

Acknowledgments

We thank Ivan Diaz and Omaira Rodriguez for their support of the fieldwork and Juan Camilo Roncallo for his laboratory support.

This study was supported by funding from US Department of Defense Health Agency, Armed Forces Health Surveillance Division, Global Emerging Infections Surveillance Branch (work unit no. 800000.82000.25GB. B0016; ProMIS ID: 20160390211).

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Fecal Excretion of *Mycobacterium leprae*, Burkina Faso

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DOI: <https://doi.org/10.3201/eid2706.200748>

Mycobacterium leprae was detected by optical microscopy, fluorescent in situ hybridization, and molecular detection in feces collected for the diagnosis of *Entamoeba coli* enteritis in a leprosy patient in Burkina Faso. This observation raises questions about the role of fecal excretion of *M. leprae* in the natural history and diagnosis of leprosy.

Leprosy caused by *Mycobacterium leprae* remains endemic in Burkina Faso, a West Africa country with a level of disability 2 of 31.2% among new patient cases (1). Laboratory diagnosis of leprosy is determined by observation of acid-fast bacilli after microscopic examination of a Ziehl-Neelsen-stained nasal smears and cutaneous lesions (1). Recently, fluorescence in situ hybridization (FISH) was introduced as a complementary approach to increase the specificity of microscopic observations (1,2). We report on the specific microscopic detection of *M. leprae* in the stool specimen of a patient in Burkina Faso.

A 20-year-old man originating from the village of Bama in Burkina Faso sought care at the dermatology department at the Centre Hospitalier Universitaire Souro Sanou (Bobo-Dioulasso, Burkina Faso) for multiple infiltrated papules and nodules on his face and ear pavilions. These symptoms were accompanied by rhinitis and nosebleeds, which had been evolving for >2 months. Clinical examination further showed nasal enlargement (papulonodular), ulcerative-crusts lesions on the limbs, ulnar nerve hypertrophy, and a sausage-like appearance of the fingers, all of which suggested a lepromatous form of leprosy. A nasal smear and skin biopsy were performed on an infiltrative lesion (right arm), and 3 swab specimens were collected from a skin wound (left forearm), crusted lesions (elbow of right arm), and ulcerative papules (left arm). All samples were microscopically examined after Ziehl-Neelsen staining and revealed acid-fast bacilli in all 5 samples. Acid-fast bacilli were further identified as *M. leprae* by partial PCR amplification sequencing of the *rpoB* gene using a validated protocol (1).

The patient also had abdominal pain, and stool samples were collected to check for parasites. Microscopic examination (at 400× magnification) of fresh stool specimens mixed with Lugol's solution revealed

cysts containing >6 nuclei, suggesting cysts of *Entamoeba coli*. Microscopic examination of the stool specimens filtrate after Ziehl-Neelsen staining (at 60× magnification) revealed 2 acid-fast bacilli per 300 microscopic fields (Figure).

Identification of the pathogens was confirmed by a PCR-based method and FISH for *M. leprae* (Appendix, <https://wwwnc.cdc.gov/EID/article/27/6/20-0748-App1.pdf>). Because *M. leprae* has been identified as an intra-amoebal pathogen (3), we tested the intracystic location of *M. leprae* by FISH in clarified stool specimens using sucrose-density gradients. In brief, the cyst wall was permeabilized by incubating stool specimen in 1 mL of cellulase (Sigma Aldrich, <https://www.sigmaaldrich.com>) for 48 hours at 45°C (4). After cellulase activity was stopped by washing with physiologic water and 5 minutes of centrifugation at 3,000 g, the pellet was incorporated into 4',6-diamidino-2-phenylindole-FISH staining. Observation of 8 *Escherichia coli* cysts disclosed nuclei stained with 4',6-diamidino-2-phenylindole and an absence of any detectable *M. leprae* by FISH (Figure). Dynamic, dormant, and dead staining to identify the viability of mycobacteria (5) revealed dead mycobacteria in the skin biopsy, the 3 cutaneous swab specimens, and stool specimens, whereas 8 bacilli out of a total of 22 observed in a series of 6 microscopic fields in the nasal smear were dynamic (Appendix Figure).

Previous reports relied only on Ziehl-Neelsen staining to assess the presence of acid-fast bacilli in stool specimens collected from patients in whom leprosy was diagnosed, without any further formal identification (6,7). In the patient we report, stool-borne acid-fast bacilli were identified as *M. leprae* by 2 independent methods in the presence of negative controls. These *M. leprae* organisms were possibly swallowed by the patient along with blood or upper respiratory secretions during leprosy rhinitis and epistaxis (7). This observation correlates with

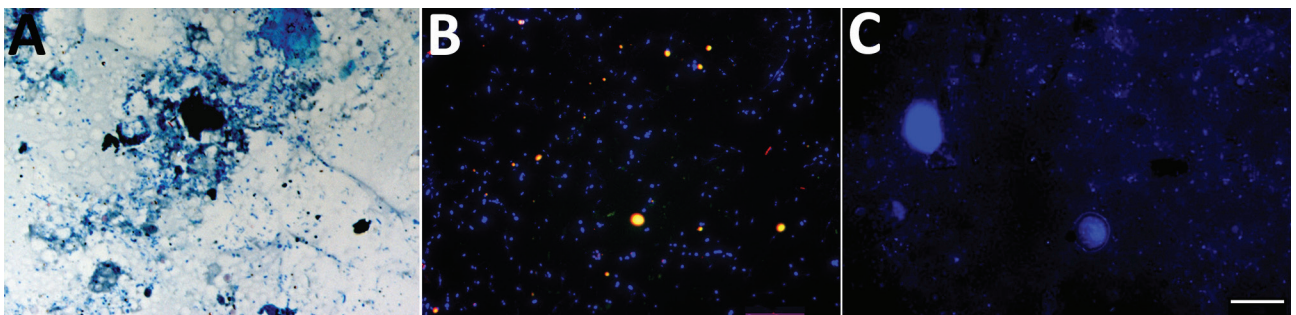


Figure. Optical microscopy observation of *Mycobacterium leprae* in the stool specimens of a leprosy patient in Burkina Faso. A) Ziehl-Neelsen staining; B) fluorescence in situ hybridization. No mycobacteria were observed inside the *Entamoeba coli* cysts (C). Scale bars represent 10 (A), 20 (B), and 20 (C) microns.

a study in armadillos, an *M. leprae* host in some leprosy-endemic regions, in which experimental infection results in the extensive involvement of the intestine and the presence of *M. leprae* in stools (8). In the stool specimens of the patient described in this study, only dead *M. leprae* cultures were observed using dynamic, dormant, dead staining, whereas dynamic mycobacteria were detected in the nasal smear (9).

On the basis of this research, further studies are required to confirm the prevalence of fecal excretion of *M. leprae* in various leprosy populations. Because stools are a noninvasive specimen, they could be collected for the positive diagnosis of leprosy using appropriate laboratory methods, as reported for the positive diagnosis of pulmonary tuberculosis (10). This diagnostic approach is easy to implement, including in children, in contrast to the current biopsy procedure, which requires a qualified staff and post-surgical management.

This work was supported by the government of France under the Investments for the Future Program managed by France's National Research Agency (reference: Méditerranée Infection [project no. 10-IAHU-03]). This work also was supported by the Région Le Sud, Provence Alpes Côte d'Azur, and European 95 funding (grant no. FEDER PA 0000320 PRIMMI).

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Correction: Vol. 26, No. 12

GenBank accession numbers have been added for the sequenced viral sequences from Lymphocytic Choriomeningitis Virus Infections and Seroprevalence, Southern Iraq (H. Alburkatet al.). The article has been corrected online (https://wwwnc.cdc.gov/eid/article/26/12/20-1792_article).