Mixed Mycobacterium tuberculosis Lineage Infection in 2 Elephants, Nepal

Sarad Paudel,1 Chie Nakajima,1 Susan K. Mikota, Kamal P. Gairhe, Bhagwan Maharjan, Suraj Subedi, Ajay Poudel, Mariko Sashika, Michito Shimozuru, Yasuhoiko Suzuki, Toshio Tsubota

Author affiliations: Hokkaido University, Sapporo, Japan (S. Paudel, C. Nakajima, M. Sashika, M. Shimozuru, Y. Suzuki, T. Tsubota); Elephant Care International, Hohenwald, Tennessee, USA (S.K. Mikota); Department of National Parks and Wildlife Conservation, Kathmandu, Nepal (K.P. Gairhe); German Nepal Tuberculosis Project, Kathmandu (B. Maharjan); National Trust for Nature Conservation, Lalitpur, Nepal (S. Subedi); Chitwan Medical College, Chitwan, Nepal (A. Poudel)

DOI: https://doi.org/10.3201/eid2505.181898

1These first authors contributed equally to this article.

Mycobacterium tuberculosis is a primary cause of tuberculosis (TB) in elephants (1). Culture of trunk wash samples is regarded as the standard method for the diagnosis of TB in elephants; however, this method has many limitations (2). We previously reported TB in 3 elephants in Nepal that was caused by M. tuberculosis of Indo-Oceanic lineage (3). Here, we report on mixed M. tuberculosis lineage infection in 2 captive elephants from Chitwan National Park (CNP) in Nepal.

Elephant A was a female elephant ≈65–70 years old. She had been in retirement for 3 years before she died in February 2013. We observed TB-like lesions in the lungs postmortem (Appendix Figure 1, https://wwwnc.cdc.gov/EID/article/25/5/18-1898-App1.pdf). Elephant B was a 32-year-old male. His body condition had substantially deteriorated for several months before he died. We found extensive TB-like lesions in the lungs at postmortem.

We performed the DPP VetTB Assay (Chembio Inc., http://chembio.com), a serologic test, on the postmortem lung fluid (an off-label use) of elephant A and the serum of elephant B; results were reactive in both cases, indicating the presence of antibodies to TB. We processed the suspected lung lesions according to standard guidelines (4) and performed culture by using Löwenstein–Jensen media.

We performed genetic analyses on the 2 M. tuberculosis isolates by using spoligotyping and large-sequence polymorphism (LSP) as described previously (5). We amplified the direct-repeat region with a primer pair and hybridized the PCR products to a set of 43 oligonucleotide probes corresponding to each spacer covalently bound to the membrane. We identified the spoligo-international type by comparing spoligotypes with the international spoligo typing database (SpolDB4) (6). We performed LSP on the isolates by using specific primers for respective lineages, as described previously (7).

We identified the elephant isolates as a mixture of 2 strains based on uneven spoligotyping color development (suggesting mixture) and LSP detection PCR results (2 bands were observed). The spoligotyping results showed that the elephant A isolate had a new spoligotype that was not found in the international spoligotyping database. The elephant B isolate belonged to the Indo-Oceanic lineage (East African–Indian 5 spoligo-international type 1365) (Table). The prevalence of the Indo-Oceanic lineage among human TB patients in Nepal is only 11.5% (8). The drug
resistance–associated region sequences \textit{rpoB}, \textit{katG}, \textit{inhA} promoter region, and \textit{gyrA} were all wild types in both isolates. Similarly, LSP results showed that elephant A was infected by the Indo-Oceanic and East African–Indian lineages (CAS-Delhi) (Appendix Figure 2), whereas elephant B was infected with the East Asian type (Beijing type) (Appendix Figure 3). The prevalence of CAS-Delhi and Beijing type lineages in Nepal in human TB patients is 40.6% and 32.2%, respectively (8). In the \textit{gyrA} sequence, both of the samples showed a mixed peak of T231C, suggesting that the East African–Indian type is a Nepal-specific lineage.

Our study shows that the first elephant was infected with the Indo-Oceanic and East African–Indian (CAS-Delhi) \textit{M. tuberculosis} lineages, whereas the second elephant was infected with the Indo-Oceanic and East Asian (Beijing) lineages. We previously identified the Indo-Oceanic lineage in 3 elephants from Nepal (3). We suspect that this lineage might be well adapted in elephants in Nepal.

We diagnosed the mixed lineage infection postmortem in both elephants. However, a successful antemortem diagnosis of mixed infection in a single elephant would enable a precise TB diagnosis and selection of an appropriate anti-TB treatment, which could eventually lead to the control of this disease at the herd level.

The source of these mixed infections is unknown and could be from humans or elephants infected with these lineages. Infected elephant handlers who have daily close contact would be a likely human source. Genotyping of additional isolates from elephants and their handlers will help to determine the source of infection. We recommend regular TB screening of elephant handlers to safeguard human health and help prevent transmission of TB from humans to elephants.

**Acknowledgments**

We thank Arjun Pandit, Chitra B. Khadka, and Kiran Rijal for helping with the collection of elephant samples; technicians at the German Nepal Tuberculosis Project in Kathmandu for performing the laboratory work; and Yukari Fukushima and Haruka Suzuki for technical support at the Hokkaido University Research Centre for Zoonosis Control.

The National Trust for Nature Conservation Nepal and the Department of National Parks and Wildlife Conservation of Nepal’s Ministry for Forests and Soil Conservation supported this work.

**References**


**About the Author**

Mr. Paudel is an assistant professor in the Department of Cell Physiology, Faculty of Medicine and Graduate School of Medicine, Hokkaido University, Sapporo, Japan. His research interests include the mechanism of TB bacteria entry into cells, TB in elephants, and development of TB diagnostic tools for elephants.