Retrovirology

SHORT REPORT

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Bovine leukemia virus proviral load is more strongly associated with bovine major histocompatibility complex class II *DRB3* polymorphism than with *DQA1* polymorphism in Holstein cow in Japan

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Abstract

Bovine leukemia virus (BLV) causes enzootic bovine leukosis and is closely related to the human T-lymphotropic virus. Bovine major histocompatibility complex (BoLAs) are used extensively as markers of disease and immunological traits in cattle. For BLV diagnosis, proviral load is a major diagnosis index for the determination of disease progression and transmission risk. Therefore, we investigated the frequency of *BoLA-DRB3* alleles, *BoLA-DQA1* alleles, and haplotypes of *BoLA class II* isolated from the heads of 910 BLV-infected cows out of 1290 cows assessed from BLV-positive farms, in a nationwide survey from 2011 to 2014 in Japan. Our aim was to identify *BoLA* class II polymorphisms associated with the BLV proviral load in the Holstein cow. The study examined 569 cows with a high proviral load and 341 cows with a low proviral load. Using the highest odds ratio (OR) as a comparison index, we confirmed that *BoLA-DRB3* was the best marker for determining which cow spread the BLV (OR 13.9 for *BoLA-DRB3*, OR 11.5 for *BoLA-DQA1*, and OR 6.2 for *BoLA* class II haplotype). In addition, *DRB3*002:01*, **009:02*, **012:01*, **014:01*, and **015:01* were determined as BLV provirus associated alleles. *BoLA-DRB3*002:01*, **009:02*, and **014:01* were determined as resistant alleles (OR > 1), and *BoLA-DRB3*012:01* and **015:01* were determined as susceptible alleles (OR < 1). In this study, we showed that *BoLA-DRB3* was a good marker for determining which cow spread BLV, and we found not only one resistant allele (*BoLA-DRB3*009:02*), but also two other disease-resistant alleles and two disease-susceptible alleles. This designation of major alleles as markers of susceptibility or resistance can allow the determination of the susceptibility or resistance of most cows to disease. Overall, the results of this study may be useful in eliminating BLV from farms without having to separate cows into several cowsheds.

Keywords: *BoLA-DRB3*, *BoLA-DQA1*, Bovine leukemia virus, Proviral load, Japanese Holstein

Bovine leukemia virus (BLV), which is the causative agent of enzootic bovine leukosis (EBL), belongs to the family Retroviridae (genus *Deltaretrovirus*), together with human T-lymphotropic virus types 1 and 2 (HTLV-1 and -2) [1]. At present, BLV is widely distributed in cow populations [2–7]. The virus was identified in 1969 as an infectious retrovirus, and it induces $CD5^{+}$B- cell leukemia/lymphoma in 1% to 5% of infected cows of 5 to 10 years of age [1]. Therefore, the virus was categorized as a non-severe infectious disease in several regions, including USA, South America, and some Asian countries [8]. This decision has resulted in the number of BLV-infected cows increasing in these areas; for example, in Japan, almost 40% of cows are infected [9], and in the USA, 80% of farms have become BLV-positive [8]. At present, BLV elimination is quite difficult, due to its high infection rate and the existence of heavily infected cows. Moreover, recently emerged BLV infections can

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cause earlier EBL onset, high accident rates, and low rates of conception and low milk production [6, 10–15]. Therefore, techniques for identifying high-risk cows are urgently needed to mitigate economic losses. It has been posited that cows classified as high-risk show a proviral load of over 14,000 copies/10^5 cells and 18,000 copies/10^5 cells in blood samples secreting BLV provirus into nasal and saliva, respectively [16]. It has been suggested that these cows may cause a high-risk for BLV transmission via coming into direct contact with healthy cows. In addition, it appears that proviral load correlates not only with BLV infection, but also with BLV disease progression [17–19]. Thus, BLV proviral load is an important index for estimating the stage of BLV infection.

Studies on BLV-associated host factors identified polymorphisms within the bovine major histocompatibility complex (MHC) (BoLA) [20–29]. BoLA is a highly polymorphic and tightly linked gene cluster [30]. Functionally, the *BoLA* class II gene is classified into two groups, DR and DQ. The DR molecule was constructed from a single *DRA* locus and a single *DRB3* locus, and DQ molecules were constructed from at least two *DQA* loci and two *DQB* loci [31]. To date, 136 *DRB3*, 65 *DQA*, and 87 *DQB* alleles have been registered on the IPD-MHC database (http://www.ebi.ac.uk/ipd/mhc/bola). Recently BLV proviral load quantification methods have been developed [17, 32] and several studies have successfully identified SNPs or *BoLA-DRB3* alleles that are associated with increasing or suppressing the BLV provirus load [27–29, 33–36]. However, the results of association studies that compared the frequencies of *BoLA* alleles in low proviral load cows with those in high proviral load cows were strongly affected by the allele frequencies in normal cows, and there is little information on how the allele frequencies were stable year-on-year in a countries.

For BLV diagnosis, proviral load is one of the major diagnosis indices for determining disease progression and transmission risk. Therefore, in this study, we investigated *BoLA-DRB3* and *BoLA-DQA1* allele frequencies in Japan over 4 years, performed an association study using the specific *BoLA* class II allele to determine BLV provirus load in Holstein cows, and determined proviral load-associated polymorphisms using cow which collected among 4 years.

We collected blood samples from 1290 cow heads over 6 months old from BLV-positive farms in a nationwide survey in Japan from 2011 to 2014, isolated genomic DNA and sera from peripheral blood. Cows determined as BLV-positive by anti-BLV gp51 antibody ELISA kit using sera (JNC Corporation, Kanagwa Japan) (Table 1) and the BLV proviral load measured by the BLV-CoCoMo-qPCR-2 method [32] using genomic DNA. First, we confirmed the allele frequencies of *BoLA-DRB3* gene in each 4 years and confirmed the allele frequency is stable in these 4 years in Holstein in Japan (Fig. 1). Next, our previous report showed that cows with a detected proviral load of over 14,000 copies/10^5 cells (as determined by the BLV-CoCoMo-qPCR-2 method) secreted BLV provirus into nasal secretions [16]. Thus, these cows may be high-risk transmitters. Therefore, we here categorized the 910 BLV-infected cows into two groups, as follows: (i) cows with proviral load over 10,000 copies/10^5 cells—high-risk BLV spreader cows, and (ii) cows with proviral load under 10,000 copies/10^5 cells—low-risk BLV spreader cows (Table 1). The 910 cow heads tested were separated into 341 heads of "low-risk spreaders" and 569 heads of "high-risk spreaders."

Next, these 910 cows were subjected to *BoLA-DRB3* genotyping using a PCR-sequence-based typing (PCR-SBT) method [37]. From 910 BLV-positive cows, a total of 1820 *BoLA-DRB3* alleles were detected, which were classified into 23 types of known *BoLA-DRB3* alleles (Fig. 2). *BoLA-DRB3* allele frequencies of these two groups, i.e., 682 alleles originating from low-risk spreaders and 1138 alleles originating from high-risk spreaders, were calculated, and estimated p values and odds ratios (ORs) for each *BoLA-DRB3* allele in the two spreader groups were compared (Fig. 2). If the allele

Table 1 Samples collected from Japanese BLV-positive farms (over 60% positive rate in the test prior to collection for this study) and distribution of proviral load in BLV-positive cow

	Year				Total
	2011	2012	2013	2014	
Number of animals					
Collected	322	390	290	288	1290
BLV-positive	222	275	199	214	910
High risk spreader*	137	160	133	139	569
Low risk spreader**	85	115	66	75	341
Distribution of age in BLV positive cow (month)					
Average of age	57.1	58.0	56.0	55.3	56.7
Maximum age	148	191	142	134	191
Minimum age	12	11	15	17	11
Standard deviation of age	24.2	28.6	24.7	22.2	25.3
Distribution of proviral load in BLV positive cow (copies per 10^5 cells)					
Average	33564	27,927	42,850	42,014	35,878
Maximum	137,905	135,662	154,306	135,950	154,306
Minimum	0	0	0	0	0
Standard deviation	32,786	28,945	38,382	39,536	35,285

* High risk spreader; proviral load > 10,000/10^5 cells

** Low risk spreader; proviral load ≤ 10,000/10^5 cells

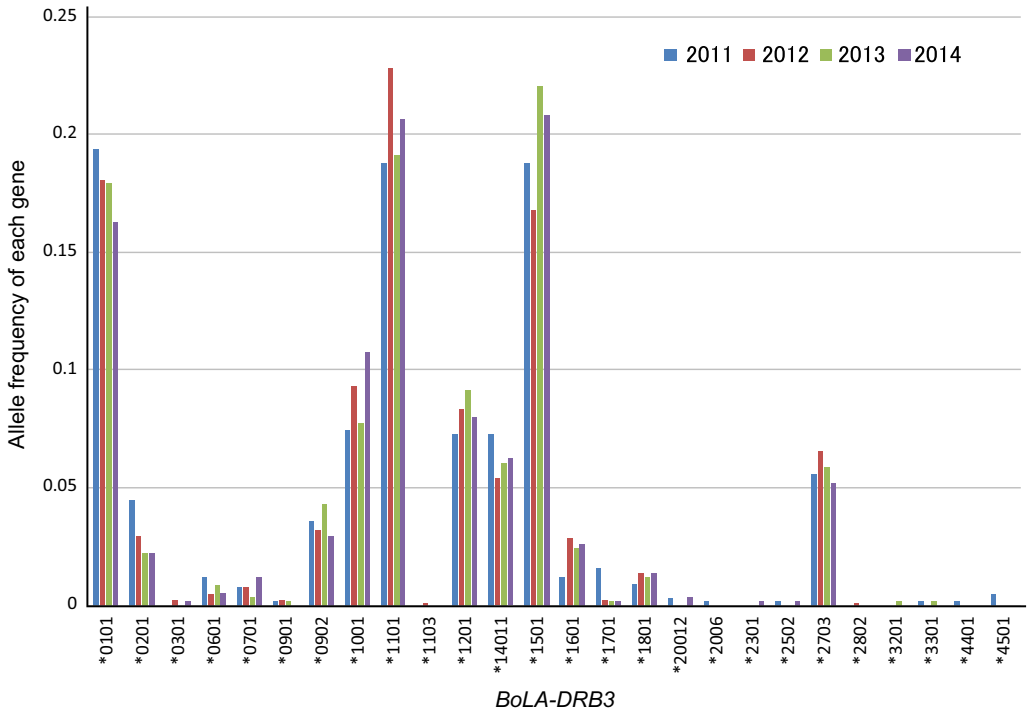

Fig. 1 Transition of *BoLA-DRB3* allele frequencies of Holstein cow in Japan from 2011 to 2014. DNA samples were collected from the blood of 1290 Holstein cows belonging to BLV-positive commercial dairy farms located in the 23 prefectures of Japan, from 2011 to 2014 by Ohno et al. [18] and genotyped for *BoLA-DRB3* alleles with PCR-sequence based typing (SBT) developed previously [37]. In total, 644 alleles were detected at 322 cows in 2011, 780 alleles at 390 cow in 2012, 580 alleles at 290 cow in 2013 and 576 alleles at 288 cow in 2014

which significantly low frequency in low risk spreader than high risk spreader (OR > 1), we determined that the allele was resistant allele. Moreover, in the case that the allele which significantly high frequency in high risk spreader than low risk spreader (OR > 1), the allele was determined as susceptible allele. From these 23 *BoLA-DRB3* alleles, *DRB3*002:01*, *DRB3*009:02*, *DRB3*012:01*, *DRB3*014:01:01*, and *DRB3*015:01* were determined as BLV provirus-associated alleles. *BoLA-DRB3*002:01*, *DRB3*009:02*, and *DRB3*014:01:01* were determined to be alleles associated with BLV resistance (OR > 1), whereas *BoLA-DRB3*012:01* and *DRB3*015:01* were determined to be alleles associated with BLV susceptibility (OR < 1).

There are *DR* and *DQ* genes embedded in the *BoLA class II* region, and these genes were closely linked to each other [30]. Indeed, we previously identified 39 *DRB3-DQA1* haplotypes in 507 Japanese Black cows [38]. Therefore, to determine the effect of other class II genes, we genotyped the second polymorphic class II genes, such as the *DQA1* gene. The 910 Japanese Holstein cow heads were subjected to genotyping of the *BoLA-DQA1* gene using a PCR-SBT method [39] and 899 cows were succeeded to genotyping for *BoLA-DQA1* alleles. These 899 cows were divided into low-risk (N = 336) and high-risk spreaders (N = 563), based on whether their proviral load was under or over 10,000 copies/10^5 cells, respectively. In total, 1798 *BoLA-DQA1* alleles were detected, and these alleles were assigned as one of 14 kinds of known *BoLA-DQA1* alleles (Fig. 3). *BoLA-DQA1* allele frequencies of these two groups (672 alleles originating from low-risk spreaders and 1126 alleles originating from high-risk spreaders), were analyzed using Fisher's exact test. Three kinds of B*oLA-DQA1* allele—*DQA1*002:04*, *DQA1*012:01:01*, and *DQA1*014:02*—were significantly associated with the high proviral load (Fig. 3). *DQA1*002:04* and *DQA1*014:02* showed ORs > 1 (12.8 and 2.47, respectively), as these two alleles were disease resistant. Conversely, the OR of *DQA1*012:01* was 0.34 and the allele indicated disease susceptibility.

Notably, *DRB3* and *DQA1* were highly linked [38]: for example, *DRB3*009:02* was linked with *DQA1*002:04*

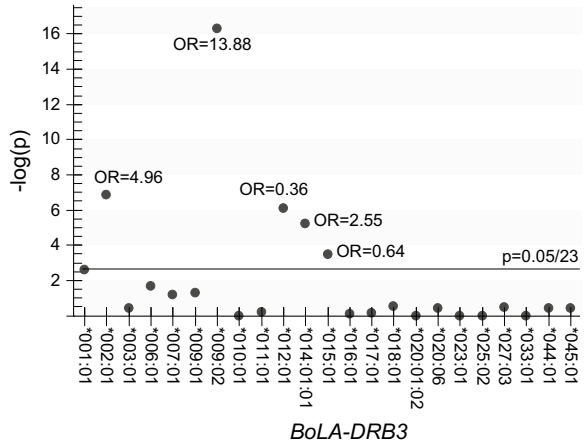

Fig. 2 Association study to determine the significance of the detection frequency of the *BoLA-DRB3* allele compared with those of 682 alleles from low-risk spreaders and 1138 alleles from high-risk spreaders.. BLV-positive cows were diagnosed using an anti-BLV antibody ELISA Kit (JNC, Tokyo, Japan), which is used to detect anti-Env gp51 antibodies from serum samples, according to the manufacturer's instructions. BLV-positive cows were subjected to BLV proviral load calculation using the BLV-CoCoMo-qPCR-2 system (RIKEN Genesis, Kanagawa, Japan) [32]. The 910 BLV-infected cows were classified into two groups: (i) cows with proviral load over 10,000 as high-risk BLV spreader cows (N = 569), and (ii) cows with proviral load under 10,000 as low-risk BLV spreader cows (N = 341). Fisher's exact test was performed for estimating *p* values and odds ratios (ORs) were used for detecting the association of each BoLA-DRB3 allele and BLV proviral load, using R version 3.4.2 statistical analysis software [43]. The X-axis shows the allele number for *BoLA-DRB3* and the Y-axis shows the minus multiplied common logarithm for *p* values. The alleles with *p* values < 0.00217 (0.05/23 alleles, the statistical significance threshold) were determined to be resistant when OR > 1 and susceptible when OR < 1

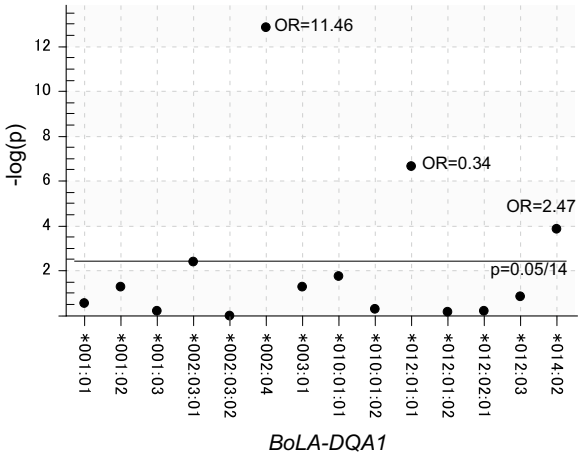

Fig. 3 Association study to determine the significance of detection frequency of the *BoLA-DQA1* allele compared with 672 alleles from low-risk spreaders and 1126 alleles from high-risk spreaders. The same samples as described in Fig. 1 were genotyped for *BoLA-DQA1* alleles with PCR-sequence based typing (SBT) developed previously [39]. Fisher's exact test was performed for estimating *p* values and odds ratios (ORs) were used to detect the association of *BoLA-DQA1* alleles with BLV proviral load. The X-axis shows the allele number for *BoLA-DQA* and Y-axis shows the minus multiplied common logarithm for *p* values. The alleles with *p* values < 0.00357 (0.05/14 alleles) were determined as resistant when OR > 1 and susceptible when OR < 1

and *DRB3*014:01:01* was linked with *DQA1*014:02* in Japanese Holstein cows [35]. Therefore, we identified that *DRB3*009:02-DQA1*002:04* and *DRB3*014:01:01-DQA1*014:02* haplotypes were indicated disease resistance. Table 2 shows that animals with the resistant haplotype were detected at a significantly higher level in the low proviral load group compared with the high proviral load group. However, the OR was lower when the *DRB3-DQA1* haplotype was used as a marker (OR 6.16) than when the *DRB3* allele alone was used (OR 13.88).

In this study, we used three markers, *BoLA-DRB3*, *BoLA-DQA1*, and *BoLA class II* haplotypes, to determine the risk of BLV spread through cows in the farm environment. Using the biggest OR as a comparison index, we confirmed that *BoLA-DRB3* was the best marker for determining which cow spread the BLV (OR 13.9 for *BoLA-DRB3*, OR 11.5 for *BoLA-DQA1*, and OR 6.2 for *BoLA class II* haplotype).

The most strongly associated allele was *BoLA-DRB3*009:02*, which was determined to be a BLV-resistant allele in our study, and was also detected in several studies, such as those by Julliarena et al. [36], Miyasaka et al [35], Forletti et al [40], Lutzelscheab et al. [21], Carignano et al [34], and Hayashi et al [33]. Moreover, we explored the other resistant alleles, *BoLA-DRB3*002:01* and *DRB3*014:01:01*, and susceptible alleles, *DRB3*012:01* and *DRB3*015:01*. The effects of these alleles were weaker than that of *BoLA-DRB3*009:02*, but they were more frequently detected in the farm [41]. Therefore, obtaining information about these common alleles is more important than obtaining information about rare alleles. It is true that the PVL we determined is only about single time point, the PVL may be changing in future. However, in our limited data in lab, the PVL tends to be stable at least 6 months. Needs more research to confirm how long the PVL shows stable. As the BLV PVL is the most variable quantitative index for assessing the risk of BLV transmission [42], the information about

Table 2 Association between cows with BLV-resistant *DRB3-DQA1* haplotypes and cows without BLV-resistant *DRB3-DQA1* haplotypes (p value = 9.029×10^{-15}, odds ratio = 6.168086)

		Category of proviral load	
		Low (≤ 10,000 copies/10^5 cells)	High (> 10,000 copies/10^5 cells)
Number of *DRB3-DQA1* haplotype	BLV resistant*	544	1192
	Non BLV resistant	62	22

* BLV resistant haplotype; *BoLA-DRB3*009:02-DQA1*002:04* or *BoLA-DRB3*014:01:01-DQA1*014:02*

disease susceptible and resistant alleles may be useful to eliminate BLV from the farm without separating cows into several sheds.

Abbreviations

BLV: bovine leukemia virus; BoLA: bovine leukocyte antigen; EBL: enzootic bovine leukosis; HTLV: human T-lymphotropic virus; MHC: major histocompatibility complex; OR: odds ratio; PCR-SBT: PCR-sequence based typing; PVL: proviral load.

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Authors' contributions

Study conception and design: YA and ST. Data acquisition, analysis, and interpretation: ST and AO. Contribution of reagents/materials/analysis tools: YA. Drafting and revising the manuscript: ST and YA. All authors read and approved the final manuscript.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article and its additional file.

Ethics approval and consent to participate

All animals were handled by veterinarians from the veterinary officers of the prefectural Livestock Hygiene Service Centers, and RIKEN, Japan in strict accordance with good animal practice following the guidelines of RIKEN. The study was approved by the RIKEN Animal Experiments Committee (approval number H29-2-104).

Consent for publication

Signed informed consents were obtained from the study subjects

Competing interests

The authors declare that they have no competing interests.

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References

1. Aida Y, Murakami H, Takahashi M, Takeshima SN. Mechanisms of pathogenesis induced by bovine leukemia virus as a model for human T-cell leukemia virus. Front Microbiol. 2013;4:328.
2. Erskine RJ, Bartlett PC, Byrem TM, Render CL, Febvay C, Houseman JT. Using a herd profile to determine age-specific prevalence of bovine leukemia virus in michigan dairy herds. Vet Med Int. 2012;2012:350374.
3. Polat M, Ohno A, Takeshima SN, Kim J, Kikuya M, Matsumoto Y, Mingala CN, Onuma M, Aida Y. Detection and molecular characterization of bovine leukemia virus in Philippine cattle. Arch Virol. 2015;160:285–96.
4. Polat M, Takeshima SN, Hosomichi K, Kim J, Miyasaka T, Yamada K, Arainga M, Murakami T, Matsumoto Y, de la Barra Diaz V, et al. A new genotype of bovine leukemia virus in South America identified by NGS-based whole genome sequencing and molecular evolutionary genetic analysis. Retrovirology. 2016;13:4.
5. Polat M, Moe HH, Shimogiri T, Moe KK, Takeshima SN, Aida Y. The molecular epidemiological study of bovine leukemia virus infection in Myanmar cattle. Arch Virol. 2017;162:425–37.
6. Yang Y, Fan W, Mao Y, Yang Z, Lu G, Zhang R, Zhang H, Szeto C, Wang C. Bovine leukemia virus infection in cattle of China: association with reduced milk production and increased somatic cell score. J Dairy Sci. 2016;99:3688–97.
7. Polat M, Takeshima SN, Aida Y. Epidemiology and genetic diversity of bovine leukemia virus. Virol J. 2017;14:209.
8. APHIS: Bovine leukosis virus (BLV) on U.S. dairy operations, 2007. *Veterinary services* 2008.
9. Murakami K, Kobayashi S, Konishi M, Kameyama K-I, Tsutsui T. Nationwide survey of bovine leukemia virus infection among dairy and beef breeding cattle in Japan from 2009–2011. J Vet Med Sci. 2013;75:1123–6.
10. Emanuelson U, Scherling K, Pettersson H. Relationships between herd bovine leukemia-virus infection status and reproduction, disease incidence, and productivity in Swedish dairy herds. Prev Vet Med. 1992;12:121–31.
11. Da Y, Shanks RD, Stewart JA, Lewin HA. Milk and fat yields decline in bovine leukemia virus-infected Holstein cattle with persistent lymphocytosis. Proc Natl Acad Sci USA. 1993;90:6538–41.
12. Norby B, Bartlett PC, Byrem TM, Erskine RJ. Effect of infection with bovine leukemia virus on milk production in Michigan dairy cows. J Dairy Sci. 2016;99:2043–52.
13. Nekouei O, VanLeeuwen J, Stryhn H, Kelton D, Keefe G. Lifetime effects of infection with bovine leukemia virus on longevity and milk production of dairy cows. Prev Vet Med. 2016;133:1–9.

14. Sandev N, Koleva M, Binev R, Ilieva D. Influence of enzootic bovine leukosis virus upon the incidence of subclinical mastitis in cows at a different stage of infection. Vet Arh. 2004;74:411–6.
15. Bartlett PC, Norby B, Byrem TM, Parmelee A, Ledergerber JT, Erskine RJ. Bovine leukemia virus and cow longevity in Michigan dairy herds. J Dairy Sci. 2013;96:1591–7.
16. Yuan Y, Kitamura-Muramatsu Y, Saito S, Ishizaki H, Nakano M, Haga S, Matoba K, Ohno A, Murakami H, Takeshima SN, Aida Y. Detection of the BLV provirus from nasal secretion and saliva samples using BLV-CoCoMo-qPCR-2: comparison with blood samples from the same cattle. Virus Res. 2015;210:248–54.
17. Jimba M, Takeshima SN, Matoba K, Endoh D, Aida Y. BLV-CoCoMo-qPCR: quantitation of bovine leukemia virus proviral load using the CoCoMo algorithm. Retrovirology. 2010;7:91.
18. Ohno A, Takeshima SN, Matsumoto Y, Aida Y. Risk factors associated with increased bovine leukemia virus proviral load in infected cattle in Japan from 2012 to 2014. Virus Res. 2015;210:283–90.
19. Sato H, Watanuki S, Murakami H, Sato R, Ishizaki H, Aida Y. Development of a luminescence syncytium induction assay (LuSIA) for easily detecting and quantitatively measuring bovine leukemia virus infection. Arch Virol. 2018;163:1519–30.
20. Nikbakht Brujeni G, Ghorbanpour R, Esmailnejad A. Association of BoLA-DRB3.2 alleles with BLV infection profiles (Persistent Lymphocytosis/Lymphosarcoma) and Lymphocyte subsets in Iranian Holstein Cattle. Biochem Genet. 2016;54:194–207.
21. Lutzelschwab CM, Forletti A, Cepeda R, Esteban EN, Confalonieri O, Gutierrez SE. Co-infection with Mycobacterium bovis does not alter the response to bovine leukemia virus in BoLA DRB3*0902, genetically resistant cattle. Res Vet Sci. 2016;109:10–6.
22. Lewin HA, Wu MC, Stewart JA, Nolan TJ. Association between BoLA and subclinical bovine leukemia virus infection in a herd of Holstein-Friesian cows. Immunogenetics. 1988;27:338–44.
23. Lewin HA, Russell GC, Glass EJ. Comparative organization and function of the major histocompatibility complex of domesticated cattle. Immunol Rev. 1999;167:145–58.
24. Zanotti M, Poli G, Ponti W, Polli M, Rocchi M, Bolzani E, Longeri M, Russo S, Lewin HA, van Eijk MJ. Association of BoLA class II haplotypes with subclinical progression of bovine leukaemia virus infection in Holstein-Friesian cattle. Anim Genet. 1996;27:337–41.
25. Xu A, van Eijk MJ, Park C, Lewin HA. Polymorphism in BoLA-DRB3 exon 2 correlates with resistance to persistent lymphocytosis caused by bovine leukemia virus. J Immunol. 1993;151:6977–85.
26. Lewin HA, Bernoco D. Evidence for BoLA-linked resistance and susceptibility to subclinical progression of bovine leukaemia virus infection. Anim Genet. 1986;17:197–207.
27. Brym P, Bojarojc-Nosowicz B, Olenski K, Hering DM, Rusc A, Kaczmarczyk E, Kaminski S. Genome-wide association study for host response to bovine leukemia virus in Holstein cows. Vet Immunol Immunopathol. 2016;175:24–35.
28. Takeshima SN, Sasaki S, Meripet P, Sugimoto Y, Aida Y. Single nucleotide polymorphisms in the bovine MHC region of Japanese Black cattle are associated with bovine leukemia virus proviral load. Retrovirology. 2017;14:24.
29. Carignano HA, Roldan DL, Beribe MJ, Raschia MA, Amadio A, Nani JP, Gutierrez G, Alvarez I, Trono K, Poli MA, Miretti MM. Genome-wide scan for commons SNPs affecting bovine leukemia virus infection level in dairy cattle. BMC Genom. 2018;19:142.
30. Elsik CG, Unni DR, Diesh CM, Tayal A, Emery ML, Nguyen HN, Hagen DE. Bovine Genome Database: new tools for gleaning function from the Bos taurus genome. Nucleic Acids Res. 2016;44:D834–9.
31. Aida Y. Characterization and expression of bovine MHC class II genes. Bull Soc Fr Jpn Sci Vet. 1995;6:17–24.
32. Takeshima SN, Kitamura-Muramatsu Y, Yuan Y, Polat M, Saito S, Aida Y. BLV-CoCoMo-qPCR-2: improvements to the BLV-CoCoMo-qPCR assay for bovine leukemia virus by reducing primer degeneracy and constructing an optimal standard curve. Arch Virol. 2015;160:1325–32.
33. Hayashi T, Mekata H, Sekiguchi S, Kirino Y, Mitoma S, Honkawa K, Horii Y, Norimine J. Cattle with the BoLA class II DRB3*0902 allele have significantly lower bovine leukemia proviral loads. J Vet Med Sci. 2017;79:1552–5.
34. Carignano HA, Beribe MJ, Caffaro ME, Amadio A, Nani JP, Gutierrez G, Alvarez I, Trono K, Miretti MM, Poli MA. BOLA-DRB3 gene polymorphisms influence bovine leukaemia virus infection levels in Holstein and Holstein x Jersey crossbreed dairy cattle. Anim Genet. 2017;48:420–30.
35. Miyasaka T, Takeshima SN, Jimba M, Matsumoto Y, Kobayashi N, Matsuhashi T, Sentsui H, Aida Y. Identification of bovine leukocyte antigen class II haplotypes associated with variations in bovine leukemia virus proviral load in Japanese Black cattle. Tissue Antigens. 2013;81:72–82.
36. Juliarena MA, Poli M, Sala L, Ceriani C, Gutierrez S, Dolcini G, Rodriguez EM, Marino B, Rodriguez-Dubra C, Esteban EN. Association of BLV infection profiles with alleles of the BoLA-DRB3.2 gene. Anim Genet. 2008;39:432–8.
37. Takeshima SN, Matsumoto Y, Miyasaka T, Arainga-Ramirez M, Saito H, Onuma M, Aida Y. A new method for typing bovine major histocompatibility complex class II DRB3 alleles by combining two established PCR sequence-based techniques. Tissue Antigens. 2011;78:208–13.
38. Miyasaka T, Takeshima SN, Sentsui H, Aida Y. Identification and diversity of bovine major histocompatibility complex class II haplotypes in Japanese Black and Holstein cattle in Japan. J Dairy Sci. 2012;95:420–31.
39. Takeshima S, Miki A, Kado M, Aida Y. Establishment of a sequence-based typing system for BoLA-DQA1 exon 2. Tissue Antigens. 2007;69:189–99.
40. Forletti A, Juliarena MA, Ceriani C, Amadio AF, Esteban E, Gutierrez SE. Identification of cattle carrying alleles associated with resistance and susceptibility to the Bovine Leukemia Virus progression by real-time PCR. Res Vet Sci. 2013;95:991–5.
41. Takeshima S, Saitou N, Morita M, Inoko H, Aida Y. The diversity of bovine MHC class II DRB3 genes in Japanese Black, Japanese Shorthorn, Jersey and Holstein cattle in Japan. Gene. 2003;316:111–8.
42. Juliarena MA, Barrios CN, Ceriani MC, Esteban EN. Hot topic: bovine leukemia virus (BLV)-infected cows with low proviral load are not a source of infection for BLV-free cattle. J Dairy Sci. 2016;99:4586–9.
43. Team RC: R: A Language and Environment for Statistical Computing. (Team RDC ed.: R Foundation for Statistical Computing; 2017.

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