Comparison of Antibody Titer Immunized by KU-VAC1(rBm95+rSerpin)between Thai Native and Western Breed Cattle

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ABSTRACT

Cattle ticks, *Rhipicephalus microplus*, are the most important ectoparasites of livestock in tropical countries, and are responsible for severe economic losses through their infestations and as vectors of pathogens. In Thailand, the conventional control of tick based on the use of acaricides had adverse drug effects due to the increasing costs, contaminated animal products and environment. Therefore, anti-tick vaccine was developed as an alternative tool to resolve the problems. The objective of this study is to compare the antibody titers between Thai native cattle (Khao Lamphun-KL) and Western cattle (Angus) immunized by using KU-VAC1 (rBm95+rSerpin). These animals were vaccinated 3 times at 21-day interval and boosting after 6 month from the first immunization. Antibody (Ab) responses were detected and measured by ELISA. The Ab titer was found higher in Thai native group than Western group. By ELISA analysis, specific antibody levels from immunized animals increased at the first week after the first vaccination and prolonged until the 34th week of experiment while no specific Ab titer of the control group was rising. The Ab was recognized rBm95 and rSerpins by Western blot assay. This result indicated that KU-VAC1 has evidently induced an antibody response of native and the other Western cattle breed. Therefore, the other benefits of this vaccine have shown more promising to protect all cattle breed from tick infestations in Thailand.

Key words: Native and Western Breed Cattle, *Rhipicephalus microplus*, Immunization and KU-VAC1

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INTRODUCTION

The cattle tick, *Rhipicephalus (Boophilus) microplus*, is a one-host tick, almost monospecific for cattle, and is an important ectoparasite in tropical and subtropical countries. Heavy tick burdens on animals can decrease production and damage health. The cattle tick can also transmit major tick-borne diseases such as babesiosis and anaplasmosis (CFSPH, 2007). This tick cause economic losses due to their direct effects on the preferred hosts and by the pathogens they transmit. Global economic losses caused by *R. microplus* ticks have been estimated in 7 US$ per animal per year (McCosker, 1979). The Western cattle (*Bos taurus*) were more suffered from this tick compared to the native local cattle (*Bos indicus*) due to the difference of their innate immunity (Constantinoiu *et al.*, 2010).

Tick control has been conventionally relied on chemical acaricides. However, the occurrence of increasing acaricide resistance within a single isolate of ticks, poses great problems for the effective chemical control of this parasite. Alternative biological control methods including pasture spelling, breed resistance, and anti-tick vaccines have been developed. Tick control by the anti-tick vaccine, such as TickGARD; the first ectoparasite vaccine has been developed in Australia (Willadsen *et al.*, 1995). The major effect of this vaccine is on a successive reduction in tick numbers because of reduction of tick fertility (Rodriguez *et al.*, 1995).

Thai anti-tick vaccine by cloning and expression of recombinant protein of Bm95 and Serpins was developed in 2010 (Jittapalapong *et al.*, 2010). Antibody responses were induced by KU-VAC1 and GAVAC were compared based on the Bm86 homologue. Enzyme-linked-immunosorbent serologic assay (ELISA) was used to measure the humoral antibody specific to Thai rBm95. Cattle immunized with either KU-VAC1 or GAVAC showed significantly greater antibody production than the controls. These results indicated that KU-VAC1 and GAVAC had the similarly immunogenic (Jittapalapong *et al.*, 2010). Serine protease inhibitors (Serpins) secreted from tick salivary glands might be also used as an anti-tick vaccine against tick feeding. The immunogenicity of recombinant Serpins (rSerpins) was using rabbits as the host model (Kaewhom *et al.*, 2009).

The anti-tick vaccine is now being considered as alternative control of cattle tick in Thailand. Given the benefits of a recombinant protein vaccine over traditional chemical control, it is important to explore the potential effects of KU-VAC1(rBm95+rSerpins) vaccination. Therefore, the objective of this study was to compare the immunizing power of Thai anti-tick vaccine (KU-VAC1) between Thai native and Western breed cattle. The different antibody response between Thai native and Western breed cattle will help understanding the vaccine efficacy based on specific and non-specific immunity.
MATERIALS AND METHODS

1. Antigen preparation

The Bm95 and Serpins recombinant proteins were prepared with Bm95 and Serpins genes expressed in yeast as previously reported (Jittapalapong et al., 2008 and Kaewhom et al., 2009) and used as antigens. KU-VAC1 was prepared by thoroughly mixing the emulsified antigen with equal volumes of 10% Montanide (ISA 50V; Seppic, Paris, France) in mineral oil using an ultra-homogenizer.

2. Animals

A total of 21 native (Khao Lamphun, KL) and 14 mixed 50% Western breed (Mixed Angus, MA) with more than 1 year old were examined for health. The health status of the animals was monitored by determining the packed cell volume (PCV) and the hemoglobin. This examination was performed for screening of gastro-intestinal and blood parasites. During the preceding three months, these animals had not been treated with acaricides. Food and water were available ad libitum feeding.

These animals were randomly assigned into 5 groups. Each 7 KL was selected into three groups (KL1, KL2 and KL3), and each 7 MA was assigned into two groups (MA1 and MA2). KL1 and MA1 were immunized by KU-VAC1 as the treatment group, KL2 was immunized by adjuvant only as the adjuvant control group, and KL3 and MA2 were immunized by PBS as the control group. Animals were weekly collected for blood from the jugular vein. Blood samples were transferred to sterile and citrate tubes and their sera were separated and stored at -20°C until further analysis.

3. Immunization protocol

The protocol of immunization, KL1 and MA1 had a total of 4 intramuscular immunizations with KU-VAC1 (200 µg rBm95 and rSerpins in 1ml of adjuvant) (Table1). The second and third immunizations were given 3-weeks interval and the last immunization was given at 6 month after the first immunization. The KL2 was inoculated with adjuvant only at the same time of immunization in the treatment group. KL3 and MA2 groups had received PBS only as the control group. After 6 months, each group was again subjected to the last immunization with the same antigen and adjuvant as described above.

4. Detection of antibody responses

Serum of each animal was collected weekly, and antibody levels were determined by ELISA. The protocol for the ELISA to detect immunized antibodies was modified from Jittapalapong et al. (2000). Briefly, microtitre plates were coated with 0.3 µg of rBm95 and rSerpins per well in 0.1 M carbonate buffer (pH 9.6), washed with PBS containing 0.05% (v/v) Tween-20, blocked with 0.1% bovine serum albumin, and antisera used as primary antibody was diluted (1 : 200). Horseradish
peroxidase-conjugated goat anti-bovine IgG (ICN, Aurora, USA) was diluted 1:5000 to serve as secondary antibody, and antigen-antibody complexes were detected with 0.05% 2,2'-amino-di-[3-ethylbenzthiazoline sulphonate] (ICN) containing 0.03% H₂O₂. Optical densities at 405 nm were measured. Additional controls without antigen, primary antibody, secondary antibody or substrate are read to ensure that the colorimetric reaction is because of the formation of antigen-antibody complexes and not because of non-specific reactions.

Table 1 Experimental design and immunizations schedule

<table>
<thead>
<tr>
<th>Animal group</th>
<th>Type of treatment</th>
<th>The 1st immunization</th>
<th>The 2nd immunization</th>
<th>The 3rd immunization</th>
<th>Boost after 6 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>KhaoLamphun (KL1)</td>
<td>KU-VAC1</td>
<td>week 0</td>
<td>week 3</td>
<td>week 6</td>
<td>week 33</td>
</tr>
<tr>
<td>KhaoLamphun (KL2)</td>
<td>Adjuvant</td>
<td>week 0</td>
<td>week 3</td>
<td>week 6</td>
<td>week 33</td>
</tr>
<tr>
<td>KhaoLamphun (KL3)</td>
<td>PBS</td>
<td>week 0</td>
<td>week 3</td>
<td>week 6</td>
<td>week 33</td>
</tr>
<tr>
<td>Mixed Angus (MA1)</td>
<td>KU-VAC1</td>
<td>week 0</td>
<td>week 3</td>
<td>week 6</td>
<td>week 33</td>
</tr>
<tr>
<td>Mixed Angus (MA2)</td>
<td>PBS</td>
<td>week 0</td>
<td>week 3</td>
<td>week 6</td>
<td>week 33</td>
</tr>
</tbody>
</table>

5. Determination of reactivity between antibody and antigen by Multi screen analysis

5.1. SDS-PAGE Analysis

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed as described by Jittapalapong (2000) on 70 x 80 x 1.0-mm continuous polyacrylamide slabs using Mini-PROTEAN® 3 cells (BIO-RAD, USA). Samples were dissolved in 50 mMTris-HCl buffer (pH 6.8) containing 2% SDS, 20% glycerol, 0.02% bromophenol blue diluted 1:1 in Tris-Glycine electrode buffer (25 mMTris, 190 mM glycine; pH 8.3) containing 1% SDS. When reducing condition was required, 5% β-mercaptoethanol were added. The samples were loaded into their correspondent gel lanes. The electrophoresis was carried out for 1 hour at a constant 200 volts in PAGE buffer (1% SDS Tris-glycine). After the electrophoresis process, one of SDS-PAGE gel was preserved in transfer buffer (20% Methanol Tris-glycine buffer, pH 8.3).

5.2. Multi screen (Mini-PROTEAN® II Multiscreen Apparatus Instruction Manual, BIO-RAD)

rBm95+rSerpin protein are separated by SDS-PAGE with the comb that contains one large sample well and one reference well following the SDS-PAGE analysis method provided above. Proteins were transferred from gel to PVDF membrane (0.2 μm pore size Immobilon™ - PSQ, Millipore Corporation, USA.) The transfer was performed at 100 volt for 100 minutes in Mini Trans-Blot® Electrophoretic Transfer Cell (Bio-Rad) containing cool transfer buffer. After finishing the transfer, the membrane was washed in washing buffer (TBS, pH 7.5, 0.05% Tween-20) for 5 minutes, transferred
to blocking buffer (TBS, pH 7.5, 3% (w/v) BSA; albumin from bovine serum, minimum 98% electrophoresis, SIGMA-ALDRICH™), incubated overnight at room temperature. After that, membrane was washed by washing buffer. The multiscreen apparatus (Mini-Protean® II Multi Screen; Bio-Rad) and gaskets prior to assembly were prepared. The sera that collected in every week were diluted (1:100 dilution of each serum) in antibody buffer (TBS, pH 7.5, 0.05% Tween-20, 1% (w/v) BSA). Then loaded 600 µl of samples into each channel and incubated for 2 hour. After incubation, remove the solution individually from the channel with a pipette and took the membrane out. The membrane was washed by washing buffer and probed with the secondary antibody (1:10,000 dilution of goat anti-bovine IgG conjugated with horseradish peroxidase; KPL, USA) in antibody buffer, and incubated for 1 hour. After that, membrane was washed by washing buffer and added DAB substrate (3,3’– Diaminobenzidine Enhanced Liquid Substrate system, SIGMA-ALDRICH™) to generate color within 5 minutes.

**RESULTS AND DISCUSSION**

1. ELISA

Specific antibody levels in sera from immunized animals in KL-1 increased at the first week after the first immunization, while antibody level of the control group remained no rising (Figure 1). The highest antibody titer was found after the 2nd immunization in KL-1 group while the peak antibody of MA-1 was found after the seventh week. The last booster of KU-VAC1 at the 34th week resulted in maintain the high level of response. The trends of specific antibody levels increased then gradually declined and raised after every immunization in both group.

2. Multi Screen

Multi-screen was performed using two proteins, rBm95 and rSerpins, immunized animals, demonstrated that the weekly-taken accumulate data of antisera of these animals bound to the proteins used in the rBm95 and rSerpins antigens formulation. Band at a molecular weight of 80kDa, which is consistent with the rBm95 protein and 45kDa, which is consistent with the rSerpins protein (Figure 2) from the Thai R. microplus strain, were visible. Both bands were recognized from the 3rd week after the previous immunization until the end of the study. Furthermore, the analysis indicated that in pre-immune sera from the same immunized animal did not recognize this band.

Specific antibodies against the rBm95 or rSerpins immunogens were not detected in pre-immunization sera, while the anti-Bm95 antibody levels of native cattle (KL) immunized with rBm95 were significantly higher compared to the control native cattle. These results indicated that rBm95 was immunogenic and induced production of antibodies. Thus, these antibody responses in
vaccinated animals indicated that KU-VAC1 is capable of invoking high antibody titers in both native and Western cattle.

The immunized native animals developed a strong and specific humoral antibody response expressed by high anti-Bm95 antibody levels, which increased immediately after the first immunization. The result of antibody response was similar to Jittapalalong et al. (2010) and Lambertz et al. (2012) confirmed by the results of ELISA (Figure 1) and multi screen Western blot (Figure 2).

**Figure 1** Recognition of rBm95 (upper) and rSerpins (lower) by antisera from cattle immunized with different antigens. Mean optical densities obtained from ELISA results of serum samples collected weekly from five groups of immunization at 0, 3, 6 and 33 weeks. KL1: KU-VAC1 in native cattle (solid line), KL2: adjuvant in native cattle (squares), KL3: phosphate buffered saline in native cattle (triangle), MA1: KU-VAC1 in 50% mixed Angus (dashed line) and MA2: phosphate buffered saline in mixed Angus (diamond shape). All line was accumulated data for each week of antisera from cattle immunized with different antigens.
Figure 2 Multi screen of sera from rBm95 (upper) and rSerpins (lower) immunized native cattle using KU-VAC1 in week 0-40. All line was accumulated data of sera from Thai native cattle immunized with different antigens.

CONCLUSION

Thai anti-tick vaccine is promising to immunize different types of cattle and confer the protection from cattle tick infestations. In Thailand, an anti-tick vaccine has not yet been considered as another method of cattle tick control since it will need to educate Thai farmers, local veterinarians, and government veterinarians to get more understanding on the advantages of this vaccine. Development of vaccines against tick offers cost-effective, friendly to environment, and safety for human health. Anti-tick vaccines for control of cattle ticks can create more benefit for Thai farmers to produce organic food that have a better value.

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