Fecal Excretion of Mycobacterium leprae , Burkina Faso

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References

1. 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
2. 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
3. Sakkas H, Bozidis P, Franks A, Papadopoulou C. Oropouche fever: a review. Viruses. 2018;10:175. https://doi.org/10.3390/v10040175
4. 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.
5. Groot Liévano H. Studies on arthropod-transmitted viruses in Colombia [in Spanish]. Revista de la Academia Colombiana de Ciencas Exactas, Físicas y Naturales. 2017;4:12–33.
6. Hoyos-López R, Suaza-Vasco J, Rúa-Uribe 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
7. Parra-Henao G, Suárez L. Mosquitoes (Diptera: Culiciadae) 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
8. 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
9. 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
10. 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

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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-crusted 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

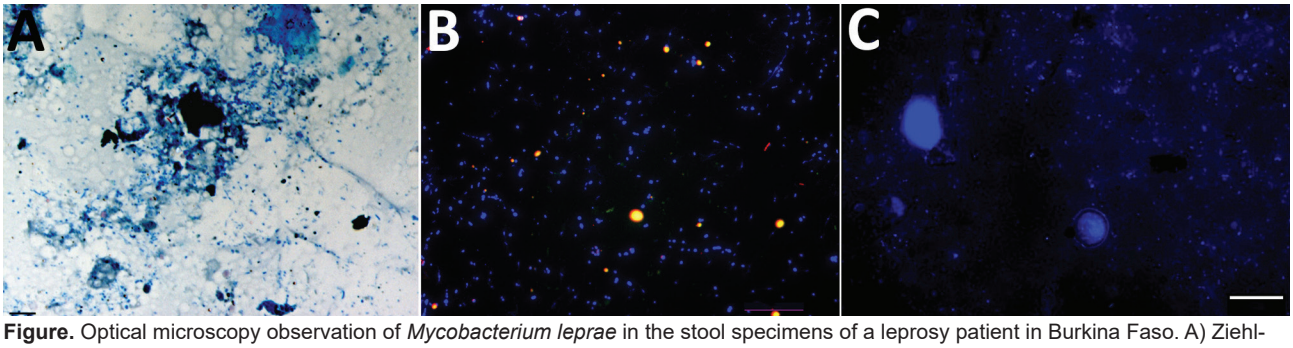

Figure. Optical microscopy observation of *Mycobacterium leprae* in the stool specimens of a leprosy patient in Burkina Faso. A) Ziehl-Neelsen staining; B) fluorescence in situ hybridization. No mycobacteria were observed inside the *Entamoeba coli* cysts (C). Scale bars represent 10 (A), 20 (B), and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

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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, Rovery 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. Leprologia. 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

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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).