IMMUNIZATION OF NEW ZEALAND WHITE RABBITS AGAINST RHIPICEPHALUS HAEMAPHYSALOIDES USING MIDGUT ANTIGEN

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Abstract: New Zealand White rabbits were experimentally immunized with crude and partially purified midgut antigen of Rhipicephalus haemaphysaloides. The midgut antigen was found to be immunoprotective as the weight of engorged females, egg masses and egg rate conversion efficiency reduced by 40 and 47.4, 70 and 75, 49.31 and 56.27 percent respectively in the case of crude midgut antigen 54.56 and 40.1,76 and 78,46.62 and 65 percent respectively in the case of partially purified midgut antigen on both the 28th and 63rd day post immunization (DPI) challenge.

Keywords: Immunization, Midgut Antigen, Rhipicephalus haemaphysaloides, Engorged tick weight.

I. Introduction

The global economic losses from ticks and tick born diseases are estimated to be in billions of dollars annually (Sonenshine, 1993). Immunological control of ticks is attaining great importance due to the apprehension of emergence of acaricidal resistant tick strains and environmental pollution. Using a vaccine against ticks has an advantage of being environmentally sustainable, economically viable, ecologically safe and socially acceptable (Banerjee et al., 1995). A series of elegant studies led to the isolation, identification and purification of the protective antigen Bm 86 from tick extract (Willadsen et al., 1989). The Bm 86 was identified, cloned and expressed in Escherichia coli (Rand et al., 1989) and in the Pichia pastoris (Rodriguez et al., 1994) vector for production in large quantities. This has led to the production of commercial vaccine TickGARD TM, TickGARD PLUS TM and GAVAC TM (Rodriguez et al., 1995) against the one host tick, Boophilus microplus in Australia and Cuba respectively.

The present work was carried to explore the feasibility of an immunological control of Rhipicephalus haemaphysaloides with tick derived midgut antigen.

II. Materials and Methods

Experimental Animals

The experimental trials were conducted using New Zealand white rabbits of 3-4 months age with no previous tick exposure. They were fed with Bengal gram, Lucerne and adlibitum water during the study.

Rearing of ticks

Pathogen free Rhipicephalus haemaphysaloides colonies were maintained using experimental rabbits. The larval, nymphal and adult stages were allowed on ears of these rabbits using thick cotton ear bags. The unfed stages were maintained in the laboratory in BOD incubator (Forma Scientific Inc., USA) at 20 ± 2ºC using Potassium hydroxide solution for maintenance of 85 per cent relative humidity.

Preparation of midgut antigen

The midgut antigen was prepared following the methods of Johnston et al. (1986) with some modifications as follows:

Partially fed female R.Haemaphysaloides ticks were placed in 0.01 M phosphate buffered saline, pH 7.2. The midguts were removed, freed of other tissues and placed in 0.01 M phosphate buffered saline at 4ºC. The isolated midguts were homogenized at 1500 cycles per minute for 10 minutes in Potter S Homogenizer (B – Braun Biotech international) in ice bath. The homogenates were sonicated using a standard probe at 8µ amplitude for 10 minutes, each cycle lasting for 2.5 minutes with an interval of one minute each, in a sonicator (B – Braun, Biotech International). The sonicated homogenates were centrifuged at 15000 g for 30 minutes at 4º in a refrigerated centrifuge (Remi C-24). The supernatant were pipetted out and the sediments were discarded. Phenyl methyl sulfonyl fluoride (PMSF) at 1 mM was added to the supernatant antigen to inhibit the proteolysis (Ghose and Khan 1997) and stored at -20ºC in 1.5 ml storage vials. The protein concentration of the antigen was determined (Lowry et al., 1951).
Purification of midgut antigen
The midgut antigen of *R. haemaphysaloides* was purified by gel filtration chromatography using sephadex G-200 column (Ghosh and Khan, 1997).

Preparation of Gel Bands
It was done according to Amero *et al.* (1994) with minor modification. It was used in the immunization trial as partially purified midgut antigen.

Immunization
Immunization trials were conducted involving eighteen New Zealand white rabbits comprising of 3 groups of 6 rabbits each for crude (Group I), partially purified (Group II) and control (Group III) and the immunization schedule was as in Table I.

**TABLE – I: IMMUNIZATION SCHEDULE**

<table>
<thead>
<tr>
<th>Group</th>
<th>Immunization Trial</th>
<th>Challenge Protocol</th>
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<tbody>
<tr>
<td>Control (N=6)</td>
<td>The control group animals received only FCA or FIA</td>
<td>28&lt;sup&gt;th&lt;/sup&gt; day and 63&lt;sup&gt;rd&lt;/sup&gt; day</td>
</tr>
<tr>
<td>Crude Midgut Antigens (N=6)</td>
<td>A total dose of 1 mg was administered in 3 divided doses per rabbit</td>
<td>28&lt;sup&gt;th&lt;/sup&gt; day and 63&lt;sup&gt;rd&lt;/sup&gt; day</td>
</tr>
<tr>
<td>Partially Pure Antigens (N=6)</td>
<td>A total dose of 400µg was administered in 3 divided doses</td>
<td>28&lt;sup&gt;th&lt;/sup&gt; day and 63&lt;sup&gt;rd&lt;/sup&gt; day</td>
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</tbody>
</table>

### III. Results and Discussion
The effect of *R.haemaphysaloides* crude and partially purified midgut antigen on the feeding and fertility parameters of ticks fed on immunized and unimmunized control animals were observed on both 28<sup>th</sup> and 63<sup>rd</sup> DPI (Table II). The percentage of rejection, reduction in engorged tick weight, egg mass weight and ERCE of ticks from immunized animals over controls were presented in (Table III).

**TABLE – II: Analysis of variance of the effect of immunization on the feeding and fertility parameters of *R. haemaphysaloides* ticks (Mean+ S.E.)**

<table>
<thead>
<tr>
<th>Sl.No</th>
<th>Variables (in days)</th>
<th>Crude antigen</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>F ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Feeding period</td>
<td>8.31±0.29</td>
<td>7.25±0.10</td>
<td>8.26±0.11</td>
<td>7.57±0.2</td>
<td>5.50±0.1</td>
</tr>
<tr>
<td>2.</td>
<td>Engorged tick weight</td>
<td>71.4±1.83</td>
<td>74.6±3.20</td>
<td>148.9±4.75</td>
<td>98.38±4.75</td>
<td>283.19±283.19</td>
</tr>
<tr>
<td>3.</td>
<td>Pre oviposition</td>
<td>4.62±0.42</td>
<td>3.44±0.20</td>
<td>4.79±0.30</td>
<td>3.75±0.30</td>
<td>2.52±0.10</td>
</tr>
<tr>
<td>4.</td>
<td>Oviposition period</td>
<td>23.85±1.80</td>
<td>25.22±0.21</td>
<td>24.67±0.14</td>
<td>25.37±0.10</td>
<td>20.96±0.10</td>
</tr>
<tr>
<td>5.</td>
<td>Egg mass weight (mg)</td>
<td>53.14±6.09</td>
<td>21.56±4.28</td>
<td>44.08±3.29</td>
<td>19.32±2.29</td>
<td>177.49±177.49</td>
</tr>
<tr>
<td>6.</td>
<td>Incubation period</td>
<td>26.88±2.052</td>
<td>0.00±0.00</td>
<td>27.42±0.00</td>
<td>0.00±0.00</td>
<td>24.03±0.00</td>
</tr>
<tr>
<td>7.</td>
<td>Fertility efficiency</td>
<td>0.32±0.03</td>
<td>0.29±0.05</td>
<td>0.28±0.03</td>
<td>0.20±0.01</td>
<td>0.19±0.01</td>
</tr>
<tr>
<td>8.</td>
<td>Egg rate conversion efficiency</td>
<td>3.49±3.67</td>
<td>29.2±5.50</td>
<td>28.03±2.75</td>
<td>28.03±2.75</td>
<td>19.43±1.95</td>
</tr>
<tr>
<td>9.</td>
<td>Feed efficiency</td>
<td>19.96±2.96</td>
<td>7.32±1.45</td>
<td>16.7±1.12</td>
<td>11.14±1.10</td>
<td>52.48±2.44</td>
</tr>
</tbody>
</table>

**TABLE – III: Over all effect of Immunization**

<table>
<thead>
<tr>
<th>Sl.No</th>
<th>Parameter (in days)</th>
<th>Crude Group I</th>
<th>Partially Purified Group II</th>
<th>F ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Percentage Rejection</td>
<td>3.3</td>
<td>20</td>
<td>13.0</td>
</tr>
<tr>
<td>2.</td>
<td>Percentage reduction in engorgement weight</td>
<td>40</td>
<td>47.4</td>
<td>54.56</td>
</tr>
<tr>
<td>3.</td>
<td>Percentage reduction in Egg mass weight</td>
<td>70</td>
<td>75</td>
<td>76</td>
</tr>
<tr>
<td>4.</td>
<td>Percentage reduction in ERCE</td>
<td>49.31</td>
<td>56.27</td>
<td>46.62</td>
</tr>
</tbody>
</table>
No significant variation in the percentage attachment and rejection was observed. The mean feeding period of ticks challenged on 28 and 63 DPI was significantly extended in group I, group II when compared to group III. A highly significant variation (P<0.01) in engorged tick weight was observed in groups I and II compared to group III on both 28 and 63 DPI. Wikel (1996) stated that the inflammatory cells may undergo degranulation leading to histamine release which may also adversely affect the engorged tick weight. Similarly Ghosh and Khan (1997) observed a moderate increase of 2 – 3 days in the feeding period of R.h.antolicum ticks immunized rabbits. The mean feeding efficiency index also decreased significantly in group I and II compared to group III on both 28 and 63 DPI.

The mean preoviposition period was significantly extended in group I and group II on 28 DPI, where as it was not significant on 63 DPI. Similarly Kimaro and Opdebeek (1994) reported prolonged preoviposition and oviposition period of ticks fed on animals immunized with purified midgut antigen of Boophylus microplus. No significant difference in oviposition period was observed in all the three groups on both 28 and 63 DPI. The incubation period was significantly different on 28 DPI in group I and group II compared to group III. The variation was not significant on 63 DPI.

A highly significant reduction (P<0.01) in egg mass weight was observed in group I and II compared to group III between 28 and 63DPI. A reduction percentage of 70, 75 and 76, 78 in the case of group I and group II on both 28 and 63 DPI was observed. The mean incubation period did not significantly vary between group I and II, when compared to group III on 28 DPI. Ticks fed on animals immunized with crude extract of R.sanguineus showed insignificant variation in the incubation period compared to ticks fed on unimmunized animals (Bechara et al., 1994). Interestingly the egg mass of ticks belonging to group I and II allowed to feed on 63 DPI failed to hatch the larvae.

A highly significant reduction in fertility efficiency index was observed in group I and group II compared to group III on both 28 and 63 DPI. The reduction was also significant within groups between 28 and 63 DPI. Similar results have been recorded by Sahibi et al., (1997). The effect of immunization significantly reduced the egg rate conversion efficiency in group I and II compared to group III on both 28 and 63 DPI. It also differed significantly within group I, II and III on both 28 and 63 DPI.

This adverse effect on egg mass weight and ERCE could be because of the blood meal volume ingested. The ticks collected from immunized animals were shrunken and smaller in size in comparison with the normal control ticks (Fig.1). The egg mass laid by ticks fed on immunized animals, differed in weight and appearance when compared to those which fed on control animals (Fig.2). Few ticks, following engorgement turned black in colour and died in a few days time without ovipositing (Fig.3). This was also reported by Kemp et al., (1986) on immunized cattle with Boophylus microplus adult extracts. Similarly Szabo and Bechara (1997) reported that many engorged ticks from gut extract and Freuds adjuvant immunized dogs were darker and so distended that they could not move properly. These ticks did not oviposit and after death became black and acquired a hard consistency. This could be due to the mode of action of host antibodies and compliments resulting in lysed cells, ruptured tissues and leakage of blood and its components in to the haemolymph (Sonenshine, 1993).

Figure - 1

**CONTROL** **CRUDE** **PARTIALY PURIFIED**

Figure - 2

**CONTROL** **CRUDE** **PARTIALY PURIFIED**
IV. References


V. Acknowledgments

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