HOKKAIDO UNIVERSITY

Title	Molecular investigation of tuberculosis in Asian elephants in Nepal
Author(s)	PANDIT, Arjun
Description	この博士論文の全文は利用可能日以降に公開されます。利用可能日以前の閲覧方法は https://www.lib.hokudai.ac.jp/dissertations/copy-guides/ をご参照ください。
Degree Grantor	北海道大学
Degree Name	博士(獣医学)
Dissertation Number	甲第16657号
Issue Date	2025-09-25
DOI	https://doi.org/10.14943/doctoral.k16657
Doc URL	https://hdl.handle.net/2115/98267
Type	doctoral thesis
File Information	Arjun_Pandit_summary.pdf, 全文の要約

Hokkaido University Collection of Scholarly and Academic Papers : HUSCAP

Molecular investigation of tuberculosis in Asian elephants in Nepal

(ネパールにおけるアジアゾウの結核に関する分子調査)

Elephant Tuberculosis (ETB), primarily caused by *Mycobacterium tuberculosis* (*Mtb*), is a common zoonotic disease with transmission dynamics that can occur from humans to elephants or within the elephant population. ETB is becoming a major conservation threat to Asian elephants in Nepal, raising serious concerns. Although the Indo-Oceanic lineage remains dominant, strains from the Beijing and CAS-Delhi lineages have also been identified. In some cases, mixed infections of two lineages have also been identified.

Diagnosis of ETB presents considerable challenges, as it requires postmortem examination followed by culture of *Mtb* from lung specimens. In live animals, we need to establish some reliable tests because the Elephant TB STAT-PAK and the Dual Path Platform Veterinary Tuberculosis test (DPP VetTB assay) are antibody-based and less confirmatory. The trunk wash culture method, regarded as the gold standard for antemortem diagnosis, is labor-intensive, often yields false-negative results, and requires high-containment laboratory facilities. Molecular diagnostic techniques such as Polymerase Chain Reaction (PCR) have been used with some success; however, limitations in sensitivity and sample quality remain. These diagnostic challenges underscore the need for a more reliable, sensitive, and field-appropriate diagnostic approach.

The main goal of this thesis is to promote elephant health management, elephant conservation, and public health by integrating conventional and molecular diagnostic tools and contributing to the existing body of knowledge on the molecular epidemiology of ETB, interspecific and intraspecific transmission dynamics, drug resistance patterns, and the diagnosis framework of ETB. To achieve that goal, Chapter I of the study aims to confirm tuberculosis (TB) as the major cause of elephant death and verify the lineage and drug-

resistance status of elephants to determine possible intraspecific and interspecific TB transmission, reinforcing the importance of a One Health approach to ETB surveillance and control in elephant management systems. Molecular genotyping aimed to gain insights into the possible transmission links between elephants and humans, a finding of critical importance given the frequent and close human-elephant interactions in Nepal's protected areas. Similarly, Chapter II aims to compare the Loop-mediated Isothermal Amplification (LAMP) and PCR to underscore the superior sensitivity of the LAMP assay, particularly at low DNA concentrations. The application of LAMP to fecal samples aims to demonstrate its potential as a non-invasive and field-adaptable tool for ETB detection.

To achieve the abovementioned goals and aims, the present thesis is structured into two chapters, each addressing a distinct aim with an aspect of ETB in Nepal. Chapter I focuses on the molecular epidemiology of ETB based on postmortem lung samples collected from nine previously deceased elephants. Various diagnostic methods were employed, including culture, PCR, and LAMP, followed by molecular characterization through Spoligotyping, Mycobacterial Interspersed Repetitive Unit-Variable Number of Tandem Repeats (MIRU-VNTR), Large Sequence Polymorphism (LSP) analysis, and *gyrA* gene sequencing. Chapter II is dedicated to the evaluation of the LAMP assay as a diagnostic tool. This chapter includes a comparative sensitivity assessment of LAMP and PCR using serially diluted DNA from BCG subcultures, followed by molecular screening of fecal samples from DPP-positive and DPP-negative elephants to assess the field applicability of LAMP as a non-invasive diagnostic method.

Chapter I analyzed lung tissues from nine deceased elephants (2019–2022) using bacterial culture, PCR, and LAMP. Besides this, a Drug sensitivity test (DST) was performed by both phenotypic and genotypic methods in the case of four culture-positive isolates, and in the case of one isolate, which was contaminated in the culture test, DNA extracted from

the tissue sample was used to analyze drug resistance-associated mutations in the encoding region of *rpoB*, *katG*, and the promoter region of *inhA*. Besides this, genotyping of five PCR-positive isolates was performed by Spoligotyping, Large Sequence Polymorphism (LSP), *gyrA* sequencing, and MIRU-VNTR. *Mtb* was detected in 6 samples by LAMP, 5 by PCR, and 4 by culture. DST revealed an isoniazid resistance-associated mutation in *katG* (Ser315Thr) in one elephant isolate. Spoligotyping in combination with LSP revealed the presence of two Beijing lineages and three Indo-Oceanic lineages, with the T231C mutation among five PCR-positive isolates. MIRU-VNTR, followed by Phylogenetic analysis and the emergence of isoniazid resistance in elephants of Nepal, suggested possible TB transmission between elephants and potential transfer from humans. These results highlight the need for reliable, non-invasive diagnostic methods for antemortem TB diagnosis in elephants and a one-health approach to control the disease.

Chapter II focused on optimizing a non-invasive technique for ETB detection based on two phases of study. The first study is based on serial dilution of BCG Tokyo-172 subcultures from 10^0 to 10^{-5} concentration, followed by LAMP and PCR test for DNA extracted from each of the concentrations of those BCG Tokyo-172 subcultures using Qiagen stool kit, with four replicates of experiments. In the first study, LAMP showed 100 times greater sensitivity than PCR, detecting as few as 150 colony-forming units (CFUs) compared to 15,000 for PCR. In the second study, a longitudinal fecal sampling (2022–2025) for three consecutive days with annual repetition of 18 captive elephants, including 11 DPP-positive and 7 DPP-negative elephants in the DPP VetTB assay in Chitwan National Park, was performed. Sample collection was followed by LAMP and PCR tests of DNA extracted from those fecal samples using the Qiagen stool kit. In this study, among 11 DPP-positive elephants, two LAMP-positive cases were detected with consistent results during the study period, while PCR detected none. LAMP results were further corroborated by identifying culture-positive ETB in a LAMP-positive elephant that died two years into the study. The

consistent result throughout the study period, along with the culture result of the dead elephant, strengthens the likelihood of true infection.

The study provides molecular evidence of *Mtb* transmission dynamics among elephants, including zoonotic risks, and is the first report of drug-resistant ETB in elephants in Nepal. It also demonstrates the potential of LAMP as a sensitive, non-invasive field diagnostic tool.