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Research Article

COMPARATIVE PROTEIN PROFILES OF SALIVARY GLAND EXTRACTS OF HYALOMMA A. ANATOLICUM AND HYALOMMA DROMEDARII (ACARI: IXODIDAE) TICKS

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ABSTRACT

The salivary glands of partially fed adult females of *Hyalomma a. anatolicum* and *H. dromedarii* collected from cattle, buffaloes and camel were dissected out and extracts were processed by homogenizing them in 0.1 M phosphate buffer solution containing protease inhibitors. One-dimensional gel electrophoresis was used to characterize the salivary gland proteins from ticks. The number of protein bands in the molecular weight range of 10 kDa to 116 kDa were 20 and 22 in *H. a. anatolicum* and *H. dromedarii*, respectively. While there were one and three bands, respectively in the range greater than 116 kDa. The Rf values and the molecular weights of the additional peptide bands obtained in *H. dromedarii* were 0.02083, 0.05208, 0.26042, 0.6667 and 0.70833 (MW 463.8 kDa, 185.5 kDa, 53.9 kDa, 22.5 kDa and 19.8 kDa), which were distinguishable from *H. a. anatolicum* in which these bands were absent and a protein band with Rf value 0.6875 (MW 21.9 kDa) was present. It indicated polymorphism in salivary gland proteins of these two species of *Hyalomma* ticks. The differences in the electrophoretic finger prints of peptides reported here may be used for identification of these species and strains of ticks by using the image analysis system.

Key words: *Hyalomma a. anatolicum*, *H. dromedarii*, salivary gland extracts, protein profiles

The ticks *Hyalomma a. anatolicum* and *H. dromedarii* (Family *Ixodidae*) naturally infest many host species such as cattle, buffalo, camel etc. The salivary glands of these ixodid ticks are vital organs, playing several key roles during the arthropod's life cycle. When attaching to a host, the salivary glands of most species produce cement that binds the tick's mouthparts to the host's skin (Moorhouse and Tatchell, 1966). Subsequently, a cocktail of enzymes and other bioactive molecules are secreted throughout the extensive feeding period (7–14 days). The bioactive saliva maintains blood flow to the feeding site, antagonizes host haemostatic and inflammatory mediators, and helps the tick evade the host's rejection responses (Ribeiro, 1989; Wikel, 1996; Nuttall,1998). All of the salivary gland functions are likely to be associated with specific proteins, some constitutive and some secreted.

However, the significance of most of the numerous bands detected by polyacrylamide gel electrophoresis (SDS-PAGE) of salivary gland extracts (SGE) remains obscure. New insights on salivary gland bioactive molecules are emerging through molecular biological approaches. However, the mechanisms that enable these to feed on such a wide range of hosts are unclear. One possibility is that a tick population maintains molecular (genotypic and/or phenotypic) diversity such that these species vary in their competency in taking blood meals under different feeding conditions. As the feeding success of ixodid ticks depends on the efficiency of the cocktail of anti-inflammatory, immunomodulatory, antithrombotic, antiplatelet aggregating substances etc. in saliva, the variations if any, in expression of the proteins in the salivary gland could serve as an anglestone in identification of these two species. Hence, it was planned to generate the finger prints of salivary gland proteins of partially engorged females of the *Hyalomma a. anatolicum* and *H. dromedarii* ticks collected from field conditions.

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MATERIALS AND METHODS

Collection of Ticks and Their Processing: Partially fed adult female *H. a. anatolicum* and *H. dromedarii*

ticks were collected from cattle, buffaloes and camels from the villages around Hisar. Properly washed and sterlised female ticks were glued to the bottom of a Petri dish with their dorsal surface upward and placed on ice for 20 min. Using fine scalpel blade and fine tip forceps, ticks were incised along the dorsal-lateral margin, and the dorsal integuments were removed under a stereoscopic dissection microscope. The salivary glands were then removed and transferred into 0.1 M phosphate buffer solution (PBS) containing 5% glycerol, protease inhibitor cocktail (Sigma, P2714), pH 6.0 (Wu *et al*., 2010).

Extract Preparation: The salivary glands were homogenized under ice using a tissue homogenizer (IKA T10 basic Ultra-Turrox). The homogenate was centrifuged at 10000 rpm for 15 min at 4°C. The supernatant was used afresh to separate the peptides on 15% discontinuous SDS-PAGE by the method of Hames (1998) on vertical gel apparatus (Scie-Plus Max Fill) to study the differences in protein profiles between the two species of ticks.

SDS-PAGE Analysis: Resolving (15%) and stacking gels (4%) were polymerized. The samples to be analyzed and the unstained protein molecular weight marker (Fermentas, #SM0431, 116kDa-14.4kDa) were mixed with sample buffer in 1:1 ratio. The mixtures were heated at 95°C for 5 minutes and then cooled before loading. A constant electric field (90 volt) was then applied depending on their size, each protein moved differently through the gel matrix. Then the gel was stained with 0.25% Coomassie Brilliant Blue R-250 for 1hr with continuous shaking and was destained by overnight incubation in destaining solution. The molecular weights of the peptides were estimated by using their relative flow rates ($R_f$value).

RESULTS AND DISCUSSION

The electrophoretic pattern of the proteins extracted from the SGE of *H. a. anatolicum* and *H. dromedarii* is shown in Fig.1. The two species under investigation showed differences in the number of protein bands, their relative flows, quantitative expressions and their molecular weights (Table 1). The

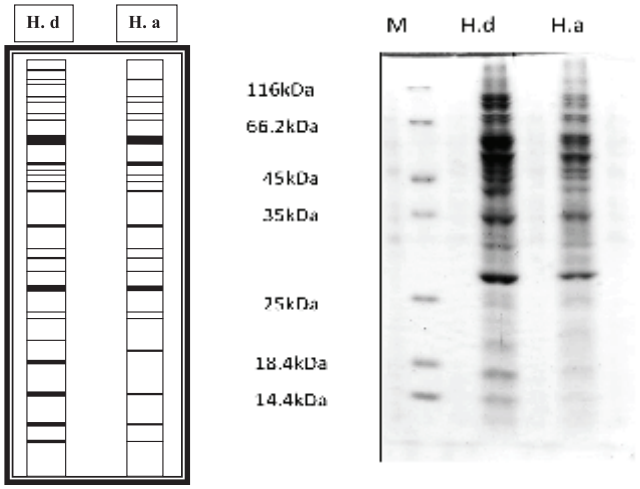

Fig 1. Protein profile of salivary gland extracts (SGE) of *Hyalomma dromedarii* and *Hyalomma a. anatolicum* separated by SDS-PAGE on 15% acrylamide gel. Lane M = bands of molecular weight marker; Lane H.d = SGE of *H. dromedarii* engorging females; Lane H.a. = SGE of *H. a. anatolicum* engorging females.

numbers of protein bands were 20 and 22 in the range of 10 kDa to 116 kDa and 1 and 3 in the range of greater than 116 kDa in *H. a. anatolicum* and *H. dromedarii*, respectively. The presence of the additional bands with R_f values 0.02083, 0.05208, 0.26042, 0.6667 and 0.70833 (molecular weights 463.8 kDa, 185.5 kDa, 53.9 kDa, 22.5 kDa and 19.8 kDa) from *H. dromedarii* were distinguishable from *H. a. anatolicum* where the bands were absent. A protein band with $R_f$value 0.6875 (molecular weight 21.9 kDa) was present only in *H. a. anatolicum*.

The qualitative differences observed between female ticks' SGE of the two species could be explained on the basis of differences in biochemical mechanisms in the two species. Genetic variations, protein polymorphism and protein variations have been reported by others between tick species and populations (Healy, 1979; Bull *et al*. 1984; Hilburn and Sattler, 1986; Davey and Hilburn, 1991; Gomes and Wouters, 1991). These different molecular bases of salivary gland proteins could also have implications for pathogen-vector-host interactions and for analytical strategies in molecular biology research. Such diversities between these two species might also be relevant to the mechanism (s) that might have provided *H. dromedarii* to suck large amount of blood from the

Table 1
Relative flow and approximate molecular weight of the protein bands of *H. a. anatolicum* and *H. dromedarii* salivary gland extracts

Molecular weight in kDa				
Band No. rate (Rf)	Relative flow marker	Mol. wt.	*H. dromedarii anatolicum*	*H. a.*
1.	0.02083	-	463.8	-
2.	0.05208	-	185.5	-
3.	0.0625	-	154.6	154.6
4.	0.0833	116.0	-	-
5.	0.09375	-	103.1	103.1
6.	0.11458	-	96.3	96.3
7.	0.13542	-	81.5	81.5
8.	0.15625	-	70.6	70.6
9.	0.1667	66.2	-	-
10.	0.1875	-	58.8	58.8
11.	0.25	-	56.3	56.3
12.	0.26042	-	53.9	-
13.	0.27083	-	51.9	51.9
14.	0.29167	-	48.2	48.2
15.	0.3125	45.0	-	-
16.	0.32292	-	42.9	42.9
17.	0.39583	35.0	35.0	35.0
18.	0.4479	-	33.7	33.7
19.	0.46875	-	32.2	32.2
20.	0.5	-	30.2	30.2
21.	0.54167	-	27.8	27.8
22.	0.59375	-	25.4	25.4
23.	0.60417	25.0	-	-
24.	0.61458	-	24.6	24.6
25.	0.6667	-	22.5	-
26.	0.6875	-	-	21.9
27.	0.70833	-	19.8	-
28.	0.76042	18.4	-	-
29.	0.78125	-	17.9	17.9
30.	0.84375	14.4	14.4	14.4
31.	0.88542	-	13.7	13.7

host very successfully.

The extent to which the diverse molecular masses revealed here by SDS-PAGE might have derived variations at either the genetic level, transcription/ translation regulations, post-translational modifications or a combination of these which have not been determined in present study. The electrophoretic finger printing of peptides reported here may be used to identify the *H. a. anatolicum* and *H. dromedarii* ticks if their morphological characters and adjacent structures are damaged due to any reason. The reported finger prints could also be useful in the classification and evolutionary studies.

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