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ORIGINAL ARTICLE

Detection of acaricidal resistance in *Hyalomma anatolicum anatolicum* from Banaskantha district, Gujarat

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Abstract The resistance status in *Hyalomma anatolicum anatolicum* collected from Banaskantha district, Gujarat (India) was estimated by larval packet test (LPT) with different concentrations of amitraz (125, 250, 500, 750 and 1,000 ppm) and cypermethrin (100, 200, 300, 400 and 500 ppm). The regression graphs of mean mortality of larvae ticks were plotted against values of progressively increasing concentrations of amitraz and cypermethrin for the estimation of LC_{95} values and were determined as 1,529.39 and 351.84 ppm, respectively. Further a resistance of level I was determined against cypermethrin whereas, a comparatively higher resistance level (II) was recorded against amitraz. The current study appears to be the pioneer report of amitraz and cypermethrin resistance in *H. a. anatolicum* from the Gujarat state, India.

Keywords Amitraz · Cypermethrin · *Hyalomma anatolicum anatolicum* · Resistance

Introduction

Hyalomma anatolicum anatolicum is a widely distributed multi host tick infesting cattle, buffaloes, sheep and goats responsible for transmitting *Theileria annulata*, *T. buffeli* and *T. lestocardi* (*T. hirci*) in India (Ghosh et al. 2008). Apart from its vectorial potential, the direct effect of this tick on livestock production leads to a significant economic losses. A recent study shows that the cost of management of ticks and tick borne diseases (TTBDs) in livestock of India is as high as US$ 498.7 million per annum (Minjauw and McLeod 2003). The direct application of acaricides on host animals is the most widely used method for the control of the ticks in the country. In this regard, the commonly available acaricides like synthetic pyrethroids (cypermethrin and deltamethrin) and formamidines (amitraz) are being used indiscriminately for control of ticks by the livestock owners. Further, the application of the acaricides is poorly supervised, resulting in economic problems produced by phenomenon such as acaricide resistance (Rodriguez Vivas et al. 2007). Although, there is a lot of information available on acaricidal resistance in one host cattle tick *Rhipicephalus (Boophilus) microplus* (Singh et al. 2010, 2012; Kumar et al. 2011, 2013; Sharma et al. 2012), limited studies had shown the presence of resistance in *H. a. anatolicum* in India (Sangwan et al. 1993; Shyma et al. 2012; Singh et al. 2013a) despite the fact that it is one of the major ticks infesting livestock of the country. Hence, the current study was undertaken to detect the development of resistance against commonly used acaricides viz. amitraz and cypermethrin in *H. a. anatolicum*.

Materials and methods

Study area

Fully engorged females ticks were collected in August, 2012 from cattle sheds at village Jethi, district Banaskantha, Gujarat in vials, closed with muslin cloth to allow air

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and moisture exchange and brought to the Entomology Laboratory, Department of Veterinary Parasitology, GADVASU, Ludhiana. After identification, the ticks were held individually at 28 ± 1 °C and 85 ± 5 % relative humidity in labeled plastic tubes for oviposition. The eggs laid were allowed to hatch to larvae under similar conditions of incubation.

Acaricides

Technical grade (100 % pure) amitraz and cypermethrin (AccuStandard® Inc. USA) were used for conducting larval packet test (LPT). The acaricides were dissolved in methanol for preparation of stock solutions and different concentrations of the amitraz and cypermethrin were prepared in distilled water from the stock solutions for testing against *H. a. anatolicum*.

Larval packet test (LPT)

The LPT was conducted according to FAO (1971) guidelines with minor modifications. Briefly, 0.4 ml of different concentrations of amitraz (125, 250, 500, 750 and 1,000 ppm) and cypermethrin (100, 200, 300, 400 and 500 ppm) in water were used to impregnate 7.0 × 7.0 cm filter paper (541 Whatman), dried and then folded diagonally and sealed on one side with adhesive tapes, forming an open-ended triangular packet to place approximately 100 larvae. Then, the open end of each packet was sealed and placed in a desiccator in BOD incubator maintained at 28 ± 1 °C and 85 ± 5 % R.

H. for 24 h. For each concentration of acaricide the test was conducted in quadruplet and in control group distilled water was used. The larval mortality was calculated by counting the live and dead larvae from the each packet.

Estimation of resistance status

LC_{95} values of amitraz and cypermethrin against *H. a. anatolicum* were calculated by regression curve of mortality plotted against values of acaricide concentrations (Finney 1962). Resistance factors (RF) were worked out by the quotient between LC_{95} of field isolates and LC_{95} of susceptible isolate. On the basis of RF, the resistance status of *H. a. anatolicum* was classified as susceptible (RF < 1.4), level I resistant (RF = 1.5–5.0), level II resistant (RF = 5.1–25.0), level III resistant (RF = 25.1–40) and level IV resistance (RF > 40.1) (Shyma et al. 2012).

Results and discussion

The ticks collected were identified as *H. a. anatolicum*. Data on the effects of different concentrations of various acaricides on the larvae of *H. a. anatolicum* are presented in Table 1. The mean mortality of larvae treated with various concentration of amitraz varied from 22.36 ± 3.78 to 70.33 ± 8.60 %. The slope of mortality was 0.04921 ± 0.005934 whereas, the value of goodness of fit (R^2) was 0.9582. From the regression equation, the LC_{95} value was calculated as 1,529.39 ppm with a resistance factor of 12.23 which indicated level II resistance status against amitraz. Reports of resistance against amitraz are available against *R. (B.) microplus* ticks from various parts of world (Jonsson et al. 2000; Ducornez et al. 2005) but published reports of amitraz resistance in *H. a. anatolicum* are currently not available. As regards Indian scenario, resistance against amitraz has been scarcely reported probably because the use of amitraz for tick control started late in near past after the development of resistance against organophosphates and synthetic

Table 1 Dose dependent response of *H. a. anatolicum* against various acaricides by LPT

Acaricide	Conc. (ppm)	No. of replicates	Mortality (%) (mean ± SE)	Slope, R^2, LC_{95}, RF
Amitraz	125	4	22.36 ± 3.78	Slope-0.04921 ± 0.005934
	250	4	37.77 ± 5.46	R^2 = 0.9582
	500	4	43.12 ± 5.99	LC_{95} = 1,529.39 ppm
	750	4	54.31 ± 1.46	RF = 12.23
	1,000	4	70.33 ± 8.60	
Cypermethrin	100	4	73.97 ± 3.44	Slope-0.06559 ± 0.01667
	200	4	86.47 ± 1.72	R^2 = 0.8377
	300	4	97.54 ± 1.05	LC_{95} = 351.84 ppm
	400	4	100 ± 0.0	RF = 3.51
	500	4	100 ± 0.0	
Control		4	2.77 ± 0.77	

RF resistance factor, R^2 goodness of fit pyrethroids acaricides. But now upon its indiscriminate and incessant use for past few years for the tick control the problem of resistance against amitraz is emerging and has also been recently reported in *R.* (*B.) microplus* ticks from Gujarat (Singh et al. 2013b).

The slope of mortality of *H. a. anatolicum* against increasing concentrations of cypermethrin was 0.06559 ± 0.01667 whereas; the value of R^2 was 0.8377. From the regression equation, the LC_{95} value was calculated as 351.84 ppm with a resistance factor of 3.51 which indicated level I resistance status. Almost negligible mortality (2.77 ± 0.77) was recorded in larvae of control group treated with distilled water. Recent reports indicate cypermethrin as one of the most extensively used acaricide in many parts of the country leading to development of resistance level in *R. (B.) microplus* (Singh et al. 2010, 2012; Sharma et al. 2012; Kumar et al. 2013). The development of resistance leads to decrease in efficacy of the drug as reported by the end users from this part of the country thus compelling them to shift to other effective drug class particularly formamidines (amitraz) for control of ticks mainly due to its easy availability and on field cost effectiveness. This has led to the decrease in the use of cypermethrin in the field condition in this particular geographical area which probably may be the reason for the lesser resistance level encountered in the current study.

The standard bioassay recommended by the FAO for testing resistance to acaricides is the LPT originally described by Stone and Haydock (1962). In bioassays, technical grade amitraz and cypermethrin were selected over commercial formulation as commercial products are prepared with many proprietary ingredients and it is difficult to assess the responses due to active ingredients (Shaw 1966). Among the various acaricides used in India for the control of ticks in livestock, resistance has been reported against most of the acaricides in *R.* (*B.) microplus* (Chaudhary and Naithani 1964; Khan and Srivastava 1977; Basu and Haldar 1997; Singh et al. 2010, 2012, 2013b; Kumar et al. 2011, 2013; Sharma et al. 2012). However, reports of development of resistance in multi host tick *H. a. anatolicum* are limited (Sangwan et al. 1993; Shyma et al. 2012; Singh et al. 2013a). The incidence of acaricide resistance is higher in one host ticks because a much larger fraction of the total population of such species remains under chemical challenge at any one time than multi host ticks. In addition to it, a single generation of multi-host tick may extend over up to 3 years compared to 2–3 months in one host tick (Harley 1966).

The results revealed development of resistance in *H. a. anatolicum* to both acaricides in which resistance to amitraz was higher. Based on the data obtained on the emerging problem of resistance in *H. a. anatolicum*, an alert on good practices regarding judicious use of chemical acaricides for tick control is required to be recommended. This will further prevent the development of resistance and at the same time will decrease environmental pollution, thus also causing reduction in the residual effect of acaricides in the animal products like milk and meat.

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