Mixed *Mycobacterium tuberculosis* Lineage Infection in 2 Elephants, Nepal

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resistance-associated region sequences rpoB, katG, inhA promoter region, and 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 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) 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.

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Table. Genotypic characteristics of Mycobacterium tuberculosis isolates from 2 elephants, Nepal*

<table>
<thead>
<tr>
<th>Source</th>
<th>Spoligotype† binary code</th>
<th>SIT</th>
<th>Clade</th>
<th>gyrA‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elephant A</td>
<td>11100000111111111111011100111111111</td>
<td>New§</td>
<td>New§</td>
<td>T231C</td>
</tr>
<tr>
<td>Elephant B</td>
<td>00000000000000000000011100111111111</td>
<td>1365</td>
<td>EAI5</td>
<td>T231C</td>
</tr>
</tbody>
</table>

*EAI5, East African–Indian 5; SIT, spoligo-international type.
†Spoligotype was determined as previously described by Brudey et al. (6).
‡Mutation in a partial sequence of gyrA. The gyrA sequence of both elephant isolates had a synonymous single nucleotide polymorphism from T to C at position 231.
§Not found in the international spoligotyping database (SpolDB4).

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.

References

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Appendix

**Appendix Figure 1.** Granulomatous tuberculosis lesion with caseous mass in lungs of Elephant A.
Appendix Figure 2. Large Sequence Polymorphism (LSP) results of Elephant A isolate. Gel electrophoresis was run in isolate A using primers for Indo-oceanic lineage (lineage 1) and East-African-Indian lineage (lineage 3). Lanes: 1, 50bp DNA ladder; 2, Elephant A isolate; 3, positive control for Indo-Oceanic lineage; 4, H37Rv (Euro-American lineage, lineage 4); 5, negative control; 6, 50bp DNA ladder; 7, elephant sample; 8, positive control for East-African-Indian lineage; 9, H37Rv; 10, negative control.

Appendix Figure 3. Large Sequence Polymorphism (LSP) results of Elephant B isolate. Gel electrophoresis was run in isolate B using primers for East-Asian-Beijing (lineage 2). Lanes: 1, 2-log DNA ladder; 2, Elephant B isolate; 3, positive control for East-Asian-Beijing; 4, BCG; 5, negative control.