ELECTROPHORETIC AND IMMUNOLOGICAL CHARACTERIZATION OF SPECIFIC ANTIGENS INDUCING IMMUNE RESISTANCE DERIVED FROM CATTLE TICK BOOPHILUS ANNULATUS

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ABSTRACT

SDS- PAGE analysis was carried out on salivary gland antigen (SGA) of the cattle tick Boophilus annulatus . A protein profile was observed with various polypeptide bands.On the other hand,has been determined the presence of the specific antigen on salivary gland extract by means of immunoblotting analysis using hyperimmune serum of rabbit inoculated with extract. Higher reactivity was observed at three polypeptides (48,42 and 32 KD).

INTRODUCTION

The Acquisition of immunity in cattle to tick infestation was first described by ***Johnson and Bancroft (1918). Trager (1939)*** described a similar phenomenon in rabbits infested with *Dermacenter variabilis*. Attempts have been made to determine the bases of antitick immunity ***(Riek 1962, Allen et. al. 1977, Allen and Humphreys 1979, Askenase 1980, Brown et. al. 1982, Kemp et. al. 1986 and Mongi et. al. 1986).***

Several workers induced resistance to ticks using salivary glands: *Hyalomma anatolicum* ***(Kohler et. al. 1967)*** , *Ripicephalus sanguinen* ***(Garin and Grabarev ; 1972)***, *Dermacenter andersoni* in guinea pigs and cattle ***(Allen and Humphreys,1979)***and *Ammblyomma americanum* in guinea pigs ***(Brown , et al., 1984).***The immunogenicity of the salivary

gland was demonstrated firstly by ***Allen and Humphreys (1979)*** and according to ***Brown et. al. (1984)*** and ***Brown and Askenase (1986 a, b)*** the antigens responsible for the induction of host resistance appear to be of salivary gland origin.

MATERIALS AND METHODS

Ticks: About 2000 adult ticks(*Boophilus annulatus*)were collected from naturally infested cows from different investigated farms and identified according to ***Hoogstraal (1956).***Ticks were kept in a biological incubator at constant temperature 28 °C and 75 % relative humidity.

Preparation of salivary gland antigens (SGA): Salivary glands were obtained from 1000 adult male and female *B.annulatus* after feeding for 4 to 5 days on rabbits as described by ***Martinod et. al. (1985).*** The salivary glands were harvested in 2 ml phosphate buffered saline (PBS) without protease inhibitors. The glands were sonicated at amplitude of 22 (Mm)for 2 minutes at 4°C.The sonication process was repeated four times at 3 minutes intervals. A soluble fraction of the extract was obtained after centrifugation at 1000 xg for 15 minutes.The protein content in the soluble fraction was determined by the method of ***Lowry et. al. (1951).***

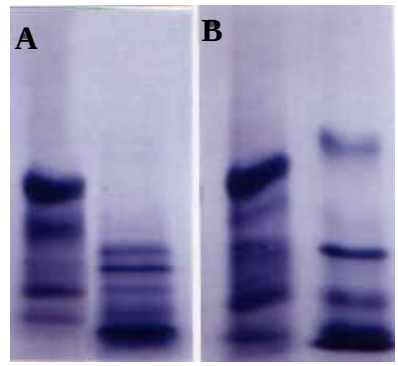
Preparation of hyperimmune serum: Three successive injections with SGA in a group of 5 Boscat rabbits was performed. One week after the completion of the 2nd and one week after completion of the 3rd injection, blood was collected from the central ear vein of each rabbit and allowed to clot at room temperature for 1 hour. After incubation overnight at 4 °C, serum was collected by centrifugation and stored at -20 °C until use.

Detection of specific antigens in salivary glands by the western blot technique. Antiserum to SGA was used to detect tick antigens in the SGA extracts. The extract was analyzed on 5 to 15 % polyacrylamide gradient gel in the presence of sodium dodecyl sulfate (SDS – PAGE) by the method of ***Laemneli and Favre (1973).*** The separated proteins were electrophoretically transferred into nitrocellulose membrane as described

by ***Burnette (1981).*** Non - specific reactive sites on the nitrocellulose paper were blocked by 5% fat - free milk in Tris - buffered saline (TBS) for 2.5 hours at room temperature 25 ^{O}C. Strips were incubated with the respective primary antisera diluted 1:400 for 4 hours at room temperature under constant shaking. After a series of washing in 5% fat - free milk in TBS containing goat - antirabbit IgG horseradish peroxidase (HRP) conjugate(SIGMA - ALDRICH)diluted 1:1000.The strips were incubated for 2 hours in the conjugate at room temperature with constant shaking. The conjugate was decanted and the strips were washed in several changes of TBS. Finally, the strips were developed in 4 chloro -1-naphthol in methanol at room temperature.

RESULTS

Separation on SDS-PAGE and identification by the western Blotting technique of the different polpeptides detectable on SGA by the antiserum revealed the presence of many polypeptides. Polypeptides recognized by antiserum to SGA ranged in molecular weight from 18.5 to 85.0 KD. Three polypeptides (48,42 and 32 KD) were the most prominent (Fig.1 and Table.1).



Fig(1): A) SDS - PAGE of antigen purified from salivary glands of *Boophilus annulatus* .

B) Immunoblotting analysis of antigen purified from salivary glands of *Boophilus annulatus.*

Lane (1): Molecular weight marker.
Lane (2): Salivary gland antigen of B.annulatus.

Table : 1

Band	Molecular weight marker (KD)
1	**97.4**
2	**66**
3	**45**
4	**36**
5	**19**
6	**14**

DISCUSSION

In a study on *Ripicephalus. appendiculatus*, ***Fawcett et. al. (1986)*** showed an enormous increase in types 11 and 111 alveoli in the course of feeding. The cells became enlarged and the alveolar lumenae diluted filling with salivary secretions. In a related study on *Amblyomma .americanum*, ***Shelby et. al. (1987)*** showed biochemical differentiation in the course of feeding of this tick. In an earlier study, ***McSwain et. al. (1982)*** showed the synthesis of a new polypeptides in *A. americanus* and a 25 fold increase in the size of cells and protein content.

Gill et. al. (1986) showed in *Hyalomma anatolicum* (Koch) that several polypeptides found in native ticks increase in quantity with feeding.

Brown et. al. (1982, 1984) identified in the salivary glands of the three - host tick *A. americanum* a 25-kd polypeptide that they considered to be responsible for the induction of antitick immunity in guinea pigs. In a study using *Dermacenter andersoni* Stiles SGA and tick resistant rabbit serum, ***Gordan and Allen (1987)*** identified several polypeptides ranging from 18 to 172 KD. The polypeptide of 172 Kd was considered immuno-dominant in the SGA preparations. ***Shapiro et. al. (1987)*** reported that *R.*

appedicculatus SGA contained a 90 - KD polypeptide and considered it responsible for the induction of antitick resistance in rabbits.

Our study has identified a common polypeptide in SGA responsible for the elicitation of antitick immunity where three distinct polypeptide bands were prominent at 48, 42 and 32 KD. This disparity may reflects difference in experimental approach.

Recently, ***Willadsen (1987)*** speculated on different immunological approaches to the control of ticks and pointed out that mechanisms in tick resistance arising from infestations are different from those generated by vaccination with tick antigens.The most important difference was that tick resistance as a result of infestation is affected by immediate hypersensitivity reactions; whereas antibody mediated responses are responsible for immunity after vaccination. The latter responses were shown to cause gross damage to *B. microplus* ticks feeding on cattle immunized with tick derived antigens ***(Agbede and Kemp 1986).***

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التحليل الكهربي والصفة المناعية لتحديد الانتيجين المناعي للغدد اللعابية الخاصة بقراد الأبقار نوع بوفيلس انيولات

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**** معهد بحوث صحة الحيوان– المعمل الفرعي بمحمية الجميل الطبيعية ببورسعيد**

تم استخدام اختبار الاستقطاب الكهربي للتحليل الانتيجينى لمستخلص الغدد اللعابية لقراد الأبقار نوع بوفيلس انيولاتس0 وقد أظهرت النتائج وجود أكثر من نوع لعديد الببيتيد وبأجراء اختبار الطبع المناعي لهذه الانتيجينات ضد المصل عالي المناعة المحتوى على مضادات تلك الانتيجينات ، أظهر مستخلص الغدد اللعابية تفاعلات مناعية متخصصة . وكان أعلى تفاعل مناعي عند عديد الببيتيد كان وزن الجزئيي عند 48 ، 42 ، 32 كيلودالتون.