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学位論文内容の要旨
Abstract of the dissertation

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学位論文題名
The title of the doctoral dissertation

Development of a molecular tool for the differentiation of *Mycobacterium bovis* and molecular characterization of *Mycobacterium bovis* isolates in Malawi

(ウシ型結核菌遺伝子診断法の開発とマラウイにおけるウシ型結核菌分離株の遺伝学的解析)

Bovine tuberculosis (bTB) caused by *Mycobacterium bovis* is a neglected tropical and zoonotic disease of animals and humans. BTB burden is high in developing countries because of the presence of multiple risk factors maintaining the circulation of the disease. The lack of surveillance tools and limited laboratory support has significantly contributed to the underestimation of the disease's burden. Hence there is a critical need for simplified and low-cost methods for the detection of *M. bovis* that can easily be integrated for use in developing countries. Additionally, molecular epidemiological studies become essential to provide information of circulating *M. bovis* strains necessary for formulating optimal bTB control approaches.

In chapter I, a loop-mediated isothermal amplification (LAMP) assay was developed for specific detection of *M. bovis* by targeted the region of difference 4 (RD4), a 12.7kb locus that is deleted in all *M. bovis* strains but inserted in other *Mycobacterium tuberculosis* complex (MTBC) species. The assay's specificity was assessed by 139 isolates comprising 65 *M. bovis* isolates, 40 *M. tuberculosis* isolates, seven MTBC reference strains, 22 non-tuberculous mycobacteria and five other bacteria. All *M. bovis* isolates were tested positive, while all other bacteria were negative. The LAMP assay detected 10 copies of *M. bovis* genomic DNA within 40 minutes.

Considering that wet LAMP requires cold chain maintenance of reagents especially

enzymes. In chapter II, dry LAMP assay was developed based on wet LAMP method established in previous study. Field evaluation was performed on cattle samples collected during routine post-mortem examination at the three regional abattoirs in Malawi. Additionally, clinical samples from the National TB reference laboratory in Lilongwe were also subjected to dry LAMP assay. A total of 1,547 cattle were examined, out of these 146 had tuberculosis - like lesions and samples were collected. Dry LAMP assay detected 82 (65.6%) as *M. bovis* while multiplex PCR performed on the same samples detected 78 (62.4%) as *M. bovis.* Out of 86 clinical isolates, only one was positive as *M. bovis.*

In chapter III, the molecular epidemiology of *M. bovis* in central parts of Malawi was elucidated to gain insights into the transmission dynamics, sources, and circulating population of *M. bovis* strains. Samples were collected during the previous study. Molecular typing tools; deletion analysis, spoligotyping and MIRU-VNTR were performed. Our findings show that European 1 clonal complex (81%) and spoligotype SB0131 (56%) are dominant. We found a high genetic diversity of *M. bovis* in the area. The isolated strains showed genetic relationships with *M. bovis* isolates previously reported in neighbouring countries.

The LAMP assay developed in this study provides a better option the detection of *M. bovis* in developing countries where the burden of bTB is high. The molecular findings of *M. bovis* in central parts of Malawi significantly increases the understanding of bTB in the area and suggesting the need for the development of new control strategies or enhance the current control programs to control the disease.