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Abstract of the dissertation

博士の専攻分野の名称：博士（獣医学）

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Studies on genotype and diagnosis of tuberculosis in Asian elephants (*Elephas maximus*) of
Nepal

（ネパールのアジアゾウにおける結核の遺伝子型別および診断に関する研究）

Tuberculosis (TB) has been increasingly seen in the captive elephants around the world. TB is a re-emerging disease in the elephants basically caused by *Mycobacterium tuberculosis*, a human form of TB. Culture of trunk wash is regarded as a gold standard for diagnosis of TB in elephants; however, this technique has many limitations. There are about 200 captive elephants in Nepal and TB was first documented in 2002. Only very few studies on the genotyping of the *M. tuberculosis* isolates from the elephants has been performed till now.

As a part of my PhD work, I performed the molecular characterization of *M. tuberculosis* isolates from three elephants (Elephant A, B and C) of Nepal. Spoligotyping, TbD1 detection and multi-locus variable number of tandem repeat analysis (MLVA) results suggested that three isolates belonged to a specific lineage of Indo-Oceanic clade, EAI5 SIT138. One of the elephant isolates had a new synonymous single nucleotide polymorphism (SNP) T231C in the *gyrA* sequence, and the same SNP was also found in human isolates in Nepal. MLVA results and transfer history of the elephants suggested that

two of them might be infected with *M. tuberculosis* from the same source.

Furthermore, the genotyping of the *M. tuberculosis* isolates from other two elephants (Elephant D and E) that died of suspected TB was performed by spoligotyping and large sequence polymorphism (LSP). The spoligotyping showed that Elephant D isolate had a new spoligotype that were not found in the SpolDB4 database while Elephant E isolate belonged to Indo-Oceanic lineage (EAI5, SIT1365). LSP results showed that Elephant D isolate belonged to two lineages of *M. tuberculosis* i.e. Indo-oceanic lineage and East-African-Indian (CAS-Delhi) lineage while Elephant E isolate belonged to East-Asian (Beijing) lineage.

Similarly, I developed an interferon- γ release assay (IGRA) specific for Asian elephants (*Elephas maximus*). The peripheral whole blood collected from forty captive elephants from three protected areas of Nepal was stimulated with three different mitogens ie. phytohaemagglutinin (PHA), pokweed mitogen (PWM) and phorbolmyristateacetate/ionomycin (PMA/I). Sandwich ELISA was performed using anti-elephant IFN- γ rabbit polyclonal antibodies as capture antibodies and biotinylated anti-elephant IFN- γ rabbit polyclonal antibodies as detection antibodies which was able to recognize the rEIFN- γ as well as native interferon- γ from the elephants. The results also showed that PMA/I was the best mitogen as a positive control in elephant IGRA.

In conclusions, our study provides valuable information on the genotypes of on *M. tuberculosis* isolates from elephants of Nepal. These findings indicated the source of *M. tuberculosis* infection of those elephants were local residents, presumably their handlers.

Our study has also reported the mixed infection of *M. tuberculosis* isolates in Asian elephants of Nepal. Mixed infection in both of these elephants might be due to double infection from human patients or from infected elephants with similar lineages. Future study including the genotyping of elephant and human isolates will help to explain the epidemiology, source and route of TB transmission between the elephants and humans. Similarly, our study on the development of Asian elephant specific interferon gamma release assay (IGRA) has shown that PMA/I was the best mitogen as a positive control in elephant IGRA. ELISA system developed was able to detect the native interferon- γ from the Asian elephants. This preliminary study showed that it can be a potential tool for the early diagnosis of diseases like tuberculosis in Asian elephants after validation in a larger population of TB infected and non-infected elephants.