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VACCINATION OF DOGS AGAINST *RHIPICEPHALUS SANGUINEUS* TICKS USING SALIVARY GLAND ANTIGEN

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SUMMARY

The present study aimed to induce experimental resistance in dogs against *Rhipicephalus sanguineus* ticks using salivary gland antigen. Four dogs were vaccinated by salivary gland antigen obtained from partially fed adult ticks then challenged with 10 pairs of unfed adult ticks on the 35th day. The fifth dog remained as a control inoculated with adjuvant only. The results of vaccination showed a high protection in terms of decreased the percentage of tick attachment and significant reduction in the weight of engorged female ticks. SDS-PAGE analysis of salivary gland antigen revealed 14 bands ranging from 119.7 to 38 KDa. Immunoblotting with hyper-immune sera against salivary gland antigen showed 4 polypeptides. In contrast only three bands were detected with naturally infested dog sera. Three common polypeptides were recognized in both sera with molecular weights 97, 75 and 38 KDa, but two of them (97 and 38 Kda) were strongly recognized with hyper immune sera and faintly in naturally infested sera. One band of 88 KDa was recognized by hyper-immune sera only. Therefore, tick salivary gland antigen seem to have promising potentials in inducing resistance in dogs.

INTRODUCTION

Rhipicephalus sanguineus (the brown dog tick) is probably the most widely distributed tick species in the world and has been linked to tick-borne diseases in both humans and animals. *R. sanguineus* is a well known vector of many infections of dogs such as Babesia canis, *Hepatozoon, Ehrlichia canis, Rickettsia rhipicephali, Rickettsia Conorii, crimean- congo hemorrhagic fever* virus and *Thogoto* virus. High levels of infestation can cause skin irritation, anemia and damage in dogs. Prevalence of *R. sanguineus* in Egypt is very high. **ElKammah et al. (2001)** observed that, *R. sanguineus*-were found on dogs with 89.9%. The principal tick control method is the application of acaricides. This approach is associated with a number of disadvantages such as environmental pollution, resistance to the acaricides in addition of its high cost **(Rodriguez-Vivas et al. 2006)**. At present, there is no vaccine directed against *R. sanguineus* in dogs.

Several investigators are concerned with the problem of developing an efficient and safe tick- control by immunizing animals against ticks using salivary gland antigens of different species *Hyalomma anatolicum* **(Kohler et al., 1967)** *Amblyomma americanum* **(Brown et al., 1984)**. The acquired resistance to *R. sanguineus* ticks began with **(Garin and Grabarev, 1972)** who found that, rabbits could be immunized against adults *R. sanguineus* by injection with a suspension of tick salivary glands.

Resistance was accompanied with the production of anti-tick antibodies. **Martin Hernandez et al. (1994)** observed that, antibody levels were higher with female salivary glands and female mid gut extracts than those of male of *R. sanguineus*. The same authors in (1995) were analysed antigens from *R. sanguineus* by electrophoresis and Western blotting. They recorded that, the higher reactivity with anti-adult sera was observed against female salivary gland extracts. **Inokuma et al. (1999)** found that, 24 KDa protein from *R. sanguineus* was common to larvae, nymphs, adults and salivary gland extract in the sera of the repeatedly infested dogs with adult *R. sanguineus*. **Jittapalapong et al. (2000)** observed that, dogs immunized with salivary gland extract of *R. sanguineus* had the greatest impact on the feeding period and weight of engorged female ticks.

The present study is a trial to control *R. sanguineus* ticks by vaccination of dogs with salivary gland antigen, as well as to characterize salivary gland antigen using SDS-PAGE and immunoblot.

MATERIALS AND METHODS

Ticks: Ticks were obtained from the laboratory colonies of *R. sanguineus* maintained on rabbits in the Animal Health Research Institute, Dokki, Egypt.

Dogs: Five male dogs aged 4 months old and proved to be free from previous tick infestation.

Antigen preparation: Salivary glands of *R. sanguineus* were dissected from both partially fed male and female (4-5 days) into cold phosphate buffered saline (PBS pH 7.2). This extract was sonicated by ultrasonicator for 5 min in ice bath. Sonicated tissues were centrifuged at 14,000 rpm for 45 min at 4°C. The protein content of the supernatant was determined using Lowry's method **(Lowry et al., 1951)**. The salivary gland antigen was aliquoted and saved at -70°C until used.

Immunization of dogs: Dogs were immunized according to **Ferreira et al. (1996)** Four dogs were injected subcutaneously with salivary gland antigen (250 µg protein per animal) emulsified in equal volume in complete Freundís adjuvant. Two boosters were given on day 14 and 28 later using incomplete Freundís adjuvant. The fifth dog was injected with PBS emulsified in adjuvant and used as a control.

Hyper-immune sera: Sera of immunized dogs were collected on day 35 PI.

Naturally infested sera: 50 stray dogs were collected, positive infested cases with *R. sanguineus* ticks were 20 (40%). Blood samples were obtained from radial vein and sera were pooled for the immunoblotting.

Challenge of dogs: All dogs (immunized and control) were challenged with 10 pairs of unfed adult *R. sanguineus* ticks on day 35 PI using cotton ear bag.

Electrophoretic analysis of salivary gland antigen by SDS-PAGE: Ten µg of salivary gland antigen were electrophoresed using 10% SDS-PAGE under reducing conditions **(Laemmli, 1970)**. The fractionated antigen was visualized by Coomassie blue stain. The gel was soaked overnight in the Coomassie blue R (0.25% Coomassie blue powder dissolved in destaining solution). The gel was then destained with destaining solution (45% methanol, 5% glacial acetic acid and 50% distilled water) with several changes till the bands became clear.

Enzyme immunoblot: Salivary gland antigen was electrophoresed using 10% SDS-PAGE under reducing conditions. The fractionated antigen was electrically transferred onto nitrocellulose (NC) membrane. NC sheets were cut into 0.5 cm strips **(Towbin et al., 1979)** followed by blocking in 5% BSA in PBS for 2 h on rocker platform. Sera from immunized dogs and naturally infested dogs were diluted at 1:100 in 5% BSA/PBS-T, and reacted with fractionated salivary gland NC strips separately for 2 h on rocker platform. Following washing, protein A peroxidase as conjugate diluted at 1:1000 (Sigma Co.) in PBS-T was added to NC strips for 1h on rocker platform. The chromogen AEC substrate (Sigma Co.) was added to NC strips and allowed to develop for 30 min. The reaction was visualized by the naked eye.

RESULTS

The results presented in table (1) indicated that, the percentage of tick attachment in the immunized dogs with salivary gland antigen was 17.5% while in the adjuvant treated dog was 95%. The attached ticks on the immunized dogs did not complete their feeding and may be died or trapped in the inflammatory exudate. A high reduction in the weight of feeding ticks on the immunized dogs was observed as compared to control dog. Female ticks collected from immunized dogs were not fed enough to become completely engorged so, could not be able to lay eggs whereas all female ticks collected from control dog became completely engorged and laying eggs with percentage 100% (table 1).

Table (1): Effect of vaccination on some biological parameter of *R. sanguineus* ticks.

Treatment	Mean % of tick attachment	Mean weight of fed female ticks (mg)	Mean % of female completely engorged and laying eggs
Vaccinated dogs with salivary gland antigen	17.5%	105±3.4	0%
Control dog	95%	487	100%

Coomassie blue staining of electrophoretically migration proteins from salivary gland antigen revealed 14 bands ranging from 119.7 to 38 KDa (119.7, 116.3, 112.91, 108.67, 104.72, 97, 88, 81.5, 75, 72, 65, 64, 56 and 38 KDa) (plate 1 figs.1 and 2).

Separation of salivary gland antigen on SDS-PAGE and identification by Western blotting technique revealed the presence of 4 polypeptides with hyper-immune sera and 3 polypeptides only with natural infested sera. Three polypeptides 97, 75 and 38 KDa were common in both sera. 97 and 38 KDa were strongly recognized in hyper immune sera and faintly in naturally infested sera. One band of 88 KDa was recognized by hyper-immune sera not by naturally infested sera (Plate 2 figs. 3 and 4).

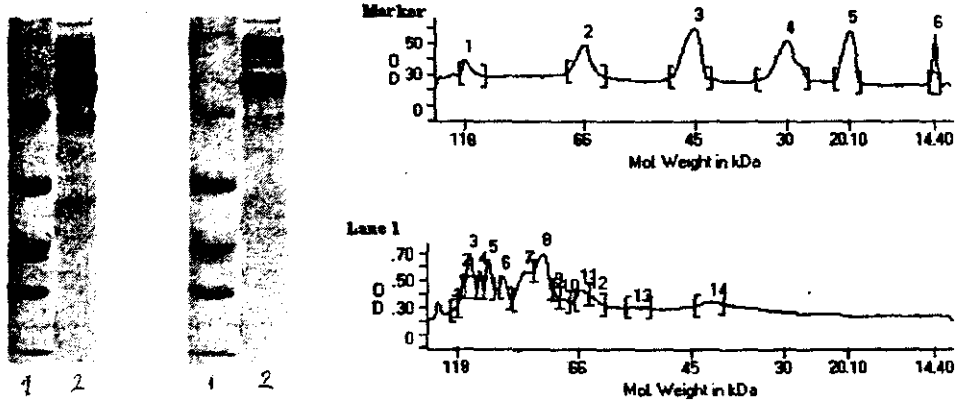

Plate (1): Characterization of salivary gland antigen by SDS-PAGE.
Lane (1): Unstained low molecular weight markers (Amersham Pharmacia).
Lane (2): Salivary gland antigen.
Fig. (1): Electrophoretic analysis of the molecular weight markers.
Fig. (2): Electrophoretic analysis of salivary gland antigen.

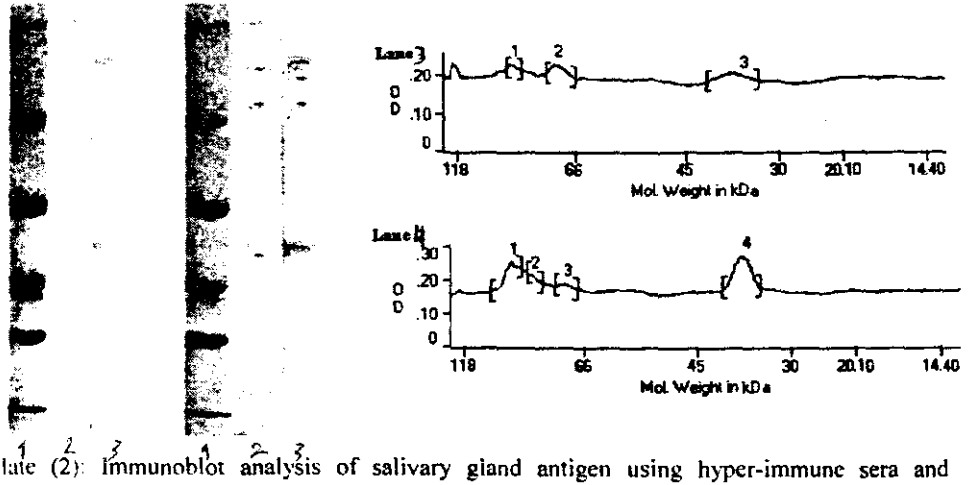

Plate (2): Immunoblot analysis of salivary gland antigen using hyper-immune sera and naturally infested sera.
Lane (1): Unstained low molecular weight markers (Amersham Pharmacia).
Lane (2): Immunoblot analysis of salivary gland antigen using naturally infested sera.
Lane (3): Immunoblot analysis of salivary gland antigen using hyper-immune sera.
Fig (3): Immunoblot analysis of salivary gland antigen using naturally infested sera.
Fig (4): Immunoblot analysis of salivary gland antigen using hyper-immune sera.

DISCUSSION

The present study is a trial to control *R. sanguineus* through vaccination of dogs with salivary gland antigen. The resistance of vaccinated dogs was elucidated through decreasing the percentage of tick attachment and interfering ticks to complete their engorgement. The percentage of tick attachment was 17.5% in immunized dogs while, in control dog was 95%. Significant reduction in the weight of fed female ticks was cleared. So, the mechanism of immune response to salivary gland antigen can be explained by interfered the fixation and feeding of ticks. These results indicated that immunity had been developed and prevented ticks from attachment and feeding properly. These results were in agreement with the result of **Jittapalapong et al. (2000)** who found that, dog immunized with tick salivary gland extract of *R. sanguineus* had the greatest impact on engorgement weight of female ticks. Similar results were also obtained by **Abdul-Rahman et al. (1992)** who demonstrated that, rabbits immunized with different antigens of *R. sanguineus* reduced number of tick recovery, weight of engorged females and egg mass. Also **Sahibi et al. (1997)** reported that, vaccination of calves with salivary gland extracts of *Hyalomma marginatum marginatum* affected all the feeding characteristics.

The result of electrophoretic migration of protein from salivary gland antigen revealed 14 bands ranging from 119.7 to 38 KDa. Identification of salivary gland antigen by Western blotting technique revealed the presence of 4 polypeptides recognized by prepared antiserum to salivary gland antigen

and three polypeptides with naturally infested sera. Hyper-immune sera recognized 88 KDa. This may indicate that, ticks succeeded in evading immune system and reducing antibody synthesis because of immunosuppressive components present in tick saliva or salivary gland antigen that could be more successful source for immunization.

The present study revealed that, the common polypeptides in both sera were 97, 75 and 38 KDa. This result agree with **Brown (1988)** who observed by Western blots a protein at 38 KDa recognized in egg, larval, nymphal, and female salivary gland antigen of *Amblyomma americanum*. **Wakler et al. (1990)** reported that polypeptides at Mw 36 to 38 KDa were antigenic in salivary gland of *R. appendiculatus*. **Ferreira et al. (1996)** found that 97 KDa polypeptide was strongly recognized by infested or immunized guinea pigs with whole tick adult or larval homogenate of *R. sanguineus* while, **Inokuma et al. (1999)** recorded that 24 KDa polypeptide common in larvae, nymphs whole body and salivary gland extract of *R. sanguineus*. Also, Martin **Hernandez et al. (1995)** reported that, a prominent band of 45 KDa was determined in all stages and salivary gland of *R. sanguineus*, **Bergman et al. (2000)** identified a 36 KDa from salivary gland and saliva of *Dermacentor andersoni* using immunoblot. The present results and those of others who studied other genera of ticks have not identified a common polypeptide in salivary gland antigen. This disparity may be due to difference of species and strains of ticks.

The results of this experiment suggest that salivary gland antigen has potential as a vaccine against *R. sanguineus* ticks.

Characterization of the two common polypeptides 97 and 38 KDa would be an important step in the development a *R. sanguineus* tick immunogen by methods involving recombinant DNA technology and monoclonal antibody techniques.

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تحصين الكلاب ضد قراد الريباسيفليس سنجوينس بإستخدام مستضد الغدد اللعابية

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كلية الطب البيطرى جامعة القاهرة ومعهد بحوث صحة الحيوان الدقى جيزة

الهدف من هذه الدراسة هو إحداث مقاومة فى الكلاب ضد قراد الريباسيفليس سنجوينس بإستخدام مستضد الغدد اللعابية وقد تم تحصين أربعة من الكلاب بمستضد الغدد اللعابية المحضر من الطور البالغ الغير مكتمل التغذية، أما الكلب الخامس فقد تم إستخدامه كضابط حيث تم حقنه بمساعد الدواء فقط . تم عمل التحدى بعشرة أزواج من الطور البالغ غير المغذى عند اليوم ٣٥ من بداية التحصين على كل كلب وقد أظهرت نتيجة التحصين حماية عالية تتمثل فى إنخفاض نسبة التصاق القراد ووزن الإناث المغذاة ، وبتحليل مستضد الغدد اللعابية بإستخادم الإستقطاب الكهربى أظهر عن وجود ١٤ حلقة ببتيدية تتراوح أوزانها بين ١١٩,٧ إلى ٣٨ كيلو دالتون . وبعمل الطبع المناعى بإستخدام المصل العالى المناعة أظهر وجود ٤ حلقات بينما ثلاث حلقات فقط ظهرت مع مصل الكلاب المعدية طبيعياً بالقراد وقد ظهر بوضوح حلقتان (٩٧و٣٨ كيلو دالتون) مع المصل العالى المناعة وخافته مع المصل الطبيعى أما الحلقة ٨٨ كيلو دالتون فقد تم تحديدها مع المصل العالى المناعة فقط ولذلك فإن مستضد الغدد اللعابية يبدو أن له قدرة عالية على إحداث مقاومة للقراد فى الكلاب .