Form No.5

Abstract of Thesis/Dissertation

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Title : Epidemiological study of bovine leukemia virus infections in eastern Hokkaido, Japan and northern Vietnam（日本の北海道東部とベトナムの北部における牛白血病感染の疫学的研究）

Abstract

Bovine leukemia virus (BLV) is an oncogenic virus belonging to genus *Deltaretrovirus* in *Retroviridae* family. BLV is the etiological agent of enzootic bovine leucosis, and it closely related to Human T-cell leukemia virus type I and type II (HTLV-I and II) in terms of its genetic structure, sequence, and regulation of expression. Two copies of single-stranded genomic RNA, and two enzymatic proteins: reverse transcriptase and integrase (RT-IN) are packaged in the viral particle. The complete sequence of the integrated BLV is 8,714 nucleotides long, and the 5' and 3' extremities of the integrated BLV present the same nucleotide sequence called long terminal repeat (LTR). BLV's genome structure can be divided into two main parts including the essential part which consists four open reading frames (orfs), encoding for the capsid proteins (*gag*), the viral protease (*pro*), the reverse transcriptase polymerase (*pol*), and the envelope proteins (*env*). The regulation part encodes for at least four proteins including the *R3*, *G4, rex*, and *tax* proteins. BLV is not very pathogenic or infectious in natural conditions. However, the commercial exchanges of cattle led to the spread of the infection around the world. Phylogenetic analysis using the complete BLV *gp51 env* gene sequence demonstrated that BLV is more divergent than previously thought. Recent studies reported

that BLV can be classified into ten genotypes (G1-G10). Some European countries have successfully eradicated BLV infections by the national control program. On the other hand, many countries are now still dealing with BLV infections among their cattle population including Japan. In the recent years, BLV infections have been rapidly increased in Japan, and it is a notifiable disease which has been subjected to passive surveillance since 1998. In contrast, BLV infection has not been paid much attention in Vietnam, and the understanding of the disease is very limited. So far, no study on the BLV infection has been conducted in Vietnam. Therefore, to understand a status of BLV infection among cattle in Japan and Vietnam, I conducted an epidemiological study of BLV infections in eastern Hokkaido, Japan, and Hanoi, Vietnam.

The study in chapter 1 evaluated the in-house absolute quantitative real-time PCR targeting the BLV *pol* gene and monitored the current status of BLV infection among cattle in eastern Hokkaido from 2016-2017. All cattle in two selected farms (named K and U) in eastern Hokkaido were screened for BLV infections by serological and molecular detection methods. The positive rates determined by ELISA were higher than those by the real-time PCR in both farms. Overall, the seroprevalence of BLV in K farm was high at 46.44%, while low in U farm at 17.65%. Molecular characterization demonstrated that BLVs belonging to genotype 1 are circulating among cattle in eastern Hokkaido. The comparison of in-house absolute quantitative real-time PCR targeting the BLV *pol* gene with the commercial absolute quantitative real-time PCR targeting the BLV *tax* gene suggested that in-house real-time PCR assay can be applied to the detection of BLV DNA provirus in the infected cattle. The results also demonstrated that continuing surveillance and finding the route of horizontal transmission in Japanese farming system, as well as elimination and controlling of risk factors in order to prevent new infections are critical.

The study in chapter 2 provided the information on BLV infection in Vietnam for the first time. The result presented that, BLV infections were detected among cattle in 8 out of the 22 tested farms in 4 districts in Hanoi, Vietnam in 2017. The BLV infected prevalence was high at 35.48 % as determined by both ELISA and in-house absolute quantitative real-time PCR targeting the BLV *pol* gene. One remarkable finding is that the commercial absolute quantitative real-time PCR targeting the BLV *tax* gene failed to determine Vietnamese BLVs genotype 6, similar to a previous report on Myanmar BLVs

genotype 10. Phylogenetic analysis indicated that at least two genotypes groups, G1 and G6, were circulating among cattle population in Hanoi, Vietnam. Among them, a new sub-genotype 6 named G6f was found. In addition, phylogenetic analysis showed that Vietnamese BLVs could be clustered with Thai BLVs in both genotypes (G1 and G6). It was suggested that Vietnamese BLV may have the same origin as Thai BLVs as the consequence of cattle trading between two countries. Considering the management of Vietnamese farming system, cattle replacement from different herds of farms, crossbreeding between native cattle and imported cattle, and extended calving interval may be the factors that caused the genetic diversity of this virus in this country. Moreover, phylogenetic analysis demonstrated that the complete *env gp51* gene sequences (903 bp) are recommend to differentiate BLV G6 from G10 instead of the partial *env gp51* (444 bp) that has been usually used to genotype BLVs.

The study in chapter 3 evaluated the SureSelect Target Enrichment System, which is an efficient and useful method for deep sequencing the whole BLV genome from DNA provirus samples. BLV is an exogenous virus, which integrates its genome into the genome of host B cells, and coexists with the host B cells as a provirus. Therefore, sequencing the complete BLV genome by Sanger sequencing or conventional next generation next-generation sequencing (NGS) requires sensitive PCR techniques and plenty of time for sequence processing, since genomic DNA extracted from the BLV-infected animals contains a large amount of the host cell genome. Four Vietnamese complete BLV genome were successfully sequenced, which is the first report in Vietnam. Analyzing the genetic diversity of BLV *pol* and *tax* genes of Vietnamese BLVs revealed that the BLV G6VN *tax* gene is more diverse than its *pol* gene. In addition, comparing the BLV G6VN *tax* gene sequences with the BLV G6VN reference *tax* gene sequences, total 7 specific nucleotide mutations were exclusively found in the BLV *tax* gene sequences of the BLV G6 strains including the BLV G6VN and G10 strains. It is assumed that any of the 7 nucleotide mutations in the BLV *tax* gene possibly abrogate the gene detection by the commercial tax-rt-PCR assay. The results obtained may contribute to improving the reliability of such detection systems. The phylogeographic analysis illustrated that Vietnamese BLVs were clearly distinguished into two groups. One group had the same origin as Japanese and Korean strains, while another group had the same origin as the strains isolated in Brazil, Argentina, Paraguay, Thailand, and China. Moreover, the results

of Google Earth software analysis demonstrated that BLV was transmitted to Thailand from the USA in 1905, and 45 years later Thai BLVs began to spread to Vietnam cattle. On the other hand, BLV was imported to Japan from the USA in 1982, and then it started to spread to Vietnamese cattle in 2004.

In conclusion, this study provides the informative information of current BLV infection status among cattle population in eastern Hokkaido, Japan, and northern Vietnam. The results supported that BLV is more divergent than previously thought. A new sub-genotype G6f was found in this study. In addition, the study showed the SuperSelect Target Enrichment System is an efficient and useful method for preparing DNA provirus samples for NGS to sequence the whole BLV genome. The results were obtained in less than one week. The phylogeographic analysis in this study gave an overview for better understanding of the temporal history of BLV spread due to spatial molecular epidemiology not only in Vietnam but also in other countries. The genetic diversity analysis of the BLV *tax* genes may contribute to improving the reliability of the commercial *tax*-rt-PCR assay. The findings obtained herein would be useful for further epidemiological studies on BLV infection not only in Japan and Vietnam but also in other countries that require better cattle husbandry practices.

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