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Prospect of Antigenic Protein Whole protein Ekstrak of *Rhipicephalus sanguineus* Larva for The Development of Anti-tick Vaccine in Dogs

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Abstract.

The aim of this research is to know antigenic protein of tick *Rhipicephalus sanguineus and* determine the humoral immune response in rabbits immunized with protein larvae of *R. sanguineus* which were expected to be used as a basic for the development of anti-tick vaccine. This research used larvae of ticks of *Rhipicephalus sanguineus* obtained from dog skin for making whole extract. Whole tick extract protein was obtained by sonication technique then the protein was analyzed using SDS-PAGE technique and continued with identification of antigenic proteins by Western blot technique. Antibodies obtained from the blood of rabbits immunized whole extract larvae of *R. sanguineus*. Blood sampling performed on day 14, 21, 28 and 35. Antibodies and antigens obtained are used for the Indirect ELISA test then read by an ELISA reader at a wavelength of 405 nm. The results of the ELISA reader in the form of OD values. OD values analysis by descriptive statistics using SPSS. Analysis result whole extract protein *R. sanguineus* with SDS-PAGE technique gained 3 protein bands with molecular weight of 95.3 kDa, 45.2 kDa and 24.4 kDa, respectively. The results of identification whole extract protein *R. sanguineus* by Western blot technique acquired 2 antigenic protein bands with molecular weight of 95.3 kDa and 45.2 kDa, respectively. The proteins are potential to be developed material of anti-tick vaccine. Statistical analysis showed OD values at blood sampling day 14, 21, 28 and 35 was 0.060 ± 0.009; 0.201 ± 0.038; 0.147 ± 0.025 and 0.296 ± 0.035. In the graph shown increasing OD values at days 21 and 35, while a decrease in OD values occurred on day 28. The conclusion of this study is immunization with whole extract of *R. sanguineus* larvae can cause humoral immune response in rabbits with the highest OD value on day 35.

Keywords: *Rhipicephalus sanguineus, Whole protein extract, SDS-PAGE, Western blot, Indirect ELISA.*

1. INTRODUCTION

Rhipicephalus sanguineus or often called as "*brown dog tick*" can be found in almost the whole world. *R. sanguineus* infestation is a problem frequently experienced by dog owners. Difficulties in completely control *R. sanguineus* are due to its three host tick type. During their life cycles they are mostly found in the surrounding environment where the dogs mostly spend, that causes reinfestation. Cases of acaricide resistance have increased tick population (Winkel, 2014). An alternative method in controlling *R. sanguineus* tick is the use of vaccine. This method is efficient and cheap for controlling tick infestation and in addition vaccine does not contaminate and has potency in various hosts (Willadsen, 2004). Vaccination or immunization has been broadly developed for the control of tick infestation (Sasmita *et al*., 2011). Vaccination in animals was intended to stimulate protective immune response. Basic requirement in vaccine production was the known ability of antigen protein to stimulate immune response (antibody) (Tizard, 2009). Parasites express many different antigens; therefore a research on molecular based antigenic protein is needed (Lydyard *et al*., 2004). Vaccination is a method that is quite effective to control parasites; however, the identification of antigenic protein of larvae stadium *R. sanguineus* has not been conducted. It is expected that antigenic protein profile of larvae stadium *R. sanguineus* can be utilized as vaccine candidate to reduce *R. sanguineus* tick infestation in host by using Western blot, then the titer of antibody raised as immune humoral response in rabbit after immunization *R. sanguineus* larvae protein using Indirect ELISA technique.

2. MATERIALS AND METHODS

This research used *R. sanguineus* larvae *whole protein extract resulted from rearing* female tick collected from dogs in Universitas Airlangga Animal Hospital. Chemicals used were Phosphate Buffered Saline (PBS) as the media for tick larvae *whole protein extract*, meanwhile materials for SDS-PAGE technique were *Acrylamide*, *Tris HCl* pH 8.8, 10% SDS, *aquadest*, TEMED (*N,N,N,N-Tetramethylethylenediamine*), APS (*amonium persulfat*), *Tris HCl* pH 6.8, *Laemmli buffer* (*mercaptoetanol, bromfenolblue, gliserin*), *electrophoresis buffer* (*glycine, SDS, Tris aminomethan*), *methanol, acetic acid*, *Coomassie Brilliant Blue*. Materials for Western Blot were *Tris aminomethane, glycine, methanol, aquadest, ethanol,* primary antibody, conjugate (AP labeled anti-rabbit IgG), *phospatase* substrate (BCIP-NBT). Experimental animals in this research were two male rabbits weighing ± 2 Kg. Materials used for immunization were PBS, *Complete Freund's Adjuvant* (CFA), *Incomplete Freund's Adjuvant* (IFA). Meanwhile for *Indirect* ELISA were PBS-*Tween, buffer coating, washing buffer, blocking buffer, conjugate*, antigen, antibody, substrate buffer, substrate p-NPP and NaOH 3N.

A sample of 50 µl *R. sanguineus* larvae *whole protein extract* was measured its protein concentration using Bradford method based *spectrophotometry* at 590 nm wave length and ready to be separated by means of SDS-PAGE and to be determined the immunization dose.

Polyclonal antibody production was conducted by injecting 20.4 µL *R. sanguineus* larvae *whole protein extract* in 0.5 mL *Complete Freund's Adjuvant* (CFA) and 459.2 µg PBS per immunization subcutaneously for each of the two male rabbits. Second immunization was performed two weeks after the first immunization using the same protein amount in Incomplete *Freund's adjuvant* (IFA) and PBS of the same amount of the first one. The third and fourth immunizations were performed one week later using the same amount of materials and methods. Two weeks after the last injection rabbit blood was collected to obtain anti-*R. sanguineus* serum and followed with blood collection at day-14, -21, -28 and -35. Protein molecular weight calculation after SDS-PAGE and Western blot was performed by comparing with standard marker through calculating Retardation factor *(*Rf) value of each protein band (Rantam, 2003). Rabbit serum was utilized as test sample to determine antibody titer using Indirect ELISA. Negative control serum in Indirect ELISA was obtained from blood collection before the first immunization. Results of blood collection were read using ELISA *reader* at 405 nm wave length.

3. RESULTS

Separation of *R. sanguineus* larvae *whole protein extract* using SDS-PAGE resulted three protein bands (Figure 1). Identification of *R. sanguineus* larvae whole protein extract molecular weight using SDS-PAGE resulted three protein bands with molecular weight (MW) of 95.3 kDa, 45.2 kDa and 24.4 kDa.

Antigenic protein identification of *R. sanguineus* larvae *whole protein extract* using *Western blot* resulted two protein bands with molecular weight (MW) of 95.3 and 45.2 kDa. *Western blot* technique used polyclonal antibody due to its high affinity to an antigen. Therefore it is the only protein that has high antigenicity that can be bound by antibody. The protein with a molecular weight of 24.4 kDa did not appear in the *Western blot* result because this protein had a low antigenicity.

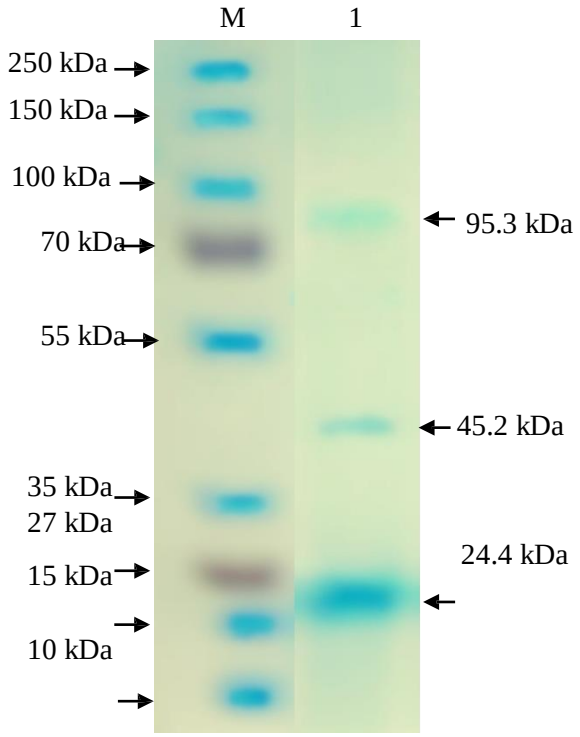

Figure 1. Identification result of *R. sanguineus* larvae whole protein extract using SDS PAGE. M, marker; 1, *R. sanguineus* larvae whole protein extract.

Antigenic analysis of *R. sanguineus* larvae *whole protein extract* using *Western blot* resulted two protein bands (Figure 2).

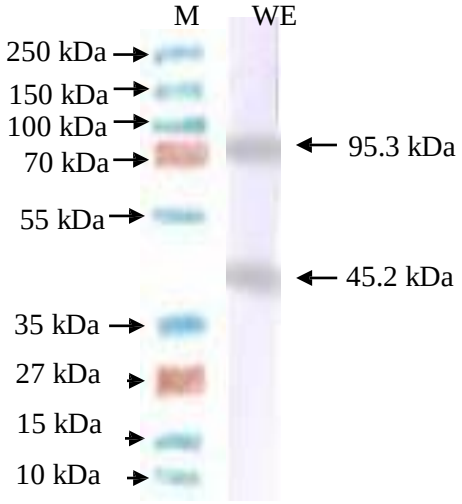

Figure 2. Antigenic protein identification result of *R. sanguineus* larvae *whole protein extract* using *Western blot*. M, marker; WE, *R. sanguineus* larvae *Whole protein extract*.

ELISA results showed Optical Density (OD) value of 0.060 ± 0.009; 0.201 ± 0.038; 0.147 ± 0.025 and 0.296 ± 0.035 respectively from blood collected at day -14, 21, 28 and 35 (Table 1).

Table 1. OD value (Means ± SD) of rabbits immunized with *R. sanguineus* larvae protein at different blood collection time

Time	OD value
day-14	0.060 ± 0.009
day-21	0.201 ± 0.038
day-28	0.147 ± 0.025
day-35	0.296 ± 0.035

Those results indicated that the rabbits immune system had responded the entering antigen in their bodies after the immunization. From the analysis results above it is seen that rabbit immune response experienced an increase at day -21 blood collection and the highest result was obtained at day-35 despite the prior decline. This is in line with the research results of Arifin (2008) that rabbit humoral immune response increased at the second immunization and the highest humoral immune response was obtained at the fourth immunization. Tizard (2009) stated that rabbit antibody was initially generated 10-14 days after the first immunization. According to Artama (1992) cited by Arifin (2008) repeated immunization with certain time interval would increase humoral immune response in experimental rabbit through the stimulation of a number of cell B clones to generate antibody and also as memory cells.

4. CONCLUSION

1) Identification of *R. sanguineus* larvae protein profile using SDS-PAGE resulted three protein bands with molecular weight of 95.3, 45.2 and 24.4 kDa.
2) Identification of *R. sanguineus* larvae protein profile using *Western blot* resulted two protein bands with molecular weight of 95.3 and 45.2 kDa.
3) *Rhipicephalus sanguineus* larvae protein were able to induce humoral immune response in rabbits with the highest optical density value at day -35.

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