rajasthan using Adult Immersion test (AIT) and Larval packet test (LPT). Recommended concentration of acaricides for AIT and LPT were used. Deltamethrin (0.075 g L^{-1}) and Cypermethrin (0.05 g L^{-1}) in AIT and Deltamethrin (0.06%) and Cypermethrin (0.2%) in LPT were used to evaluate acaricidal resistance. The overall analysis of data of the two tests with reference drug wise resistance for all three villages revealed that resistance to the deltamethrin was statistically higher than Cypermethrin. The drug wise results of AIT for deltamethrin for Vallabhnagar, Ranchorpura and Kikawas villages was 50%, 45% and 40% and for cypermethrin the resistance was 15%, 10% and 10% respectively. The results of LPT revealed that highest resistance in Vallabhnagar (27.5%), followed by Ranchorpura (21%) and Kikawas (19%) for deltamethrin while cypermethrin showed 19%, 11.5% and 9.5% resistance in Vallabhnagar, Ranchorpura and Kikawas, respectively.

Keywords: Resistance, Deltamethrin, Cypermethrin, Adult Immersion test (AIT), Larval packet test (LPT)

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1. Introduction

"India's livestock sector is one of the largest in the world and plays an important role in country's economy. In India, 70% of the rural households own livestock for generating additional employment through milk, meat, wool and eggs production" (Ali, 2007). "The livestock sector especially the dairy sector comprising of approximately 199 million cattle in

India is an important part of the rural agribusiness Indian economy" (Ghosh et al., 2014). "Cattle rearing being supplementary to agriculture is part of social and cultural heritage of Indian civilization. Cattle rearing is one of the main source of income for small, marginal and landless farmers, whose majority live below the poverty index" (Gandhi et al., 2015). "Ecto-parasites such as ticks and mites transmit different pathogens, which lead to a number of threatening diseases" (Aslam et al., 2015). "Ticks are obligate blood feeding ecto-parasites. The most emerging infectious diseases arise from zoonotic pathogens, and majority of them are transmitted by ticks. Ticks are among the most competent and versatile vectors of pathogens and are second to mosquitoes as vectors of a number of human pathogens, like viruses, bacteria, rickettsia, spirochetes, etc, and the most important vector of pathogens affecting cattle worldwide" (Peter et al., 2005). "Globally the ticks are second to mosquitoes as vector of infectious pathogens to humans and animals. They transmit important haemoprotozoan diseases" (Jongejan and Uilenberg, 2004). "Ticks and tick borne diseases (TTBD) of cattle pose serious threats on the growth of dairy industry and cause a significant reduction in profit by severe loss in lactation" (McLeod and Kristjanson, 1999). "Cattle producers thus encounter a serious threat to manage TTBDs, largely due to the progressive evolution of resistance of ticks to almost every acaricides available in the market" (George et al., 2004). "Indirect effects are related to the transmission of tick borne diseases like babesiosis and anaplasmosis" (Sharma et al., 2012). "Single tick consumes a minimum of 30 drops of blood for completion of its life cycle and hence the most important adverse effects of tick infestation in animals are the anaemia and retardation of growth" (Mondal *et al.*, 2013). "In India, almost all the cattle population suffers from tick infestations and besides adverse effects on growth and production, tick infestation causes 20-30% reduction in the cost of leather due to tick bite marks" (Biswas, 2003). "The global loss due to ticks and tick borne diseases (TTBDs) was estimated to be between US$ 13.9 and 18.7 billion annually" (De Castro, 1997). "In India, the cost of controlling TTBDs has been estimated to be up to US$ 498.7 million per annum" (Minjauw and McLeod, 2003). "Tick-borne infectious diseases are growing steadily considering the establishment of the tick vector in urban areas, posing serious threat to the world health problem. Synthetic pyrethroids have been introduced in the 1970s (Graf, 2004) and are currently widely used". "They are less toxic to mammals and are highly biodegradable. Currently, tick control is more difficult due to the presence of resistant populations to major families of acaricides" (Fernandez-Salas et al., 2012). "The use of

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acaricides is the most common tick control method adopted by cattle owners in India. Indiscriminate application and use of sublethal concentrations of acaricides has probably contributed to the development of resistance in the ticks as resistance to the organophosphorous compound “diazinon” has recently been experimentally validated in Indian isolates of *Rhipicephalus (B.) microplus”* (Kumar et al., 2011). “Resistance has evolved to the three classes of acaricides used most extensively in the continent, namely fourth-generation synthetic pyrethroids (SP), organophosphates (OP) and amidines (AM), in virtually all countries in which they have been deployed across the globe” (Githaka et al., 2022). “The development of acaricide resistance in ticks infesting cattle is a major problem in the livestock industry in tropical and subtropical regions worldwide” (Dzemoet et al., 2022). “The synthetic pyrethroids, deltamethrin and cypermethrin, are commercially available in India and at present are two predominant acaricides used for tick control in the country. In addition, continued use of acaricides for long periods exerts selective pressure on the ticks resulting in the development of resistance” (Jonsson et al., 2000). Periodic monitoring of the ticks for development of resistance against commonly used acaricides in the respective region is essential for appropriate recommendation effective tick and tick borne disease control measures.

2. Material and methods

The present study was conducted from the month of May, 2018 to November, 2018 (7 month) in three villages (Vallabhnagar, Ranchorpura and Kikawas) of **Udaipur District (Rajasthan)**.

2.1 Acaricides

The commercial acaricides used in this study, 1.25%Butox® (Deltamethrin) and 10% w/v DERMEEZ® (Cypermethrin) were purchased from Udaipur. Their application was based on previous recommendations recommended (FAO, 2004).

2.2 Collection, Transportation and Laboratory handling of ticks

The fully engorged female cattle ticks were collected randomly from three villages (Vallabhnagar, Ranchorpura and Kikawas) of **Udaipur District (Rajasthan)** in the morning hours into small boxes with a few small holes allowing air to circulate. They were taken to the

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Parasitology laboratory in the Department of Veterinary Parasitology, Navania, Vallabhnagar, Udaipur College, where they were subjected to mounting and morphological identification. Engorged female cattle ticks were subjected to AIT and larval hatching for LPT. The engorged female ticks collected from a particular area were labelled and kept individually in labelled glass tubes covered with muslin cloth and kept in desiccators maintained at room temperature and 85±5% relative humidity (RH) for oviposition. The eggs were collected after 7 days from commencement of incubation. Each tube containing the first week egg production was labelled to ensure the selection of more uniform batch of larvae for each LPT. The eggs laid were allowed to hatch under uniform conditions of incubation and 14-21days old unfed larvae were utilized for the performance of Larval Packet Test (LPT) for detection of resistance status against deltamethrin and cypermethrin acaricides.

2.3 Adult Immersion Test (AIT)

The Adult Immersion Test with a Discriminating Dose (AIT-DD) was conducted as per the protocol described by FAO (2004). The following procedure was adopted for AIT-DD. The ticks were immersed in 20 ml recommended concentration (0.075 g L^{-1}) of Deltamethrin and Cypermethrin (0.05 g L^{-1}) for 30 minutes at about 27°C and containers were gently agitated during the period. The control group for deltamethrin and cypermethrin was immersed in 20 ml of distilled water. After 30 minutes, the acaricide solution was poured off into a safe storage container and the ticks were gently dried on a clean filter paper. The ticks from each container were pasted with ventral side up onto double-sided sticky tape on a glass plate. The glass plates with pasted ticks were incubated in white enamel tray at room temperature (25 - 30°C) for 7 days. During this period, the trays were provided with wet muslin cloth to maintain humidity. After 7 days, the ticks from treatment and control groups were observed and the number of ticks that have laid eggs were counted and recorded. (Plate. 1)

Ticks that were treated with acaricide but still laid eggs were considered as resistant, while ticks that were treated with acaricide and did not lay eggs were considered susceptible. The percentage resistance was calculated as:

$$\text{Resistance (\%)} = \frac{Nt}{Nw} \times 100 \qquad (1)$$

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Where, *Nt*= number of treated ticks laying eggs

Nw= number of untreated ticks laying eggs

2.4 Larval Packet Test (LPT)

The LPT was conducted according to the guidelines of FAO, (2004) with minor modifications. A control and test packets to be used in the procedures were prepared with Whatmann no. 1 filter paper, each having size of 7.0×7.0 cm. The papers were impregnated with recommended sublethal concentrations of deltamethrin (0.06%) and cypermethrin (0.2%) and test doses of deltamethrin (0.09%) and cypermethrin (0.3%). The working solution of acaricides was dried by keeping the filter paper for 30 min in incubator at 37ºC to allow trichloroethylene to evaporate. The filter papers and the fabric were then folded in half and the sides were sealed with adhesive tape, forming an open ended square packet to place tick larvae. The control packets were impregnated with solutions of trichloroethylene (2 parts) and olive oil (1 part) and dried by keeping the filter paper for 30 min in incubator at 37ºC to allow trichloroethylene to evaporate. After preparation of packets, all the packets were handled with forceps for further procedure. 100 larvae were placed into each packet with fine brush and the top open end of each packet was sealed with white adhesive tape. The closed packets were laid on a white enamel tray before its placement in the incubator. The use of a white tray enables observation of accidentally fallen larvae and subsequently trap them on adhesive tape. The treatment and control packets were then placed in an incubator at 27˚C ± 2˚C temperature with relative humidity (RH) of 85 ±5 %. For each acaricide, the test was conducted in duplicate. The packets impregnated with deltamethrin and cypermethrin were removed after 24 hours, the live and dead larvae counts and larval mortality was calculated.

Detailed counting was not done if the larvae in a packet were all dead and, such packets were automatically considered as having 100 % mortality. If counting reveals mortality to be very low less than 5 % then the direct mortality figures can be utilized. If they are found to be low (5-10 %) in the control, then percentage mortality in all the treated larval packets will have to be corrected by applying Abbott's formula.

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$$\text{Corrected percent mortality} = \frac{\text{Percent Test Mortality} - \text{Percent Control Mortality}}{100 - \text{percent control Mortality}} \times 100$$

2.5 Statistical Analysis

The results of AIT and LPT were analysed using to complete randomized design (CRD).

3. Results and Discussion

3. 1 Deltamethrin and Cypermethrin resistance status

The results of AIT for deltamethrin for Vallabhnagar, Ranchorpura and Kikawas villages was 50%, 45% and 40% and for cypermethrin the resistance was 15%, 10% and 10% for Vallabhnagar, Ranchorpura and Kikawas villages respectively. The AIT resistance of ticks against both durgs was found to be significant ($p<0.05$). Singh *et al.*, (2010) conducted AIT as per FAO (2004) for deltamethrin and cypermethrin drug using commercial formulations against *R. (B.) microplus* in Punjab where they found high frequency of resistance against deltamethrin (96.67%) than cypermethrin (93.33). Mathivathani et al., (2011) also reported 64.72% resistance to deltamethrin in *R. sanguineus* ticks in Chennai by AIT. Qassim et al. (2022) conducted "study on the resistance level of Rhipiciphalus microplus against trichlorfon and deltamethrin. The probit analysis of egg hatch assay showed the highest hatching percentage in zone 1 on both dilutions (67-76% on two-fold and 68-88% on ten-fold dilution) while lethal concentration (LC95) was found to be 2.187 ppm and discriminating dose (DD) as 4.374 ppm for trichlorfon".

Table 1 : Acaricidal resistance of ticks after employing AIT

Acaricides	Village	Acaricide concentration		Number of Females-Acaricide^{-1} Replicate^{-1}	Number of Females oviposited		Percent Mean Resistance
					Ist Replicate	IInd Replicate	
Deltamethrin	Vallabhnagar	Tr.	0.075gm L^{-1}	10	04	06	50
		Co.	-	10	10	10	-
	Ranchorpura	Tr.	0.075gm L^{-1}	10	05	04	45
		Co.	-	10	10	10	-
	Kikawas	Tr.	0.075gm L^{-1}	10	05	03	40
		Co.	-	10	10	10	-
Cypermethrin	Vallabhnagar	Tr.	0.05gm L^{-1}	10	02	01	15
		Co.	-	10	10	10	-
	Ranchorpura	Tr.	0.05gm L^{-1}	10	00	02	10
		Co.	-	10	10	10	-

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	Kikawas	Tr.	0.05gm L^{-1}	10	01	01	10
		Co.	-	10	10	10	-

3.2 Acaricidal resistance of ticks after employing LPT

The results of LPT revealed that highest resistance in Vallabhnagar (27.5%), followed by Ranchorpura (21%) and Kikawas (19%) for deltamethrin. For cypermethrin 19%, 11.5% and 9.5% resistance were observed in Vallabhnagar, Ranchorpura and Kikawas, respectively. The resistance of ticks against deltamethrin and cypermethrin was found to be significant ($p<0.05$). Rodriguez et al., (2006) who reported 61.2% resistance to deltamethrin and 59.2% resistance to cypermethrin in Mexico. Mendes et al., (2011) reported 86.36% to deltamethrin and 82.6% resistance to cypermethrin in *Rhipicephalus (Boophilus) microplus* ticks from small farms of the State of Sao Paulo, Brazil. Sousa et al., (2022) in a similar study found tick mortality of 2.5%, 7.5%, and 0% for Amitraz, Cypermethrin, and Deltamethrin, respectively. The percentage of larval hatching was 53.7% for Amitraz, 88.7% for Cypermethrin, and 80.0% for Deltamethrin.

Table 2 : Acaricidal resistance of ticks after employing LPT

Acaricides	Village	Acaricide concent-ration		Number of larvae $packet^{-1}$	Larval Mortality count		Percent Mean Mortality	Percent Mean Resistance
					I st Replicate	II nd Replicate		
Deltamethrin	Vallabhnagar	Tr.	0.06%	100	75	70	72.5	27.5
		Co.	-	100	00	00	00	-
	Ranchorpura	Tr.	0.06%	100	78	80	79	21
		Co.	-	100	00	00	00	-
	Kikawas	Tr.	0.06%	100	80	82	81	19
		Co.	-	100	00	00	00	-
Cypermethrin	Vallabhnagar	Tr.	0.2%	100	80	82	81	19
		Co.	-	100	00	00	00	-
	Ranchorpura	Tr.	0.2%	100	87	90	88.5	11.5
		Co.	-	100	00	00	00	-
	Kikawas	Tr.	0.2%	100	90	91	90.5	9.5
		Co.	-	100	00	00	00	-

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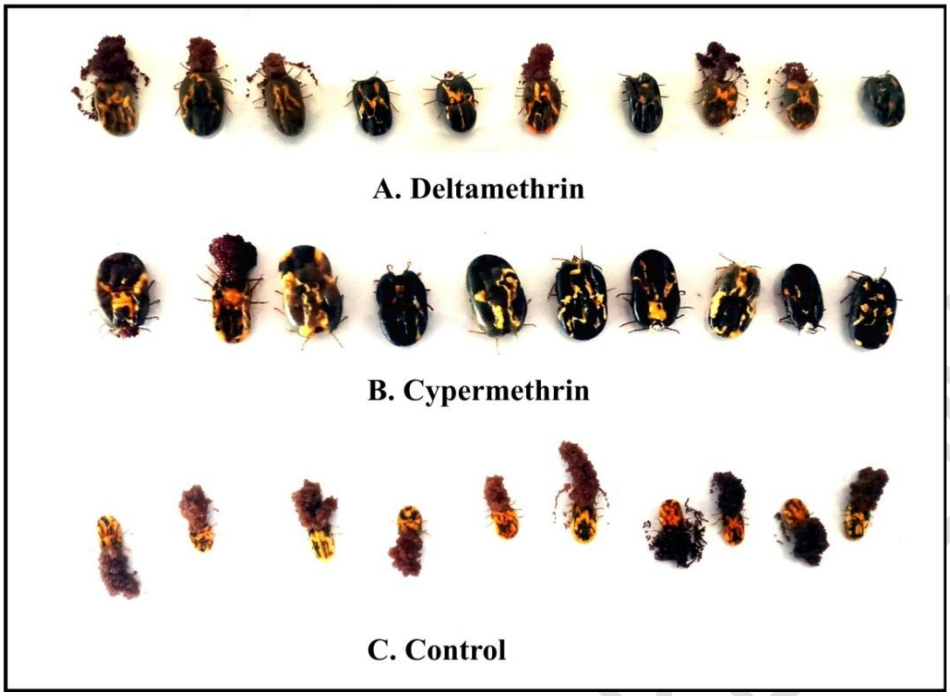

PLATE 1: Resistance status of ticks by Adult Immersion test (AIT)

Conclusion

Adult Immersion Test (AIT) and Larval Packet Test (LPT) of the present study showed similar trends of resistance for deltamethrin and cypermethrin acaricides. Synthetic pyrethroids are also easily available commercial acaricides and their application methods are easy which leads to indiscriminate use of these products. Probably unscientific and casual approach towards use of these acaricides, application of sub lethal doses and sequential use of the same chemical group for long periods has favoured the development of resistance.

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