

IMMUNE RESPONSE AND TICK REJECTION PATTERN OF MIDGUT AND SALIVARY GLAND VACCINES AGAINST LOCALLY PREVALENT *BOOPHILUS MICROPLUS* TICKS

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ABSTRACT

Midguts and salivary glands were isolated from partially engorged female ticks and processed for a vaccine candidate. The protein contents were measured and two different oil-based vaccines were prepared; midgut vaccine (MGV) and salivary gland vaccine (SGV). These vaccines were administered sub-cutaneously on days 0, 14 and 21. Their efficacy was monitored on the basis of antibody titers as determined by agar gel precipitation test. Ticks rejection on immunized hosts was evaluated in terms of attachment, mortality, oviposition, and hatchability of eggs. MGV offered significantly better control of *B. microplus* as compared to SGV. The highest antibody titers were observed in animals given MGV with a GMT of 5.5 which was 45.3% higher than animals given SGV.

Key words: Immunization, *Boophilus microplus*, rabbits, midgut, salivary glands

INTRODUCTION

Tick infections are responsible for huge economic losses all over the world but especially in tropical and subtropical countries. Their bites are debilitating, annoying and responsible for depletion of hide quality. Tick bite wounds can become infected and cause tick pyaemia or predispose to screw worm myiasis. Heavy tick infestation can result in significant blood loss, reduced productivity and weight gain (Richard and Shearer, 1997). Tick bites may cause toxicosis and paralysis and is an important cause of death in sheep, goat and cattle (Blood *et al.*, 1994).

The most serious threat of ticks is their potential ability to transmit a large number of bacterial, viral, protozoan and rickettsial diseases both in humans and animals (Blood *et al.*, 1994; Nuttall *et al.*, 2006; De Fuente and Kocan, 2006). Economic losses in the tune of billions of dollars have been ascribed to tick and tick borne diseases (De Castro, 1997). No such comparable information is available from Pakistan about the economic losses attributed to ticks or tick borne diseases.

Control of tick infestations has been difficult due to their diverse habitat. A major component of integrated tick control methods is the use of acaricides. Acaricides are useful only for a short-term and do not offer a permanent solution to tick control. Furthermore, the development of new acaricides is a long and expensive process, which reinforces our resolve for alternative approaches to control tick infestations (Frisch *et al.*, 1999; Mulenga *et al.*, 1999; De Fuente and Kocan, 2006). Among the several alternative tick control measures considered to-date, only the host vaccination

against ticks has proved promising (Mulenga *et al.*, 1999).

Keeping in view the importance of ticks and tick borne diseases, new alternatives for their control are urgently needed. Present work was conducted to study and compare the immune responses of antigens prepared from midguts and salivary glands of locally prevalent *B. microplus* in rabbits as a simulation model for further studies relying on immunization as alternative tick control measure.

MATERIALS AND METHODS

Partially engorged female *B. microplus* ticks, previously fed only on rabbits, were used as a source of an antigen. Midguts and salivary glands of the ticks were collected and processed for preparation of vaccines. Vaccines from two sources were tried in different groups of experimental rabbits and then challenged with ticks.

Maintenance of tick colony: *B. microplus* ticks were collected from the tick colony exclusively maintained for this purpose in University of Veterinary and Animal Sciences Lahore. Ticks were reared on rabbit ears in tightly fitted bags (Ghosh and Khan, 1996). Partially engorged adult female and male ticks were collected from the bags. These ticks were carefully removed so as to prevent any damage to their mouthparts. Ticks were maintained at 4°C in Petri dishes in humid environment.

Isolation of midguts and salivary glands: Ticks were brought to the room temperature and were surface sterilized by submersion and then intermittent agitation in 0.5 % benzalkonium chloride solution followed by

washing with 70% alcohol until they were cleared of benzalkonium chloride, and then rinsed thrice in sterilized distilled water (Akhtar *et al.*, 1999). Sterilized partially engorged female ticks were dissected individually to harvest salivary glands and midguts. Both the salivary glands and midguts were separately washed with three changes of phosphate buffer saline (PBS) by centrifuging at 1000 rpm for 5 minutes. The supernatant was removed and 5 ml PBS was added to each test tube (Opdebeeck *et al.*, 1988). The samples were then sonicated for 2 minutes and subsequently centrifuged at 5000 rpm for 30 minutes at 4°C. The supernatant was collected and pellets discarded (Kumar and Kumar, 1997).

Total protein contents of both the samples were determined by Biuret method using kit from Bio Systems. The samples were reconstituted in PBS (pH 7.2) to achieve a final protein concentration of 10 mg mL⁻¹ (Kumar and Kumar, 1997). Antibiotics (Potassium Penicillin 100 IU mL⁻¹) and Streptomycin Sulphate (100 µg mL⁻¹) were added to both the samples to prevent bacterial growth (Ghosh and Khan, 1996).

Preparation of vaccine: The vaccine was prepared by adding oil-based adjuvant to protein sample. One part of sample was admixed with four parts of oil-base adjuvant which composed of liquid paraffin, Span-80 and Tween-80 (95:4:1, Kumar and Kumar, 1997).

Experimental design: Eighteen adult rabbits were used in the study and were randomly divided into three groups, each comprising of six animals. The rabbits of first group were injected with midgut vaccine (MGV). The animals of second group were administered with salivary gland vaccine (SGV) while third group acted as control (CTR) and received only PBS along with adjuvant in parallel to the immunization schedule.

Immunization: The first dose of 1 ml was given sub-cutaneously on day-0. Two booster doses (1 ml each) were also given sub-cutaneously on day 14 and 21 (Kumar and Kumar, 1997).

Challenging of rabbits with ticks: After seven days of last injection, animals of each group were challenged with ticks. Each animal was infested with 10 pairs of adult *B. microplus*. The number of dead ticks was recorded. The surviving ticks were collected and maintained in the laboratory to monitor the effect of immunization on ticks.

Measurement of antibody titers: Blood samples were collected from rabbits after 7 days of last injection and sera were obtained. The antibody levels were measured by agar gel precipitation test (AGPT). Antigen sample was put in the central well and the serum samples were poured in the surrounding wells with two-fold dilutions of serum (1:2, 1:4, 1:8, 1:16, 1:32, 1:64 and 1:128) in

sequence. These plates were incubated at 37°C and the results were recorded after 48 hours.

Statistical analysis: The data were analyzed using one-way analysis of variance (ANOVA) and statistical difference among the immunized and non-immunized groups was determined by Least Significant Difference (LSD). Analysis was made with the help of SPSS software for Windows.

RESULTS AND DISCUSSION

Different organs of the ticks have been used by researchers as the antigen sources for tick vaccines. The most widely used tick organs for preparation of vaccine is midgut and are considered as source of concealed antigens (Khalaf-Allah, 1999). Exposed antigens from salivary glands are also employed for vaccination (Mulenga *et al.*, 1999). Male ticks were not used as they are too small to work with and females were allowed to engorge on the rabbits. The performance of tick vaccines was evaluated not only on the basis of antibody titers in immunized hosts but their protective capability was evaluated by monitoring various tick rejection parameters. The percent attachment of ticks in three groups of rabbits was 83.34%, 86.67% and 85.0% and there was no difference in the immunized and control groups (Table I). *B. microplus* is a one host tick of cattle and this may have attributed to the lack of difference in their attachment to this animal model. Not all species of ticks can infest laboratory animal models easily and *Boophilus specie* requires larger animal hosts for feeding (Gosh *et al.*, 2007). Tick Homogenate tick vaccine generated immune response in buffalo calves against *Hyalomma specie* (Ali *et al.*, 2009).

Significant difference in the mortality of ticks was recorded in both vaccinated groups as compared with control groups ($p \leq 0.05$). Within the vaccinated groups, administration of MGV has significantly higher mortality as compared with SGV (Table I). No mortality was observed in the control group. Ticks feeding on vaccinated animals have impaired body functions as compared to ticks feeding on non vaccinated animals and experienced greater mortality. Much higher mortality has been reported by other workers i.e. 56% by Mulenga *et al.*, (1999) and 78% by Khalaf-Allah (1999). Still higher mortality of 88% is observed in other study (Kimaro and Opdebeeck, 1994). All the ticks could not attach to the rabbits; moreover, they failed to engorge properly. Thus sufficient antibodies could not be sucked from immunized rabbits and that led to low mortality. Another possible reason for this disparity could be the tick specie and host variation in these studies. As already mentioned there was no difference in the attachment of ticks in three groups.

Egg laying capacity of the ticks survived on vaccinated animals was significantly reduced as compared to control group (Table I). Average eggs laid by MGV, SGV and control groups were 753.36, 918 and 1174.08 respectively. Two vaccinated groups laid 35.86% and 17.97% less eggs as compared with control group. No effect on hatchability of eggs was recorded in this study. Although no effect on the hatchability of eggs was observed but an increased mortality and reduced egg laying capacity will result in reduced tick exposure to susceptible population. These results are consistent with the findings of Mulenga *et al.*, (1999) but are contrary to that of Abdul-Rahman and Jagannath (1992) who found significant decrease in hatchability of eggs after vaccination. Much higher decrease up to 95% is reported in the egg laying but these studies were carried out on cattle (Opdebeeck *et al.*, 1988). Damages to other organs by antibodies may have contributed to this and antibodies not only destroyed candidate cells but also cross-react with other organs like ovaries and resulted in damages to eggs and their hatchability. This can be explained on the occurrence of common antigens in different organs of the ticks (Wang and Nuttall, 1999).

The individual antibody titers were recorded and then the geometric mean titer (GMT) was calculated for each group. No antibody titer was recorded in sera of control animals. In MGV group the GMT was 5.50 while mean titer of 3.01 was recorded for SGV group. Thus the MGV group had a higher (45.28%) titer than of SGV group. Agar gel precipitation test (AGPT) was found to be sensitive enough to detect trends in immune response / differences in antibody titer of three groups. Higher titers have been reported in different tests like Enzyme-Linked Immunosorbent Assay (ELISA) by Wong and Opdebeeck (1990), Kimaro and Opdebeeck (1994), Ghosh and Khan (1995).

Table-1: Effects of MGV and SGV on various parameters of *Boophilus microplus*.

Parameter	MGV Group	SGV Group	Control Group
Attachment	83.34 ^a	86.67 ^a	85 ^a
% (X ± S.E.)	± 3.94	± 3.33	± 4.28
Mortality	21.82 ^c	11.20 ^b	0 ^a
% (X ± S.E.)	± 4.44	± 2.53	
Eggs Laid	753.36 ^c	918 ^b	1174.08 ^a
(X ± S.E.)	± 8.85	± 16.43	± 25.53
Hatchability	95.11 ^a	97.67 ^a	98.21 ^a
% (X ± S.E.)	± 2.95	± 3.02	± 2.17
Titer (GMT)	5.50 ^b	3.01 ^a	0 ^a

MGV = Midgut Vaccine SGV = Salivary Gland Vaccine
 X = Mean S.E. = Standard error a, b, c, = Any two means carrying same superscript in a row are not significantly different from each other at 5% probability level, using LSD test.

The results of the study are very encouraging and show that a vaccine prepared from the midgut of *B. microplus* is better than that prepared from salivary glands in control of ticks. Immunization strategy against ticks has the advantages of being cost-effective, reduce environmental pollution, prevent drug resistance and most importantly it will reduce the chances of residues in animal products. Inclusion of multiple antigens in candidate vaccine that could target a broad range of tick species and also prevent transmission of pathogens will be a desirable strategy (De Fuente and Kocan, 2006). Recent developments in molecular biology and biotechnology can help us in candidate antigen identification and their availability.

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