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Comparative study of esterases in deltamethrin and diazinon resistant *Rhipicephalus microplus* and *Hyalomma anatolicum* ticks collected from the Trans-Gangetic plains of India

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Abstract A comparative analysis of esterases in susceptible and resistant ticks revealed six types of esterases (EST-1b, EST-2b, EST-3b, EST-4b, EST-5b and EST-6b) in *Rhipicephalus microplus* and four types (EST-1h, EST-2h, EST-3h, EST-4h) in *Hyalomma anatolicum* using α-naphthyl acetate substrate. Inhibition studies with eserine sulfate, p-chloromercuribenzoate, copper sulphate and phenylmethylsulfonyl fluoride revealed a marked variation in band intensity between susceptible and resistant ticks, with the latter being more intense. Qualitative expression of EST-4b along with an extra band of EST-5b and EST-6b were indicative of deltamethrin and diazinon resistance in *R. microplus*, whereas qualitative expression of EST-4h was probably responsible for diazinon resistance in *H. anatolicum*. The data suggest that increased esterase activity may represent a detoxification strategy leading to the development of resistance in these tick populations.

Keywords *Rhipicephalus microplus* · *Hyalomma anatolicum* · N-PAGE · Esterase and Resistance

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Introduction

Ticks and tick-borne diseases (TTBD) of cattle pose serious threats on growth of the dairy industry (FAO 1996) and cause a significant reduction in profit by severe loss in lactation (McLeod and Kristjanson 1999). Ticks are widely distributed in most regions of the world particularly in tropical and subtropical regions and are classified into two major families, the Ixodidae, or hard ticks, and the Argasidae, or soft ticks (Simlimger 2005). In India, the control cost of TTBDS in the dairy sector has been estimated in the tune of $498.7 million per annum (Minjauw and McLeod 2003). Ticks cause severe economic losses through anorexia, toxicosis, decreased milk yield, decreased meat production, damaged hides and by transmission of disease-causing agents, babesiosis and anaplasmosis (Rodríguez-Vivas et al. 2004; Ghosh et al. 2007).

Acaricides such as synthetic pyrethroids (SPs) and organophosphates (OPs) are currently the most used method of tick control but the prolonged, incorrect and indiscriminate use of these compounds over the years has led to the selection of resistant ticks (FAO 2004). Resistance in ticks can be attributed to several mechanisms, including reduction in pesticide absorption, enhanced metabolic detoxification by esterases, oxidases and glutathione S-transferases and also pesticide target insensitivity (He et al. 2002; Vatsya and Yadav 2011; Kumar et al. 2013). Among the esterases, carboxylesterases (CaEs) and acetylcholinesterases (AChEs) have been associated with pesticide resistance in ticks.

Hyalomma anatolicum is a widely prevalent tick of livestock in India which transmits theileriosis. Development of resistance to drugs is an evolutionary adaptation that puts at risk every parasiticidal agent (Shoop 1993). Large-scale development of resistance to the OP diazinon has been reported in 20 Indian isolates of the one-host cattle tick *Rhipicephalus microplus* collected from six agro-climatic regions (Kumar et al. 2011) and SPs (Sharma et al. 2012), and moderate resistance in *H. anatolicum* to both OPs and SPs (Shyma et al. 2012). A high resistance level to the SP deltamethrin is also recorded in the Indian isolates of *R. microplus* (Sharma et al. 2012). Shyma et al. (2012) studied the acaricide resistance status in Indian isolates of *H. anatolicum* to deltamethrin, cypermethrin and diazinon in 20 areas located in three agro-climatic regions. Efficacy of market-available formulations of two SPs, namely cypermethrin and fenvalerate, was estimated against *R. microplus* (Abdullah et al., 2013). Ravindran et al. (2014) compare the in vitro acaricidal effects of commercial preparations of cypermethrin and fenvalerate against *Rhipicephalus annulatus.* A comparative study on cypermethrin resistance in *R. microplus* and *H. anatolicum* from Punjab (India) was done to evaluate cypermethrin resistance in these ticks collected from Muktsar and Mansa districts of Punjab state, by using an adult immersion test (AIT) (Singh et al. 2014a, b). Baxter and Barker (2002) have demonstrated the relationship between the resistance to OPs and enhanced AChEs activity in Australian *R. microplus* and Jamroz et al. (2000) have shown increased CaE activity in a resistant pyrethroid Mexican strain of *R. microplus*. Carboxylesterase and AChEs gene point mutations have also been detected in these ticks (Hernandez et al. 2000, 2002).

The α and β esterases have been extensively studied in insects and are known to be involved in different physiological processes, such as regulation of juvenile hormone levels (Kort and Granger 1981), reproduction (Richmond et al. 1980), functioning of the nervous system and development of resistance to insecticides (Guillemaud et al. 1997). Among the esterases, CaEs and AChEs have been associated with pesticide resistance in ticks. In *R. microplus*, the resistance mechanisms associated with esterase detoxification have been investigated by several authors. AChEs and CaEs are closely related enzymes and ubiquitous among ticks. Both the enzymes specialize in hydrolyzing ester bonds. Both AChEs

and CaEs work simultaneously on OP and SP acaricide resistance in ticks. The tick esterases also play important roles in host immunomodulation against tick antigens, tick mediated transmission and establishment of pathogens to the host (Temeyer and Tuckow 2016). So, esterase profile in acaricide resistant and susceptible ticks will aid to support the chemical basis of acaricide resistance.

Knowledge of the underlying mechanism of resistance in ticks to acaricides would lead to the development of more rapid and accurate diagnostic tools for early detection of resistance which are essential for planning long-term effective control measures. The emergence of tick populations resistant to acaricides used for their control continues to pose the most serious threat to stable chemical tick control strategies. Hence it is essential to understand resistance mechanism. Therefore, in the present study, esterase enzyme patterns are compared between acaricide susceptible or resistant adult females of *H. anatolicum* and *R. microplus* against a synthetic pyrethroid (deltamethrin) and an organophosphate (diazinon) seeking to identify differences in enzyme activity that might reflect acaricide resistance mechanisms associated with hydrolytic esterase activity.

Materials and methods

Collection of tick sample

Dropped down fully engorged females of *R. microplus* and *H. anatolicum* were collected from farms, villages and gaushalas around Hisar and brought to the laboratory and identified (Miranpuri 1979) for esterase profiling. Freshly dropped engorged ticks were set aside for adult immersion tests (AIT). On the base of this AIT we categorised our ticks in resistance and susceptible groups which were further used in esterase profiling. For AIT, the ticks were then placed individually in cleaned, labelled and separate vials, closed with muslin cloth to allow air and moisture exchange, placed in a desiccator kept at 10 °C in an incubator and 85 ± 5% RH maintained by KOH (10 g/100 ml) till the experiment was conducted.

Adult immersion test (AIT)

Adult immersion test was conducted according to the methods of Drummond et al. (1973) and Benavides et al. (1999) with minor modifications. Different concentrations of working solutions of acaricides were prepared from stock solution in distilled water to conduct the dose–response bioassay. The pre-weighed (90–120 mg in case of *R. microplus* and 270–330 mg in case of *H. anatolicum*) engorged female ticks ($3 \times 5 = 15$) were immersed in different concentrations of acaricides for 2 min and then dried on filter paper placed in the Petri dishes. Within 24 h individual ticks were transferred to the glass tubes covered with muslin cloth and were kept in desiccator kept in incubator maintained at 28 ± 1 °C and 85 ± 5% RH. The ticks which did not oviposit after 14 (*R. microplus*) or 20 (*H. anatolicum*) days were considered as dead. The control group were treated in similar manner in distilled water. The following parameters are compared:

- Mortality: recorded up to 14 or 20 days post treatment (dpt)
- Egg masses laid by the live ticks
- Reproductive Index (RI) = egg mass weight (EW)/engorged female weight (EFW)
- Inhibition of oviposition (IO%) = (RI control − RI treated/RI control) × 100.

Test acaricides

Technical grade 100% pure deltamethrin and diazinon (AccuStandard, USA) were used to to prepare the stock solutions of 5000 ppm in acetone and 50,000 ppm in methanol, respectively. For esterase enzyme pattern eight groups (A–H; Table 1) were made from previously used batches of *R. microplus* and *H. anatolicum*, which were experimentally proved to be susceptible or resistant (to deltamethrin and diazinon) by conducting an AIT.

Statistical analysis

Dose–response data were analyzed by probit method (Finney, 1962) using GraphPad Prism 4 software. The LC_{50} and LC_{95} values of deltamethrin and diazinon were determined by applying regression equation analysis to the probit transformed data of mortality. Resistance factors (RF) for field isolates were worked out by the quiescent between LC_{50} of field isolates and LC_{50} of IVRI-I strain of *R. microplus* (Castro-Janer et al. 2009). On the basis of RF, the resistance status in the field population of *R. microplus* was classified as susceptible ($RF < 1.4$) or resistant at level I ($1.5 < RF < 10.0$), level II ($10.1 < RF < 25.0$), level III ($26 < RF < 40$) or level IV ($RF > 41$) (Kumar et al. 2011; Sharma et al. 2012).

Extract preparation for esterase assay

For esterase pattern analysis, five ticks ($\approx$120 mg each) from each group were used and individually analysed. A longitudinal incision was made in the abdomen of each tick and the excess blood was removed with distilled water. Then the ticks were homogenized under ice using tissue homogenizer (IKA T10 basic Ultra-Turrox) in buffer containing 10 mM of sodium phosphate, pH 6.5 (10% w/v). Then the homogenate was centrifuged at 10,000 rpm for 15 min at 4 °C. Then the supernatant was filtered with 0.22 µm syringe filter (MILLEX-GV). The filtrate was stored at −40 °C with protease inhibitor cocktail (Sigma P, 2714) and used for esterase profiling.

Table 1 Groups of *Rhipicephalus microplus* and *Hyalomma anatolicum* ticks used for esterase enzyme pattern analysis

Tick group		Resistance status	
		Resistance factor (RF)	Resistance level (RL)
A	Deltamethrin susceptible *R. microplus*	1.02	I
B	Deltamethrin resistant *H. anatolicum*	1.8	I
C	Diazinon susceptible *R. microplus*	0.88	S
D	Diazinon resistant *H. anatolicum*	2.46	I
E	Deltamethrin resistant *R. microplus*	3.5	I
F	Deltamethrin susceptible *H. anatolicum*	1.0	S
G	Diazinon resistant *R. microplus*	2.38	I
H	Diazinon susceptible *H. anatolicum*	0.64	S

Estimation of protein concentrations

Extracted protein from tick homogenates was quantified at 595 nm using the Bradford (1976) method and the average protein concentration of each homogenate determined from a standard curve using bovine serum albumin (BSA, Himedia) as the standard protein.

Native PAGE for esterase profiling

The extracts from whole ticks were used for esterase profiling. Non-denaturing polyacrylamide gel electrophoresis (PAGE) was performed using the buffer system without sodium dodecyl sulfate (SDS) and a gel system consisting of a 4% (w/v) stacking gel and a 10% (w/v) separating gel. Equal amounts (50 µg) of extracted protein per sample were loaded in each well and electrophoresis was conducted at a constant voltage of 90 V for 4 h at 4 °C in pre-chilled buffer containing 87 mM Tris and 13 mM glycine (pH 8.3).

Substrate staining with α-naphthyl acetate

Gels were stained by pre-incubation in 100 mM sodium phosphate buffer (pH 6.5) for 30 min at 37 °C, followed by incubation in 100 mM phosphate buffer containing 3.2 mM α-naphthyl acetate and 2.4 mM Fast Blue R/R Salt for 60 min in the dark at 37 °C. In the presence of α-naphthyl acetate substrates, gels stain black, indicating preferential hydrolysis by α-naphthyl acetate. The α-naphthyl acetate stock solution was prepared in 1 ml of acetone to aid their solubility in the phosphate buffer.

Inhibition assay

Inhibition tests (Baffi et al. 2005) for the biochemical characterization of esterases, 1.0 mM copper sulfate, 1.0 mM p-chloromercuribenzoate (pCMB), 1.0 mM eserine sulfate and 1.0 mM phenylmethylsulfonyl fluoride (PMSF) were used. Gels were pre-incubated for 30 min in the dark in phosphate buffer (100 mM, pH 6.5) containing inhibitor and then stained for esterase activity in the presence of inhibitor. PMSF was dissolved in 1 ml methanol and pCMB was dissolved in 1:1 mixture of ethanol and water prior to use. Eserine sulfate and copper sulfate were added directly to the pre-soaking and staining solution. Eserine sulfate was used to determine activity attributed to AChEs. PMSF is an inhibitor of serine esterases. Copper sulfate and pCMB were used as arylesterase, i.e. hydrolase, inhibitor.

Results

Esterase profile pattern

Esterase activity in susceptible and resistant *R. microplus* and *H. anatolicum* against α-naphthyl acetate dubbed as EST-1b to EST-6b and EST-1h to EST-4h, respectively, is shown in Fig. 1, according to their migration from the anode and further explained in Table 2.

In *R. microplus* EST-1b, EST-2b, EST-3b and EST-4b were found in deltamethrin susceptible and resistant groups. Among them the expression of EST-4b was more intense

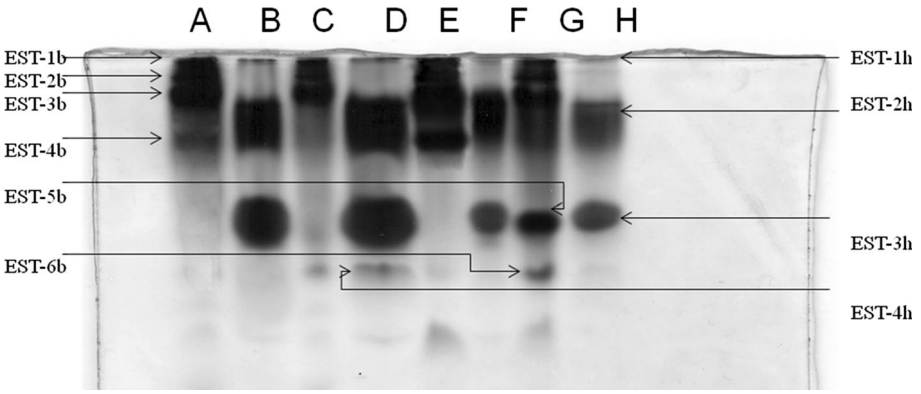

Fig. 1 Pattern of esterases in extracts of *Rhipicephalus microplus* and *Hyalomma anatolicum* ticks susceptible and resistant to diazinon and deltamethrin. Protein extracts were separated on 10% (w/v) polyacrylamide gel and stained in the presence of α-naphthyl acetate. *Lanes A–H* see Table 1

Table 2 Esterase activity in susceptible and resistant *Rhipicephalus microplus* and *Hyalomma anatolicum* ticks to deltamethrin and diazinon in presence of α-naphthyl acetate

	Esterase	Deltamethrin		Diazinon	
		Susceptible	Resistant	Susceptible	Resistant
R. microplus	EST-1b	√	√√	√	√√
	EST-2b	√	√√	√	√√
	EST-3b	√	√√	√	√√
	EST-4b	√	√√	N	N
	EST-5b	N	N	N	√√
	EST-6b	N	N	N	√√
H. anatolicum	EST-1h	N	√	N	√
	EST-2h	√	√√	√	√√
	EST-3h	√	√√	√	√√
	EST-4h	N	N	N	√√

√ moderate activity, √√ more intense activity, N not detected

in the deltamethrin resistant (Fig. 1, column E) than in the deltamethrin susceptible (Fig. 1, column A) group. Similarly EST-1b, EST-2b and EST-3b were found to be present in diazinon susceptible (Fig. 1, column C) and resistant (Fig. 1, column G) ticks. EST-5b and EST-6b was exclusively expressed in diazinon resistant (Fig. 1, column G) ticks.

In all groups of *H. anatolicum* EST-1h, EST-2h, EST-3h were found to be present: in deltamethrin susceptible (Fig. 1, column F) and resistant (Fig. 1, column B) and in diazinon susceptible (Fig. 1, column H) and resistant (Fig. 1, column D), but expression was more intense in both resistant groups. In the diazinon resistant group expression of EST-4h was slight (Fig. 1, column D).

Inhibitor effects on esterase activity

Inhibition was performed by using inhibitors—copper sulphate, p-chloromercuribenzoate (pCMB), eserine sulphate, phenylmethylsulfonylfluoride (PMSF)—against susceptible and resistant *R. microplus* and *H. anatolicum*. The inhibitor effects are shown in Figs. 2, 3, 4, 5 and further explained in Table 3 (eserine sulphate) and Table 4 (PMSF).

When different inhibitors were used for *R. microplus* it was found that EST-4b, EST-5b and EST-6b were strongly inhibited by eserine sulphate and PMSF, whereas pCMB and copper sulphate do not show any inhibition. Our inhibition assays shows that PMSF almost totally inhibited all the bands. PMSF is a serine-hydrolase inhibitor, suggesting that these enzymes contain serine residues in their active sites. These observations suggest that EST-4b (Fig. 1, column E), EST-5b (Fig. 1, column G) and EST-6b (Fig. 1, column G) may be an AChE, as this enzyme was totally inhibited by PMSF (Fig. 4) and strongly inhibited by eserine sulphate (Fig. 2). Other esterases, viz. EST-1b, EST-2b, and EST-3b of the deltamethrin susceptible and resistant groups, were totally inhibited by PMSF (Fig. 4), not by eserine sulphate (Fig. 2), and observations suggest that these may be CaEs containing an active serine. None of the esterases are inhibited by pCMB (Fig. 5) and copper sulphate (Fig. 3) which means no arylesterases are present in any group of *R. microplus*.

When different inhibitors were used for *H. anatolicum* it was found that EST-4h was strongly inhibited by eserine sulphate (Fig. 2) and totally by PMSF (Fig. 4). Hence, EST-4 h is said to be an AChE whose qualitative expression was responsible for diazinon resistance in *H. anatolicum* (Fig. 1, column D). Other esterases, viz. EST-1h, EST-2h and EST-3h of deltamethrin susceptible and resistant groups, were totally inhibited by PMSF and not by eserine sulphate; observations suggest that these may be CaE containing an active serine. None of the esterases are inhibited by pCMB (Fig. 5) and copper sulphate (Fig. 3) which means no arylesterases are present in any group of *H. anatolicum.*

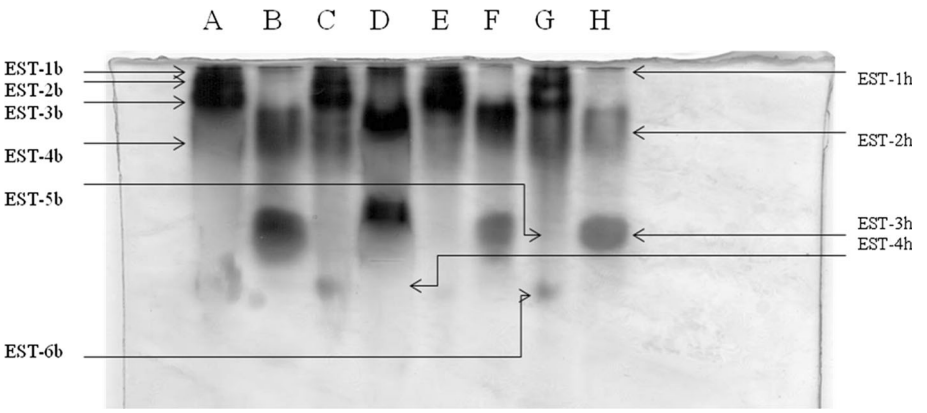

Fig. 2 Effect of eserine sulphate inhibitor on esterase pattern in extracts of *Rhipicephalus microplus* and *Hyalomma anatolicum* ticks susceptible and resistant to diazinon and deltamethrin. Protein extracts were separated on 10% (w/v) polyacrylamide gel and stained in the presence of α-naphthyl acetate. *Lanes A–H* see Table 1

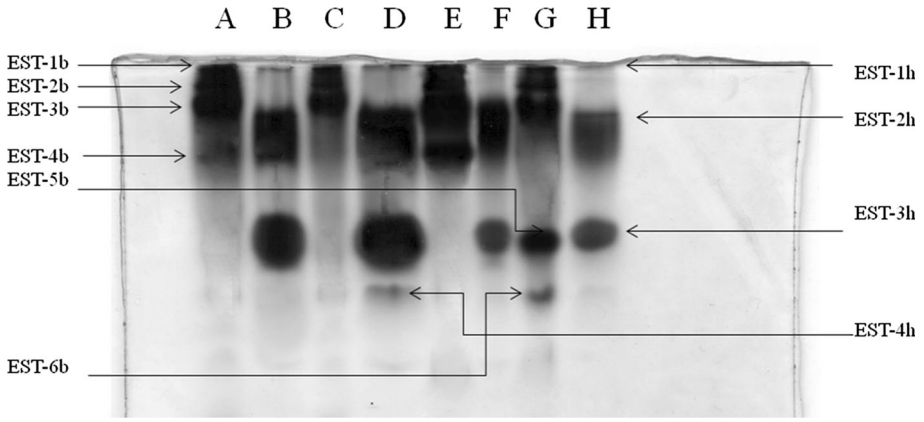

Fig. 3 Effect of copper sulphate inhibitor on esterase pattern in extracts of *Rhipicephalus microplus* and *Hyalomma anatolicum* ticks susceptible and resistant to diazinon and deltamethrin. Protein extracts were separated on 10% (w/v) polyacrylamide gel and stained in the presence of α-naphthyl acetate. *Lanes A–H* see Table 1

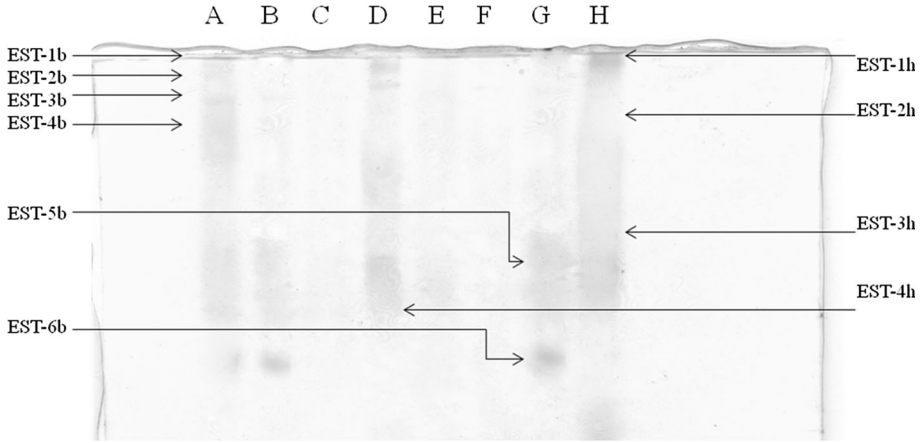

Fig. 4 Effects of phenylmethylsulfonylfluoride (PMSF) inhibitor on esterase pattern in extracts of *Rhipicephalus microplus* and *Hyalomma anatolicum* ticks susceptible and resistant to diazinon and deltamethrin. Protein extracts were separated on 10% (w/v) polyacrylamide gel and stained in the presence of α-naphthyl acetate. *Lanes A–H* see Table 1

Discussion

The *R. microplus* and *H. anatolicum* populations have immense potential for rapidly developing resistance due to their biological and behavioural characteristics and the level of resistance to various active ingredients as has been reported in almost all countries where these ticks occur (Alonso-Diaz et al. 2006). The overall goal of this research is to identify mechanisms involved in resistance to acaricides (specifically, deltamethrin and diazinon) and to develop rapid, accurate, and sensitive diagnostic methods for early detection and assessment of resistance status in tick populations so that an effective management strategy can be designed.

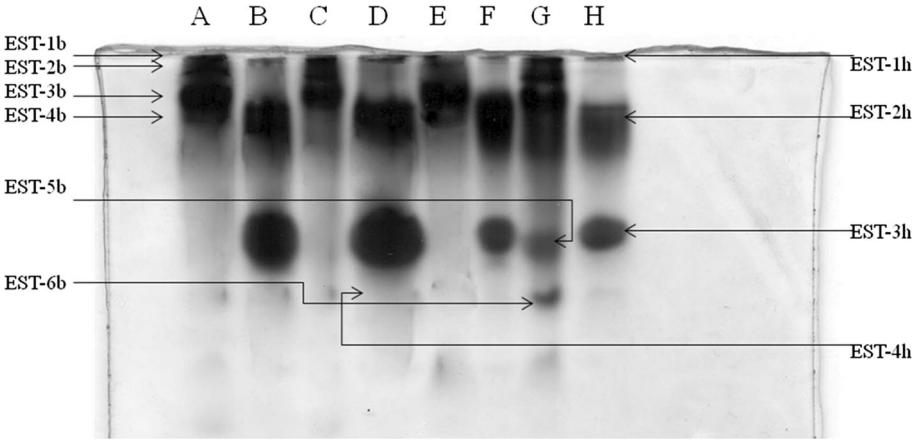

Fig. 5 Effects of p-chloromercuribenzoate (pCMB) inhibitor on esterase pattern in extracts of *Rhipicephalus microplus* and *Hyalomma anatolicum* ticks susceptible and resistant to diazinon and deltamethrin. Protein extracts were separated on 10% (w/v) polyacrylamide gel and stained in the presence of α-naphthyl acetate. *Lanes A–H* see Table 1

Table 3 Effect of eserine sulphate inhibitor on esterase activity in susceptible and resistant *Rhipicephalus microplus* and *Hyalomma anatolicum* ticks to deltamethrin and diazinon in the presence of α-naphthyl acetate

	Esterase	Deltamethrin		Diazinon	
		Susceptible	Resistant	Susceptible	Resistant
R. microplus	EST-1b	√	√√	√	√√
	EST-2b	√	√√	√	√√
	EST-3b	√	√√	√	√√
	EST-4b	+++	+++	N	N
	EST-5b	N	N	N	+++
	EST-6b	N	N	N	+++
H. anatolicum	EST-1h	N	√	N	√
	EST-2h	√	√√	√	√√
	EST-3h	√	√√	√	√√
	EST-4h	√	√√	N	+++

√ moderate activity, √√ more intense activity, N not detected, +++ serine sulphate inhibition

Abdullah et al. (2012) showed the esterase profile of *R. microplus* populations collected from Northern India exhibiting varied susceptibility to deltamethrin. Here, esterase profile of larval extracts using native PAGE, revealed five bands of esterase activity designated EST-5 to EST-1A. Inhibitory tests recognized EST-1, EST-2 and EST-3 as AChEs, and EST-4 and EST-5 as CaEs. The band intensity varied between tick populations of various locations, being more intense in case of the resistant populations. An extra band of esterase activity (EST-1A) was obtained in larval extracts of ticks from three locations. This

Table 4 Effect of phenylmethylsulfonylfluoride (PMSF) inhibitor on esterase activity in susceptible and resistant *Rhipicephalus microplus* and *Hyalomma anatolicum* ticks to deltamethrin and diazinon in the presence of α-naphthyl acetate

	Esterase	Deltamethrin		Diazinon	
		Susceptible	Resistant	Susceptible	Resistant
R. microplus	EST-1b	+++	+++	+++	+++
	EST-2b	+++	+++	+++	+++
	EST-3b	+++	+++	+++	+++
	EST-4b	+++	+++	N	N
	EST-5b	N	N	N	+++
	EST-6b	N	N	N	+++
H. anatolicum	EST-1h	N	+++	N	+++
	EST-2h	+++	+++	+++	+++
	EST-3h	+++	+++	+++	+++
	EST-4h	N	N	N	+++

$\surd$ moderate activity, $\surd\surd$ more intense activity, N not detected, +++ PMSF inhibition

increased esterase activity might be involved in the resistance development in these tick populations.

Present data differ from a study of a population of Mexican *B. microplus* larvae which detected 22 regions with α-naphthyl acetate esterase activity (Jamroz et al. 2000), but it should be remembered that in our study we used engorged adult females and because tick development is controlled by direct and indirect changes in the pattern of gene expression, esterase profiles can be very different at different stages in the life cycle. Carboxylesterases can serve a protective role for the target AChEs during OP intoxication because the CaEs are alternative phosphorylation sites (Watson and Chambers 1996). Villarino et al. (2003) purified three esterases from the integument of resistant adult Texan *B. microplus* and characterized the esterases as CaEs based on inhibitor effects and also identified the presence of different amounts of esterase activity in resistant and susceptible ticks. This result indicates that these enzymes could be present at a higher copy number in the resistant ticks, but this hypothesis can only be confirmed through a quantitative analysis. EST-4b, EST-5b and EST-6b were also strongly inhibited by serine sulphate and almost totally by PMSF, results which putatively indicate that they represent AChEs activity. However, it is unlikely that these enzymes represent distinct AChEs and they probably correspond to alternative forms of AChEs, as they were detected as double bands much closer to each other on the gels. Likewise, EST-4h was classified as AChE because it was strongly inhibited by eserine sulphate and PMSF. However, these bands may represent different gene products from the same allele which may have suffered glycosylation or some other kind of post-translational modification, producing esterase bands with a slightly different migration pattern. Esterases with molecular weights ranging from 66 to 68 kDa have been attributed to differential glycosylation and associated with insecticide resistance in the brown planthopper *Nilaparvata lugens* (Small and Hemingway 2000). Acaricide resistance is a multi-factorial phenomenon, thus other causes of increased resistance like sodium channel mutation and reduced drug penetration (e.g. cuticle thickening) and

behavioral changes (e.g. avoiding the pesticides) are to be tested in future in order to confirm the cause of resistance development in these acaricide-resistant tick populations.

Instant study detected EST-4b, EST-5b and EST-6b enzymes only in the resistant (deltamethrin and diazinon) *R. microplus* and EST-4h in diazinon-resistant *H. anatolicum.* This may indicate additional AChEs as an important defense mechanism used by ticks to develop deltamethrin and diazinon resistance. Acetylcholinesterase is the target enzyme for the pesticide attack and an altered AChEs level with enhanced metabolic detoxification was identified as the primary mechanism used by *B. microplus* to develop resistance to OP pesticides in Mexican strains (Hernandez et al. 1999). Acetylcholinesterase has been identified at synapses in the central nervous system of arthropods. This enzyme terminates nerve impulses by catalyzing the hydrolysis of the neurotransmitter acetylcholine at the synaptic junction after which the nerve impulse is transmitted to the axonium membrane where it stimulates the opening of the sodium channels. Pyrethroids normally act by keeping the sodium channels open and causing death by hyperexcitation.

Organophosphates and carbamates act as AChE inhibitors, and in the present experiment we detected an OP and pyrethroid resistance mechanism mediated by AChEs. It is possible that these AChEs compensate for the inhibitory effect of sodium channels. In addition, we used field ticks which were exposed to several types of acaricides, including pyrethroids and OPs over time. This could indicate that our resistant field ticks are resistant to other acaricides and that the AChEs are related to OP resistance or that there is another pyrethroid resistance mechanism undetected by us. Abbas et al. (2014), found that esterase activity is directly linked with acaricide resistance also.

Acaricide resistance is a dynamic process resulting from multiple mechanisms, including point mutations, gene amplification, duplication events, and post-translational modifications, which contribute toward the overexpression and enhanced activity of detoxifying enzymes, including CaEs and AChEs (Hemingway et al. 2002). This work is the first study describing esterase profile of *R. microplus* and *H. anatolicum* collected from various areas of Hisar and adjoining districts and results suggests that increased expression of AChEs have contributed toward the development of resistance to OPs and SPs in the ticks. Fewer esterase bands were expressed in *H. anatolicum* than in *R. microplus*. This indicates that these ticks were more susceptible to acaricides than *R. microplus* in the Indian condition. These data comprise information that may be useful for future management programs involving resistance to acaricides and their more effective utilization.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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