Characterization of *Phlebotomus orientalis* salivary antigens

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Children with visceral leishmaniasis

Sand fly female taking the bloodmeal

LEISHMANIASIS IN ETHIOPIA

Leishmaniasis is caused by the protozoan parasite of the genus *Leishmania* (Kinetoplastida: Trypanosomatidae). There are 12 million people affected by this disease and 350 million people live at high risk areas.

Ethiopia, Kenya and Sudan are the most affected countries with visceral leishmaniasis (VL) in Africa. VL in Africa is caused by *Leishmania donovani*, which is assumed to be an antroponosis. However, it is not excluded that animals are also involved in the transmission cycle. Similar to endemic areas of *L. infantum*, dogs are the most suspected reservoir hosts for *L. donovani*.

Warning sign against VL in Mykadra

Sand fly salivary glands (Nieves et al. 2010)

Phlebotomus orientalis is one of the main vectors of *L. donovani* in Africa. Its saliva contains proteins with antihaemostatic, immunomodulatory and antigenic properties that are injected into the host skin during the bloodmeal.
Saliva induces antibody response in repeatedly bitten hosts. Antibodies are highly specific to sand fly species and reflect an exposure intensity.

The antibody amount decreases in time so it indicates a recent exposure (Hostomská et al. 2008, Vlková et al. 2011).
According to all these characteristics, antibodies against sand fly saliva can be used in epidemiological studies as exposure markers, indicating vector exposure and thus a risk of *Leishmania* transmission.

RESULTS

1) Salivary protein, MW = 27.56, kDa, pI = 4.78

```
MIVKSLLGVFLVILLVSVTEQDRGVDGHNTDHEEYDYSHVDEYDDENRHHVVNGDEEEHEMARG   64
HTKSHEHDDDDYLFSHGYDGYDDGEDEDEQQSYSRGGDRAGSHRRGSQEQSYDPHSGORAPGYA  128
ESSEYEHSGDYDNSHYQQYSSTPSTANQIDHYLNQIQLHSVPADLAQYAESYLKHSKDSIRYYAAH  194
AKDFEKIRPCLEAVMKYFNILNDDLAKEYVRCQRQCFLDRLNSYTTAISQFTVTTNNCINNRLH  258
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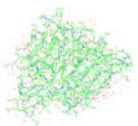
2) + 3) Unidentified salivary proteins

5) Salivary apyrase A, Apyrase family, MW = 35.53 kDa, pI = 8.95



3D model of a human apyrase

```
MILKLCAIAVIFLLIGDGEAAPRPTRFIPFAIISDLH   37
RKAMHDEKNRFTSIVKYGQLKYNGEKYTLSIRS       70
ENLHYFTQDTYKGTGAEMSELIYFNNKLYTLND      103
ETGTIYEVKHGGELIPWVTLKNDDGNDKDGFKA      136
KWATVKGDKLIVGSAGMAFLDAKTMNIDRDAL       168
WVKEISESGHITNKYWDSEYKKVRDAMGLFSGF      201
VWHGAVNWSPRKNVWVFIPRKCTNEPYTVRLD       233
KNTGCNQIITANEDFSDIKTIKIEGDIKDQASGFSS   269
FKFIPGTKNNDIFALKTIEKNGKIATYGTVIDING    304
KTLMPERRIIDDKYEGLVFFKHPIGIK            331
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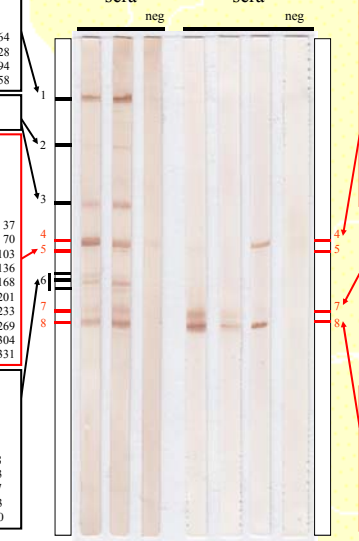
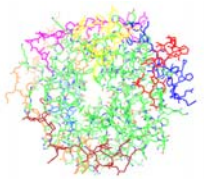
6) Salivary apyrase B, Apyrase family, MW = 35.09, pI = 8.99

```
MILKLCAIAVACLLIGEAEAAPRATKFIPFALIADFDKKSIKEDQKSFTSIVKYGELKHNGERYTLSL   68
KSENLHYFTRYAYNGRGAELSELLYFNDKLYTIGDKTGIVFEVKHGGDLIPWVILSNGPGNQKDG   133
FKAEWATVKDDKLYVGSTGMTFLDKRTGNISKNALWVKEINKEGEVISINWENQYKKVKDAMGI   197
SSGFVWHEAVNWSPRKNIWVFMPRKCSKQQFSAQIEENTGCNQIITANENFSDVKAINIDRAAVDP   263
ASGFSSFKFIPNTRNNDIFAIKTIERNGQTATYGTVIDINGKTLLPDKRILDDNMKELHFSGSQSIK   330
```

SIGNAL PEPTIDE, PROTEIN DOMAIN or FAMILY, B-CELL EPITOPES,
N-GLYCOSYLATION SPOT



4) Yellow-related salivary protein, MRJP domain, MW = 42.31 kDa, pI = 8.07



```
MKIFLCIIAVVSLQGVVAFHVEREYAWKNITYEG   34
IDPASYNIENSIPTAFAHDAASKKIFITVPRRNQIP   70
FTLTELDTTKHPERSPPLSKFPGSDKLISVYQPVI   105
DECRRLWIADVGQVEYKGDEQKYPKQKAAIIAY   138
DLTKDNYPEIDRYEIPNNVAGNSLGFGGFAVDV   171
TNPKEGCGNTFVYITNFEDNTLIVYDQEKKDSW   204
KISHGSFKPEHDSTLSHNGEQYKYRVGIFGIALG   238
DRDPEGNRPAYYIAGSSTKLFEISTKILKQKGAKF   273
DPVNLGNRGPHSEAIALAYDPKTKVIFFAEADSR   307
QISCWNIQKPLNHKNTDVIYASSKFIFGTDISVDS   342
ESQLWFLSNGQPPIDNLKLTFDKPHIRLMRVDTA   376
KAIRRTKCEVKRVKP   391
```

7) Antigen 5-related salivary protein, SCP family, MW = 28.78 kDa, pI = 8.94

```
MLQIKHFLFFVVLFVVVHANDYCQPKLCTNGKTVRPHIGCKNNGDFDRSACPNDAELLEMTQER   64
KNLFLKIHNRLRDRFARGSVPNFKSAAKMPVLKWDNELAKLAEYNVRTCKFAHDQCRATTACP   127
YAGQNLGQMLSSPDYLDPGYAIKNITREWFLEYQWANQERTNTYTAGSGKNGKQIGHFTAFVH   190
EKSDKVGCAVAKLTNRQFNMKQYLIACNYCYTNMMNERVYSTGAPCSKCQSKKCDSKYKNLC   252
DASEKVEPIPDIFLKKPRT   271
```

8) D7-related salivary protein, PBP_GOBP family, MW = 26.79 kDa, pI = 9.21

```
MNNLLTIFGLLYFLGFATSLQFPRDPDQTRWAEKTCLREFSRAPPSLLKKWQELDFPNTNLTHCFI   66
RCFTSYLGVYNETTRKFNVDGIKTQFESQGIPPPQGLETLRKTSKGTCKDIYLMTVDLIKKNKLPF   132
AKAFHGISAEAAKWYIDNKGNVKGKYQKASEFCKSKDDECRLHCRFYYYRFVDEDYQIFKRNIK   196
IPGISNAQLEQCRNRASQAKGCQVAKVLRHCLKEINPENLKATLRELDEISAK   249
```

SIGNAL PEPTIDE, PROTEIN DOMAIN or FAMILY, B-CELL EPITOPES,
N-GLYCOSYLATION SPOT

Phlebotomus orientalis salivary antigens were identified by SDS-PAGE and immunoblot. Based on the cDNA library (36 different proteins) and proteome, we determined eight antigens from four protein families: **apyrases**, **antigen 5-related proteins**, **yellow-related proteins** and **D7-related proteins**. Salivary antigens 4, 5, 7 and 8 induce antibody response both in human and canine hosts.

Salivary antigens 1, 2, 3 and 6 are canine specific. The **D7-related protein** seems to be antigenic for all tested sera, thus can be used as exposure marker and the risk marker of *Leishmania* transmission. We propose the D7-related protein in a **recombinant form** could be utilize in larger epidemiological studies.

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Photos are from the project website http://vl-ethiopia.huji.ac.il/gallery.htm.