

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).

E.J.A. is US military service member. C.D.C., D.P., C.G., M.S. and J.S.A. are employees of the US government. This work was prepared as part of their official duties. Title 17, USC, §105 provides that copyright protection under this title is not available for any work of the US Government. Title 17, USC, §101 defines a US Government work as a work prepared by a military Service member or employee of the US Government as part of that person's official duties.

About the Author

Dr. Gómez-Camargo is the director of Tropical Medicine Doctoral Program and the UNIMOL Laboratory at the Universidad de Cartagena in Cartagena, Colombia. Her primary research interest is the molecular biology of infectious diseases.

References

- Romero-Alvarez D, Escobar LE. Oropouche fever, an emergent disease from the Americas. *Microbes Infect*. 2018;20:135–46. <https://doi.org/10.1016/j.micinf.2017.11.013>
- Wise EL, Pullan ST, Márquez S, Paz V, Mosquera JD, Zapata S, et al. Isolation of Oropouche virus from febrile patient, Ecuador. *Emerg Infect Dis*. 2018;24:935–7. <https://doi.org/10.3201/eid2405.171569>
- Sakkas H, Bozidis P, Franks A, Papadopoulou C. Oropouche fever: a review. *Viruses*. 2018;10:175. <https://doi.org/10.3390/v10040175>
- Travassos da Rosa JF, de Souza WM, Pinheiro FP, Figueiredo ML, Cardoso JF, Acrani GO, et al. Oropouche virus: clinical, epidemiological, and molecular aspects of a neglected Orthobunyavirus. *Am J Trop Med Hyg*. 2017;96:1019–30.
- Groot Liévano H. Studies on arthropod-transmitted viruses in Colombia [in Spanish]. *Revista de la Academia Colombiana de Ciencias Exactas, Físicas y Naturales*. 2017;4:12–33.
- Hoyos-López R, Suaza-Vasco J, Rúa-Urbe G, Uribe S, Gallego-Gómez JC. Molecular detection of flaviviruses and alphaviruses in mosquitoes (Diptera: Culicidae) from coastal ecosystems in the Colombian Caribbean. *Mem Inst Oswaldo Cruz*. 2016;111:625–34. <https://doi.org/10.1590/0074-02760160096>
- Parra-Henao G, Suárez L. Mosquitoes (Diptera: Culicidae) as potential vectors of arboviruses in the Urabá region, Northwest of Colombia [in Spanish]. *Biomedica*. 2012;32:252–62. <https://doi.org/10.7705/biomedica.v32i2.667>
- Forshey BM, Guevara C, Laguna-Torres VA, Cespedes M, Vargas J, Gianella A, et al.; NMRCD Febrile Surveillance Working Group. Arboviral etiologies of acute febrile illnesses in Western South America, 2000–2007. *PLoS Negl Trop Dis*. 2010;4:e787. <https://doi.org/10.1371/journal.pntd.0000787>
- Saeed MF, Wang H, Nunes M, Vasconcelos PFC, Weaver SC, Shope RE, et al. Nucleotide sequences and phylogeny of the nucleocapsid gene of Oropouche virus. *J Gen Virol*. 2000;81:743–8. <https://doi.org/10.1099/0022-1317-81-3-743>
- Djikeng A, Halpin R, Kuzmickas R, Depasse J, Feldblyum J, Sengamalay N, et al. Viral genome sequencing by random priming methods. *BMC Genomics*. 2008;9:5. <https://doi.org/10.1186/1471-2164-9-5>

Address for correspondence: Doris E. Gómez-Camargo, calle 29 #50-50, Laboratorio UNIMOL, Universidad de Cartagena, Campus de la Salud, Cartagena, Colombia; email: dmtropical@unicartagena.edu.co

Fecal Excretion of *Mycobacterium leprae*, Burkina Faso

Anselme Millogo, Ahmed Loukil, Coralie L'Ollivier, Diakourga Arthur Djibougou, Sylvain Godreuil, Michel Drancourt

Author affiliations: Centre Hospitalier Universitaire Souro Sanou, Bobo-Dioulasso, Burkina Faso (A. Millogo); Aix-Marseille-Université, Institut de recherche pour le développement, Institut Hospitalo-Universitaire Méditerranée Infection, Marseille, France (A. Millogo, A. Loukil, M. Drancourt); Université de Montpellier, Institut de Recherche pour le développement, Montpellier, France (A. Millogo, S. Godreuil); Aix-Marseille-Université, IRD, Assistance Publique-Hopitaux de Marseille, Service de Santé des Armées, Marseille (C. L'Ollivier); Institut de Recherche en sciences de la Santé, Bobo-Dioulasso (D.A. Djibougou); Centre MURAZ, Bobo-Dioulasso (D.A. Djibougou)

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

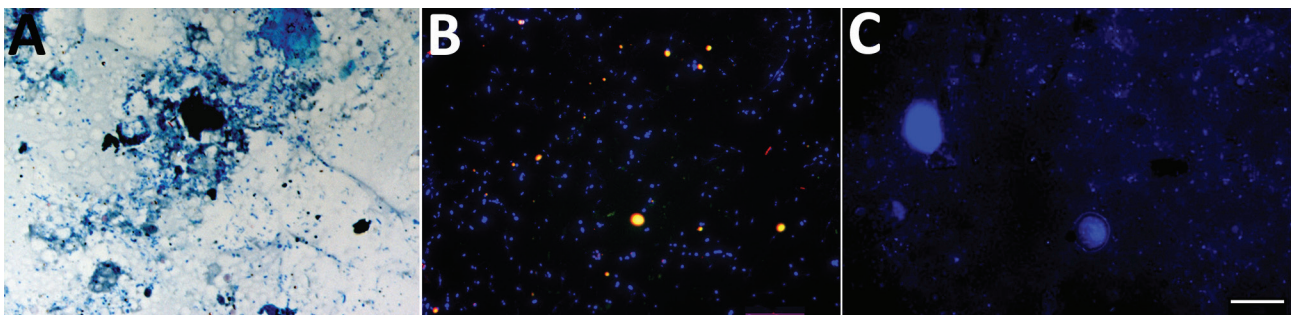


Figure. Optical microscopy observation of *Mycobacterium leprae* in the stool specimens of a leprosy patient in Burkina Faso. A) Ziehl-Neelsen staining; B) fluorescence in situ hybridization. No mycobacteria were observed inside the *Entamoeba coli* cysts (C). Scale bars represent 10 (A), 20 (B), and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

About the Author

Mr. Millogo is a biology doctoral student at the University of Montpellier, France, and Institut Hospitalo-Universitaire Méditerranée Infection, Marseille, France, and a biologist at the Souro Sanou University Hospital in Bobo-Dioulasso, Burkina Faso. His primary research interests include cutaneous mycobacterioses.

References

1. Millogo A, Loukil A, Fellag M, Diallo B, Ouedraogo AS, Godreuil S, et al. Fluorescent hybridization of *Mycobacterium leprae* in skin samples collected in Burkina Faso. *J Clin Microbiol*. 2020;58:e02130–19. <https://doi.org/10.1128/JCM.02130-19>
2. Musso D, Rovey C, Loukil A, Vialette V, Nguyen NL. Leprosy in French Polynesia. *New Microbes New Infect*. 2019;29:100514. <https://doi.org/10.1016/j.nmni.2018.10.010>
3. Lahiri R, Krahenbuhl JL. The role of free-living pathogenic amoeba in the transmission of leprosy: a proof of principle. *Lepr Rev*. 2008;79:401–9. <https://doi.org/10.47276/lr.79.4.401>
4. Brahim Belhaouari D, Baudoin JP, Gnankou F, Di Pinto F, Colson P, Aherfi S, et al. Evidence of a cellulosic layer in *Pandoravirus massiliensis* tegument and the mystery of the genetic support of its biosynthesis. *Front Microbiol*. 2019;10:2932. <https://doi.org/10.3389/fmicb.2019.02932>
5. Loukil A, Darriet-Giudicelli F, Eldin C, Drancourt M. Pulmonary tuberculosis conversion documented by microscopic staining for detection of dynamic, dormant, and dead mycobacteria (DDD staining). *J Clin Microbiol*. 2018;56:e01108–18. <https://doi.org/10.1128/JCM.01108-18>
6. Manzullo A, Manzi RO, Lefevre A, Oteiza ML. Investigation of acid-fast bacilli in the digestive tract of leprosy patients. *Leprolgia*. 1965;10:14–6.
7. Koshy A, Karat ABA. A study of acid-fast bacilli in the urine, gastric juice and feces of patients with lepromatous leprosy. *Lepr India*. 1971;43:3–7.
8. Kirchheimer WF, Storrs EE, Binford CH. Attempts to establish the armadillo (*Dasypus novemcinctus* linn.) as a model for the study of leprosy. II. Histopathologic and bacteriologic post-mortem findings in lepromatoid leprosy in the armadillo. *Int J Lepr Other Mycobact Dis*. 1972;40:229–42.
9. Palomino JC, Falconi E, Marin D, Guerra H. Assessing the viability of *Mycobacterium leprae* by the fluorescein diacetate/ethidium bromide staining technique. *Indian J Lepr*. 1991;63:203–8.
10. Drancourt M. Culturing stools to detect *Mycobacterium tuberculosis*. *J Clin Microbiol*. 2018;56:e02033–17. <https://doi.org/10.1128/JCM.02033-17>

Address for correspondence: Michel Drancourt, Aix-Marseille-Université, IRD, MEPHI, IHU Méditerranée Infection, 19-21 Boulevard Jean Moulin, 13005 Marseille, France; email: michel.drancourt@univ-amu.fr

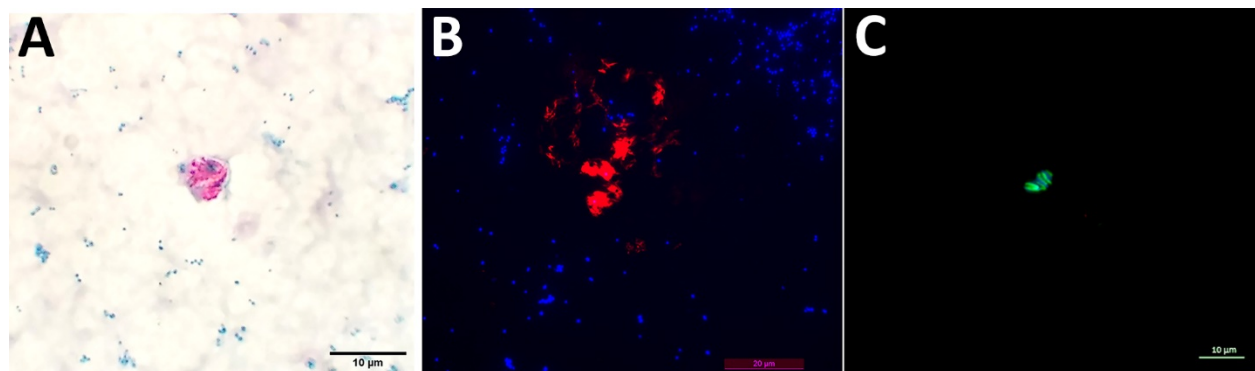
Correction: Vol. 26, No. 12

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).

Fecal Excretion of *Mycobacterium leprae*, Burkina Faso

Appendix

DNA was extracted from stool by combining chemical lysis, glass powder, heating at 56°C for 2.5 hours, sonication, and EZ1 automatic elution (QIAGEN, <https://www.qiagen.com>). *Escherichia coli* 18S rRNA partial PCR was performed using AmpliTaq Gold™ 360 Master Mix (Applied Biosystems, <https://www.thermofisher.com>) and incorporating forward primer: 5'-CGAGGAATAAGGGTTCGACA3' and reverse primer: 5'-ATCGCTTTCTCACGATGGTT-3' picked using Primer3Plus software (<https://primer3plus.com/cgi-bin/dev/primer3plus.cgi>) based on the reference *E. coli* sequence (GenBank accession no. MH133210.1) following the following thermal cycle: initial denaturation at 95°C for 15 minutes, 35 cycles of 30 seconds at 95°C, 30 seconds at 64°C and 1 minute at 72°C. Final extension at 72°C performed for 5 minutes. Molecular identification by real-time glbO PCR of *M. leprae* incorporated 6-FAM CGCGAGCCCGTCAGAATCTCCG6TAMRA, Mycoblep_1203 F: 5'-GGAATTTTCGTCACAATTCCACAA-3', Mycoblep_1203 R: 5'-TCGTCTCGTATCCGCCAATC-3' in the presence of negative controls.



Appendix Figure. Optical microscopy observation of *Mycobacterium leprae* in the nasal swab of a Burkinabe patient after Ziehl-Neelsen staining (A), FISH staining (B), and DDD staining (C).