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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)

Tuberculosis (TB) poses a growing threat to the conservation of Asian elephants in Nepal and has emerged as a leading cause of mortality. In Nepal, there is the possibility of interspecific and intraspecific disease transmission, with the Indo-Oceanic lineage being the major lineage. However, other lineages like the Beijing lineage and the CAS-Delhi lineage have also emerged. This study investigates the epidemiology, molecular characterization, and diagnostic advancement of *Mycobacterium tuberculosis* (*Mtb*) in elephants.

In Chapter I, lung tissue samples from nine deceased elephants (2019–2022) were analyzed using bacterial culture, conventional PCR, and loop-mediated isothermal amplification (LAMP). *Mtb* was detected in 6/9 samples by LAMP, 5/9 by PCR, and 4/9 by culture. Genotyping of five PCR-positive isolates revealed infections with both Indo-Oceanic and Beijing lineages, including an isoniazid-resistant Beijing strain. Phylogenetic analysis using MIRU-VNTR typing, alongside *gyrA* and *katG* sequencing, indicated potential elephant-to-elephant transmission of the Indo-Oceanic lineage and suggested possible zoonotic transmission of the drug-resistant Beijing lineage from humans. These findings underscore the urgent need for improved, non-invasive diagnostic approaches.

Chapter II focused on optimizing a non-invasive diagnostic tool to enhance TB surveillance in elephants. This was conducted in two phases. In the first study, *Bacillus Calmette-Guérin* (BCG Tokyo-172) cultures were used to compare the sensitivity of PCR

and LAMP. LAMP demonstrated superior sensitivity, detecting as few as 1.5×10^2 colonies, compared to PCR's limit of 1.5×10^4 colonies. In the second study, longitudinal fecal sampling was conducted on 18 captive elephants (11 DPP-positive, 7 DPP-negative) in Chitwan National Park from 2022 to 2025. DNA extracted from feces was tested using PCR and LAMP. Among the DPP-positive elephants, only two were LAMP-positive, and none were PCR-positive. This disparity likely reflects differences in diagnostic targets; DPP detects antibodies indicative of prior exposure, while LAMP and PCR identify active infection via bacterial DNA. Postmortem confirmation of TB by culture in one LAMP-positive elephant further validated LAMP as a viable, non-invasive diagnostic tool.

In conclusion, this study presents the molecular evidence of *Mtb* transmission dynamics in elephants in Nepal, including zoonotic implications and the first reported drug resistance in elephants from Nepal. Moreover, it highlights the potential of LAMP as a sensitive, field-applicable diagnostic method for the non-invasive detection method for surveillance of TB in elephants, thereby contributing to more effective disease management and conservation strategies in the captive-wild interface.