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NOTE

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A survey of bovine leukemia virus resistant bovine leukocyte antigen (*BoLA*)-*DRB3*009:02* allele-carrying Japanese Black cattle in two prefectures in Japan

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ABSTRACT. The bovine leukocyte antigen (*BoLA*) *DRB3*009:02* allele is strongly associated with a low/undetectable bovine leukemia virus (BLV) proviral load. Understanding the status of cattle possessing *DRB3*009:02* allele is key for BLV control by breeding. We performed a survey of *DRB3*009:02*-carrying cattle in two prefectures in Japan using a TaqMan assay developed previously. The allele was found in 3.8% (confidence interval (CI): 3.3–4.3) of 6020 Japanese Black female cattle. A prefecture-level difference was found: the allele was observed in 8.6% CI: 7.5–9.9) of 2242 cattle of the birth prefecture B in Kyushu/Okinawa region, and this percentage was significantly higher than those of prefecture C in Kyushu/Okinawa region (1.3% (CI: 0.4–3.4) of 319) and prefecture A in Chugoku region (0.9% (CI: 0.6–1.4) of 2741), respectively. Consideration on the difference in possession of *DRB3*009:02* allele is needed to establish the more efficient control strategy of BLV infection in Japanese Black cattle.

KEYWORDS: bovine leukemia virus, bovine leukocyte antigen (*BoLA*)-*DRB3*009:02*, breeding, regional survey

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The cattle industry suffers from economic losses caused by bovine leukemia virus (BLV) infection. BLV belongs to the genus *Deltaretrovirus* of the Retroviridae family and causes a malignant B cell lymphoma, enzootic bovine leukosis, as a direct economic loss [1, 3]. BLV infection is also known to reduce milk production and carcass weight [19, 20, 23].

BLV is transmitted via transfer of infected lymphocytes from infected animals to uninfected ones. Iatrogenic factors, such as the repeated use of contaminated needles, dehorning, and rectal palpation with used rectal palpation sleeves, contributes to BLV transmission, as well as direct physical contact of cattle [9, 10, 21]. The quantity of BLV provirus determines the transmissibility of infected individuals [14]. Cattle with high proviral load have a strong potential for BLV transmission, while cattle with low/undetectable proviral load possibly disrupt the chain of BLV transmission [7].

Bovine leukocyte antigen (*BoLA*), which is related to host immune response, partially contributes susceptibility to diseases [25]. *BoLA-DRB3* (*DRB3*) haplotype is associated with susceptibility to BLV infection. Cattle with *DRB3*015:01* and **016:01* are associated with high proviral load in Holstein and Japanese Black species, respectively [8, 16, 26]. Cattle with *DRB3*002:01*, **010:01* and **014:01:01* in Holstein, and **009:02* and **011:01* in both Holstein and Japanese Black species are associated with low proviral load [8, 13, 15, 16, 26]. Focusing these allele-carrying cattle in herd management and breeding is important for BLV transmission control in addition to measurement of proviral load. Especially, *DRB3*009:02*-carrying cattle has outstanding capacity of suppressing proviral load to very low or undetectable level regardless of homozygote or heterozygote [2, 4, 6, 12]. Thus, it can be used as a key marker of BLV resistance.

The breeding system has an important impact on disease susceptibility at a population level. In Japan, each prefecture uses its own protocol for the management of beef cattle [5]. To improve the growth rate and meat quality, and satisfy the requirements of beef

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consumers, breeding is generally performed by artificial insemination using frozen semen of bulls with higher productivity. Such a system could lead to the localization of certain genomic characteristics of a cattle population in a region, resulting in specific *DRB3* alleles in the region. The objective of this study was to investigate the regional differences of *DRB3*009:02*-carrying Japanese Black cattle in different birth prefectures to develop a strategy for determining *DRB3*009:02*-carrying cattle for BLV transmission control.

In total, 6,020 samples from Japanese Black female cattle in two prefectures (prefecture A and B) in Japan were analyzed. Of these samples, 4,043 DNA samples from whole blood collected during 2016–2021 were provided by an animal husbandry experiment station in prefecture A. The other 1,977 peripheral blood samples were collected from 1,040 farms in prefecture B during August to October 2021. All samples were stored at −20°C until analysis.

Genomic DNA from whole blood was extracted using one of the following methods: a Wizard® Genomic DNA Purification Kit (Promega Corp., Madison, WI, USA), a MagDEA Dx SV reagent (Precision System Science Co., Ltd., Chiba, Japan) with an automated nucleic acid extraction system (magLEAD 12gC; Precision System Science Co., Ltd.), or a MagMAX™ CORE Nucleic Acid Purification Kit (Thermo Fisher Scientific Inc., Waltham, MA, USA) with an automated nucleic acid extraction system (KingFisher Duo Prime; Thermo Fisher Scientific Inc.), in accordance with the manufacturer's instructions. Cattle carrying *DRB3*009:02* were identified using a pooled testing system based on the *DRB3*009:02*-TaqMan assay we previously reported [22]. For the screening, DNA extracted from batches containing 5–30 individual blood samples or a DNA mixture of ten samples were tested. When pools were PCR positive, individual testing was performed to identify the *DRB3*009:02*-carrying cattle. Individual testing-positive indicates either of *DRB3*009:02* homozygous or heterozygous including this allele. These assays were performed using QuantStudio3 system (Thermo Fisher Scientific Inc.).

The information on the birth prefectures of the cattle were provided from the prefectural animal husbandry experiment station in prefecture A. To identify the birth prefectures of cattle sampled in prefecture B, we traced their between-farm movement history using the Search Service of Individual Identification Information of Cattle managed by the National Livestock Breeding Center (NLBC) using the ear tag number of each animal. As the result, 30 prefectures were identified.

Pairwise comparison of proportions was used to judge the significant differences in the percentage of *DRB3*009:02*-carrying cattle by birth regions and prefectures using R software (v. 3.6.2; www.r-project.org). We classified each prefecture into one of eight regions by the location according to previous report [18]. Prior to statistical analysis, the birth regions/prefectures of less than 139 cattle were excluded based on an inclusion criterion of sufficient sample size to determine 10% of the designated prevalence (assumed based on the percentage of *DRB3*009:02*-carrying cattle in Kyushu Island, Japan [22]) calculated by Epitools (precision value=0.05, confidence level (CI)=0.95) [24]. *P* values <0.05 were considered statistically significant.

We found that 228 (3.8%, 95% CI: 3.3–4.3) of 6,020 cattle possessed the *DRB3*009:02* allele. Based on the criterion mentioned above, Chugoku, Kyushu/Okinawa, and Hokuriku/Chubu regions and prefectures A, B, and C were statistically analyzed, respectively. As compared by birth regions, the percentage of *DRB3*009:02*-carrying cattle in Kyushu/Okinawa region was significantly higher than other regions ($P<0.05$, Table 1). Remarkably, the percentage of *DRB3*009:02*-carrying cattle in birth prefecture B in Kyushu/Okinawa region was significantly higher than the other birth prefectures ($P<0.05$, Table 2) and it contributed the higher percentage of *DRB3*009:02*-carrying cattle in this region. According to our previous studies, the percentage of *DRB3*009:02*-carrying cattle in the region including birth prefecture B was 10.6% (19/180) [22] or 6.8% (57/835) [6]. A similar percentage of *DRB3*009:02*-carrying cattle in birth prefecture B was confirmed in this study (8.6%, 95% CI: 7.5–9.9). Thus, although present result was based on the convenient sample, it might particularly reflect actual situation. Interestingly, however, prefecture-level differences were also observed between prefecture B and C in the same region of Kyushu/Okinawa.

Genetic variation and localization of *DRB3.2 exon 2* (*DRB3.2*) has been reported worldwide. The presence of *DRB3*009:02*-carrying cattle has been investigated in some countries/regions, including 6.4% of Holstein in Argentina, 15.4% of Holstein in Bolivia, 7.2% of Holstein in Paraguay, 5.6% of Holstein in Peru, 8.0% of Holstein in Chile [28], 5.2% of Hanwoo (native beef cattle) and 13.6% of Holstein in Korea [11]. Takeshima *et al.* investigated the genetic variation of *DRB3.2* alleles in Japan and reported the percentage of *DRB3*009:02*-carrying cattle by species: 5.9% in Holstein, 4.5% in Japanese Shorthorn, 0% in Jersey, and 7.2% in Japanese Black [27]. Miyasaka *et al.* indicated the farm-level differences of the percentage of *DRB3*009:02*-carrying cattle: 2.8%, 5.8%, and 2.0%

Table 1. The percentage of *DRB3*009:02*-carrying cattle by birth region

Birth region	Total n (heads)	*DRB3*009:02*-carrying n (heads)	*DRB3*009:02*-NOT carrying n (heads)	Percentage of *DRB3*009:02*-carrying % (95% confidence interval)
Chugoku	2,965	27	2,938	0.9 (0.6–1.3)[a]
Kyushu/Okinawa	2,716	198	2,518	7.3 (6.4–8.3)[b]
Hokuriku/Chubu	157	2	155	1.3 (0.2–5.0)[a]
Tohoku	108	1	107	0.9 (0.0–5.8)
Kanto	38	0	38	0 (0.0–11.4)
Hokkaido	27	0	27	0.0 (0.0–15.5)
Kinki	7	0	7	0.0 (0.0–43.9)
Shikoku	2	0	2	0 (0.0–80.2)
Total	6,020	228	5,792	3.8 (3.3–4.3)

a–b: $P<0.05$.

Table 2. The percentage of *DRB3*009:02*-carrying cattle by birth prefecture

Birth prefecture	Region	Total n (heads)	*DRB3*009:02*-carrying n (heads)	*DRB3*009:02*-NOT carrying n (heads)	Percentage of *DRB3*009:02*-carrying % (95% confidence interval)
A	Chugoku	2,741	25	2,716	0.9 (0.6–1.4)[a]
B	Kyushu/Okinawa	2,242	193	2,049	8.6 (7.5–9.9)[b]
C	Kyushu/Okinawa	319	4	315	1.3 (0.4–3.4)[a]
D	Kyushu/Okinawa	105	0	105	0.0 (0.0–4.4)
E	Chugoku	99	1	98	1.0 (0.1–6.3)
F	Chugoku	95	1	94	1.1 (0.1–6.6)
G	Tohoku	88	0	88	0.0 (0.0–5.2)
H	Hokuriku/Chubu	66	0	66	0.0 (0.0–6.9)
I	Hokuriku/Chubu	57	0	57	0.0 (0.0–7.9)
J	Hokuriku/Chubu	32	2	30	6.3 (1.1–22.2)
K	Hokkaido	27	0	27	0.0 (0.0–15.5)
L	Kanto	23	0	23	0.0 (0.0–17.8)
M	Kyushu/Okinawa	19	1	18	5.3 (0.3–28.1)
N	Tohoku	18	1	17	5.6 (0.3–29.4)
O	Chugoku	16	0	16	0.0 (0.0–24.1)
P	Chugoku	14	0	14	0.0 (0.0–26.8)
Q	Kyushu/Okinawa	12	0	12	0.0 (0.0–30.1)
R	Kanto	11	0	11	0.0 (0.0–32.1)
S	Kyushu/Okinawa	10	0	10	0.0 (0.0–34.5)
T	Kyushu/Okinawa	7	0	7	0.0 (0.0–43.9)
U	Kinki	6	0	6	0.0 (0.0–48.3)
V	Kanto	3	0	3	0.0 (0.0–69.0)
W	Tohoku	2	0	2	0.0 (0.0–80.2)
X	Kyushu/Okinawa	2	0	2	0.0 (0.0–80.2)
Y	Shikoku	1	0	1	0.0 (0.0–94.5)
Z	Kanto	1	0	1	0.0 (0.0–94.5)
AA	Hokuriku/Chubu	1	0	1	0.0 (0.0–94.5)
AB	Hokuriku/Chubu	1	0	1	0.0 (0.0–94.5)
AC	Shikoku	1	0	1	0.0 (0.0–94.5)
AD	Kinki	1	0	1	0.0 (0.0–94.5)
Total		6,020	228	5,792	3.8 (3.3–4.3)

a–b: $P<0.05$.

in each Japanese Black farm and 7.2%, 5.2%, and 0.0% in each Holstein farm [17]. In this study, we found significant differences in the percentage of *DRB3*009:02*-carrying cattle by birth prefecture. One of possible reason is an impact of current Wagyu breeding strategy in each prefecture. The improvement of growth rate and meat quality by artificial insemination, generally performed by prefecture independently, results production of local-specific bulls. Distribution of the frozen semen of *DRB3*009:02*-carrrying bull possibly contributes higher percentage of *DRB3*009:02*-carrying cattle in birth prefecture B. The impact of the localization of heifer is also considerable despite the limited information. Also, it is important to evaluate the correlation between the percentage of the allele possession and BLV prevalence at regional/prefectural level to determine if the allele is truly useful as the criterion for the population based BLV control or not. In this point of view, beside the allele possession status clarified in this study, the present BLV prevalence should be investigated because the latest nationwide BLV prevalence survey was conducted more than a decade ago [18]. If the allele status and BLV prevalence show a clear correlation, the genetical approach including the utilization of *DRB3*009:02* allele in BLV control measure would be more promising. Further investigations on these matters are needed.

CONFLICT OF INTEREST. The authors have no conflicts of interest to the content of this article.

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