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Research Report

## COMPARATIVE STUDY ON THE INTENSITY OF *Mycobacterium leprae* EXPOSURE BETWEEN HOUSEHOLD AND NON-HOUSEHOLD CONTACT OF LEPROSY

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### ABSTRACT

*Leprosy stills a public health problem in West Sulawesi which has a Case Detection Rate (CDR) around 43.69/100.000 population. Household contacts of leprosy are a high risk group to be infected, due to droplet infection mode of transmission of the disease. A nose swab examination and serological study was conducted to detect exposure of M. leprae of people who live in leprosy endemic area. Detection of M. leprae in the nasal cavity will represent the exposure rate from outside and the measurement of specific antibody is represented the result of exposure to the immune system. Two group of inhabitants (30 household contacts of leprosy and 30 non-household contacts) were involved in the study. They live in Banggae district, a leprosy endemic area of Majene Regency, West Sulawesi. Sixty nose swab samples and sixty capillary blood samples from the same individuals of the two groups were collected and sent to Leprosy laboratory of the Institute of Tropical Disease, Airlangga University Surabaya. A Polymerase Chain Reaction (PCR) was performed to the nose swab samples for detection of M. leprae. The blood samples were examined serologically to measure the level of anti PGL-1 antibody. PCR examination of nose swab samples showed 1/30 positive result in the household contact group and also 1/30 positive result in non-household contact of leprosy (statistically no significant difference,  $p > 0.05$ ). Serological study showed higher sero-positive result in the household contact group (15/30 or 50%) compared to non-household contact (11/30 or 36%), but statistical calculation revealed no significant difference between the two groups ( $p > 0.05$ ) on sero-positive results of leprosy. It is concluded that household and non-household contact in leprosy have the same risk to be affected by the disease. The term of household and non-household contact need to be redefined. The possible role of exposure from the environment was also discussed, especially from non-human resource of M. leprae.*

**Key words:** leprosy – sero epidemiology – PGL-1 antibody

### INTRODUCTION

Leprosy is a chronic infectious disease caused by *M. leprae* and primarily affect the peripheral nerves, secondary to skin and other organs. The complication of the disease can cause some disabilities and social problem in the community. Close contact is one condition that increased the risk of transmission. From several contact surveys, it is reported that more leprosy patients found and live in the same house, indicates that household member of leprosy patient is a high risk group for affected the disease.<sup>1</sup> Droplet infection mode of transmission seems the main

route of transmission.<sup>2</sup> After the lepra bacilli enter the body, the immune response will be induced to eradicate the microorganism. Specific antibody to *M. leprae*, the anti Phenolic Glycolipid-1 (PGL-1) antibody is also developed. The level of antibody is correlated with the antigen load of the bacilli, which means that level of antibody is represented the amount of *M. leprae* in the body.<sup>3</sup> From this point of view, the intensity of *M. leprae* exposures to individual could be measured by examining the presence of *M. leprae* in the nasal cavity and study the level of specific antibody on the bacilli.

## AIM OF STUDY

The aim of this study is to compare the intensity of *M. leprae* exposure between the healthy household contacts group and the non-household contacts of leprosy, by detection of *M. leprae* in the nasal cavity and measurement the specific antibody to *M. leprae* of the same individuals as an immunologic response to the infection.

## MATERIAL AND METHOD

Sixty adult healthy individuals from Banggae subdistrict, Majene, West Sulawesi, (figure 1) consisted of 30 household contacts of leprosy patients (live in the same house with the leprosy patients) and 30 non-household contacts were involved in the study.

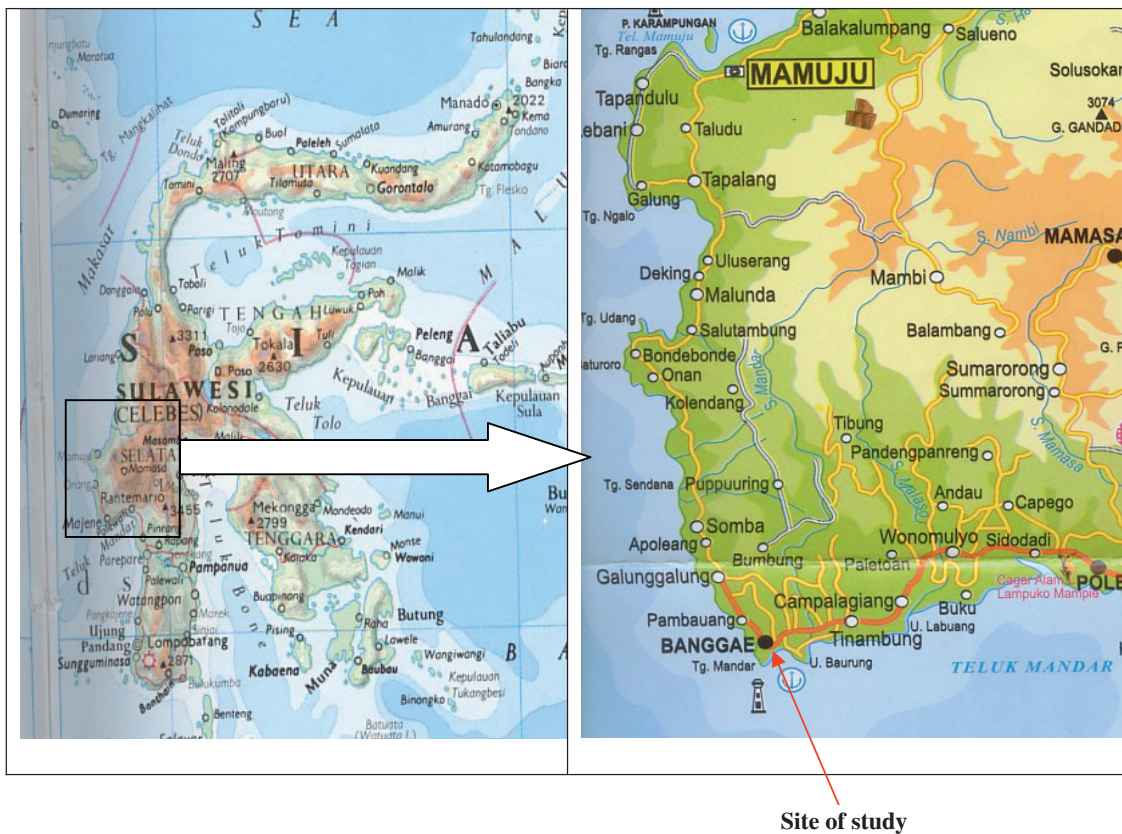
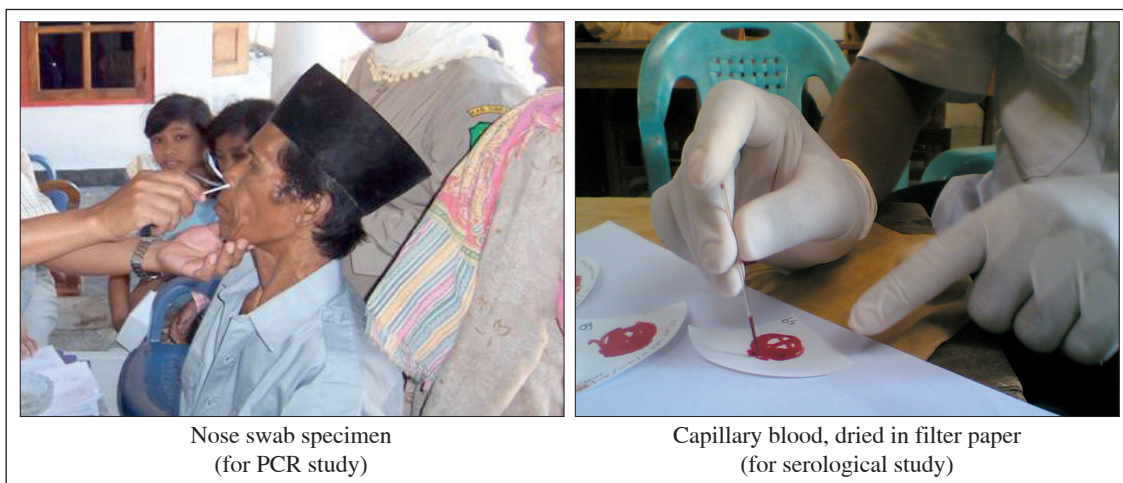


Figure 1. Geographic area of the study



Nose swab specimen  
(for PCR study)

Capillary blood, dried in filter paper  
(for serological study)

Figure 2. Collection of specimens

From each patient a nose swab specimen was collected and 100 ul capillary blood was collected by finger tip punctured and dried in the filter paper (figure 2). These 60 pairs of specimens were brought to Leprosy lab in the Institute of Tropical Disease, Airlangga University, Surabaya. A Polymerase Chain Reaction (PCR) test were performed to detect *M. leprae* in the nose swab specimens, while the dried capillary blood was examined serologically to measure the level of anti PGL-1 antibody using the ELISA technique.<sup>4</sup> The results will be analyzed to compare the positive PCR results of the nose swab specimens and also to compare the immunologic response to *M. leprae* between the two groups. The dried blood in filter paper are diluted in distilled water for two hours and shaken. This diluted blood was used as a specimens for ELISA study to measure the level of IgM anti PGL-1 antibody and using the conversion value, the results were converted to serum equivalence value.<sup>5</sup> By Biolise program in computer, the Optical Density (OD) value was converted to unit.ml. Using cut off value 605 u/ml, sero-positive result were established.<sup>6</sup>

## RESULTS

Using the Lp1-Lp4 nested primer that amplify the *Rlep* region of *M. leprae* DNA (99 bp), the household contact group showed 1/30 positive PCR result, compared to 1/30 positive result in the non-household contact group. Statistically there is no significant difference between the two groups in the positive PCR results ( $p > 0.05$ ). In serological examination, after a conversion to achieve the serum equivalency and using the cut off 605 u/ml for IgM anti PGL-1 (ELISA), 15/30 samples from the household contact group showed sero-positive results, compared to 11/30 sero-positive in the non-household contact group. Although the number of sero-positive is higher in household contact group, statistically no significant difference between the two groups in the sero-positive results. Also when the two datas (PCR and serology of leprosy) are combined, still no significant difference between the two groups ( $p > 0.05$ ).

## DISCUSSION

The route of transmission in leprosy mainly by droplet infection, since multibacillary leprosy case will harbour many lepra bacilli in his nasal cavity.<sup>7</sup> Prolonged contact, intimate and continuously with leprosy patients are the condition for affected the disease.<sup>8</sup>

The existence of *M. leprae* in the nasal cavity could be either from outside, aspirated during respiration, or secretion from the nasal mucous as a secretion from leprosy lesion in the nasal cavity.<sup>9,10</sup> Household contacts of leprosy fulfill these criteria and become the high risk group. When the bacilli enter the body, the immune response will develop. Although the anti PGL-1 antibody is not effective

to eradicate the *M. leprae* infection, it is a useful parameter for monitoring the infection.<sup>11</sup> The level of this specific antibody is correlated with the amount of *M. leprae* in the body.<sup>12</sup> Based on previous serological surveys in endemic and non-endemic areas, the cut off IgM and IgG anti PGL-1 (ELISA) can be calculated. The level 605 u/ml for IgM anti PGL-1 and 615 for IgG anti PGL-1 was used as the cut value. Those who have the IgM anti PGL-1 level  $>605$  are considered as a sero-positive case. Most of serological studies use serum samples, which originally from venous blood samples. The use of capillary blood which is dried on the filter paper has been introduced since 2007 and very useful for collecting blood samples from field that located long distance from the laboratory.<sup>13,14</sup> Using a conversion coefficient, the equivalence value of anti PGL-1 antibody in serum can be obtained.<sup>15</sup>

Sub-clinical leprosy is a term for healthy individual who live in leprosy endemic area, with high level of IgM anti PGL-1 in serological examination. These sub-clinical leprosy cases still show no sign of clinical leprosy, but they are potential to progress toward manifest leprosy.<sup>16</sup> In this study the serological examination result showed around 43% of the inhabitants showed sero-positive, which means that they are already exposed to *M. leprae* and induced the humoral response. Since the level of antibody correlates with the antigen load, once can assume the load of bacilli in the body is also more than normal people in other areas. Although it is hypothesized that household contacts will get more *M. leprae* exposure than those non-household contact of leprosy, this study showed that by cross sectional study both groups of study only showed 1/30 PCR (+) for *M. leprae* in the nose swab cavity. This means that airborne infection of *M. leprae* in the household and non-household contacts is similar, or in other word the *M. leprae* infection source not only from leprosy patient in the house, but maybe from other patients or environment. From the serological study, the results showed the same phenomena, but the level of antibody in sero-positive cases showed a different pattern.

Household contacts with sero-positive anti PGL-1 antibody showed a higher incidence and higher (figure 1).

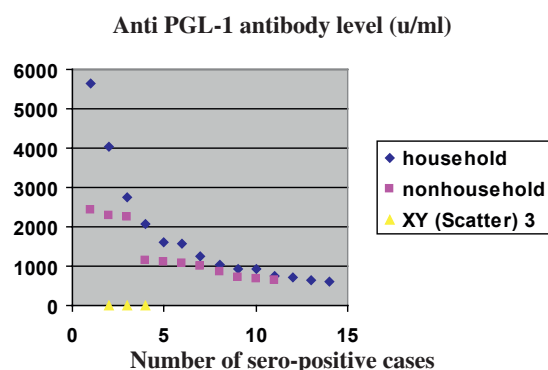


Figure 3. Distribution of serological level of sero-positive cases among household and non-household contacts of leprosy



Since the immune response need a certain duration before it is developed, once can assume that household contacts have more antigen load (*M. leprae*) in their body. Prolonged contact with leprosy patient in the same house might cause the accumulation of antigen and induce high level of specific antibody production. Those sero-positive contacts with high level of antibody (sub-clinical leprosy) need special attention to avoid progression towards manifest leprosy in the future.<sup>17</sup>

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