

RINGKASAN

Deteksi DNA *Mycobacterium leprae* Pada Sumber Air Penduduk di Daerah Endemik Kusta**Studi Epidemiologi Molekuler di Kabupaten Sumenep**

Dinar Adriaty

Sejak tahun 1993 WHO telah mencanangkan program “*Elimination of Leprosy by year 2000*”, dimana seluruh negara di dunia harus menurunkan prevalensi kusta di bawah 1 per 10.000, bertujuan agar kusta tidak lagi menjadi masalah kesehatan. Pada dasarnya, program yang dicanangkan oleh WHO telah berhasil sesuai target, tetapi di beberapa negara berkembang, termasuk Indonesia penyakit kusta masih menjadi masalah kesehatan. Saat ini meskipun angka yang dicapai Indonesia adalah 0,84 per 10.000 penduduk, namun tidak semua wilayah di Indonesia bisa mencapai angka tersebut. Masalah kusta di Indonesia, menurut disebabkan karena beberapa propinsi di Indonesia masih terdapat daerah endemis (daerah kantong) penyakit kusta yang ternyata adalah daerah sulit dijangkau dan terpencil, menyebar terutama di beberapa kawasan Indonesia Timur sehingga mempersulit penanggulangan.

Hingga pertengahan tahun 2004, angka prevalensi kusta di Propinsi Jawa Timur sebesar 1,39 per 10.000 penduduk yang menyebar pada 38 kabupaten/kota dengan jumlah penderita terdaftar sebanyak 4298 penderita. Dari seluruh kabupaten/kota tersebut Kabupaten Sampang menduduki urutan pertama prevalensi kusta yaitu 6,41, kemudian diikuti Sumenep 6,29, Pamekasan 4,01, Lamongan 3,94 dan Tuban 3,54 per 10.000 penduduk. Kabupaten Sumenep merupakan salah satu daerah endemik kusta yang masih memiliki daerah kantong dengan angka prevalensi yang sangat tinggi. Kecamatan Talango adalah salah satu daerah kantong endemik kusta dengan angka prevalensi kusta sebesar 23,6 per 10.000 penduduk. Kecamatan Talango memiliki jumlah penduduk sebesar 39.479 tersebar di 8 desa, dimana 5 dari 8 desa di kecamatan tersebut memiliki angka prevalensi diatas 20 per 10.000 penduduk.

Selama dekade terakhir pemberantasan kusta di Jawa Timur pada umumnya telah berhasil menurunkan prevalensi kusta, namun insidens kusta baru tetap bertahan terutama di daerah endemis (daerah kantong) walaupun kasus aktif sebagai sumber infeksi telah diobati. Hal ini mungkin disebabkan antara lain karena : adanya *backlog case* yakni adanya kasus yang tidak terdeteksi dan tidak mendapat terapi, adanya infeksi subklinis yang tidak terdeteksi pada populasi, dan kemungkinan adanya sumber penularan / reservoir di luar manusia, yang menyebabkan kontrol, eliminasi dan eradikasi kusta pada manusia menjadi sulit. Dari berbagai penelitian epidemiologi, timbul kecurigaan bahwa banyak individu yang terinfeksi basil kusta tanpa adanya sumber penularan yang jelas atau tidak

ditemukannya penderita kusta yang menjadi sumber penularan, terjadi antara lain disebabkan oleh penularan secara tidak langsung yakni melalui lingkungan hidup.

Pada dasawarsa terakhir, ilmu biologi molekuler telah dipergunakan untuk mendeteksi basil *M. leprae*, diantaranya adalah dengan menggunakan teknik *Polymerase Chain Reaction* (PCR). Deteksi *M. leprae* dengan PCR pertama kali dipergunakan dan dikembangkan oleh Klatser untuk mendeteksi adanya basil kusta dari spesimen biopsi penderita kusta hingga kini berkembang berbagai metode terapan PCR. PCR merupakan suatu cara *in vitro* untuk memperbanyak DNA suatu organisme dengan menggunakan enzim polimerase yang diarahkan oleh potongan urutan DNA yang spesifik bagi DNA organisme tersebut. Berbagai variasi teknik PCR telah dilaporkan, meliputi amplifikasi berbagai rangkaian DNA target yang telah digunakan untuk deteksi *M. leprae*. Umumnya terdapat rangkaian DNA yang mengkode sebagian besar antigen seperti 18 kDa, 36 kDa, 65 kDa, atau rangkaian penyandi non antigen seperti *M. leprae specific repetitive sequence* atau *ribosomal RNA sequences*.

Penelitian ini bertujuan untuk menganalisa kejadian positif DNA *M. leprae* dari sumber air yang dipakai penduduk di daerah endemis kusta, deteksi tersebut memakai metode PCR yang menggunakan primer Lp1, Lp2, Lp3, Lp4 dimana primer tersebut menyandi daerah 18 kDa antigen *M. leprae* regio RLEP *repetitive sequence*. Dari hasil PCR yang dilakukan terhadap 34 sampel air sumur di desa Kombang didapatkan 13 sampel positif mengandung DNA *M. leprae* (38%) dan dari 35 sampel air sumur di desa Gapurana didapatkan 6 sampel positif mengandung DNA *M. leprae* (17%). Uji *Chi-Square* menunjukkan bahwa terdapat perbedaan bermakna antara kedua daerah tersebut dalam insiden positifitas PCR pada air sumur yang digunakan penduduk $p < 0,05$ ($p = 0,045$). Hasil penelitian lebih lanjut dari sampel air sumur di desa Kombang dan Gapurana menunjukkan bahwa PCR positif terbanyak berasal dari sumur yang tidak ada penderita. Hal ini menunjukkan bahwa adanya *M. leprae* di dalam sumber air penduduk tidak tergantung ada tidaknya penderita kusta di daerah tsb dan juga tidak tergantung tipe kusta dari pengguna sumber air tsb. Tampaknya *M. leprae* memang dapat bertahan hidup di alam lingkungan sumber air tsb.

SUMMARY

Detection *Mycobacterium leprae* DNA from Water Resource in Endemic Leprosy Areas

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Since WHO (World Health Organization) proclaimed “*Elimination of Leprosy by year 2000*” in 1993, the global prevalence of leprosy has declined significantly in the last 10 years. However, this decline does not correspond to a reduction of the new leprosy cases in the world. This condition has also remained the same in Indonesia. After following the MDT (Multi Drug Therapy) treatment program, the prevalence rate in Indonesia has reduced into 0,84 per 10.000 inhabitants. In contrast to the prevalence rates, the detection rate (CDR) of leprosy, have remained unchanged over the last 10 years, this caused leprosy is still one of health problem in Indonesia. The most important unsolved problems in epidemiology of leprosy are the highly uneven geographic distribution of the disease. There are many hyper endemic “pocket” areas in some endemic countries, spreading in several provinces especially in the east of Indonesia.

East Java province is the one of many provinces which has some pockets areas of hyper endemic leprosy. Until in the middle of 2004, prevalence rate in East Java province still 1,39 per 10.000 inhabitants distribute to 38 districts with 4298 registered cases. From whole districts in East Java Province, Sampang has the highest prevalence rate (6,41), followed by Sumenep (6,29), Pamekasan (4,01), Lamongan (3,94) and Tuban (3,54) per 10.000 inhabitants. Sumenep is one of endemic areas which have many hyper endemic areas especially in the islands region that still isolated from outsider. One of them is Talango Island that has 39.479 inhabitants living in 8 villages with prevalence of leprosy is 24,1 per 10.000 inhabitants.

According to the theory, these major problems in leprosy might be caused to: backlog cases, sub clinical leprosy among the healthy people and probably due to the existence of non human reservoir in the environment of endemic leprosy areas. Some environmental factors are suspected to be an important role in *M.leprae* infection and transmission of the disease other than patients. However, there a considerable number of epidemiological and microbiological observations lead us to explore the real fact on this matter happen in those endemic places in East Java.

In order to better understand the role of *M. leprae* transmission among endemic leprosy areas, a molecular biology method called Polymerase Chain Reaction (PCR) was performed. This method can detect a 99-bp fragment of the 18 kDa antigen *M.leprae* RLEP *repetitive sequence*. This technique has specific and sensitive for DNA *M.leprae*, so it can be reliable to investigate the DNA *M.leprae* in specimens such as skin slit smear, biopsy tissue or from the environment.

From PCR detection with expected lengths were compared a comparative study on the incidence of *M. leprae* in the water between two villages that represented highly endemic leprosy area and lower endemic leprosy area is conducted. The result shows that 13 out of 34 water samples from highly endemic leprosy area positive. Lower endemic leprosy area has 6 positive PCR out of 35 water samples. The correlation between the presence of DNA *M.leprae* in the water and prevalence of leprosy in two villages was showed that there was statistically highly significant $p < 0,05$ ($p = 0,045$).

Analysis data using Chi-square also shows that there was no statistically significant difference between the number of patients who used the water and positive PCR DNA *M.leprae* from water samples. Another result shows that there was no statistically significant difference between the type of leprosy and positive PCR of DNA *M.leprae*.

All the information shows that possible epidemiological roles of the bacilli in the environment influence to transmission of leprosy in endemic leprosy area. Although the results seems strongly suggest water as a probable source of infection, more investigation still need to explain the existence of live bacilli and how can they infect to human. However, this information has benefit in order to show that the leprosy eradication program which is still ongoing, such as early detection of *M.leprae*, prevention, promotion among the inhabitants in endemic leprosy area and it must be continuously conducted by public health official.

ABSTRACT

Detection *Mycobacterium leprae* DNA from Water Resource in Endemic Leprosy Areas

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One of the most important unsolved questions in epidemiology of leprosy is the highly uneven geographic distribution of the disease. There are many hyper endemic “pocket” in endemic countries. Little is known about the reason why leprosy is still hyper endemic in these areas.

Some environmental factors were suspected to be an important role in *M.leprae* infection and transmission of the disease other than patients. However, there is considerable number of epidemiological and microbiological observations leads us to explore the real happened in those endemic places in East Java.

The aim of this research is analyzing statistically using Chi-square, correlation between the presence of DNA *M.leprae* in the water and prevalence of leprosy in two villages. Water is being taken from well in highly endemic leprosy area and lower endemic leprosy area in Kecamatan Talango, Kabupaten Sumenep; a small island which has prevalence rate more than 20 per 10.000. All PCR products has expected length about 99bp come from region 18 kDa antigen *M.leprae* RLEP repetitive sequence using primers Lp1, Lp2, Lp3, Lp4 recommended by Donoghue et.al. The result shows that 13 out of 34 water samples from highly endemic leprosy area positive. Lower endemic leprosy area has 6 positive PCR out of 35 water samples. The correlation between the presence of DNA *M.leprae* in the water and prevalence of leprosy in two villages was showed that there was statistically highly significant $p < 0,05$ ($p = 0,045$).

Keywords: *Mycobacterium leprae*, DNA, water, endemic leprosy