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**<Short Communication>**

# Genetic analysis of *env* and *gag* gene fragments of bovine leukemia virus identified in cattle from Korea

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**Abstract :** Bovine leukemia virus (BLV) is the causative agent of enzootic bovine leukosis. This study was conducted to clarify the molecular characteristics of BLVs obtained from a specific region in Korea. Proviral BLVs were detected in anti-BLV antibody-positive blood samples by PCR. *Env* and *gag* fragments were sequenced and compared to previously published reference sequences. Analysis of the *env* gene sequence revealed that the YI strain was highly similar to genotype 1, including United States and Japanese strains. The *gag* gene sequence had the highest degree of similarity with a Japanese strain.

**Keywords :** bovine leukemia virus, cattle, *env* gene, *gag* gene

Bovine leukemia virus (BLV) belongs to the family *Retroviridae,* and it is the causative agent of enzootic bovine leukosis (EBL) [7]. Although most countries in western Europe are EBL-free, BLV infection has a worldwide distribution and sero-epidemiological studies have reported high prevalence in some countries [1]. Since transmission of BLV may occur via contact with infected animals, parturition, mechanical transmission by insects, blood transfusion, and the use of common needles, it is important to carry out active surveillance to ensure control of the disease [10].

The seroprevalence of BLV in dairy and native cattle herds has been reported in South Korea. In 2003, sera obtained from 1,413 Korean-native cows in 8 provinces and from 2,415 dairy cattle in 9 provinces were analyzed by enzyme-linked immunosorbent assay (ELISA). Anti-BLV antibodies were found in 0.14% of the Korean-native cows and 54.2% of the dairy cows [13]. Although no eradication program exists at the national level, a large dairy farm in Chungnam was applied to “test and segregate” program and established into BLV-free dairy herd [12].

BLV infection is life-long and 30~35% of infected cattle develop persistent lymphocytosis; the presence of antibodies or integrated proviral DNA is an indicator of virus exposure [3]. To diagnose BLV infection, a variety of tests, including agar gel immunodiffusion (AGID), virus neutralization assay, polymerase chain reaction (PCR), and ELISAs have been developed [6, 9].

Partial *env* and *gag* sequences have been used to reveal genetic variation and to characterize BLVs [7]. A recent phylogenetic study of the BLV *env* gene from strains isolated worldwide demonstrated that the virus can be divided into eight genotypes [10]. Molecular analysis of BLV infections in domestic ruminants have been reported in many countries, including Argentina and Japan [7, 8, 10]. However, the BLV provirus has not been characterized in cattle in South Korea.

The aim of this study was to characterize the *env* and *gag* gene fragments of BLV identified from specific region in South Korea. The genetic characteristics of BLVs were confirmed by genomic analysis, including sequencing and sequence alignment.

In December 2013, total of 110 whole bloods and sera collected from a dairy cattle farm in Gyeonggi province were tested for BLV. An AGID kit (BLV AGID; MEDIAN Diagnostics, Korea) was used for the detection of anti-BLV antibodies in the sera. Total DNA was extracted from whole blood using the QIAamp DNA Mini kit (Qiagen, Germany) according to manufacturer’s instructions.

For *env* gene amplifications, env-1 (5'-TCTGTGCCAAGT CTCCCAGATA-3') and env-2 (5'-AACAACAACCTCTGG-GAAGG G-3') were used as outer primers; env-3 (5'-CCCA-CAAGGGCGGCGCCGGTTT-3') and env-4 (5'-GCGAGGC CGGGTCCAGAGCT GG-3') were used as inner primers, as previously described [15]. For *gag* gene amplifications, gag-1 (5'-CCAATCATATCTGAAGGGAA-3') and gag-2 (5'-

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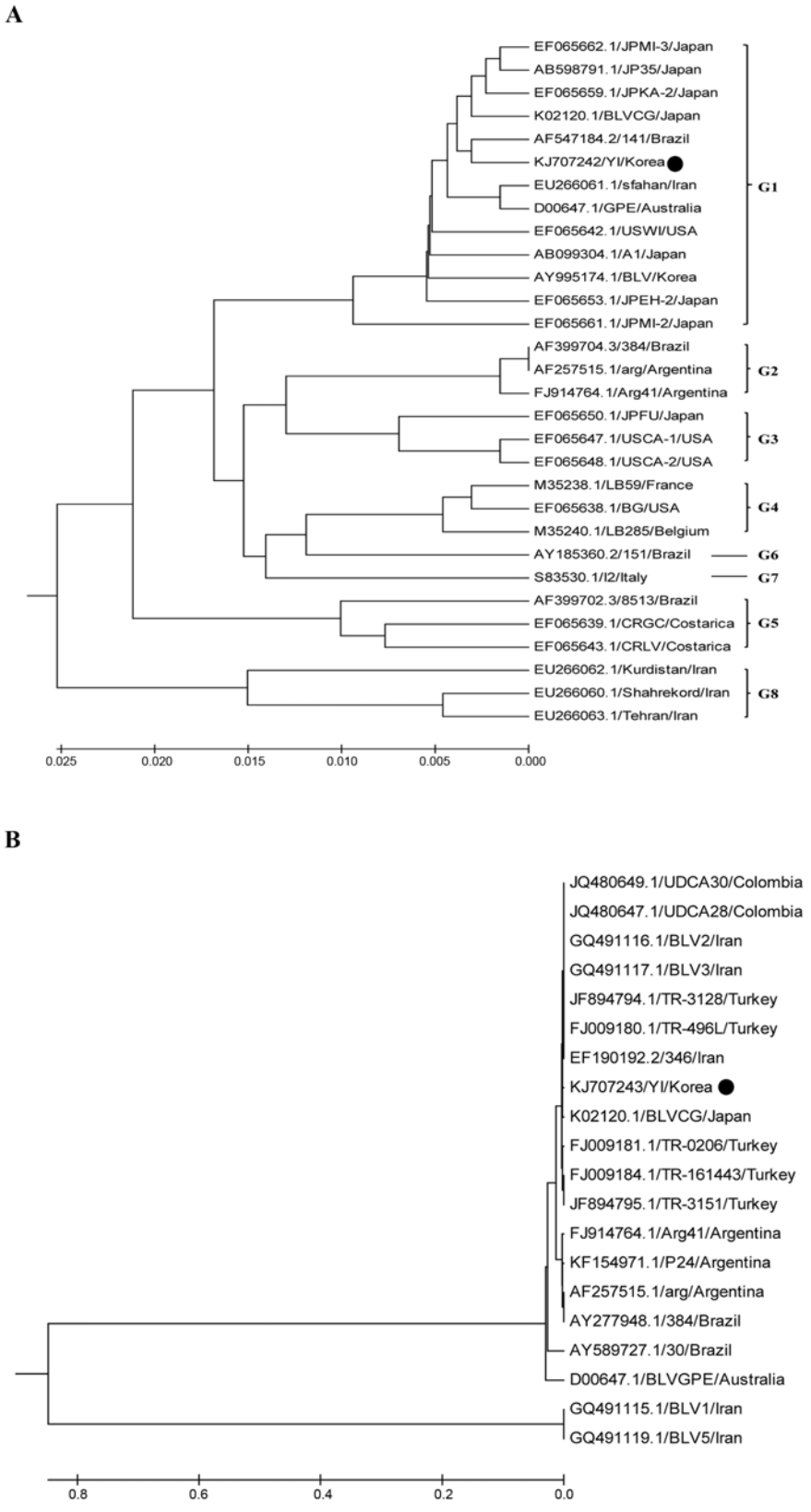


**Fig. 1.** Phylogenetic analysis of partial sequences of bovine leukemia virus (BLV) *env* (A) and *gag* (B) gene. One thousand bootstrap replicates were performed to determine the nucleotide sequence distance. The consensus phylogenetic trees were created using the unweighted pair group method with arithmetic mean.

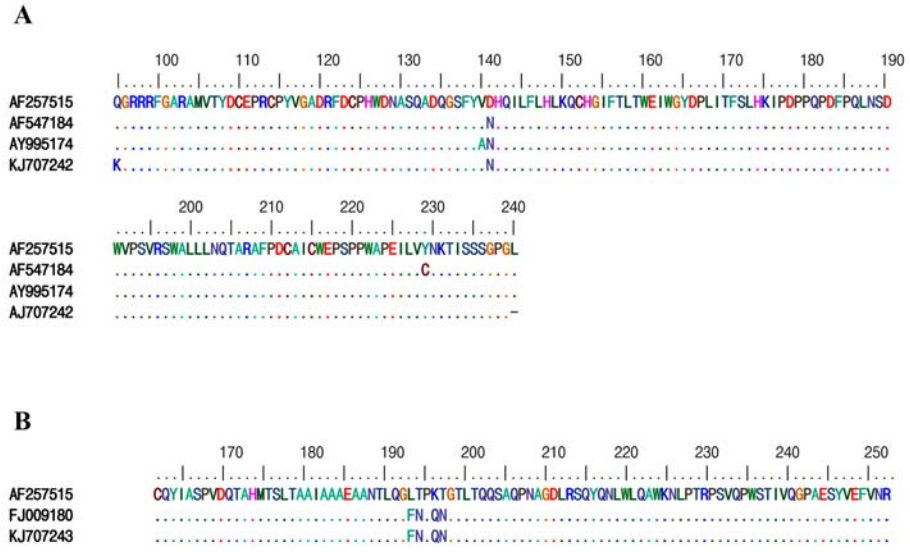


**Fig. 2.** Alignment of the partial *env* (A) and *gag* (B) deduced amino acid sequences from the YI bovine leukemia virus (BLV) strain. Dots indicate identity to the AF257515 sequence. The numbers above the AF257515 sequence refer to the complete amino acid sequence.

CAGAAGT GCAGGCTGTTTCA-3') were used as outer primers; gag-3 (5'-AACACTACGACTT GCAATCC-3') and gag-4 (5'-GGTTCCTTAGGACTCCGTCG-3') were used as inner primers, as previously described [5]. The amplified DNA fragments were purified by Agarose Gel DNA Extraction kit (iNtRON Biotechnology, Korea) and sequenced (Macrogen, Korea). For phylogenetic analysis, sequences were aligned in BioEdit software (ver. 7.1.9; Ibis Biosciences, USA) and analyzed using MEGA 6 software with bootstrap values calculated from 1,000 replicates [14]. The sequences obtained in this study were submitted to GenBank (National Center for Biotechnology Information, USA) under accession numbers KJ707242 for the *env* gene and KJ707243 for the *gag* gene.

The AGID test showed that 59.3% of samples were positive for anti-BLV antibodies. BLV was also detected by PCR, and the amplified 444 bp *env* gene, 380 bp *gag* gene products were visualized by electrophoresis (data not shown). Among the positive samples in both AGID and PCR tests, *env* and *gag* gene were sequenced from 5 blood samples and aligned. The sequences of the 5 samples were completely identical.

The nucleotide sequences of each gene fragment were also compared to those of other previously characterized BLV strains. Since BLV infection is almost restricted to cattle, the evolutionary rate of the virus is assumed to be constant [10]. Therefore, the unweighted pair group method with arithmetic mean algorithm was used for building a phylogenetic tree of BLV.

Phylogenetic analysis revealed that the partial *env* gene sequence obtained from the Korean BLV isolates belonged to genotype 1, together with isolates from Japan, USA, Australia, and Brazil (Fig. 1). The sequences were almost identical (98.1~99.4%) within genotype 1 strains. The partial *env* deduced amino acid (aa) sequence was compared to the complete deduced *env* aa sequence belonging to a genotype 2 strain, the Argentinean isolate B19 (AF257515), a genotype 1 strain (AF547184), and a Korean strain (AY995174) previously submitted to GenBank. In the second neutralization domain (aa 131-149), there was one substitution ($D^{141}$→N) compared to other genotype 2 isolates (Fig. 2). There were no differences in the region comprising aa 145-253, a highly conserved region with important biological functions related to interaction with cell receptors and infection [2].

The *gag* gene sequences of all of the BLV strains used in phylogenetic analysis of the *env* genes were not available in GenBank; therefore, we could not compare the sequences of *env* and *gag* genes of the same BLVs. The *gag* gene sequence of BLV reported in this study showed 94.6~99.6% similarity to Turkish, Argentinian and Japanese strains at the nucleotide level, with lower similarity to two Iranian strains (68.6%). The partial deduced aa sequence of *gag* gene from the Korean BLV strain did not differ from the Turkish strain. Although aa differences at four residues (aa 199, 200, 202, and 203) were investigated in the complete aa sequence (AF257515), our sequences were highly conserved across the BLV *gag* region (Fig. 2B).

Licursi *et al*. [8] have reported that most Argentinian BLVs are related to the European type, while most Japanese BLVs are similar to those originating from the USA and Australia. Korean strains were highly similar to genotype 1 including the United States, Australia, Iran, Brazil, and Japanese strains.

BLV *env* encodes a single precursor protein cleaved into two subunits; the envelope protein, gp51, and the transmembrane subunit, gp30. BLV gp51 and the BLV viral capsid protein (*gag* p24) are the immunodominant viral proteins *in vivo* [7]. Although complete BLV genomes from worldwide strains have been recently reported, partial sequencing of different genes, including *env* and *gag* has been previously reported [4]. In this study, Korean BLV was classified by

phylogenetic analysis based on *env* and *gag* regions of the viral genome.

Based on the BLV characterization such as replicating slowly, the prevalence is closely related to disease outbreak. The prevalence of BLV infection in cattle was known as to be quite high on some farms in Korea, which may be due to the movement of cattle among farms. Of the case, 3~20% of calves from BLV-infected dams were infected with BLV at birth, thereby maintaining the BLV infection from one generation to the next. Moreover, the cattle management procedures involving transference of infected blood (*i.e*., ear tattooing, dehorning, the use of clipper, the use of infected needles) have been postulated as a common mode of BLV transmission [11]. This may explain why BLV infection rates remain high. Therefore, great care is necessary for the control of BLV infection in cattle.

Further studies should investigate other regions of the BLV genome and assess a greater number of samples in order to shed light on BLV transmission between herds and the molecular epidemiology of BLV infection in Korea.

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