

Detection of *Mycobacterium tuberculosis* in sputum from Suruí Indian subjects, Brazilian Amazon

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This investigation aimed at the detection of Mycobacterium tuberculosis (MTB) in the sputum of Suruí Indian subjects from Amazonia, Brazil. Polymerase chain reaction analyses were positive for 12 samples, five of which were also culture-positive (N = 147). Four MTB genotypes were identified, one of which showed resistance to rifampicin and isoniazid. The study also highlighted one village complex as of particular importance, considering the relatively high number of tuberculosis cases reported and of MTB isolates obtained.

Key words: tuberculosis - epidemiology - drug resistance - South American Indians - genotyping

An incidence rate for tuberculosis (TB) among indigenous populations of the Brazilian Amazon that stands at over 300 cases per 100,000 inhabitants/year indicates a major health problem. Recent studies show that the incidence and case fatality rates for tuberculosis in the Brazilian Amazon may be over ten times higher among Indians than those observed among non-Indians living in the same region (Sousa et al. 1997, Escobar et al. 2001, Garnelo et al. 2003, Basta et al. 2004).

As part of an ongoing epidemiological study of tuberculosis in indigenous populations of southwestern Brazilian Amazon, we investigated sputum samples obtained from subjects who were screened as TB suspects during a population-based survey carried out among the Suruí Indians (Basta et al. 2006).

STUDY POPULATION AND METHODS

At the time of the fieldwork (May-August 2003), the Suruí numbered 920 individuals living in numerous villages at the Sete de Setembro Indian Reservation in the state of Rondônia, Brazilian Amazon (60°-61°W, 10°-11°S). The average incidence of TB among the Suruí between 1991 and 2002 was 2,518.9 per 100,000 inhabitants, almost 50 times higher than the regional average, with nearly half the cases diagnosed in children < 15 years old (Basta et al. 2004).

Spontaneously expectorated sputum was collected from all willing subjects who presented respiratory symptoms. Samples were kept under refrigeration for a maximum period of ten days, until they arrived at the laboratory in Rio de Janeiro. Unprocessed samples were first submitted to standard smear microscopic examination

for acid-fast bacilli. The remaining sputum samples were decontaminated and liquefied according to the Petroff method (4% NaOH). Subsequently, the samples were centrifuged (3000 × g for 30 min) and the cell-containing sediments re-suspended in 1 ml of phosphate buffered saline (PBS). Two aliquots of 0.1 ml were seeded on Löwenstein-Jensen medium and incubated for 60 days at 37°C. MTB identification was carried out by standard biochemical and enzymatic tests (Kent & Kubica 1985). Drug resistance tests were performed for rifampicin, isoniazid, streptomycin, ethambutol, and ethionamide with critical concentrations set at 0.2, 40, 4, 2, and 20 µg/ml, respectively (Canetti et al. 1963). Resistance was diagnosed when the percentage of colonies exceeded 10% of the growth on a drug-free medium (streptomycin and ethionamide) or 1% (remaining drugs).

Sediments of processed sputum specimens were tested with AMPLICOR® *M. tuberculosis* (AMPLICOR MTB Roche Diagnostic Systems, NJ, US) following instructions of the manufacturer. However, due to the high number of inconclusive results initially obtained (44 samples), samples with inconclusive results were submitted to a modified protocol for assay-repetition by washing 100 µl aliquots three times with 1 ml of PBS containing 0.2% bovine serum albumin (known to sequester a variety of PCR inhibitors), followed by the wash step included in the standard protocol of the assay.

Ethical approval was obtained from the National Commission of Ethics in Research (Conep) of the National Health Council. A research permit was also obtained from the National Indian Foundation. Informed consent was obtained from village leaders, since most subjects were not literate in Portuguese.

Mycobacterial interspersed repetitive units-variable-number tandem repeats (MIRU-VNTR) and spoligotyping analysis - Extraction of DNA from mycobacterial strains followed Ausubel et al. (2002). Clusters were defined as groups of patients with MTB strains showing identical MIRU-VNTR (the same canonical number for 12-loci MIRU-VNTR) and spoligotype patterns. Spoligotyping

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The results suggest recent TB transmission in two subjects (A and B) from the same village, in view of their confirmed epidemiological link. Although all isolates were grouped as members of the LAM genotype MTB family (Brudey et al. 2006), the diversity in spoligopatterns and MIRU-VNTR suggests that these strains have had sufficient time to diversify over the years. Therefore, the LAM spoligotype is likely to have been introduced in the Suruí some time ago.

We have performed a search in a MTB database, available through the Institute Pasteur of Guadeloupe (The SITVIT Database available at: <http://www.pasteur-guadeloupe.fr>), which includes spoligotypes and MIRU-VNTR patterns. By comparing the spoligotype found in the isolate from patient E with spoligotypes available in the database, we found a 100% pattern match. The spoligotype shared-type (SIT) 102 and VNTR-MIRU-type (VIT) 33 has been found in 54 clinical isolates retrieved worldwide in a frequency of 11/54 (New York, US), 6/54 (Pretoria, South Africa), 5/54 (Brasília, Brazil) and 3/54 (Buenos Aires, Argentina). The VNTR-MIRU pattern was found in 139 clinical worldwide isolates, mostly in the US. The isolate from patient D matches spoligotype SIT 1758, found in 8 MTB clinical isolates from Brazil (7/8) and Portugal (1/8). No matches were found in the database for the VNTR-MIRU VIT of patient's D isolate. These observations suggest that the MTB isolates from patients D and E are distributed worldwide and participate in chains of transmission in the Brazilian general population. According to our database query, both isolates have been previously found in TB patients from Brasília.

The study also highlights the village complex of *Linha 14-Placa* as of particular importance in the epidemiology of TB in the Suruí population, considering the possible clustering of MTB isolates. As it has been noted elsewhere, this village complex that maintains intense social exchange, not only concentrates nearly all TB cases identified in this survey (Basta et al. 2006), but it also accounts for close to half of all prior cases reported among the Suruí (Basta et al. 2004). Although at present this constitutes the largest Suruí village, concentrating *circa* 20% of the total population, further studies remain to be done in order to clarify MTB transmission patterns in this population.

The study of TB transmission by genotyping in indigenous populations in Amazonia deserves more attention and should be repeated with a greater number of isolates in order to allow for a better understanding of TB epidemiology in these populations.

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