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## ***Mycobacterium tuberculosis* Isolates of Beijing Genotype in Thailand**

**To the Editor:** Anh and colleagues recently reported that in new tuberculosis (TB) cases from Vietnam, *Mycobacterium tuberculosis* isolates of the Beijing genotype are associated with younger age and, in isolates from Ho Chi Minh City, with resistance against isoniazid and streptomycin (1). However, occurrence of Beijing genotype *M. tuberculosis* strains may be different in other Southeast Asian countries such as Thailand.

From May 1999 to June 2000, we obtained 244 *M. tuberculosis* isolates from TB patients at Ramathibodi Hospital, Bangkok, Thailand (the hospital treats approximately 625 TB patients annually). The isolates have been prospectively analyzed by DNA-fingerprinting with the spoligotyping method (2). Drug-resistance testing and recording of patients' data (sex, age, geographic origin) were completed for 204 of 244 patients. The 204 patients

originated from all six regions of Thailand, although the central region (comprising the Bangkok area) and the northeast region predominated (59% and 23.5%, respectively). Altogether 111 male and 93 female patients with a median age of 34 years (1 to 89 years) were included. Status of BCG vaccination or HIV infection was not assessed.

The Beijing genotype was found in 90 (44.1%) of the 204 isolates analyzed in detail, without significant differences regarding the respective patients' geographic origin or sex. Thus in Thailand, the frequency of the Beijing type is somewhere between the frequency in Vietnam (53%) (1) and in peninsular Malaysia (estimated at 24%) (3). Using the same age groups as Anh and colleagues, we did not find an association of Beijing genotype with young age ( $p = 0.41$ ; chi-square test for trend). Although Beijing type isolates were more frequent among patients <25 years (18 [56%] of 32) than among those >25 years (69 [43%] of 161), this was not significant ( $p = 0.13$ ). This association remained not significant, if only isolates from the central or the northeast region were analyzed.

Of 204 isolates, 62 (30%) showed resistance to  $\geq 1$  of 4 drugs tested (isoniazid, 8.8%; rifampicin, 6.4%; streptomycin, 19.6%; ethambutol, 4.9%). However, overall drug resistance, resistance to single drugs, and multidrug resistance were not associated with the Beijing genotype. The frequency of resistance was similar in distribution but overall lower than reported for the Ho Chi Minh City isolates (isoniazid, 24%; rifampicin, 2%; streptomycin, 31%; ethambutol, 2%) (1).

In both studies, the highest percentage of drug resistance was found for streptomycin. In our sample, this was not associated with particular spoligotypes or with geographic origin of the patient. Furthermore, streptomycin-resistant isolates were not more frequent in older age groups, although there was a nonsignificant trend ( $p = 0.12$ ; chi-square<sub>trend</sub>). Streptomycin is still used for standard quadritherapy in Thailand, and occurrence of resistant strains can reflect selection or transmission recently or in the past. This differs from occurrence of streptomycin resistance in countries where streptomycin is no longer used in standard therapy (4).

In the original description of the Beijing family of strains in 1995, Beijing genotype isolates were found in 7 (37%) of 19 Thai isolates (5). In a subsequent IS6110 restriction fragment length polymorphism analysis, 80 (38%) of 211 isolates from central Thailand collected in 1994 to 1995 belonged to the Beijing family (6). Whether 90 (44%) of 204 among our recent isolates reflect a reliable increase in Beijing type transmission over the last 5 years, is difficult to state. However, the fact that no correlation of Beijing type with (young) age of the patient was observed in the previous analysis (6) supports the notion that increasing incidence of the Beijing strain in Thai cases is not due to recent transmission.

The *M. tuberculosis* population appears to be considerably more heterogeneous in Thailand than in the large urban areas of Vietnam. In our study, the three most common spoligopatterns besides Beijing, S156, S153, and S22 (according to the nomenclature of Soini et al. [7]), together comprised 20% of 244 isolates. However, the second most frequent spoligopattern, the "Vietnam genotype" (S10 according to Soini), reportedly shared by 27% of the Vietnamese isolates, was not found in our sample of

Thai isolates. Only 20 different spoligopatterns were found among 499 isolates in the Vietnam study, compared to 60 among 244 isolates in our study.

Although the Beijing genotype of *M. tuberculosis* has been recognized in settings of emerging drug resistance around the world, the situation in Southeast Asian countries with a high frequency of Beijing type isolates appears to be nonuniform.

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### Jungle Yellow Fever, Rio de Janeiro

**To the Editor:** Yellow fever control in Brazil through vaccination campaigns began in 1937. However, cases of jungle yellow fever still occur despite the existence of a potent vaccine and immunization campaigns focused on areas endemic for the jungle form of the disease (1). Most of these cases are in men in rural areas.

In Brazil from 1980 to 1998, 376 cases of jungle yellow fever were laboratory confirmed (by virus isolation, with or without immunoglobulin [Ig]M-capture enzyme-linked immunosorbent assay [MAC-ELISA] and immunoperoxidase stain), with 216 deaths (case-fatality rate 57.4%). Most cases were from Maranhão and Goiás States, with 99 and 41 cases, respectively; Goiás, in midwestern Brazil, reported a case-fatality rate of 95%.

During 1998 to 1999, 106 cases of jungle yellow fever were confirmed, with 40 deaths (37.7%). During 1999, 75 cases were confirmed, compared with 34 cases in 1998 and a mean of 20 cases per year from 1980 to 1998 (2). In 2000, 84 cases

were confirmed, with 40 deaths (47.6% case-fatality rate). During 2000, the probable site of infection for nearly all cases was in Goiás, with 53 confirmed cases and 23 deaths, suggesting epizootic circulation of the virus (2). These cases were in unvaccinated persons who became ill in their home states after traveling to endemic areas for tourism or work.

In Brazil, almost two thirds of the territory is considered an enzootic area (3). Rio de Janeiro State is not endemic for jungle yellow fever, but in January 2000, the Oswaldo Cruz Institute confirmed a case of yellow fever in a 24-year-old woman who had traveled to a national park in Goiás State on December 28, 1999, with a group of 17 persons. Yellow fever infections were also confirmed in tourists from other states who visited this park in late 1999.

The young woman became ill on January 3 with fever, headache, retroocular pain, prostration, anorexia, and nausea. She returned to Rio de Janeiro on January 5 and visited a private clinic on January 7, when a complete blood count, platelet count, urea, creatinine, liver function tests, and dengue serologic testing were performed. The patient had leukopenia (1,730 leukocytes/mm<sup>3</sup>), 100,000 platelets/mm<sup>3</sup>, AST 911 U/L and ALT 680 U/L, creatinine 0.90 mg/dL, urea 10 mg/dL, and normal bilirubin and protein. Anti-dengue IgM serology was negative. A blood sample was collected January 11 for yellow fever diagnosis. Reverse transcription-polymerase chain reaction (RT-PCR) test was performed on RNA extracted from the serum (4), and virus isolation was attempted on C6/36 cells, both with negative results. A MAC-ELISA test was positive for yellow fever, with a serum IgM titer 1/80,000 8 days after onset of symptoms. The patient recovered within a week. After confirmation of this case in the only person who became ill in the travel group, yellow fever IgM serologic testing was performed on the other group members, all of whom tested negative. RT-PCR and virus isolation were not attempted because the sera were taken after the viremia period.

Control measures for the *Aedes aegypti* vector were promptly taken for a radius of 300 m around the patient's home. A vaccination campaign was carried out, during which 735 neighbors were vaccinated. An epidemiologic survey was conducted in the area by using active surveillance for all symptomatic cases of fever during the period of yellow fever transmissibility. Blood samples from patients with fever were assessed for yellow fever virus and antibodies. Surveillance was intensified immediately in Rio de Janeiro State, and our laboratory examined 54 sera from patients who had traveled recently to endemic areas and who had compatible signs and symptoms (in accordance with a nationwide protocol). All these persons tested negative for yellow fever.

From January to July 2000, >16.9 million people were vaccinated against yellow fever (2); however, cases continue to occur. Unvaccinated persons who visit yellow fever-endemic areas pose a high risk of introducing jungle yellow fever cases into nonendemic areas.

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