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CORRESPONDENCE

This department is for the publication of informal communications that are of interest because they are informative and stimulating, and for the discussion of controversial matters. The mandate of this JOURNAL is to disseminate information relating to leprosy in particular and also other mycobacterial diseases. Dissident comment or interpretation on published research is of course valid, but personality attacks on individuals would seem unnecessary. Political comments, valid or not, also are unwelcome. They might result in interference with the distribution of the JOURNAL and thus interfere with its prime purpose.

Detection of *Mycobacterium leprae* DNA by PCR in Blood Sample from Nine-Banded Armadillo: Preliminary Results

TO THE EDITOR:

Identification of *Mycobacterium leprae,* the agent of leprosy, is difficult, partly due to the inability of the bacillus to grow *in vitro.* Although *M. leprae* was the first agent to be linked to an infectious disease, leprosy is still today an enigmatic disease which is not fully understood. Multibacillary patients are thought to be the main source of *M. leprae* and the transmission is mainly aerogenic; it is generally recognized that the nasal cavity is involved in the carriage and shedding of *M. leprae* ([13]). The natural transmission among armadillos in the southern parts of the United States of America has been described ([10]). This discovery suggested the possibility that the nine-banded armadillo plays a role in the transmission of human leprosy in the United States of America. Reich has proposed that clinical leprosy arises from within a pool of subclinical infections found in the majority of the population in an endemic area ([6]). The search of the *M. leprae* sources is the main point of the strategy for leprosy elimination, such as multibacillary patients and environmental sources. Recently, specific DNA probes have been developed which improve the leprosy diagnosis ([2, 3, 5, 7, 9, 11–12]). The strength of PCR (polymerase chain reaction) is its extreme sensitivity, and, with careful choice of primers, high specificity. Therefore, with PCR the investigative studies about the sources of the leprosy bacillus have become better understood than in the past. Santos, *et al.,* were able to detect DNA from *M. leprae.* The DNA was detected by CPR from 21 out-of-water sources in the leprosy endemic region of Indonesia, and strongly suggested that leprosy was transmitted by contaminated water ([4]). Many other sources of *M. leprae* were investigated by PCR. We have been studying the armadillo as a possible source of leprosy bacilli since 1999. Our survey was conducted in the rural area of Espírito Santo State, Brazil, where the blood of 14 nine-banded armadillos was collected. This region is hyperendemic for leprosy and the presence of nine-banded armadillos is frequent as well as the human's contact with the animal, direct and indirect ([1]). The captured armadillos were anesthetized with intramuscular Ketamine® and skin biopsies of the neck, ear, and traumatic foot lesions were performed and nasal mucus samples were collected. Blood was collected by intracardiac puncture and aliquoted with EDTA. In the event of an animal death, we performed a necropsy and collected liver, lung, brain, kidney, heart and lymph node fragments. All the samples were frozen at –20°C. None of the animals studied had any lesions suggestive of leprosy. The PCR cou-

pled with hybridization analysis for detection of *M. leprae*-DNA were performed by amplification of an *M. leprae*-specific sequence, with the following set of primers ML-1 (GCACGTAAGCCTGTCGGTGG) and ML-2 (CGGCCGGATCCTCGATGCAC). PCR and hybridization conditions were as described earlier by Santos, *et al.* ([9]).

The blood from 5 of 14 animals was positive for *M. leprae*-DNA by PCR. All the other samples, biopsies and nasal secretion, had a negative PCR. To our knowledge, these are the first results in the medical literature and are in accordance with the findings by American researchers who also reported the presence of *M. leprae* in armadillos from the states of Texas and Louisiana, U.S.A. ([10]). Experiments are underway for increasing the number of tested samples. However, these preliminary results suggest that in the Espírito Santo State of Brazil nine-banded armadillos could be considered a natural reservoir of *M. leprae*. Further studies should be performed in order to investigate whether these animals would be considered as animal sources for human infection.

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