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ISOLATION OF DEFENSIN GENE FROM SALIVARY GLAND OF *HYALOMMA DROMEDARII* TICKS FROM *CAMELUS DROMEDARIUS* BY POLYMERASE CHAIN REACTION

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ABSTRACT

A molecular study was carried out to isolate the defensin gene of *Hyalomma dromedarii* of camels, which is a part of innate immune response of the tick. Engorged adult *H. dromedarii* ticks (n=350) were collected from camel herds in the Bikaner district of Rajasthan. Total genomic DNA was isolated from the salivary glands of ticks. Primers were designed for amplification of defensin gene from *H. dromedarii* by using base sequence of *Ixodes ricinus*. The defensin gene of *H. dromedarii* was successfully amplified from genomic DNA and was identified on the basis of its size in agarose gel electrophoresis as 580 bp.

Key words: *Camelus dromedarius*, defensin gene, *Hyalomma dromedarii*, polymerase chain reaction, salivary gland

Tick infestation of camels is an universal problem. All age groups are prone to it. The ixodid tick *Hyalomma dromedarii* is one of the most common ticks infesting livestock in India and in other tropical countries. Hyalomma species are responsible for transmission of a wide range of pathogens to farm animals such as viruses-Dhori, CCHF, Thogoto and Wanowrie (Darwish and Hoogstraal, 1981), protozoa like *Theileria annulata* (De Kok *et al,* 1993), bacteria and tick borne infections such as *Theileria equi, T. annulata* and *Babesia caballi* (El- Kammah *et al,* 2001). These ticks are ectoparasites with irritating bites causing extensive harm to their hosts due to blood loss, damage to the skin and anorexia leading to reduction in growth and are also considered as the agents of 'tick paralysis' in man and animals, probably due to the secretion of toxic substances in their saliva. Despite the importance of ticks as vectors of diseases very little is known about their immune system. In terms of coevolution, perhaps ticks have developed multiple antimicrobial factors (like defensins) because they encounter a large diversity of pathogenic microbes and they themselves remain unaffected. Antimicrobial peptides- defensins are major components of innate immunity in ticks that have been shown to provide protection against gram-negative and gram-positive bacteria, fungi, viruses and protozoan parasites (Chrudimska *et al,* 2010). Tick control represents the most important part of the current global strategy, as tick-borne diseases are major factor limiting livestock production world-wide. Control of ticks by conventional methods using acaricides has become increasingly difficult owing to emergence of acaricide resistant strains of ticks (Nolan, 1990). This has stimulated research on alternative method, such as immunological control of ticks. The salivary gland proteins from the ticks are the important molecules as vaccine target for the ticks control programme from the various reports. This study was therefore, undertaken to provide information about isolation of the defensin gene(s) from camel tick, *Hyalomma dromedarii* so as to find out the suitable strategy for its control.

Materials and Methods

About 350 engorged adult ticks were collected from the camels in and around Bikaner city at environmental temperature of 42^{o}C and 75% relative humidity. Morphological features were examined under stereo microscope and identified using the guide to identification of species (Estrada-Pena *et al,* 2004) and morphological features of *H. dromedarii*, as described by Apanaskevich *et al* (2008). Dissection of ticks for collection of salivary glands was done under a dissecting microscope (10x-15x) following

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the method of Purnell and Joyner (1968). These salivary glands were transferred to separate labelled micro centrifuge tubes, washed 3 times with (PBS) and stored at -80°C with 100 µl of 70% ethyl alcohol until used for extraction of DNA. Then total genomic DNA was isolated from the fine suspension of ground salivary glands of adult *H. dromedarii* by the phenol-chloroform extraction followed by ethanol precipitation method as suggested by Sambrook and Russel (2001). Then the concentration and purity of DNA sample was determined by Spectrophotometric method and Agarose Gel Electrophoresis. This genomic DNA is used as a template for amplification by Polymerase Chain Reaction using following set of primers 5' ATTCAGCGCTTCCGTCCG and PCR amplified DNA was analysed by analytical agarose gel electrophoresis as per the procedure described by Sambrook and Russel (2001). The molecular sizes of the DNA bands were analysed in relation to molecular weight marker.

Results

The genomic DNA was analysed in 0.8% analytical agarose gel and was found to be intact without much shearing (Fig 1). The yield and purity (OD ratio 260/280nm) of DNA sample was 100µg/ml and 1.9, respectively. Gene specific forward and reverse primers were used for amplification and the amplicons were analysed by agarose gel electrophoresis (Fig 2). An intensely amplified DNA was seen in lane 2-7 which was absent in the control (Lane 8). The intense band did correspond to the defensin gene of *H. dromedarii*. The size of the intense band was deduced from the standard curve drawn between the log molecular sizes of the marker bands against their respective mobility and found to be 580 bp, which was the reported size of the defensin gene of *H. dromedarii*. Thus, the band corresponded to the defensin gene of *H. dromedarii*.

Discussion

The first report of defensin in ticks was a partial amino acid sequence with 100% homology to a scorpion 4 kDa defensin purified from the hemolymph of *Ornithodoros moubata* (van der Goes van Naters-Yasui *et al,* 2000). Subsequently, the full length sequences of four defensin isoforms were obtained (Nakajima *et al,* 2001a, 2002b). RT-PCR analyses of these defensin genes revealed that they were expressed in all stages from eggs to adults and appeared to be constitutively expressed. The isolation of defensin gene from *H. dromedarii* of camel has not been reported previously. In the present study, newly designed primers for amplification of *H. dromedarii* defensin gene was confirmed as specific amplicon and the two important variables such as primer annealing temperature and $MgCl_2$ concentration were considered for the optimisation of the PCR conditions. Gradient PCR was used for the optimisation of the PCR conditions. Different concentrations of $MgCl_2$ ranging from 1.0mM to 3.0mM were tried for the PCR trouble shooting. The optimum results for PCR were obtained at 59 degree and by using 1.5mM $MgCl_2$ concentration. Theoretically, the average Tm value for both the primer was found to be 63.5°C but there was no amplification of the target region at this temperature. The amplicon obtained in the PCR reaction would be the specific target region as there was no amplification in the negative control included in the reaction (negative control included all the components of the PCR mix without template). The specificity of the amplicon was confirmed by running it along with standard DNA molecular weight marker (1500bp DNA ladder). In the agrose gel DNA

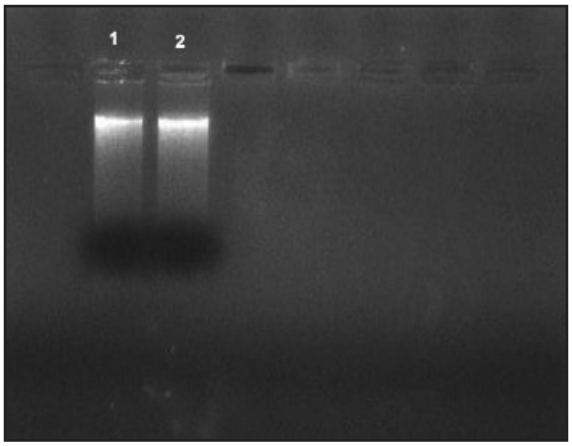

Fig 1. Lane 1, 2- intact genomic DNA in 0.8% agarose gel.

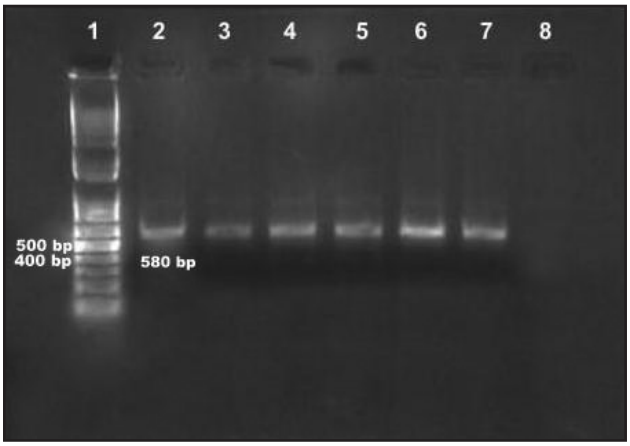

Fig 2. Lane 1- 1.5 kb DNA ladder, lane 2 to 7- amplified product of defensin gene at 580 base pairs in 1.2% agarose gel.

profile, the expected size of the amplicon (580 bp) was obtained. Absence of primer-dimer in the DNA profile was proving as the primer concentration of 100 pmole used in the PCR reaction was highly optimal. Gene encoding the members of this protein family has already been found in several species of hard and soft ticks. Noureddine *et al* (2011) reported the Ixosin as a defensin having linear DNA of size 926 bp from *Ixodes ricinus*. (GenBank accession number is GU074767) and Nene (2008) reported the Scapularisin, one of such tick defensins having a whole genome shotgun sequence of 532848 bp (GenBank accession number is DS930883) isolated from *Ixodes scapularis*. Ceraul *et al* (2003) identified an arthropod defensin named varisin A1 (vsnA1) expressed by the hemocytes of the American dog tick, *Dermacentor variabilis* of 624 bp linear mRNA. (GenBank accession number is AY181027). However, in this study, the transcripts of the gene isoforms were not categorically identified. It was proving that the DNA fragment amplified in the PCR reaction was of expected size (580bp) and highly target specific region of defensin gene of *H. dromedarii*. With the cloning and sequencing of defensin gene of *Hyalomma dromedarii* and expression of this protein it can make a great impact on the discovery of new protective antigen. It would be an ideal vaccine target or drug target in its own right for the control of Hyalomma genus or some different tick species of India.

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