Mycobacterium leprae Infection in a Wild Nine-Banded Armadillo, Nuevo León, Mexico

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Nine-banded armadillos (*Dasypus novemcinctus*) are naturally infected with *Mycobacterium leprae* and are implicated in the zoonotic transmission of leprosy in the United States. In Mexico, the existence of such a reservoir remains to be characterized. We describe a wild armadillo infected by *M. leprae* in the state of Nuevo León, Mexico.

Nine-banded armadillos (*Dasypus novemcinctus*) can be naturally infected with *Mycobacterium leprae* and have been implicated in the zoonotic transmission of leprosy in the US states of Texas, Louisiana, Alabama, Georgia, and Florida (*1*,*2*). Despite Mexico falling within the armadillos' natural geographic habitat and the report of 182 new human leprosy cases in Mexico in 2019 (*3*), only 1 report of an armadillo infected with acid-fast bacilli has occurred since 1984, and the bacterial species in that case was never fully characterized (*4*).

In 2019, a nine-banded armadillo with ataxia, dyspnea, and adynamia was captured along the Pilon River in Montemorelos in the state of Nuevo León, Mexico. The animal was euthanized, and necropsy revealed granulomatous lesions in diverse organs and tissues (Appendix Figure 1, https://wwwnc.cdc.gov/EID/article/28/3/21-1295-App1.pdf). Histopathologic examination identified acid-fast bacilli in the liver, lung, heart, striated muscle, and ear; the bacilli were especially abundant in the spleen (Figure; Appendix Figure 2). We confirmed the presence of M. leprae in tissue by PCR testing of DNA extracted from the ear, liver, and lung by using the specific repetitive element RLEP (5) (Appendix). We used bacterial DNA extracted from the liver of the infected armadillo (strain A1), harboring the highest bacilli number by microscopy, for library preparation, followed by targeted enrichment using hybridization capture and whole-genome sequencing using NextSeq 500 (Illumina, https://www.illumina.com) (Appendix).After targeted enrichment using hybridization capture, we extracted bacterial DNA from the liver of the infected armadillo (strain A1), harboring the highest bacilli number by microscopy, and conducted sequencing by using NextSeq 500 (Illumina, https://www.illumina.com) (Appendix). The mean read coverage of 87× was sufficient for further comparative analysis at the single nucleotide level with other *M. leprae* isolates (Appendix Table 1). The armadillo-derived A1 strain belongs to genotype 3I-2, similar to other *M. leprae* isolates from the United States, Venezuela, Brazil, and Mexico (*1*).

Phylogenetically, A1 branches between the US human (NHDP-98) and animal–human (I30, NHDP-63, NHDP-55) *M. leprae* strains and closely clusters with EGG (*6*), a strain isolated in 2014 from a 70 year-old man with leprosy living in Nuevo León, Mexico (Appendix Figures 4, 5). Strains A1 and EGG share 9 polymorphisms when compared with the whole-genome sequences from 295 other *M. leprae* isolates and differed from each other by only 5 single-nucleotide polymorphisms (SNPs) (Appendix Figure 6).

We submitted DNA from *M. leprae* isolates recovered from the biopsies of additional leprosy patients from the states of Nuevo León (n = 9) and Jalisco (n = 2), Mexico, to partial whole-genome sequencing (n = 4) and PCR genotyping (n = 7) (Appendix Table 2, Figure 5.), We deciphered their clustering from previously described positions specific to genotypes 3I-1 and 3I-2 (*1*) as well as new informative SNPs specific to EGG and A1 (Appendix Table 2, Figure 6). Partial genome reconstruction for all 11 isolates revealed that 4 of them belong to genotype 3I-1, whereas 7 belong to genotype 3I-2. Within genotype 3I-1, isolates F2, F6, and F11 belong to a similar cluster, named 3I-1-c2 (Appendix Figure 4, 5). Within genotype 3I-2, 4 isolates (F1, F8, F14, and F23) belong to the same cluster, named 3I-2-c3, which also encompasses A1 and EGG. Of these isolates, only F1 shared an additional common SNP with A1 but differed >1 SNP (genome position 3232319) from it

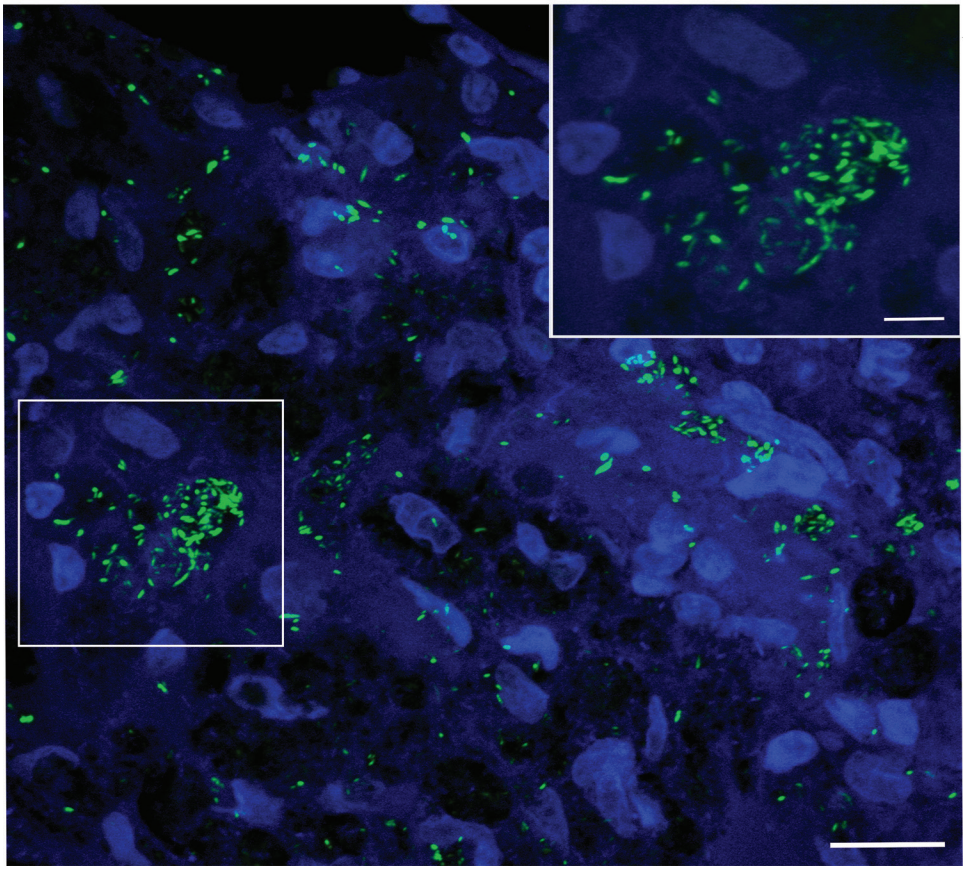

Figure. Identification and characterization of leprosy and *Mycobacterium leprae* acid-fast bacilli in the tissue in the wild nine-banded armadillo (*Dasypus novemcinctus*), Nuevo León, Mexico. SYBR gold staining shows a high density of bacilli in the spleen tissue organized in globi (boxed area at left and inset at right). Image is a merger of 16 images, 0.33 μm apart, in a z-stack taken with a 100× objective lens. Scale bars represent 20 μm (main image) and 5 μm (inset).

(Appendix Figures 4, 6). All patients infected with an *M. leprae* isolate from cluster 3I-2-c3 live in close vicinity (radius of ≈100 km) to the city of Montemorelos, where the infected armadillo was captured (Appendix Figure 5).

We describe the identification and genetic characterization of *Mycobacterium leprae* in a wild nine-banded armadillo in Mexico. In addition, we show that *M. leprae* strains belonging to different clusters are circulating in patients in Mexico. The state of Nuevo León, Mexico, shares a border with the US state of Texas, where a high density of leprosy-infected nine-banded armadillos have been reported (*4*,*7*). Nine-banded armadillos expanded their range into the United States in the mid-1800s from Mexico (*8*).

The *M. leprae* armadillo isolate from Mexico we describe belongs to the same genotype as patients and armadillo isolates from the United States but clusters separately. Isolate A1 further clusters with human isolates exclusively identified in Mexico thus far, with which it displays similar low genetic variation as observed between animal and human isolates in the United States (*1*). Therefore, our results raise concerns that wild-banded armadillos may, similarly to the situation in the United States, serve as reservoirs for the leprosy bacillus in the state of Nuevo León and call for additional surveillance across Mexico to assess the spread of the disease in the animal population and evaluate zoonosis risks associated with human contact with armadillos.

The existence of an animal reservoir hosting the leprosy bacillus in Mexico threatens the goal of leprosy elimination. In light of our results, we propose that interventions based on a One Health approach may be more efficient in achieving eradication of the disease.

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Sensitivity of *Mycobacterium leprae* to Telacebec

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The treatment of leprosy is long and complex, benefiting from the development of sterilizing, rapidly-acting drugs. Reductive evolution made *Mycobacterium leprae* exquisitely sensitive to Telacebec, a phase 2 drug candidate for tuberculosis. The unprecedented potency of Telacebec against *M. leprae* warrants further validation in clinical trials.

Leprosy, also known as Hansen disease, is a chronic infectious disease caused primarily by *Mycobacterium leprae* and to a lesser extent by *M. lepromatosis* bacteria. Both species have a strong tropism for the Schwann cells; infection causes peripheral neuropathy, which leads to the characteristic deformities and disabilities. Despite successful implementation of multidrug therapies for the treatment of leprosy, >200,000 new cases were reported globally in 2019. Drug-resistant *M. leprae* strains, although rare, are emerging in several parts of the world (*1*). Therefore, newer rapidly acting bactericidal, orally bioavailable drugs are required to shorten treatment time and reduce transmission.

The high potency of drugs targeting the cytochrome *bcc:aa*$_3$ terminal oxidase (also known as QcrB inhibitors) against *M. ulcerans* has been reported (*3*). Of particular importance is the finding that a single dose of the drug candidate, Telacebec (Q203) (*3*), eradicates infection in a mouse model of Buruli ulcer (*4*). The potency of drugs targeting the cytochrome *bcc:aa*$_3$ terminal oxidase against *M. ulcerans* is explained by the absence of a functional cytochrome *bd* oxidase, an alternate terminal oxidase that limits the potency of telacebec in *M. tuberculosis* (*5*,*6*). Like *M. ulcerans*, *M. leprae* has lost the genes encoding the cytochrome *bd* oxidase and any other alternate terminal electron acceptors (*7*). Because *M. leprae* relies exclusively on the cytochrome *bcc:aa*$_3$ terminal oxidase for respiration, Scherr et al. hypothesized that telacebec and related QcrB