

## Fractionation and Characterization of Different Protein Extracts from *Hyalomma dromedarii* (Acaris: Ixodidae).

Omnia M. Kandil and Salwa M. Habeeb

Department of Parasitology and Animal Diseases, National Research Center, Dokki, Giza, Egypt

**Abstract:** Three fractionated larval, eggs and salivary gland extract antigens obtained from engorged females of *Hyalomma dromedarii* for protection against tick feeding. Gel filtration was used to purify larval, eggs, salivary gland antigens of *Hyalomma dromedarii* ticks. Three purified peaks were obtained from crude larval protein, fractions (LPF1, 2, 3), egg protein fractions (EPF1, 2, 3) and salivary gland protein fractions (SGPF1, 2, 3). Analysis of the three purified peaks of each antigen by SDS- PAGE revealed 17, 9 & 6 polypeptide bands in EPF1, 2 & 3 with molecular weights ranging from 220-17.36 KDa; 231.66- 22.57 KDa and 222.73-32.54 KDa, respectively. Electrophoretic profile of LPF1, 2&3 resolved 10; 12 and 7 polypeptides with molecular weights ranging from 147.78- 15 KDa, respectively. SGPF 1, 2&3 were separated into 10, 11 and 6 polypeptides with molecular weight ranging from 244.55-25.42 KDa, 242-24.58 KDa and 236.36- 31.41 KDa, respectively. Enzyme linked immunosorbent Assay was used to determine the suitable proteins which were used to protect rabbits against female of *H. dromedarii*. Serum infected rabbits revealed higher antibodies titer against LPF2, EPF2 and SGPF2 than all fractions. These fractions were tested to protect rabbits against female ticks. Immunoblotting revealed greater IgG responses to LPF2, EPF2 and SGPF2 in rabbit immunized with their proteins or rabbit exposed to ticks (after third fed) compared to naïve rabbits. It was concluded Serum infected rabbits revealed higher antibodies titer against EPF2; LPF2 and SGPF2 than all fractions derived from egg, larvae and salivary gland of *H. dromedarii* extracts.

**Key words:** *Hyalomma dromedarii* % Gel filtration % SDS- PAGE % ELISA % Immunoblot % Fractionation % Immunization

### INTRODUCTION

The camel tick *H. dromedarii* is widely distributed in desert, semi desert and steppes wherever camels occur [1]. It is a vector of many disease agents such as protozoa diseases [2], bacteria [3], virus [4] and rickettsia [5]. Intensive application of chemotherapy control or acaricides to manage the ticks infestation in many regions of the world is common [6]. Problems of chemical residues environmental pollution and development of resistance by ticks to the acaricides, have been recognized and helped to stimulate interest in tick control by immunological means [6]. The primary step in vaccine development is the identification of suitable antigen targets [6]. The development of antitick vaccines represent the most promising alternative to chemical control. It has many advantages, free of residues, less cross- species action, cheaper, environmental safety and less resistance, lack of human health risk and ease of administration [7,8].

Several investigations have successfully induced host resistance by administration of tick antigen derived from salivary gland, [7,9,10]. They also showed that vaccination effects the ability of ticks to transmit diseases. [8,11] showed that tick antigens isolated from different developmental stages (larvae, nymphs and adults) and tissues (midgut, synjamgion, integument and hemolymph) have been tested for their potential to induce artificial resistance to ticks. [12] vaccinated rabbits with *H. dromedarii* tick eggs antigen. He found that a significant reduction in tick weight egg mass weight, oviposition period and percentage of eggs hatchability. The use of concealed tick antigens was considered the first step for the basis of a commercial vaccine. Therefore, several workers have reported development of acquired immunity against tick by artificial immunization with tick salivary gland antigens, *Boophilus microplus* [13]. *H. anatolicum anatolicum* [14] and *H. dromedarii* [15].

The present work aim to investigate upon the protective effect of various salivary gland, larval and egg antigens of *H. dromedarii* (semi purified) as trial for vaccination.

## MATERIALS AND METHODS

**Ticks:** About 205 engorged females of *H. dromedarii* (Koch 1818) were collected from the ground of camel pens, Burkash village, Giza governorate, Egypt and identified according to Estrada-Pena *et al.* [16]. Five engorged females were kept individually in plastic tubes and incubated at 26°C, 75% RH and photoperiod of 12:12 (L:D) h throughout oviposition.

**Antigens Preparation:** Salivary glands were collected from 200 adult ticks (semi-engorged females). Females 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.

About 500 eggs and the same number of larvae were taken from the five incubated females. Eggs and larvae were placed into PBS (pH 7.4 at 4°C).

Salivary glands, eggs and larvae were disrupted for 30 second in PBS at 4°C with a tissue homogenizer followed by sonication for 15 seconds and centrifugation according to El-Kammah and Sayed [17]. The protein content of the supernatant was determined by the Lowry method [18].

**Fractionation of Crude Extract Larval, Eggs and Salivary Gland Antigens:** The fractionated antigens were obtained by Sephadex G100 (Pharmacia Chemical Co., Sweeden). Peaks were collected 1ml in each tube at a flow rate of 1.5 ml hG<sup>1</sup> and the run was terminated the protein absorbance at 280 nm and monitored by Giford -Spedro-photometer (instrument laboratories, USA). Peak 1,2,3 from each antigen were pooled separately, concentrated and estimated as protein of lowery method [18].

**Sodium Dodecyle Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE):** All fractions from salivary glands, larvae and eggs were analysed using SDS-PAGE for determination of protein bands and their molecular weights for each fraction. as described by Hames [20].

**Antibody-antigen Interaction:** The antibody-antigen interaction was measured by using the ELISA technique according to Voller *et al.* [21]. ELISA was used to

compare the protein which fractionated from three different antigen extracts obtained by gel filtration. This assay was done by using the serum rabbits which were infested by the adult ticks of *H. dromedarii*. ELISA was used to determine the most sensitive and specific protein fractions which used to immunize rabbits.

**Experimental Design:** Twelve healthy New Zealand white male rabbits (1.5-2 kg), had no previous contact with ticks were used for immunization. Rabbits were divided into 4 groups, 3 rabbits for each group.. Groups were inoculated as follows, first group with LPF2, second group with EPF2 and third group with SGPF2. These groups were immunized intramuscularly, in the form of three injections at 21 days intervals[22]. The fourth group was kept as control (repeated infestation). Rabbits were inoculated with 150 µl protein fraction two of salivary glands (SGPF2), larval (fraction two (LPF2) and eggs fraction two (EPF2) suspended in equal volume of complete Freund's adjuvant at the first injection. while, in the second and third injections the same volume of protein fractions was suspended in equal volume from incomplete Freund's adjuvant. All rabbits in immunized groups were challenged one week after the last injection with 10 males and 10 females of *H. dromedarii* for each rabbit. One week later after the first challenged animals were challenged again according to Jittapalapong *et al.* [22]. Fourth group of rabbits were infested five times at 21 days intervals with 10 males and 10 females of *H. dromedarii* for each rabbit at each infestation.

**Rabbit Sera:** Blood samples were obtained from all groups of rabbits at zero time, after third injection; third infestation; after first and second challenge; fourth and fifth infestation. Serua were separated and stored at -20°C until used.

**SDS-PAGE and Western Blot Techniques:** To detect antibody response, peak two for each eggs, larval and salivary glands extracts were used in this assay. The proteins were electrophoretically transferred to a nitrocellulose membrane using standard method [23]. The nitrocellulose strips blotted with tick peak two were tested in two groups. The first group was allowed to react with pooled sera from three groups of rabbits immunized with peak two of larvae, salivary glands and eggs (after third injection) and from non-infested rabbits. The second group was allowed to react with serum from the previous group of rabbits after second challenge and control group (infested rabbits) after third infestation.

The molecular weights of specific and non specific polypeptides were determined using low molecular weight standard curve as described by the producer (Pharmacia Pharmaceutical Co., Sweden)

## RESULTS

**Identification of Protein Fractions:** Three absorbent peaks were resolved by gel filtration from tick-eggs, larval and salivary gland extracts, individually. The elutes corresponding to these peaks were collected and pooled into three fractions peaks 1, 2 and 3. The protein content of these fractions were 2.8, 1.9 and 1.3 mg at peak 1, 2 and 3, respectively for tick-eggs and 2.1, 1.6 and 1.1 mg and 1.5, 1.4 and 1.2 mg at peak 1, 2 and 3 for tick-larvae and salivary gland antigens, respectively.

**Identification of Polypeptides:** Electrophoretic separation of fractionated antigens with commassie blue staining showed that seventeen, nine and six polypeptides were identified from EPF1, 2 &3 with molecular weights ranging from 220-17.36 KDa; 231.66- 22.57 KDa and 222.73-32.54 KDa, respectively. Electrophoretic separation of LPF1, 2&3 resolved 10; 12 and 7 polypeptides with molecular weights ranging from 147.78- 15 KDa, respectively. SGPF 1, 2&3 were separated into 10, 11 and 6 polypeptides with molecular weight ranging from 244.55-25.42 KDa, 242-24.58 KDa and 236.36- 31.41 KDa, respectively, Table 1 and Fig. 1.

Table 1: Molecular weights (MW) of protein fractions derived from larvae, eggs and salivary glands of *H. dromedarii*

Larva		Egg			Salivary gland				
LPF1	LPF 2	LPF 3	EPF 1	EPF 2	EPF 3	SGPF 1	SGPF 2	SGPF 3	
147.78	219.39	239.09	220	231.66	310	244.55	242	263.64	
88.684	168.95	214.55	190	186.34	255.45	153.89	221	236.36	
78.684	101.97	170.91	146.25	167.12	222.73	111.11	192.5	214.55	
69.521	86.82	153.89	102.37	112.64	179.09	98.684	178.4	140.14	
55.822	64.95	120.28	85.526	84.31	132.5	85.526	160.8	124.36	
40.212	58.50	83.947	72.26	43.59	100.26	75	120		
34.561	47.98	60.274	61.301	29.39	74.658	65.753	75.00	75	
31.734	37.96		51.712	25.95	32.54	43.644	58.5		
28.305	27.96		46.059	22.57		34.798	41.26	31.411	
20.273	25.10		41.102			25.424	29.02		
	22.19		35.636				24.58		
	15		33.185						
			32.056						
			31.008						
			26.864						
			21.545						
			17.364						

**Enzyme Linked Immuno Sorbent Assay (ELISA):** Serum infested rabbits by adult females of *H. dromedarii* showed higher titer antibodies against LPF2 and EPF2 than LPF1, LPF3 and EPF1; EPF3, respectively (Fig. 2 a,b). The interaction between serum infected rabbits and SGPF1, 2&3 showed higher antibodies titer than non infested sera rabbits (Fig. 2 d) Serum infected rabbits revealed higher antibodies titer against EPF2; LPF2 and SGPF2 than all fractions derived from egg, larvae and salivary gland of *H. dromedarii* extracts. In this investigation EPF2, LPF2 and SGPF2 were tested to protect the rabbits against female of *H. dromedarii*.

**Western Blot Analyses of LPF2, EPF2 and SGPF2 with Rabbit Sera (Fig. 3 a,b):** LPF2, EPF2 and SGPF2 were analyzed by western blotting. Membranes were immunoprobed with a 1: 50 dilution of sera obtained from naïve rabbits, rabbits repeatedly infested (after third feeding), rabbits immunized with those three proteins LPF2, EPF2, SGPF2, individually. Sera obtained after second challenge with female tick *H. dromedarii* at rabbit immunized with LPF2, EPF2 and SGPF2 separately Fig. 3 a,b. The antigenic pattern of LPF2, EPF2 and SGPF2 varied according to proved serum used. Sera obtained from naïve rabbits showed 58.49 and 47.98 KDa with LPF2; 84.31, 43.59, 29.38 KDa with EPF2 and 41.26 and 29.02 KDa with SGPF2. 168.95, 101.97, 64.95, 47.98 and 37.48 KDa LPF2 antigens were detected by sera obtained from rabbits immunized with its. EPF2 antigen showed 186.34, 112.64 and 43.59 and 29.38 KDa by sera obtained from rabbits immunized with LPF2. The 192.5 KDa, 58.5 and 29.02 KDa SGPF2 antigens were recognized by sera obtained from rabbits immunized by LPF2. The 168.95, 86.81, 58.49, 47.98, 37.48, 167.12, 112.64, 43.59, 29.38 and 192.75, 75, 41.26 and 29.02 KDa were recognized in the LPF2, EPF2 and SGPF2, respectively by sera obtained from rabbits immunized with EPF2. Most of detected bands in the LPF2, EPF2 and SGPF2 by serum from rabbits immunized with SGPF2, these bands were 168.95, 64.95, 58.49, 27.95 KDa in the LPF2; 167.12 KDa, 112.64, 84.31, 75.59 and 34.95 KDa in the EPF2 and 242, 41.26 KDa in the SGPF2. After third infestation, sera from rabbits repeatedly infested with adult ticks *H. dromedarii* showed 58.49, 22.19 KDa and 41.26, 29.02 KDa in the LPF2 and SGPF2, respectively. New detected proteins from EPF2, 43.59, 25.39 KDa did not recognize by sera obtained from naïve rabbits. Two bands, 64.95, 37.49 KDa were detected in the LPF2 by sera obtained from rabbits immunized with LPF2 after second challenge. Four bands 160.0, 58.5, 41.29 and 29.02 KDa were recognized in the SGPF2 by

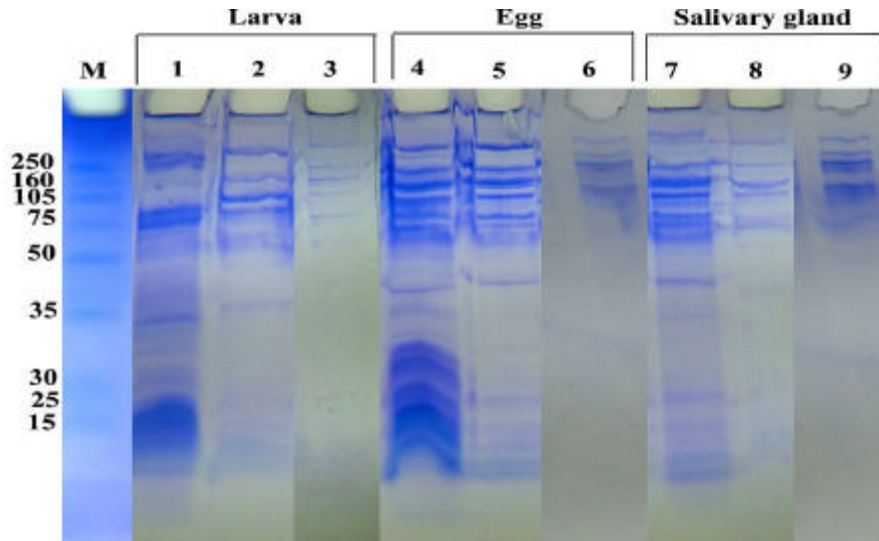


Fig. 1: Electrophoretic profile of protein fractions of larvae, eggs and salivary glands for tick *H. dromedarii*

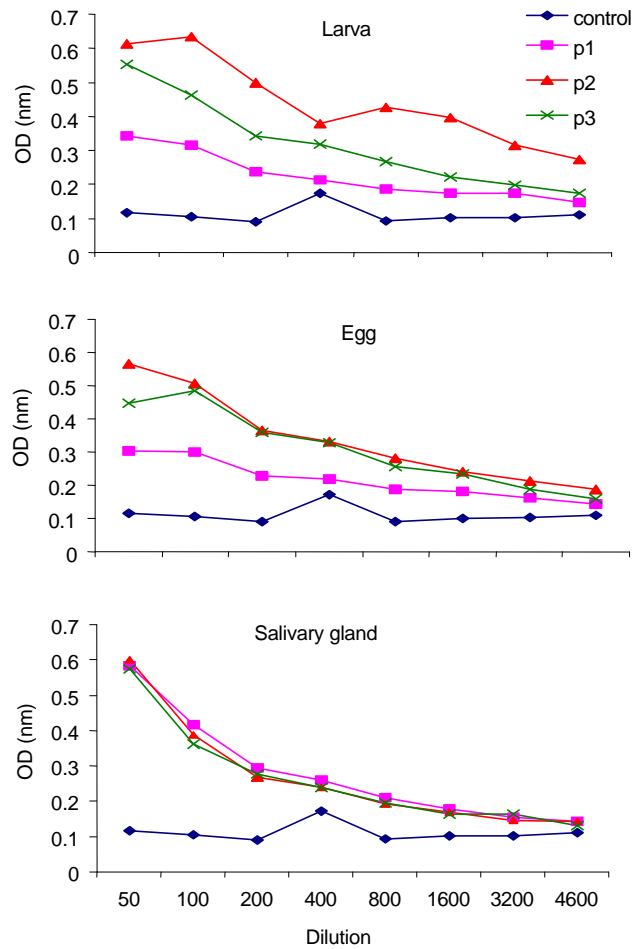


Fig. 2: Antibody protein fractions interaction for larva, egg and salivary gland of *H. dromedarii*

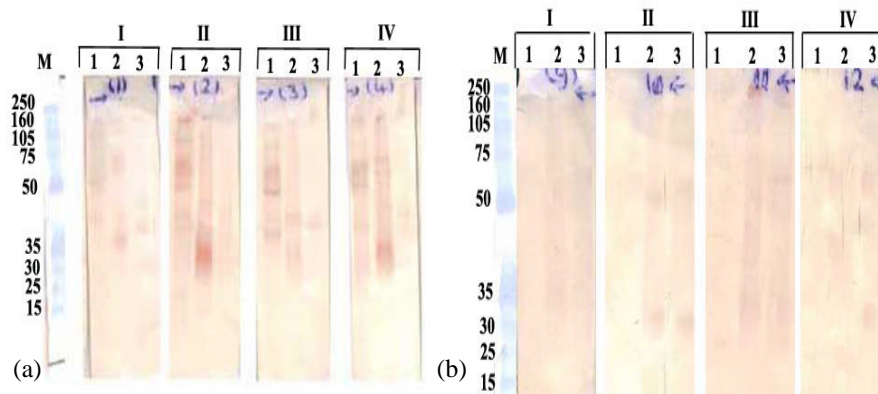


Fig. 3a,b: **a.** Showing the Western blots of serum samples collected from tick-naïve rabbits (I), immunized rabbits with LPF2 (II), immunized rabbits with EPF2 (III) and immunized rabbits with SGPF2 (IV). 1= LPF2, 2= EPF2, 3= SGPF2. **b.** shows the Western blots of serum samples collected after challenge two from: immunized rabbits with LPF2 (I), immunized rabbits with EPF2 (II), immunized rabbits with SGPF2 (III) and control group (after third infestation) (IV). 1= LPF2, 2= EPF2, 3= SGPF2.

sera obtained from rabbits immunized with LPF2 (after second challenge). Three proteins were recognized in the LPF2, EPF2 and SGPF2 by sera obtained from rabbits immunized by EPF2 (after second challenge) with female of *H. dromedarii*. The molecular weights of these proteins were 219, 64.95, 37.49KDa in LPF; 84.31,53.59 and 29.38 KDa in EPF2; 221.8, 58.5 and 29.02 KDa in SGPF2. Antigens of 86.81, 64.95,47.98, 37.49, 27.96 KDa were found in the LPF2; 167.12, 112.64,84.31,43.59,29.39 KDa were found in the EPF2, 120.8,41.26 and 29.02 KDa and 58.5 KDa were found in the SGPF2 by sera obtained from rabbits immunized with SGPF2 after second challenge with adult tick *H. dromedarii*.

## DISCUSSION

Immunization trials play an important role in tick feeding and transmitting disease agents. The use of different partial purified antigens were based on the concept that tick feeding on appropriately immunized hosts might ingest antibodies specific for target antigens within the tick, producing deleterious effects on the feeding and reproductive performance of the tick [24]. Tick salivary gland antigens therefore seem to have promising potential for inducing immunity in calves[25].

Sephadex G 100 gel filtration can be used to fractionate immune protective antigens from crude EPF, LPF and SGPF. From camel tick *H. dromedarii* extracts. Three fractions were obtained from each of EPF. LPF, SGPF. However, [10] separated two fractions from SGPF from tick *H. dromedarii* antigen and also [26] found two fractionated gut extract antigens from engorged females of *H. dromedarii* using sephadex G 100 gel filtration.

The present study demonstrated the presence of few glycoproteins common between fractions of EPF, LPF and SGPF of *H.dromedarii*. The electrophoretic pattern of the LPF1, EPF1 and SGPF2 revealed that two shared antigens between them, 34 KDa and 85 KDa. Low molecular weight band of 31KDa was detected in the two fractions (EPF1 and SGPF3) of *H. dromedarii* ticks.

ELISA assay determined the reactivity between LPF1, EPF1, SGPF1 and antisera of adult *H. dromedarii* ticks.. The presence of antibodies against LPF1,2,3 antigens in the serum of rabbit infested with adult ticks was evident. A cross reaction was found between EPF 1, 2, 3 antigens and antisera to adult ticks of *H. dromedarii* indicating the presence of antibodies against EPF1, 2, 3 in the serum of rabbits infested with adult of *H. dromedarii*. The reaction was evident between SGPF1, 2, 3 and antisera to adult ticks of *H. dromedarii*. Our results agree with Abou-El-Dobal *et al.* [27] and Ogden *et al.* [28]. This finding combined with highly significant differences between *H.dromedarii* exposed and *H. dromedarii* – naïve rabbit in their IgG ELISA results. The variations in ELISA results were mostly associated with variations in responses to *H. dromedarii* antigens rather than cross- reactive antigens.

A comparison was made between three proteins LPF2, EPF2 and SGPF2 by western blot analysis with sera derived from control rabbits, rabbits immunized with LPF2, EPF2 and SGPF2, individually, sera obtained from repeated infestation of rabbits (third fed). Sera obtained after second challenge by western blot analysis showed variations in IgG responses to *H. dromedarii* LPF2, EPF2 and SGPF2 antigens. The presence of bands on western

blots probed with sera from *H. dromedarii*-naïve-rabbit suggested the potential for rabbit to be exposed to non-*H. dromedarii* antigens that shared some epitopes with some LPF2, EPF2 and SGPF2 proteins or cross reactivity between secondary antibody and LPF2, EPF2, SGPF2 proteins. Previous studies have also suggested some cross reactivity between ticks (SGE) salivary gland extract antigens and non tick antigens in sheep [28,29]. The greater IgG responses to LPF2, EPF2 and SGPF2 in rabbit immunized with their proteins or rabbit exposed to ticks after third fed compared to naïve rabbits suggested that rabbit resistance to ticks was associated with increased specific IgG responses.

On the other hand, sera derived from rabbits immunized with LPF2 after second challenge and repeated infestation of rabbits after third infestation did not show new protein bands with EPF2 and SGPF2 respectively. It is suggested that antibodies in sera derived from rabbits immunized to react with LPF2 did not find specific protein in the EPF2 and SGPF2.

Characterization of the immunodominant polypeptide would be an important step in the development of *H. dromedarii* tick immunogenes by methods involving recombinant DNA technology and monoclonal antibody technique. In conclusion, the peak two of larval, egg and salivary gland fractions are unique secretory proteins associated with *H. dromedarii*. Therefore, our further intention to study the protective potential of this isolated protein against multi-tick infestation may consider as preliminary step for a vaccine production against different ticks.

## REFERENCES

1. Higgins, A.L., 1985. The camel in health and disease.4. Common ectoparasites of the camel and their control. Bulletin Vet. J., 141: 197-215.
2. d'Oliveira, C.M., Van der Weide, P. Jaequiet and F. Jomgejan, 1997. Detection of *Theileria annulalata* by the PCR in ticks (Acari: Ixodidae) collected from cattle in Mauritania. Experimental. App. Acarol., 21: 279-291.
3. Montasser, A.A., 2005. Gram- negative bacteria from the camel tick *Hyalomma dromedarii* ( Ixodidae) and the chicken tick *Argas persicus* ( Argasidae) and their antibiotic sensitivities J. Egyptian Soc. Parasitol., 35: 95-106.
4. Gunes, T., 2006. Crimean- Congo Hemorrhagic Fever. Microbial Bulletin, 40: 279-287.
5. Loffis, A.D., W.K. Reeves, D.E. Scumlas, M.M. Abbassy, I.M. Helmy, J.R. Moriarity and G.A. Dasch, 2006. Rickettsial agents in Egyptian ticks collected from domestic animals. Experim. Appl. Acarol., 40: 67-81.
6. Mulenga, A., C. Sugimoto and M. Onuma, 2006. Issues in tick vaccine development: Identification and characterization of potential candidate: vaccine antigens. Microbes Infection., 2: 1353-1361.
7. Wikel, S.K., 1996. Host immunity to ticks. Ann. Rev. Entomol., 41: 1-22.
8. Willadsen, P., 1997. Novel vaccines for ectoparasites. Vet. Parasitol., 71: 209-222.
9. De la Fuente, J., M. Rodriguez, M. Redonda, C. Montero, J.C. Garcia-Garcia, L. Mendez, E. Serrano, M. Valdes, A. Enriquez, M. Camales, E. Ramos, O. Bone, H. Machado, R. Lleomart. C.A. de Armas, S. Rey, J.L. Rodrigues, M. Artilles and L. Garcia, 1998. Field studies and cost – effectiveness analysis of vaccination with Gavac TM against the cattle tick *Boophilus microplus*. Vaccine, 16: 366-373.
10. Mahmoud, M.S., S. Abdel- Shafy and S.K.A. Abou El-Dobal, 2005. Evaluation of crude and purified salivary gland antigens for protection against camel tick *Hyalomma dromedarii* (Acari: ixodidae). J. Egyptian Vet. Med. Assoc., 65(4): 229-243.
11. Kinyua, J.K., E.O. Osir, D.O. Ogoyi and E.K. Nguu, 2002. Characterization of protective antigens from the midgut of *Amblyomma variegatum* ticks. Experim. Appl. Acarol., 26: 101-113.
12. Omar, S.F.A., 1999. Experimental studies on vaccination of rabbits against hard ticks *Hyalomma dromedarii*. Vet. Med. J., 47: 457-466.
13. Parmar, B.A., A.S. Greal and P. Dhillin, 1996. Immunological cross- reactivity between salivary gland proteins of *Hylomma anaticum* and *Boophilus microplus* ticks. Vet. Immunology and Immunopathology, 51: 345-352.
14. Sran, H.S., A.S. Grewal and J.K. Kondal, 1996. Enhanced immunity of *Hyalomma anaticum* ticks in cross- bred (*Bios indicus* x *Bos Taurus*) calves using ascaris extract immunonobilator with the tick salivary gland extract antigens. Vet. Immunology and Immunopathology, 51: 333-343.
15. El-Kelesh, E.A.M., 2002. Application of the *Hyalomma dromedarii* salivary gland epitopes for protection against camel ticks. PH.D. Thesis, Faculty of Veterinary of Medicine, Cairo Universty, Egypt

16. Estrada-Pena, A., A. Bouattow, J.L. Camicas and A.R. Walker, 2004. Ticks of domestic animals in the Mediterranean region. A guide to identification of species-First edition., pp: 131.
17. El-Kammah, M.K. and M.A. Sayed, 1999. Biochemical fingerprints of egg and salivary gland characterizing four common tick genera in Egypt. Arab J. Biotechnol., 2: 127-134.
18. Lowry, O.H., N.J. Rosenbrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with Folin-phenol reagent. J. Biol. Chem., 193: 265-275.
19. Kazimirova, M., V. Jancinova, M. Petrikova and M. Labuda, 2002. An inhibitor of thrombin-stimulated blood platelet aggregation from the salivary glands of the hard tick *Amblyoma variegatum* (Acari: Ixodidae). Experim. Appl. Acarolog, 28: 97-105.
20. Hames, B.D., 1987. Gel electrophoresis ceds, B. D. Hames and Rickwood, 6<sup>th</sup> edition, TRL Press, pp: 86.
21. Voller, A., D. Bidwell and A. Bartlett, 1976. Enzyme immunoassay in diagnostic medicine. Bulletin W.H.O., 53: 55-56.
22. Jittapalpong, S., R.W. Stich, J.C. Gordon, T.E. Wittum and O.O. Barriga, 2000. Performance of female *Rhipicephalus sanguineus* (Acari: Ixodidae) fed on dogs exposed to multiple infestations or immunization with tick salivary gland or midgut tissues. J. Med. Entomol., 37: 601-11.
23. Towbin, H., T. Sahelin and G. Gordon, 1979. Electrophoresis transfer of proteins from polyacrylamide gels to nitrocellulose sheets and some applications. Proceeding of National Academic Science USA., 76: 4350-4354.
24. Johnston, L.A.Y., D.H. Kemp and R.D. Pearson, 1986. Immunization of cattle against *Boophilus microplus* using extract derived from adult female ticks: Effects induced immunity on tick population. Intl. J. Parasitology, 16: 27-34.
25. Banerjee, D.P., R.R. Momin and S. Samantray, 1990. Immunization of cattle (*Bos indicus x Bos Taurus*) against *Hyalomma anatolicum anatolicum* using antigens derived from tick salivary gland extracts. Intl. J. Parasitology, 20: 969-972.
26. Abdel-Shafy, S., M.S. Mahmoud and M.M. Abdel-Aziz, 2008. Evaluation of crude and fractionated gut extract antigens for protection against camel tick *Hyalomma dromedarii* (Acari: Ixodidae). J. Entomology, 5: 91-102.
27. Abou-El-Dobal, S.K.A., A.A. Derbala, A.K. Ahmed and M.A. Hassanain, 1999. Characterization of different extracts from *Hyalomma dromedarii* (Acari: Ixodidae) SDS-PAGE electrophoresis and western blot techniques in experimentally infected rabbits. Egyptian J. Zool., 32: 459-483.
28. Ogden, N.H., A.N.J. Casey, C.H. Lawrie, N.P. French, Z. Wolehiwet and S.D. Carter, 2002. IgG responses to salivary gland extract of *Ixodes ricinus* ticks vary inversely with resistance in naturally exposed sheep. Med. Vet. Entomol., 16: 186-192.
29. Barriga, O.O., F. Andujar and W.J. Andrzejewski, 1991. Manifestations of immunity in sheep repeatedly infested with *Amblyomma americanum* ticks. J. Parasitology, 77: 703-709.