Global Veterinaria 3 (2): 125-131, 2009
ISSN 1992-6197

Evaluation of Fractionated Proteins Extracted from Different Stages of *Hyalomma dromedarii* (Acari: Ixodidae) as a Control Agent Against Female Ticks

Salwa M. Habeeb, Omnia M. Kandil and Amira H. El-Namaky

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

Abstract: This investigation was carried out to compare the effects of repeated infestations versus immunization of rabbits with tick larvae protein fraction 2 (LPF2), eggs protein fraction 2 (EPF2) or salivary gland protein fraction 2 (SGPF2) on the feeding and performances of female ticks. In each immunized group, three tick-naive rabbits were immunized three times with either LPF2, EPF2 or SGPF2 and twice challenged at 21 d intervals by allowing 10 female and 10 male adult ticks to feed on each animal. The repeated infestation group of three naive rabbits were infested five times at 21 d intervals by the same number of ticks. The repeated infestation group showed reduced tick performance after the third infestation but some of the tick performance parameters had recovered by the fifth infestations. Immunized rabbits with EPF2 showed a significant reduction in tick yield, engorgement weight and egg mass weight in addition to reduced egg production and egg viability. Immunization with LPF2 or SGPF2 resulted in the greatest effect of tick fecundity parameters, which included pre- oviposition, oviposition and egg-incubation periods. The results confirm that rabbits can become resistant to *H. dromedarii* and EPF2 induced the best protection in terms of reduced feeding and reproductive performance of the ticks.

Key words: *Hyalomma dromedarii* · Gel filtration · ELISA · Fractionation · Immunization · Egg · Larva · Salivary gland

INTRODUCTION

There is a necessary need for new ectoparasite control strategies. Immunological control of ectoparasites is a useful new technology which has enormous advantages in reducing adverse environmental effects of currently used pesticides. Also, it is cost effective and would provide long lasting acquired resistance of hosts against parasites.

The immunological control of ticks is gaining importance and encouraging results have been achieved in the past by immunizing various animals (cattle, guinea-pigs, rabbits, mice and dogs) with the respectable tick antigens against *Boophilus microplus*, *Rhipecephalus appendiculatus*, *Amblyomma americanum, Dermacentor variabilis*. *H. dromedarii*, *Ixodes ricinus* and *Rhipecephalus sanguineus* infestation [1-5].

Hosts that becomes resistant after multiple infestations often display immune response to substances found in tick saliva [6 - 10]. It has been proposed that immunization with salivary gland extract could induce resistance against infestation that resembles the immune protection observed after repeated infestations. Two protein fractions from midgut, namely DET (detergent) and AQ (aqueous) of *Amblyomma variegatum*, two protein fractions from salivary gland and two from midgut of *Hyalomma dromedarii* were used against these ticks. They have offered a significant protection in rabbits against these tick species [11 - 13].

Hyalomma dromedarii is the second most common tick in Egypt. It is suspected of playing an important role in transmitting a haemoprotozoan diseases, bovine tropical theileriosis, caused by *Theileria annulata* [14, 15].

This investigation aimed to fractionate and evaluate three proteins derived from larvae, egg and salivary gland of *H. dromedarii* and to test the protective efficacy of these fractions against challenged infestations with ticks.

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

Corresponding Author: Dr. Salwa M. Habeeb, Parasitology and Animal Diseases Department, National Research Center, Dokki, Giza, Egypt

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 (Light:Dark) hrs throughout eggs laying.

Antigen Preparation: Salivary glands were collected from 200 adult ticks (semi-engorged females). Females were placed into phosphate buffer saline pH 7.4 (PBS) and opened along their dorsal surface. Salivary glands were removed, dissected free of other tissues, placed into PBS at 4°C [17].

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 second, according to El-Kammah and Sayed [17].The protein content was determined by the Lowry method [18].Proteins were stored individually at -20 ° C until use.

Fractionation of Crude Extract Larvae, Eggs and Salivary Gland Antigens: The fractionated antigens were obtained by gel filtration method [19], concentrated and estimated as protein using Lowery method [18].

Antibody-antigen Interaction: The antibody-antigen interaction was measured by using the ELISA technique according to Voller *et al.* [20].Indirect ELISA was used to compare the protein fractions obtained from the three different antigen extracts (eggs, larvae and salivary glands) prepared by gel filtration. This assay was done by using the serum from rabbits infested by the adult ticks of *H. dromedarii*.

Experimental Design: New Zealand white male rabbits weighing 1.5-2 kg which had no previous contact with ticks were used in this study. Rabbits were divided into 4 groups, 3 rabbits for each group. First, second and third groups were immunized intramuscularly, in the form of three injections at 21 days intervals [10]. The above mentioned three groups were inoculated as follows, first group with LPF2, second group with EPF2 and third group with SGPF2.The fourth group was kept as a control (repeated infestation). At the first injection rabbits were inoculated with 150 µl protein (120µg /rabbit), protein fraction 2 of salivary glands (SGPF2), larvae (fraction 2 (LPF2) and eggs fraction 2 (EPF2) in equal volume of complete Freund's adjuvant. In the second and third injection, the same volume of protein fractions was used in equal volume from incomplete Freund's adjuvant. All rabbits in above three groups were challenged one week after the last injection with 10 males and 10 females of *H. dromedarii* for each rabbit. One week later, the first challenged animals were challenge another time according to Jittapalapong *et al*.[10]. Fourth group of rabbits were infested five times at 21 days intervals with 10 males and 10 females of *H. dromedarii* for each rabbit and each infestation. Biological parameters of *H. dromedarii* females were studied for all rabbit groups. Feeding period, weights of engorged females, pre-oviposition, oviposition, weights of egg masses, hatching period were recorded.

Statistical Analysis: Data from biological parameters were compared between treatment groups using multivariable analysis of variance (ANOVA) procedures. All analysis assessed the effect of treatment group, adjusted for the effect of multiple infestations. Pair wise comparison of LS means was accomplished with student's t-test.

The percentage of tick yield, percentage of reduction in engorged tick weight, reproductive index and the percentage of egg mass reduction were estimated as outlined by Kumar and Kumar[1] by the following formulae:

$$Tick\ weight\ reduction\ (\%) = 1 - \frac{Mean\ weight\ of\ engorged\ ticks}{Mean\ weight\ of\ engorged\ ticks\ in\ control}$$

$$\mathrm{Re}\ productive\ index = \frac{Mean\ weight\ of\ egg\ mass}{Mean\ weight\ of\ engorged\ ticks}$$

$$Egg\ mass\ reduction\ (\%) = \frac{Mean\ weight\ of\ non-hatching\ egg\ mass}{Mean\ weight\ of\ egg\ mass}$$

RESULTS

Identification of Protein Fractions: Three peaks of protein fractions were separated by gel filtration from tick-eggs, larvae and salivary glands individually. The protein content of these fractions at peak 1, 2 and 3were 2.8, 1.9 and 1.3 for tick-eggs ; 2.1, 1.6 and 1.1 for tick- larvae and 1.5, 1.4 and 1.2 µg for tick- salivary glands, respectively.

Enzyme Linked Immuno Sorbent Assay (ELISA): Serum from infested rabbits by adult females of *H. dromedarii* showed higher antibodies titer against LPF2 and EPF2 than LPF1, LPF3 and EPF1; EPF3 respectively (Fig 1) The interaction between serum infected rabbits and SGPF1,2and3 showed higher antibodies titer than sera of

Table 1: Feeding and reproductive parameters of *H. dromedarii* females after repeated feeding on rabbits

	Feeding number						
Biological parameter	1st	2nd	3rd	4th	5th	Total mean	P value
Feeding period (day)	9.16±2.32	11.0±3.06	9.5±1.87	9.71±2.56	8.0±1.0	9.5±0.89	NS
Engorgement weight (g)	0.974±0.12^{c}	0.588±0.29^{b}	0.366±0.20^{a}	0.242±0.27^{a}	0.718±0.027^{b}	0.58±0.06	<0.01
Pre-oviposition period (day)	5.6±2.40^{a}	15.0±2.00^{b}	15.20±1.90^{b}	6.4±2.6^{a}	6.0±1.0^{a}	9.6±0.95	<0.01
Oviposition period (day)	5.5±1.30^{a}	12.2±1.90^{b}	7.0±1.60^{a}	6.5±1.30^{a}	18.5±1.30^{c}	9.9±0.70	<0.01
Egg mass weight (g)	0.563±0.13^{c}	0.394±0.12^{b}	0.200±0.12^{a}	0.109±0.15^{a}	0.337±0.08^{b}	0.32±0.037	<0.01
Egg incubation period (day)	17.5±1.90^{a}	14.7±2.20^{a}	22.7±1.50^{b}	22.7±4.2^{b}	18.3±5.80^{ab}	19.2±1.50	<0.01

Superscript letters represent significant differences between feeding numbers

NS= Non significant.

Table 2: Feeding and reproductive parametersof *H. dromedarii* females fed on immunized rabbits

	Protein fraction 2 (challenge number)									
	Larva (LPF2)			Salivary gland (SGPF2)			Egg (EPF2)			
Biological parameter	1st	2nd	Total mean	1st	2nd	Total mean	1st	2nd	Total mean	P value
Feeding period (day)	8.0±0.57	9.5±0.76	8.8±0.67	8.2±0.86	9.5±0.76	8.8±0.81	8.7±0.85	9.2±1.0	9.0±0.93	NS
Engorgement weight (g)	0.427±0.12^{a}**	0.893±0.12*^{b}	0.66±0.12	0.873±0.06^{b}	0.870±0.10*^{b}	0.87±0.08	0.762±0.06^{b}	0.183±0.05**^{a}	0.47±0.06	<0.05
Pre-oviposition period (day)	15.3±1.20*	16.3±0.67	15.8±0.94	16.0±1.40**	14.4±1.1*	15.2±1.10	16.5±0.65**	17.0±0.71	16.8±0.68	NS
Oviposition period (day)	13.3±1.0**	14.2±0.95**	13.8±0.98	16.5±0.65**	14.2±0.86	15.4±0.76	17.5±0.65**	13.6±1.30	15.5±0.98	NS
Egg mass weight (g)	0.364±0.05	0.543±0.84*	0.45±0.45	0.442±0.04	0.429±0.05	0.44±0.05	0.357±0.04**	0.296±0.08	0.33±0.06	NS
Egg incubation period (day)	17.0±0.71^{a}	20.7±0.89**^{b}	18.9±0.8	17.6±0.93^{a}	18.6±1.30**^{ab}	18.1±1.10	18.0±0.82^{ab}	21.0±0.82**^{b}	19.5±0.82	<0.05

Superscript letters represent significant differences between feeding numbers for all protein fractions.

* = Significant difference with first and second feeding in control according paired t test, at P<0.05.

** = Significant difference with first and second feeding in control according paired t test at P<0.01.

NS= Non significant.

non infested rabbits (Fig 1). Serum of infected rabbits revealed higher antibodies titer against EPF2; LPF2 and SGPF2 than all protein fractions derived from egg, larvae and salivary gland of *H. dromedarii*. In this investigation EPF2, LPF2 and SGPF2 were tested to protect the rabbits against female of *H. dromedarii.*

Performance of Female *H. Dromedarii* after Multiple Infestations: Repeated infestation alone reduced all of tick feeding performance parameters, with host resistance initially expressed after the third infestation (Table 1). Repeated infestation did not have any significant affect on feeding period except infestation 2, in which the feeding period was longer than all infestations, it was 11±1.2 day. Mean of engorgement weights fell from infestation 1 to infestatiuon4 (P<0.05). Pre-oviposition period showed marked significant differences between infestation 1 and infestations 2; 3, respectively (P<0.01). However, significant differences were observed in oviposition periods between infestation 1 and infestation 2(P<0.01) and 5(P<0.001), respectively.

Mean of egg mass was significantly reduced from infestation 1 to infestation 4(P<0.05). Incubation period was lengthened from infestation 1 to infestation 3 and 4, (P< 0.01).

Observations made on the feeding performance and reproductive success of *H. dromedarii* female ticks are shown in Table 2, Fig. 2. It was clear that rabbits immunized with LPF2showed no significant differences between first challenge and second challenge in all biological parameters of *H. dromedarii* except engorgement weight and egg mass weight.. Incubation period increased from challenge 1(P<0.05) to challenge 2(P<0.01). The data in Table 2 revealed light changes in the performance of female *H. dromedarii* at challenge 1 and 2 in case of rabbits immunized with SGPF2. Rabbits immunized with EPF2 revealed that Engorgement weight revealed significant decrease from challenge 1(P<0.01) to challenge 2(P<0.05), respectively. Incubation period increased (P<0.01) from 18 d challenge 1 to 21 d challenge 2, respectively (Table 2).

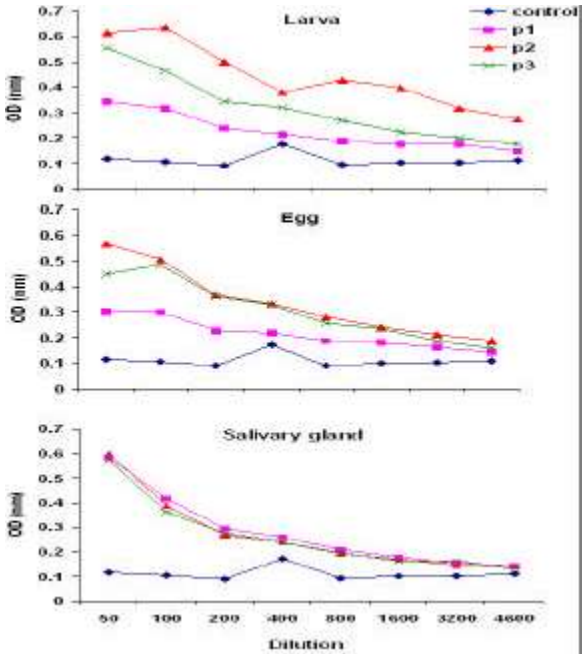

Fig. 1: Antibody protein fractions profile for larva, egg and salivary gland of *H. dromedarii*

Comparative performance of tick after feeding on immunized rabbits with LPF2; EPF2 and SGPF2: Performance of female *H. dromedarii* fed on immunized rabbits with three fractions LPF2, SGPF2 and EPF2 revealed no significant differences among these fractions except engorgement weight and incubation period. Engorgement weight increased from 0.427 g Challenge 1 to 0.893 g challenge 2 and decreased from 0.762 g challenge 1 to 0.183 challenge 2 in case of immunized rabbits with LPF2($P<0.05$) and EPF2($P<0.01$),respectively. Incubation period lengthened at all fractions from challenge 1 to challenge 2, LPF2($P<0.01$); SGPF2($P<0.01$) and EPF2($P<0.01$), respectively (Table 2.).

Comparative Performance after Multiple Infestation and after Feeding on Immunized Rabbit: Immunization with LPF2, SGPF2 and EPF2 impacted several feeding performance parameters (Tables 1, 2 and Fig. 2). The mean percentage of tick yield in the repeated infestations group was higher than those tick yield on rabbits immunized with LPF2 SGPF2 and EPF2 respectively. The mean weight of engorged female ticks from the repeated infestation

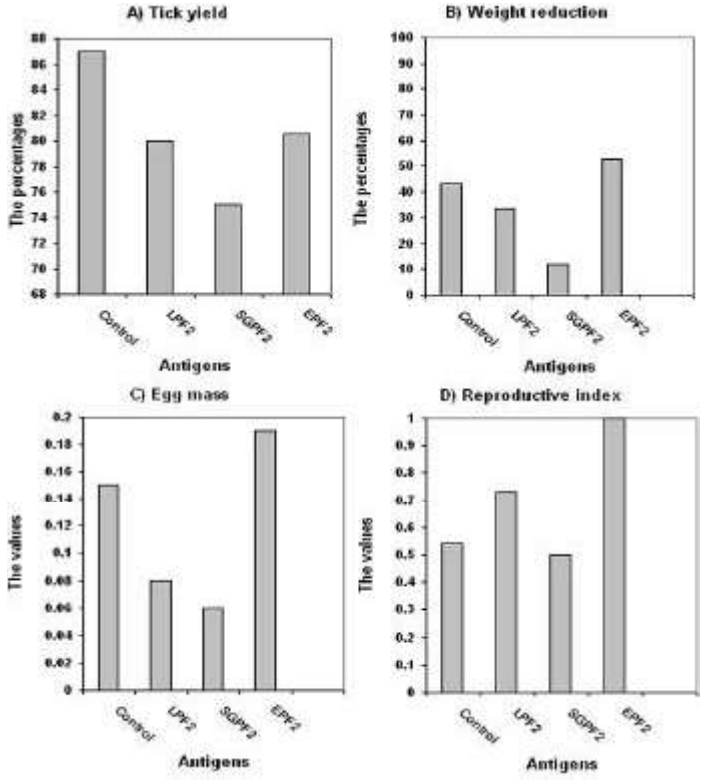

Fig. 2: Feeding and fecundity parameters of *H. dromedarii* females from all infestations fed on rabbits that were infested or immunized with fractionated tick protein (LPF2, SGPF2 and EPF2). A) The mean percentage of tick yield, B) The mean percentages of engorged weight reduction, C) The mean of egg mass reduction, D) The mean of reproductive index.

group was greater than the average of those fed on rabbit immunized with EPF2, but they were lighter than the average of those fed on rabbit immunized with LPF2and SGPF2. The mean percentage of tick weight reduction was 43.3% for the repeated infestation group which was greater than the mean percentage of those fed on rabbit immunized with LPF2 (33.6%)and SGPF2 (11.7%), but this was less than the average percent of those fed on rabbit immunized with EPF2 (52.9%).

Host immunization with LPF2; SGPF2 and EPF2 also impacted several *H. dromedarii* fecundity parameters as compared with repeated infestation alone (Table 1, 2 and Fig. 2). Pre-oviposition and oviposition periods were increased for ticks fed on immunized rabbits with three fractions than control(repeated infestation) The mean egg mass weight produced during repeated infestation was less than those fed on LPF2 and SGF2 immunized rabbits. There was no difference between egg mass weight produced by ticks fed on repeated infested and EPF2 immunized rabbits. The mean of egg mass weight reduction of repeated infestations, was greater than those fed on immunized rabbits with LPF2 and SGPF2. However, it was less than those fed on immunized rabbits with EPF2. The mean weight of reproductive index of repeated infestation, was less than those fed on immunized with LPF2and EPF2 and they were equal to the mean reproductive index produced by immunized rabbits with SGPF2 (Fig. 2).

Performance of Tick During Different Challenge Infestations: Further comparison of tick performance during the first and second challenge infestations provided insights to the sustainability of any resistance induced by immunization with tick protein fractions (Table 1 and 2). For feeding period no significant differences was observed on rabbits immunized with LPF2 and SGPF2 between challenge 1, 2 and infestation 1, 2($P<0.05$) except in case of rabbits immunized with EPF2($P>0.05$). No significant increase was observed between infestation 2 and challenge 2 (Tables 1 and 2). Engorgement weight, when ticks fed on tick LPF2 and EPF2 immunized rabbits, we observed a significant reduction between repeated infestation 1, 2 and challenge 1 and 2, respectively($P>0.01$). The decreased engorgement weights of female ticks fed on tick EPF2-immunized rabbits resulted in the lowest feeding efficiency.

The reproductive success of female *H. dromedarii* obtained from immunized rabbits is shown in Table 2 and Fig. 2. Pre-oviposition period showed significant differences between first infestation and second infestation for repeated infestation and between challenge 1, challenge 2for EPF2 and SGPF2, respectively($P>0.01$). In case of oviposition period, data illustrated in tables 1 and 2 showed marked significant differences between control group (infestation 1) and repeated infestation 2, challenge infestation 1and challenge infestation 2) for all three protein fractions($P>0.01$). The egg mass weights produced by control ticks averaged 0.563 g which dropped to 0.394 g ($P<0.01$) for repeated infestation 2 and 0.357 to 0.296 g for challenge infestation 1 and challenge infestation2 produced by ticks fed on rabbits immunized with EPF2, respectively. Egg mass weights drop significantly for ticks fed on rabbits immunized with EPF2, however, it is not drop significantly for ticks fed on rabbits immunized with LPF2 and SGPF2, respectively.

Data illustrated that marked significantly difference between egg incubation period of second infestation for control group and first, second challenge from ticks fed on rabbits immunized with EPF2, LPF2 and SGPF2, ($P> 0.05$) and ($P> 0.01$) respectively (Table 1,2).

DISCUSSION

Reduced tick performance parameters in this investigation often recovered in the subsequent infestations. This phenomenon may be explained by the ability of ticks to manipulate the host immune system [10, 21]. For example after the second challenge infestation, the engorgement weight of females recovered from rabbits immunized with EPF2 was significantly lower than those from naive controls (first infestation of control group), infestation 2 of control group and rabbits immunized with LPF2, SGPF2. However, after the first challenge infestation, the engorgement weight of females recovered from rabbits immunized with LPF2 was significantly different from those of naive controls (first infestation of control group) and the first challenge infestation for rabbits immunized with EPF2 and SGPF2 separately. Conversely marked lowest engorgement weight of females were recovered from rabbits immunized with EPF2 after the second challenge infestation, possibly because of a booster effect, during the first challenge infestation of the EPF2 immunogen (E) responsible for reducing this parameter.

The mean percentage of engorged females tick yield recovered from rabbits immunized with SGPF2 was significantly lower than those from repeated infestation, however tick yield recovered from immunized rabbits with LPF2; EPF2 were no significantly different from those of repeated infestation. Similar performance recoveries were

observed for the tick weight reduction of females tick recovered after repeated infestation, the pre-oviposition, oviposition period and egg incubation period for females fed on EPF2-immunized rabbits, egg mass weight reduction from females fed on SGPF2-immunized rabbits. However, engorgement weights of females were recovered from rabbits immunized with EPF2 was significantly lower, the reproductive index was significantly higher than the reproductive index of females from repeated infestation, LPF2, SGPF2 immunized rabbits. This indicates the excessive of mechanism that increases the reproductive index as a survival mechanism.

The current results demonstrated that three infestations of rabbits with adult of *H. dromedarii* induced significant immunity expressed as an inhibition of fertility of the ticks from a subsequent infestation, feeding period did not affect. In case of rabbits immunized with EPF2, ticks attached or completed their engorgement on the immune animals, did ingest less blood. It was presumd that the effect arm of the immune response interfered with the fixation of the tick on the host and also with their subsequent suction of blood. Finally, the ticks that succeeded in laying eggs produced fewer eggs than ticks fed on non immune animals (first infestation) from control group. It seems clear, therefore that host immunity affects the physiology of the tick in some permanent manner that persists even after the parasite detached. In this respect [22], mentioned that ingestion of host blood containing specific antibodies may lead to binding of the antibodies to the surface of the digest cells followed by lyses of these cells and drastically increased leakage of material from the gut into ticks heamolymph. However, [23] reported that these antibodies may bind a target epitopes of salivary glands or ovaries. The present results agreed with [1, 10, 13,24] as they achieved greater success in immunizing rabbits cattle and dogs with crude and purified gut antigen and salivary gland extracts derived from partly or fully fed females of *H. dromedarii*, *H. marginatum marginatum* and *Rhipicephalus sanguineus*.

The reduction of both feeding and fecundity performance after the third repeated infestation indicated that a trend of protective immunity was developing in rabbits without immunization with tick tissues. The resistance manifested by repeated infestation affected in both feeding and fecundity performances in a manner similar to that of the tick EPF2 immunization group. However, [10] found that the resistance manifested by repeated infestation affected in both feeding and fecundity performances in manner similar to that of the tick salivary gland immunization group they explained that the majority of antigens released into the host during tick feeding are probably secretary products of the tick salivary glands

This study demonstrates that rabbits vaccinated with SGPF2 and later exposed to ticks are not better protected against adult ticks than rabbits that were only infested with adult tick *H. dromedarii*, this results agreed with [3], they found that, infestation of rabbits with 60 adult ticks of *Rhipicephalus appendiculatus* leads to high protection in terms of reduction in the engorged weight against adult ticks, larvae and nymphs (88.6, 31.5 and 55.9%, respectively), vaccination with salivary gland alone provides reduction in adult, larval and nymph ticks, (53. 9, 29.7 and 35.7% respectively) and vaccination of rabbits already exposed to adult tick infestation appeared to have no additive immunological benefit above that already provided by adult ticks. This result indicates that SGPF2 may not be suitable protein in this investigation.

Fractions of LPF2, SGPF2 and EPF2 revealed that no significant differences in all biological parameters except engorgement weight and incubation period, rabbits immunized with EPF2 showed a higher significantly effect in this two parameters.

These results indicate that these rabbits developed immune resistance to female *H. dromedarii*. This study directly compared the performance of *H. dromedarii* females fed on rabbits exposed to repeated infestation or immunization with LPF2, EPF2 and SGPF2 individually. Ticks were fed on rabbits immunized with EPF2 or ticks fed on rabbits that were exposed to repeated infestations showed greater reductions in feeding and fecundity than ticks fed on rabbits immunized with LPF2 or SGPF2. Its indicate that protein EPF2 could be the suitable protein used in vaccinating rabbits against female of *H. dromedarii*.

It was concluded that, rabbits showed a resistance against female of *H. dromedarii* with EPF2, LPF2 and SGPF2.It could be that rabbits, had a more significant protection with EPF2 than LPF2, SGPF2 and repeated infestation for female of *H. dromedarii* tick. Further work will focus on purified fractions LPF1, LPF3, EPF1, EPF3, SGPF1 and SGPF3 which were obtained from larvae, eggs and salivary glands extracts of *H. dromedarii* to determine the suitable protein to use as a vaccine against tick of *H. dromedarii*.

REFERENCES

1. Kumar, R. and R. Kumar, 1995. Artificial immunization of rabbits with *Hyalomma dromedarii* tick-derived midgut antigen. Exp. Applied Acarol., 19: 319-324.

2. Dusbabek, F., I. Borsky, F. Jelinek and J. Vhlir, 1995. Immunosuppression and feeding success of *Ixodes ricinus* nymphs on BALBC mice. Med. Vet. Entomol., 9: 133-140.
3. Nyindo, M., S. Essuman, R. Chesang and P. Muteria, 1996. Immunity induced by vaccination with *Rhipic ephalus appendiculatus* salivary gland antigens dose not augment protective immunity acquired naturally by exposing rabbits to adult ticks. J. Med. Entomol., 33: 559-565.
4. Omar, S.F.A., 1999. Experimental studies on vaccination of rabbits against hard ticks *Hyalomma dromedarii*. Vet. Med. J., 47*:* 457-466.
5. 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: 86-192.
6. Willadsen, P., 1987. Immunological approaches to control of ticks. Intl. J. Parasitol., 17: 671-677.
7. Wikel, S.K., 1982a. Immune responsiveness of the bovine host to repeated low-level infestations with *Dermacentor andersoni*. Ann. Trop. Med. Parasitol., 76: 405-414.
8. Wikel, S.K., 1982b.Immune responses to arthropods and their products. Ann. Rev. Entomol., 27: 21-48.
9. Wikel, S.K. and A.C. Whelen, 1986. Ixodid host interaction, identification and characterization of relevant antigens and tick induced host immune-suppression. Vet. Parasitol., 20: 149-174.
10. Jittapalapong, 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.
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. Exp. Applied Acarology., 26(1-2): 101-113.
12. 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. Egypt Vet. Med. Assoc., 65. 4: 229-243.
13. 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. Entomol., 5(2): 91-102.
14. Bhattacharyulu, Y., R.P. Chaudhri and B.S. Gill, 1975. Transstadial transmission of *Theirleria annulata* through common ixodid ticks infesting Ind. cattle. Parasitol., 71: 1-7.
15. Abdel-shafy, S., 2002. Microbiological and control studies on ticks infesting farm animals and poultry. Ph.D thesis-Faculty. Of Agriculture Cairo University. pp: 208.
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. Lowery, 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, P. Takac and M. Labuda, 2002. An inhibitor of thrombin-stimulated blood platelet aggregation from the salivary glands of the hard tick *Amblyoma variegatum* (Acari:Ixodidae). Exp. Applied. Acarol. 28: 97-105.
20. Voller, A., D. Bidwell and A. Barttett, 1976. Enzyme immunoassay in diagnostic medicine. Bulletien. W.H.O. 53: 55-56.
21. Wikel, S.K., 1988. Immunological control of hematophagous arthropod vectors: utilization of novel antigens. Veterinary Parasitology. 29: 235-264.
22. Willadsen, P., 1997. Novel vaccines for ectoparasites. Veterinary of Parasitology., 71: 209-222.
23. Ackerman, S., F.B. Cure, T.W. Mcgill and D.E. Sonenshine, 1981. Passage of host serum components, including antibody, across the digestive tract of *Dermacentor variabilis*. J. Parasitol., 61: 737-740.
24. Sahibi, H., A. Rhalem and O.O. Barriga, 1997. Commparative immunizing power of infections, salivary extracts and intestinal extracts of *Hyalomma marginatum marginatum* in cattle. Veterinary Parasitology. 68: 359-366.

(Received: 31/12/2008; Accepted: 25/01/2009)