Antibody Responses in Naïve and Sensitised Goats Infested by *Sarcoptes scabiei*

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ABSTRAK

TARIGAN, S. 2004. Respon antibodi pada kambing naïve dan kambing sensitasi terhadap infestasi Sarcoptes scabiei. JITV 9(4): 258-265.

Tujuan dari penelitian ini adalah untuk mengkarakterisasi respon antibodi IgG and IgE pada kambing yang diinfestasi secara berulang dengan *Sarcoptes scabiei*. Sebanyak 10 ekor kambing yang diperoleh dari peternakan yang berstatus bebas skabies diinfestasi dengan 2000 tungau hidup pada daun telinga. Lima puluh hari setelah infestasi, kambing diobati dengan ivermectin. Setelah kambing sembuh total, kambing diinfestasi ulang lalu diobati lagi pada hari ke-50. Sampel darah diambil sesaat sebelum infestasi, kemudian setiap sepuluh hari sampai hari ke-270. Serokonversi untuk IgG terbentuk 30 hari setelah infestasi pertama, sedangkan titer IgG maksimum terjadi setelah hari ke-50. Pada analisis imunoblot diidentifikasi sejumlah antigen (M_r 180, 135, 43 dan 38 KDa) yang dikenali oleh IgG pada hari ke-10 dan secara terus menerus dikenali sepanjang perjalanan infestasi. Karena dikenali secara konsisten oleh serum, apabila antigen tersebut dipurifikasi diperkirakan dapat dipakai untuk mengembangkan test diagnostik imunologis untuk skabies. Titer antibodi IgE spesifik skabies naik sedikit secara perlahan-lahan dan turun dengan cepat setelah hewan diobati. Akan tetapi, pada infestasi kedua dan ketiga antibodi *reaginik* tersebut naik dengan cepat dan dengan titer yang lebih tinggi. Pada analisis imunoblot diidentifikasi sekurang kurangnya 11 antigen (M_r 130, 72, 64, 58, 44, 41 39, 27, dan 25 KDa) yang dikenali oleh IgE serum. Karena IgE diduga memainkan peranan utama dalam proteksi, antigen atu allergen tersebut kemungkinan dapat dipakai sebagai komponen utama vaksin anti skabies.

Kata kunci: Sarcoptes scabiei, antibodi, kambing

ABSTRACT

TARIGAN, S. 2004. Antibody response in naïve and sensitised goats infested by Sarcoptes scabiei. JITV 9(4): 258-265.

The purpose of this study was to characterize the IgG and IgE antibody responses in goats infested repeatedly with *Sarcoptes scabiei*. Ten goats purchased from scabies-free farms were infested with 2000 live mites on the auricles. Fifty days after the initial infestation, the goats were treated with ivermectin. After being completely recovered, the goats were reinfested then treated again at 50 days post infestation. Blood samples were collected at the time of the first infestation, then every 10 days afterwards for 270 days. Seroconversion for IgG took place after 30 days following the first infestation, whereas the maximum level of the specific IgG antibodies occurred after 50 days. Immunoblot analysis identified a number of antigens (M_r 180, 135, 43 and 38 KDa) that recognised by the IgG at 10 days and continuously recognised throughout the course of the multiple infestations. Being consistently recognised, those antigens should be essential in the development immunological diagnostic tests for scabies. The levels of scabies-specific IgE antibodies increased slowly during the first infestation and rapidly dropped following treatment of the animals with ivermectin. In the second and third infestations, however, the reaginic antibodies rose rapidly and with a grater level. On immunoblot analysis, at least 10 antigens (M_r 130, 72, 64, 58, 48, 44, 41, 39, 27 and 25 KDa) were observed to be recognised by the IgE present in the sera from scabies-infested animals. Since IgE response is considered to play a major role in the immune protection, those allergens, therefore, could be used as the main component of an anti-scabies vaccine.

Key words: Sarcoptes scabiei, antibody, goats

INTRODUCTION

Scabies or sarcoptic mange is a contagious pruritic skin disease in man and animals caused by a mite, *Sarcoptes scabiei*. The disease has a worldwide distribution affecting more than 40 species from 17 families and seven orders of mammals (ZAHLER *et al.*, 1999). The prevalence of the disease in man and animals is high. It is estimated that more than 300 million of people worldwide are affected every year (ARLIAN, 1989). Although the disease in man is not usually fatal but the itching caused by the mites is often unbearable and often leads to secondary bacterial infection which in turn resulted in cellulitis, lymphangitis or acute glomerulonephritis (BROOK, 1995; KEMP *et al.*, 2002). The prevalence of the disease in a goat population appears to fluctuate

considerably, from less than 5% to nearly 100%. The mortality rate of disease is reported be the to surprisingly high, 67-100% in young 11% in and around mature goats (BROTOWIJOYO, 1987; al., Manurung et 1987). A high prevalence of sarcoptic mange in goats is also reported in Malaysia (DORNY et al., 1994), India (PARIJA et al., 1995) and Libya (GABAJ *et al.*, 1992). In pigs, sarcoptic mange is apparently also one of the most important diseases because its prevalence is usually high. A survey conducted in Northern Spain reveals a prevalence of 33.7% (GUTIERREZ et al., 1996), in Southern Minnesota, USA 14% (DAVIES et al., 1996) and in Tanzania 88% (KAMBARAGE et al., 1990).

Although the disease has been known for thousands of years and persistently causes enormous public health and economic burden, no practical diagnostic tool and prophylactic control measure are yet available (RONCALLI, 1987). The current diagnosis of scabies which is based on clinical signs and microscopical examination of mites in the skin scraping is impractical and insensitive as the mites are found in only 20-50% of skin scraping from infested animals (GUTIERREZ et al., 1996; LOWER et al., 2001). The control of the disease which is still heavily relied on the use of acaricides has many disadvantages such as induction of mite resistance and acaricide residue in the meat. The sluggishness in the availability of the diagnostic test and vaccine seemingly associated with our limited understanding in the immunology of the disease. It has been understand for more than 60 years that the mites induce immunity in human during natural infestation (MELLANBY, 1944). Similar phenomena with the development of scabies-specific IgG antibodies have also been demonstrated in rabbits, dogs and foxes (ARLIAN et al., 1996; ARLIAN et al., 1994; LITTLE et al., 1998; TARIGAN, 2003a, b). In our previous studies we described the development of protective immunity in and sensetised goats. compared gross and histopathology of lesions developed in naïve and sensitised goats following infestation with Sarcoptes scabiei (TARIGAN, 2003a, b). In the present study we described the scabies-specific IgG and IgE antibodies in those repeated infested goats. In this study we identified a number of mite antigens that recognised by the host early and consistently and therefore they could be used to develop a sensitive diagnostic test for the disease.

MATERIALS AND METHODS

Animal, Sarcoptes scabiei and infestation

In our previous studies aiming at comparing the gross and histopathology of lesions caused by S. scabiei infestation in naïve and sensitised goats we used 30 young female goats which were allocated into 5 groups, each group contained 6 animals (TARIGAN, 2003b). Group 1, 2 and 3 goats had been infested with the mite once, twice and thrice, respectively. Group 4 and 5 goats were naïve or goats that previously had not been exposed to S. scabiei. The sera used in this present study were collected from 5 goats from each group 2 and group 3. Sample sera were collected at the time of the first infestation then every 10 days from day 0 to day 270 (group 3) or to day 180 (group 2). The second and third infestations were performed at days 80 and 160, respectively; and 50 days after the initial of each infestation the animals were treated with ivermectin (Figure 1). A detailed description regarding the sourced of mites, procedures for infestation and treatment can be found in TARIGAN (2003a).

Enzyme link immuno-sorbent assay (ELISA)

Antigen for the Elisa was prepared according to ARLIAN et al. (1988) with some modification. Briefly, mites were freeze-dried then ground to fine powder and delipidated with diethyl ether. Soluble proteins from the mites were extracted with PBS and the host immunoglobulin (IgG) contained in the extract was depleted by passing through a superose-protein-G column (Amersham Biosciences). The concentration of proteins in the suspension was determined by Bradford's method using a commercial kit (Bio Rad Laboratories). A checkerboard titration was performed to determine the optimal concentrations of coating antigen, test serum and secondary antibody. A dilutions that gave the lowest optical density (OD) with serum from non-infected (negative control) goats, and the highest OD ratio between positive serum from severelyinfested goats and the negative control serum were chosen and used in assaying the sample sera. The wells of polystyrene microtitre plates (Maxisorp, NuncTM, Denmark) were coated overnight at room temperature (25°C) with 50 μ l of the antigen diluted in 0.1 M carbonate buffer pH 9.6 (11 µg proteins/well). After washing the coated wells twice with PBST (PBS containing 0.05% Tween 20), duplicate 50 µl volume of the sera to be tested, diluted 1:1000 in PBS_{T80NaCl} (PBS containing 0.5% Tween 80 and 0.8 M NaCl) were added to the wells and incubated for 1 hour at room temperature. Following washing (5 times) in PBST, wells were incubated for 1 h with HRP-conjugateddonkey-anti-goat IgG (Jackson Immuno Research Laboratories, PA, USA) diluted 1: 20,000 in PBS_{T80NaCl}. 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 mictrotite-plate reader.

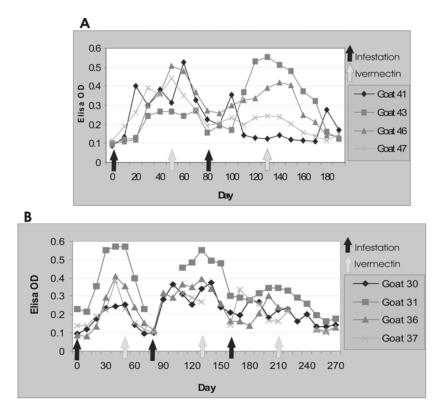


Figure 1. IgG responses of goats infested twice (A) and three times (B) with Sarcoptes scabiei

For IgE Elisa, coating of microplate was performed similarly and assay was done at room temperature. The sera to be tested were diluted 1: 10 in PBS_{T80NaCl} and added to the washed wells and incubated for 1 hour. After washing the plates five times, murine-antiovine/caprine IgE (A kind gift from Dr. J.F. Huntley, Moredun Reasearch Institute, Scotland) diluted 1: 200 in PBS_{T80NaCl} was added and incubated for 1 hour. Following washings, wells were incubated for 1 hour with biotinylated anti-mouse IgG (Dako, Denmark) diluted 1: 2 500 in PBS_{T80NaCl}. After washing the plates five times, Streptavidin (Dako, Denmark) diluted 1: 5 000 PBS_{T80NaC1} was added and incubated for 1 hour. Washed plates were developed in TMB substrate (Sigma) for 10 minutes and reaction was stopped by adding 50 μ l H₂SO₄ (0.1 *M*). Absorbance was read at 450 nm. Controls consisted of wells containing a known positive and a known negative sera sample were applied to each test Elisa plate.

Immunoblotting

Washed mites were homogenised in Laemmli reducing SDS-PAGE-sample buffer (65 mg mites/ml buffer) and heated for 10 min at 95°C. After pelletting insoluble materials by centrifugation, 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 and 20% methanol in an electrophoretic transfer cell (MiniTransblot, Bio-Rad Laboratories) at 100 volts, 350 mA for 2 hour. 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 alkalinephosphatase - labeled - anti - goat -murine-monoclonal antibody (Sigma) at 1: 10.000 dilution in PBS. After washing five times, the membrane strips were developed in NBT/BCIP substrates (Sigma-Aldrich Pte, Singapore) according to the procedures provided. For IgE detection, mouse anti goat IgE monoclonal antibody (provided kindly by Dr J.F. Huntley, Moredun Research Insitute, Scotland) was used as the secondary antibody and peroxidase-labelled rabbit anti mouse antibody as the detecting antibody. The peroxidaselabelled antibodies that bound to the antigen-IgE complex was detected by a chemiluminescence detection system (ECL plus, Amersham Biosciences).

RESULTS

IgG responses

The levels of scabies-specific-IgG antibodies, expressed as optical density of Elisa (Elisa OD) of the repeated infested goats were presented in Figure 1. The Elisa ODs of pre-infestation sera of all goats, except goats 31 and 37, were less than 0.1. Between 10-20 days after the initial infestation, a gradual increase of the OD values were recorded, and maximum level of 0.254 -0.572 were recorded at the time when the goats were treated with ivermectin. The variability in the OD values between animals was considerable despite the fact that the values were relatively similar at the start of infestation. Treatment of the mangy goats with ivermectin resulted in the gradual decreases in the OD values, reaching nearly the pre infestation level after 30 days. After the second mite infestation, the OD values were higher than those in the first infestation but some animals (goats 41 and 47) the OD values were lower than those in the first infestation. Treatment of this second infestation was also resulted in the drop of the OD values but the drops were less rapid and less intense compared to that in the first infestation. The third infestation also raised the OD but the rises were not as intensive as that in the previous infestations.

The Elisa result showing that the goats had not been in contact with S. scabiei previously was supported by the immunoblot assay as no mite protein was recognised by the pre infestation serum. The immunoblot was apparently more sensitive than the Elisa since the former could detect the presence of scabies-specific IgG antibodies as soon as 10 days after the initial infestation. At least five mite proteins of M_r \geq 220, 135, 116, 43 and 38 KDa were recognised by the serum at 10 day post infestation (Figure 2). The \geq 220-KDa protein was the most intensively recognised antigen but its recognition by the sera was disappeared at later stage of infestation. The other proteins, although faintly recognised at 10 days, become more and more intensively stained afterwards, and were consistently recognised throughout the infestation. At 20 days post infestation, the serum recognised a prominent antigen $(M_r 180 \text{ KDa})$ which was unrecognised at 10 days. This antigen was recognised consistently and one of the most intensively stained antigen throughout the infestation. At the end of the first infestation (50 days post infestation) just before the goats were treated with ivermectin, the serum recognised at least 11 antigens with estimated M_r of 180, 135, 116, 99, 87, 76, 64, 43, 40, 38 and 37 KDa. Treatment of the mangy goats resulted in the lessening the intensity of immunoblot staining. In agreement with the Elisa result, 30 days after the treatment there were hardly any reaction was seen

The antigen recognition of the serum, however, was rapidly reappeared when the sensitised animals were reinfested. Ten days after the initial second infestation, the reactivity level of the serum was higher than that of the end of the first infestation. The highest level of the IgG reactivity was observed 40 days after the second

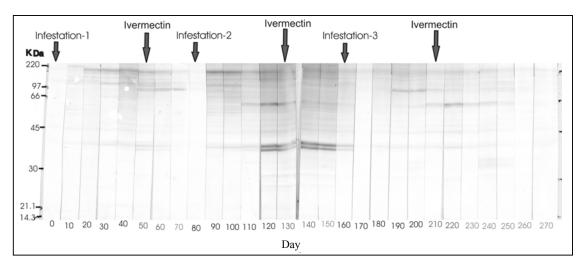


Figure 2. Immunoblot showing IgG immunoreactivity in sera from Goat 30

infestation and this high level was maintained until 20 days after the goats were treated with ivermectin. At the highest IgG-reactivity level, fused bands from 43 to 220 KDa as well as four very prominent antigens (M_r 180, 60, 38 and 37 KDa) were seen. In addition, bands at 28, 26 and 25 KDa which were unseen in the first infestation were obvious at the second infestation. Thirty days after the initial treatment of the second infestation, unlike in the first infestation, the IgG reactivity of the serum was still obvious. However, all the reactive IgG antibodies were disappeared at 40 days and only the 180-KDa antigen was faintly recognised. The third infestation, after the second infestation was completely cured, caused reappearance of the reactive IgG antibodies but the level of reactivity was considerably lower than that in the second infestation. The IgG recognition profile of the serum from the thirdinfested animals was different to that from the first infestation but the 180 and 38 KDa antigens which were consistently recognised in the first and second infestation were still recognised in the third infestation.

IgE responses

The levels of scabies-specific-IgE antibodies, expressed as optical density of Elisa (Elisa OD) of the repeated infested goats were presented in Figure 3. The mean OD value of the 4 goats before the application of the mites was 0.054 (standard deviation = 0.008). After the first infestation, the OD values were almost unchanged in two animals or slightly increase in the other two. On IgE-immunoblot assay, a band with an estimated M_r of 130 KDa was faintly recognised at 20 days and became intensively stained at 50 days (Figure 4). The OD levels were rapidly dropped following treatment of the first infestation with ivermectin but similar drop in the specific IgE levels was not seen in the immunoblot assay. Reinfestation of the animals, after being completely cured from the first infestation, resulted in the rapidly increase the OD levels. The mean OD at 50 days after the second infestation was over three times of that after the first infestation. The immunoblot recognised at least 10 antigens with estimated M_r of 130, 72, 64, 58, 48, 44, 41, 39, 27 and 25 KDa, respectively. As in previous infestation, treatment of the second infestation resulted in the rapid drop in the scabies-specific IgE. Again, infestation of the goats for the third time, after being cleared from the second infestation, resulted in the rapid increase in the OD levels and immunoblot-stained intensity. The mean OD and stain intensities caused by the third infestation were higher than those by the second infestation. This is in contrast with the IgG response in which both Elisa and immunoblot showed that the level of specific IgG in the third infestation were markedly lower than that in the second infestation.

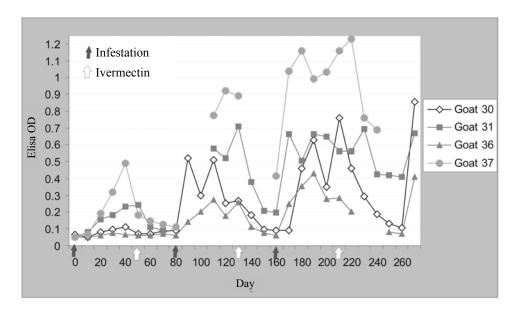


Figure 3. Level of circulating scabies-specific IgE antibodies in goats repeatedly infested with Sarcoptes scabiei

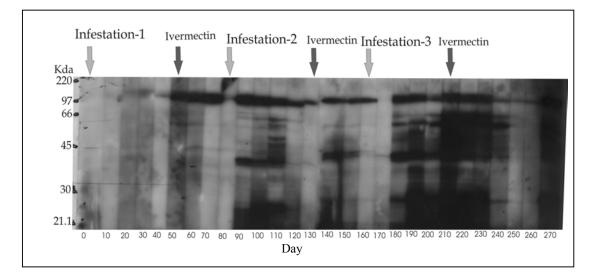


Figure 4. Immunoblot showing IgE immunoreactivity in sera from Goat 30

DISCUSSION

This study demonstrated that infestation of goats with S. scabiei resulted in the development of circulating scabies-specific IgG and IgE antibodies that followed the classical pattern for primary and secondary infection. To study immune response of goats against S. scabiei required that animals used in the experiment must be naïve or had not previously been in contact with the mites. For this reason, the goats used in this study were purchased from farmers who had not seen any sign of mange in their animals for the last two years. Although the farmers' assurance might not be totally reliable, the Elisa tests and immunoblot analyses provide sufficient evidence to indicate the naivety of the goats, as the assays showed that the pre infestation sera from the animals did not contain any detectable level of IgG and IgE antibodies specific for S. scabiei. The high OD level in the IgG Elisa in some animals (goats 31 and 37) is unlikely to be the indication of previous scabies infestation because those animals did not contain detectable scabies-specific IgE antibodies. In addition, when those animals were infected with the mites no protective immunity was observed, indicating that those animals had not been in contact with scabies before (TARIGAN, 2003a).

In general, results obtained from this study were comparable to those obtained from previous similar studies in rabbits (ARLIAN *et al.*, 1994), dogs (ARLIAN *et al.*, 1996) or red foxes (BORNSTEIN *et al.*, 1995). In the present study, seroconversion or significant level of specific-IgG antibodies and maximum level the IgG, as measured by the Elisa, were observed at 30 and 50 days post infestation, respectively. In rabbits, seroconversion took place around 40 days after the initial infestation and the specific IgG reached its maximum level at 50

days (ARLIAN *et al.*, 1994). In dogs the seroconversion and the maximum IgG level occurred slightly faster, at 16 and 49 days (ARLIAN *et al.*, 1996), whereas in red foxes the seroconversion and maximum IgG level which took place at 4 and 10 weeks post infestation are considerably slower (BORNSTEIN *et al.*, 1995).

Previous studies show that Elisa tests using crude extract of *S. scabies* have high sensitivity (80%) and high specificity (\geq 98%) when tested in scabies-free and scabies-infected farms (HOLLANDERS *et al.*, 1997). A 84.2% sensitivity and a 89.5% specificity were obtained when similar test was evaluated in scabies-positive and negative dogs (LOWER *et al.*, 2001).When used in scabies-eradication programmes in some pig industries in Europe, the Elisa using the crude antigens proved to be practical and sufficiently accurate in monitoring infestation rate in the herds (HEINONEN *et al.*, 2000; JACOBSON *et al.*, 1999).

Although having high sensitivity and specificity, Elisa tests using crude extract of *S. scabiei*, as demonstrated in this study and others, are only suitable for identifying scabies-infested animals at the late stages (ARLIAN *et al.*, 1996; BORNSTEIN *et al.*, 1995; VAN DER HEIJDEN *et al.*, 2000). The development of significant level of specific IgG antibodies measured by the Elisa lagged behind the development of skin lesions caused by the mites. Skin lesions characterised by crusted dermatitis around the site of infestation were obvious at 10 days post infestation and by 40 days the lesions had been widely spread (TARIGAN, 2003a, b). This limitation, therefore, would prevent the acceptance of the Elisa as a reliable test for scabies.

Development of an Elisa test that capable of detecting early stage of infestation seems possible provided that appropriate antigen purified from the mites is used in the Elisa. Such antigens could be

identified by immunoblot analysis of sera collected serially from infested animals as done in this study. In this study, the antigens with the estimated M_r of 180, 135, 43 and 38 KDa which were recognised at 10 days and continuously recognised at all stages of the repeated infestations would potentially be appropriate. The fact that circulating scabies-specific IgG antibodies rapidly decline in parallel with the disappearance of mite from the skin, as demonstrated in this study and others, indicate that the result of the Elisa is easy to interpret because they indicate the presence of mite infestation in the skin (ARLIAN et al., 1996; ARLIAN et al., 1994; BORNSTEIN et al., 1995). However, the severity of infestation does not parallel the level of scabies-specific IgG. As shown in this study, 40 to 50 days after the initial second infestation, the levels of specific IgG were maximum; at the same time the goats displayed minimal lesions because of the protective immunity acquired from the first infestation.

While the importance of the scabies-specific IgG antibodies in diagnosis of scabies is comprehensible, the roles played by the conferred IgG antibodies in the immunity is not clear. Since this study was not intended to cover that aspect, no evidence was obtained that may suggest the importance of the immunoglobulin in the protective immunity. However, evidence is available to suggest that the specific IgG antibodies do not play significant role in the protective immunity. Immunisation of goats with soluble mite proteins did induce the development of scabies specific IgG antibodies but when the vaccinated animals were challenged with the mites no protection was observed in spite of the high level of specific IgG (Unpublished data). The contention that the IgG does not play important role in the protective immunity is support by ARLIAN et al. (1994) who demonstrated in rabbits that the level of the scabies-specific IgG was negatively correlated with the level of protection.

Protective immunity developed during natural infestation is considered to be associated with the development of immediate hypersensitivity (DAVIS and MOON, 1990a, b; MELLANBY, 1944; TARIGAN, 2003b). One of the key elements in the immediatehypersensitive reaction is the presence of specific IgE on the surface of mast cells, which upon binding with a specific antigen causes the release of inflammatory mediators which in turn causes cutaneous anaphylaxis (TIZARD, 2000). Immediate formation of serocellular exudates consisting of serum, neutrophils and eosinophils that confronting the invading mites are assumed to be the mechanism by which the sensitised animals protect themselves (TARIGAN, 2003b). In the present study, all animals developed scabies-specific IgE antibodies upon infestation with the mites. The level of the IgE antibodies developed during the first infestation varied; in fact, some animals did not apparently developed the reaginic antibodies. In the second and third infestations, however, all animals developed high level antibodies regardless of the levels in the first infestation. Despite the variable level of the reaginic antibodies, reinfestation of the animals with the mite proved to be that all of them had an equal protective immunity. This may indicate that it is not the circulating specific IgE but the presence of specific IgE on the surface of skin mast cells which is importance in the protective immunity. Also, as demonstrated in this study the IgE antibodies were rapidly dropped following treatment of the animals. At the time the animals were infested for the second or the third times the level of the reaginic antibodies had been dropped to the pre infestation level, provided additional evidence that the circulatory IgE could not be used as the indicator of protective immunity.

The mite antigens that recognised by the immune sensitised goats shown in the immunoblot analysis are considered to be important as the protective antigens.

If it is true that the IgE play a major role in the protective immunity, appropriate immunisation of animals with those antigens should conferred protective immunity. However, our study conducted separately with the present study indicate that immunisation of goats with soluble mite proteins using intramuscular route failed to confer detectable scabies specific IgE antibodies and no protection was observed when the vaccinated animals challenge with the mites (Unpublished data). The failure in conferring the reaginic antibodies is considered to have been resulted from an inappropriate route of vaccination, dose of immunogen or inappropriate adjuvant.

CONCLUSION

To sum up, this study shows that despite living in the avascular, non living cornified outer layer of the skin, *S. scabiei* induces obvious IgG and IgE antibody responses. The antibodies were rapidly diminish paralleled disappearance of the mite infestation on the skin. A number of mite antigens recognised early in the infestation process by the host circulating IgG and continuously recognised throughout the course of infestation are supposedly importance in the development of sensitive immunological diagnostic test for scabies. Whereas, antigens stimulating the development of the reaginic antibodies identified in this study might be useful as the main component of a vaccine.

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