Detection of *Mycobacterium africanum* Lineage 6 in White-Nosed Coatis, Costa Rica

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We detected disseminated granulomatous disease with abundant acid-fast bacilli in free-ranging, white-nosed coatis in Costa Rica. Molecular characterization identified *Mycobacterium africanum* lineage 6. Our findings demonstrate the presence of *M. africanum* in free-ranging wildlife in the Americas and highlight the value of wildlife surveillance for prompting coordinated One Health investigation and response.

Mycobacterium africanum, a member of the *Mycobacterium tuberculosis* complex (MTBC), accounts for ≈50% of human tuberculosis (TB) cases in some regions in West Africa but is rarely identified in humans in the Americas (*1*,*2*). Although *M. africanum* is traditionally considered human-adapted, sporadic infections have been documented in domestic and wild animals, suggesting a broader host range and raising questions about its zoonotic potential (*3*–*5*). *M. africanum* infections in free-ranging wildlife remain poorly understood, with no confirmed infections reported in the Americas (*1*). *M. africanum* comprises MTBC lineages 5 and 6, and lineage 6 shares evolutionary relationships with animal-adapted members of the complex (*3*,*6*). We describe the detection of *M. africanum* lineage 6 in free-ranging, white-nosed coatis (*Nasua narica*) in Costa Rica by using multiple molecular approaches, including targeted next-generation sequencing (tNGS).

The Study

During 2024–2026, the National System of Conservation Areas (Costa Rica) wildlife officers identified 8 free-ranging male white-nosed coatis in Guanacaste Province with nodular or ulcerative cutaneous lesions and variable clinical deterioration and referred them to wildlife rehabilitation facilities (Figure 1; Appendix Table 1, https://wwwnc.cdc.gov/EID/article/32/12/256-0362-App1.xlsx). Clinical signs ranged from localized cutaneous masses and lymphadenitis to extensive ulcerative skin lesions, severe pneumonia, and poor body condition. Several animals received empirical amoxicillin (15 mg/kg) and enrofloxacin (10 mg/kg); however, all deteriorated and died within days of admission. We performed complete necropsies on 6 of the 8 coatis. We examined only archived tissues for the remaining 2 coatis, which died in 2024 with similar lesions.

Among the 6 coatis that underwent complete necropsy, all had nodular or ulcerative cutaneous lesions and granulomatous pulmonary disease. We identified additional lesions on serosal surfaces and other internal organs, including the diaphragm, liver, spleen, intestine, and lymph nodes. Histopathologic examination of all 6 animals revealed granulomatous inflammation consisting predominantly of histiocytes, with variable numbers of degenerate neutrophils, lymphocytes, and multinucleated giant cells surrounding central caseous necrosis. Ziehl–Neelsen staining revealed abundant acid-fast bacilli in granulomatous lesions involving various tissues in all 6 coatis (Figure 2; Appendix Table 1).

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DOI: https://doi.org/10.3201/eid3212.260362

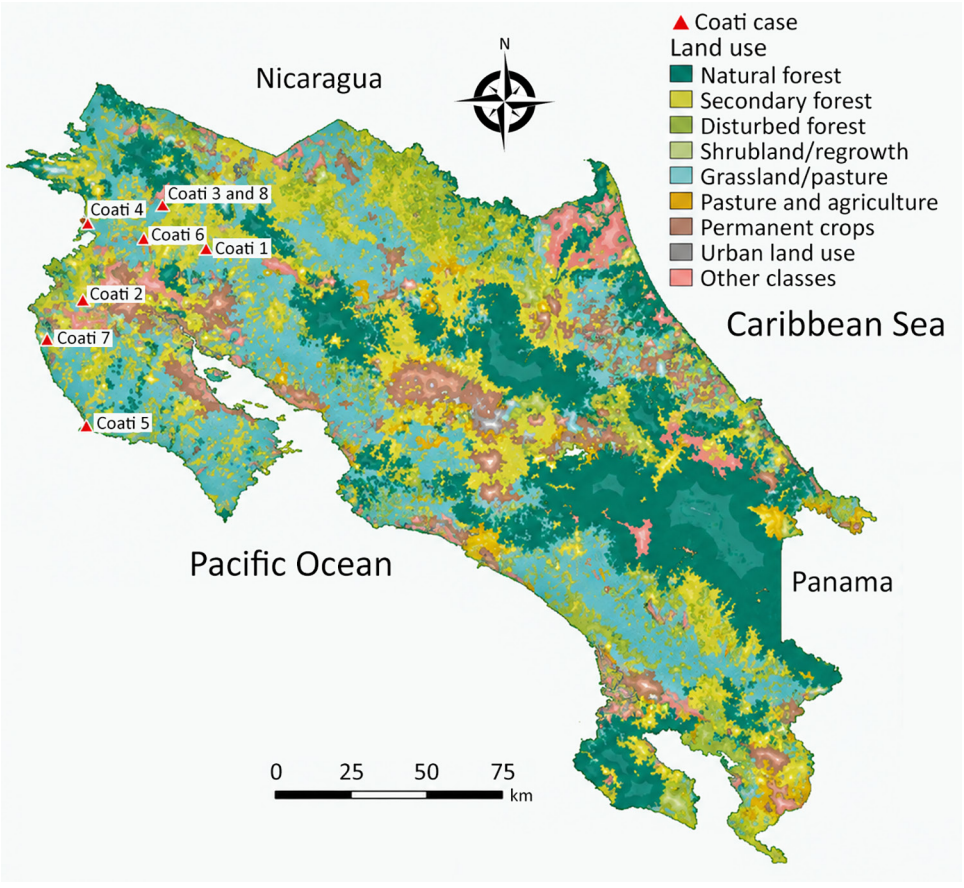


Figure 1. Geographic distribution of *Mycobacterium africanum* cases in white-nosed coatis, Costa Rica, 2024–2026. Red triangles indicate the 7 distinct geographic locations where coatis infected with *M. africanum* were identified; 1 location is shared by 2 coatis. The underlying land-use layer illustrates the diverse ecologic zones and anthropogenic landscapes across the national territory.

For each coati, we individually homogenized available skin, lung, and liver tissue specimens in sterile saline by using a sterile tissue grinder. We decontaminated tissue homogenates by using the modified Petroff method (*7*) and performed DNA extraction and mycobacterial culture on the resulting sediments. We extracted total DNA by using the RSC Viral Total Nucleic Acid Purification Kit (Promega, https://www.promega.com) with the Maxwell instrument, following the manufacturer's protocol. We inoculated all samples into Löwenstein-Jensen solid medium and MGIT liquid medium (Becton, Dickinson and Company, https:/ /www.bd.com). We incubated Löwenstein-Jensen slants at 35°C–37°C, monitored them weekly for up to 56 days, and classified cultures with no visible growth on day 56 as negative. We incubated MGIT tubes in the BACTEC MGIT 960 system (Becton, Dickinson and Company) at 35°C–37°C for up to 42 days, until the instrument flagged them as positive or negative. We classified cultures with no detectable growth by day 42 as negative.

We detected MTBC DNA by using either the Anyplex MTB/NTM Real-Time Detection Kit (Seegene, https://www.seegene.com) or the Xpert MTB/RIF Ultra cartridge (Cepheid, https://www.cepheid.com), depending on reagent availability, according to manufacturers' instructions (*8*). We speciated and typed MTBC-positive samples by using the GenoType MTBC assay (Hain Lifescience, https://www.hain-lifescience.de) and screened for resistance mutations by using GenoType MTBDR*plus* and MTBDR*sl* (Hain Lifescience) (*9*). We included negative extraction and amplification controls in all molecular runs, which yielded no detectable MTBC signal.

We performed tNGS on a lung tissue sample from coati D7264-25 to further characterize MTBC lineage assignment by using the Deeplex Myc-TB assay (Genoscreen, https://www.genoscreen.fr). We quantified, normalized, and sequenced libraries by using paired-end sequencing on the MiSeq platform (Illumina, https://www.illumina.com) and analyzed sequence data by using Deeplex software.

We independently confirmed MTBC DNA detection, by using tissue samples from 4 coatis (D4413-25, D5414-25, D0299-26, and D1753-26). We processed tissue samples separately and mechanically homogenized them by using a BeadBug-6 homogenizer (Benchmark Scientific, https://www.benchmarkscientific.com) with

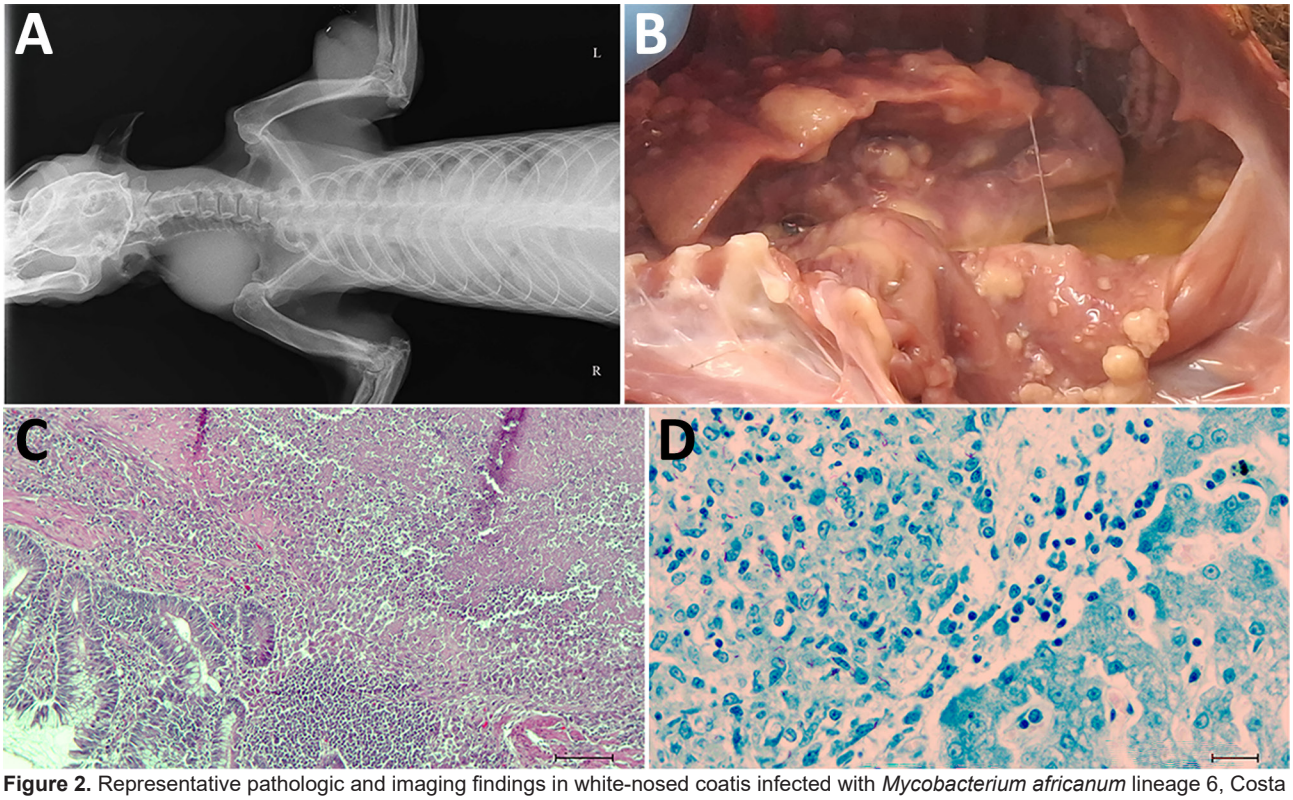


Figure 2. Representative pathologic and imaging findings in white-nosed coatis infected with *Mycobacterium africanum* lineage 6, Costa Rica. A) Dorsoventral radiograph from 1 coati revealing 2 well-defined, rounded, soft-tissue masses, 1 lateral to the right cervical region and 1 adjacent to the left elbow. Both structures exhibit homogeneous soft-tissue opacity without mineralization. The thorax shows a mild, diffuse increase in pulmonary opacity, partially obscuring vascular markings. The axial skeleton appears unremarkable. B) Image of white-nosed coati thoracic cavity showing macroscopic lesions in the pleura and lungs and multifocal whitish nodules ranging from 2 to 25 mm. C) Hematoxylin and eosin staining of the intestine showing granuloma in the submucosa extending into the mucosa, with a predominantly histiocytic and mildly neutrophilic inflammatory infiltrate and a wide central area of necrosis. Scale bar indicates 100 µm. D) Ziehl–Neelsen liver tissue staining showing abundant acid-fast bacilli within the granuloma. Scale bar indicates 20 µm.

silica glass beads (0.5 mm and 1.0 mm) in Tris-EDTA buffer. We extracted DNA by using the DNeasy Blood and Tissue Kit (QIAGEN, https://www.qiagen.com) after overnight digestion with proteinase K at 56°C before purification. For MTBC detection, we used 2 independent real-time PCRs targeting insertion sequences IS1081 and IS6110. IS1081 primers and probe were from the US Department of Agriculture, and IS6110 used the ID GENE *Mycobacterium tuberculosis* Complex Duplex kit (IDvet, https://www.innovative-diagnostics.com). We included *M. africanum* ATCC 35711 and *Mycobacterium bovis* NCTC 10772 as positive controls. We performed additional characterization by using a multiplex PCR assay targeting regions of difference (RDs; RD1, RD4, RD9, RD12, and RD1mic) (*10*). We analyzed amplified fragments by using capillary electrophoresis (QIAxcel Advanced System, Qiagen).

We did not recover viable isolates, despite repeated culture attempts. We detected MTBC DNA from all 8 coati samples. GenoType MTBC line-probe assays revealed hybridization patterns consistent with *M. africanum*, and GenoType MTBDR*plus* and MTBDR*sl* did not detect mutations associated with first- or second-line antimicrobial drugs. TNGS of the lung tissue sample from coati D7264-25 assigned the sample to *M. africanum* lineage 6 (Figure 3, panel A). We confirmed MTBC DNA in tissue specimens from 4 coatis. Multiplex PCR analysis of RDs detected RD1, RD4, RD12, and RD1mic but not RD9, a profile compatible with *M. africanum* (Figure 3, panel B; Figure 4.

Conclusion

We report *M. africanum* infection in 8 free-ranging, white-nosed coatis from northwestern Costa Rica from 2024–2026. Affected animals had disseminated granulomatous disease, involving skin and multiple internal organs, with abundant acid-fast bacilli. GenoType MTBC assays, NGS, and RD analysis identified *M. africanum* lineage 6. *M. africanum* is primarily associated with human TB and remains largely restricted to West Africa (*3*). Although sporadic infections have

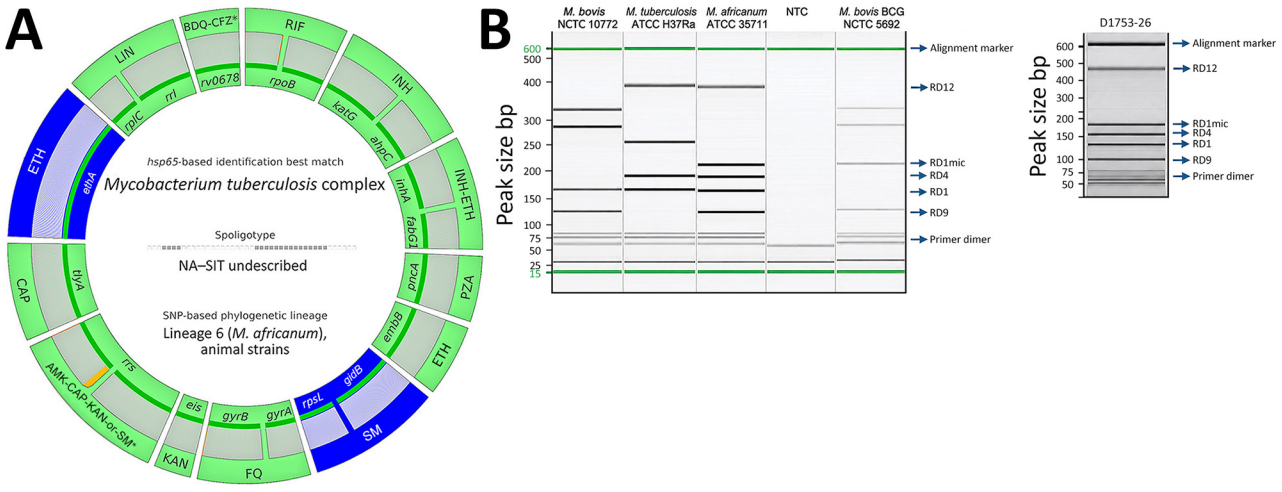


Figure 3. Molecular characterization of *Mycobacterium africanum* lineage 6 in free-ranging, white-nosed coatis, Costa Rica. A) Targeted next-generation sequencing result showing assignment to lineage 6 (*M. africanum*) within the *Mycobacterium tuberculosis* complex. Asterisks indicate a documented resistance-conferring mutation in targets of interest. B) RD analysis demonstrating a genomic profile consistent with *M. africanum* in coati D1753-26 (bottom) compared with controls (top). AMK, amikacin; BDQ, bedaquiline; CAP, capreomycin; CFZ, clofazimine; ETH, ethambutol; FQ, fluoroquinolone; INH, isoniazid; KAN, kanamycin; LIN, linezolid; NA–SIT, not applicable, spoligotype international; NTC, no template control; PZA, pyrazinamide; RD, regions of difference; RIF, rifampin; SM, streptomycin; SNP, single-nucleotide polymorphism.

been reported in domestic animals and a few wildlife species, including hyraxes, no definitive animal reservoir has been identified (*3*–*5*). Our findings expand the recognized host range of *M. africanum* in free-ranging wildlife in the Americas (*1*,*3*).

Because no viable isolates were recovered for whole-genome sequencing, we cannot exclude an undescribed or highly divergent MTBC variant (*6*,*11*). Isolate recovery and whole-genome sequencing will help resolve phylogenetic position and support future investigations of transmission pathways and infection sources.

Cases occurred in areas with mixed agricultural and peridomestic land use, suggesting opportunities for contact among wildlife, livestock, and humans. The source of infection remains uncertain; exposure to infected humans, domestic animals, or contaminated fomites cannot be excluded (*3*). Whether a sylvatic transmission cycle exists within local coati populations is unknown. In Costa Rica, *M. africanum* infections in humans have only been documented sporadically (*2*).

Because bovine TB surveillance in Costa Rica is primarily focused on detection of *M. bovis*, a member of the MTBC, other MTBC members, including *M. africanum*, are not routinely investigated in productive animals (*12*). Consequently, infections caused

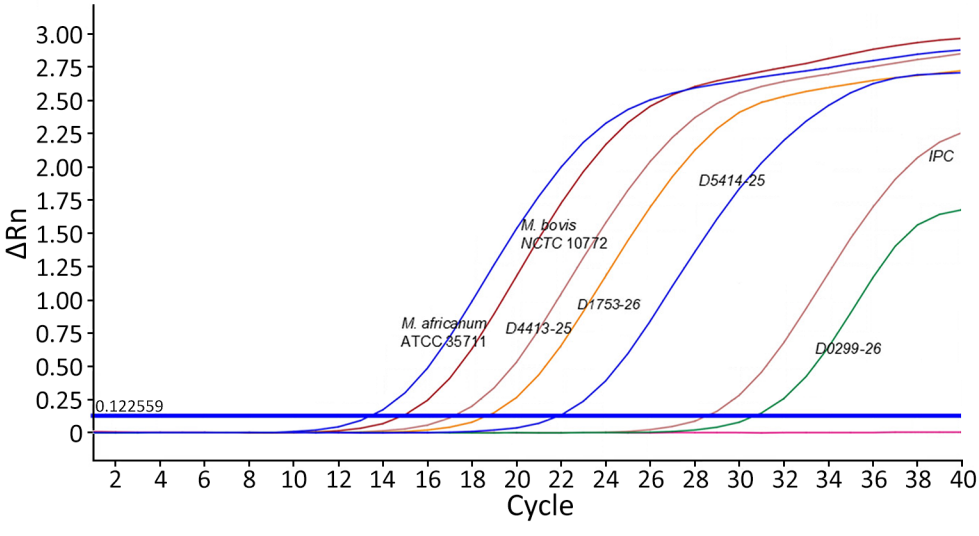


Figure 4. Real-time PCR amplification curves of *Mycobacterium tuberculosis* complex DNA in representative tissue samples in study of *Mycobacterium africanum* lineage 6 in free-ranging, white-nosed coatis, Costa Rica. D4413-25, D5414-25, D0299-26, and D1753-26 indicate samples from individual coatis; Internal positive control (IPC), *M. africanum* ATCC 35711, and *Mycobacterium bovis* NCTC 10772 were positive controls. Blue horizontal line indicates the real-time PCR threshold to distinguish amplification signal from background noise and baseline drift.

by less-recognized MTBC lineages could remain undetected in domestic animal populations. Our findings highlight the need to include a broader range of MTBC members in national TB surveillance.

Our findings triggered a coordinated One Health response involving wildlife, veterinary, public health, and academic institutions. Activities included evaluating persons in close contact with affected coatis, informing wildlife professionals and facility personnel, training staff to recognize and report compatible lesions, and reinforcing passive wildlife disease surveillance. The response also strengthened interinstitutional coordination, MTBC diagnostic preparedness, and risk communication without causing unnecessary alarm. The investigation found no evidence of epidemiologic linkage to human TB cases. Our findings illustrate the value of wildlife disease surveillance for detecting unusual health events and prompting interinstitutional coordination and highlight the need for more comprehensive One Health investigations integrating wildlife, livestock, environmental, and human health sectors (*13*,*14*).

Acknowledgments

We thank the National Veterinary Services Laboratory of the National Animal Health Service, and the Instituto Costarricense de Investigación y Enseñanza en Nutrición y Salud (INCIENSA), the Tropical Diseases Research Programme of Universidad Nacional, Costa Rica, for their institutional support. We are also grateful to the staff of the National System of Conservation Areas, the wildlife rescue centers, and the participating laboratories, who reported cases, collected samples, and ran the diagnostic tests.

This study received no external funding. The participating organizations paid for it from their own resources.

About the Author

Dr. Vargas is a veterinarian and epidemiologist who coordinates the Pathology Laboratory at the National Animal Health Service, Costa Rica. His research covers diagnostic pathology, infectious and zoonotic diseases, and wildlife health surveillance, mainly through epidemiological investigations and a One Health approach.

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