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RESEARCH ARTICLE

IMMUNOGENICITY OF SALIVARY GLAND AND MIDGUT HOMOGENATE ANTIGENS AGAINST CAMEL TICK HYALOMMA DROMEDARII (IXODOIDEA: IXODIDAE) AND RELATED BIOLOGICAL IMPACTS AND CONTROL POTENTIAL : IN VIVO STUDY.

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Abstract

Salivary gland (SgAg) and midgut (MgAg) antigens derived from partially fed female *H. dromedarii* were used to vaccinate rabbits at a dose of 100 µg/kg protein. The immunized hosts were challenged with unfed female ticks. Data revealed a significantly decreased feeding percent and duration, engorged tick and egg mass weights, number of eggs laid, hatching percent and fertility compared to control groups. Overall vaccine efficacy was evaluated as 67% and 43.79% for SgAg and MgAg, respectively, that means tick Ags used induced best protection against *H. dromedarii* population. Immunogenicity of experimental vaccine homogenates revealed a strong and specific immune response against both antigens. Western blot analysis revealed that 7 polypeptide bands ranging in their molecular weights from 95.8 to 32.3 kDa were recognized in SgAg, whereas 10 polypeptide bands ranging from 93.7 to 8.6 kDa were identified in MgAg.

Introduction:-

Ticks are important ectoparasites of domestic animals **(Roberts & Janovy, 2005)**. In Egypt, *Hyalomma dromedarii* is an important pest of livestock parasitizing chiefly camels and sometimes attack man. As they feed for extended periods of time on their hosts, they cause substantial economic loss to camel production through anaemia, toxicoses and severe physical damage to hides **(Ibrahim *et al.*, 2001)**. This tick species plays an important role in transmitting haemoprotozoan disease, bovine tropical theileriosis **(Bhattacharyulu *et al.*, 1975)** and camel theileriosis and babesiosis **(Mazyad & Khalaf, 2002)**. The idea of developing immuno-based prophylactic measures against tick infestations is based on the concept that ticks feed on immunized hosts may ingest antibodies specific for a target antigen(s) within the tick, thereby exerting deleterious effects on feeding and reproduction **(Allen & Humphreys, 1979; Pruett, 1999)**. **Trager (1939a)** observed the development of natural resistance to *Dermacentor variabilis* after a single infestation of the host. Several researchers attempted to induce immune resistance by the inoculation of the host with different tick extracts. Salivary glands are employed for vaccination as they appear intuitively as an obvious source of naturally exposed antigens injected by the tick into the host during feeding **(Mulenga *et al.*, 1999)**. However, successful immunization of hosts against tick infestations using gut and other non-salivary extracts

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(Concealed antigens) of ticks was assessed for the first time by **Allen & Humphreys (1979)**. Concealed antigens are preferred over exposed ones since natural antigens were supposed to have co-evolved with the host and lost part of their antigenicity **(Tellam *et al.*, 1992)**, mediated largely by immediate type hypersensitivity **(Willadsen, 1987)** and the vaccine that caused allergy would be undesirable. However, their limitations are many such as they does not prevent feeding of ticks, therefore, does not stop the damage to the hides or the transmission of the tick-borne pathogens. Moreover, immunity conferred by them is not boosted by natural infestations like natural antigens, and require periodic revaccination especially in endemic areas **(Sahibi *et al.*, 1997)**. So that, identification of potent antigens especially those exposed ones in order to overcome these limitations must continue. Literature dealing with the immunization of hosts with tick-derived salivary gland and midgut reports their great interference with feeding and reproductive performances of females belonging to various species (**Allen & Humphreys, 1979**; **Brown and Askenase, 1986**; **Manohar and Banerjee, 1992**; **Kumar and Kumar, 1995; Sahibi *et al.*, 1997; Szabó and Bechara, 1997; Jittapalapong *et al.*, 2000 & 2004; Abdel-Shafy *et al*., 2008; Asif *et al.*, 2011)**. The present study was undertaken to assess the induced effects in the tick *H. dromedarii* after feeding on hosts injected by three subcutaneous inoculations of 100 µg/kg protein of both SgAg and MgAg on 0, 14 and 28 day. The work includes the main biological parameters affecting reproduction and development such as feeding period, amount of ingested blood, oviposition, hatchability and fertility. The dose was further used to investigate the humoral immune response (Total IgG) and to determine the immunoreactive protein bands in both tested antigens following their fractionation.

Materials and methods:-

Tick origin and colonization:-

Engorged *H. dromedarii* females collected from infested camels at Imbaba market, Giza governorate, Egypt were used to start a colony in the Parasitology Department laboratories, Animal Health Research Institute, Dokki, Giza, Egypt. The rabbit *Oryctolagus cuniculus* (2.5-3 kg weight) not previously attacked with ticks and free from coccidian infection was used as a host. Ticks were kept at 28±1^{0}C and relative humidity of 75% as described by **Berger *et al.* (1971)**.

Preparation of salivary gland and midgut antigens:-

Salivary gland and midgut tissues from approximately 200 semi-fed *H. dromedarii* females were prepared. Living semi-fed females were washed with 0.01 M Phosphate-buffered saline (PBS, pH 7.2) and embedded in a Petri-dish containing a mixture of paraffin wax and charcoal. The dorsal integument of the tick was removed and the internal organs were immersed with PBS. Salivary glands and midgut were removed, placed in cold PBS (4^{0}C) and stored at -20^{0}C. They were subsequently thawed and sonicated by an ultra-sonicator in ice bath at 55,000 cycle/sec five times, one minute each, followed by one minute as cooling interval. The extracts were centrifuged at 4^{0}C for 1 h at 14000 rpm, using high speed cooling centrifuge, according to **Heller-Haupt *et al.* (1996)**. The resulting supernatant of each tissue was separated and used as antigenic material. The protein content of SgAg and MgAg, was measured using the micro total protein (MT-P) Pyrogallol-Red kit (Egyptian company for Biotechnology (S.A.E).

Immunization:-

Rabbits were artificially immunized against *H. dromedarii* tick infestation according to the protocol of artificial immunization **(Sran *et al.*, 1996)**. Rabbits were divided into 4 groups,(3/ each). Group I was kept as negative control, group II inoculated with aluminium hydroxide adjuvant (Alum) while groups III and IV inoculated with MgAg and SgAg, respectively. Rabbits of groups III and IV received three subcutaneous inoculations of 100 µg/kg protein **(Abdel-Shafy *et al.*, 2008)** emulsified with Alum in equal volumes. The first immunization dose was administered on day 0 post emulsification with Alum. The second and third doses were given on days 14 and 28 respectively same as the first dose. On the other hand, group II received three inoculations of 1ml PBS with an equal volume of Alum at the same days as groups III and IV. On day 36 post-immunization, rabbits were infested with adult unfed *H. dromedarii* ticks. The infestation was carried out using 10 males and 10 females per rabbit. Daily observation was performed to detect some biological parameters of females.

Biological parameters:-

The biological performance of females was examined by determination of their feeding percent and period, oviposition, hatchability and fertility. Feeding % was determined by dividing the number of fed females / total number of females x 100. Feeding period is the time taken from starting feeding to engorgement. The amount of blood ingested by females was determined by the weight prior to feeding subtracted from the weight directly post feeding. Oviposition was represented by oviposition percent (number of ovipositing females / total number of females x100), pre-oviposition period (days from engorgement to onset of egg laying), and the number of eggs for

the oviposited female. Hatchability was represented by the pre-hatching period (days from onset of egg laying to onset of hatching) and hatching percent (number of hatched eggs / total number of eggs X100). Fertility is the weight of egg mass divided by the weight of replete female **(Khalil *et al.*, 1984)** in addition to the weight of one egg (Weight of egg mass/Total number of eggs). The efficiency of the tested vaccines was assessed by determining the number of engorged females (%DT), the egg laying capacity (%DO) and the efficacy (%E) **(Galaï *et al.*, 2012)** where %DT = $100[1-\left(\frac{NTV}{NTC}\right)]$ (NTV is the mean number of engorged females collected from vaccinated groups and NTC is the mean number of engorged females collected from the control group), %DO = $100[1-\left(\frac{PATV}{PATC}\right)]$ (PATV is the average weight of eggs per replete female of the vaccinated groups and PATC is the average weight of eggs per replete female of the control group) and %E = $100[1-(CRT\ x\ CRO)]$ [CRT is the reduction in the mean number of females in vaccinated groups compared to the control group (NTV/NTC) and CRO is the reduction in the egg laying capacity of vaccinated females compared to the control group (PATV/PATC)].

Immune response:-

Blood samples were collected weekly from the ear vein of rabbits from all groups till the 7^{th} week post-immunization. Sera were separated, aliquoted and stored at - 20˚C till use. The antibody levels against SgAg and MgAg were measured using indirect ELISA technique according to **Voller *et al.* (1976)** with some modifications. Initially, a checkerboard titration was used to optimize the reaction conditions regarding the sensitizing antigen concentration, antibody and conjugate dilutions. Post optimization, the tested antigens were applied in a concentration of 20 µg/ml coating buffer (0.1 M carbonate-bicarbonate buffer, pH 9.6), and 4% bovine serum albumin (BSA) in PBS buffer was used to block nonreactive sites on the microtiter plates. Serum samples were dispensed in triplicates and the starting dilution of sera samples was 1:100 prepared in 1% BSA/PBS, while anti rabbit conjugate was used as 1/1000 in 1% BSA/PBS. Ortho-phenylene diamine (OPD) was used as a substrate and allowed to react in the dark at room temperature for 15-20 minutes. The reaction was stopped with 1N H_2SO_4 and optical density (OD) was measured at 450 nm by ELISA reader (BioRad, USA).

Polyacrylamide Gel Electrophoresis (SDS-PAGE):-

Electrophoretic analysis of SgAg and MgAg was performed using the Mini-Protein II Dual-Slab Cell (BioRad, USA) and 10% SDS-PAGE under reducing conditions according to the method described by **Laemmli, 1970**. Protein bands were visualized by Commassie blue stain.

Western blot analysis:-

The profile of reactive bands of the tested antigens was recognized by hyperimmune sera collected from rabbits on day 36-post primary immunization. The electrophoresed antigens were transferred from gels to nitrocellulose membranes for immunoblotting using a Bio-Rad Semi-dry transfer cell according to the manufactures protocol. Membranes were cut into 0.5 cm stripes, blocked with 5% skim milk/PBS-Tween and probed with rabbit anti-sera 1:100 diluted for $1\frac{1}{2}$ h over a shaker at room temperature. After four wash cycles with hot PBS-Tween (65˚C), membranes were incubated with anti-rabbit IgG peroxidase conjugate at a dilution of 1:500 in 5% skim milk/PBS-Tween. After three wash cycles with hot PBS-Tween (65˚C) and two times with PBS alone, chromogen DAB/PBS-30% H_2O_2 was added to the nitrocellulose stripes for 10-15 minutes allowing the colorimetric reaction to develop. The reaction was stopped using distilled H_2O and visualized by the naked eye.

Statistical analysis:-

Data were statistically analyzed by **SAS (2004)** using general linear model procedure (GLM) classification, followed by Duncan Multiple Range Test to examine the significance between means.

Results:-

Immunization of rabbits using MgAg and SgAg affected markedly the ability of females to attach and feed. It resulted in a substantial reduction ($P < 0.01$) in the feeding percentage to 83.33% and 70%, respectively, versus 100% and 95% of negative control and Alum-inoculated groups [Table 1]. On the other hand, the time required for full engorgement was significantly reduced ($P < 0.05$) post immunization. The shortest feeding period was observed in group IV being 6.21 days compared with the other groups recording 6.94, 6.73 and 6.44 days for groups I, II and III, respectively. The amount of blood ingested by females fed on immunized rabbits exhibited a significant decrease ($P < 0.01$) compared to those fed on unvaccinated one. Females of groups III and IV ingested 633.50 and 572.00

mg, respectively, whereas those of the control ones (I and II) consumed 868.63 and 774.33 mg respectively [Table 1].

Table 1:- Effects of salivary gland and midgut antigens on feeding percent, feeding period and amount of ingested blood by female *H. dromedarii*.

Biological parameter	Group I	Group II	Group III	Group IV
Feeding percent % ± SE	**100.00 ± 4.082^{a}**	**95.00 ± 4.082^{b}**	**83.33 ± 4.082^{c}**	**70.00 ± 4.082^{d}**
Feeding period (day) (Mean ± SE)	**6.94 ± 0.15^{a}**	**6.73 ± 0.15^{ab}**	**6.44± 0.14^{bc}**	**6.21 ± 0.16^{c}**
Ingested blood volume (mg) (Mean ± SE)	**868.63 ± 40.41^{a}**	**774.33 ± 41.74^{a}**	**633.50 ± 38.10^{b}**	**572.00 ± 43.20^{b}**

* Group I: Negative control, Group II: Alum-inoculated rabbits, Group III: MgAg-immunized rabbits and Group IV: SgAg-immunized rabbits.

* Figures followed by the same letters are statistically similar ($p > 0.05$); those followed by different letters are significantly different ($p < 0.05$ - $p < 0.01$).

Fully engorged females were collected, weighed and put individually in glass vials, then placed in an incubator and checked daily for egg laying. The ratio of ovipositing females was 100% in groups I and II, which decreased significantly ($P < 0.01$) to 76.6% and 60% in groups III and IV, respectively. No significant difference was observed in the preoviposition period among the different groups. Salivary gland antigen-immunized group only exhibited a significant prolongation ($P < 0.05$) in their prehatching period being 23.5 days compared with the negative control group which recorded 22.17 days. On the other hand, females fed on rabbits immunized with SgAg have the most prolonged oviposition time being 24.62 days which decreased to 23.55, 22.87 and 22.33 days in MgAg-immunized, Alum-inoculated and negative control groups, respectively. Meanwhile, the number of eggs laid by females and the hatching percent decreased significantly ($P < 0.01$) as a result of immunization by both antigens [Table 2]. The mean weights of replete females, egg masses laid and fertility were presented in table (3). Data revealed that all these parameters decreased significantly as a result of immunization of rabbits with the tested antigens. The mean weight of replete females of group III (754.55 mg) was statistically similar ($P > 0.05$) to those of group II (782.67 mg). On the other hand, it differed significantly ($P < 0.01$) when compared to the negative control which recorded 877.50 mg. The lowest mean weight (651.25 mg) of replete females was observed among group IV compared to all other groups. Egg mass laid by females of group I presented the maximum weight being 623.33 mg which significantly decreased to 533.33, 457.27 and 343.75 mg in groups II, III and IV, respectively. Fertility (weight of egg mass/weight of replete female) was reduced significantly ($P < 0.01$) from 0.71 and 0.68 in groups I and II, respectively, which are insignificantly differ; to 0.59 and 0.52 in groups III and IV, respectively. On the other hand, the mean weight of one egg showed a diverse trend since it is increased significantly ($P < 0.01$) from 0.06649 and 0.06670 mg in control groups I and II respectively, to 0.06721 mg in SgAg-immunized group. Whereas, MgAg-immunized group which is statistically similar to the Alum-inoculated and SgAg-immunized group, exhibited a significant increase when compared with the negative control since it was 0.06686 mg [Table 3].

Table 2:- Effects of salivary gland and midgut antigens on oviposition and hatchability of female *H. dromedarii*.

Biological parameter	Group I	Group II	Group III	Group IV
Oviposition percent% ±SE	**100.00± 5.773^{a}**	**100.00± 5.773^{a}**	**76.67± 5.773^{b}**	**60.00± 5.773^{c}**
Preoviposition period (day) (Mean ± SE)	**6.17± 0.17^{a}**	**6.27± 0.15^{a}**	**6.36± 0.18^{a}**	**6.63± 0.21^{a}**
Oviposition period (day) (Mean ± SE)	**22.33± 0.45^{b}**	**22.87± 0.40^{b}**	**23.55± 0.47^{ab}**	**24.62± 0.55^{a}**
No. of eggs (Mean ± SE)	**9373.67± 348.96^{a}**	**7993.53± 312.12^{b}**	**6838.27± 364.48^{c}**	**5114.25± 427.39^{d}**
Prehatching period (day) (Mean ± SE)	**22.17± 0.38^{b}**	**22.73± 0.34^{ab}**	**23.00± 0.40^{ab}**	**23.50± 0.47^{a}**
Hatching percentage % ± SE	**96.83± 0.46^{a}**	**94.07± 0.41^{b}**	**91.40± 0.48^{c}**	**88.94± 0.56^{d}**

Group I: Negative control, Group II: Alum-inoculated rabbits, Group III: MgAg-immunized rabbits and Group IV: SgAg-immunized
rabbits. Figures followed by the same letters are statistically similar ($p > 0.05$); those followed by different letters are significantly different
($p < 0.05$ - $p < 0.01$).

Table 3:- Effects of salivary gland and midgut antigens on fertility of female *H. dromedarii*.

Biological parameter		Group I	Group II	Group III	Group IV
Mean weight of replete female (mg) (Mean ± SE)		**877.50± 23.97^{a}**	**782.67± 21.44^{b}**	**754.55± 25.03^{b}**	**651.25± 29.35^{c}**
Mean weight of egg mass (mg) (Mean ± SE)		**623.33± 23.25^{a}**	**533.33± 20.79^{b}**	**457.27± 24.28^{c}**	**343.75± 28.47^{d}**
Fertility	**Weight of egg mass (mg)/ female weight (mg)(Mean ± SE)**	**0.71± 0.01^{a}**	**0.68± 0.01^{a}**	**0.59± 0.01^{b}**	**0.52± 0.01^{c}**
	Weight of one egg (mg) (Mean ± SE)	**0.06649± 0.00012^{c}**	**0.06670± 0.00011^{bc}**	**0.06686± 0.00012^{ba}**	**0.06721± 0.00014^{a}**

Group I: negative control, Group II: Alum-inoculated rabbits, Group III: MgAg-immunized rabbits and Group IV: Sg Ag-immunized rabbits.

Figures followed by the same letters are statistically similar ($p > 0.05$); those followed by different letters are significantly different ($p < 0.05$- $p < 0.01$).

The effect of the two prepared experimental vaccines on the reproductive index was determined by calculating the number of engorged female ticks (%DT), the egg laying capacity (%DO) and the vaccine efficacy [%E]. The percentage DT of females dropped from SgAg-immunized rabbits was significantly reduced when compared with those dropped from MgAg-immunized ones which records 40 % and 23%, respectively. Additionally, females fed on SgAg-immunized rabbits recorded 44.85 %DO which significantly reduced to 26.64 %DO for MgAg-immunized group. The overall vaccine efficacy revealed that vaccination with SgAg was superior inducing impairment of feeding and reproductive performance. The efficacy percent equals to 67% and 43.7% for SgAg and MgAg respectively [Fig. 1].

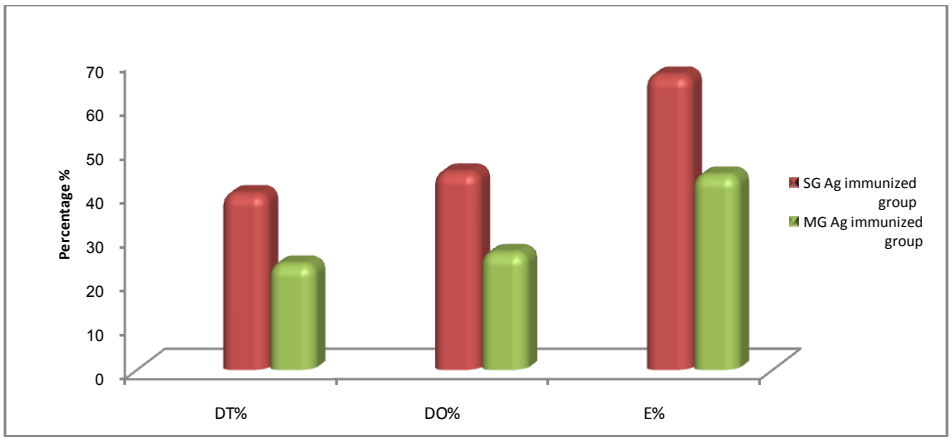

Fig. 1:- Effects of salivary gland and midgut antigens on the number of engorged females, the egg laying capacity and the overall vaccine efficacy.

The level of antibodies elicited post rabbit vaccination using SgAg and MgAg was monitored using ELISA. Specific IgG antibody titers were initially detected one week post the priming dose. This response was enhanced by subsequent boosting, rapidly increasing during the 2nd week, following the second antigen inoculation. Antibody levels in MgAg-immunized rabbits reached its maximum on 28th day post-primary inoculation, while those in SgAg-immunized rabbits reached the maximum value one week post the third antigen dose (on day 36). No significant difference was observed in the antibody titers post challenge-infestation. The antibody levels remained similar and at high levels throughout the experiment. No significant differences between the antibody levels of the two antigens except on the days 21 and 28 ($P < 0.01$). Also, no specific IgG antibodies were detected in the pre-immunization sera or sera from the negative control group.

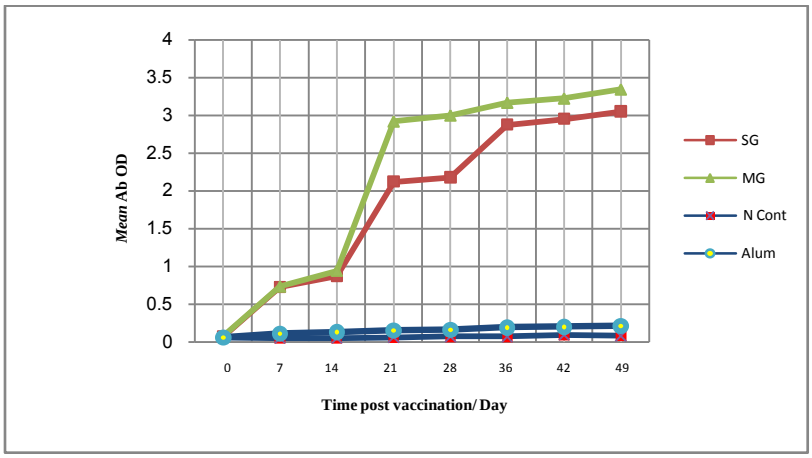

Fig. 2:- Comparative evaluation of immune response of rabbits post-vaccination with salivary gland and midgut experimental vaccines and Alum as adjuvant relative to time using ELISA.

Electrophoretic separation of SgAg and MgAg components was shown in [Fig. 3 A & B]. Approximately 12 polypeptide bands were resolved from SgAg as well as MgAg. The respective molecular weights were 95.84, 83.41, 74.18, 54.4, 45.84, 36.26, 32.23, 25.74, 21.67, 19.55, 16.11 and 15.14 kDa for SgAg [Fig. 3A] and 93.73, 81.99, 52.3, 42.4, 38.67, 30.03, 19.06, 17.89, 15.37, 14.41, 12.72 and 8.62 kDa for MgAg [Fig. 3B]. [Fig. 4] showed the profile of reactive bands of SgAg and MgAg recognized by hyperimmune sera from immunized rabbits. SgAg included 7 reactive bands ranging in their molecular weights from 95.8 to 32.3 kDa (95.84, 83.41, 74.18, 54.4, 45.84, 36.26 and 32.23 kDa). Most of the 7 polypeptides are faintly stained and only two (95.84 and 54.4 kDa) have a moderate reaction. On the other hand, MgAg revealed 10 reactive bands ranging in their molecular weights from 93.7 to 8.6 kDa (93.73, 81.99, 52.3, 42.4, 38.67, 30.03, 19.06, 14.41, 12.72 and 8.62 kDa). However, polypeptides with the 93.73 and 42.4 kDa have a strong reaction. Meanwhile, polypeptides with the 52.3, 38.67 and 8.62 kDa

exhibited a moderate reactions while the remaining polypeptides are faintly stained. No colour reaction was observed between hyperimmune sera collected from Alum-inoculated and negative control groups and tested antigens.

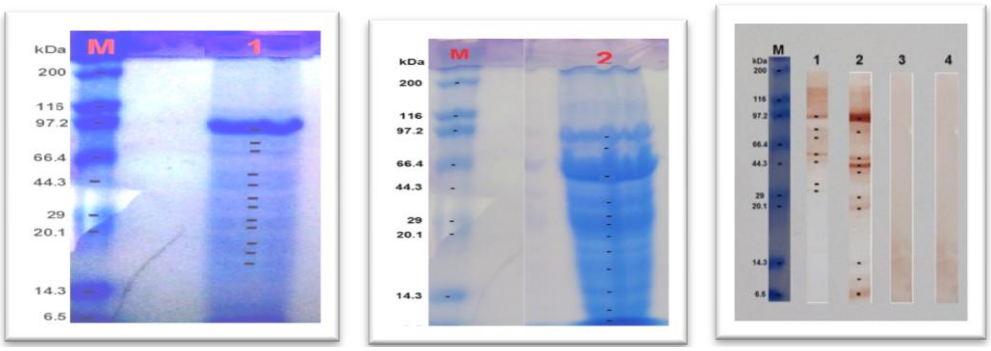

Fig. 3:- SDS-PAGE electrophoretic profile of salivary gland (A) and midgut (B) antigens derived from semi-fed female *H. dromedarii*. Lane (M): Molecular weight marker, Lane (1): Salivary gland antigen and Lane (2): Midgut antigen. [Fig.4]: Western blot analysis showing the reactive bands of salivary gland recognized by anti-SG Ag serum (1), Midgut antigen recognized by anti-MgAg serum (2) and SgAg and MgAg antigens recognized by pool of anti-Alum and negative control sera (3 and 4 respectively).

Discussion:-

Adult *H. dromedarii* as most ixodid ticks spend most of their life on the host. Therefore, the present study was directed to vaccinate hosts with experimentally prepared tick salivary gland and midgut homogenates to avoid severe drawbacks of using acaricides in controlling ticks, since the development of host immunity was considered as a new vision / alternative tick control measure. The effect of host vaccination was first investigated by **Trager (1939b)** and subsequently it was considerably dealt with. Data of the present investigation showed that immunization of hosts with tick SgAg and MgAg disrupt feeding and reproductive development of female *H. dromedarii*. According to **Sran *et al.* (1996)**, it was reported that salivary gland antigens are involved in the acquisition of resistance to *H. anatolicum* as an important vector of bovine tropical theileriosis. The gut antigen also induced the best protection in terms of reduced feeding and reproductive performance of ticks **(Kumar & Kumar, 1995)**. The significantly decreased percentage of fed females observed in the present study may be attributed to the ability of ticks to manipulate the hemostatic effects of the mammalian hosts. Attenuation of fibrinolytic or anti-hemostatic agents of tick saliva causes disruption of blood flow and blocks successful feeding of the blood meal of ticks **(Maritz-Olivier *et al.*, 2007)**. Also, the moderate reduction in the feeding percentage of females fed on SgAg-immunized rabbits was recorded, which was in agreement with the results obtained by **Kumar and Kumar (1995)** and **El-Kelesh (2002)** as they reported a significant reduction in the attachment rate of *H. dromedarii* using gut supernate and salivary gland antigens, respectively. Similar results were obtained by **Sran *et al.* (1996)** on immature *H. anatolicum* using salivary gland extract. **Sahibi *et al.* (1997)** found that immunization of cattle with salivary gland extract against *H. marginatum* reduced the percentage of attachment by 47%, while intestinal extract-generated immunity had no effect (0%) on the attachment percentage. In contrary, **Szabó and Bechara (1997)** reported that the lowest tick recovery was obtained from guinea pigs immunized with gut extract and from dogs immunized with the gut extract emulsified in Freund‘s adjuvant instead of saponin adjuvant. Moreover, the attachment percent was found to be similar among rabbits immunized with both salivary gland and midgut antigens against *Boophilus microplus*, with no significant difference compared to control group (86.67%, 83.34% and 85%, respectively) **(Asif *et al.*, 2011)**. The variable reduction percentages may be attributed to the tick species, host variation and even variable tolerance among individuals of the same species. It was found that, rejection of large ticks represents a lower percentage than that of small ones as observed by **Banerjee *et al.* (2003)** who recorded 34% rejection against adult *H. antolicum* versus 87.8% rejection against the smaller adult *Rhipicephaus sanguineus* **(Szabó and Bechara, 1997)**. The authors suggested that larger ticks are more tolerant. Tick evasion mechanisms might also affect coevolved host-parasite relationships when, even though there is an effective resistance to ticks, immunosuppressed state of hosts may prevail **(Wikel and Allen, 1982); Willadsen *et al.*, 1993)**. Another possible reason is the efficiency of Freund‘s adjuvant which is known to stimulate Th1 type immune response **(Audibert and Lise, 1993)** which may have a predominated effect. This suggestion was also demonstrated by **Szabó and Bechara (1997)** who reported that dogs immunized with Freund‘s adjuvant only, produced lower mean egg mass weight than ticks from dogs inoculated with gut extract and saponin. It seems generally that the effector arm of the immune

response interfered with the fixation of the ticks on the host and also with their subsequent suction of blood. In the present study, *H. dromedarii* females fed on vaccinated hosts reacted to both antigens by expressing short feeding period with ingestion of small amounts of blood and consequently lower body weights. This shortening of the feeding time is also reported by **Abdel-Shafy *et al.* (2008)** using gut antigens and **Sahibi *et al.* (1997)** who concluded that stronger immunization using salivary gland antigens can probably inhibit feeding totally and subsequent transmission of tick-borne pathogens. On the other hand, **Jittapalapong *et al.* (2000)** reported that immunization of dogs using tick salivary gland extract significantly prolonged the feeding period of *R. sanguineus* females, suggesting a slower suction of blood. Impairment of tick feeding in terms of tick yield and engorgement weight observed from the present results was in agreement with the findings of **kumar (1990)** and **Banerjee *et al.* (2003)** against *H. anatolicum*, **Kumar and Kumar (1995**) and **El-Kelesh (2002)** against *H. dromedarii*, **Sahibi *et al.* (1997)** against *H. marginatum*, **Szabó and Bechara, 1997** against *R. sanguineus* and **Jittapalapong *et al.* (2004)** against *B. microplus*. Retarded feeding or complete rejection of ticks from immunized hosts was attributed to the influence of anti-saliva response not to the response against gut components that have been regurgitated during blood meal **(Connat, 1991)**. In the present study, the number of females that have the ability to oviposit was greatly reduced by 40% and 23% for SgAg- and MgAg-immunized groups, respectively, and those that did, took a considerably longer time to complete oviposition with no significant change in the pre-oviposition period. In ticks, the actual volume or weight of condensed blood meal exerts its effects on reproductive development **(Diehl *et al.*, 1982)**. However, at suboptimal levels, oogenesis may not be initiated or, if it begins, will not be sustained. A further general reduction in the number of eggs produced may be due to prolongation of the period between feeding and oviposition. In the present study, ticks fed on SgAg- and MgAg-vaccinated rabbits and succeeded in laying eggs, produced few number that took longer time before the hatched larvae emerged when compared to control groups. The prehatching period of MgAg-immunized group was similar to that of the Alum-inoculated one but slightly longer than that of the negative control group. Meanwhile, the egg laying capacity, hatchability and fertility of both vaccinated groups were significantly reduced. Similar observations were also reported by **Kumar and Kumar (1995)**, **El-Kelesh (2002)** and **Abdel-Shafy *et al.* (2008)** on *H. dromedarii*, **Sahibi *et al.* (1997)** on *H. marginatum*, **Jittapalapong *et al.* (2000)** on *R. sanguineus* and **the same author *et al.* (2004)** and **Asif *et al.* (2011)** on *B. microplus*. It seems clear that, the reduction in the production indices may be attributed simply to impairment in meal processing or to the host immune response which affects directly the physiology of ticks in some permanent way that persists even after the parasite detachment. **Ben-Yakir *et al.* (1986)** and **Wang and Nuttall (1994)** cleared that midgut is permeable to host immunoglobulins, and that these antibodies enter the tick hemolymph and can bind to antigens associated with other internal organs of the tick. These host immune effectors present in the blood meal not only destroying the candidate cells as the damage caused to the gut cells and leakage of gut contents into the haemocoel in response to immunization with gut antigens against *B. microplus* **(Agbede and Kemp, 1986)**, but also could bind to antigens associated with the vital organs such as the reproductive organs and could have detrimental effects on the oogenesis and egg hatchability of ticks. This can be explained on the basis of the presence of common antigens in different organs of the ticks **(Wang and Nuttal, 1999)**.

The drastic effects of immunizing hosts with SgAg- and MgAg on the feeding and reproductive performance of the tick *H. dromedarii* observed in the present study may based on the concept that ticks feeding on appropriately immunized hosts might ingest antibodies specific for target antigens within the tick. This suggests that these antibodies may apply their effect through certain routes, possibly the nervous system and specifically via the neuroendocrine or endocrine mechanism essential in the regulation of feeding and consequently oocyte differentiation and/or vitellogenesis **(Pound & Oliver, 1979).** In the present study, a strong antibody response was rapidly detected in vaccinated rabbits. The immunogenic capacity of these antigens and their characterized specific proteins has been extensively documented through a high number of immunization trials (**Sran *et al.* 1996**; **Jittapalapong *et al.* 2000**; **Trimnell *et al.*, 2005; Prevot *et al.*, 2007; Abdel-Shafy *et al.* 2008**; **Madani *et al.*, 2008**; **Habeeb *et al.*, 2009**; **Asif *et al.* 2011**; **Kumar *et al.*, 2012**; **Jiang *et al.*, 2014**; **Ali *et al.*, 2015**). Moreover, high antibody level was suggested to correlate with the protective efficacy against tick infestations **(Willadsen, 2004)**. This protective efficacy was observed as a physical damage to ticks **(Imamura *et al.*, 2006)** and as a reduction of the biotic potential **(Barriga, 1999)** which would likely result in progressive reduction of tick populations, while in turn can enhance reduction of acaricides use. Also, MgAg-immunized group had a slightly higher antibody titer than SgAg-immunized one. In the present study, western blotting analysis revealed that SgAg and MgAg could induced polyclonal antibodies against the two Ags polypeptides, and MgAg was more immunogenic. This may be attributed to the gut proteins content that enhance random stimulation of immune system than that of the SgAg, which was supported by the reactivity to 10 and 7 bands of proteins, respectively. The protective efficacy, impairment of feeding and reproduction were influenced by vaccination. This may be attributed

to the different concentrations, antigens content and feeding duration. Also, the possible reasons could be due to some proteases are abundant in the various tissues of the tick and that they exist as multiple isoenzymes, therefore a high concentration of antibodies directed against them could be necessary to induce a significant injury to the tick **(Imamura *et al.*, 2009)**. Additionally, some saliva proteolytic enzyme inhibitors such as Iris was found to be up-regulated during the blood meal and their concentration increased at the end of feeding **(Leboulle *et al.*, 2002)** when tick ingest the great amount of blood **(Sonenshine, 1991)**, suggesting its particular usefulness in this late feeding step as it could be ingested with the blood meal to the midgut and could facilitate both blood intake and protection of the midgut walls **(Prevot *et al.*, 2007)**. **Kemp *et al.* (1989)** suggested that *B. microplus* can, to a significant extent, repair immunologically induced damage to its gut. Perhaps the balance between the damage and repair will determine the outcome of vaccination, which may differ from species to species. The degree of damage and oviposition capacity may depends also on the amount of blood ingested, and hence the amount of antibodies developed post vaccination. Finally it can be concluded that experimentally immunization of rabbits with salivary gland extract of *H. dromedarii* homogenates effectively impaired tick feeding and reproduction much better than vaccination with midgut homogenate suggesting further investigations to characterize the protective antigens involved in the protection process.

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