Effectiveness of integrated bovine leukemia virus eradication strategies utilizing cattle carrying resistant and susceptible histocompatibility complex class II DRB3 alleles

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ABSTRACT

Bovine leukemia virus (BLV) has spread worldwide and causes serious problems in the cattle industry owing to the lack of effective treatments and vaccines. BLV is transmitted via horizontal and vertical infection, and cattle with high BLV proviral load (PVL), which is a useful index for estimating disease progression and transmission risk, are considered major infectious sources within herds. PVL strongly correlates with highly polymorphic bovine lymphocyte antigen (BoLA)-DRB3 alleles. The BoLA-DRB3*015:01 and *012:01 alleles are known susceptibility-associated markers related to high PVL, and cattle with susceptible alleles may be at a high risk of BLV transmission via direct contact with healthy cows. In contrast, the BoLA-DRB3*009:02 and *014:01:01 alleles comprise resistant markers associated with the development of low PVL, and cattle with resistant alleles may be low-risk spreaders for BLV transmission and disrupt the BLV transmission chain. However, whether polymorphisms in BoLA-DRB3 are useful for BLV eradication in farms remains unknown. Here, we conducted a validation trial of the integrated BLV eradication strategy to prevent new infection by resistant cattle and actively eliminate susceptible cattle in addition to conventional BLV eradication strategies to maximally reduce the BLV prevalence and PVL using a total of 342 cattle at 4 stall barn farms in Japan from 2017 to 2019. First, we placed the resistant milking cattle between the BLV-positive and -negative milking cattle in a stall barn for 3 years. Interestingly, the resistant cattle proved to be an effective biological barrier to successfully block the new BLV infections in the stall barn system among all 4 farms. Concomitantly, we actively eliminated cattle with high PVL, especially susceptible cattle. Indeed, 39 of the 60 susceptible cattle (65%), 76 of the 140 neutral cattle (54%), and 20 of the 40 resistant cattle (50%) were culled on 4 farms for 3 years. Consequently, BLV prevalence and mean PVL decreased in all 4 farms. In particular, one farm achieved BLV-free status in May 2020. By decreasing the number of BLV-positive animals, the revenue-enhancing effect was estimated to be ¥5,839,262 for the 4 farms over 3 years. Our results suggest that an integrated BLV eradication program utilizing resistant cattle as a biological barrier and the preferential elimination of susceptible cattle are useful for BLV infection control.

Key words: bovine leukemia virus, resistant, susceptible, proviral load, BLV prevalence, BLV eradication

INTRODUCTION

Bovine leukemia virus (BLV) belongs to the family Retroviridae (genus Deltaretrovirus), together with human T-cell leukemia virus types 1 and 2 (HTLV-1 and -2), and causes enzootic bovine leukosis (EBL), the most common neoplastic disease affecting cattle worldwide (Aida et al., 2013). BLV is transmitted through the transfer of infected lymphocytes via horizontal or
vertical routes (Evermann et al., 1986). Horizontal transmission of BLV occurs primarily by close contact with infected animals or via blood-sucking insects, such as tabanids and stable flies (Bartlett et al., 2014; Kohara et al., 2018; Panei et al., 2019) or iatrogenic procedures, including the repeated use of individual needles, syringes, rectal palpation gloves, and de-horners (Lassauzet et al., 1990; Kohara et al., 2006). Meanwhile, vertical transmission occurs via dam–calf contact through intrauterine infection of the fetus and/or ingestion of milk and colostrum from BLV-infected dams (Mekata et al., 2015; Ruiz et al., 2018; Watanuki et al., 2019; Borjigin et al., 2021). Infection by BLV may remain clinically silent and asymptomatic at the aleukemic stage, whereas approximately 25–30% and 1–5% of BLV-infected cattle develop persistent lymphocytosis and B cell lymphoma, respectively, after several years of latency (Aida et al., 2013).

In 2012, 51 countries or territories regularly reported the presence of EBL infections in The World Organization for Animal Health (OIE) (Panel and Health, 2015). After decades of systematic control and eradication approaches, most European countries and Oceania have eradicated BLV from their dairy herds (Gillet et al., 2007; Panel and Health, 2015). However, in several countries where compulsory eradication or control strategies have not been implemented, the spread of BLV infection continues, owing to the absence of effective treatments or vaccines. Recently, a high BLV prevalence has been reported in the United States (US), China, Canada, Japan, and other countries (Murakami et al., 2013; Ohno et al., 2015; Nekouei et al., 2015; Yang et al., 2016; Ladrónka et al., 2018). Thus, BLV infection commonly affects the cattle industry worldwide and causes considerable economic loss owing to the premature death of animals by lymphomas (Rhodes et al., 2003), carcass condemnation at slaughter (White and Moore, 2009), reductions in milk yield (Da et al., 1993; Sargeant et al., 1997; Otta et al., 2003; Erskine et al., 2012; Nekouei et al., 2016), daily gain (VanLeeuwen et al., 2010), decreased immunity (Konmai et al., 2017), and through effects on reproductive capacities (VanLeeuwen et al., 2010) and longevity (Erskine et al., 2012).

The BLV proviral load (PVL), which represents the amount of retroviral genome integrated into the host genome, correlates strongly with disease progression (Jimba et al., 2010; Somura et al., 2014; Ohno et al., 2015; Kobayashi et al., 2019), BLV infectivity as assessed via syncytium formation (Sato et al., 2018; Sato et al., 2019; Bai et al., 2021), the lymphocyte count (Ohno et al., 2015), viral biokinetics (Panei et al., 2013), virus shedding into the salivary, nasal secretions (Yuan et al., 2015), milk (Watanuki et al., 2019; Nakatsuchi et al., 2022b), and the biodistribution of BLV in the organs during the early stages of experimentally infected cattle (Kohara et al., 2023). Thus, PVL is considered a major diagnostic index for estimating the BLV transmission risk (Mekata et al., 2015; Julierana et al., 2016). In particular, previous studies have shown that the BLV provirus can be detected in the milk, nasal mucus, and saliva of dairy cattle with PVLs of >10,000, 14,000, and 18,000 copies/10⁵ cells in their blood samples, respectively (Yuan et al., 2015; Watanuki et al., 2019). These results suggest that a PVL of approximately 10,000 copies/10⁵ cells in the blood might be an indicator of efficient BLV spreading throughout the body, which is a relatively high number. Therefore, a BLV PVL of 10,000 copies/10⁵ cells was set as the threshold to distinguish between high- and low-PVL cows (Takeshima et al., 2019; Lo et al., 2020). Furthermore, PVL is strongly associated with the highly polymorphic bovine leukocyte antigen (BoLA)-DRB3 (Juliarena et al., 2008; Takeshima et al., 2017; Takeshima et al., 2019). In addition to PVL, BoLA-DRB3 polymorphism in BLV-infected cattle appears to be related to infectivity in the blood (Bai et al., 2021), lymphoma (Nikbakht et al., 2016; Lo et al., 2021a, 2021b), and PVL, infectivity, and anti-BLV antibody levels in milk (Nakatsuchi et al., 2022a, 2022b). A total of 384 alleles were registered in the Immuno Polymorphism Database (IPD)-MHC database (https://www.ebi.ac.uk/ipd/mhc/group/BoLA/, accessed on September 2, 2022). Among the alleles, the BoLA-DRB3*015:01 and DRB3*012:01 alleles (Takeshima et al., 2019; Lo et al., 2020) are known susceptibility-associated markers related to high PVL and infectivity of milk (Nakatsuchi et al., 2022b). In contrast, the BoLA-DRB3*009:02 allele (Juliarena et al., 2016; Lützelschwab et al., 2016; Takeshima et al., 2019; Lo et al., 2020), DBR3*014:01:01 allele (Takeshima et al., 2019; Lo et al., 2020), and DRB3*002:01 allele (Lo et al., 2020) are resistant markers associated with low PVL in the blood. Other BoLA-DRB3 alleles are not significantly associated with PVL in vivo (Juliarena et al., 2008). Therefore, in addition to the individual genetic specificity of disease susceptibility, BLV-resistant cattle, which are BLV-positive cattle carrying resistant BoLA-DRB3*009:02 or *014:01:01 with <10,000 copies/10⁵ cells of provirus in the blood, are unviable as a source of horizontal and vertical transmission, while BLV-susceptible cattle, which are BLV-positive cattle carrying susceptible BoLA-DRB3*012:01 or *015:01 with >10,000 copies/10⁵ cells of provirus in the blood, are considered the primary risk factors for horizontal and vertical transmission. Therefore, reducing BLV prevalence and PVL via the preferential culling of cattle with susceptible alleles and high PVL, and
selective breeding of cattle with resistant alleles and low PVL for breeding is suggested as a low-cost and high-efficiency BLV eradication strategies. However, whether polymorphisms in BoLA-DRB3 alleles are useful for BLV eradication in farms is not known. Currently, segregating or culling infected animals from herds is considered the most effective prevention or eradication strategy against BLV (More et al., 2017). However, these costly eradication approaches cannot be thoroughly implemented in herds with high BLV prevalence (Gillet et al., 2007). In addition, separation approaches are impossible to implement on some farms due to farm-scale, facilities, management factors, or economic and technical shortages. Thus, many farmers desire an effective approach that can reduce BLV transmission or can achieve BLV eradication while minimizing culling cattle numbers.

There are 4 types of dairy cattle rearing systems in Japan, including “stall barn,” “free-stall,” “free-barn,” and “Pasturing” systems. By 2020, there were about 14,000 dairy cattle farms in Japan and stall barn systems are common in Japan because of the advantages of individual observation, adequate management, and ease of feeding, representing approximately 70–90% of all dairy farms. From January 2017 to December 2019, we implemented a validation study for an integrated BLV eradication strategy, adding a new perspective on genetic specificity for disease susceptibility in addition to conventional BLV eradication strategy in 4 stall barn dairy farms in Japan. In this study, first, we placed cattle carrying resistant BoLA-DRB3 alleles with a PVL of <10,000 copies/10⁵ cells in the blood between BLV-positive and -negative populations as a biological barrier to block new horizontal transmission. Concomitantly, we actively eliminated cattle with high PVL, in particular cattle carrying susceptible BoLA-DRB3 allele with PVL >10,000 copies/10⁵ cells in the blood. Finally, we evaluated the effects of this integrated strategy by periodically checking for newly infected cattle and reducing BLV prevalence and PVL.

MATERIALS AND METHODS

Information regarding farms, animals and samples collection

From January 2017 to December 2019, peripheral blood samples were consecutively collected 7 times from 261 to 354 Holstein-Friesian cattle from 4 different dairy farms (A, B, C, and D) located in Chiba, Saitama, and Tochigi prefectures in Japan (Table 1). Serum or plasma was separated from whole blood or EDTA-treated blood samples. Milking herds were raised in a stall barn on all 4 farms. All farms were generally managed as follows: (i) using individual, single-use needles and syringes during vaccination or therapeutic protocols, and (ii) using disposable equipment (or at least cleaning, disinfecting, or sterilizing reusable materials and surgical instruments) in procedures such as dehorning, tattooing, implanting, cauterizing, castration, or ear-tagging.

Ethics approval

This study was approved by the Animal Ethical Committee and Animal Care and Use Committee of RIKEN (approval numbers H29-2-104 and W2019-1-001, respectively).

Extraction of genomic DNA and separation of serum or plasma

Genomic DNA was extracted from EDTA (EDTA)-treated peripheral blood samples using the Wizard Genomic DNA Purification Kit (Promega Corporation, Madison, WI, USA) according to the manufacturer’s instructions. Serum or plasma was separated from whole blood or EDTA-treated blood samples by centrifugation.

Enzyme-linked immunosorbent assay (ELISA) for anti-BLV gp51 antibody

Anti-BLV gp51 antibodies were measured in serum or plasma samples using an anti-BLV antibody ELISA kit (JNC, Tokyo, Japan), according to the manufacturer’s instructions.

Quantification of BLV provirus using BLV-CoCoMo-qPCR-2 assay

BLV PVLs were quantified from genomic DNA samples using BLV-CoCoMo-qPCR-2 (RIKEN Genesis, Kanagawa, Japan) and THUNDERBIRD Probe qPCR Mix (Toyobo, Tokyo, Japan), as described previously (Jimba et al., 2010, 2012; Takeshima et al., 2015). In brief, a 183 bp sequence of the BLV LTR gene was amplified using the degenerate primer set “CoCoMo-FRW and CoCoMo-REV” and detected with a 15 bp 6-carboxyfluorescein (FAM)-labeled LTR probe. As an internal control, the BoLA-DRA gene was amplified using the primer set “DRA-F and DRA-R,” and detected with the FAM-labeled DRA probe. Finally, the number of PVL copies in 10⁵ cells was calculated using the following formula: (number of BLV LTR copies/number of BoLA-DRA copies) × 10⁵ cells.
**BoLA-DRB3 Genotyping**

BoLA-DRB3 alleles were typed using the PCR-sequencing-based typing (SBT) method (Takeshima et al., 2011). Briefly, we amplified exon 2 of BoLA-DRB3 with PCR using primers DRB3FRW and DRB3REV. Thereafter, the first PCR fragments were purified using an ExoSAP-IT PCR Product Purification Kit (USB Corp., Cleveland, OH, USA) and sequenced using an ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA, USA). Finally, the sequence data were analyzed using the Assign 400ATF ver. 1.0.2.41 software (Conexio Genomics, Fremantle, Australia).

**Implementation of integrated BLV eradication strategy**

**(i) Definition of cattle by first total herd test.** In January 2017, the first total herd test was performed. As shown in Figure 1a, we first identified BLV-positive and BLV-negative cattle using a combination of BLV Env gp51 specific antibody detection and provirus quantification as follows. (i) In cattle populations over 6 mo of age, BLV-positive cattle were defined as those that were positive for either proviruses or antibodies. (ii) In cattle populations less than 6 mo of age, provirus-positive cattle were defined as positive cattle because antibody tests are not meaningful since the antibodies transferred from their mother are still present at this time. Furthermore, we identified the BoLA-DRB3 alleles of all cattle using the PCR-SBT method and classified the BLV-infected cattle based on combined BoLA-DRB3 allele data and PVL (Figure 1b). (i) Resistant cattle are BLV-positive cattle with PVL of <10,000 copies/10^5 cells in blood carrying at least the resistant BoLA-DRB3*009:02 or *014:01:01 allele. (ii) Susceptible cattle are BLV-positive cattle with PVL of >10,000 copies/10^5 cells in blood carrying the susceptible BoLA-DRB3*012:01 or *015:01 allele, but not the resistant allele. (iii) Neutral cattle are BLV-positive cattle that do not carry susceptible or resistant alleles.

<table>
<thead>
<tr>
<th>Sampling times</th>
<th>Cattle</th>
<th>Farm A</th>
<th>Farm B</th>
<th>Farm C</th>
<th>Farm D</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jan. 2017</td>
<td>BLV-positive</td>
<td>51 (72.9)</td>
<td>26 (49.1)</td>
<td>102 (81.0)</td>
<td>62 (66.7)</td>
<td>241 (70.5)</td>
</tr>
<tr>
<td></td>
<td>Resistant</td>
<td>8 (15.7)</td>
<td>3 (11.5)</td>
<td>18 (17.6)</td>
<td>12 (19.3)</td>
<td>41 (17.0)</td>
</tr>
<tr>
<td></td>
<td>Susceptible</td>
<td>15 (29.4)</td>
<td>5 (19.2)</td>
<td>33 (32.4)</td>
<td>7 (11.3)</td>
<td>60 (24.9)</td>
</tr>
<tr>
<td></td>
<td>Neutral</td>
<td>28 (54.9)</td>
<td>18 (69.2)</td>
<td>51 (50.0)</td>
<td>43 (69.4)</td>
<td>140 (58.1)</td>
</tr>
<tr>
<td></td>
<td>BLV-negative</td>
<td>19 (27.1)</td>
<td>27 (50.9)</td>
<td>24 (19.1)</td>
<td>31 (33.3)</td>
<td>101 (29.5)</td>
</tr>
<tr>
<td>May. 2017</td>
<td>BLV-positive</td>
<td>35 (63.6)</td>
<td>21 (42.0)</td>
<td>91 (82.0)</td>
<td>39 (50.0)</td>
<td>186 (63.3)</td>
</tr>
<tr>
<td></td>
<td>Resistant</td>
<td>6 (17.1)</td>
<td>3 (14.3)</td>
<td>18 (19.8)</td>
<td>10 (25.6)</td>
<td>37 (19.9)</td>
</tr>
<tr>
<td></td>
<td>Susceptible</td>
<td>9 (25.7)</td>
<td>4 (19.0)</td>
<td>31 (34.1)</td>
<td>8 (20.5)</td>
<td>52 (28.0)</td>
</tr>
<tr>
<td></td>
<td>Neutral</td>
<td>20 (57.1)</td>
<td>14 (66.7)</td>
<td>42 (46.2)</td>
<td>21 (53.8)</td>
<td>97 (52.2)</td>
</tr>
<tr>
<td></td>
<td>BLV-negative</td>
<td>20 (36.4)</td>
<td>29 (58.0)</td>
<td>20 (18.0)</td>
<td>39 (50.0)</td>
<td>108 (36.7)</td>
</tr>
<tr>
<td>Nov. 2017</td>
<td>BLV-positive</td>
<td>38 (62.3)</td>
<td>18 (38.3)</td>
<td>94 (79.7)</td>
<td>41 (48.2)</td>
<td>191 (61.4)</td>
</tr>
<tr>
<td></td>
<td>Resistant</td>
<td>6 (15.8)</td>
<td>3 (16.7)</td>
<td>19 (20.2)</td>
<td>9 (22.0)</td>
<td>37 (19.4)</td>
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<tr>
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<td>Susceptible</td>
<td>8 (21.1)</td>
<td>2 (12.5)</td>
<td>29 (30.9)</td>
<td>7 (17.1)</td>
<td>46 (24.1)</td>
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<tr>
<td></td>
<td>Neutral</td>
<td>24 (63.2)</td>
<td>13 (72.2)</td>
<td>46 (48.9)</td>
<td>25 (61.0)</td>
<td>108 (58.5)</td>
</tr>
<tr>
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<td>BLV-negative</td>
<td>23 (37.7)</td>
<td>29 (61.7)</td>
<td>24 (20.3)</td>
<td>44 (53.8)</td>
<td>120 (38.0)</td>
</tr>
<tr>
<td>Apr. 2018</td>
<td>BLV-positive</td>
<td>41 (63.1)</td>
<td>16 (33.3)</td>
<td>91 (74.0)</td>
<td>39 (41.9)</td>
<td>187 (56.8)</td>
</tr>
<tr>
<td></td>
<td>Resistant</td>
<td>8 (19.5)</td>
<td>2 (12.5)</td>
<td>19 (20.9)</td>
<td>9 (23.1)</td>
<td>38 (20.3)</td>
</tr>
<tr>
<td></td>
<td>Susceptible</td>
<td>10 (24.4)</td>
<td>3 (18.8)</td>
<td>33 (36.3)</td>
<td>8 (20.5)</td>
<td>54 (28.9)</td>
</tr>
<tr>
<td></td>
<td>Neutral</td>
<td>23 (56.1)</td>
<td>11 (68.8)</td>
<td>39 (42.9)</td>
<td>22 (56.4)</td>
<td>95 (50.8)</td>
</tr>
<tr>
<td></td>
<td>BLV-negative</td>
<td>24 (36.9)</td>
<td>32 (66.7)</td>
<td>32 (26.0)</td>
<td>54 (58.1)</td>
<td>142 (43.2)</td>
</tr>
<tr>
<td>Nov. 2018</td>
<td>BLV-positive</td>
<td>37 (57.8)</td>
<td>8 (17.0)</td>
<td>87 (67.4)</td>
<td>42 (41.2)</td>
<td>174 (50.9)</td>
</tr>
<tr>
<td></td>
<td>Resistant</td>
<td>7 (18.9)</td>
<td>1 (12.5)</td>
<td>16 (18.4)</td>
<td>10 (23.8)</td>
<td>34 (19.5)</td>
</tr>
<tr>
<td></td>
<td>Susceptible</td>
<td>7 (18.9)</td>
<td>2 (25.0)</td>
<td>31 (35.6)</td>
<td>9 (21.4)</td>
<td>49 (28.2)</td>
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<tr>
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<td>23 (62.2)</td>
<td>5 (62.5)</td>
<td>40 (46.0)</td>
<td>23 (54.8)</td>
<td>91 (52.3)</td>
</tr>
<tr>
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<td>BLV-negative</td>
<td>27 (42.2)</td>
<td>39 (83.0)</td>
<td>42 (32.6)</td>
<td>60 (58.8)</td>
<td>168 (49.1)</td>
</tr>
<tr>
<td>Apr. 2019</td>
<td>BLV-positive</td>
<td>34 (53.1)</td>
<td>5 (10.0)</td>
<td>83 (58.5)</td>
<td>40 (40.8)</td>
<td>162 (48.5)</td>
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<tr>
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<td>6 (17.6)</td>
<td>1 (20.0)</td>
<td>13 (15.7)</td>
<td>10 (25.0)</td>
<td>30 (18.5)</td>
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<tr>
<td></td>
<td>Susceptible</td>
<td>7 (20.6)</td>
<td>2 (40.0)</td>
<td>28 (33.7)</td>
<td>9 (22.5)</td>
<td>46 (28.4)</td>
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<tr>
<td></td>
<td>Neutral</td>
<td>21 (61.8)</td>
<td>2 (40.0)</td>
<td>42 (50.6)</td>
<td>21 (52.5)</td>
<td>86 (53.1)</td>
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<tr>
<td></td>
<td>BLV-negative</td>
<td>30 (46.9)</td>
<td>45 (90.0)</td>
<td>59 (41.5)</td>
<td>58 (59.2)</td>
<td>192 (54.2)</td>
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<tr>
<td>Nov. 2019</td>
<td>BLV-positive</td>
<td>35 (51.5)</td>
<td>1 (2.4)</td>
<td>82 (55.7)</td>
<td>33 (34.0)</td>
<td>151 (42.7)</td>
</tr>
<tr>
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<td>Resistant</td>
<td>7 (20.0)</td>
<td>0 (0.0)</td>
<td>12 (14.6)</td>
<td>6 (18.2)</td>
<td>25 (16.0)</td>
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<tr>
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<td>7 (20.0)</td>
<td>0 (0.0)</td>
<td>24 (29.3)</td>
<td>8 (24.2)</td>
<td>35 (23.8)</td>
</tr>
<tr>
<td></td>
<td>Neutral</td>
<td>26 (70.1)</td>
<td>1 (10.0)</td>
<td>46 (56.1)</td>
<td>11 (57.6)</td>
<td>87 (57.6)</td>
</tr>
<tr>
<td></td>
<td>BLV-negative</td>
<td>33 (48.5)</td>
<td>40 (97.6)</td>
<td>66 (44.3)</td>
<td>64 (66.0)</td>
<td>203 (57.3)</td>
</tr>
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</table>
alleles, PVL of >10,000 copies/10^5 cells in blood carrying a resistant allele, or PVL of <10,000 copies/10^5 cells in blood carrying a susceptible allele without a resistant allele.

(ii) Visualization of cattle. To visualize the cattle as defined above, we tagged the cattle with ear tags of different colors. Resistant cattle were green, susceptible cattle were red, neutral cattle were yellow, and BLV-negative cattle were blue (Figure 2a). To effectively manage milking cattle during the strategy period, we arranged a magnetic sheet of the same color as the ear tags of each cattle on the whiteboard by imaging the cattle position in the stall barn (Figure 2b).

(iii) Sorting milking cattle in stall barn utilizing resistant cattle as a biological barrier. For 3 years from May 2017 to December 2019, we first placed the resistant cattle between the BLV-positive and -negative cattle as a biological barrier in the stall barn to block horizontal infection routes according to each farm’s status (Figure 1c and Figure 2c①). In addition, BLV-negative resistant cattle were used as biological barriers in farms without positive resistant allele (Figure 1c). During the 3 years, these sorting plans were partially modified in line with the farm status after the total herd test (Figure 3).

(iv) Actively culling susceptible cattle with high PVLs. For 3 years from May 2017 to December 2019, we also suggested that the 4 farmers cull animals with higher PVLs, especially cull priority from susceptible cattle (Figures 1c and 2c②).
(vi) Supplementary strategy. At the same time, we implemented the above strategies and suggested that the 4 farmers implement supplementary strategies, as summarized in Table S1.

(vi) Three-year follow-up total herd tests for the integrated BLV eradication strategy. After the first total herd test in January 2017, a total of 6 times for 3 years from May 2017 to December 2019, we repeated follow-up total herd tests to validate the effect of the biological barrier using resistant cattle in stall barns and preferentially culling susceptible cattle by investigating the reduction in BLV prevalence and average PVLs in each farm.

Estimation of revenue-enhancing effect

The annual revenue enhancement generated by decreasing the number of BLV-positive animals and increasing the number of BLV-negative animals during the integrated BLV eradication strategy was estimated using the following equations:

In Canada, annual net revenue was estimated to be 1.0831 times higher in BLV un-infected dairy cattle than in infected dairy cattle (Kuczewski et al., 2019). The annual revenue per dairy cattle in Japan was calculated as ¥306,300 in 2017 (https://www.maff.go.jp/j/chikusan/kikaku/lin/1_hosin/attach/pdf/index-155.pdf).

1). Thus, we hypothesized that the annual revenue per BLV negative dairy cattle in Japan is ¥306,300.

2). Then, based on the data and hypothesis as mentioned above, the annual revenue of BLV-positive dairy cattle was estimated as follows:
**Figure 3.** The sorting of milking herds and the prevention effect of new infections by resistant cattle placed as a biological barrier in stall barns among 4 farms (A, B, C and D). Susceptible cattle (■); resistant cattle (■); neutral infected cattle (■); un-infected cattle (■); un-infected cattle with resistant alleles (■); newly infected cattle (●); biological barrier area using resistant cattle (□); unoccupied chamber (open rectangle with diagonal line). The number of newly infected cattle in either stall barn or outside were shown for 3 years from May 2017 to December 2019.
Annual revenue per BLV-positive dairy cattle = annual revenue per BLV negative cattle × (1–0.0831) = 280,846

3). Revenue-enhancing effect on 4 farms (A, B, C, and D) during the strategic period = (Number of BLV-positive cattle after strategy × average annual revenue per BLV-positive cattle + Number of BLV-negative cattle after strategy × average annual revenue per BLV-negative cattle) – (Number of BLV-positive cattle before strategy × average annual revenue per BLV-positive cattle + Number of BLV-negative cattle before strategy × average annual revenue per BLV-negative cattle)

4). Furthermore, the annual revenue-enhancing effect in all stall barn in Japan was estimated as follows:

Annual revenue-enhancing effect in all stall barns in Japan = Total number of stall barns in Japan × 1/4 revenue enhancement in 4 farms during this strategic period × 1/3 (strategy year)

**Statistical Analysis**

Following the ANOVA, Tukey’s test was used to determine the significance of the PVL of farms at differ-

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**Figure 4.** During 3-year follow-up total herd tests, sequential changes in BLV prevalence (a) and the average BLV proviral loads (b) of total herds at 4 farms (A, B, C and D) due to the implementation of this integrated BLV eradication strategy. The BLV prevalence was compared the total herd test results which before and after the strategy. *P* < 0.05 represents statistically significant results.
ent sampling times. The pairwise.prop.test was used to
determine the significance of BLV prevalence in farms
at different sampling times. Statistical significance was
set at $P < 0.05$ was considered significant.

RESULTS

Basic situation of each farm before this strategy

In January 2017, before starting the strategy, 342
dairy cattle from 4 farms were investigated for the
presence of *BoLA-DRB3* alleles using the PCR SBT
method, and for BLV prevalence using a combination
of ELISA to detect anti-BLV gp51 antibody and the
CoCoMo-qPCR-2 method to detect BLV provirus.
The PVLs were quantified using the CoCoMo-qPCR-2
method (Figure 1 and Table 1).

Among all herds, BLV prevalence ranged from
49.1~81.0% in the first total herd test, in which one-
half or more animals were infected with BLV among
the 4 farms (Table 1 and Figure 4a). The mean BLV
PVLs ranged from 10,521 to 18,239 copies/10^5 cells,
which was higher than the 10,000 copies/10^5 cells on all
farms (Figure 4b). Among them, the BLV prevalence
in milking herds was in the range of 67.9~97.1%, and
2-thirds to almost all animals were infected with BLV
(Figure 5a). The mean BLV PVLs in milking herds
ranged from 15,288 to 26,897 copies/10^5 cells, which
was higher than the 10,000 copies/10^5 cells on all farms
(Figure 5b).

Figure 5. During 3-year follow-up total herd tests, sequential changes in BLV prevalence (a) and proviral loads (b) of milking herds at 4 farms (A, B, C and D) due to the implementation of this integrated BLV eradication strategy. The BLV prevalence was compared among the total herd test results which before and after the strategy. $P < 0.05$ represents statistically significant results.
Although the distribution of BoLA-DRB3 alleles was slightly different among the 4 farms, there were 17 different types of BoLA-DRB3 alleles on the 4 farms and BoLA-DRB3*011:01 (frequency, 21.4%), *015:01 (20.6%), *001:01 (16.3%), *010:01 (10.7%), *014:01:01 (8.3%), *012:01 (6.8%), *007:01 (4.5%), *027:03 (3.4%), and *009:02 (3.0%) alleles accounted for 95.0% of the total alleles collectively (Table S2). Among them, the frequencies of the susceptible alleles of BoLA-DRB3*015:01 and *012:01 (accounting for 27.4%) were higher than those of the resistant alleles of BoLA-DRB3*014:01:01 and *009:02 (accounting for 11.3%) (Table S2). There were 57 different BoLA-DRB3 genotypes in the 4 farms (Table S3). Among them, genotypes containing resistant alleles (BoLA-DRB3*014:01:01 or *009:02) accounted for 21.6%, genotypes containing susceptible alleles (BoLA-DRB3*015:01 or *012:01) accounted for 45.3%, and neutral/neutral genotypes accounted for 38.6% (Table S3).

Based on combined BoLA-DRB3 allele data and PVLs, we successfully identified and defined resistant, susceptible, and neutral cattle. As shown in Table 1, at 4 farms in January 2017, we identified resistant cattle with PVL of <10,000 copies/10⁵ cells in blood (41 cattle, 17.0%), susceptible cattle with PVL of >10,000 copies/10⁵ cells in blood (60 cattle, 24.9%), neutral cattle (140 cattle, 58.1%), and BLV-negative cattle (101 cattle, 29.5%).

**Efficacy of the integrated BLV eradication strategies using resistant cattle as a biological barrier to block horizontal BLV transmission in addition to conventional BLV eradication strategies**

After defining cattle by the first total herd test in January 2017, we performed a secondary total herd test in May 2017 and then formulated a sorting plan using resistant cattle as a biological barrier for each farm according to the total herd test results and farm status, where each farmer was requested to place the resistant cattle between the BLV-positive and -negative milking cattle with provirus, 15,763 copies/10⁵ cells, from August to October 2019 (Figure 3Bb, third and fourth rows from right). Other than the 2 abnormal infections mentioned above, the remaining cattle were not newly infected during the strategy period. Interestingly, at the end of this strategy (May 2020), the within-milking herd BLV prevalence reached 0% (Figure 4).

Farm C comprises a small-scale, family owned dairy farm with 126 Holstein cattle, consisting of 90 milking cattle and 36 calves and heifers, and uses a stall barn system and mobile milker as in farms A and B. At the start of this strategy, the within-milking herd BLV prevalence was 95.1% and PVL had 20,819 copies/10⁵ cells (Figure 5). In farm C, no new infections occurred during the first 2 years (Figure 3C). However, in the third year, 3 new infections occurred at an outside stall barn due to the mixed rearing of BLV-negative and -positive dams in the calving paddock during the calving season, after which the 3 newly infected milking
Farm D is a small-scale, family owned dairy farm with 93 Holstein cattle, consisting of 60 milking cattle and 33 calves and heifers. This farm uses a stall barn system with space allocated for each cattle and mobile milker. At the start of this strategy, the within-milking herd BLV prevalence was 73.2% and PVL had 15,288 copies/10^5 cells (Figure 5). In the first year (May–October 2017), 2 different types of cattle sorting were performed on farm D (Figure 3D, third row from the left). In the area from the entrance of the barn to the horizontal aisle, the BLV-negative population was located on the right of the vertical aisle and the BLV-positive population on the left, separated into 2 populations by a 2 m vertical aisle. On the other hand, in the area from the horizontal aisle that ran from the middle to the back of the barn, the resistant cattle were located between BLV-positive and negative populations as suggested by us. In the second year (May–November 2018), only the opposite types of sorting were performed (Figure 3D, fourth row from left). However, 8 new infections were recorded in the first year (Figure 3D, third row from left) and 7 new infections were recorded in the second year (Figure 3D, third row from right) occurred at an outside stall barn, from which newly infected milking cattle were returned to the stall barn. These new infections occurred because BLV-negative and BLV-positive dry cattle frequently mixed in the paddock during the strategy period. Therefore, in the third year (May–December 2019), the resistant cattle were located between BLV-positive and BLV-negative populations as suggested by us, and the dry cattle were separated in the paddock by BLV-positive and -negative. Interestingly, no newly infected cattle were detected in the area where new infections had occurred in the past 2 years (Figure 3D, first row from the right).

Taken together, our results indicate that resistant cattle have efficacy as a biological barrier to block the new infections of BLV in addition to conventional BLV eradication strategies.

**Prioritizing culling of susceptible cattle**

While implementing the above infection control strategy utilizing resistant cattle, we suggested that farmers cull the individuals with high PVL, especially susceptible cattle. Although susceptible cattle were not all culled during this strategic period owing to management factors and financial reasons, they were culled preferentially (Table 2). Thirty-nine of the 60 susceptible cattle (65.0%), 76 of the 140 neutral cattle (54.3%), and 20 of the 40 resistant cattle (50.0%) were culled across all farms. However, at the same time as culling, susceptible, neutral, and resistant cattle were newly categorized because they were newly introduced in the stall barn or because of increasing PVLs in some cattle that initially had low PVL. As a result, the number of susceptible, neutral, and resistant cattle decreased to 39, 87, and 25 cattle, respectively, and the number of uninfected cattle increased to 203 cattle across the 4 farms in December 2019.

**Reduction of BLV prevalence and average PVLs**

By utilizing resistant cattle to prevent new infections and actively eliminate susceptible cattle in addition to conventional BLV eradication strategies, the BLV prevalence in total (Figure 4) and milking herds (Figure 5) was significantly decreased in all 4 farms. The reduction rates in BLV prevalence within milking herds were 34.2% in farm A, 93.4% in farm B (December 2019), 16.2% in farm C, and 32.9% in farm D (Figure 5a). The reduction rates in BLV prevalence within the total herds were 29.4% in farm A, 95.1% in farm B (December 2019), 31.2% in farm C, and 49.0% in farm D (Figure 4a).

Although there were no statistically significant differences owing to the large variations in PVLs on farms A, C, and D, they decreased numerically on all

<table>
<thead>
<tr>
<th>Cattle types</th>
<th>Cattle number before the strategy</th>
<th>Culling cattle number during the strategy</th>
<th>Total cattle number after the strategy</th>
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<tr>
<td></td>
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</tr>
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<td>20</td>
<td>12</td>
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<tr>
<td>Neutral cattle</td>
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<td>8</td>
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<tr>
<td>Total</td>
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</tbody>
</table>
4 farms. The decreasing rates of PVLs within milking herds were 37.3%, 98.8%, 9.7%, and 32.7% on farms A, B, C, and D, respectively (Figure 5b). Among all the herds, the decreasing rates of PVLs were 37.9%, 98.9%, 27.3%, and 29.5% in farms A, B, C, and D, respectively (Figure 4b).

On farm B, only one infected cattle was culled in May 2020, and a BLV-free status was achieved.

Partial revenue-enhancing effect and testing cost by this strategy

As shown in Table 3, by the effect of the integrated BLV eradication strategies, the number of BLV-negative cattle increased from 101 before the strategy to 203 after the strategy, with a rate of increase of 101.0%. Therefore, annual partial revenue enhancement was generated by decreasing the number of BLV-positive animals and increasing the number of BLV-negative animals during this integrated BLV eradication strategy. In Canada, the annual income was 1.083 times higher in BLV-negative dairy cattle than in BLV-positive dairy cattle (Kuczewski et al., 2019). The annual partial revenue per dairy cattle in Japan was calculated as ¥306,300 (2017). So, we hypothesized as the annual revenue per BLV-negative dairy cattle is equivalent to ¥306,300. Thus, annual revenue of BLV-positive cattle = the annual income per BLV-negative cattle × (1–0.083) = ¥280,846. Using this strategy, the number of BLV-positive cattle was reduced to 151 from 241 cattle, while the number of BLV-negative cattle increased to 203 from 101 cattle among the 4 farms. As a result, ¥5,839,262 partial revenue enhancements were calculated for 4 farms during this strategy period. Furthermore, if this strategy is applied to stall barns throughout Japan, the annual revenue enhancement is estimated to be ¥5,352,656,954 (Table 3).

As shown in Table S4, we estimated the testing costs incurred during the 3 years of the strategy. Testing costs primarily include PVL quantification, antibody testing, BoLA-DRB3 typing, labor, and consumables costs. A total testing cost of ¥4,332,043 was estimated for the 4 farms during the strategy period.

DISCUSSION

The present study comprises the first field trial approach to “an integrated BLV eradication strategy,” adding a new perspective of genetic disease susceptibility in addition to the conventional BLV eradication strategy. This integrated BLV eradication strategy using a combination of the biological barrier using resistant cattle in the stall barn system and preferentially culling BLV-susceptible cattle was able to reduce BLV prevalence and PVL to the maximum. At the same time, the 4 farmers implement supplementary strategies, as summarized in Table S1. Many conventional BLV eradication strategies have been adopted to control BLV transmission by segregating the BLV-negative population from the BLV-positive population, culling BLV-positive cattle, and increasing the number of BLV-negative offspring to replace the BLV-positive population (Panel and Health, 2015; Ruggiero and Bartlett, 2019). The benefits of controlling BLV on farms was estimated to be highest in strategies of “test and segregate” and “test and cull” as compared with not controlling BLV (Kuczewski et al., 2019), and many European countries have succeeded in controlling and eradicating EBL as a result of decades of systematic test and cull approaches (Gillet et al., 2007; Panel and Health, 2015; More et al., 2017). However, the “test and segregate” approach is inappropriate for implementation in farms with smaller areas where it is difficult to segregate animals by BLV-positive and -negative status. Feasibility of the “test and cull” approach will depend on the level of BLV prevalence and the culling rate in the herd, and it can be successful in dairy herds with low or moderate
within herd BLV prevalence (Panel and Health, 2015; More et al., 2017; Ruggiero and Bartlett, 2019). Therefore, it is important to implement optimal BLV eradication programs based on farm size, management factors, facilities, and economic sustainability. This integrated BLV eradication strategy for stall barn systems may solve these problems. This strategy is suitable and feasible for stall barns with low to high BLV prevalence, where it is impossible to cull all BLV-positive animals or difficult to segregate the BLV-negative animals from the positive animals because of the total area of farms, facilities, work circumstances, and economic sustainability. This strategy can gradually achieve BLV eradication while minimizing economic absorption for farmers by implementing an annual cull plan for susceptible cattle according to farm status, including farm size, number of infected cattle or susceptible cattle, and financial capability. Thereafter resistant cattle should be placed between the BLV-positive and BLV-negative populations as a biological barrier to block the horizontal transmission route within barns, without having to segregate cattle into several cowsheds.

Here, we demonstrated the effectiveness of resistant cattle as a biological barrier to block new infections of BLV in addition to conventional BLV eradication strategy. Several groups have previously reported that BoLA-DRB3 resistant alleles suppress PVL to a lower level (Miyasaka et al., 2013; Takeshima et al., 2019), resulting in resistance to disease progression (Hayashi et al., 2017; Lo et al., 2020b) and the reduction of in utero infection in calves (Borjigin et al., 2021). In particular, it was also reported that the cattle with resistant alleles and low PVL are able to disrupt the BLV transmission chain (Juliarena et al., 2016). As mentioned above, there is evidence that resistant cattle can prevent horizontal BLV transmission. Among the 4 farms included in this study, 74 (21.6%) cattle with resistant alleles were present. Of these, 40 (11.7%) resistant cattle were identified using our definition criteria, which means that there were sufficient resistant cattle for this strategy. Indeed, the efficacy of the strategy using a combination of conventional BLV eradication strategy and resistant cattle as a biological barrier to prevent new infections was demonstrated in farms where the cattle could be sorted, as suggested by us, and which segregated the dry cattle by BLV-positive and BLV-negative in outside paddocks. Because there were no controlled experiments in this study, it is difficult to determine whether the comprehensive strategy affects the conventional strategies and the resistant cattle as biological barriers or one of the strategies. It is also difficult to determine whether the new infection prevention effect observed in farm B was due to the empty pen or the biological barrier, as the empty pen was installed adjacent to the biological barrier due to the circumstances of the farm. However, the biological barrier was considered effective for the following reasons: (i) The Apr–Dec 2019 results for farms A and D and the May–Oct 2017 and May–Nov 2018 results for farm C in Figure 3 show that no new infections occurred within the sorting areas with biological barriers. (ii) Although the PVL and BLV prevalence did not decrease at these 4 farms, even though they had taken conventional strategies before the implementation of this strategy, after implementing the strategy using resistant cattle as a biological barrier and actively culling susceptible cattle, both PVL and BLV prevalence decreased significantly in this experiment. In particular, farm B was able to become BLV-free, suggesting the effectiveness of this biological barrier. In addition, farm A, which had a 73% infection rate, was BLV-free 3 years after this trial ended in 2023. To confirm the effectiveness of biological barriers, it will be necessary to implement controlled experiments in the future using only conventional strategies without biological barriers.

Among all 4 farms, milking cattle accounted for approximately 53% of the total population, and if resistant cattle are placed as biological barriers, it is unnecessary to use the facilities to segregate milking cattle into BLV-positive and BLV-negative. The remaining 47% comprised dry cattle, heifers, and calves, and, if only these cattle are segregated into BLV-positive and -negative groups, this strategy is convenient. Therefore, this is an economically beneficial and labor-saving BLV eradication strategy compared with the conventional strategy that segregates all BLV-positive and BLV-negative cattle.

The present study demonstrated that newly infected cattle occurred on all farms, and most were infected in dry or calving cattle paddocks outside the stall barn. In contrast, the newly infected case at farm B between May–Oct 2017 show that Y18 was infected by contacting Y29 for a short period in a stall barn. We also obtained interesting data from farm C, in which 3 BLV-infected cattle carrying resistant alleles were group-housed with 16 BLV-negative cattle at a rate of 15.8% for 15 weeks in the paddock, and no newly infected cattle were detected (data not shown). These results indicate that the risk of infection was likely unrelated to the rearing system. These results also show that neutral cattle with high PVL and susceptible cattle are at a high risk of infection.

From our results, we can conclude several benefits and flaws from our methods. First, results from farms A, C, and D showed that it is vital to separate BLV-negative and -positive cattle when milking cattle are moved from the stall barn into the dry cattle paddock during the dry milk period. Indeed, in farm B, the only
farm in which BLV-free status was achieved, all dry cattle had been placed in their original milking area and were not moved into the outside paddock. This result demonstrates that movement restrictions of dry cattle are important for the success of this strategy. This also agrees with previous results that movement restrictions are an effective tool for preventing BLV transmission between herds (More et al., 2017). Second, the results from farm B showed that it is important for the success of this strategy to not let infected cattle freely enter and wander into cleanup areas where un-infected cattle are present in the intra-stall barn. Third, the results of farm B also showed that milking need to performed in order from BLV-negative cattle to BLV-positive cattle, or the milker that milked the BLV-infected cattle must be cleaned and sterilized before milking the next cattle. Fourth, many calves had been protected from BLV by being segregated into individual calf-hatches and by providing a heat-sterilized colostrum derived from the mothers or commercial milk replacers. Further, heifers were protected from BLV through the isolation of BLV-positive and -negative cattle or by being deposited on BLV-free public pastures. In addition to these strategies, using resistant cattle as a biological barrier to prevent new infections is effective for faster BLV eradication in stall barn systems. It is simple and economical because there is no need for additional facilities, and it can be performed simply by sorting cattle in the same stall barn.

Under current conditions, it is impossible to cull all infected animals on farms with heavily infected animals to eradicate BLV. Thus, it is particularly important that the preferential elimination of susceptible cattle is useful for BLV infection control on farms with high infection rates. Our results demonstrate that this integrated BLV eradication strategy, which achieves the preferential elimination of susceptible cattle, can maximally reduce BLV prevalence, PVL, and infection risk while culling infected cattle to a minimum. Cattle with high PVL, especially susceptible cattle, are a source of infection and risk factors for progression to EBL, and culling cattle with high PVL results in an efficient reduction of BLV transmission and BLV prevalence (Ruggiero and Bartlett, 2019; Taxis et al., 2023). Our previous data showed that the vertical transmission risk (Borjigin et al., 2021), PVL, and infectivity of milk (Nakatsuchi et al., 2022a) were higher in susceptible cattle than that in resistant cattle. As mentioned above, there is scientific evidence that culling susceptible cattle can reduce BLV prevalence, PVL, and horizontal and vertical transmission risks.

In the present study, 136 cattle carrying susceptible alleles (39.8% in total) and 132 cattle with neutral alleles (38.6% in total) were present on 4 farms (Table S3). Of these, 60 (24.9%) susceptible cattle and 140 (58.1%) neutral cattle were identified as BLV-positive cattle according to our definition criteria, as shown in Figure 1. Among the 140 neutral cattle, 53 (37.9%) had a PVL of >10,000 copies/10^5 cells. These results showed that there were 113 (33.0%) cattle (60 susceptible cattle and 53 neutral cattle) with a higher risk of BLV transmission among the 4 farms. Indeed, in this case, the spread of BLV is may be suppressed by actively culling only 33.0% of the animals. Finally, by this strategy period, the susceptible and neutral cattle were eliminated at rates of 65.0% and 54.3%, respectively (Table 2), and the BLV-negative population increased from 101 to 203 at a rate of 101.0% (Table 3). Thus, our strategy comprises an economical BLV eradication strategy when compared with the conventional strategy of eliminating all positive cattle.

Following the implementation of this strategy, a large revenue-enhancing effect was estimated with a reduction in BLV prevalence and PVL. Within a herd, BLV prevalence is negatively associated with milk production and cattle longevity (Erskine et al., 2012; Bartlett et al., 2013; Nekouei et al., 2016; Norby et al., 2016; Benitez et al., 2022), and slaughtering BLV-infected cattle results in a good milk yield in a commercial dairy herd of approximately 500 cattle (Ohshima et al., 1988). The economic loss due to reduced milk production by BLV-infected cattle alone is estimated to be 525 million USD annually in the US dairy industry (Otta et al., 2003). It was also reported that the yearly mean partial net revenue of BLV-negative dairy cattle (Can$8,276) is 1.083 times higher than that in BLV infected cattle (Can$7,641) (Kuczewski et al., 2019). Implementing this strategy, the BLV-negative population increased from 101 to 203 and the BLV-positive population decreased from 241 to 151 among the 4 farms, and \$5,839,262 of revenue enhancement was calculated in this strategy period. If this strategy is implemented on stall barns all over Japan, \$5,352,656,954 of the annual revenue enhancement is estimated.

However, testing costs of approximately \$4,332,043 were estimated for the 4 farms during this strategy period. Although cattle were checked experimentally using a combination of antibody testing and PVL quantification, an antibody test was not required for the actual strategy. Thus, we can save an additional \$473,760 in this study. Although subtracting the testing costs from the above revenue reduces the actual revenue, only partial benefits are estimated. Many other benefits can be obtained by reducing the prevalence of BLV and PLV in herds. The benefits from the extended longevity of milking cattle would be very large because the benefit is not only milk yield, but also the number of milking years. The lifespan of BLV-negative cattle is...
longer than that of BLV-positive ones (Bartlett et al., 2013; Benitez et al., 2022). Furthermore, the conception rate tends to be lower ($P = 0.06$) in BLV-positive cattle than in BLV-negative cattle (VanLeeuwen et al., 2010). As a result of this strategy, the number of BLV-negative cattle significantly increased on the 4 farms. This result indicates that the longevity, calving rate, milking period, and conception rate of the population on the 4 farms improved. Therefore, the unquantifiable revenue from this strategy is incalculable.

The combination of the biological barrier using resistant cattle in the stall barn system and preferentially culling susceptible cattle, and the additional strategies mentioned above increased the BLV-negative population from 101 cattle to 203 cattle among the 4 focal farms. This strategy not only directly reduced the BLV prevalence and PVL within herds, but also indirectly reduced infection risk. Especially on farm B, there was only one infected cattle remaining in December 2019, which was culled in May 2020 to achieve BLV-free status on the farm. Recently, farm A, which had a 73% infectious rate, recently achieved BLV-free after 3 years of this trial ended at 2023. Therefore, based on the results of the above 4 farms, on the assumption that this strategy is broadly applicable, we estimated the number of years until the achievement of BLV eradication at the stall barn with different BLV prevalence in Japan. Farms with BLV prevalence of <50% can be BLV-free within 3 years, 50–70% can be BLV-free within 5 years, 70–80% can be BLV-free within 6 years, and 80–100% can be BLV-free within 9 years. BLV has been eradicated in most New Zealand and most European countries have achieved BLV free by 10 to decades of eliminating program (Gillet et al., 2007; Panel and Health, 2015; More et al., 2017). Although BLV prevalence, farm size, facilities, management factors, environment, and the minds of farmers affect the time to BLV eradication, if our strategies are thoroughly implemented, small-sized farms with approximately 50–200 cattle in Japan could achieve BLV eradication within 10 years. These results strongly demonstrate the effectiveness of this strategy in maximally reducing BLV prevalence and PVL, even when group housing BLV-positive and -negative cattle in stall barns. Furthermore, in free stalls, free barns, or grazing pastures, it is possible to reduce the frequency of contact between BLV-negative cattle and cattle carrying high PVL by gradually increasing the proportion of resistant cattle in the herds.

CONCLUSIONS

By adding a new perspective of individual specificity on disease susceptibility to the conventional BLV eradication strategy, this “integrated BLV eradication strategy” is an effective strategy to maximally reduce BLV prevalence and PVL even in group housing BLV infected and un-infected cattle in stall barns.

ACKNOWLEDGMENTS

The authors thank all farmers for providing the samples, their help with sampling, and the collection of epidemiological data. We thank the veterinarians and our collaborators for their kind assistance with sampling from many farms. We are also grateful to all members of the Viral Infectious Diseases Unit, RIKEN, for their assistance and helpful suggestions. We would like to thank Kaltech Co., Ltd. (https://kaltec.co.jp/en/) for helping with the organization of our laboratory, and Editage (www.editage.com) for English language editing. This research was supported by grants from the Projects of the NARO Bio-oriented Technology Research Advancement Institution (the special scheme project on regional developing strategy) and by grants from the Livestock Promotional Subsidy of the Japan Racing Association (JRA).

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