

The Potential of Antigenic Protein of *Sarcoptes scabiei* as a Serological Diagnostic Candidate for Scabies in Goats

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Abstract: In Indonesia, the prevalence of scabies on goat cattle is still high, which shows that scabies is still not handled properly. It is now considered as an emerging/re-emerging parasitic disease that threatens human and animal health globally. The scabies disease has been known for a thousand years, and it is a persistent problem on public health and also livestock. Yet, until today a serological diagnostic tool is sensitive and specific is not available, especially for goat cattle in Indonesia. In recent years, an indirect antibody enzyme-linked immunosorbent assay (ELISA) has been available, which has higher sensitivity and specificity than traditional diagnostic methods. In order to overcome those problems preventive actions need to be undertaken through research for serological diagnostic development as an alternative for scabies prevention in goats in Indonesia. The purpose of this research is doing sensitivity and specificity tests towards the antigenic protein of *Sarcoptes scabiei* isolated from goat as diagnostic material for scabies in goats. The method was comprised of deciding the gold standard for positive and negative scabies, checkerboard test toward antigenic protein, and ELISA test for measuring sensitivity and specificity. The research results showed that *S. scabiei* antigenic protein with molecular weight of 57.3 kDa can be recognized by serum antibody of goat that infested with scabies with sensitivity level of 96% and specificity of 86,6%. From that result, it can be recommended that *S.scabiei* with a molecular weight of 57.3 kDa is the specific antigenic protein that can be used as a candidate of serological diagnostic material for scabies isolated from Indonesian local goat.

1 INTRODUCTION

Scabies disease is already known for thousand years ago and is persistently harming the health of people as well as cattle. However, until today a sensitive and specific tool for serological diagnostic which is specially provided for goat livestock in Indonesia has not been found yet. Diagnosis of scabies nowadays is still conventional, which is based on clinical symptoms and microscopic examination from the skin scraping results done by scraping until the deep layer skin is peeled off. The diagnosis is not very practical if the number of livestock is high and the livestock is less sensitive because the clinical symptoms are similar to other skin disease like caused by other mites (psoroptes, notoedres and chorioptes), fungus, and ticks, which cause atopic dermatitis, itching, and alopecia (Soulsby, 1986; Walton and Currie, 2007; Yu Zheng, 2016). A

definite diagnosis (skin scraping) by finding *Sarcoptes scabiei* mites will meet difficulties, especially if the number of mites is low in the infected animals and the success level is only 30-50% (Arlian, 2000; Lower *et al.*, 2001; Tarigan, 2004; Walton and Currie, 2007). As an effort to resolve the problem, it is necessary to develop diagnostic material serologically to enable early therapy to prevent broader transmission. Some countries, such as Australia, Germany and the United States, have developed serological diagnosis (ELISA) for dogs and pigs, it is very possible because *S.scabiei* could induce a humoral antibody response on the infected host (Lower *et al.*, 2001; Arlian *et al.*, 2004; Tarigan, 2004; Vercruysse, 2004; Walton and Currie, 2007; Lastuti, 2017; Lastuti, 2018).

2 MATERIAL AND METHODS

2.1 Determination of Gold Standard

The determination of gold standard for positive control and negative controls was based on microscopic examination of goat skin scraping infected with scabies. Positive results were declared if the researchers found *S.scabiei* mites under the goat skin by scraping examination, which would then be used as positive control. Meanwhile, the negative control comes from healthy goats, which were previously examined by skin scraping and did not contain any mites. The number of samples for gold standard was 40 samples consisting of 25 positive samples of scabies and 15 negative samples of scabies (Lastuti, 2017).

2.2 Indirect ELISA assay

The checkerboard result of antigenic protein of *S.scabiei* (57.3 kDa) was examined through indirect ELISA test to test the ability to detect antibody reaction towards positive control and negative control performed as follows: a microtiter plate consisting of 96 wells, with each well being coated with 100 µl of antigen solution with a concentration of 10 µg/ml in buffer coating, was incubated at 4 °C overnight. The next day, the well was washed with buffer washing (NaCl-Tween) 200µl three times. Furthermore, the well was blocked using a 4% creamer (in PBS-Tween) of 200 µl/well and incubated at 37 °C for one hour, then microtiter plate will be washed the same way three times. The next step is that the well was added with goat serum from positive control and negative controls and also added with PBS tested on 1/100 dilution for as much as 100 µl per well. Work was done *in duplo*. As per the standard for counting antibody titers, the dilution of antibody for which the positive control antibody titer was known was done at dilutions of 1/25 to 1/51200 and 100 µl was added per well. Then, the plate was incubated at 37 °C for an hour and washed again. The next step is the addition of anti-goat conjugate (IgG anti-goat) at 1/5000 dilution for as much as 100 µl each well and incubated at 37 °C for one hour. The plate was washed again and added pNPP substrate in substrate buffer (diethanolamine 1 mg/ml) of 100 µl per well. Then, the well was incubated at room temperature in a dark room within 15 to 45 minutes. The reaction was stopped by the addition of 50 µl NaOH 3N solution per well, then the plate was read using the ELISA reader with a wavelength of 405 nm (Lastuti, 2018). The value of

OD obtained in positive and negative controls would determine the sensitivity and specificity of the tested antigens.

3 RESULTS AND DISCUSSION

The results of the indirect ELISA test showed that the antigenic protein identified by the gold standard antibody sample was a molecular weight protein of 57.3 kDa. The number of samples was 40, which consists of positive controls with 25 samples and negative controls with 15 samples. The average value of Optical Density (OD) and the antibody titer were listed in table 1 below.

Table 1. Average OD values and gold standard sample antibody titer which recognized the *S.scabiei* protein 57.3 kDa

Sample	Value of OD (mean ± SD)	Antibody Titer (mean ± SD)
Positive	0.281 ^a ± 0.096	712,000 ^a ± 451,220
Negative	0.166 ^b ± 0.020	26,666 ^b ± 70,373

Annotation: A different superscript on the same column showed a very significant difference (p <0.01).

Based on the ELISA indirect test, the value of OD and antibody titer was used as the basis for testing the sensitivity and specificity of proteins with a molecular weight of 57.3 kDa. The test results showed that 57.3 kDa protein serum antibodies could be recognized by a goat, with the following results: out of 25 positive samples of gold standard, 24 samples were positive (true positive) and one sample was negative (false positive). Meanwhile, from 15 negative samples of gold standard, 13 negative samples and 2 positive samples (false negative) were found. The results are summarized in Table 2 below.

Table 2: Sensitivity and Specificity Tests of *S.scabiei* Protein 57.3 kDa.

	Skin Scraping Examination			Total
	+	-		
Elisa test	+	24	2	26
	-	1	13	14
Total	25	15	40	

Note: Skin scraping: conventional method for scabies diagnostic ELISA test: serological test used to develop scabies diagnostic.

The calculation results of sensitivity test, which is the positive number of ELISA test divided by the positive number of scraping examination, is: 24/25 =

96%, while the specificity test result is the negative number of ELISA test divided by the number of total negative examination of gold standard scraping is: $13/15 = 86.6\%$. These results indicated that *S.scabiei* antigen protein with a molecular weight of 57.3 kDa could be identified by scabies infected goat serum antibody with 96% sensitivity level and specificity level of 86.6%. From the results, it can be recommended that the *S.scabiei* protein goat isolates with a molecular weight of 57.3 kDa is a specific antigenic protein that could be used as a serological diagnostic candidate for scabies in goats.

Based on the results of Tarigan's research (2004a) that goat infected with *S.scabiei* showed a high IgG response ten days after infection and the high level of IgG could be maintained for up to 20 days after receiving ivermectin. The antibody was able to recognize antigen with molecular weight of 43 to 220 kDa with four highly prominent antigens being 180, 60, 38 and 37 kDa. Sensitivity test results of 96% and a specificity of 86.66% showed an accurate diagnosis result exceeding 80% (Bornstein, 2006; Lower *et al.*, 2001). The development of serological diagnostic tests that have been performed to diagnose scabies in various animals have been undertaken by researchers, including a diagnosis developed by ELISA techniques to detect *S.scabiei* antibody. Serological test results showed not much different levels of sensitivity and specificity as they did Lower *et al* (2001) for serologic diagnosis in dogs with ELISA assay, which found a sensitivity level of 84.2% and specificity of 89.5%, in which antigen was used to detect antibodies in dogs who had received scabies treatment for 1 to 4.5 months and the ELISA test was recommended for the diagnosis of scabies in dogs. The same test had been evaluated by serological test with ELISA indirect test against scabies in red fox (*Vulpes vulpes*), which showed a sensitivity level of 95.4% and specificity level of 100% and based on these results it was concluded that ELISA test was used for diagnosing and studying the epidemiology on scabies on red fox (Bornstein, 2006). Similarly, Rambozzi *et al* (2004) performed a serological test for detecting antibodies induced by *S. scabiei* in chamois (*Rupicapra* spp) with asymptomatic symptoms in the outbreak region of the scabies showing 97% sensitivity level with ELISA assays. Based on the exploratory results of *S.scabiei* protein, it has been proved that *S.scabiei* contains a protein, which is capable of inducing humoral and cellular immune responses and has high sensitivity and specificity level (> 80%), which can be used as serological diagnostic candidate kits for scabies in goats.

4 CONCLUSIONS

Antigenic protein of *S. scabiei* with a molecular weight of 57.3 kDa could be recognized by serum antibody of goat infested with scabies and had high sensitivity and specificity level (> 80%). From the results, it can be recommended that *S.scabiei* with a molecular weight of 57.3 kDa is the specific antigenic protein that can be used as a candidate of serological diagnostic material for scabies isolated from Indonesian local goat.

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