PN-ABU-108
93505

8.700

SERO-EPIDEMIOLOGY OF LEPROSY

FINAL REPORT TO USAID

REC'D IN R&D/R
FEB 28 1995

INVESTIGATORS:

VINAI SURIYANON, MD
KRIANGSAK PRAPUTPITTAYA, PHD
NAMTIP SRIRAK, MS
TASANAI WONGCHAK, MPH

JANUARY 1995

Research Institute For Health Sciences (RIHES)
The Center For Public Health Research
Chiang Mai University
PO Box 80 CMU, Chiang Mai 50200, Thailand
Tel: [053] 221966 Fax: 6653 221849

FINAL REPORT

Investigators:

Vinai Suriyanon, MD
Kriangsak Praputpittaya, PhD
Namtip Srirak, MS
Tasanai Wongchak, MPH

Period Covered: 01.08.88 to 30.06.94.

Submitted to: Office of the Science Advisor

U S Agency for International Development

5041

Sero-Epidemiology of Leprosy

Principal Investigator: Vinai Suriyanon, MD

Grantee Institution: Research Institute for Health Sciences (RIHES)

Chiang Mai University

Project Number: 8.700

8046 S

Grant Number: 936-5542-G-00-8049-00

AID Grant Project Officer: Mr Karun Ragwanit

Project Duration: 01.08.88 to 30.06.94.

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Executive Summary:

Leprosy is still a health problem causing chronic disability in many parts of the world. New cases of leprosy continue to occur in endemic areas despite the availability of effective chemotherapeutic agents, which, if used effectively, should interrupt transmission from infectious cases. Delay in diagnosis and treatment is one of the main reasons for failure to eradicate the disease. The purpose of this project is to evaluate the efficacy of an ELISA test for antobodies to *M. leprae* specific antigens as a tool for early diagnosis of leprosy.

A longitudinal study of household contacts of leprosy cases was undertaken between August 1988 and December 1993. One thousand three hundred and forty (1340) household contacts of leprosy cases were enrolled and followed-up yearly.

During the study 11 contacts developed leprosy. The incidence rate was 3.5 per 1000 person years. Four (4) of the incident cases and 75 of those who did not develop leprosy were positive for antibodies to *M. leprae* antigen by ELISA. The others were negative. These data showed that the sensitivity of the test was 36.4% and the specificity of the test 94.4%. The positive predictive value of the test was 5.1%. The seropositive contacts had 9.1 fold higher risk of developing leprosy than the seronegatives. It was also found that 3 of the incident cases were seropositive several months before recognition of the disease. These findings suggest that even though the ELISA test for antibodies against *M. leprae* specific antigen is not sensitive and has low predictive value, it still might be useful. Diagnosis of leprosy could be made earlier by testing household contacts of leprosy cases for the antibodies periodically and by close observation of the seropositives. Following diagnosis, the disease could be treated earlier and its transmission thereby prevented.

Studies on people living in villages where there were no known cases of leprosy were done in 1989, 1992 and 1994. Eighty-one (81) out of 155 villagers (5.2%) were seropositive. During the study none of the villagers developed leprosy. The normal village studies confirm that the ELISA test for antibodies to *M. leprae* antigen is specific.

Research Objectives:

Leprosy is a human disease which is responsible for a large burden of illness and disability in persons living in underdeveloped or developing countries. During the past eight years the numbers of estimated and registered cases of leprosy in the world have fallen from 10-12 million to 2.7 million and from 5.4 million to 1.9 million respectively.[1] In Thailand the prevalence rate of leprosy has fallen from 0.9/1000 in 1984 to 0.23/1000 in 1990.[2] The decrease in numbers of leprosy probably results from the implementation of standard WHO multidrug (MDT) regimens. The detection rate of new cases in the world on the other hand, has not changed significantly. Approximately 690,000 cases were detected in 1992.[1] The reason for unchanged case detection rate is not clear. MDT may in reality decrease the incidence of leprosy but

the downward trend has been obscured by intensified case finding efforts.[1] On the other hand, it is conceivable that MDT may have little or no effect on the incidence of leprosy. This can be explained by the general delay in diagnosis and treatment. Although leprosy can be cured by MDT, the spread of disease which has already occurred before the start of the treatment, cannot be stopped. Control of leprosy would be more successful if there is a method for diagnosis at an early stage of infection.

The possibility of developing a highly specific and sensitive assay for antibodies to *M. leprae* was improved with studies of Hunter and Brennan on the chemical composition of the cell wall of *M. leprae*.[3,4] The successful artificial infection of the nine-banded armadillo[5] provided the opportunity for isolation of rather large quantities of *M. leprae*, so these elegant biochemical studies could be carried out. Using organisms from infected armadillos and the infected tissues themselves, Hunter and Brennan isolated the major glycolipid (PGL-1) that contains the unique trisaccharide. This saccharide moiety is linked to a phenolic lipid.[3,4] Sera from nearly all patients with multibacillary leprosy and about 50% of paucibacillary leprosy reacted to PGL-1 when tested in ELISA assays.[6] In studies in Micronesia and Sri Lanka 37 out of 39 cases from whom sera had been collected prior to clinical onset of the disease had elevated antibodies to PGL-1 from 3 months to 2 years before the development of clinical signs of the disease.[7] Importantly, many investigators have shown there was no cross-reactivity in patients infected with *M. tuberculosis* or a variety of other mycobacteria.[6,8,9,10] Serum antibodies are present primarily in the IgM fraction. Antibodies persist for several years after infection in patients with multibacillary disease and their levels may be an indirect measure of bacillary load of *M. leprae*.[6-8]

Young and Buchanan reported the use of a deacylated preparation of PGL-1 which they felt gave improved performance due to increased solubility of the antigen in aqueous media.[8] However, PGL-1 itself or the deacylated version have recently been replaced with neoglycoproteins containing the chemically synthesized epitope of PGL-1[11] and Cho et al. have synthesized and reported on the use of an artificial antigen containing the epitope coupled to bovine serum albumin (BSA) in the serodiagnosis of leprosy.[12] This was followed by the synthesis of the disaccharides and trisaccharides containing the unique carbohydrate structure found in *M. leprae* PGL-1.[13,14] Then, by conjugating these saccharides to bovine serum albumin two neoglycoproteins were produced, namely, Natural Disaccharide-Octyl-Bovine Serum Albumin (ND-O-BSA) and Natural Trisaccharide-Octyl-Bovine Serum Albumin (NT-O-BSA). These synthetic neoglycoproteins have been used for serologic studies in leprosy patients and household contacts.[13,14,15] Douglas et al. found in a longitudinal study that those who were seropositive to ND-O-BSA had an approximately twenty fold higher risk of developing disease than the seronegatives.[15]

Another cell wall component of *M. leprae* called lipoarabinomannan B (LAM-B) has been isolated by Hunter et al.[16] This antigen has been hypothesized to be a possible early marker of lepromatous disease.[16]

These advances in the understanding of biochemical structure of *M. leprae* and human serological responses to infection with these organisms have raised possibilities for using serologic methods to detect early leprosy. The general objective of this project is to evaluate the efficacy of ELISA test for antibodies to *M. leprae* specific antigens as a tool for early diagnosis of leprosy.

Specific Objectives:

1. To determine the prevalence of serum antibodies to *M. leprae* specific antigens (PGL-l, ND-O-BSA and NT-O-BSA) in household contacts of leprosy patients and controls without known household exposure to leprosy.

2. To determine the prevalence of serum antibodies to the highly antigenic lipopolysaccharide of *M. leprae*, lipoarabinomannan B (LAM-B) among household contacts of leprosy cases and controls without known household exposure to leprosy.

3. To determine the comparative efficacy of filter paper strips containing dried blood in detecting antibodies to *M. leprae* specific antigens in leprosy patients and their household contacts.

4. To determine the predictive value of seropositivity of household contacts of leprosy cases and controls.

Methods and Results:

All leprosy patients who have been first diagnosed and treated either at the McKean Rehabilitation Center or at the Ministry of Public Health's Leprosy Control Center, Region 10, between 1982 and 1992 were recruited. These leprosy patients were examined by physicians and classified by leprosy type according to the five group scheme of Ridley and Jopling. Ten cc of blood for serologic tests and 4 mm punch biopsies of skin for histological examination were obtained from each patient. Slit skin smears from six sites to measure bacteriological index (BI) and morphological index (MI) were also done. Arrangements were made to visit the household of each patient. Household members who were living with the patients as well as others who at sometime in the past within the period starting 5 years back from the first diagnosis of leprosy had lived in association with the index case(s) for at least one year were enrolled in the study. Each of these household residents were examined by the field team for evidence of leprosy. All contacts with suspicious skin lesions were brought to McKean hospital where a complete physical examination and skin biopsy were done by physicians. From each contact 10 cc of venous blood were drawn for serologic studies. Small children from whom it was difficult or not possible to obtain blood specimens by venepucture, fingerstick specimens were obtained instead.

Blood specimens from leprosy patients and their household contacts were tested for antibodies to *M. leprae* PGL-1, ND-O-BSA, NT-O-BSA and LAM-B by ELISA techniques using the methods as described by Cho et al.[6], with some

modification. Each study subject was followed-up once a year. All studies done on the first visit except skin biopsy were repeated in the follow-up visits.

Similar studies were done on villagers living in four (4) villages in Sunpatong District where there were no known cases of leprosy. Due to budget and manpower constraints, the villagers could not be followed-up every year, and instead, they were followed up only twice, 2 years and 4 years after the initial study.

Between August 1988 and December 1993, 1340 houshold contacts of 435 leprosy patients were enrolled and each subject was followed-up once a year. During the study 11 contacts developed leprosy. Two cases were classified as BL, six BT, two TT and one indeterminate. The incidence rate was 3.5 per 1000 person years (table 1).

Studies on normal villagers were done three times. The first study was done from November 1989 to April 1990, the second from January to February 1992 and the third from February to March 1994. The total number of normal villagers enrolled in these three studies was 1554 (table 2). During the study none of them developed leprosy.

At the beginning, blood specimens from each subject were tested for antibodies to all *M. leprae* antigens proposed in the grant, namely, PGL-1, ND-O-BSA, NT-O-BSA and LAM-B. Later we decided to confine our test for antibodies to only one antigen (NT-O-BSA) because our interim analysis had demonstrated that the data on antibodies to the first three antigens were similar, and the antibodies to LAM-B were not specific (see our previous reports).

The following are the results of our studies using NT-O-BSA as the test antigen.

1. The prevalence of serum antibodies to NT-O-BSA (table 2):

   a. in household contact of leprosy = 5.90%
   b. in controls or normal villagers = 5.2%

The seropositive rates among household contacts and among normal villagers by visit are shown in tables 3 and 4.

2. Four (4) of the incident cases and 75 of those who did not develop leprosy were positive for antibodies to NT-O-BSA. Others were seronegative. The sensitivity and specificity of the ELISA test for antibodies to NT-O-BSA were therefore equal to 36.4% and 94.4% respectively. The predictive value of the test was 5.1% The seropositive contacts had 9.1 fold higher risk of developing leprosy than the seronegatives (table 5). Three of the incident cases were found seropositve several months before diagnosis of leprosy were made clinically (table 6).

3. Various factors that might be associated with an increased risk of developing leprosy among household contacts were analyzed (table 7). The types of leprosy of

index cases and the serostatus of the household contacts were found to be risk factors. Contacts of multibacillary leprosy patients had a higher risk of developing leprosy than contacts of paucibacillary leprosy patients (R.R. = 4.6). The risk of developing leprosy was increased in contacts with positive leprosy serology (R.R. = 9.1).

4. The *M. leprae* antibody levels in blood colected on filter paper by fingerstick correlated very well with the antibody levels in sera collected by venepuncture (see our previous report and reference number 17 below). Therefore, in small children from whom it is difficult or impossible to obtain blood by venepuncture, we can use fingerstick specimens instead for the determination of antibodies to *M. leprae* antigens.

Table 1

Incidence Rates of leprosy Among Houshold Contacts of Leprosy Cases

No. of Index Cases	No. of Household Contacts	Person-Years at Risk	No. of Incident Cases	Incidence Per 1000 Person Years
435	1340	3100	11	3.5

Table 2

Prevalence of Antibody to NT-O-BSA in the Sera from Leprosy Patients, Household Contacts and Normal Villagers

Subjects	Total Number	Number Positive†	Percentage Positive
All Patients			
MB	209	113	54.1
PB	219	14	6.4
I	7	1	14.3
Total	435	128	29.4
New Patients‡			
MB	55	41	74.5
PB	37	1	2.7
I	2	0	0
Total	94	42	44.7
Contacts of			
MB	665	46	6.9
PB	661	33	5.0
I	14	0	0
Total	1340	79	5.9
Normal Villagers	1554	81	5.2

Notes:

MB = multibacillary PB = paucibacillary I = Indeterminate

‡ MB patients who have been treated less than six months before first blood drawing or PB (and I) patients less than 3 months.

† The serum is considered positive to NT-O-BSA if its OD_{492} value exceeds 0.117 (or +3 SD of normal).

Table 3

Positivity Rates of Antibody to NT-O-BSA among Household Contacts of Leprosy by Visit

Visit	All Contacts			Incident Cases	
	No. Tested	No. Positive	Percent Positive	No. Tested	No. Positive
1	1340	35	2.6	11	1
2	916	24	2.6	9	2
3	807	29	3.6	9	2
4	732	20	2.7	8	0
5	561	24	4.3	9	3
6	74	1	1.4	1	0

Table 4

Positivity Rates of Antibody to NT-O-BSA among Normal Villagers by Visit

Visit	No. Tested	No. Positive	Percent Positive
1	1179	34	2.9
2	1032	29	2.8
3	783	34	4.3

Visit 1 - November 1989 - April 1990
Visit 2 - January - February 1992
Visit 3 - February - March 1994

Table 5

Sensitivity, Specificity and Predictive Value of ELISA Test for Antibodies to NT-O-BSA

	Developed Leprosy		**Total**
ELISA Test	**Yes**	**No**	
Positve	4 (a)	75(b)	79
Negative	7(c)	1254(d)	1261
Total	11	1329	1340

Sensitivity $= \frac{a}{a+c} = \frac{4}{11} = 36.4\%$

Specificity $= \frac{d}{b+d} = \frac{1254}{1329} = 94.4\%$

Predictive Value $= \frac{a}{a+b} = \frac{4}{79} = 5.1\%$

R.R. $= \frac{a/(a+b)}{c/(c+d)} = \frac{4/79}{7/1261} = 9.1$

Table 6

ELISA Data from Eleven (11) Houshold Contacts Who Later Developed Leprosy

Contacts	Specimen Number					
	1	2	3	4	5	6
C 0019†	0.043 (neg)	0.035 (neg)	0.127 (pos)	N.D.	0.487 (pos)	ND
C 0033	0.018 (neg)	0.020 (neg)	0.071 (neg)	0.060 (neg)	N.D.	0.025 (neg)
C 0035	0.043 (neg)	0.025 (neg)	0.017 (neg)	0.013 (neg)	0.008 (neg)	ND
C 0169	0.070 (neg)	N.D.	N.D.	N.D.	0.270 (pos)	ND
C 0201	0.012 (neg)	0.009 (neg)	0.009 (neg)	0.019 (neg)	0.022 (neg)	ND
C 0395‡	0.020 (neg)	0.154 (pos)	0.075 (neg)	0.064 (neg)	0.068 (neg)	ND
C 0467	0 (neg)	ND	0.020 (neg)	0.015 (neg)	ND	ND
C 0631	0 (neg)	0 (neg)	0.003 (neg)	0.011 (neg)	0.001 (neg)	ND
C 0640	0.075 (neg)	0.051 (neg)	0.041 (neg)	0.033 (neg)	0.043 (neg)	ND
C 0766	0 (neg)	0.002 (neg)	ND	ND	ND	ND
C 1063§	0.118 (pos)	0.119 (pos)	0.124 (pos)	0.096 (neg)	0.126 (pos)	ND

NOTES:

The serum is considered positive to NT-O-BSA if its OD_{492} value exceeds 0.117.

ND = not done.

† C0019 was seropositive 22 months before recognition of disease.

‡ C0395 was seropositive 4 months before recognition of disease.

§ C1063 was seropositive 8 months before recognition of disease.

Table 7

Risk Factors for Incident Leprosy among Houshold Contacts of Leprosy Cases

Factors	No. of Contacts Who Later:		Total Number	p Value
	Developed Leprosy	Did Not Develop Leprosy		
1. Age (years)				
<10	1	268	269	
11-20	1	260	261	
21-30	2	300	302	
31-40	3	180	183	
41-50	2	82	84	
51-60	2	104	106	
61-70	0	61	61	
>70	0	37	37	0.23
2. Gender:				
Male	2	590	592	
Female	9	703	712	0.07
3. Duration of Contact (years)				
1 - 3	8	1059	1067	
4 - 6	3	173	176	
> 6	0	1	1	0.45
4. Closeness of Contact				
Not close	2	419	421	
Close	5	455	460	
Very close	4	418	422	0.45
5.a. History of BCG Vaccination				
Yes	3	665	668	
No	8	624	632	0.11

Table 7 (Continued)

Risk Factors for Incident Leprosy among Houshold Contacts of Leprosy Cases

Factors	No. of Contacts Who Later:		Total Number	p Value
	Developed Leprosy	Did Not Develop Leprosy		
5.b. BCG Scar				
Yes	3	671	674	0.20
No	8	636	644	
6. Types of Leprosy Index Cases				
MB	9	656	665	0.03
PB	2	673	675	
7. No of Index Cases in the Household				
1	9	1088	1097	0.49
2	1	135	136	
3 or more	1	37	38	
8. No of Persons Per Room in Housing Unit				
1 - 2	4	602	606	0.33
3 - 4	4	536	540	
> 4	3	162	165	
9. Socio-Economic Status				
High Class	0	87	87	0.21
Middle Class	5	807	812	
Low Class	6	406	412	
10. ELISA Test for Antibodies to NT-O-BSA				
Positve	4	75	79	0.002
Negative	7	1254	1261	

Impact, Relevance and Technology Transfer:

It was found in this study that ELISA test for antibodies to NT-O-BSA was highly specific for leprosy. The test was not sensitive and had low predictive value in diagnosing leprosy. The seropositive household contacts had about 9 fold higher risk of developing leprosy than the seronegative. Some incident cases were found seropositive several months before recognition of the disease. These findings suggest that even though the ELISA test for antibodies to NT-O-BSA has low sensitivity and low predictive value, it might still be useful, especially in countries or areas where prevalence of leprosy is higher than Thailand (prevalence rate in Thailand in 1990 = 0.23/1000). In endemic areas, the predictive value of the test could be higher than was observed in this study. By testing household contacts of leprosy cases or other risk groups periodically for the antibodies and by close obervation of the seropositive persons, leprosy might be detected earlier. Following diagnosis the disease could be treated earlier and its transmission thereby prevented.

This study was done in collaboration with Dr Kenrad E Nelson (director of the Infectious Diseases Program, the Johns Hopkins University School of Hygiene and Public Health), Dr J T Douglas (Department of Microbiology, University of Hawaii), Dr P J Brennan (Department of Microbiology, Colorado State University), Mr Wanchai Teppawan (late director of the Royal Thai Ministry of Public Health Leprosy Control Center, Region 10), Mrs Sribut Thepsri (director of the MOPH Leprosy Control Center, Region 10), and Dr T C Smith (medical director, the McKean Rehabilitation Center). The coordination of patient care activities between the McKean Rehabilitation Center and the MOPH's Leprosy Control Center, Region 10, and Chiang Mai University has improved considerably during this research project. As a result of the field research activities, surveillance for new cases, active and effective followup treatment of leprosy and treatment of other medical problems have been facilitated in northern Thailand.

The transfer of the ELISA techniques for leprosy serology from the laboratories of our overseas collaborators has enhanced the technological expertise of the Research Institute for Health Sciences (RIHES), Chiang Mai University. Furthermore, the research capability of local scientists working on this project has been strengthened by contacts with overseas collaborators and by hands-on experience with handling a community-based project with a large data base.

Project Activities/Outputs:

Publications and Presentations:

1. Suriyanon V, Praputpittaya K, Hirunpetcharat C, Suphavilai C and Masarat A: Serological reactivity against *Mycobacterium leprae* antigens in leprosy patients. Abstracts of Second National Seminar on Leprosy Control in Thailand, 1988, page 185.

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6. Praputpittaya K, Suriyanon V, Hirunpetcharat C, Rungreungthanakit K and Suphavilai C. Comparison of IgM, IgG and IgA responses to M. leprae specific antigens in leprosy. Asian Pacific J Allergy Immunol 1990;8:19-25.

7. Rungruengthanakit K, Apichartpiyakul C, Kantamala D, Suriyanon V and Vithayasai V. A comparative study of serodiagnosis of leprosy using a particle agglutination test and an enzyme linked immunosorbent assay. Abstracts of the Ninth Annual Health Sciences Meeting, Research Institute for Health Sciences, Chiang Mai University 1991, pages 55-56.

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11. Rungruengthanakit K, Apichartpiyakul C, Kingkaew D, Suriyanon V and Vithayasai V. A comparsion of serodiagnosis of leprosy using a particle agglutination test and an enzyme-linked immusorbent assay. Proceedings of the Second Meeting of the scientific Working Groups on Leprosy Research in Thailand, 1991. p 61.

12. Rungruengthanakit K, Kantamala D, Srirak N, Praputpittaya K and Suriyanon V. Effect of BCG vaccination on the serum levels of antibodies to mycobacterial antigens (NT-O-BSA and LAM-B) in normal people. Proceedings of the Second Meeting of the Scientific Working Group on Leprosy Research in Thailand, Chiang Mai 1991, p 60.

13. Rungruengthanakit K, Apichartpiyakul C, Kingkaew D, Suriyanon V and Vithayasai V. A comparative study on serodiagnosis of leprosy using a particle agglutination test and an enzyme-linked immusorbent assay. Chiang Mai Med Bulll 1993;32(1):1-7.

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15. Nelson KE, Suriyanon V, Taylor E, Wongchak T, Kingkeow C, Srirak N, Lertsrimongkol C, Cheewawat W, Celentano DD. The incidence of human immunodeficiency virus Type-1 infections in village populations in northern Thailand. AIDS 1994;8:951-955.

Training and Travel:

Dr Kriangsak Praputpittaya, co-principal investigator, was invited to attend a workshop, sponsored by BOSTID, at Catholic University of America, 22-29 September 1990, on techniques of polymerase chain reaction. Workshop was useful, but too short for in-depth training. After this workshop, Dr Kriangsak visited the laboratory of Dr James T Douglas, professor of microbiology, University of Hawaii. Dr Douglas is a consultant/collaborator on this project and he assisted project investigators to set up the ELISA assay tests and to help monitor and advise on the laboratory results.

At the invitation of National Academy of Science, National Research Council, BOSTID, Dr Diane Taylor, Associate Professor, Department of Biology, Georgetown University, made a site visit to Chiang Mai, 11-14 November 1990, to review programs, principally those supported by USAID. This was a useful visit for us, despite being arranged at fairly short notice.

Ms Namtip Srirak, the leader of the field team on this project, was awarded a TDR fellowship in October 1991 for advanced study leading to a PhD in health

behavior/medical anthropology with specific reference to behavior patterns among leprosy reaction patients. Ms Namtip is currently enrolled as a PhD student at the University of Queensland Medical School's Tropical Health Program and will finish her doctoral studies in June 1995.

Project Productivity:

Yes, the project fulfilled all objectives.

Future Work:

In this project all serum samples collected from leprosy patients, their houshold contacts and normal controls were aliquoted and stored in a -20°C freezer. We will keep an eye on the world's progress in development of new antigens of *M. leprae*. In the future we might have new antigens that promise to be more sensitive indicators of *M. leprae* infections than the antigens we used in this project. At that point we will test those promising antigens with the specimens stored in our serum bank

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