Volume 72, Number 3
Printed in the U.S.A.
(ISSN 0148-916X)

INTERNATIONAL JOURNAL OF LEPROSY
and Other Mycobacterial Diseases

VOLUME 72, NUMBER 3 SEPTEMBER 2004

An Approach to Understanding the Transmission of *Mycobacterium leprae* Using Molecular and Immunological Methods: Results from the MILEP2 Study[1]

W. Cairns S. Smith, Christine M. Smith, Ian A. Cree, Ruprendra S. Jadhav, Murdo Macdonald, Vijay K. Edward, Linda Oskam, Stella van Beers, and Paul Klatser[2]

ABSTRACT

Background. The current strategy for leprosy control using case detection and treatment has greatly reduced the prevalence of leprosy, but has had no demonstrable effect on interrupting transmission.

Methods. Three leprosy endemic communities in India were recruited, examined, and followed up sequentially over 2 yrs using nasal swabs and saliva collections. The nasal swabs were tested by polymerase chain reaction for the presence of *M. leprae* and the saliva was assayed for anti-*M. leprae* IgA.

Findings. Only 1.6% of 2552 nasal swabs were PCR positive, and 68% of saliva samples were positive for ML-IgA. BCG and household contact status was associated with the mucosal immune response, but not with PCR positivity. PCR positivity did not persist and most PCR positive results were in the wet season.

Interpretation. The findings contribute to our understanding of the epidemiology of *M. leprae* and the possible periods of greatest likelihood of exposure and transmission.

[1] Received for publication on 18 July 2003. Accepted for publication on 31 March 2004.

[2] MILEP2 Study Group: Smith, C. M.; Smith, W. C. S. (Study coordinator), Department of Public Health, University of Aberdeen, Foresterhill, Aberdeen, U.K.; Cree, I. A.; Macdonald, M., Department of Pathology, Institute of Ophthalmology, University of London, London, U.K; Klatser, P.; Oskam, L.; van Beers, S., KIT (Royal Tropical Institute), Biomedical Research, The Netherlands; Harboe, M.; Beyene, D.; Wieles, B.; Ryon, J., Armauer Hansen Research Institute, Ethiopia; Edward, V. K.; Rao, J. R.; Jadhav, R. S.; Fernando, A.; Shinde, V.; Kamble, R., Richardson Leprosy Hospital, Miraj, India; Bjune, G., Centre for International Health, Haukeland Hospital, Bergen, Norway.

Reprint requests to: Professor W. C. S. Smith, Department of Public Health, University of Aberdeen, Polworth Building, Foresterhill, Aberdeen AB9 2ZD, Scotland. Email: w.c.s.smith@abdn.ac.uk

RÉSUMÉ

Contexte: La stratégie actuelle pour le contrôle de la lèpre, qui utilise la détection de nouveaux cas et la polychimiothérapie, a grandement diminué la prévalence de la maladie, mais n'a eu aucun effet tangible pour interrompre la transmission.

Méthode: Trois communautés indiennes où la lèpre est endémique furent enrôlées, examinées et suivies séquentiellement pendant 2 ans en utilisant des écouvillons de fosses nasales et des prélèvements de salive. Les écouvillons de nez furent testés par la réaction de polymérisation en chaîne (PCR) pour la présence des gènes de *M. leprae* et la salive fut testée pour la présence d'immunoglobulines de type A (IgA) dirigées contre *M. leprae*.

Résultats: Il n'y eut que 1,6% des 2552 écouvillons de fosses nasales qui furent positive par le test de PCR, et 68% des prélèvements de salive étaient positifs pour les IgA anti-*M. leprae*. La vaccination par le bacille de Calmette et Guérin (BCG) et le statut des individus vivant sous le même toit furent associés à la réaction immunitaire muqueuse, mais pas aux résultats positifs à la PCR. La positivité par la PCR n'a pas persisté et la majorité des résultats positifs par la PCR furent obtenus durant la saison humide.

Interprétation: Ces données contribuent à une meilleure compréhension de l'épidémiologie de *M. leprae* ainsi que des périodes possibles pour la probabilité la plus grande d'exposition et de transmission de la lèpre.

RESUMEN

Panorama. La estrategia actual para el control de la lepra basada en la detección y tratamiento de los casos, ha reducido grandemente la prevalencia de la enfermedad pero no ha tenido un efecto demostrable en la interrupción de la misma.

Métodos. Se reclutaron los pacientes de 3 comunidades con lepra endémica de la India, se examinaron, y se siguieron durante 2 años analizando los exudados nasales y la saliva. Los exudados nasales se probaron por la reacción en cadena de la polimerasa para buscar la presencia de *M. leprae*, y la saliva para buscar anticuerpos IgA anti-*M. leprae*.

Hallazgos. Sólo el 1.6% de 2552 exudados nasales fueron PCR-positivos mientras que el 68% de las salivas colectadas tuvieron anticuerpos IgA anti-ML. El BCG y el estatus de contacto intrafamiliar fueron asociados con la respuesta inmune de la mucosa, pero no con la positividad por PCR. La positividad por PCR no persistió y la mayoría de los resultados positivos por PCR fueron observados en la estación húmeda.

Interpretación. Los hallazgos contribuyen a nuestro conocimiento sobre la epidemiología de la lepra y de los periodos de mayor posibilidad de exposición y transmisión.

Important developments have taken place over the past decade in tackling the global leprosy burden. There has been a very significant reduction of over 90% in the total number of leprosy patients registered for treatment through the implementation of multidrug therapy regimes ([19]) that have dramatically reduced the duration of treatment. However, despite this reduction in registered prevalence of leprosy, there has been no parallel reduction in the global case detection ([18]). This is a serious barrier to aspirations to eradicate leprosy in the future.

Mycobacterium leprae was the first bacterium shown to be associated with disease in humans through the work of Hansen ([15]) in the 1870s, yet the sources of infection, mode of transmission and early pathogenesis of infection remain an enigma ([9]). The inability to cultivate *M. leprae in vitro* and the limitations of *in vivo* cultivation mean that our current understanding of the transmission is largely based on circumstantial evidence. Observations from studies of household contacts show that households with leprosy patients have higher attack rates of leprosy than those without such exposure, and the attack rates are higher where the index case has a higher bacterial load ([7]). Despite this, most new patients do not report a history of contact, which raises questions of the nature of the exposure, the pattern of responses, and the possibility of other reservoirs of infection. Careful epidemiological study can demonstrate contacts for most cases in certain settings although most patients may be unaware of such contact ([16]). The very slow rate of multiplication of *M. leprae*, the long incubation period (years rather than days) and the variable host immune response make it extremely difficult to link exposure to disease

outcome. Earlier work clearly demonstrated that the nose is a major route of exit for *M. leprae* ([13]), and the nose may also be a major route of entry ([14]).

The immunological response to exposure to *M. leprae* is complex. Measurements of cell-mediated immunity are the most relevant in leprosy, as *M. leprae* is an intracellular pathogen, however findings are difficult to interpret ([2]). However it has been postulated that if the earliest lesions are in the nasal mucosa then mucosal IgA responses may be the most important markers of early exposure and infection ([1]). Recent methodological developments now make it possible to study these mucosal responses in populations ([10]).

Improved understanding of the transmission of leprosy is an important key to developing approaches that may interrupt transmission, leading to eradication, rather than the current approach of disease control based on early case detection and treatment ([4]). The development of genetic and molecular methods creates the potential to address some of these critical questions about transmission. Polymerase chain reaction (PCR) methods have been used in the study of *M. leprae* for a number of years but in the early work there were serious concerns about the validity of the methods and detection systems. Recent research, which analyzed nasal swabs taken in the community in leprosy endemic and non-endemic countries along with blinded spiked laboratory samples, has answered many of the earlier concerns about validity of findings ([10]). Currently, a series of studies in a number of countries have produced interesting findings on the distribution of *M. leprae* in the community that challenge conventional theories. In these studies healthy individuals in leprosy endemic countries have been shown to have nasal swabs that have tested positive for *M. leprae*. The evidence suggests that positivity may persist in communities but not in individuals ([8, 11, 12, 17]).

In this study on the Mucosal Immunology of Leprosy (MILEP2), we have combined both PCR and immunological techniques to study three communities in India repeatedly over a period of 2 yrs. The aim of the study was to establish the relationship between *M. leprae* infection and the development of immune responses in a community in which multidrug therapy (MDT) had been used for more than 10 yrs and to elucidate the pathogenesis of primary nasal infection in leprosy.

METHODS

Three communities in India with similar characteristics and leprosy endemicity were selected for the study to test the consistency in findings and offer the potential to conduct community based intervention studies. The three communities were villages in southern Maharashtra of between 1000 and 1500 population and within a day's travel from the Stanley Browne Research Laboratory in the Richardson Leprosy Hospital at Miraj, Maharashtra. Prior to commencement of the main study, pilot studies for development of methods and logistical studies were undertaken in a fourth village. The presence of *M. leprae* DNA in the nose of participants was measured by PCR, while mucosal immunity was assessed by enzyme-linked immunosorbent assay (ELISA) for anti-*M. leprae* IgA antibodies.

Community surveys. The villages were selected from all villages within the Sangli District where a MDT-based leprosy control program had been in operation for more than 10 yrs. Data on the size, socio-demographic characteristics and leprosy epidemiology of all villages was collated and three villages of roughly equal size (1000 to 1500 population), similar average incidence rate of leprosy over the previous 10 yrs of around 1 per 1000 per year, and physically separate but within a 50 mile radius from the laboratory, were selected. Each village was then visited in turn by the investigators and meetings with the village elders and the village community convened. The basis of the study was explained, the proposed methods described, and agreement for the participation was secured. Thereafter, a census of each village was conducted which included examination of all those present. A number of visits were conducted to achieve a high coverage of each village. Details of the residents were collected by household and these included: age, sex, marital status, occupation, BCG immunization, history of leprosy, previous contact with leprosy, tuberculosis and other diseases. All household members in households with a case of leprosy in the previous 10 yrs were classi-

fied as household contacts. In addition, each resident was clinically examined for signs of leprosy. Nasal swabs (2 specimens per person from right and left nostril) were obtained from all individuals over three years of age for the PCR analysis and a sample of saliva collected to assay mucosal IgA. The survey was repeated on 2 to 3 further occasions over a two-year period, when those present were re-examined for signs of leprosy and further nasal swabs and saliva samples were taken.

Laboratory methods. A pilot study was conducted in a separate village to test out the procedures for specimen collection and transportation. Nasal swabs and saliva samples were transported on the same day they were taken, from each of the villages to the laboratory in Miraj. The samples were numbered in the field to link to the survey data and given a unique identifier on receipt of the specimens in the laboratory.

PCR assays of nasal swabs. DNA extracted from nasal swabs was analyzed by PCR. A simple method for the rapid screening of PCR samples using the Peptide-Nucleic-Acid (PNA) hybridization technique with an ELISA endpoint was employed and is described in detail in a previous publication ([11]). This method using Ready-to-Go™ PCR Beads (Pharmacia) was designed specifically to be simple and robust enough to be used in leprosy-endemic areas where there may be difficulties in maintaining a reliable cold chain for the transport and storage of various components.

*Salivary IgA anti-*M. leprae *(ML-IgA) immunoassay.* A previously developed ELISA method with whole *M. leprae* as antigen was used ([14]), and stringent statistical criteria were applied to the results to ensure the reproducibility and uniformity of the ELISA and the positivity of the samples for *M. leprae*. The salivary IgA assay is semi-quantitative so the results are presented as negative (–) or positive (+), with those found in the top 10% of the distribution considered highly positive (++)

Data analysis. Results from the baseline survey and laboratory assays were analyzed to describe the frequency of PCR and IgA positivity in the three communities and their relationship to demographic variables. The cross sectional data from the baseline survey in all three villages was analyzed to assess the relationship between PCR and IgA results with household contact status, BCG immunization, and seasonal patterns. The data from the two follow-up surveys were analyzed to assess changes in both PCR and IgA status and their persistence over time in individuals and communities. The statistical methods used were differences in proportions and the chi square test.

Study funding and approval. The study was funded by the European Commission as part of the Health Research with Developing Countries Program (INCO-DC). The Indian Council for Medical Research granted approval for the study.

RESULTS

Surveys were carried out four times in village 1 (between November 1997 and July 1999), three times in village 2 (between March 1998 and May 1999), and three times in village 3 (between July 1998 and September 1999). The findings of the baseline surveys for each of the three villages are presented in Table 1. The total enumerated population of the three villages was 3725 and examinations were conducted in 3034 (81.4%), with slightly higher coverage in females 1513 (84.3%) than in males 1521 (78.8%). Three new cases of leprosy were diagnosed during the surveys (all male and aged 7, 25, and 60 yrs) and overall 6.7% of those surveyed were household contacts of current or previous leprosy patients (46 cases). Overall BCG coverage was 50.1%, and demonstrated an age effect due to the implementation of BCG programs in the under 35 yrs age groups, but 95% coverage in the 10 to 15 yrs age group.

Strict quality control criteria were applied and valid PCR analysis was obtained on 2552 of 3034 (84.1%) samples tested. In the baseline survey, only 42 of these 2552 (1.6%) nasal swabs were found to be PCR positive. There was no clear pattern by age or sex but there was variation by village ranging from 0.1% to 3.3%. In the saliva samples, 2890 out of 3034 (95.2%) met the quality control criteria for the salivary IgA assay. Of those assays meeting the criteria, 67.7% were positive for salivary ML-IgA, this varied from 53% to 81% between villages. This variation was also seen in the top 10% of ML-IgA high titres but no dif-

TABLE 1. *Baseline survey results by village.*

Village	Enumerated	Examined	% BCG	% Household Contact	Nasal PCR			Salivary IgA		
					Valid	+	(%)	Valid	+ (%)	++(%)
Village 1										
Male	792	641	49.6	7.3	450	7	(1.6)	606	76.6	18.5
Female	748	657	37.9	8.5	457	8	(1.8)	632	80.9	19.8
Village 2										
Male	615	462	58.4	9.7	439	1	(0.2)	423	53.2	2.4
Female	561	451	56.5	9.1	422	0	(0)	427	60.9	2.8
Village 3										
Male	523	418	61.7	1.2	402	15	(3.7)	409	58.0	2.2
Female	486	405	42.2	2.0	382	11	(2.9)	393	65.7	5.6
All villages										
Male (Total)	1930	1521	55.6	6.4	1291	23	(1.8)	1438	64.4	9.1
Female (Total)	1795	1513	44.6	6.9	1261	19	(1.5)	1452	70.9	11.0
Total	3725	3034	50.1	6.7	2552	42	(1.6)	2890	67.7	10.0

ference was found between age groups (Table 1).

There was a highly statistically significant association ($p < 0.01$) between higher ML-IgA and household contact status with higher levels being observed in household contacts, but no evidence of an association between PCR positivity and household contact status (Table 2). A greater proportion (12.5%) of those with no record of BCG immunization had high ML-IgA titres compared to those with BCG (Table 3); this was highly statistically significant ($p < 0.01$). Of the 42 individuals who tested positive for PCR, only 6 (14.3%) were also IgA strongly positive, which was not significantly different from those who were negative for nasal PCR, and 86% of the surveyed population from the 3 villages were negative for both nasal PCR and salivary ML-IgA (Table 4).

There was, however, a seasonal pattern to PCR positivity with a peak between August and November coinciding with the monsoon season (Table 5), with ML-IgA strongly positive results increasing to 37% in November.

Follow up surveys. An 80% response rate was achieved in the baseline surveys conducted in all three villages. The first

TABLE 2. *Nasal PCR and salivary ML-IgA in household contacts.*

			Nasal swab PCR				Salivary IgA	
		Total	Valid	+ ve	(%)	Valid	+ ve (%)	++ ve (%)
Men	Household contacts	97	78	1	(1.28)	96	64 (66.7)	18 (18.8)
	Non contacts	1424	1190	22	(1.85)	1313	839 (63.9)	108 (8.2) **
Women	Household	105	79	0	(0)	105	74 (70.5)	19 (15.2)
	Non contacts	1408	1172	19	(1.62)	1333	949 (71.2)	140 (10.5) *
Total	Household contacts	202	157	1	(0.6)	201	138 (68.7)	34 (16.9)
	Non contacts	2832	2362	41	(1.7)	2646	1788 (67.6)	248 (9.4)

Differences in proportions
**$p < 0.01$
*$p < 0.05$

TABLE 3. *PCR and salivary IgA by BCG status.*

		Total	PCR			IgA		
			Valid	+ ve	(%)	Valid	+ ve (%)	++ ve (%)
Male	BCG	846	742	14	(1.89)	802	509 (63.5)	49 (6.1)
	No BCG	676	549	9	(1.64)	636	417 (65.6)	72 (11.3) **
Female	BCG	675	582	8	(1.37)	652	438 (67.2)	52 (8.0)
	No BCG	838	679	11	(1.62)	800	591 (73.9)	107 (13.4) **
Total	BCG	1521	1324	22	(1.7)	1454	947 (65.1)	101 (6.9)
	No BCG	1514	1228	20	(1.6)	1436	1008 (70.2)	179 (12.5) **

Differences in proportions
**p <0.01
*p <0.05

follow-up in village 1 was low (25%) due to the selection of subjects who were identified as PCR-positive. This led to some difficulties for these individuals and in subsequent follow-up surveys all available villagers were re-tested regardless of previous PCR status. The participation rate for the first and second follow-up surveys was 43–56% and higher among females than males. The percentage of PCR positive individuals in these two follow-up examinations varied by village, but was consistent with the seasonal pattern observed in the baseline survey (Table 6). High levels of ML-IgA found in saliva tended to follow the periods of PCR positivity. There was also evidence of a lack of persistence of PCR positivity and IgA over time. Of the 42 individuals who were PCR positive in the baseline survey, 24 were successfully tested in the first follow-up survey and 23 in the second follow-up survey. Of these none were positive in the first follow-up survey and only one in the second follow-up survey (Table 7).

DISCUSSION

This study is the largest and most comprehensive investigation of the role of the upper respiratory tract in the transmission of *M. leprae* using a combined molecular and immunological approach. This paper represents the culmination of 10 yrs of development work establishing a laboratory in a leprosy endemic country, and the development of robust methods under field conditions to study leprosy transmission. The current global leprosy control strategy, based on early detection of leprosy and treatment using MDT, has had a dramatic effect of the numbers of leprosy patients registered for treatment but has not had a demonstrable effect on interrupting transmission. The approach adopted in this study combines the use of PCR methods to identify small numbers of *M. leprae* with salivary tests for mucosal immune responses to *M. leprae*. The findings of this large scale population based study tracking three communities over a two year period are therefore relevant and timely.

The initial analyses of the PCR and IgA results looked at age and sex distributions, geographical patterns, temporal trends, relationships to BCG immunizations and tuberculosis infections, associations with contact status and the persistence over time of the findings in individuals. The baseline and follow-up surveys revealed that there was considerable variation between the villages in levels of nasal PCR positivity, but there was no clear age or sex pattern, or association with recent leprosy contact or BCG status. Conversely, high ML-IgA responses demonstrated possible associations with

TABLE 4. *Association between nasal PCR status and salivary ML-IgA.*

High salivary IgA	Nasal PCR result		
	+ ve	– ve	Total
Yes	6	284	290
No	36	2226	2262
Total	42	2510	2552

TABLE 5. *Monthly pattern of nasal PCR and salivary ML-IgA.*

Month	Nasal swab PCR			Salivary ML-IgA		
	Valid PCR	+ve	%	Valid IgA	+ve	++ve
January	156	0	0	175	63.4	0.57
February	42	0	0	40	77.5	5.0
March	—	—	—	—	—	—
April	659	0	0	632	55.7	2.05
May	146	1	0.7	162	57.4	3.7
June	56	0	0	56	69.6	5.4
July	93	1	1.1	89	56.2	3.4
August	604	23	3.8	627	64.4	6.1
September	87	2	2.3	86	46.5	2.3
October	—	—	—	—	—	—
November	370	14	3.8	624	97.1	37.3
December	339	1	0.3	399	56.6	0.25

contact status and lack of BCG immunization.

The major, new finding from this study is the strong seasonal pattern of nasal PCR and high titre ML-IgA positivity. This suggests that there may be high levels of exposure to *M. leprae* within these populations during the wet season. The peak rainfall period in the area of the study being July to October, this would fit with the high ML-IgA levels in November at the end of the wet period. The rates of PCR positive nasal swabs are low at the start of the wet period and continue high in the period immediately after the monsoon rains. It has long been considered that transmission of leprosy infection was easier in a wet environment; *M. leprae* does not survive prolonged drying ([5]), and the disease is common in tropical areas of high humidity ([6]). One might then postulate that endemic areas of relatively constant high humidity might not show the same degree of seasonal transmission as appears to be present in Maharashtra. The long incubation period between infection and disease makes it difficult to assess seasonal patterns of transmission from any season pattern in the incidence or indeed the detection of disease. While the possibility of an environmental reservoir cannot be excluded, this may not be necessary if there is sufficient and repeated transient infection of non-immune individuals as we have previously suggested from earlier studies ([4]). An alternative explanation of the seasonal pattern observed in this study is that of closer contact and increased exposure within the household as a result of longer periods of time spent indoors and poorer ventilation.

The transient nature of nasal PCR positivity is also striking and has been observed in previous studies ([8]), and it appears to be associated with the development high ML-IgA titres. Studies of the carriage of other bacteria in the upper respiratory tract have

TABLE 6. *Percentage nasal PCR and salivary ML-IgA positive at each survey.*

Village		Nasal PCR			High salivary ML-IgA		
		Baseline	First follow-up	Second follow-up	Baseline	First follow-up	Second follow-up
Village 1	Male	1.6	1.6	2.0	18.5	0.6	2.3
	Female	1.8	5.1	3.9	19.8	0.5	5.3
Village 2	Male	0.2	5.2	0.9	2.4	2.3	21.7
	Female	0.0	8.4	1.4	2.8	4.6	25.3
Village 3	Male	3.7	1.6	2.7	2.2	21.3	37.1
	Female	2.9	2.3	1.6	5.6	25.1	47.6

TABLE **7**. *Follow-up of the 42 individuals nasal PCR positive at baseline.*

Baseline PCR Positive		First follow-up PCR results		Second follow-up PCR results	
42		Positive	0	Positive	1
		Negative	24	Negative	22
		No data	18	No data	19
Salivary ML-IgA results					
Baseline ML-IgA		First follow-up ML-IgA		Second follow-up ML-IgA	
Negative	13	Negative	8	Negative	11
ML-IgA +	24	ML-IgA +	17	ML-IgA +	12
ML-IgA ++	5	ML-IgA ++	1	ML-IgA ++	9
No data	0	No data	16	No data	10

shown similar transient occurrence in individuals with persistence in the community ([3]). Unlike the divergence of cell-mediated and humoral responses seen in the systemic immune response to *M. leprae* infection, mucosal IgA responses appear to be induced with systemic cell-mediated immunity, suggesting that the development of mucosal immunity to *M. leprae* may be protective ([4]). The data presented here support this but there was no measurement of cell-mediated immune responses in this large epidemiological study.

The authors of this paper are aware that there were a number of limitations of this study since not all of the follow-up surveys were of the same individuals and some of the intervals between surveys were not linked to seasons. It also may be argued that the PCR positivity could be due to the presence of other non-leprosy mycobacteria sharing the same sequences as the primers used in this study. The two primers are considered to be specific to *M. leprae*, based on known sequenced mycobacteria. We used two different sets of specific primers, based on the sequences of the pra gene and the repetitive element RLEP. The processing of large numbers of PCR and IgA assays has demanded the development of rigorous internal and external quality control procedures, and batches failing quality control (up to 50%) were required to be re-run. PCR results need to be interpreted with caution and laboratories need to demonstrate rigorous quality control procedures. External quality control based on nasal swabs from a non-endemic country ([10]) demonstrates that it is possible to achieve good sensitivity and specificity. The development of these methods and their application to field studies provide tools for studying the transmission of leprosy and direct methods for testing innovative interventions.

Validation of these findings requires careful follow-up a cohort every month for a year to confirm if there is a relationship between the transient nature of PCR positivity and seasonal patterns. Studies of contacts of new untreated patients and studies to investigate environmental sources in the wet season would be of value. It would be possible to examine first exposure by serial examination of new-born infants and previously unexposed visitors to leprosy-endemic areas. Confirmation of the seasonal nature of *M. leprae* infection would have major implications for our understanding of leprosy transmission and for interrupting transmission as it may be possible to protect populations for a relatively short period timed to coincide with the period of greatest likelihood of exposure.

Contributions. All the study partners contributed to the development of the study proposal co-ordinated by Smith, the study builds on an earlier developmental program led by Cree and also funded by the European Commission. Cree, Macdonald, and Jadhav developed the PCR methods with guidance and technical advice from Klatser, Van Beers, and Oskam. The PCR quality control was organized and assessed by Oskam and Klatser. The ML-IgA methods were developed by Cree, Jadhav, and Fernando, with guidance and technical advice for Bjune. The fieldwork was designed and developed by Rao, Edward, Macdonald,

and Jadhav with technical advice from W. Smith, C. Smith, and van Beers. Jadhav, W. Smith, and C. Smith conducted the collation of data and analysis. All authors contributed to the drafting process in the preparation of this paper and approved the final version. There are no conflicts of interest.

Acknowledgment. The MILEP2 study group acknowledges the co-operation of the participating village communities in India without whom this study would not have been possible, and also the survey team and laboratory staff and the European partners who have spent time at the study center. The work is funded by the European Commission as part of the International Co-operation with Developing Countries Program (INCO-DC) contract number IC18*CT960047. We are grateful to The Leprosy Mission for their support for this project and for the assistance of their staff in Delhi and at Miraj. We thanks Dr. S. D. Sontakke for her support to the laboratory staff at Miraj. We acknowledge the assistance from Professor Anand Job from CMC Vellore who conducted nasal examinations on those found to be PCR positive. We are grateful to Professor Patrick Brennan, Colorado State University for the free supply of whole *M. leprae* for the ML-IgA assay.

REFERENCES

1. Barton, R. P. Importance of nasal lesions in early lepromatous leprosy. Annals of the Royal College of Surgeons of England **57** (1975) 309–312.
2. Britton, W. J. Immunology of leprosy. Trans. Royal Society Trop. Med. Hygiene **87** (1993) 508–514.
3. Burr, M. L., Gray, S. J., and Howells, C. H. A survey of nasal *Streptococcus pneumoniae* in children. J. Hygiene **88** (1982) 425–431.
4. Cree, I. A., and Smith, W. C. Leprosy transmission and mucosal immunity: towards eradication?. Lepr. Rev. **69** (1998) 112–121.
5. Desikan, K. V. Viability of *Mycobacterium leprae* outside the human body. Lepr. Rev. **48** (1977) 231–235.
6. Desikan, K. V., and Sreevatsa. Extended studies on the viability of *Mycobacterium leprae* outside the human body. Lepr. Rev. **66** (1995) 287–295.
7. Fine, P. E., Sterne, J. A., Ponnighaus, J. M., Bliss, L., Saui, J., Chihana, A., Munthali, M., and Warndorff, D. K. Household and dwelling contact as risk factors for leprosy in northern Malawi. Am. J. Epidemiol. **146** (1997) 91–102.
8. Hatta, M., van Beers, S. M., Madjid, B., Djumadi, A., de Wit, M. Y., and Klatser, P. R. Distribution and persistence of *Mycobacterium leprae* nasal carriage among a population in which leprosy is endemic in Indonesia. Trans. Royal Society of Trop. Med. Hygiene **89** (1995) 381–385.
9. Jacobson, R. R., and Krahenbuhl, J. L. Leprosy. Lancet **353** (1999) 655–660.
10. Jadhav, R. S., Macdonald, M., Bjune, G., and Oskam, L. MILEP2 Study Group. Simplified PCR detection method for nasal *Mycobacterium leprae*. Int. J. Lepr. Other Mycobact. Dis. **69** (2001) 299–307.
11. Klatser, P. R., van Beers, S., Madjid, B., Day, R., and de Wit, M. Y. Detection of *Mycobacterium leprae* nasal carriers in populations for which leprosy is endemic. J. Clin. Microbiol. **31** (1993) 2947–2951.
12. Pattyn, S. R., Ursi, D., Ieven, M., Grillone, S., and Raes, V. Detection of *Mycobacterium leprae* by the polymerase chain reaction in nasal swabs of leprosy patients and their contacts. Int. J. Lepr. Other Mycobact. Dis. **61** (1993) 389–393.
13. Pedley, J. C., and Geater, J. G. Does droplet infection play a role in the transmission of leprosy? Lepr. Rev. **47** (1976) 97–102.
14. Ramaprasad, P., Fernando, A., Madhale, S., Rao, J. R., Edward, V. K., Samson, P. D., Klatser, P. R., de Wit, M. Y., Smith, W. C., and Cree, I. A. Transmission and protection in leprosy: indications of the role of mucosal immunity. Lepr. Rev. **68** (1997) 301–315.
15. Rees, R. J. W. The Microbiology of Leprosy. In: *Leprosy*. Edinburgh: Churchill Livingstone, 1985.
16. van Beers, S. M., Hatta, M., and Klatser, P. R. Patient contact is the major determinant in incident leprosy: implications for future control. Int. J. Lepr. Other Mycobact. Dis. **67** (1999) 119–28.
17. van Beers, S. M., Izumi, S., Madjid, B., Maeda, Y., Day, R., and Klatser, P. R. An epidemiological study of leprosy infection by serology and polymerase chain reaction. Int. J. Lepr. Other Mycobact. Dis. **62** (1994) 1–9.
18. World Health Organization. Leprosy: global situation. Weekly Epidemiological Record **77** (2002) 1–8.
19. World Health Organization. Report on third meeting of the WHO technical advisory group on elimination of leprosy. WHO/CDS/CPE/CEE/2002.29