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CHARACTERISATION OF SALIVARY GLAND PROTEINS AND P-18 GENE OF CAMEL TICKS FROM BIKANER

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ABSTRACT

The main objective of this study was to investigate the various salivary gland proteins and gene analysis of camel ticks *Hyalomma dromedarii* found on one-humped camels (*Camelus dromedarius*) in Bikaner city, Rajasthan, India. For this purpose, Salivary glands were collected from 5 adult ticks (engorged females). Various protein analysis from salivary gland using SDS-PAGE for determination of protein bands and their molecular weights for each fraction was done. The molecular weight of resolved major bands ranged from 14.4 to 96.0 KDa. Out of them large majority of protein molecular weight was around 18 KDa. This low molecular weight band of around 18 KDa was detected in the all 5 fractions of salivary gland of *Hy. dromedarii* ticks. The protein gene P–18 of *Hy. dromedarii* of present study had 90.4% sequence identity with that of *Hy. asiaticum* from China.

Key words: Camel *Hyalomma dromedarii*, P-18 Gene salivary gland proteins

Hyalomma dromedarii ticks were the object of several studies seeking to characterise the molecules isolated from salivary gland extracts and saliva. In order to avoid host defenses, ticks secrete saliva at the bite site that contain many biologically active molecules that display anticoagulation, antiplatelet, vasodilatory, anti-inflammatory and immunomodulatory activities (Kazimirova and Stibraniova, 2013; Chmelar *et al,* 2012; Simo *et al,* 2017).

The tick saliva has hundreds of different proteins (Chmelar *et al,* 2016) which are multipotent and had pharmacological features (Steen *et al,* 2006). Accordingly, several transcript and protein profiles of tick salivary glands were carried out in different stages of development, for both genders and feeding behaviour (Francischetti *et al,* 2011; Tirloni *et al,* 2014; Tan *et al,* 2015). The field of vector–host interaction has gained tremendously by high–throughput analysis of salivary gland transcripts and proteomes, collectively called the sialome (Ribeiro and Francischetti, 2003). More interestingly, sialo transcriptomic analyses improved proteomic studies of unknown genome species that seek to identify pharmaceutically active proteins (Evans *et al,* 2012; Mudenda *et al,* 2014).

Only few reports have explored *Hy. dromedarii* salivary glands. The characterisation of salivary gland proteins of *Hy. dromedarii* at protein level was done (Kandil and Habeeb, 2009). The molecular characterisation of Bm86 orthologue from *Hy. dromedarii* was carried out (Ben Said *et al,* 2012). Compared to other haematophagous parasites, relatively little information exists about the molecular composition of *Hy. dromedarii* salivary glands (Ibrahim and Masoud, 2018; Marzouk and Darwish, 1994).

In present study the various salivary gland proteins and gene analysis of camel ticks *Hyalomma dromedarii* found on one-humped camels (*Camelus dromedarius*) in Bikaner city were investigated.

Materials and Methods

Collection of ticks from camels

Five engorged adult female ticks were collected from the ground of camel pens at herd of National Research Centre on Camel (NRCC), Bikaner, India. These were morphologically identified (Apanaskevich *et al,* 2008) and kept individually in plastic tubes, incubated at 26°C, 75% RH and photoperiod of 12:12 (L: D). The ticks were cleaned several times in sterile 1 x PBS (pH 7.2).

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Isolation of salivary glands and proteins analysis

Salivary glands of collected *Hyalomma dromedarii* (engorged females) were isolated as per the described by EL–Kammah *et al* (2005). These were placed into phosphate buffer saline (PBS) (pH 7.4) and opened along their dorsal surface. Salivary glands were removed, dissected free of other tissues, placed into PBS at 4°C. The salivary gland proteins were analysed using SDS-PAGE for determination of protein bands and their molecular weights for each fraction as described by Hames (1987).

RNA Isolation and cDNA Synthesis

Total cellular RNA was extracted from tick salivary glands using Total RNA isolation kit–Cells and Tissues (Bangalore GeNei Pvt. Ltd, India). Using total cellular RNA extracted from the tick salivary glands as a template, cDNA was synthesised by Easyscript First Strand cDNA Synthesis Kit according to the manufacturer's instructions from the resultant cDNA synthesised.

Characterisation of salivary gland protein gene (P-18)

The P–18 protein gene of *Hyalomma dromedarii* was amplified using the forward primer of 5′ GAG CGG ATC CAT GAT TTT ATG GGC GCT TTG 3′ and reverse primer of 5′ CGC GCT CGA GTT ACC ACT CAA TCT TGA CTG 3′. The sequences of the primers were deduced from the Gen Bank Accession No. EU000252. PCR amplification was performed with following thermal profiles: initial denaturation of 94°C for 3 min. followed by 35 cycles of denaturation at 94°C for 1 min., 57°C for 1 min for P–18 gene and extension at 72°C for 1 min. and final extension at 72°C for 10 min. The PCR amplified products were checked on 1% agarose gel.

Cloning and sequencing of P-18 Gene

The purified amplicons corresponding to genes encoding P–18 of *Hyalomma dromedarii* were cloned into pGEM–T Easy vector (Promega Corp., Medison, USA). The ligated mixtures for P-18 gene was individually transformed into *Escherichia coli* DH 5α (Sambrook *et al,* 1989). The positive clones were confirmed by colony PCR using gene–specific primers and restriction analysis with EcoRI. The positive clones were sequenced at the sequencing facility, Delhi University (South campus), Delhi. Since pGEM–T easy vector was used for the cloning purpose, universal T7 and SP6 primers were used for the sequencing of recombinant clones. The primer sequences used for the sequencing were based on respective promoter sequences.

Sequence analysis of P-18 Gene

Nucleotide identity and comparison of sequences with published sequences of members of *Ixodidae* available in the GenBank database were carried out using the computer software Bio Edit version 7.0.9. These sequences were compared in Clustal X (Thompson *et al,* 1997) and a phylogenetic tree was constructed based on the amino acid sequences by the neighbour–joining method using Mega 4 (Molecular Evolutionary Genetics Analysis software with bootstrap values calculated for 1, 000 replicates (Tamura *et al,* 2007).

Results

The salivary glands isolated under light microscope were significantly larger and were observed having white folds and normal acini.

Salivary glands protein (P-18) analysis by SDS-PAGE

The protein analysis from salivary gland using SDS-PAGE for determination of protein bands and their molecular weights for each fraction. A total number of 11 major bands of salivary gland proteins with molecular marker were seen in the gel (Fig 1). The molecular weight of the resolved major bands ranged from 14.4 to 96.0 KDa. Out of them, the intense band of protein having the molecular weight of around 18.0 KDa was observed in all the salivary glands of five ticks examined.

Genes encoding P–18 of *Hyalomma dromedarii* were cloned and the length of the P–18 gene sequenced was 461 bp (Fig 2).

Sequence comparison of P-18 Gene

BLAST search analysis in NCBI database showed that it closely matched with P–18 gene of *Hyalomma asiaticum* (Accession No. EU000252), which

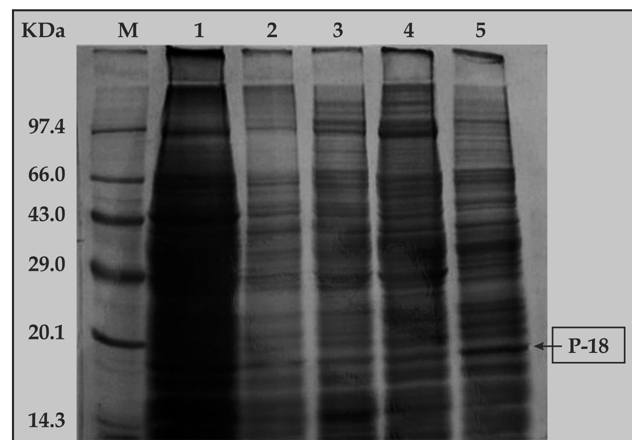

Fig 1. SDS-PAGE photograph showing protein profile of salivary glands of *Hyalomma dromedarii.*

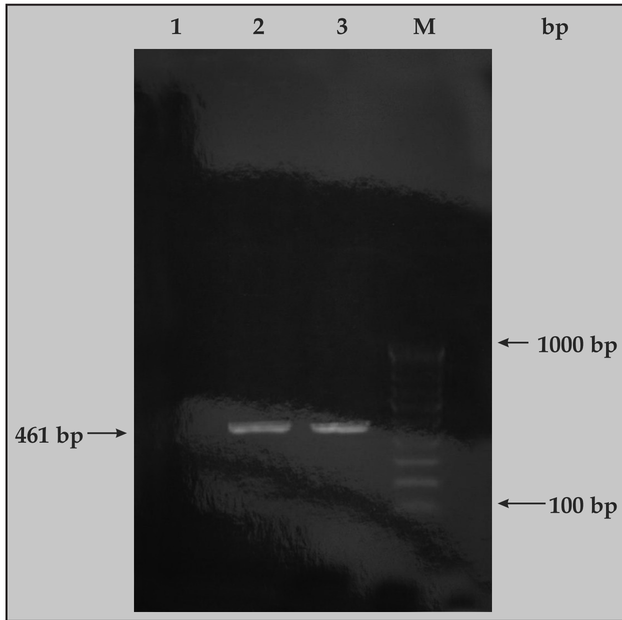

Fig 2. Ethidium bromide stained 1.2% agarose gel showing amplification of salivary gland protein (P18) gene of *Hyalomma dromedarii*.
Lane M-100 bp DNA. Ladder Lane 1- Negative control. Lane 2-3-Desired amplicons.

is the only one available sequence in NCBI database. Pair–wise comparison of these 2 sequences showed that 461 bp of *Hy. dromedarii* from India was matching with the nucleotide base, 26 to 486 of P–18 gene of *Hy. asiaticum* from China (Fig 3). These partial 461 bp gene sequences compared with the corresponding nucleotide sequences of P–18 gene of *Hy. asiaticum* (Gen Bank Accession No. EU000252) revealed that they had 90.4 % sequence identity with *Hy. asiaticum*. The nucleotide sequences of the salivary gland protein (P–18) gene of *Hy. dromedarii* were submitted to GenBank, NCBI database and assigned accession number HM051110.

Discussion

Hyalomma dromedarii is a very characteristic tick closely associated with camels and widely distributed in desert and steppes, wherever, camels are found.

Several investigations have successfully induced host resistance by administration of tick antigen derived from salivary gland (Wikel, 1996; de la Fuente *et al,* 1998; Mahmoud *et al,* 2005). The use of concealed tick antigens was considered the first

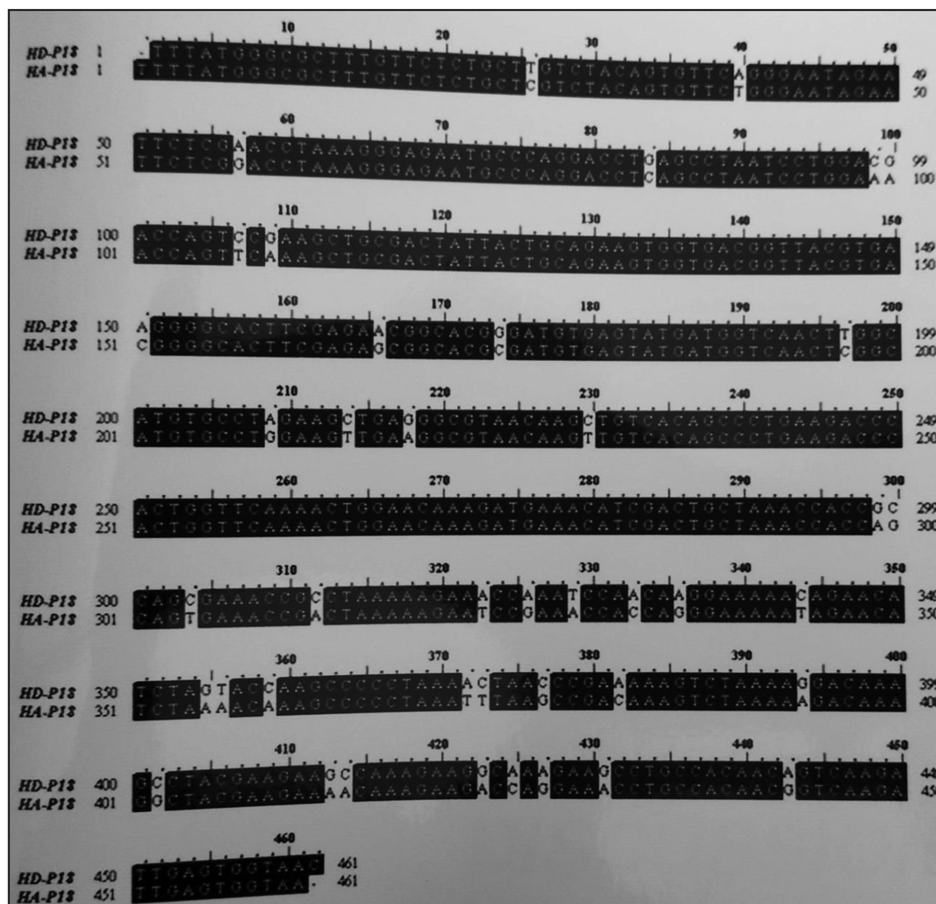

Fig 3. Multiple alignment of nucleotide sequence of P18 gene of *Hy. dromedarii* from India and *Hy. asiaticum* from China.

step for the basis of a commercial vaccine. Therefore, several workers have reported development of acquired immunity against tick by artificial immunisation with tick salivary gland antigens of *Boophilus microplus* (Parmar *et al,* 1996); *Hy. anatulicum anatolicum* (Sran *et al,* 1996) and *Hy. dromedarii* (El-Kelesh, 2002; Kandil and Habeeb, 2009).

The first proteomics study informed by transcriptomics to identify *Hy. dromedarii* salivary gland proteins in both genders using LC-MS/MS was reported by Bensaoud *et al* (2019). Only few reports have explored *Hy. dromedarii* salivary glands.

The nucleotide sequences of the salivary gland protein (P–18) gene of *H. dromedarii* were submitted to GenBank and the assigned accession number was HM051110.

The baseline information about the salivary gland protein P–18 gene of the present study would help the exploration of sialomics of *Hy. dromedarii* and other ixodid ticks from different geographical areas of India and thereby the development of a new generation vaccine for control of ticks would be feasible in India.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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