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Artificial immunization of rabbits with *Hyalomma dromedarii* tick-derived midgut antigen

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ABSTRACT

New Zealand white rabbits were immunized with partly fed *Hyalomma dromedarii* tick-derived midgut concealed antigens (supernate and pellet fractions) and Freund's complete adjuvant (FCA). The rabbits received three inoculations subcutaneously on days 0, 14 and 21 at a dose rate of 1 mg antigen per animal. The effects of the immunity induced was determined by infesting the rabbits with adult *H. dromedarii* ticks. In immunized rabbits a significant reduction in tick yield, engorgement weight, oviposition period, egg mass weight and percentage of egg hatchability was found. The gut supernatant antigen fraction induced the best protection in terms of reduced feeding and reproductive performance of the ticks.

Key words: Vaccination, ticks, *Hyalomma dromedarii*, midgut antigens, rabbits.

Ticks cause large economic losses directly as pests and indirectly by transmitting diseases and pose a major threat to the livestock industry of the world. At present, chemical acaricides are used for the control of ticks but due to the emergence of resistance to acaricides in ticks, meat and milk residual problems, environmental pollution, alternative control strategies are required. The immunological control of ticks is gaining importance and encouraging results have been achieved in the past by immunizing various animals (cattle, guinea-pigs and rats) with the respective tick antigens against *Boophilus microplus*, *Rhipicephalus appendiculatus*, *Amblyomma americanum* and *Dermacentor variabilis* infestations (Ackerman *et al.*, 1980; Brown and Askenase, 1983; Johnston *et al.*, 1986; Opdebeeck *et al.*, 1988; Willadsen *et al.*, 1989; Wong *et al.*, 1990; Essuman *et al.*, 1991).

Hyalomma dromedarii is the second most common tick among *Hyalomma* species in India and is suspected of playing an important role

in transmitting a haemoprotozoan disease, bovine tropical theileriosis, caused by *Theileria annulata* (Bhattacharyulu *et al.*, 1975). In the present study immunity was induced in rabbits as a model with midgut antigens, since it has been reported by various workers that the immunologically potent antigens are localized on the digest cells of the midgut of the tick (Agbede and Kemp, 1986; Kemp *et al.*, 1986, 1989; Tracey-Patte *et al.*, 1987). However, the feasibility of immunizing animals against *H. dromedarii* ticks has remained unexplored so far.

Hyalomma dromedarii tick colonies were maintained in the laboratory according to the methods of Walker *et al.* (1985). Ticks were fed on the ears of rabbits and, in the laboratory, they were kept at 28°C and 85% relative humidity (RH). New Zealand white rabbits, aged 3–4 months of both sexes, were used in the experiments. Rabbits were kept individually in cages which were placed over bottom trays filled with water to prevent entry of tick infestations from any external source.

Partially fed (4–5 days) *H. dromedarii* female ticks were used for antigen preparation. Ticks were surface sterilized with 1% merthiolate solution and embedded in low melting points wax (52–54°C) for dissection. Dissections were performed according to Purnell and Joyner (1968). The midgut diverticula were harvested and placed in cold 0.1 M phosphate buffered saline (PBS) of pH 7.2 and stored at −20°C until used. Subsequently, midgut diverticula were homogenized, sonicated with a 100 W probe for 2 min, centrifuged at $10000 \times g$ for 30 min at 4°C and separated into supernate and pellet fractions. The pellet fraction was reconstituted in PBS. Protein concentrations were determined according to Lowry *et al.* (1951) and the concentration was adjusted to 1 mg protein per ml.

The experimental rabbits were split into five groups of four animals each. The animals in the first two groups (groups I and II) were inoculated with gut supernatant antigen (GSA) with or without Freund's complete adjuvant (FCA), while groups III and IV were immunized with gut pellet antigen (GPA) with or without FCA. The animals of the fifth group were used as controls. All animals received injections on days 0, 14 and 21, the first two injections being with FCA, while the third injection was administered without FCA in groups I and III. Fourteen days after the last inoculation the animals were challenged with ten pairs of adult *H. dromedarii* ticks. The effects of induced immunity on feeding and reproductive performance of female adult ticks were monitored and data were statistically analysed using the Student's *t*-test. The percentage of tick

rejection, percentage reduction in engorged tick weight and the reproductive index were estimated by the following formulae:

$$\text{percent tick rejection} = 1 - \frac{\text{mean percentage of tick yield on immunized animals}}{\text{mean percentage of tick yield on control animals}} \times 100$$

$$\text{percent tick weight reduction} = 1 - \frac{\text{mean tick engorgement weight on immunized animals}}{\text{mean tick engorgement weight on control animals}} \times 100$$

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

Observations made on the feeding performance of *H. dromedarii* female ticks are shown in Table 1. It was revealed that rabbits of group I significantly ($p < 0.01$) reduced the number of engorged female ticks, while no significant reduction was observed on rabbits in the other groups. Furthermore, ticks harvested from the rabbits of group I and II were found to be significantly ($p < 0.01$) reduced in their engorgement weights, whereas this reduction was not significant with the other groups. In general, there was no effect of immunization on the duration of the feeding period of the female ticks.

The reproductive success of female *H. dromedarii* obtained from immu-

TABLE 1

Feeding performance of *H. dromedarii* female ticks on immunized rabbits.

Group number	Immunization regimen	Number of animals	Engorgement period (days)	Tick yield (%)	Engorgement weight (mg)	Weight reduction (%)	Tick rejection (%)
I	Midgut supernate with FCA	4	9.2 ±0.3 (7–12)	47.5 ±2.5**	154.8 ±14.8**	70.7	24
II	Midgut supernate without FCA	4	8.7 ±0.2 (7–11)	57.5 ±4.8	343.7 26.2**	34.9	8
III	Midgut pellet with FCA	4	8.6 ±0.2 (7–10)	57.5 ±2.5	455.00 ±32.6	13.8	8
IV	Midgut pellet without FCA	4	8.7 ±0.4 (7–12)	62.5 ±2.5	517.5 ±28.1	2.0	0
V	Control	4	8.7 ±0.2 (7–12)	62.5 ±2.5	527.9 ±40.3	0	0

Each values denotes the mean ± SE and the values in parentheses represent the range.
**$p < 0.01$.

TABLE 2

Reproductive success of *H. dromedarii* female ticks fed on immunized rabbits.

Group number	Immunization regiment	Number of animals	Pre-oviposition period (days)	Oviposition period (days)	Egg mass weight (mg)	Egg mass reduction (%)	Engorgement weight (mg)	Reproductive index	Egg incubation period (days)	Egg hatchability (%)
I	Midgut supernate with FCA	4	10.4 ±0.4** (8–13)	33.2 ±1.2**	76.2 ±6.6**	74.9	154.8 ±14.8**	0.47	34.2 ±0.3	87.6 ±0.3**
II	Midgut supernate without FCA	4	9.4 ±0.4** (7–12)	31.3 ±1.0**	169.4 ±12.7**	44.2	343.7 ±26.3**	0.49	34.3 ±0.2	88.2 ±0.3**
III	Midgut pellet with FCA	4	8.7 ±0.3 (8–11)	35.4 ±1.2**	238.5 ±16.8**	21.5	455.0 ±32.6	0.53	34.2 ±0.2	92.7 ±0.3**
IV	Midgut pellet without FCA	4	8.4 ±0.2 (7–10)	39.1 ±1.1**	283.1 ±15.0	6.8	517.5 ±28.1	0.55	34.4 ±0.2	93.0 ±0.3**
V	Control	4	8.2 ±0.2 (7–10)	44.1 ±1.3	303.7 ±23.0	0	527.9 ±40.3	0.57	34.4 ±0.3	95.0 ±0.3

Each value denotes the mean ± SE and the values in parentheses represent the range.
**$p < 0.01$.

nized rabbits is shown in Table 2. Significant differences ($p < 0.01$) (except for the egg incubation period) were found among ticks obtained from animals in groups I and II, while the ticks from animals in groups III and IV showed a significant reduction ($p < 0.01$) only of their oviposition period, reproductive index and percentage of egg hatchability. Egg masses laid by ticks obtained from group III were also significantly reduced. The egg incubation period also remained unaffected in groups III and IV. No significant difference ($p > 0.05$) was observed in the pre-oviposition period of female ticks obtained from animals of groups III and IV.

Our results confirm the findings of Allen and Humphreys (1979) who achieved greater success in immunizing guinea-pigs and cattle with tick extracts 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 the maximum (Agbede and Kemp, 1986).

Vaccination with GSA together with FCA gave the highest protection of animals against adult ticks in terms of a significant alteration in feeding as well as reduced reproductive success of these ticks. The findings revealed that impaired feeding in terms of reduced tick yield and engorgement weights of *H. dromedarii* female ticks can be induced by inoculating GSA with FCA. These results are in agreement with the findings of Kumar (1990) using *Hyalomma anatolicum* ticks fed on cattle immunized with gut antigens. Similar findings were observed by other workers using *D. andersoni* (Allen and Humphreys, 1979; Ackerman *et al.*, 1980). *A. americanum* (Wikel *et al.*, 1987) and *B. microplus* (Willadsen *et al.*, 1989), when fed on animals immunized with various tick antigens. It is likely that these tick species elicit the same immunological response as induced by *H. dromedarii*. The observation of a reduced egg mass in *H. dromedarii* in response to GSA is in agreement with the findings of Opdebeeck *et al.* (1988) and Wong and Opdebeeck (1989), for *B. microplus* on cattle immunized with tick gut antigen suggesting that the function of the tick reproductive organs is impaired. Our findings should be confirmed in cattle by immunizing them with *H. dromedarii* tick derived midgut antigen and further studies are required to define the nature of the antigens capable of eliciting a protective response.

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