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Ten-year absence of new bovine leukemia virus infections under integrated bovine leukemia virus control measures, including 4.8-m spatial separation, in a Japanese Black cattle breeding herd

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Research Article

Keywords: bovine leukemia virus, horizontal transmission, physical separation, proviral load, real-time polymerase chain reaction

Posted Date: October 30th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11371318/v1

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Additional Declarations: No competing interests reported.

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Abstract

Bovine leukemia virus (BLV) is primarily transmitted through infected lymphocytes. When vertical and iatrogenic routes are effectively controlled, horizontal transmission is the major driver of within-farm spread. Although physical separation has been proposed to reduce insect-mediated horizontal transmission, long-term field evidence of this approach in actual farming conditions remains limited. In this 10-year observational study, we evaluated whether maintaining a fixed 4.8-m physical separation between BLV-positive and BLV-negative cattle could prevent horizontal BLV transmission in a commercial Japanese Black cattle breeding herd. On the study farm, BLV-positive and BLV-negative cattle were housed in separate sections of the same barn. A 4.8-m corridor and a 4.8-m vacant pen were consistently maintained as buffers between the cattle groups. Vertical and iatrogenic transmission was minimized through colostrum management, calf segregation, and strict hygiene protocols. BLV testing was performed 21 times over the 10-year observation period. Cows in the test barn were tested twice annually using real-time polymerase chain reaction (PCR) and proviral load (PVL) was quantified. Across the 21 rounds of PCR testing, no BLV-negative cows became PCR-positive, even though high-PVL cattle were present throughout the study, blood-sucking insects were abundant, and no insect control measures were implemented in the test barn. These findings suggest that a 4.8-m physical separation, combined with strict compartmental segregation and accurate diagnostic testing, may help to

prevent horizontal BLV transmission under specific farm conditions.

Key words: bovine leukemia virus; horizontal transmission; physical separation; proviral load; real-time polymerase chain reaction

Introduction

Enzootic bovine leukosis (EBL) is a malignant B-cell lymphoma caused by the bovine leukemia virus (BLV), an oncogenic delta-retrovirus that integrates into the genome of host lymphocytes and persists lifelong in infected cattle (Zhao et al. 2025). In most infected cattle, BLV infection remains subclinical, whereas approximately one-third develop persistent lymphocytosis, and a small proportion progress to EBL after years of latency (Murakami et al. 2013). This results in substantial economic losses in both the dairy and beef industries. The recently developed BLV vaccine is safe and effective (Suárez Archilla et al. 2022), but is not yet globally available, and BLV control still relies primarily on preventing new infections through the prioritized removal of infected cattle as well as implementation of other transmission-blocking strategies.

BLV spreads through both vertical and horizontal routes. Vertical transmission occurs in utero, during parturition, and via colostrum or milk, whereas horizontal transmission occurs through the transfer of infected lymphocytes during iatrogenic procedures or via blood-sucking insects, such as tabanids and stable flies (Ohshima et al. 1981; Buxton et al. 1985; Manet et al. 1989; Panei et al. 2019).

Although BLV has multiple transmission routes (Kuczewski et al. 2021), practical measures can be implemented to limit mother-to-calf transmission (Mekata et al. 2015) as well as iatrogenic transmissions through colostrum management, calf segregation, and strictly

83 ensuring hygiene during veterinary procedures. When these routes are effectively controlled,
84 horizontal transmission becomes the primary pathway for within-farm BLV spread (Nekouei
85 et al. 2015). Under such conditions, strategies aimed at reducing horizontal transmission are
86 essential to prevent further within-farm spread of BLV.

87 Insect-mediated transmission occurs when BLV-infected lymphocytes attached to
88 insect mouthparts and remain infectious until the mouthparts dry (Hopkins and DiGiacomo
89 1997). This implies that physical separation of BLV-positive and BLV-negative cattle may
90 reduce the risk of transmission by prolonging the interval between feeding sessions.
91 However, no long-term field studies have rigorously evaluated whether physical separation
92 can prevent BLV transmission under real farm conditions when vertical and iatrogenic
93 transmission routes are appropriately managed. Previous field observations have shown that
94 short-distance separation within the same barn does not fully prevent BLV transmission, as
95 seroconversion is observed to occur when BLV-positive cattle are not comprehensively
96 identified or strictly segregated from the BLV-negative herd (Oshima et al. 2014; Matsuda et
97 al. 2018). These findings highlight the importance of accurate detection and thorough
98 separation of BLV-positive cattle when evaluating distance-based transmission control.

99 In this study, we addressed these issues by evaluating BLV transmission over a
100 10-year period on a Japanese Black cattle breeding farm. While complete barn-level isolation
101 of BLV-positive cattle is often recommended, many Japanese beef breeding farms lack the

physical space to implement full separation. Therefore, a practical “semi-separation” approach, which involved continuously maintaining a 4.8-m buffer space between BLV-positive and BLV-negative cattle, was adopted in this study. The 4.8-m distance corresponds to the standard pen-width in Japanese Black breeding barns and represents the maximum feasible separation within the same barn under typical Japanese farm infrastructure. During the study period, the cows in the test barn were tested twice annually using real-time polymerase chain reaction (PCR), and strict measures were taken to minimize the risk of iatrogenic and vertical transmission.

Materials and Methods

Farm demographics and environmental conditions

Sixty Japanese Black cows were housed in the experimental barns. On average, the cows on the farm calved six times and were maintained there for approximately 8 years.

The study farm is located in a mountainous area where tabanid flies and other blood-sucking insects are abundant from early June to late October.

Observation period

The observation period spanned from November 2015 to November 2025. The 10-year observation period was designed to reflect realistic fluctuations in herd turnover and seasonal

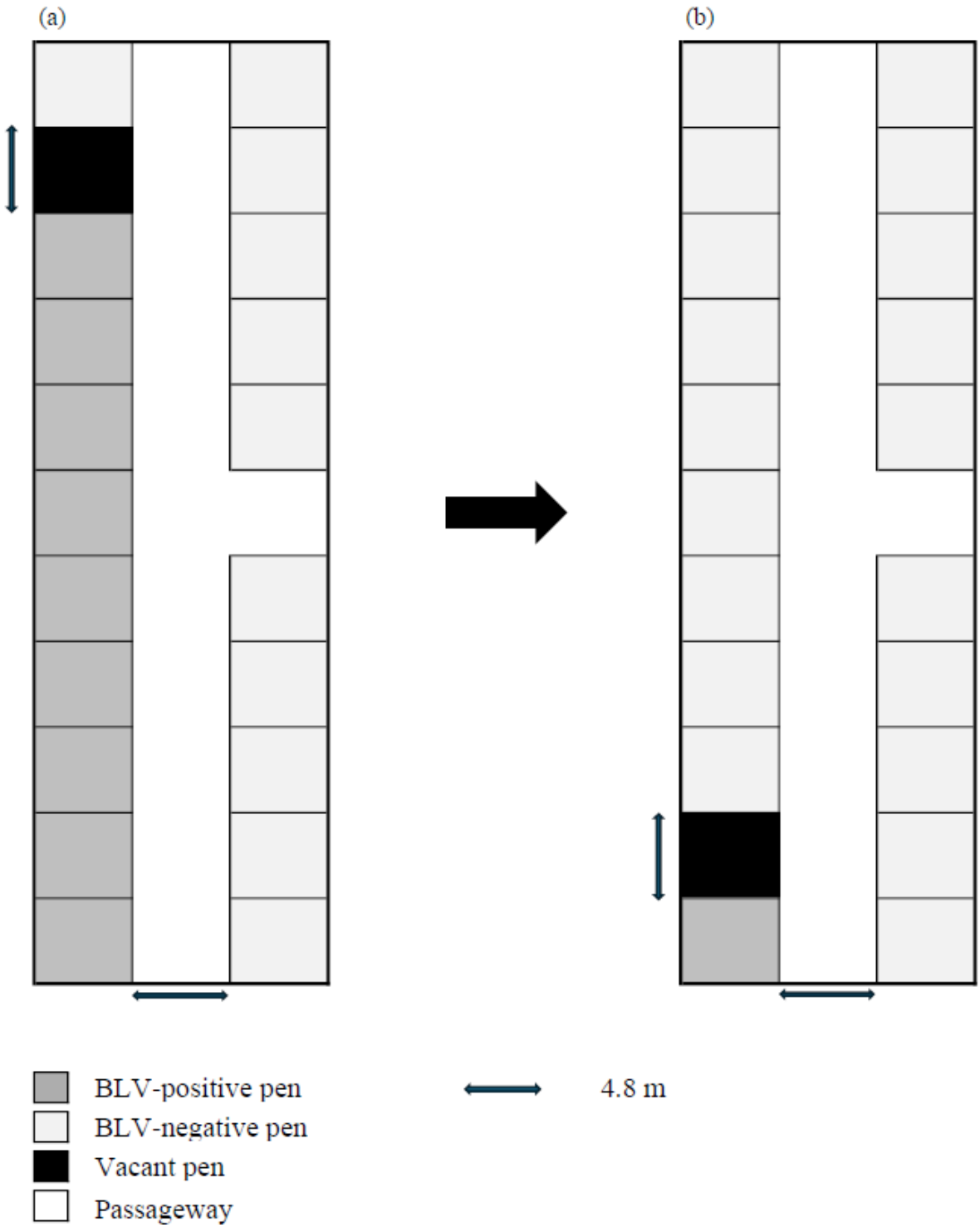
exposure to hematophagous insects.

Initial BLV status

At the beginning of the program, 25 BLV-positive and 35 BLV-negative cattle were present (BLV-positive rate: 41.6%). About 10 foundation cows were introduced annually to replace animals that were culled or retired from the herd, and approximately 30% of the introduced cows tested positive for BLV.

Initial pen allocation

The barn consisted of two opposing rows of pens, separated by a passageway: the west row contained 11 pens and the east row contained 10 pens. At the beginning of this study, the western row of pens in the test barn was designated as the BLV-positive section. It comprised nine BLV-positive pens, one empty buffer pen, and one BLV-negative pen (Fig. 1a). The east row contained 10 BLV-negative pens, resulting in an initial configuration of 9 positive pens, 11 negative pens, and 1 empty pen. Each pen housed three adult Japanese Black cows, the standard stocking density, throughout the study period.



140

141 **Fig. 1** Barn layout before and after implementation of the 4.8-m spatial segregation scheme

142 (a) Before implementation

143 (b) After implementation

144

Distance management

The opposing rows of pens were separated by a 4.8-m-wide central passageway (Fig. 2). The empty pen on the west side also measured 4.8-m (Fig. 3), matching the width of the passageway. When BLV-positive and BLV-negative pens were located on the same side of the barn, a 4.8-m-wide vacant pen was consistently maintained between them to prevent direct adjacency. High-proviral load (PVL) cattle were not specifically positioned within the BLV-positive section; cattle placement followed routine herd management and was not based on PVL category. The separation protocol was maintained throughout the entire 10-year observation period.

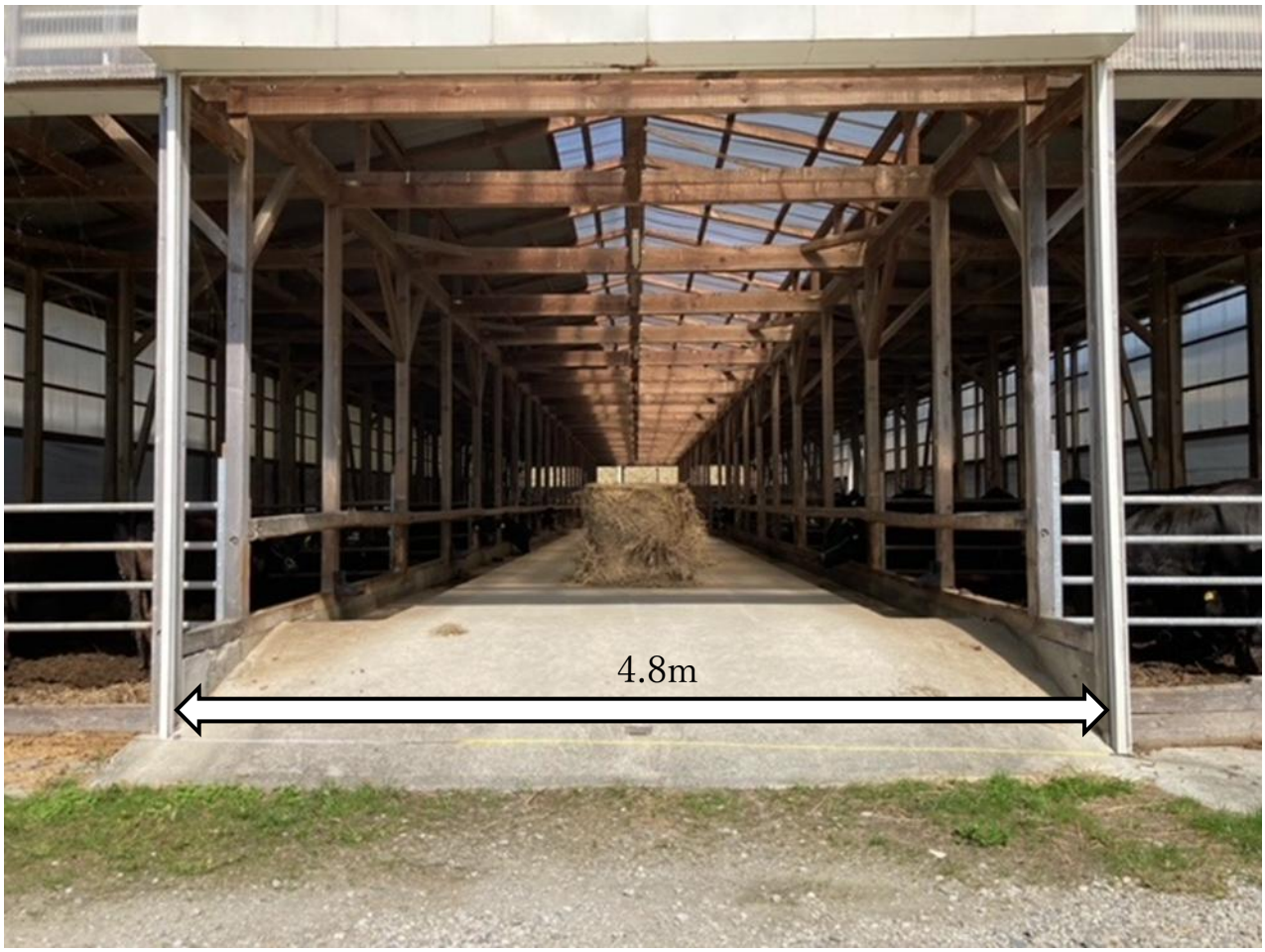


Fig. 2 The 4.8-m central corridor separating bovine leukemia virus (BLV)-positive and BLV-negative pens



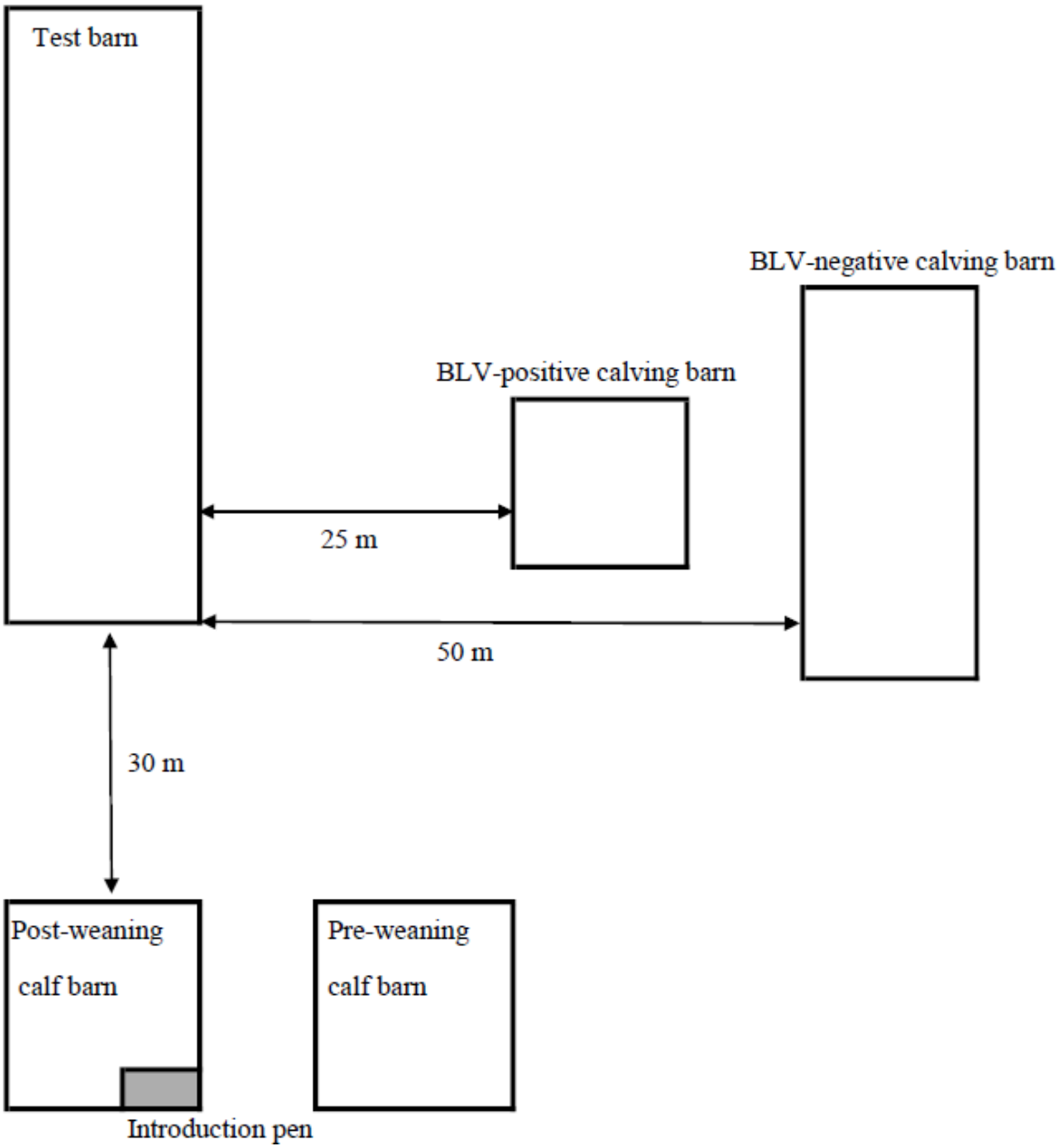
Fig. 3 The 4.8-m-wide vacant pen used as a buffer between bovine leukemia virus (BLV)-positive and BLV-negative pens

Changes in the number of BLV-positive cattle

BLV-positive cattle were progressively culled as part of the herd replacement, with removal generally prioritized for animals with high-PVLs. However, cows with high genetic value were exempt from this rule and were retained even when classified as having a high-PVL; consequently, at least one cow with a high-PVL remained in the BLV-positive group throughout the observation period.

Movement of cattle between barns

Calves were not housed in the test barn (Fig. 4). Pregnant BLV-negative cows were moved from the test barn to a BLV-negative calving barn located 50 m away, by 14 days before their expected calving dates. After birth, the calves were allowed to suckle maternal colostrum for 1 week and were then transferred to a pre-weaning calf barn located 30 m away from the test barn, which housed the pre-weaning calves, without undergoing PCR testing. Pregnant BLV-positive cows calved in a separate BLV-positive calving barn located 25 m away from the test barn. These calves were immediately separated from the cows, fed a colostrum replacement (Matsuda et al. 2026), and underwent PCR testing. Calves that tested negative by PCR were transferred to the pre-weaning calf barn. Calves born to BLV-negative cows, as well as PCR-negative calves born to BLV-positive cows, were moved to a post-weaning calf barn for rearing after weaning. PCR-positive calves were moved to an external calf-rearing facility at Iwate University and were raised until shipment; they did not return to the study farm.



197

198 **Fig. 4** Overall test farm layout showing the spatial arrangement of barns used for bovine

199 leukemia virus infection-based segregation of cattle

200

201 **Elimination of iatrogenic transmission**

202 Iatrogenic transmission is a major horizontal infection route in BLV epidemiology, and strict

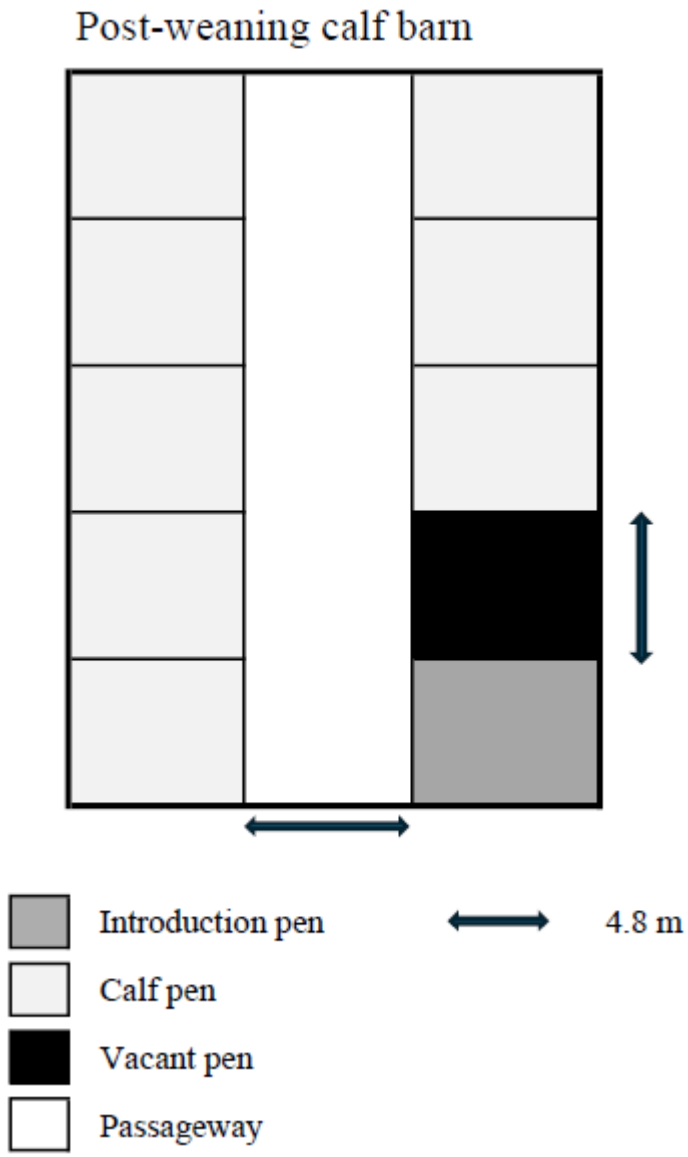
203 hygiene is essential for successful eradication programs (Kuczewski et al. 2021). To prevent

horizontal iatrogenic transmission, single-use needles, syringes, and rectal examination gloves were used for each cow during all veterinary procedures. The instruments used for hoof trimming and castration were disinfected between animals to avoid cross-contamination.

Management of introduced cattle

All newly introduced cattle were housed in a dedicated introduction pen located at one end of the post-weaning calf barn for approximately 14 days after introduction, while awaiting the results of PCR testing (Murakami et al. 2013) (Figs. 5, 6). As the introduced cattle included BLV-positive animals, strict adherence to the 4.8-m separation rule was maintained to prevent potential insect-mediated transmission to BLV-negative candidate animals housed in the post-weaning calf barn. This pen was separated from the calf-rearing pens by a fixed 4.8-m distance created by a passageway and a vacant pen, following the same spatial-segregation principle used in the test barn. An anti-insect net was installed only while introduced cattle were kept in the introduction pen to protect post-weaning calves in the adjacent calf barn from insect bites and was removed after the introduced cattle were transferred out of the pen.

Before entering either section of the test barn, each introduced animal was tested for BLV by real-time PCR. PCR-positive cattle were transferred to BLV-positive sections and PCR-negative cattle were transferred to BLV-negative sections of the test barn.



221

222 **Fig. 5** Layout of the post-weaning calf barn within the 4.8-m spatial segregation scheme



Fig. 6 Layout of the introduction pen, which was separated from calf pens by a 4.8-m buffer zone and a net barrier

Insect control measures

Insect nets, fly traps, and tabanid traps were not used in the test barn, with the exception of a temporary net barrier installed between the introduction pen and the adjacent vacant pen (Fig. 6), which was used only during the short period of introduction of new cattle (approximately 14 days). Tabanid flies and other hematophagous insects were abundant from early June through late October every year. Thus, the 4.8-m distance served as the sole barrier against insect-mediated horizontal transmission in the test barn throughout the study.

Diagnostic method: real-time PCR

PVL is strongly correlated with infectivity and horizontal transmission risk of BLV (Matsuda et al. 2018; Panei et al. 2019), making quantitative real-time PCR (qPCR) the most sensitive and important diagnostic tool for BLV surveillance (Bai et al. 2021). All PCR tests, including the diagnosis of newborn calves born to BLV-positive dams, were performed using the qPCR assay described below.

Blood samples were collected from the jugular vein into EDTA tubes (Venoject II VP-DK052K, Terumo Corp., Tokyo, Japan). DNA was extracted from the blood samples by using an automated nucleic acid extraction device (Magtration System 12GC PLUS; Precision System Science, Chiba, Japan). The DNA concentration was measured at 260 nm using a spectrophotometer (NanoDrop One, Thermo Fisher Scientific, Waltham, MA, USA). BLV proviral DNA was quantified using a commercial qPCR reagent (Bovine Leukemia Virus qPCR Detection Kit, Takara Bio, Shiga, Japan), which targets the BLV *pol* gene and has a diagnostic sensitivity comparable to that of other validated assays (Pluta et al. 2024). Bovine *RPPH1* served as the internal control. qPCR was performed using a QuantStudio 3 system (Thermo Fisher Scientific K.K., Tokyo, Japan), with quantification based on 10 ng of extracted DNA.

253 **Definition and interpretation of BLV test results**

254 BLV-infected cattle were defined as those in which BLV proviral DNA was detected by PCR.
255 PVL was quantified using real-time PCR. The tested cattle were categorized into three
256 categories according to the grouping criteria defined in previous studies that demonstrated
257 that cattle with a high-PVL exhibited increased infectivity (Matsuda et al. 2018; Kuczewski
258 et al. 2021), as follows:

259 Low-PVL (< 100 copies per 10 ng DNA),

260 Medium-PVL (100–499 copies per 10 ng DNA), and

261 High-PVL (≥ 500 copies per 10 ng DNA).

262

263 **BLV testing schedule**

264 BLV testing was performed 21 times over the 10-year observation period. Testing was
265 conducted twice annually: in May, prior to the active insect season, and in November, after
266 the active insect season ended.

267

268 **Ethical approval**

269 All study procedures were approved by the Miyagi Prefectural Agricultural Mutual Aid
270 Association (approval no. MN20170008) and were conducted in accordance with the
271 institutional guidelines for the care and use of agricultural animals.

Results

Twenty-five BLV-positive cattle were identified in the herd at the beginning of the study. These included 4 high-PVL cattle, 6 medium-PVL cattle, and 15 low-PVL cattle.

BLV-positive cattle were progressively culled as part of the herd replacement. The number of BLV-positive cattle gradually decreased over time. Thus, the number of BLV-positive pens decreased from nine to one over the course of this study (Fig.1b). Conversely, the number of BLV-negative pens increased from 11 to 19 as positive cattle were removed, and their pens were reassigned as negative sections. By the end of the observation period, 2 BLV-positive cattle and 58 BLV-negative cattle remained in the herd. The annual changes in the numbers of high-, medium-, and low-PVL cattle are shown in Online Resources 1 and 2.

Removal by culling generally prioritized animals with a high-PVL. The number of high-PVL cattle thus decreased from the initial 4 to 2 in year 2, and to 1 in year 4. One high-PVL cow was newly introduced in year 5 and another in year 8, resulting in the presence of 2 high-PVL cattle in years 5 and 8. The number of high-PVL cattle decreased to 1 in years 6, 9, and 10. Thus, at least 1 cow with a high-PVL was present every year during the 10-year observation period (Online Resource 1).

In the 21 BLV PCR rounds conducted over the 10-year period, no BLV-negative cow became PCR-positive.

Discussion

In this 10-year study, under the experimental conditions, namely, a fixed 4.8-m physical separation and a barn layout that prevented any direct adjacency between BLV-positive and BLV-negative pens, no new BLV infections were detected among BLV-negative Japanese Black cattle. Across 21 PCR testing rounds; thus, no BLV-negative cows converted to BLV-positive. These findings indicated that physical distance plays an important role in reducing the risk of horizontal BLV transmission under certain farming conditions.

A high-PVL is a well-established risk factor for BLV transmission. Previous studies have shown that cattle with a high-PVL shed larger numbers of infected lymphocytes and act as major transmission sources within herds (Murakami et al. 2013; Ooshiro et al. 2013; Mekata et al. 2015; Nekouei et al. 2015). At the beginning of the study, the herd contained 25 BLV-positive cattle, including 4 high-PVL cattle (≥500 copies/10 ng DNA) and 6 medium-PVL cattle (100–499 copies per 10 ng DNA). As high-PVL cattle remained present throughout the observation period, the separation strategy was evaluated under sustained transmission pressure in this study. In addition, the farm was located in a mountainous area where tabanid flies and other hematophagous insects are abundant from early June to late October every year. Moreover, no insect control measures, including nets, were implemented in the test barn itself, although a net barrier was installed in the introduction pen where

310 introduced cattle remained for approximately 14 days until their PCR results were available.
311 Our findings under these conditions, i.e., persistent presence of high-PVL cattle, and seasonal
312 exposure to blood-sucking insects in the absence of insect control measures, indicate that
313 maintaining a 4.8-m physical separation may be an effective measure for preventing BLV
314 infection in BLV-negative cattle via horizontal transmission.

315 Previous on-farm studies evaluating the effectiveness of physical separation between
316 BLV-infected and non-infected cattle reported BLV seroconversion even when separation
317 distances of approximately 4–5 m were maintained (Oshima et al. 2014; Matsuda et al. 2018).
318 Thus, the differences between the findings of our study and those of these earlier studies
319 cannot be attributed to the distancing alone. Instead, the key distinction lies in the accuracy of
320 BLV detection and the thoroughness of transmission-blocking management. In previous
321 studies, BLV-positive cattle may have gone undetected because antibody-based screening
322 methods, such as enzyme-linked immunosorbent assays or phytohemagglutinin assays were
323 used, which are less sensitive for detecting early infections. In addition, vertical transmission
324 and iatrogenic exposure were not fully controlled in the earlier studies. In contrast, the
325 present study minimized the influence of transmission routes other than horizontal infection
326 via insect-mediated spread. This was achieved by preventing vertical transmission through
327 managing colostrum intake and segregating infected calves (Matsuda et al. 2026), reducing
328 iatrogenic transmission via strict hygiene protocols (Kuczewski et al. 2021), and ensuring

329 accurate identification of BLV-infected cattle by using real-time PCR, which is more
330 sensitive than antibody-based assays for detecting early infection (Bai et al. 2021). Under
331 these conditions, maintaining a 4.8-m separation, equivalent to a single pen-width, was
332 sufficient to prevent horizontal BLV transmission. These findings suggest that appropriate
333 diagnostic testing and calf management measures are essential prerequisites for evaluating
334 distance-based control and that physical separation becomes effective only when these
335 foundational measures are in place.

336 In addition, as no insect control measures were used in the test barns, the results
337 imply that the 4.8-m separation served as the primary barrier against insect-mediated
338 transmission throughout the study. A net barrier was used in the introduction pen to protect
339 the BLV-negative post-weaning calves in the adjacent calf barn, only while the introduced
340 cattle were housed there while awaiting PCR test results. This net represented an isolated
341 management measure rather than being a part of the distance-based separation strategy. These
342 methodological differences likely explain why some distance separation failed to prevent
343 BLV transmission in previous barn-based studies, but succeeded in the present study.

344 Furthermore, the relatively small number of high-PVL cattle in this study may have
345 contributed to the reduced transmission risk. High-PVL cattle are a major source of infected
346 lymphocytes, and farms with a large number of high-PVL animals generally experience
347 greater infection pressure. Consequently, the opportunity for horizontal transmission

increases and maintaining effective physical separation within the same barn becomes more difficult. Thus, the low prevalence of high-PVL cattle in the study herd likely supported the effectiveness of the 4.8-m separation under the specific conditions of this study.

Taken together, these findings suggest that preventing vertical transmission, eliminating iatrogenic transmission, and ensuring accurate identification of BLV-positive cattle are prerequisites for evaluating distance-based control. When these measures are in place, maintaining the physical separation of at least 1 pen-width (4.8-m) within the same barn may be sufficient to prevent horizontal BLV transmission under certain farming conditions. Although the present study demonstrated the effectiveness of 4.8-m separation, further research is needed to determine the minimum effective distance under varying insect burdens and barn structures.

This study had some limitations. As the investigation was conducted on a single farm, the reproducibility of the study findings remains uncertain. Farm-specific factors, including insect burden, barn structure, herd turnover, and the frequency of high-PVL cattle movement, may influence transmission dynamics. Thus, the present findings should not be interpreted as evidence that a separation distance of 4.8-m will universally prevent horizontal BLV transmission across all farm environments. Nevertheless, the presence of high-PVL cattle throughout the study period and the farm's location in an area with abundant blood-sucking insects provide contextual support for the conclusion that a 4.8-m separation distance

may be an effective control measure under conditions similar to those at this farm. Furthermore, the 10-year observation period captured variations in management practices and environmental conditions, thereby strengthening the reliability of the findings.

In conclusion, the results of this study offer valuable practical insights for BLV eradication programs. Physical separation is a low-cost infrastructure-based strategy that can be implemented without the need for specialized equipment. Our results indicate that, when combined with accurate diagnostic testing and strict compartmental segregation, distance management may be a useful component of integrated BLV eradication strategies, particularly in herds in which vertical and iatrogenic transmission routes are effectively controlled.

Statements and Declarations

Ethics: All study procedures were approved by the Miyagi Prefectural Agricultural Mutual Aid Association (approval no. MN20170008).

Availability of data and materials: The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Conflicts of interest: The authors have no relevant financial or non-financial interests to disclose.

Funding: The authors declare that no funds, grants, or other support were received during the preparation of this manuscript

Authors' contributions: Keiichi Matsuda conceived and designed the study, collected and analyzed the data, and drafted the manuscript. Takehisa Yamamoto contributed to data interpretation and manuscript preparation. Mayumi Shiobara performed proviral load measurements. Takenori Arai contributed to sample collection. Sonoko Kondo contributed to data interpretation and manuscript revision. Atsushi Kimura and Yoshiyuki Tsuchiya contributed to manuscript revision and scientific discussion. Toshihiro Ichijo supervised the study and critically revised the manuscript. Kenji Murakami contributed to study design, supervised the project, and critically revised the manuscript. All authors read and approved the final manuscript.

Acknowledgements: The authors thank the staff of the participating farm for their

cooperation throughout the study period.

AI Statement: Generative artificial intelligence was not used for data collection, statistical analysis, or interpretation of results. AI tools were used only to assist with language editing during manuscript preparation, and all scientific content was written, verified, and approved by the authors.

References

Bai L, Borjigin L, Sato H et al (2021) Kinetic study of BLV infectivity in BLV susceptible and resistant cattle in Japan from 2017 to 2019. Pathogens 10(10):1281. https://doi.org/10.3390/pathogens10101281

Buxton BA, Hinkle NC, Schultz RD (1985) Role of insects in the transmission of bovine leukosis virus: Potential for transmission by stable flies, horn flies, and tabanids. Am J Vet Res 46(1):123–126. https://doi.org/10.2460/ajvr.1985.46.01.123

Hopkins SG, DiGiacomo RF (1997) Natural transmission of bovine leukemia virus in dairy and beef cattle. Vet Clin North Am Food Anim Pract 13(1):107–128. https://doi.org/10.1016/S0749-0720(15)30367-4

Kuczewski A, Orsel K, Barkema HW, Mason S, Erskine R, van der Meer F (2021) Invited review: Bovine leukemia virus—Transmission, control, and eradication. J Dairy Sci 104(6):6358–6375. https://doi.org/10.3168/jds.2020-18925

Manet G, Guilbert X, Roux A, Vuillaume A, Parodi AL (1989) Natural mode of horizontal transmission of bovine leukemia virus (BLV): The potential role of tabanids (Tabanus spp.). Vet Immunol Immunopathol 22(3):255–263. https://doi.org/10.1016/0165-2427(89)90012-3

Matsuda K, Arai T, Shiobara M, Kimura A, Ichijo T, Murakami K (2026) Effect of colostrum replacer containing bovine leukemia virus antibodies on infection and antibody level in

423 young calves. J Dairy Sci 109(1):584–591. https://doi.org/10.3168/jds.2025-27090
424 Matsuda K, Satou M, Oohashi S, Endou S, Muramatsu Y, Koida Y, Suzuki K (2018)
425 Measures and problems to clean bovine leukemia at clinical site. J Farm Anim Infect Dis
426 7(4):153–162. (In Japanese, English abstract available)
427 Mekata H, Sekiguchi S, Konnai S et al (2015) Evaluation of the natural perinatal transmission
428 of bovine leukaemia virus. Vet Rec 176(10):254. https://doi.org/10.1136/vr.102464
429 Murakami K, Kobayashi S, Konishi M, Kameyama K, Tsutsui T (2013) Nationwide survey of
430 bovine leukemia virus infection among dairy and beef breeding cattle in Japan from
431 2009–2011. J Vet Med Sci 75(8):1123–1126. https://doi.org/10.1292/jvms.12-0374
432 Nekouei O, VanLeeuwen J, Sanchez J, Kelton D, Tiwari A, Keefe G (2015) Herd-level risk
433 factors for infection with bovine leukemia virus in Canadian dairy herds. Prev Vet Med
434 119(3–4):105–113. https://doi.org/10.1016/j.prevetmed.2015.02.025
435 Ohshima K, Okada K, Numakunai S, Yoneyama Y, Sato S, Takahashi K (1981) Evidence on
436 horizontal transmission of bovine leukemia virus due to blood sucking tabanid flies. Jpn
437 J Vet Sci 43(1):79–81. https://doi.org/10.1292/jvms1939.43.79
438 Ooshiro M, Konnai S, Katagiri Y et al (2013) Horizontal transmission of bovine leukemia
439 virus from lymphocytotic cattle, and beneficial effects of insect vector control. Vet Rec
440 173(21):527. https://doi.org/10.1136/vr.101833
441 Oshima I, Kiyama T, Matsumoto S et al (2014) The effect of segregation in a cowshed on

horizontal transmission of bovine leukemia virus of Japanese Black steers. J Warm Reg Soc Anim Sci, Japan 57(1):31–36. (In Japanese, English abstract available)

Panei CJ, Larsen AE, Fuentealba NA, Metz GE, Echeverría MG, Galosi CM, Valera AR (2019) Study of horn flies as vectors of bovine leukemia virus. Open Vet J 9(1):33–37. https://doi.org/10.4314/ovj.v9i1.6

Pluta A, Jaworski JP, Droscha C et al (2024) Inter-laboratory comparison of eleven quantitative or digital PCR assays for detection of proviral bovine leukemia virus in blood samples. BMC Vet Res 20(1):381. https://doi.org/10.1186/s12917-024-04228-z

Suárez Archilla G, Gutiérrez G, Camussone C et al (2022) A safe and effective vaccine against bovine leukemia virus. Front Immunol 13:980514. https://doi.org/10.3389/fimmu.2022.980514

Zhao Y, Wang J, Chen J, Chen Y, Hu C, Chen X, Guo A (2025) Bovine leukemia virus: Origin, prevalence, phylogenetic diversity, risk factors, and strategies for control. Animals 15(9):1344. https://doi.org/10.3390/ani15091344

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