

## Effect of Tick Gut Extract on Protection of Cattle against *Hyalomma anatolicum anatolicum*

### Short Communication

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### **Summary**

To develop immunity against *Hyalomma anatolicum anatolicum*, the protective effect of tick gut extract antigen was studied during a period of two years. Ten male Holstein-Freisian calves were randomly divided to two groups of five each. The test group was inoculated three times with the adjuvanted antigen. Control group was delivered Freund's adjuvant. They were challenged with fifty pairs of homologous adult and characteristics representing tick. Feeding and fertility were recorded and compared with control group. A significant decrease in percent of engorgement, engorged weight, feeding index, percentage of oviposition, egg mass and fertility index were observed in the ticks fed on the test calves ( $p < 0.05$ ).

**Key words:** immunization, tick, *Hyalomma anatolicum*, midgut, antigen, cattle

### **Introduction**

*Hyalomma anatolicum anatolicum* is the most common tick vector of bovine and ovine tropical theileriosis in Iran (Hooshmand-rad & Hawa 1973, Hashmi-fesharaki 1987). There are considerable production losses in the livestock industry as results of disease. Without effective tick control it would be virtually impossible to improve livestock production. Although control of ticks by chemical acaracides is a practical method, the increasing problem of resistance to acaracides has stimulated research on alternative methods of control in recent years. Immunization of cattle against

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ticks is a promising alternative to expensive and laborious acaricide treatment. To date, only the midgut Bm86 vaccine that affects *Boophilus microplus* feeding on cattle has successfully been commercialized in Australia (Willadsen *et al* 1995) and Cuba (Dela Fuente *et al* 1998). Acquired immunity against *H.anatolicum anatolicum* has been studied previously in rabbits (Manohar & Banerjee 1992) and cattle (Sran *et al* 1996, Sangwan *et al* 1998, Ghosh *et al* 1999). In these researches, the extracts from salivary gland, whole tick, whole larvae tick and whole nymph tick have been used for immunization. Artificial immunization of rabbits using midgut tick antigen has been successful (Razmi *et al* 2003). In the present study, induction of immunity in calves against *H.anatolicum anatolicum* using tick gut antigen was studied.

### **Materials and Methods**

**Tick.** Laboratory colonies of *H.anatolicum anatolicum* were used. Larvae were fed on ears of rabbits and in the laboratory. Ticks were kept at 28°C and 85% relative humidity.

**Experimental animal.** The Holstein-Freisian male calves, 6-8 months ages, were born and raised in the farm of Ferdowsi University and tick free herd.

**Antigen preparation.** Semiengorged adult female ticks were used for preparation of gut antigens. Ticks were surface sterilized by washing in 30% hydrogen peroxide followed by rinses in 70% alcohol and in phosphate buffered saline (PBS) pH7.2 with 100IU/ml penicillin and 100mg/ml streptomycin. According to Opdebeeck *et al* (1988) the guts were dissected from 100 female ticks under a binocular stereomicroscope. Dissected gut was held in cold PBS and stored at -70°C until used. The gut was sonicated (Soniprep-150, MSE England) at 200 W for 3min, while cooling in ice. The homogenate was centrifuged at 10,000g for 30min at 4°C (Soravel, 6000B). The pellet was discarded and protein concentration was determined 4.2mg/ml according to Lowry *et al* (1951).

**Immunization procedure.** Ten male calves were divided randomly into two groups of five. 5ml of the prepared antigen was mixed thoroughly with equal volume of Freund's complete adjuvant (FCA) and 2ml of the adjuvanted antigen was inoculated subcutaneously into each calf in test group at day 0. They were delivered the combined antigen with FCA and Freund's incomplete adjuvant (FIA) at days 14 and 28. Control calves were injected with saline in both adjuvants in a parallel inoculation regime.

**Challenge trial.** Two weeks after the third inoculation each calf of test and control groups were challenged with fifty pairs (25♀ and 25♂) of adult *H.anatolicum anatolicum*. The bags were examined daily and the observation was recorded up to seven days. After engorging the detached ticks were collected and placed individually in glass tubes in an incubator at 28°C and 85% relative humidity. These ticks were observed daily and their oviposition and oviposition periods were recorded. After oviposition was completed, the egg mass was weighed.

**Biological parameters.** The following biological parameters related to female tick feeding and reproductive performance were observed during each infestation: percentage of engorgement, feeding length, female weight detachment, percentage of oviposition, preoviposition period, egg mass also feeding efficiency index (weight in mg divided by length of feeding in days) and fertility efficiency index (weight of the egg mass divided by the weight of the engorged female).

**Statistics.** The data were analyzed by student's *t*-test.

### ***Results and Discussion***

The result of the experiment is summarized in table 1. As shown in the table, immunization with gut antigen significantly affected some feeding characteristics including percent of engorgement, engorged weight and feeding index, and some fertility attributes including egg mass and fertility index as compared with the control group ( $P<0.05$ ).

Table 1. *Effects on the feeding and reproductive parameters of Hyalomma anatolicum anatolicum female ticks fed on calves (mean±SD)*

Parameter	Group	
	Test	Control
%Engorgement	44.44±12.1*	62.41±14.23
Engorgement period (day)	7±1.15	7±0.64
Engorged weight (mg)	410±122.5*	625±3.38
Feeding Index	50.27±14.39*	88±4.31
%Oviposited	55.33±26.35	97.5±2.5
Preoviposition (day)	7±1.15	7.2±0.9
Oviposition (day)	17.25±0.25	19.5±0.86
Egg mass (mg)	223.33±113.48*	383.33±18.55
Fertility Index	42.5±21.3*	56±0.5

\*Significantly difference from control groups by Student's *t*-test ( $P<0.05$ )

The principle of vaccination against ectoparasites using concealed antigens is now well established (Willadsen & Kemp 1988). The Concept has led to commercial development of a vaccine against the tick *Boophilus microplus*, named as TickGARD and TickGARD Plus (Willadsen *et al* 1995). The Bm 86, a glycoprotein located in microvilli membranes of the midgut epithelium, is the main antigen of TickGARD (Kemp *et al* 1986). According to *in vivo* and *in vitro* investigations, cell mediated immune reactions do not appear to play a part in this vaccine (Kemp *et al* 1986 and 1989). Immunoglobulin G with or without complement system is sufficient to cause the damage in the tick gut. However, this work stimulates the further development of vaccines against other tick species.

Immunization trails conducted in the present study using tick-derived midgut antigen from *H.anatolicum* were based on the assumption that ticks feeding on appropriately immunized hosts might ingest specific antibodies for target antigen in midgut of tick, producing deleterious effect on the feeding and reproductive performance of the tick (Allen & Humphreys 1979, Johnston *et al* 1986). In the present study, the supernatant antigen produced high protection and affected feeding

and fertility of adult ticks. Our results confirm the finding of Opdebeeck *et al* (1988) who dissected tick midguts from partially engorged females and vaccinated Hereford cattle. These results are in agreement with findings of Kumar and Kumar (1995) using *H.dromedari* tick fed on rabbits Immunized with gut supernatant antigens. Similar findings were reported when *Rhipicephalus sanguineus* (Szabo *et al* 1997) and *H.marginatum marginatum* (Sahibi *et al* 1996) fed on animals immunized with gut antigen. We believe that gut-concealed antigen derived from *H.aanatolicum anatolicum* can be strongly protective.

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