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Molecular characterization of HAO3, the homologue of the Bm86 tick vaccine antigen, from the Iranian isolate of *Hyalomma anatolicum anatolicum*

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ABSTRACT

Hyalomma anatolicum anatolicum tick is widely distributed in many parts of Iran and while the commercial vaccines based on the application of midgut-derived recombinant Bm86 antigen are used for its control, limited information about the efficiency of this vaccination in Iran is available. Herein, with the final aim of evaluation of Bm86-based heterologous vaccination, as the primary step the Bm86 homologue of the *H. a. anatolicum* (*Hao3*) from an Iranian isolate was characterized and compared with the commercialized Bm86 and other Bm86 homologoue sequences available in GenBank.

Our *in silico* predictions resulted in the identification of seven epidermal growth factor (EGF)-like domains, one hydrophobic transmembrane region, one leader sequence and several glycosylation sites within the structure of both Hao3 and Bm86 proteins, which suggested the pattern of extracellular membrane-bound glycoproteins with the role of regulation in cell growth for both proteins. Moreover, while the nucleotide and amino acid sequences corresponding to Bm86 homologue showed a high level of conservation among the Iranian isolates (Hao3, Hao3-1 and Hao3-2, more than 99%), the Hao3 amino acid sequence had a homology of around 89%, 64% and 65% with that of Indian, Australian and Argentinean isolates, respectively. This indicated a considerable variation between commercial Bm86 antigen and *H. a. anatolicum* Bm86-like protein of Iranian and Indian isolates. Taking together, these results imply that the efficiency of commercial Bm86-based vaccine against the Iranian *H. a. anatolicum* may be under the question and indicates the value of the development of Hao3-based recombinant vaccines and further planning for their *in vivo* evaluation.

1. Introduction

Ticks, as the specialized group of obligate, blood-sucking, ecto-parasites of mammals, birds and reptiles, because of their major role in spreading different diseases among the livestock including human being are of special research interests. Approximately one billion cattle, most of which are in the tropical and subtropical countries, are under the threat of various tick species (Pegram et al., 1993) and this can economically affect their production by reducing the weight and production of milk. *Hyalomma anatolicum anatolicum* is one of the most important tick species with a wide range of host as well as geographical distribution that transmits *Theileria annulata*, the infectious agent of tropical theileriosis, which is mostly observed in South Europe, East Africa, Middle and South Asia, Middle East (Uilenberg, 1981; Young, 1981; Azhahianambi et al., 2009). Control of ticks has been centralized on the use of acaricides. However, continuous application of acaricide has led to the natural selection of the acaricide-resistant ticks (Nolan, 1990), while this strategy is not cost-effective (Chema, 1990; Pegram et al., 1991). These consequences, in addition to the public concerns over the chemical residues in livestock products and the undesirable effects of chemicals in the general environment, have led to the search for alternative methods of control. Accordingly, development of naturally resistant hosts, as well as application of vaccines to induce an immunological response against tick infestations, has been conducted (Rodriguez et al., 1994). A major milestone in vaccine development against the different parasites was the application

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of *E. coli*-expressed Bm86 tick gut antigen as a recombinant vaccine, which was directed against the cattle tick, *Boophilus microplus* in Australia and commercialized under the name of TickGARD (Willadsen et al., 1995). A similar recombinant vaccine, produced in *Pichia pastoris* yeast, was also developed and commercialized in Cuba under the name of GAVAC (Canales et al., 1997; Garcia-Garcia et al., 2000), which has been successfully applied in the countries of South America and Australia in *B. microplus* controlling programs (De la Fuente et al., 2007).

H. a. anatolicum is widely distributed in many parts of Iran, but limited data is available about it. Control of the tick infestation in Iran has been prioritized to reduce its direct effect on the host (Nabian et al., 2007). Although one report on the detection of Bm86 homologue of the Iranian *H. a. anatolicum* (Hao3 glycoprotein) is published (Nourouzi et al., 2007), however, no information is available regarding the specific immunization against the common Iranian tick species, *Hyalomma anatolicum anatolicum* and *Boophilus microplus*.

Success of the vaccine-based *H. a. anatolicum* controlling program greatly depends on the application of antigenic molecules with an important physiological role in the tick life cycle (Imamura et al., 2005). However, available data suggest that the tick physiological differences in addition to the sequence variability in Bm86 antigen of various species can affect the efficacy of the vaccines (Garcia-Garcia et al., 2000; De La Fuente et al., 2000), indicating the value of identification and characterization of potent species-specific antigens (De La Fuente and Kocan, 2003).

Considering that no reliable data regarding the protectivity of TickGARD and GAVAC vaccines against the Iranian ticks are available, in the present study we elected to perform a molecular characterization and homology assessment of the *Bm86* homologue of Iranian *H. a. anatolicum* tick isolate (*Hao3*) in comparison with the *Bm86* of *B. microplus* (the antigen of TickGARD and GAVAC) and the *Bm86* homologue of Indian *H. a. anatolicum* tick isolates, as the two previously described sequences (Azhahianambi et al., 2009; Rand et al., 1989), to introduce a new potential target for vaccine development against Iranian ticks.

2. Materials and methods

2.1. In silico data analysis

The primers used for gene cloning were designed based on the published sequence information (FJ160586.1) and analyzed by DNAMAN (version 4.13, Lynnon Biosoft) and Oligo (version 5, National Biosciences, Inc.) softwares.

Amino acid sequence of the *Hao3* gene, its molecular weight, and isolelectric point (pI) were predicted by MacVector (version 7.2, Accelrys, Inc.) software. Homology search was performed using the BLAST server (http://www.ncbi.nlm.nih.gov/pubmed). Transmembrane domain and the putative signal peptide cleavage site were determined using the ISERC (http://www.ch.embnet.org/software/TMPRED_form.html) and SignalP V2.0.b2 (http://www.cbs.dtu.dk/services/SignalP) servers, respectively. *N* and *O*-glycosylations were analyzed using software-based services available at http://www.cbs.dtu.dk/services/NetNGlyc and http://www.cbs.dtu.dk/services/NetOglyc, respectively. Sequences were aligned by MacVector software.

2.2. Construction and characterization of HAO3 gene in pTZHAO3 plasmid

One hundred nano-grams of the cDNA corresponding to the 1995-bp *Hao3* gene (*Bm86* homologue of *H. a. anatolicum* isolated from Boushehr province of Iran that was obtained from Biotechnology Department, Razi Vaccine and Serum Research Institute, Tehran, Iran) was PCR-amplified in a 50 μl reaction containing 5 μl of 10X buffer, 12 μl of mixed dNTPs (2.5 mM each), 15 pmol of each of the upstream (5′-GAATTCATGTGCAGCCCACCTTTG-3′) and downstream (5′-GCTCTAGATGCGGCGGCAGCTGCAA-3′) primers and 2 units of *Taq* DNA polymerase (Mannheim, Roche, Germany).

The thermal program was set at 95 °C for 5 min, followed by 30 cycles of 94 °C for 1 min, 65 °C for 1 min and 72 °C for 2 min, and a final extension at 72 °C for 20 min.

The PCR product was T/A cloned into the prokaryotic cloning vector of pTZ57R/T (Fermentas, Lithuania) and the recombinant

Table 1
Deduced amino acid sequence of EGF-like domains of Bm86 (Accession No. M29321), Hao3 (Accession No. FJ160586.1) and Haa86 (Accession No. EU665682).

EGF-like domain number	Bm86/Hao3/Haa86	Deduced amino acid sequence of EGF-like domains
1	Bm86	ESSI**C**SDFGNEF**C**RNAE**C**EVVPGAEDDFV**C**K**C**PRDNMYFNAAEKQ**C**E
	Hao3	TSSV**C**SDFGKQF**C**QSAE**C**EVIPGTEDDFV**C**K**C**PKDDMYYNAAEKR**C**E
	Haa86	TSSV**C**SDFGEQF**C**QNAE**C**EVIPGREDDFV**C**R**C**PKDDMYYNAAEKQ**C**E
2	Bm86	YKDT**C**KTRE**C**SYGR**C**VESNPSKAS**C**V**C**EASDDLTLQ**C**K
	Hao3	YKRT**C**KTVK**C**SYGH**C**IQVGPGRTA**C**G**C**QGVDTLTLK**C**G
	Haa86	YKGT**C**KTVE**C**SYGN**C**VQISPGRTD**C**G**C**QGVDTLTLK**C**G
3	Bm86	VPTT**C**LRPDLT**C**KDL**C**EKNLLQRDSR**CC**QGWNTAN**C**S
	Hao3	VPTT**C**IRPDLT**C**KDL**C**EKNLLGKDTR**CC**QGWNSTD**C**S
	Haa86	VPTT**C**IRPDLT**C**KDL**C**EKNLLGKDTR**CC**QGWNPTD**C**S
4	Bm86	CINA**C**KTKEAGFV**C**KHG**C**RSTGKAYE**C**T**C**PSGSTVAEDGIT**C**K
	Hao3	CKDA**C**TTKEALLL**C**KGG**C**IKGQKPGKAYE**C**I**C**PHGYEIAEDGIT**C**K
	Haa86	CIDA**C**TTKEALLL**C**KDG**C**IKGQKPGKAYK**C**I**C**PHGYEIAEDGIT**C**K
5	Bm86	HTVS**C**TAEQKQT**C**RPTED**C**RVHKGTVL**C**E**C**PWNQHLVGDT**C**I
	Hao3	GIVD**C**TEEQKAA**C**LPGQQ**C**RVHKENSV**C**E**C**PSDQQLLDGK**C**A
	Haa86	GIVD**C**TEEQKAA**C**LPGQQ**C**RVHKENSV**C**E**C**PSDQQLLDGK**C**A
6	Bm86	CISD**C**VDKK**C**HEEFMD**C**GVYMNRQS**C**Y**C**PWKSRKPGPNVNINE**C**L
	Hao3	CASE**C**VENS**C**HENFTD**C**GVYMNKQG**C**Y**C**PWNTRKPPRGVEISR**C**V
	Haa86	CASE**C**VDNR**C**HENFTD**C**GVYMNKQG**C**Y**C**PWTTRKPPGGVEISR**C**M
7	Bm86	ERTT**C**NPKEIQE**C**QDKKLE**C**VYKNHKAE**C**E**C**PDDHE**C**Y
	Hao3	ARTT**C**NPKEIRE**C**EDKKKE**C**VYRDQKAE**C**K**C**PQGTVDDGQG**C**S
	Haa86	ARTT**C**NPKEIRE**C**EDKKKE**C**VYRDQKAE**C**Q**C**PEGTVDYGQG**C**S