

## A novel marvelous evolutionary detection: Lack of a large 2888 bp intron region within HA03 gene from *Hyalomma anatolicum anatolicum* unlike its commercial recombinant anti-tick orthologue, Bm86, from *Boophilus microplus*

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**H**yalomma anatolicum anatolicum (H. a. anatolicum) as the most widespread tick species in Iran and other parts of the middle east is responsible for the hugely serious economic losses in livestock industry. This study was conducted to investigate genetic variability of Bm86 orthologous gene, HA03, in different Iranian H. a. anatolicum isolates including Kordan, Qom, Boiazahra, Lorestan and Bushehr. Likewise, a number of analyses were performed in order to predict the possible impact of the amino acid substitutions on antigenicity of the protein. Comparative sequence analysis of the orthologous gene sequence among five tick isolates allowed for identification of four non-synonymous single nucleotide polymorphisms (SNPs) including c.995A>C, c.1150G>C, c.1151A>C/T and c.1152G>T which would result in p.Asn 332 r, p.Glu 384 Leu and p.Glu 384 Ala substitutions. As much as antigenicity is concerned, based on *in silico* studies, the amino acid position 384 was located in a putative antigenic peptide of the protein. Our subsequent physicochemical and structural analyses illustrated that two out of three amino acid substitutions including p.Glu 384 Leu and p.Glu 384 Ala considerably influenced the 3-dimensional structure and physicochemical properties of HA03 protein including hydrophobicity, amphiphilicity and net charge; thus, they might affect the antigen-antibody reaction and consequently immunogenicity of the antigen. In conclusion, it is a rational measure not only to replace Bm86 with