ANALYSIS OF THE PHYSICAL ENVIRONMENT OF THE HOUSE AND THE EXISTENCE OF *MYCOBACTERIUM LEPRAE* DNA IN THE SOIL OF THE HOUSE FLOOR IN LEPROSY ENDEMIC AREAS, MADURA, INDONESIA IN 2013

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Abstract

Leprosy is an infectious disease caused by *Mycobacterium leprae*. Five districts with the highest number of leprosy events, including the Camplong Subdistrict, have reported a continuous rise in the number of leprosy cases. This study aimed to analyze the relationship between the physical environment of the house, the presence of *M. leprae* DNA on the floor of the house and the presence of leprosy patients in Camplong Subdistrict, Sampang District. This study used a cross-sectional design. We collected data regarding 40 houses. The presence of *M. leprae* DNA in the floor samples was analyzed using the polymerase chain reaction (PCR) technique; 10% of soil samples showed the presence of *M. leprae* DNA. Variables associated with the presence of leprosy patients were temperature and the wall of the house. We concluded that that the presence of *M. leprae* does not depend on the presence of leprosy patients in the house although, theoretically, the soil may be a transmission medium for *M. leprae*. Therefore, everyone residing in an endemic area has the same risk of *M. leprae* exposure from the environment. We recommend that programs be conducted in endemic areas to raise the knowledge of the population about what constitutes a healthy house.

Keywords*: Mycobacterium leprae*, Leprosy, House Sanitation, Polymerase Chain Reaction

Introduction

Leprosy is a chronic infectious disease caused by *Mycobacterium leprae* (1, 2). In the primary phase, it attacks the peripheral nerves and in the secondary phase, it affects the skin and the organs (1). Leprosy remains an important public health problem worldwide, especially in developing countries with poor hygiene and sanitation (3). Leprosy causes not only medical, but also psychosocial and economic problems (4). One country highly endemic for leprosy is Indonesia (1); it had the third-highest leprosy prevalence worldwide during 2004-2011. During that time, East Java was ranked as the leading contributor to leprosy cases in Indonesia (5), the number of new cases of leprosy being 5.284 and the total number of registered cases being 6.650 (6). Sampang has the highest prevalence of leprosy in Madura. As per the criteria, the Camplong Subdistrict is also an endemic area for leprosy, with a high prevalence rate of 11.65/10,000 among its habitants and a Case Detection Rate of 72.81/100,000 population (7). Multi drug therapy (MDT) is believed to prevent the transmission of leprosy by patients with multi bacillary (MB) leprosy, hence leading to a reduction in new cases of leprosy. Thus, the question that arises is what is the source of the infection that contributes to the continuous emergence of new cases. Animals, plants, and the environment are the suspected potential sources of infection (8).

Various research reports have stated that *M. leprae* bacteria can survive and live in the environment and may be a pathway for leprosy transmission. Using the polymerase chain reaction (PCR) technique, we can analyze the existence of specific microorganisms in complex media with good sensitivity and specificity (9-11). House environments that do not meet the health requirements are potential sources of transmission of various diseases, especially diseases that are spread through environmental factors (12). Some studies have suggested a link between the risk of leprosy occurrence and housing conditions (13-17). Houses with physical environmental factors not meeting the health requirements can cause leprosy bacteria to develop optimally, and this developmental rate increases with the presence of other factors (18). Thus, we aimed to analyze the relationship between the physical environment of the house, the presence of *M. leprae* DNA on the house floor and the presence of leprosy patients in Camplong Subdistrict, Sampang District.

Materials and Methods

Sample collection

This observational, analytical, cross-sectional study was conducted in two populations. The experimental population included houses with leprosy patients in areas highly endemic for leprosy, namely in Camplong, Sampang, Madura, Indonesia. The comparative population included houses without leprosy patients in the village with the lowest leprosy prevalence in Camplong, Sampang, Madura, Indonesia. The study sample was divided into cases and comparison groups. The sample cases included houses with leprosy patients registered as new cases of leprosy from January 2012 to December 2013, as per the health center medical records in Camplong, Sampang, Madura, Indonesia. Comparison samples included houses without leprosy patients in the village with the lowest prevalence of leprosy in Camplong, Sampang, Madura, Indonesia. This research was conducted in 2013.

The study included 40 houses, 20 houses with leprosy patients and 20 houses without leprosy patients (the comparator group). The selection of sample cases and comparison cases was performed using the simple random sampling technique. The independent variables in this study were the physical environment of the house, including temperature, natural light, humidity, walls, and the existence of *M. leprae* DNA in the soil of the house floor. The dependent variable was the presence of leprosy patients in the house.

Instruments used for measuring the physical environment of the house included a checklist, thermohygrometer, lux meter, digital anemometer, and soil moisture meter. Primary data included the physical environment of the house, comprising the temperature, house wall, house floor, house floor soil moisture, air humidity, and lighting, which were collected through field observation of the respondent's house using a checklist. Secondary data were the number of registered patients and number of new cases of leprosy during 2012, and the Camplong Subdistrict profile obtained through document observation at the Camplong Clinic, Sampang, Madura, Indonesia.

Data analysis

The data were collected carefully to avoid any errors in data processing. The data were then coded and entered in accordance with the purpose of the research to simplify data analysis. Analysis was conducted in the following ways:

1. Descriptive analysis. This analysis was conducted to illustrate the state of the variables studied. Data was presented in the form of frequencies and percentages and was cross tabulated.
2. Analytical analysis. This analysis was performed to illustrate the effect of the independent variable on the dependent variables by using multiple logistic regression tests to determine the association between the physical environment of the house and the existence of *M. leprae* DNA in the soil of the house floor and a leprosy event.
3. DNA extraction techniques in PCR examination
    a. DNA extraction from soil samples. The pelletized soil sample was extracted using a Qiagen miniprep kit.
    b. DNA amplification by PCR method. DNA amplification was performed by the nested PCR technique, beginning with the first PCR, by inserting PCR mixture, primer LPF-LPR and DNA template into a 0.2 ml PCR tube. The 0.2 ml tube was then inserted in a cycler PCR thermal machine with a denaturation of 98 °C, annealing of 56 °C, and a 72 °C extension, repeated for 35 cycles. The first PCR product with a 280 bp size became the template DNA for the second PCR process using the LP3-LP4 primer with the same PCR conditions but repeated as many as 30 cycles. The second PCR product was the result of a 99 bp amplicon which could be seen using a 3% agarose gel run in the electrophoresis field. The gel was then stained with ethidium bromide 0.1 µg/ml and viewed with a transilluminator (UV light) and then photographed using a Kodak EDAS 290 digital camera. The results were considered positive when bands were found at the alignment corresponding to the band of the positive control of *M. leprae*, 99 bp on the 100 bp DNA ladder.

Results

The frequency of existence of *M. leprae* DNA in the soil of the house floor can be seen in Table 1. Based on the data presented in Table 1, of the 40 studied soil samples, 4 (10%) were positive for *M. leprae* DNA, while the remaining 36 (90%) were negative. The soil selected as the sample in this study was soil with high humidity and protected

from the sun. These environmental conditions are ideal for the growth of *M. leprae* which grows well in a humid environment. This study used the nested PCR technique to detect the DNA of *M. leprae*. Nested PCR involves the use of two pairs of primers for the DNA locus; therefore, the amplification is performed twice.

Table 1: The frequency of existence of *M. leprae* DNA in the soil of the house floor in Camplong Subdistrict, Sampang, Madura

***M. leprae* DNA**	**Frequency**	**Percentage (%)**
Exist	4	10
Not Exist	36	90
Total	40	100

Based on the data in Table 2, 17 (42.5%) houses did not have a healthy house temperature; there was a leprosy patient in 14 of these 17 houses (82.4%), while there were no leprosy patients in the remaining three houses (17.6%). There were 23 (57.5%) houses with a healthy house temperature, of these six houses (26.1%) had a leprosy patient, and 17 houses (73.9%) did not. The results of the statistical test yielded a p value = 0.001 and PR = 3.157, which means that respondents who lived in a house with temperature conditions that did not meet health requirements were 3.157 times more likely to suffer from leprosy than respondents living in a house with a room temperature that met these requirements.

Table 2: Distribution of existence of leprosy according to temperature in the Camplong Subdistrict, Sampang, Madura

Temp	**Existence of Leprosy**				**Total**		**P-Value (PR 95% CI)**
	Exist		Not Exist				
	N	%	N	%	N	%	
Not Qualify	14	82	3	17	17	43	0.001 (3.157)
Qualify	6	26	17	74	23	57	
Total	20	50	20	50	40	100	

Based on the data in Table 3, 38 houses (95%) had natural light that did not meet health requirements; of these, 20 houses (52.6%) had a leprosy patient, and 18 houses (47.4%) did not. Two houses had natural lighting as per the health requirement (5%); these houses did not have a leprosy patient. With a confidence level of 95% and a p value = 0.478 derived from Fisher's Exact test, we can conclude that there was no correlation between the natural light in a house and the presence of a leprosy patient in the same house in Camplong, Sampang, Madura. Based on the data in Table 4, 33 houses (82.5%) did not have a wall that met the health requirement; of these, 13 houses (39.4%) had a leprosy patient while 20 houses (60.6%) did not. There were seven (17.5%), houses with walls that did not meet health requirements, and all (100%) had a leprosy patient. Statistical test results yielded a p value = 0.008, thus we can conclude that there is a relationship between the walls of a house and the presence of a leprosy patient in that house; there was also a prevalence ratio (PR) = 0.394.

Table 3: Distribution of existence of leprosy according to natural lighting in Camplong Subdistrict, Sampang, Madura

Natural Lighting	**Existence of Leprosy**				**Total**		**P-Value (PR 95% CI)**
	Exist		Not Exist				
	N	%	N	%	N	%	
Not Qualify	20	52	18	47	38	95	0.487 (0.474)
Qualify	0	0	2	100	2	5	
Total	20	50	20	50	40	100	

Table 4: Distribution of existence of lepros according to wall in Camplong Subdistrict, Sampang Madura

Wall	**Existence of Leprosy**				**Total**		**P-Value (PR 95% CI)**
	Exist		Not Exist				
	N	%	N	%	N	%	
Not Qualify	13	40	20	60	33	82.5	0.008 (0.394)
Qualify	7	100	0	0	7	17.5	
Total	20	50	20	50	40	100	

Based on the data presented in Table 5, four floor soil samples (10%) were positive for *M. leprae* DNA; one (25%) was from a house with a leprosy patient, and three (75%) were from houses without a patient. The soil samples from 36 (90%) houses were not positive for *M. leprae* DNA; 19 (52.8%) of these were from houses with a leprosy patient and 17 (47.2%) were from those without a patient. Thus, *M. leprae* DNA was 75% more common in the soil samples collected from the floor of houses without a leprosy patient than in those from houses with a leprosy patient. With a confidence level of 95%, a Fisher's Exact p value = 0.605 and PR = 0.474, we can conclude that there was no relationship between the existence of *M. leprae* DNA in the soil samples from the house floor and the presence of a leprosy patient in Camplong, Sampang, Madura, Indonesia.

Table 5: Distribution of existence of leprosy by *M. leprae* DNA in the soil of house floor in the Camplong Subdistrict, Sampang, Madura

M. leprae DNA	Existence of Leprosy				Total		P-Value (PR 95% CI)
	Exist		Not Exist				
	N	%	N	%	N	%	
Exist	1	25	3	75	4	10	0.605 (0.474)
Not Exist	19	53	17	47	36	90	
Total	20	50	20	50	40	100	

Discussion

This study used the nested PCR technique for detecting *M. leprae* DNA. Nested PCR involves the use of two primer pairs for the DNA locus; therefore, the amplification is performed twice. This method is used because it is theoretically expected to increase sensitivity and specificity (19).

The soil selected as the sample in this study was that which had a high humidity and was protected from sunlight. These environmental conditions are ideal for the growth of *M. leprae,* which grows well in a humid environment. Based on the PCR test results of 40 soil samples taken from the floor of the houses in the Camplong Subdistrict of Sampang Regency, we found four (10%) samples tested positive for *M. leprae* DNA. This is the most important finding reported thus far from among all the studies conducted in Indonesia regarding the existence of *M. leprae* DNA in soil samples. However, our result percentages were smaller than those reported in the Ghatampur (India) study (33.3% and 37.5%) (10, 18). The differences in the number of soil samples evaluated and soil quality factors, such as temperature and humidity, between the soil in Sampang, especially in Camplong, and that in Ghatampur (higher temperature and moisture) could have caused the difference in the results.

The present results indicate that environmental factors, especially temperature, play a role in a disease outbreak among house residents. This result is in line with several previous reports on leprosy that also showed a relationship between temperature and leprosy incidence (16, 20, 21). The prevalence ratio was 3.157, indicating that individuals living in homes with improper room temperature conditions were at a greater risk (3.157 times higher risk) of developing leprosy than individuals living in homes with healthy environmental conditions.

The statistical analyses showed no relationship between the natural lighting of a house and the presence of leprosy patients in a home. This can be attributable to the homogeneous conditions of the houses with and without leprosy patients (the temperature requirements were not fulfilled). However, the results showed that more leprosy patients lived in houses with natural lighting that did not meet the health requirements.

This indicates that natural lighting factors can play a role in individual health conditions for leprosy. The present results are similar to those reported by Ellyke, who also concluded that there was no relationship between natural light and leprosy incidents in Jenggawah Subdistrict, Jember Regency (22). Hartanti stated that there was no difference in the lighting of bedrooms in the houses with and without leprosy patients in Padas District, Ngawi Regency (14).

The results showed that all the houses studied had moisture conditions that did not meet the health requirements (> 60% Rh). The statistical test found no relationship between the humidity of the room and the presence of leprosy patients. This result is similar to some previous reports that have shown no relationship between indoor air humidity and leprosy incidence (17, 22). Although statistical results do not prove the relationship between the humidity of the room and the presence of leprosy patients, the observations indicate that all (100%) of the houses of leprosy patients had air humidity conditions that did not meet health requirements, making air humidity a physical environmental factor of the house which needs attention for leprosy control. This is particularly important considering that the leprosy-causing bacteria *M. leprae* grows optimally in high-humidity environments. Humidity is also influenced by climate and weather factors. Hence, climate conditions can be one of the factors that influence the incidence of disease. For example, research by Mazrura et al. proves that climate change factors influence the incidence of dengue fever (23).

This study is in line with the research by Faturrahman that also showed a relationship between house walls that did not meet health requirements and leprosy incidence (16). The poor condition of the walls of a house can contribute to the creation of moisture and a temperature that allows the disease to spread. Lubis stated that one of the factors affecting the humidity in a room is the walls of the room because walls made of materials that are not water-resistant would allow water seepage, contributing to the humidity. Moist housing conditions support the growth of disease germs, including the *M. leprae* bacteria that can cause leprosy (24). The result of this observation indicates that houses with walls that do not meet the health requirement have fewer leprosy patients than those with walls that meet the requirement. However, it should be remembered that the incidence of leprosy is influenced by various factors; in addition to environmental factors, there are behavioral factors, such as age and frequency of contact with leprosy patients, which also play an important role in determining leprosy incidence. Thus, if the house walls do not meet the health requirements but other house environmental factors do, and behaviors for leprosy prevention are practiced, this may help prevent leprosy. However, the present results also showed that houses with walls that failed to meet the health requirements more commonly had leprosy patients.

The present results are contrary to previous reports, in terms of a higher number of soil and water samples

showing positivity for bacterial contamination from houses without leprosy patients. However, this finding is in line with the research by Wahyuni and Adriyati, who also found that the number of positive soil and water samples containing *M. leprae* DNA was higher in houses without leprosy patients (25, 26). Moreover, Mudatsir also managed to find *M. leprae* DNA in soil from homes where there were no patients with leprosy (26). However, the finding that *M. leprae* DNA was more common in houses without leprosy patients can be attributed to subclinical leprosy cases. According to the theory, in addition to active cases, subclinical leprosy can be a transmission source because, at this stage, someone can secrete bacterial secretions from nasal (27).

However, whether the *M. leprae* DNA originates from leprosy patients or subclinical leprosy patients, the DNA of *M. leprae* should be more common in areas with leprosy. Thus, these explanations can be ruled out. One of these explanations includes the research conducted by Nursidah who found that seropositive leprosy in endemic areas with a low prevalence is greater than that in high-prevalence endemic areas (28). These results suggest that exposure to the surrounding environment plays a role in seropositive leprosy, further leading to the hypothesis that the environment is the source of *M. leprae*. In other words, the leprosy germs present in the environmental components are not only sourced from leprosy patients, but also from the environment itself. This hypothesis is also supported by the research by Arsyad et al. who conducted serological tests of Household and Non-Household Contact of Leprosy showing no significant differences between the two groups in the seropositive leprosy results, suggesting that Household and Non-Household Contact of Leprosy had the same risk for leprosy (29). Thus, it is possible that in addition to leprosy patients, another potential source of infection transmission is the environment itself. Hence, promotion by health workers to overcome the various problems related to the physical environment of the house is important. Similarly, research by Hassali et al. highlights the importance of the role of health workers in promoting health and the importance of community participation (30).

Conclusion

Based on the present results, we can conclude the following: *M. leprae* DNA was found in soil samples from the house floors of four of the 40 houses (10%) in a district in a leprosy-endemic area in Camplong, Sampang, Madura. Temperature and condition of the house walls were associated with the presence of leprosy patients in Camplong, Sampang, Madura.

We recommend that the Sampang District Health Office and District Health Clinics in Camplong improve awareness programs that can support healthy house improvement and sanitation to prevent the development of germs in the house. Early detection of leprosy involving serological examination is important to prevent subclinical cases of leprosy. There is also a need to increase public awareness to support the creation of a healthy house environment.

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Competing Interests

The authors declare that they have no conflict of interest.

Ethical Clearance

This research, before it was implemented, went through an ethical assessment by the Health Research Ethics Committee, Airlangga University and was declared to have received ethical clearance with certificate number: 54-KEPK.

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