



NOTE

Virology

Epidemiological evidence for early-onset of enzootic bovine leukosis by L233-Tax-carrying bovine leukemia virus in Japanese Black cattle

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ABSTRACT. Bovine leukemia virus (BLV) is the causative agent of enzootic bovine leukosis (EBL), of which annual number has rapidly increased in Japan, and it can be divided into two categories based on the amino acid at position 233 in the Tax protein. Here, we conducted a nationwide surveillance of Japanese Black cattle between 2008 and 2021 in Japan. Among 237 tumor samples, 131 (55.3%) and 101 (42.6%) were harbored L233- and P233-Tax, respectively. Onset of EBL under the age of 3 years by L233-Tax-carrying BLV was frequently observed, especially in the animals born via embryo transfer. We also found that L233-Tax-carrying BLV was more prevalent in dairy areas than non-dairy areas. These findings give insight into prevention of EBL.

KEYWORDS: bovine leukemia virus, enzootic bovine leukosis, Japanese Black cattle, Tax

Bovine leukemia virus (BLV), a member of the family *Retroviridae* and genus *Deltaretrovirus*, is the causative agent of enzootic bovine leukosis (EBL). Although most countries in the European Union successfully eradicated EBL via “test and elimination” [1, 11, 16], BLV infection is widely distributed in cattle-raising countries, including Japan, in which the number of cases has increased annually with extensive economic loss. Because BLV is found in the cellular fraction of various body fluids, transmission of the virus may occur via contact with affected animals, parturition, feeding with colostrums, mechanical transmission by insects, blood transfusion, and the use of shared needles [6, 10, 13, 18]. Most BLV-infected animals are asymptomatic carriers, and approximately one-third of infected animals exhibit benign polyclonal proliferation of B cells, called persistent lymphocytosis, which is usually stable for several years without any other apparent clinical signs. Fatal lymphoma or lymphosarcoma occurs in 0.1–5% of infected animals aged 3–5 years or older [5, 9]. Because signs depend on the site of the tumors and include anorexia, indigestion, and chronic bloat, many cases are diagnosed following parturition and found in meat hygiene inspection centers after the cattle are slaughtered [4]. Therefore, it is important to improve the prognostication of tumors development in asymptomatic carriers.

The Tax protein plays an essential role in leukemogenesis induced by BLV [2, 3, 19]. We demonstrated through a surveillance study mainly involving Holstein (Hol) cattle that BLV can be divided into two categories based on the amino acid at position 233 in Tax and that P233-Tax-carrying BLV had a 2-year longer incubation period for EBL than L233-Tax-carrying BLV [7]. Recently, we also reported that L233-Tax-expressing cells secrete a number of molecules that play critical roles in vesicular development and angiogenesis at greater levels than P233-Tax-expressing cells and that L233-Tax had greater tumorigenic potential than P233-Tax through a murine subcutaneous xenograft study [14, 21]. It was also reported that the proviral load (PVL) in the blood of Hol cattle infected with L233-Tax-carrying BLV was significantly higher than that in animals infected with P233-Tax-carrying BLV [17]. Thus, amino acid position 233 in Tax is anticipated to have clinical value as a prognostic factor for BLV infection.

Japanese black (JB) cattle, a breed of Wagyu cattle that has long been raised in Japan, produce high-quality beef throughout the country. Embryo transfer (ET) technology is utilized to both produce highly genotypic JB beef cattle and impregnate cows in Japan through surrogate birthing by Hol dairy cows, in which the seroprevalence of BLV was higher than that in both fattening and breeding beef cattle [15]. Moreover, almost all BLVs infecting Hol cattle carry L233-Tax, whereas the viruses infecting JB cattle possess both forms of Tax equally [7, 17]. The purpose of this study was to investigate the characteristics of EBL among JB cattle with a focus on Tax harbored in the tumors and the relation with Hol cattle.

Lymphocytic sarcomas were collected from 237 JB cattle at various slaughterhouses in Japan in 2008–2021. The date and place

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of birth, breeds of the dams of the host animals, and place of slaughter were traced using the Information for Individual Identification of Cattle provided by National Livestock Breeding Center, Japan. The Tax type was determined by multiplex PCR as described previously [7].

A total of 237 tumor samples were examined to determine which Tax was retained. Out of them, 131 (55.3%) and 101 (42.6%) samples harbored L233- and P233-Tax-carrying BLV, respectively (Table 1). Meanwhile, 62 of 82 samples (75.6%) collected in the Hokkaido region carried L233-Tax-carrying BLV, and 19 samples (23.2%) carried P233-Tax-carrying BLV. Conversely, 51 of 78 samples (65.4%) collected in the Tohoku region harbored P233-Tax-carrying virus. The chi-squared test demonstrated that L233-Tax- and P233-Tax-carrying viruses were present in significantly more samples collected in the Hokkaido and Tohoku regions, respectively ($P < 0.05$). These findings suggest that the BLV type prevailing among JB cattle varied by region in Japan.

Because a large number of fattening beef cattle move across regions in Japan, tumor samples were examined by birthplace of the host animals, which had not moved across regions during their lifetime. As presented in Fig. 1, among 54 samples obtained from animals born in the Hokkaido region, where the number of dairy cows (mainly Hol cattle), heifers, and Hol beef cattle was more than 5-fold greater than that of JB cattle in 2019 [12], 45 samples (83.3%) carried L233-Tax. Conversely, in the Tohoku region, where JB cattle

Table 1. Prevalence of Tax types associated with enzootic bovine leukosis among Japanese Black cattle

	Number of cattle (%)			Total
	L233-Tax	P233-Tax	ND ^a	
Hokkaido	62 (75.6)	19 (23.2)	1 (1.2)	82 (100)
Tohoku	26 (33.3)	51 (65.4)	1 (1.3)	78 (100)
Kanto	36 (56.3)	25 (39.1)	3 (4.7)	64 (100)
Tokai	0 (0)	1 (100)	0 (0)	1 (100)
Kinki	3 (75.0)	1 (25.0)	0 (0)	4 (100)
Chugoku	1 (100)	0 (0)	0 (0)	1 (100)
Kyushyu/Okinawa	3 (42.9)	4 (57.1)	0 (0)	7 (100)
Total	131 (55.3)	101 (42.6)	5 (2.1)	237 (100)

a. Not determined.

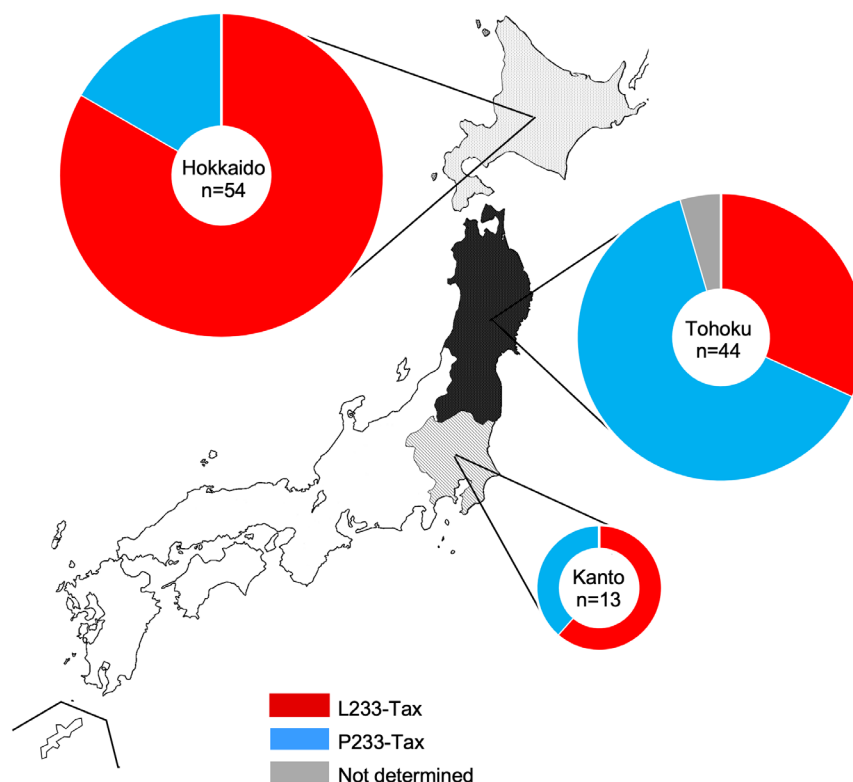


Fig. 1. Regional comparison of prevailing viruses among Japanese Black cattle. Tumor samples were examined for Tax by birthplace of the host animals.

were more common than dairy cows, 28 of 44 samples (63.6%) harbored P233-Tax. Similarly, L233-Tax-carrying BLV was dominant in Kanto, in which dairy cows were more common than JB cattle. The chi-squared test demonstrated that L233-Tax- and P233-Tax-carrying viruses more commonly infected samples derived from animals born in the Hokkaido and Tohoku regions, respectively. It was reported that most viruses detected in Hol cattle possessed L233-Tax nationwide [7, 17] and that the prevalence of BLV was higher in dairy cows than in beef cattle [15]. A significant number of farms fatten JB cattle together with JB-Hol crossbred cattle and Hol beef cattle, and ET is widely conducted in Japan, especially in dairy areas. In fact, 14 of 23 ET cases (60.9%) investigated in this study were detected in the Hokkaido region. Transmission of the virus may occur via contact with the affected animals, parturition, and mechanical transmission by insects [6, 13, 18]. Therefore, it is suspected that L233-Tax-carrying BLV had spread from Hol cattle to JB cattle in at least dairy areas, such as Hokkaido region.

We investigated the age distribution of JB cattle affected by EBL by dividing them into two groups: 182 animals assigned individual identification numbers at birth and 55 animals, which were born before issuing the numbers in Japan and assigned them after birth. As presented in Fig. 2A, 110 of 182 animals (60.4%) were infected with L233-Tax-carrying BLV. The age distribution at diagnosis of EBL associated with L233-Tax exhibited the largest and sharpest peak around 30 months of age, followed by 60 months of age. The median and first quartile of the 110 animals were 59.0 and 29.0 months, respectively. Conversely, the age distribution of 69 animals infected with P233-Tax-carrying virus (37.9%) had a gentle peak at 73 months old, and the median and first quartile were 77.0 and 55.0 months, respectively. Wilcoxon rank sum test demonstrated a significant difference of the median age between the L233-Tax and P233-Tax groups ($P=0.0091$). Excluding early onset before 40 months old, no significant difference was observed between L233-Tax (84.5 months old, $n=70$) and P233-Tax (83.0 months old, $n=59$) ($P=0.9209$). These findings indicate that most cases of early onset before 40 months of age were caused by L233-Tax-carrying BLV. On the other hand, P233-Tax-carrying BLV was dominant in animals assigned numbers after birth (Fig. 2B). There was no significant difference between median ages for P233-Tax group (138.5 months old, $n=35$) and L233-Tax group (129.5 months old, $n=20$) ($P=0.9209$), suggesting that early-onset EBL by L233-Tax-carrying virus had already occurred among cattle born before issuing the numbers.

Out of the 237 samples collected in this study, 171 samples were identified with the breed of dam of the host animal. Thus, they were examined for the L233/P233 ratio of Tax and age of EBL onset by dividing them into two groups: 23 samples derived from animals born to Hol or JB-Hol crossbred cattle (ET group) and 148 samples from those born to JB cattle (non-ET group). As presented in Table 2, 17 samples (73.9%) in the ET group and 91 samples (61.5%) in the non-ET group harbored L233-Tax. Although the L233/P233 ratio tended to be higher in the ET group, no significant difference was observed by Fisher's exact test ($P=0.2823$). Figure 3A

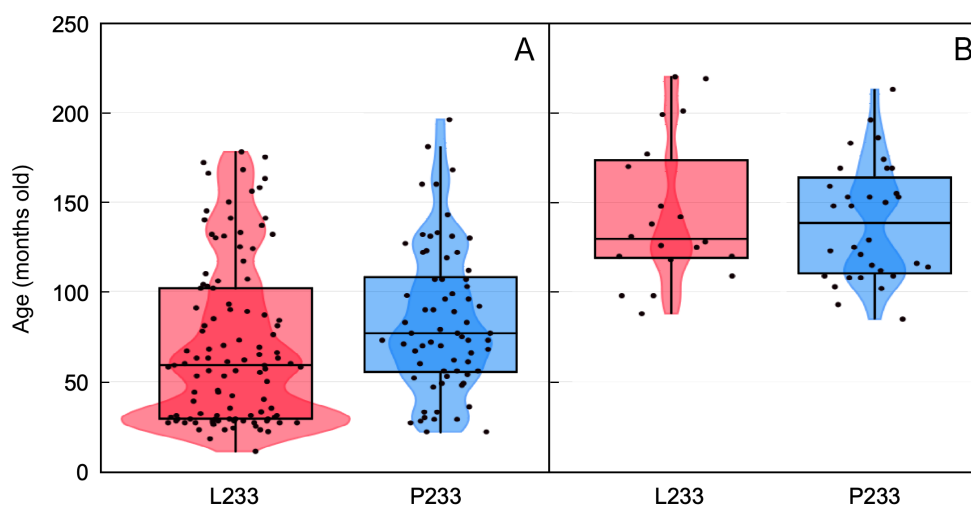


Fig. 2. Comparison of the distribution of age at diagnosis of enzootic bovine leukosis between Japanese Black cattle infected with L233-Tax- or P233-Tax-carrying bovine leukemia virus. Violin plot analyses were conducted using data from animals assigned individual identification numbers at birth (A) and those assigned numbers after birth (B).

Table 2. Impact of embryo transfer (ET) on the prevalence of each Tax type

	Number of cattle (%)			
	L233-Tax	P233-Tax	ND ^a	Total
ET	17 (73.9)	6 (26.1)	0 (0)	23 (100)
Non-ET	91 (61.5)	55 (37.2)	2 (1.4)	148 (100)

a. Not determined.

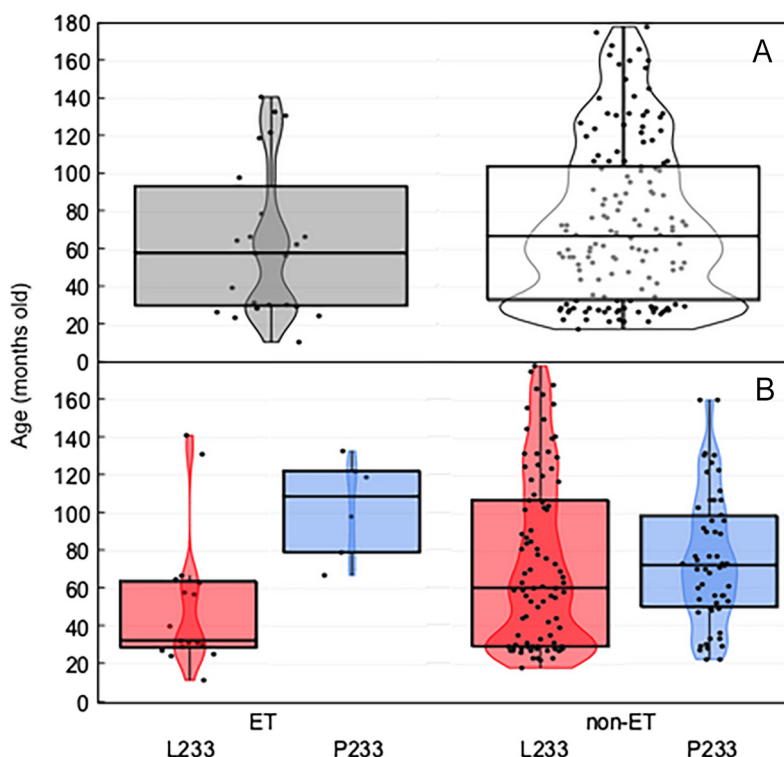


Fig. 3. Comparison of the distribution of age at diagnosis of enzootic bovine leukosis between the embryo transfer (ET) and non-ET group. Violin plot analyses were performed irrespective of the Tax type (A) and in consideration of the Tax type (B).

also reveals no significant difference of the overall median age of EBL onset between the ET (58.0 months) and non-ET groups (67.0 months) ($P=0.3253$). As presented in Fig. 3B, however, the median age of EBL onset caused by L233-Tax-carrying BLV markedly differed between the ET and non-ET groups (32.0 months vs. 60.0 months) despite the lack of significance ($P=0.0750$). On the other hand, the median age of onset of EBL associated with P233-Tax was significantly lower in the non-ET group (72.0 months) than in the ET group (108.5 months) ($P=0.0483$). It is notable that more than half (9/17) of the animals infected with L233-Tax-carrying virus in the ET group developed tumors at no more than 32 months of age, which is the end of the shipping term, whereas no cases of EBL were observed in animals infected with P233-Tax-carrying BLV in the ET group.

Kolmogorov-Smirnov test demonstrated that the age distribution of cattle affected by L233-Tax-associated EBL in the ET group followed a log-normal distribution. Sartwell [20] stated that the incubation periods for infectious diseases originating from pointsource exposure follow a log-normal distribution. Thus, it is suggested that the animals acquired the virus from surrogate mothers at perinatal period. Because PVL in the blood of animals infected with L233-Tax-carrying virus was significantly higher in Hol cattle than in JB cattle [17], animals born from surrogate dams infected with L233-Tax-carrying BLV experienced perinatal infection. Previous studies reported that high PVL in infected animals was a risk factor for BLV transmission [8, 13, 22]. It was also reported that the PVL of Hol cattle infected with P233-Tax-carrying BLV was significantly lower than that infected L233-Tax-carrying BLV [17]. The lower PVL in surrogate dams might play a passive role in perinatal infection. Possible perinatal infection, preferentially by L233-Tax-carrying BLV through ET, might increase the risk of EBL itself as well as early onset.

In conclusion, we demonstrated that L233-Tax-carrying BLV could cause the early onset of EBL among JB cattle. The higher PVL in dams infected with L233-Tax-carrying BLV than in those infected with P233-Tax-carrying BLV might play a role in virus transmission to offspring. It is also suggested that the virus spread from Hol cattle to JB cattle through ET and co-raising. Early shipment of fed cattle infected with L233-Tax-carrying BLV is essential to avoid economic loss associated with EBL. Elimination in decreasing order of PVL is recommended for breeding cows infected with L233-Tax-carrying virus. Segregation from Hol cattle is also important, and surrogate mothers for ET should at least be free of L233-Tax-carrying BLV. After the preferential elimination of cattle infected with L233-Tax-carrying virus, eradication of BLV from JB cattle will be completed by slaughtering infected animals and using extreme care in introducing cattle.

CONFLICT OF INTEREST. The authors declare that there are no conflicts of interest.

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