Haryana Vet. (Dec., 2019) **58(2),** 228-231

Research Article

DEVELOPMENT STAGE SPECIFIC AND SEX RELATED CHANGES IN ESTERASE PATTERN OF *HYALOMMA ANATOLICUM* AND *RHIPICEPHALUS* (*BOOPHILUS*) *MICROPLUS* TICKS OF HARYANA

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Received: 21.02.2019; Accepted: 10.07.2019

ABSTRACT

Species and sex specific esterases were characterized in *Hyalomma anatolicum* and *Rhipicephalus* (*Boophilus*) *microplus* ticks susceptible to deltamethrin and coumaphos. Native electrophoretogram revealed a total of 10 types of esterases, EST-1h to EST-10h in *H. anatolicum* and 9 types, EST-1b to EST-9b in *R.* (*B.*) *microplus* including adult, larvae and nymph. In *H. anatolicum* larvae, 5 types of esterases were found while in *H. anatolicum* male, 3 types of esterases were observed. Female *H. anatolicum* exhibited 9 types of esterases. EST-8h was found to be male specific while EST- 6h, EST-9h and EST-10h were female specific. In comparison, *R.* (*B.*) *microplus* males exhibited 7 types of esterases while in female, 6 types of esterases were present. EST-2b was present throughout their life cycle but EST-7b was present only in larvae, nymph and adult male. EST-5b was found to be nymph specific; EST-6b as adult female specific; EST-4b as adult male specific and EST-9b as adult male and female specific. Expression of esterases varied during different stages of life cycle of ticks and it also varies with species revealing the importance of exploiting these esterases for control of ticks.

Keywords: Esterases, *Hyalomma anatolicum*, *Rhipicephalus* (*Boophilus*) *microplus*

Esterases in ticks hydrolyse the ester bonds present in a wide range of acaricides (Montella *et al.*, 2012), used to control tick infestation. Organophosphates and carbamates acts through inhibiting the activity of these esterases. Over the years, several tick researchers working in the field of acaricide resistance have reported esterase as an important agent for development of acaricide resistance (Baffi *et al.*, 2008). Acetylcholine esterase (AChEs) and carboxylesterase (CaEs) were closely related enzymes and well reported from ticks by electrophoretic profiling followed by inhibitor studies (Abdullah *et al.*, 2012; Gupta *et al.*, 2016). It has also been reported that the pattern of esterases varies among species and among different stages of same species (Baffi *et al.*, 2007). Therefore, the present work was undertaken to compare esterase electrophoretogram during different stages of the ticks' life cycle as well as sex related variations in commonly available cattle ticks of Haryana i.e. *Hyalomma anatolicum* and *Rhipicephalus* (*Boophilus*) *microplus*.

MATERIALS AND METHODS

Collection of ticks: Fully engorged freshly dropped adult female ticks of *H. anatolicum* and *R.* (*B.*) *microplus* were collected from Gaushala located in Charkhi Dadri district of Haryana. Adult ticks were kept for laying at 28±1 °C and 75-85 % RH in a BOD incubator. Male ticks and nymphs were directly collected from body of farm animals. Identification of ticks was carried out using standard literature (Miranpuri and Gill, 1983).

Adult Immersion Test with discriminating dose (AIT-DD): AIT-DD was conducted according to the methods of FAO (2004) with minor modifications. Ten healthy engorged female ticks were immersed in freshly prepared acaricide concentration of 59.2 ppm for deltamethrin (Shyma *et al.*, 2013) and 262 ppm for coumaphos (Kumar *et al.*, 2015) for 30 minutes at RT with gentle shaking. After 30 minutes, the ticks were dried gently on filter paper, sticked with ventral side up onto double-sided sticky tape in a Petri dish and placed in desiccators (85 to 95% RH, 25 to 30 °C) for 7 days. The tests were performed in triplicate. After 7 days, number of ticks that have laid eggs were counted and the percentage resistance was calculated as under:

$$\text{Resistance (\%)} = (N_t / N_w) \times 100$$

Where, N_t = number of treated ticks laying eggs; N_w = number of untreated ticks laying eggs

Preparation of homogenate for esterase assay: For preparation of female ticks' extract, a longitudinal incision was made in the abdomen; excess blood was removed with distilled water. One hundred larvae of 10-14 days old were used for preparation of larval extracts. Approximately 40-50 nymphs of ticks were used for preparing the extract. Tissue samples were homogenized in 200 µl chilled 0.1M sodium phosphate buffer, pH 6.5, containing 20% sucrose under ice using tissue homogenizer (IKA T10 basic Ultra-Turrox). Then the homogenate was centrifuged at 15,000 × g for 15 min at 4° C and collected supernatant was filtered through 0.22 µm syringe filter (MILLEX-GV). The filtrates of various extracts were used for esterase profiling.

Estimation of protein concentrations: Total protein concentration in tick homogenates were estimated using

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the Bradford (1976) method by plotting a standard curve using bovine serum albumin.

Native PAGE for esterase profiling: Non-denaturing polyacrylamide gel electrophoresis (PAGE) was performed using a gel system consisting of 4% (w/v) stacking gel and 10% (w/v) separating gel. Samples having the same quantity of protein (40 µg) were loaded in each well and electrophoresis was run 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 of gel with α-naphthyl acetate: Native gels after run were stained by the method followed by Baffi *et al.* (2005) by pre-incubating 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. The bands showing esterase activity in the gel stained black, as esterase hydrolysed α-naphthyl acetate into α-naphthol which gave black colour after staining with Fast Blue R/R salt. It was followed by washing of gel twice and keeping it overnight submerged in clean water for clearing of the background. Next day recording of gel was done using gel documentation system.

RESULT AND DISCUSSION

Native PAGE analysis revealed a total of 10 bands showing esterase activity during the different developmental stages of *H. anatolicum* ticks' life cycle which were numbered as EST-1h to EST-10h while in *R.* (*B.*) *microplus*, 9 bands were detected numbered as EST-1b to EST-9b and are presented in Tables 1 and 2 and Fig. 1 and 2, respectively. Activities of two esterases namely EST-3h and EST-5h were found to be present in all the developmental stages as well as adult male and female of *H. anatolicum* ticks indicating their functional importance in the metabolism and metamorphosis during their life cycle as during development, metabolic needs get changed because of changes in the activity of certain genes. While in larvae of *H. anatolicum* ticks, 3 more bands namely EST-2h, EST-4h and EST-7h of esterase activity were detected. In adult male, esterase activity bands i.e. EST-2h, EST-4h and EST-7h disappeared while an additional activity band EST-8h appeared but, in case of adult female in addition to larval esterase activity, EST-1h, EST-6h, EST-9h and EST-10h appeared indicating expression of additional esterases in females as compared to males. EST-8h was to be male specific while EST- 6h, EST-9h and EST-10h were female specific.

The pattern of esterase expression of susceptible *R.* (*B.*) *microplus* ticks was entirely different in larvae, nymph, adult male and adult female. The data related to *R.* (*B.*) *microplus* showed that EST-2b was present throughout their life cycle in male and female but EST-7b was present only in larvae, nymph and adult male. EST-5b was found to be nymph specific. EST-6b was adult female specific, whereas EST-4b was adult male specific. EST-9b was found to be adult male and female specific. Sex specific esterases have also been detected in some species of Drosophila (Oakeshott *et al.*, 1993). A control mechanism of the parasite via sterile males could be the subject of research, starting from the manipulation of the

Table 1

Status of esterase activities in larvae, nymph, adult male and female of *R. (B.) microplus* ticks susceptible to deltamethrin and coumaphos

	Stages	Est-1b	Est-2b	Est-3b	Est-4b	Est-5b	Est-6b	Est-7b	Est-8b	Est-9b
R. (B.) microplus	Larvae	“	“	“	-	-	-	“	“	-
	Nymph	-	“	-	-	“	-	“	-	-
	Adult male	“	“	“	“	-	-	“	“	“
	Adult female	“	“	“	-	-	“	-	“	“

“ - esterase activity present, - = esterase activity absent

Table 2

Status of esterase activities in larvae, adult male and female of *H. anatolicum* ticks susceptible to deltamethrin and coumaphos

	Stages	Est-1h	Est-2h	Est-3h	Est-4h	Est-5h	Est-6h	Est-7h	Est-8h	Est-9h	Est-10h
H. anatolicum	Larvae	-	“	“	“	“	-	“	-	-	-
	Adult male	-	-	“	-	“	-	-	“	-	-
	Adult female	“	“	“	“	“	“	“	-	“	“

“ - esterase activity present, - = esterase activity absent

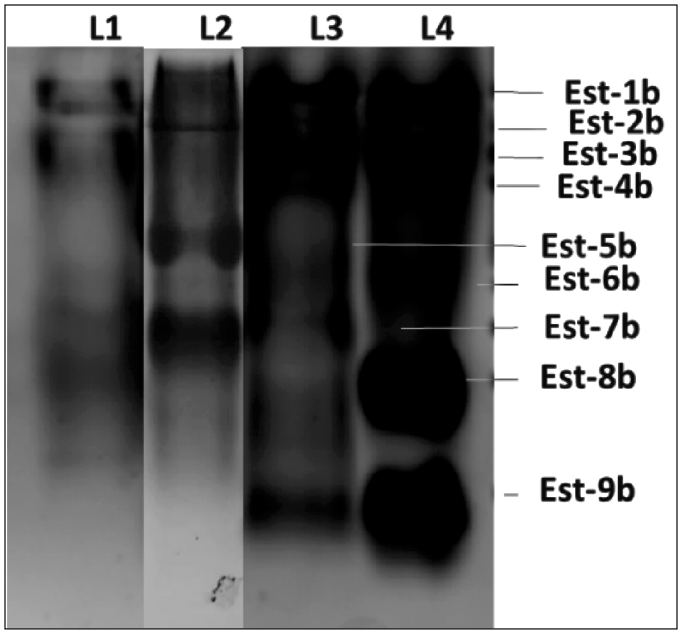

Fig. 1. Pattern of esterases in extracts of different stages of *R.* (*B.*) *microplus* ticks separated on 10% (w/v) native polyacrylamide gel electrophoresis and stained using α-naphthyl acetate as substrate. L1-larvae; L2-nymph; L3-adult male; L4-adult female

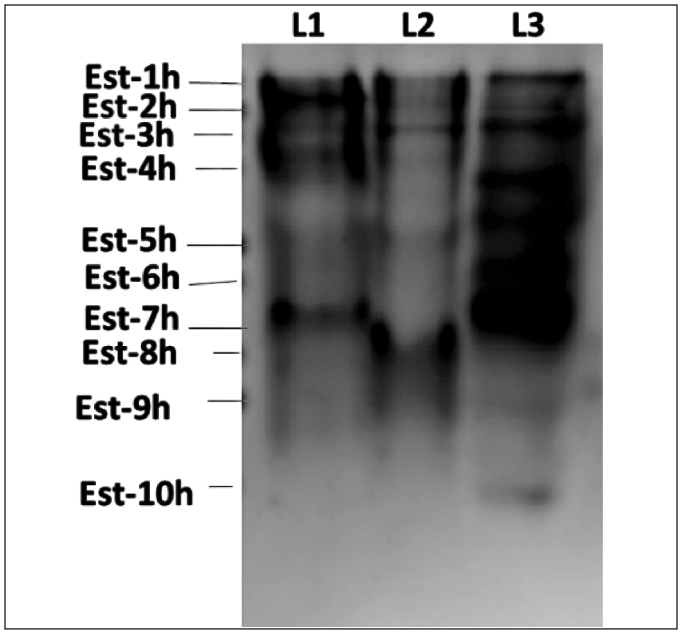

Fig. 2. Pattern of esterases in extracts of different stages of *H. anatolicum* separated on 10% (w/v) native polyacrylamide gel electrophoresis and stained using α-naphthyl acetate as substrate. L1-larvae; L2-adult male; L3-adult female

esterase expression.

Variation in esterase bands from larvae to nymph indicates that the additional esterases may be playing an important role in molting from one stage to other. The adult specific EST-9b esterase enzymes might be playing role in digestion, nervous system, reproductive system, external reproductive apparatus, and gonadal development (Baffi *et al.*, 2007). Similarly Ghosh *et al.* (2017) has also reported significant variation in esterase activity with the increasing age of *R.* (*B.*) *microplus* larvae. However, Baffi *et al.* (2005, 2008) and Gupta *et al.* (2016) have reported only three esterase bands in adult female of *R.* (*B.*) *microplus* collected from susceptible ticks lines maintained at Uruguay (Brazil) and IVRI (India), respectively. The increased number of esterase activity bands in heterogeneous Indian tick population of *R.* (*B.*) *microplus* and *H. anatolicum* in the present study might be due to exposure to multiple acaricides as compared to homogenous lines maintained by Uruguay (Brazil) and IVRI (India).

Variation in the esterase activities in different stages of tick life cycle have also been reported both qualitatively and quantitatively during ontogenetic development in many species of insects (Bitondi and Mestriner, 1983) resulting into the synthesis of different proteins which are important for triggering the processes of metamorphosis. In the present study also, differences in the expression of esterases in the different phases of life cycle of both *H. anatolicum* and *R.* (*B.*) *microplus* was observed. Characterization of these esterases could aid in the diagnosis of resistance in order to allow more effective tick control strategies.

ACKNOWLEDGEMENT

The authors would like to thank the Head of Department of Veterinary Physiology and Biochemistry, LUVAS, Hisar and ICMR for generation of funds for the present study.

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