



INTERNATIONAL LEPROSY CONGRESS

Hidden challenges

BRUSSELS, 16th-19th SEPTEMBER 2013



World Health
Organization

031

FINAL PROGRAMME AND BOOK OF ABSTRACTS

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World Health
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FINAL PROGRAMME AND BOOK OF ABSTRACTS

TABLE OF CONTENTS

PROGRAMME AT A GLANCE

5	WELCOME ADDRESS FROM THE PRESIDENT - WELCOME TO BRUSSELS	
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INFORMATION

	Committees	9
	Acknowledgements	9
	Instructions for Presenters	10
	- Oral Presentations	10
	- ePoster Presentations	10
	Social Programme	11
	Student Awards	11
	General Information	12
	Plenary Speaker Biographies	14

17 PROGRAMME MONDAY

	Day at a Glance	18
--	-----------------------	----

19 PROGRAMME TUESDAY

	Day at a Glance	21
	List of abstracts for Tuesday	22
	Abstracts - Plenary Speakers	36
	Abstracts - Symposium Leaders	37
	Abstracts - Oral presentations	39
	Abstracts - ePoster presentations	69

115 PROGRAMME WEDNESDAY

	Day at a Glance	117
	List of abstracts for Wednesday	118
	Abstracts - Plenary Speakers	132
	Abstracts - Symposium Leaders	133
	Abstracts - Oral presentations	134
	Abstracts - ePoster presentations	165

213 PROGRAMME THURSDAY

	Day at a Glance	215
	List of abstracts for Thursday	216
	Abstracts - Plenary Speakers	228
	Abstracts - Symposium Leaders	229
	Abstracts - Oral presentations	230
	Abstracts - ePoster presentations	252

299 INDEX OF AUTHORS

304 SPONSORS & EXHIBITORS

	List of Sponsors	304
	List of Exhibitors	304

VENUE MAP

Management Centre Europe (MCE)

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ACKNOWLEDGEMENTS

Palma Galdas, José Augusto Da Costa Neto, S. Carlos Da Costa, Vera Dasso, Ebenezer Daniel, Maria Aires, J. de S. Gomes, E. Barros, Engelen, Sunil Daggak, Sunil Datta, V. de S. D. C. Rodrigues, Memiah Ebenezer, Jannick, A. de S. L. Engelbreksson, Paul Fine, Marco Andrey, Francisco António Garbino, Bob Gelber, Anniamak Galuk, Tom Gillis, Aakramo Gonçalves, P.K. Gopal, Jacques Grosset, Jörg Heuvelinkhous, Margaret Hogeweg, Noodle H. Holmes, Abdellatif Inoussi, Azzouzi, Eliane Ignotti, Keri Ingits, Christian Johnson, John Justice, Indira Karayawa, H.K. Kar, Joseph Kawuma, Raj Datta Lahiri, Anwei Law, Linda Lehman, Maria Leide, Daniel Lodi, Clovis Lombardi, M. Matsuoaka, Marcelo Mira, Alice Miranda, Eliane Mahrudi, Mogadam, Jean Norbet Mambou, J. de S. Sandra Nuvid Aranas, Ben Naars, Manstik, Nasun, N. de S. Peter Nicholls, Sarah Nikita, Gert Norman, Milton, S. de S. W. Pan, V. Panikar, Maria Pena, Maria Ludia, S. de S. P. Portaele, Erik Post, Mathian Arokia Rajan, Jose Ramalho, P.V. Ranganadha Rao, Jh. Richardus, Jo Robertson, Arsenio Bates, Claudio Salgado, Ferritoria Sammarco Rosa, S. de S. Sarno, Paul Saunderson, Pieter Schreuder, David S. de S. Rashi Sharma, W. Smith, Lucia Helena Soares Camargo, Douglas Sauter, Samba Sow, H. de Soyagim, John Spelman, H. Sunivasan, James Staples, Marlene Stenzel, Beremik Tarekagne Tsagave, Maria Angela Trindade, Wim van Brakel, Marcos Virmond, Magnus Vollser, Steve Walker, Cassandre White, Diana Wilfrains, Hany Ziyadi.



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330



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PROGRAMME AT A GLANCE

10:30 – 11:00

Best Clinical Practice**Screen 1, 10:30 - 10:40** P-001

POST CALAZAR DERMAL LEISHMANIASIS AND ERYTHEMA NODOSUM LEPROSUM: CASE REPORT AND LITERATURE REVIEW

Presenter: *Maria Angela Trindade***Screen 1, 10:40 - 10:50** P-003

IRON-CONTAINING PROTEINS AS A MARKER OF M. LEPRAE PERSISTENCE IN LEPROSY PATIENTS IN THE CLINICAL REGRESSION STAGE

Presenter: *Prof. Dr Oleg Degtyarev***Screen 1, 10:50 - 11:00** P-005

PREVALENCE AND CHARACTERISTICS OF NEUROPATHIC PAIN IN TREATED LEPROSY PATIENTS IN A TERTIARY CARE REFERENCE HOSPITAL

Presenter: *Artur Gosling***Epidemiological Surveillance****Screen 2, 10:30 - 10:40** P-161

CLINICAL AND EPIDEMIOLOGICAL LEPROSY PROFILE AMONG CHILDREN BELOW 15 YEARS OLD DIAGNOSED AT THE FUNDAÇÃO ALFREDO DA MATTA IN MANAUS, BRAZIL FROM JANUARY 2006 TO DECEMBER 2011

Presenter: *Maria Da Graca Cunha***Screen 2, 10:40 - 10:50** P-168

THE ACTUAL STATE OF LEPROSY IN ESTONIA – AN UPDATE REPORT AFTER 20 YEARS

Presenter: *Attyla Drabik***Screen 2, 10:50 - 11:00** P-163

A SYSTEMATIC REVIEW ON THE EPIDEMIOLOGICAL DATA OF ERYTHEMA NODOSUM LEPROSUM, A TYPE 2 LEPROSY REACTION

Presenter: *Erik Post***Epidemiological Surveillance****Screen 3, 10:30 - 10:40** P-211

SEROPOSITIVITY ANTI PGL-1 IN HOUSEHOLD CONTACTS OF CASES DIAGNOSED WITH LEPROSY

Presenter: *Ana Paula Carvalho***Screen 3, 10:40 - 10:50** P-214

DEGREE OF DEFORMITY IN LEPROSY CASES DIAGNOSED IN CHILDREN UNDER 15 YEARS OLD AND ITS RELATIONSHIP WITH OPERATIONAL AND EPIDEMIOLOGICAL FACTORS

Presenter: *Angélica Fabri***Screen 3, 10:50 - 11:00** P-213

DEATHS BY LEPROSY AS THE UNDERLYING CAUSE MORTO GROSSO FROM 2000 TO 2007.

Presenter: *Eliane Ignotti***Prevention of Disability****Screen 4, 10:30 - 10:40** P-225

KEY MODALITIES OF FIELD AREA DISABILITY CARE

Presenter: *Dr Atul Shah***Screen 4, 10:40 - 10:50** P-226

DPMR CAMPS - A PRAGMATIC APPROACH TO RENDER DISABILITY CARE SERVICES AND TRAIN HEALTH CARE STAFF

Presenter: *Dr Atul Shah***Screen 4, 10:50 - 11:00** P-227

MONITORING OUTCOMES AT THE END OF ANTIBIOTIC TREATMENT USING BU01, POD AND BUFLS FORMS WITH 23 NEW CASES IN 2012 AT KUKUOM HEALTH CENTER ASUNAFU SOUTH DISTRICT, BRONG AHAFO REGION OF GHANA

Presenter: *Linda Lehman***Leprosy Control - Urban and Special Populations****Screen 5, 10:30 - 10:40** P-038

CHILD CARE CAMPS FOR DISABILITY PREVENTION AND CARE FOR RFT CASES

Presenter: *Dr Atul Shah***Screen 5, 10:40 - 10:50** P-039

IMPARTING AWARENESS ABOUT LEPROSY AMONG CHILDREN OF MADRASAS SCHOOLS, AS A NEW CASE DETECTION METHOD

Presenter: *Abraham Selvasekar***Screen 5, 10:50 - 11:00** P-041

RELAPSE OF HANSEN'S DISEASE DIAGNOSED BY LEPROMA IN NASAL CAVITY-A CASE REPORT.

Presenter: *Yoshiko OKANO***Leprosy Control - Urban and Special Populations****Screen 6, 10:30 - 10:40** P-058

EPIDEMIOLOGICAL SITUATION OF LEPROSY IN URBAN AREAS IN INDIA - A RAPID ASSESSMENT STUDY

Presenter: *Venkata Ranganadha Rao Pemmaraju***Screen 6, 10:40 - 10:50** P-059

TREND OF SMEAR POSITIVE CASES IN THE URBAN SLUMS OF MUMBAI - A FIELD STUDY IN MUMBAI

Presenter: *Vivek Pai***Screen 6, 10:50 - 11:00** P-060

TWO WOMEN FROM THE SAME FAMILY WITH SIMILAR EXPERIENCES WITH LEPROSY

Presenter: *Nicole Holmes***History of Leprosy****Screen 7, 10:30 - 10:40** P-124

HISTORY OF LEPROSY IN SPAIN

Presenter: *Jose Terencio de las Aguas***Screen 7, 10:40 - 10:50** P-125

DIVERSITY OF MYCOBACTERIUM LEPRAE ON THE BASIS OF REPETITIVE SEQUENCES OF TTC FROM ANCIENT BONES FOUND IN BALI AND EAST NUSA TENGGARA, EAST INDONESIA

Presenter: *Bimo aksono*

PRESENTATIONS

access to services. Considering the above results of the rapid assessment, leprosy control model in urban settings needs to include activities like training of all health staff in leprosy, improving awareness on leprosy in the community and access to health services particularly in inadequately served areas like urban slums and peri-urban villages.

P-059

Presentation Time: Tuesday 17/09/2013 at 10:40 – 10:50
Abstract Topic Name: Leprosy Control – Urban and Special Populations
Presentation Screen Number: 6
Presenter: Vivek Pai

TREND OF SMEAR POSITIVE CASES IN THE URBAN SLUMS OF MUMBAI – A FIELD STUDY IN MUMBAI

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Introduction: Bombay Leprosy Project (BLP) covers an urban population of 2 million comprising mainly of slums including Dharavi one of the biggest slums in Asia. How leprosy case detection and treatment is managed in the post integration scenario in Mumbai was reported earlier in 2008 (Ganapati et al 2008). We present our experience in BLP in the post integration period (July 2004 to 2012) pertaining to the study of occurrence of new smear positive patients in urban slums of Mumbai.

Methods: The leprosy programme was integrated with the Health Posts (HP) of the General Health Care System (GHC) in Mumbai (population: 12 million) in July 2004. Health delivery in the city of Mumbai is highly complex. The health structure primarily comprises of HP, medical colleges besides the non teaching hospitals. There are also General practitioners and Practicing dermatologists besides several specialists and corporate and private hospitals. Keeping in mind this backdrop, BLP has been offering services after reorganization post integration through few satellite clinics and extension units in public hospitals. These clinics are being strengthened and retained at the ward level and services sustained. Monitoring of detection of new cases with special emphasis on smear positive cases was undertaken and analyzed for the period after integration in the city of Mumbai.

Results: From July 2004 till December 2012, a total of 158 smear positive cases were detected out of 945 total new cases detected and registered for treatment in the Project area in a population of 2 million giving a trend of an average of 17 (16.7%) new smear positive cases in a year. Most of these cases reported directly to the satellite clinics and referral centre and teaching medical colleges and a few practicing dermatologists. These cases as well as those identified through catchment clinics of BLP were confirmed by senior supervisory staff.

Conclusion: It is observed that there is a static trend in detection of new smear positive cases in project area during the study period indicating a constant pool of reservoir of infection in the community. Though the practice of taking skin smear is done away with in the routine programme, BLP has been continuing the practice of taking skin smears to identify the quantum of reservoir of infection responsible for chain of transmission of infection in the slums.

P-060

Presentation Time: Tuesday 17/09/2013 at 10:50 – 11:00
Abstract Topic Name: Leprosy Control – Urban and Special Populations
Presentation Screen Number: 6
Presenter: Nicole Holmes

TWO WOMEN FROM THE SAME FAMILY WITH SIMILAR EXPERIENCES WITH LEPROSY

N. H. Holmes¹

¹IDEA, Rex, United States

Introduction: My aunt, Michelle McKenzie, and I were born in Trinidad, but while she grew up there, I immigrated to the United States and lived in Brooklyn, NY. Although we were separated by hundreds of miles, we both contracted leprosy, and were the only ones in our family to do so. We may have had different experiences, but many similar themes related to stigma, social isolation and rejection, as well as self image and depression, came about in the course of our illness.

Methods: Michelle and I would share our experiences with each other, and she was a constant reminder to me, that I would someday be well again. In preparation for this presentation, I came up with a list of questions that I emailed to my aunt in order to determine her experiences related to her diagnosis, treatment, and recovery; as well as her mental and emotional health, physical, and social changes. I also collected photos of my aunt and myself before, during, and after treatment to further document the transformations we both endured in all aspects of our lives.

Results: My aunt and I were both diagnosed in our late teens while in higher education. We noticed changes in our bodies that prompted us to seek medical attention, and resulted in us being diagnosed with leprosy. Michelle describes that "I was devastated, depressed and angry. Asking God why he did this to me, and thinking I was being punished for my sins." I too felt this

way, would often recreate in my mind my life's actions, and which thing I did in particular that warranted this punishment.

I was impacted by how people treated me, in particular, some medical professionals who regarded me as a specimen instead of a person, or did not want to interact or deal with me because of my illness. I still think about the doctor who did not want to come into his office to greet me, but instead choose to speak to me from across the room, in the safety of the doorway. Michelle explains that "I was treated with scorn and neglected. Nurses didn't want to touch me, but this was because of the Steven Johnson's Syndrome." In addition to my aunt coping with a diagnosis of leprosy, she also suffered from the effects of having an allergic reaction to Dapsone. There were also times we had to pick and choose who we shared our diagnosis with, in fear of the response we would receive. My aunt's boyfriend left her shortly after he learned about her diagnosis, and I rarely told anyone about my illness in the beginning.

We were both active, pretty, young women at the time we were diagnosed, who were admired and looked up to by family and friends. As a result of the side effects of the medications we took, our complexions changed, we both had scars from nodules, and we gained weight. This resulted in changes in how we now saw ourselves, and in our self-confidence. We both endured periods of depression, but were able to seek out and get help through counseling.

Conclusion: My aunt and I may have lived in different countries, but our stories are similar. Michelle and I share the same message because of our struggles with leprosy, and our acceptance and appreciation for how it has shaped our lives for the better. My aunt expresses this best when she says: "My experience with this disease has made me stronger and closer to God. It has taught me that looks are not all in life, and that you must love yourself first, before you can love others."

P-124

Presentation Time: Tuesday 17/09/2013 at 10:30 – 10:40
Abstract Topic Name: History of Leprosy
Presentation Screen Number: 7
Presenter: Dr Jose Terencio de las Aguas

HISTORY OF LEPROSY IN SPAIN

J. Terencio De Las Aguas¹

¹Jose Terencio de las Aguas, DENIA, Spain

Introduction: Leprosy arrived in Spain first through Phoenicians who came to Andalusia, and later through Roman soldiers and civil servants who came from the Middle East and remained in Spain for 600 years. Then the Muslim presence for 8 centuries, being Valencia, Murcia and Andalusia the most affected areas.

Methods: The first leper colonies were founded in the Christian Spain, Barcelona, Asturias Galicia; and after the Reconquest Valencia, Granada, Sevilla, Málaga and Canarias Island. Avicenna and Averroes from Muslim Spain and Arnau de Vilanova from the Christian Spain are quoted to be among the most important medical figures.

Results: It is discussed the growing of leprosy during the 19th century, especially in Valencia, fact that caused the foundation of the Fontilles sanatorium in 1909, commenting its important medical attention, investigation and teaching roles; in the same way the great work of the Spanish dermatology in the diagnosis and treatment of sick people what has led to the fact that of the 600 cases that there were in the sixties, with an annual rate of 300 new cases, in the last 10 years are only diagnosed 10 to 14 new cases, being immigrants the 90%.

Conclusion: Nowadays leprosy is not a danger for the Spanish Public Health and it will never be an emergent disease.

P-125

Presentation Time: Tuesday 17/09/2013 at 10:40 – 10:50
Abstract Topic Name: History of Leprosy
Presentation Screen Number: 7
Presenter: Bimo Aksono

DIVERSITY OF MYCOBACTERIUM LEPRAE ON THE BASIS OF REPETITIVE SEQUENCES OF TTC FROM ANCIENT BONES FOUND IN BALI AND EAST NUSA TENGGARA, EAST INDONESIA

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Introduction: Excavations of the genetic material of pathogenic organisms in the ancient bones provide important information for the study of certain infectious diseases in ancient populations. In addition, the identification of bacterial DNA provides direct evidence and the frequency of occurrence of infectious diseases in ancient populations and may provide information about the evolution of microorganisms and related diseases. Several recent reports have succeeded in

isolating several *Mycobacterium* by using PCR technique, because the PCR technique, although very small amount of DNA in ancient biomaterials such as bone or soft tissue but can be identified. This new approach not only of knowledge related to the evolution of different strains of *Mycobacterium*, but may also provide correlative data on the influence of environment on the development of *Mycobacterium* and biodiversity. However until now has never reported any *Mycobacterium* especially *Mycobacterium leprae* was found in a ancient bones from Indonesia, so too has not been widely reported throughout the world of *M. leprae* from ancient bones found on the old 2990 +/- 160 BP. The purpose of this study was performed diversity analysis of *M. leprae* on the basis of repetitive sequences of TTC from ancient bones found in Bali and East Nusa Tenggara, East Indonesia.

Methods: One of ancient bones who lived 2990 +/-160 BP from Lembata Island-Flores, Indonesia (code LL 1/5) and the one of ancient bones who lived Paleometallic period derived from Semawang-Bali, Indonesia (code SMW/III/1990). The DNA extraction was performed using a kit from Qiagen products and its TTC repeating pattern were seen with the method of direct sequencing.

Results: The inner part of the ancient bone from Lembata Island-Flores, Indonesia (code LL 1/5) was obtained by 13 repetitions TTC and the one derived from Semawang-Bali was obtained by 20 repetitions TTC. The different number of TTC repetitions have showed the different isolates of *M. leprae* between in the ancient bone from Lembata-Island-Flores, Indonesia and from Semawang-Bali, Indonesia

Conclusion: The result towards of TTC. Its commonly show that 13X TTC motif was found of ancient bone from Flores, Indonesia. Whereas 20X TTC motif was found of ancient bone from Bali, Indonesia. If it was related to leprosy spreading in Indonesia. That alot of them were found in East Indonesia. Whereas in the middle area was few relatively and it West area, it was none relatively, except in Aceh. In historical, if it was indeed so leprosy always follows in human migration from Asia continent to Indonesia. So it shouldn't empty space of leprosy in the middle area. in spite of it was estimated that also interrelated to the influences of Wallacea area, that covered Sulawesi, Maluku and Papua which have different environment like in West area excited.

P-127

Presentation Time: Tuesday 17/09/2013 at 10:50 - 11:00
Abstract Topic Name: History of Leprosy
Presentation Screen Number: 7
Presenter: Mamina Turegano

THE RISE AND FALL OF CHAULMOOGRA OIL

S. A. Norton *

*Dermatology, Children's National Medical Center, Washington, DC, United States

Introduction: For many decades, chaulmoogra oil was the treatment of choice for leprosy but in the past 75 years, chaulmoogra has vanished from our formularies and very nearly from our memories. This presentation will review chaulmoogra: its botany, its use in traditional Asian medicine, its entry into Western medicine, and its place on the history, lore, art, and literature of leprosy.

Methods: Chaulmoogra oil (CO) is extracted from seeds of several closely related trees in the genus *Hydnocarpus*, found in scattered areas in southeast Asia. CO entered Western medicine in the mid-1800's when British Army physicians reported that traditional healers in India's Western Ghats treated leprosy with oil made from crushed seeds of an unknown tree. For the next half-century, Western supplies of CO were obtained from bazaars in India and Indochina but the product was scarce and often of poor quality. Any doubts about CO's efficacy, however, were regarded as the fault of adulterated supplies of CO.

Results: To provide reliable oil for leprosy patients in the US and territorial Hawaii, the United States government decided to create its own chaulmoogra plantation. At this time, only a few remote hill tribes knew which trees produced the desired seed so the US Department of Agriculture sought a botanist for a chaulmoogra collecting expedition. They chose Joseph Rock, instructor of botany and Chinese languages at Honolulu College in Hawaii, Viennese by birth and sinophile by nature, Rock learned to speak several Chinese dialects as a youth and emigrated to Hawaii in 1907, where he gained recognition as an authoritative tropical botanist. In 1920, the USDA dispatched Rock to southeast Asia, where he traveled among hill tribes of Burma, Siam, and Assam for nearly 2 years before identifying which trees produced chaulmoogra seeds. Rock's expedition through Southeast Asia and his early explorations of Tibet are described in *Lamas, Princes, and Brigands* by Michael Aris, (deceased) husband of Burmese Nobel laureate, An Song Su Kyi.

With Rock's seeds, the USDA started a chaulmoogra plantation in Oahu's rugged Waiahole Valley. Within a few years, maturing trees provided enough seed to treat American leprosy-patients in Carville and Kalaupapa. Soon the valley supplied much of the world's CO and major pharmaceutical corporations used Waiahole seed to formulate new anti-leprotics. Treatment with CO was painful, however - up to 5ml was injected subcutaneously or intramuscularly daily for months. Many patients found this unacceptably painful and considered letting their disease go untreated.

Arthur Dean, president of University of Hawaii and a distinguished biochemist, studied chaulmoogra's properties and identified the chemical structures of its allegedly active components, chaulmoogrin and hydnocarpin. There were several theories on CO's mechanism

of action but proper studies on its efficacy and safety were never conducted. Although *in vitro* investigations of chaulmoogra still appear in the biochemistry literature, we still do not know if it had any clinical efficacy. In the 1930s, sulfone medications were developed and now, dapsone (along with rifampin, clofazimine, & thalidomide) have made leprosy an entirely treatable disease.

Conclusion: One hundred years ago, the chaulmoogra branch symbolized international efforts to combat leprosy. Now chaulmoogra appears mostly as footnotes in quaint and curious volumes of forgotten lore.

P-143

Presentation Time: Tuesday 17/09/2013 at 10:30 - 10:40
Abstract Topic Name: Microbiology
Presentation Screen Number: 8
Presenter: Yuji Miyamoto

METABOLOME ANALYSIS OF MYCOBACTERIUM LEPRAE

Y. Miyamoto ^{1,*}, M. Matsuoka ¹, Y. Fukutomi ¹, T. Mukai ¹, M. Kai ¹, Y. Maeda ¹, M. Makino ¹

¹National Institute of Infectious Diseases, Higashimurayama, Japan

Introduction: Mycobacteria have a characteristic feature that the cell envelope is composed of complex molecules. Among them, glycolipids are abundantly present on the cell surface, and they are reported to be associated with pathogenicity of mycobacteria. On the other hand, the functional and quantitative profiles of intracellular metabolites such as organic acids, amino acids, nucleic acids, which are responsible for maintaining the cellular metabolism of the bacteria, are still unclear. *Mycobacterium leprae*, causative agent of leprosy, is known as the only bacteria that cannot proliferate *in vitro*. Thus, it is predicted that *M. leprae* possesses the specific metabolic systems that are different from other bacteria. In this study, to elucidate the specific metabolism involved in pathogenicity of *M. leprae*, the metabolome analysis, which is a powerful tool for clarification of comprehensive metabolism, was performed.

Methods: *M. leprae* Thai-53 strain was inoculated into the foot-pad of nude mouse and was grown for 9 months. According to the procedure previously reported, foot-pad were homogenized by Hanks' balanced salt solution (HBSS), and incubated in 0.05% trypsin at 37°C for 60min and 1% sodium hydroxide at 37°C for 15min, resulting in isolation of *M. leprae*. *Mycobacterium bovis* BCG Tokyo was harvested from 14 day-culture of 7H9 broth supplemented with 10% ADC enrichment. *M. bovis* BCG was further mixed with foot-pad of non-infected nude mouse and the resulting mixture was also subjected to above procedure of *M. leprae* isolation. For preparation of intracellular metabolites, harvested mycobacterial cells were exposed to methanol followed by water extraction and ultrafiltration. Resulting extracts were subjected to Capillary electrophoresis-mass spectrometry (CE-MS) analysis. Relative quantity was estimated by peak area of detected cationic or anionic metabolites compared with that of internal standard and also calibrated by equal amount of mycobacterial cells.

Results: Since *M. leprae* was isolated from nude mouse foot-pad by specific procedure including treatment of alkaline solution, it was necessary to ascertain that the following possibilities could be eliminated: (i) the remnants of mouse tissue metabolites from foot-pad was not completely excluded during *M. leprae* isolation procedure and consequently could be contaminants (ii) components of intracellular metabolites were altered by the isolation procedure. Firstly, as control mycobacteria, *in vitro*-grown *M. bovis* BCG was used for analysis. Next, we prepared the mixture of above *M. bovis* BCG and intact nude mouse foot-pad, followed by isolation procedure in a similar way to *M. leprae* (designated *M. bovis* BCG-foot-pad). CE-MS analysis was performed on intracellular extract from *M. leprae*, *M. bovis* BCG and *M. bovis* BCG-foot-pad. Comparison of their mass spectrum with those from known compounds revealed that quantitative ratio of several amino acid species were around 10-40 fold higher than those of both *M. bovis* BCG and *M. bovis* BCG-foot-pad. Their elevated level in *M. leprae* was assumed to be due to intrinsic metabolism, and not an artifact of the isolation procedure described above, because the level in *M. bovis* BCG is approximately the same as that in *M. bovis* BCG-foot-pad.

Conclusion: CE-MS analysis for retrieving the quantitative profiles of intracellular metabolites demonstrated that several species of amino acid were significantly accumulated in the extract of *M. leprae*, compared with that of *M. bovis* BCG.

334



DIVERSITY OF MYCOBACTERIUM LEPRAE ON THE BASIS OF REPETITIVE SEQUENCES OF TTC FROM ANCIENT BONES FOUND IN BALI AND EAST NUSA TENGGARA, EAST INDONESIA

B. Aksono¹, D. Adriaty¹, R. Wahyuni¹, Iswahyudi¹, I. Agusni¹, S. Izumi², Nasronudin², M. I. Lusida¹, T. Koesbardiat^{1, 2}

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INTRODUCTION

Excavations of the genetic material of pathogenic organisms in the ancient bones provide important information for the study of certain infectious diseases in ancient populations. In addition, the identification of bacterial DNA provides direct evidence and the frequency of occurrence of infectious diseases in ancient populations and may provide information about the evolution of microorganisms and related diseases. Several recent reports have succeeded in isolating several *Mycobacterium* by using PCR technique, because the PCR technique, although very small amount of DNA in ancient biomaterials such as bone or soft tissue but can be identified. This new approach not only of knowledge related to the evolution of different strains of *Mycobacterium*, but may also provide correlative data on the influence of environment on the development of *Mycobacterium* and biodiversity. However until now has never reported any *Mycobacterium* especially *Mycobacterium leprae* was found in a ancient bones from Indonesia, so too has not been widely reported throughout the world of *M. leprae* from ancient bones found on the old 2990 +/- 160 BP. The purpose of this study was performed diversity analysis of *M. leprae* on the basis of repetitive sequences of TTC from ancient bones found in Bali and East Nusa Tenggara, East Indonesia.

OBJECTIVES

The purpose of this study was performed diversity analysis of *M. leprae* on the basis of repetitive sequences of TTC from ancient bones found in Bali and East Nusa Tenggara, East Indonesia.

METHODS & MATERIAL

One of ancient bones who lived 2990 +/- 160 BP from Lembata Island-Flores, Indonesia (code LL 1/5) and the one of ancient bones who lived Paleometallic period derived from Semawang-Bali, Indonesia (code SMW/III1990). The DNA extraction was performed using a kit from Qiagen products and its TTC repeating pattern were seen with the method of direct sequencing.

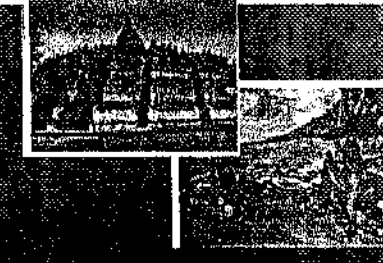
Figure 2. PCR products of DNA *M. leprae*. Lane 1-4: PCR products (99 bp) using primers Lp1-Lp2 (outer) and Lp3-Lp4 (inner). Lane 1: PCR products using Kit Qiagen methods. Lane 2: PCR products using conventional methods. Lane 3: Negative controls. Lane 4: Positive controls (DNA *M. leprae* Thais53). Lane 5-8: PCR products (129 bp) using primers LpF-LpR (outer) and Lp1-Lp2 (inner). Lane 5: PCR products using Kit Qiagen methods. Lane 6: PCR products using conventional methods. Lane 7: Negative controls. Lane 8: Positive controls (DNA *M. leprae* Thais53). Lane 9: 100bp DNA ladder.

CONCLUSION

The result towards of TTC, its commonly show that 13X TTC motif was found of ancient bone from Flores, Indonesia. Whereas 20X TTC motif was found of ancient bone from Bali, Indonesia. If it was related to leprosy spreading in Indonesia. That alot of them were found in East Indonesia. Whereas in the middle area was few relatively and in West area, it was none relatively, except in Aceh. In historical, if it was indeed so leprosy always follows in human migration from Asia continent to Indonesia. So it shouldn't empty space of leprosy in the middle area. In spite of it was estimated that also interrelated to the influences of Wallacea area, that covered Sulawesi, Maluku and Papua which have different environment like in West area excited.

RESULTS

The inner part of the ancient bone from Lembata Island-Flores, Indonesia (code LL 1/5) was obtained by 13 repetitions TTC and the one derived from Semawang-Bali was obtained by 20 repetitions TTC. The different number of TTC repetitions have showed the different isolates of *M. leprae* between in the ancient bone from Lembata Island-Flores, and from Semawang Bali-Indonesia.



ACKNOWLEDGMENT

This study was supported by the Directorate General of Higher Education, Department of National Education, Indonesia, Rector of Airlangga University and Mr. Rusyad Adi Suriyanto



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