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Protein and esterase profile patterns of the camel tick *Hyalomma* (*Hyalomma*) *dromedarii* (Koch) (Acari: lxodidae) in Hail and Qassim Regions, Saudi Arabia

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ABSTRACT

Electrophoresis for the total protein (SDS-PAGE) of the camel tick *Hyalomma dromedarii* (Koch) (Acari: Ixodidae) collected from infested camels in the animals resting house from Hail and Qassim regions, Saudi Arabia was investigated. Analysis indicated the presence of 14 protein bands in *H. dromedarii* collected from Hail region and 11 protein bands in ticks collected from Qassim region. The number of polymorphic bands was five, number of monomorphic bands was nine in ticks from Hail region and six monomorphic bands in ticks from Qassim region. Similarity % was 80.21 and commonality % was 20.00 between the camel tick collected from Hail and Qassim regions. Esterase isozyme pattern after electrophoresis (EST-PAGE) showed presence of nine esterase band, the polymorphic bands was nine, while, no monomorphic bands appeared. Similarity % was 78.05 and commonality % was 50.00. SDS-PAGE and EST-PAGE may help to detect the differences and similarity in the same species, which may help to detect sibling species.

1. INTRODUCTION

Ticks are important ectoparasites to human and animals because they transmit many dangerous diseases (Aeschliman *et al*., 1990). The camel tick, *H. dromedarii* is one of the most important ectoparasites of livestock, both in the tropics and subtropics in general. This parasite causes 65% of direct damage and 35% of indirect damage to cattle raising. Direct damage includes losses in milk production, fatality and weight along with increased mortality and acaricide consumption as well as damage to the leather industry due to punctures by this tick. Indirect damages are due to the fact that also *H. dromedarii* acts as a disease vector transmitting for animal dermatophilosis and causing human cases of hemorrhagic fever in Saudi Arabia due to a flavivirus, alkhurma hemorrhagic fever virus (Charrel *et al*., 1971). *H. dromedarii* is the second most common tick in Egypt. It is suspected of playing an important role in transmitting a haemoprotozoan disease, bovine tropical theileriosis, caused by *Theileria annulata* (Bhattacharyulu *et al*., 1975).

Protein electrophoresis has been an effective technique for the detection of genetic polymorphism for over three decades. Enzymatic polymorphism detected by electrophoresis, including esterase isozyme has been widely used in ticks and mites (Navajas and Fenton, 2000).

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In ticks, several enzymatic systems can be resolved from single individuals, however, diverse studies have reported a low polymorphism of the resolved loci (Delaye *et al*., 1997; Kain *et al*., 1997).

The aim of this work is to determine protein (SDS-PAGE) and esterase (EST-PAGE) profile patterns of the camel tick *Hyalomma dromedarii* (Koch) (Acari: Ixodidae) collected from Hail and Qassim region in Saudi Arabia.

2. MATERIALS AND METHODS

2.1. Collection of Ticks:

Engorged females of the camel tick *H. dromedarii* collected from infested camels in the animals resting house from Hail and Qassim regions in Saudi Arabia.

2.2. Preparation of protein profile pattern (SDS-PAGE):

Preparation for total protein assay was carried out according to the method of Lowry *et al*., (1951). Electrophoresis was carried out as described by Laemmli (1970) using pre-stained high molecular weight standard marker in comparison with molecular weights 200.00, 116.25, 97.40, 66.2, 45.00, 31.00, 21.5, 14.40 and 6.5 (KDa = Kilo Dalton). After the electrophoresis process the gels were stained with silver stain and distained according to method of Hitchcock and Brown (1983). The stained gels were photographed and examined for the presence and absence of visualized bands.

2.3. Preparation of esterase profile pattern (EST-PAGE):

The same steps were followed for esterase electrophoresis using α – naphthyl propionate as substrate according to Sims (1965).

Concentration of protein and esterase bands (Conc. %), relative mobility of bands or relative fragmentation (Rf) and similarity coefficient (Sim co.) were calculated according to Nei and Li (1979) and commonality percentage (Com. %) was calculated according to Haymer and McInnis (1994) as following:

$$\text{Conc. \%} = \frac{\text{O. D. of sample}}{\text{O. D. of standard}} \times \text{Conc. of standard}$$

Where: O. D. = Optical density

$$\text{Rf value} = \frac{\text{Distance of migrated band}}{\text{Distance of migrated tracked gel}}$$

$$\text{Sim. co.} = 1 - \frac{NXY}{NX+NY}$$

Where:

NXY= The number of common bands in samples X and Y

NX= The number of bands in sample X

NY= The number of bands in sample Y

$$\text{Com. \%} = \frac{\text{Number of common bands in samples X and Y}}{\text{Number of total bands of both samples X and Y}}$$

Arabic 30 g, chloral hydrate 200 g, glycerin 20 ml) and covered with a cover slip. Slides were placed in an incubator adjusted at 42^{o}C for another one day to remove air bubbles. Slides were examined by light microscope and identified according to Zaher (1986).

3. RESULTS

The obtained data revealed that the camel tick *H. dromedarii* collected from Hail and Qassim regions differed in both protein and esterase patterns. Results in Figs. (1 and 2) and Table (1) show SDS- PAGE of protein and clearly indicate the differences and similarities in the camel tick collected from Hail and Qassim. SDS-PAGE gave 14 visualization bands for the camel tick collected from Hail region, where these bands had molecular weights of 109.31, 98.61, 95.21, 85.70, 76.50, 73.70, 40.82, 29.21, 21.50, 17.16, 14.45, 12.03, 9.44 and 8.01 KDa. Only 11 visualization bands were obtained from ticks collected from Qassim region, where the bands had molecular weights of 109.65, 106.82, 91.93, 74.78, 39.57, 27.40, 23.94, 18.50, 14.91, 10.74, 9.28 KDa. Number of polymorphic bands (shared and common bands) was five bands (bands number 1, 4, 6, 10 and 19). These polymorphic bands varied in their molecular weights, although in the same sites on the lanes of SDS-PAGE pattern, where their molecular weights averaged between 109.31 KDa and 109.65 KDa for the polymorphic bands 1, 95.21 KDa; 91.93 KDa for the polymorphic bands 4, 76.50 KDa; 74.78 KDa for the polymorphic bands 6, 40.82 KDa; 23.94 KDa for the polymorphic bands

10 and 9.44 KDa and 2.60 KDa for the polymorphic bands 19 for the ticks collected from Hail and Qassim regions, respectively.

The number of monomorphic bands was nine bands for Hail tick and six bands for Qassim tick. The relative mobility (Rf) of the bands ranged from 0.13 to 0.89.

Results in Fig. (5) also confirm these data and show the concentration of protein bands which ranged from 23.04% and 2.25 % for the camel ticks collected from Hail, 11.85% and 2.60 % for the camel tick collected from Qassim. Sim. % was 80.61 and com. % was 20.00%.

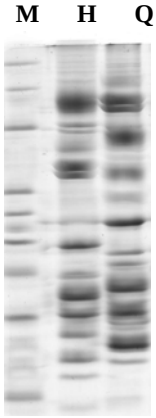

Fig. 1: SDS-PAGE (The poly peptides profile) of the camel tick *Hyalomma dromedarii* collected from Hail and Qassim regions, Saudi Arabia. H = lane of camel tick collected from Hail, Q = lane of camel tick collected from Qassim and lane M represents the known molecular size marker.

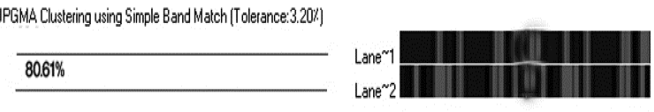

Fig. 2: Similarity relationships among protein bands in the camel tick *Hyalomma dromedarii* in Hail and Qassim regions, Saudi Arabia.

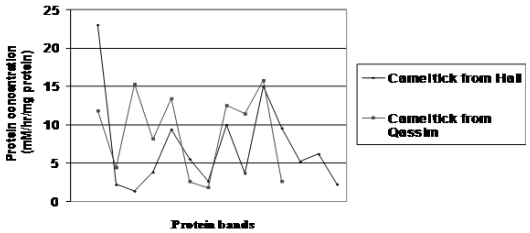

Fig. 5: Activity levels of protein and banding patterns of the camel tick *Hyalomma dromedarii* collected from Hail and Qassim regions, Saudi Arabia.

Table 1: Banding patterns, molecular weights and relative mobility (Rf) of protein for *Hyalomma dromedarii* collected from Hail and Qassim regions, Saudi Arabia.

Band number	Mol. W. (KDa)	Rf.	Band occurrence of *Hyalomma dromedarii*	
			Hail	Qassim
1	109.31- 109.65	0.13	+	+
2	106.82	0.20	-	+
3	98.61	0.21	+	-
4	95.21-91.93	o.24	+	+
5	85.70	o.29	+	-
6	76.50- 74.78	0.34	+	+
7	73.70	0.36	+	-
8	39.57	0.49	-	+
9	27.40	0.56	-	+
10	40.82- 23.94	0.60	+	+
11	29.21	0.63	+	-
12	18.50	0.65	-	+
13	14.91	0.69	-	+
14	21.50	0.71	+	-
15	17.16	0.75	+	-
16	10.75	0.79	-	+
17	14.45	0.82	+	-
18	12.03	0.83	+	-
19	9.44-2.60	0.85	+	+
20	8.02	0.89	+	-

Mol. W.= Molecular weight in KDa, Rf= Relative fragmentation, (+) Present , (-) Absent, Sim. %= 80.61 and Com. %= 20.00.

EST-PAGE has 9 esterase bands for each camel tick collected from both of Hail and Qassim regions, these nine bands have the same relative mobility ranged betwwen 0.19 and 0.93. (Table (2) and Figs. 3 &4).

Table 2: Banding patterns, molecular weights and relative mobility (Rf) of esterase isozyme for *Hyalomma dromedarii* collected from Hail and Qassim regions, Saudi Arabia.

Band number	Rf	Band occurrence of *Hyalomma dromedarii*	
		Hail	Qassim
1	0.19	+	+
2	0.24	+	+
3	0.40	+	+
4	0.46	+	+
5	0.52	+	+
6	0.69	+	+
7	0.72	+	+
8	0.90	+	+
9	0.93	+	+

Rf= Relative fragmentation, (+) Present, Sim. %= 78.05 and Com. %= 50.00.

Fig. 3: EST-PAGE (Banding patterns of esterase isozyme) for the camel tick *Hyalomma dromedarii* collected from Hail and Qassim regions, Saudi Arabia. H = lane of camel tick collected from Hail, Q = lane of camel tick collected from Qassim.

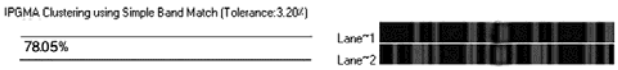

Fig. 4: Similarity relationships among esterase bands in the camel tick *Hyalomma dromedarii* collected from Hail and Qassim regions, Saudi Arabia.

The curve in Fig. (6) reveals the concentration % of each band from the 18 esterase bands which can be used easily to differentiate between the camel tick species collected from Hail and Qassim region although there are similarity in band numbers and relative mobility of each band, The concentration % was 9.19, 5.65, 22.46, 14.06, 5.03, 4.41, 2.85, 10.53 and 22.26 for the camel ticks collected from Hail and 12.58, 1.33, 23.73, 13.10, 1.48, 7.14, 2.62, 12.42, 25.61 for the camel ticks collected from Qassim. The higher peaks in the curve found in Fig (6) represent the dense bands which appeared clearly in Fig. (3). All the obtained bands were of polymorphic types and no monmorphic bands appeared in EST-PAGE for the camel tick collected from Hail and Qassim regions. Similarity % 78.05 and commonality % was 50.00%.

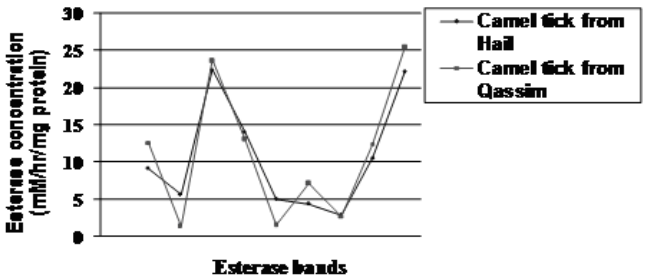

Fig. 6: Activity levels of esterase and banding patterns of the camel tick *Hyalomma dromedarii* collected from Hail and Qassim regions, Saudi Arabia.

4. DISCUSSION

In this work we detected 14 visualization bands for protein profile for *H. dromedarii* collected from Hail region, Saudi Arabia. These bands had molecular weights that ranged from 109.31 to 8.01 KDa. Only 11 visualization bands were obtained from ticks collected from Qassim region, Saudi Arabia. These bands had molecular weights that varied between 109.65 and 9.28 KDa. Number of polymorphic bands was five bands. These polymorphic bands varied in their molecular weights, although in the same sites on the lanes of SDS-PAGE pattern had molecular weights averaged between 109.31 KDa and 109.65 KDa The number of monomorphic bands was nine bands for Hail tick and six bands for Qassim tick. The relative mobility (Rf) of the bands ranged from 0.13 to 0.89. Esterase pattern had 9 bands for each *H. dromedarii* collected from Hail and Qassim regions, these nine bands have the same relative mobility that ranged between 0.19 and 0.93. Morever there were similarity in band numbers and relative mobility of each band, the band concentration % was different for the camel ticks collected from Hail about the camel ticks collected from Qassim regions.

Future work will focus on the characterization of these mechanisms to provide an accurate diagnosis of resistance in order to allow more effective tick control strategies. Partially purified pyrethroid hydrolyzing esterases have been previously isolated from Australian tick, *B. microplus* strains (Riddles *et al*., 1983 and De Jersey *et al*., 1985). A trans-permethrin hydrolyzing carboxylesterase was identified from the pyrethroid resistant strain that had an approximate molecular weight of 89 kDa (De Jersey *et al*., 1985). These biochemical means for species identification by proteins and enzymes throughout electrophoretic analysis separate proteins and enzymes into fractions that have species-specific mobility (Nilima *et al*., 1987). Isozyme were used for species identification of acarines. The most commonly studied enzymatic system in mites is that of the esterases (Avanzati *et al*., 1994). In ticks, several enzymatic systems can be resolved from single individuals however, diverse studies havereported a low polymorphism of the resolved loci (Delaye *et al*., 1997; Kain *et al*., 1997). Furthermore, electrophoresis is the most simple and quite reliable tool for species identification (Zhijian *et al*., 1997). The identification of tick species has always been based on morphological key characters of the mouth parts and adjacent structures. These methods can not be applied to damaged specimens. Recently, protein and esterase electrophoresis, beside molecular genetic studies were introduced to differentiate among the different genera and/or species (El Kammah and Sayed, 1999; Poucher *et al*., 1999). In the same trend, Das *et al*., (2000) reported that SDS-PAGE revealed three antigenic proteins of 100, 59.4 and 37 kDa responsible for induction of resistance to *H. dromedarii* in the host. Currently, the genetic origin of pesticide resistance has been widely studied and some authors have

shown that this phenomenon can be due to gene amplification and point mutations in resistance- related genes (Hemingway, 2000). The partial inhibition of EST-2A and EST-2B by eserine in the resistance tick strain may indicate acetylcholinesterases with enhanced insensitivity to acaricide. This insensitivity suggests a AChE-mediated resistance mechanism possibly involving gene duplication and esterase over expression. Gene duplication events have previously been proposed to explain pyrethroid resistance involving carboxylesterases in Mexican ticks (Jamroz *et al.*, 2000). For over three decades. Enzymatic polymorphism detected by electrophoresis has been widely used in ticks and mites (Navajas and Fenton, 2000). Protein electrophoresis has been an effective technique for the detection of genetic polymorphism Acaricides such as pyrethroids and organophosphates are currently the most used method of tick control but the indiscriminate use of these compounds over the years has led to the selection of resistant ticks. Resistance in arthropods 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 insensivity (He *et al.*, 2002). Kumar *et al*., (2002) found 26 discrete polypeptide bands with molecular weights ranging from 25 to 208kDa with SDS-PAGE of gut supernatant antigen derived from partly fed *H. anatolicum anatolicum* adult females. Pruett *et al*., (2002) described the isolation of a 62.8 kDa protein and enrichment of permethrin esterase activity that is thought to be responsible, in part, for the observed pyrethroid resistance associated with the Mexican tick strain, *oophilus microplus* collected near Coatzacoalcos, Veracruz and Mexico in Maxis, This tick possesses permethrin hydrolytic activity and contains the enriched 62.8 kDa protein is only partially pure, containing a prominent 51.4 kDa contaminant, the permethrin esterase activity of the 62.8 kDa protein. El-Fiky *et al*., 2003 used esterase an RAPD-PCR to genetically identify five Egyptian tick species. Organogenetic eggs of identified *Argas hermanni*, *Argas persicus*, *Boophilus annulatus*, *Hyalomma dromedarii* and *Hyalomma excavatum* were used. The results showed that there are a variation in esterase activity levels and a high polymorphism within and between genera and species of *Argas* and *Hyalomma*. Eight esterase markers out of 10 (88.8%) were found to be useful as species-specific markers. Baffi *et al*., (2005) described acaricide resistance related to esterase activity in a Brazilian tick, *Boophilus microplus* population. Norouzi *et al*., (2007) carried out detection of the midgut antigens of *H. anatolicum anatolicum* tick using SDS-PAGE, also Rachinsky *et al.*, 2007 used gel electrophoresis and mass spectrometry to investigate differences in protein expression in ovarian tissues from *Babesia bovis* infected and uninfected southern cattle tick, *Rhipicephalus* (*Boophilus*) *microplus*. Soluble and membrane proteins were extracted from ovaries of adult female ticks, and analyzed by isoelectric focusing and one-dimensional or two-dimensional (2-D) gel electrophoresis. Protein patterns were analyzed for differences in expression between infected and uninfected ticks. 2-D separation of proteins revealed a number of proteins that appeared to be up- or down-regulated in response to infection with *Babesia*, in particular membrane/membrane-associated proteins and proteins in a low molecular mass range between 6 and 36 kDa; Al-Shammery *et al.,* (2011) utilized protein and esterase electrophoresis to discriminate between *H. dromedarii* and *Boophilus annulatus* from Hail and Qassim regions in Saudi Arabia. Their results showed that SDS-PAGE gave 16 visualization bands for *H. dromedarii*. These bands that ranged between 109.38 KDa and 5.96 KDa, had relative fragmentation (Rf) ranged from 0.012 and 0.917 and concentration varied between 24.44 and 1.23. On the other hands, *B. annulatus* has 15 bands located between 108.24 KDa and 9.31 KDa, had Rf values

ranged from 0.167 and 0.875 and concentration varied from 6.32 and 3.44. The common reactive bands between *H. dromedarii* and *B. annulatus* were nine with molecular weight that ranged from 109.38 KDa and 9.46 KDa . Similarity % was 77.14% and the commonality % was 29.03. Esterase profile pattern of *H. dromedarii* and *B.annulatus* had eight different esterase bands, with Rf values ranged from 0.19 to 0.93 and concentration ranged from 8.78 to 36.14 in *H. dromedarii* and from 8.59 to 35.04 in *B. annulatus*. Similarity % in esterase bands of ticks' species was 82.93% and the commonality % was 50.00%. The above results indicated existence of multiple forms of resistance in these ticks. Future work will focus on the characterization of these mechanisms to provide an accurate diagnosis of resistance in order to allow more effective tick control strategies.

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