Protective Value of Immune Responses Developed in Goats Vaccinated with Insoluble Proteins from *Sarcoptes scabiei*

SIMSON TARIGAN

Balai Penelitian Veteriner, PO Box 151, Bogor 16114

ABSTRAK

TARIGAN, S. 2005. Nilai proteksi respon imun pada kambing yang divaksin dengan protein nirlarut dari *Sarcoptes scabiei*. *JITV* 10(2): 118-126.

Vaksin yang dikembangkan dari protein membran yang berasal dari permukaan lumen usus artropoda telah dibuktikan efektif untuk mengendalikan beberapa artropoda. Pendekatan yang sama kemungkinan dapat juga diterapkan pada *Sarcoptes scabiei* karena parasit ini juga telah terbukti menghisap imunoglobulin induk semangnya. Untuk mengevaluasi protektif imunitas dari protein membran *S. scabiei*, protein 'nirlarut' tungau diekstraksi berturut-turut dalam larutan: 1.14 *M* NaCl, 2% *SB 3-14 Zwitterion detergent*, 6 *M* urea, 6 *M* guanidine-HCl dan 5% SDS. Lima kelompok kambing (6 atau 7 ekor per kelompok) masing-masing divaksin dengan fraksi protein tersebut. Vaksinasi dilakukan sebanyak 6 kali, masing-masing dengan dosis 250 µg protein dengan interval 3 minggu antara vaksinasi. Kelompok 6 (7 ekor) diberikan hanya PBS dan adjuvan saja, dan bertindak sebagai kontrol yang tidak divaksin. Satu minggu setelah vaksinasi terakhir, semua kambing ditantang dengan 2000 tungau hidup pada daun telinga. Perkembangan lesi diamati pada hari pertama dan ke dua, lalu setiap minggu dari minggu pertama sampai ke delapan. Setiap kambing ditimbang dan sampel darah diambil setiap minggu, dan pada akhir percobaan kerokan kulit diambil untuk menetapkan populasi tungau. Respon antibodi akibat vaksinasi dan infestasi tantang diperiksa dengan ELISA dan *Western Blotting*. Hasil penelitian ini menunjukkan bahwa vaksinasi dengan fraksi protein nirlarut tungau menyebabkan pembentukan antibodi dengan titer yang sangat tinggi terhadap protein tersebut, namun antibodi tersebut sama sekali tidak memberikan proteksi terhadap tantangan tungau. Uji tantang menyebabkan lesi pada kelompok kambing yang divaksin yang keparahannya sama dengan yang terdapat pada kambing yang tidak divaksin.

Kata Kunci: *Sarcoptes scabiei*, Protein Nirlarut, Kambing, Vaksinasi

ABSTRACT

TARIGAN, S. 2005. Protective value of immune responses developed in goats vaccinated with insoluble proteins from *Sarcoptes scabiei*. *JITV* 10(2): 118-126.

Vaccines developed from certain membrane proteins lining the lumen of arthropod's gut have been demonstrated effective in the control of some arthropod ectoparasites. A similar approach could also be applied to *Sarcoptes scabiei* since this parasite also ingests its host immunoglobulins. To evaluate immune protection of the membrane proteins, insoluble mite proteins were fractionated by successive treatment in the solutions of 1.14 *M* NaCl, 2% SB 3-14 Zwitterion detergent, 6 *M* urea, 6 *M* guanidine-HCl and 5% SDS. Five groups of goats (6 or 7 goats per group) were immunised respectively with the protein fractions. Vaccination was performed 6 times, each with a dosage of 250 µg proteins, and 3 week intervals between vaccination. Group 6 (7 goats) received PBS and adjuvant only, and served as an unvaccinated control. One week after the last vaccination, all goats were challenged with 2000 live mites on the auricles. The development of lesions were examined at 1 day, 2 days, and then every week from week 1 to 8. All animals were bled and weighed every week, and at the end of the experiment, skin scrapings were collected to determine the mite burden. Antibody responses induced by vaccination and challenge were examined by ELISA and Western blotting. This experiment showed that vaccination with the insoluble-protein fractions resulted in the development of high level of specific antibodies but the responses did not have any protective value. The severity of lesions and mite burden in the vaccinated animals were not different from those in the unvaccinated control.

Key Words: *Sarcoptes scabiei*, Insoluble Protein, Goat, Vaccination

INTRODUCTION

Ivermectin, the drug of choice for scabies, is so effective and practical that has thwarted our endeavour to search for a better means of disease control. However, the consumer of food products are getting increasingly demanding that the food supply should be free of all chemical residues, whether or not they are known to be harmful to their health or environment (DONALD, 1994). Therefore, sooner or later, ivermectin will face strong consumer resistance because this acaricide is not environmentally friendly, and has a long withdrawal period. It has been demonstrated that animals injected with ivermectin contained the drug in

their excretes that kills a variety of important dung-colonising insects (WALL, 1992). Prolonged and improper use of any pesticide have the potential to promote resistance of the parasite against the pesticide. The occurrence of resistance of *S. scabiei* against ivermectin has been reported recently (CURRIE *et al.*, 2004). New pesticide become increasingly difficult to be available in the market because the cost of product discovery and development, registration and market introduction are expensive; whereas, market for insecticides against livestock pests is small (WILLADSEN, 1997). In addition, ivermectin is expensive and unaffordable by most farmers in Indonesia. Other pesticides especially those applied topically are either ineffective or unpractical for use in the control of scabies in animals.

In view of the negative aspect of the acaricides, alternative methods of control have to be sought. Vaccination is considered to be the most appropriate because it is safe for the consumers and environment, has long live immunity and potentially cheaper. However, developing vaccine against the parasites is much more difficult because of the complex interaction between the parasites and the immune system of the host. For *S. scabiei*, the difficulty is further exaggerated by the microscopic size and unavailability of culture system to produce sufficient amount of mites for the isolation of vaccine antigens. Because of those difficulties, the availability of practical anti-scabies vaccine will require considerable lengthy of time.

Developing an anti-arthropod vaccine comprises at least three main steps: (i) identification and purification of protective antigens, (ii) production of functional recombinant version of the protective antigen, and (iii) formulation of the functional recombinant antigen into practical vaccine. Based on the exposure to the host immune system, vaccine antigens for ectoparasite could either be conventional antigens *ie.* parasite molecules to which the host is exposed during a normal host-parasite interaction, or 'concealed' antigens *ie.* parasite molecules to which the host is not exposed during natural infestation but when the host is immunised artificially with the molecules, it may confer protective immunity (WILLADSEN, 1997).

Our previous studies showed that vaccination of goats with soluble mite proteins that contained the conventional antigens did not conferred protective immunity, despite the high titre of specific IgG (TARIGAN and HUNTLEY, 2005). The lack of protective immunity is considered to be associated with the fact that vaccination fail to induce specific IgE. Experience with other multicellular parasites such as *Boophilus microplus, Haemonchus contortus,* and *Lucilia cuprina* indicated that a more complete protection was obtained by vaccination with concealed antigens than that with conventional antigens (WILLADSEN, 2001). Similar

approach may also be feasible with *S. scabiei* since our previous studies indicated that *S. scabiei* does ingest its host immunoglobulin, despite the fact that this mite is not blood-feeding arthropod and reside on the avascular, cornified layer of skin (TARIGAN, 2005). In this present study, the insoluble mite proteins that expected to contain the concealed antigens were fractionated by successive solubilisation in the various solution of surfactants and chaotrophic agents of increasing solubility, then the capacity of each fraction to confer protective immunity was determined by vaccination and challenge experiment.

MATERIALS AND METHODS

Preparation of immunogen

Sarcoptes scabiei mites were cultivated and harvested according to a procedure described in our previous study (TARIGAN, 1998). Briefly, goats severely infested by the mites, were euthanised with ether and chloroform, and skin showing encrustation dermatitis was scraped deeply. The scraping was chopped into about 2-mm^3 pieces, mixed thoroughly and kept at 4°C overnight. To harvest the mites, the skin scrapings were placed at the edge of a Petri disc, then a beam of light was directed to the centre of the Petri disc. Mites migrating toward the light were collected by sucking into a special tube that attached to a vacuum pump.

After washing in 1% SDS, the mites, approximatey 15 gram in wet weight, were homogenised in PBS using a glass homogeniser. The homogenate was centrifuged at 8 000 x g for 30 minutes at 4°C, then the pellet was collected and washed twice with PBS. Extraction of insoluble or membrane proteins from the mite pellet was performed by a previously described procedure with some modification (RIDING *et al.*, 2000). Briefly, the pellet was homogenised and incubated sequentially in the extraction solutions of 1.14 M NaCl for 2 hours, 2% SB 3-14 zwitterion detergent (N-tetradecyl-N, N-dimethyl-3-ammonio-1-propane sulphonate) for 2 hours, 6 M urea for 18 hours, and 6 M guanidine-HCl for 18 hours. Following each extraction, the homogenates were subjected to ultracentrifugation (100 000 x g for 1 hour at 4°C) followed by resuspension of the pellet in the next extraction solution. Supernatants obtained after each centrifugation were collected and referred to as fractions F_{NaCl}, F_{Zwit}, F_{urea}, F_{Guan}, respectively. The pellet obtained after the extraction with guanidine solution was washed twice with double distilled water and twice with 50 mM Tris-HCl, pH 7.5. After the washings, the pellet was homogenised in 50 mM Tris-HCl, pH 7.5 containing 5% SDS and 10 mM dithiothreitol (DTT). The homogenate was heated at 95°C for 30 minutes then centrifuged at 25°C for 1 hour at 100 000 x g at 25°C. The supernatant was collected

and referred to as F_{SDS}. After determination of protein concentrations with a commercial Lowry protein assay kit (RC^{TM} protein assay, BioRad), each fraction was aliquoted and stored at -20ºC until used.

Animals and vaccination

Forty goats were allocated randomly into 6 groups, group 1 (7 goats), group 2 (7 goats), group 3 (7 goats), group 4 (6 goats) and group 5 (6 goats) were vaccinated respectively with F_{NaCl}, F_{Zwit}, F_{urea}, F_{guan} and F_{SDS}. Group 6 (7 goats) were injected with PBS and served as an unvaccinated control. Vaccination was conducted 6 times, 3 week intervals between vaccination, and a dose of 250 µg protein per vaccination. Quil A with a dose of 0.5 mg was used in all vaccination.

Challenge and post challenge examination

One week after the last vaccination, all animals were challenged with approximately 2000 live mites on the left auricles using a procedure described previously (TARIGAN, 2003). Briefly, skin scraping was collected from a mangy goat, chopped into about 2-mm^3 pieces, and mixed thoroughly. The number of mite per gram of skin scraping was determined by placing the skin scraping at the edge of a Petri disc. A beam of light was directed to the centre of the Petri disc, and mites migrating toward the light were counted under a microscope after 6 hours. A piece of cloth (6 x 4 cm^2) was placed on the convex surface of a goat auricle, the bottom, left and right rims of the cloth were attached to the auricle by adhesive tapes. Skin scraping containing approximately 2000 mites was inserted under the cloth through the top edge then the top edge was attached to the auricle.

After 48 hours, the cloth together with the skin scraping were removed and infestation was allowed to progress. Skin lesions caused by the mite challenge were photographed and assessed their severity at 1 day, 2 days, then at weekly intervals from one to eight week. The severity of lesions were determined by the following scores: score 0 if no lesion, 1 if <10% of the infected auricles affected by dermatitis, score 2 if 10-50% were affected, score 3 if >50-100 %, and 4 if the lesions affected not only the infested auricles but also skin of other parts of the body. The effect of mite infestation on body condition was assessed from the changes in the body weight which were measured weekly before and after challenge.

Enzyme-linked immuno-sorbent assay (ELISA)

The development of specific antibodies in animals was quantified by ELISA using antigens containing protein fraction with which the tested animals were vaccinated. A checkerboard titration was performed to determine the optimal concentration of coating antigen, test serum and secondary antibody. A dilutions that gave the lowest optical density (OD) with serum from non-infected (control) goats, and the highest OD ratio between positive serum (from severely-infested goats) and the control serum were chosen and used to assay the sample sera.

The wells of polystyrene microtitre plates (Maxisorp, Nunc™, Denmark) were coated overnight at room temperature (28ºC) with 100 µl of the antigen diluted in 0.1 M carbonate buffer pH 9.6 (4-8 µg proteins/well). After washings the wells twice with PBST (PBS containing 0.05% Tween 20), duplicate 100 µl volume of the sera to be tested, diluted 1:200 in PBS, were added to the wells and incubated for 1 hour at room temperature. Following washings, wells were incubated for 1 hour with HRP-conjugated-donkey-anti-goat IgG (Jackson Immuno Research Laboratories, PA, USA) diluted 1: 20,000 in PBS. Washed plates were developed in the dark for 30 minutes with a chromogenic/substrate of ABTS and the resulting green colour was quantitated at 405 nm using a microtitre-plate reader.

Immunoblotting

Washed mites were homogenised in Laemmli reducing SDS-PAGE sample buffer (65 mg of mites/ml buffer) and heated for 10 minutes at 95ºC. Insoluble materials were pelletted by centrifugation and a 75-µl portion of the supernatant was loaded into the large well of a two-well, 0.75-mm-thick comb of *Mini-Protean 3 cells* (BioRad Laboratories). The small well was loaded with 5 µl prestained-molecular-weight marker (Amersham Biosciences). The stacking and the separating gels contained 4 and 12% monomer of acrylamide, respectively. Electrophoresis was carried out through the gels at 100 volts until the leading dye reach about 1.5 cm above the bottom of the gel. After electrophoresis, proteins from the acrylamide gel were transferred onto a nitrocellulose membrane using transfer buffer consisting of 25 mM Tris, 192 mM glycine, 20% methanol in an electrophoretic transfer cell (MiniTransblot, Bio-Rad Laboratories) at 100 volts, 350 mA for 2 hours. After the transfer, the membrane was cut into 5-mm-wide strips then soaked for 2 hours in 0.1% solution of non-fat skim milk to blocked unoccupied sites in the membrane. The strips were reacted for 2 hours with goat sera at 1:1000 dilution in PBS for 2 hours. After washing five times, the membrane strips were incubated for 2 hours in alkaline-phosphatase-labelled-anti-goat-mouse-monoclonal antibody (Sigma) at 1: 5, 000 dilution in PBS. After washing, the membrane strips were developed in

NBT/BCIP substrates according to the procedures provided (Sigma-Aldrich Pte, Singapore).

Mite burden

At the end of challenge schedule (8 weeks after the initial mite infestation), the auricles were cut off at 5 cm from the tip, after previously had been infiltrated with a local anaesthetic, Lidocaine-HCl. After the biopsy, the incision site was sutured, smeared with an anti-septic solution (Betadine®) and sprayed with a screw-worm-and-wound-dressing spray (Gusanex®). The detached auricles was stored at -20ºC to be used for mite count. The skin from the auricles that stored at -20ºC were scraped on an area of 2 cm^2 and the scrapes were suspended in 20% KOH for 30 minutes. The scrape suspension was poured on a 2 mm^2-grid and the number of mites were counted under a light microscope and mite burden was expressed as number of mite per cm^2 skin surface.

Statistical analysis

Protective value of immune response elicited by the vaccination was based on the severity of lesions caused by the mite challenge, mite densities, titre of specific antibody and changes in body weight. Differences in the score of lesions between group of goats were analysed using a non-parametric analysis, Kruskal-Wallis one-way ANOVA (PETRIE and WATSON, 2001). Differences in the antibody titres, body weight changes and mite densities were analysed with one-way ANOVA (PETRIE and WATSON, 2001). The data of mite densities which were expressed in number of mites/cm^2 were first transformed into their logarithmic value before analysed with one-way ANOVA (PETRIE and WATSON, 2001). When differences between groups were statistically significant at P<0.05, Tukey's multiple comparison analysis were performed.

RESULTS

The SDS-PAGE profile of fraction of insoluble proteins from the *S. scabiei* is presented in Figure 1. Each fraction that contained a large number of proteins with molecular weights ranged from less than 10 to over 100 kDa has a distinct profile. All of the fractions were immunogenic as high level of specific antibodies were conferred by animals vaccinated with the fractions.

In general, the prevaccinated and unvaccinated control animals did not recognised the insoluble mite proteins (Figure 2). Some animals, however, recognised faintly same of the proteins but the recognition was not intensified by the vaccination and mite challenge. This stable reaction, therefore, argued the possibility that those goats had previously been exposed to scabies. Sera collected after the last vaccination recognised a great number of mite proteins and differences were observed between group of animals. Blots reacted with pre-challenge vaccinated sera produced signals which were obviously stronger than those with post-challenge unvaccinated sera, indicating that the level of
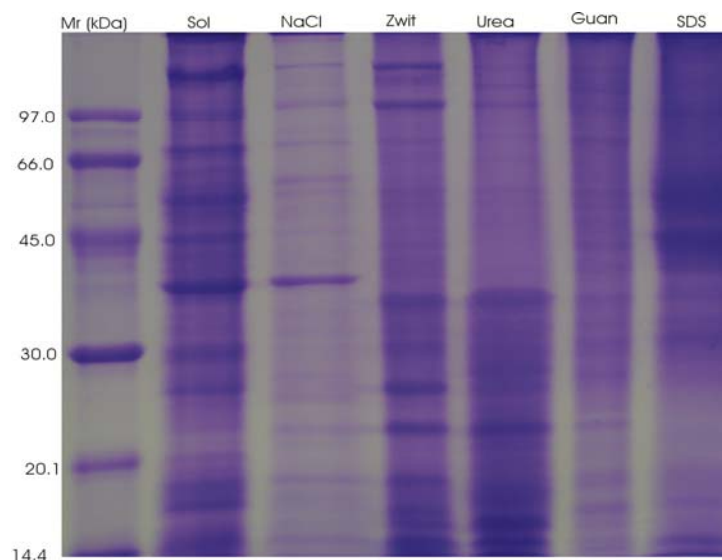

Figure 1. SDS-PAGE protein profile of insoluble-mite-protein fractions. Sol = soluble mite proteins, NaCl = F$_{NaCl}$, Zwit = F$_{Zwit}$, Guan = F$_{Guan,}$ SDS= F$_{SDS}$
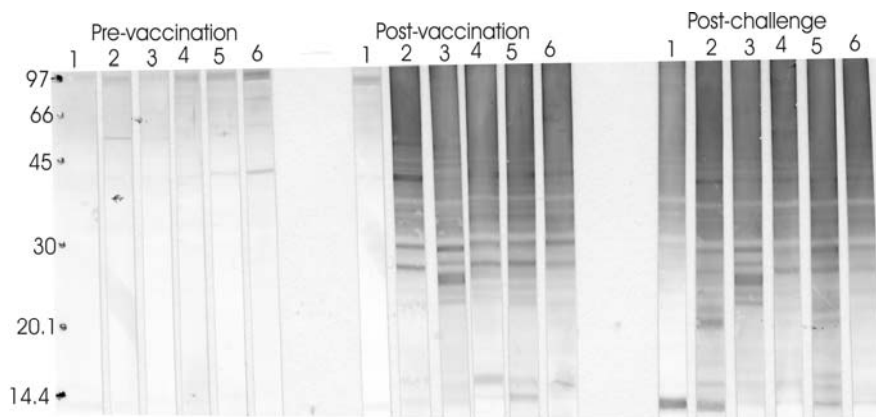
Figure 2. Immunoblot of SDS-PAGE-fractionated total mite proteins against sera of animals collected before vaccination (Pre-vaccination), after the 6th vaccination (post-vaccination) and 8 week post mite challenge (post-challenge). Line 1: the mite proteins were reacted with pooled sera from unvaccinated control goats, line 2, 3, 4, 5 and 6 with pooled sera of group of goats respectively vaccinated with F_{NaC}l, F_{zwit}, F_{urea}, FGuan and F_{SDS}

antibodies conferred by the vaccination were very high because they were higher than that induced by severe natural infestation. Although the majority of antigens recognised by the pre-challenge-vaccinated sera were also recognised by the post-challenge-unvaccinated controls, differences in the antigen recognition by the two groups were obvious. Antigens with estimate M_r of 47, 32 and 29 KDa which were recognised strongly by all groups of pre-challenge vaccinated animals were not recognised by any of the post-challenge-unvaccinated animals. These antigens, therefore, were considered to be major, novel or concealed antigens of *S. scabiei*. A protein with an estimate M_r of 53.6 KDa which was recognised strongly by the post-challenge unvaccinated group and pre-challenge F_{NaCl} group was only recognised faintly by the pre-challenge vaccinated-animals. This may indicate that this protein was likely to be highly water soluble and one of the major antigens in the normal parasite-host interaction.

Quantification of the specific antibodies by the ELISA showed that the level of specific antibodies, as indicated by OD increases, were increased after each vaccination. However, the OD increases were small with the maximum increases of only two or three folds over the base line (Figure 3). Also with unknown reason the ODs dropped in all vaccinated animals after the fourth and fifth vaccination, and the drop varied significantly between groups. These phenomena, however, were unseen in the immunoblot assay.

Despite strong immune responses developed in the vaccinated animal, the immune responses did not have any protective value. Challenge of the goats with *S. scabiei* one week following the last vaccination resulted in the development of severe acute dermatitis at the infested auricles in all goats. Twenty-four to 48 hours following infestation, the infested auricles in most goats

became severely swollen (Figure 4A). When palpated, the affected auricles were tender, soft in consistency, and appeared much hotter than the opposite auricles. Serous exudates were observed at the site of infestation especially on auricles previously severely swollen, and numerous small papules under the coat at or around the site of infestation were perceived when the auricles were palpated. Differences in nature or severity of the lesions, however, were not discernible between unvaccinated control and vaccinated groups, or within the vaccinated groups. Examination at 1-week revealed that the swelling and exudation had disappeared from all animals leaving patches of alopecia or thinning or hair. The infested auricles were covered by either dried serum crusts or thin parakeratotic scales. The crusts and scales were thickened and spread rapidly and more than halve the auricle surface was affected by two weeks post infestation in most animal (Figure 4B). By 3 weeks, practically the entire surface of the infested auricles had been covered by thick crusts or scab formation, and the lesions started to spread to other regions of the body, particularly to the shoulder, neck, head and opposite auricle (Figure 4C). At the end of experiment (8 week post infestation), practically the whole skin was affected. Again, no differences in severity of lesions could be discernible between unvaccinated control and vaccinated groups (Figure 4D).

The effects of mite challenge on body weight changes in the unvaccinated-control and vaccinated groups are presented in Table 1. Except for F_{zwit}-group goats at two week post challenge, no reduction in body weight was observed in any group of the goats. No significant differences in the body weight changes between vaccinated and non vaccinated goats were observed. The mean body weight increases in goats of

F_{NaCl}-group were found to be significantly higher than that of F_{zwit}-group but those differences did not associated with severity of lesions as no differences in the severity were observed.

In agreement with severity of lesions and body-weight changes, the mite burdens or mite densities in the vaccinated groups were not different from that in the unvaccinated control (Figure 5). For unknown reason, the number of eggs in the F_{SDS}-group was significantly higher than that in the F_{Guan}-group.
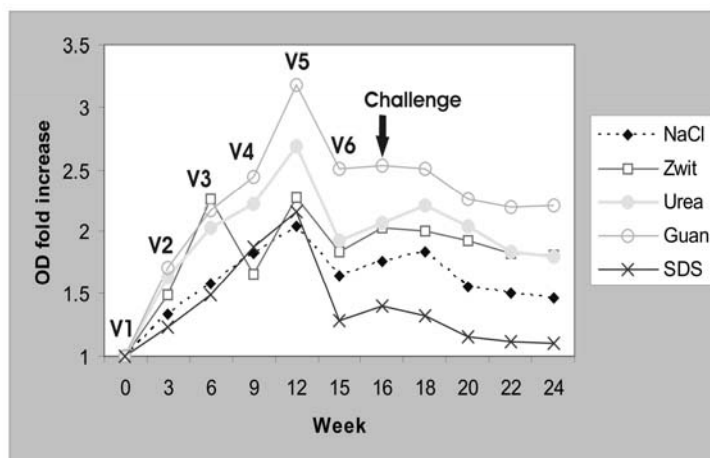

Figure 3. Antibody response of goats monitored with ELISA against vaccination and mite challenge. NaCl = F_{NaCl}, Zwit = F_{Zwit}, Guan = F_{Guan}, SDS= F_{SDS}. V1 to V6 represent first to sixth vaccinations respectively
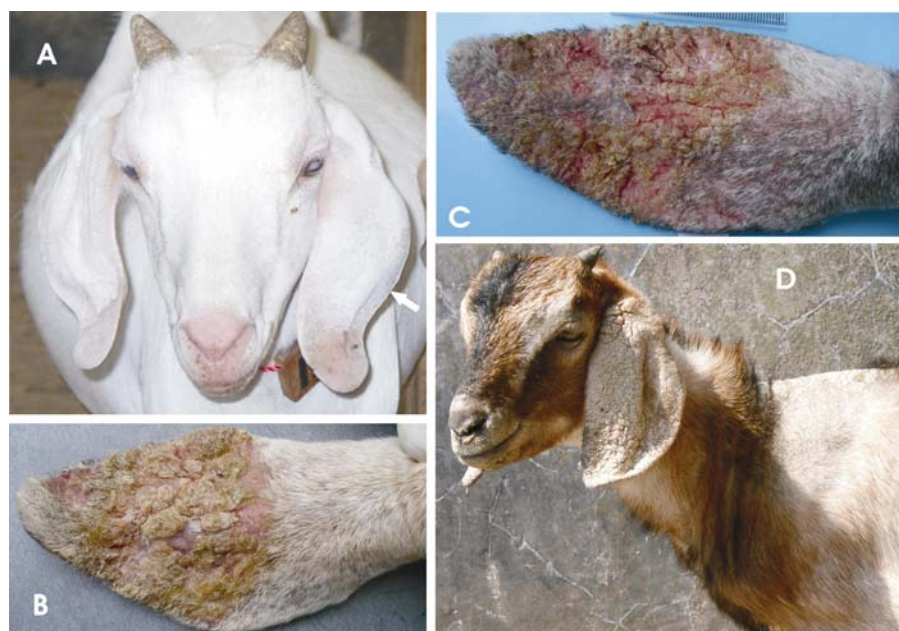

Figure 4. Lesions developed after mite challenge. Severe swelling in the infested auricle (arrow) two days post challenge (A), dermatitis with thick sero-cellular exudates two-weeks (B) and 3 weeks post challenge (C). Severe encrustation dermatitis affecting almost the entire skin (D). Note: no differences in the severity of lesions between vaccinated and unvaccinated groups

Table 1. Change in the body weight of goat after challenge

Group	Time			
	2 week	4 week	6 week	8 week
Unvaccinated	0.52	1.05	1.02	1.45
FNaCl	1.43[b]	2.10[b]	2.08[b]	2.00
FZwit	-0.06[a]	0.71[a]	0.40[a]	0.54
FUrea	0.91	1.66	2.03[b]	2.20
FGuan	1.07	1.66	1.90	2.43
FSDS	1.02	1.45	1.58	1.78

Note: different superscript indicate significantly different (P<0.05)
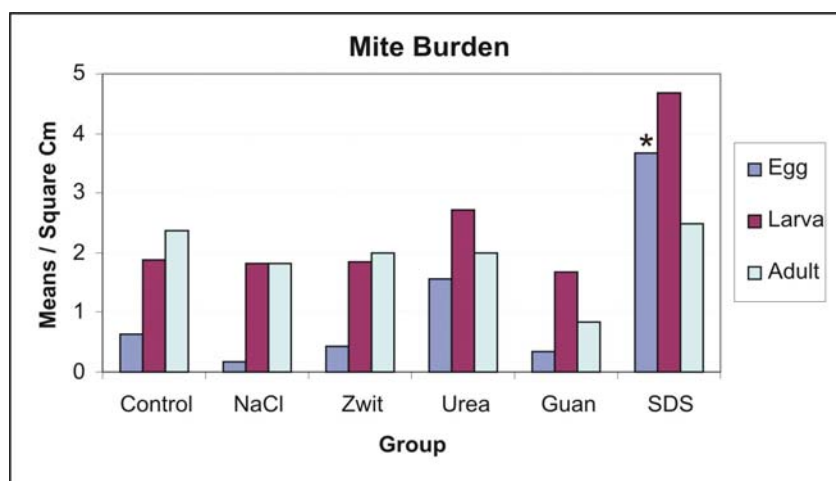

Figure 5. The density of mites (number of mites/cm^2) on the right auricle. No differences between groups were found, except that the density of eggs in the SDS group was significantly higher (P=0.002). NaCl = F$_{NaCl}$, Zwit = F$_{Zwit}$, Guan = F$_{Guan}$, SDS= F$_{SDS}$.

DISCUSSION

These studies show that immune responses elicited by immunisation of goats with insoluble proteins extracted from *S. scabiei* did not protect the animals from mite challenge. The lack of protective immunity in any group of animals after immunizing with the insoluble mite proteins was disappointing. We had assumed that membrane proteins lining the lumen of the mite's gut would be extracted by the surfactants or chaotrophic agents, and therefore the proteins should be present in one or more of the fractions. Therefore, it was expected that animals vaccinated by the fraction should induce specific antibodies, and when ingested by mites, the antibodies would inactivate the function of the membrane proteins which in turn caused mortality or severe damage to the mites. This expectation was based on our previous experiment which indicates that *S. scabiei*, despite residing not deeper than the cornified

layer of the skin, does ingest its host immunoglobulin (TARIGAN, 2005).

At the time of challenge, all vaccinated animals had a high level of antibodies. This is not a surprise since the animals had been vaccinated six times. The high number of vaccination is to anticipate the possibility of low abundance of protective antigens in the fractions. The high level of antibodies were obvious when examined by immunoblot analysis but not so clear when examined with the ELISA. The inaccuracy of the ELISA is thought to be resulted from the inadequate binding of the mite-insoluble proteins to the polysterene-microplate wells. This limited binding is probably associated with the insoluble nature of the proteins in the coating buffer used.

The lack of protection conferred by the vaccination protocol may result from a number of possibilities. First, the protective membrane proteins might have not been extracted by any of the surfactants or chaotrophic agents although the method has been successfully used

in the extraction of protective membrane proteins from screw-worm fly (RIDING *et al.*, 2000). This possibility seems unlikely since a number of surfactants and chaotrophic agents were used included those with high solubilisation capacity. All membrane proteins should be solubilised at least in the SDS solution. Second, the protective membrane may have been degraded by proteolytic enzymes. During homogenisation of the mites, anti serine-proteases, anti cysteine-protease and anti metallo-proteases were included in the extraction buffer (TARIGAN and HUNTLEY, 2005). Therefore, soluble proteases should have been inactivated. However, during fractionation of the water-insoluble proteins the anti proteases, for technical reasons, were not included. It is also unlikely that the remaining proteases should be still active under such high concentration of the surfactants and chaotrophic agents. Third, protective membrane proteins underwent coagulation during fractionation which in turn resulted in the loss of protective capacity. Fourth, the mites did not ingested the host immunoglobulin sufficiently or the protective membrane proteins exist in such low abundance that incapable of conferring sufficient protective immunity. If this later possibility is true, then, the protective proteins must exist in an extremely low concentration since six time vaccination is still unable to induce immune protection. The contention that protective concealed antigens exist in extreme low concentration is supported by the findings in *Boophilus microplus*. To purified several milligram of protective concealed antigen, *Bm 86*, required nearly 1 kg of ticks as the starting materials (WILLADSEN *et al.*, 1989). If such low concentration also happened in *S. scabiei*, the amount of protective antigen received by each vaccinated animals must have been too low to confer detectable protective immunity.

The criteria for the protective immunity as used in this study was the reduction in the severity of lesions and mite burden in the vaccinated individuals, as compared to those in the unvaccinated control. These criteria may not be satisfactory since ectoparasite vaccines that have been developed so far are not intended to protect the vaccinated individual nor to knock down invading parasite immediately but rather to reduce ectoparasite population over successive parasite generation in an area-wide management programme (PRUETT, 1999). Nevertheless, there is no reason to believe that repeated vaccination with the mite-insoluble protein will cause reduction in the mite population since no difference in mite burden between vaccinated and unvaccinated animals.

CONCLUSSION

To sum up, the protective-concealed antigens from *S. scabiei* are either very labile or exist in extreme low concentrations that their protective capacities are undetectable by conventional biochemical fractionation and vaccination-challenge experiment.

ACKNOWLEDGMENTS

REFERENCES

CURRIE, B. J., P. HARUMAL, M. MCKINNON and S. F. WALTON. 2004. First documentation of *in vivo* and *in vitro* ivermectin resistance in *Sarcoptes scabiei. Clin. Infect. Dis*. 39: 8-12.

DONALD, A. 1994. Parasites, animal production and sustainable development. *Vet. Parasitol*. 54: 27-47.

PETRIE, A. and P. WATSON. 2001. Statistics for veterinary and animal science. Blackwell Science Ltd, London.

PRUETT, J. H. 1999. Immunological control of arthropod ectoparasites-a review. *Int. J. Parasitol*. 29: 25-32.

RIDING, G., S. MUHARSINI, R. PEARSON, SUKARSIH, E. SATRIA, G. WIJFFELS and P. WILLADSEN. 2000. Fractionation, identification and vaccination efficacy of native antigens from the screwworm fly, *Chrysomya bezziana. JITV (Edisi Khusus)* 5: 150-159.

TARIGAN, S. 1998. Metode pengembangbiakan dan pemanenan tungau *Sarcoptes scabiei*. Pros. Seminar Nasional Peternakan dan Veteriner, Bogor, 1-2 Desember 1998. Puslitbang Peternakan, Bogor. hlm. 1009-1017.

TARIGAN, S. 2003. Dermotopathology of caprine scabies and protective immunity in sensitised goats against *Sarcoptes scabiei* infestation. *JITV* 7: 265-271.

TARIGAN, S. 2005. Ingestion of host immunoglobulins by *Sarcoptes scabiei. JITV* 10: 35-40.

TARIGAN, S. and J.F. HUNTLEY. 2005. Failure to protect goats following vaccination with soluble proteins of *Sarcoptes scabiei:* Evidence for a role for IgE antibody in protection. *Vet. Parasitol. In Press.*

WALL, R. 1992. The environment impact of avermectin use in livestock. *Parasitol. Today* 8: 255-256.

WILLADSEN, P. 1997. Novel vaccines for ectoparasites. *Vet. Parasitol.* 71: 209-222.

WILLADSEN, P. 2001. The molecular revolution in the development of vaccines against ectoparasites. *Vet. Parasitol.* 101: 353-367.

WILLADSEN, P., G. A. RIDING, R. V. MCKENNA, D. H. KEMP, R. L. TELLAM, J. N. NIELSE, J. LAHNSTEIN, G. S. COBON and J. M. GOUGH. 1989. Immunologic control of a parasitic arthropod- identification of a protective antigen from *Boophilus microplus*. *J. Immunol.* 143: 1346-1351.