

SEROPREVALENCE OF *SARCOPTES SCABIEI* VAR *CANIS* ANTIBODIES AMONG ABORIGINES IN PENINSULAR MALASIA

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Abstract. The Aborigines or Orang Asli in Peninsular Malaysia who are still seminomadic are known to have a close association with dogs. In this study, enzyme-linked immunosorbent assay (ELISA) was used to detect anti-*Sarcoptes scabiei* var *canis* antibodies in this community as a measure of exposure to the mite. Out of 312 Orang Asli tested, 24.7% were positive for polyvalent anti-*Sarcoptes* antibodies. No significant difference was found between the positive rates in males (26.1%) and females (23.6%). Only 1.9% were positive for IgA and none was positive for IgE anti-*Sarcoptes* antibodies. Since there were very few patients with clinical manifestation of scabies, there is a possibility that continuous exposure to the dogs mite confers cross-protective immunity in the community against human scabies.

INTRODUCTION

Sarcoptes scabiei causes the disease called "scabies" or mange in mammals. Human scabies is caused by *Sarcoptes scabiei* var *hominis*. The *Sarcoptes* sp. which affect wild and domesticated animals are closely related to *Sarcoptes scabiei* var *hominis*. Humans occasionally become infested with animal scabies strains when they handle or come in close contact with the infested animals. A case of crusted or Norwegian scabies due to *Sarcoptes scabiei* var *canis* in a 14-year-old girl with Turner's Syndrome was reported (Ruiz-Maldonado *et al*, 1977).

Although it is generally believed that most human and animal cross-infestations are self-limiting, there is evidence to suggest that the canine mite which infests human naturally can complete its life cycle in human skin (Schwartzman, 1983). Transfer experiments indicated that canine mites are capable of burrowing, feeding and producing eggs in human skin (Estes *et al*, 1983). An outbreak of *Sarcoptes scabiei* involving goats, cattle, sheep, dogs and which eventually spread to man was reported in two adjacent villages in West Bengal State, India (Mittra *et al*, 1993).

The Aborigines or Orang Asli in Peninsular Malaysia who are still seminomadic are known to

have a close association with dogs as they are particularly useful for hunting. As they are allowed to frequent and sleep inside houses, the risk of acquiring the infestation from dogs is present. This study is to determine the seroprevalence of anti-*Sarcoptes scabiei* var *canis* antibodies among this community.

MATERIALS AND METHODS

Test sera

Two ml of blood was withdrawn intravenously from each patient and the accompanying relative admitted to Gombak Hospital (a hospital for the aborigines). It was then centrifuged at 5,000 rpm for 10 minutes and the serum obtained was stored at -70°C until used. The age and sex of each patient were recorded. Only three patients were clinically diagnosed as having scabies.

Control sera

Two ml of blood was also collected from each of the 30 presumably negative volunteers from the Institute for Medical Research for use as control sera.

Sarcoptes scabiei var *canis* soluble antigens

Sarcoptes scabiei var *canis* mites were obtained from naturally infested dogs at the Centre of the Society for Prevention of Cruelty to Animals (SPCA) in Ampang, Kuala Lumpur. The suspected scabies

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infested skins were dissected from the dogs and brought back to the laboratory in moist screw capped bottles. The specimens were examined under a dissecting microscope for the presence of *Sarcoptes scabiei*.

Skins infested with *Sarcoptes scabiei* mites were used in the experimental infection of rabbits (*Oryctolagus cuniculus*). Infested skin pieces were placed on the ear pinna which was folded closed and secured with porous adhesive tape and left overnight. Rabbits with established heavy infestations were sacrificed for mite antigens and for transfer to other rabbits. The crusted rabbit skins were left under the light source. The mites that gathered in the light beam were collected using an applicator stick. The collected mites were freeze thawed several times, defatted in anhydrous ethyl ether, homogenized and sonicated and left overnight at 4°C for elution of the antigens. It was subsequently centrifuged and the supernatant was recovered as crude soluble antigen.

ELISA test

The ELISA was carried out following the method published previously with modification (Voller *et al*, 1976). The optimal antigen, serum and conjugates dilution were determined by checkerboard titration using positive and negative sera samples. Two hundred µl of antigen diluted in coating buffer were added into each well of the microtiters plate (Micro-ELISA plate, Dynatech Laboratories). The plates were then left overnight at 4°C for coating to occur. The plates were then washed thrice with PBS-Tween, the wash solution being left in the wells for three minutes each time. Two hundred µl of each test serum diluted with PBS-Tween were added into two wells (duplicates) of the antigen coated plates and incubated at room temperature for two hours. Each plate contained PBS-Tween, positive and negative control sera. After incubation, the plates were washed as before. Subsequently 200 µl of enzymed-labeled goat anti-human polyvalent (IgG, IgA and IgM), IgA and IgE horseradish peroxidase conjugates diluted in PBS-Tween were added into each well and incubated for 3 hours at room temperature. After washing, 200 µl of substrate solution containing orthophenylenediamine (OPD) were added to each well and incubated in the dark for half an hour. The enzyme reaction was stopped using 50 µl per well of

2.5 M sulphuric acid. The absorbance values of the tests were read at 492 nm using an automatic micro ELISA reader.

RESULTS

The mean ELISA optical density (OD) ± standard deviation (SD) for 30 control sera from the healthy subjects for polyvalent, IgA and IgE was 0.304 ± 0.171 , 0.429 ± 0.176 and 0.071 ± 0.216 respectively. The OD + 3SD of the control sera were taken as the cut-off points. The cut-off point for polyvalent, IgA and IgE was 0.817, 0.957 and 0.719 respectively. Of the 312 sera tested, 72 (24.7%), 6 (1.9%) and 0 were positive for polyvalent, IgA and IgE antibodies respectively.

Out of the 312 tested samples, 126 were males and 186 were females. Thirty-three (26.1%) of the tested males were positive for anti-*Sarcoptes scabiei* antibodies while 44 (23.6%) females were positive. Only 6 (1.9%) of the patients tested were positive for IgA antibodies and none was positive for IgE antibodies.

Fig 1 shows the distribution of anti-scabies polyvalent antibodies by age groups. The polyvalent antibodies positive rate was highest at the age group 11 to 20 years and declined in the older age groups. The mean OD ± SD of polyvalent antibodies in the 6 IgA positive subjects is 1.34 ± 0.81 , while the mean value for the IgA negative subjects is 0.6464 ± 0.451 . There is a significant difference in the mean OD of the polyvalent antibodies between the IgA positive and negative subjects (*t*-test, $p < 0.01$).

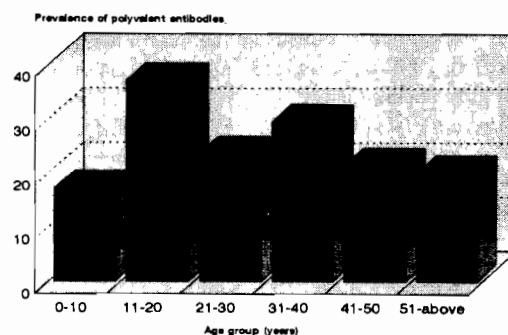


Fig 1—Prevalence of anti-scabies polyvalent antibodies with age group.

DISCUSSION

Scabies continues to be an important parasitic disease which persists through out the world despite the availability of various acaricides to control it. Human scabies is caused by *Sarcoptes scabiei* var *hominis*. Although the scabies mite is somewhat host specific, there is evidence that indicates animal scabies strain can also produce skin disease in man. An epidemic of scabies with interspecies transmission affecting various domestic animals and human has been reported (Mittra *et al*, 1993). Naturally acquired canine scabies in man was also found to be able to progress to a severe form of the disease known as crusted scabies or formerly known as Norwegian scabies (Estes *et al*, 1983). It is unclear whether the human and the canine mites are the same organism which had adapted to different hosts. It has been suggested that the variability of *S. scabiei* is the result of continuous interbreeding in the strains infecting man and domestic animals (Parish and Lomholt, 1976). Although canine mite can complete its life cycle in human skin, the infestations were usually transitory (Schwartzman, 1983).

The majority of the Aborigines of Peninsular Malaysia still live in underprivileged conditions in the new settlements or forest fringes. They use dogs for hunting and are therefore in close association with dogs. It is not easy to measure the degree of exposure to this mite clinically due to the transient nature of the infestation. Therefore detection of anti-*Sarcoptes scabiei* var *canis* antibodies in the community is an alternative measure of exposure to this mite.

This study shows that 24.7% of the Aborigines in Peninsular Malaysia were positive for anti-*Sarcoptes scabiei* var *canis* polyvalent antibodies. There was no significant difference between the positive rate in males (26.1%) and females (23.6%). This indicates that both sexes share the same degree of exposure to this mite. The positive rate is highest in the group 11 to 20 years but this tended to decline with age.

Seroprevalence studies of anti-*Sarcoptes scabiei* antibodies in community using *Sarcoptes scabiei* antigens have not been reported before. Almost all reported surveys were based on the clinical manifestation of the disease. The available serological data from several reported studies on scabies were

the measurements of serum immunoglobulin values (Hancock and Milford, 1974; Falk, 1980; Morsy *et al*, 1993). The mean value for serum IgG and IgM were found to be significantly higher in patient with scabies than the control group. The levels were observed to be higher during pre-treatment compared to post-treatment levels. Conflicting results were reported on the level of serum IgE in patients with scabies (Hancock and Milford, 1974; Falk, 1980; Morsy *et al*, 1993). None of the patients tested in this study had elevated level of anti-*Sarcoptes* IgE antibodies. Only 6 (1.9%) of the patients tested were positive for anti-*Sarcoptes* IgA antibodies. In various previously reported studies, serum IgA levels were noted to be lower in patients with scabies before treatment compared to after treatment (Hancock and Milford, 1974; Falk, 1980; Morsy *et al*, 1993). None of the clinically positive patients had positive titer for IgA antibodies. However, the mean value of polyvalent anti-*Sarcoptes* antibodies in the IgA positive individuals was significantly higher than the negative ones. Whether concurrent increase in the levels of IgA and polyvalent anti-*Sarcoptes* antibodies indicate greater protection of just a measure of degree exposure to the disease is unknown.

Measurement of serum immunoglobulin levels in previously reported studies gave no indication as to whether the observed changes in immunoglobulin levels represent a non-specific or a specific immunological reaction against the scabies mite. The successful transfer of *Sarcoptes scabiei* var *canis* into rabbit has provided adequate supply of antigen for measurement of specific anti-*Sarcoptes scabiei* antibodies to be carried out (Arlian *et al*, 1984; 1985).

This study shows that the number of the Aborigines with clinical diagnosis of scabies is surprisingly low. Only three of them were considered positive clinically. Two were children aged one and nine years. Both of them did not show elevated anti-scabies antibodies levels. The third was an adult aged 28 years, with positive polyvalent anti-scabies antibodies but negative for IgA and IgE. Due to the low number of patients with clinical scabies, correlation between the level of antibodies and clinical disease could not be elucidated. However, it has been reported previously that antibody levels do not correlate with clinical parameters (Hancock and Milford, 1974; Falk, 1980). Based on the number of patients with positive antibodies

titers that did not have any clinical symptoms observed in this study, it is suggestive that such a correlation does not exist.

There is a possibility that continuous natural exposure to canine mite confers some protective immunity in the community. The immunity would also cross-protect against human scabies. This probably explains the low prevalence of clinical scabies in the community. Since provision of adequate supply of *Sarcoptes scabiei* var *canis* is now possible, further studies to identify the fraction of the antigen extracts involved in the development of protective immunity can be carried out.

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