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EXPERIMENTAL STUDIES ON VACCINATION OF RABBITS AGAINST HARD TICKS (*HYALOMMA DROMEDARII*)

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SUMMARY

Boscat white rabbits were vaccinated with *Hyalomma dromedarii* tick eggs antigen (Antigen I) and sex organs with midgut antigen (Antigen II). Both antigen were exposed to electrophorytic analysis . The rabbits received two inoculations subcutaneously on 0 day and after 2 weeks. The effect of the immunity induced was determined by enzyme linked immunosorbent assay (ELISA) and by infesting the rabbits with adult *H.dromdarii* ticks. In immunized rabbits, the antibody titer was detected in serum. A significant reduction in tick weight, egg mass weight, oviposition period and percentage of eggs hatchability were found. Three specific immunogenic bands were detected for antigen I and four for antigen II. Sex organs with midgut antigen induced best protection than eggs antigen.

INTRODUCTION

To speak of vaccination for the control of ectoparasites is to look to the future rather than the present. Ticks are responsible for substantial economic losses to livestock industry, necessitating intensive use of chemical acaricides in many parts of the world. Problems of chemical residues, environment pollution, cost of acaricides and development of resitance by ticks have long been recognized and consequently stimulated interest in tick control by immunological means. Acquired immune resistance to infestation with ticks has been intenesively investigated during the last several decades (Nelson et al., 1977; Mc-gowan & Barker., 1980; Barriga et al. 1991; Sahibi et al., 1993 and Willadsen et al., 1996). Many experiments with laboratory animals and cattle demonstrated that tick vaccine can effectively induce some degree of protective immunity against *Boophilus microplus. Rhipicephalus appendiculatus, Amblyomma americanum* and *Dermacentor variabilis* (Ackerman et al., 1980;

Brown and Askenase, 1983; Johnston et al., 1986; Willadsen et al., 1989; Wong et al., 1990 and Essuman et al., 1991). Also, Allen and Humphrey (1979) immunized bovines with extracts of intestinal and genital tracts of *Dermacentor andersoni* ticks and found that the vaccinated animals inhibited the fertility of the ticks that fed on them. It has been reported that the immunologically potent antigens are localized on the digest cells of the ticks midgut (Agbede and Kemp, 1986; Kemp and Agbede, 1986 and Tracey-Patte and Joyner., 1987). The effect of tick vaccine was experimentally measured by Willadsen et al., (1995) as reduction in engorgement weight, strong inhibition of egg laying by the engorged adult female ticks which survived as well as partial decrease in the hatchability of that eggs. This study was designed to investigate a tick vaccine prepared from *H.dromedarii* midgut, reproductive ogans and tick eggs. Its effect on feeding, reproductive performance of reared ticks and antibody production in rabbits was evaluated. Also, the most sensitive and specific antigenic band of tick antigen were determined.

MATERIAL AND METHODS

Ticks and rabbit

Laboratory colonies of *H. dromedarii* were maintained by feeding all 3 parasitic stages on the ear of Boscat rabbits to obtain pure colonies as described by Walker and Fletcher. (1985).

Antigen preparation

For preparation of egg antigen (Antigen I), engorged females of *H.dromedarii* were kept individually in a vial held at 24°C and relative humidity of 85%. Egg mass produced by females just after termination of egg laying were collected in ice-cold PBS 7.2 . homogenized, sonicated in ice at 1 minute interval for 5 minutes, centrifuged at 10.000 g for 30 minutes and the supernatant kept at 4°C. Protein concentration was determined according to Lowry et al. (1951) and the concentration was adjusted to 1mg protein/ml.

Midgut and reproductive organs (Antigen II) were dissecated from females adult ticks which were fed for 5 days on rabbits. Ticks surfaces were sterilized with merthiolate solution. Dissection was performed according to Purnell and Joyner (1968). Dissected organs were held in ice-cold PBS 7.2PH. The tissues were homogenized, sonicated and centrifuged as before. The protein concentration was determined according to Lowry et al. (1951), the concentration was adjusted to 1.2mg protein/ml and kept at 4°C.

Rabbit vaccination

Fifteen Boscat rabbits weight 1250-1500gm were kept in 3 separate groups (each 5 rabbit). Each rabbit of group I received a dose of 1.0mg/kg body weight from egg antigen (antigen I) in freunds complete adjuvent (FCA) on day 0

and 14. Animals in group II were immunized with a dose of 1.2 mg/kg body weight of sex organ and midgut antigen in freunds complete adjuvent (Antigen II). Control group (group III) received similar injections but lacking tick antigen. All injections were subcutaneously given in different sites of the animal body. Sera were obtained on 0,2,4,8,12 week post vaccination. Each rabbit was infested with 10 adult males and 10 unfed adult females *H.dromedarii* fourteen days after the booster dose, ticks were removed from rabbits 2 weeks later, each female tick was weighted and individually placed in vials held at 26°C in 85% RH. Egg mass produced by each female was weighed and the number of hatched larvae within 60 day of tick engorgement was recorded The pre- oviposition period, oviposition period egg, mass reduction were recorded and statistically analyzed using the Student's t-test. The percent of tick weight reduction and reproductive index were also determined according to the following equations (Kumar and Kumar, 1995).

$$\text{Percent of tick weight reduction} = \frac{\text{mean tick engorgement weight on immunized animal}}{\text{mean tick engargement weight on control animal}} \times 100$$

$$\text{Reproduction index} = \frac{\text{mean egg mass weight}}{\text{mean tick engorgement weight}}$$

Polyacrylamide gel electrophoresis

Polyacrylamide gel electrophoresis for antigen I and II were carried out according to Laemmli (1970). Fifty *ug* of total protein from each antigen were loaded in each lane. The gel was rum at 100 V in 3% stacking gel at 200 V in 8% resolving gel. Protein bands were stained by silver stain (Blum et al., 1987).

Electrophoretic transfer of proteins bands from polyacrylamide gels to nitrocellulose sheets.

Immunologic detection of antigens blotted to nitrocellulose sheet was performed essentially by the method of Towbin et al (1979). The nitrocellulose strip corresponding to gel lanes were cut and untreated sites were blocked by incubation in 5% bovine serum albumin in PBS-T 0.3%. The strip were incubated with tested sera (diluted 1:100 in washing buffer) for one hour at room temperature and another one hour with anti-rabbit peroxidase as conjugate (Accurate chemical & scientific Corporation, Westbury. N.Y-11590). (1:750 dilution in washing buffer) Bounded enzyme was detected using 4 CN peroxidase substrate.

Enzyme Linked Immunosorbent Assay to detect antibody response (ELISA).

Optical density value (OD) of sera samples obtained from vaccinated rabbits with antigen I and II were determined by ELISA according to the method described by Voller et al., (1979).

200ul of 0.5ug/ml antigen I and II were used to coat the ELISA microtiter plate wells. The optimum concentration of rabbit anti-peroxidse conjugate was 1/800 and sera dilution at 1/200. An absorbance reading equal or more than twice the mean value of all negative control sera was considered positive. (Iacona et al. 1980).

RESULTS

Two antigenic extracts were prepared; antigen I from ticks eggs, and antigen II from the midgut and sex organs. Observation made on the feeding performance of *H.dromedarii* female ticks are shown in table 1. It was revealed that both groups of vaccinated rabbits were affected through significant reduction ($P<0.001$) in engorgement weight, egg mass and number of hatched larvae. These reductions were prominent in group vaccinated with antigen II.

The reproductive success of female *H.drimedarii* obtained from immunized rabbits shown in table 2. Significant differences were found among

Table (1): Some parameter of ticks fed on rabbit vaccinated with antigen I and II (mean ± S.E.).

Type of antigen	Rabbit Number	Mean wt of fed female tick (mg)	Mean wt of egg mass/female tick (mg)	Mean No of larvae/female tick
Controls	1	355.9±10.5	214.3±7.5	
	2	311.2±17.3	213.2±12.1	
	3	496.2±3.9	257.2±13.0	
	4	387.9±24.5	219.1±5.5	
	5	378.2±9.2	214.8±9.2	
Antigen I	1	190.8±11.9*	43.1±7.2	107
	2	173.2±12.2*	0	0
	3	207±14.6*	0	0
	4	193.1±9.2*	0	0
	5	229.6±12.9*	48.3±9.3	152
Antigen II	1	105±4.2*	24±3.1	0
	2	107±4.3*	24.1±4.2	0
	3	108.7±4.8*	0	0
	4	92±3.5*	0	0
	5	99.5±3.8*	0	0

* Significantly different at $P<0.001$ compared with controls.
Mean weight of fed female tick of all control = 379.2
Mean weight of egg mass of all tick control = 214.2
Mean reproductive index of tick control = 0.56
Each rabbit was infested with 10 adult males and 10 unfed adult females.

Table (2): Reproductive success of *H.dromedarii* female ticks on immunized rabbit.

Type of antigen	Rabbit number	% of Tick weight reduction	Pre. O.P.	O.P	Egg mass reduction (%)	Reproductive index
Antigen I	1	48.6	10.9	19.6	80.9	0.21
	2	69.1	0	0	100	0
	3	45.9	0	0	100	0
	4	70.5	0	0	100	0
	5	31.2	11	21.2	72.5	0.23
Antigen II	1	70.5	11.2	20.9	92.5	0.14
	2	72.3	11.4	23.2	92.0	0.16
	3	74.2	0	0	100	0
	4	79.4	0	0	100	0
	5	76.5	0	0	100	0

Pre. O.P.: Pre Oviposition period (days) # O.P = Oviposition period (days)
Mean pre-oviposition period of control ticks = 5.4 day
Mean oviposition period of control ticks = 14.2 day

Table (3): Electrophoretic analysis of antigen I and II

Lanes / Band	Lane 1 Mol.W	Protein amount ug/dl	Lane 2 Mol.W	Protein amount ug/dl	Lane 3 Mol.W	Protein amount ug/dl
1	614.7	17.5	753.36	17	817.20	44.8
2	205	14.6	272.52	6.36	392.99	11.2
3	97	14.9	108.69	8.28	154.21	11.3
4	66	17.3	80.012	12.9	80.827	8.72
5	45	20.7	36.822	30.2	55.413	9.01
6	29	15	24.890	25.3	25.370	14.9
Sum		100		100		100
In lane		100		100		100

Lane 1: Molecular weight marker
Lane 2: Antigen I (tick egg antigen).
Lane 3: Antigen II (Sex organs and midgut antigen).

Table (4): OD value of sera samples obtained 0,2,4,8 and 12 weeks post vaccination with antigen I (egg antigen).

Sample No. \ Weeks	0	2	4	8	12
1	0.195	1.590	1.809	1.627	1.202
2	0.147	0.720	0.870	0.671	0.676
3	0.117	0.690	0.708	0.541	0.576
4	0.050	0.521	0.642	0.491	0.406
5	0.009	0.282	0.536	0.342	0.363

OD Value of 0.590 or moe were considered as positive result.

Table (5): OD value of sera samples obtained 0,2,4,8 and 12 weeks post vaccination with antigen II (Sex organs and midgut antigen).

Sample No. \ Weeks	0	2	4	8	12
1	0.153	0.510	2.291	1.199	1.193
2	0.098	0.434	1.936	0.914	0.776
3	0.074	0.397	0.969	0.605	0.641
4	0.060	0.321	0.749	0.442	0.483
5	0.045	0.309	0.376	0.396	0.325

OD Value of 0.402 or moe were considered as positive result.

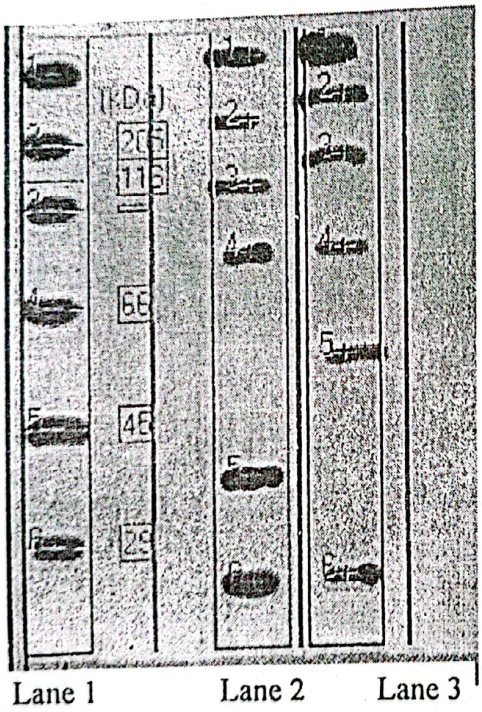

Fig. (1): Silver stained polyacrylamid gel electrophoresis of tick antigen
Lane 1: Molecular weight marker.
Lane 2: Antigen I (tick egg antigen).
Lane 3: Antigen II (sex organs and midgut antigen).

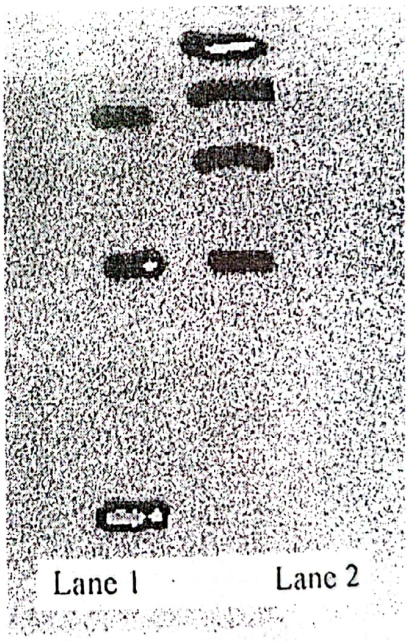

Fig. (2): Immunoblot using egg antisera with egg antigen (lane 1) and sex organs with midgut antisera against sex organs and midgut antigen (lane 2).

ticks reared on rabbits vaccinaed with antigen I and II. Tick weight reduction, increased pre-oviposition period, increased oviposition period, egg mass reduction and decrease of reproductive index were detected.

Electrophoretic analysis of both antigens in Sodium dodecyl sulphate (SDS polyacrylamid gel which ran at 100 V in 3% stacking gel at 200 V in 8% resolving gel showed different size bands from top to bottom. Protein marker was run in lane 1 while antigen I and II in lane 2 and 3 respectively (Fig.1, table 3). Immunoblotting of antigen I and II recognized three specific bands for antigen I and for 4 antigen II (Fig.2).

To detect antibody response against the antigen I and II, enzyme linked immunosorbent assay (ELISA) was carried out on sera collected from rabbits on 0,2,4,8,12 week post vaccination. The results showed that the maximum antibody level had occurred 2 week post boostering (4^{th} week) (table 4 & 5).

DISCUSSION

Immunization trials reported here using antigens from the tick eggs (Antigen I), sex organs and midgut (Antigen II) were based on the crude concept that ticks feeding on appropriately immunized host might ingest antibodies specific for antigens within the alimentary tract and reproductive organs of the ticks leading to deleterious effect on the feeding and reproductive behavior of the ticks. Two antigenic extracts were prepared from partly fed female *H.dromedarii* ; antigen I from tick eggs and antigen II from the sex organs and midgut. It seems that feeding and preproductive performances of ticks infesting immunized rabbits were significantly reduced. It seems reasonable to speculate that more impressive results were obtained in rabbits immunized with antigen II.

These results agreed with Willadsen et al ., (1989) and Willadsen (1997). They 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 lysis of these cells and drastically increased leakage of material from the gut into the ticks haemolymph. However Ackerman et al., (1980) mentioned that these antibodies may bind to a target epitopes of salivery glands or ovaries. Our results confirmed the findings of Allen and Humphreys (1979) who achieved greater success in immunizing guinea pigs and cattle with tick extract derived from partly fed than from unfed females of *Dermacentor andersoni*. This is likely to be due to the fact that partial feeding of ticks increases the number of gut cells to its maximum (Agbede and Kemp, 1986).

Vaccination with antigen II gave highest protection of rabbits against hard ticks in terms of significant alteration in feeding and reduce reproductive success of these ticks. These results are in agreement with the finding observed by other workers using *Hyalomma anatolicum* ticks, *Dermacentor andersoni* (Allen and Humphreys, 1979; Ackerman et al., 1980 and Kumar, 1990) *Amblyomma americaum* (Wikel et al., 1987) *B. microplus* (Willadsen et al., 1989) and *H.dromedariii* midgut antigens in rabbits (Kumar and Kumar. 1995).

The observation of reduced egg mass in *H.dromedarri* in response to vaccination is in agreement with the findings of Opdebeeck et al., (1988) and Wong et al., (1990) for *B.microplus* and for *Rhipicephalus appendiculatus* (Essuman et al., 1991) on cattle immunized with tick gut antigen suggesting that the function of the tick reproductive organs is impaired . Concerning the level of protection against ticks, Binnington and Kemp (1980) and Wang and Nuttall (1995) support the suggestion that ticks has a mechanism for eliminating host immunoglobulin via saliva and store host immunoglobulin in the haemocoel but other ticks can gradually break down the harmful host proteins. These findings may explain why some ticks can survive after feeding on vaccinated host.

Electrophorytic analysis of antigen I and II in SDS polyacrylamid gel showed six band for each antigen. Immunoblotting provides a mean of identifying the most specific antigenic bands to female *H.dromedarii* eggs and sex organs with midgut. Regarding to our results tick egg antisera recognized three bands (272.52 KDa, 80.012 KDa and 36.822 KDa) with tick egg antigen while sex organs and midgut antisera recognized four bands (817.20 KDa, 392.99 KDa, 154.21 KDa and 80.827 KDa). All the above mentioned bands did not appear with the control sera indicating 100% specificity.

For detection of anti-tick antibody, the enzyme-linked immunosorbent assay (ELISA) carried out on sera samples collected on 0,2,4,8,12 weeks post vaccination of rabbits (Voller et al., 1979). The maximum antibody titer was reached on the 4th week post vaccination on using both types of antigen. Although the immunoglobulin in rabbits sera was reached high level but the protection against ticks was not the same. These may be due to the ability of some tick species to pass the host immunoglobulin through its gut wall into the hemolymph (Ackerman et al., 1980 and Minoura et al., 1985).

It is recommended that vaccination of animals heavily infested with ticks should receive a simultaneous treatment with acarcid; inspite of its side effects; to keep the ticks number under some control until the tick vaccine could be effective to give better overall control.

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